Spatial, but not temporal, aspects of orientation are controlled by the fine-scale distribution of chemical cues in turbulent odor plumes

Paul A. Moore\(^1,\)*, David Edwards\(^1\), Ana Jurcak-Detter\(^2\) and Sara Lahman\(^3\)

**ABSTRACT**

Orientation within turbulent odor plumes occurs across a vast range of spatial and temporal scales. From salmon homing across featureless oceans to microbes forming reproductive spores, the extraction of spatial and temporal information from chemical cues is a common sensory phenomenon. Yet, given the difficulty of quantifying chemical cues at the spatial and temporal scales used by organisms, discovering what aspects of chemical cues control orientation behavior has remained elusive. In this study, we placed electrochemical sensors on the carapace of orienting crayfish and measured, with fast temporal rates and small spatial scales, the concentration fluctuations arriving at the olfactory appendages during orientation. Our results show that the spatial aspects of orientation (turning and heading angles) are controlled by the temporal aspects of odor cues.

**KEY WORDS:** Chemical orientation, Crustacean, Odor plume, Strategy

**INTRODUCTION**

Organisms from a large range of taxa utilize chemical cues to extract meaningful information about different aspects of an environment at both spatial and temporal scales. At the smallest scales, chemical signals can cause single-celled organisms to gather and create a super organism during reproduction (Bobek et al., 2017). At the largest scales, Arctic terns and salmon perform large-scale orientation using the spatial and temporal distribution of cues to find food or return to natal streams (Nevitt et al., 2008; Ueda, 2014). Across these extreme scales, the singular task of extracting meaningful spatial and temporal information from chemical signals is unique within the field of sensory ecology. Unlike visual, auditory, magnetic or thermal cues, the mechanism of dispersion of the cue across habitats is disconnected from the stimulatory properties of the cue. For example, blue and red light are affected differentially by scattering (blue) and absorption (red) (Cronin et al., 2014). In a similar fashion, high- and low-frequency sounds reflect off surfaces differently (Denny, 1993). Chemical cues are primarily dispersed by the mechanical movement of air or water. Exactly how these chemicals are moved through an environment depends on the spatial and temporal scales involved (Webster and Weissburg, 2001; Moore and Crimalli, 2004; Weissburg, 2010).

For organisms that move and orient at larger spatial scales (>1 cm), the primary mechanism that disperses chemical signals is bulk flow, and, in natural habitats, that bulk flow is likely to be turbulent in nature. Although diffusion primarily determines chemical cue distribution at smaller space and longer time scales, most macroscopic organisms are faced with turbulent odor plumes as their chemosensory landscape. Because turbulence is a chaotic process (Ottino, 1990), the resulting chemical cue is perceived as a series of fluctuating signals (Moore and Atema, 1991; Weissburg, 2010). In addition, the spatial distribution of a chemical cue within a landscape is perceived as temporal patches or pulses of varying concentrations (Webster and Weissburg, 2001). These fluctuations have a number of different components that have been quantified and reported throughout the literature (Moore and Atema, 1991; Moore et al., 1992; Webster and Weissburg, 2001; Vickers, 2006). A single plume can consist of odor patches (a temporal period in which the concentration is above background levels), as well as periods in which the concentration component of the cue is beneath background (Moore and Atema, 1991). Intermittency is the time between patches (Murlis, 1997). Even within a single patch, the concentration fluctuations can be divided into patch length, concentration level of various peaks within a patch, the rising slope of concentration changes and even different frequency components (Moore and Atema, 1991). From an engineering or signal processing point of view, there are a large number of components within a turbulent odor plume that can provide directional cues to orienting animals (Webster and Weissburg, 2001; Hayes et al., 2002).

Despite this seemingly daunting task, animals use turbulent odor plumes successfully across different physical habitats for homing, mating and foraging, demonstrating the ability to extract meaningful information (Weissburg, 2010). Moths, flying upwind in pheromone plumes, use visual cues, odor concentrations and the timing between odor patches to sustain a search strategy (Vickers, 2000; Cardé, 2016). In a process known as optimotor anemotaxis, they use the temporal nature of pulses combined with optical flow to sustain the upwind flying behavior (Vickers and Baker, 1994). The presence or absence of odor patches and their concentration determine a zig-zag pattern if the temporal pattern of pulses arrives slower than 300 ms (Vickers and Baker, 1996). A similar strategy has been found within blue crabs; temporal components of the odor plume seem to stimulate the walking speed of the animal, and the concentration components determine the spatial aspects (turning and heading angles) of the orientation behavior (Page et al., 2011a,b). Applying a slightly different strategy, crayfish and clawed lobsters do not exhibit the characteristic zig-zag pattern of insects (Moore et al., 1991b; Grasso and Basil, 2002; Moore et al., 2015). It appears as if these two crustaceans use the temporal components of the odor plume (intermittency, concentration slopes or frequency components) to determine distances from odor sources and some yet
unknown concentration components to control the spatial aspects of their orientation strategy (Moore et al., 2015). All this work clearly shows that the spatial aspects of orientation strategies (e.g. turning and heading) are more influenced by different informational components of the turbulent odor plume than are the temporal aspects (e.g. walking speed, movement up wind or up current) (Cardé, 2016; Grasso and Basil, 2002; Weissburg, 2010).

In between this input (odor plume) and output (behavior) are two other aspects concerning the processes by which animals extract information. First, and unique to the chemical signals, many of these animals actively sample their chemosensory landscape (Koehl et al., 2001). The most prominent example in the terrestrial realm is the sniffing performed by vertebrates (Zhao et al., 2006). In the aquatic environment, fish also create currents that carry odor molecules to receptors (Neivitt, 1991). Within crustaceans, antennular flicks create periodic samples of odor plumes (Koehl, 2006). Both the periodic nature of the flicking and the local distribution of fluid mechanics reshape the concentration and timing of odor molecules that arrive at the receptor cells (Moore et al., 1991a; Reidenbach and Koehl, 2011; Pravin et al., 2012). This periodic sampling is further processed for concentration and temporal information by olfactory cells and neural circuits designed to extract both pieces of information (Gomez and Atema, 1996; Gomez et al., 1999; Nagel and Wilson, 2011). Although the research on the temporal coding of odor cues has lagged behind the work on mixture detection, a number of studies have demonstrated that both terrestrial and aquatic organism have chemosensory systems sensitive to the temporal nature of chemical cues (Gomez and Atema, 1996; Gomez et al., 1999). Using this neural work as a basis, neural models have shown that the encoding of temporal information in real turbulent odor plumes can provide meaningful spatial information to orienting animals (Moore and Atema, 1988; Moore and Shao, 2000; Leathers et al., 2020; Michaelis et al., 2020).

By combining measurements of turbulent odor plumes with the periodic flicking of olfactory appendages and neural models, researchers have started to make predictions about the different strategies employed by animals to orient using chemical cues (Leathers et al., 2020; Michaelis et al., 2020). Given the recent advances in laser techniques to quantify dye plumes, simultaneous measurement of input (odor plume concentration) and output (behavior) has been achieved (Page et al., 2001a,b). The results of this work reinforce the concept that orientation strategies may have temporal and spatial components that are influenced by different aspects of the turbulent odor plume. Despite all these advances, the direct measurement of odor fluctuations on an orienting animal remains a significant gap in our understanding of chemical orientation. This experiment is designed to fill this missing gap in chemical orientation. Placing electrochemical sensors on orienting crayfish allows us to simultaneously record orienting behavior and chemical signal structure arriving at the olfactory appendages of crayfish. The direct measurement of input and output will provide insight into what aspects of the odor plume are controlling the spatial and temporal aspects of orientation behavior. In particular, we seek to understand which informational aspects of turbulent odor fluctuations control the behavioral decisions and movement of animals during chemical orientation.

MATERIALS AND METHODS

Animals
Male and female rusty crayfish, *Faxonius rusticus* (Girard 1852) (carapace 3.1±0.01 cm, mean±s.e.m.), were collected from three branches of the Portage River in Wood County, OH, USA (41.379255, −83.476134). All crayfish used in this study had fully intact walking and sensory appendages. Both male and female crayfish were used because previous work has demonstrated no difference in their chemical orientation abilities (Kozlowski et al., 2003; Moore and Grills, 1999; Moore et al., 2015). Crayfish were individually housed in a recirculating aerated system within an environmental chamber. Temperature was maintained at 23±1°C, and crayfish were kept on a 12 h:12 h light:dark cycle. Crayfish were fed one commercial rabbit pellet three times per week until chosen for the experimental trial. At that point, crayfish were starved 1 week before trials to ensure motivation to orient to a food source. Only one orientation pathway (see ‘Orientation trials’ section) for each crayfish was analyzed. A total of 91 orientation trials were run to obtain 34 orientation pathways. Of the 57 trials that were excluded, 15 were removed owing to problems with the electrode recording, 23 were removed because the crayfish touched the side walls of the flume, and the final 19 were removed because the crayfish failed to find the odor source within the time limit (see ‘Orientation trials’ section for the criteria of success). The 34 successful trials all had unique crayfish as no animal was used more than once.

Flume
 Trials were conducted in a 2000 l recirculating flume (Kraus-Epley and Moore, 2002; Kozlowski et al., 2003; Lahman et al., 2015) (Fig. 1; complete dimensions, 568×57 cm; working section, 244×57×61 cm). The end tanks and frame for the working section of the flume were constructed from stainless steel, and the sides and bottom of the working channel were glass. A thin layer of gravel (0.73±0.04 cm diameter), a substrate found in their natural habitats, was glued with silicone onto a Plexiglas sheet and positioned on the bottom of the flume. White gravel was used to provide visual contrast between the animal and the background for later digitization of the orientation pathway. Average flow velocity in the flume was kept constant at 5±0.03 cm s−1 by a centrifugal pump (WEG, model #005180P3E184JM) regulated by an adjustable speed drive (Baldor, model #ID15H205-E). The water depth within the working section was maintained at 25 cm and the water temperature was ~25°C. A jacuzzi filter system (Emerson motor, model #S55CXPED-7500 CAT; Landslide filter, model #LS40-S1LTG9-3-HK6-LH) was run during and between trials to eliminate background accumulation of food odors and dopamine. The flume and filter system were emptied, cleaned and refilled after every three trials. Statistical analysis showed no effect to trial order on behavioral results. The hydrodynamics parameters describing the flow within the flume are published elsewhere (Kraus-Epley and Moore, 2002; Kozlowski et al., 2003).

Electrochemical measurements and sensory placement
An electrochemical detection system (Epsilon, Bioanalytical Systems, West Lafayette, IN, USA) was used to quantify the finescale distribution of a 0.001 mol l−1 dopamine tracer (see ‘Odor delivery’ section). This system has been used to quantify the finescale distribution of chemicals in both field and laboratory settings (Weighman and Moore, 2020; Harrigan and Moore, 2018; Edwards and Moore, 2014). A triple-carbon-fiber microelectrode was constructed in-house using standard procedures (Moore and Gerhardt, 2000; Moore et al., 1992). Three carbon fibers were inserted into a small ball (~1 mm diameter) of graphite epoxy and a small copper wire was inserted into the opposite end of the carbon epoxy ball. These materials were baked in an oven at 125°C for 24 h. This triple-fiber electrode was sealed within a molded silicone
(Sylgard™ 184 Silicone Elastomer Kit) container to waterproof the electrode. A small silicone backpack was glued to the back of the crayfish, and the waterproof electrode was secured to the carapace of the crayfish using the silicone backpack (Fig. 2). This backpack was connected to the Epsilon system by a copper shield thin wire that allowed free movement of the crayfish in the flume. Two Ag/AgCl reference electrodes were placed in the flume water at the upstream and downstream end of the flume to create a reference for the electrode system. An additional ground wire was added to help eliminate electrical noise from both pumps. Finally, a mini-Faraday cage built out of copper wire was placed around the downstream end of the flume and Epsilon system to further shield the detection system from outside electrical noise.

We used standard methods for the quantification of chemical signals within the turbulent odor plume (Moore and Atema, 1991; Moore and Gerhardt, 2000; Edwards and Moore, 2014; Weighman and Moore, 2020). In general, DC potential amperometry was used to measure chemical concentrations arriving at the triple-carbon-fiber electrode. The DC voltage was set to 1000 mV, and a 100 Hz low-pass noise filter was employed to eliminate electrical noise from the flume and jacuzzi pump. Chemical measurements were quantified at a sampling rate of 20 Hz for the entire orientation trial. Electrodes were used only once. These values were chosen because of the known temporal sampling resolution of the chemosensory systems of several benthic invertebrates. The electrode was calibrated with flume water and known dopamine concentrations (500–1000 µmol l\(^{-1}\)). Calibrations were completed by introducing a sequence of three known dopamine concentrations into a 50 ml beaker, mixing thoroughly, and monitoring the resultant electrical current at the electrode. The response curves exhibited a linear relationship and could later be used to convert measurements of electrical current taken within the flume into corresponding dopamine concentrations arriving at the electrode.

**Odor delivery**

Crayfish are benthic omnivores and are attracted to carrion odors. Thus, the odor stimulus utilized in this study was made from homogenized commercial canned sardines (Beach Cliff Sardines). The canned sardines (92 g) were liquefied and mixed with 500 ml deionized water. Then, 100 ml sardine juice was mixed with 1 l of 0.001 mol l\(^{-1}\) dopamine solution. Dopamine is neither an attractive nor an aversive stimulus for crayfish (Kozlowski et al., 2003), which allowed us to measure the instantaneous concentrations of dopamine without disrupting the natural orientation behavior of crayfish. It is important to note that both the Reynolds number of the...
Based on this theoretical work and the previous experimental work, of important turning and movement data (Tourtellot et al., 1991). Conversely, selecting too large a digitization rate can cause the loss numbers, which emphasizes the variability within a data set. In using one point (Moore and Grills, 1999; Kozlowski et al., 2003; Moore et al., 2015). Without detailed movement decisions within this 1 s time frame (Moore and Grills, 1999, Kraus-Epley and Moore, 2002, Lahman et al., 2015). Data analysis – electrochemical signals Electrochemical data were trimmed to eliminate any portion of the trial that did not coincide with the time of the behavioral trial of the crayfish (excess recordings at the beginning and end of the trial). All electrochemical measurements were calibrated using the predetermined calibration curve to produce odor measurements in micromolar. Because the electrochemical measurements were recorded at a faster rate (20 Hz) than the orientation trials were digitized (1 Hz), two different odor by time profiles were calculated. As the purpose of the experiment is to correlate or connect fluctuations in odor concentrations with behavioral decisions, we selected to downsample the 20 Hz odor signal to 1 Hz. This choice was based on the need to have time records of odor profiles that had the same temporal frequency of behavioral digitization rates. From a theoretical point of view, crayfish appear to make behavioral and movement decisions within this 1 s time frame (Moore and Grills, 1999; Kozlowski et al., 2003; Moore et al., 2015). Without detailed analysis of flicking mechanics and neural processing rates, the behavioral decisions that occur every second provide the clearest insight into decision making based on odor information. Thus, to have identical time records of behavior and odor fluctuations, we

**Orientation trials**
After the electrode had been calibrated and attached to the carapace, crayfish were held 1 m downstream from the odor delivery tube in a flow-through shelter (13×13×13 cm) constructed from plastic grating (egg crating, 2×2 cm square holes). Crayfish were acclimated in this shelter for 10 min before the start of the trial. Delivery of the odor and dopamine stimulus began 5 min before the start of each trial to ensure that the odor had reached the downstream end of the flume before a crayfish was released. Following the 10 min acclimation period, the shelter was removed and crayfish could move freely around the flume. Crayfish were given 20 min to orient the 1 m distance upstream to where the odor stimulus was located. Recording of electrochemical signal began as soon as the shelter was removed. All trials were videotaped from above using a Panasonic HDC-H250 Digital Recorder (frame rate: 30 frames, 60 fields s⁻¹). Once a crayfish crossed a virtual line exactly 1 m downstream from the source, a mechanical stimulus (visible on the camera) was applied to the ground wire of the Epsilon system that produced a spike in the recording. This electrical spike and mechanical stimulus was used to synchronize the video analysis with the electrical recording in time. Only trials during which the crayfish oriented within 2 cm of the Tygon tube within the 20 min time period were considered successful. Additionally, behavioral trials during which crayfish walked along the side of the flume with their chelae against the flume walls were eliminated from analysis as this behavior is thigmotaxis. As such, the crayfish are deriving spatial information from the mechanical stimulus and not the chemical distribution. A total of 91 individual trials were run to produce a total of 34 trials (with 34 different animals) that fit this definition of success.

**Data analysis – orientation pathways**
Orientation pathways of successful trials were digitized using EthoVision XT 8.5 (Noldus Information Technology, Wageningen, The Netherlands). For each pathway, a single point on the middle of the crayfish’s carapace was digitized for x,y coordinates at 1 point s⁻¹ (Tourtellot et al., 1991). This method has been used in past orientation trials because crayfish can move in two dimensions without changing their body orientation relative to flow. As the directional movement of crayfish is independent of body orientation, the orientation pathway through space can be tracked using one point (Moore and Grills, 1999; Kozlowski et al., 2003; Moore et al., 2015). Higher tracking rates are possible, but problematic issues arise when digitizing pathways at rates unrelated to movement of the animal (Tourtellot et al., 1991). In particular, at higher digitization rates, turning angles and walking speeds approach zero, and the entire data set is biased toward low numbers, which emphasizes the variability within a data set. Conversely, selecting too large a digitization rate can cause the loss of important turning and movement data (Tourtellot et al., 1991). Based on this theoretical work and the previous experimental work, a digitization rate of 1 point s⁻¹ appears to provide the most robust resolution of crayfish orientation pathways. Trials were analyzed for heading angle toward the source, turning angle toward the source, heading angle relative to upstream, walking speed toward the odor source and straightness of path (net:gross). These orientation parameters were defined and utilized to classify orientation in previous research (Fig. 3) (Moore et al., 1991a,b, Moore and Grills, 1999, Kraus-Epley and Moore, 2002, Lahman et al., 2015).
downsampled the odor signal at the same rate at which behavioral data were collected. The first 1 Hz data set simply cut all of the measurements between the 1 s time points. Thus, the original recording was downsampled from 20 to 1 Hz. The second 1 Hz data set used a time average model to produce a 1 Hz sample. This was done by averaging 20 samples (20 Hz signal) to produce a single sample for any single data point. The next averaging period started at the beginning of the next 20 samples. Thus, this is not a rolling average, but a discrete average taken every second. The first 1 Hz data set provided an instantaneous measure of odor dynamics that occur every second, and the second 1 Hz (time averaged) data set produced a 1 s average of the odor signal. Additional signals were also produced in order to run through statistical models. Each of these additional signals were calculated as comparisons between the current odor concentration and a previous one in time. To calculate these new odor profiles, the current odor concentration was simply subtracted from the previous measurement at 1, 2, 4, 6, 8 and 10 s. This provided the ability to analyze a number of odor profiles to feed into models to find a best-fit relationship between the odor concentration and behavior.

In addition, the instantaneous 1 s odor signal was subjected to signal processing analysis in the statistical and graphic software package Origin (2018b, OriginLab Corporation). Each odor signal associated with a successful orientation trial was transformed to produce a fast Fourier analysis. This analysis produced a frequency plot that included both the intensity of the signal within different frequencies as well as the power density estimate (using the mean squared amplitude method). Because of different background levels and signal sensitivity of differing electrodes (all were calibrated though), all transformations were normalized to the maximum within each transformed signal. This was performed separately for the magnitude and power density estimates. The intensity and power density were averaged across all 34 successful trials and the 95% confidence interval was calculated from these averages.

**Data analysis – statistical treatment**

All data analysis took place within the programming language of R (https://www.r-project.org/). The beginning steps of the data conditioning follow those typically done in mixed model analysis (Zuur et al., 2009). The first step in the analysis was to produce histograms, qplots and normality tests of all the behavioral variables. Walking speed data were not normally distributed and showed a Poisson distribution. These data were transformed using a square root transformation, which created a normal data set. All subsequent statistical tests were performed on the transformed walking speeds. Cleveland dotcharts were used to examine both the behavioral and odor plume data for potential outliers. Although several points in the odor plume data initially appeared to be outliers, an examination of the raw data files showed that the concentration values fit within the fluctuations of these turbulent odor plumes.

Finally, to check for collinearity within both the behavioral and odor plume variables, independent regressions were performed between all of the independent (odor plume) and dependent (behavioral) variables. None of the behavioral measures showed any significant correlations, except for walking speed and speed to source, which had a correlation coefficient of 0.6. The statistical findings for this correlation showed a significant *P*-value (*P*<0.0001), but the adjusted *r*² value was quite low, indicating poor predictability (*r*²=0.018, *F*₁,₄⁹₂=37.71). Conversely and quite expectedly, many of the odor plume variables were significantly correlated with each other, although the correlation coefficients were not that large, except for one comparison. The correlation between the instantaneous concentration and 1 s average was 0.95 (*P*<0.001, *F*₁,₄⁹₂=43.96). Between the different time delays, the adjusted correlation coefficient ranged from 0.25 to 0.4 with all significant *P*-values. Consequently, odor plume models were constructed out of singular variable sets as opposed to different combinations of odor plume parameters. Thus, we did not analyze any possible interactions between the odor plume variables and only include a single predictor and the random effects within each model.

All statistical models were performed using generalized linear mixed models (Zuur et al., 2009). All models run in R used the lmer function from the lmerTest package in R (Kuznetsova et al., 2017; https://cran.r-project.org/package=lmerTest). Following model construction, the outputs were extracted using the anova function from the car package in R (https://cran.r-project.org/web/packages/car/index.html). For the models, the behavioral measure served as the dependent variable and the odor plume record served as the independent measure. Finally, the trial number was used as a random factor within each model. Given the findings of collinearity above, models were constructed using a single odor plume parameter. Model selection was based on Akaike’s information criterion (AIC) (Akaike, 1974). The selection of best model was based on the lowest AIC value while removing all models with a ΔAIC greater than 2 from our selection process (Burnham and Anderson, 2002). The statistical effect of each model was determined by extracting Cohen’s *D* (Cohen, 2013) using the effectsize package in R (https://cran.r-project.org/package=effectsize). Cohen’s *D* is a statistical measure that indicates the strength of the apparent relationship indicated by the statistical analysis and can be used to interpret the importance of statistical significance. Values for Cohen’s *D* can generally be interpreted as small (<0.2), medium (0.5) or large (0.8).

**RESULTS**

**Odor plume dynamics**

The raw odor plume examples (Fig. 5) as well as all other successful orientation trials displayed a similar magnitude and power density spectrum (Fig. 4). The spectrum displayed is the signal as it is being recorded by the working electrode on the back of the moving crayfish. Thus, the spectrum is a result of both the odor plume dynamics associated with the hydrodynamics of this flume as well as the movement of the crayfish (which can be up and down current as well as sideways). Despite the variability of orientation tracks and plume dynamics, a characteristic fluctuation in odor intensity appears to create an increased fluctuation of odor signals at 0.14, 0.29 and 0.43 Hz.

**Raw orientation tracks**

A total of 4938 data points were collected across 34 successful orientation trials (Table 1). All 4938 data points were not independent of each other, thus mixed models were selected for analysis with trial number as a random factor to control for the non-independence. Orientation trials lasted 154±15.6 s (mean±s.e.m.) and ranged from 42 to 330 s (Table 1). The mean, s.e.m., range, and minimum and maximum values for all the behavioral parameters are reported in Table 1.

Crayfish demonstrated a wide variety of orientation tracks in response to the turbulent odor plume structure (Fig. 5). Unlike the zig-zag pheromone orientation in insects, there was not an obvious behavioral pattern displayed by any of the crayfish that successfully found the odor source. Crayfish exhibited patterns that included sections where the crayfish moved downstream (Fig. 5, top left) as...
well as patterns that were almost straight lines toward the source of the odor (Fig. 5, top right). Some animals had orientation tracks that contained significant sideways deviations from the center of the plume that were reminiscent of small-scale zig-zag patterns (Fig. 5, bottom left). Finally, some crayfish had loops in their tracks as well as sections where significant overlap occurred in their positions (Fig. 5, bottom right).

Behavioral parameters

Walking speed

The best-fit model for walking speed was the difference between the current concentration of dopamine tracer at the electrode compared with the concentration 6 s previously ($F_{1,492}=5.44$, $P=0.019$; Table 2). The closest-fit model to the selected model had a ΔAIC of 4 (Table 3). The slope of the linear fit was negative, indicating that the walking speed decreased in speed as the concentration of the odor tracer increased. Although the $P$-value for the model was significantly less than 0.05, the effect size was rather small (0.0008).

Speed to source

Speed to source had a best-fit model when the instantaneous 1 s signal was used as the predictive parameter ($F_{1,492}=25.3$, $P<0.0001$; Table 2). Unlike the walking speed statistical model, small ΔAICs indicated that similar fits existed with the 1 s average and the 10 s difference (Table 3). Contrary to the fits for walking speed, speed to source significantly increased as the odor concentration increased. The effect size for this (and the other two models) was also small (0.0025).

Turning angle

The best-fit model for the turning angle included the 8 s difference in odor concentration ($F_{1,365}=18.3$, $P<0.0001$; Table 2). The ΔAICs for this model compared with the other odor parameters were greater than 2 (Table 3). The slope of this model indicated that turn angles decreased in size as the odor concentration increased in size. Finally, the effect size for this model was large enough to be considered nontrivial (0.81).

Heading angle relative to odor source

The best-fit model for the heading angle relative to the odor source used the 1 s average as its odor parameter ($F_{1,207}=14.22$, $P<0.0002$; Table 2). The ΔAICs for this model compared with the other odor parameters were greater than 3 (Table 3). The slope of this model indicated that heading angle relative to the source decreased in size as the odor concentration increased in size. Finally, the effect size for this model was large enough to be considered nontrivial (0.90).

Heading angle relative to upstream

All models for the heading angle relative to upstream resulted in non-significant fits (Table 2).

DISCUSSION

For decades, researchers have been examining chemical orientation behavior in a large array of animals across numerous terrestrial and aquatic conditions (for reviews see Vickers, 2000; Weissburg, 2010; Wyeth, 2019). One of the most daunting challenges in answering questions on how animals extract information from the spatial and temporal distributions of chemicals is quantifying the signal structure during orientation trials (Moore and Atema, 1991; Koehl et al., 2001; Webster and Weissburg, 2001; Atema, 2012). In this study, we have quantified the temporal pattern of chemical information while simultaneously recording orientation behavior. The results of this work demonstrate that the fine-scale fluctuations of chemicals significantly alter both the spatial aspects of orienting (turning and heading angles) as well as the temporal aspect of orienting (walking speed and speed toward the source).

Although all the behavioral parameters quantified in this study produced statistically significant models with different odor parameters, only two of the models that included spatial behaviors generated effect sizes that were large enough to be considered important (Table 2). The turning angle of crayfish decreased significantly as odor concentration increased, and the heading angle relative to the source also decreased significantly as odor concentration increased. The overall relationship between these spatial parameters and odor dynamics was similar; however, the best predictors of those behavioral parameters were different. Whereas the heading angle was influenced by the current concentration of chemicals (1 s average), the turning angle was based on the difference between the current concentration arriving at the crayfish and a concentration arriving 8 s previously (Table 2). This result, regarding turn angles, indicates that crayfish are making 8 s temporal comparisons of concentrations and determining the angle to turn in their pathway based on that concentration difference. It is important to note here that the spatial patchiness of odor plumes is often perceived or sampled as temporal pulses (Moore and Atema, 1991). In many ways, these temporal differences are a result of the spatial patchiness of odor plumes. If that 8 s difference in concentration is relatively large, the crayfish exhibits larger turns and vice versa. Thus, course alterations or corrections change based on some previous concentration and those corrections result in heading angles that also decrease.

Although temporal differences in odor concentrations appear to control turns, it is likely that crayfish are incorporating other sources of information to help guide their search patterns. Whereas lobsters appear to follow odor patches, blue crabs incorporate flow into their decision making during orientation (Basil and Atema, 1994; Weissburg et al., 2003). Not much research has been done on optic flow as a source of information for aquatic animals, but visual flow fields are critical for flying insects (see Vickers, 2000 for review). Crayfish also use thigmotaxis to navigate unfamiliar environments.
surroundings (Basil and Sandeman, 2000). Thigmotaxis is unlikely in the present case as trials in which crayfish touched the side of the flume with their chelae were removed from the analysis. The behavioral measure, heading angle upstream, should provide some indication of the use of hydrodynamic cues; however, this was the only behavioral parameter to not produce any significant correlation. This result could arise from our attempt to correlate this parameter with odor cues instead of the local hydrodynamic cues. Previous work has shown that changes in the hydrodynamic environment alter orientation in both crayfish and blue crabs (Moore and Grills, 1999; Weissburg et al., 2003). Despite the lack of statistical significance of this behavioral parameter, it is quite likely that hydrodynamic cues, and possibility visual cues, play an important role in orientation in natural settings. In most cases, changes in hydrodynamics or odor cues appear to change the movement rates of crustaceans.

In congruence with previous work on crustaceans, the temporal patterns of movement (walking speed and speed to source) were significantly influenced by odor (Moore et al., 1991a,b; Weissburg et al., 1994; Grasso and Basil, 2002; Moore et al., 2015). However, in contrast to this previous work, the effect sizes found in this study suggest that the statistical significance is probably not biologically or behaviorally significant. Work on lobsters, blue crabs and crayfish demonstrated changes in these temporal patterns of behavior associated with either the frequency signature of odor plumes or levels of intermittency within the concentration fluctuations (Basil and Atema, 1994; Moore and Grills, 1999; Page et al., 2011b). Most recently, studies using neural modeling of

![Dopamine concentration and odor plume recording](image)

**Fig. 5. Four examples of orientation pathways of *F. rusticus*.
**Orientation pathways digitized at 1 point s⁻¹ (bottom diagram of each pair) and the odor plume recording (top graph of each pair) are shown. These trials were chosen explicitly to demonstrate the range of behaviors exhibited by crayfish during orientation trials. Some pathways showed almost a straight line to the odor source (top right), others demonstrated motion downstream as well as circular sections (top left and bottom right), and some even showed minor zig-zag patterns similar to insect orientation (bottom left).

Table 1. Raw data parameters from *Faxonius rusticus* behavioral trials

<table>
<thead>
<tr>
<th>Behavioral parameter</th>
<th>Mean</th>
<th>s.e.m.</th>
<th>Range</th>
<th>Minimum value</th>
<th>Maximum value</th>
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<tr>
<td>Trial length (s)</td>
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<td>288</td>
<td>42</td>
<td>330</td>
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<td>Walking speed (cm s⁻¹)</td>
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<td>Speed to source (cm s⁻¹)</td>
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<td>Turning angle (deg)</td>
<td>45.07</td>
<td>0.76</td>
<td>180</td>
<td>0</td>
<td>179.8</td>
</tr>
<tr>
<td>Heading angle toward source (deg)</td>
<td>51.9</td>
<td>0.71</td>
<td>179</td>
<td>0.2</td>
<td>178</td>
</tr>
<tr>
<td>Heading angle upstream (deg)</td>
<td>48.6</td>
<td>1.38</td>
<td>180</td>
<td>0</td>
<td>180</td>
</tr>
</tbody>
</table>

* N=34 trials using unique crayfish, resulting in 4938 different data points.
olfactory receptor neurons have demonstrated that these temporal components of concentration fluctuations are extracted and could influence orientation behavior (Michaelis et al., 2020). The lack of any large effect sizes of odor concentrations on the temporal patterns of behavior are likely to be due to the direct correlation of instantaneous odor concentrations and orientation behavior as opposed to mapping spatial behaviors onto a whole plume (Page et al., 2011b). These findings seem to show some convergence of orientation mechanisms across habitats, organisms and odor plume structure.

Work within the insect community has shown that the spatial and temporal patterns of pheromone orientation are controlled by different aspects of the odor plume dynamics (Vickers, 2000). Moths use optimotor anemotaxis to locate the source of calling females (Vickers and Baker, 1994, 1996). This work has demonstrated that the spatial components of turning and timing between turns (zig-zags) are controlled by the presence of pheromone cues arriving at the antennae (Baker and Vickers, 1997; Cardé and Mafra-Neto, 1997). Even elements concerning the magnitude of the turns of male moths are influenced by pheromone concentrations (Vickers, 1999). The upwind movement of the moth (surge) is controlled both by optical flow and the intermittent arrival of pheromone pulses (Baker and Haynes, 1996). The timing of the pulse arrival rather than the concentration of the pulses appears to be what dictates upwind movement (Vickers and Baker, 1994). Combining previous work on crustacean models and the results of our study indicate similar, yet different, contrasting strategies of chemical orientation (Basil and Atema, 1994; Page et al., 2011a,b; Moore et al., 2015).

The temporal components of crustacean orientation appear to be controlled or influenced by the temporal components of the odor plume. Just as orientation parameters can be divided into spatial and temporal parameters, odor plume fluctuations can be divided into intensity aspects (instantaneous concentration, peak-to-mean ratios) and temporal aspects (frequency measures, periodicity, intermittency; Moore and Crimaldi, 2004). Walking speeds, movement toward the source, and the periodic start and stop of movement are influenced by intermittency, temporal frequencies and the periodicity of pulses in odor plumes (Keller et al., 2003; Kozlowski et al., 2003; Michaelis et al., 2020), whereas the spatial components (turning, heading and even rerouting of behavior) are heavily influenced by the concentration of the odor plume as they arrive at the animal (Moore and Grills, 1999, Weissburg et al., 2003). Thus, orientation strategies in aquatic crustaceans appear to be like those in terrestrial insects in that the spatial and temporal components of behavior are influenced by different aspects (intensity and temporal) of the odor plume. Even more so, the strategies of chemical orientation might demonstrate a convergence of a similar solution to the problem of extracting spatial and temporal information from a turbulent odor plume. In both insects and crustaceans, the spatial aspects of behavior (turning, zig-zags and headings) appear to be influenced most heavily by concentration aspects of the odor plume (Willis and Avondet, 2005; Vickers, 2006; Weissburg, 2010) (Tables 2 and 3). The results of this study demonstrate that the temporal aspects of orientation (surges, walking speed and progress to the source) are influenced more by the temporal elements in concentration fluctuations.

More neural and neural modeling evidence suggests that temporal patterns within turbulent odor plumes can be encoded by the olfactory receptor/organ system (Gomez and Atema, 1996; Gomez et al., 1999; Bazhenov et al., 2005). At the behavioral level, crustaceans flick their antennules to sample odor plumes (Pravin et al., 2012; Reidenbach et al., 2008). Between flicks, the fluid dynamics in the microspace of the sensory hairs prevents odor from arriving at the receptor cells (Koehl et al., 2001; Koehl, 2006). So, the very nature of the behavioral sampling of turbulent odor plumes creates temporally disparate samples (Reidenbach et al., 2008). Although not as prevalent as neural work on concentration and mixture coding, more recent work has demonstrated that crustacean (and insect) receptor cells are sensitive to the temporal nature of odor presentation (Nagel and Wilson, 2011). Using this neural work as a foundation, the processing of turbulent odor cues using models has shown that temporal features, such as the scope of the concentration or intermittency, can be encoded by the crustacean olfactory system (Park et al., 2016; Michaelis et al., 2020). When these models are applied to larger-scale turbulent plumes, intermittency of the plume pulses arises as a key source of information for behavioral search strategies (Michaelis et al., 2020).

### Table 2. Statistical output of the best-fit mixed models (as selected by the AIC) for predicting *F. rusticus* orientation behavior

<table>
<thead>
<tr>
<th>Behavioral parameter</th>
<th>Best-fit odor parameter</th>
<th>F</th>
<th>P</th>
<th>Slope of model</th>
<th>Effect size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Walking speed</td>
<td>6 s difference</td>
<td>F&lt;sub&gt;1,4902&lt;/sub&gt;=5.44</td>
<td>0.019</td>
<td>−0.00844</td>
<td>0.0008</td>
</tr>
<tr>
<td>Speed to source</td>
<td>1 s average</td>
<td>F&lt;sub&gt;1,4928&lt;/sub&gt;=25.3</td>
<td>&lt;0.0001</td>
<td>+0.0050</td>
<td>0.0025</td>
</tr>
<tr>
<td>Turning angle</td>
<td>8 s difference</td>
<td>F&lt;sub&gt;1,3657&lt;/sub&gt;=18.27</td>
<td>&lt;0.0001</td>
<td>−0.10</td>
<td>0.81</td>
</tr>
<tr>
<td>Heading angle</td>
<td>1 s average</td>
<td>F&lt;sub&gt;1,2073&lt;/sub&gt;=14.22</td>
<td>&lt;0.0002</td>
<td>−0.11</td>
<td>0.90</td>
</tr>
<tr>
<td>Heading angle upstream</td>
<td>2 s difference</td>
<td>F&lt;sub&gt;1,3680&lt;/sub&gt;=0.192</td>
<td>0.192</td>
<td>0.005</td>
<td>NA</td>
</tr>
</tbody>
</table>

Significant P-values are highlighted in bold. NA, not applicable.

### Table 3. ΔAIC values for the mixed models predicting *F. rusticus* behavioral patterns as a function of different odor plume parameters

<table>
<thead>
<tr>
<th>Odor plume parameter</th>
<th>Walking speed</th>
<th>Speed to source</th>
<th>Turning angle</th>
<th>Heading angle relative to source</th>
<th>Heading angle upstream</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 s average</td>
<td>5</td>
<td>0.3</td>
<td>3</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Instantaneous</td>
<td>4</td>
<td>0</td>
<td>4</td>
<td>7</td>
<td>2</td>
</tr>
<tr>
<td>1 s difference</td>
<td>5</td>
<td>6</td>
<td>5</td>
<td>10</td>
<td>4</td>
</tr>
<tr>
<td>2 s difference</td>
<td>5</td>
<td>4</td>
<td>5</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>4 s difference</td>
<td>0</td>
<td>5</td>
<td>3</td>
<td>9</td>
<td>4</td>
</tr>
<tr>
<td>6 s difference</td>
<td>0</td>
<td>5</td>
<td>3</td>
<td>10</td>
<td>4</td>
</tr>
<tr>
<td>8 s difference</td>
<td>5</td>
<td>3</td>
<td>0</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>10 s difference</td>
<td>5</td>
<td>0.7</td>
<td>4</td>
<td>7</td>
<td>4</td>
</tr>
</tbody>
</table>
Taken together with the results of this study, this indicates that the spatial behavior of orienting animals is guided by or influenced by the temporal features of turbulent plumes. This control over turning patterns has been inferred in other studies on crustaceans.

In a pair of insightful studies, Page et al. (2011a,b) demonstrated that the fine-scale orienting behavior of blue crabs was controlled by specific elements of the odor plume. Blue crab walking speeds showed acceleration within 0.25 s of receiving an odor filament. This time frame is similar to that of moths and flies initiating upwind surges in turbulent odor plumes (Cardé and Willis, 2008; Vickers, 2000). Similar to our findings, blue crabs appear to make turning decisions based on concentrations received within the last 2 s (Page et al., 2011a,b). In our results, crayfish are adjusting their turning angles based on a concentration received 8 s ago. This time aspect has not been quantified within lobsters but appears to be more in line with the results in crayfish as opposed to those in moths or blue crabs (Basil and Atema, 1994). In gastropods, which move significantly slower than any of the crustaceans, odor comparisons appear to occur over even longer time scales, but the detailed work to make exact claims is missing (Wyeth, 2019). Temporal comparisons and behavioral shifts are probably additionally influenced by flicking kinematics as well as overall body movements (Reidenbach and Koehl, 2011; Waldrop et al., 2015). If animals are using temporal comparisons to change their behavior, then there needs to be a neurological substrate for this comparison to take place.

The temporal range over which odor plume fluctuations influence the spatial aspect of orientation is in the order of seconds, with the 8 s difference in concentration having the best-fit model. This time range is similar to that found in neurophysiological studies of both insects (Galizia and Szszyzka, 2008; Riffell et al., 2008) and crustaceans (Gomez et al., 1999). Although it may be attractive to generalize the time frame of the crayfish in our study to those in other organisms or turbulent plume environments, the exact nature of the 8 s time range is likely to be a product of the average moving speed of the animal through the environment, the temporal rate of sampling (flicking) and the hydrodynamic conditions that construct the dynamic nature of turbulent plumes. Both crayfish and lobsters have maximum flicking rates of 4–6 times s$^{-1}$ and move through plumes at a rate of 1–3 cm s$^{-1}$ (Moore et al., 2015; Moore and Kraus-Epley, 2013). Blue crabs, which tend to orient with their body posture sideways to the flow, have similar flicking rates (2–4 flicks s$^{-1}$; Pearson et al., 1979) but faster walking rates (6–10 cm s$^{-1}$; Page et al., 2011a,b). Changes in the hydrodynamics underlying plume development also change movement speeds in crayfish (Moore et al., 2015). The temporal encoding of information and its subsequent transference into behavior is likely to be dependent upon the sensory landscape that is present (Michaelis et al., 2020).

The complete comparison, even across benthic crustaceans, should be cautioned though. The structure of odor plumes from which these animals are extracting information varies quite highly based on odor input kinematics as well as the hydrodynamics of laboratory flumes (Moore and Crimald, 2004; Weissburg, 2010). Indeed, mesocosm conditions have demonstrated that, even in similar flow conditions, odor plume dynamics are quite distinct (Moore et al., 2000). In a similar vein, although benthic crustaceans have tufted appendages that are flicked to sample odor plumes, the differences in both morphology and flicking behavior across the three orientation models (blue crabs, lobsters and crayfish) are quite significant and can influence the perception of odor cues (Koehl, 2006; Reidenbach et al., 2008). These distinctions further enforce the need for simultaneous measurement of odor dynamics and animal behavior in order to make direction connections between the two. Given the diversity of olfactory appendages and sampling, the next step that is needed is the placement of chemical sensors directly within the tufts of olfactory hairs. Thus, the influence of bilateral flicking on the perception of odor cues during actual chemical orientation can be quantified.

Often when drawing conclusions on animal orientation, particularly with chemical cues, the field is drawn to distinctions and definitions based on differing elements. For example, orientation strategies can be separated by whether comparisons are made across spaced sensors (taxis) or through time (kinesis; Kennedy, 1986). Adding confusion to the definition of strategies, elements of a strategy can include the measurement of concentrations, temporal changes in behavioral patterns, the incorporation of other sensory information like vision, or whether steering is self-controlled (i.e. zig-zags) or stimulus controlled (Vickers, 2000; Weissburg, 2010). Despite one of the clearest sets of definitions of orientation strategies, decades ago, Kennedy (1986) acknowledged that there is a lot that is unknown about chemical orientation because of the turbulent nature of chemical plumes. Since that seminal piece by Kennedy (1986), technological advances in lasers have led to significant work on understanding the spatial and temporal nature of odor dynamics; however, the direct coupling of input (odor) and output (orientation) has remained elusive. The work here, coupled with more recent studies (Michaelis et al., 2020), indicates that temporal features, such as intermittency and concentration changes, are important sources of information for guiding animals orienting in turbulent odor plumes.

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Author contributions

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Data availability
Raw data are available through the University of Michigan Biological Station’s research portal: https://mfld.umich.edu/dataset/resource/604461b9-46ed-4039-be7c-fd63593a108f/

References


