Insect olfactory landmark navigation

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Abstract

The natural world is full of chemical signals - organisms of all scales and taxonomic classifications transmit and receive chemical signals to guide the full gamut of life's processes: from helping forming mother-infant bonds [23], to identifying potential mates [14] and even signalling their own death [57]. Insects are particularly reliant on chemical cues to guide their behaviour [27, 20] and understanding how insects respond to and use chemical cues in their environment is a high active research area.

In a series of recent studies [41, 43, 42] Steck et al. produced evidence that foragers of the Saharan desert ant species *Cataglyphis fortis* are able to learn an association between an array of odour sources arranged around the entrance to their nest and the relative location of the nest entrance and later use the information they receive from the odour sources to help them navigate to the visually inconspicious nest entrance. This ability to use odour sources as *olfactory landmarks* had not been previously seen experimentally in insects, and is a remarkable behaviour given the extremely complex and highly dynamic nature of the olfactory signals received by the ants from the turbulent odour plumes the chemicals travel in from the sources.

After an introductory chapter covering some relevant background theory to the work in this project, the second chapter of this dissertation will detail a field study conducted with the European desert ant species *Cataglyphis velox*. As in the studies of Steck et al. the ants were constrained to moving a linear channel and so the navigation task limited to being one-dimensional, the aim of this study was to see if there was any evidence supporting the hypothesis that *Cataglyphis velox* ants are able to use olfactory landmarks to navigate in a more realistic open environment. The results of the study were inconclusive, due to the low sample sizes that were collected and small effect size in the study design used, however it is proposed that the study could be considered usefully as pilot for a full study at a later date, and an adjusted study design is proposed that might overcome a lot of the issues encountered in the current study.

In the third and final chapter of this dissertation, a modelling study of what information is available in the olfactory signal received from a turbulent odour plume about the location of the source of that plume is presented, with this work aiming to explore the information which may being used by *Cataglyphis* desert ants when using olfactory landmarks to navigate. The details of the plume and olfactory sensor models used are described and the results of an analysis of the estimated mutual information between the modelled olfactory signals and the location of odour source presented. It is found that the locational informational content of individual signal segment statistics seems to be low, though combining multiple statistics does potentially allow more useful reductions in uncertainty.

Acknowledgements

Declaration

I declare that this thesis was composed by myself, that the work contained herein is my own except where explicitly stated otherwise in the text, and that this work has not been submitted for any other degree or professional qualification except as specified.

(Matthew Graham)

Table of Contents

Chapter 1

Introduction

1.1 Olfaction: definition and properties

Chemosensation, the sensing of external chemical stimuli, is perhaps the most universal of senses, with organisms from the scale of single cellular bacteria to the largest vertebrates being able to detect and utilise chemical signals in their environments [44]. Within this broad definition a variety of different subdivisions of chemical senses can be made, with delineations including anatomical differences such as the location of the chemosensory receptors within the organism or neural pathway the transduced signals take, and differences in the properties of the stimuli themselves such as the fluid-phase of the chemical or carrier.

This work will focus on olfaction, here taken to mean any chemosensation mechanism which gives the host organism information about remote chemical sources, i.e. those not in direct physical contact with the organism (in contrast to contact chemosensation modalities such as gustation).

1.1.1 Odour transport

A strongly distinguishing feature of olfaction, is that the transmission of the signal from source to receptor is in general a much more complex process than the wave propagation based transmission of sound and light. Odour chemical transport can be considered as the combination of two sets of physical processes: advection and diffusion [29]. Advection is the bulk transport of chemicals by the vector flow field in the fluid while (molecular) diffusion is the spread of the chemical across gradients in the scalar concentration field.

Figure 1.1 provides a simple overview of the processes involved in odour transport, showing indicative representations of the effect of diffusion and advection on the concentration field of a small patch of odour in a fluid.
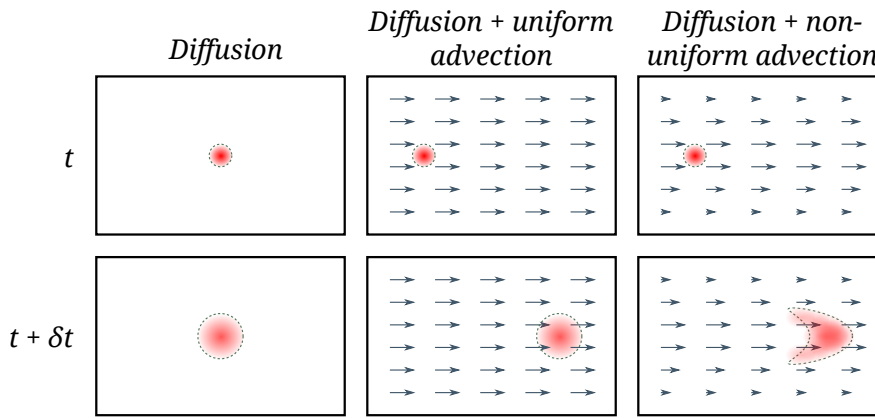
Figure 1.1: Illustration of the some of the processes involved in odour transport.

First the simplest case of odour transport due to diffusion alone when the carrier fluid is stationary, illustrated in the leftmost column of figure 1.1 will be considered. Qualitatively, diffusion has the effect of causing variations in a concentration field to smooth out over time. More quantitatively, Fick's distinct first law of diffusion states that the rate of flux of a diffusing quantity across some (infinitesimal) boundary is proportional to the negative of the concentration gradient across that boundary, with the proportionality constant being the diffusion coefficient D_m (the subscript m indicating molecular diffusion). Thus whenever there is a gradient present in the concentration field, the chemical will tend to diffuse down the gradient. In terms of figure 1.1, the strong radial gradient present in the odour patch in the top left panel will facilitate a proportional rate of diffusion in the radial direction leading to the odour patch to become more spread out at the later time shown in the bottom left panel.

For a given length scale L, the characteristic diffusion time, roughly the expected interval for a particle of diffusant to move a distance L, is $T_D = \frac{L^2}{2D_m}$. The diffusion coefficients of organic chemical vapours in air at standard ambient temperature and pressure (298 K / 10 kPa) tend to be on the order of 10^{-5} m^{2}s^{-1} [25]. For a length scale of 1 m, which is an appropriate scale for the distances from a source over which an insect might need to detect an odour, this gives a rough estimate of the characteristic diffusion time as 50 000 s or approximately 14 hours. Clearly this is an infeasibly long time for an animal to wait to gain information about an odour source. Equally however it is obvious that animals do respond to odours emitting from sources over distances of a metre and more over much shorter time-scales.

The solution to this (contrived) quandary is the already mentioned advection. In virtually all real situations the carrier fluid will not be stationary and therefore alongside diffusive odour transport, the odour will be transported by bulk motion of the fluid. Analogously to diffusion, a characteristic time-scale can be defined for advection - if

the mean flow velocity is of magnitude U then the expected time for a small patch of odour chemical to be transported over some relevant length scale L is $T_A = \frac{L}{U}$. For even a very low mean flow speed of $U = 0.1\,\text{ms}^{-1}$, the characteristic advection time scale is $T_A = 10\,\text{s}$ for the same length scale of $L = 1\,\text{m}$ as previously - from this it is clear at macroscopic length scales advection will be the dominant odour transport mechanism in the vast majority of situations.

The middle column of panels in figure 1.1 demonstrates the combined effects of diffusion and advection in a *uniform* velocity field. With comparison to the diffusion-only mediated transport illustrated in the previously discussed left panels, the odour patch now undergoes a translation downstream due the velocity field, while also spreading out due to diffusion along the radial concentration gradient as previously. Note that in this case of a completely uniform velocity field there is no interaction between the diffusive and advective processes, every point in the odour patch undergoing the same translation due the constant velocity field and hence the concentration distribution and so gradients within the patch being unaffected by advection.

Such uniform velocity fields rarely occur in reality. The right column of panels in figure 1.1 illustrate a slightly more realistic situation in which the velocity field is non-uniform, here the simplistic case in which the non-uniformity is in the magnitude of the velocity profile in the cross-stream direction, as might be encountered for example in low velocity flow through a pipe due with viscous friction with the pipe walls. As different points in the odour patch now experience different flow velocities, the patch undergoes shearing due the *differential advection* as it progresses downstream leading to a distortion in the shape of the patch as shown indicatively in the bottom right panel of figure 1.1.

As well as this direct effect, differential advection by changing the spatial concentration distribution and so gradients of the odour patch affects the diffusion based transport of the odour. The stretching of the contours of the odour patch lead to an increase in the boundary area across which there is a strong concentration gradient and so leads to increased diffusion. This sort of interaction means that although diffusion on its own is a relatively minor contributor to odour transport at macroscopic scales, in interaction with advection it is still an important effect that needs to be accounted for.

Although useful as a toy example to illustrate the effects of differential advection, the velocity field shown in the right panels of figure 1.1 is still far from realistic. In most real situations, fluid flow is turbulent [11, p.3]. Turbulent flow is characterised by chaotic dynamics whereby small deviations in initial conditions lead to widely divergent evolutions of the system, and results in complex flow velocity fields with highly irregular,

multi scale structure and seemingly random velocity fluctuations [11, p.12-13].

An example of the complex structures which turbulence results in can be seen in figure 1.2 which shows a cross-section through a low-velocity ($< 0.5\,\text{ms}^{-1}$) plume[1] of fine water droplets imaged using a laser sheet. Even this relatively low velocity plume shows examples of the filamentous structures and eddies which are characteristic of turbulent flow. The distortion of the concentration field contours into these intricate shapes promotes diffusion as explained previously for the simple differential advection case, with this enhanced dispersion of the fluid termed turbulent mixing [29].
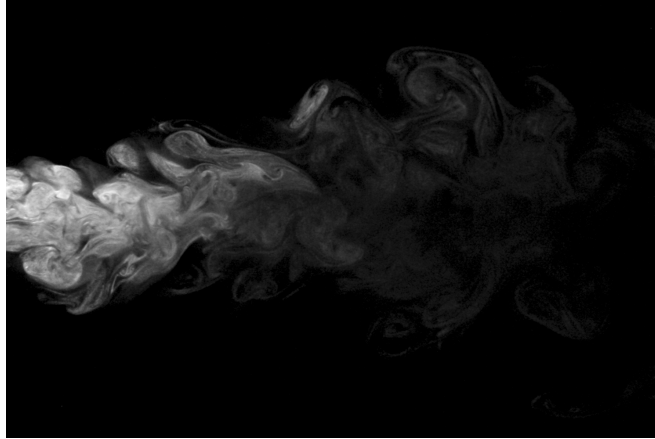

Figure 1.2: Cross section through a turbulent plume

An important quantity in determining whether a flow will be turbulent or not is the dimensionless Reynolds number,

$$Re = \frac{UL}{\nu}$$

where U and L are characteristic velocities and length scales of the flow and ν the kinematic viscosity of fluid, with $\nu = 1.6 \times 10^{-5}\,\text{m}^2\text{s}^{-1}$ for air at $298\,\text{K}$ / $10\,\text{kPa}$. The Reynolds number is a measure of the inertial to viscous forces in a particular flow field. Viscous forces tend to damp out velocity fluctuations so that flows with low Reynolds flows where viscous forces are dominant tend exhibit smooth laminar flow while at high Reynolds numbers the flow transitions to turbulence.

An important feature of turbulent flow which has been briefly mentioned already is its multi-scale structure. The energy in a turbulent flow is distributed over a range of spatial scales - it is common to relate these scales to different sizes of eddies in the flow as described in the seminal work of Kolmogorov on turbulence in high Reynolds number flows [19]. Energy enter the flow through largest eddy structures and then

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[1]As it is a term which will crop up regularly, it is worth at this point clearly defining what is meant by a plume: a plume is a flow structure that forms from the movement of one fluid within another due to advective and diffusive effects.

cascades down through successively smaller scale eddies as their motion is initiated by the larger structures.

The gross motion of a plume moving in a turbulent flow, for example the sinuous meandering commonly seen in industrial smoke stacks, is determined by the largest eddy structures. Smaller scale eddies produce the whorls and filamentous type structures seen in figure 1.2. As energy progresses down to increasingly small scales, the effect of viscous forces becomes increasingly dominant until eventually a point is reached where the viscous forces overwhelm the structured motion produced by inertial forces giving a lower limit to turbulent eddy size and so the scale at which turbulent mixing occurs . There is therefore a minimum scale of the components of a turbulent plume - i.e. a rough discretisation. This combined with the growth of the plume in the cross-stream directions due to the dispersive effects of medium size eddy structures leads to a key characteristic of turbulent odour plumes - they are highly intermittent with patches of odour of a scale related to the lower limit just mentioned being separated by increasingly large gaps of zero odour as the plume expands [31].

This intermittency property of turbulent plumes in well illustrated in 1.3, another cross section snapshot of a low velocity turbulent plume imaged using the same techique as in figure 1.2. Data from field experiments indicates that an odour levels within a turbulent plume are below detectable levels 60% of the time at $2\,\mathrm{m}$ from the source, falling to 90% at $20\,\mathrm{m}$ from the source [44, 31].
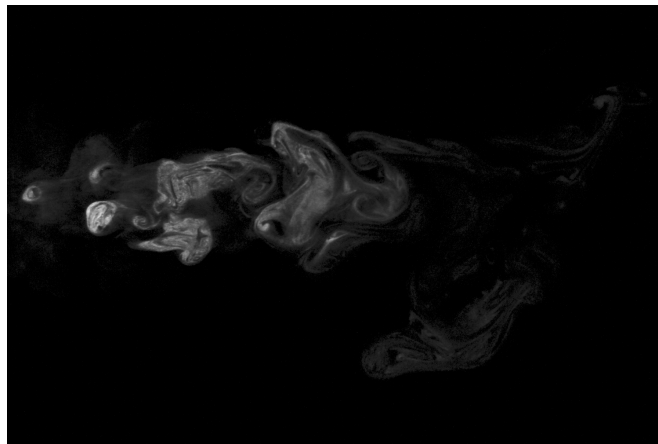
Figure 1.3: Cross section through a turbulent plume showing a strongly intermittent concentration field

The presence of animal in a plume and their movement through it will affect the local flow conditions and this can have important effects on the final odour signal received by the animal [44]. Viscous fluids form boundary layers at their interface with solid substrates, with frictional forces leading to a zero flow velocity at the surface and

a increasing velocity gradient with a logarithmic profile until the free-stream velocity is reached [33, 44]. The boundary layer which forms at the interface of the olfactory sensory appendage(s) of an animal with the fluid flow from which they are sensing, strongly shapes the properties of the sensed odour signal with these effects depending on the morphology of the sensory appendages [38, 39] and any active processes involving the appendages such as the antennule flicking shown exhibited by some crustaceans or mammalian nose sniffing [10].

In conclusion, the highly complex spatial structures of turbulent plumes, their intermittent nature and complex effects of the animal themselves on the local flow conditions means using olfactory sensory input to gain information about an odour source is a highly non-trivial task and puts strong demands on the capabilities of the sensory system in terms of having sufficient spatial and temporal resolution to be able to resolve the highly complex temporal and spatial concentration field patterns.

1.2   Insect olfaction

Given the many challenges utilising olfactory information presents, it is all the more remarkable that it so widely employed by animals from widely divergent evolutionary paths and for such varied purposes. While in humans olfaction is often viewed as a weak sibling to vision and audition as a modality for sensing remote stimuli we are more the exception than the rule. In many if not most organisms the olfactory system is a central component of how they perceive the environment around them [1] and in insects in particular the olfactory system plays a key part in many behaviours.

Pheromones, species specific chemicals secreted to modulate social responses of members of the same species, are used by insects to govern a wide range of behaviours, varying from triggering aggressive or defensive behaviours in case of attack by a predator to aiding in mate selection [27]. Pheromone-based odour signals may be single simple monomolecular compounds or mixtures of multiple components with the blend needing to have species specific ratios to elicit a response [17]. Pheromones may have different behavioural results depending on the context they are experienced in have been observed in insects, with 11-cisvaccenyl acetate acting to promote aggregation in *Drosophilla melanogaster* when encountered as a plant volatile, while inhibiting mating when experience by a male in the presence of other males or mated females [27].

As well as using pheromones, insects also utilise a variety of other chemical cues from the environment around them. For example honeybees are able to detect and distinguish between a range of different floral odours [36] especially impressive given the complex multi-component nature of most floral odours. As well as being able to

discriminate between the floral odours of different plant species, bees are also able to distinguish fine variations between the odours of different plant individuals of the same species which relate to its pollination status [36]. Parasitic insects such as mosquitoes and midges use the plume of carbon dioxide emitted by a respiring potential host to track their target while the fruit fly *Drosophilla melanogaster* innately avoids carbon dioxide while walking but appears to be track carbon dioxide plumes when flying [47].

1.2.1 Insect olfactory system

Underlying the wide repertoire of olfaction driven behaviour, in insects is a olfactory system which is to a large degree conserved across the massive range of insect species and in fact demonstrates many similarities in the principles of its organisation to vetebrate olfactory systems [1]. The top-level organisation of the first stages of the insect olfactory system is shown in schematic form in figure 1.4.
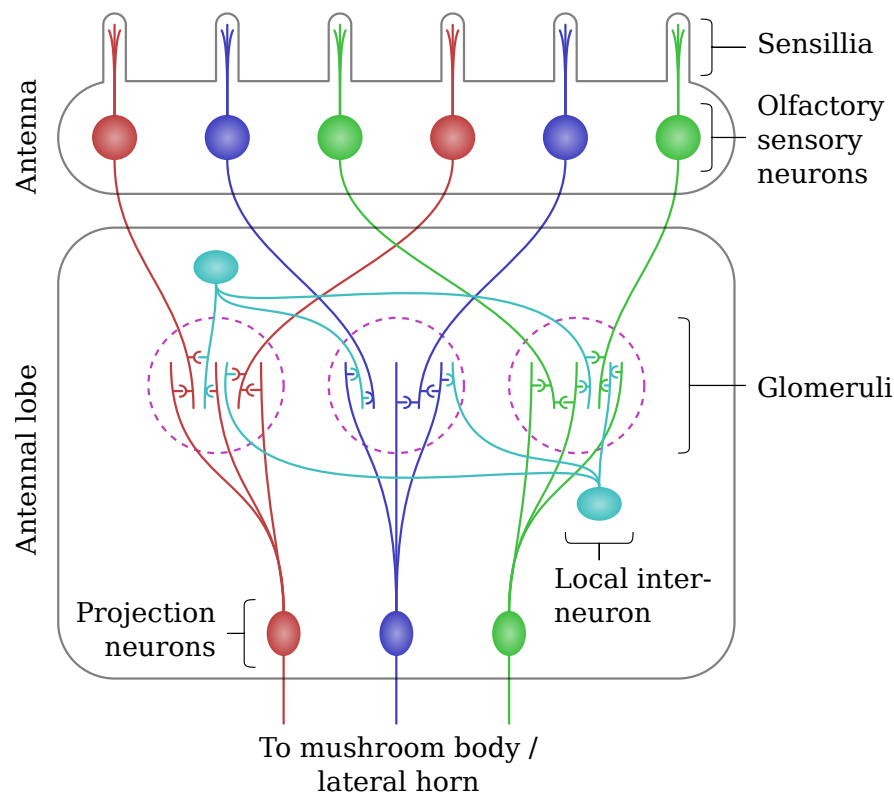
Figure 1.4: Schematic overview of the initial stages of the insect olfactory system.

Insect chemosensation is driven by olfactory receptor proteins which are contained in the dendrites of olfactory sensory neurons and are where the initial transduction from chemical to (electrical) neuronal signal occurs. The olfactory sensory neurons are located in head appendanges such as antennae or palps, with the dendrites extending into sensilla, hair like protrusions on the surface of the olfactory sensory organ. There

are various sub-types of olfactory receptor protein with differing affinities to volatile odour chemicals, these variations in response forming the basis of the coding of odour quality. Each olfactory sensory neuron typically expresses a single type of olfactory receptor though in some species, some olfactory receptor cells express multiple receptor types [27].

In the more typical first case of one receptor type per olfactory sensory neuron, the axons of the cells expressing the same receptor type converge on distinct glomeruli in the antennal lobe, which are neuropil structures i.e. regions of high density of synaptic connections between neuronal processes but relaitvely low density of cell bodies. Connections between the glomeruli are formed by local interneurons which shape the glomeruli population activity, with lateral inhibition (more common) sharpening the population response and lateral excitation leading to broadened population responses. The output from the antennal lobe is formed by projection neurons which carry the shaped glomeruli population activity on to higher brain regions including the mushroom bodies and lateral horn, were further processing of the olfactory input is performed and integration with input from other sensory modalities.
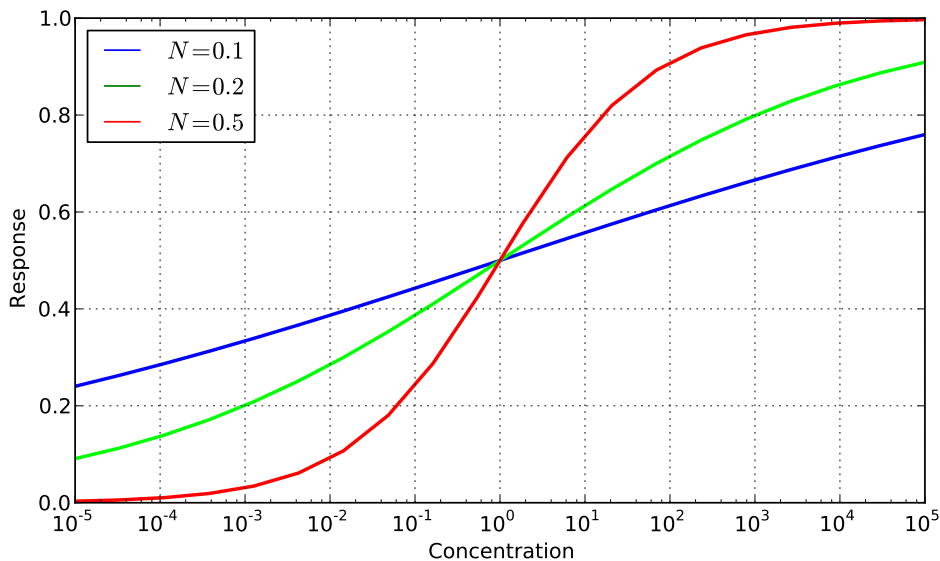
Figure 1.5: Plots of the Hill equation response function for different values of the Hill coefficient N with $R_{\mathrm{max}} = 1$ and $K = 10^0$.

A calcium imaging study of the antennal lobe in honeybees by Sachse and Galizia [35] found that the number of active glomeruli and their individual activity levels appear to increase with increasing odour stimuli concentration, with the activities of individual glomuleri having a Hill equation response function form to the odour stimuli

concentration. The Hill equation has the form

$$r = f_{\text{Hill}}(c;\ R_{\max}, K, N_{\text{Hill}}) = R_{\max} \left(\frac{c^{N_{\text{Hill}}}}{c^{N_{\text{Hill}}} + K^{N_{\text{Hill}}}} \right)$$

where r is the response value, c the input value (concentration here), $R_{\max}$ is the maximal response value, K the half-saturating concentration and N_{Hill} the Hill coefficient which determine the slope of the response function. As illustrated in figure 1.5 the Hill equation is equivalent to a sigmoidal response function on the logarithm of the input and so for concentrations near to K the response will scale nearly linearly with the logarithm of the input with the relationship becoming increasingly non-linear at the extremes.

The shape of the response functions of individual glomeruli to changing odour concentration differed such that the relative glomeruli population activity changed as a function of odour concentration, seemingly complicating the simultaneous decoding of odour intensity and quantity. The glomeruli activity levels just described however corresponded to the afferent input from the olfactory sensory neurons to the antennal lobe glomeruli. In the Sachse and Galizia study alongside these input activities, the projection neuron outputs from the glomeruli were also imaged with a second calcium-sensitive dye, thus allowing the effect of local computation due to lateral connection within the antennal lobe to be observed. It was found, that unlike the glomeruli input activity, the output of the most responsive glomeruli for a particular odour showed near-invariant relative activity levels across a odour concentration range of up to 4 $\log_{10}$ units. Thus the authors conclude that the local computation in the antennal lobe network acts to contrast enhance the output and improve the concentration-invariant encoding of odour quality, with the odour stimuli concentration being represented in overall activity levels and the odour quality by the relative activity levels across the glomeruli population.

For analysing the temporal resolution of the insect olfactory system, electrophysiological techniques are more appropriate than calcium imaging which is limited by slow indicator dynamics. In a study measuring the olfactory sensory neuron output from the sensillia of an American cockroach (*Periplaneta americana*) antenna, it was found that high concentration odour pulses of down to 25 ms of a simple monomolecular odour, hexanol, could be resolved in the firing rate output of the sensory neurons with pulses of down to 100 ms being able to resolved reliably at low concentrations [21]. Testing with a more complex mixed odour, coconut oil, in the same study, found pulses of 50 ms could be reliably resolved in the neuronal output at high concentrations, this increasing to 200 ms for lower concentrations.

These results are limited in their direct interpretation due to the recordings being at

the periphery of the nervous system and so not accounting for any latency and/or loss of temporal resolution resulting from the transmission and processing of the signals within the central nervous system, however they provide a useful benchmark for the temporal performance of the insect olfactory system. Further, evidence from behavioural studies of various moth species tracking plumes of sex-pheromone has shown that the moths are able to respond to the impingement of a filament of pheromone on their antennae with a latency of 0.15-0.3 s [5, 45], suggesting that the delays introduced by central processing are not overly severe.

1.3   Insect navigation

Navigation is a term which is used in various different contexts with different varying levels of specificity in what is being referred to, here the fairly inclusive definition will be used that navigation is the process of an organism directing its movement from its current location to another fixed location using external (allocentric) and/or internal (ideothetic) cues. Navigation has several distinct advantages for studying as a model behaviour: it is a task which is virtually universal applicable with most if not all motile organisms having to navigate as part of their normal behaviour and has a clear and easily measured behavioural output, the organisms location in space over time [56].

Of particular interest in studies of navigation are social insects, with the most familiar perhaps being members of the order *Hymenoptera* which includes ants, wasps and bees. Such insects tend to live in large colonies and have a strict division of labour, with specialist foragers leaving to find food sources which they then return to the colony with (known as central-place foraging). This offers a distinct advantage for behavioural research that providing the foragers can be correctly identified, once away from the vicinity of the nest or hive they will have a known motivation [56].

1.3.1   Cataglyphid desert ants: a model for insect navigation

Desert ants of the genus *Cataglyphis* have become a particularly well studied model organism for insect navigation. They inhabit desert and scrubland environments, with their centre of distribution being north Africa but their range also extending to southern Europe and the Middle East [22, 2]. The ants tend to be scavengers mainly feeding on dead arthropods [22]. *Cataglyphis* species are generally highly heat-tolerant and tend to exhibit thermophilic behaviour [22], foraging workers leaving their nest during the hottest part of the day both minimising the risk of encountering predators while also presumably being an ideal time to find the dead carcasses of less heat resistant arthropods.

The high temperatures and still present risk of predation during foraging runs have exerted a strong evolutionary pressure on *Cataglyphis* species to develop accurate and reliable navigation methods to return to quickly their nest once a food item has been collected. Many ant species use a pheromone trail system to control navigation to food sources: on the return leg of a successful foraging run an ant will lay a chemical trail. Other ants will subsequently be recruited to the trail and further reinforce the chemical signal on their return journeys. When a food source becomes depleted, ants which return without food items will not reinforce the trail leading to the chemical marker decaying as it naturally dissipates and thus the trail falling out of use.

Cataglyphis species have not however been observed to utilise this form of chemically driven navigation [22]. This is thought to be the result both of the typically small size and unpredictable distribution of their natural food sources which does not provide a strong motivation for mass recruitment to a single location and the high temperatures of their natural habitats meaning pheromone chemicals along a trail would be prone to quickly evaporating away [55, 22, 41]. Instead *Cataglyphis* ants forage singly, following circuitous routes on the outward leg of their runs while searching for food items, then once a food item has been located using their highly sophisticated navigation capabilities to return by near direct return routes.

This characteristic foraging behaviour of *Cataglyphis* individuals is largely enabled by their impressive path integration system [48, 30, 49] by which the ants continually track their estimated vector position with respect to the nest entrance during the search phase of a foraging run and are then able to use this home vector to guide their return run. This path integration system is supported by a celestial compass whereby the ants use celestial cues such as the sun's position and sky polarisation patterns to provide a continual absolute estimate of their orientation [50, 48], and an odometery capability thought to be based on step counting [54] which enables tracking of the distance travelled in different directions. Together these elements allow *Cataglyphis* ants to perform impressive feats of dead reckoning; the ants of the species *Cataglyphis fortis* which are found in the flat and feature-deficient salt-pans of Tunisia have been found to be able to trace a near direct route back to the vicinity of their nest entrance after extremely circuitous foraging runs covering distances of up to 1 km and maximum straight line displacements from the nest entrance of 100-200 m [30, 55, 40].

Although the *Cataglyphis* path integration system is remarkably accurate, any such egocentric positioning system will necessarily accumulate errors which increase with the distance covered. Therefore alongside the path integrator *Cataglyphis* are also able to utilise an impressive array of cues from the environment as geocentric landmarks, with the cues used by *Cataglyphis* ants so far found to include visual [52], olfactory

[7, 40], vibrational and magnetic [6]. Here landmark will be taken to mean any fixed feature of the environment which is able to be used by an animal to aid in navigation.

It seems that the two navigation systems, egocentric path integration and geocentric landmark based, work together in a complementary fashion. In cases where the ants habitat provides plentiful natural landmarks, landmark cues will be the dominant method used to navigate, however the ants will still maintain an estimate of the home vector allowing them to switch back to path integration based navigation in cases were they come off a landmark based route or the landmarks have changed from the previously remembered configuration [49]. Similarly even when being predominantly guided by the path integration system, ants are able to use information from landmarks to help reduce accumulated errors [51] and will usually switch to landmark based navigation for the final stage of a return journey to the nest, due to uncertainty in the homing vector meaning it can only be relied on to guide the ant to the vicinity of the nest entrance [55, 40].

Visual landmarks are though to be the most commonly used geocentric environmental cue by *Cataglyphis* ants - in fact until relatively recently the only form of landmarks known to be used by *Cataglyphis* species were visual [55]. The nest entrances of *Cataglyphis* species are by themselves often visually inconspicuous, and so the ants need to use visual cues from the surrounding environment rather than the nest entrance itself for nest-defining landmarks. One model proposed for insect visual landmark navigation, is that 'snapshots' of the panoramic view seen by the insect at particular locations and are stored neurally and later compared to the current visual input, with the maximisation of some similarity metric, typically a simple sum of absolute difference between intensity levels, between the current visual input and stored snapshot, being proposed as the method by which the insect navigates to the remembered visual landmark. This snapshot model, orginally proposed for bee navigation [9], has seen many subsequently applied to other insects including *Cataglyphis* ants [51, 28] and has seen various extensions including models to account for using visual landmarks sequentially to follow a route rather than navigate to a single point [3].

Alongside their use of visual landmarks it appears that *Cataglyphis* ants are able to utilise cues from other sensory modalities to assist when navigating, of particular interest for this project being their use of olfactory information. *Cataglyphis fortis* ants were shown in [55] to use the odour plume emanating from a repeatedly visited food source to guide their final approaches, using a combination of olfactory and anemotactic (wind-based) information (the task of plume following will be considered in more detail in the following section). Further the ants appeared to adjust their path integration guided incoming trajectories such that they consistently arrived downwind of the food

source and thus were able to use the odour plume to guide their final pinpointing of the food source. *Cataglyphis fortis* ants are also able to use odour plumes emitted from their nest entrances to aid in their final approach to the nest, with a plume of carbon dioxide, which is produced by the ants during respiration being identified as sufficient to elicit this plume-following behaviour [7]. Plume following only seems to occur when the path integration system of the ant indicates they are close to their nest, this possibly being driven by the non-specificity of carbon dioxide plumes and the danger of following a plume into an enemy nest [7] (which has a strong probability of resulting in the death of the ant).

As well as these odour plume following behaviours, *Cataglyphis fortis* ants have also been shown to be able to use single or multiple odour sources at fixed positions relative to the nest to guide their navigation to the nest entrance, that is they are able to use odour sources as *olfactory landmarks*. This remarkable ability was demonstrated in a series of papers by Kathrin Steck and colleagues [41, 43]. Given these studies formed the immediate motivation for this project, they will be covered in more detail below, but first a fuller treatment of odour plume following behaviour will be presented as this can be viewed as a specialised form of olfactory landmark navigation where there is a single odour landmark collocated with the target, and the large existing body of research on this topic is thus relevant to the more general task of olfactory landmark navigation.

1.3.2 Odour plume following

Plume following or tracing is a navigation task in which the aim of the animal is to locate and move to the source from which an odour plume is emanating. This is a common requirement for many animals particularly insects - for example when searching for a pheromone releasing mate, food source or potential oviposition site [44, 8].

The previously discussed highly intermittent spatial distribution of an odourant in a turbulent plume over time means that using olfactory information alone to follow a plume to its source is very challenging with the odour signal being absent for much of the time. The lack of reliable concentration gradients has supported the proposition that many, perhaps all, insects which show plume-following behaviour rely on the sensation of flow (wind) velocity as their primary directional cue [31, 44, 8].

The methods available to animals to sense flow velocity information depends mainly whether they primarily move by walking on a fixed surface (e.g. non-flying insects and bottom-dwelling marine arthropods) or they move through a fluid without direct support from any surface (e.g. flying insects and fish) [44]. In the former case as the animal (when stationary) has a fixed inertial frame of reference with respect to the fluid

flow the magnitude and direction of fluid flow can be estimated by mechanoreceptive means - for example the deflections of mechanoreceptor sensilla. To be able to estimate absolute wind velocity while moving the animal must have some independent means of estimating and compensating for their own motion, for example from proprioception or optical flow. For flying and swimming animals the task of fluid flow velocity detection is more difficult as they must rely on non-mechanical means to estimate a surface-fixed frame of reference. It is thought flying insects achieve this visually, with results from the experimental manipulation of the optical flow of plume following moths and other flying insects supporting this hypothesis [44].

Sensation of the local flow direction gives plume following animals a more continuous cue of what direction to follow the plume upstream compared to the usually highly intermittent olfactory signal. However in the usual case of a fluctuating flow velocity field, the distribution of odour patches downstream of the source is determined by the whole history of the flow velocities experienced by the patches since their release. Therefore the instantaneous local flow velocity experienced by animal will only rarely coincide with the direction of the plume centreline (defined by the centre of mass of the concentration distribution across lateral cross sections through the plume [24]) at its closest point and so flow direction will only give a rough indication of which direction to move in to follow the plume upstream to its source [8].

Flying insects have characteristic zigzagging trajectories when following an airborne odour plume [44, 8], as they fly up the plume they making a counter-turns across the wind stream directions at regular intervals. Closely related is the 'casting' behaviour shown by insects who have lost contact with a plume whereby they conduct increasingly large amplitude oscillatory flights in the cross wind direction (vertical oscillatory motion amplitude also increasing) until they re-establish contact with the plume. Importantly the former counter-turning behaviour is exhibited even in cases when the insects trajectory remains wholly within a continuous plume structure and so it does not appear to be the case that turns are simply initiated by loss of odour signal [8]. In combination with this oscillatory motion, plume tracking insects also undergo upwind surges upon making contact with odour filaments [44, 8]. It has been proposed [4] that these two behaviours, surging and counterturning are the result of two control systems running in parallel, one guided by an internal oscillator which creates the counterturning behaviour at a frequency driven by the frequency of encountered odour filaments and a controller producing upwind surges on each odour filament detection event with a higher gain such that it dominates the counter-turning control signals when active.

Interestingly the previously mentioned food [55] and nest plume [7] following studies with *Cataglyphis fortis* noted similar zigzagging behaviour as the foragers tracked the

plumes upwind to the food source and demonstrated the necessity of both olfactory and wind sensation by the ants for successful plume following to occur. This suggests the possibility that ants may use similar strategies to flying insects when plume following.

Given the incomplete information offered by both local olfactory and flow velocity measurements, it seems logical that insects would gain from using both together when plume following and this indeed appears to be the case from the observed surge-counterturn behaviour of flying insects and the suggested two controller model. Importantly however in this case the olfactory signal is limited to being used in a binary manner - a odour filament is present or not. Whether or not insects are able to use additional information from the odour signal and how they might do so if so is not well understood.

There is some indirect evidence of support of such abilities. Several species of moth when released in to a pheromone plume in a wind tunnel in which the flow is stopped after the start of the moth's flight but prior to the moth reaching its target are still able to navigate to the target in the still air conditions, suggesting they are able to use none flow-based cues for directional information although the mechanisms for this are not clear [8]. Some species of bottom-dwelling marine arthropods including the blue crab *Callinectes sapidus* and American lobster *Homarus americanus* have been shown to use odour concentration gradient information when following a marine odour plume potentially using both bilateral comparisons across spatially separate olfactory sensors and sequential comparisons of the output of the same sensor [44], though the large scale of these animals (and so separation of their olfactory sensors) and marine environment with its different scaling factors on the fluid dynamics mean it is not clear if such abilities could also be present in smaller air-borne insects.

1.3.3 Olfactory landmark navigation

In contrast to odour plume following, there has been little evidence for a more general ability of using one or more odour sources as landmarks to direct navigation to a point in space which may not be coincident with any of the source locations; in fact the previously mentioned work of Kathrin Steck and colleagues is the only firm evidence in support of such an ability in any species [40]. Although there has been some suggestion that pigeons may be able to use 'olfactory scenery', to navigate [32, 46] these results are controversial [37] and relate to chemical gradients over much larger scales of hundreds of kilometres versus just metres for the results with ants. The studies of Steck et al. on the olfactory landmark navigation abilities of *Cataglyphis fortis* will now be summarised to motivate the extent of the claimed capabilities.

1.3.3.1   Smells like home: desert ants, Cataglyphis fortis, use olfactory landmarks to pinpoint the nest, Steck et al. (2009)

In this initial study, the authors first demonstrated the presence of features in the *Cataglyphis fortis'* natural environment that have the required properties to be used as olfactory landmarks. They showed that there are distinct odour blends associated with different ground structures of the natural salt-pan habitat of *Cataglyphis fortis* that are both perceivable by them, as indicated by measurable electroantennogram [2] responses to components of the odour blends, and had relatively stable mix ratios over two consecutive days of collection.
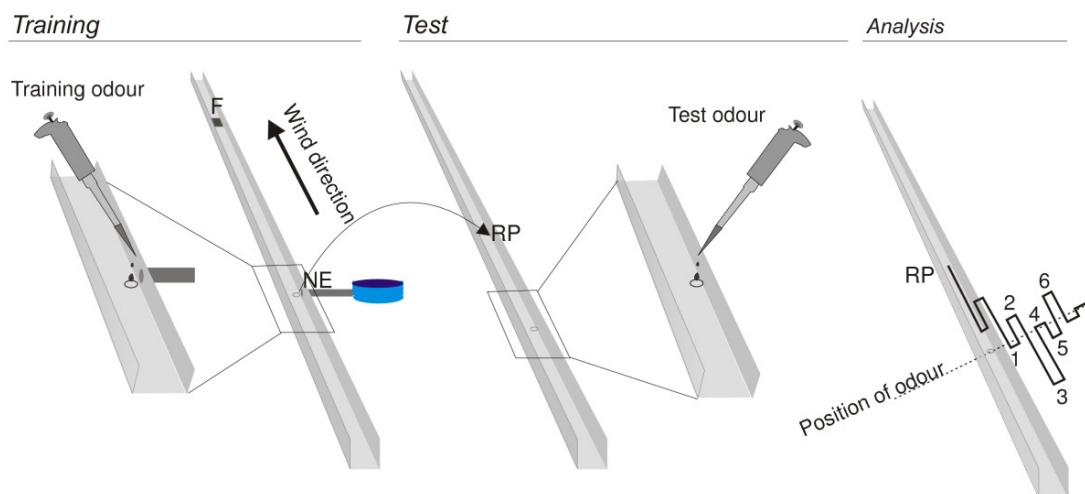

Figure 1.6: Experimental set up used in Steck et al. (2009) study. Taken from [41].

They then moved on to a behavioural experiment designed to test if the ants could learn an association between a specific odour cue and the location of their nest entrance, the diagrammatic overview from the paper of the protocol used shown in figure 1.6. *Cataglyphis fortis* foragers were trained to forage in open-topped linear channels, aligned with their long axis along the prevailing wind direction and with a feeder at 8 m downwind of a visually inconspicuous hole given them access to their nest from the channel. A single monomolecular odour landmark was periodically applied at the location of their nest access during training. Four different odour chemicals were used in separate training periods, two of the four chemicals having been found as components in the location specific odour blends identified in the ants natural environment and all four eliciting a electroantennogram response and being neither innately attractive or repulsive. Trained foragers returning to the nest access point with a food item

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[2]A method of recording the average electrical response of an insect antennae to an odour stimuli, measured by inserting electrodes in to either end of a detached antennae and amplifying the potential across them.

were then captured and introduced into a separate test channel with an odour stimulus applied at a point under several different conditions:

1. The same odour as used in training.
2. A different odour to that used in training.
3. A blend of four monomolecular odours, including the training odour.
4. The solvent used to create the odour solutions in isolation.

Ants which were tested with the same odour as they were trained with had a search pattern about the odour location that was tighter by a statistically signficant amount over the search patterns of ants tested with non-trained odours, an odour blend or solvent control. Additionally the ants tested with the odour blend showed a significantly tighter search pattern compared to when tested with the solvent control, suggesting that they were still able to pick out the trained odour within the blend but that the confounding background odours introduced extra uncertainty.

1.3.3.2 Do desert ants smell the scenery in stereo?, Steck et al. (2010)

In this follow up study the authors considered whether *Cataglyphis fortis* ants are able to use olfactory cues from a two-dimensional array of odour sources to navigate with and whether their ability to do so was affected by the availability of bilateral olfactory input from both antennae.
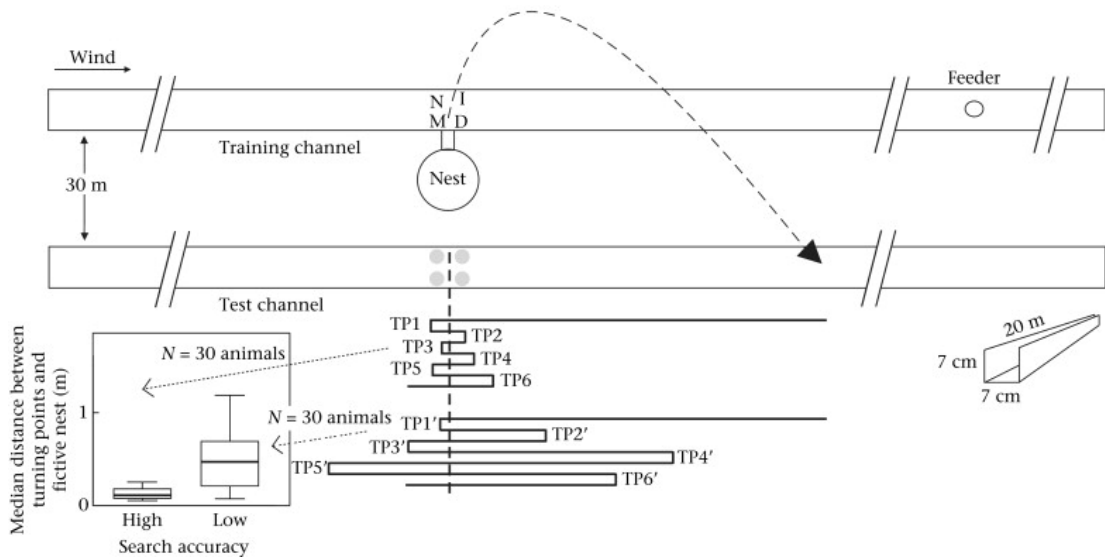

Figure 1.7: Experimental set up used in Steck et al. (2010) study. Taken from [43].

The experimental set up, shown in figure 1.7, largely followed that in the just described previous study, with the ants being trained in open-top linear channels with a feeder provided downwind of a inconspicuous hole in the channel giving access to and

from their nest. Here however rather than using a single odour stimulus applied at the nest access location, a square array of four different monomolecular odours (the same four odours as used in the previous study) were applied around the nest access point.

To check the odour concentration distribution produced by their array and thus the olfactory information available to ants within its vicinity, the authors measured the concentration of a tracer gas released from a point on a section channel equivalent to the location of one of the odours in the array and measured its concentration over a 1 cm grid using a *photo ionisation detector* (PID). They then constructed an indicative representation of the (normalised) joint concentration distributions resulting from all four odours in the array by applying suitable translation and mirroring transformation to the measured tracer concentration distribution. From these results the authors argue that there will be place specific blends of odour within the channel and that the concentration distributions differ over a spatial scale as small as the ants antennal separation (this distance is not stated explicitly but from the accompanying figure it appears a separation of approximately 1 cm was used). Although providing some useful information about the likely spatial extent of the odour plumes emanating from the odour array, it should be noted that the authors interpretation of this data in terms of the olfactory environment experienced by the ant seems a bit of a stretch as the smooth continuous concentration distribution measured would seem to indicate the temporal resolution of the the PID used was insufficient to resolve the intermittent temporal structure of the tracer plume.

The behavioural tests were conducted similarly to the previous study, with ants trained with the odour array in place captured before entering the nest access point with a food item and placed in a separate test channel. Here alongside the manipulations of the odour stimuli in the test channel, with either the original array configuration being used or one in which each pair of odours across the channel in the array swapped positions (hence what was previously on the left of the ant when moving downwind along the channel was now on the right and vice versa), the authors also compared the performance of ants which were both trained and tested after having one antenna surgically removed to those with both antennae intact. To control for general changes in search accuracy caused by the antenna removal, results where also collected with ants with one and two antennae when trained and tested with a blend of odours applied to a single point, i.e. equivalent to the protocol in the previous study.

The authors found that the search accuracy of ants when tested with original odour array configuration they were trained with was significantly higher than the accuracy of the ants tested with the swapped array configuration, suggesting the ants were somehow using the relative locations of the odour sources to either their left or right when

navigating. Ants with only a single antenna showed significantly lower search accuracy when tested with the odour array configuration they had been trained with compared to ants with both antennae intact, but showed no significant difference in performance when tested with a single odour source they had been trained with compared to the performance of intact ants in an equivalent test with a single odour. This suggests the simultaneous input from both antennae was important to the ants when navigating using the array of odours; in the terminology of the title of the paper, they are able to 'smell in stereo'.

Tests where also conducted on intact ants trained with the full odour array, with only two of the four odours present during testing. It was found that all changes from the training arrangement lead to a significant decrease in search accuracy other than when the two odours applied upwind of the nest access point remained in their original positions with the two downwind odours removed. In this case search accuracy was not significantly different from the case where all four odours where present in their original configuration suggesting these two odours along provided a sufficient landmark to guide the ants navigation.

1.4 Aims of this work

I will conclude this chapter by setting out what the aims of the work which will be described in this dissertation were.

In the studies of Steck et al. just discussed, the ants were confined to linear channels aligned with the wind in both training and testing. Although useful (and as will be discussed later perhaps essential) as a method for controlling the visual surroundings of the ants between training and testing and simplifying tracking of the ants during testing, this set up is obviously not representative of the environment that would be experienced by the ants naturally.

The narrow width of the channels meant the ants search was effectively reduced to being in one-dimension, this reflected in the results recorded with the ants search trajectories only being recorded along the longitudinal axis of the channels. This restricted the complexity of the task when compared to searching in the open conditions that would more typically experienced by the ants.

Further it is unclear from the current studies how important having the channels aligned with the wind direction is. It is not stated in the papers how the wind direction was measured for aligning the channels or whether the orientation of the channels needed to be changed at all during training or testing to maintain alignment with the wind (though it seems unlikely the channels would have been moved during training at

least given the fixed connection to the ants nest). The extremely flat and featureless nature of the salt-pan environment the fieldwork was conducted in may have meant the wind direction was relatively constant. In the paper of Wolf and Wehner [55] on *Cataglyphis fortis'* use of odour plumes arising from food sources, it was noted that 'a fairly reliable southeastern wind blows in the desert habitat', with this study having being conducted in the same Tunisian salt-pan environment as the Steck et al. studies. The paper does however later goes on to discuss the effect of changes in the wind direction during a foraging run on an ant's navigation. Irrespective of whether the wind direction with respect to the channels was kept constant, the presence of the channels in itself will have affected the flow conditions and may have led to the production of odour plumes that where not particularly representative of those that would be experienced by the ants in reality.

These factors place some limitations on how much the behaviour observed in the studies can be interpreted as being applicable to the behaviour of the ants in their natural conditions (note that this is not intended to suggest the results of the original studies are in anyway invalid). The first part of the work that will be described in this thesis will be of a field study conducted in an attempt to further explore the olfactory landmark navigation abilities of *Cataglyphis* ants under less restricted conditions. Specifically the aim of the study was to try to identify if ants of another species of the *Cataglyphis* genus, *Cataglyphis velox* are able to use olfactory landmarks when navigating in two-dimensions in an open environment.

A further question posed by the results of the Steck et al. studies is what sensory information from the olfactory landmarks is used by the *Cataglyphis fortis* to guide their navigation. As noted above the constructed odour concentration distribution presented in the the second study by Steck et al. based on the PID measurements of a tracer gas, did not seem to reflect the known highly intermittent concentration distribution within odour plumes. Therefore its use as a justification for there being place specific odour blends that the ants could use to navigate with seems invalid as it does not appear to represent the sensory information available to the ants at a behaviourally relevant time-scale.

Further the large body of research on the strategies used by insects in the related task of odour plume following seems to suggest mainly the use of wind-direction information and binary detection of the presence of odour plume filaments by the olfactory system rather than the use of odour concentration levels. The second part of this project was therefore aimed at attempting to model the signals received by an ant's olfactory system from a set of olfactory landmarks and analysing what information would be available to the ant from these signals to guide their navigation.

Chapter 2

Can Cataglyphis ants use olfactory landmarks to navigate in two dimensions?

2.1 Introduction

In the 2010 study *Do deserts ants smell the scenery in stereo?* of Steck et al. [43] it was demonstrated that *Cataglyphis fortis* ants are able to use a square array of odour sources as olfactory landmarks when attempting to navigate to their nest entrance while within a narrow linear channel. This result naturally suggests the question of whether *Cataglyphis* ants are also able to use this remarkable ability to aid navigation in a less constrained open two-dimensional environment. Here a field study designed to try to answer this question will be presented.

2.2 Materials and Method

2.2.1 Field site

The fieldwork was conducted in an area of disused scrub-land to the south of Seville, Spain (37°19'58"N, 5°59'23"W). A photograph of the environment at the field site is shown in figure 2.1. As can be seen the environment is visually cluttered, with relatively dense coverage of grass and shrub patches. There are also a number of large man-made visual landmarks in the immediate vicinity including buildings, electrical distribution pylons and road lighting.

Figure 2.1: View of the cluttered scrub-land habitat at the field site.

Figure 2.2: Typical example of *Cataglyphis velox* forager used in this study with mm scale.

2.2.2 Animal model

Rather than the Saharan desert ant, *Cataglyphis fortis*, used in the studies of Steck et al., the ants used in this experiment were members of the related European species *Cataglyphis velox*. *Cataglyphis velox* members share many of the common features of the *Cataglyphis* genus being heat-tolerant, central place solitary foragers who mainly feed on dead arthropod carcasses and do not rely on the use of pheromone trails [22, 26].

Uniquely among *Cataglyphis* species they exhibit worker polymorphism, with there being a large range of worker sizes (lengths 4.5–12 mm [22]). Workers of different sizes forage at different times, with the less heat tolerant smaller ants mainly being outside the nest during the cooler hours of the morning and larger individuals foraging in the hotter near-midday period [22]. Within this study the foragers seen during both the training and testing periods were typically on the larger end of the typical size scale, an example being shown in figure 2.2. Thus the ants tested here were of a comparable size to *Cataglyphis fortis* ants, with a typical body length range of 5.5–9.6 mm [18].

Figure 2.3: Inconspicuous nest entrance of *Cataglyphis velox* colony used in this study.

The cluttered environment *Cataglyphis velox* ants exist in is very different from the feature-deficient salt-pans that *Cataglyphis fortis* are found in, with the large number of plants acting as both potential visual landmarks but also obstacles. This environment poses different navigational challenges to the ants and *Cataglyphis velox* are thought mainly to use visual landmark route memories as their main means of navigation [26] over long distance as opposed to the mainly path integration based navigation of *Cataglyphis fortis*. The range of foraging runs of *Cataglyphis velox* is also reduced, with a maximum observed search distance of 60 m [26] compared to hundreds of metres for *Cataglyphis fortis*. Similar to *Cataglyphis fortis* however, the nest entrances of *Cataglyphis velox* tend to be visually inconspicuous (for an example see figure 2.3), thus they face a more similar navigational challenge to *Cataglyphis fortis* for the final part of a return run to the nest entrance, having to rely on visual and other landmarks around the nest entrance to pin point its exact location.

As well as the differences in the visual experience of *Cataglyphis velox* compared to
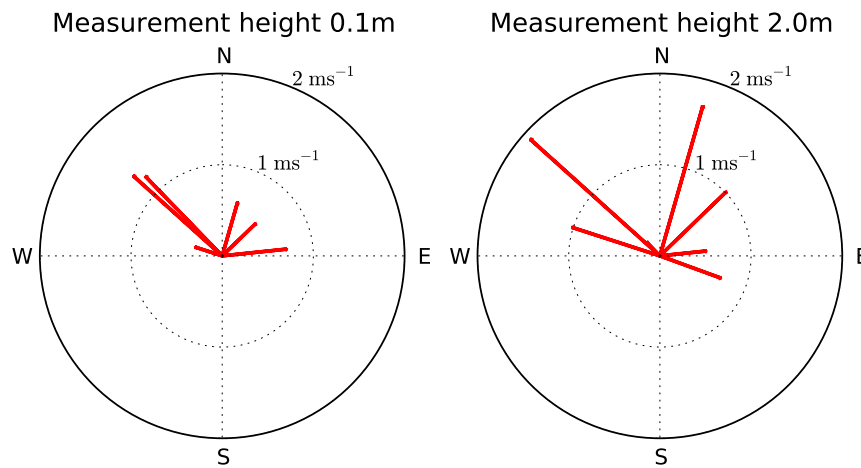
Figure 2.4: Polar plots of wind velocities measured at approximately 20 minute intervals between 12:00 and 14:00 at field site.

Cataglyphis fortis, it seems likely that the olfactory experience of the animals also differs in some significant ways. The wind velocity was highly variable during the time spent at the site with the velocities measured over a typical two-hour period shown in figure 2.4 (measured using a hand-held anenometer oriented along the direction indicated by a thread tell-tale, with each measurement being a 5 s average to smooth out high frequency variations). As can be seen both the magnitude and direction of the wind velocity varies significantly over this relatively short period, with the direction of the wind covering an almost 180° range at the higher measurement height.

This contrasts with the fairly constant wind velocity that seems to be the norm in the Tunisian salt-pan environment of *Cataglyphis fortis* [55], and it is likely that such dynamic flow field will have made learning an association between olfactory landmarks and a nest location much more difficult. Although this level of variability had not been anticipated, in retrospect it seems apparent that much more cluttered environment at the field site, will lead to highly turbulent wind flow with eddies being produced at a wide range of scales corresponding to the variety of structures present. The large amount of plant life and also other competing ant species at the field site also mean the range of odour volatiles naturally present at the field site is likely to have been significantly larger than in the environment of *Cataglyphis fortis*, on the one hand potentially providing a greater range of natural olfactory landmarks but also making their use more difficult due to the high level of background olfactory 'noise'.

2.2.3 Odour chemicals

Three of the four monomolecular odour chemicals used by Steck et al. in their studies were used for the olfactory landmarks in this study - methyl salicylate, nonanal and decanal. Although it would have been preferable to include the fourth chemical used, indole, as well, its classification as a dangerous good due to its toxicity meant that it was not possible to transport the chemical by air to Spain. Although there is no direct evidence that the three odour chemicals used are perceivable to *Cataglyphis velox* individuals, it was assumed that the evolutionary closeness of *Cataglyphis velox* and *Cataglyphis fortis* means that their olfactory systems will be responsive to the same chemicals.

The hexane solvent used by Steck et al. as the base of the odour solutions is a highly flammable liquid and so also has severe restrictions on its transport. Therefore instead an alternative solvent propylene glycol was used. Propylene glycol was chosen due it having the required property of the three odour chemicals being soluble in it, while also being a non-flammable and non-toxic chemical that was practical to transport. It has no strong odour to humans, though unfortunately it was not possible to test if it elicited a strong sensory response in *Cataglyphis* ants due to time and resource constraints.
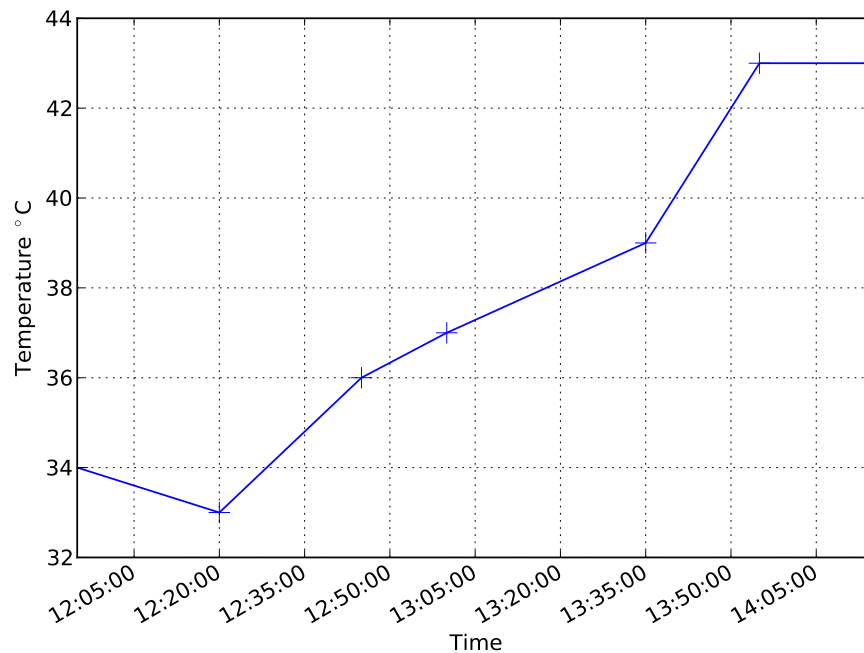

Figure 2.5: Time course of the temperatures at the field site location over the a typical midday period when the large *Cataglyphis velox* foragers were active.

The solutions were prepared in the same 1:50 mass ratio of solute (odour chemical) to solvent as used by Steck et al. Compared to hexane, propylene glycol is much less

volatile (a necessary consequence of using a non-flammable solvent) and so the odour solutions used here were likely to be evaporate at a reduced rate compared to those in the Steck et al. studies, the lower ambient temperatures (a typical temperature time course at the site is shown in figure 2.5) likely further adding to this. This issue may have been ameliorated slightly by the application of the odour solutions to raised pegs which will have increased the velocity of the local airflow around the odour sources and so hopefully the rate of odour dissipation. Rather than applying $20\,\mu\ell$ of the odour solutions every 20 minutes as done in the Steck et al. studies, instead a larger volume of $40\,\mu\ell$ (measured using capillary micropipettes) was applied every 30 minutes, largely for convenience reasons.

2.2.4   Olfactory landmark arrangement

In common with the Steck et al. 'Do desert ants smell the scenery in stereo?' study a square array of four odour sources was used as the set of artificial olfatory landmarks that it was attempted to train the ants to associate with the nest location. As only three distinct odour chemicals were available, methyl salicylate solution was applied to two diagonally opposite points in the array, with the choice of this odour solution as being the one to repeat arbitrary. Although using four distinct odours would have been preferable, in the Steck et al. study it seemed that the ants were still able to successfully localise where the nest entrance should be during testing when only the two upwind odour sources were present. It was hoped therefore that it would still be feasible for the ants to be able to navigate using three olfactory landmarks instead of four.

2.2.5   Overview of experimental protocol

An overview of the set up of the experimental protocol used is shown in figure 2.6. Two sets of removable, visually identical pegs were arranged in square arrays around the nest entrance, each having the same side length of $40\,\mathrm{cm}$ but offset from each other in a diagonal direction by $30\,\mathrm{cm}$. During training each of the pegs in the square arrays indicated by a filled circle in figure 2.6 had one of the three odour solutions regularly applied to it forming a set of 'with-odour' pegs. The other set of pegs, had no odour applied at any point during training or testing and so will be termed the 'without-odour' pegs.

The intention was that the with-odour pegs would form the set of olfactory landmarks around the nest entrance that it was hoped the ants would learn to associate with the nest location, with the ants being encouraged to forage in the immediate surrounds of the nest during the training period by the placement of artificial feeders to
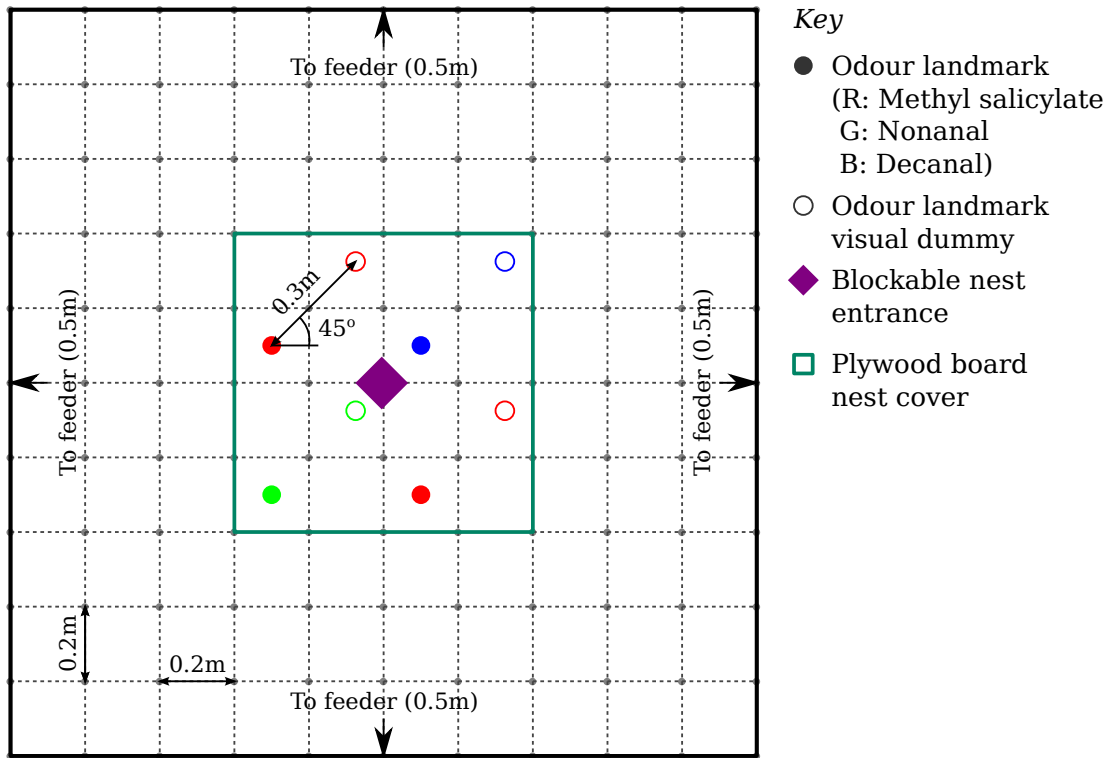
Figure 2.6: Diagrammatic plan view of experimental set up used.

facilitate the learning of this association.

During testing access to the nest entrance was blocked, and the path taken by a forager which had collected a food item recorded by noting its position within a reference grid at regular time intervals. Due to it holding a food item the ant should have been motivated to try to return to the nest and so started to search for the (blocked) nest entrance. The grid-binned positions of the recorded ant's path form a measure of the amount of time the ant spent searching at any particular bin in the grid - a search density map.

Search density data was collected for two separate test conditions. In the CON-TROL condition, the with-odour pegs were left in their original positions during the test period, hence the olfactory 'scenery' experienced by the ant was the same as that in training. In the SHIFT condition, after blocking the nest entrance and before recording was begun, each of the with-odour and without-odour pegs at the corresponding positions in their square arrays were swapped. In this case the olfactory scenery experience by the ant during testing was now shifted by 30 cm, with the visually identical nature of the pegs meaning the visual scenery has remained unchanged.

The expectation was that in the CONTROL conditions the ants search density distribution should show a peak at or near to the nest entrance, with both visual and olfactory landmarks in this case guiding the ants to the correct nest location. The

hypothesis being tested was that: *Cataglyphis velox* foragers are able to form an association between the provided olfactory landmarks and the location of the nest entrance, are able to use the sensory information they receive from the olfactory landmarks to navigate in two dimensions *and* will still use the information they receive from olfactory landmarks to some degree when navigating in the presence of other conflicting cues. If this hypothesis is correct then it should be possible to observe an offset in the peaks of the search density distributions of the SHIFT condition ants, relative to the peaks of the search distributions of the CONTROL condition ants, towards the location where the shifted olfactory landmarks would suggest the nest entrance is.

2.2.6 Site preparation

Figure 2.7: Photograph of prepared experimental site.

A single colony of *Cataglyphis velox* ants was used for all testing. Vegetation within a 1.5 m radius of the nest entrance was first cleared back to improve ease of access and minimise changes in the ants visual scenery over the course of training and testing due to the inevitable damage to the plants that would be caused by regular movement of myself and others around the nest entrance.

Coarse sand was laid around the nest entrance to form a level surface and then two plywood boards fitted in place on top of this levelled area, with the boards fixed in place with long nails driven directly in to the hard ground surface below the sand. A small square section was cut out from the middle of the boards at the location of the nest entrance and retained to use to block the ants access to nest during testing.

The boards primarily served to ensure there was only one, blockable, access point to the nest. They were also used to account for the possibility of the odour solutions becoming impregnated in the ground during their repeated application to the with-odour pegs during training, due to drips or spillages. As this would later provide conflicting olfactory cues when the locations of the with-odour pegs were shifted during testing, a previously unused set of boards was laid down before beginning the SHIFT condition tests. To try to ensure the ants could easily gain access on to the board even when carrying a large food item, sand was used to create smooth ramps up to the external edges of the board.

A 20 cm spaced square grid was marked around the nest entrance (extending 1 m in all directions from the nest entrance point), with pen marks being used on the plywood boards and small nails driven in to the ground in the surrounding uncovered area.
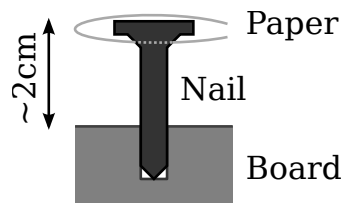

Figure 2.8: Diagram showing construction of olfactory landmark 'pegs'.

Two sets of four holes, each defining the corners of a 40 cm square, were made into the nest plywood cover boards. Into each of these, nails with a square of paper folded around the head were placed as shown in figure 2.8, these forming the two sets of with-odour and without-odour swappable peg arrays explained above.

Due to the large disruption caused to the ants by this preparation of the nest location, no actual training or testing was carried out till the days subsequent to that in which the site was prepared.

2.2.7 Training

During training the odour solutions were applied at 30 minute intervals to the paper carriers on the with-odour set of pegs, at this point these being positioned at the locations defined by the filled colour circles in figure 2.6.

To encourage the ants to forage in the immediate vicinity of the nest and so gain experience of the olfactory 'scenery' produced by the odour array, four feeder sites were established at 1.5 m distance along each cardinal direction from the nest entrance. The feeder sites consisted of crumbled dry biscuits with the ants being allowed to freely forage at them and the feeder sites regularly restocked.

The ants were not individually marked, therefore to try to ensure as large a propor-

tion of ants as possible had gained some experience of the olfactory landmarks while on foraging runs, two days were spent training the ants before any testing was carried out. On these days the odours would start to be applied at around 11:00 before the peak foraging period began and continue to be applied till the foraging activity dropped again to a low level, typically around 14:00.

2.2.8   Testing

Testing was carried out on two consecutive days following the initial training period. All CONTROL condition tests were carried out on the first day of testing and all SHIFT condition tests on the second. Ideally testing of the two conditions would have been interleaved to control for changes in the environmental conditions over the two days. However it was judged necessary to place new boards over the nest for the SHIFT condition, to eliminate any odour cues coming from the surface immediately below where the with-odour pegs had been placed during training as a result of the odour chemicals from the pegs becoming impregnated in the wood due to drips from the pegs, accidental spillages, or absorption from the air. This removal and laying down of new boards needed to be carried out before peak foraging activity began to minimise the disturbance to the ants, and so it was not feasible to swap between the two conditions on the same test day.

During the first hour of foraging activity on both of the test days, the odour solutions were applied at regular intervals to the with-odour pegs in their original positions. This was intended to provide recent experience of the olfactory landmark array to the foraging ants. After this initial retraining period, testing began.

Testing was initiated when a forager was identified as having picked up a food item at one of the feeder locations and was about to return to the nest. At this point the nest entrance was blocked and the current wind velocity recorded. For the SHIFT condition tests the with-odour and without-odour pegs were also swapped at this point. During testing any other ants which had also been outside of the nest foraging at the time the nest was blocked were also unable to regain access. Although it would have been beneficial to have multiple people recording from different returning ants simultaneously and this had originally been planned, in practice the ants did not pick up food items from the feeding site regularly enough for there to be times when two ants began a return run within a short time of each other and so could be recorded simultaneously.

Recording of the ant's grid position was begun after it first arrived on to the board covered area, recording beginning at this point rather than when it first entered the grid to reduce differences in the initial search path due to the location of the feeder the search was started from. The grid square the tracked ant was currently within was

manually marked on a corresponding paper grid at two second intervals, with timing being directed by an audible metronome. Recording was intended to be carried out for 300 s giving 150 recorded positions per test ant. However in practice it was not possible to use a consistent recording time for all of the ants. This was a result of several factors:

- Human error in the timing process due to difficulties in coordinating all the steps required at the beginning of the tests.
- Ants dropping the food item they were carrying mid-test mean recording had to be halted as they no longer had a clear motivation for returning to the nest.
- Recorder losing track of a food carrying ant due to another food carrying ant passing in close proximity.

In the results that follow only test runs in which a minimum of two-minutes of data, i.e. 60 points, were recorded will be included.

After a test run was finished, the nest block was removed and in the case of SHIFT condition tests the with-odour and without-odour pegs returned to their original positions. Ideally a substantial time period would have been left between sequential tests to allow the ants time to restrengthen any association formed between the nest location and olfactory landmarks, with this possibly being weakened during testing due to the conflicting information provided by the blocked nest entrance (and shifted olfactory landmarks in the case of the SHIFT condition). Time constraints and a low number of ants successfully picking up food items from the feeder locations however meant that recording was restarted as soon as a viable ant was identified.

2.3 Results

Unfortunately due to the time it took to establish a usable protocol and problems with the ants showing poor motivation to forage at the feeders, only a small number of valid search density data sets were able to be collected for each of the CONTROL ($N = 5$) and SHIFT ($N = 3$) conditions. The data collected from two of the SHIFT condition ants was excluded from analysis:

- One due to the ant dropping the food item mid-test before the minimum of two-minutes of test data had been collected.
- One due to the ant having remained stationary for a long period in the test after the particularly large food item it was carrying became stuck in a gap between the edge of the plywood board and sand ramp, leading to a spurious strong peak in its search density distribution at this location.

Along with several remaining methodological issues the lack of data limited the useful analysis or interpretation could be made from the results.
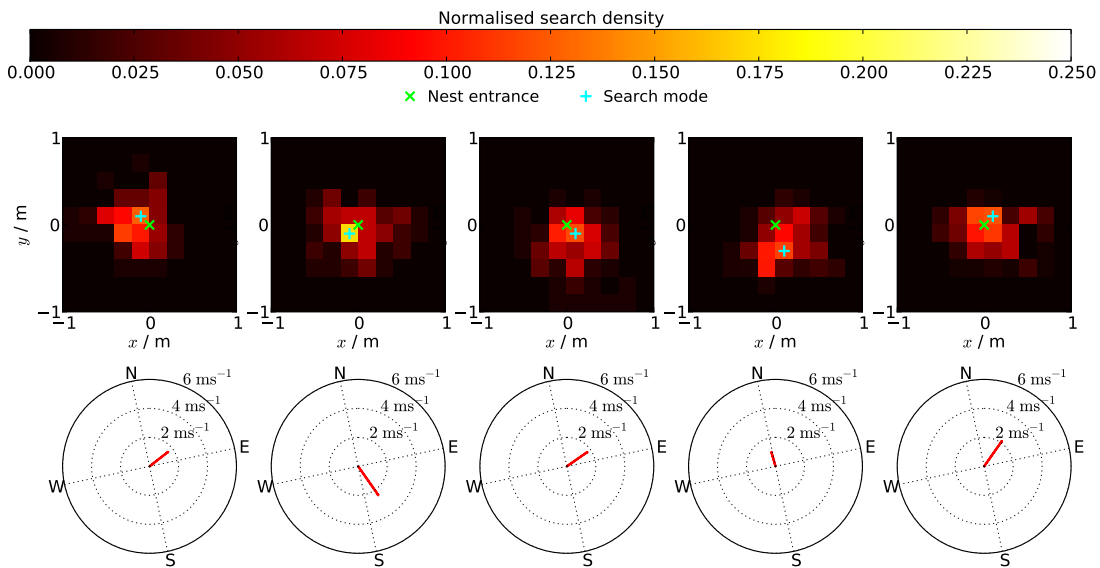
Figure 2.9: Results from CONTROL condition ants with odour landmarks in the same positions as training. Top row of boxes show heat maps of normalised search density of ant within a 0.2 m spaced grid around the nest entrance (the origin of coordinate system and marked with a green ×), with the mode search density being marked with a cyan +. The bottom row of polar plots shows the wind velocity measured just before search density recording was begun.

Figure 2.9 shows heat maps of the normalised search densities (i.e. the proportion of time spent in each grid box) of the five ants results obtained for the CONTROL condition. As can be seen all of the ants performed a search which was generally concentrated around the nest entrance location, with the mode of the search distribution of all but one of the ants lying in one of the grid squares adjacent to the nest entrance. The search distributions generally seem to be skewed such that the centre of masses of search densities and modes do not coincide, suggesting the ants are preferentially searching on on one side of the nest. There does not however appear to be any discernible pattern to this skew, and in particular it does not seem to be dependent on the wind direction recorded at the start of the test as might have been expected from the results in [55] and [7] which showed *Cataglyphis fortis* ants preferentially approached a target on the downwind side to try to pick up an odour plume. The wind directions recorded at the start of each test vary widely agreeing with the earlier observations of a highly variable wind velocity field.

Figure 2.10 shows the search density results for the SHIFT condition ants. It is immediately apparent that there is not any perceivable shift in the ants search distribution towards the apparent nest entrance location due to the shifted olfactory landmark array. Qualitatively the distributions seems to be very similar to those recorded for the
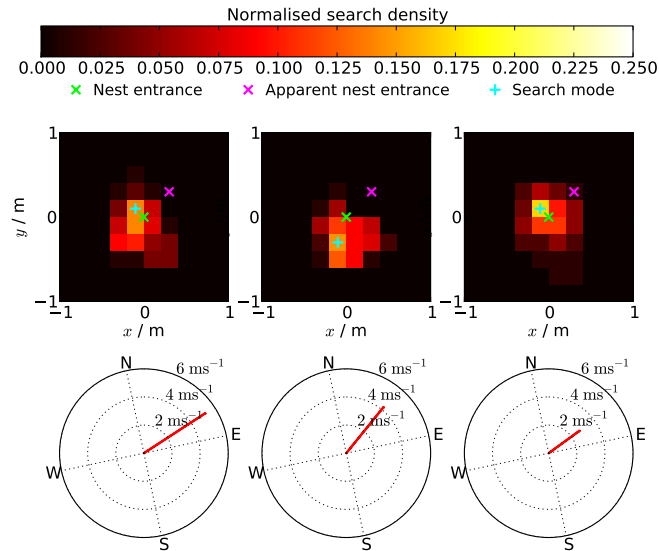
Figure 2.10: Results from SHIFT condition ants with odour landmarks shifted by 0.3 m NE. Top row of boxes show heat maps of relative search density of ant within a 0.2 m spaced grid around the nest entrance (marked with a green ×), with the mode of the search density being marked with a cyan +. The apparent location of the nest entrance according to the shifted olfactory landmark array is shown with a magenta ×. The bottom row of polar plots shows the wind velocity measured just before search density recording was begun.

control condition, with the search density modes being in grid squares adjacent to the actual nest entrance location in two of the three results sets recorded, and again the search distributions seeming to show some skew suggestive of the ants preferentially searching on a particular side of the nest entrance, but once more with no obvious pattern to this skew.

Quantitatively, the group mean of the distances of the search distribution modes (i.e. the centre of the grid square where the ants spent the most time during recording) from the apparent nest location due to the shifted olfactory array was 0.35 ± 0.06 m (mean ± standard error) for the CONTROL condition ants and 0.42 ± 0.09 m for the SHIFT condition ants. Therefore the peaks of the SHIFT condition ants search distributions were in fact *further* on average from where the olfactory landmark array suggested their nest entrance was compared to the CONTROL ants, though this negative effect was non-significant ($p = 0.30$ one-sided Welch's t-test).

It is worth noting that the wind velocities recorded prior to SHIFT condition tests are both consistently larger in magnitude and much less variable in direction than for the CONTROL condition tests. This substantial change in the quality of the wind flow between the first and second test days is likely to have lead to quite different characteristics in the plumes formed by the olfactory landmark odour sources and

highlights the issues inherent in separating the test conditions over different days.

2.4 Discussion

The results gathered in this study did not provide any support for the proposed hypothesis. The negative result however cannot be interpreted as evidence against the tested ants being able to use olfactory landmarks to navigate in two dimensions however. Any observable effect of navigation using the olfactory landmarks was likely to be weak due to the conflicting effects of navigation using the other sensory cues available to the ants. The extremely small sample sizes recorded for mean the test was not statistically powerful enough to provide evidence for such an effect. Further the variation in the wind conditions between the days the CONTROL and SHIFT tests may have influenced the ants ability to use the olfactory landmarks, making the results even less interpretable.

2.4.1 Conflicting effects

From my own observation of the ants while recording the test runs, it seemed very apparent that even though the nest block fitted tightly into the nest access hole that ants clearly still recognised the location of the nest entrance. Both the ant being tested and the other ants still present outside the nest when the block was put in place, focussed their movements very tightly around the nest block and at points even seemed to be attempting to push into the thin gaps around the edge of the nest block, with this behaviour being apparent in both the CONTROL and SHIFT condition tests.

That the results suggest the ants' searches were not completely focussed at the nest entrance is perhaps more a result of the ants attempting to find an alternative route in to the nest rather than any uncertainty as to the actual nest entrance location. In support of this suggestion was the discovery upon removing the board at the end of the testing that the ants had in fact opened up an alternative nest access via a route underneaths the boards with initial access from a small gap which they opened up between the sand ramp and board edge.

In terms of the cues that could have been being used by the ants to locate the nest entrance with the block in place, there are a variety of possibilities. Firstly and most obviously the visual landmarks around the nest, including the artificial ones introduced by the nest site preparation, remained in place during both the CONTROL and SHIFT conditions, and may have been sufficiently powerful in their effect on the ants navigation to completely overpower any effect due the ants use of olfactory landmark navigation.

An alternative, or additional, signal the ants may have been using to locate the nest entrance with the nest block in place is some form of vibrational cue from the

movement of ants below the boards. As mentioned in the introduction, it has been found previously that *Cataglyphis fortis* ants are able to use vibrational cues to navigate to their nest entrance [6] and other species of ant have been known for a long time to use communicate via vibration sent through solid substrates [34]. It is therefore feasible that the *Cataglyphis velox* ants being tracked during testing which showed clear recognition of the nest location with the block in place were responding to vibration cues due to the activity of their nest mates below the plywood board they were walking on. Indeed whenever the nest block was removed following a test there was a large amount of activity in the vicinity of the nest entrance, probably elicited by the disturbance caused by having the nest block put in place, which may well have being proving some signal to the ants above.

There may have also been some tactile cues from the board surface and seams at the edge of the nest block. This is supported by the observation of the ants sometimes appearing to 'feel' around the edge of the nest block during testing.

Another alternative still is that there may have been some none artificially introduced olfactory cues the ants were able to use to identify the nest location during testing. Ants, including *Cataglyphis* species are known to mark the immediate surrounding of their nests with various secretions [22] and it could well be that the *Cataglyphis velox* ants had marked the area around the nest entrance with some form of scent cue during the long training period, although this would seem to be less likely as an explanation for the continued ability of the ants to locate the nest entrance during the SHIFT condition tests, at the beginning of which new boards where laid down. Another possibility is that the nest block was not acting as a good enough seal to prevent a perceivable plume of carbon dioxide emerging from the respiration of ants within the nest, as discussed previous *Cataglyphis fortis* ants having been shown to use such plume to aid in their final pinpointing of the nest location [7].

The conflicting effects of some or all of these cues meant that even if the hypothesis was true, any effect due to the manipulated variable, the olfactory landmark array position, was likely to be very weak to the point of possibly being non-observable even with a much larger sample size. This was anticipated as being a potential issue before starting with the experiments, however attempts to find some way of overcoming this problem during the initial exploratory work at the field site did not produce any solutions.

My original plan before arriving in Seville had been to conduct training and testing in two separate locations with a circular visual barrier in place around both locations to prevent the ants using visual landmarks to navigate with, this being directly comparable to the method used by Steck et al. However there were a greater number of large visual

landmarks in the vicinity of field site than I had anticipated and it did not seem that it would be feasible to construct a high enough barrier to prevent any of these being visible to the ants at both locations. Building such a high barrier would also have created in issues in disrupting the wind flow.

Further to this in some early attempts at capturing foragers returning to the nest with a food item and transporting them to a distinct site, it was found that the ants generally did not seem to be motivated to even begin searching in the location they were moved to, instead dropping their food item and running off in a random direction. This suggested that the general concept of performing testing a separate site from training was potentially problematic.

This motivated the original decision to change to the experimental method finally used with training and testing taking place at the same location. Importantly this method also inherently meant most of the relevant extraneous factors remained constant between the CONTROL and SHIFT conditions. This meant if a positive result was found it would have provided strong evidence in support of the hypothesis.

2.4.2 Other potential design flaws

The side length of the olfactory array was chosen as 40 cm as this was twice the 20 cm resolution of the recording grid being used, with the intention of making it possible to identify during testing if the ants were able to gain enough information from the olfactory landmarks to search more densely at the grid square corresponding to the location of the nest entrance within the array. In retrospect this was overly ambitious given the likely small effect size. Further by positioning the odour sources much further apart than the 7 cm spacing used in the Steck et al. studies, the overlap between the plumes arising from each will have been smaller or even non-existent. This may have limited the ants ability to navigate using them if this is based on the simultaneous sensing of multiple sources.

The use of odour sources which were elevated above the ground (by approximately 2 cm) may have also created some important differences compared to the placement of the sources on the surface of the channel in the Steck et al. studies. Viscous effects mean the wind velocity field at the ground surface will be zero and will increase gradually through a boundary layer until some limiting free stream velocity is reached. In the Steck et al. studies the plume formed may have therefore remained laminar (non-turbulent) for a longer distance due to the lower velocities at the level it was situated. Further as already mentioned the channels used in the Steck et al. studies will have had some effect on the local wind flow and may have facilitated the flow transitioning to a turbulence at a higher velocity due to their smooth uniform nature while also

potentially having a sheltering effect and so reducing the wind flow speeds. This could have meant that was more useful information available to the ants in the Steck et al. studies in the immediate vicinity of the odour landmarks as the smoother concentration fields in laminar flow would potentially lend themselves more naturally to chemotaxis type behaviours.

These differences from the Steck et al. studies may be part of the reason no effect was observed here. It could be argued however that if olfactory landmark navigation is a naturally occurring behaviour rather than being limited to arising in artificial test conditions, it would seem necessary for the ants to be able to utilise landmarks that are sparsely distributed and at varying heights. Therefore the arrangement in this study although in some ways non-ideal is perhaps more representative of some of the challenges the ants would face naturally, with testing under such conditions one of the motivating factors for this study.

2.4.3 Environmental factors

As well as the issues with the experimental design used, there were some problems experienced during the study which were more unavoidable consequences of working in an uncontrolled field environment.

An issue throughout the training and testing was that foragers from another nearby *Cataglyphis velox* colony as well as foragers of other indigenous ants species tended to compete with the *Cataglyphis velox* foragers of the test colony for the food at the feeders. The foragers of the second *Cataglyphis velox* colony were particularly troublesome during testing, as at several points recording was accidentally begun with an alien forager after it was spotted picking up a food item from a feeder, only for it to run straight through the grid and off towards its own colony. The foragers of other ants species caused their own issues, with the test colony *Cataglyphis velox* foragers being deterred from using feeder sites which other ants species were using, this agreeing with the known behavioural submissiveness of *Cataglyphis velox* ants to other indigenous species in their habitat [22].

As well as these problems caused by other ants, the foragers of the test colony in general seemed to be poorly motivated to collect food from the provided feeders. It was suggested by one of the post-doctoral researchers I was working with at the field site that this may have been due to over-saturation of the colony with high-sugar foods, with the foragers being no longer motivated to collect more food of the same nutritional content, but instead that they would be seeking high-protein food items. Some impromptu experiments at establishing a feeder on the last day of testing using small flakes of cooked meat seemed to support this hypothesis, with this feeding site

receiving a much higher rate of foragers arriving from the test colony than the normal feeders using biscuit crumbs.

In addition to already mentioned problems with wind direction variability between the tests of the two conditions, which could have been potentially controlled for with more careful planning of the experiments, other issues were also experienced with weather conditions at the field site which would have been more difficult to account for. Thick cloud cover and the resulting low temperatures on one of the days lead to limited foraging activity occurring at the test colony for most of the day, a significant loss of time when only six days were available on site. Further the wind speeds encountered at the site during my stay seemed to be unusually high according to one of the researchers who has worked regularly at the location, this being quantitatively backed up by a seasonal wind speed average of $1\,\mathrm{ms}$-1 being measured at a nearby weather station for June when the field work was conducted [53] compared to a mean of $1.8\,\mathrm{ms}^{-1}$ (standard deviation $0.5\,\mathrm{ms}^{-1}$) for the CONTROL condition tests and $4.0\,\mathrm{ms}^{-1}$ (standard deviation $1.3\,\mathrm{ms}^{-1}$) for the SHIFT condition tests. This suggests even if the variability had been controlled for the high magnitude of the wind velocities may have meant the olfactory 'landscape' experienced by the ants may have been more challenging than they would usually have to face, perhaps further disincentivising the use of olfactory landmark information for navigation.

2.4.4   Choice of animal model

As a final point it should be noted that it may well be the case that for *Cataglyphis velox* olfactory landmark based navigation is simply not a advantageous enough behavioural asset for them to have developed the abilities shown by their *Cataglyphis fortis* brethren. As noted at the beginning of this chapter, *Cataglyphis velox* ants exist in a much more visually rich environment than *Cataglyphis fortis* and seem to be much more dependent on visual information for navigation [26] and so may not have been subject to same evolutionary pressure as *Cataglyphis fortis* to use such a wide range of sensory modalities for navigation purposes.

2.4.5   Proposed set up for a future study

Although as discussed in some detail above there were several problems in the implementation of the experimental method used here, the key limitations which prevented useful interpretation of the data were a small expected effect size of the olfactory landmark positions due to the presence of strong conflicting effects from other sensory cues and the small sample sizes of the data collected. The basic idea however did have merit in terms of effectively controlling changes in extraneous factors between the two

test conditions and so if the relative effect size of the manipulated variable could be increased and more data gathered it would seem viable for some useful results to be achieved. What follows are some suggestions of how the experimental set up could be improved, with the proposed changes summarised in figure 2.11.
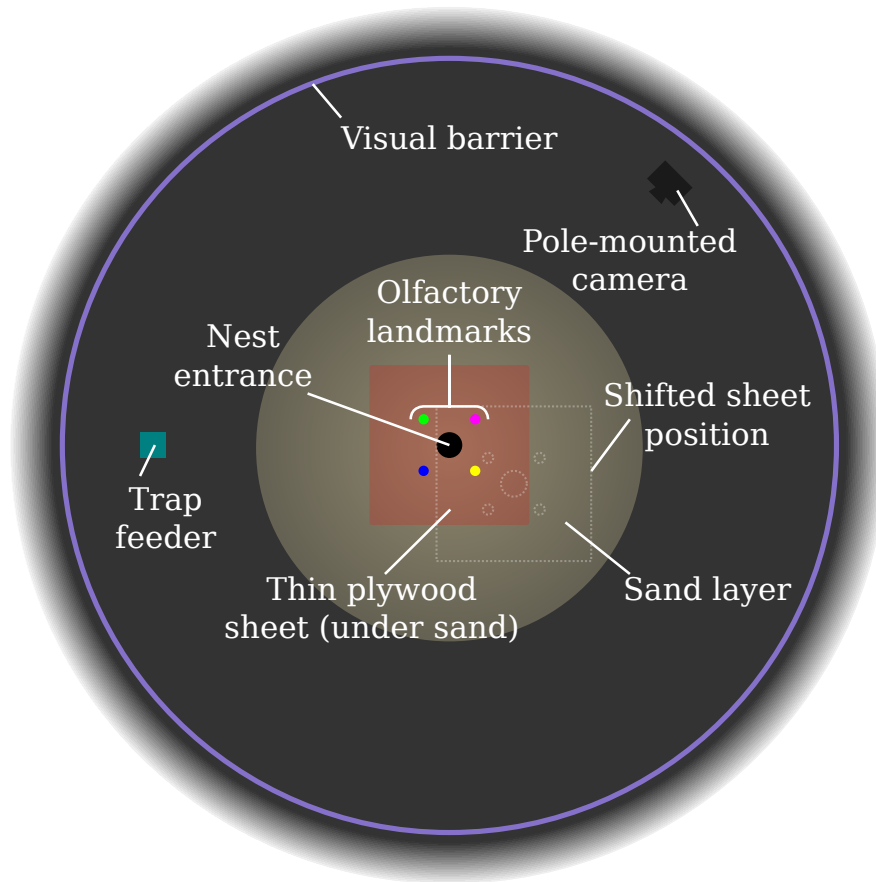

Figure 2.11: Plan view detailing layout of proposed field study method.

Increasing the relative effect size of the manipulated variable, the olfactory landmark array position, on the measured outcome, the shift in the position of the peak of the search distribution of the ants, could be achieved in three main ways - decreasing the conflicting effects from other sensory cues, increasing the effect exerted by the manipulated variable directly or decreasing the noise in the measurement of the outcome.

2.4.5.1 Reducing conflict effects

A relatively simple change that could be made would be to use a field site location where there are fewer large man-made visual landmarks in the immediate vicinity. If such a site was found it would then be more feasible to construct a visual barrier of a practical height in a circular configuration around the test colony nest that provides a uniform horizon at ant-level, with skyline contours thought to be an important visual

feature used by ants when navigating [13]. Such a barrier would also serve to discourage alien ants entering the test area and encourage the test colony ants to stay within the immediate vicinity and so have more time to learn an association between the nest location and olfactory landmarks. Ideally a barrier would be as low and as far as possible from the nest entrance while still eliminating (most) skyline cues to minimise the effect it has on the wind flow at the nest and to reduce the possibility of the ants using perspective based distortions of the horizon as a navigational cue. Within the barrier it would also be important to remove as many obvious visual landmarks as possible.
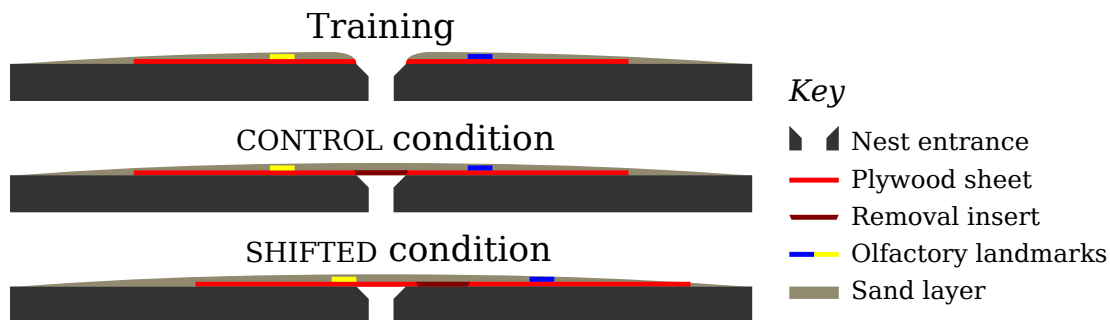
Figure 2.12: Cross sectional view of proposed field study method showing how position of landmarks and cover sheet would differ between training and the two test conditions.

The plywood nest cover used in the current study method itself potentially provided both tactile and visual positional cues to the ants due to the distinct texture of its surface compared to the surrounding ground, sharp edges and remaining seam when the nest block was in place. The odour 'pegs' also could have been used by the ants as visual landmarks. A potential solution to all these issues is to cover the sheet and surrounding area with a layer of coarse sand as shown in cross-section in figure 2.12. As with the previous method a section would be cut out of the plywood sheet and the hole placed over the colony nest entrance to allow the foragers egress and ingress during training. The cut section would retained and used as an insert to block access to the nest during testing. Rather than having the odours on removable pegs, the olfactory landmarks array would be constructed by applying the odour solutions directly on to the board / sand layer. Changing between the CONTROL and SHIFT conditions would then involve sliding the board including sand layer on top by the desired offset as shown schematically in figure 2.12.

To make the process of sliding the board easier, cord handles could be attached to the edges of the board and concealed under the sand when not being used. It might be possible to simply shift the board with the sand completely loose on top if done carefully, but if this is not feasible, the sand movement could be minimised using a

system of rails on top of the board or even by using adhesive to completely immobilise the sand layer above the board. Some levelling and smoothing of the sand at the edges of the board would be needed after a shift. Sand would also be spread over the nest block insert location when in place during testing to make it visually and tactically indistinguishable from the the surrounding area during testing. The layer of sand would hopefully also help to dampen any vibrations coming from the movement of ants in the nest below and to minimise the escape of any carbon dioxide or odour volatiles being emitted from the nest.

Although this proposed method would inevitably lead to some disturbance to nest during the shifting, importantly as there would now be no need to lay a new board down for the SHIFT condition and fix it in place with nails, it would feasible to interleave CONTROL and SHIFT conditions on the same day, thus helping to reduce the effect day-to-day changes in the wind conditions acting as a confounding factor due to conditions being tested on different days.

2.4.5.2 Increasing the effect due to the olfactory landmarks

The absolute effect size of the manipulated variable could be amplified in several ways. One possibility might simply be to use more concentrated odour solutions or use a greater volume in each application, though preferably the rate of odour molecule emission would still be plausible relative to the emission rates that would be expected of naturally occurring odour sources. Shifting the array by a larger offset between the SHIFT and CONTROL conditions could increase the effect size on the measured outcome, though there are practical limits to this in terms of the board and test area size. It is also possible that beyond a certain point increased shifts could have a detrimental effect due to the ant starting to weight residual cues from other sensory inputs more heavily if the information they provide appears more 'plausible' to the ants in some way.

A related issue that would be worth more exploration on site, is what the optimal olfactory array spacing is. It would be useful to use some form of visible tracer such as smoke to gain an idea of the scale of the plume structures formed under the flow and surface conditions at the field site. This could be then used to judge how wide the olfactory cues can be spaced while still allowing some overlap in the plumes they emit. Alternatively if time was available, the effect of the size of the olfactory array could itself be explored as a further manipulated variable.

2.4.5.3   Reducing noise in the measured outcome

The recording grid used in the current study only allowed relatively coarse search density measurements (equivalently it added a high level of quantisation noise) to be recorded. Additionally the use of nails to mark out the grid in this study also provided further potential visual landmarks, acted as a physical obstacle to the ants and would interfere with the sliding technique for shifting the olfactory array. An obvious alternative which would overcome the latter two issues would be to use nails only at the edges of the grid and create a overhead string lattice from these fixed at these edge points. This was in fact how the grid was originally constructed in the current study, however it was found that the uneven height of the ground meant it was difficult to ensure adequate clearance between the string and ground at all points within the grid and further that it was very difficult to move about within the resulting grid in order for example to apply the odour solutions or block the nest entrance.

There is also the issue of the inevitable human errors introduced with a manual grid based tracking system. I found it very difficult to monitor the position of an ant and transfer this position to the paper grid at even the relatively coarse spatial and temporal resolution used here. Several recording runs had to be abandoned after I completely lost track of an ant and in other situations mistakes will have been made when I temporarily lost track of the ant when looking down to record a position or when marking an incorrect grid square.

Some form of camera based tracking system would potentially allow search tracks to be recorded at a much higher temporal and spatial resolution and with greater accuracy, though it would not be without its own issues. Manually post-processing the frames would be extremely time consuming, while automatic tracking of small and quickly moving ants within video footage is non-trivial. Additionally the camera would in itself constitute a fairly strong visual landmark - the magnitude of this effect could be assessed by testing if the ants search distributions are altered by changing the cameras position, and the camera could be camouflaged to some degree if necessary.

2.4.5.4   Increasing sample size

The small sample sizes collected in this study were partly simply due to the limited time that was available for testing so the simplest improvement here would be to leave more time available to conduct tests. However the lack of data was also exacerbated by the non-streamlined nature of the testing procedure which led to a number of missed recording opportunities due to returning ants not being spotted in time and the presence of multiple ants in testing meaning it was difficult to keep track of which ant was being recorded. This situation could potentially be improved by the use of one or more trap

feeders (a feeder which foraging ants can enter but not leave independently, constructed from for example a plastic container in an excavated hole so its lip is at ground level) rather than freely accessible feeders used in this study. This would allow the number of ants moving around the nest during testing to be limited and for the timing of the start of an ant return journey to be controlled. This control of timing would possibly be essential with the added complexity created by using the sliding system to shift the odours in the SHIFT condition as the set of steps which it was required to complete at the beginning of each test in the current method (identifying a returning ant, measuring the wind velocity, putting nest block in place and swapping odour pegs if necessary, starting timer and metronome for recording) was already difficult to manage in time.

Chapter 3

What and how much information is available from olfactory landmarks?

3.1 Introduction

That Steck et al. found evidence in support of *Cataglyphis fortis* ants being able to extract location information from their sensation of olfactory landmarks is truly remarkable. As discussed in the first chapter of this thesis, in virtually all real environments the fluid flow governing the transmission of an odour signal from source to recipient will be turbulent. The highly chaotic nature of the dynamics controlling such flows are reflected in the highly complex structure of the received odour signals.

The description of the sensory input received by the ants as an olfactory signal here is not accidental - it is highly instructive to think of olfaction (and other sensory modalities) in signal processing terms. Olfactory landmark navigation can be considered as a decoding problem - the message being (some aspect of) the location of the odour source relative to the navigating animal and the medium of transmission the carrier fluid the odour chemicals are contained in.

Decoding this location message from the highly variable odour signal is an extremely non-trivial task. The intermittent nature of turbulent plumes means the signal is absent (zero) for much of the time and when it is present it tends to be in high amplitude and short duration bursts. Over long time periods averages of the odour signals measured at different points in a turbulent plume do show dependence on location relative to the source [24], however the time-scales of minutes or more required to perform some averages mean they are not behaviourally relevant to animals such as *Cataglyphis fortis* which are constantly moving.

In this chapter an information theoretic approach will be used to attempt to analyse what location information is available to *Cataglyphis fortis* in the odour signals

they receive from olfactory landmarks under the constraint of only receiving short sections of odour signal 'transmission' at any particular location. The advantage of using an information theory based approach is that it allows analysis of the how much information about location is fundamentally available to ants in the odour signals they receive without requiring strong assumptions about how the ants subsequently process and use that information when navigating. Given the lack of data on this topic such assumptions would necessarily need to be largely based on conjecture.

As discussed previously, it is clear that insects including *Cataglyphis* ants use sensation of the local wind velocity when following a turbulent plume to its source in addition to the olfactory signal they receive due to the odour concentration field. It seems very likely therefore that *Cataglyphis fortis* ants also use information from the local wind velocity field when performing the highly related task of olfactory landmark navigation.

Here however I will consider the information available in from the odour concentration field in isolation. This is partly simply due to practical constraints on the scope of what can be covered in this project. However it is also motivated by the fact that while the use of wind direction and binary odour presence information by insects when following a plume has been extensively studied previously and is thought to be understood at some level, there has been so far limited evidence for insects being able to use information from their perception of local odour concentrations to aid in navigation and even less in the way of proposed explanations for such an ability. The evidence from the studies of Steck et al. with *Cataglyphis fortis* strongly suggest that the ants are able to use such information however, hence the aim here to try to explore what aspects of the odour signal might contain the information they are using.

Although the use of an information theory approach was motivated above in terms of not requiring any assumptions about how the ants process the information they acquire from their sensation of the local concentration field, some attempt will be made to model the signal processing performed by the initial stages of the ants olfactory sensory system. It is clear that the ants are not able to measure the local concentration fields with infinite temporal resolution or to perceive the very wide range of concentration values present in plume concentration field with infinite precision. Further the peripheral nature of the initial stages of the olfactory system of insects and its ease of manipulation mean that it has been extensively studied and the results from this research can be used to construct a simple data-driven model of the initial stages of signal processing in the insect olfactory system that captures the more basic aspects of how it shapes the odour signal. From a more pragmatic point of view, to make an information theoretic analysis of the modelled concentration data feasible computationally it will be necessary

to reduce the dimensionality of the input space (technically infinite for a continuously valued signal) and it seems logical to achieve as much of this reduction as possible based on known limitations of the sensory systems involved rather than in a completely arbitrary manner.

3.2 Materials and methods

3.2.1 Plume model

In order to analyse what information is available to ants from olfactory landmarks, a necessary first step is identifying some suitable model for how the odour chemical released from a source is dispersed. In particular given the interest in the challenges inherent in using information from odours transported in turbulent plumes, some way of producing representative samples of how the odour concentration fields within a turbulent plume evolve over time is required.

As already noted however the fluid dynamics governing turbulent flow are highly non-linear and chaotic and *computational fluid dynamics* (CFD) simulations of turbulent plumes are incredibly computationally intensive even when significant simplifying assumptions are made about the flow properties. That even the forward problem of computing the expected time-varying concentration field arising from a specific spatial arrangement of odour sources and flow conditions is so computationally demanding in itself serves to emphasise the challenge posed by the inverse problem of trying to recover that spatial distribution of sources from only a very limited data about the concentration field. Given the computational resources required for CFD simulations, specialist software and technical expertise I decided against attempting to use them to model the turbulent plumes in this project.

Part of the challenge of CFD simulation of turbulent flow is the wide scale of spatial structures which are present in the flow fields as discussed in the introduction. Accurate simulation of the small-scale structure requires that a fine discretisation of the simulated spatial region is used and so large number of simulated elements, while the large-scale structures represent the presence of dependencies between the properties of simulated elements at long distances from each other which impedes the ability to parallelise the computations. However, although there are often clear patterns to the large-scale structure of the plume, such as sinuous meandering of the whole plume and the gradual increase in the cross sectional area of the plume envelope at increasing distance from the source, at a fine scale the chaotic nature of the dynamics mean the fluctuations in concentration values appear almost random.

This suggests a viable approach to producing a model which replicates some of

the main observed characteristics of a turbulent plume would be to use a physically-based dynamic model to simulate the variation in the flow field over a large scale while modelling the finer scale variations with stochastic processes which reflect the observed distributions of these variations. This allows a large-element size and so lower total number of elements to be used for the computationally intensive fluid dynamics modelling while still producing fine scale variation in plume structure which although not completely physically realistic reflects the overall statistics of the true small scale structure.

3.2.2   Farrell et al. (2002) puff-based odour plume model

The approach just described is that used in the odour plume model of Farrell et al. described in [12]. The authors use a simplified fluid dynamical model (in particular assuming a stratified flow such that their is negligible mean wind velocity along the vertical axis, a reasonable approximation for the flow field near to a solid surface, and neglecting viscous and Coriolis forces) to simulate the mean wind flow velocity field across a coarse grid discretisation of the simulated region. The boundary conditions for the grid are provided by a coloured noise process the parameters of which determine the size and frequency of any plume meandering.

The mean advective transport of small 'puffs' of odour[1] is determined by this wind velocity field. On top of this mean velocity, the odour puffs are modelled as having a further velocity component representing the mid-scale turbulent stirring of puffs. This velocity component is modelled as a white noise stochastic process, such that each puff is conducting a random walk on top of its mean wind driven advective transport which cause the puffs to disperse out from the plume centre line as they move downstream. This dispersion occurs in all three dimensions which is important as although it may be that the concentration values are only required at across a plane at a fixed height (as here), the dispersion of the puffs in the vertical direction has a significant effect on the plume structure.

As well as the advection based transport processes, the model simulates the diffusion based transport of the odour within the puffs causing them to spread out becoming more diffuse as time progresses. The concentration distribution within each puff is modelled as having an isotropic Gaussian form, with a radius parameter which grows with time determining both the current width and amplitude of the distribution such

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[1] The authors of the paper switch between using 'puff' and 'filament' to describe the parcels of odour, with making a distinction in terms of a puff being composed of multiple filaments released at the same time in their paper, but in their implementation of the model somewhat confusingly using puff to refer to what they termed a filament in the paper. I will simply refer to a single parcel of odour as a puff: the use of filament seems slightly confusing given the isotropic density distribution assumed for the parcels which is not at all filamentary.

that the total odour mass represented by each puff remains conserved over time. Again importantly a three-dimensional formulation is used for the puff concentration distribution model so that the model correctly reflects that the diffusive spread of the odour will occur out of the horizontal plane as well as within it.

Within their paper the authors compare the temporal and amplitude statistics of simulated concentration fields produced using their model against the concentration statistics measured for a turbulent plume in field conditions in [16] using an ion tracer system. They find generally good agreement between the lower order amplitude statistics of the model and field data and between the distributions of the temporal statistics, validating that model generates plume concentration fields which capture many of the basic properties of the concentration distributions within true turbulent plumes.

Although this model by no means provides a fully accurate representation of the olfactory signals that would be experience by an ant in a turbulent plume, I decided to use it to generate the plume concentration data in this study as I judged it to be a good compromise between the level of realism provided versus computational complexity and so difficulty in generating the large data sets needed.

3.2.3 Implementation overview

The authors provide binaries and `C++` source code for an implementation of their model integrated in to a demonstration Microsoft Windows application. Unfortunately the code did not appear to be particularly reusable, with tight coupling between the demo application code and the model implementation. Therefore I decided to create my own implementation of the model in `python` using the `numpy` scientific computing package. The spatial partial derivatives required for updating the wind velocity field are approximated using centred first and second order finite difference methods and all time integrations are performed with a forward Euler method. The underlying calculations performed by this implementation match the authors as far as could be seen from code comparisons and a qualitative comparison of the fields produced by the demo application to those produced by own implementation with the same parameters appeared to support this. Unfortunately the authors demo application does not provide any way to set the seed used for the pseudo-random number generator so direct comparisons of model runs under exactly the same conditions were not possible. One small change made was to allow to have the puffs generated stochastically at the source using a Poisson process rather than at uniform rate as it was felt this more accurately portrayed the variability seen in the rate of emission from an odour source in reality.

Figure 3.1 shows an example instantaneous concentration field produced by the model implementation overlaid with the instantaneous wind velocity field. The effects
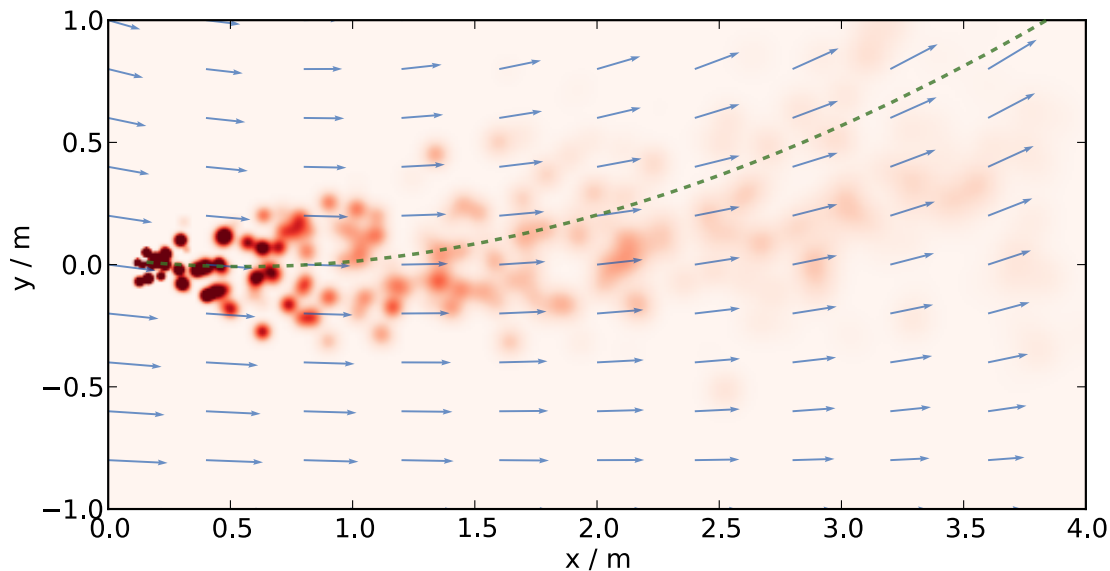
Figure 3.1: Example of the concentration fields (red colour-mapped image, dark red high indicate concentration, white low) and wind velocity fields (blue arrows show magnitude and direction of wind at tail point) produced with puff-based plume model across a plane at source height. The source is at position ($x = 0.1\,$m, $y = 0\,$m). The dashed green curve shows the plume centre-line, the path which would be followed by the puffs if there was no noise process causing them to disperse as they move downwind; note that it does not necessarily align with the local instantaneous wind velocities as it depends on the whole history of the wind velocity field up to this instant. The plume model used to produce this plot had a higher puff release rate (200 puffs per second) than used for simulations the subsequent analyses are based on, to make the plume structure more visible.

of the three modelled transport processes can all be seen - the plume centre-line follows a curved path due to the oscillations of the wind mean velocity field, the puffs originally start off tightly distributed around this centre line and become increasingly dispersed the further downstream they move due to the random walk dispersion model component while the distribution associated with each individual puff becomes increasingly diffuse as they move downstream.

An example concentration time course recorded from a point $1\,$m downwind of the plume source generated using the model is shown in figure 3.2. It can be seen that raw concentration signal has the required intermittent character, being near zero for most of the $5\,$s interval shown, with these periods of signal absence interspersed with bursts of strong concentration signal values.
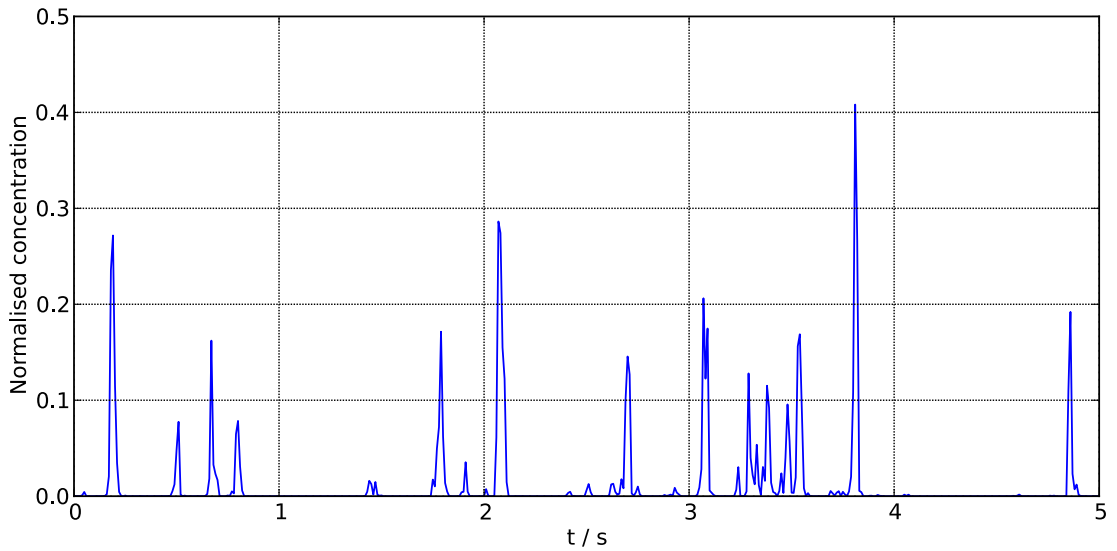
Figure 3.2: Example of the simulated concentration time course at a point 1 m downwind of the source generated using the puff-based plume model. It can be seen that the concentration signal is highly intermittent over time with bursts of concentration peaks followed by long (near-)zero periods.
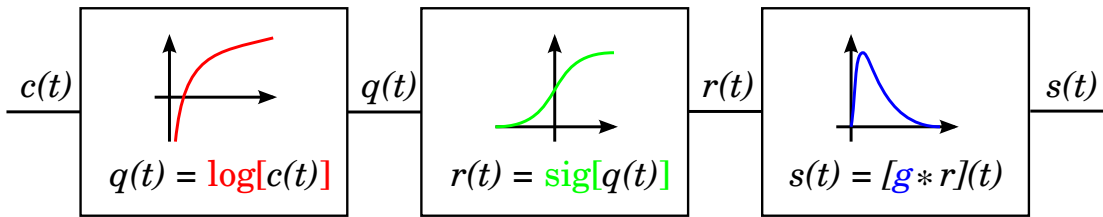

Figure 3.3: Block diagram showing overview of sensor model used. $\mathrm{sig}[\cdot]$ indicates a generalised sigmoid response function, $[\cdot * \cdot](t)$ indicates a convolution operation. The first two blocks together constitute a Hill equation response function (i.e. a sigmoid response on the logarithm of the input).

3.2.4 Sensor model

A simple sensor model design was chosen which attempted to reflect some of the main characteristics of the olfactory system of insects described in chapter 1. The basic form of the sensor was an initial non-linearity to model the concentration response of the olfactory system followed by a temporal filtering operation to model the temporal dynamics.

The non-linearity chosen was the Hill equation response function (equivalent to a sigmoidal response to the logarithm of the input) described in chapter 1. This was motivated by the previously discussed study of Sachse and Galizia [35] on odour coding in the honeybee antennal lobe, which showed that the glomeruli activity levels had a

Hill equation type response to the concentration of the presented odour stimuli. A Hill coefficient of NHill $= 0.3$ was chosen which was in the middle of the range found for the curves fitted to the glomeruli responses in the Sachse and Galizia study. The gain parameter R_{max} was set to one, the lack of any additive output noise meaning this parameter has no effect on the results. The half-saturation concentration was set to $K = 0.01$ based on a coarse histogram-equalisation based fitting using a sample set of concentration values produced using the plume model. This was motivated in terms of being representative of the contrast enhancement that would be performed by adaptation in the olfactory system, though here the fitting was performed only once and then the parameter left static rather than being dynamically varied to adapt to the recent sensory inputs.

The temporal dynamics were modelled with a simple mass-spring-damper type linear time-invariant system with a second order response, as described by the differential equation

$$\tau^2 \ddot{s}(t) + 2\zeta\tau\dot{x}(t) + x(t) = Gr(t)$$

where $s(t)$ is the time-dependent output, $r(t)$ the time-dependent input, ζ the damping coefficient and τ the time constant (inverse of bandwidth of system) and G the input gain. This form of response is very commonly used for modelling the temporal dynamics of physical systems, being able to simulate a more realistic range of temporal behaviours than first order systems while still having minimal free parameters.
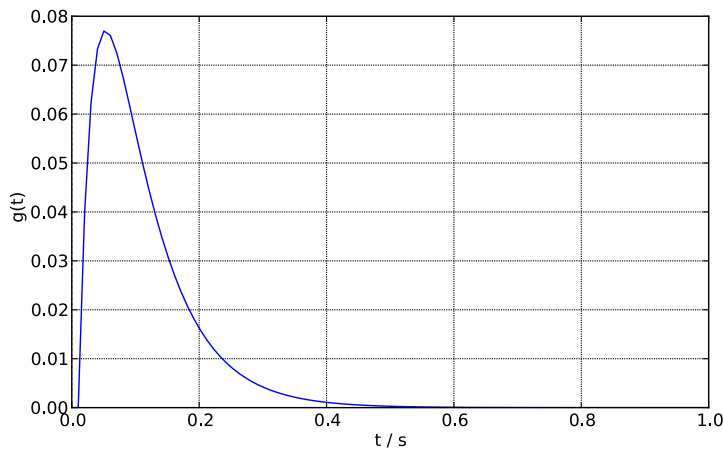

Figure 3.4: Impulse response of sensor model.

Based on the study of Lemon and Getz [21] of the temporal resolution of the cockroach olfactory sensory neurons, a time constant of $\tau = 50$ ms was chosen, this being the shortest pulse length it was found that the response of the sensory neurons could accurately follow for both a monomolecular and mixed odour stimuli. The gain parameter G was set to unit because of the arbitrary amplitude scaling in this model. The

damping coefficient was chosen as $\zeta = 1$, this representing a critically damped system. This choice was somewhat arbitrary but was based on the lack of observation of any oscillatory nature to the response in the Lemon and Getz study (suggesting $\zeta \geq 1$) and a desire to be as optimistic as possible with the temporal response properties of the sensor model to avoid losing information from the raw input concentration values that might be accessible to the ants. The impulse response of a system with these parameters is shown in figure 3.4 and the smoothing effects of the system on the sensor output are illustrated with example traces in figure 3.5.
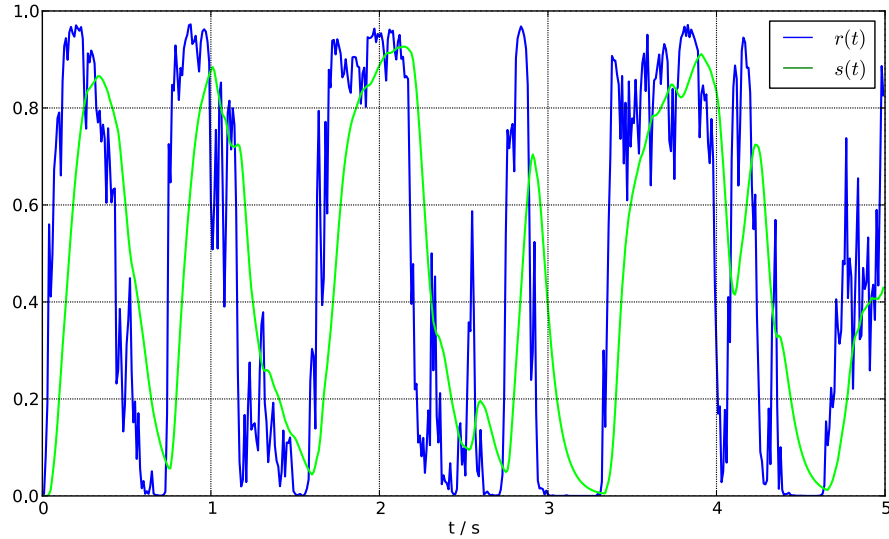

Figure 3.5: Example pre- and post-filtering outputs of the sensor model to illustrate the temporal smoothing effect of the filtering operation. The blue trace shows the result of applying a Hill equation response function to the raw concentration values calculated at a point 0.5 m downwind from the source using the puff-based plume model and the green trace the output of the sensor after convolving with the impulse response function in figure 3.4.

3.2.5   Information content analysis

The approach used to analyse the location information content of the modelled olfactory sensory signals was as follows.

The plume and sensor models were used to produce large datasets of the simulated olfactory signals measured at a set of discrete positions $\mathcal{P} = \{p_1 \ldots p_M\}$ in the plume. The sampling positions were in all cases regularly spaced along some axis, with the spacing between them the finest resolution at which the analysis would allow it to be established that the information from the odour signal was sufficient for the ants to be able decode their position with. As it was expected (and found) that any information

in the odour plume signal would only be able to provide a very coarse indication of location, the choice of a particular spacing was not likely to be a major limiting factor on the analysis and so arbitrarily chosen as 0.5 m.

Sets of N non-overlapping segments $\mathbf{s}_{i,j} \ \forall \ i = 1 \ldots M, \ j = 1 \ldots N$ of the simulated olfactory signals at the points $\mathcal{P}$ were then extracted. These segments are intended to model the olfactory signal that would be experienced by a navigating ant when at a particular discrete position p_i and so the segment duration $T_s = K\delta t$ (δt being the simulation time step and so K the number of time points in a segment) represents some rough measure of the time the ant would be remain close enough to the location to be able to neglect the effect of their movement. Although it would be more realistic to model the signal received by a sensor moving through the plume this was an added level of complication I did not feel would be viable to explore in the time available. Rather than use a fixed value for T_s where it was feasible to do so a range of values were used with the aim of exploring how the information available to the ants depends on this parameter roughly corresponding to how quickly they move when navigating.

From these olfactory signal segments feature vectors of $L \geq 1$ discrete values $f[\mathbf{s}_{i,j}] = \mathbf{v}_{i,j} \ \forall \ i = 1 \ldots M, \ j = 1 \ldots N$ were then extracted - a simple example is the quantised time average of the olfactory signal across the segment duration

$$f[\mathbf{s}] = \text{quantise}\left(\frac{1}{K} \sum_{k=1}^{K} \left\{ s^{(k)} \right\} \right)$$

The features vector values are constrained to lie in some set $\mathcal{F}$ with a finite size $|\mathcal{F}| = D \ \ D \in \mathbb{N}$, so that each feature vector can be mapped to some integer $V \in \{1 \ldots D\}$. Various choices for the features to extract from the segments were considered with the aim of finding the features which give the most information about which position p_i the segment the feature vector was extracted from was measured at.

Each of the feature vectors $\mathbf{v}_{i,j}$, constitutes a sample from the probability distribution $p(V \mid P = p_i)$. Providing the number N of sample feature vectors collected is large enough we can use the normalised counts of the number of feature vectors collected corresponding to each possible integer value of V to approximate the conditional probability distribution $p(V \mid P = p_i)$ for each $p_i \in \mathcal{P}$, which together with the fact that $p(P = p_i) = \frac{1}{M}$ by design (as an equal number of signal segments are collected at each p_i) means an approximation for the joint probability distribution $p(V, P) = p(V \mid P) p(P)$ is obtained.

From this approximation of the joint distribution, information theoretic quantities can be estimated. Of particular interest here is $\mathcal{I}[P, V]$ the mutual information between positions and odour signal features. The higher the value of $\mathcal{I}[P, V]$ the greater the amount of information, or equivalently reduction in uncertainty, about P knowing

V gives. Although the mutual information can be computed by 'plugging-in' the approximated join distribution in to the formula defining mutual information, in practice this is known to gives poor results in most cases, as the standard formula is a biased estimator for the mutual information when the joint probability distribution has been approximated from a finite number of samples. This is a well researched problem due to its relevance in the application of information theory techniques to problems such as neural coding, and there have been a number of improved schemes proposed for estimating mutual information from a limited set of samples. Of particular interest here is the `python` based `pyentropy` package developed by Ince et al. and described in [15], which provides easy to use implementations of these methods that are well suited for use with the `python` based models in this study, and so all information based quantities reported below where calculated with this package.

The reason for using some feature-based description of the signal segments rather than the simulated values themselves is that it is necessary to limit the size of the set of possible values the samples can take such that getting enough samples to be able to approximate the joint probability distribution is a feasible task. A $1\,\mathsf{s}$ segment of odour signal simulated with a time step of $\delta t = 0.01$ would contain 100 floating point values. If each of these values were quantised to $\ell geq 2$ levels, the set of all possible values will be of size ℓ^{100} which for any valid ℓ is an infeasibly large number. Therefore a massive reduction in the size of the set of possible values is required. Although $\mathcal{I}\left[P, V\right]$ will be limited by the loss of information on going from $\mathbf{s}_{i,j} \rightarrow \mathbf{v}_{i,j}$ it still provides a useful lower bound on the information available about location and further also allow comparison of how useful different features of the odour signal would be to the ants.

3.3 Results

3.3.1 Single statistic feature vectors

To begin with a number of different feature 'vectors' composed of single quantised statistics of the signal segments were analysed. In particular the following statistics of the signal segments were investigated: mean, variance, maximum value, ratio of maximum value to mean, maximum gradient magnitude and intermittency (here defined as the proportion of time the signal value < 0.3), these being chosen fairly arbitrarily but with the intention of reflecting a variety of aspect of the signal segments. In all cases samples where taken from $1000\,\mathsf{s}$ of simulated plume data. Two plume conditions were analysed - no meander (i.e. constant mean wind velocity field) and small meander (produced with boundary condition noise parameters bandwidth $0.2\,\mathsf{rad\,s^{-1}}$, gain 5 and damping 0.2), in both cases the mean flow velocity being $1\,\mathsf{ms^{-1}}$. The set of positions

$\mathcal{P}$ either was composed of 8 positions at $0.5\,\mathrm{m}$ spacing along the axis aligned with the mean wind flow direction and at zero cross-wind offset (streamwise positions) or 5 positions at $0.5\,\mathrm{m}$ spacing along the axis perpendicular to the mean wind flow direction symmetrically distributed about the plume axis and $1\,\mathrm{m}$ downstream from the source (lateral positions). Changing the spacing between positions had negligible effect on the results. The statistic values where quantised to 50 discrete levels.
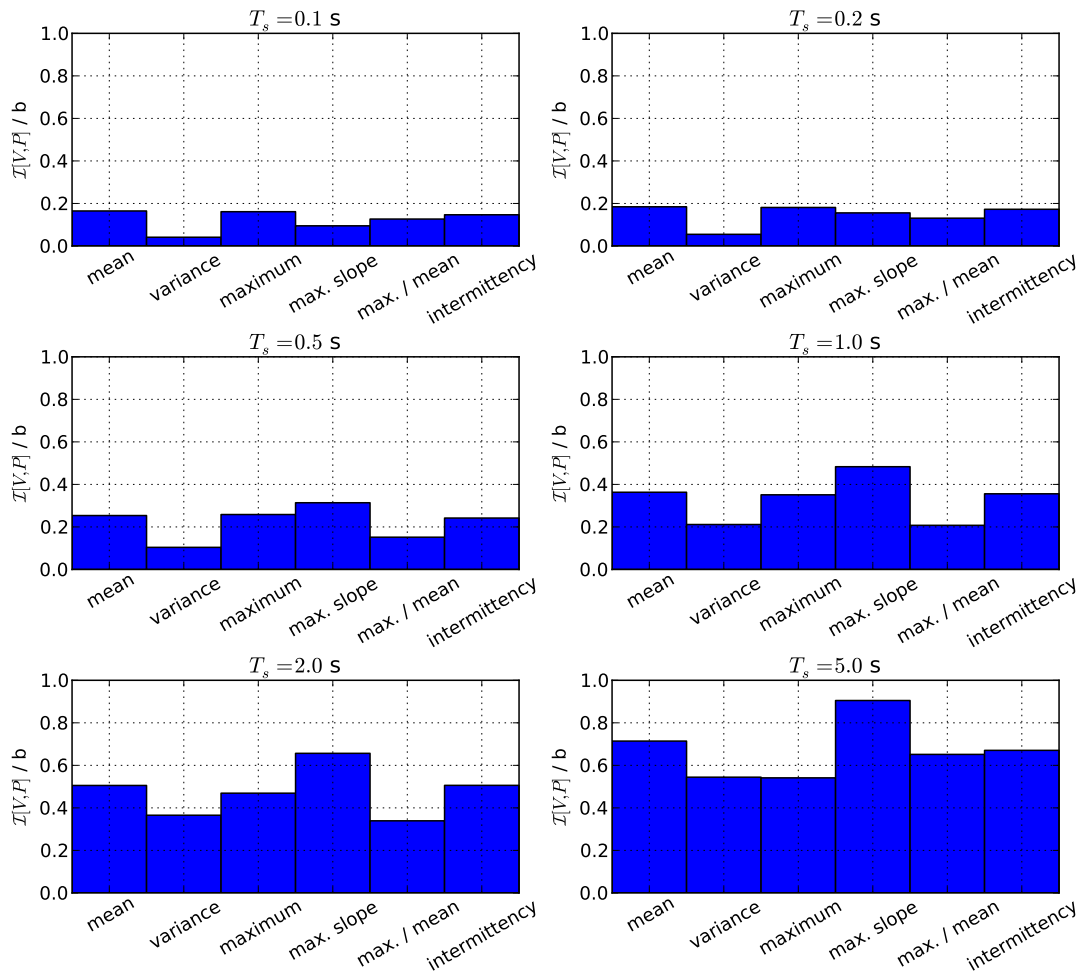

Figure 3.6: Estimated mutual informations between single statistic features and streamwise position in plume (no meander).

Figure 3.6 show the results for the streamwise positions with no plume meander. It can be immediately be seen that even with relatively long signal segment durations ($5\,\mathrm{s}$ probably being unfeasibly long for an ant to remain close to one position while navigating) the maximum mutual information between these single statistics and position in the plume is relatively low at just over 0.9 bits at best (maximum absolute signal gradient), not even quite enough information to reduce narrow down the location to four of the eight positions.

Interestingly the best statistic to use appears to depend on the signal segment duration, with for example the maximum slope statistic performing relatively poorly for shortest two T_s values while being the most informative four the longer four. Somewhat surprisingly the mean statistic performs relatively well for the shorter segment durations. This runs counter to the case for analyses with signals in which the filtering stage of the sensor model was not included, in these situations the maximum and intermittency statistics performing significantly better than the mean statistic for shorter segment durations (results not shown).
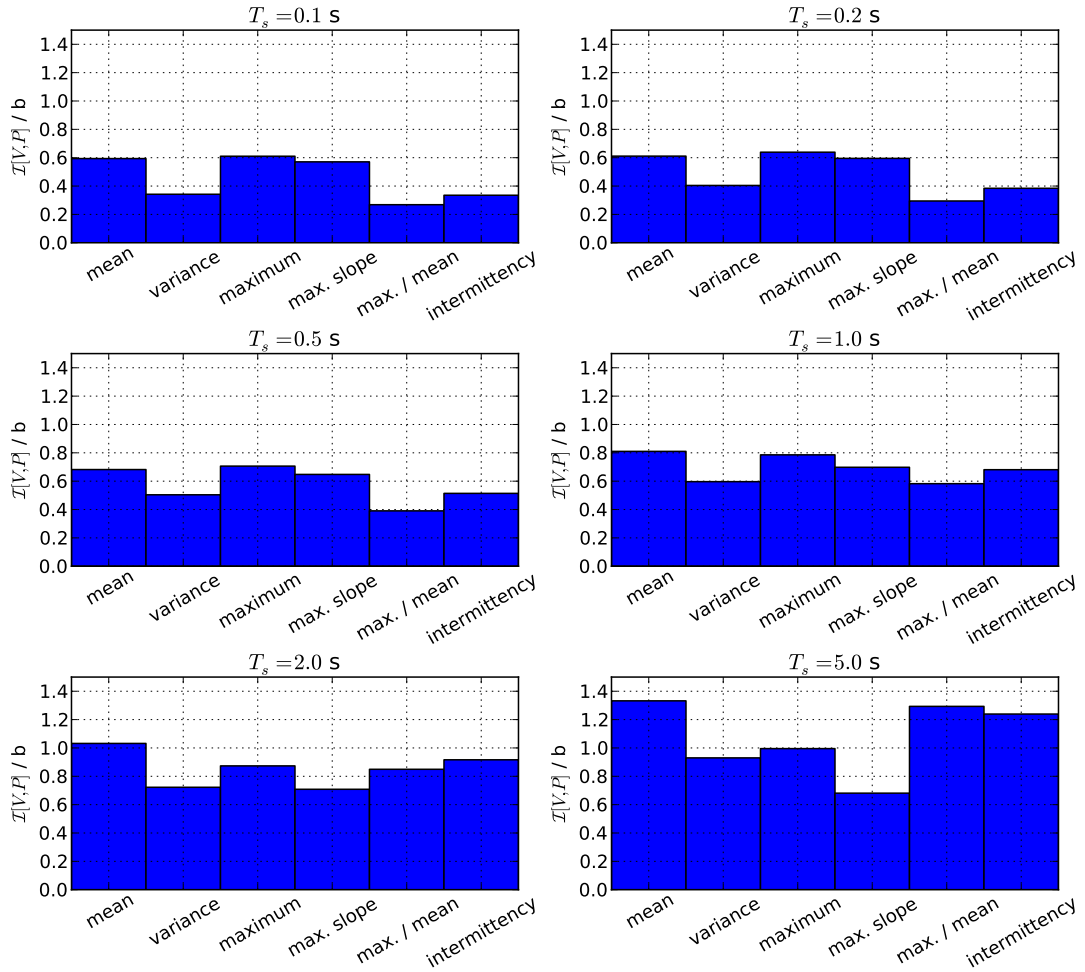

Figure 3.7: Estimated mutual informations between single statistic features and lateral position in plume (no meander).

Considering now the results shown in figure 3.7 for the lateral positions with no plume meander it can be seen that overall these statistics seem to be significantly more informative about lateral position within the plume than for the streamwise direction, this effect being noticeable across all segment durations but particularly so for the smallest T_s where the information content of the best statistic is four times as informa-

tive as the best statistic for the streawise positions. There are also significant changes in the relative performances of the different statistics across the different segment duration lengths, with the mean being more informative at higher durations for the lateral than streamwise positions while the opposite being true for the maximum slope.

Figures 3.8 and 3.9 show the results for a meandering plume for the streamwise and lateral positions respectively. It can be seen that as would be expected the extra variability and so uncertainty introduced by plume meandering reduces the information content of the statistics across the board for both the lateral and streamwise positions. Generally however the relative performance of the different statistics for a given segment duration remains constant, though the performance of the mean does drop for the longer segment durations, reflecting the longer times for such averaging statistics to converge when extra variability is introduced.
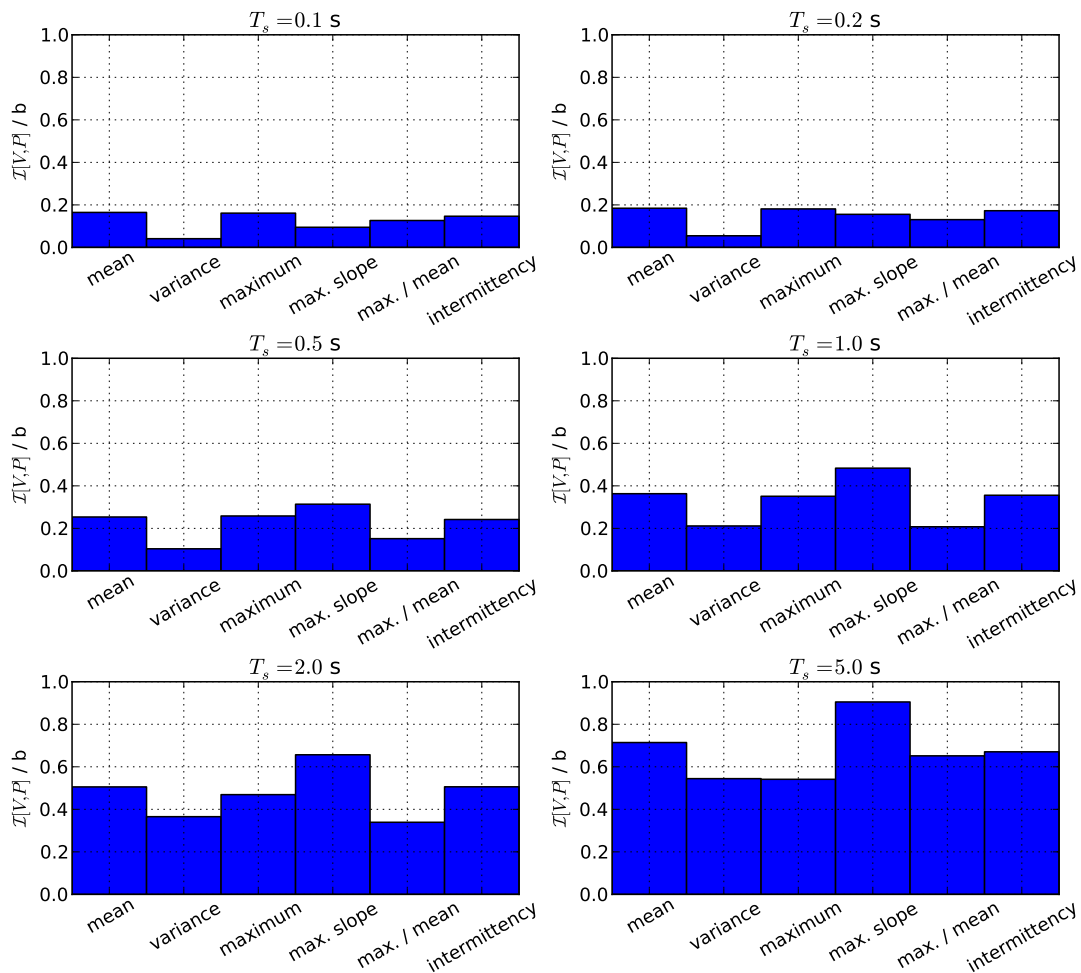

Figure 3.8: Estimated mutual informations between single statistic features and streamwise position in plume (low meander).
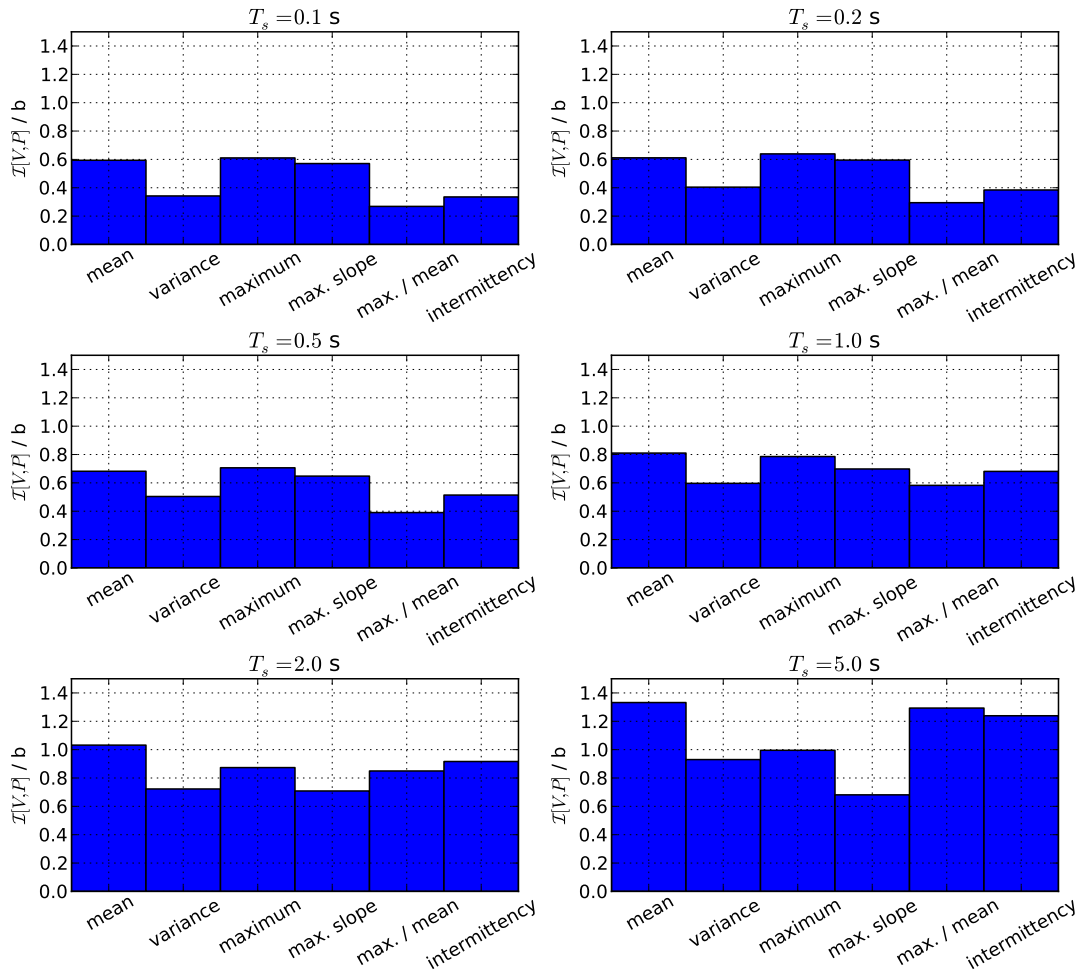
Figure 3.9: Estimated mutual informations between single statistic features and lateral position in plume (low meander).

3.3.2 Combinations of multiple statistics

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Next it was attempted to see if the different statistics contained independent location information such that combining several of them together into the feature vector lead to an increase in the mutual information. In this case only one signal segment duration was considered $T_s = 0.5\,\text{s}$, only the no meander plume condition and only for the streamwise positions. The results are shown in figure 3.10 which shows the mutual information as an increasing number of statistics are added to the feature vector. It can be seen that the statistics do indeed appear to contain independent information, the addition of each successive statistic leading to gain in the mutual information between the feature vector values and the plume position. Although it may seem somewhat surprising at first that the addition of the maximum to mean ratio adds further information over the top of the already present mean and maximum statistics, it needs to

be remembered that the values are quantised after the statistics are calculated so it is possible that the ratio was quantised as a finer scale and so offers further information previously lost in the individual statistics.
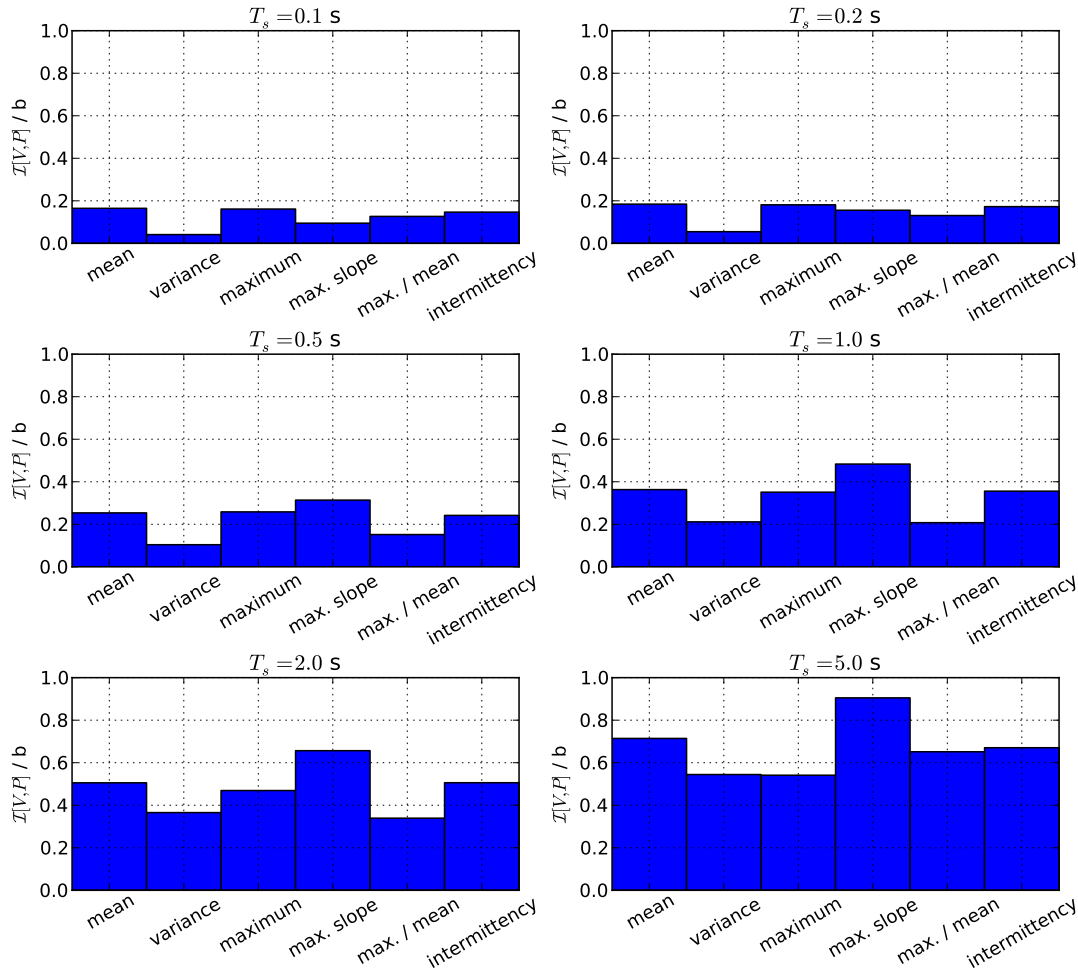

Figure 3.10: Estimated mutual informations between different combinations of statistic features and streamwise position in plume (no meander) with $T_s = 0.5$ s. Horizontal axis label indicates number of statistics included in feature vector, with the order of inclusion being [maximum slope, mean, maximum, intermittency, maximum to mean ratio]

3.3.3   Downsampled and quantised feature vector

Finally the mutual information between streamwise position and feature vectors composed of coarsely downsampled and quantised versions of the raw signal segments was analysed. Specifically segments of duration $T_s = 1$ s where downsampled (with a prior antialiasing filtering step) from 128 time points to 8 (with δt having being changed from the normal value of 0.01 s in this case to allow an integer downsampling factor) and quantised to 8 levels. This produced a set of possible values of size $8^8 = 16777216$, with

this being judged to be near to the limit of what it would be feasible to computationally analyse on the desktop machine being used. Signal segments extracted from 64000 s of simulation data were used to generate the samples - although this means the sample set was still massively below the size of set of possible values, even producing this amount of simulation data involved a 12 hour simulation run. The estimated mutual information between these feature vectors and the 8 lateral plume positions was 1.49 bits, corresponding approximately being able to make a 1 in 3 choice correctly.

3.4 Discussion

Overall it is clear that the information content of the signal segment features considered was generally fairly low, reaching a maximum of 1.49 bits for the final downsampled example. This value will still not reflect the full information content of the (modelled) signal segments due to the information loss in mapping from segments to the feature vectors. However it does suggest the total positional information available in the odour signals may be fairly low.

It does need to be born in mind of course that the signals here are based on models that neglect most of the fine scale structure of the plumes, which may contain information that can be used for localisation. However this is itself in some ways informative as it suggests the information used by ants may need to rely on subtler properties of the signal than modelled here.

An ideal way of producing more realistic odour concentration fields would be to physically model the formation of a plume under field conditions for example by imaging the scattered light intensity from a laser light sheet illuminating a plane through a turbulent plume of smoke or water vapour. Preliminary work was completed within this project to implement such a system however equipment problems meant that only indoor tests possible in the time available, producing the turbulent flow photos included in the introduction.

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