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RESEARCH ARTICLE

One rhinophore probably provides sufficient sensory input for odour-based navigation by the nudibranch mollusc *Tritonia diomedea*

Gregory B. McCullagh, Cory D. Bishop and Russell C. Wyeth*

ABSTRACT

Tritonia diomedea (synonymous with *Tritonia tetraquetra*) navigates in turbulent odour plumes, crawling upstream towards prey and downstream to avoid predators. This is probably accomplished by odour-gated rheotaxis, but other possibilities have not been excluded. Our goal was to test whether *T. diomedea* uses odour-gated rheotaxis and to simultaneously determine which of the cephalic sensory organs (rhinophores and oral veil) are required for navigation. In a first experiment, slugs showed no coherent responses to streams of odour directed at single rhinophores. In a second experiment, navigation in prey and predator odour plumes was compared between animals with unilateral rhinophore lesions, denervated oral veils, or combined unilateral rhinophore lesions and denervated oral veils. In all treatments, animals navigated in a similar manner to that of control and sham-operated animals, indicating that a single rhinophore provides sufficient sensory input for navigation (assuming that a distributed flow measurement system would also be affected by the denervations). Amongst various potential navigational strategies, only odour-gated positive rheotaxis can produce the navigation tracks we observed in prey plumes while receiving input from a single sensor. Thus, we provide strong evidence that *T. diomedea* uses odour-gated rheotaxis in attractive odour plumes, with odours and flow detected by the rhinophores. In predator plumes, slugs turned downstream to varying degrees rather than orienting directly downstream for crawling, resulting in greater dispersion for negative rheotaxis in aversive plumes. These conclusions are the first explicit confirmation of odour-gated rheotaxis as a navigational strategy in gastropods and are also a foundation for exploring the neural circuits that mediate odour-gated rheotaxis.

KEY WORDS: Chemosensation, Gastropod, Navigation, Odour-gated rheotaxis, Predator odour, Prey odour

INTRODUCTION

Many animals rely on odour and flow cues during navigation. In higher Reynold's number environments (>>1), where inertial hydrodynamic forces dominate, animals primarily respond to turbulent odour plumes to navigate with respect to odour sources (Vogel, 1994; Weissburg, 2000). Our understanding of both the behavioural strategies and the sensory modalities used is based largely on faster-moving arthropods and vertebrates, such as blue crabs, lobster, cockroaches, moths and fish (e.g. Baker et al., 2002; Grasso and Basil, 2002; Vickers, 2006; Weissburg and Zimmer-Faust, 1994). By contrast, aquatic gastropods also often have a primary reliance on odour-based navigation (Croll, 1983; Cummins and Wyeth, 2014), yet have more narrowly spaced sensory structures and a lower typical speed of locomotion that might lead to differences in navigation adaptations when compared with the faster taxa. Our objective then was to build on recent efforts (Ferner and Weissburg, 2005; Wilson and Weissburg, 2012; Wyeth and Willows, 2006b; Wyeth et al., 2006) to explore the navigational strategies used by gastropods in turbulent odour plumes. A parallel motivation was to isolate the sensory structures that contribute to odour-based navigation in the nudibranch *Tritonia diomedea* (Bergh 1894) (synonymous with *Tritonia tetraquetra*; Martynov, 2006). *Tritonia diomedea* is a neuroethological model system that is well suited for exploring the neural control of navigation (Murray et al., 2006). As in a number of gastropods, the central nervous system has relatively few and re-identifiable neurons, and is particularly amenable for electrophysiological study. Moreover, previous work in this species identifying the odour cues that primarily guide navigation (Wyeth and Willows, 2006b; Wyeth et al., 2006) is combined with a number of studies identifying the motor neurons that control both the ciliary locomotion and turning behaviours required for navigation (Cain et al., 2006; Popescu and Willows, 1999; Redondo and Murray, 2005). Thus, our objective for this study was also to confirm the source(s) of sensory afference that are used in navigation as a key step towards reconstructing the central neural circuit that integrates sensory input and controls output to the motor neurons.

Tritonia diomedea primarily uses odour plumes to navigate, crawling upstream towards prey and conspecifics, and downstream away from predators (Wyeth and Willows, 2006a). The high Reynolds number conditions found in their habitat create turbulent flow and therefore suggest that the slugs are likely to use odour-gated rheotaxis to find attractive odour sources, based on what as has been found previously with many other faster moving species (e.g. Cardé and Willis, 2008; Pasternak et al., 2004; Zimmer-Faust et al., 1995). However, other strategies have not been specifically excluded in the slow-moving slugs. In particular, their slower pace suggests that temporally integrated chemosensation might lead to some measurement of average odour concentrations, possibly creating circumstances when the animals could follow the time-averaged chemical gradients found in odour plumes (Webster and Weissburg, 2001; Webster and Weissburg, 2009). Although evidence has been gathered that is consistent with gastropods using odour-gated rheotaxis (Ferner and Weissburg, 2005; Wilson and Weissburg, 2012; Wyeth and Willows, 2006b; Wyeth et al., 2006), there has been no comprehensive experimental confirmation that this or any other particular navigational strategy is used. Furthermore, there has been little exploration of navigational strategies to avoid aversive odour sources in gastropods or other taxa (but see Rochette et al., 1997; Wasserman et al., 2012). Our first goal was therefore to use *T. diomedea* as a representative slow-moving gastropod and to test whether it uses odour-gated rheotaxis in both attractive and aversive odour plumes.

[1]Department of Biology, St Francis Xavier University, PO Box 5000 Antigonish, NS, Canada B2G 2W5.

*Author for correspondence (rwyeth@stfx.ca)

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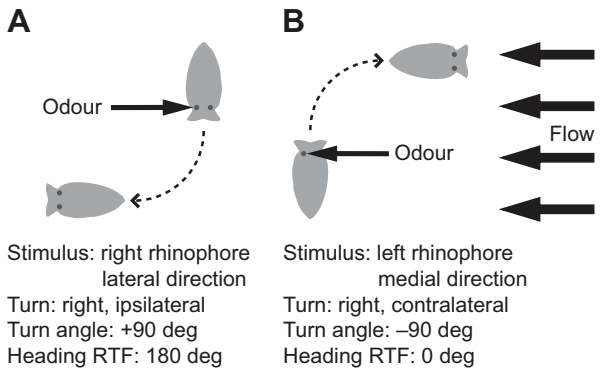

Fig. 1. Schematic diagram of direct rhinophore stimulation and analysis methods. (A) The right rhinophore is stimulated by a stream of odour applied from the lateral direction. The resulting ipsilateral turn is measured as +90 deg, with a final downstream heading relative to flow (RTF) of 180 deg. (B) The left rhinophore is stimulated by a stream of odour applied from the medial direction. The resulting contralateral turn is measured as −90 deg, with a final upstream heading RTF of 0 deg.

Determining the number of sensory inputs used during navigation can lead to insights into the navigational strategies available to the animals. Odour-gated rheotaxis requires only a single odour sensor (to determine if odour is present) and a single flow sensor (to determine flow direction). In contrast, although chemotaxis can be accomplished with one or two odour sensors, it is only with two sensors that animals can make the spatial comparisons necessary to follow relatively straight paths up chemical concentration gradients. With only one sensory structure, chemotaxis uses serial comparisons and a biased random walk, as has been shown in a number of taxa living in low Reynold's number regimes (e.g. Berg and Brown, 1972; Miller, 1985; Pierce-Shimomura et al., 1999). Although chemotaxis based on instantaneous chemosensory inputs in *T. diomedea* is unlikely because turbulent flow destroys chemical gradients (Weissburg, 2000), the slow movement of the slugs raises the possibility of time-averaged chemosensation. Given adequate integration time, chemotaxis might be used for both finding odour plume midlines and the direction of the odour source along the midline (Ferner and Weissburg, 2005; Webster and Weissburg, 2001; Wilson and Weissburg, 2012). A third strategy, plume edge following, has similar dependence on the number of sensory inputs because it relies on chemotaxis to find the location of the plume edge through bilateral spatial comparisons of widely spaced sensors (Page et al., 2011; Weissburg et al., 2002). Thus, the differing consequences of single versus paired odour sensory inputs imply that by manipulating the sensory organs we can gain insight into which strategies are used by the slugs during navigation.

Tritonia diomedea has two cephalic sensory organs that are likely to be used for navigation. The bilaterally paired rhinophores extend dorsally up into the flow above the substrate. The single oral veil is composed of bilaterally paired lobes that collectively span the entire width of the animal and is held anteriorly, just above or brushing the substrate (Wyeth and Willows, 2006a). The rhinophores have been shown to be chemosensory and necessary for navigation inside prey and predator odour plumes (Wyeth and Willows, 2006b). In addition, earlier evidence suggests that the rhinophores are rheosensitive in flow alone (Field and Macmillan, 1973). In contrast, more recent and comprehensive studies of the oral veil found that it only detects flow (Murray and Willows, 1996; Willows, 1978). However, none of these studies of the oral veil properly controlled both odours and flow throughout the experiments, and thus the roles of the rhinophores and oral veil in flow detection in odour plumes is unclear. Nonetheless, on the basis of the more recent results, we hypothesized that if flow detection is necessary for navigation in odour plumes, then the oral veil would be the primary rheosensitive organ.

To test our hypotheses, we used controlled odour and flow conditions in laboratory experiments that would be otherwise difficult to create in the field. We first applied streams of odour in seawater to directly stimulate one rhinophore on the animals. Despite using prey and predator odour solutions that triggered normal navigational responses in odour plumes, no clear pattern of responses emerged. In a second experiment with flow conditions designed to be more similar to those occurring in nature, we created turbulent odour plumes in a flow tank and then compared navigation amongst slugs with various cephalic sensory organ manipulations, as well as with intact and sham-operated controls. Navigation was found to be consistent across all surgical treatments, including animals with a single rhinophore and complete oral veil lesions. These results provide strong evidence that a single rhinophore provides sufficient input for navigation via odour-gated rheotaxis in *T. diomedea*.

RESULTS

Experiment 1: direct rhinophore stimulation

When *T. diomedea* individuals received a stream of prey, predator or control odours in seawater that were directed towards a single rhinophore, turn responses (Fig. 1) to each of the odour treatments were not significantly different. This was the case regardless of whether the odours were applied laterally or medially, whether application occurred in still or flowing water, or whether the maximum turn or total turn during odour application was analyzed [maximum turn repeated measures (rm)MANOVA $F_{2,12}$=0.18, P=0.84; total turn rmMANOVA $F_{2,12}$=0.35, P=0.71]. In still water, slugs showed no consistent turn direction in response to any odour stimulation from either the medial or lateral direction (Fig. 2). In

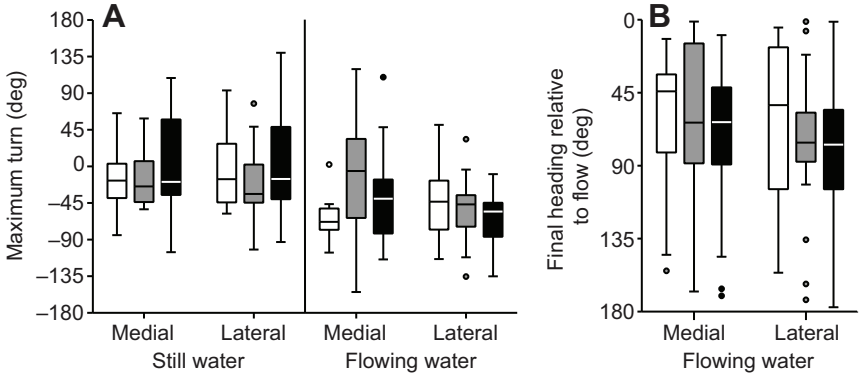

Fig. 2. Turns by *T. diomedea* show no consistent response to different odour streams applied to the rhinophore. Control seawater, white; prey (*P. gurneyi*) odour, grey; predator (*P. helianthoides*) odour, black. (A) Maximum turn angles during either medial or lateral odour stream application to rhinophores (positive values, ipsilateral turns; negative values, contralateral turns) in a tank with either still or flowing water. (B) Final headings relative to flow direction in flowing water only. The box represents the 25th and 75th quartiles, the line represents the median, the whiskers represent the minimum and maximum, and circles represent outliers that are >1.5 times the interquartile range.

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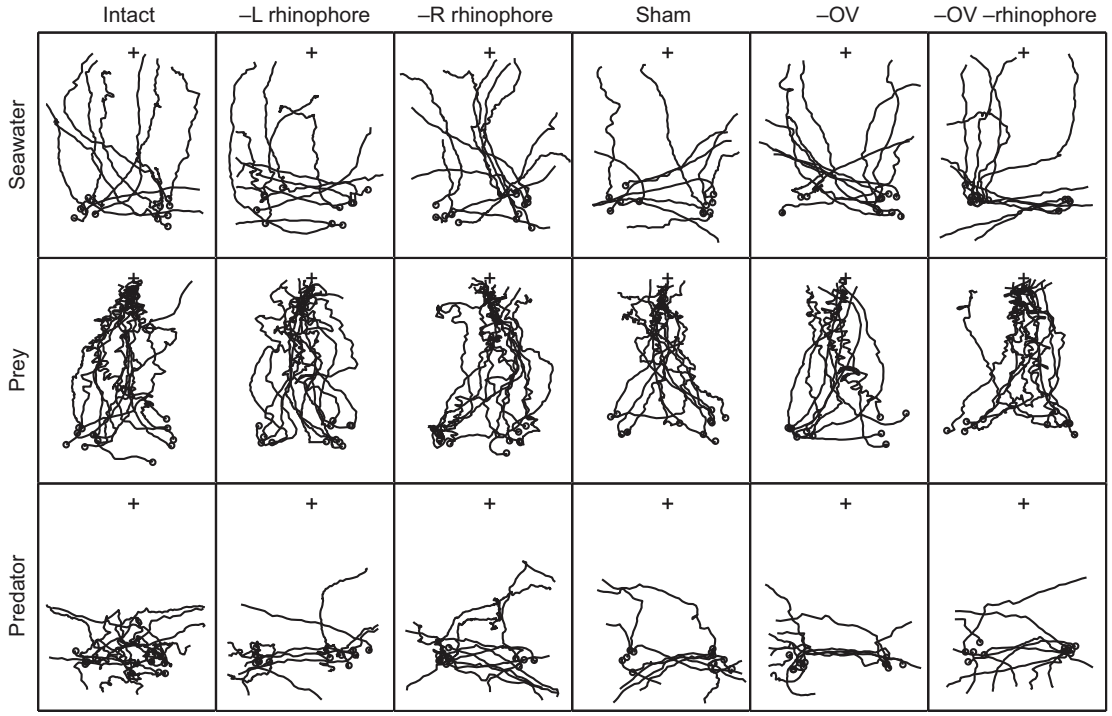

Fig. 3. Movement tracks for *T. diomedea* show no substantial effect of different cephalic sensory organ manipulations during navigation in control seawater, prey (*P. gurneyi*) or predator (*P. helianthoides*) odour plumes. Each black line represents the path of one slug crawling (open circles mark the starting locations) relative to an odour plume (+ indicates plume source) generated in unidirectional turbulent flow. Surgical treatments (columns) involved the removal or denervation of the rhinophores or oral veil, including no surgery (Intact; *N*=12), right rhinophore removed (–R rhinophore; *N*=12), left rhinophore removed (–L rhinophore; *N*=12), sham denervation surgery (Sham; *N*=11), oral veil denervation (–OV; *N*=10) and combined denervation of one rhinophore and the oral veil (–OV –rhinophore; *N*=11). All slugs were tested separately in the three odour treatments (rows). Behavioural arena dimensions: 88 cm×64 cm.

flowing water, slugs had a slight tendency to turn contralaterally in all treatments, including in response to control seawater (Fig. 2). After the stimulus ended, slugs tended to have final headings facing upstream (and perhaps also affected by stimulus direction), but again no consistent differences in the final heading relative to flow occurred when any of the different odours were applied (rmMANOVA $F_{2,12}$=3.3, P=0.07; Fig. 2).

Experiment 2: cephalic sensory organ manipulations

Our next approach for determining the sensory inputs for navigation was to compare navigation performance in odour plumes among surgical treatments designed to limit sensory input from different cephalic sensory organs. Our results showed no qualitative differences in navigational performance amongst any of the surgical treatments in each odour type (Fig. 3). Normal navigation was observed even when both the oral veil and one of the rhinophores were fully denervated. In all surgical treatments, slugs crawled upstream in prey odour plumes, concentrating their movements towards the odour source, whereas in predator odour plumes slugs consistently turned downstream. In control plumes without odours, some slugs in all surgical treatments crawled cross-stream and then upstream, whereas others crawled only in a cross-stream direction.

Quantitative analyses of navigational metrics confirmed that surgical manipulations had minimal effects on performance. In all but one case (slug speed), odour treatment had a significant effect on the metrics, but critically there was no significant interaction between the surgical and odour treatments (Table 1). Thus, differences among odour treatments in the mean and final headings (Fig. 4) did not depend on the surgical treatment. Regardless of the surgical treatment, slug headings were significantly closer to upstream in prey plumes and significantly closer to downstream in predator plumes when compared with those in control seawater plumes (based on odour treatment contrasts, Table 1). Similarly, differences among odour treatments in the minimum distance relative to the odour source and the mean distance from the estimated odour plume midline did not depend on surgical treatment (Fig. 5). Irrespective of the surgical treatment, slugs stayed significantly closer to the midline and navigated closer to the odour source in prey plumes compared with controls, whereas slugs in predator plumes stayed further away from the source (odour treatment contrasts, Table 1). The only metric that did not differ between the different odour treatments was slug speed, which showed no significant differences amongst any treatments (Table 1). This presumably then led to the significantly different trial durations in prey odour plumes (odour treatment contrasts, Table 1) due to the different paths slugs chose in the different odour treatments, but again with no significant interaction with the surgical treatments.

Specific analysis of behaviours in predator and prey plumes also showed slugs were unaffected by the surgical treatments. In predator odour plumes, maximum downstream turn angles (68±5.5 deg; mean ± s.e.m.) were significantly greater in comparison with the maximum downstream turn angles in control seawater plumes (38±3.3 deg; rmMANOVA $F_{1,62}$=23.0, P<0.001), and surgical treatments had no significant interaction with this difference (rmMANOVA $F_{5,62}$=0.68, P=0.64). In prey plumes, a side-to-side head-sweeping movement was apparent, and to characterize this, we analyzed the angular differences between successive headings. When analyzing entire trials, significantly higher differences between subsequent headings were observed for both predator and

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Table 1. Repeated measures MANOVA statistics testing the effect of odour and surgical treatments on various metrics of navigation in odour plumes by *T. diomedea*

Metric and odour treatment	Mean ± s.e.m.	Test	Statistic	*P*-value
(1) Mean heading (deg)		Interaction	$F_{10,116}$=1.2	0.31
Seawater	52±4	–	–	–
Prey	29±2	Contrast	$F_{1,58}$=26.4	<0.001
Predator	81±5	Contrast	$F_{1,58}$=19.4	<0.001
(2) Final heading (deg)		Interaction	$F_{10,122}$=0.8	0.59
Seawater	53±4	–	–	–
Prey	27±2	Contrast	$F_{1,61}$=30.4	<0.001
Predator	103±5	Contrast	$F_{1,61}$=54.1	<0.001
(3) Mean distance from midline (cm)		Interaction	$F_{10,122}$=1.2	0.28
Seawater	14±1	–	–	–
Prey	8±0	Contrast	$F_{1,61}$=63.0	<0.001
Predator	14±1	Contrast	$F_{1,61}$=0.30	0.59
(4) Minimum distance from source (cm)		Interaction	$F_{10,122}$=0.8	0.67
Seawater	36±2	–	–	–
Prey	5±1	Contrast	$F_{1,61}$=175.2	<0.001
Predator	55±1	Contrast	$F_{1,61}$=53.3	<0.001
(5) Movement speed (cm min^{-1})		Interaction	$F_{10,122}$=1.8	0.16
Seawater	7.9±0.5	–	–	–
Prey	7.2±0.2	Contrast	$F_{1,61}$=3.7	0.06
Predator	7.6±0.3	Contrast	$F_{1,61}$=0.3	0.6
(6) Trial duration (min)		Interaction	$F_{10,122}$=0.6	0.73
Seawater	9.1±0.6	–	–	–
Prey	15.5±0.8	Contrast	$F_{1,61}$=45.3	<0.001
Predator	7.5±0.7	Contrast	$F_{1,61}$=3.7	0.06
(7) Head sweeping all (deg)		Interaction	$F_{10,122}$=1.2	0.32
Seawater	9±0	–	–	–
Prey	12±1	Contrast	$F_{1,61}$=33.2	<0.001
Predator	10±0	Contrast	$F_{1,61}$=9.4	0.003
(8) Head sweeping final min (deg)		Interaction	$F_{10,122}$=1.9	0.048
Seawater	9±0	–	–	–
Prey	12±1	Contrast	$F_{1,61}$=16.1	<0.001
Predator	10±0	Contrast	$F_{1,61}$=0.6	0.45

Two types of test are presented for each of the eight metrics: interactions between odour (within-subjects effect) and surgical treatments in order to establish whether the responses to odour depended on surgery for each metric, and within-subjects contrasts to test for the effects of odour alone relative to control seawater plumes.

prey odour plumes, but again with no significant interaction with surgical treatments (Table 1). However, when analysis was limited to just the final minute of trials (when the metric is less likely to be affected by the sharp downstream turn in predator plumes, and thus a better measure of solely head-sweeping behaviour), this metric was only significantly greater in prey plumes (Table 1). In this case, there was a marginally significant interaction between surgical and odour treatments (Table 1). However, analyzing the head sweeping solely in prey plumes showed no significant differences among surgical treatments (Fig. 6A; entire trials, one-way ANOVA, $F_{5,62}$=0.73, P=0.61; final minute of trials, one-way ANOVA, $F_{5,62}$=0.82, P=0.54). We therefore conclude that cephalic sensory organ manipulations also had little or no effect on head-sweeping behaviours in prey odour plumes. Unsurprisingly then, total crawling distances in prey odour plumes were unaffected by surgery either (Fig. 6B; one-way ANOVA, $F_{5,62}$=0.53, P=0.76).

DISCUSSION

This study demonstrates that in *T. diomedea* the rhinophores are sufficient to recapitulate normal attractive and aversive responses to prey and predator odour plumes. When sensory input was isolated to the rhinophores alone, navigation in each odour treatment (seawater, prey or predator) was found to be qualitatively and statistically indistinguishable to intact control and sham-surgery slugs (Fig. 3; Table 1). Because removing the rhinophores disables navigation in odour plumes (Wyeth and Willows, 2006b), the rhinophores must therefore provide sufficient sensory input for the behaviour. Moreover, our data are all consistent with odour-gated rheotaxis based on odours and flow, which are both detected by the rhinophores.

Only the rhinophores are needed for odour-based navigation

The direct application of prey and predator odour streams to the rhinophores produced no clear responses (Fig. 2). Three possibilities can explain this result. First, odour concentrations may not have reached a threshold for detection or behavioural response. We consider this option to be unlikely because pre-tests indicated that the odour mixtures elicited normal odour plume navigational responses. Second, *T. diomedea* might integrate odour and flow information from both the rhinophores and the oral veil (or other body regions). In that case, stimulation of one rhinophore in combination with either absent or contradictory flow cues received by the other contributors to flow detection resulted in erratic behaviours. However, in this case, we expected at least normal responses when direct stimulation of the rhinophore paralleled ambient flow, which did not happen. Finally, it is possible that the

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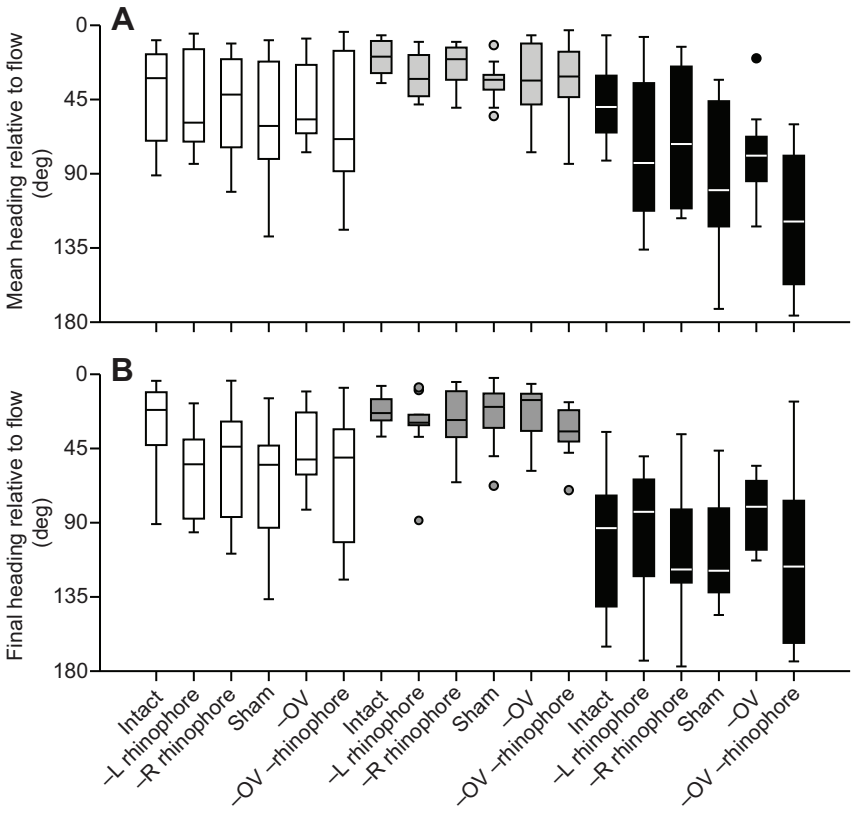

Fig. 4. *Tritonia diomedea* headings relative to flow depend on the odour plume treatment but vary little amongst cephalic sensory organ surgical treatments. Control seawater, white; prey (*P. gurneyi*) odour, grey; predator (*P. helianthoides*) odour, black. (A) Mean headings. (B) Final headings. For both metrics, prey and predator odour treatments were significantly different compared with seawater trials, but there was no significant interaction between surgical and odour treatments (Table 1). See Fig. 3 caption for surgical treatments and Fig. 2 for box and whisker plot explanation.

rhinophores can detect both odours and bulk flow direction, and that stimulation by a fine odour stream is not a sufficiently similar stimulus to that created by normal flow. The odour stream is likely to be quite dissimilar to turbulent bulk flow in normal odour plumes for several reasons: not all of the tuft is stimulated, substantial flow shear and vortices are created at the edge of the stimulus stream, and reduced pressure is created across the entire rhinophore, affecting how the rhinophore bends (among other possibilities). Any of these

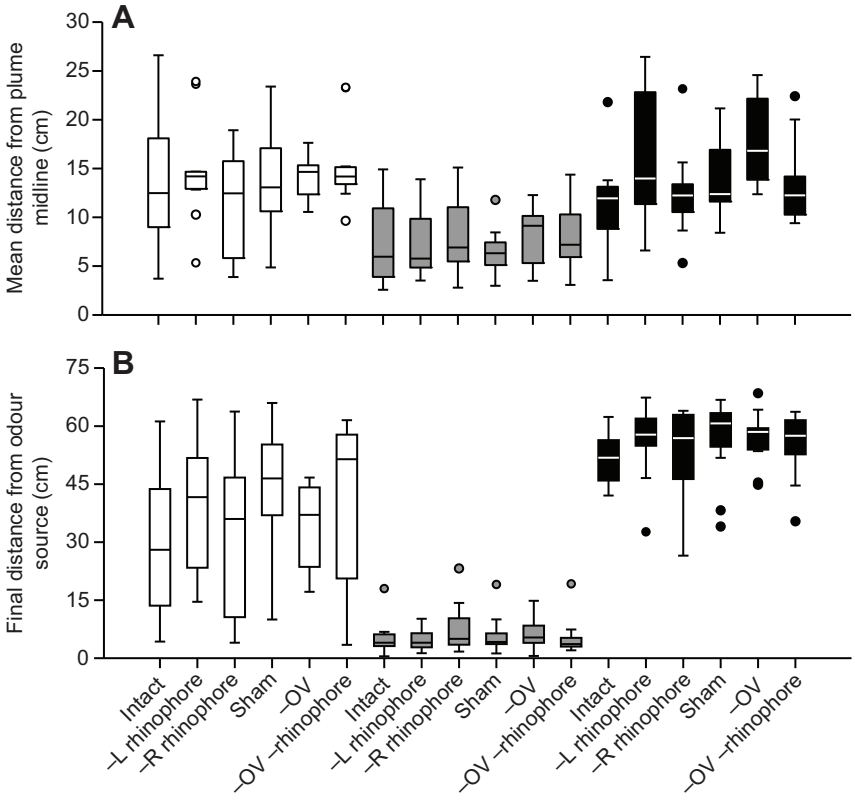

Fig. 5. *Tritonia diomedea* distances from odour plume features depend on the odour plume treatment but vary little amongst cephalic sensory organ surgical treatments. Control seawater, white; prey (*P. gurneyi*) odour, grey; predator (*P. helianthoides*) odour, black. (A) The distance from the tank midline in prey odour but not predator odour is significantly lower than that in controls. Note that odour plume sources were placed on the tank midline, and thus, on average, odour plumes were approximately centred in the tank. (B) The final distance from the plume source was significantly lower in prey odour and significantly higher in predator odour when compared with controls. For both metrics (A and B), there was no significant interaction between surgical and odour treatments (Table 1). See Fig. 3 caption for surgical treatments and Fig. 2 for box and whisker plot explanation.

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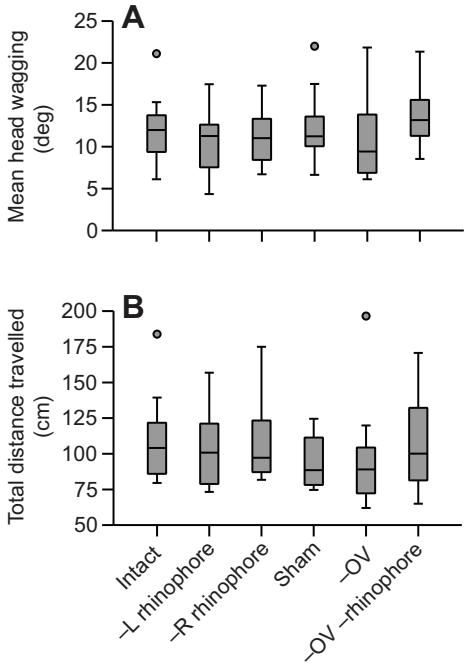

Fig. 6. *Tritonia diomedea* movements specific to prey (*P. gurneyi*) odour plumes showed little or no difference amongst surgical treatments. (A) Head-sweeping behaviour, measured by averaging differences between successive headings over the final minute of each trial. (B) Total distance crawled over each trial. No significant differences between surgical treatments were observed for either metric (one way ANOVAs). See Fig. 3 caption for surgical treatments and Fig. 2 for box and whisker plot explanation.

factors could compromise the flow information that is detected by the rhinophore in this first experiment, and thus produce erratic behaviours. The results from the second experiment, in which we manipulated the cephalic sensory organs, support this final option (the rhinophores detect both odours and bulk flow), but the first option (that odour concentrations in the direct stimulation streams were not sufficiently high to elicit responses) cannot be entirely ruled out.

In the second experiment, manipulating the cephalic sensory organs provided strong evidence that the rhinophores can be the sole source of sensory information for navigation inside odour plumes (Figs 3–5). In a previous study, *T. diomedea* with the rhinophores removed and intact oral veils were unable to navigate normally relative to upstream prey or predator odour sources, thus showing that rhinophores are necessary for navigation (Wyeth and Willows, 2006b). Here, we demonstrated that slugs with intact rhinophores and lesioned oral veils were capable of locating upstream prey odour sources and avoiding upstream predator sources. Therefore, the combined conclusion is that the rhinophores are necessary and sufficient for navigation in prey and predator odour plumes (assuming that only the specialized sense organs provide the sensory inputs for flow detection).

Bilateral cue comparisons are not necessary for navigation in odour plumes

Our results also indicate that a bilateral cue comparison between the rhinophores was not a requirement for navigation in odour plumes. Directing an odour stream onto one rhinophore did not result in turns based on a simple concentration comparison between rhinophores (ipsilateral turns for prey odours and contralateral turns for predator odours). Moreover, removing one rhinophore or eliminating all cephalic sensory input except for afference from a single rhinophore had little effect on navigation in odour plumes. Thus, the integration of cues detected by both rhinophores was not necessary to navigate inside the odour plumes presented in this study. Larger aquatic animals that are known to use bilateral comparisons have wide spatial separations between sensory organs, and are thus likely to be capable of taking advantage of steeper chemical gradients along plumes (Atema, 1996; Weissburg and Zimmer-Faust, 1994). The narrow spacing between *T. diomedea* rhinophores (1–4 cm) and the turbulent mixing in their habitat are likely to make any instantaneous bilateral comparison between odour concentration a poor strategy for determining the direction towards (or away from) an odour source. In contrast, temporal averaging of odour concentration can theoretically be used to follow chemical gradients to an odour plume source (Ferner and Weissburg, 2005; Webster and Weissburg, 2001; Wilson and Weissburg, 2012), and such a strategy could be accomplished by bilateral comparisons of the averaged odour concentrations detected by the rhinophores. Our results do not exclude this possibility, but they do indicate that it is unnecessary for navigation in odour plumes by *T. diomedea*.

Rhinophores provide both inputs for odour-gated rheotaxis

Our data further support the hypothesis that *T. diomedea* uses odour-gated rheotaxis in attractive turbulent odour plumes. As in previous studies, slugs moved upstream in the presence of prey odour (Wyeth and Willows, 2006b; Wyeth et al., 2006). Alternative chemotactic strategies via time-averaged sampling with either bilateral or serial comparisons of odour concentration could still occur in *T. diomedea*, but are unlikely to have been the primary navigational mechanism used here. As noted above, bilateral comparisons are largely excluded because *T. diomedea* navigated in an essentially normal manner with a single rhinophore. Serial comparisons involving a single detector (one rhinophore) are also unlikely because they rely on a random walk to follow the concentration gradient (Webster and Weissburg, 2009) and should therefore result in relatively erratic paths leading to an attractive odour source. Instead, the slugs had consistent paths directed towards the prey odour source. Moreover, time-averaged sampling should necessarily involve a delayed initial response to odour, and yet slugs responded almost immediately (albeit slowly) to contact with odour plumes (anecdotal observations). In contrast, odour-gated rheotaxis is entirely consistent with the paths taken by the slugs inside the prey odour plumes and with upstream movement shortly following contact with the odour plume. Odour-gated rheotaxis is a strategy held in common by many other faster moving aquatic animals that navigate inside turbulent odour plumes (e.g. Baker et al., 2002; Grasso and Basil, 2002; Weissburg and Zimmer-Faust, 1994), and our data provide strong evidence that such a strategy is also used in a slower-moving gastropod.

If *T. diomedea* uses odour-gated rheotaxis, then the rhinophores are most probably rheosensitive as well as chemosensitive. Odour-gated rheotaxis requires the detection of flow and odour cues, and slugs navigated normally with sensory input from the rhinophores alone. The conclusion that rhinophores detect both odours and flow depends on the assumption that the body wall or gills do not also contribute sensory inputs to navigation inside odour plumes. We were unable to experimentally exclude this possibility with surgical treatments (because the required nerve cuts for sensory deprivation would have too many other effects); however, we believe the assumption is reasonable for several reasons. First, no other body parts are clearly specialized as sense organs. Second, the alternative

requires a complex distributed flow detection system that must both exclude input from the cephalic sensory organs (otherwise our treatments should have affected it) and compensate for sensory afference from a soft body that is capable of substantial twists and bends, and that can be partially buried in the sediment (R.C.W., personal observation). Finally, because odour-gated rheotaxis relies on detecting the direction of flow carrying odours, little or no spatial separation between the two modalities may well be adaptive. Although information on sense organs used specifically during odour-gated rheotaxis is limited, there is evidence for the detection of both flow and chemicals by a single sense organ from a range of other taxa (Basil et al., 2005; Bicker et al., 1982; Mellon, 2007; Ruth et al., 2002). Moreover, when *T. diomedea* crawl, the dorsally extended rhinophores are probably better placed for detecting cues associated with odour plumes, rather than the oral veil which is held close to the substrate (and therefore more strongly affected by substrate boundary layers). Accordingly, we suggest that the detection of odours and flow by the rhinophores without any input from the oral veil or other body regions is best supported by the available evidence and that this would provide the most accurate information for odour-gated rheotaxis as a navigational mechanism for slugs experiencing turbulent flow.

This conclusion contradicts our original hypothesis that the oral veil detects flow while *T. diomedea* navigates in odour plumes. Our hypothesis was based on laboratory studies that showed that the oral veil detected flow in a race-track flume or Y-maze (Murray and Willows, 1996; Willows, 1978). However, these earlier experiments did not properly control for odours and thus are both harder to interpret and less applicable to slug navigation in nature (Wyeth and Willows, 2006a). In particular, both studies were conducted such that conspecific odours (which are attractive; Wyeth and Willows, 2006b) may or may not have been present during trials that were meant to test slugs in flow alone. Willows (Willows, 1978) conducted Y-maze experiments with slugs that were sometimes tested singly and, at other times, in groups. Murray and Willows (Murray and Willows, 1996) used a recirculating flume that was not drained and washed between all trials. Thus, it is in fact unclear if the earlier studies indicate whether the oral veil mediated responses to flow without odours, flow with conspecific odours or both. Moreover, our recent experiments in flow that was properly controlled to exclude any conspecific, prey or odours (our results) (Wyeth and Willows, 2006b) show that *T. diomedea* has a minor tendency to crawl upstream in flow alone, and this behaviour is consistent with the rheotactic responses observed in the earlier studies. All the various results can be reconciled in three ways. First, it is possible that although the oral veil can detect flow leading to rheotactic responses in the absence of odours, flow detection by the oral veil is not involved in orientation in odour plumes. We favour this first option as the most parsimonious. Alternatively, the oral veil may be involved in flow detection during orientation to conspecific odour but not prey and predators. Finally, because both earlier studies used laminar rather than turbulent flow, the oral veil may be involved in orientation to odours only in very low-flow environments (which may sometimes occur in nature for some populations of *T. diomedea*, but rarely for those studied here) (Wyeth and Willows, 2006a).

Behaviours in prey versus predator odour plumes

In prey odour plumes, *T. diomedea* displayed lateral head-sweeping behaviours during upstream crawling, a behaviour also observed in the field (R.C.W., personal observation). This behaviour did not occur either in predator or control plumes and did not change in response to surgical treatment (Fig. 6). Other animals behave similarly, including snails (Ferner and Weissburg, 2005; Townsend, 1974) and *Drosophila melanogaster* larvae (Gomez-Marin et al., 2010). Based again on the fact there was no difference in navigation performance between treatments with one or two rhinophores, we conclude that head sweeping in *T. diomedea* is not involved in expanded bilateral spatial comparisons, but could be involved in improving the sensitivity of odour detection for odour-gated rheotaxis by increasing the search area for odours. The rhinophores are separated by only 1 to 4 cm, and by swinging them from side-to-side the animals probably increase the probability of encountering odour filaments carried in the turbulent flow. Importantly, our methods could not distinguish whether head sweeps occurred in response to particular aspects of odour plumes or whether they were simply a stereotypical behaviour used throughout attractive odour plumes. Alternatively, head sweeps may have little or no function during navigation, but instead could be a stereotypic behaviour to increase the tactile contact rate with prey, facilitating predatory strikes (Wyeth and Willows, 2006a). Thus, further work is needed to understand the role of head sweeping in navigation by *T. diomedea*.

In predator odour plumes, *T. diomedea* turned downstream, as has been noted previously (Wyeth and Willows, 2006b; Wyeth et al., 2006). This behaviour does not appear to be a switch from positive rheotaxis in attractive odour plumes to an exactly inverted negative rheotaxis in aversive odour plumes. Rather, instead of orienting precisely downstream, the slugs seemed to turn in a downstream direction when the predator odour was detected, which may or may not lead to them facing exactly downstream. The result then is a pattern of more dispersed headings for negative rheotaxis, ranging between cross-stream and downstream, which is likely to be accounted for by animals receiving varying amounts of predator odour stimulation due to turbulence and the width of the plume when they first detect the odour. Importantly, there are multiple potential locations and directions that will serve to avoid an odour source. Thus, it is not necessarily surprising that there is more variability in predator avoidance than prey attraction.

Further work

If one rhinophore is sufficient for navigation in odour plumes, then why have two rhinophores? Constraints imposed by a bilaterally symmetric ancestry could account for paired structures, as could redundancy in the event of loss of a single organ. However, the two simultaneous inputs may still be beneficial for navigation, even if only one is sufficient. Paired organs could enhance the sensitivity of both chemosensation (Gomez-Marin et al., 2010) and rheosensation, and thereby improve the effectiveness of odour-gated rheotaxis. We suggest more challenging behavioural assays (longer distances, obstacles, etc.) would better test for possible advantages of two sensory inputs over just one input. In addition, there remains considerable potential for time-averaged sampling or other modalities contributing to sensory inputs during navigation by *T. diomedea* and other slower moving gastropods (Ferner and Weissburg, 2005; Wilson and Weissburg, 2012; Wyeth, 2010). Although both theory and our data suggest time-averaging is not necessary for navigation in *T. diomedea* in our flow tank, further work is needed to explore other circumstances where time averaging to detect odour plume concentration gradients could be beneficial. At the same time, the possibility of time-averaged rheosensation helping to detect the bulk-flow direction must also be considered. Finally, isolation of sufficient input for navigation to a single rhinophore is a key step towards designing further neuroethological

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experiments to trace the afferent pathways and central circuits that respectively transmit and integrate odour and flow information to generate odour-gated rheotaxis in *T. diomedea*.

MATERIALS AND METHODS

Animals

Tritonia diomedea (8–20 cm) and *Ptilosarcus gurneyi* were collected by SCUBA from Yellow Bank (49°14′00″N, 125°55′30″W) in Clayoquot Sound, Canada. *Pycnopodia helianthoides* were collected from several sites near Bamfield Marine Sciences Centre (BMSC), Canada (48°50′06″N, 125°08′10″W). Slugs were fed *P. gurneyi ad libitum*, unless noted, and *P. helianthoides* were starved for the duration of this study. All animals were maintained in flow-through seawater at BMSC, and all procedures were approved as compliant with Canadian Council for Animal Care regulations by the BMSC animal care committee.

Flow tank

We tested *T. diomedea* navigational performance in a non-recirculating Plexiglas flow tank (156×64×15 cm) designed to create odour plumes in which animals navigated in a manner similar to that recorded in previous field and laboratory observations (Wyeth and Willows, 2006a; Wyeth and Willows, 2006b). Seawater (20 l min^{-1}) was piped into a tilted tray (55 cm width) that spilled into the upstream end of the flow tank, and then flowed through a 0.5 cm thick Plexiglas baffle drilled with 0.75 cm holes into a behavioural arena (88 cm×64 cm), before spilling over the downstream wall (cut 3 cm lower than the rest of the flow tank). Water depth was 12 cm and flow speeds 1.0–1.2 m min^{-1}, as measured before each trial by fluorescein dye transport. To promote slug attachment to the floor of the tank, a thin layer of beach sand was added. The sand was removed and the tank drained, scrubbed and refilled between all trials. An overhead video camera (Model HDR-CX560V, Sony, Toronto, ON, Canada) was fixed 150 cm above the flow tank to record slug behaviours at 60 frames s^{-1} and 1440 by 1080 pixel resolution.

Experiment 1: direct rhinophore stimulation

Slug turning responses were measured following direct stimulation of a rhinophore while manipulating odour type, the direction of flow stimulation, and the ambient flow conditions.

Treatments: odour type, direction stimulation and ambient flow conditions

Each day, odour solutions were generated by filling three separate tanks with 6 l of seawater, adding six *P. gurneyi* (prey), four *P. helianthoides* (predator), or nothing (control), respectively. After 1 h, the water in each bin was mixed for 30 s, transferred to 50 ml aliquots with 0.05 mg ml^{-1} fluorescein sodium salt (F6377, Sigma-Aldrich, Canada) added, and subsequently kept cool in the BMSC seawater system. As a pre-test, short odour plumes were created in the flow tank from 30 ml aliquots of the odour treatments, and navigational responses were observed for three slugs not otherwise involved in testing that day. If all slugs displayed a tendency for positive rheotaxis in seawater alone, distinct upstream turns and crawling in prey odour plumes, and downstream turns in predator odour plumes, then the treatments were deemed sufficient to evoke normal odour-plume responses. On one occasion, the prey odour treatment did not meet this criterion, and all odour treatments were restarted. For direct rhinophore-stimulation tests, the three odour treatments (prey, predator and control) were applied (blind) to the rhinophore tufts, either medially or laterally, and in surrounding seawater that was either still (5 min without flow before testing) or flowing. Our goal was to test all slugs (N=18) separately in all treatments, subjecting each slug to 12 different treatments (three different odours with two odour-stimulation directions and two ambient flow conditions). Errors in assigning slugs to trials resulted in six animals receiving duplicate trials for up to four treatments (and correspondingly not receiving up to four treatments). However, the repeated measures statistical analyses we used accommodate this unbalanced design (through reduced degrees of freedom and partitioning of between-subjects and within-subjects variation), and most importantly, a minimum of 12 animals were tested in each treatment.

Direct rhinophore-stimulation protocol

Equal numbers of slugs were randomly selected to receive stimuli directed at their left (N=9) or right rhinophore (N=9). Slugs were placed midway along one side of the flow tank behavioural arena, 2.5 cm from the wall, facing cross-stream, such that their downstream rhinophore would be stimulated (to ensure only one rhinophore was stimulated; in still water trials, slugs were placed in a similar manner for consistency). Stimuli were applied after slugs began crawling and the rhinophores were fully extended. If the slugs turned more than 45 deg prior to stimulation then their heading was adjusted and we again waited for rhinophore extension. The odour stimulus was gravity-fed from an open 10 ml syringe with a polyethylene tubing nozzle (4 ml, lasting 108±24 s) directed into the appropriate rhinophore ‘tuft’.

Analysis of slug turns

Slug headings were measured in videos based on a line perpendicular to the oral veil. Both maximum and total turns were calculated using either maximum deviation or final deviation from the heading at the start of the odour application. Turns were distinguished (Fig. 1) between ipsilateral (0 deg to +180 deg) and contralateral (0 deg to −180 deg). For trials in flowing water, final headings relative to flow were calculated as the magnitude of the difference between the slug heading at the end of the odour application and the upstream direction (Fig. 1; upstream: 0 deg, downstream: 180 deg). For all three metrics, repeated measures factorial MANOVAs (rmMANOVAs) were used to test comparisons amongst the odour, stimulus direction and ambient flow condition treatments (O’Brien and Kaiser, 1985).

Experiment 2: cephalic sensory organ manipulations

Navigational performance in control, prey and predator odours was compared amongst surgical treatments limiting sensory input from different cephalic sensory organs.

Treatments: odours and cephalic sensory organ lesions

Odour mixtures were created in separate flow-through header tanks (13 l) containing either 10–12 *P. gurneyi* (prey), 6–8 *P. helianthoides* (predator) or no animals (control). Odour mixtures (with added fluorescein dye) were delivered into the upstream end of the flow tank behavioural arena through vinyl tubing (2 cm diameter, at the tank midline, 7 cm downstream from the upstream grille and 5 cm above the tank floor). A constant head pressure was maintained in the header tanks by placing them 1.25 m above the flow tank, and allowing inflows to slightly exceed the odorant outflow to the flow tank (with consequent overflow diverted to the drain for the whole system). Thus, odour solution flow (restricted with a valve to 180 ml min^{-1}) was constant throughout trials and was delivered such that bulk flow transported odours downstream (i.e. no odorant jet was created). Qualitative observations of dye showed the plume occupied the full depth of the water column beyond 5 cm downstream of the odour source, and spread outwards at ~31 deg, reaching ~45 cm width at 80 cm downstream. Turbulence was such that plume edges usually shifted by up to 10 cm across the width of the tank (in 10 s or less), and the dye occasionally spanned as little as 30 cm and as much as 60 cm across the behavioural arena at 80 cm downstream of the source. Meanwhile, observations of dye showed boundary effects extended ~5 cm from the sidewalls.

To test the roles of the cephalic sensory organs, we compared the navigational performances in the odour treatments amongst animals with lesioned rhinophores or oral veil versus intact control and sham-operated animals (72 slugs total, six surgical treatments, N=12 in each). All slugs were anaesthetized in 0.125% 1-phenoxy-2-propanol (484423, Sigma-Aldrich, Canada) for 1.5 h before surgery (Wyeth et al., 2009). To test the role of bilateral comparisons between rhinophores, we removed either the left or right rhinophore (Wyeth and Willows, 2006b). To test the role of the oral veil, we denervated it by combining procedures that have been explained in previous reports (Murray and Willows, 1996; Willows et al., 1973; Wyeth et al., 2009). Briefly, the brain was exposed and pinned by connective tissue to a wax-covered platform. Cerebral nerves (CeN) 2 and 3 were cut bilaterally, the pins and platform removed, and the incision closed with surgical adhesive (A0-002/R2, Gluture, Abbot Laboratories, USA). To further test the role of just one rhinophore, we denervated all cephalic sensory organs with the exception of a single rhinophore by bilaterally cutting CeN2, CeN3 and CeN4 (a

conservative precaution since CeN4 innervates the mouth region) and unilaterally cutting one CeN1. For controls, intact slugs underwent anaesthetic and recovery procedures, whereas sham slugs underwent surgery without nerve cuts. For recovery, slugs were segregated in flow-through containment units (15×15×15 cm) for a minimum of three days. All surgeries were verified after trials were complete by anaesthetizing the animals, opening the surgical incision and confirming the appropriate nerves were cut and that nerves had not regenerated. On this basis, two animals (one with an oral veil lesion and one with a combined oral veil and single rhinophore lesion) were excluded. In addition, when given the opportunity to feed after all trials were complete, the majority of animals after all surgical treatments consumed *P. gurneyi* pinnae (intact 83%, sham 82%, unilateral rhino removal 92%, oral veil removal 80%, oral veil and unilateral rhino removal 82%). This provides evidence that anaesthesia and surgery and had little or no non-specific effects (e.g. on feeding motivation, motor capabilities in non-target organs, etc.), consequently all animals were included in analyses whether or not they fed.

Odour-plume trial protocol

Each slug was tested on separate days in each of the three odour treatments (prey, predator and seawater), systematically varying the order of odour treatments and slug placement (left or right side of the flow tank) within each surgical treatment. In each trial, the slug was placed facing cross-stream, 80 cm downstream from the odour source and 20 cm from the plume midline (with a 3 cm gap from the side wall). The odour source valve was opened, creating the odour plume with the animals placed just outside or on its edge, and the trial continued until the animal contacted a tank wall or crawled upstream past the odour source. Trials were repeated if the slug did not crawl further than its own body length or if it immediately turned and contacted the tank wall (thus, either lacking motivation to crawl or failing to encounter the odour plumes).

Tracking and analysis of movements inside odour plumes

Slug positions (midpoint between the two rhinophores) and headings (a line perpendicular to the oral veil) were recorded every 10 s. All headings were calculated as absolute values relative to flow direction (Fig. 1) and are therefore linear (on the continuous interval [0,180]). Consequently, to better summarize or test for significant differences amongst treatments (e.g. box plots or tests accommodating repeated measures designs are not available for circular data), we used linear descriptive statistics and tests of significance rather than circular statistics (Zar, 2010). We used eight movement metrics for each trial: (1) mean heading while inside the odour plume, averaging headings between 2 and 4 min after the slug began crawling; (2) final heading, averaging headings in the final minute of each trial; (3) minimum distance relative to the odour source over the entire trial; (4) mean distance over the entire trial between the slug and the midline of the tank (as a proxy for the average centre line of the odour plume); (5) slug speed over the entire trial; (6) trial duration; (7) greatest downstream turn achieved between 1 min average headings measured stepwise across the duration of the trial; and (8) mean change in heading (absolute value) between each subsequent pair of headings within each trial (used to assess head-sweeping behaviour). This head-sweeping metric was calculated for both entire trials and the final minute of trials in an attempt to better distinguish head-sweeping behaviour from single sharp turns that occurred earlier in predator plumes.

All metrics were analyzed similarly using rmMANOVAs to test for significant effects of odour (the within-subjects effect) and an interaction effect between surgery and odour treatments (Pillai's trace statistic) (O'Brien and Kaiser, 1985; Zar, 2010). Treatment contrasts were then used to assess whether responses in specific treatments were different from controls. Because head-sweeping behaviours only occurred in prey plumes, we performed one way ANOVAs to compare the effect of different surgeries on both the head-sweeping metric and the total distance travelled by the slugs in just prey odour plumes. This latter comparison also served to test whether differences in navigation performance occurred between animals with one rhinophore and those with two rhinophores.

Software

Video was processed in VoltaicHD (v3.0.1, Systemic Pty Ltd) and tracking was completed in ImageJ software (v1.46, National Institutes of Health, Bethesda, Maryland, USA). Tracks were plotted in MATLAB (7.10.0. MathWorks), whereas statistical analyses and other figures were completed in SPSS (v20, IBM).

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Competing interests

The authors declare no competing financial interests.

Author contributions

G.B.M. contributed to funding, conception, design and execution of the experiments, and manuscript preparation. C.D.B. contributed to funding and design of the experiments. R.C.W. contributed to funding, conception, design and execution of the experiments, and manuscript preparation.

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References

Atema, J. (1996). Eddy chemotaxis and odor landscapes: exploration of nature with animal sensors. *Biol. Bull.* **191**, 129-138.

Baker, C. F., Montgomery, J. C. and Dennis, T. E. (2002). The sensory basis of olfactory search behavior in banded kokopu (*Galaxias fasciatus*). *J. Comp. Physiol. A* **188**, 553-560.

Basil, J. A., Bahctinova, I., Kuroiwa, K., Lee, N., Mims, D., Preis, M. and Soucier, C. (2005). The function of the rhinophore and the tentacles of *Nautilus pompilius* L. (Cephalopoda, Nautiloidea) in orientation to odor. *Mar. Freshwat. Behav. Physiol.* **38**, 209-221.

Berg, H. C. and Brown, D. A. (1972). Chemotaxis in *Escherichia coli* analysed by three-dimensional tracking. *Nature* **239**, 500-504.

Bicker, G., Davis, W. J., Matera, E. M., Kovac, M. P. and Stormogipson, D. J. (1982). Chemoreception and mechanoreception in the gastropod mollusc *Pleurobranchaea californica.* 1. Extracellular analysis of afferent pathways. *J. Comp. Physiol.* **149**, 221-234.

Cain, S. D., Wang, J. H. and Lohmann, K. J. (2006). Immunochemical and electrophysiological analyses of magnetically responsive neurons in the mollusc *Tritonia diomedea*. *J. Comp. Physiol. A* **192**, 235-245.

Cardé, R. T. and Willis, M. A. (2008). Navigational strategies used by insects to find distant, wind-borne sources of odor. *J. Chem. Ecol.* **34**, 854-866.

Croll, R. P. (1983). Gastropod chemoreception. *Biol. Rev. Camb. Philos. Soc.* **58**, 293-319.

Cummins, S. F. and Wyeth, R. C. (2014). Olfaction in gastropods. In *Neuroecology and Neuroethology in Molluscs: The Interface between Behaviour and Environment* (ed. A. Di Cosmo and W. Winlow), pp. 45-72. Hauppauge, NY: Nova Science Publishers, Inc.

Ferner, M. C. and Weissburg, M. J. (2005). Slow-moving predatory gastropods track prey odors in fast and turbulent flow. *J. Exp. Biol.* **208**, 809-819.

Field, L. H. and Macmillan, D. L. (1973). An electrophysiological and behavioural study of sensory responses in *Tritonia* (Gastropoda, Nudibranchia). *Mar. Behav. Physiol.* **2**, 171-185.

Gomez-Marin, A., Duistermars, B. J., Frye, M. A. and Louis, M. (2010). Mechanisms of odor-tracking: multiple sensors for enhanced perception and behavior. *Front. Cell. Neurosci.* **4**, 6.

Grasso, F. W. and Basil, J. A. (2002). How lobsters, crayfishes, and crabs locate sources of odor: current perspectives and future directions. *Curr. Opin. Neurobiol.* **12**, 721-727.

Martynov, A. V. (2006). Nudibranchia. In *Marine and Brackish Water Gastropoda of Russia and Adjacent Countries: an Illustrated Catalogue* (ed. Y. I. Kantor and A. V. Sysoev), pp. 268-294, plates 131-138. Moscow: KMK Scientific Press.

Mellon, d., Jr (2007). Combining dissimilar senses: central processing of hydrodynamic and chemosensory inputs in aquatic crustaceans. *Biol. Bull.* **213**, 1-11.

Miller, R. L. (1985). Sperm chemo-orientation in the metazoa. In *Biology of Fertilization: Biology of the Sperm*, Vol. 2 (ed. C. V. Metz and A. Monroy), pp. 275-337. London, UK: Academic Press.

Murray, J. A. and Willows, A. O. D. (1996). Function of identified nerves in orientation to water flow in *Tritonia diomedea*. *J. Comp. Physiol. A* **178**, 201-209.

Murray, J. A., Estepp, J. and Cain, S. D. (2006). Advances in the neural bases of orientation and navigation. *Integr. Comp. Biol.* **46**, 871-879.

O'Brien, R. G. and Kaiser, M. K. (1985). MANOVA method for analyzing repeated measures designs: an extensive primer. *Psychol. Bull.* **97**, 316-333.

Page, J. L., Dickman, B. D., Webster, D. R. and Weissburg, M. J. (2011). Staying the course: chemical signal spatial properties and concentration mediate cross-stream motion in turbulent plumes. *J. Exp. Biol.* **214**, 1513-1522.
Pasternak, Z., Blasius, B. and Abelson, A. (2004). Host location by larvae of a parasitic barnacle: larval chemotaxis and plume tracking in flow. *J. Plankton Res.* **26**, 487-493.
Pierce-Shimomura, J. T., Morse, T. M. and Lockery, S. R. (1999). The fundamental role of pirouettes in *Caenorhabditis elegans* chemotaxis. *J. Neurosci.* **19**, 9557-9569.
Popescu, I. R. and Willows, A. O. D. (1999). Sources of magnetic sensory input to identified neurons active during crawling in the marine mollusc *Tritonia diomedea*. *J. Exp. Biol.* **202**, 3029-3036.
Redondo, R. L. and Murray, J. A. (2005). Pedal neuron 3 serves a significant role in effecting turning during crawling by the marine slug *Tritonia diomedea* (Bergh). *J. Comp. Physiol. A* **191**, 435-444.
Rochette, R., Dill, L. M. and Himmelman, J. H. (1997). A field test of threat sensitivity in a marine gastropod. *Anim. Behav.* **54**, 1053-1062.
Ruth, P., Schmidtberg, H., Westermann, B. and Schipp, R. (2002). The sensory epithelium of the tentacles and the rhinophore of *Nautilus pompilius* L. (Cephalopoda, Nautiloidea). *J. Morphol.* **251**, 239-255.
Townsend, C. R. (1974). The chemoreceptor sites involved in food-finding by the freshwater pulmonate snail, *Biomphalaria glabrata* (Say), with particular reference to the function of the tentacles. *Behav. Biol.* **11**, 511-523.
Vickers, N. J. (2006). Winging it: moth flight behavior and responses of olfactory neurons are shaped by pheromone plume dynamics. *Chem. Senses* **31**, 155-166.
Vogel, S. (1994). *Life in Moving Fluids: the Physical Biology of Flow*. Princeton, NJ: Princeton University Press.
Wasserman, S., Lu, P., Aptekar, J. W. and Frye, M. A. (2012). Flies dynamically anti-track, rather than ballistically escape, aversive odor during flight. *J. Exp. Biol.* **215**, 2833-2840.
Webster, D. R. and Weissburg, M. J. (2001). Chemosensory guidance cues in a turbulent chemical odor plume. *Limnol. Oceanogr.* **46**, 1034-1047.
Webster, D. R. and Weissburg, M. J. (2009). The hydrodynamics of chemical cues among aquatic organisms. *Annu. Rev. Fluid Mech.* 41, 73-90.
Weissburg, M. J. (2000). The fluid dynamical context of chemosensory behavior. *Biol. Bull.* **198**, 188-202.
Weissburg, M. J. and Zimmer-Faust, R. K. (1994). Odor plumes and how blue crabs use them in finding prey. *J. Exp. Biol.* **197**, 349-375.
Weissburg, M. J., Dusenbery, D. B., Ishida, H., Janata, J., Keller, T., Roberts, P. J. W. and Webster, D. R. (2002). A multidisciplinary study of spatial and temporal scales containing information in turbulent chemical plume tracking. *Environ. Fluid Mech.* **2**, 65-94.
Willows, A. O. D. (1978). Physiology of feeding in Tritonia I. Behaviour and mechanics. *Mar. Behav. Physiol.* **5**, 115-135.
Willows, A. O. D., Dorsett, D. A. and Hoyle, G. (1973). The neuronal basis of behavior in *Tritonia*. I. Functional organization of the central nervous system. *J. Neurobiol.* **4**, 207-237.
Wilson, M. L. and Weissburg, M. J. (2012). Temporal and spatial sampling strategies maintain tracking success of whelks to prey patches of differing distributions. *Anim. Behav.* **84**, 1323-1330.
Wyeth, R. C. (2010). Should animals navigating over short distances switch to a magnetic compass sense? *Front. Behav. Neurosci.* **4**, 42.
Wyeth, R. C. and Willows, A. O. D. (2006a). Field behavior of the nudibranch mollusc *Tritonia diomedea*. *Biol. Bull.* **210**, 81-96.
Wyeth, R. C. and Willows, A. O. D. (2006b). Odours detected by rhinophores mediate orientation to flow in the nudibranch mollusc, *Tritonia diomedea*. *J. Exp. Biol.* **209**, 1441-1453.
Wyeth, R. C., Woodward, O. M. and Willows, A. O. D. (2006). Orientation and navigation relative to water flow, prey, conspecifics, and predators by the nudibranch mollusc *Tritonia diomedea*. *Biol. Bull.* **210**, 97-108.
Wyeth, R. C., Croll, R. P., Willows, A. O. D. and Spencer, A. N. (2009). 1-Phenoxy-2-propanol is a useful anaesthetic for gastropods used in neurophysiology. *J. Neurosci. Methods* **176**, 121-128.
Zar, J. H. (2010). *Biostatistical Analysis*. 5th edn. Upper Saddle River, NJ: Pearson Prentice Hall.
Zimmer-Faust, R. K., Finelli, C. M., Pentcheff, N. D. and Wethey, D. S. (1995). Odor plumes and animal navigation in turbulent water flow: a field-study. *Biol. Bull.* **188**, 111-116.