

## Olfaction in a viscous environment: The “color” of sexual smells in *Temora longicornis*

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**Abstract** We investigate chemical aspects of mating in the marine copepod *Temora longicornis* (Copepoda, Calanoidea). Our emphasis is the female pheromone signaling in form of well-defined trails for males to follow, observed in Doall et al. (1998). The viscous environment and the properties of the odorants play important roles as the spread of the pheromone trail limits the time during which it is useful for tracing. A key observation from our earlier work is the ability of a searching male to detect the direction of the female and to correct its swimming direction if necessary. We propose a simple mathematical model for the spread of a pheromone from a moving source and carry out numerical simulations of two possible detection mechanisms. We find that a searching agent that is capable to detect a ratio outperforms a searcher that depends on the gradient of a single compound. This suggests that copepod sex pheromones consist of blends of chemical compounds and that a ratio detection mechanism similar to that in airborne insects is at work.

**Keywords** Animal behavior · Olfaction · Mathematical modeling

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## 1 Introduction

The color “ochre” has the coordinates (204, 119, 34) in the standard Red-Green-Blue (RGB) color space (Wikipedia, 2017). Small changes in the ratio of these components result in visible changes of the color. Here we address the question whether the fresh signal of a sexually mature female copepod *Temora longicornis* has a similar location in the space of semiochemicals.

Animals from all phyla use chemoreception to orient themselves in their surroundings and to communicate (Wyatt, 2014). The purposes of chemoreception are the location of food and mating partners, the establishment of social structures and the avoidance of predators or defense against them. In many examples of intraspecific communication semiochemicals are emitted intentionally by some individuals in order to be specifically received and understood by others. In this case the semiochemicals are called pheromones (Karlson and Lüscher, 1959), a term that encompasses single odorants and multicomponent blends. The most well-known examples of pheromone communication are the advertisement of sexual receptiveness in many species and communication among social insects such as bees, ants and termites.

It has been estimated that there are  $3.2 \times 10^{21}$  individual copepods alive, a number only slightly smaller than Avogadro’s constant (Humes, 1994). They constitute an important link between autotrophs and higher trophic levels in the earth’s freshwater and marine food webs. With the crustacean fossil record dating back over 500 million years, copepods have been recorded since the Early Cretaceous, 146 million years ago. This geological time stretch has led to the evolution of extremely fine sensory and locomotory mechanisms that allow the animals to thrive in virtually all freshwater and oceanic habitats on earth. The book by Louis Jurine (1820) contains a remarkable 200 year old drawing of copulating copepods where the male grasps the female using his long antennae. Since the groundbreaking papers of Katona (1973) and Hamner and Hamner (1976), it has been demonstrated that various copepod species rely on chemical signaling mechanisms for mating and food searches. Regarding the reproductive search, the generally accepted understanding is that female copepods leave traces of pheromones that males are able to detect and to follow (Bagøien and Kiørboe, 2005; Doall et al., 1998; Seuront, 2013; Weissburg et al., 1998; Tsuda and Miller, 1998; Yen et al., 2004). The pheromones emitted by the female must remain intact for a sufficiently long time for the male to follow it. Multiple strategies exist to imprint a sense of directionality to a pheromone trail. Pharaoh’s ants *Monomorium pharaonis* create networks of trails in which angles of about  $60^\circ$  at a Y-shaped junction point away from the nest and towards the food source (Jackson et al., 2004). Female garter snakes of the genus *Thamnophis* mark the anterolateral surfaces of environmental objects with pheromone deposits from glands in their ventral and lateral body surfaces. Males then use their highly sensitive tongues to determine the direction of the female from these surface deposits (Ford and Low, 1984; Ford, 1986). In marine polychaete worms *Nereis succinea* cysteine-glutathione disulfide (CSSG) is known to elicit accelerated swimming, trail

following and release of sperms in males in a concentration-dependent manner (Ram et al., 1999, 2008). The observations in Doall et al. (1998) showed male copepods turning around if they were initially following the female’s track in the wrong direction with great fidelity. Thus the mixture of chemical compounds may convey a sense of directionality to aid the male encountering the female. The problem of finding an odor source in environments of different degrees of turbulence is faced by many species of aquatic and terrestrial arthropods (Atema, 1996; Balkovsky and Shraiman, 2002; Koehl and Reidenbach, 2011) as well as tracking dogs (Wells and Hepper, 2003; Hepper and Wells, 2005).

In this paper we revisit the evidence for mate-tracking in the copepod *Temora longicornis* and investigate some of the chemical and physical background of pheromone communication. We focus on the encoding of directionality in the chemical signal. We propose a simple yet efficient mathematical approach to investigate whether the pheromones emitted by the females consist of one or more compounds. Our simulations show that an agent that is able to detect a ratio of concentrations outperforms a searcher on a gradient of a single compound. We argue that the chemical trail consists of multiple organic compounds that decay at different rates. The ratio of the individual chemical compounds then gives a sense of the distance along the trail and changes in this ratio indicate the direction of the source. At the end of the paper, we discuss directions for future research into the chemical properties of the odorants in the pheromone blend and the required neuronal circuitry needed to decode those signals.

## 2 Materials and Methods

The mating behavior of the copepod *Temora longicornis* (O. F. Müller, 1785) was observed in 1994 and 1996 as described in (Doall et al., 1998). Briefly, the animals were collected from Stony Brook Harbor, NY and cultured in filtered seawater. Mating interactions in a 1.5 L experimental vessel were captured by laser photography providing simultaneous orthogonal views (Strickler and Hwang, 1999). The digital footage recorded at 30 Hz frame frequency was then processed with video-analysis software (Optima).

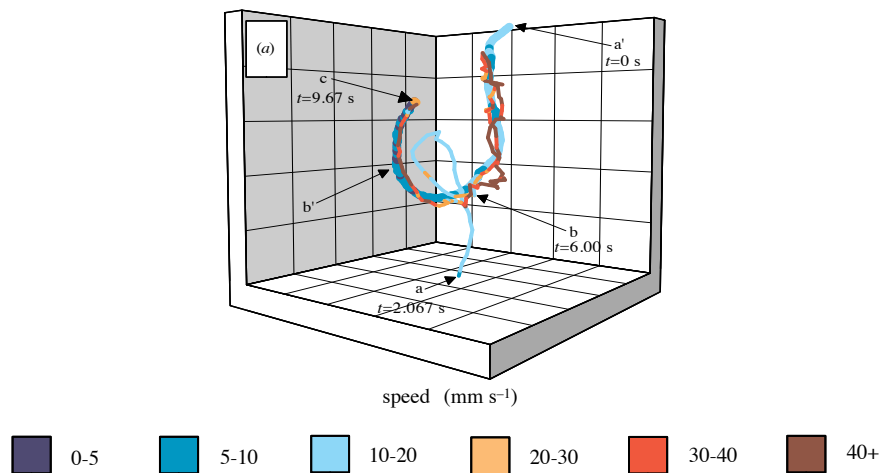
Numerical simulations were carried out with the Open Source computational package SCILAB (Scilab, 2017). The codes are available from the corresponding author upon request.

## 3 Results

### 3.1 Observations of Pheromone-mediated Pursuits

*T. longicornis* is a marine copepod of typical prosome length 1-2 mm that is most abundant in the tempered coastal waters of the Northern hemisphere.

Doall et al. (1998) found that males are able to detect trails of females and to follow them closely at up to five times higher swimming speeds compared to their average cruising speed. Once they reach the female they capture it with their antennae to deposit their spermatophores. The authors observed that on occasion the male went in the incorrect direction upon the detection of the trail (the one the female came from), but was quickly able to correct this and turn around on the path, see Figure 1. This correction was observed in 22 out of 27 pursuit events in which the male had started in the wrong direction (81%). Males were able to follow trails that were 10 s old or more. This indicates that the directional cue must be a chemical gradient, whether of one compound or more, as any hydrodynamical disturbances would have been obliterated (Yen and Lasley, 2011).



**Fig. 1** Schlieren photography is used to visualize trails of females and males in Doall et al. (1998). The “fat” trail starting at  $a'$  is that of the female and the “thin” trail starting slightly later at  $a$  that of the male. The male encounters the female’s trail after 6 s at  $b$  and proceeds for the next  $\approx 1.2$  s in the wrong direction before turning around and finally capturing the female after 9.67 s at  $c$ . Note that the male’s trail is much smoother when it follows the undisturbed female’s trail in region  $b'$ . The grid unit is 1 cm.

Not all pheromone trails left by the females were created equal for pursuit. Those best for pursuit were laid by cruising females that moved with a relatively constant velocity and with only gradual changes in direction. Females that made abrupt motions threw off their male pursuers from the track. This may be explained by “gaps” in the pheromone track. Later Yen et al. (2004) created and visualized pheromone tracks using the Schlieren optical technique. There it was observed that a male swimming along a pheromone track disturbs the structure of the track and therefore has greater difficulties to follow the track after making a turn. Naturally, this also helps to throw off a competing male from following the same female pheromone trail. The prevalent mode of locomotion of zooplankton at low Reynolds numbers is by

rotation and translation along a helix (Crenshaw, 1996). Bagøien and Kiørboe (2005) reported similar behavior for the marine copepod *Centropages typicus*.

Seuront and Stanley (2014) have argued that while pheromones clearly play a role in communication, the concept of well defined tracks is confined to the laboratory setup where the tank water is at rest. Seuront and Stanley cite a critical dissipation rate of  $10^{-9} \text{ m}^2\text{s}^{-3}$  above which chemical trails erode. They state that in the surface ocean (the habitat of *T. longicornis*) and estuarine waters the relevant quantity has the value of  $10^{-6} - 10^{-4} \text{ m}^2\text{s}^{-3}$ . Female pheromones are thus present in form of clouds that change the general statistics of the search processes (Seuront and Stanley, 2014). Inhabitants of surfs where waves are breaking, e.g. *Acartia tonsa*, are known to have fewer aesthetascs for chemoreception (Huys and Boxshall, 1991; Lenz, 2016). This suggests that search strategies may be different for species inhabiting the open ocean than for those living in turbulent surf. To the best of our knowledge, so far all experimental observations in copepods were done in water at rest.

Peters and Marrasé (2000) carried out an extensive survey of the literature and collected turbulence measurements in observations of plankton in both laboratory and the field. They found a large range of values for the kinetic energy dissipation rate  $\epsilon$ , ranging over eight orders of magnitude, with a center of the spectrum at  $10^{-4} \text{ m}^2\text{s}^{-3}$ . A very interesting observation is that the majority of planktonic organisms have body sizes just below the likely Kolmogorov length scales of their environments (Peters and Marrasé, 2000, Figure 6). The Kolmogorov microscale is the length at which viscosity becomes dominant and the turbulent kinetic energy is dissipated into heat (Pope, 2000). Thus the hydrodynamic environment may have been a factor in the evolution of these species to their present sizes.

Communication via sex pheromones has been studied extensively in airborne insects, mostly moths and other lepidopterans (quite commonly severe agricultural pests). In these works it has been found that many pheromones are mixtures of volatile organic compounds and that other factors such as pheromone-binding proteins are involved in chemoreception (Chang et al., 2015; Honson et al., 2003; Renou et al., 2015).

### 3.2 Hiking on a Binary Mixture Trail

We consider the motion of the female laying a track of chemicals under laminar environmental flow conditions. This problem is similar to modeling a constant source of, say, smoke in air or a pollutant in a water body subject to diffusion and transport (Csanady, 1973). We assume that the female lays out a straight trail of a chemical compound that is produced at a rate  $Q$  ( $\text{mol s}^{-1}$ ), has a molecular diffusion rate  $D$  ( $\text{cm}^2\text{s}^{-1}$ ) and a decay rate  $k$  ( $\text{s}^{-1}$ ). We fix the frame of the moving female so that the constant velocity  $U$  ( $\text{cm s}^{-1}$ ) points in the direction of the positive  $x$ -axis. The advection-diffusion equation for the

concentration  $c(x, y, z, t)$  is given by

$$\frac{\partial c}{\partial t} + U \frac{\partial c}{\partial x} = D \Delta c - kc \quad (1)$$

where  $\Delta$  denotes the Laplace operator in cartesian coordinates  $\Delta = \frac{\partial^2}{\partial x^2} + \frac{\partial^2}{\partial y^2} + \frac{\partial^2}{\partial z^2}$ . The fundamental solution of equation (1) with an instantaneous point source at  $(x, y, z) = (0, 0, 0)$  and  $t = 0$  is given by

$$c(x, y, z, t) = \frac{Q}{(4\pi Dt)^{\frac{3}{2}}} \exp\left(-\frac{(x - Ut)^2 + y^2 + z^2}{4Dt} - kt\right). \quad (2)$$

The steady state solution of a constantly emitting point source (comparable to a chimney plume in highly idealized laminar air flow conditions) is obtained as an integral of the fundamental solution from (2) as follows

$$\begin{aligned} c(x, y, z) &= \int_0^\infty \frac{Q}{8(\pi Ds)^{\frac{3}{2}}} \exp\left(-\frac{(x - Us)^2 + y^2 + z^2}{4Ds} - ks\right) ds \\ &= \frac{Q}{8(\pi D)^{\frac{3}{2}}} \exp\left(\frac{xU}{2D}\right) \int_0^\infty s^{-\frac{3}{2}} \exp\left(-\frac{r^2}{4Ds} - \frac{U^2 + 4Dk}{4D}s\right) ds \quad (3) \\ &= \frac{Q}{4\pi Dr} \exp\left(-\frac{r\sqrt{U^2 + 4Dk} - Ux}{2D}\right), \end{aligned}$$

where we have set  $r^2 = x^2 + y^2 + z^2$ . As we can see from the last expression in (3), the concentration decays much faster in the directions orthogonal to the  $x$ -axis than along the negative  $x$ -axis. We therefore simplify our problem to a one-dimensional search along the trail by only considering points with  $r = x$ .

If there are two or more compounds in the pheromone blend, we assume that all are produced at an equal rate  $Q$  and have the same molecular diffusion rate  $D$ . This is plausible if they are structurally similar organic compounds; see (Honson et al., 2003; Lassance et al., 2013) for some structural formulas of components of moth pheromones. The differences are assumed to lie in the decay rates  $k_i$  of each compound. The concentrations of the individual compounds are then given by

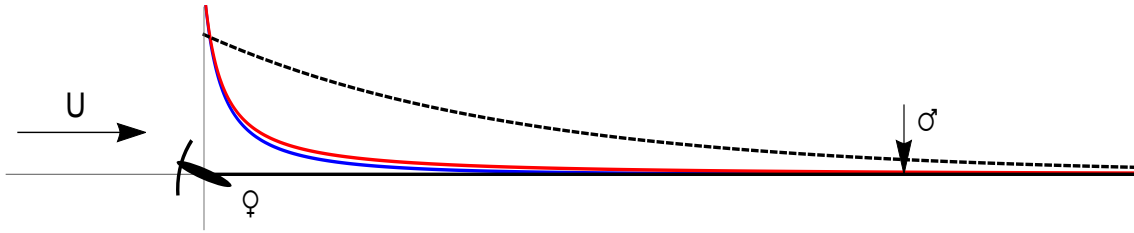
$$c_i(x) = \frac{Q}{4\pi Dx} \exp\left(-\frac{x(\sqrt{U^2 + 4Dk_i} - U)}{2D}\right). \quad (4)$$

The typical value for the diffusion rate of small organic molecules in water has been given as  $D = 10^{-5} \text{ cm}^2\text{s}^{-1}$ , (Bagøien and Kjørboe, 2005). As the female swimming speed we select  $U = 0.5 \text{ cm s}^{-1}$ . With this choice of parameters, the expression (4) can be considerably simplified to

$$c_i(x) = \frac{Q}{4\pi Dx} \exp(-4k_i x). \quad (5)$$

Selander et al. (2015) recently reported an odorant production rate from the copepod *Calanus finmarchicus* of  $Q = 30 \text{ pmol d}^{-1}$ .

The male copepod searcher has to interpret the signal depending on whether one or multiple compounds are present. On the one hand it can seek for changes of concentrations along the trail of a single compound. However, these can be quite small, as they are set by the production rate  $Q$ . On the other hand, if the male is capable of determining the ratio of two compounds in the blend, then that ratio is of the order of unity, as the compounds are produced at the same rate. Thus a gradient in the ratio of two compounds may be easier to be detected than a gradient in the absolute concentration of a single compound. A schematic picture of the compound concentrations on the trail is given in Figure 2.



**Fig. 2** The pheromone trail in the fixed frame of the female copepod. Shown are the concentrations of two compounds (red and blue lines) at the center of the trail and the ratio “blue/red” (dashed black line). The entrance point of the male is indicated by an arrow (not drawn to scale). Though the absolute concentrations of the compounds are small, their ratio is of order one.

We implement a searcher that begins the search process at some  $x_0 \in [0, L]$ , where  $L$  is the trail length. The male has a very short memory, it can only detect the signal strength at its present location, denoted by  $v_0$ , and remember the signal strength at one immediately past location, denoted by  $v_{-1}$ . There is only one reaction to a change in signal strength, namely if  $v_0 - v_{-1} < -\delta$ , then the walker changes its direction. Here  $\delta > 0$  is the minimum detectable change in signal strength. The male moves a certain number  $m$  of steps of length  $l$  in its current direction, where  $m$  is a Poisson-distributed random variable. The mean of this random variable,  $\mu$ , indicates how often the signal strength measurements are compared.

In the first set of simulations the male can read only one signal and has to determine its direction from it. To fix the constants, we work with

$$c(x) = \frac{1}{10x} \exp\left(-\frac{x}{20}\right), \quad (6)$$

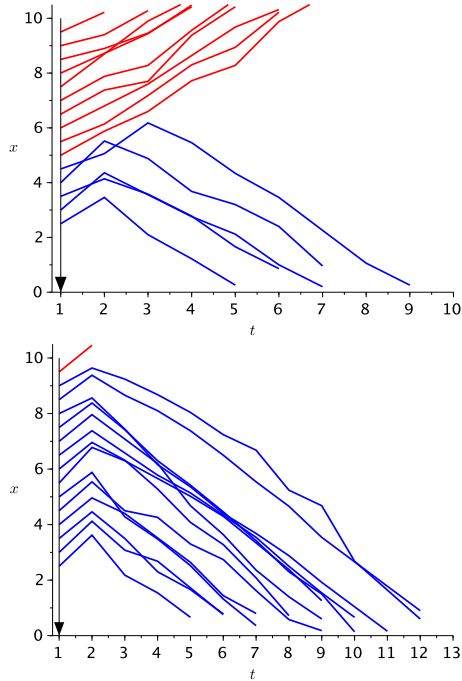
and we fix the length of the trail to  $L = 10$ . Note that here we do not insert actual production and diffusion rates into expression (5). In the second set of simulations the male can record the ratio of two concentrations. We select

$$c_1(x) = \frac{1}{10x} \exp\left(-\frac{x}{20}\right), \quad c_2(x) = \frac{1}{10x} \exp\left(-\frac{x}{10}\right). \quad (7)$$

Thus substance 2 decays faster than substance 1. The ratio of the fleeting versus the lasting compound is

$$v(x) = \frac{c_2(x)}{c_1(x)} = \exp\left(-\frac{x}{20}\right). \quad (8)$$

Figure 3 shows the success or failure of a number of searches depending on the starting point on the trail. Initially, the male is set to move in the wrong direction. When only one substance is detected, the detection threshold is set at  $\delta = 2 \cdot 10^{-3}$ , when the ratio is detected, the detection threshold is set at  $\delta = 6 \cdot 10^{-3}$ . In spite of this reduced sensitivity, the fraction of successful searches is greater in the ratio detection mode. In both cases, the signal is recorded on average every  $\mu = 12$  steps and the step size is 0.08 cm. The performance of the singular signal detector improves as  $\mu$  is increased, that is, as the comparison time points become further apart (data not shown).



**Fig. 3** The outcomes of 15 simulated search processes depending on the starting point on the trail. The direction towards the female is indicated by the arrow. Successful searches are marked in blue while unsuccessful ones are marked in red. (*Top*) The searcher can detect only an absolute concentration, given in equation (6). The detection threshold is  $\delta = 2 \cdot 10^{-3}$ . (*Bottom*) The searcher detects the ratio of two concentrations, given in equation (8). The detection threshold is  $\delta = 6 \cdot 10^{-3}$ .

The choice of the minimum detectable change in signal strength  $\delta$  in the two simulations is of course open to discussion. Clearly, a mechanism to de-



tect a change in a ratio has to be implemented by a more complicated mechanism in both sender and receiver, starting with the need for cells to produce multiple compounds. However, even if only one absolute concentration is used to detect the direction along the trail, it is still valuable to have a second odorant present that decays slower. In that case, the male could use the slower decaying compound as a general guidance in space and the faster decaying compound as the “direction field”.

The parameters in equation (4) determine certain desirables of chemical compounds that are part of a pheromone blend whose purpose is to establish a trail. If the compounds diffuse or decay too quickly, the trail cannot be established by the female. If, on the other hand, the compounds decay too slowly or do not get dispersed by diffusion, a gradient cannot be established, and no directional information can be encoded. Rittschof and Cohen (2004) have argued that the short half-lives and the many possible compounds from even short amino acid sequences make them ideal carriers of information. There are numerous ways by which peptides can be removed from the water column, including microbial consumption (Pantoja et al., 2009). The animal also must weigh the metabolic costs of creating compounds with complicated structures or large molecular weights. Conversely, compounds that consist of only a few atoms are unlikely to allow species-specific coding. Bagøien and Kiørboe (2005) have argued that amino acids or small peptides are likely candidates for odorants in sex pheromones. If the odorants are indeed amino acids, then it is unlikely that the pheromone is made of just a single one of them. This would not allow to convey any information about the species that laid out the trail, although the species and gender specific mating system in copepods is not always failsafe (Heuschele and Selander, 2014). Only quite recently a component of the female sex pheromone of the European green crab *Carcinus maenas* has been identified, namely uridine diphosphate (UDP) which has a molar mass of  $404 \text{ g mol}^{-1}$  (Hardege et al., 2011). Note that UDP signals the general presence and receptivity of the female after a molt and is not used to create a well-defined trail. Various chemical substances that induce toxic reactions in copepods’ phytoplankton prey (so-called copepodamides) are found in mass range of  $400 - 700 \text{ g mol}^{-1}$ , (Selander et al., 2015, 2016).

#### 4 Discussion

Our simulations in Section 3.2 indicate that it is preferable to have a pheromone that is a binary mixture of odorants. One lasting component acts as a “rail” along which the male copepod needs to stay in any case. The second, more fleeting component provides the sense of the direction on the path. The time it takes to detect that the initial direction was wrong and a turn has to be made depends on a number of factors. First, it is necessary to make a comparison of signals that are recorded a certain time apart, and second the change in the signal must exceed a certain threshold to be noticed. Recordings

that are too close to each other are likely not going to result in a detectable change in the signal. Recordings that are spaced too far apart may result in a loss of the trail at its far end. We emphasize that a difference in decay rates is not the only way to create a change in concentrations of different blend components. Furthermore, ambient temperature, light and turbulence conditions may affect the decay rates of the compounds and this may change significantly the length of the trail that is usable for following the female. Our “one-compound/two-compound” theory depends on differences in decay and diffusion rates of the components of a pheromone blend. The concept of “sensitivity threshold” needs careful definition. It is often understood as “What is the minimum signal strength to elicit a reaction?”. In our theory, the sensitivity threshold  $\delta$  means “What is the minimum change in the signal strength that elicits a reaction?”. For example, it may be that a copepod can very well detect a compound at a concentration of 1 nM or even less, but not a change in concentration from 1 to 0.9 nM. This can even be different in a newly encountered signal or one that the animal has adapted to (very similar to our human senses). Different detection thresholds  $\delta$  were chosen in the two simulations in Figure 3, where an agent with ratio detection mechanism could afford a bigger value and still exceed the performance of the single-compound searcher. A context is necessary to correctly compare these sensitivities. Ultimately, the output of any sensing mechanism is the firing rate of a neuron or a group of neurons.

Clearly, the values of the constants in the Section 3.2 are chosen for demonstration. Future research needs to elucidate

1. the chemical structure of the odorant(s) in the pheromone (Hardege et al., 2011; Hardege and Terschak, 2011; Selander et al., 2015, 2016; Heuschele et al., 2016), as well as potential differences in their decay and diffusion rates in sea water,
2. the mechanism of ratio detection in the olfactory system (Zavada et al., 2011; Langhoff et al., 2017),
3. the integration of the pheromone ratio and other signals, e.g. hydromechanical signals, resulting in mate tracking behavior (Seuront, 2013).

With or without the knowledge of the pheromone components a valuable experimental technique is the laying of synthetic trails from animal-conditioned water in microfluidic devices (Yen et al., 2004).

Even in such minute animals as planktonic crustaceans the nervous system has a center to which information is conveyed and from which subsequently commands are passed on to the motor cells. As in higher crustaceans and insects, the meeting point of ascending and descending pathways is called a “brain”. Precise studies of the anatomy of the copepod brain are rare (Andrew et al., 2011), yet the evidence suggests that a high level of complexity was already present in an ancestral pancrustacean, the clade comprising crustaceans and hexapods (Andrew et al., 2011). Thus in future work we will be guided by the well-studied architecture of the olfactory system in insects. The olfactory system in insects can be subdivided into two subsystems. The

“generalist” subsystem recognizes food odors, while the “specialist” subsystem recognizes sex pheromones (Masson and Mustaparta, 1990; Hansson and Stensmyr, 2011). Part of the specialist subsystem are the olfactory receptor neurons (ORNs) and the local neurons (LN). Every type of olfactory receptor neuron is capable of recognizing a particular odorant. The olfactory receptor neurons excite their associated specialist local neurons as well as generalist local neurons. The output of the generalist neurons is then processed by higher centers of the brain. These groups of neurons are organized in the macroglomerular complex (MGC) which is located in the antennal lobe in insects (Kuebler et al., 2011). A sexual dimorphism is present in some insect orders where males possess more elaborate macroglomerular complexes.

A similar problem is solved by specially trained tracking dogs that have to find the direction of an odor trail left by a human (Hepper and Wells, 2005). Tracking dogs follow a trail with their nose close to the ground. This suggests that they take the odor cues directly from the footsteps and are not influenced by the prevailing wind direction. Hepper and Wells (2005) showed that dogs were able to catch the right direction in five footsteps but not in three. While comparisons across different species are always to be made with caution (for the dogs, the odor cues are discrete), Hepper and Wells (2005) argue that it is likely a gradient resulting from decay that conveys the direction in which the human went.

## 5 Conclusion and Outlook

More animals communicate by chemical signals than by any other sensory modality (Wyatt, 2014), a fact that is sometimes insufficiently appreciated by us highly auditory and visually oriented humans. Here we have revisited the evidence for sex pheromone communication among copepods and their behavior of laying and following trails. We propose that communication via a blend of multiple substances with differing chemical kinetics is advantageous over communication with a single semiochemical compound. Of course, copepods possess more than just the ability to sense chemicals. Equally important is their ability to sense hydrodynamical disturbances in the near flow field. In mating searches, the search is often completed with a jump towards the female, once the male has gotten within a few body lengths (Seuront, 2013). While the hydrodynamical signal is quickly dissipated in water, the same viscosity keeps the chemical trail in shape for a much longer time. In the viscous environment these senses evolved to complement each other.

The chemical world in which animals live consists of a multitude of overlapping and confusing signals and requires a remarkable sensory apparatus to extract only the useful information. Increasing anthropogenic chemical pollution and pH changes of the water have detrimental effects on the chemical sensing ability of crustaceans and fish (Olsén, 2011). Due to their prominent location between autotrophs and heterotrophs, it is of high value

to understand the chemical sensing mechanisms in copepods and how they may respond to contemporary challenges.

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## References

- Andrew, D. R., Brown, S. M., and Strausfeld, N. J. (2011). The minute brain of the copepod *Tigriopus californicus* supports a complex ancestral ground pattern of the tetraconate cerebral nervous systems. *J Comp Neurol*, 520:3446–3470.
- Atema, J. (1996). Eddy chemotaxis and odor landscapes: exploration of nature with animal sensors. *Bull Biol*, 191:129–138.
- Bagøien, E. and Kiørboe, T. (2005). Blind dating: mate finding in planktonic copepods. I. Tracking the pheromone trail of *Centropages typicus*. *Mar Ecol Prog Ser*, 300:105–115.
- Balkovsky, E. and Shraiman, B. I. (2002). Olfactory search at high Reynolds number. *Proc Natl Acad Sci USA*, 99:12589–12593.
- Chang, H., Liu, Y., Yang, T., Pelosi, P., Dong, S., and Wang, G. (2015). Pheromone binding proteins enhance the sensitivity of olfactory receptors to sex pheromones in *Chilo suppressalis*. *Sci Rep*, 5:13093.
- Crenshaw, H. C. (1996). A new look at locomotion in microorganisms: Rotating and Translating. *Amer Zool*, 36:608–618.
- Csanady, G. T. (1973). *Turbulent Diffusion in the Environment*. Reidel, Dordrecht, Boston.
- Doall, M. H., Colin, S. P., Strickler, J. R., and Yen, J. (1998). Locating a mate in 3D: the case of *Temora longicornis*. *Phil Trans R Soc Lond B*, 353:681–689.
- Ford, N. B. (1986). The role of pheromone trails in the sociobiology of snakes. In Duvall, D., Müller-Schwarze, D., and Silverstein, R. M., editors, *Chemical Signals in Vertebrates 4*, pages 261–279. Plenum Press, New York, London.
- Ford, N. B. and Low, J. R. (1984). Sex pheromone source location by garter snakes: A mechanism for detection of direction in nonvolatile trails. *J Chem Ecol*, 10:1193–1199.
- Hamner, P. and Hamner, W. M. (1976). Chemosensory tracking of scent trails by the planktonic shrimp *Acetes sibogae australis*. *Science*, 195:886–888.
- Hansson, B. S. and Stensmyr, M. C. (2011). Evolution of insect olfaction. *Neuron*, 72:698–711.

- Hardege, J. D., Bartels-Hardege, H. D., Fletcher, N., Terschak, J. A., Harley, M., Smith, M. A., Davidson, L., Hayden, D., Müller, C. T., Lorch, M., Welham, K., Walther, T., and Bublitz, R. (2011). Identification of a female sex pheromone in *Carcinus maenas*. *Mar Ecol Prog Ser*, 436:177–189.
- Hardege, J. D. and Terschak, J. A. (2011). Identification of crustacean sex pheromones. In Breithaupt, T. and Thiel, M., editors, *Chemical Communication in Crustaceans*, pages 373–393. Springer Verlag, New York, Dordrecht, Heidelberg, London.
- Hepper, P. G. and Wells, D. L. (2005). How many footsteps do dogs need to determine the direction of an odour trail? *Chem Senses*, 30:291–298.
- Heuschele, J., Nemming, L., Tolstrup, L., Kiørboe, T., Nylund, G. M., and Selander, E. (2016). The sex specific metabolic footprint of *Oithona davisae*. *J Sea Res*, 117:1–6.
- Heuschele, J. and Selander, E. (2014). The chemical ecology of copepods. *J Plankton Res*, 36:895–913.
- Honson, N., Johnson, M. A., Oliver, J. E., Prestwich, G. D., and Plettner, E. (2003). Structure-activity studies with pheromone-binding proteins of the Gypsy moth, *Lymantria dispar*. *Chem Senses*, 28:479–489.
- Humes, A. G. (1994). How many copepods? *Hydrobiologia*, 292:1–7.
- Huys, R. and Boxshall, G. A. (1991). *Copepod Evolution*. The Ray Society, London.
- Jackson, D. E., Holcombe, M., and Ratnieks, F. L. W. (2004). Trail geometry gives polarity to ant foraging networks. *Nature*, 432:907–909.
- Jurine, L. (1820). *Histoire de Monocles*. J J Paschoud, Geneva, Paris.
- Karlson, P. and Lüscher, M. (1959). ‘Pheromones’: a new term for a class of biologically active substances. *Nature*, 183:55–56.
- Katona, S. K. (1973). Evidence for sex pheromones in planktonic copepods. *Limnol Oceanogr*, 18:574–583.
- Koehl, M. A. R. and Reidenbach, M. A. (2011). The spatial and temporal patterns of odors sampled by lobsters and crabs in a turbulent plume. *J Exp Biol*, 214:3138–3153.
- Kuebler, L. S., Olsson, S. B., Weniger, R., and Hansson, B. S. (2011). Neuronal processing of complex mixtures establishes a unique odor representation in the moth antennal lobe. *Front Neural Circuit*, 5:7.
- Langhoff, W., Hinow, P., Strickler, J. R., and Yen, J. (2017). Chemosensation and potential neuronal mechanism of ratio detection in a copepod. In Uttereri, M., editor, *Biology, Distribution and Ecology of Copepods*, 15 p. (to appear). Nova Science Publishers, Inc., Hauppauge, NY.
- Lassance, J.-M., Liénard, M. A., Antony, B., Qian, S., Fujii, T., Tabata, J., Ishikawa, Y., and Löfstedt, C. (2013). Functional consequences of sequence variation in the pheromone biosynthetic gene pgFAR for *Ostrinia* moths. *Proc Natl Acad Sci USA*, 110:3967–3972.
- Lenz, P. (2016). Personal communication.
- Masson, C. and Mustaparta, H. (1990). Chemical information processing in the olfactory system of insects. *Physiol Reviews*, 70:199–245.

- Olsén, H. K. (2011). Effects of pollutants on olfactory mediated behaviors in fish and crustaceans. In Breithaupt, T. and Thiel, M., editors, *Chemical Communication in Crustaceans*, pages 507–529. Springer Verlag, New York, Dordrecht, Heidelberg, London.
- Pantoja, S., Rossel, P., Castro, R., Cuevas, L. A., Daneri, G., and Córdova, C. (2009). Microbial degradation rates of small peptides and amino acids in the oxygen minimum zone of Chilean coastal waters. *Deep-Sea Res II*, 56:1055–1062.
- Peters, F. and Marrasé, C. (2000). Effects of turbulence on plankton: an overview of experimental evidence and some theoretical considerations. *Mar Ecol Prog Ser*, 205:291–306.
- Pope, S. B. (2000). *Turbulent Flows*. Cambridge University Press, Cambridge.
- Ram, J. L., Fei, X., Danaher, S. M., Lu, S., Breithaupt, T., and Hardege, J. D. (2008). Finding females: pheromone-guided reproductive tracking behavior by male *Nereis succinea* in the marine environment. *J Exp Biol*, 211:757–765.
- Ram, J. L., Müller, C. T., Beckmann, T., and Hardege, J. D. (1999). The spawning pheromone cysteine-glutathione disulfide (‘nereithione’) arouses a multicomponent nuptial behaviour and electrophysiological activity in *Nereis succinea* males. *FASEB J*, 13:945–952.
- Renou, M., Party, V., Rouyar, A., and Anton, S. (2015). Olfactory signal coding in an odor background. *BioSystems*, 136:35–45.
- Rittschof, D. and Cohen, J. H. (2004). Crustacean peptide and peptide-like pheromones and kairomones. *Peptides*, 25:1503–1516.
- Scilab (2017). *Scilab: Free and Open Source software for numerical computation*. Scilab Enterprises, Orsay, France. [www.scilab.org](http://www.scilab.org).
- Selander, E., Heuschele, J., Nylund, G. M., Pohnert, G., Pavia, H., Bjærke, O., Pender-Healy, L. A., Tiselius, P., and Kiørboe, T. (2016). Solid phase extraction and metabolic profiling of exudates from living copepods. *PeerJ*, 4:e1529.
- Selander, E., Kubanek, J., Hamberg, M., Andersson, M. X., Cervin, G., and Pavia, H. (2015). Predator lipids induce paralytic shellfish toxins in bloom-forming algae. *Proc Natl Acad Sci USA*, 112:6395–6400.
- Seuront, L. (2013). Chemical and hydromechanical components of mate-seeking behaviour in the calanoid copepod *Eurytemora affinis*. *J. Plankton Res.*, 35:724–743.
- Seuront, L. and Stanley, H. E. (2014). Anomalous diffusion and multifractality enhance mating encounters in the ocean. *Proc Natl Acad Sci USA*, 111:2206–2211.
- Strickler, J. R. and Hwang, J.-S. (1999). Matching spatial filters in long working distance microscopy of phase objects. In Cheng, P. C., Hwang, P. P., Wu, J. L., Wang, G., and Kim, H., editors, *Focus on Multidimensional Microscopy*, pages 217–239. World Scientific, River Edge, NJ.
- Tsuda, A. and Miller, C. B. (1998). Mate-finding behaviour in *Calanus marshallae* Frost. *Phil Trans R Soc Lond B*, 353:713–720.

- Weissburg, M. J., Doall, M. H., and Yen, J. (1998). Following the invisible trail: kinematic analysis of mate-tracking in the copepod *Temora longicornis*. *Phil Trans R Soc Lond B*, 353:701–712.
- Wells, D. L. and Hepper, P. G. (2003). Directional tracking in the domestic dog, *Canis familiaris*. *Appl Anim Behav Sci*, 84:297–305.
- Wikipedia (2017). Ochre. [en.wikipedia.org/wiki/Ochre](https://en.wikipedia.org/wiki/Ochre). Accessed: March 2017.
- Wyatt, T. D. (2014). *Pheromones and Animal Behavior*. Cambridge University Press, Cambridge, 2nd edition.
- Yen, J. and Lasley, R. (2011). Chemical communication between copepods: Finding the mate in a fluid environment. In Breithaupt, T. and Thiel, M., editors, *Chemical Communication in Crustaceans*, pages 177–197. Springer Verlag, New York, Dordrecht, Heidelberg, London.
- Yen, J., Prusak, A. C., Caun, M., Doall, M., Brown, J., and Strickler, J. R. (2004). Signaling during mating in the pelagic copepod, *Temora longicornis*. In Seuront, L. and Strutton, P. G., editors, *Handbook of Scaling Methods in Aquatic Ecology: Measurement, Analysis, Simulation*, pages 149–159. CRC Press, Boca Raton.
- Zavada, A., Buckley, C. L., Martinez, D., Rospars, J. P., and Nowotny, T. (2011). Competition-based model of pheromone component ratio detection in the moth. *PLoS One*, 6:e16308.