

Orientation and Navigation Relative to Water Flow, Prey, Conspecifics, and Predators by the Nudibranch Mollusc *Tritonia diomedea*

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Abstract. Progress in understanding sensory and locomotory systems in *Tritonia diomedea* has created the potential for the neuroethological study of animal navigation in this species. Our goal is to describe the navigational behaviors to guide further work on how the nervous system integrates information from multiple senses to produce oriented locomotion. Observation of *T. diomedea* in its habitat has suggested that it uses water flow to navigate relative to prey, predators, and conspecifics. We test these hypotheses in the field by comparing slug orientation in time-lapse videos to flow direction in circumstances with and without prey, predators, or conspecifics upstream. *T. diomedea* oriented upstream both while crawling and after turning. This trend was strongest before feeding or mating; after feeding or mating, the slugs did not orient significantly to flow. Slugs turned downstream away from an upstream predator but did not react in control situations without an upstream predator. These data support the hypothesis that *T. diomedea* uses a combination of odors (or some other cue transported downstream) and water flow to navigate relative to prey, predators, and conspecifics. Understanding the context-dependent choice between upstream and downstream crawling in *T. diomedea* provides an opportunity for further work on the sensory integration underlying navigation behavior.

Introduction

The accessible nervous systems and easily studied behaviors of gastropod molluscs (Chase, 2002) present opportu-

nities for study of the sensory integration underlying navigation. Locomotory and sensory systems have been studied in the opisthobranchs *Aplysia* spp. (e.g., Audesirk and Audesirk, 1977; Lederhendler *et al.*, 1977; Fredman and Jahan-Parwar, 1980; Teyke *et al.*, 1992; Levy *et al.*, 1997), *Navanax inermis* (Paine, 1963; Susswein *et al.*, 1982; Leonard, 1992), and *Pleurobranchaea californica* (Lee *et al.*, 1974; Bicker *et al.*, 1982a, 1982b), among others, and descriptions of field behaviors are also available for several species: *Aplysia* spp. (Kupfermann and Carew, 1974; Susswein *et al.*, 1984; Leonard and Lukowiak, 1986), *Bursatella leachii* (Ramos *et al.*, 1995), and *Navanax inermis* (Leonard and Lukowiak, 1984). However, the nudibranch *Tritonia diomedea* Bergh is the only species for which a description of navigation (Wyeth and Willows, 2006a) is paired with work on both locomotory and sensory systems (e.g., Willows, 1978; Lohmann *et al.*, 1991; Murray *et al.*, 1992; Popescu and Willows, 1999; Wang *et al.*, 2003; Redondo and Murray, 2005; Cain *et al.*, 2006). Observation in *T. diomedea*'s habitat (Wyeth and Willows, 2006a) generated three navigational hypotheses that we now test: *T. diomedea* crawls upstream to find mates, it crawls upstream to find prey, and it moves downstream away from predators. Our goal here is to use behavioral observations to determine the cues integrated during navigation.

Adults of *T. diomedea* may use several guidance cues as they navigate by crawling over the sediment substratum of their habitat. *T. diomedea* detects water flow (Willows, 1978; Murray *et al.*, 1992), and current direction can guide crawling both in the field (Murray, unpubl. data) and in the laboratory (Murray and Willows, 1996). In the absence of other stimuli in the laboratory, *T. diomedea* crawls upstream, exhibiting positive rheotaxis (Murray and Willows, 1996). However, in the field (Wyeth and Willows, 2006a),

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Abbreviations: RTF, relative to flow.

flow direction apparently orients slugs crawling toward mates or prey, and away from predators. Odors may be important in detecting these upstream organisms (Willows, 1978). Downstream flow patterns may also be characteristic of upstream animals (Atema, 1996). Finally, *T. diomedea* can detect the geomagnetic field (Lohmann and Willows, 1987; Lohmann *et al.*, 1991; Popescu and Willows, 1999; Wang *et al.*, 2004). Preliminary evidence suggests that shoreward orientation or other adaptive behaviors may depend on magnetosensation (Lohmann and Willows, 1987; Willows, 1999). Accordingly, here we study crawling with respect to water flow, to surrounding environmental features that could act as odor sources or flow disruptors, and to magnetic bearings.

Our goals were to learn whether positive rheotaxis is continuous for *T. diomedea*, whether the presence or absence of positive rheotaxis is correlated with other sensory cues, and whether circumstances with other orientations to flow or consistent geomagnetic orientation occur. Our time-lapse video records of *T. diomedea* navigating through its natural habitat show that the slugs crawled upstream toward mates and prey, but did not do so after mating or feeding. Conversely, the slugs moved downstream (negative rheotaxis) away from a predator. We did not find any consistent magnetic heading preferences.

Materials and Methods

Behavior camera setup

We recorded time-lapse video of *T. diomedea* navigating in beds of the sea pen *Ptilosarcus gurneyi*, its natural habitat (Birkeland, 1974; Wyeth and Willows, 2006a) at Dash Point (47°19.28'N, 122°25.22'W) in southern Puget Sound, Washington, USA. These videos were also used to describe *T. diomedea* field behaviors (Wyeth and Willows, 2006a). Three underwater video cameras were each attached to a pole driven into the sediment. Cameras were arranged in a triangle, angled down towards the substratum, with fields of view overlapping slightly at the top left of each camera. Video was cabled to the research vessel and digitized to hard disk at 2.5 frames s⁻¹ and 320 × 240 pixel resolution. Camera orientation was calibrated by holding a compass in the field of view. We placed slugs on the substratum under the cameras at densities consistent with those found in the surrounding habitat, and recorded slug movements without further disturbance. We recorded video on 11 days, spanning 9 flood, 9 slack, and 5 ebb tides.

Behavior measurements

All behavior videos were reviewed to track *T. diomedea* within and between cameras. We tracked 103 slugs, but we conservatively estimate that 10% of these left and then returned to the field of view, and 25% were re-used on a

subsequent day. Thus, our data are estimated to be based on behaviors from about 70 different slugs.

We digitized slug positions (pixel coordinates) and orientations (magnetic headings calibrated by the compass video) every 30 s. For each position and orientation, we also recorded slug identity, time, and behavior (see Wyeth and Willows, 2006a, for behavior definitions, durations, and locomotory distances). Behaviors within 15 min of disturbance by a diver were omitted from analyses, except for data relating to mating, where reproductive activity is assumed to indicate that the animal was not disturbed. We excluded behaviors when the slugs were in contact with obstructions to crawling (conspecifics, prey, algae, gear, etc.) or were laying eggs.

Current-heading measurements

A “current camera” was deployed adjacent to the behavior cameras (Wyeth and Willows, 2006a), less than 3 m away from any slug in the behavior videos. We measured current headings (not speed) every 6 s by tracking small suspended particles in videos from the current camera (Wyeth and Willows, unpubl. data). Each heading was an average over both time (particles were tracked between 150 frame pairs over the 6 s) and space (the measurement volume was ~14 l). By averaging such a large set of particle movements, we were able to measure the heading of bulk flow in the region. The flow data corresponded well with the direction of dye plumes in the behavior videos, and it predicted the flow experienced by the slugs, as indicated by the movement of slugs swept downstream after swimming or being dislodged by flow (Wyeth and Willows, unpubl. data). Thus, this method of flow measurement provides an accurate and independent set of flow headings to compare to slug orientations from the behavior videos.

Flow-variation quantification

Flow experienced by *T. diomedea* is affected by both the direction of bulk flow and the turbulence generated as the flow passes over the benthic habitat. Variation in bulk flow affects its utility as a guidance cue during navigation. Positive rheotaxis can reach a stationary upstream target only if flow heading changes little over the course of the behavior. Smaller scale turbulence spread dye plumes from a point source by $32 \pm 6.6^\circ$ (mean \pm st. dev.; $n = 7$). This suggests that for a slug downstream of a target of interest, a 30° change in flow will eliminate any cues transported downstream from the target. We therefore quantified both long-term and short-term flow variation with respect to this 30° value.

Long-term variation was assessed by “interval heading changes.” We divided each day into a series of identical intervals. Mean headings were calculated for each interval, and angular distances between subsequent interval means

were then averaged for each day. The resulting daily interval heading change gives a measure of how much flow headings changed, on average, from one interval to the next. The same calculations were performed for intervals of 1, 2, 5, 10, 15, 30, and 60 min (limited to days with ≥ 5 intervals to average). These data provide information on long-term variability by measuring how much average current direction changes over different lengths of time.

Short-term variation was quantified by “sector residence duration.” We measured how long subsequent current headings remained within an angular sector centered on each current heading. Averaging the durations of sector residence over the entire day measures how long, on average, current headings stayed wholly within a sector span. The same calculations were performed for sector angles of 10°, 20°, 30°, 45°, 60°, 90°, and 180°. These data provide information on short-term variability by measuring how quickly, on average, the current heading changes over different angular distances.

Tidal data

We used measured tide heights available every 6 min for Commencement Bay, 6.1 km from Dash Point (NOAA/NOS, 2004; Wyeth and Willows, 2006). We converted tidal height to tidal state by finding the sign of differences between successive tidal heights (positive = flood and negative = ebb). Slack tide was assigned when the magnitude of the difference was below an arbitrary threshold set such that the mean duration of all slack tides was 1 h.

Orientation analysis

Three angular data types were calculated for each behavioral measurement: slug magnetic bearing, water-flow magnetic bearing (paired with behavior measurements by averaging over the 30 s before the behavior measurement), and slug heading relative to flow (RTF, Fig. 1). For each be-

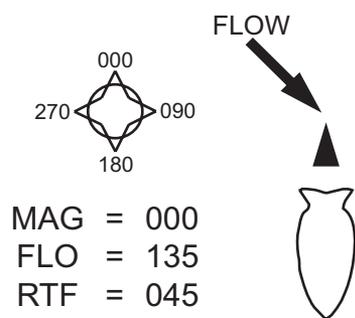


Figure 1. Orientation calculations. *Tritonia diomedea* orientation (arrowhead, MAG) and water flow direction (arrow, FLO) were compass-calibrated for magnetic bearing. Slug heading relative to flow (RTF) was calculated as the angular difference between slug orientation and directly upstream.

havior with multiple measurements, mean angles were calculated for slug bearing, flow bearing, and heading RTF. The single values for the three data types calculated for each behavior were then used for subsequent orientation analyses.

We analyzed slug orientation during behaviors grouped by circumstantial criteria. For example, behavior groups included crawling, before turns, after turns, and crawling before feeding, among others. These groups of behaviors defined sets of angles for each of the three data types. The mean angles for each set were then tested for significance (Rayleigh test; Zar, 1999). For example, for crawling behaviors, we tested the significance of the mean magnetic bearing of all crawling slugs, the mean flow bearing during those behaviors, and the mean slug heading RTF while crawling. A significant mean angle for headings RTF implies that slug orientation was nonrandom with respect to flow for that group of behaviors. A significant magnetic bearing for the slugs suggests orientation to the earth’s magnetic field if flow does not also show a significant mean magnetic bearing in the opposite direction.

Rayleigh test *P* values (Greenwood and Durand, 1955) were calculated using a numerical integration algorithm and checked against tables in Zar (1999). Differences in angular dispersion between two groups of behaviors were tested for significance using either a Wilcoxon ranked sum test for paired measurements on the same animals or a Mann-Whitney test otherwise (Zar, 1999).

Special considerations for orientation before and after mating or feeding

Most mating pairs occur with one slug (the initiator) clearly initiating contact with another (the initiate; Wyeth and Willows, 2006). For analyses of orientation before mating, we considered behaviors by initiators and also those by slugs that approached and made contact with mating pairs (Wyeth and Willows, 2006a). After mating, we considered behaviors by both initiators and initiates. In addition, we pooled crawling (mean headings) and turn behaviors (final measurements, our best estimate of the slug’s preferred orientation) for analysis of orientation relative to mating or feeding. As a result, these data sets contain some paired and some independent measurements; we therefore use Wilcoxon tests to analyze the paired data, and Mann-Whitney tests to analyze the independent data.

Predator-avoidance experiment

We tested *T. diomedea* responses to the predatory sea star *Pycnopodia helianthoides* in a different *P. gurneyi* bed at MacIntosh Rocks (49° 12.60’N, 125° 57.45’W), North of Vargass Island, British Columbia, Canada. *T. diomedea* individuals were video recorded (30 frames s⁻¹) by a behavior camera arranged vertically about 1 m above the slug.

Flow direction was determined by a scuba diver observing marine snow and fluorescein dye throughout the trial. Slug activity was recorded for at least 2 min before stimuli were presented by the diver either upstream or downstream of the slug. Activity was videoed for at least another 2 min, or until the slug's response became clear. We used three stimuli: Control A ($n = 5$)—an empty dive glove of similar diameter and height to *P. helianthoides*, ~30 cm upstream, controlled for upstream physical disturbance; Control B ($n = 6$)—*P. helianthoides*, ~30 cm downstream, controlled for diver disruption of flow; Experimental ($n = 10$)—*P. helianthoides* ~30 cm upstream (5 different sea stars). The stimuli were repositioned to maintain an upstream or downstream location. After three downstream control presentations of *P. helianthoides*, we also briefly moved the sea star upstream. Two downstream presentations of *P. helianthoides* were not analyzed because erratic flow prevented the diver from maintaining a downstream stimulus location. We also found three *P. helianthoides* individuals that failed to elicit any distant responses in any slug. Trials with these sea stars ($n = 9$) as stimuli were excluded from analysis.

From the video of each trial, slug and current headings were recorded every 5 s during stimulus presentations. Headings RTF for control (A and B grouped together) and experimental groups were tested for mean directions (Rayleigh tests). The change in heading RTF after each presentation was also calculated, and control and experimental groups were compared with a Mann-Whitney test (Zar, 1999).

Software

All analyses and calculations were performed using either custom software designed in Matlab 6.5 and 7.0 (The Mathworks Inc., Natick, MA), Excel 11.6 (Microsoft, Redmond, WA), JMP 5.1 (SAS Institute Inc., Cary, NC), or SPSS 13.0 (SPSS Inc., Chicago, IL).

Results

Flow in *Ptilosarcus gurneyi* beds

Currents measured in the *P. gurneyi* bed were variable (Fig. 2). Flow sometimes changed 90° or more in less than 3 min, and at other times remained stable for an hour or more. Flow direction showed little correspondence with tidal state (Fig. 3). Headings varied across all magnetic bearings during flood and slack tides. Only ebb tides showed any consistency in flow direction; however, our sample of ebb tides was smaller than that for flood and slack tides.

Our quantitative measures of flow variation show that flow direction changed by 30° or more every 5 to 15 min. Long-term changes in flow heading, measured as daily means for interval heading changes (angular distances be-

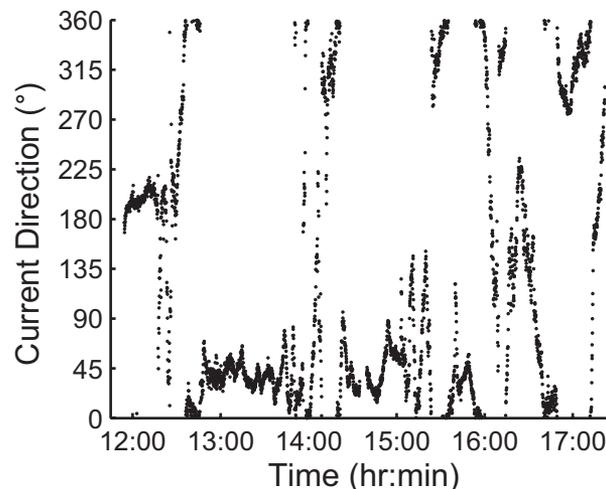


Figure 2. Example of flow during a flood tide in a *Ptilosarcus gurneyi* bed, recorded at Dash Point on 24 April 2001. Flow magnetic bearings varied widely over the afternoon, with periods of stability at about 040° magnetic and other periods when flow wandered erratically with no consistent compass heading.

tween headings averaged over different time intervals), increased rapidly to a mean of 31° for intervals of 15 min, and continued to increase for longer intervals (Fig. 4A). Short-term variation, measured as daily means for sector residence durations (the duration headings remained inside different sector spans), was 5 min for a 30° sector and 23 min for a 90° sector (Fig. 4B).

Orientation and navigation relative to flow

Tritonia diomedea oriented to flow. Crawling slugs' mean heading relative to flow (RTF, Fig. 1A) was directly upstream (Table 1, Fig. 5A). Conversely, we observed no significant mean magnetic bearing for the same crawling behaviors (Table 1, Fig. 5B). The mean bearing of 212° magnetic, although nonsignificant, is inside the confidence limits for the true upstream direction during the behaviors (Table 1, Fig. 5B). Thus, it appears that slugs orient to flow while crawling but have no consistent magnetic orientation. This remained true when the crawling behaviors were grouped by tidal state. All three groups (flood, slack, and ebb) showed mean headings with confidence limits including upstream (Table 1). Only crawling during ebb tides showed a significant mean magnetic bearing. Here again, the confidence limits for the mean crawling bearing include the mean upstream magnetic bearing for flow during those behaviors (Table 1), consistent with orientation to flow.

T. diomedea also oriented to flow by turning upstream. Initial orientations at the start of a turn showed no significant mean heading RTF (Table 2, Fig. 6A). The final measurements at the end of the turn, however, showed a significant mean heading, facing directly upstream (Table 2,

Fig. 6B). Final measurements RTF were significantly less dispersed than first measurements (paired Wilcoxon rank sum test, $n = 111$, $T_- = 1714$, one-tailed P value = 0.000021). Although the final measurements also showed a significant magnetic bearing (Table 2), the confidence limits for the mean bearing include the mean upstream bearing at the time of the turn behaviors.

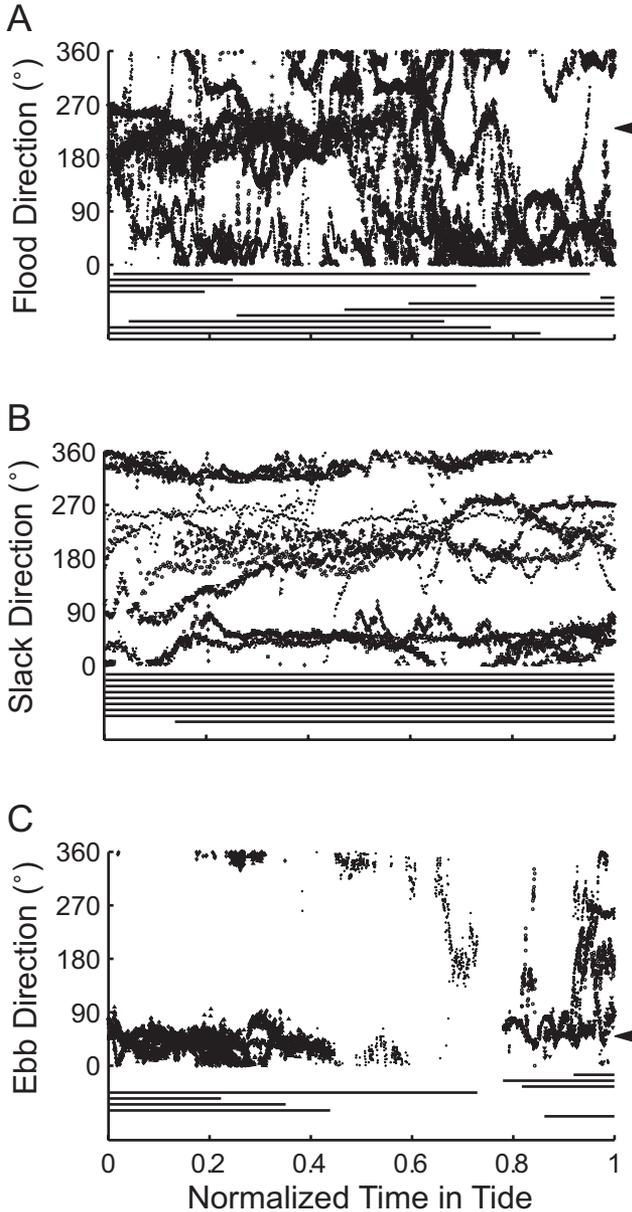


Figure 3. Flow magnetic bearings during flood (A), slack (B), and ebb (C) tides reveal that flow direction varies widely. *Tritonia diomedea* in *Ptilosarcus gurneyi* beds can therefore experience flow from any direction, regardless of tidal state. Time values are normalized to the fraction of the tide duration (time = 0 at the start, time = 1 at the end of the tide). The horizontal lines at the bottom of the axes indicate the time sampled on each of 11 days. In A and C, the parallel-to-shore flood and ebb directions are indicated by the arrowhead on the right.

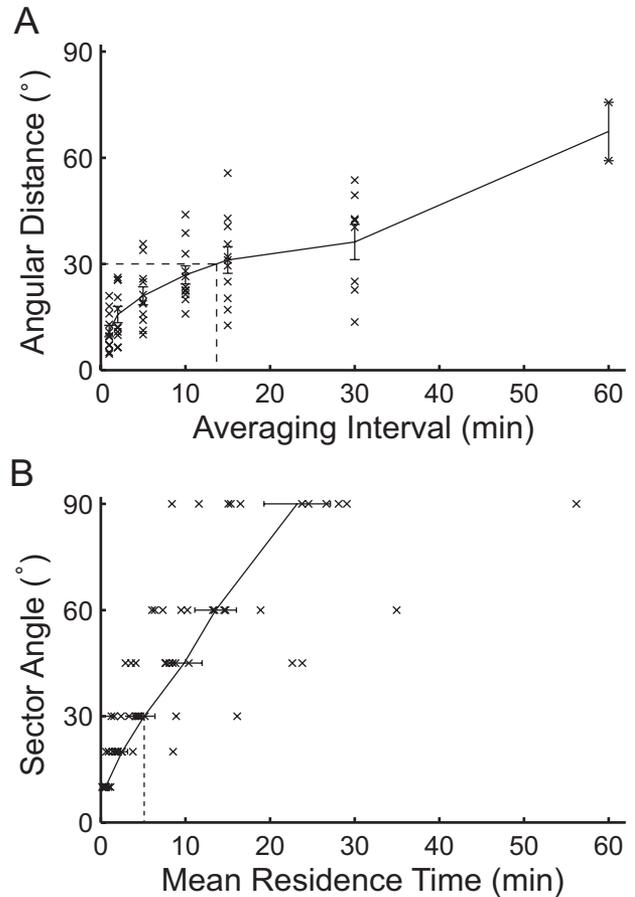


Figure 4. Long-term and short-term flow variability in *Ptilosarcus gurneyi* beds suggests that *Tritonia diomedea* has, on average, between 5 and 15 min of stable flow as a navigation cue. (A) Long-term interval heading changes: Current data were divided into serial intervals, and mean flow direction was calculated for each interval. Angular distances between each interval mean and the next were then averaged over each day. Daily means for intervals of 1, 2, 5, 10, 15, 30, and 60 min are plotted, with overall means (solid line) and standard errors. Data limited to days with five or more intervals. (B) Short-term sector residence durations: Starting at each current datum, the period over which flow bearing remained wholly within a sector was calculated for sectors of 10°, 20°, 30°, 45°, 60°, and 90°. Daily means are plotted, with overall means (solid line) and standard errors. Our observations of dye plume spread suggest that downstream cues from an upstream feature would be removed by a current change of 30° or more. The 30° current changes and the corresponding time scales of variability are marked (dashed lines), and indicate that flow in this *P. gurneyi* bed varies sufficiently to eliminate upstream cues in 5 to 15 min on average.

Inactive slugs also initially oriented upstream. First measurements showed a mean heading RTF with confidence limits that included the upstream direction (Table 2). However, while slugs remained inactive, flow continued to vary, and thus there was no relationship between orientation and flow for the final measurements at the end of each period of inactivity (Table 2). Neither first nor

Table 1

Tritonia diomedea crawling orientations

Group	<i>n</i>	Data	θ (°)	<i>r</i>	z_n	<i>P</i> value	CL (°)
All	200	RTF	359	0.33	21.9	0.00018	343, 016
		MAG	211	0.12	2.72	0.066	ns
		FLO	041	0.22	10.1	0.00012	195, 246
Flood	137	RTF	360	0.31	13.1	0.000076	338, 022
		MAG	213	0.08	0.94	0.39	ns
		FLO	028	0.10	1.31	0.27	ns
Slack	27	RTF	352	0.48	6.26	0.0014	320, 024
		MAG	323	0.18	0.91	0.40	ns
		FLO	159	0.20	1.12	0.33	ns
Ebb	45	RTF	007	0.28	3.57	0.027	321, 054
		MAG	193	0.30	4.15	0.015	151, 236
		FLO	042	0.67	20.3	0.000038	206, 237

Crawling behaviors were grouped according to tidal state (flood, slack, and ebb). Mean direction (θ , *r*) of crawling relative to flow (RTF), magnetic bearing of crawling (MAG), and magnetic bearing of flow during crawling (FLO) were all analyzed for significance using a Rayleigh test (z_n statistic). Confidence limits (CL) of 95% are given for data sets with significant mean headings at $\alpha = 0.05$. For behavior sets with a significant bearing for mean flow, the 95% CL for the *upstream* magnetic bearing for flow are given to compare with crawling behaviors' mean magnetic bearings. The slugs consistently oriented to flow (significant headings RTF with confidence intervals overlapping upstream). The only significant magnetic orientation (during ebb tides) was consistent with upstream orientation since the confidence limits for crawling bearing and upstream direction overlap. ns, not significant.

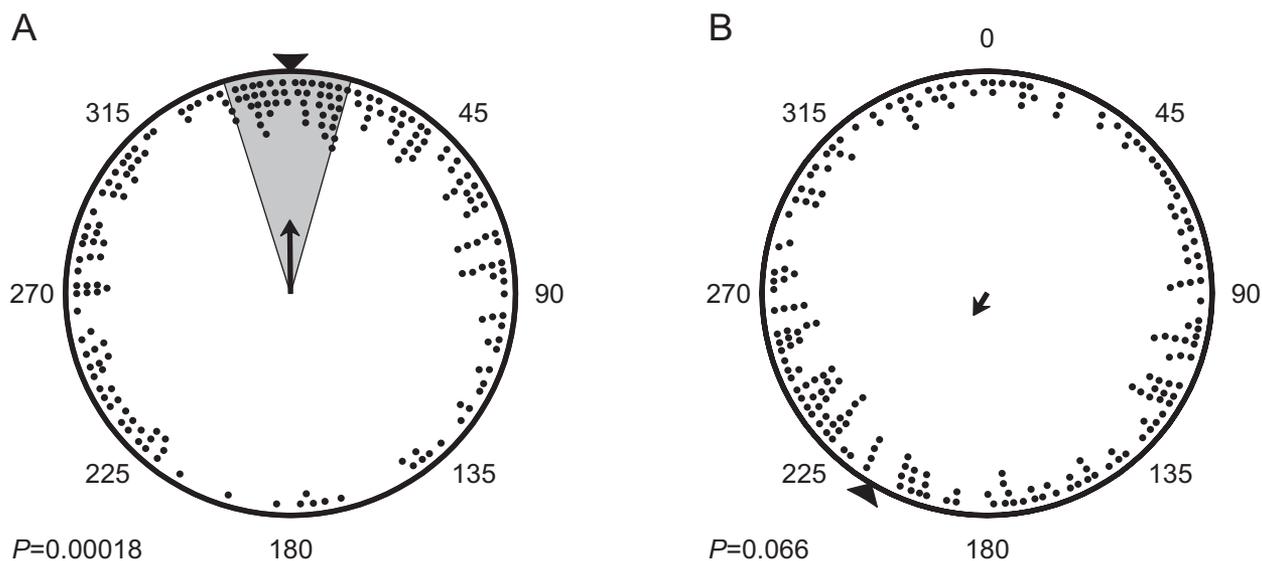


Figure 5. *Tritonia diomedea* tends to crawl upstream but has no preferred magnetic bearing while crawling. (A) Mean headings relative to flow (RTF) for crawling slugs. Each point indicates mean slug heading RTF (top arrowhead indicates upstream = 000° RTF), the central arrow indicates mean heading (θ) for all points, the length (*r*) measures dispersion, the outer circle indicates minimal dispersion (*r* = 1), and the gray sector indicates the 95% confidence limits for the true mean heading RTF. The mean heading RTF is significant and oriented upstream (confidence limits include 000° RTF). (B) Mean magnetic bearings for the same behaviors. There was no significant departure from random orientations. The sample mean bearing (central arrow) is directed towards the mean upstream bearing for flow (arrowhead). See Table 1 for statistics.

Table 2

Tritonia diomedea orientations during turns and inactivity

Behavior	<i>n</i>	Data	θ (°)	<i>r</i>	z_n	<i>P</i> value	CL (°)
Turn First	111	RTF	344	0.16	2.97	0.051	ns
		MAG	276	0.12	1.53	0.22	ns
		FLO	073	0.21	4.74	0.0085	214, 292
Turn Last	111	RTF	357	0.49	26.6	0.00012	342, 012
		MAG	216	0.21	4.92	0.0071	178, 255
		FLO	062	0.27	7.8	0.0004	213, 271
Inactive First	95	RTF	355	0.36	12.2	0.000052	333, 018
		MAG	331	0.08	0.65	0.52	ns
		FLO	052	0.02	0.03	0.97	ns
Inactive Last	95	RTF	330	0.15	2.04	0.13	ns
		MAG	308	0.15	2.25	0.11	ns
		FLO	081	0.21	4.16	0.015	219, 304

The first and last measurements of turn behaviors and periods of inactivity were analyzed for mean direction (θ , r) relative to flow (RTF), magnetic bearing (MAG), and magnetic bearing of flow at the time of the behavior measurement (FLO). Significance of the means was assessed using a Rayleigh test (z_n statistic). Confidence limits (CL) of 95% are given for data sets with significant mean headings at $\alpha = 0.05$. For behavior sets with significant mean flow, the 95% CL for the *upstream* magnetic bearing for flow are given to compare with the behaviors' mean magnetic bearing. ns, not significant.

final measurements showed a significant mean magnetic bearing (Table 2).

T. diomedea oriented to flow before mating and feeding. We pooled mean headings for crawling preceding mating or feeding with the final measurement of turns preceding mat-

ing or feeding for these analyses. The pooled orientations showed significant mean headings RTF, whether analyzed relative to mating, feeding, or both in combination (Table 3, Fig. 7A). Conversely, pooled orientations after mating or feeding showed no significant mean heading RTF (Table 3,

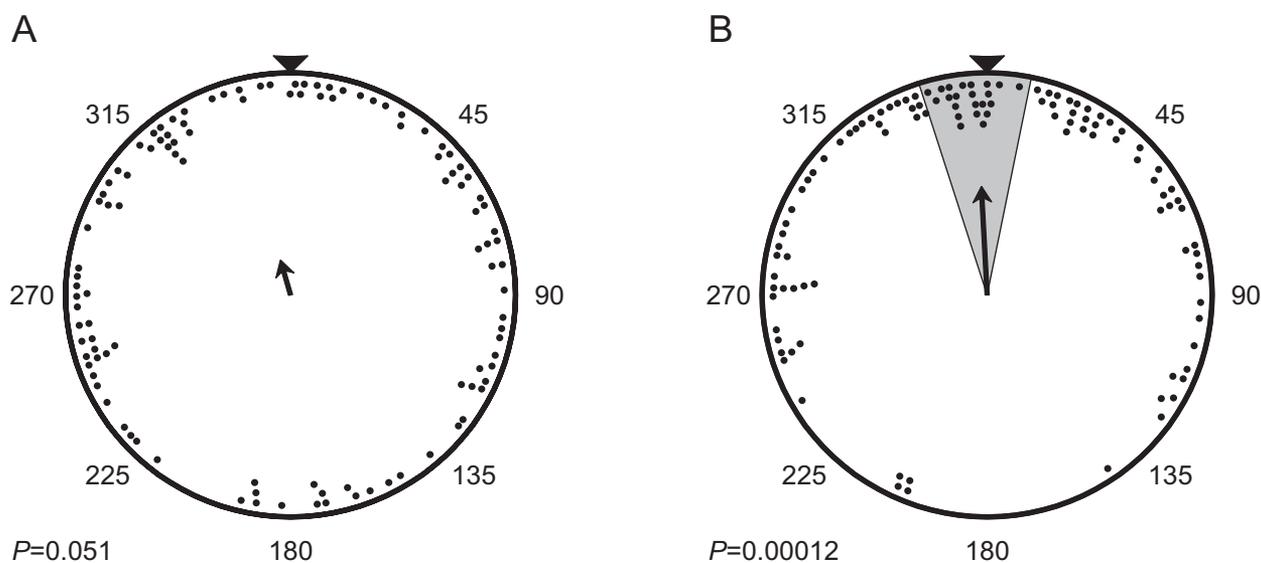


Figure 6. *Tritonia diomedea* tends to turn upstream. Slug heading was measured every 30 s for each turn in the behavior videos. First measurement indicates the turn origin, and the final measurement indicates turn endpoint. (A) Original headings relative to flow (RTF) do not significantly depart from random. (B) Final headings RTF have a significant upstream mean heading RTF (confidence limits include 000° RTF). See Table 2 for statistics.

Table 3*Tritonia diomedea* orientations before and after mating or feeding

Behavior	<i>n</i>	Data	θ (°)	<i>r</i>	z_n	<i>P</i> value	CL (°)
Before							
Bite strike	13	RTF	360	0.58	4.31	0.011	320, 039
		MAG	222	0.19	0.46	0.64	ns
		FLO	038	0.56	4.03	0.015	177, 260
Mate	24	RTF	359	0.59	8.24	0.00014	332, 026
		MAG	134	0.29	2.07	0.13	ns
		FLO	258	0.03	0.02	0.98	ns
Combined	37	RTF	359	0.58	12.6	0.000021	338, 020
		MAG	153	0.20	1.53	0.22	ns
		FLO	034	0.18	1.21	0.30	ns
After							
Bite strike	13	RTF	040	0.41	2.23	0.11	ns
		MAG	288	0.26	0.88	0.42	ns
		FLO	029	0.56	4.01	0.015	168, 250
Mate	25	RTF	099	0.14	0.43	0.66	ns
		MAG	166	0.20	0.96	0.39	ns
		FLO	334	0.11	0.29	0.75	ns
Combined	38	RTF	061	0.21	1.56	0.21	ns
		MAG	211	0.11	0.46	0.63	ns
		FLO	015	0.25	2.22	0.11	ns

Pooled crawling and final turn measurements before and after mating and feeding were analyzed for mean direction (θ , r) relative to flow (RTF), magnetic bearing (MAG), and magnetic bearing of flow at the time of the behavior measurement (FLO). Significance of the means was assessed using a Rayleigh test (z_n statistic). Confidence limits (CL) of 95% are given for data sets with significant mean headings at $\alpha = 0.05$. For behavior sets with significant mean flow, the 95% CL for the *upstream* magnetic bearing for flow are given to compare with the behaviors' mean magnetic bearing. ns, not significant.

Fig. 7B). In addition, dispersion in orientation RTF for headings before mating was significantly lower than the dispersion in headings after mating (paired data: Wilcoxon rank sum test, $n = 11$, $T_- = 54$, one-tailed P value = 0.031; unpaired data: Mann-Whitney test, $n_1 = 13$, $n_2 = 12$, $U = 111$, one-tailed P value = 0.038). We found no significant difference in dispersion when headings before feeding were compared to headings after feeding (paired Wilcoxon rank sum test, $n = 13$, $T_- = 104$, one-tailed P value > 0.10). However, in combination, the dispersion was significantly less before feeding and mating than after (paired Wilcoxon rank sum test, $n = 24$, $T_- = 65$, one-tailed P value = 0.0076). The dispersion in orientation RTF before feeding or mating was also significantly lower than dispersion during crawling in general (Mann-Whitney test, $n_1 = 37$, $n_2 = 172$, $U = 4242$, one-tailed P value = 0.00067). We found no significant magnetic bearing for any of these behaviors (pooled crawling and final measurements of turns, relative to feeding, mating, or both, Table 3). The consistent trend was thus for *T. diomedea* to be oriented upstream before mating or feeding, but not afterward.

Magnetic orientation

Slugs did not show any consistent magnetic orientation across behavior types. No preferred magnetic bearing was observed for slugs that recently settled onto the substratum after being handled by divers (data not shown).

Predator avoidance

All 11 slugs responded to upstream presentation of the sea star *Pycnopodia helianthoides*. They either turned and then crawled downstream ($n = 7$, Fig. 8A) or swam and thus drifted downstream ($n = 4$). Prior to stimulation, 10 of these 11 slugs were inactive, and one was crawling. The latter turned about 160° to crawl downstream after stimulation. After the turns, crawling was directed downstream (Rayleigh test, $\theta = 192^\circ$ RTF, $r = 0.86$, $n = 7$, $z = 5.18$, P value = 0.0020, 95% confidence limits include downstream; Fig. 8A). In control trials, all slugs were inactive before stimulation, no response was observed, and no significant subsequent mean heading RTF was observed (Rayleigh test, $\theta = 164^\circ$ RTF, $r = 0.20$, $n = 9$, $z = 0.36$, P

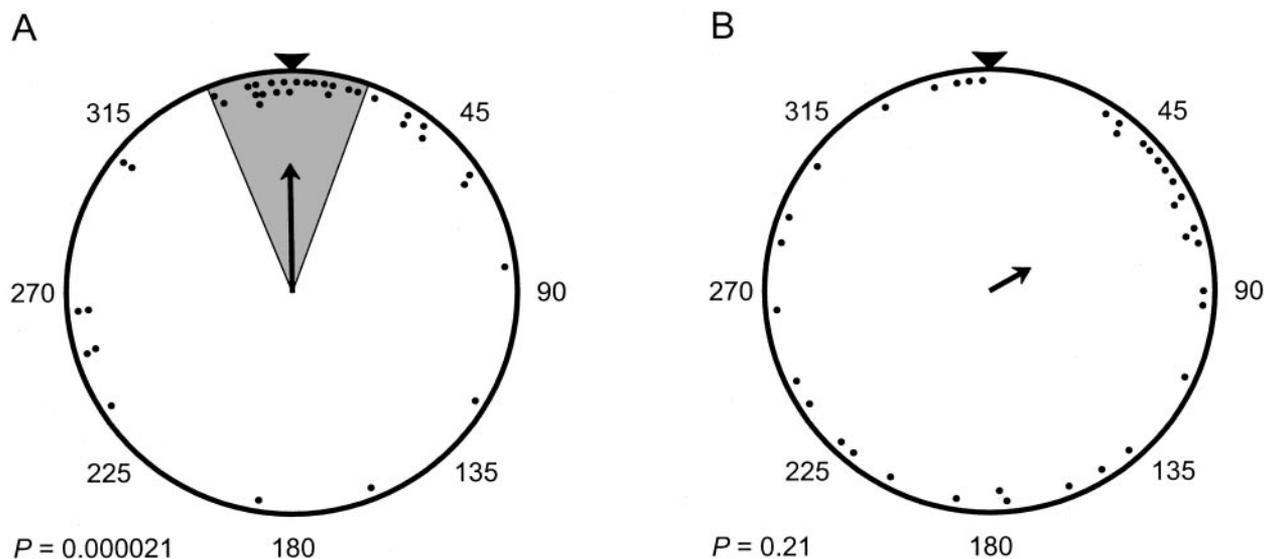


Figure 7. *Tritonia diomedea* crawls upstream towards mates and food, but not after mating or feeding. (A) Headings relative to flow (RTF) for crawling and turning behaviors before mating or feeding. The mean heading RTF is significant and oriented upstream (confidence limits include 000° RTF). (B) Headings relative to flow for crawling and turning behaviors after mating or feeding. There was no significant departure from random orientations. For turning behaviors, we used endpoints as best estimates of slugs' preferred orientation.

value = 0.71). The changes in heading RTF over the course of the experimental trials ($88 \pm 32^\circ$ RTF, due to both slug movements and variations in flow) were significantly larger than changes in headings RTF for controls ($29 \pm 6^\circ$ RTF, due entirely to variations in flow; Mann-Whitney test,

$n_1 = 7$, $n_2 = 9$, $U = 58$, one-tailed P value = 0.0025, Fig. 8B). After three downstream presentations of *P. helianthoides*, the sea star was briefly presented upstream. All elicited responses from the slugs were similar to the responses shown by the experimental slugs. *T. diomedea*

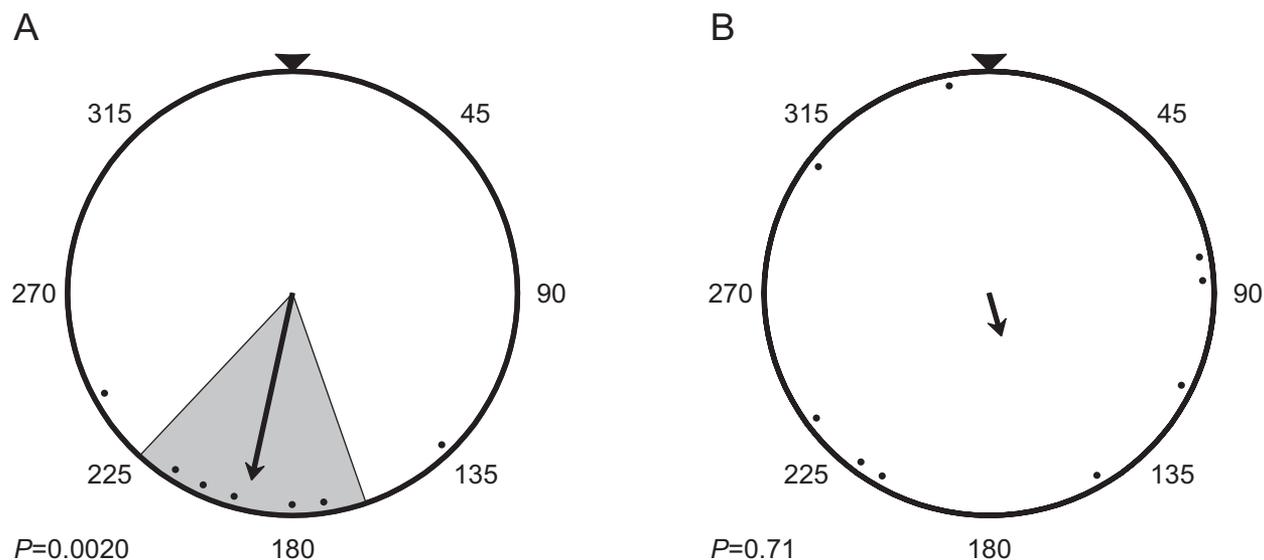


Figure 8. *Tritonia diomedea* crawls downstream in the presence of an upstream predator. (A) Final headings relative to flow (RTF) for crawling behaviors after presentation of upstream *Pycnopodia helianthoides*. The mean heading RTF is significant and oriented downstream (confidence limits for the true mean include 180° RTF). (B) Final headings RTF for stationary *T. diomedea* following control presentations of either an upstream dive glove upstream or downstream *P. helianthoides*. There was no significant departure from random orientations.

therefore consistently responded to distant *P. helianthoides* by turning and then either crawling or swimming, both resulting in downstream movement away from the sea star.

Discussion

Navigation relative to odors and flow

Tritonia diomedea used flow direction to navigate. Non-random orientation to flow for crawling, turns, and inactive periods (Figs. 5 and 6; Tables 1 and 2) all suggest active orientation behavior. Orientation to some other cue correlated with flow, although theoretically possible, seems unlikely given prior evidence that *T. diomedea* orients to flow (Murray and Willows, 1996; Murray, unpubl. data). Our ability to choose criteria that either strengthened or removed the relationship to flow suggests that the slugs orient to flow in certain circumstances. Thus, turns, which suggest navigational choices and often precede mating (Wyeth and Willows, 2006a), were initiated without significant orientation to flow, but ended with the slugs, on average, facing upstream (Fig. 6). Slugs crawled upstream to find targets of interest: both food and mates. However, after mating or feeding, when motivation to find another target of interest may be low, slugs crawled randomly relative to flow (Table 3 and Fig. 7). When an upstream predator was present, slugs crawled or swam away downstream (Fig. 8). All these observations point to an orientation relative to flow that is based on context, rather than simply on constant positive rheotaxis.

Odors may be an important contextual cue modulating responses to flow. Sensation by a downstream slug probably relies on either distinctive odors or downstream flow characteristics of upstream features. Downstream flow characteristics would depend on feature distance, shape, size, and orientation, as well as on flow speed, turbulence, and other factors (Vogel, 1994). Yet flow in the habitat is highly variable (Figs. 2–4). Furthermore, both predators and conspecifics vary greatly in size, so their morphology does not seem likely to disrupt flow in any consistent way. A consistent downstream flow pattern is more plausible for the sea pen *Ptilosarcus gurneyi*, because its feeding mechanism relies on creating a distinctive flow pattern (Best, 1988; Vogel, 1994). In contrast, odor is a well-known cue for modulating responses to flow in marine organisms (Vickers, 2000; Weissburg, 2000; Zimmer and Butman, 2000; Grasso and Basil, 2002), including several gastropods (Lee *et al.*, 1974; Lederhendler *et al.*, 1977; Bousfield, 1979; Brown and Rittschof, 1984; Ferner and Weissburg, 2005). Our observations in the natural habitat are consistent with odors as a cue affecting navigation relative to flow. Furthermore, *T. diomedea* responds to prey, predator, and conspecific odor plumes in the laboratory (Willows, 1978; Wyeth and Willows, 2006b). Thus, we suggest that odors are a primary

cue used by *T. diomedea* for orientation and navigation, and should be a focus for future experimentation.

If odors released by other slugs are a navigational cue for *T. diomedea*, slug headings relative to flow might be expected to depend on the presence or absence of upstream conspecifics. Unfortunately, the camera field of view limited our ability to properly distinguish between slugs behaving with or without upstream slugs. Slugs may head upstream with greater fidelity in the presence of conspecific odor. In the absence of upstream conspecifics they may also use cross-current crawling, a viable search strategy under certain flow conditions (Sabelis and Schippers, 1984). However, to pursue the details of navigational responses to the presence or absence of upstream conspecifics, more complete data is needed on relative slug positions.

Flow variability

Changes in flow direction affect navigation by *T. diomedea*. Since dye plumes disperse 30° from a point source, changes in flow greater than 30° will, on average, eliminate a slug's ability to use odor-triggered positive rheotaxis to find an upstream target. Our analysis of flow variability indicates that changes in flow of 30° occur, on average, every 5 to 15 min (Fig. 4). Crawling behaviors, which do not include large changes in direction, lasted just 8 min on average (Wyeth and Willows, 2006), and mate search sequences lasted 10 min ± 4 (mean ± st. dev.). Thus, behavior durations correlate well with expected durations if slugs are following currents only while they remain constant enough to provide useful information about upstream targets. In addition, the variable flows in the habitat suggest that the time constant of changes in navigational heading in response to changes in flow heading may need to be finely tuned to optimize upstream navigation. Flow variability also affects search strategy when no upstream target is detectable. Cross-stream, upstream, or downstream crawling can all be effective search strategies, depending on the degree of flow variability (Sabelis and Schippers, 1984; Dusenbery, 1989, 1990). Future work should consider slug navigation relative to recent flow history and to the relative positions of nearby prey and conspecifics.

Magnetic orientation

We did not observe any consistent orientation to the earth's magnetic field in slugs exposed to water currents. Only two groups of behaviors (after turns and crawling during ebb tides) showed significant mean magnetic bearings, but both may be accounted for by currents impinging from the opposite direction. This failure to observe magnetic orientation in the natural habitat, where flow is continuous, is consistent with laboratory observations that responses to flow supersede magnetic responses (Murray, unpubl. data).

What, then, does *T. diomedea* use its magnetic sense for? Previous hypotheses have suggested that either spiral or shoreward crawling could return slugs to their habitat after they are swept away by currents (Lohmann and Willows, 1987; Willows, 1999). We saw no indication of such behaviors here: no preferred magnetic heading after slugs were swept off the bottom or disturbed by divers (data not shown). However, the slugs were not removed from the *P. gurneyi* bed, and spiral or shoreward crawling may be triggered only when habitat cues are absent. What is clear from our data is that the current is always flowing in the habitat and is a critical cue controlling the behavior of *T. diomedea*. Further work on the functional role of magnetosensation in this species must integrate hypotheses with the known rheosensitive behaviors, as well as control for the effects of flow during experimentation.

Summary

Our analysis of behavior provides quantitative evidence to corroborate our qualitative observations of navigation in *T. diomedea* (Wyeth and Willows, 2006a). We have shown navigation in relation to three major habitat features: *T. diomedea* crawls upstream to find mates and prey, and crawls or swims downstream away from predators. Only one of these behaviors (attraction to the prey species *P. gurneyi*) has moderate support from prior experimentation in the laboratory (Willows, 1978). However, no previous experiments replicated the natural flow conditions observed here, and thus the absence of behaviors in the laboratory is inconclusive. Further laboratory experimentation should be designed with both the flow conditions and sensory capabilities of *T. diomedea* in mind (Wyeth and Willows, 2006b). Furthermore, recognition that a behavioral choice, based on different upstream cues, determines whether *T. diomedea* crawls upstream or downstream provides an opportunity for neuroethological study. Establishing the sensory basis of this choice will allow further investigation into the sensory integration underlying navigation.

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Literature Cited

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

- Audesirk, T. E., and G. J. Audesirk. 1977.** Chemoreception in *Aplysia californica*. II. Electrophysiological evidence for detection of the odor of conspecifics. *Comp. Biochem. Physiol. A* **56**: 267–270.
- Best, B. A. 1988.** Passive suspension feeding in a sea pen: effects of ambient flow on volume flow rate and filtering efficiency. *Biol. Bull.* **175**: 332–342.
- Bicker, G., W. J. Davis, E. M. Matera, M. P. Kovac, and D. J. Stormogipson. 1982a.** Chemoreception and mechanoreception in the gastropod mollusc *Pleurobranchaea californica*. 1. Extracellular analysis of afferent pathways. *J. Comp. Physiol.* **149**: 221–234.
- Bicker, G., W. J. Davis, and E. M. Matera. 1982b.** Chemoreception and mechanoreception in the gastropod mollusc *Pleurobranchaea californica*. 2. Neuroanatomical and intracellular analysis of afferent pathways. *J. Comp. Physiol.* **149**: 235–250.
- Birkeland, C. 1974.** Interactions between a sea pen and seven of its predators. *Ecol. Monogr.* **44**: 211–232.
- Bousfield, J. D. 1979.** Plant extracts and chemically triggered positive rheotaxis in *Biomphalaria glabrata* (Say), snail intermediate host of *Schistosoma mansoni* (Sambon). *J. Appl. Ecol.* **16**: 681–690.
- Brown, B., and D. Rittschof. 1984.** Effects of flow and concentration of attractant on newly hatched oyster drills, *Urosalpinx cinerea* (Say). *Mar. Behav. Physiol.* **11**: 75–93.
- Cain, S. D., J. H. Wang, and K. J. Lohmann. 2006.** Immunochemical and electrophysiological analyses of magnetically responsive neurons in the mollusc *Tritonia diomedea*. *J. Comp. Physiol. A* **192**: 235–245.
- Chase, R. 2002.** *Behavior and Its Neural Control in Gastropod Molluscs*. Oxford University Press, New York.
- 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 M. J. Weissburg. 2005.** Slow-moving predatory gastropods track prey odors in fast and turbulent flow. *J. Exp. Biol.* **208**: 809–819.
- Fredman, S. M., and B. Jahan-Parwar. 1980.** Processing of chemosensory and mechanosensory information in identifiable *Aplysia* neurons. *Comp. Biochem. Physiol.* **66A**: 25–34.
- Grasso, F. W., and J. A. Basil. 2002.** How lobsters, crayfishes, and crabs locate sources of odor: current perspectives and future directions. *Curr. Opin. Neurobiol.* **12**: 721–727.
- Greenwood, J. A., and D. Durand. 1955.** The distribution of length and components of the sum of *n* random unit vectors. *Ann. Math. Stat.* **26**: 233–246.
- Kupfermann, I., and T. J. Carew. 1974.** Behavior patterns of *Aplysia californica* in its natural environment. *Behav. Biol.* **12**: 317–337.
- Lederhendler, I. I., K. Herriges, and E. Tobach. 1977.** Taxis in *Aplysia dactylomela* (Rang, 1828) to water-borne stimuli from conspecifics. *Anim. Learn. Behav.* **5**: 355–358.
- Lee, R. M., M. R. Robbins, and R. Polovcik. 1974.** *Pleurobranchaea* behavior: food finding and other aspects of feeding. *Behav. Biol.* **12**: 297–315.
- Leonard, J. L. 1992.** Patterns of neural and behavioral activity in freely moving *Navanax inermis* (Mollusca, Opisthobranchia). *Acta Biol. Hung.* **43**: 329–342.
- Leonard, J. L., and K. Lukowiak. 1984.** An ethogram of the sea slug, *Navanax inermis* (Gastropoda, Opisthobranchia). *Z. Tierpsychol.* **65**: 327–345.
- Leonard, J. L., and K. Lukowiak. 1986.** The behavior of *Aplysia californica* Cooper (Gastropoda; Opisthobranchia). I. Ethogram. *Behaviour* **98**: 320–360.
- Levy, M., S. Blumberg, and A. J. Susswein. 1997.** The rhinophores sense pheromones regulating multiple behaviors in *Aplysia fasciata*. *Neurosci. Lett.* **225**: 113–116.

- Lohmann, K. J., and A. O. D. Willows. 1987. Lunar-modulated geomagnetic orientation by a marine mollusk. *Science* **235**: 331–334.
- Lohmann, K. J., A. O. D. Willows, and R. B. Pinter. 1991. An identifiable molluscan neuron responds to changes in earth-strength magnetic fields. *J. Exp. Biol.* **161**: 1–24.
- Murray, J. A., and A. O. D. Willows. 1996. Function of identified nerves in orientation to water flow in *Tritonia diomedea*. *J. Comp. Physiol. A* **178**: 201–209.
- Murray, J. A., R. S. Hewes, and A. O. D. Willows. 1992. Water-flow sensitive pedal neurons in *Tritonia*: role in rheotaxis. *J. Comp. Physiol. A* **171**: 373–385.
- NOAA/NOS Center for Operation Oceanographic Products and Services. 2004. NOAA/NOS/CO-OPS water level data retrieval page. [Online]. Available: http://co-ops.nos.noaa.gov/data_res.html. [18 November 2004].
- Paine, R. T. 1963. Food recognition and predation on opisthobranchs by *Navanax inermis* (Gastropoda: Opisthobranchia). *Veliger* **6**: 1–9.
- Popescu, I. R., and A. O. D. Willows. 1999. Sources of magnetic sensory input to identified neurons active during crawling in the marine mollusc *Tritonia diomedea*. *J. Exp. Biol.* **202**: 3029–3036.
- Ramos, L. J., J. L. L. Rocafort, and M. W. Miller. 1995. Behavior patterns of the aplysiid gastropod *Bursatella leachii* in its natural habitat and in the laboratory. *Neurobiol. Learn. Mem.* **63**: 246–259.
- Redondo, R. L., and J. A. Murray. 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.
- Sabelis, M. W., and P. Schippers. 1984. Variable wind directions and anemotactic strategies of searching for an odour plume. *Oecologia* **63**: 225–228.
- Susswein, A. J., M. S. Cappell, and M. V. L. Bennett. 1982. Distance chemoreception in *Navanax inermis*. *Mar. Behav. Physiol.* **8**: 231–241.
- Susswein, A. J., S. Gev, Y. Achituv, and S. Markovich. 1984. Behavioral patterns of *Aplysia fasciata* along the Mediterranean coast of Israel. *Behav. Neural Biol.* **41**: 7–22.
- Teyke, T., K. R. Weiss, and I. Kupfermann. 1992. Orientation of *Aplysia californica* to distant food sources. *J. Comp. Physiol. A* **170**: 281–289.
- 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 ed. Princeton University Press, Princeton, NJ.
- Wang, J. H., S. D. Cain, and K. J. Lohmann. 2003. Identification of magnetically responsive neurons in the marine mollusc *Tritonia diomedea*. *J. Exp. Biol.* **206**: 381–388.
- Wang, J. H., S. D. Cain, and K. J. Lohmann. 2004. Identifiable neurons inhibited by Earth-strength magnetic stimuli in the mollusc *Tritonia diomedea*. *J. Exp. Biol.* **207**: 1043–1049.
- Weissburg, M. J. 2000. The fluid dynamical context of chemosensory behavior. *Biol. Bull.* **198**: 188–202.
- Willows, A. O. D. 1978. Physiology of feeding in *Tritonia*. I. Behaviour and mechanics. *Mar. Behav. Physiol.* **5**: 115–135.
- Willows, A. O. D. 1999. Shoreward orientation involving geomagnetic cues in the nudibranch mollusc *Tritonia diomedea*. *Mar. Freshw. Behav. Physiol.* **32**: 181–192.
- Wyeth, R. C., and A. O. D. Willows. 2006a. Field behavior of the nudibranch mollusc, *Tritonia diomedea*. *Biol. Bull.* **210**: 81–96.
- Wyeth, R. C., and A. O. D. Willows. 2006b. Odours detected by rhinophores mediate orientation to flow in the nudibranch mollusc, *Tritonia diomedea*. *J. Exp. Biol.* **209**: 1441–1453.
- Zar, J. H. 1999. *Biostatistical Analysis*, 4th ed. Prentice-Hall, Upper Saddle River, NJ.
- Zimmer, R. K., and C. A. Butman. 2000. Chemical signaling processes in the marine environment. *Biol. Bull.* **198**: 168–187.