

SHORT COMMUNICATION

Sea turtle hatchlings can distinguish between coastal and oceanic seawaters

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ABSTRACT

Following their emergence on land, sea turtle hatchlings need to travel through the open ocean. Whether hatchlings can detect ecologically and functionally relevant chemical cues released in the marine habitat is unknown. We collected seawater at 6 and 27 km off the Brazilian coast, i.e. within and beyond the continental shelf. In a two-choice flume, we exposed post-emergent (<24 h old) loggerhead (*Caretta caretta*) turtles to these seawaters. Based on their life history, we posited that if hatchlings could distinguish between the seawater from these regions, they should prefer the oceanic seawater and/or avoid the coastal seawater. Hatchlings were tested singly and could access any parts of the flume. We recorded the seawater plume first visited and the time spent in each plume. Of all the first choices and time spent in a plume, nearly 70% involved the oceanic seawater. The ability of hatchlings to distinguish between seawaters could provide goal-recognition information.

KEY WORDS: Sensory ecology, Chemoreception, Experimental ecology, Seascape, Goal recognition, Chelonian ecology

INTRODUCTION

Long-distance migrations impose tough demands on the sensory guidance of animals (Akesson and Hedenstrom, 2007; Bauer et al., 2011). This reality may be particularly acute for marine organisms that journey in a seemingly featureless environment (i.e. without landmarks or beacons), often for hundreds or thousands of kilometres (Block et al., 2011; Costa et al., 2012; Luschi, 2013). Given their precarious conservation status (Anonymous, 2018) and their necessity to travel great distances to reach precise locations to complete essential parts of their life cycle, the mechanisms by which sea turtles accomplish this feat have been of great interest to ecologists and conservationists (Koch et al., 1969; Lohmann et al., 2008a; Mazar et al., 2016).

Following their emergence on land, sea turtle hatchlings migrate offshore and into the open ocean using a suite of guidance mechanisms that primarily rely on wave direction (Salmon and Lohmann, 1989; Lohmann and Lohmann, 1992; Goff et al., 1998; Wang et al., 1998) and Earth's magnetic field (Brothers and Lohmann, 2018; Lohmann and Lohmann, 2019; Lohmann et al., 2001, 2008a,b; Luschi et al., 2003; Southwood and Avens, 2010). However, how the movement decisions of sea turtle hatchlings may

be influenced by their detection of chemical cues is comparatively far less understood. Although geomagnetic and hydrodynamic cues provide broad-scale guidance, chemosensory cues emanating from, for example, native beaches, landmasses and feeding 'hotspots' may need to be detected at a closer range. If detected, these cues could confirm the arrival at an intended destination or the function of goal recognition (*sensu* Endres et al., 2009, 2016; Grassman et al., 1984; Kedzif and Salmon, 2016).

Although sea turtles can detect several chemosensory cues, determining the functional significance of this process requires that ecologically relevant substances (in composition and concentration) be used in controlled experiments. Most experiments on sea turtle olfaction have been conducted under laboratory conditions and provided compelling evidence that specific water-borne (Grassman and Owens, 1987; Manton et al., 1972; Owens et al., 1982) and/or air-borne cues (Endres and Lohmann, 2012, 2013; Endres et al., 2009; Kedzif and Salmon, 2016) may be detected. However, these experiments typically relied on substances that were either artificial and/or with their concentration unknown relative to what sea turtles may encounter *in situ*. Whether animals may detect a given chemical cue (e.g. odour) enabling a response is contingent on the nature (Brown et al., 2010) and the intensity of that cue (its concentration), the latter requiring being above a minimum threshold (Brown et al., 2006; Mirza et al., 2006). Thus, a better understanding of the functional significance of the chemosensory abilities of sea turtles could be enhanced by testing ecologically relevant substances, such as those found in their habitat.

Here, we tested whether loggerhead (*Caretta caretta* Linnaeus 1758) sea turtle hatchlings can distinguish between seawater types from two regions of their natural habitat. Specifically, we used seawater collected from within and beyond the continental shelf (thereafter 'coastal' and 'oceanic', respectively), and experimentally exposed hatchlings to these. We attempted to enhance the ecological relevance of our methodology by testing the ability of these post-emergent hatchlings to distinguish among seawater types without providing prior exposure to these seawater types. As reaching the open ocean (oceanic province) is thought to be the goal of loggerhead hatchlings (Bolten, 2003), we posited that if hatchlings were able to distinguish between these seawater types, hatchlings would be attracted by the oceanic seawater and/or avoid the coastal seawater. Regardless, this possibility would imply that hatchlings may distinguish between the coastal and oceanic regions based on ecologically relevant concentrations of chemosensory cues present in seawater. Such an ability could imply the occurrence of a goal-recognition function, informing hatchlings of having reached their destination.

MATERIALS AND METHODS

Hatchling collection and maintenance

Thirty loggerhead (*C. caretta*) hatchlings, less than 24 h old, were obtained from the Tamar Project (www.tamar.org.br), a non-

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governmental Brazilian organization involved in sea turtle conservation. Specifically, eggs were directly collected by field technicians of the Tamar Project from ovipositing females at beaches near Praia do Forte, Bahia State, Brazil. These eggs were incubated until hatching at the Tamar Project's nursery beach. On 2 and 28 June and 17 July 2019, we collected recently hatched turtles; each of these three groups contained 10 hatchlings that were the offspring of three females (hereafter groups 1–3). After their transport to the laboratory in a 20×15×20 cm icebox overlaid with sand from their native beach, hatchlings were held in a 60×25×25 cm aquarium, also overlaid with this sand and kept at 30°C, following recommendations by Mrosovky (1980). Hatchlings remained in the laboratory for a maximum of 36 h before their release at their native beach. All collections and handling procedures were authorized by the national Brazilian agency involved in the conservation of biota (Sisbio License 66006-1). Furthermore, all experimental activities (detailed below) were authorized and complied with the requirements of the Ethics Committee on the Use of Animals from the Federal University of Bahia (project number 08/2019).

Seawater collection

We collected seawater from two locations offshore from Praia do Forte (coordinates: 12°31'44.2"S, 37°49'30.1"W and 12°35'57.7"S, 37°53'39.0"W). We used a 10 m motorized vessel to access these two regions, specifically within and beyond the continental shelf, i.e. at 6 km (depth of ~40 m) and 27 km (depth of ~1000 m) from shore, respectively (Fig. 1). Seawater sampling occurred on the same days as obtaining hatchlings, thus on three occasions. We used 20-liter plastic containers to collect and transport this water to the laboratory. Prior to usage, these containers were cleaned following the procedure detailed in Lewin and Chen (1973). Specifically, we soaked these containers for 5 days with chlorine-diluted tap water (concentration of 2 ml l⁻¹). We subsequently rinsed and soaked

these containers for an additional 5 days with an anti-chlorine-based solvent (i.e. sodium thiosulfate 100 mg l⁻¹). Following this period, we performed a second rinsing and soaking process for a period of 2 days, this time with dechlorinated tap water mixed with 10 ml of a neutral detergent [i.e. sodium dodecylbenzenesulfonate (5.0–15.0%), sodium lauryl ether sulphate (1.0–10.0%) and bronopol (<0.2%)]. Finally, we rinsed each container five times with local surface seawater before collecting the seawater used in the present experiment. Water collection was done by filling the containers with surface seawater from the bow of the vessel, with the latter being adrift and its motor stopped. We used this procedure to reduce potential chemical interferences that could emanate from the vessel. Throughout the experiment, each container was used for only one type of seawater (i.e. coastal or oceanic). At the laboratory, each seawater type was transferred into one of two 100-liter reservoirs, which, along with its tubing system (e.g. hoses and valves), had also been washed as described above.

Experimental apparatus and procedures

We used a two-choice flume, which allows testing for a preference (or avoidance) between one of two water plumes (*sensu* Jutfelt et al., 2017). Our glass-made flume was 200 cm in length and its sides (i.e. 30 cm in height) were covered from the outside by opaque dark-blue paper (Fig. S1). The base of the flume (i.e. the outflow) was equipped with a 30×45 cm shelf positioned a few centimeters above the water flow and covered with sand from the hatchlings' native beach. This miniature 'beach' was set to reduce the stress inherent to manipulating hatchlings and served for acclimation, whereby each test subject could move freely from the terrestrial to the aquatic environment. This beach gently sloped toward the flowing water, with this characteristic known to provide hatchlings with a means of orientating toward the sea (Lohmann and Lohmann, 1996; Salmon et al., 1992). Upstream (i.e. 20 cm) from the forward edge of the beach, the flume's outflow channel splits into two inflow channels

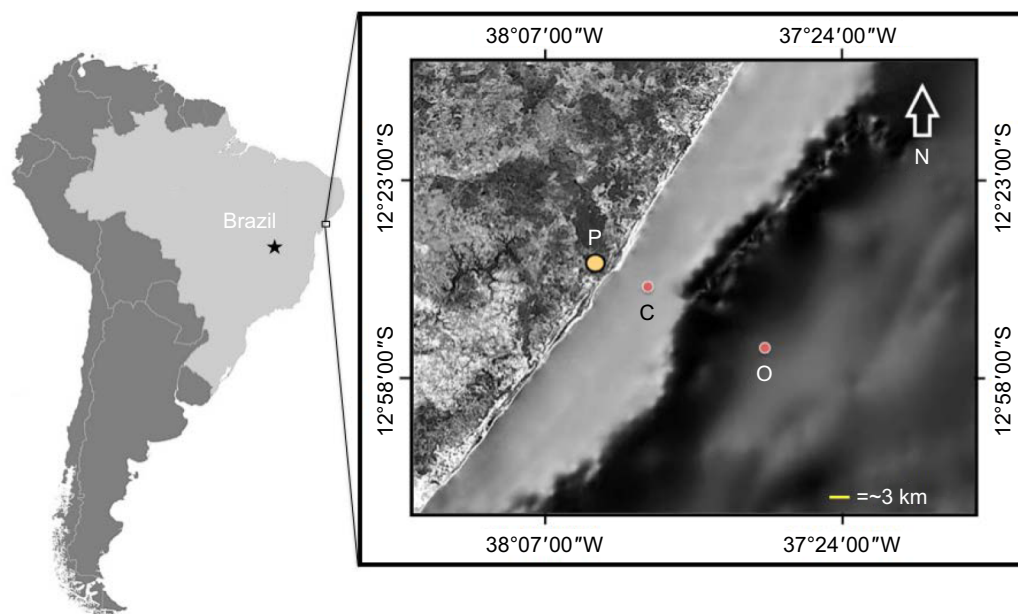


Fig. 1. Map of Brazil and the seawater collection points. The location of the seawater collection points, within (C, coastal) and beyond (O, oceanic) the continental shelf, are indicated by the red circles (inset map, right). The continental shelf is represented by the light-grey region that is contiguous to the coast. The coastal and oceanic sampling points were each distanced by 6 and 27 km from the coast, and their approximate depths were 40 and 1000 m, respectively. P, the beach of Praia do Forte (State of Bahia) from where hatchlings originated. The star on the main map (left) indicates the location of Brasilia, Brazil's capital. Source: Google Earth Pro 2021.

(left and right; each 45×135 cm; Fig. 1), in each of which flowed one of the two seawater types described above.

Each of the two seawater storage reservoirs was equipped with a dual outflow system (i.e. valves and tubing) that could supply each of the two channels on demand. Flow resulted from gravity and was regulated by predetermining the aperture of valves and by keeping the reservoirs at least half full. We conducted dye tests to assess flow speed and linearity in each of the two channels, with this procedure allowing us to measure the time required for the dye to reach the base of each channel (Fig. S2). After determining the proper valve aperture to obtain the desired flow, we repeated the dye test at the onset of each trial session; this allowed us to ensure that flow was comparable among channels and through repetitions. In each channel, the water current was set at 16 ml min⁻¹, which was sufficient to create a directional flow while allowing hatchlings to swim freely. To reduce potential interference of aerial chemical cues (i.e. odours; Endres et al., 2009) emanating from the laboratory, we covered the channels with a removable glass plate, positioned at 15 cm above the water flow. No other activities (experimental or other) took place in the lab during the experiment.

To reduce potential interference from extraneous light sources that might affect the behaviour of the sea turtles (Witherington and Martin, 2005), we conducted this experiment under low light conditions (*sensu* Leduc et al., 2010). Specifically, under otherwise dark conditions, we relied on two lights equipped with a 5 W red lamp, each placed diagonally at a distance of 1.2 m from the upstream end of each channel (Fig. S3). Such dimly lit conditions appeared to be adequate for turtles to behave 'normally' (i.e. readily swimming in the flume) and allowed us to document the behaviour of the hatchlings, as detailed below.

The experimental testing occurred on three dates (i.e. the day that followed the collection of hatchlings and seawater, as detailed above), each with 10 hatchlings. We used this number of hatchlings per testing session given the logistical constraints of keeping a greater number of individuals in captivity at a given time. Furthermore, to provide greater ecological relevance, we considered that trials needed to occur within 24 h after the hatchlings' emergence. During this period of hyperactivity (so-called 'frenzy'; Wyneken, 1997), hatchlings are characterized by their continuous swimming behaviour (Musick and Limpus, 1997).

A testing session started by filling each of the flume's channels with one of two seawater types (randomly chosen) to a depth of ~15 cm and letting this water flow for 5 min. Each hatchling was placed in an identified container, which allowed us to randomize the testing order while knowing the identity of each individual. To initiate a trial, we placed an individual on the sand-covered beach at the starting point (Fig. S3). Specifically, hatchlings were placed ~10 cm ahead of the back wall of the flume, at the mid-point of the beach's width, and at a 90 deg angle (i.e. hatchling's head-to-tail body plan) to the flume's back wall. After entering the water, hatchlings were allowed to freely explore the flume for a period of 10 min and were video-recorded with a GoPro Hero 4 camera (24 frames s⁻¹ and 1080 dpi; San Mateo, CA, USA) placed overhead. After all 10 hatchlings were tested, we drained the flume, reversed the flow of each seawater type into each of the two channels (i.e. left versus right) and repeated the procedures presented above. In other words, each hatchling was tested twice, each time with each of the two seawater plumes on a different side of the flume. Afterward, the remaining seawater was discarded and hatchlings were returned to their native beach and released at the water's edge.

Data extraction and statistical analysis

After the completion of all trials, an observer (G.S.) analysed the videos in random order, thus without knowledge of the treatment. Videos were watched on a 31.0×17.5 cm computer screen, from which we extracted: (1) the time required to leave the beach and enter the water, (2) the time in the water before entering a channel, (3) whether a hatchling first entered the left or right channel, and (4) the time spent in each channel. A choice was defined when a hatchling had entirely passed beyond the channels' bifurcation. We also noted (5) the number of times a hatchling switched sides (e.g. leaving the left to enter the right channel of the flume), indicating whether it had been in direct contact with both seawater plumes.

As only one experimenter conducted the trials, this person was required to know the order of the treatments and thus could not conduct blind tests. If unconscious biases by the experimenter occurred relative to the initial position and orientation of hatchlings when introduced into the flume, such biases could potentially influence their initial direction and affect the channel in which they subsequently entered. To control for this possibility, a second observer (A.O.H.C.L., blind to the treatments) verified the position and orientation of hatchlings upon being introduced onto the flume's beach. This was done by visualizing the video records and measuring the initial position of the hatchlings and the direction they faced using a ruler and a protractor, respectively, placed against the computer screen. Specifically, the starting position of hatchlings was measured in relation to the midpoint (i.e. of the width) of the flume's beach (e.g. 25 out of 50 cm, or 50%), whereas the starting angle involved measuring the deviation of hatchlings' longitudinal body plan (i.e. from head to tail) relative to the flume's lengthwise direction (e.g. 0 deg, or no deviation). These data then were used in our statistical model to control for any deviation from neutral starting conditions.

To determine whether hatchlings preferred a seawater plume (i.e. coastal or oceanic) or one side of the tank (i.e. left or right), we compared the percentage of time they passed in each of the plumes and sides, using separate generalized linear models (GLMs) with a linear error distribution. The first and second analyses considered the fixed factors plume and side, respectively, both with the fixed factor repetition, and an interaction between the two terms. We also considered the fixed factor group, as testing was done with three groups of 10 individuals, each of a different female. As covariates, we used the variables position, orientation, time to channel and the number of side switches. We also tested whether the number of the first choices toward the oceanic plume differed among repetitions, using a GLM with a binary logistic error distribution, whereby the number of the first choice (whether between plumes or sides) could change or remain constant between the first and second repetitions. A preference (for a plume or side) would be indicated by a lack of significant change between the first and second repetitions, thus being maintained across repetitions. Statistical analyses were conducted using IBM SPSS v. 21.

RESULTS AND DISCUSSION

The starting conditions of hatchlings on the flume's beach were neutral/unbiased (i.e. at the midpoint, and 0 deg of deviation) in all but 23% of trials (14/60), with these deviations being minute. Specifically, in six trials, hatchling position deviated by up to ±2% from the 50% midpoint (±1 cm; mean±s.e.m.=50.1±0.08%; means are presented ±s.e.m. throughout). In eight other trials, hatchling orientation deviated by up to ±3 deg from the 0 deg starting angle (mean 0.07±0.06 deg). No statistical differences between groups or repetitions existed for these two variables. When the oceanic plume

was presented on the left and right sides of the flume, the mean initial position and orientation of hatchlings were $50.1 \pm 0.08\%$ with 0.1 ± 0.11 deg, and $49.9 \pm 0.09\%$ with $<0.1 \pm 0.13$ deg, respectively. On average, hatchlings took 21.4 ± 1.29 s following their release onto the flume beach to enter a channel with no statistical effect of groups or repetitions. In most trials, hatchlings swam in both seawater plumes (mean number of side switches = 2.1 ± 0.16), with no difference between groups or repetitions. To further verify whether the minute deviations from neutrality at the onset of these 14 trials could have affected our overall conclusions, we verified the side in which these hatchlings subsequently entered. For example, orientating or placing a hatchling toward the right side of the flume suggests a higher propensity to enter the right-side channel, and vice versa for left-side placements. However, such outcomes unfolded in less than 43% of these cases (6 out of 14; Table S1).

Hatchlings initially chose the oceanic plume in 66.7% and 70.0% of the trials for the first and second repetitions, respectively (mean = 68.3%; Fig. 2), with no statistical difference in the number of the first choice for a seawater plume between repetitions (Wald Chi-square = 0.077, d.f. = 1, $P = 0.781$). Thus, the hatchlings' first choice was maintained among repetitions. By contrast, hatchlings initially chose the left side of the flume in 66.7% and 30.0% of the first and second repetitions, respectively, with this choice statistically differing between repetitions (Wald Chi-square = 7.858, d.f. = 1, $P = 0.005$).

The mean percentage of time that hatchlings spent in the oceanic seawater was 69.3% and 68.1% for the first and second repetitions, respectively (mean = $68.8 \pm 2.22\%$) (Fig. 2). Alternatively, the percentage of the time spent on the left side of the flume was 55.6% and 44.9% for the first and second repetitions, respectively (mean = $50.3 \pm 3.20\%$). We observed a significant difference in the percentage of time spent between plumes, but not between sides (Table 1). When comparing the interaction terms plume \times repetition and side \times repetition, the former yielded no significant difference, suggesting that no change occurred among repetitions, whereas the latter yielded a significant difference. Thus, hatchlings did not appear to prefer a distinct side of the flume but rather a plume type. The variable group had no statistically significant effect on these outcomes. The position and orientation of hatchlings upon introduction onto the beach, the time to reach the channels, and the number of times hatchlings switched sides had no effects on these results (Table 1).

A functional role for hatchlings' chemosensory abilities

Here, we tested whether post-emergent loggerheads could distinguish between seawater types collected within and beyond the continental shelf. Testing for the ability to distinguish between distinct water masses provides crucial information on the abilities of sea turtles to sense the components of their habitat. Furthermore, using ecologically relevant substances may further our understanding of the functional role such abilities may serve at sea. Based on this, our experiment's rationale hinged on the fact that post-emergent loggerheads migrate to the oceanic (offshore) province (Type 2 life history; Bolten, 2003), presumably to avoid predators that could abound coastward (Carr, 1987). Thus, if hatchlings could distinguish between these naturally occurring seawater types, we expected to observe a preference for the oceanic seawater and/or avoidance of the coastal one. In agreement with our prediction, in nearly 70% of trials, hatchlings initially chose the oceanic plume and spent significantly more time in that plume.

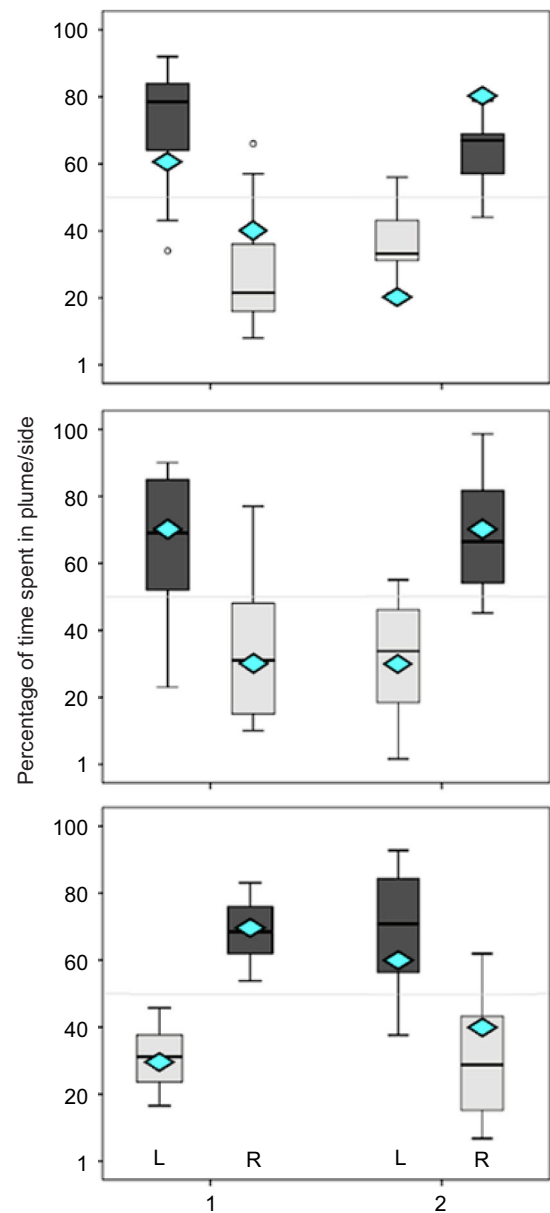


Fig. 2. Mean percentage of time spent in each of the two seawater plumes and sides of the flume, and the first choices made by hatchlings. The percentage of time spent in each of the two seawater plumes (dark- and light-grey boxplots corresponding to oceanic and coastal seawater, respectively) and sides of the flume during the first (1) and second (2) repetitions for each of the three groups of hatchlings ($N = 10$, top to bottom panels, respectively). L and R stand for the left and right channels of the flume, respectively, in which the seawater of the two sampled regions flowed sequentially. The boxplot lines are the mean for the measured parameters, the boxes range from the 25th to the 75th percentile, whiskers are $1.5 \times$ interquartile range and circles are outliers. The blue diamonds indicate the percentage of hatchlings that initially entered the left or right channel of the flume. For the first and second groups, the oceanic seawater flowed in the left channel (randomly determined) during the first repetition. For the third group, this seawater initially flowed in the right channel. During the second repetition, we reversed the flow of the seawater among channels to control for a potential side bias. In each panel, the grey horizontal line represents the 50% mark, which indicates no preference for a side and/or a plume.

Sea turtles can detect chemicals emanating from various substances, including food (Endres et al., 2009; Kedzif and Salmon, 2016), plastic debris (Pfaller et al., 2020), dimethyl sulfide (Endres and Lohmann,

Table 1. Results of generalized linear models, with a linear error distribution that yielded no over-dispersion of residues

	Wald Chi-square	d.f.	P
Plume			
Plume	155.24	1	<0.001
Repetition	0.00	1	1.00
Interaction	0.18	1	0.67
Group	0.00	2	1.00
Initial position	0.00	1	1.00
Initial orientation	0.00	1	1.00
Time to enter a channel	0.00	1	1.00
No. of side switches	0.00	1	1.00
Side			
Side	0.14	1	0.90
Repetition	0.00	1	1.00
Interaction	5.88	1	0.01
Group	0.00	2	1.00
Initial position	0.00	1	1.00
Initial orientation	0.00	1	1.00
Time to enter a channel	0.00	1	1.00
No. of side switches	0.00	1	1.00

These models compared the percentage of time that hatchlings spent in a seawater plume (coastal or oceanic), and the side of the flume (left or right). As fixed factors, we used the variables plume or side, repetition, their interaction, and group. Covariates included hatchlings' initial position and orientation, the time required to reach the water, and the number of side switches. Bold numbers indicate a statistically significant effect.

2012), mud (Endres and Lohmann, 2013; Endres et al., 2009) and sand (Grassman et al., 1984). Investigations into these substances have provided insights into the chemosensory abilities of turtles. However, the functional significance of such sensory ability has remained rather elusive, i.e. whether turtles use chemosensory abilities for orientation, navigation and/or goal recognition. At least partly, this may be the result of the nature and/or concentration of the substances tested, which may not represent what turtles encounter in their habitat. Uncovering the functional role of the chemosensory functions of sea turtles may be furthered by testing substances that occur in the wild, and at their ecologically relevant concentrations. For example, when considering feeding at sea, seawater collected from an oceanic productive 'hotspot' would represent a more ecologically relevant cue compared with arbitrary use of seafood fragments. Although collecting such seawater for its subsequent laboratory use is logistically challenging, the results obtained should further our understanding of the chemosensory functions of sea turtles in the open ocean.

Laboratory experiments using ecologically relevant cues paired with *in situ* monitoring may help to understand the role of chemosensory functions in sea turtle navigation and orientation. Throughout their ontogeny, sea turtles may rely on active-oriented swimming to reach and remain in favourable habitats (Hays et al., 2020; Luschi, 2013; Luschi et al., 1998; Putman and Mansfield, 2015; Putman et al., 2012a,b). Orientating at sea is based on wave direction and a geomagnetic compass and map (Bauer et al., 2011; Goff et al., 1998; Lohmann et al., 2008c, 2012, 2022; McNamara et al., 2011). However, *in situ* investigations of sea turtles equipped with satellite transmitters allowed tracking their movements relative to an oceanic island goal (e.g. Ascension Island). Contrary to upwind sea turtles, those located downwind from this island were able to reach it, suggesting that olfaction could play a role in orientation and goal recognition (Hays et al., 2003, 2020; Luschi et al., 2001; but see Girard et al., 2006). Although an island constitutes a tangible landmark, reaching a precise marine destination (e.g. gyre), by contrast, lacks such a feature. Reaching and remaining in such a destination may require relying on more locally restricted cues, such

as chemicals emanating from that location. In their experiment, Polovina et al. (2006) monitored loggerheads equipped with satellite tags. These sea turtles remained in a pelagic hotspot (i.e. Kuroshio Extension Bifurcation Region) when the surface water contained a high concentration of chlorophyll. However, when these waters became vertically stratified and the chlorophyll concentration dropped, these sea turtles moved to a distinct location with high surface chlorophyll concentration. If local chemical cues from productive oceanic regions are detected, that may indicate that a specific region has been reached and trigger context-appropriate behaviour (e.g. feeding, *sensu* Kezduf and Salmon, 2016). Paired with our experiment, this evidence underscores the possibility that sea turtles may distinguish between different seawater masses, which could provide goal-recognition information.

Our oceanic sampling was 15 km from the edge of the continental shelf, 21 km from the coastal sampling point and 27 km from the coast. Although the distance separating these points is relatively short considering the size of the marine realm, their respective depth drastically differed, specifically from ~40 to ~1000 m. This region is located within the bifurcation zone of the South Equatorial Current, which gives origin to two western boundary currents: the southbound Brazil Current and the northbound North Brazil Undercurrent, which partly flow above the continental shelf (Peterson and Stramma, 1991; Pereira et al., 2014; Rodrigues et al., 2007). Monitoring in the region has revealed that shelf break upwellings, mainly driven by wind processes, also occur (Thévenin et al., 2019). Paired with vertical and horizontal water movements, the abrupt change in depth could result in distinct concentrations of suspended sediments among adjacent regions. Thus, distinct concentrations of suspended sediments could provide, at least partly, a mechanism that enabled hatchlings to distinguish between the seawater plumes presented here.

How marine organisms may use ecologically relevant chemosensory cues is not always well understood because their precise composition and/or concentrations that may be detected to elicit a response are often poorly known (Costa et al., 2012). Nonetheless, while the ability to detect odours may be important, it may also provide an activational role, whereby organisms become more attuned to other sensory cues and display the most context-appropriate behaviour (Jorge et al., 2009). Here, we have provided evidence that sea turtle hatchlings may distinguish between distinct seawater masses that they are likely to encounter during their seaward migration. This detection, along with other sensory mechanisms, may allow these organisms to determine their arrival at favourable destinations when at sea.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: G.S., A.O.H.C.L.; Methodology: G.S.; Formal analysis: A.O.H.C.L.; Investigation: G.S.; Resources: E.M.d.S.; Data curation: G.S.; Writing - original draft: A.O.H.C.L.; Writing - review & editing: G.S., E.M.d.S., A.O.H.C.L.; Visualization: G.S.; Supervision: A.O.H.C.L.; Project administration: E.M.d.S.

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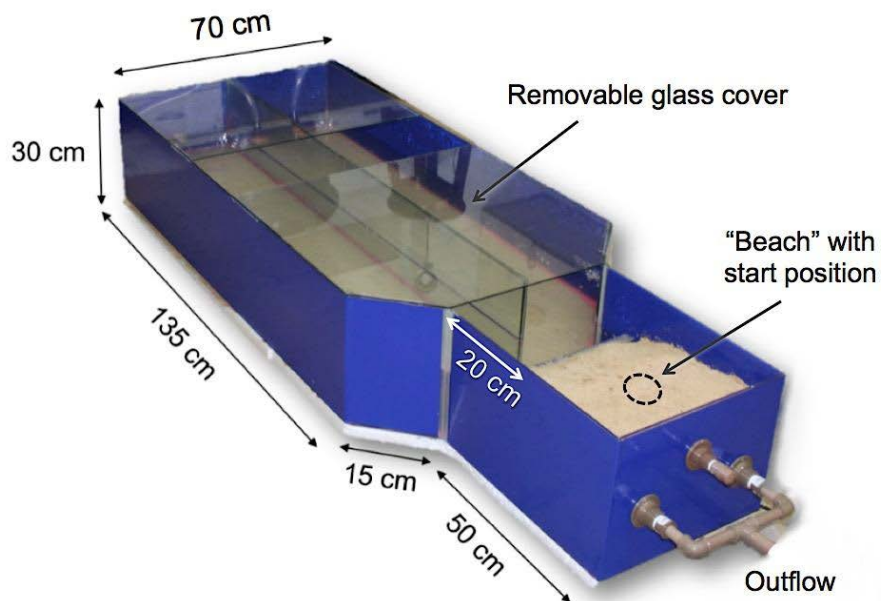


Fig. S1. Picture of the two-choice flume used in the present experiment. This flume was equipped with a starting shelf, covered with sand (i.e., “Beach”), which aimed to simulate the environment that hatchlings face before reaching the sea. To reduce potential aerial chemical interferences (e.g., airborne odours), the flume’s channels were covered by a removable glass cover (in this picture, this cover is partly opened). The flume’s walls were covered with opaque dark blue paper to minimize external lateral visual cues. Each of the two seawater plumes flowed through either the left or right channel (randomly chosen at the onset of testing); to control for a side bias, the testing procedure was repeated after reversing the side in which each of the seawater plumes flowed.

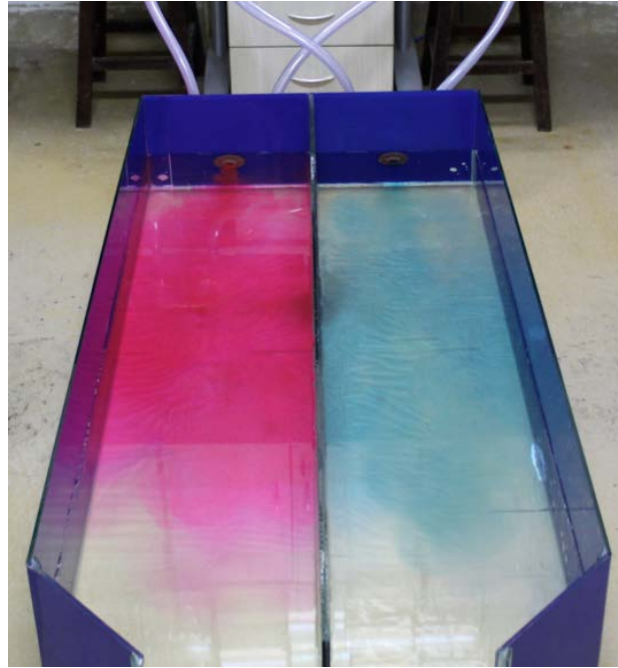


Fig. S2. An example of a dye test conducted to ensure that flow linearity and velocity were similar among the left and right channels and through repetitions. We observed no back-flow into the channels, with the water in each channel flowing at $16 \text{ ml} \cdot \text{min}^{-1}$. After establishing the aperture of valves to obtain the desired flow velocity, we conducted this procedure at the onset of each testing day (i.e., three times), which yielded comparable results.



Fig. S3. An example of a hatchling from its starting position on the ‘beach’ facing the channels and the flume’s flow. At the onset of each trial, we aimed to place a hatchling at the midpoint of the flume’s beach and with an orientation that was parallel to the flume’s lengthwise direction (see the dashed lines). A distance of 20 cm separated the edge of the ‘beach’ from the bifurcation of the flume, which lead to the left and right channels. The ‘beach’ gently sloped toward two dim red light-sources (not shown), each of these placed at approximately at 1.2 m from the inflow of each channel. Beach slope and light are known guidance cues for hatchlings after emergence, which we aimed to replicate to mimic the conditions encountered in the wild.

Table S1. List of the trials in which a hatchling's initial placement onto the flume's beach deviated from neutrality, in either the orientation angle (degree) or position relative to the beach's midpoint (cm). No individual's placement deviated from neutrality in both angle and position. Groups and repetitions relative to these placements are presented. In such biases (angle or position), negative values indicate that individuals were oriented or placed slightly toward the left side of the flume, suggesting a higher propensity of hatchlings to enter the left-side channel. Contrarily, positive values would suggest entering the right-side channel. Such occurrences are presented in bold (rightmost column) and account for less than 43% of these cases (i.e., 6 out of 14). Thus, most biased starting conditions did not guide hatchlings toward a specific side of the flume. Blank spaces indicate neutrality (i.e., a value of '0') of the starting parameter (orientation angle or position).

Turtle ID	Group	Repetition	Biased angle (degree)	Biased position (cm)	Channel entered
2	1	1		-1	Left
3	1	1	-1		Left
3	1	2	-1		Left
6	1	1		+1	Left
9	1	1	+1		Left
10	1	2		-1	Right
11	2	2	-1		Right
12	2	2		-1	Right
17	2	1		+1	Left
18	2	2	-2		Right
19	2	2	+1		Right
22	3	2		+1	Left
23	3	1	+3		Right
29	3	1	+2		Right