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# **Title:** How do fishes find the shore? Evidence for orientation to bathymetry from the non-homing sea lamprey

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Orientation to a shoreline is the critical first step for aquatic organisms that navigate to coastal waters, estuaries, and rivers to feed or reproduce. Most studies of animal migration has focused on homing-based navigation while non-homing navigation is poorly understood. We quantified the navigation behavior of sea lamprey during their non-homing return migration to a coastline in the Laurentian Great Lakes. Acoustically-tagged sea lamprey were displaced 3.3 km from shore into the center of an acoustic listening array that provided high resolution (30s intervals, <5m accuracy) 3-dimensional paths. 81% of individuals arrived at the nearest coast by moving towards shallower water. A biphasic sequence of movement was documented for most individuals, a more tortuous movement closer to the bottom associated with orientation, and a faster more linear movement we associate with directed search. Sea lamprey oriented to shallow water even when that was not the shoreward direction, and did not appear to rely on memory or recognition of the nearest coast. We postulate that individuals specifically performed barokinesis, whereby individuals assessed the gradient in absolute hydrostatic pressure on the bottom and choose a heading towards shallower water. Repeated excursions to the bottom may confirm progress, while time spent at the surface is likely associated with surface-linked olfactory cues that indicate proximity to river water entrained along the coast. This is the first evidence that suggests the shoreward gradient in hydrostatic pressure may be used during shoreward orientation, and may represent a class of sensory information not previously considered in aquatic animal navigation.

**Keywords:** Orientation, Non-homing Migration, Sea Lamprey, *Petromyzon marinus,* Barokinesis, Hydrostatic Pressure

Each year a remarkable diversity of species undertake migrations that range from a few to thousands of kilometers. Much progress has been made in understanding the navigational mechanisms that guide natal homing and breeding site philopatry (Able 1991;Alerstam 2006). However, many aquatic animals undertake relatively large movements, including search for feeding and reproductive sites, which do not involve homing (Block et al. 2011). Comparatively little is known about the environmental features and sensory mechanisms that guide the migratory movements of searching organisms over a large distance. Homing based navigation typically involves an animal ascertaining its position relative to a geographically specific goal, and movement towards the goal through the intervening topography (e.g., via map and compass); thus, the true ‘map sense’ includes a learned or inherited geomagnetic- or landmark- based map. The ability to ‘map’ has recently been extended to include any orientation mechanism that allows a migrant to orient to a specific geographic target, including other mechanisms that rely on odor-labeled paths or piloting with landmarks. Non-homing movements may rely on simple navigation, defined here as the ability to determine and maintain a course relative to environmental information (e.g., compass direction in association with a sensory cue or gradient), that leads to a characteristic habitat vs. a geographically specific site (Huijbers et al. 2012).

The ability to navigate in response to local environmental information in unfamiliar areas is key to the success of non-homing migrations (Huijbers et al. 2012). A searching animal may locate a distant target by orienting to geographically-stable environmental features (referents) that reveal the direction toward a region likely to contain the target, undertake directed movements in accordance with the referent(s) (Able 2001;Åkesson and Hedenström 2007), and use additional cues (e.g., odor) to recognize the target’s attributes upon arrival (Dittman and

Quinn 1996;Walker et al. 2002). In particular, aquatic animals that undertake non-homing movements to offshore feeding grounds and return to estuaries or rivers to spawn are faced with the task of orienting to shore. Sometimes referred to as Y-axis orientation, the ability to perform movements perpendicular to a coastline is frequently observed in freshwater and marine organisms, particularly when hydrologic conditions are unstable, and may be informed by several orientation cues (e.g., (Kough et al. 2014;Landler and von Oheimb 2013).

Orientation research has focused extensively on the use of celestial and geomagnetic compass cues, with lesser emphasis placed on currents, physical landmarks or odor (Åkesson and Hedenström 2007;Collett and Graham 2004;Lohmann et al. 2008;Nosal et al. 2016;Walker et al. 2002). Only recently has orientation to general environmental gradients, like shoreward chemical gradients associated with land-derived waters, been demonstrated in leopard sharks (*Triakis semifasciata*) (Nosal et al. 2016) and reef fishes (Paris et al. 2013). The vertical attributes of the underwater environment (e.g., hydrostatic pressure, topography) can also produce persistent gradients, but have received less attention outside of diel vertical migration research (Huebert 2008). In terrestrial systems, non-homing organisms utilize stable three-dimensional geophysical gradients to locate distinct geophysical regions associated with high resource availability. For example, the butterfly *Melitaea trivia* navigates upslope to topographical summits to locate mates (Pe'er et al. 2013), a common mate-searching strategy in insects. Interestingly, the butterflies orient and maintain course in response to the average slope using simple behavioral rules to avoid entrainment onto local ‘high spots’ (Pe'er et al. 2013). In aquatic systems, bathymetry (i.e., water depth and the shape of underwater terrain) is analogous to terrestrial topography and may similarly inform a migrant of its position with respect to the shoreline, whereby a migrant would only need to move up gradient until reaching a general target.

Although the purpose and nature of three dimensional fish movements have been studied in several cases (Arnold and Cook 1984;Barbin et al. 1998;Klimley et al. 2002;Willis et al. 2009), navigation research has remained focused on geomagnetic and visual orientation mechanisms that operate principally in association with two-dimensional surface movement.

Bathymetry comprises a variety of stimuli that indicate stable shoreward gradients including the substrate slope (via body plane orientation) (Schöne et al. 1976), overall depth, wave direction (Nishimoto and Herrnkind 1978), wave derived topographic features (e.g., sand waves) (Auster et al. 2003), and hydrostatic pressure (Blaxter 1980;Burt de Perera et al. 2005;Cain 1995). The ability to orient with respect to hydrostatic pressure (barotaxis and barokinesis) has been implicated in three-dimensional tracking movements by fishes in the lab, and in one case dominates decision-making when horizontal and vertical information come into conflict (Holbrook and Burt de Perera 2009). Determining the full set of sensory information an animal uses to orient in nature can be difficult as redundant cues are common and the animal may switch among referents depending on where the animal is during the migration (e.g., ocean, coast, estuary, or river) and proximity to the target. Orientation behavior has most commonly been studied by limiting the information available, either by altering the animals senses (e.g., blinding) or by displacing the animal into a scenario that limits the set of potential features that could be used during orientation (Emlen 1975;Fuxjager et al. 2011;Lohmann and Lohmann 1996). Recently, there has been a general call to describe orientation tactics from the statistical properties of observed movement paths in nature that reveal the potential sensory and behavioral mechanisms (Nathan et al. 2008).

This study investigates the referent(s) used for orientation to the shoreline by the landlocked sea lamprey, *Petromyzon marinus*. After a prolonged parasitic feeding stage, this nocturnal species navigates to and selects spawning rivers based on sensory cues contained in the river water, making their reproductive migration a useful lens into non-visual navigational strategies used by non-homing organisms (Waldman et al. 2008). Shoreward orientation after displacement is a natural feature of the sea lamprey's reproductive migration (Cheung et al. 2007). As a parasite, the sea lamprey is a passenger on its prey; thus, the starting location of the migration for each individual is likely determined by the host's movements, though sea lamprey may coarsely control start location through host selection or the timing of release. Evidence from an anadromous population suggests the animal arrives at the coastline nearest the feeding grounds of its host, indicating an efficient orientation mechanism towards shallow water (Lanca et al. 2014). How lampreys orient and move shoreward in lakes and oceans is undescribed. Sea lamprey could simply employ a common habit, such as westward movement for animals migrating in to the western Atlantic basin that would return individuals to shore. The shoreward migration takes place in early spring, a time when coastal waters can reach more than 3 kilometers from shore (November to June) and before a hydrographically distinct coastal zone that limits mixing with offshore waters is established (Auer and Gatzke 2004;Rao and Schwab 2007). Consequently, shore-derived cues (e.g., odors) could potentially form a rough gradient from offshore to inshore that could aid in navigation towards shore. Once reaching the coast, migrating sea lamprey move parallel to the coastal edge (i.e., coastline), casting from surface to bottom while searching for river plumes (Meckley et al. 2014b;Vrieze et al. 2011). River selection is based on an ecological legacy rather than a natal preference acquired at birth (Waldman et al. 2008); the odor released by larvae growing in the river that were deposited by previous generations of migrants acts as a cue to new migrants at the river mouth, and informs them that the river contains suitable reproductive sites upstream (Teeter 1980).

To examine our hypothesis that sea lamprey orient and return to the coast by following the bathymetric gradient, we evaluated six research questions, informed by the examination of 23 high resolution three-dimensional movement paths collected in the offshore environment of Lake Huron, USA. We first assessed whether migrants arrive at the nearest coast, indicating the occurrence of orientation to the nearest coast (Q1). We then statistically evaluated the movement tracks for evidence of two distinct movement processes (biphasic pattern) indicative of orientation followed by directed-movement (Q2), and whether the directed movement phase heading was related to where the sea lampreys were captured (i.e., prior experience) (Q3), associated with movement in a consistent direction (i.e., entrainment on a common default cue) (Q4), or counter to the local bathymetric gradient (versus the nearest coastal edge) (Q5). Orientation based on some form of memory (Q3), awareness of the nearest edge through an unknown feature (e.g., olfactory gradient, auditory or visual cues), or entrainment on an innate default heading (Q4), all represent alternative hypotheses. Use of a shoreward olfactory gradient was not independently tested (i.e., no olfactory occlusion was performed and evidence of the direction of shoreward odor gradients was not measured). Finally, we evaluated the timing, locations and extent of vertical movements through the water column, a known sea lamprey behavior near shorelines (Meckley et al. 2014b;Vrieze et al. 2011), to assess the potential use of hydrostatic pressure by the lamprey (Q6). If sea lamprey utilize pressure to move towards the coast and then search for other cues that are stratified at the surface, we would expect sea lamprey initially to spend time on the bottom before moving vertically through water column, spending time at the surface and bottom, after a direction was selected. We chose a study location within an embayment where the direction to the nearest shore was approximately orthogonal to the local bathymetric gradient, rather than the normal circumstance where the nearest distance to shore correlates with the bathymetric gradient towards shallower water. Confirmation of both biphasic movement and movement towards shallower water, but not towards the nearest coastal edge, was deemed necessary to support the hypothesis of bathymetric orientation.

## Materials and Methods

### *Telemetry Array*

We deployed a 43-receiver acoustic telemetry positioning array (VPS, Vemco, Nova Scotia, Canada) in Hammond Bay (Lake Huron, Michigan, USA, 45.527799 N, -84.044466 W) in an area centered 3.3 km offshore with shallow bathymetric contours leading to the south and west (depth range, 21-39 m; Figure 1), which creates a scenario where the nearest direction to the coast does not correlate with the direction to shallower water. Vemco VR2W receivers were spaced 275 m apart in diamond formations with an internal array area of 3 km$^2$. Each receiver was anchored 3 m from the lake bottom and its position determined via high-precision GPS (Trimble GeoXH and Tornado antennae) at deployment and retrieval and one additional occasion during low wave conditions. Nine synchronization transmitters (VEMCO model V16-2H, 69 kHz, 160 db, 500 to 700 s transmission interval) were co-located with receivers to maintain time synchronization of the receiver clocks. Bathymetry in the detection region of the array was measured along transects spaced 50 m apart leading northwest to southeast, and then repeated crisscrossing the first grid in a southwest to northeast direction. 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.

In addition to the VPS data that provided 3-D transmitter positions, transmitters were detected by receivers located on the coast that only record transmitter depth and indicated a relative proximity to that receiver, but did not provide positions. Transmitter detections were obtained from six additional receiver locations along the coastline of Lake Huron and operated by the Great Lakes Acoustic Telemetry Observation System (GLATOS; Figure 1). Solitary receivers were positioned near the mouths of the Black Mallard (BM, 45.532888, -84.120801) and Ocqueoc (OCQ, 45.491893, -84.071879) Rivers. Receiver strings extending perpendicular from shore were treated as single detection points and were located near the mouth of the Cheboygan River (CHB, 45.67239, -84.429368, 5 receivers), 40 mile point (FMP, Outer receiver: 45.507563, -83.901379, 3 receivers), Presque Isle (PRS, Outer receiver: 45.333842, -83.458343, 3 receivers), and Detour Pass, which leads into the St. Mary's River (SMR, West side of Drummond Island, centered at: 45.984929, -83.891787, 6 receivers). 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 of a single tag in Hammond Bay under very 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 for V9 tags are unlikely (F. Smith, VEMCO, Personal Communication, 20 June 2014), and none were observed during fixed range testing. The detection range of a receiver placed in < 3 m of water directly in front of the Ocqueoc and Black Mallard River outlets, as was done in this study, is likely lower than observed in the range test, because the receiver was located in the wave zone, an acoustically noisy area with reduced line of sight to receivers. We do not expect this was a substantial problem as both of these river mouths were small (2-10 m wide). A nearest neighbor filter of 30 minutes, which removes a position from the dataset if it does not occur within 30 minutes of another position for the same tag, was used to remove spurious detections and rare detections near the maximum extent from the GLATOS detection data.

### *Experimental Subjects*

Sixty-seven female sea lamprey were released in total. Only female sea lamprey had a large enough body cavity to accommodate the acoustic tag, due to their large interstitial area designated for gonadal development. Fifty-seven sub-adult (post-feeding, sexually immature) actively migrating 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)). An additional ten parasitic-phase lampreys were captured while attached to fish in the lake (Lake Huron, Hammond Bay, captured January 03 - February 28, 2012). River capture locations were chosen to include a range of east-, west-, and south-discharging rivers to allow for the examination of past migratory experience on the observed movement patterns when the animals were displaced into a common location. All Sea lamprey were held from the time of capture in 150 L flow through tanks that cycled ambient Lake Huron water (5.2-12.3 ˚C; 100% water exchange every 2 h) and experienced a natural light cycle.

### *Tagging and Release Procedures*

We surgically implanted 67 subjects with one of two types of acoustic transmitters that emitted signals every 15-45 s (mean = 30 s). Forty-nine animals received a tag that transmitted

2D position and depth via a pressure sensor (Vemco model V9P-2H, 9 mm D x 47 mm L; mass, 6.4 g in air, 3.5 g in water; power output 150 dB (re 1 μPa at 1 m); pressure sensor (depth) 0.37 m accuracy, 0.22 m resolution). The remaining 18 received a tag that transmitted horizontal position only (Vemco model V9-2H; 9 mm D, 29 mm L; mass, 3.6 g in air, 2.2 g in water; power output 151 dB (re 1 μPa at 1 m)). Sea lampreys ranged 273-577 mm in length (mean 499 mm) and weighed 139-398 g (mean 274 g). Prior to surgery, each lamprey was anesthetized by immersion in 0.2 $\mathrm{mL \cdot L^{-1}}$ clove oil solution (minimum 84%-88% eugenol, Lot No. HB9387, Hilltech Canada Inc. Vankleak Hill. Ontario, Canada). 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 ± 1 SE, 559 ± 14.2 s, maximum 846 s) (Keene et al. 1998). The surgery was performed in a plastic pipe with continuous water flow to maintain gill irrigation by completely submerging the head and gills. The transmitter was inserted 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 monocryl monofilament) and each knot was sealed with veterinary adhesive (Vetbond, *n*-butyl cyanoacrylate adhesive). The surgical procedure took an average of 283 ± 4 s to complete (mean ± 1 SE, range 220-409 s). Each subject was monitored in a postoperative holding tank until the animal regained equilibrium and began natural swimming movements (recovery time, mean ± 1 SE, 360 ± 28 s, maximum 1240 s). Transmitter-implanted subjects were held for 72 h prior to release to ensure metabolism of stress compounds (Close et al. 2003).

On each release day, three tagged sea lampreys were transported to the field site in aerated coolers and released into the center of the acoustic array at ~15:00 (09:00 - 17:30 EST) after being lowered in a release cage at a rate of 6 m·min$^{-1}$. The decent rate was approximately one third that observed from three free-swimming lampreys released at the surface during preliminary trials. The cage opened at a fixed depth of 30 m (within 5.7-7.2 m of the bottom) when pressure-sensitive release clamps were automatically triggered.

*Data Processing*

The VEMCO VPS system provides a two-dimensional position for each tag transmission, derived from raw detections of a coded signal at multiple receivers by a proprietary hyperbolic positioning algorithm (Meckley et al. 2014a;Smith 2013). Each recorded transmission consists of a transmitter code that is unique to each fish tag, the time of the signal transmission, and the water pressure experienced by the transmitter at the time of transmission, which indicates the depth of the transmitter. Transmitter readings are in whole PSI values (no decimals) equating to a 0.22 m resolution. Transmitters were pressure tested in a pressurized PVC pipe to five PSI levels (0, 5, 10, 15, 20, 25), equivalent to depths of 0-18 m. Transmitters were tested to ensure accuracy prior to fish implantation and had an average accuracy of 0.39 m, with a max of 0.88 m error. Error values were usually consistently erroneous across depths, taking the form of a consistent offset. The horizontal position accuracy of this VPS array was tested by comparing the VPS position estimates to post-processed GPS measured positions (Trimble Geo XH) 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 potential for positioning error to artificially increase 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 positions in which position confidence is low (Meckley et al. 2014a;Roy et al. 2014). The average step length (i.e., the distance between two consecutive points), was 22 m in calm wave conditions based on sea lamprey movement speeds and transmitter transmission rate, 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 (See Meckley et al. 2014a for more information). We selected an HPE filter of 15.17 for the fish data, removing all positions that exceeded the criteria (3223 positions of actively moving sea lamprey were removed representing 22% of all active positions; see S1 for more detail). For those lampreys fitted with pressure-sensing transmitters, total water column depths at fish positions were interpolated from the georeferenced depth data using inverse distance weighting ("itnterp", package Akima) (Gebhardt et al. 2013).

Once individual positions were evaluated, a decision was made on whether to retain each animal's movement path for analysis based on the animal's behavior following release. We analyzed in detail the movements of those animals that stopped in the array and exited within three nights (N=22). The individuals that were not observed exiting the array because they left during the day (N=30), moved to the edge of the array (i.e., those animals that moved normally but produced inaccurate tracks too short to determine behavior; N=5), or did not move on the first night (N=10), were dropped from the study. Those that did not exit the array on the first night were removed under suspicion of post-tagging effects, analogous to dropback in riverine tracking studies (Frank et al. 2009). Of 10 fish that did not exit the array on the first night, 7 were from the Manistee and Betsie rivers. The remaining 22 fish used in analyses came from the

Cheboygan river (N=8/21), Manistique river (N=6/21), Ocqueoc river (N=4/4), or were trapped as large parasites and held until spring (N=3/10).

To classify sea lamprey positions as active or stopped we performed a first passage-time classification method with R (R Development Core Team 2016). 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 (“fpt”, package adehabitatLT) (Calenge 2006). Initial values were selected based on the sea lamprey’s known movement capacity and the performance of the array. The accuracy of labeling each position as “moving” or “stationary” 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.

### *Data Analysis*

*(Q1)* Do migrants arrive at the nearest shoreline?

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. The time frame was selected to ensure that subjects could not have reached another coastline prior to being observed on the nearest coast. Lamprey observed on the southern coast, were classified as successfully navigating, with the null expectation that absent an orientation mechanism and ability to maintain course, the probability of random success would be 50%.

*(Q2)* Do migrants exhibit two-phase movement consistent with navigation, including orientation and directed movement?

To test for biphasic movement (sinuous movements followed by extensive straight-lime swimming), we used sinuosity to separate the phases then evaluated whether trajectory parameters (i.e., speed and sinuosity) significantly differed between the postulated phases. We evaluated the paths of 22 sea lamprey from the commencement of movement following a settled state until exiting the detection range of the VPS array. 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 (Figure 2). A broken stick model was fit estimating four shape parameters (initial slope ($\beta_1$), break point ($\tau$), sigma (σ), and final slope ($\beta_2$)) and a first order autocorrelation in the residuals. In the broken stick model backward path length ($x$) is the explanatory variable for estimating the response variable backward beeline distance ($y$). The y intercept ($\beta$ ) is fixed at 0 due to the nature of the parameter always returning to (0, 0). The model used:

$$y = y + \varepsilon; \;\; \varepsilon \sim \text{AR1}$$

$$y = \beta_1 x_1 + \beta_2 x_2 + \varepsilon$$

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

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

When $x \leq \tau$ the model is linear with slope $\beta_1$ and when $x > \tau$ the model is linear with slope $\beta_2$ 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 (Webber et al. 2008). Serial autocorrelation in the residual term is accounted for by fitting the first-order autoregression process (AR(1)). We used generalized least squares estimation (Pinheiro and Bates 2000) to estimate the parameters of this model with gnls package in the nlme R package (Pinheiro et al. 2013).

We first tested for evidence of a broken stick model against a linear ($\beta_1 = \beta_2, \tau = 0$) null model with a likelihood ratio test (2 degrees of freedom). Those individuals for which the broken stick fit the track better than a linear model were further analyzed (n=20). We individually characterized differences in trajectory parameters that were not directly associated with track sinuosity (e.g. ground speed, turning bias, average depth, and variation in depth) between phase one (before break point) and phase two (after break point) using two sample t-tests. To make more general inferences about behavioral changes, we fit nonlinear mixed-effects models with the respective covariates as fixed effects and individual lamprey as a random effect. We predicted that if a distinct post-settlement orientation phase occurred (phase one), the initial behavior would be accompanied by slower more sinuous movement followed by straighter faster movement associated with directed search (phase two).

*(Q3) Did the river of origin associate with the direction of orientation?*

To determine if the lamprey's capture river influenced orientation, the distribution of Phase 2 headings between sea lamprey from different river sources were compared using a Watson-Wheeler test ("Watson.wheeler.test", package circular) (Lund et al. 2013) and the differences in the distance traveled during Phase one were considered (two sample t-test). We compare the sea lamprey trapped in the Manistique River and Cheboygan River. If the river they were trapped in has 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 would be that the Phase two heading would be more southerly towards the local coast, than the Manistique source subjects. If a sea lamprey relies on local information to orient, we predict there will be no effect of river source on orientation.

*(Q4) Is there evidence for orientation towards a consistent direction?*

To determine if sea lamprey are entrained in any significant mean direction, we utilized a Raleigh test based on an unspecified mean ("raleigh.test", package circular, (Lund et al. 2013)) or a significant non-uniform distribution via a Watson's test ("watson.test", package circular) (Lund et al. 2013) for the portion of tracks between the transition point and departure point from the array. Together, these test provide evidence, respectively of a significant non-uniform distribution of headings (goodness of fit to a circular uniform distribution - Watson's test), and significance of a mean heading given a non-uniformity (z-test statistic - Raleigh test). If sea lamprey orient to a consistent feature, such as a shoreward odor gradient or less likely through seeing or hearing the coastline, we would predict sea lamprey to have a significantly clustered mean direction and a non-uniform distribution. If sea lamprey are not orienting, are orienting to multiple cues at different times, or are orienting to a locally varying feature such as bathymetry in our array, we predict sea lamprey will not have a significantly clustered mean heading or a non-uniform distribution.

*(Q5) Do sea lamprey orient according to a bathymetric gradient, regardless of the direction to towards the nearest coast?*

To evaluate if sea lamprey orient to shallower water with an aspect of the local bathymetry (i.e., slope, water depth, terrain) vs. the nearest shoreline (odor gradients, visual, or auditory cues), we evaluated the orientation bearing of each individual during phase 2, defined as the portion of tracks between the transition point and departure point from the array. We tested to see if sea lamprey move towards either the nearest coastline (coast test) or towards shallower water (bathymetry test). For the bathymetry test, the bathymetric heading, or in other words the bearing of the local bottom slope around the sea lamprey's track, 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 last measured position to capture the general bottom slope around each sea lamprey track leading to the exit of the array. After all bathymetric headings were verified visually on a map, the heading was subtracted from each subject's phase two headings (directed swimming phase), so that a bathymetry test heading of near $0^{\circ}$ (or $360^{\circ}$) indicated that the sea lamprey was moving in the direction towards shallower water and $180^{\circ}$ represented moving in the direction of deeper water. If sea lamprey are orienting in the direction of the local bathymetric slope towards shallower water we predict the Rayleigh test with specified mean of 0 will be significant and a Raleigh test with a specified mean of $172^{\circ}$ for the test of movement towards the nearest coast, will be non-significant.

*(Q6) Is the timing of vertical casting behavior related to information used during navigation?*

When moving alongshore and in river plumes, sea lamprey have been observed swimming vertically from the surface to bottom (Meckley et al. 2014b;Vrieze et al. 2011). We characterized the onset and nature of this vertical component of movement. First, we evaluated how vertical movements contrasted across the different phases of movement. Second, we evaluated if the timing of onset of vertical movements was consistent with orientation to information at the bottom (i.e., initial movement on the bottom). Third, if a directional phase was observed, then we evaluated if the vertical movement is comprised by consistent oscillations through the entire water column suggesting that the movement could be metering some aspect of depth, which would require the rate or angle of ascent and descent to be similar (e.g., Klimley et al. 2002). Alternatively, if sea lamprey appear to spend time principally at the surface and bottom during the directional phase, it would indicate information at the surface and bottom is most important to navigation to the coast.

To assess differences between phase one and two, whether movement was initially at the bottom, and whether the vertical movements represented constant and consistent oscillations, we had to classify vertical movements. To classify the vertical movements of sea lamprey through the water column, each step (two consecutive positions) was assigned to one of three classes of vertical movement (ascent, descent, or horizontal movement) based on pitch (descent $< -5^{o}$, horizontal $\geq -5^{o}$ and $\leq 5^{o}$, ascent $> 5^{o}$), which is the acute angle between a horizontal axis and the step length slope (i.e., angle of climb). Because sea lamprey move from surface to bottom during each phase, as defined by the broken stick model, we characterized vertical movement with the time spent moving horizontally within each phase, the depth of sea lamprey when moving horizontally in each phase, and the number of dives, defined as the number of times sea lamprey ascended and descended at least 10 m in succession.

To characterize differences between phase one and phase two and to consider if horizontal movement was principally on the bottom during phase one. Individual two-sampled t-tests for each sea lamprey subject were checked for differences in depth between Phase 1 and 2 during horizontal movements (horizontal $\geq -5^{o}$ and $\leq 5^{o}$), whereby replicates are comprised by each step length for the individual. Significant differences for most individuals would be indicative of a common pattern of difference between phase one and phase two and we would expect most individuals to spend more time moving horizontally during phase one and for the average depth to be near the bottom if absolute pressure (i.e., information at the bottom) is being used.

To test whether sea lamprey could be metering the depth (i.e., based on distance or time) with consistent ascents and descents, we tested if there was a difference in the rate of vertical displacement or pitch during ascent and descent (i.e., evidence against behaviors for metering depth, See Klimley et al 2002), we used three individual logistic regression analyses with a single response variable each, including time moving horizontally, pitch angle ($^{o}$), or vertical displacement rate (m/s), respectively, that was fit using a generalized linear mixed effects modeling framework using individual lamprey as a random factor. For testing for differences in time spent moving horizontally in each analysis, we used the movement phase as a fixed effect, and we weighted the model by the number of observations to emphasize the cases where we had more extensive observations. For testing displacement rates and pitch during ascent and descent, absolute value of ascent and descent (pitch and vertical speed) were fixed effects. In addition, a visual evaluation of where sea lamprey were located in the water column was completed to review whether sea lamprey appeared to spend equal time throughout the water column during phase two or if time appeared to be mostly at the surface and bottom.

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, following the observation during a preliminary review of the data that some sea lamprey did move vertically during the day but did not move the entire way to the surface. This was the only aspect in which data for sea lamprey that did not stop immediately after release were used, and was done to consider the condition of these individuals. All analyses were performed in R (R Development

Core Team 2016), including the lme4 package (Bates et al. 2014), for fitting the mixed effects model.

*Ethics Statement*

All handling, tagging, and release procedures were consistent with ethical treatment guidelines and approved by the Michigan State University Institutional Animal Use and Care Committee via animal use permit 04/07-033-00.

## Results

Of the 67 tagged sea lamprey, all of the individuals were analyzed for arrival on the coast, while the path trajectories were only evaluated for the 22 sea lamprey that stopped in the array following release and left on the first night. Sea lamprey that did not stop (n=30), included eight sea lamprey that exited the array on the bottom and 22 that showed vertical movements of at least 10 m off of the bottom. These 22 individuals moving vertically during the day were included in one additional post hoc analysis of proximity to surface while swimming.

### *(1) Migrants arrived at the nearest shoreline*

Of the 22 sea lamprey that rested in the array, 17 (81%) 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 1). If considering all subjects, 43 of the 67 (61%) sea lamprey were detected on the nearest coast (Table 1). Sea lamprey appear to be orienting to information leading to the nearest coast.

*(2) Two-phase movement occurred, consistent with orientation and directed movement*

The analysis revealed a biphasic pattern consistent with navigation, for 20 of the 22 sea lamprey (Figure 2, S2). The remaining two individuals immediately departed in an apparent straight line without any sinuous movement (T26 and T44, S2) and a broken stick model did not fit better than a linear model in our backward beeline analysis (Table 2). Phase one persisted for an average track length of 805 m ± 126 m (mean ± 1SE, interquartile range -IQR- 456-880 m). Phase two lasted 1038 m ± 125 m (IQR 700-1341 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 2, Table 3). During phase one, 8 of the 20 total individuals turned with a left-bias while 3 individuals were right-biased. The magnitude of persistence in turning of biased individuals was greater before than after the transition (Figure 3, Table 2, Theta). Sea lamprey turned less during the phase two though this could be confounded by the fact that the break point was defined in terms of the path sinuosity (Table 2). Mean depth was shallower after the break point for 14 of 16 individuals and the standard deviation in depth was significantly greater for 5 of the 16 individuals vs significantly reduced for 2 of 16 individuals, who appeared to swim near the surface without surface to bottom movements during the directed search phase. Lastly ground speed was significantly faster for 17 of 20 individuals during phase two (mean: 0.50 $m \cdot sec^{-1}$) when compared to phase one (mean: 0.35 $m \cdot sec^{-1}$) (Table 3). The mixed effects models on movement behaviors across the two phases with individual lamprey as a random effect and accounting for serial autocorrelation in the residuals (S3), confirmed these patterns. In phase one, lamprey moved significantly more slowly (0.13 ± 0.1 m/s slower (coef. mean ±1 se); $p < 0.01$), at significantly greater depths (6.8 ± 1.2 m deeper; $p < 0.01$), and with significantly less standard deviation in depth (-1.1 m/s ± 0.4 m; $p < 0.01$).The array captured two phases of movement consistent with orientation followed by directed search.

*(3) The river of origin did not relate to the direction of orientation*

If past experience related to orientation, sea lamprey trapped in the same river should orient in a similar direction due to common behavior or common experience and fish trapped from a river with a coastline facing a similar direction as our study site should orient more quickly. The Watson-Wheeler test of homogeneity of angles found no significant difference effect of what river the sea lamprey were trapped in, although the strength of this conclusion is not high when sample size is less than 10 for each group ($W = 1.2$, $p = 0.56$). There was no significant difference between the distances traveled prior to the break point for Cheboygan source (mean ± 1SE, 965 m ± 207 m) or Manistique source fish (mean ± 1SE, 1140 m ± 333 m) ($p$=0.66). The direction sea lamprey headed during phase two was not related to the origin of the sea lamprey in this study.

*(4) Directed movement was not in a consistent direction*

If sea lamprey were preprogrammed with a navigational map, sea lamprey would head in a consistent direction based on magnetic information. During phase two, sea lamprey assumed an average absolute heading of $251 \pm 96^{\circ}$ (95 % CI: $213\text{-}289^{\circ}$). Sea lamprey did not show an absolute orientation in any consistent direction ($r = 0.37$, $p = 0.06$; Rayleigh Test, unspecified mean), although the p value was near significant and their circular distribution was not significantly different from a random (test statistic = 0.17, critical value at a significance of 0.05 = 0.19; Watson's Test). Displaced sea lamprey did not move in a common heading during phase two, although the average heading that was nearly significant. The average heading was generally towards shore (not near the nearest coastal point) and appears in the average direction towards shallower water if visually assessing bathymetric contours across the entire array.

*(5) Sea lamprey moved towards shallower water but not the nearest point on the coast*

In the region of the coast sea lamprey were released, if sea lamprey moved toward the nearest point to the coast we would expect they are relying on visual, auditory, or olfactory information associated with the coastline, while if they just moved towards shallower water, it would indicate they are relying on a feature of bathymetry (e.g., pressure). There was no evidence that sea lamprey were oriented towards the nearest coast ($r = 0.10$, $p = 0.27$; Rayleigh Test, specified mean: 173°; Figure 4). By contrast, phase two headings (mean: 357°, 95% CI: 295-54 °), were not randomly distributed with respect to bathymetry (Watson's Test: test statistic= 0.58, critical value= 0.19, reject null hypothesis; Figure 4) and were significantly oriented to local bathymetry in the region where they exited the array ($r = 0.41$, $p < 0.01$; Rayleigh Test, specified mean: 0°). Combined this information indicates that sea lamprey moved towards shallower water during phase two, but not towards the nearest coast.

*(6) Vertical excursions increase after the orientation phase*

During phase one, sea lamprey primarily moved on the bottom and made occasional vertical excursions to the surface (Figure 5). During phase two, sea lamprey made more vertical excursions and spent more time at the surface than the bottom (Table 3), however most observations occurred with sea lamprey either at the surface or bottom rather than traversing through the water column (Figure 5). At the transition between phase one and phase two, the animal begins to ascend to the surface and return to the bottom, building from partial to full excursions through the water column (e.g., Figure 6).

Mixed effects logistic regression modeling revealed that sea lamprey spent significantly less time moving horizontally in the water column during the phase two than during phase one (-0.40 (40% less time), $p < 0.01$), and more time moving vertically through the water column. Mixed effects logistic regression revealed that there was no significant difference in the pitch of sea lamprey during ascent or descent ($p = 0.093$), but there was a difference in the vertical rate of ascent and descent, as sea lamprey descended more quickly than they ascended ($p < 0.01$). 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 ± SD; Day: 10.4 m ± 5.4m, Night: 2.6 m ± 3.6 m). Sea lamprey moved vertically throughout the entire water column at night (e.g. Figure 6). On average they made 7 ascents or descents of at least 10 meters (6.7 ± 3.2 dives per h during orientation; 7.3 ± 4.5 dives per h during directed search). Sea lamprey were observed maintaining a straight course (no turns $>15^{o}$) 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 (S4). Sea lamprey ascended at a pitch of $15.0 \pm 7.8\ ^{o}$ and vertical speed of $5.1 \pm 2.8\ m\cdot min^{-1}$ during the phase one and a pitch of $13.0 \pm 6.8\ ^{o}$ and vertical speed of $5.6 \pm 2.5\ m\cdot min^{-1}$ during phase two. Sea lamprey descended at a pitch of $14.9.0 \pm 8.0\ ^{o}$ and vertical speed of $7.1 \pm 7.9\ m\cdot min^{-1}$ during phase one and a pitch of $14.9 \pm 7.7\ ^{o}$ and vertical speed of $8.3 \pm 5.2\ m\cdot min^{-1}$ during phase two.

## Discussion

Establishing how animals perceive and make use of environmental information to orient in nature is important to understanding how sensory capabilities contribute to the evolution of navigation strategies. This study takes the first step toward understanding the contribution of sensory information gathered at depth to the common challenge of shoreward orientation, using the non-homing nocturnal sea lamprey as a model. High-resolution telemetry observations revealed the sea lamprey undertakes a biphasic movement process each evening consistent with orientation towards shallower water, but not necessarily the nearest point on the coast, followed by directed swimming, resulting in arrival at the coastline. At the onset of movement (phase one), sea lamprey undertook a relatively slow, sinuous path on the bottom, moving across the local bathymetric gradient, followed by directed swimming counter to the bathymetric gradient (phase two). At the transition between orientation and directed swimming, the sea lamprey began to cast vertically through the water column, building from partial to full excursions that persisted until the animal passed out of the detection range of the array. During directed swimming, the sea lamprey also increased ground speed and steered a straight course. We postulate that sea lamprey used the spatial gradient in absolute hydrostatic pressure sampled on the bottom during the orientation swim to choose a heading towards shallower water, and partly maintained orientation during directed swimming with repeated excursions to the bottom to confirm ever-shallowing depth (i.e., barokinesis). Further, we suggest the time spent at or near the surface was associated with the search for surface-constrained olfactory cues that indicate entry into the hydrographically distinct coastal zone that contains river-derived odors, and may also contribute to avoidance of habituation of the depth-sensing apparatus. Persistent gradients in hydrostatic pressure associated with the sloped bathymetry of the coastal zone may represent an important class of sensory information not previously considered in fish movement studies.

The observed shoreward orientation movements were most consistent with barokinesis, whereby individuals moved across the local depth gradient until they perceived the direction towards shallower water by detecting changing hydrostatic pressure. Animals may achieve shoreward orientation at depth through a variety of environmental referents that exhibit stable shoreward patterning including detection of changing depth via hydrostatic pressure (Blaxter 1980;Burt de Perera et al. 2005;Cain 1995), detection of the substrate slope via body plane orientation (Schöne et al. 1976), or orientation to features that are consistently aligned with the shoreline (e.g., sand waves, an innate or learned magnetic direction preference)(Auster et al. 2003;Schlegel and Renner 2007). Orientation to the substrate slope via body plane or topographic features was unlikely. Orientation involved active swimming over tens to hundreds of meters, whereas organisms that orient via body plane are often walking on the substrate (Craig 1973;Schöne et al. 1976). Further, the study site contained a monotonous landscape that lacked consistent shoreward-aligned topographic features, like sand waves, and the movement took place in darkness, precluding visual orientation. Finally, the study generated no statistical evidence for a consistent tendency toward a dominant compass bearing across individuals, nor for selection of a consistent direction in animals taken from the same source (river). The orientation movements instead appear consistent with a kinesis in response to hydrostatic pressure, as hydrostatic pressure is a non-directional feature and requires movement across a gradient of depths to determine directionality. This stands in contrast to a taxis that includes orientation to a cue that instantaneously provides directional information (e.g., flow direction via rheotaxis). Barokinesis falls within the general category of klinokinesis (Benhamou and Bovet 1989;Kennedy 1976), as sea lamprey changed turning frequency in response to movement across the depth gradient.

Though appealing, our proposed explanation hinges on the animal's ability to detect changing absolute hydrostatic pressure. Other 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 (Bleckmann and Zelick 2009;Fraser 2002;Fraser et al. 2008). Sea lamprey lack a swim bladder and have a primitive lateral line, and an inner ear that lacks a lagena (otolith endorgan). However, the sea lamprey inner ear does exhibit semicircular canals in an orthogonal configuration that is structurally similar to the vestibular apparatus of other pressure-sensitive fishes (Fraser et al. 2008;Hammond and Whitfield 2006;Khorevin 2008). Such configurations, can detect pressure changes on the order of 0.5-2.0 kPa in small aquaria, corresponding to a change in 5-20 cm of depth (Blaxter 1980). At greater depths, the ability to detect changes in hydrostatic pressure may be subject to Weber's Law via the magnitude effect (Gallistel and Gelman 2000), whereby the detectable difference in pressure is directly related to the magnitude of the absolute pressure. If operating, the animal would be required to move over a greater depth range in deep versus shallow water in order to detect differences in pressure, potentially explaining the large displacements observed during the orientation swim.

At the transition from the orientation swim to directed movement, periodic dives from surface to bottom were typical, with more time spent on the surface than near the bottom. This behavior has been previously observed close to shore and in river plumes (Meckley et al. 2014b;Vrieze et al. 2011), but appears to cease once the animal enters a river (Holbrook et al. 2015). Such vertical movements have generally been associated with locating or using a search cue (Klimley et al. 2002;Westerberg 1982;Willis et al. 2009). Specifically, it has been suggested the sea lamprey casting behavior is associated with a search for river plumes that contain a conspecific cue (larval odor) that labels rivers with suitable spawning and rearing habitats (Meckley et al. 2014b;Vrieze et al. 2011). In the coastal ocean and in estuaries, where this behavior likely evolved, fresh and mesohaline plumes are reliably constrained to the surface due to strong density differentials driven by salinity. In the Great Lakes, density differentials are generally weaker and driven by temperature; plumes are generally well-mixed vertically, but constrained nearshore by a thermal bar that establishes between the open lake and the shallow coastal zone in spring (Auer and Gatzke 2004). Our array was positioned close enough to shore to be exposed to shore-derived waters. Thus, it is likely the subjects of this study were subject to some river-borne odorants, but likely did not encounter odor from the nearest river plume during telemetry observation. We cannot preclude the possibility that sea lamprey rely on orientation toward persistent shoreward chemical gradients associated with land-derived waters, as was recently demonstrated in leopard sharks (*Triakis semifasciata*) (Nosal et al. 2016) and reef fishes (Paris et al. 2013). Sea lamprey have a remarkable ability to discriminate chemical signals at very low concentrations, including river water (Siefkes and Li 2004;Sorensen and Vrieze 2003). However, the lack of observed movement towards the nearest coast and the dynamic nature of the currents in spring (Rao and Schwab 2007) suggest against orientation to an odor gradient.

We postulate the dives to the bottom were associated with the animal monitoring its progress by sampling hydrostatic pressure at the bottom to ensure a shoreward course, and the greater time spent on the surface was associated with a search for river plumes. Diving to evaluate whether depth is decreasing should entail energetic costs as the overall path length is increased, and subjects did not spend more time falling than rising, precluding any obvious biomechanical or thermoregulatory advantage (Carey et al. 1990;Katz 2002;Klimley et al. 2002). Whether lampreys exhibit this behavior in deep waters, where the swimming costs may be substantial, is unknown. The use of hydrostatic pressure to maintain track orientation is appealing as it also offers an alternative explanation for the previously observed vertical casting that occurs when the animal moves along the coast. Specifically, the hypothesis that vertical casting is related to a search for river plumes constrained to the surface or the bottom (Vrieze et al. 2011). Sea lamprey maintain tracks that are remarkably parallel to the coastline at night (Meckley et al. 2014b). If the animal utilizes hydrostatic pressure to guide navigation, it may switch from moving towards shallow water to moving along a fixed depth contour after it arrives at the shoreline, allowing for maintenance of an efficient course in concert with the shoreline without the use of visual cues. It also would provide the animal with a means to combat unintentional displacement towards or away from shore due to shifting currents; a problem common to a wide variety of migrating aquatic organisms (Lohmann et al. 2008;Luschi 2013).

During the directed swimming phase, the lampreys maintained a straight course towards shallower water despite moving vertically through the water column and away from the bottom. Absent consistent external directional information, animals are often incapable of maintaining a straight course toward a target (Åkesson and Hedenström 2007;Cheung et al. 2007). Geomagnetic fields and water current direction are the two most common nocturnal navigational stimuli for maintaining a straight heading (Able 1991;Alerstam et al. 2003). However, water currents in the Great Lakes are wind-driven and highly variable in direction (Beletsky et al. 1999), making for an inconsistent directional guide. Current velocities are also typically weak (< 2 cm/s) (Beletsky et al. 1999), which may have contributed to the straight movement paths as the animals were less likely to be deflected during the observation periods. It is unknown whether sea lamprey are capable of magnetic compass orientation, though utilizing a magnetic compass sense to maintain a selected heading would be energetically beneficial even at relatively small spatial scales (Wyeth 2010). A geomagnetic compass sense is frequently implicated in homing navigation exhibited by fishes and sea turtles (Lohmann et al. 2008), and should be tested for in this basal vertebrate. If sea lamprey can maintain course using a geomagnetic compass, a frequently observed capacity in migrating marine organisms at both small and large scales (Lohmann et al. 2008), it would provide a persistent and depth-independent cue for maintaining a straight course.

If sea lamprey are relying on pressure, transiting between the bottom and surface to monitor depth along the movement track requires individuals to recall pressures experienced during previous dives over time spans of minutes to tens of minutes. This task is well within the general cognitive abilities of fishes (Odling-Smee et al. 2007). For example, the electric elephantnose fish (*Gnathonemus petersii)* is capable of learning hydrostatic pressures associated with vertical landmarks and using the pressure gradient to orient to the landmark (Cain 1995). Some fishes have been shown to be 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). The strongest evidence for the ability of fishes to remember short term experiences may be that fishes contain similar forebrains to more advanced vertebrates, a region responsible for spatial memory (Rodríguez et al. 2002). Sea lamprey observed moving on two subsequent nights reoriented each night, suggesting they did not retain a spatial map associated with water pressure.

Limited observations of ocean run lamprey during the return migration are consistent with counter-bathymetry movement when returning to the coast. Both Pacific (*Entosphenus tridentatus*) and sea lampreys are neither panmictic nor philopatric (Hess et al. 2013;Spice et al.

2012;Waldman et al. 2008), suggesting that some aspect of their life cycle constrains them to a region but another disperses them, termed non-specific homing (Bett and Hinch 2016). There is additional evidence of restricted movement, as North American and European sea lamprey populations do not mix (Genner et al. 2012;Rodriguez-Munoz et al. 2004), yet move broadly throughout the ocean when parasitizing other fishes, in some cases moving over 815 km from the nearest coast (Silva et al. 2014). Little is known about the fate of the sea lamprey observed traveling a great distance from shore. It is possible that there are additional mechanisms to prevent navigational mistakes that result in the mixing of populations within the Atlantic basin sea lamprey, such as some control over when a parasite will release from a host to prevent large displacements or that those individuals simply fail to reproduce. The strongest evidence of efficient transiting to shore by ocean run sea lamprey comes from the observation that parasitic sea lamprey feeding within three separate abyssal plains near the Iberian peninsula typically migrated to the nearest river basin along the shoreline (Lanca et al. 2014). This suggests that the parasitic phase spreads individuals, while the return migration involves a direct migratory path to a nearby coast and localization of a river containing larvae.

Finally, it is important to consider those sea lamprey that swam directly out of the array during daylight release (30 animals), which we distinguish from those animals that immediately swam but stopped too close to the edge of our detection range to generate useful tracks during subsequent nocturnal movement (5 animals). Telemetry studies of migrating fishes frequently report ‘fallback’ in the period immediately following post-tagging release, defined generally as aberrant movement in an anomalous direction (e.g., downstream for an anadromous fish) as a probable consequence of tagging-induced stress, displacement, and/or disorientation (Caudill et al. 2014;Frank et al. 2009). Here, two general deviations (vs. nocturnal biphasic movement) were observed: movement during daylight hours, and vertical casting that did not reach the surface. Specifically, lampreys that exited the release cage and immediately began swimming exhibited an inconsistent array of tendencies (slow swimming on the substrate or an apparent orientation swim without vertical casting or straight-line swimming with vertical casting). Those lampreys that exhibited vertical casting (73% of fallback fish) undertook truncated casts, never moving into the upper 10 m of the water column. Because we did not explicitly examine the effects of tagging and handling on behavior immediately after release, we cannot determine conclusively whether these animals behaved aberrantly, or if migratory activity during daylight hours is more common in deeper waters than has been observed in shallow water studies (Meckley et al. 2014b;Vrieze et al. 2011). We suspect fallback, as those lampreys that exhibited daylight exiting of the array and were subsequently observed (i.e., after more than 24 h at large) were only observed at night. Regardless of this observation, our data are insufficient to develop a reasonable hypothesis for the observed daytime movement. Consequently, our results should be taken as a clear depiction of typical nocturnal orientation, but not necessarily precluding the possibility of the existence of typical daylight orientation, which may exhibit different patterns.

In summary, this study extends observations of topography-guided search from principally terrestrial cases to large aquatic environments. Bathymetry (underwater topography), likely through the lens of water pressure, was relevant at intermediate to long distances from the coast (2-5 km), as compared to other navigation cues that tend to be relevant at smaller (< 1 km, e.g., vision, acceleration, water chemistry) or larger scales (> 5 km, e.g., geomagnetism, celestial) (Kingsford et al. 2002). Sea lamprey actively navigated to the local coast through a biphasic pattern of movement, most consistent with orientation to a bathymetric gradient followed by directed movement to the coast. The parasitic stage of the life cycle may displace sea lamprey away from the coast, and this flexible strategy based on simple movement rules would allow them to find the local coast, and contributes to our understanding of  why sea lamprey are neither panmictic nor philopatric. Of the bathymetric cues that could be used for orientation, hydrostatic pressure was best supported (i.e., barokinesis). It remains unknown whether sea lamprey employ other navigational behaviors farther from the coast or if bathymetric navigation would be effective at greater distances from the coast. Though barokinesis is the most parsimonious hypothesis, this study did not address how sea lamprey sense hydrostatic pressure or maintain a straight course towards shallow water while moving vertically throughout the water column. The potential for sea lamprey to utilize a shoreward odor gradient should receive further consideration. 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. This is the first evidence of a navigation tactic consistent with the use of a bathymetric gradient, and should be carefully evaluated in other species, especially the numerous aquatic organisms that undertake similar offshore-inshore migrations without natal homing or breeding site philopatry that exhibit vertical movements through the water column (e.g., (Lohmann et al. 2008;Nakamura et al. 2011)).

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## References

Able, K.P. 1991. Common themes and variations in animal orientation systems. Amer Zool **31**(1): 157-167.

Able, K.P. 2001. The concepts and terminology of bird navigation. J. Avian Biol. **32**(2): 174-183.

Åkesson, S., and Hedenström, A. 2007. How migrants get there: migratory performance and orientation. BioScience **57**(2): 123-133.

Alerstam, T. 2006. Conflicting evidence about long-distance animal navigation. Science **313**(5788): 791-794.

Alerstam, T., Hedenstrom, A., and Akesson, S. 2003. Long-distance migration: evolution and determinants. Oikos **103**(2): 247-260.

Arnold, G.P., and Cook, P.H. 1984. Fish migration by selective tidal stream transport: first results with a computer simulation model for the european continental shelf. *In* Mechanisms of Migration in Fishes. *Edited by* J.D. McCleave, G.P. Arnold, J.J. Dodson and W.H. Neill. Springer US, Boston, MA. pp. 227-261.

Auer, M.T., and Gatzke, T.L. 2004. Exploring Superior, the spring runoff Event, thermal bar formation, and cross margin transport in Lake Superior. J. Gt. Lakes Res. **30**: 64-81.

Auster, P.J., Lindholm, J., Schaub, S., Funnell, G., Kaufman, L.S., and Valentine, P.C. 2003. Use of sand wave habitats by silver hake. J. Fish Biol. **62**(1): 143-152.

Barbin, G.P., Parker, S.J., and McCleave, J.D. 1998. Olfactory clues play a critical role in the estuarine migration of silver-phase American eels. Environ. Biol. Fishes **53**(3): 283-291.

Bates, D., Maechler, M., Bolker, B.M., and Walker, S. 2014. lme4: Linear mixed-effects models using Eigen and S4. *In* R package version 1.1-7.

Beletsky, D., Saylor, J.H., and Schwab, D.J. 1999. Mean circulation in the Great Lakes. J. Gt. Lakes Res. **25**(1): 78-93.

Benhamou, S., and Bovet, P. 1989. How animals use their environment: a new look at kinesis. Anim. Behav. **38**(3): 375-383.

Bett, N.N., and Hinch, S.G. 2016. Olfactory navigation during spawning migrations: a review and introduction of the Hierarchical Navigation Hypothesis. Biol. Rev. **91**: 728-759.

Blaxter, J.H.S. 1980. The effect of hydrostatic pressure on fishes. *In* Environmental Physiology of Fishes. *Edited by* M.A. Ali. Springer US. pp. 369-386.

Bleckmann, H., and Zelick, R. 2009. Lateral line system of fish. Integr. Zool. **4**(1): 13-25.

Block, B.A., Jonsen, I.D., Jorgensen, S.J., Winship, A.J., Shaffer, S.A., Bograd, S.J., Hazen, E.L., Foley, D.G., Breed, G.A., Harrison, A.L., Ganong, J.E., Swithenbank, A., Castleton, M., Dewar, H., Mate, B.R., Shillinger, G.L., Schaefer, K.M., Benson, S.R., Weise, M.J., Henry, R.W., and Costa, D.P. 2011. Tracking apex marine predator movements in a dynamic ocean. Nature **475**(7354): 86-90.

Burt de Perera, T., de Vos, A., and Guilford, T. 2005. The vertical component of a fish's spatial map. Anim. Behav. **70**(2): 405-409.

Cain, P. 1995. Navigation in familiar environments by the weakly electric elephantnose fish, *Gnathonemus petersii L.* (Mormyriformes, Teleostei). Ethology **99**(4): 332-349.

Calenge, C. 2006. The package "adehabitat" for the R software: A tool for the analysis of space and habitat use by animals. Ecol. Model. **197**(3-4): 516-519.

Carey, F.G., Scharold, J.V., and Kalmijn, A.J. 1990. Movements of blue sharks *(Prionace glauca)* in depth and course. Mar. Biol. **106**(3): 329-342.

Caudill, C.C., Jepson, M.A., Lee, S.R., Dick, T.L., Naughton, G.P., and Keefer, M.L. 2014. A field test of eugenol-based anesthesia versus fish restraint in migrating adult Chinook Salmon and Steelhead. Trans. Am. Fish. Soc. **143**(4): 856-863.

Cheung, A., Zhang, S., Stricker, C., and Srinivasan, M.V. 2007. Animal navigation: the difficulty of moving in a straight line. Biol. Cybern. **97**(1): 47-61.

Close, D.A., Fitzpatrick, M.S., Lorion, C.M., Li, H.W., and Schreck, C.B. 2003. Effects of intraperitoneally implanted radio transmitters on the swimming performance and physiology of Pacific Lamprey. North Am. J. Fish Manage. **23**(4): 1184-1192.

Collett, T.S., and Graham, P. 2004. Animal navigation: path integration, visual landmarks and cognitive maps. Curr. Biol. **14**(12): R475-R477.

Craig, P.C. 1973. Orientation of the sand-beach amphipod, . Anim. Behav. **21**(4): 699-706.

Dittman, A., and Quinn, T. 1996. Homing in Pacific salmon: mechanisms and ecological basis. J. Exp. Biol. **199**(1): 83-91.

Emlen, S. 1975. The stellar-orientation system of a migratory bird. Sci. Am. **233**(2): 102.

Frank, H.J., Mather, M.E., Smith, J.M., Muth, R.M., Finn, J.T., and McCormick, S.D. 2009. What is "fallback"?: metrics needed to assess telemetry tag effects on anadromous fish behavior. Hydrobiologia **635**(1): 237-249.

Fraser, E.J., and Stacey, N. E. 2002. Isolation increases milt production in goldifish. J. Exp. Biol. **293**: 511-524.

Fraser, P.J., Cruickshank, S.F., Shelmerdine, R.L., and Smith, L.E. 2008. Hydrostatic pressure receptors and depth usage in crustacea and fish. Navigation **55**(2): 159-165.

Fuxjager, M.J., Eastwood, B.S., and Lohmann, K.J. 2011. Orientation of hatchling loggerhead sea turtles to regional magnetic fields along a transoceanic migratory pathway. J. Exp. Biol. **214**(15): 2504-2508.

Gallistel, C.R., and Gelman, R. 2000. Non-verbal numerical cognition: from reals to integers. Trends in Cognitive Sciences **4**(2): 59-65.

Gebhardt, A., Petzoldt, T., and Maechler, M. 2013. akima: Interpolation of irregularly spaced data. *In* R Package Version 0.5-11.

Genner, M.J., Hillman, R., McHugh, M., Hawkins, S.J., and Lucas, M.C. 2012. Contrasting demographic histories of European and North American sea lamprey (*Petromyzon marinus*) populations inferred from mitochondrial DNA sequence variation. Mar. Freshwat. Res. **63**(9): 827-833.

Hammond, K.L., and Whitfield, T.T. 2006. The developing lamprey ear closely resembles the zebrafish otic vesicle: otx1 expression can account for all major patterning differences. Development **133**(7): 1347-1357.

Hess, J.E., Campbell, N.R., Close, D.A., Docker, M.F., and Narum, S.R. 2013. Population genomics of Pacific lamprey: adaptive variation in a highly dispersive species. Mol. Ecol. **22**(11): 2898-2916.

Holbrook, C.M., Bergstedt, R., Adams, N.S., Hatton, T.W., and McLaughlin, R.L. 2015. Fine-scale pathways used by adult sea lampreys during riverine spawning migrations. Trans. Am. Fish. Soc. **144**(3): 549-562.

Holbrook, R.I., and Burt de Perera, T. 2009. Separate encoding of vertical and horizontal components of space during orientation in fish. Anim. Behav. **78**(2): 241-245.

Holbrook, R.I., and Burt de Perera, T. 2013. Three-dimensional spatial cognition: freely swimming fish accurately learn and remember metric information in a volume. Anim. Behav. **86**(5): 1077-1083.

Huebert, K.B. 2008. Barokinesis and depth regulation by pelagic coral reef fish larvae. Mar. Ecol. Prog. Ser. **367**: 261-269.

Huijbers, C.M., Nagelkerken, I., Lössbroek, P.A.C., Schulten, I.E., Siegenthaler, A., Holderied, M.W., and Simpson, S.D. 2012. A test of the senses: Fish select novel habitats by responding to multiple cues. Ecology **93**(1): 46-55.

Katz, S.L. 2002. Design of heterothermic muscle in fish. J. Exp. Biol. **205**(15): 2251-2266.

Keene, J.L., Noakes, D.L.G., Moccia, R.D., and Soto, C.G. 1998. The efficacy of clove oil as an anaesthetic for rainbow trout, *Oncorhynchus mykiss* (Walbaum). Aquacult. Res. **29**(2): 89-101.

Kennedy, J.S. 1976. Behaviourally discriminating assays of attractants and repellents. Pesticide Science **7**(6): 632-632.

Khorevin, V.I. 2008. The lagena (the third otolith endorgan in vertebrates). Neurophysiology **40**(2): 142-159.

Kingsford, M.J., Leis, J.M., Shanks, A., Lindeman, K.C., Morgan, S.G., and Pineda, J. 2002. Sensory environments, larval abilities and local self-recruitment. Bull. Mar. Sci. **70**(1): 309-340.

Klimley, P.A., Beavers, S.C., Curtis, T.H., and Jorgensen, S.J. 2002. Movements and swimming behavior of three species of sharks in La Jolla Canyon, California. Environ. Biol. Fishes **63**(2): 117-135.

Kough, A.S., Paris, C.B., and Staaterman, E. 2014. In situ swimming and orientation behavior of spiny lobster (*Panulirus argus*) postlarvae. Mar. Ecol. Prog. Ser. **504**: 207-219.

Lanca, M.J., Machado, M., Mateus, C.S., Lourenco, M., Ferreira, A.F., Quintella, B.R., and Almeida, P.R. 2014. Investigating population structure of Sea Lamprey (*Petromyzon marinus, L.*) in Western Iberian Peninsula using morphological characters and heart fatty acid signature analyses. Plos One **9**(9): 14.

Landler, L., and von Oheimb, P.V. 2013. Y-axis orientation in the South American freshwater snail species Chilina patagonica (*Gastropoda: Chilinidae*). Molluscan Res. **33**(2): 98-103.

Lohmann, K., and Lohmann, C. 1996. Orientation and open-sea navigation in sea turtles. J. Exp. Biol. **199**(1): 73-81.

Lohmann, K.J., Lohmann, C.M.F., and Endres, C.S. 2008. The sensory ecology of ocean navigation. J. Exp. Biol. **211**(11): 1719-1728.

Lund, U., Agostinelli, C., and Agostinelli, M.C. 2013. Package ‘circular’.

Luschi, P. 2013. Long-distance animal migrations in the oceanic environment: orientation and navigation correlates. ISRN Zoology **2013**: 23.

Meckley, T.D., Holbrook, C., Wagner, C.M., and Binder, T.R. 2014a. An approach for filtering hyperbolically positioned underwater acoustic telemetry data with position precision estimates. Anim. Biotelem. **2**(1): 7.

Meckley, T.D., Wagner, C.M., and Gurarie, E. 2014b. Coastal movements of migrating sea lamprey *(Petromyzon marinus)* in response to a partial pheromone added to river water: implications for management of invasive populations. Can. J. Fish. Aquat. Sci. **71**(4): 533 - 544.

Nakamura, I., Watanabe, Y.Y., Papastamatiou, Y.P., Sato, K., and Meyer, C.G. 2011. Yo-yo vertical movements suggest a foraging strategy for tiger sharks *Galeocerdo cuvier*. Mar. Ecol. Prog. Ser. **424**: 237-246.

Nathan, R., Getz, W.M., Revilla, E., Holyoak, M., Kadmon, R., Saltz, D., and Smouse, P.E. 2008. A movement ecology paradigm for unifying organismal movement research. Proc. Natl. Acad. Sci. U.S.A. **105**(49): 19052-19059.

Nishimoto, R.T., and Herrnkind, W.F. 1978. Directional orientation in blue crabs, *Callinectes sapidusRathbun*: Escape responses and influence of wave direction. J. Exp. Mar. Biol. Ecol. **33**(2): 93-112.

Nosal, A.P., Chao, Y., Farrara, J.D., Chai, F., and Hastings, P.A. 2016. Olfaction contributes to pelagic navigation in a coastal shark. PLoS ONE **11**(1): e0143758.

Odling-Smee, L., Simpson, S.D., and Braithwaite, V.A. 2007. The role of learning in fish orientation. *In* Fish Cognition and Behavior. Blackwell Publishing Ltd. pp. 119-138.

Paris, C.B., Atema, J., Irisson, J.-O., Kingsford, M., Gerlach, G., and Guigand, C.M. 2013. Reef odor: a wake up call for navigation in reef fish larvae. PLOS ONE **8**(8): e72808.

Pe'er, G., Saltz, D., Münkemüller, T., Matsinos, Y.G., and Thulke, H.-H. 2013. Simple rules for complex landscapes: the case of hilltopping movements and topography. Oikos **122**(10): 1483-1495.

Pinheiro, J., and Bates, D. 2000. Mixed-effects models in S and S-PLUS. Springer New York.

Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., and R Development Core Team. 2013. Linear and nonlinear mixed effects model.

R Development Core Team. 2016. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Rao, Y.R., and Schwab, D.J. 2007. Transport and mixing between the coastal and offshore waters in the Great Lakes: a review. J. Gt. Lakes Res. **33**(1): 202-218.

Rodriguez-Munoz, R., Waldman, J.R., Grunwald, C., Roy, N.K., and Wirgin, I. 2004. Absence of shared mitochondrial DNA haplotypes between sea lamprey from North American and Spanish rivers. J. Fish Biol. **64**(3): 783-787.

Rodríguez, F., López, J.C., Vargas, J.P., Broglio, C., Gómez, Y., and Salas, C. 2002. Spatial memory and hippocampal pallium through vertebrate evolution: insights from reptiles and teleost fish. Brain Res. Bull. **57**(3–4): 499-503.

Roy, R., Beguin, J., Argillier, C., Tissot, L., Smith, F., Smedbol, S., and De-Oliveira, E. 2014. Testing the VEMCO Positioning System: spatial distribution of the probability of location and the positioning error in a reservoir. Anim. Biotelem. **2**: 1.

Schlegel, P.A., and Renner, H. 2007. Innate preference for magnetic compass direction in the Alpine newt, Triturus alpestris (Salamandridae, Urodela)? J. Ethol. **25**(2): 185-193.

Schöne, H., Neil, D., Stein, A., and Carlstead, M. 1976. Reactions of the spiny lobster,*Palinunis vulgaris*, to substrate tilt (I.). J. Comp. Physiol. **107**(1): 113-128.

Siefkes, M.J., and Li, W. 2004. Electrophysiological evidence for detection and discrimination of pheromonal bile acids by the olfactory epithelium of female sea lampreys (*Petromyzon marinus*). J. Comp. Physiol. A -Neuroethol. Sens. Neural Behav. Physiol. **190**(3): 193-199.

Silva, S., Araújo, M.J., Bao, M., Mucientes, G., and Cobo, F. 2014. The haematophagous feeding stage of anadromous populations of sea lamprey Petromyzon marinus: low host selectivity and wide range of habitats. Hydrobiologia **734**(1): 187-199.

Smith, F. 2013. Understanding HPE in the VEMCO positioning system (VPS). Halifax, NS: VEMCO.

Sorensen, P.W., and Vrieze, L.A. 2003. The chemical ecology and potential application of the sea lamprey migratory pheromone. J. Gt. Lakes Res. **29**: 66-84.

Spice, E.K., Goodman, D.H., Reid, S.B., and Docker, M.F. 2012. Neither philopatric nor panmictic: microsatellite and mtDNA evidence suggests lack of natal homing but limits to dispersal in Pacific lamprey. Mol. Ecol. **21**(12): 2916-2930.

Teeter, J.H. 1980. Pheromone communication in sea lampreys (*Petromyzon marinus*): implications for population management. Can. J. Fish. Aquat. Sci. **37**: 2123-2132.

Vrieze, L., Bergstedt, R., and Sorensen, P. 2011. Olfactory-mediated stream-finding behavior of migratory adult sea lamprey (*Petromyzon marinus*). Can. J. Fish. Aquat. Sci. **68**: 523 - 533.

Waldman, J., Grunwald, C., and Wirgin, I. 2008. Sea lamprey, *Petromyzon marinus*: an exception to the rule of homing in anadromous fishes. Biol. Lett. **4**(6): 659-662.

Walker, M.M., Dennis, T.E., and Kirschvink, J.L. 2002. The magnetic sense and its use in long-distance navigation by animals. Curr. Opin. Neurobiol. **12**(6): 735-744.

Webber, D., White, P., and Helvin, A. 2008. Modelling structural change using broken sticks. University of the West of England: http://carecon.org.uk/DPs/0801.pdf.

Westerberg, H. 1982. Ultrasonic tracking of Atlantic salmon (*Salmo salar L.*), 2: Swimming depth and temperature stratification. Report-Institute of Freshwater Research, Drottningholm (Sweden).

Willis, J., Phillips, J., Muheim, R., Diego-Rasilla, F.J., and Hobday, A.J. 2009. Spike dives of juvenile southern bluefin tuna (*Thunnus maccoyii*): a navigational role? Behav. Ecol. Sociobiol. **64**(1): 57-68.

Wyeth, R.C. 2010. Should animals navigating over short distances switch to a magnetic compass sense? Front. Behav. Neurosci. **4**(42).

**Table 1.** The number of sea lamprey detected by receivers located near shore for those sea lamprey that were observed stopping in the array and moving on the first night (n=22) and for all individuals released (n=67), is shown. The number of hours it took sea lamprey to be observed after release at each receiver is shown under the column "Time to reach receiver in hours".

| Receiver/ detection Site | Distance from array (km) | No. of 1st Detections in 72 hours (N=22, N=67) | Time to reach receiver hours (mean ±SE) (N=22) | Time to reach receiver hours (min, max) (N=22) | 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 19/22 and 46/67. 10/23 and 19/67 sea lamprey entered the Ocquoec River. 1/ 22 and 2/65 entered the Black Mallard River. 4/23 and 7/67 were trapped in the Ocqueoc River and 1/ 67 was trapped in the Cheboygan River.

**Table 2.** Summary statistics of individual track analyses (Theta, Rho, and Line Test) for n = 22 sea lamprey are shown. P1 and P2 indicate first and second phases of movements. Average Turn angle (i.e., Theta) ranges from -$\pi$ to $\pi$, indicating left and right turns respectively. Individual t-tests for each individual revealed that more sea lamprey had a bias of consistent left or right turns during phase 1 than during phase 2. Turning bias was more commonly left or counterclockwise. Rho is a measure of straightness ranging from 0-1, where 0 is complete randomness and 1 is a straight line. Most fish swam a straighter course during phase 2 (Rho *p*). The line test evaluated whether a broken stick model fit each track better than a straight line. Only those better fit by broken stick regression are included in analyses (T26 and T44 were not included). No break point was identified for T26 (Line Test, *p=0.283), not in table*. Bold values are significant.

| **ID** | **Theta (Average Turn Angle)** | | | | **Rho (Straightness Index)** | | | **Line Test** |
|---|---|---|---|---|---|---|---|---|
| | P1 | **P1 *p*** | P2 | **P2 *p*** | P1 | P2 | ***p*** | ***p*** |
| T02 | -0.03 | 0.488 | -0.08 | **0.039** | 0.81 | 0.83 | 0.615 | **<0.01** |
| T04 | -0.06 | 0.494 | 0.00 | 0.996 | **0.70** | **0.86** | **<0.01** | **0.048** |
| T05 | -0.17 | 0.214 | 0.01 | 0.792 | 0.74 | 0.86 | 0.18 | **<0.01** |
| T07 | -0.81 | **<0.01** | -0.21 | 0.287 | 0.74 | 0.65 | 0.469 | **<0.01** |
| T08 | 0.07 | 0.689 | 0.04 | 0.458 | 0.48 | 0.84 | **<0.01** | **<0.01** |
| T12 | -0.58 | **<0.01** | 0.06 | 0.480 | 0.61 | 0.77 | 0.183 | **<0.01** |
| T17 | 0.14 | 0.625 | -0.10 | 0.484 | - | 0.75 | 0.662 | **<0.01** |
| T22 | -0.43 | **<0.01** | -0.04 | 0.508 | **0.54** | **0.88** | **<0.01** | **<0.01** |
| T25 | 0.50 | **<0.01** | -0.07 | 0.274 | **0.59** | **0.81** | **0.012** | **<0.01** |
| T27 | -0.26 | **<0.01** | -0.06 | 0.668 | 0.64 | 0.75 | 0.176 | **<0.01** |
| T31 | -0.47 | **<0.01** | 0.14 | 0.244 | 0.61 | 0.73 | 0.356 | **<0.01** |
| T32 | 0.07 | 0.792 | 0.04 | 0.667 | 0.61 | 0.74 | 0.124 | **<0.01** |
| T36 | -0.58 | **<0.01** | 0.01 | 0.876 | 0.65 | 0.75 | 0.188 | **<0.01** |
| T37 | 0.02 | 0.878 | 0.33 | **<0.01** | 0.67 | 0.85 | 0.122 | **<0.01** |
| T42 | 0.16 | **0.043** | -0.19 | 0.116 | 0.69 | 0.78 | 0.362 | **<0.01** |
| T44 | -0.28 | 0.836 | 0.03 | 0.61 | -0.28 | 0.79 | 0.210 | 0.417 |
| T47 | 0.68 | **0.028** | -0.08 | 0.301 | 0.62 | 0.84 | 0.143 | **<0.01** |
| T54 | -0.12 | 0.107 | 0.01 | 0.764 | 0.72 | 0.81 | 0.102 | **<0.01** |
| T56 | -0.11 | **<0.01** | -0.21 | **<0.01** | 0.70 | 0.70 | 0.933 | **<0.01** |
| T61 | -0.01 | 0.903 | 0.06 | 0.557 | 0.75 | 0.73 | 0.816 | **<0.01** |
| T63 | -0.20 | **<0.01** | -0.07 | **0.024** | **0.65** | **0.86** | **<0.01** | **<0.01** |
| Total ( $p < 0.05$): | | 11/ 20 | | 4 | | | 5 | 20 |
| Total Possible: | | | | 20 | | | 20 | 22 |

**Table 3.** Individual two-sampled t-tests indicate if there was a difference in the mean depth, standard deviation in depth, mean depth when maintaining vertical depth, or ground speed between phase 1(P1) and phase 2 (P2) for each sea lamprey. The tests support our observations that these two phases are different. Bold values are significant.

| | Mean Depth (m) | | | Standard Dev. Depth (m) | | | Hz Mean Depth (m) | | | Ground Speed (m/s) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ID | P1 | P2 | ***p*** | P1 | P2 | ***p*** | P1 | P2 | ***p*** | P1 | P2 | ***p*** |
| T02 | 32.85 | 17.98 | **<0.01** | 4.86 | 9.38 | **<0.01** | 35.1 | 14.6 | 0.052 | 0.38 | 0.49 | **<0.01** |
| T04 | 32.69 | 15.85 | **<0.01** | 6.60 | 9.89 | 0.083 | 36.8 | 7.1 | **<0.01** | 0.38 | 0.48 | **<0.01** |
| T05 | 29.80 | 21.59 | **<0.01** | 8.79 | 11.45 | 0.382 | 34.1 | 20.3 | 0.158 | 0.38 | 0.49 | **<0.01** |
| T07 | 33.86 | 23.76 | **<0.01** | 0.15 | 8.30 | **<0.01** | 33.9 | 24.8 | 0.207 | 0.33 | 0.50 | **<0.01** |
| T08 | 35.07 | 28.09 | **<0.01** | 0.92 | 6.84 | **<0.01** | 35.3 | 27.5 | 0.231 | 0.29 | 0.51 | **<0.01** |
| T12 | 21.38 | 8.04 | **<0.01** | 10.54 | 6.69 | 0.141 | 26.5 | 4.4 | **<0.01** | 0.33 | 0.51 | **<0.01** |
| T17 | 23.52 | 14.67 | 0.087 | 8.42 | 10.96 | 0.623 | 32.0 | 6.2 | **<0.01** | 0.59 | 0.69 | 0.084 |
| T22 | 22.72 | 4.97 | **<0.01** | 12.08 | 3.62 | **<0.01** | 24.2 | 3.8 | **<0.01** | 0.30 | 0.52 | **<0.01** |
| T25 | 32.91 | 5.99 | **<0.01** | 3.90 | 3.85 | 0.955 | 34.4 | 4.8 | **<0.01** | 0.17 | 0.30 | **<0.01** |
| T27 | 23.16 | 7.51 | **<0.01** | 11.88 | 6.93 | **0.031** | 25.0 | 3.9 | **<0.01** | 0.35 | 0.47 | **<0.01** |
| T31 | 27.31 | 13.95 | **<0.01** | 6.51 | 11.37 | 0.135 | 25.8 | 4.7 | **<0.01** | 0.34 | 0.57 | **<0.01** |
| T32 | 17.84 | 8.33 | **<0.01** | 3.44 | 9.39 | **<0.01** | 21.5 | 3.4 | 0.065 | 0.34 | 0.42 | **<0.01** |
| T36 | 31.98 | 31.06 | 0.129 | 1.79 | 5.61 | **<0.01** | 32.1 | 32.5 | 0.345 | 0.36 | 0.43 | **<0.01** |
| T37 | 28.93 | 26.25 | **0.04** | 2.90 | 3.11 | 0.868 | 30.2 | 26.1 | 0.283 | 0.40 | 0.44 | 0.150 |
| T42 | - | - | - | - | - | - | - | - | - | 0.41 | 0.73 | **<0.01** |
| T47 | - | - | - | - | - | - | - | - | - | 0.56 | 0.69 | 0.076 |
| T54 | - | - | - | - | - | - | - | - | - | 0.37 | 0.60 | **<0.01** |
| T56 | - | - | - | - | - | - | - | - | - | 0.36 | 0.50 | **<0.01** |
| T61 | 15.02 | 3.84 | **<0.01** | 9.06 | 2.14 | 0.064 | 8.6 | 2.3 | **<0.01** | 0.46 | 0.51 | **<0.01** |
| T63 | 26.67 | 5.94 | **<0.01** | 9.94 | 7.45 | 0.280 | - | 4.5 | - | 0.27 | 0.53 | **<0.01** |
| Total Significant ($p < 0.05$): | | | 14 | | | 7 | | | 8 | | | 17 |
| Total Possible: | | | 16 | | | 16 | | | 14 | | | 20 |

## Figure Captions

**Figure 1.** An acoustic array was deployed in Hammond Bay, located in Lake Huron (a). Each sea lamprey was released near the bottom in the middle of a 43 receiver array (●,b) with 3 km$^2$ of array coverage (c). Individual receivers provided detection data along multiple locations on the coast, including 40 mile point (▲), Presque Isle (▲), and in front of as well as in the Black Mallard (■), Ocquoec (■), and, St. Mary's rivers (▲) (b). The nearest coastline was 172$^o$ (3 radians; SSE) at the release location. Sea lamprey (n=20) settled in the array during the day, began moving at night, and were fit well by the backward beeline analysis (d). The array depth varied (20-39 m) and is represented by 5 m increments (d). Fish tracks are color coded by source including those from the Ocqueoc River (Orange), Cheboygan River (Black), Manistique River (red), and parasites (blue) (d).

**Figure 2.** The backward-beeline analysis (BBA) characterizes when individual sea lamprey transitioned from sinuous to straighter movements that led out of the array. Two key measures are made at each position in a path including the backward beeline distance (BBD) and the backward path length (BPL). The BBD is the distance measured from each position to the final measured position in the track. The BPL is the remaining cumulative path of each step from the current to the final position. The unclassified track of sea lamprey T04 depicts how the variables BPL and BBD would be calculated for the first position in the track. To complete the BBA, a broken stick model was fit and optimized using generalized least squares estimation with "gnls" in R across four parameters (initial slope ($\beta_1$), break point (Tau), final slope ($\beta_2$), and sigma). An example of the broken stick model fit is shown (red line depicts standard error of

each slope (black line)). The break point separates phase one (orange) from phase two (blue) in a classified track. An overall heading can then be estimated for the straight path from the start to the end of the period, we term the orientation bearing (OB).

**Figure 3.** Dotted lines connect phase 1 (orange) and phase 2 (blue), data points for each sea lamprey subject. Both the mean value (dot) and standard deviation in each axis (+) is shown.

**Figure 4.** The heading of each sea lamprey during phase two (a), and the turn angle between heading and the direction towards shallow water (b), is depicted as a black dot within one of 60 bins of the circle. If sea lamprey were moving towards the coast, points would be clustered at 172 degrees (coast test, a). The bathymetry test does not indicate cardinal direction, but indicates whether each fish heading was opposite the local contour around that individual fish. In the bathymetry test, a $0^{o}$ angle would indicate a fish moving perfectly towards shallow water while $180^{o}$ would be movement opposite, toward deeper water (b). Average heading (gray arrow) and 95% confidence interval (shaded) are shown. These images highlight the pattern that sea lamprey did not appear to go in a consistent direction (a), however sea lamprey did tend to move opposite the local bathymetry towards shallower water (b).

**Figure 5.** The vertical profile of where sea lamprey (n=20) were observed swimming in the water column, in terms of the total number of observations within each 20% depth bin. These are shown for all monitoring (left), only during phase one (center), and only during phase 2 (right). The surface is 0% and the bottom is 100%, but the actual depth varied across the array (20-39 m). All 20 tracks are shown in S4.

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**Figure 6.** 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 20 tracks are shown in S2.

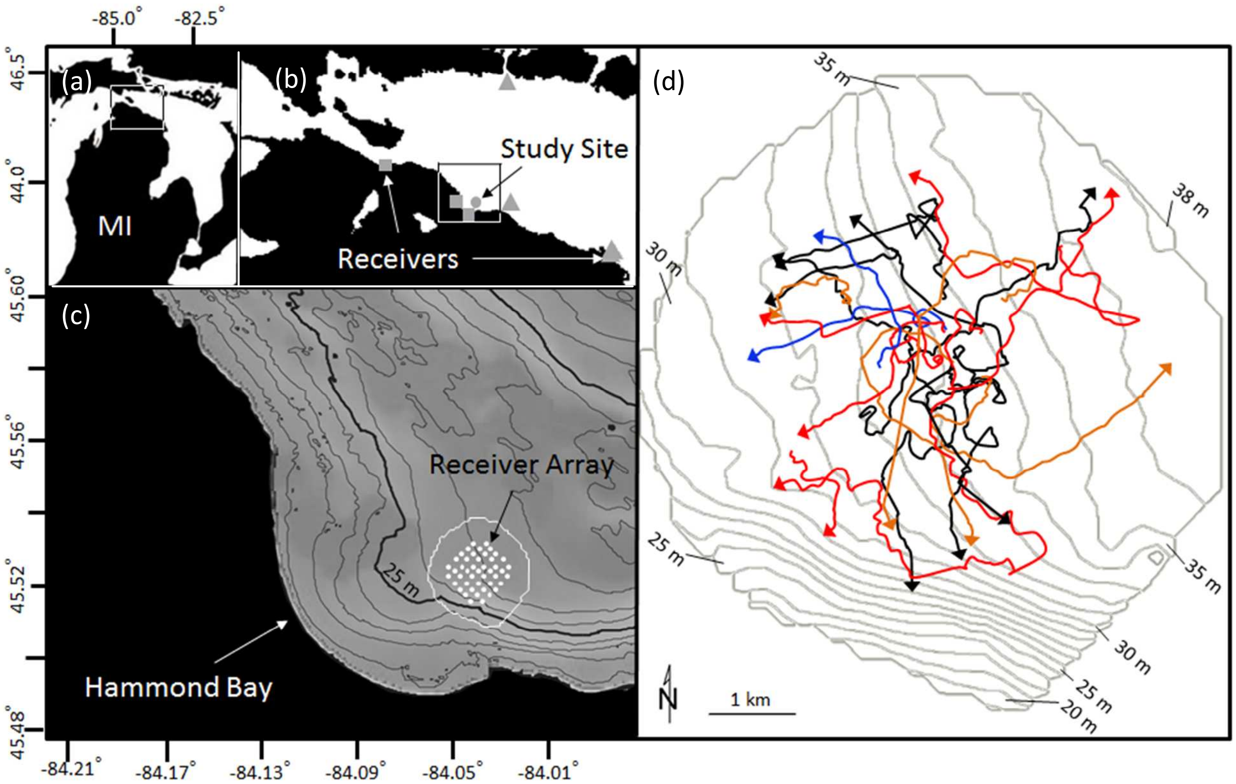

**Figure 1.** An acoustic array was deployed in Hammond Bay, located in Lake Huron (a). Each sea lamprey was released near the bottom in the middle of a 43 receiver array (●,b) with 3 km$^2$ of array coverage (c). Individual receivers provided detection data along multiple locations on the coast, including 40 mile point (▲), Presque Isle (▲), and in front of as well as in the Black Mallard (■), Ocquoec (■), and, St. Mary's rivers (▲) (b). The nearest coastline was 172$^o$ (3 radians; SSE) at the release location. Sea lamprey (n=20) settled in the array during the day, began moving at night, and were fit well by the backward beeline analysis (d). The array depth varied (20-39 m) and is represented by 5 m increments (d). Fish tracks are color coded by source including those from the Ocqueoc River (Orange), Cheboygan River (Black), Manistique River (red), and parasites (blue) (d).

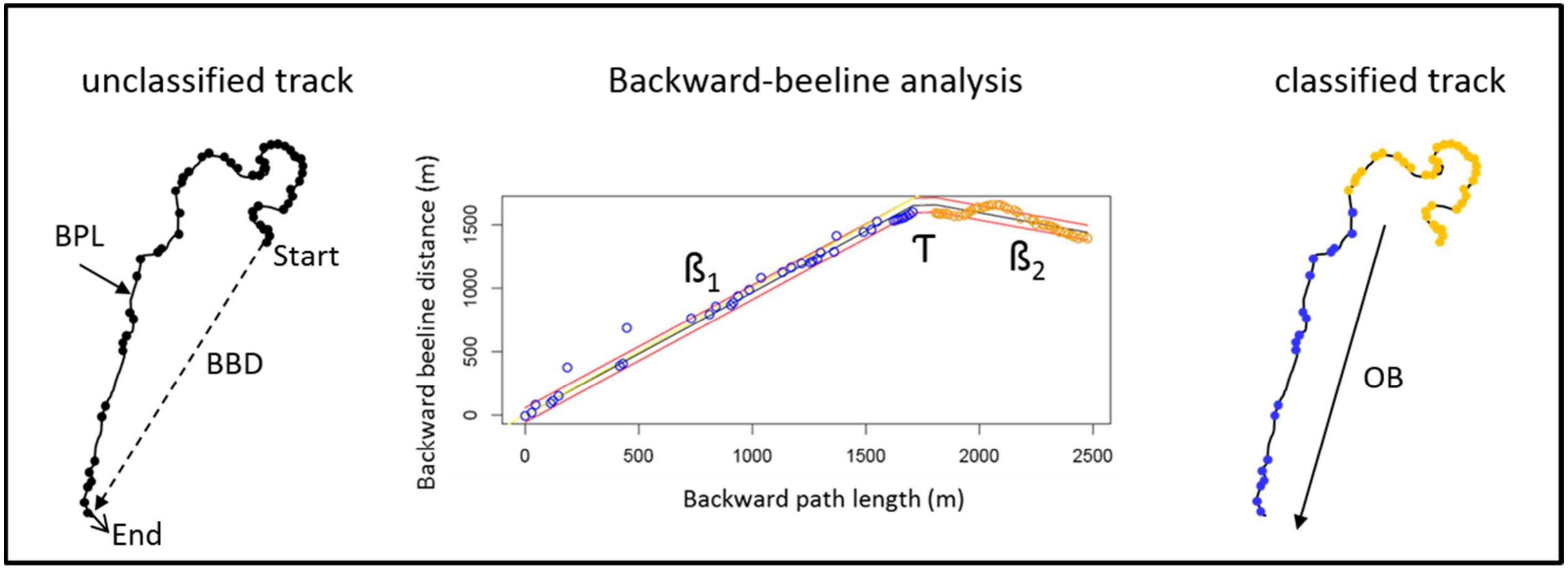

**Figure 2.** The backward-beeline analysis (BBA) characterizes when individual sea lamprey transitioned from sinuous to straighter movements that led out of the array. Two key measures are made at each position in a path including the backward beeline distance (BBD) and the backward path length (BPL). The BBD is the distance measured from each position to the final measured position in the track. The BPL is the remaining cumulative path of each step from the current to the final position. The unclassified track of sea lamprey T04 depicts how the variables BPL and BBD would be calculated for the first position in the track. To complete the BBA, a broken stick model was fit and optimized using generalized least squares estimation with “gnls” in R across four parameters (initial slope ($\beta_1$), break point (Tau), final slope ($\beta_2$), and sigma). An example of the broken stick model fit is shown (red line depicts standard error of each slope (black line)). The break point separates phase one (orange) from phase two (blue) in a classified track. An overall heading can then be estimated for the straight path from the start to the end of the period, we term the orientation bearing (OB).

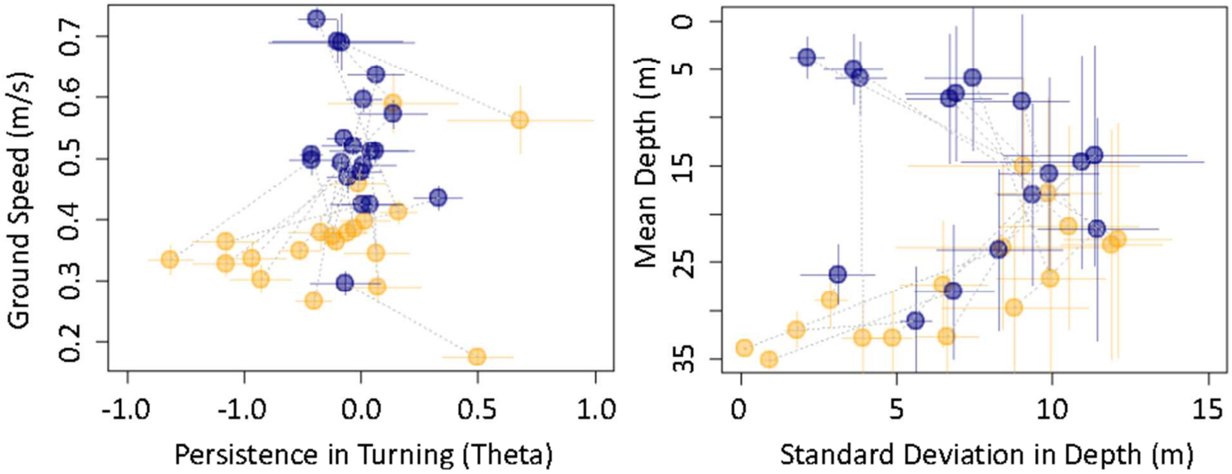

**Figure 3.** Dotted lines connect phase 1 (orange) and phase 2 (blue), data points for each sea lamprey subject. Both the mean value (dot) and standard deviation in each axis (+) is shown.

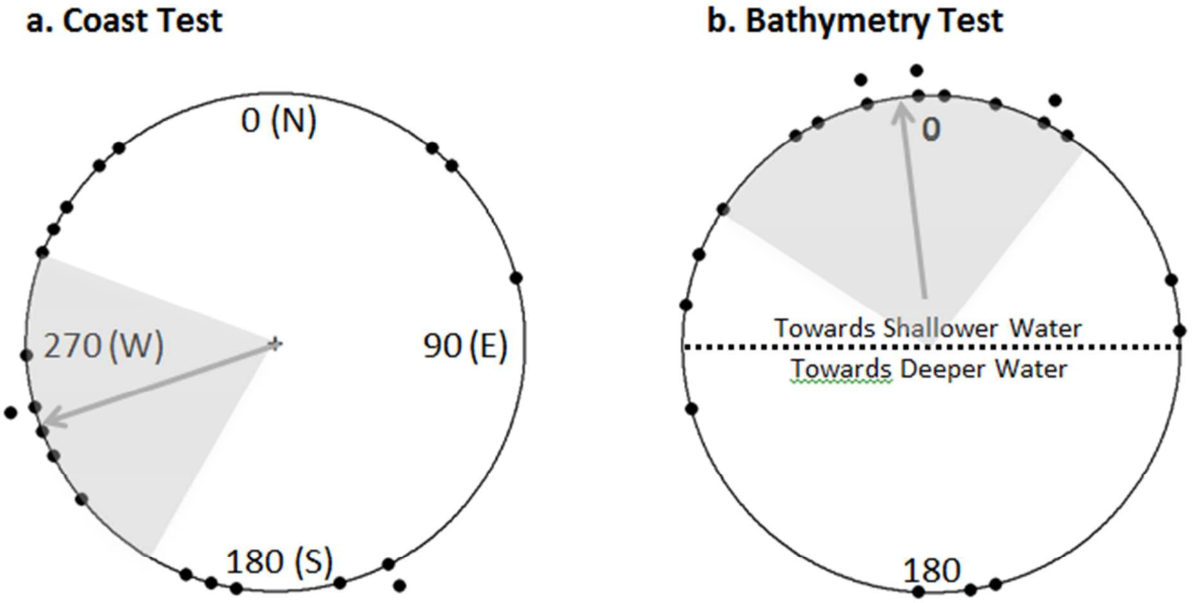

**Figure 4.** The heading of each sea lamprey during phase two (a), and the turn angle between heading and the direction towards shallow water (b), is depicted as a black dot within one of 60 bins of the circle. If sea lamprey were moving towards the coast, points would be clustered at 172 degrees (coast test, a). The bathymetry test does not indicate cardinal direction, but indicates whether each fish heading was opposite the local contour around that individual fish. In the bathymetry test, a 0 ° angle would indicate a fish moving perfectly towards shallow water while 180 ° would be movement opposite, toward deeper water (b). Average heading (gray arrow) and 95% confidence interval (shaded) are shown. These images highlight the pattern that sea lamprey did not appear to go in a consistent direction (a), however sea lamprey did tend to move opposite the local bathymetry towards shallower water (b).

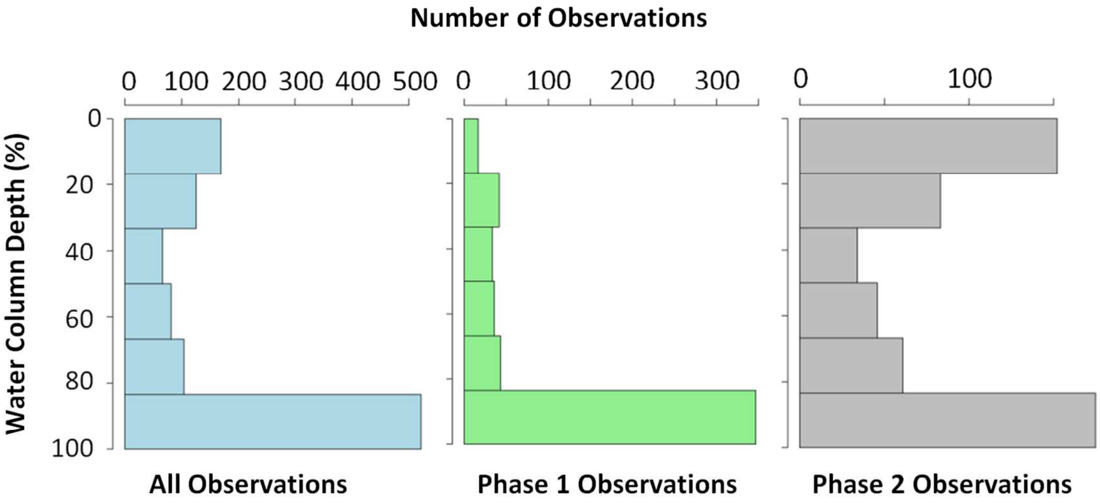

**Figure 5.** The vertical profile of where sea lamprey (n=20) were observed swimming in the water column, in terms of the total number of observations within each 20% depth bin. These are shown for all monitoring (left), only during phase one (center), and only during phase 2 (right). The surface is 0% and the bottom is 100%, but the actual depth varied across the array (20-39 m). All 20 tracks are shown in S4.

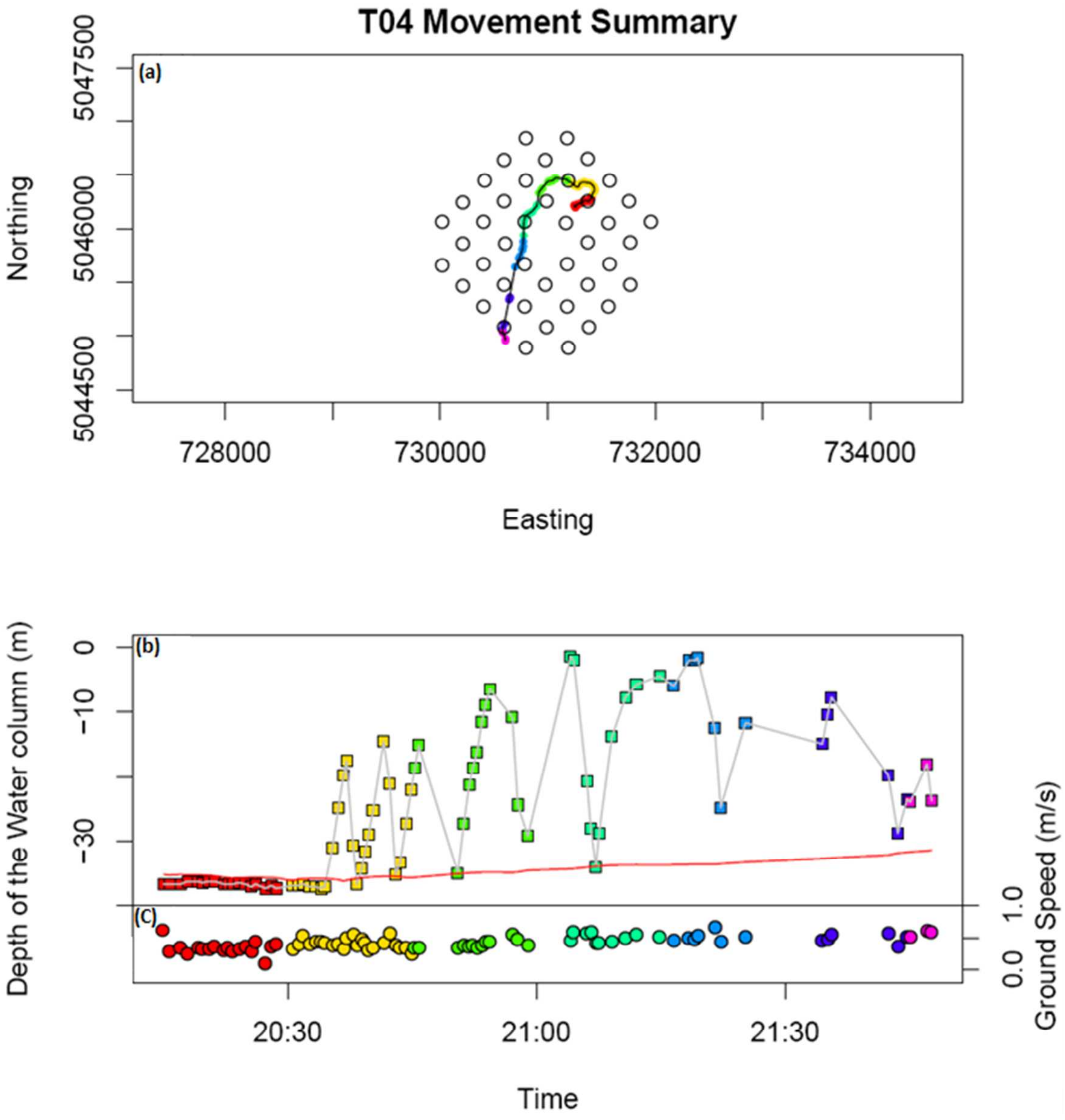

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**Figure 6.** The track of sea lamprey T04 is depicted as it exited the array (a), and below is the water column depth (b) and ground speed during the track (c). The graphs are color coded by 15 minute intervals. In inset "a" small circles represent 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 20 tracks are shown in S2.