

## SMELLING VORTICES: ANIMAL TRACKING OF CHEMICAL SCENTS IN TURBULENT, UNIDIRECTIONAL FLOWS

Elena Tricarico<sup>(1)</sup>, Matthew Johnson<sup>(2)</sup>, Jan Kubec<sup>(3)</sup>, Miloš Buřič<sup>(3)</sup>, Iva Johovic<sup>(1)</sup> Stuart J. McLelland<sup>(4)</sup>, Thomas Breithaupt<sup>(5)</sup>

- (1) Department of Biology, University of Florence, Italy, E-mail: <a href="mailto:elena.tricarico@unifi.it;">elena.tricarico@unifi.it;</a> <a href="mailto:iva.johovic@gmail.com">iva.johovic@gmail.com</a>
- (2) School of Geography, University of Nottingham, Nottingham, UK, E-mail: <a href="mailto:M.Johnson@nottingham.ac.uk">M.Johnson@nottingham.ac.uk</a>
  (3) Faculty of Fisheries and Protection of Waters, University of South Bohemia, Vodňany, Czech Republic, E-mail: <a href="mailto:kubecj@frov.jcu.cz">kubecj@frov.jcu.cz</a>; buric@frov.jcu.cz
  - (4) Department of Geography, Geology and Environment, University of Hull, Hull, UK, E-mail: S.J.McLelland@hull.ac.uk
    - (5) Department of Biological and Marine Sciences, University of Hull, Hull, UK, E-mail: T.Breithaupt@hull.ac.uk

Aquatic animals rely on chemical scents to identify food, conspecifics and potential predators. Climate change can alter the conditions in rivers, affecting the ability to track chemical scents. We conducted laboratory experiments at the Total Environment Simulator, University of Hull (UK) to assess the reception by the American signal crayfish *Pacifastacus leniusculus* of chemical signals in a range of ambient velocities and substrate roughnesses (also simulating the effects of climate change). Experiments showed that *P. leniusculus* was more successful tracking the odour on gravel substrate, at low velocity conditions (0.16 m/s), irrespective of low or high-water depth (0.15 m and 0.26 m). Results highlight the ability of this species to detect chemical scent in lower velocity but not flood conditions.

## 1. INTRODUCTION

In aquatic animals, chemical scents can come from a food-source or a predator or a conspecific, and can be used to attract a mate or deter a competitor (Aquiloni & Gherardi, 2010; Breithaupt & Thiel, 2011; Tricarico et al., 2011). They are transmitted, diluted and filtered by the flow. Consequently, the characteristics of the flow can exert a control over the structure of the chemical plume and its dispersal and, therefore, the response of receiving organisms (Webster & Weissburg, 2009). However, research on this phenomenon has been primarily conducted in marine environments where conditions are different from river environments. Experiments with low roughness and low velocities (< 10 cm/s) have shown that turbulent mixing dilutes waterborne chemical stimuli, creating an intermittent distribution of chemical patches and filaments that are interspersed with scent-free water (Weissburg et al., 2000). This affects the ability of animals to track odour plumes to their source. However, there is a lack of studies that have assessed plume tracking behaviour with velocity, roughness or turbulence levels that correspondent to river environments, even during low flows, or assessed the distance over which animals can find scent sources.

Climate change is expected to impact rivers in severe and pervasive ways, altering water levels and changing the frequency and magnitude of drought and flood conditions in Europe (Vörösmarty et al., 2000). In particular, alterations in water depth and velocity during summer drought conditions are likely to affect the ability of animals to distinguish and interpret chemical signals because of the role of the flow in mixing, transporting and diluting signals. Ecohydraulics has the potential to inform us about these issues and, more specifically, about the relationships between animals and habitats to better inform how we can manage aquatic animals, particularly if they are species introduced by humans, i.e. alien species (Johnson & Rice, 2014).

The American signal crayfish (*Pacifastacus leniusculus*, Dana 1852) is a widespread invasive alien species across Europe, Japan and localities in the USA outside its original distribution, inhabiting a variety of aquatic environments, and exerting relevant impacts on local biodiversity (Lodge et al., 2013). In this study we use signal crayfish as a model to understand how hydrological conditions



(simulating the effects of climate change) affect the reception of chemical scent by freshwater organisms.

### 2. METHODS

The work was carried out in July-September 2018 at the Total Environment Simulator, University of Hull (UK). The first three weeks were devoted to the construction of the flume set-up (building four channels, two with sand, two with gravel, insertion at the substrate surface of an airstone attached to a peristaltic pump to diffuse the odour), to the conditions deployed in the flume for the experiments (e.g. flow, velocity, lighting, camera set-up, odour type, release, testing crayfish). Flow conditions were set up for all experimental configurations used in the following behavioural essays (see below). Animals were collected from Gaddesby Brook, Leicesterhire, UK, permitted by the Environment Agency of England. During the first week, crayfish were acclimated in cages (100x60x39cm; water temperature: 16°C) downstream of the experimental area in two channels of the flume. A total of 39 crayfish were measured, sexed, marked with a numbering code and reversibly blindfolded to ensure that they do not respond to visual cues. We discarded crayfish with missing claws or antennules (used for chemo-reception). Various sources of odour and set up's were tested during week 2 and 3. Week 4 was dedicated to testing crayfish tracking behaviour: after some trials, cat food was used as the source of food odour and was placed in a porous bag (made from a dishcloth), within a mesh cube and placed 70 cm from the crayfish (Figure 1). Two water depths (15 cm and 26 cm), two velocities (16 cm/s and 26 cm/s), two different substrates (gravel, sand) were used in the presence or absence of odour, for a total of eight configurations. In the control (=no cat food), the porous bag was filled only with stone. Ten crayfish were tested per condition. Crayfish were allowed two minutes to acclimate in a Perspex enclosure that enabled water flow through the chamber. After the two minutes, the porous bag with cat food or with only stone was placed into the channel and the whole enclosure was removed immediately afterwards. Behaviour, movements and directions of test crayfish were video-recorded for 15 minutes. For each environmental combination, the physical environment was characterised (surface roughness, velocity with depth and spatially) and a detailed hydraulic assessment was made using an array of Vectrinos. Here, we present the main results related to successful detection of scent in different conditions.



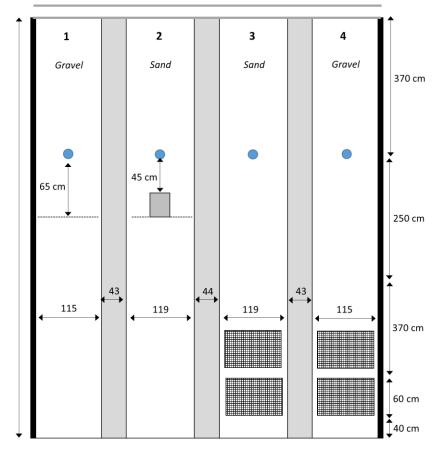


Fig. 1. The experimental set-up: crayfish were acclimatized at the end of the channels 3 and 4; experiments were carried out in the channels 1 and 2. Blue dots indicate the location of the odour source.

# 3. RESULTS AND DISCUSSION

Crayfish did not find the bag in the absence of odour, while in the presence of cat food more successful attempts were recorded in the channel with gravel (overall, 8 vs 2,  $\chi^2$ =4.12, p=0.04; Figure 2), in the presence of low velocity conditions (overall, 7 vs 3, not statistically significant,  $\chi^2$ =1.83, p=0.18) and in both water depths (overall, low 4 vs 6 high,  $\chi^2$ =0.46, p=0.50).



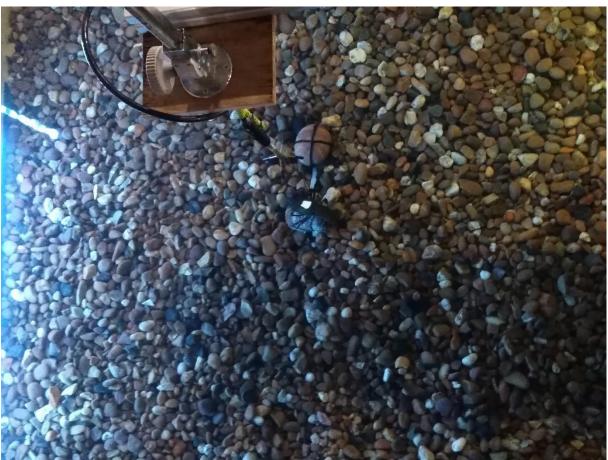


Fig. 2. A test crayfish successfully reached the porous bag with cat food in the gravel substrate.

Table 1. Number of test crayfish that successfully reached the porous bag with cat food in different conditions. LD=Low Depth, LV= Low Velocity, HD= High Depth, HV=High Velocity.

	LD, LV	LD, HV	HD, HV	HD, LV
Gravel	2	1	2	3
Sand	1	0	0	1

Signal crayfish are found in a variety of aquatic environments, ranging from still-water lakes, rapid upland streams and gravel shelves in large rivers (e.g. Lewis, 2002). However, they tend to favour and be active on relatively stable substrates and in areas of low velocity, because at higher velocities crayfish activity can become impeded (e.g. Johnson et al., 2014) with animals seeking shelter in macrophyte stands, marginal areas or burrows to prevent entrainment into the flow. In our experiments, odour detection seems to be more successful in the presence of gravel substrate, corroborating the findings by Moore & Grills (1999) on rusty crayfish (*Orconectes rusticus*): in their experiments crayfish located food quicker in artificial streams (velocity: 5 cm/s) when cobbles were placed on uniform, planar sand substrates, which the authors state to be because turbulence generated by cobbles spread the plume further than when cobbles were not present. Finally, different water depths seem not to affect significantly the successful detection of odour by *P. leniusculus*.

Overall, successful detection was low across all treatments, with a maximum success rate of 30% on gravel substrates with high depth but low flow velocity. We hypothesis that this may be due to two factors. The first is that the complexity of the hydraulic environment in the flume makes plume tracking challenging for animals. Given that the experiments represent a more complex hydraulic environment than many previous experiments, but still a simplified environment relative to a river,



this has important implications for our understanding of how animals in small, gravel-bedded streams, may use chemo-reception. Certainly, our results suggest that chemoreception is likely to be significant only over short distances (> 10 cm) in shallow, gravel-bedded streams. The second is that crayfish may have felt exposed in the channels, preferring to remain at the margins, in areas of shadow rather than venturing into central areas. Therefore, it is possible that animals did perceive the scent but chose to not track it because of a fear of predation. Future work investigating the behaviour and movement pathways of crayfish will enable us to develop these hypotheses further. The fact that animals did not track as successfully on sand is also likely to relate to the threat of expose, as well as impeded movement because near-bed flows were higher over smoother substrates.

In conclusion, our experiments show that, as expected, gravel is a more suitable substrate to spread the chemical scent and to favour its detection that high velocity impedes the normal movement of crayfish (thus in case of floods the species is not able to successfully reach the odour source), while in case of droughts, here represented by low level of water, we can expect that the species would be able to detect and reach the food.

### **ACKNOWLEDGEMENT**

We thank Brendan Murphy and Hannah Williams for their valuable help during the experiments. This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 654110, HYDRALAB+. IJ was covered by the Aquainvad-ED project (2020 Marie Sklodowska-Curie ITN-2014-ETN-642197).

### REFERENCES

- Aquiloni, L. and Gherardi, F. (2010). Crayfish females eavesdrop on fighting males and use small and sight to recognize the identity of the winner. Animal Behaviour 79: 265–269.
- Breithaupt, T. and Thiel, M. (2011) Chemical communication in crustaceans: research challenges for the twenty-first century. In: T. Breithaupt, M. Thiel (eds.) Chemical communication in crustaceans. Springer.
- Johnson, M.F. and Rice, S.P. (2014). Animal perception in gravel-bed rivers: Scales of sensing and environmental controls on sensory information. Canadian Journal of Fisheries and Aquatic Sciences 71: 945–957.
- Johnson, M.F., Rice, S.P. and Reid, I. (2014). The activity of signal crayfish (*Pacifastacus leniusculus*) in relation to thermal and hydraulic dynamics of an alluvial stream, UK. Hydrobiologia 724: 41–54.
- Lewis, S.D. (2002). *Pacifastacus*. In Holdich, D.M. (ed.) Biology of freshwater crayfish, pp. 511-540
- Lodge, D.M., Deines, A., Gherardi, F. et al. (2012). Global introductions of crayfishes: Evaluating the impact of species invasions on ecosystem services. Annual Review of Ecology, Evolution, and Systematics 43: 449–472.
- Moore, P. and Grills, J.L. (1999). Chemical orientation to food by the crayfish *Orconectes rusticus*: influence of hydrodynamics. Animal Behaviour 58: 953–963.
- Tricarico, E., Breithaupt, T. and Gherardi, F. (2011). Interpreting odours in hermit crabs: a comparative study. Estuarine, Coastal and Shelf Science 91: 211–215.
- Vörösmarty, C.J., Green, P., Salisbury, J. and Lammers, R.B. (2000). Global water resources: vulnerability from climate change and population growth. Science 289: 284–288.
- Webster, D.R. and Weissburg, M.J. (2009). The hydrodynamics of chemical cues among aquatic organisms. Annual Review of Fluid Mechanics 41: 73–90.
- Weissburg, M.J. (2000). The fluid dynamical context of chemosensory behavior. The Biological Bulletin198: 188–202.