

## CHANGES IN ODOUR INTERMITTENCY INFLUENCE THE SUCCESS AND SEARCH BEHAVIOUR DURING ORIENTATION IN THE CRAYFISH (*ORCONECTES RUSTICUS*)

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Aquatic organisms may use different mechanisms to locate an odour source, including odour-gated rheotaxis and chemotaxis. However, the effects of spatial and temporal information contained within odour signals in guiding orientation are under debate. In nature, turbulence and plume meandering give rise to signal intermittency, which organisms must overcome to locate an odour source successfully. In this study, crayfish were presented with odour plumes formed by a continuously released jet or a pulsed jet ranging from 0.5 to 3 Hz. Crayfish oriented significantly differently in pulsed odour plumes differed significantly from than in a continuously released jet plume. In a continuous odour plumes, crayfish walked faster, had more accurate heading and turning angles, and stopped less often. The results suggest that crayfish are able to orient in a pulsed odour plumes, but that the spatial and temporal complexity of a continuous released odour plume allowed them to be more successful to find the odour source.

*Keywords:* Pulsed odour plumes; Orientation behaviour; Crayfish; *Orconectes rusticus*

### INTRODUCTION

The ability to perceive and extract relevant information from the environment is important for the survival of all animals. For many organisms, chemoreception is the most important sensory modality. Aquatic organisms use chemosensory information to localize food (Moore and Grills, 1999), mates (Ameyaw-Akumfi and Hazlett, 1975; Tierney and Dunham, 1982; Dunham and Oh, 1992), predators (Hazlett, 1989), and shelters (Tamburri *et al.*, 1996), and to convey their social status (Bushman and Atema, 1997; Karavanich and Atema, 1998; Zulandt-Schneider *et al.*, 1999).

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Typically, orientation has been classified as either a taxis or kinesis. Originally defined by Fraenkel and Gunn (1961), the exact definition of these terms has been debated throughout the literature (see Bell and Cardé, 1984; Schöne, 1984; Kennedy, 1986; Dusenbery, 1992). One of the clearest explanations of these terms to date has been by Kennedy (1986). In general terms, Kennedy delineates the two terms based on how stimulus quantities are sampled and compared. If a kinesis is the driving behaviour successive stimulus quantities are compared in time, whereas taxis is performed if stimulus quantities are sampled and compared in space then the organism. For the purpose of this article, we will adhere to these definitions. In recent years, work on chemical orientation has focused more precisely on the role that stimulus distributions play in guiding search behaviour. This has been achieved by a better understanding of the stimulus distribution or even direct or proxy measurements of stimuli in experimental tanks (Murlis and Jones, 1981; Moore *et al.*, 1989; Moore and Atema, 1991). Studies on the orientation behaviour of terrestrial animals have led to the idea of an odour-modulated optomotor anemotaxis (Vickers and Baker, 1992; Mafra-Neto and Cardé, 1994; Vickers, 2000). In this type of behaviour, male moths are determining steering direction based on the local wind direction. Thus, the term anemotaxis is applied. The motor output of the moth (or flying speed) is controlled by visual flow field information and thus, the behaviour is termed optomotor. Finally, the presence and absence of odour filaments “switches” the moth between two types of flying behaviour. When an odour filament is sensed, the moth surges directly upwind. In the absence of odour, the moth performs a series of counter-turns in order to locate the next filament of pheromone (Vickers and Baker, 1992; Mafra-Neto and Cardé, 1994; Vickers, 2000). In a series of elegant experiments, Vickers and Baker (1994) showed that the intermittency of odour signals is ultimately driving the search strategy. When the odour intermittency matches the adaptation and disadaptation rate of the moth’s nervous system, successful searches are performed. At higher or lower intermittencies, moths are unable to locate the odour source effectively.

In the aquatic habitats, experiments on lobsters, blue crabs, and crayfish seem to point to two different types of mechanisms. Lobsters and crayfish use the spatio-temporal distribution of odours to regulate both walking speed and turning angles (Devine and Atema, 1982; Moore *et al.*, 1991; Moore and Grills, 1999). When deprived of bilateral information crayfish are not able to locate odour sources (Kraus-Epley and Moore, 2002); lobster also make bilateral comparisons when making directional decisions (Reeder and Ache, 1980; Devine and Atema, 1982). This would indicate that a taxis-type of mechanism is in place. Changes in the temporal structure of odour information alter orientation behaviour, which would indicate that the temporal information is also important for some aspects of the orientation behaviour (Moore and Grills, 1999; Keller *et al.*, 2001). By increasing the temporal complexity of the odour signals through decreasing intermittency, studies have shown that crayfish are more successful and locate the odour source faster (Moore and Grills, 1999; Keller *et al.*, 2001; Wolf *et al.*, in review). Thus, it appears, as with moths, that intermittency is a critical piece of information for benthic decapods during chemical orientation.

In blue crabs, changes in flow velocity inhibit the ability of animals to locate odour sources, leading to the conclusion that crabs are using an odour-gated rheotaxis (Weissburg and Zimmer-Faust, 1994; Finelli *et al.*, 1999). Changes in flow velocity

have the capability to alter the intermittency in odour signals, although recent modelling work has shown that other mechanisms can explain the behaviour exhibited in those experiments (Weissburg and Dusenbery, 2002). Regardless of the exact orientation mechanism in place for lobsters, blue crabs, or crayfish, it is clear that the temporal information in odour plumes can influence orientation behaviour.

To understand how temporal information in odour plumes can influence orientation behaviour, it is important to understand how this information is structured by the physical environment. At size scales of less than 10 mm, molecular diffusion determines the distribution of odour within the environment. At larger scales, fluid flow dominates, and turbulent dispersal processes give rise to a patchy odour signal, even when the signal is continuously released (Moore and Atema, 1991). Therefore, an animal located downstream of an odour source experiences patches of odour significantly above and below the mean concentration. In highly turbulent environments, odour patches are smaller and occur more frequently than in environments with lower turbulence (Moore *et al.*, 2000). This leads to shorter periods of intermittency, steeper stimulus onsets, and larger odour peak heights. In contrast, environments with lower turbulence have smaller odour patches resulting in longer periods of intermittency, shallower onset slopes, and smaller peak heights (Moore *et al.*, 2000). To overcome this, animals must employ effective strategies that will allow them to extract relevant information from the spatial and temporal characteristics of the odour patches, including source distance, source direction, and size.

Results from crayfish, lobsters, and blue crabs show that the temporal aspects of odour plumes are important for certain orientation behaviours, but there has not been any systematic study of the impact of intermittency on the ability of benthic crustaceans to orient. We hypothesize that, as in moths, changes in intermittency will alter the temporal aspects of orientation behaviour, and in particular, increasing intermittency will result in more successful searches and higher walking speeds. We hypothesize this because past results have shown that movement up-current and search strategies are often correlated by the temporal aspects in turbulent odour plumes. Thus, to investigate the role of intermittency in aquatic orientation, we examined the ability of the crayfish, *Orconectes rusticus*, to orient in pulsed odour plumes of frequencies ranging from 0.5 to 3 Hz. Turbulent odour plumes in nature consist of a large range of odour frequencies. By presenting controlled intermittencies through changing odour pulse frequency, we hope to provide some insight into the role that intermittency plays in regulating orientation behaviour.

## MATERIALS AND METHODS

### Animals

Female crayfish, *Orconectes rusticus*, were caught in the Portage River, located near Bowling Green, Ohio. Experimental animals were kept in communal tanks with an average water temperature of 20°C and on a L:D cycle of 14:10 h for at least 3 weeks. Each crayfish was placed on a reduced diet to increase its attraction to the odour source, and was fed approximately 2 g of food (Commercial rabbit chow). Each animal was weighed, and its carapace length was measured. A piece of reflective tape was placed on the carapace of the crayfish to aid in motion analysis. The average

length of the animals was  $3.1 \pm 0.1$  cm, and the average weight was  $10.7 \pm 3.5$  g. Each crayfish was tested only once, and 10 animals were used in each treatment.

### Experimental Trials

All trials were conducted in a 760 L recirculating flume (complete measurements: 200 cm  $\times$  62 cm  $\times$  52 cm, working section: 104 cm  $\times$  53 cm  $\times$  27 cm). The flume was constructed using clear Plexiglas sheets for the sides of the chamber and the frame was constructed of wood beams. Two sheets of fluorescent light grating (egg crates, 169 mm<sup>2</sup> holes) wrapped with plastic screen (1 mm<sup>2</sup> holes) were placed upstream and one placed downstream to serve as collimators. The average flow speed was  $5.0 \pm 0.5$  cm/s as measured in the middle of the flume with a Marsh-McBirney<sup>®</sup> Model 2000 Portable Flow Meter. The air-powered motor (Eclipse Systems, Inc.<sup>®</sup>, model # 9-4300-14A) was allowed to run for at least one hour before the first acclimation period. The bottom of the flume was lined with a thin layer of gravel (approximately 1 cm deep). Gravel was chosen as the substrate because crayfish perform better with a rough surface for walking and have the highest success for orientation on a gravel substrate (Moore and Grills, 1999).

Before the start of each trial, the crayfish was allowed to acclimate in the flume for 20 min. After the acclimation period, the crayfish was placed in a shelter (18 cm  $\times$  10 cm  $\times$  7.5 cm PVC pipe) approximately 1.5 m downstream from the source. After 5 min the shelter was opened, and the crayfish was exposed to one of six odour plume conditions, odour continuously released or pulsed at 0.5, 0.7, 1, 2, or 3 Hz. The flume was re-filled after 3 trials to avoid stimulus background build-up.

Odour was prepared of frozen haddock ( $50.4 \pm 0.24$  g/L of tap water), a very attractive odour for crayfish. Thawed haddock was blended and strained through a steel filter (250  $\mu$ m). Stimulus was gravity fed through a glass pipette (3 mm I.D.) positioned 5 cm above the bottom of the flume and 80 cm from the shelter. Odour was pulsed by a small valve (General valve 3-111-900) driven by a Grass<sup>®</sup> stimulator unit. Continuously released odour was gravity fed at 2 mL/min. Each trial began when the shelter was opened and ended when the animal either came within one carapace length of the pipette tip, or after 15 min had passed. All trials were videotaped from above and recorded using a Panasonic VCR (AG-1980).

### Data Analysis

Paths of the animal were digitized at 1 Hz using the Peak Motus Motion Analysis System<sup>®</sup>. From these paths, walking speeds, heading and turning angles were calculated (Moore *et al.*, 1991; Moore and Grills, 1999). Only those animals that successfully found the odour source in the allotted time were used in subsequent analysis. The success rate was  $n = 5$  for 0.5 Hz,  $n = 7$  for 0.7 Hz, and  $n = 10$  for all other frequencies as well as the continuous odour source. Walking speed was defined as the distance travelled per second. Heading angles were defined as the angle between a path taken and the direction toward the source. Turning angles were measured as the change in direction, i.e., the angle between the path connecting the previous position to the present and the next position. Overall differences between treatments were determined using an ANOVA (Statistica<sup>®</sup>), and Tukey HSD post-hoc test. Percent success was analyzed using a multiple comparisons for proportions contingency table ( $q_{0.05, \infty, 4} = 3.633$ )

that allows for testing analogous to the Tukey or Student–Newman–Keuls tests (Zar, 1999).

### Chemical Stimulus Profile

An In Vivo Electrochemistry Computer System (IVEC-10; Medical Systems, Greenvale, NY) was used to quantify the characteristics of plumes formed by pulsed and continuously released odour at a small spatial and a high temporal resolution (Moore *et al.*, 1989, 1991). Electrodes consisting of a 30  $\mu\text{m}$  carbon fibre measured encounters of the tracer on the electrode's surface per unit time. Recordings were made at a sampling rate of 10 Hz. Electrodes were calibrated in solutions of dopamine and exhibited a high linearity over a concentration range of 0.5–100  $\mu\text{mol/L}$  (correlation coefficient;  $r^2 > 0.97$ ). Chemical signals for each of the treatments were measured 5 cm above the substrate and 22 cm from the odour source. This distance was chosen as 25% of the distance between odour source and average start location from all of the orientation paths. The stimulus consisted of 0.4 mM dopamine (tracer), 0.05 mM ascorbic acid (anti-oxidant), and 0.05 mM fluorescein dye in aged tap water. Fluorescein was used to visualise the stimulus which was released into the flume in the same manner as the odour in orientation trials. All trials were recorded for one minute, and at least two minutes separated each recording. The stimulus profiles were analysed using an in-house QBASIC program. Odour patches in space translate into tracer peaks in time. The signal parameters most relevant to orientation trials were peak height, maximum slope, absolute slope, length of the signal, and intermittency (Moore and Atema, 1991). A general MANOVA analysed differences between the mean values of the odour-plume parameters, and a Tukey post-hoc test determine differences among the parameters.

## RESULTS

### Orientation Behaviour

Overall, crayfish were significantly less successful finding the odour source in plumes pulsed at low rates than at higher rates, or with continuous release (Fig. 1). Crayfish

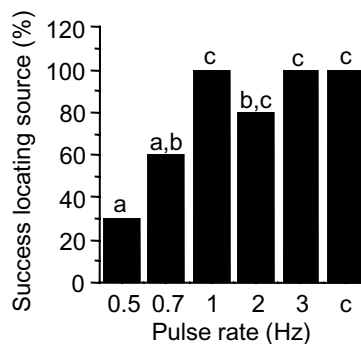


FIGURE 1 Percentage of crayfish tested that came within one body length of the odour source under all plume conditions. Different letters indicate a significant difference using a Tukey proportions test ( $P < 0.05$ ).

in plumes pulsed at 0.5 Hz found the source significantly less often than continuous plumes or plumes pulsed at 1, 2 and 3 Hz. Qualitatively the orientation paths for animals that successfully found the odour source looked similar regardless of odour pulse rate (Fig. 2).

Crayfish walked slower in odour plumes pulsed at low frequencies when compared with odour pulsed at high frequencies or continuously released plumes (Fig. 4). Specifically, when compared to crayfish orienting in continuously released plumes, the average walking speed of crayfish was significantly slower in all pulsed odour plumes except for 3 Hz (Tukey HSD test,  $P < 0.05$ ).

Crayfish spent more time stopped at pulse rates lower than 2 Hz (Fig. 3). In addition, animals stopped farther away and closer to the source (Fig. 4). In the middle section, the time stopped was quite low (20–60 cm away from the source; Fig. 5). However, at pulse rates of 0.5 and 0.7 Hz, stops near the odour source were less obvious. The results for time spent stopped was mirrored by number of stops (not shown). Thus, the distributions and values were due to a greater number of periods where no movement took place, and are not due to increased periods of no movement.

Differences were also found in the average heading angle towards the odour source, a measure of the accuracy of orientation (Fig. 6). Specifically, crayfish orienting in

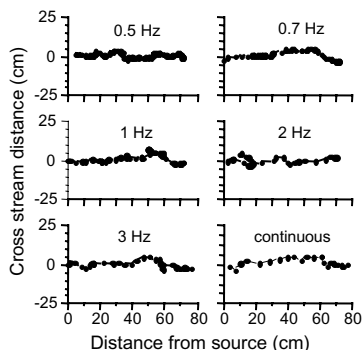


FIGURE 2 Examples of orientation paths of crayfish under all plume conditions ( $c$  = continuous release). Each orientation path was digitized at 1 Hz. The odour source was located at 0, 0 cm, and the shelter was located at 80, 0 cm. The first point on the right indicates the crayfish emerging from the shelter.

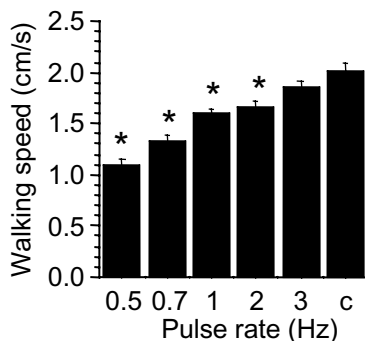


FIGURE 3 Average walking speed ( $\pm$  SE) ( $N=261$  for 0.5 Hz,  $N=379$  for 0.7 Hz,  $N=879$  for 1 Hz,  $N=748$  for 2 Hz,  $N=654$  for 3 Hz,  $N=441$  for continuous plume). Asterisks indicates significant differences between pulsed odour plumes compared to continuous release ( $c$ ) (Tukey HSD test,  $P < 0.05$ ).

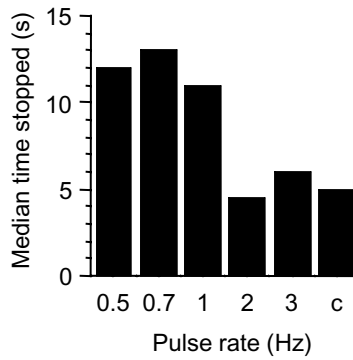


FIGURE 4 Median time crayfish stopped while locating the odour source ( $N=3$  for 0.5 Hz,  $N=6$  for 0.7 Hz,  $N=10$  for 1 Hz,  $N=8$  for 2 Hz,  $N=10$  for 3 Hz,  $N=10$  for continuous release).

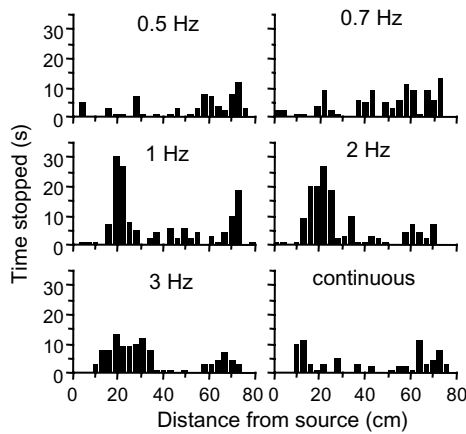


FIGURE 5 Distance at which crayfish stopped while locating the odour source. Number of stops was counted in 3 s intervals. The odour source was located at 0, 0 cm, and the shelter was located at 80, 0 cm.

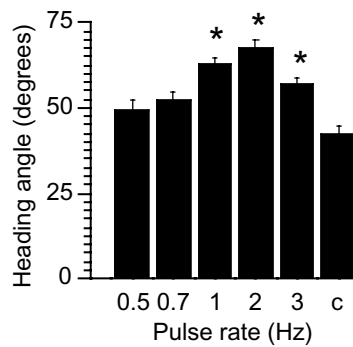


FIGURE 6 Average heading angle towards source ( $\pm$  SE) ( $N=255$  for 0.5 Hz,  $N=378$  for 0.7 Hz,  $N=874$  for 1 Hz,  $N=743$  for 2 Hz,  $N=641$  for 3 Hz,  $N=439$  for continuous plume). Asterisks indicate significant differences between pulsed odour plumes compared to continuous release (c) (Tukey HSD test,  $P < 0.05$ ).

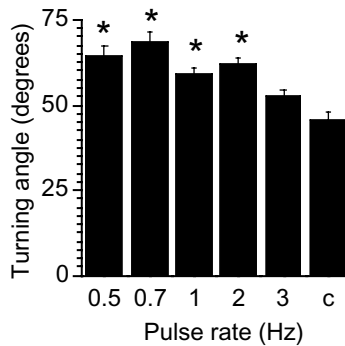


FIGURE 7 Average turning angle ( $\pm$ SE) ( $N=248$  for 0.5 Hz,  $N=372$  for 0.7 Hz,  $N=863$  for 1 Hz,  $N=731$  for 2 Hz,  $N=621$  for 3 Hz,  $N=427$  for continuous release). Asterisks indicate significant differences between pulsed odour plumes compared to continuous release (c) (Tukey HSD test,  $P < 0.05$ ).

plumes formed by odour pulsed at 1, 2 and 3 Hz had significantly larger heading angles than animals in continuous odour plumes (Tukey HSD test,  $P < 0.05$ ). There were no significant differences between the other pulse treatment and the continuous treatment (Tukey HSD test,  $P > 0.05$ ).

Crayfish showed larger turning angles at all pulse rates below 3 Hz (Tukey HSD test,  $P > 0.05$ ). Turning angles in continuous odour plumes were not different from odour plumes pulsed at 3 Hz (Fig. 7).

Overall, these results show that crayfish tended to take longer, less direct paths, and walked slower, when orienting in pulsed odour plumes. Furthermore, they were less successful at locating the source at pulse rates less than 1 Hz, and had larger heading and turning angles when compared to animals orienting in continuous released odour plumes.

### Stimulus Parameters

Visualisation of the odour plumes with dye showed differences between pulsed and continuous plumes. Filaments in the continuous plume were easily visible at the downstream end of the flume and were a result of the interaction between the dynamics of odour release and turbulent conditions. Filaments in plumes of odour pulsed at 1, 2 and 3 Hz were actually in patches that were caused by the pulsed delivery. Fewer dye patches were visible downstream in the two low frequency conditions (0.5, 0.7 Hz).

As in other measurements (Moore *et al.*, 2000) the odour profiles consisted of a number of patches (peaks) separated by periods of no signal. The number of peaks was highly variable between the continuously released and pulsed odour. The number of peaks counted in 60 s was highest at lower frequencies (0.5, 0.7 Hz), and continuous release, but decreased at 2 or 3 Hz delivery rate. The average intermittency, or time of no signal, was significantly longer at 0.5 Hz compared with both the 3 Hz and the continuous signal (Fig. 8) (Tukey HSD test,  $P < 0.05$ ).

Pulse lengths were significantly shorter at 0.5, 0.7 and 1 Hz, than pulse lengths in the continuous signal, and at 3 Hz pulses were significantly longer than in the continuous signal (Tukey HSD test,  $P < 0.05$ ). Additionally, the 2 Hz, 3 Hz, and continuous signals had significantly longer pulses than the 0.5, 0.7 and 1 Hz treatments (Tukey

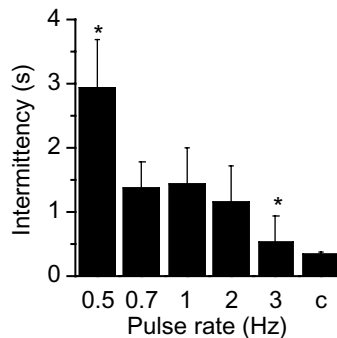


FIGURE 8 Effect of pulse rate on the average intermittency of odour peaks under all plume conditions (*c* = continuous release). Asterisks indicate significant differences between pulsed odour plumes compared to continuous release (Tukey HSD test,  $P < 0.05$ ).

HSD test,  $P < 0.05$ ). The average maximum height of the odour signals in all of the pulsed treatments, except at 3 Hz, were significantly smaller than those of the continuous signal (Tukey HSD test,  $P < 0.05$ ). The rise time (measured from threshold to maximum pulse height) was significantly shorter at 0.5, 0.7 and 1 Hz than at 3 Hz (Tukey HSD test,  $P < 0.05$ ). None of the pulsed signals was significantly different from the continuous signal.

These results illustrate the heterogeneous nature of the odour signal in a turbulent flow. The pulsed signals contained shorter, smaller peaks, and rise times than the continuous signal. These differences were more pronounced in the signals pulsed at lower frequencies than at higher frequencies signals.

## DISCUSSION

First, the temporal aspects of crayfish search strategy are altered. Since walking speed increased and time spent stopping decreased, it appears as if the increased odour frequency stimulated walking speed. These results are consistent with the theory that locomotion toward the source is stimulated by the presence of an odour filament. This is similar to the odour-modulated optomotor anemotaxis in some male moths where the presence of a pheromone filament stimulates an upwind surge (Vickers, 2000). Although crayfish do not surge up-current for large distances or increase walking speeds, it does appear that continued movement toward an odour source is stimulated by the presence of odour filaments.

Second, the spatial aspects of the crayfish search strategy are altered by increasing odour pulse frequencies. Heading angles are largest (and least accurate) at high odour pulse frequencies compared to intermediate pulse frequencies. Turning angles are decreased as pulse frequencies increase. Altogether, it becomes apparent that the presence or absence of odour filaments is guiding the spatial aspects of the crayfish search strategy. This result stands in strong contrast to the findings in moths and blue crabs: changes in crayfish orientation are most likely a result of increasing intermittency in odour plumes formed by low frequency pulses and indicates that the temporal information within plumes is necessary for some aspects of the orientation strategy used by crayfish.

By using IVEC-10 to quantify the chemical signal, we were able to measure the intermittency in each plume condition. The most striking feature of our odour plumes is a change in odour signal intermittency as a function of increased odour frequency. Intermittency was greatest in low frequency plumes, and decreased with an increase in odour pulse frequency. Combining the quantification of sensory signals in our behavioural trials with the detailed analysis of the behavioural results suggests that crayfish are extracting information about intermittency from odour plumes and using this information to guide their orientation behaviour.

### **Possible Search Strategy**

As outlined in detail in the Introduction, several different behavioural mechanisms have been proposed for chemical orientation to an odour source. It is important to note that moths operate in a three-dimensional world in regard to orientation to pheromone sources. By flying, moths lose contact with a substrate that would provide absolute information regarding the direction and strength of ambient wind currents. Thus, it becomes necessary for moths to use visual flow-field information to correct for drift caused by ambient wind conditions. Conversely, crayfish, lobsters, and blue crabs have contact with the substrate and have absolute measures of current direction and strength.

In odour-gated optomotor anemotaxis, temporal information contained within an odour signal, coupled with visual information, controls upwind flight behaviour (Murlis *et al.*, 1992; Vickers and Baker, 1994; Vickers, 2000). When locating a female that is releasing pheromone, a male moth in contact with an odour filament will surge briefly upwind. Loss of contact the odour filament causes the moth to halt upwind flight and will fly to cast horizontally (zigzag) until another filament is located (Willis and Baker, 1984; Mafra-Neto and Cardé, 1995; Vickers and Baker, 1992, 1996; Vickers, 2000). Visual flow-field information is essential to control flight direction and velocity. A change in the visual flow-field will often result in changes in the upwind flight of the moth. In turbulent, continuously released or pulsed odour plumes, male moths significantly altered their tracking behaviour. They flew faster, turned less frequently, exhibited smaller heading angles, and flew straighter upwind than those in continuous narrow plumes or slowly pulsed plumes. In addition, male moths (*Cadra cautella*) are able to locate a pheromone source pulsed at a high frequency more efficiently than a continuously released odour source, i.e., flew straighter upwind, zigzagged less frequently, and were quicker to initiate walking, wing fanning, and flight behaviour than moths in both continuously released and pulsed odour plumes (Kennedy, 1983; Mafra-Neto and Cardé, 1995). Therefore, the intermittency of an odour plume appears to control the behavioural programme that is executed by the moth: surge or cast, but does not control the spatially specific behavioural output such as flight direction (Mafro-Neto and Cardé, 1994; 1995). The spatial aspects of the moths behaviour is controlled by both visual and mechanical information.

The American lobster (*Homarus americanus*) orients differently in pulsed odour in comparison with moths. Like moths, the percentage of lobsters locating an odour source decreased in plumes formed by odour pulsed at low frequencies compared to higher frequencies of release. While animals in continuous and pulsed plumes had similar heading and turning angles, mean walking speed and path linearity decreased with

an increase in signal intermittency (Kozłowski *et al.*, 2001). These results are similar to the orientation behaviour of crayfish as observed in this study.

In general, the orientation paths of crayfish can be divided into three phases: initial search phase, tracking phase, and final approach, which is similar to lobster tracking behaviour (Moore and Atema, 1991). In this study, these phases are most obvious under continuously released plume conditions. Animals stopped far from the odour source (60–80 cm, near the shelter), stopped less during the tracking phase (20–60 cm), and stopped more frequently near the source (0–20 cm). Crayfish seem to stop more frequently during the tracking phase at 0.7 Hz. This might indicate that the information contained within the temporal plume structure is not sufficient for orientation and the animals have to sample more often while not moving. Furthermore, at these lower pulse rates, the animals stopped less often near the odour source which might indicate that the signal strength appears lower than at higher pulse rates.

In this study, the majority of crayfish located the odour source, suggesting that they need a minimum plume signal encounter rate near 1 Hz or co-pulsed at higher rates to successfully complete tracking behaviour. Similarly, moths (*Heliothis virescens*) must encounter receive odour filaments at pulse frequencies of 4 Hz and above to sustain upwind flight, and to locate the source (Vickers and Baker, 1992). Crayfish in high-frequency pulsed plumes are able to locate odour sources, but did so less successfully than in continuously released plumes. Our findings imply that a multitude of intermitencies are needed for the crayfish to locate an odour source more efficiently in contrast to previous work on insects. This range of intermitencies is what we would define as increased temporal complexity of the odour signal. Other work on crayfish support the hypothesis that increased complexity actually increases the ability of crayfish to locate odour sources (Keller *et al.*, 2001; Wolf *et al.*, in review). Using the terminology outlined by Kennedy (1983), crayfish appear to be using a combined taxis and kinesis mechanism. The temporal information of intermitency is controlling the temporal aspects of orientation (walking speed, time spent moving). Kennedy has termed this as orthokinesis. Unlike a classic kinesis where the organism has a self-steering component, the turning and heading angles of the crayfish are also influenced by bilateral information and intermitency. This would indicate a tropotaxis, where the turns are controlled by a spatial gradient of some aspect of the odour signal. We are proposing that crayfish are using these two mechanisms and that both mechanisms are influenced strongly by intermitency in an odour signal.

### **Neural Mechanisms of Temporal Information Encoding**

Neurophysiological studies on lobsters indicate that olfactory chemoreceptor cells have a temporal resolution, or flicker-fusion frequency between 1 and 5 Hz (Gomez *et al.*, 1999). At 1 Hz, lobster chemoreceptor cells discriminate odour pulses less efficiently and intensity discrimination begins to disappear. At 2 Hz, all but the first responses are indistinguishable (Gomez *et al.*, 1999). This study indicates that olfactory receptor cells have different rates of adaptation and disadaptation, and that these rates can help to encode different aspects of the temporal information in odour signals.

Neural models of olfactory cell function show that different rates of adaptation and disadaptation will allow receptor cells to become temporal filters for incoming dynamic signals. Different time constants of receptor cells result in a concentration-frequency specific response profile (Moore and Shao, 2000). Receptor cells with smaller

adaptation and disadaptation time constants will resolve shorter periods of stimulation, and are able to follow higher frequency stimulation. Conversely, receptor cells with larger adaptation and disadaptation time constants will respond greater to a lower frequency of odour stimulation. This type of signal analysis may be important for crayfish that use the temporal aspects of odour signals to orient to odour sources.

### **Stimulus Parameter**

The ability of crayfish to orient to turbulent odour plumes is influenced by altering the pulsatile nature of the chemical signal. Crayfish find the odour source more often when the frequency of odour pulses is increased. In general, as the frequency of odour pulses increased, crayfish walked faster, spent less time stopping, and had smaller turn angles. It appears as if two different aspects of the crayfish's search strategy are altered by the frequency of odour pulses.

### **Summary**

Odours in a turbulent environment are patchy in nature and have a dynamic range of temporal information within the odour signals. Our study demonstrates that crayfish use this temporal information, and in particular the intermittency, to control both the spatial (turning and heading angles) and the temporal (walking speed) aspects of their search strategy. Thus, crayfish are using a combination of a kinesis and taxis mechanism to effectively orient in their environment. The results presented here differ from moth studies which show that moths use the temporal information in odour signals to control which motor output programs are used and those in blue crabs which use the temporal information to initiate an up-current movement. These results are also different from lobsters which appear to be using a classical taxis behaviour where all aspects of movement are guided by the spatial distribution of turbulent odour plumes. One possible explanation why crayfish are using a different mechanism compared to other benthic crustaceans is that they are often found in habitats with uni-directional flow (streams) as opposed to the habitats of moths, lobsters, and blue crabs which are only periodically uni-directional. Further work needs to investigate the role of habitat differences in orientation mechanisms and the neural mechanisms of encoding the temporal information that is guiding behaviour.

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