The Journal of Experimental Biology 209, 1441-1453
Published by The Company of Biologists 2006
doi:10.1242/jeb.02164

# Odours detected by rhinophores mediate orientation to flow in the nudibranch mollusc, *Tritonia diomedea*

Russell C. Wyeth* and A. O. Dennis Willows

*Department of Biology, University of Washington, Seattle, WA 98195-1800, USA and Friday Harbor Laboratories, 620 University Road, Friday Harbor, WA 98250, USA*

*Author for correspondence at present address: Department of Physiology and Biophysics, Dalhousie University, Halifax, NS, B3H 1X5, Canada (e-mail: russell.wyeth@dal.ca)

*Accepted 8 February 2006*

## Summary

***Tritonia diomedea*** **is a useful neuroethological model system that can contribute to our understanding of the neural control of navigation. Prior work on both sensory and locomotory systems is complemented by recent field experiments, which concluded that these animals primarily use a combination of odours and water flow as guidance cues. We corroborate these field results by showing similar navigation behaviours in a flow tank. Slugs crawled upstream towards both prey and conspecifics, and turned downstream after crawling into a section of the flow tank downstream of a predator. Controls without upstream odour sources crawled apparently randomly. We then tested whether these behaviours depend on odours detected by the rhinophores. Outflow from a header tank was used to generate prey, predator and unscented control odour plumes in the flow tank. Slugs with rhinophores crawled upstream towards a prey odour plume source, turned downstream in a predator odour plume, and showed no reaction to a control plume. Slugs without rhinophores behaved similarly to controls, regardless of odour plume type. Finally, we used extracellular recordings from the rhinophore nerve to demonstrate that isolated rhinophores are chemosensitive. Afferent activity increased significantly more after application of all three odour types than after unscented control applications. Responses were odour specific. We conclude that rhinophores mediate orientation to flow, and suggest that future work should focus on the integration of mechanosensation and chemosensation during navigation in** ***T. diomedea*****.**

Supplementary material available online at
http://jeb.biologists.org/cgi/content/full/209/8/1441/DC1

Key words: *Tritonia diomedea*, nudibranch, gastropod, behaviour, neuroethology, navigation, orientation, sensory cues, chemosensation, odours, rheotaxis, mechanosensation.

## Introduction

The nudibranch *Tritonia diomedea* provides an opportunity to study how a nervous system integrates multiple sensory cues to produce oriented locomotion. A combination of easily observed behaviours with a relatively simple and accessible nervous system has promoted a variety of neuroethological studies (e.g. Willows et al., 1973; Hume et al., 1982; Willows, 1978; Beck et al., 2000; Popescu and Frost, 2002). Field studies have reported the slugs' navigational behaviours, suggesting the importance of odours and water flow as navigational cues (Wyeth and Willows, in press; Wyeth et al., in press). Progress has also been made studying both sensory and locomotory systems (e.g. Willows, 1978; Lohmann et al., 1991; Murray et al., 1992; Murray and Willows, 1996; Willows et al., 1997; Popescu and Willows, 1999; Wang et al., 2003; Redondo and Murray, 2005; Blackwell and Murray, 2005), making possible investigations into the neural basis of navigation in this species. Our goals here are to observe navigation in the laboratory, to confirm odours and water flow as guidance cues, and to study chemosensation in *T. diomedea*, which appears to be important for navigation in the field.

Analyses of crawling behaviours in the natural habitat suggested that *T. diomedea* uses odours and water flow to navigate relative to prey, predators and conspecifics (Wyeth et al., in press). The slugs crawl upstream before feeding and mating, and downstream when an upstream predator is present. In experiments considering flow alone, upstream crawling (positive rheotaxis) was observed in the laboratory (Field and Macmillan, 1973; Willows, 1978; Murray and Willows, 1996), and in the field (Murray et al., in press). However, behavioural responses to odours in controlled experiments were observed in only one laboratory study, where *T. diomedea* located prey but not conspecifics in a Y-maze (Willows, 1978). With such limited and sometimes contradictory data, we wished to clarify the navigational responses to prey, predators and mates by

reproducing in the laboratory the navigational behaviours observed in the field.

Information regarding the sensory structures used for navigation is also incomplete. The oral veil appears to be the primary source of mechanosensory input used for flow orientation (Murray and Willows, 1996). The rhinophores are likely chemosensory, as they are required to find prey in the Y-maze (Willows, 1978). Rhinophores in other opisthobranchs, including *Aplysia* sp. (Audesirk, 1975; Audesirk and Audesirk, 1977; Levy et al., 1997), *Pleurobranchaea californica* (Bicker et al., 1982a; Bicker et al., 1982b) and *Phestilla sibogae* (Croll, 1983; Murphy and Hadfield, 1997), have been shown to be chemosensory and important for odour based navigation. In *T. diomedea*, chemosensory responses have only been recorded from nerves innervating the anterior foot, oral veil and mouth regions (Field and Macmillan, 1973; Audesirk and Audesirk, 1980), with no experiments testing the rhinophores. Since odours appear to be used in navigation, our goal was to determine if the rhinophores are necessary for the navigational behaviours and to test their chemosensitivity.

We use experiments in a flow tank with upstream odour sources to reproduce all three navigational behaviours. Furthermore, we confirm that odours alone are sufficient to trigger upstream navigation towards prey and mates, and downstream navigation away from predators. Removing the rhinophores eliminates orientation to prey and predators, and extracellular recordings reveal that rhinophores are chemosensory, responding selectively to all three odours.

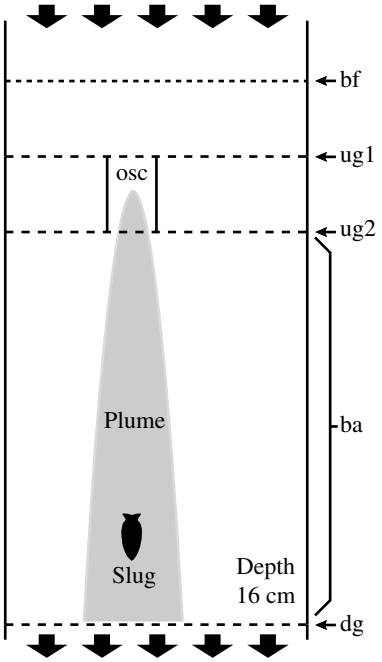


Fig. 1. Flow tank schematic. Seawater was first piped into a tilted inflow tank (95 cm wide, not shown), passing over or under four barriers, before spilling into the flow tank across its full width (black arrows). Flow continued through a 0.75 cm thick Plexiglas baffle (bf) drilled with 0.75 cm holes, two upstream grilles (ug1 and ug2; 1 cm square mesh, 0.75 cm thick), a behavioural arena (ba), and a downstream grille (dg) to prevent slugs being swept out of the tank, before spilling over the downstream end wall (cut 2 cm lower than the other tank walls). Plexiglas walls spanning the gap between the upstream grilles created an odour stimulus chamber (osc). This design created unidirectional flow through the behavioural arena with enough turbulence to spread fluorescein dye plumes (grey approximates the average plume shape) similarly to plumes in the field (Wyeth and Willows, in press). Odor plumes will be similar to these dye plumes since flow dominates chemical transport under such conditions (Vogel, 1994). The 2nd upstream grille also served to obscure any downstream flow patterns characteristic of the odour sources (Vogel, 1994). Flow tank width, 1 m.

## Materials and methods

### *Animals*

*Tritonia diomedea* Bergh and *Ptilosarcus gurneyi* were collected by SCUBA and maintained in sea tables at Friday Harbor Laboratories, Washington, USA. Slugs were fed *P. gurneyi ad libitum*, unless noted. *Pycnopodia helianthoides* were collected off the Friday Harbor Laboratories dock.

### *Navigation relative to prey, predators and conspecifics*

We tested *T. diomedea*'s ability to navigate relative to odour sources in a flow tank (Fig. 1). Slug activity in an arena downstream of odour sources placed in upstream flow-through odour stimulus chambers was recorded using a video camera mounted above the tank (Model CVC-320WP, Speco Technologies, Amityville, NY, USA). Video was digitized at 2.5 frames $s^{-1}$ and 320×240 pixel resolution by a PC computer digital video recording system [Novex 2000, Novex (Canada) Ltd., North York, ON, Canada]. Flow speeds, measured by fluorescein dye transport before every trial, were 1.1±0.01 m $min^{-1}$ (mean ± s.e.m.). Between all trials, the tank was drained and all surfaces scrubbed and rinsed unless noted.

Navigation relative to prey (*P. gurneyi*) was tested in nine slugs starved for ~2 months (*T. diomedea* remains healthy for months without food). We positioned two 12 cm wide odour stimulus chambers at the tank midline. We tested each slug twice in random order: an experimental trial, with a *P. gurneyi* in one randomly chosen odour stimulus chamber, and a control trial, with both chambers empty. Slugs were placed ~1.1 m directly downstream of the chambers, facing upstream. Each trial lasted 1.5 h after the animal settled onto the tank bottom.

Navigation relative to conspecifics was tested in 20 slugs kept celibate by isolation for ~1 month. Methods were similar to the prey experiment, modified to promote both conspecific odour release and response. In experimental trials, two slugs were placed in one randomly chosen odour stimulus chamber and one slug in the other (both chambers 15 cm wide). We forced the tested slug to remain downstream of the odour stimulus chambers for 15 min by placing it in a flow-through box with a removable upstream gate. Each trial lasted 2–4 h after the gate was lifted, with identical control and experimental durations for each slug. Four experimental trials

and three controls were excluded from orientation analyses due to continuous contact with the box.

For analysis of differences between control and experimental treatments in the prey and conspecific experiments, slug positions and headings (the direction perpendicular to a line connecting the rhinophores) were digitized every 2 min. Each slug's path was mapped onto a standard coordinate system. Slug headings relative to flow (RTF), while unobstructed by the arena edges, were averaged for each trial. Significance of the pooled mean headings RTF for both treatments was then assessed using Rayleigh tests (Zar, 1999). In addition, the differences between treatments in both angular dispersion around upstream (Wilcoxon matched pair test) and mean distance from the odour stimulus chambers (paired *t*-test) were assessed.

Navigation relative to the predatory sea star, *P. helianthoides* (Wyeth and Willows, in press; Murray et al., in press), was tested in 13 slugs. Trials lasted 1 h, with 30 cm wide odour stimulus chambers placed at the tank edges. We used latex tubing stars in control trials because sea stars affected flow at the tank edges. Sand was sifted into the behavioural arena because it appeared to facilitate cross-stream turns in preliminary experiments. Slugs were placed ~65 cm downstream of the chambers, at the midline of the tank, facing upstream. Experimental and control trials were separated by overnight flushing of the odour stimulus chambers.

For analysis of navigation relative to predators, slug positions and headings were digitized every 10 s until the arena bounds were contacted. Downstream turning upon entry into a putative odour plume was calculated as the angle between averaged slug headings RTF for the 2 min before and 2 min after crossing a line 14 cm medial to the medial odour stimulus chamber walls (metric based on preliminary data). In addition, we calculated the change in distance from the odour stimulus chamber while the slugs were inside this putative plume region. To assess differences in downstream turns and movement, we used paired *t*-tests. However, only six slugs crawled into the plume region in both control and experimental trials, and thus we also used unpaired *t*-tests to include data from all slugs that moved into the plume region.

### *Navigation with or without rhinophores relative to prey and predators*

Twenty slugs were anaesthetized separately for 1.5 h each in a bath of 0.125% 2-phenoxy propanol in seawater (Runham et al., 1965; Norton et al., 1996). We cut both rhinophores at their base from half the animals (randomly chosen). Slugs were allowed to recover for a month, during which time each slug was given one opportunity to feed overnight, monitored by video recordings.

Orientation tests were conducted on eight slugs with rhinophores and seven without (five were not tested due to a protist infection unrelated to surgery). We randomly assigned three odour stimuli (four *P. helianthoides* individuals; six *P. gurney*; empty control) to three header tanks supplied by the same source as the flow tank. We placed the slug on one side of the flowing tank, facing cross-stream. After 4 min, we introduced a header tank outflow between the upstream grilles (3 ml $s^{-1}$, dyed with a single 0.5 ml dose of 50% fluorescein in seawater), positioned so that the slug encountered the resulting dye plume. Behaviours were video recorded for 10 min. Each slug received all three odour stimuli in separate trials (order varied systematically, along with which side of the tank the slug was placed). Five trials were repeated because the slugs made contact with the flow tank walls before the header tank flow could be introduced.

For analysis, we used motion enhanced video recordings, thresholding mean subtracted frames every 10 s (Wyeth and Willows, in press). The centre of an ellipse approximating the largest white region in the arena marked the slug position, and headings were calculated from vectors between positions. For each trial, we calculated mean headings RTF for a 2 min interval, 30 s after the dye plume was encountered. We tested each treatment combination (three odour stimuli, two slug groups) for a significant second order mean heading RTF (Hotelling test; Zar, 1999).

### *Extracellular recordings from rhinophore nerves in response to odour stimuli*

#### *Dissection and recording*

We isolated rhinophores with the rhinophore nerve cut near the brain. Rhinophores were pinned in a Sylgard (Dow Corning, Midland, MI, USA)-lined dish in a seawater perfusion bath. We recorded extracellular activity in the rhinophore nerve with an *en passant* suction electrode. Several electrode applications were usually necessary before the subset of axons recorded included neurons responsive to prey or predator odours. Conspecific odour responses were often present in the first electrode application. The signal was amplified with an A-M Systems Differential AC Amplifier, Model 1700 (Carlsborg, WA, USA) with 10 000 gain and 1 kHz low-pass and 10 Hz high-pass filters. For larger animals, we increased extracellular spike amplitudes by briefly digesting the nerve sheath with protease in seawater. Recordings were digitized with a Micro1401 MkII and Spike 2 software (Cambridge Electronic Design, Cambridge, England).

#### *Odour tests*

We tested three odours in seawater sampled from separate tanks holding prey, predators, or conspecifics. Odours were isolated from between the pinnea (leaves) of *P. gurneyi*, from the between the arms of *P. helianthoides*, and from near swollen genitalia of *T. diomedea*. For each odour type a similar empty tank provided a paired control stimulus. All odour solutions were 0.22 μm filtered.

Odours were delivered to the apical sensory tuft of the rhinophore in continuous flow generated by a peristaltic pump. Thin walled polyethylene (PE) tubing immersed in the perfusion bath equilibrated the temperature of the odour flow with the bath seawater. A valve switched the odour flow between two sources: (1) a reservoir fed from the perfusion seawater, or (2) an odour tube inside, but sealed from, the

reservoir. Odour solution reached the rhinophore 10–20 s after the valve was switched, depending on pump speed. This system isolated odour stimuli responses from any temperature or mechanical effects of switching between the sources.

Each odour test consisted of five trials, separated by at least 5 min. Paired control and odour stimuli, each ~1.1 ml, were applied blindly and in random order, interspersed by washes to flush the odour system. Seven rhinophores from six slugs were responsive to prey odour, seven rhinophores from six slugs to predator odour, and ten rhinophores from five animals to conspecific odour. During these experiments, we tested at least two odours during single electrode applications in 11 different rhinophores. Both rhinophores from one animal were unresponsive to any odour.

*Response analysis*

We recorded times of spikes that exceeded one voltage level, but did not exceed a second level within 50 ms (levels identical for all trials in a test). Predator responses tended to be larger amplitude, low frequency spikes, that were not always isolated by this metric. However, the responsive waveform shape could be qualitatively distinguished from other activity, and could be isolated by template matching (Lewicki, 1998; Wheeler, 1999) in Spike 2 software.

Whether level functions or template matching were used, we counted spikes inside windows encompassing delivery of odour and control stimuli, as well as a baseline window 2 min prior to each trial. To allow for solution mixing, the analysis window started 12.5% of the mean odour delivery time (ODT) before the expected start of odour delivery to the sensory tuft and ended 25% of the ODT after the expected end of odour delivery. To make comparisons across rhinophores, counts were normalized to the maximum count in each test and arcsin transformed. The different treatments were then compared with a repeated measures multivariate analysis of variance (MANOVA) (O'Brien and Kaiser, 1985), followed by baseline *versus* control and control *versus* odour treatment comparisons ($P$=0.05, with Šidák's adjustment). We tested both the complete dataset for each odour type and a dataset including just one rhinophore per animal (randomly chosen if both rhinophores were tested).

*Rhinophore responses to mating and non-mating conspecifics*

Eight rhinophores known to be responsive to conspecific odour were post-tested for responses to mating and non-mating pairs of conspecifics. Different pairs were used for each test, one pair from a group kept celibate for ~1 month, and the other from a group with potential mates available *ad libitum*. The two pairs were placed in separate holding tanks, and after the previously celibate pair mated, 10 ml seawater samples from next to the genital openings of the mating and non-mating pairs were isolated. A control sample was isolated from an empty tank. Rhinophore responses to the three filtered samples were then recorded in three repeated trials, as above.

To test whether rhinophores responded differently to odours from mating and non-mating pairs, we analysed responses for each rhinophore separately, in each case using the same parameters as the original conspecific odour test. Spike counts during the three odour stimuli and baseline windows were normalized and arcsine transformed before comparison using an ANOVA, followed by Tukey's pair-wise comparison of mean responses (Zar, 1999).

*Software*

Analyses were performed in Premiere 6.0 (Adobe Systems Inc., San Jose, CA, USA), Excel (Microsoft, Redmond, WA, USA), Matlab 6.5 and 7.0 (The Mathworks Inc., Natick, MA, USA), Spike2 4.x (Cambridge Electronic Design, Cambridge, England), SPSS 13.0 (SPSS, Inc., Chicago, IL, USA) or JMP 5.1. (SAS Institute Inc., Cary, NC, USA).

## Results

*Upstream navigation toward upstream prey and conspecifics*

*Tritonia diomedea* crawled upstream towards prey in the flow tank (Fig. 2). In contrast, during control trials, slugs crawled apparently randomly relative to the odour stimulus

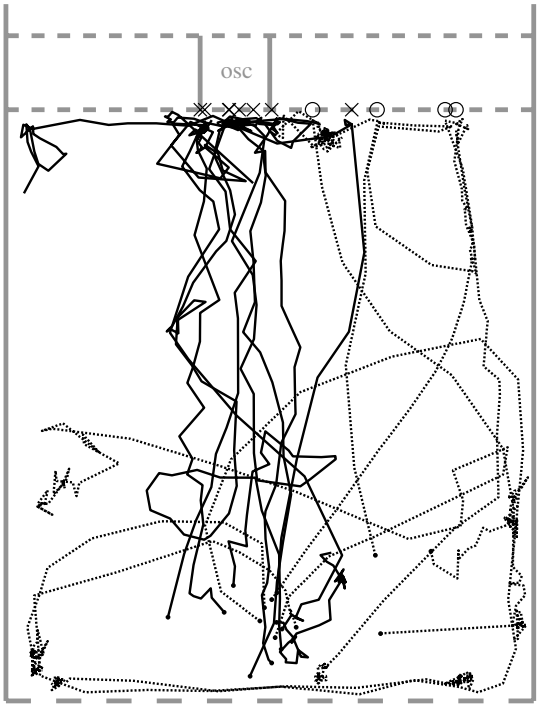


Fig. 2. *Tritonia diomedea* detect and locate upstream prey odour sources in the flow tank. *T. diomedea* tracks with (solid lines) and without (dotted lines) their prey in an upstream odour stimulus chamber (osc). With upstream prey, eight of nine slugs crawled upstream to make direct contact with the upstream grille, and seven of nine found the osc (× marks point of first contact with upstream grille). In controls, only one slug crawled directly upstream, three eventually reached the upstream grille crawling after contact with the sidewalls, and none found the empty osc (o marks point of first contact with upstream grille). Tracks are projected onto a scaled and simplified flow tank diagram. Flow tank width, 1 m.

chambers. Slugs oriented upstream during unobstructed crawling in both control and experimental trials (Table 1). However, with upstream prey, slugs showed significantly less dispersion around the upstream direction (Table 1). Over an entire trial slugs were, on average, significantly closer to the odour stimulus chamber with upstream prey than without (Table 2).

*T. diomedea* also crawled upstream towards conspecifics in the odor stimulus chambers (Fig. 3 and Fig. 3 movie in supplementary material). Only five slugs did not leave the starting box during experimental trials. In contrast, ten slugs remained in the box during control trials, and those that crawled moved apparently randomly relative to the odour stimulus chambers. Slugs faced upstream during unobstructed orientation in both control and experimental trials, but showed significantly less dispersion around the upstream direction with upstream conspecifics (Table 1). On average, slugs were significantly closer to the odour stimulus chamber with upstream conspecifics (Table 2).

*Downstream navigation away from upstream predators*

*T. diomedea* turned downstream when downstream of predators in odour stimulus chambers (Fig. 4). Slugs in control trials also turned slightly downstream; however, the turn magnitude was significantly greater with upstream predators (paired *t*-test, $N$=6, $t$=2.19, one-tailed $P$-value=0.04; unpaired *t*-test, $n_1$=9, $n_2$=8, $t$=2.41, one-tailed $P$-value=0.015). As a result of these turns, there is moderate evidence that slugs moved further away from the odour stimulus chamber with upstream predators than without (Table 2).

Table 1. *Tritonia diomedea showed stronger orientation into flow with upstream prey or conspecifics than into flow without upstream odour sources in the flow tank*

| | | | | | Rayleigh | | | Paired Wilcoxon | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Odour | Treatment | $N$ | θ (deg.) | $r$ | $z_n$ | $P$-value | 95% CL (deg.) | Pairs | $T$ | $P$ |
| Prey | Experimental | 9 | 0 | 0.98 | 8.69 | <0.0001 | 008, 352 | 9 | 8 | 0.05 |
| | Control | 9 | 342 | 0.78 | 5.42 | 0.002 | 014, 310 | | | |
| Conspecific | Experimental | 17 | 357 | 0.96 | 15.7 | <0.0001 | 005, 348 | 14 | 20 | 0.025 |
| | Control | 16 | 329 | 0.71 | 8.08 | 0.0001 | 021, 329 | | | |

Slug orientations were measured with (Experimental) and without (Control) prey or conspecifics in the upstream odour stimulus chamber.

The mean headings are relative to flow (θ, $r$; 0°=upstream) while unobstructed by tank walls etc. Rayleigh test $z$ statistic and $P$-value assess the significance of the mean heading. We also tested if the dispersion of headings around upstream was reduced in experimentals over controls for those animals with unobstructed orientation during both trials (Wilcoxon paired-sample test $T$-statistic and one-tailed $P$-value).

Under all conditions, slugs were oriented significantly upstream [95% confidence limits (CL) include upstream], and for both odour types, slugs in experimental trials had significantly lower dispersion than controls, i.e. stronger orientation into flow with upstream prey or conspecifics than without.

Table 2. *Tritonia diomedea move closer to prey and conspecific odour sources and may move further away from predator odour sources in the flow tank*

| | | | | Comparison | |
|---|---|---|---|---|---|
| Odour | Treatments | $N$ | Distance (cm) | Statistic | $P$-value |
| Prey | Experimental | 9 | 55±10 | 3.84 | 0.0025 |
| | Control | 9 | 110±12 | | |
| Conspecific | Experimental | 20 | 92±8.6 | 3.44 | 0.0014 |
| | Control | 20 | 120±4.9 | | |
| Predator | Experimental | 6 | 30±14 | 1.11 | 0.16 |
| | Control | 6 | 11±9.2 | | |
| Predator (not paired) | Experimental | 8 | 30±10 | 2.02 | 0.033 |
| | Control | 9 | 4.2±7.2 | | |

For tests with prey and conspecifics, distance from the upstream odour stimulus chamber (averaged over the entire trial) was compared to controls without odour stimuli (paired *t*-test, d.f.= number of pairs –1).

Distance values are means ± s.e.m.

For both prey and conspecific odours, slugs in experimental trials were, on average, significantly closer to the odour source. For tests with predators, the change in distance from the odour stimulus chamber was calculated while the slugs were in a section of the flow tank downstream of the chamber. Paired data were limited to six slugs that crawled into this section of the tank during both trials, and thus we also tested for differences without pairing. Only the latter test is significant, and we therefore suggest there is moderate evidence that *T. diomedea* moves downstream away from predators in this experiment.

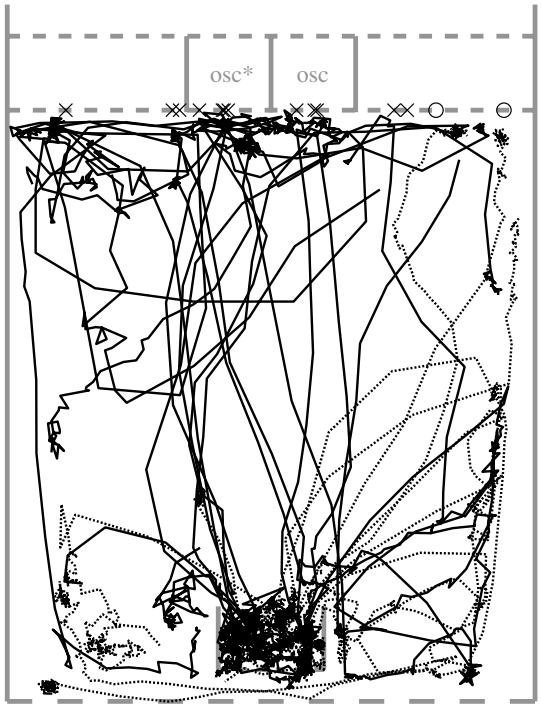


Fig. 3. *Tritonia diomedea* often detect and locate upstream conspecific odour sources in the flow tank. *T. diomedea* tracks with (solid lines) and without (dotted lines) conspecifics in an upstream odour stimulus chamber (one slug in osc, two slugs in osc*). With upstream conspecifics, 12 of 20 slugs crawled upstream to make direct contact with the upstream grille and seven found the osc (× marks point of first contact with upstream grille). Only two slugs in controls crawled upstream to make contact with the upstream grille without first contacting the side walls of the tank, and none found the empty osc (o marks point of first contact with upstream grille). Tracks are projected onto a scaled and simplified flow tank diagram. Flow tank width, 1 m. See Fig. 3 movie in supplementary material for an example movie.

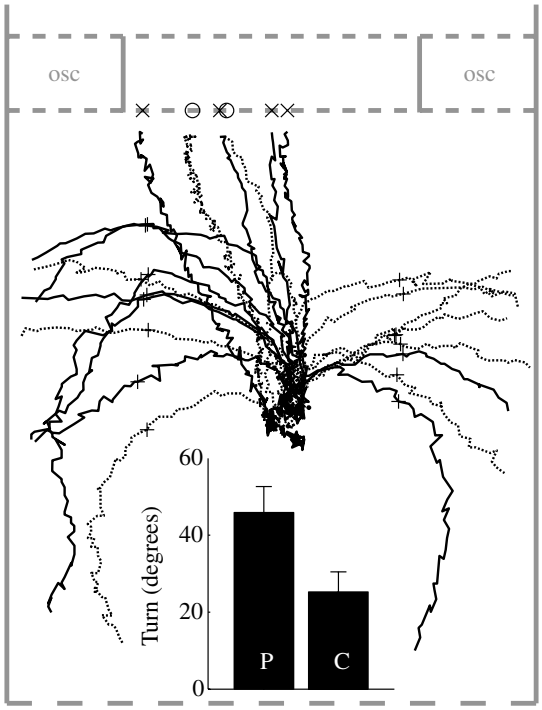


Fig. 4. *Tritonia diomedea* detects and turns away downstream from upstream predators in the flow tank. *T. diomedea* tracks with a predator (solid lines) and controls (dotted lines) with similarly shaped latex tubing in the odour stimulus chambers (osc). With an upstream predator, those slugs that crawled into a plume zone downstream of the predator had a greater tendency to turn downstream. The inset shows the downstream turn magnitude (P=predator; C=control) measured by comparing mean crawling headings for the 2 min on either side of the estimated plume zone entry points (+). Slugs in four experimental trials (×) and two control trials (o) crawled upstream and contacted the second upstream grille without entering the plume zone. A further three animals (2 controls, 1 experimental) reached neither the plume zone nor the upstream grille. Tracks are projected onto a scaled and simplified flow tank diagram. Flow tank width, 1 m.

### *Upstream versus downstream turning is mediated by the rhinophores*

*T. diomedea* with rhinophores turned and crawled upstream into an odour plume created by outflow from a header tank containing prey (Fig. 5). Mean heading RTF was significantly upstream over a 2 min interval, 30 s after encountering the plume (Table 3). At some point, six of eight slugs with rhinophores headed directly upstream towards the odour source. One slug missed the plume source by ~5 cm after crawling upstream in the plume, and the remaining slug remained stationary throughout the trial.

If the header tank contained predators, we observed downstream turns, but not necessarily downstream crawling (Fig. 5). Three slugs crawled downstream after turning downstream, but five others exited the plume with headings between downstream and cross-stream. As a group, mean heading RTF was significantly downstream if a single animal was omitted from analyses (Table 3). This slug was crawling upstream when it encountered the predator plume close to the upstream grille. The slug stopped, turned left, and rapidly exited the narrow plume, crawling cross-stream. We interpret this response as a downstream turn away from predator odour, with subsequent crawling cross-stream once the animal was outside the plume.

When the header tank was empty, we saw no consistent response to the dye plume. Slugs continued crawling upstream, cross-stream or downstream after encountering the plume. As a group, there was no significant mean heading RTF (Table 3).

If the rhinophores were removed, orientation to odour plumes was eliminated (Fig. 5). Slugs without rhinophores behaved similarly to control trials with rhinophores, regardless of header tank contents. No significant mean headings RTF were observed after encountering prey, predator or control plumes (Table 3). All animals crawled during controls and there was no significant difference in crawling speeds between animals with rhinophores (6.3±0.87 cm $min^{-1}$) and those

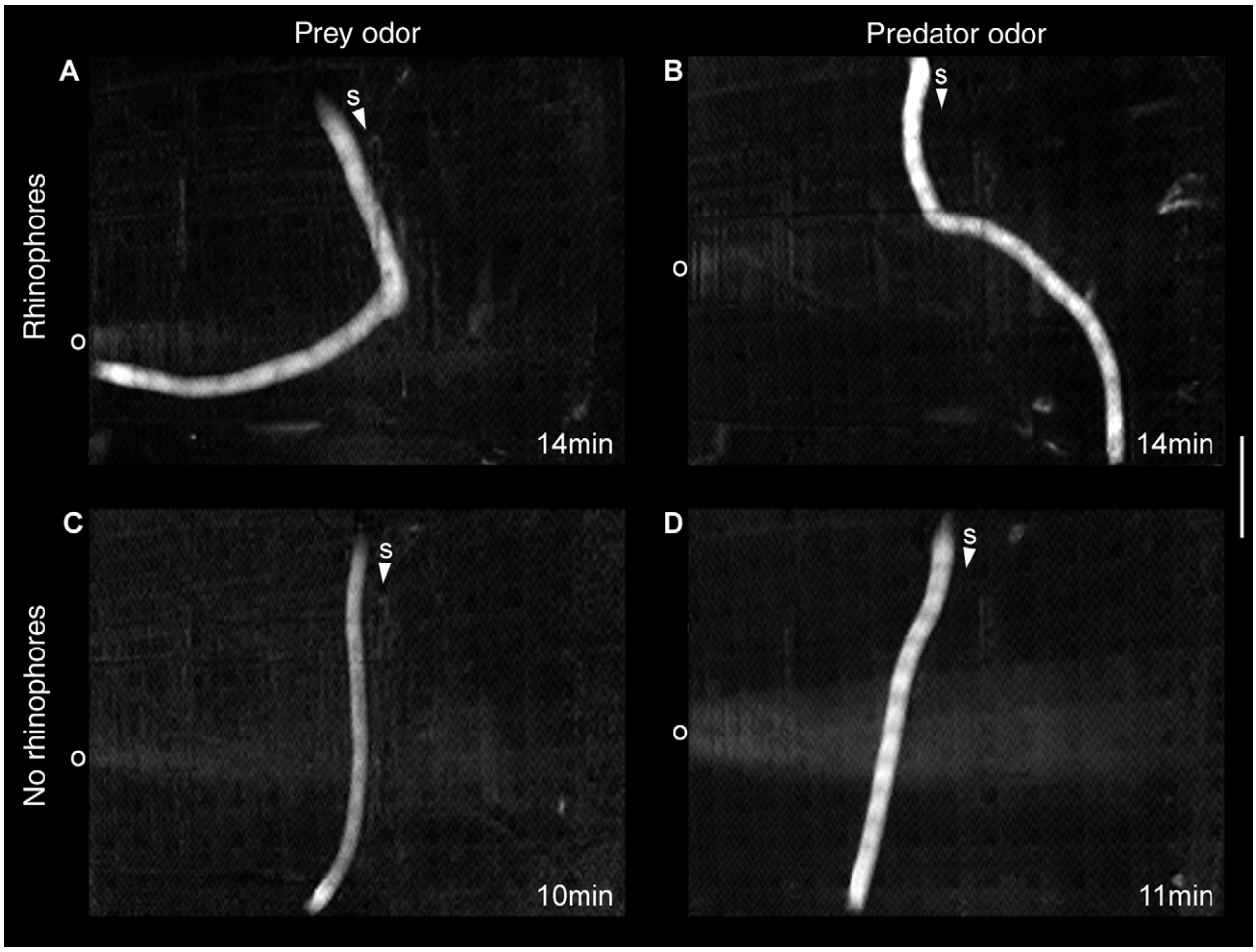


Fig. 5. *Tritonia diomedea* without rhinophores do not show navigational responses to prey and predator odour plumes. Each image is a mean projection of a motion enhanced video recording of a slug (s) crawling in the flow tank, showing slug movement (direction shown by arrowheads) and the dyed odour plume ('o', odour plume source; flow is from left to right). With rhinophores, slugs crawling cross-stream responded with upstream crawling when encountering a prey odour plume (A) or a downstream turn away from predator odour (B). Without rhinophores, slugs showed no response to odour plumes (C,D). Elapsed time (min) is shown in A–D. Scale bar, 25 cm. See Fig. 5 movies in supplementary material for the videos corresponding to A–D in this figure.

without rhinophores (7.0±0.60 cm min$^{-1}$; *t*-test, $t_{13}$=0.616, two-tailed *P*-value=0.55). Five of ten slugs without rhinophores fed when given the opportunity, three mouthed the prey, and two did not feed; eight of ten slugs with rhinophores also fed, and two did not.

### *Rhinophores respond to odours from prey, predators and conspecifics*

Isolated rhinophores responded to odours from prey, predators and conspecifics. Extracellular recordings from the rhinophore nerve showed increases in afferent spike activity when flow over the sensory tuft was switched to seawater containing these odours. When multiple odours were tested during a single electrode application, only a single odour type elicited responses, with one exception when responses to prey and predator odours were recorded without changing the electrode position. For prey and conspecific odour, the increased activity consisted of a high frequency burst of small (<15 μV) spikes, which then slowly declined in frequency over the duration of the odour stimulus (Figs 6 and 7). Rhinophores also responded to control seawater applications; however, the burst of activity had consistently fewer spikes. Normalized response magnitudes showed significantly greater responses for prey and conspecific odour than control seawater applications (Fig. 8, Table 4), using data from all rhinophores, or just one rhinophore per animal. Control applications consistently triggered a slight increase over baseline for small amplitude spikes (Figs 6–8), although this effect was only significant if all rhinophores were considered (Table 4).

For predator odour a much larger (typically >20 μV), low frequency, unit was most often responsive. In six of seven cases (Fig. 9, rhinophores i–vi), this unit could be qualitatively distinguished from other large spikes occurring in the response window (Fig. 9A), as well as quantitatively sorted using template matching (Fig. 9B). In one experiment (Fig. 9vii), activity with similar size was too frequent for easy qualitative

Table 3. *Comparison of Tritonia diomedea orientations to odour plumes, with and without rhinophores*

| Rhinophores | Odour | *N* | θ (deg.) | *r* | $F_{2,N-2}$ | *P* | 95% CL (deg.) |
|---|---|---|---|---|---|---|---|
| Present | Prey | 8 | 003 | 0.58 | 8.83 | 0.016 | 063, 304 |
| | Predator | 8 | 171 | 0.70 | 5.03 | 0.052 | ns |
| | Predator* | 7 | 171 | 0.90 | 420 | <0.0001 | 142, 202 |
| | Control | 8 | 119 | 0.30 | 1.08 | 0.40 | ns |
| Absent | Prey | 7 | 076 | 0.23 | 0.48 | 0.64 | ns |
| | Predator | 7 | 177 | 0.14 | 0.16 | 0.85 | ns |
| | Control | 7 | 042 | 0.26 | 0.38 | 0.70 | ns |

Hotelling tests of 2nd order mean headings relative to flow (θ, *r*; 0°=upstream) were used to assess the significance of mean slug orientations over the 2 min interval, 30 s after encountering a prey, predator or control odour plume in the flow tank. CL, 95% confidence limits for sample means significant at $P$=0.05; ns, not significant.

Two sets of values are presented for orientation relative to predator odour, the first is the complete dataset, the second (*) does not include a single exceptional animal (see text). ns, not significant.

visualization; however, template matching confirmed that a consistent waveform shape was more frequent during predator odour application. The predator odour responsive unit responded only 1–4 times during predator odour application, with a longer latency to the first response than for the high frequency units observed for prey and conspecifics. Control seawater applications triggered no response or just a single spike from this unit, significantly lower than during predator odour application (Fig. 8, Table 4). In four rhinophores, we also recorded smaller amplitude, high frequency responses to predator odour, similar to prey and conspecific odour responses, although increases over controls were not as great as for prey and conspecific stimuli (data not shown).

### *Conspecifics inconsistently trigger responses in rhinophores*

Rhinophores known to be responsive to conspecific odours acquired from multiple individuals had variable responses to odours from specific pairs of conspecifics (Fig. 10). Responses to mating or non-mating pairs could be either similar or greater than controls. In five cases, mating pair seawater triggered significantly stronger responses than control seawater. Responses to seawater from non-mating pairs were significantly greater than controls only once, although two cases with similar, but non-significant, trends were observed.

## Discussion

### *Navigation relative to odours and flow*

We have confirmed all three odour-based navigation behaviours observed in the natural habitat for *Tritonia diomedea* (Wyeth and Willows, in press; Wyeth et al., in press). With upstream prey, *T. diomedea* crawled upstream to find the odour source (Fig. 2). The slugs headed into flow with less angular dispersion (Table 1) and moved closer to the odour source location than without prey odour (Table 2). Conversely, with an upstream predator, *T. diomedea* turned downstream, possibly moving further away from the odour source location than without predator odour (Fig. 4). Although there is only moderate evidence from one flow tank experiment that slugs move away from the upstream predator as a result of the downstream turn (Fig. 4, Table 2), if the results from the header tank experiment (Table 3) and the field (Wyeth et al., in press) are considered, we suggest that in the presence of predator odour *T. diomedea* orients with headings between cross-stream and downstream, and thus crawls away from upstream predators. Finally, at least some of the time, *T. diomedea* crawled upstream to find conspecific odour sources (Fig. 3), again heading into the currents with less dispersion (Table 1) and moving closer to the odour source location than without conspecific odour (Table 2). We conclude that navigation in *T. diomedea* is based primarily on odours and water flow.

How does *T. diomedea* orient to flow without odours? Evidence for positive rheotaxis has been found (Field and Macmillan, 1973; Willows, 1978; Murray and Willows, 1996; Murray et al., in press); however, none of these experiments provided both reasonably natural flow conditions *and* proper control of upstream odour sources. Our recent field work was inconclusive with regard to navigation without upstream conspecifics (Wyeth et al., in press). All four groups of controls in the flow tank experiments tended to wander from the initial heading, regardless of whether the animals were first oriented upstream (Figs 2–4) or cross-stream (data not shown). Significant mean upstream headings for controls in the flow tank (Table 1) may be a legacy of initial upstream orientation, since slugs initially facing cross-stream oriented randomly to flow (Table 3). Theoretical work suggests a variety of different headings relative to unscented flow may be optimal for finding odour plumes, depending on flow variability (Sabelis and Schippers, 1984; Dusenbery, 1989; Dusenbery, 1990). Thus, we feel that further work is needed to understand *T. diomedea* navigation in the absence of odour cues.

### *Rhinophores are necessary for odour based navigation*

When the rhinophores were removed, orientation to flow based on the presence of either prey or predator odour was abolished (Fig. 5, Table 3). Since slugs without rhinophores still preyed on *P. gurneyi*, the lack of orientation suggests a loss of ability, not a lack of motivation. Murray and Willows (Murray and Willows, 1996), using nerve cuts that included

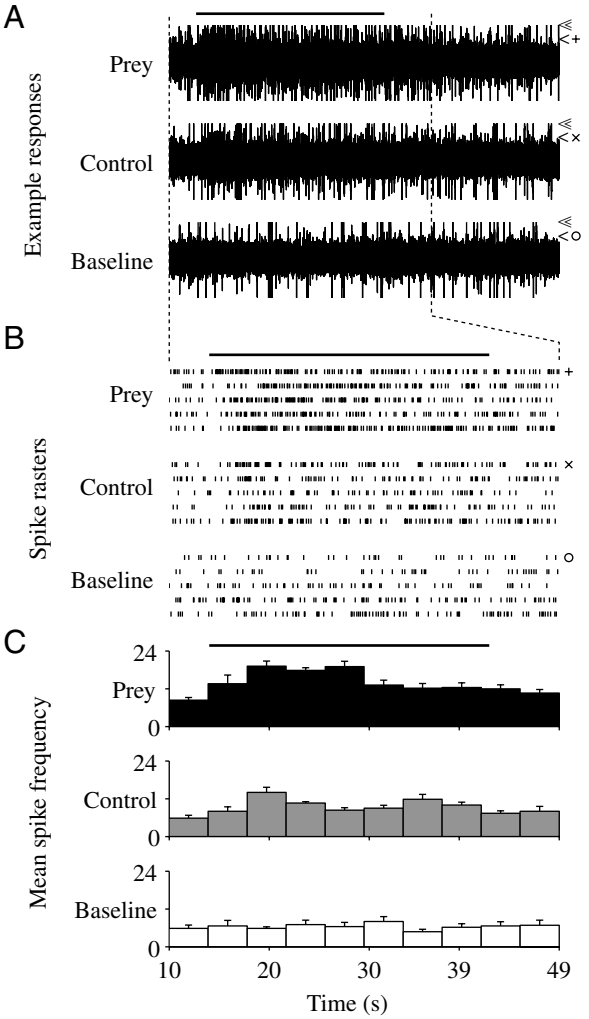


Fig. 6. A greater response in the rhinophore nerve to prey odour than to control. (A) Extracellular activity was greatest in response to perfusion of prey odour through the sensory tuft, with a lesser increase over baseline in response to control seawater. Bar indicates odour stimulus duration. Scale: 10 μV between < and ⩽ marks. (B) Raster plots of extracellular spikes surpassing +10 but not +20 μV (< and ⩽, respectively, in A) for all five trials on this rhinophore (+,×,o indicate the raster plots for the recordings in A). (C) Mean spike counts with standard error bars from all five trials, grouped into ten intervals. This rhinophore responded more strongly, on average, to seawater with prey odours than control seawater. Time for B and C is measured relative to the perfusion stream switch for prey and control applications, and an arbitrary time between trials for baseline. Broken lines show the time window used for spike count analysis.

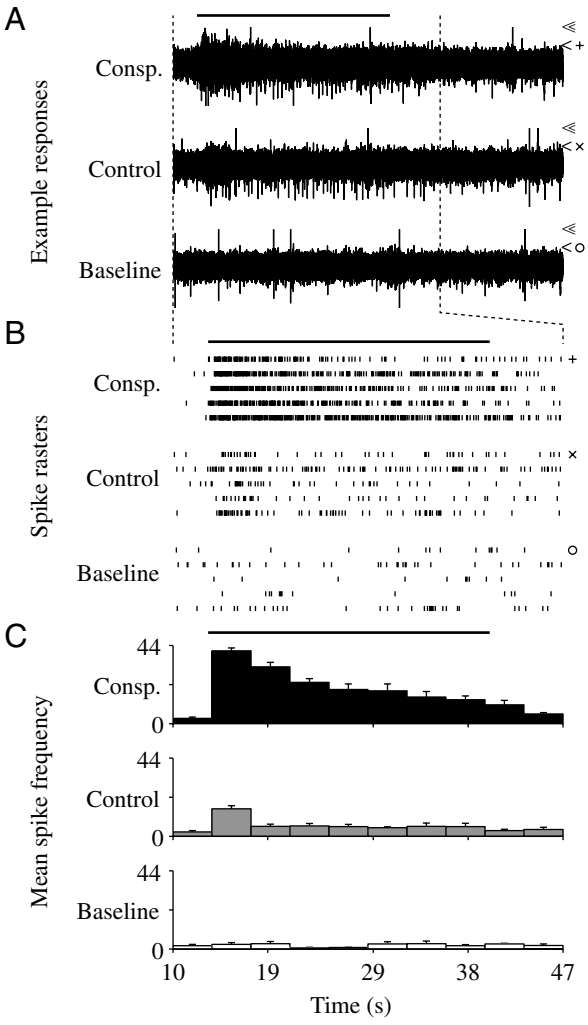


Fig. 7. A greater response in the rhinophore nerve to conspecific odour than to control. (A) Extracellular activity was greatest in response to perfusion of conspecific (consp.) odour through the sensory tuft, with a lesser increase over baseline in response to control seawater. Bar indicates odour stimulus duration. Scale: 8 μV between < and ⩽ marks. (B) Raster plots of extracellular spikes surpassing +9 μV but not +17 μV (< and ⩽, respectively, in A) for all five trials on this rhinophore (+,×,o indicate the raster plots for the recordings in A). (C) Mean spike counts with standard error bars from all five trials grouped into ten intervals. This rhinophore responded more strongly on average, to seawater with conspecific odours than control seawater. Time for B and C is measured relative to the perfusion stream switch for conspecific and control applications, and an arbitrary time between trials for baseline. Broken lines show time window used for spike count analysis.

the rhinophore nerve, concluded that the oral veil is responsible for flow orientation in *T. diomedea*. Therefore, our results suggest that removing the rhinophores eliminates the odour detection component of the navigational behaviours, rather than flow orientation. Willows made similar conclusions after tying the rhinophore sheaths closed (Willows, 1978). Thus, there is strong evidence for the rhinophores as chemosensory organs that modulate orientation to flow in *T. diomedea*.

### *Rhinophores are chemosensitive*

Extracellular recordings from the rhinophore nerve confirmed that the rhinophores are chemosensitive. Application of prey, predator or conspecific odours increased afference in the rhinophore nerve (Figs 6–9, Table 4). Similar results for prey and conspecific odours have been shown for the rhinophores of other opisthobranchs (Jahan-Parwar, 1972; Audesirk and Audesirk, 1977; Bicker et al., 1982b; Ronan,

Table 4. *Rhinophore nerve afference increases significantly after the rhinophore tuft is stimulated with prey, predator or conspecific odours*

| Odour | *N* | $F_{2,N-2}$ | *P* | Baseline | Control | Odour |
|---|---|---|---|---|---|---|
| All rhinophores | | | | | | |
| Prey | 7 | 255 | <0.001 | * | * | * |
| Predator | 7 | 72.9 | <0.001 | | === | * |
| Conspecific | 10 | 229 | <0.001 | * | * | * |
| One rhinophore/animal | | | | | | |
| Prey | 5 | 247 | <0.001 | | === | * |
| Predator | 5 | 31.2 | <0.001 | | === | * |
| Conspecific | 5 | 73.7 | 0.003 | | === | * |

Spike counts from extracellular rhinophore nerve recordings were compared between baseline, control, and odour treatments. The MANOVA *F* statistics and *P*-values test for differences amongst the treatments and the subsequent pairwise comparisons ($P$=0.05 is significant; *significantly different treatments; === links treatments that were not significantly different).

In addition, we provide statistical results for data sets limited to just one rhinophore per animal, for each odour type. In all cases, odours stimulated significantly greater spike counts than controls. Responses to controls, although consistently greater than baseline (Fig. 8), were significantly greater than baseline only in two of the six tests.

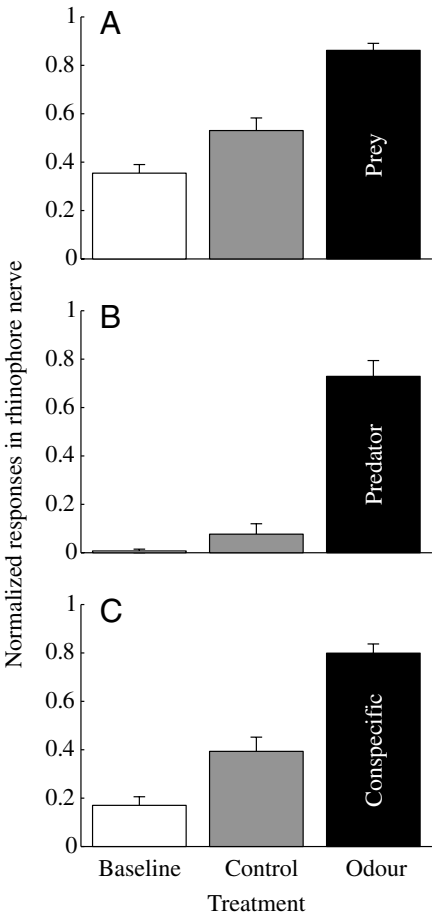


Fig. 8. Rhinophores are responsive to prey (A), predator (B) and conspecific odours (C). Mean normalized spike counts are shown with standard error bars, for seven rhinophores tested for responses to prey odour, seven for predator odour, and ten for conspecific odour. Responses to all three odour types are significantly greater than controls (Table 4). Controls were consistently greater than baseline for prey and conspecific odours, although significant in only 2 of 6 tests (Table 4).

1989; Levy et al., 1997). We observed small amplitude, high frequency responses to prey and conspecific stimuli, and less frequently to predator odour. More often, a large amplitude, low frequency, and longer latency response to predator odour was observed. Furthermore, responses to different odours were only recordable (with one exception) during different *en passant* electrode applications to the rhinophore nerve. Thus, responses to different odours appear to be carried by different subsets of axons. Evidence from other opisthobranchs suggests afference from chemosensitive organs with peripheral ganglia can be the result of integration in the ganglia (Bicker et al., 1982b; Murphy and Hadfield, 1997; Boudko et al., 1999). Whether the differences in latency and frequency in response to different odour types in *T. diomedea* reflect primary *versus* higher order neurons in different chemosensory pathways remains unknown. Regardless, the responses we observed provide evidence that the rhinophores are chemosensitive organs in *T. diomedea*.

### *Intermittently attractive conspecifics suggest a mating pheromone*

Several pieces of evidence suggest that *T. diomedea* is only intermittently attractive to conspecifics. In the field, not all stationary animals are approached by downstream conspecifics; in particular, slugs laying eggs were never approached (Wyeth et al., in press). In the laboratory, not all slugs in the flow tank crawled upstream towards conspecifics, and performance was more erratic than with upstream prey (compare Figs 2 and 4). Moreover, extracellular units responsive to odours acquired from multiple conspecifics may or may not respond to tests with odours acquired from specific pairs of slugs (Fig. 10). Responses occurred more often if the pair was mating. Similarly, we also consistently observed downstream slugs approaching already mating pairs in the field (Wyeth and Willows, in press). These observations all suggest intermittent release of an attractive odour, in addition to

intermittent motivation to find conspecifics. Other gastropods are known to use pheromones (Peters, 1964; Audesirk, 1977; Levy et al., 1997; Chase, 2002; Susswein and Nagle, 2004), and thus we hypothesize that *T. diomedea* releases a pheromone before and during mating to attract conspecifics.

### *Implications and future directions*

What are the mechanisms behind odour based navigation in *T. diomedea*? Flows in habitats such as that of *T. diomedea* make chemotaxis (gradient following) unlikely (Weissburg, 2000). Crawling upstream in the presence of an attractive odour is the norm (Weissburg, 2000; Webster and Weissburg, 2001; Vickers, 2000), using counter turns (movement back and forth across the plume) or edge following (Atema, 1996; Vickers, 2000; Grasso and Basil, 2002). However, *T. diomedea* (Fig. 6; Wyeth and Willows, in press) and other gastropods (Bousfield, 1978; Cook, 1980) do not counter turn. Nor are their chemosensors as widely spaced as in other animals that may use bilateral comparisons to follow the plume edge (Zimmer-Faust et al., 1995; Webster et al., 2001; Keller et al., 2003; Ferner and Weissburg, 2005). Thus, we suggest two possible mechanisms for further study: *T. diomedea* and other slow moving gastropods may measure flow direction, integrating mechanosensory input over time (Murray and Willows, 1996; Blackwell and Murray, 2005), and then crawl upstream when prey or conspecific odour are present. Alternatively, integration of odour information alone may provide directional information about the odour source (Finelli et al., 1999; but see Webster and Weissburg, 2001).

Our results also emphasize a cautionary note for Y-maze experiments with aquatic animals: negative results are not evidence for lack of ability (Zimmer and Butman, 2000). *T. diomedea* was unable to locate conspecifics in a Y-maze (Willows, 1978), yet field observations (Wyeth and Willows, in press) and flow tank experiments here, which better replicate natural flow conditions, show that the slugs are able to detect and find each other.

Finally, our understanding of the neural control of odour based navigation in *T. diomedea* is still limited. How mechanosensory and chemosensory signals are integrated into directional crawling relative to flow is unknown. Flow direction may be constantly measured or the behaviours may be ballistic, following an initial measurement of flow direction in the presence of odour. Simpler reflexes, based on which rhinophore or which side of the rhinophores detect the odour, are also possible. Experiments manipulating impinging odour direction relative to flow direction will help understand which navigational mechanism(s) is/are used by *T. diomedea*. In addition, recognition that *T. diomedea* uses odours and water flow for navigation and that turns are largely controlled by the

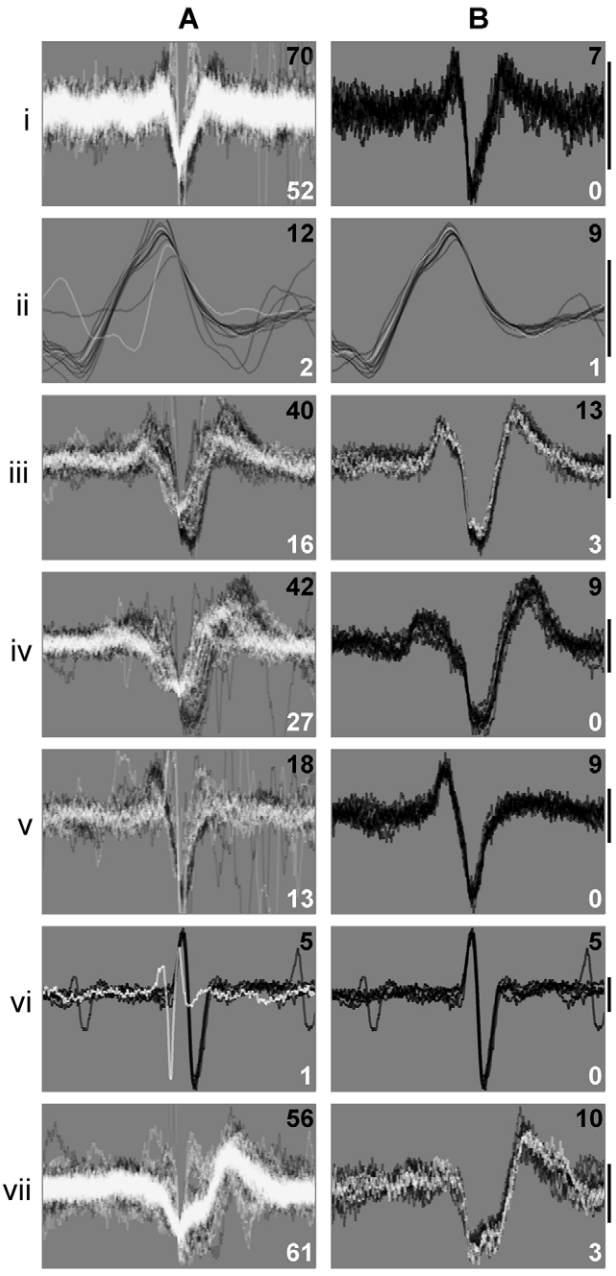


Fig. 9. Extracellular spike waveforms are responsive to predator odour. (A,B) Spikes during perfusion of control seawater (white) and seawater with predator odour (black) are overlaid. Control waveforms are drawn after predator odour waveforms and waveform transparency is scaled to the total number of waveforms displayed. Consequently, any distinct dark areas indicate waveforms with higher relative frequency during perfusion of predator odour. The number of waveforms displayed is given for each treatment type (controls, white; predator odour, black). (Ai–vii) All large amplitude spikes in the analysis window (the voltage level defining 'large' is consistent for each rhinophore). (Bi–vii) Only waveforms that matched a template using Spike2 software. For each rhinophore tested (i–vii), amongst the various large magnitude waveforms recorded (A), a single group of dark waveforms with distinct shape can be seen, and can be sorted using templates (B). This waveform occurred either not at all, or at much lower frequency during perfusion of control seawater. In rhinophore vii, spontaneous unresponsive activity was too great to visualize the responsive waveform, and therefore waveforms matching the six most frequent templates are not drawn in A to avoid obscuring rarer waveforms. Scale bars (20 μV) apply to A and B for each rhinophore.

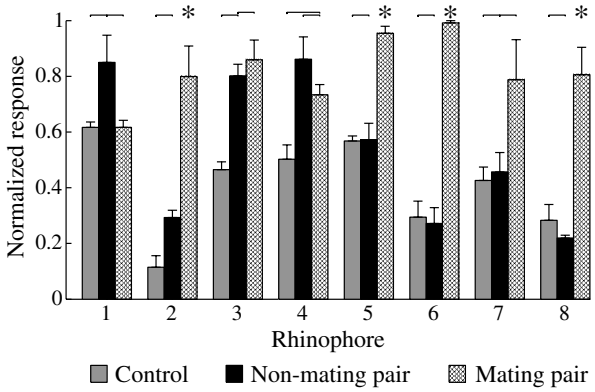


Fig. 10. *Tritonia diomedea* are inconsistent in their ability to stimulate rhinophore responses. Eight rhinophores were established as responsive to seawater with conspecific odour isolated from multiple slugs. Responses were then recorded to odours from a pair of non-mating slugs and a pair of mating slugs. Shown are the mean normalized spike counts with standard errors, for each rhinophore for applications of control seawater, non-mating pair seawater, and mating pair seawater. Lines link non-significant pairwise comparisons between treatments within each rhinophore (ANOVA, followed by Tukey's pairwise mean comparisons, $P$=0.05); *means significantly different from both other treatments. Conspecific responsive units in the rhinophores did not respond equally to odours isolated from specific pairs of *T. diomedea*, suggesting that the odours are only intermittently released.

Pd3 neuron (Redondo and Murray, 2005), suggest that applying different odours to the rhinophores may reveal how turn choices are made based on chemosensory inputs to these motor neurons. Thus, we can begin to investigate the neural integration of chemosensation and mechanosensation underlying navigation in *T. diomedea*.

We are grateful to O. M. Woodward, S. D. Cain, W. Moody, R. R. Strathmann, G. VanBlaricom, S. Hardy, S. Jung, M. Tam, the staff of Friday Harbor Laboratories (FHL) and two anonymous reviewers for help and suggestions that contributed to this manuscript. O. M. Woodward, M. Baltzley, D. Duggins, D. Thoreson, K. Britton-Simmons, R. Foley, and S. Hardy helped collect animals. Support was provided by the Packard Foundation. R.C.W. thanks C. P. Holmes, harem j of FHL, and acknowledges support from the National Sciences and Engineering Research Council (Canada) and the Conchologists of America.

## References

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

**Audesirk, G. and Audesirk, T.** (1980). Complex mechanoreceptors in *Tritonia diamedea.* I. Responses to mechanical and chemical stimuli. *J. Comp. Physiol.* **141**, 101-109.

**Audesirk, T. E.** (1975). Chemoreception in *Aplysia californica*. I. Behavioral localization of distance chemoreceptors used in food-finding. *Behav. Biol.* **15**, 45-55.

**Audesirk, T. E.** (1977). Chemoreception in *Aplysia californica*. III. Evidence for pheromones influencing reproductive-behavior. *Behav. Biol.* **20**, 235-243.

**Audesirk, T. E. and Audesirk, G. J.** (1977). Chemoreception in *Aplysia californica*. II. Electrophysiological evidence for detection of the odor of conspecifics. *Comp. Biochem. Physiol.* **56A**, 267-270.

**Beck, J. C., Cooper, M. S. and Willows, A. O. D.** (2000). Immunocytochemical localization of pedal peptide in the central nervous system of the gastropod mollusc *Tritonia diomedea*. *J. Comp. Neurol.* **425**, 1-9.

**Bicker, G., Davis, W. J. and Matera, E. M.** (1982a). Chemoreception and mechanoreception in the gastropod mollusc *Pleurobranchaea californica*. 2. Neuroanatomical and intracellular analysis of afferent pathways. *J. Comp. Physiol.* **149**, 235-250.

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

**Blackwell, J. S. and Murray, J. A.** (2005). Neuronal responses to water flow in the marine slug *Tritonia diomedea*. *Impulse* **2005**, 1-10.

**Boudko, D. Y., Switzer-Dunlap, M. and Hadfield, M. G.** (1999). Cellular and subcellular structure of anterior sensory pathways in *Phestilla sibogae* (Gastropoda, Nudibranchia). *J. Comp. Neurol.* **403**, 39-52.

**Bousfield, J. D.** (1978). Rheotaxis and chemoreception in the freshwater snail *Biomphalaria glabrata* (Say): estimation of molecular-weights of active factors. *Biol. Bull.* **154**, 361-373.

**Chase, R.** (2002). *Behavior and its Neural Control in Gastropod Molluscs*. New York: Oxford University Press.

**Cook, A.** (1980). Field studies of homing in the pulmonate slug *Limax pseudoflavus*. *J. Mollus. Stud.* **46**, 100-105.

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

**Dusenbery, D. B.** (1989). Optimal search direction for an animal flying or swimming in a wind or current. *J. Chem. Ecol.* **15**, 2511-2519.

**Dusenbery, D. B.** (1990). Upwind searching for an odor plume is sometimes optimal. *J. Chem. Ecol.* **16**, 1971-1976.

**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.

**Finelli, C. M., Pentcheff, N. D., Zimmer-Faust, R. K. and Wethey, D. S.** (1999). Odor transport in turbulent flows: constraints on animal navigation. *Limnol. Oceanogr.* **44**, 1053-1071.

**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.

**Hume, R. I., Getting, P. A. and del Beccaro, M. A.** (1982). Motor organization of *Tritonia* swimming. I. Quantitative analysis of swim behavior and flexion neuron firing patterns. *J. Neurophysiol.* **47**, 60-74.

**Jahan-Parwar, B.** (1972). Central projection of chemosensory pathways of *Aplysia*. *Physiologist* **150**, 180.

**Keller, T. A., Powell, I. and Weissburg, M. J.** (2003). Role of olfactory appendages in chemically mediated orientation of blue crabs. *Mar. Ecol. Prog. Ser.* **261**, 217-231.

**Levy, M., Blumberg, S. and Susswein, A. J.** (1997). The rhinophores sense pheromones regulating multiple behaviors in *Aplysia fasciata*. *Neurosci. Lett.* **225**, 113-116.

**Lewicki, M. S.** (1998). A review of methods for spike sorting: the detection and classification of neural action potentials. *Network-Comp. Neural* **9**, R53-R78.

**Lohmann, K. J., Willows, A. O. D. and Pinter, R. B.** (1991). An identifiable molluscan neuron responds to changes in earth-strength magnetic-fields. *J. Exp. Biol.* **161**, 1-24.

**Murphy, B. F. and Hadfield, M. G.** (1997). Chemoreception in the nudibranch gastropod *Phestilla sibogae*. *Comp. Biochem. Physiol.* **118A**, 727-735.

**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., Hewes, R. S. and Willows, A. O. D.** (1992). Water-flow sensitive pedal neurons in *Tritonia*: role in rheotaxis. *J. Comp. Physiol. A* **171**, 373-385.

**Murray, J. A., Links, A. C. and Willows, A. O. D.** (in press). Orientation of the nudibranch *Tritonia diomedea* to tidal water flow in nature. *Mar. Freshwater Behav. Physiol.*

**Norton, J. H., Dashorst, M., Lanksy, T. M. and Mayer, R. J.** (1996). An evaluation of some relaxants for use with pearl oysters. *Aquaculture* **144**, 39-52.

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

**Peters, R. S.** (1964). Function of the cephalic tentacles in *Littorina planaxis* Philippi. *Veliger* **7**, 143-148.

**Popescu, I. R. and Frost, T. M.** (2002). Highly dissimilar behaviors mediated by a multifunctional network in the marine mollusk *Tritonia diomedea*. *J. Neurosci.* **22**, 1985-1993.

**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*. *J. Comp. Physiol. A* **191**, 435-444.

**Ronan, M. P.** (1989). A review of opisthobranch rhinophores with a study of *Aplysia* rhinophore structure. MSc thesis, University of West Florida, Pensacola, USA.

**Runham, N. W., Isarankura, K. and Smith, B. J.** (1965). Methods for narcotizing and anaesthetizing gastropods. *Malacologia* **2**, 231-238.

**Sabelis, M. W. and Schippers, P.** (1984). Variable wind directions and anemotactic strategies of searching for an odour plume. *Oecologia* **63**, 225-228.

**Susswein, A. J. and Nagle, G. T.** (2004). Peptide and protein pheromones in molluscs. *Peptides* **25**, 1523-1530.

**Vickers, N. J.** (2000). Mechanisms of animal navigation in odor plumes. *Biol. Bull.* **198**, 203-212.

**Vogel, S.** (1994). *Life in Moving Fluids: The Physical Biology of Flow* (2nd edn.). Princeton: Princeton University Press.

**Wang, J. H., Cain, S. D. and Lohmann, K. J.** (2003). Identification of magnetically responsive neurons in the marine mollusc *Tritonia diomedea*. *J. Exp. Biol.* **206**, 381-388.

**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., Rahman, S. and Dasi, L. P.** (2001). On the usefulness of bilateral comparison to tracking turbulent chemical odor plumes. *Limnol. Oceanogr.* **46**, 1048-1053.

**Weissburg, M. J.** (2000). The fluid dynamical context of chemosensory behavior. *Biol. Bull.* **198**, 188-202.

**Wheeler, B. C.** (1999). Automatic discrimination of single units. In *Methods for Neural Ensemble Recordings* (ed. M. A. L. Nicolelis), pp. 61-77. Boca Raton: CRC Press.

**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.

**Willows, A. O. D., Pavlova, G. A. and Phillips, N. E.** (1997). Modulation of ciliary beat frequency by neuropeptides from identified molluscan neurons. *J. Exp. Biol.* **200**, 1433-1439.

**Wyeth, R. C. and Willows, A. O. D.** (in press). Field behavior of the nudibranch mollusc *Tritonia diomedea*. *Biol. Bull.* **210**.

**Wyeth, R. C., Woodward, O. M. and Willows, A. O. D.** (in press). Orientation and navigation relative to water flow, prey, conspecifics and predators by the nudibranch mollusc *Tritonia diomedea*. *Biol. Bull.* **210**.

**Zar, J. H.** (1999). *Biostatistical Analysis* (4th edn.). Upper Saddle River, NJ: Prentice-Hall.

**Zimmer, R. K. and Butman, C. A.** (2000). Chemical signaling processes in the marine environment. *Biol. Bull.* **198**, 168-187.

**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.