Sensory structures and neural mechanisms underlying navigational behaviours in the nudibranch mollusc *Tritonia diomedea*

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Introduction

Neuroethology links the behavioural interactions between an animal and its environment to the underlying mechanisms that constitute the neural control of behaviour. Our interest is how animals navigate through their environment, with the ultimate goal of understanding the neurophysiology of sensory systems, central processing, and motor systems that create this behaviour performed by all motile animals. Many aquatic animals navigate using a combination of flow direction and odours to travel upstream (positive rheotaxis) or downstream (negative rheotaxis) depending respectively upon cues from either attractive or aversive navigational goals (1,2). This behaviour, odour-gated rheotaxis (OGR), is the optimal strategy for most animals because flow transports the odours from the odour source, and thus finding or avoiding the odour source is best accomplished by responding to the flow. The nudibranch, *Tritonia diomedea*, uses OGR to navigate upstream in the presence of prey odour and downstream in the presence of predator odour (3,4). In addition, these sea slugs have a number of characterstics that make them amenable for the neuroethological study of navigation (5). Prior research has shown that odours are detected by *T. diomedea's* rhinophores, and that upstream turns in the absence of odours depend on flow detection by the oral veil (4,6). Our goal here is to use behavioural experiments as an initial exploration of how upstream and downstream turns required for OGR could be generated. We are testing three possibilities (see Hypotheses and Predictions).

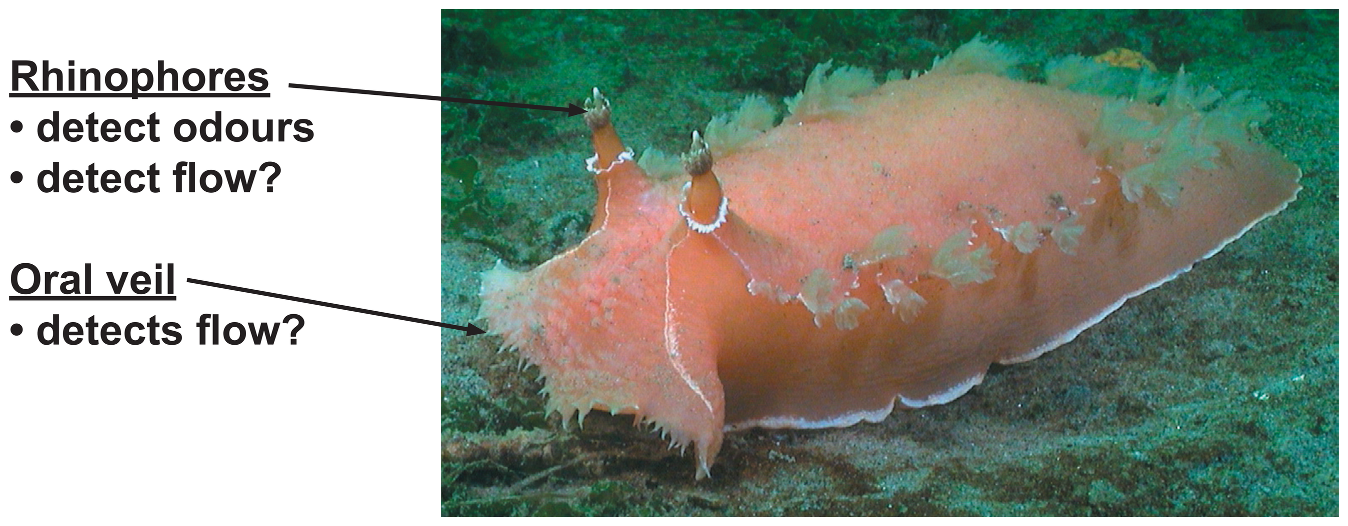

Fig 1. Primary sense organs of the nudibranch *Tritonia diomedea*.

Methods

We designed a series of treatments with different predicted results for each of our three hypotheses for how *T. diomedea* navigates. In all treatments, we applied localized streams of odour stimuli medially to one rhinophore and subsequently measured both turn angles and final headings of the animals from digital video of the slugs' responses. Slugs (n = 9) were each tested under 9 different treatment combinations (thus, 81 total trials). Treatments crossed 3 odours (control seawater, prey, and predator) with 3 rhinophore stimulation conditions (still water, in the same direction to bulk flow in a flow tank, and in opposition to bulk flow in a flow tank). Slugs in the flow tank were tested only if they were crawling across the flow, to ensure the possibility of either an upstream or downstream turn occurring. Treatment orders were randomized, and applied and analyzed blind to the odour treatment.

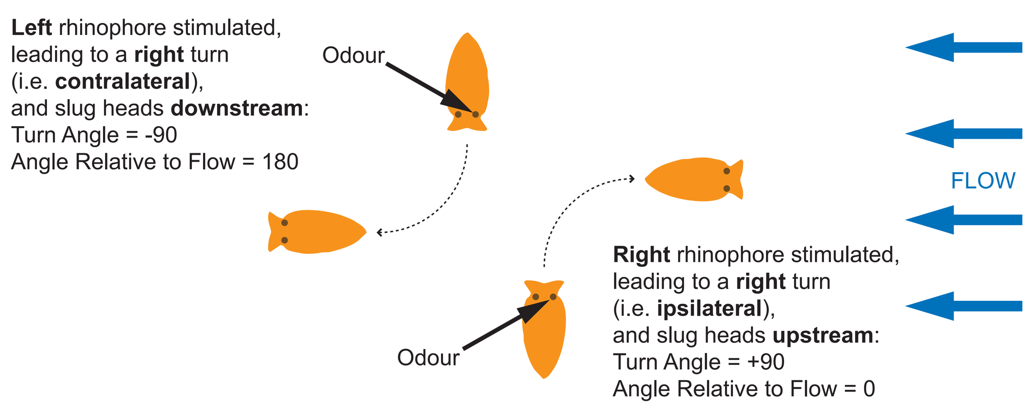

Fig 2. Schematic diagram showing measurements for slugs turning in response to odour and flow stimuli. Turn angles were measured as ipsilateral (positive values: 0° to +180°) or contralateral (negative values: 0° to -180°). Angles relative to flow were measured at the end of the turn, between upstream (0°) and downstream (180°).

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Hypotheses and Predictions

1
Reflexive turns based on odour type
turn towards prey odours and turn away from predator odours; turn direction depends on which rhinophore is stimulated

2
Turns based on odour flow and odour type
turn upstream in presence of prey odours and downstream in presence of predator odours; rhinophores detect flow and odours

3
Turns based on bulk flow and odour type
turn upstream in presence of prey odours and dowstream in presence of predator odours; oral veil detects flow; rhinophores detect odours

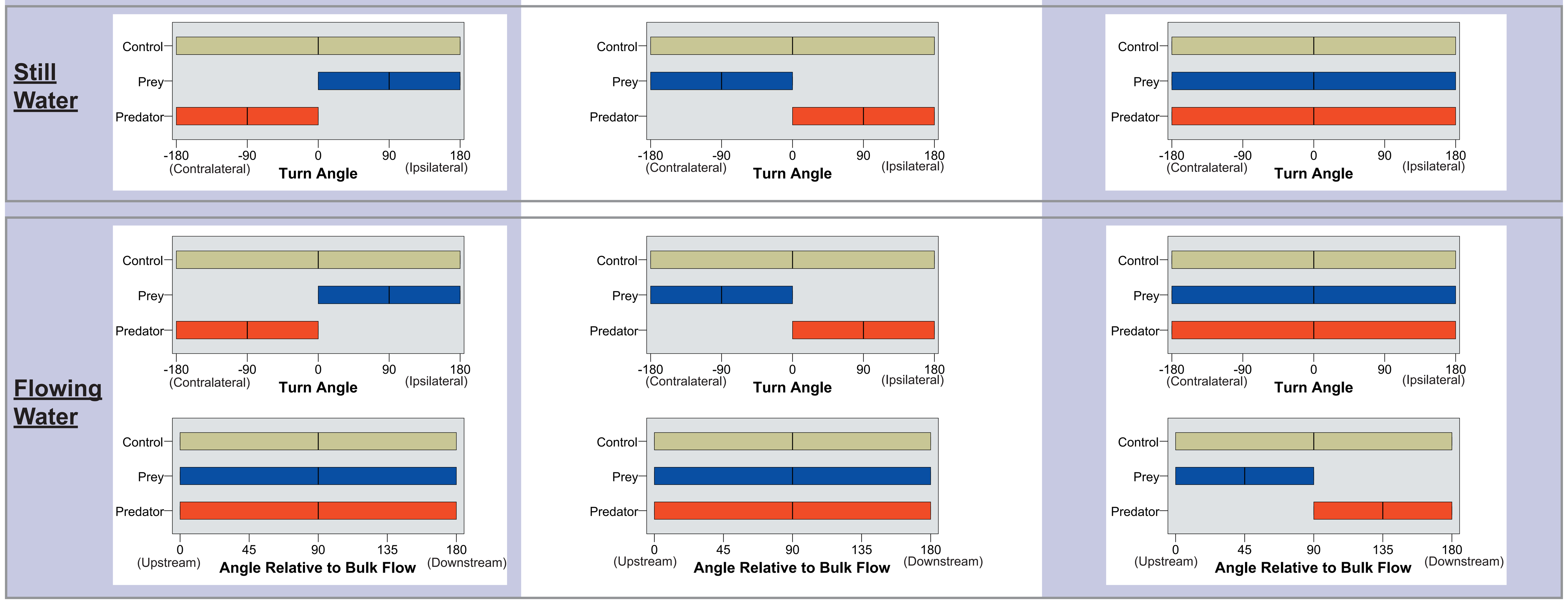

Fig 3. Three hypotheses lead to three different sets of predictions for how slugs will turn in response to odours applied in still or flowing water.

Preliminary Results

Our initial results show no significant trends that correspond to any of our hypotheses. The responses in still water suggest that a reflexive turn response is not used, however, our sample size is still small, and we have identified a problem with our stimulation protocol which will confound our results. Our attempts to stimulate just the upstream rhinophore in the flow tank often led to inadvertent stimulation of the downstream rhinophore as well. For hypotheses 1 and 2, this could lead to turns in the opposite direction than predicted. Until we solve this problem, we are drawing no conclusions based on the slight suggestion that slugs are responding most consistently with hypothesis 3.

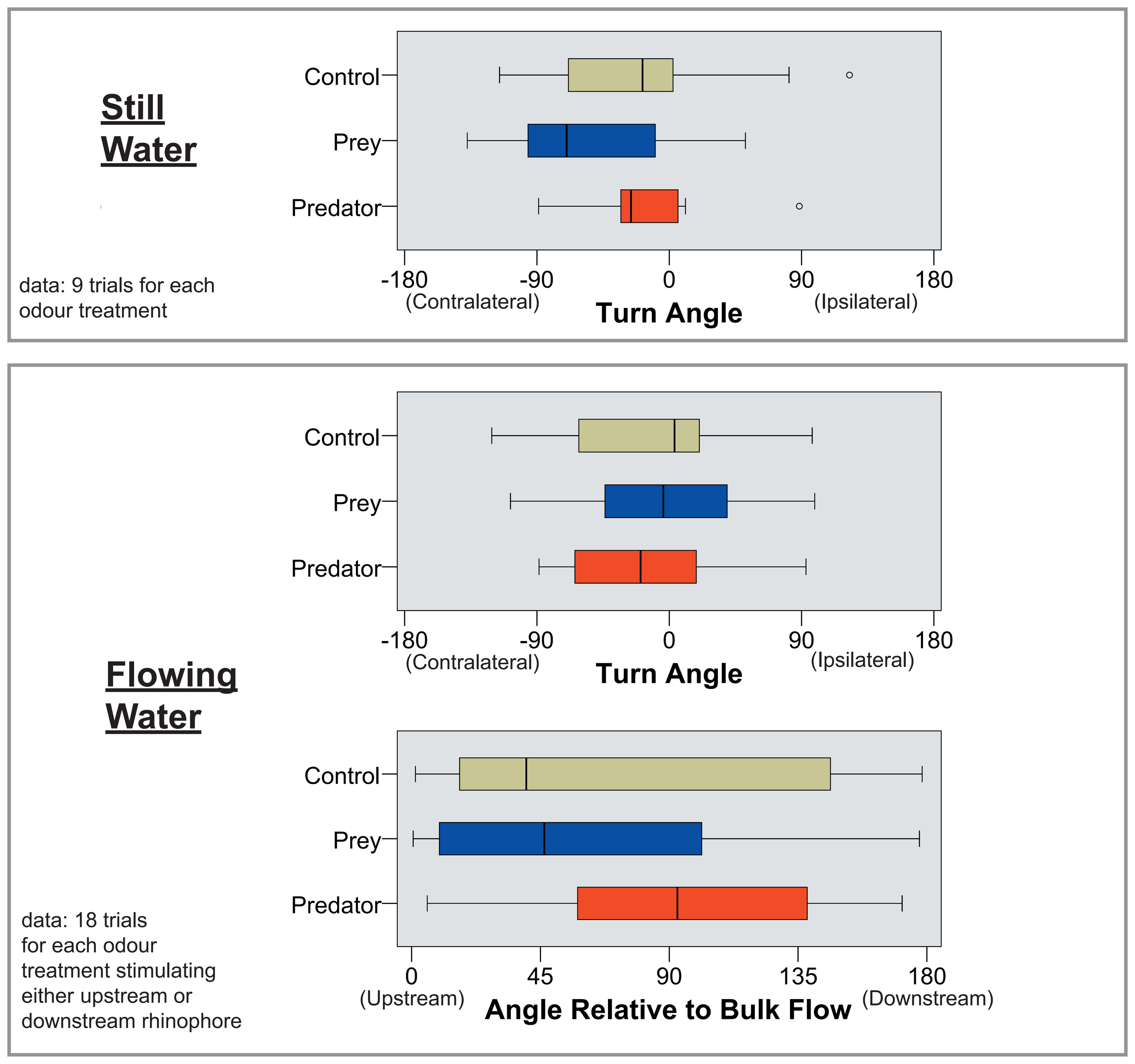

Fig 4. Turn angles in still water and both turn angles and final angles in flowing water for 9 slugs tested with control, prey, and predator odours applied medially to one rhinophore.

Future Directions & Discussion

Our immediate plan is to properly control the odour stimulation, and thus properly distinguish amongst the responses according to our predicted results. To ensure individual rhinophores are stimulated (with no stimulation of the other rhinophore), we will only stimulate the downstream rhinophore. This will require stimulation from both medial and lateral directions in the flow tank to create flow stimuli in the same direction and in opposition to bulk flow. This adds complexity to our experiment, by adding another variable (medial vs lateral stimulation), changes our predicted responses for the turn angles under hypothesis 2, and will necessitate additional analysis of turns relative to odour flow direction. We also plan to increase odour stimulus volumes, in the hopes of triggering more consistent responses.

Without further data, our expectation is still that the slugs will measure flow directions with the oral veil, and turn either upstream or downstream dependent on what odours are detected (hypothesis 3). Previous studies support this hypothesis since cutting the innervation to the lateral oral veil eliminated orientation in bulk flow (6) in the absence of odours, while removal of rhinophores disabled upstream or downstream turns in response to prey or predator odour plumes (4). However, as yet, none of the hypotheses have been explicitly refuted,, and thus we must continue to refine our methods to test all three possibilities.

Based on the future results of this study, we plan to pursue neurophysiological experiments which will expose the neural circuitry behind *T. diomedea's* navigational turns.

References

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