Electronic Supplementary Material (ESI) for Lab on a Chip.

3D Printed Porous Membrane Integrated Devices to Study the Chemoattractant Induced Behavioural Response of Aquatic Organisms

Hari Kalathil Balakrishnan [a,b], Aaron G Schultz [g], Soo Min Lee [a], Richard Alexander [a], Ludovic F. Dumée [c,d], Egan H. Doeven [g], Dan Yuan [a,h], Rosanne M. Guijt [a*]*

[a] Deakin University, Centre for Rural and Regional Futures, Locked Bag 20000, Geelong, VIC 3320, Australia

[b] Deakin University, Institute for Frontier Materials, Locked Bag 20000, Geelong, VIC 3320, Australia

[c] Khalifa University, Department of Chemical Engineering, Abu Dhabi, United Arab Emirates

[d] Research and Innovation Centre on CO_2 and Hydrogen, Khalifa University, Abu Dhabi, United Arab Emirates

[g] Deakin University, School of Life and Environmental Sciences, Locked Bag 20000, Geelong, VIC 3320, Australia

[h] The University of Queensland, School of Mechanical and Mining Engineering, Brisbane, QLD 4072, Australia

*Corresponding authors: rosanne.guijt@deakin.edu.au; d.yuan@uq.edu.au;

Fluorescein transport

The diffusion distance of fluorescein was calculated by x=√2Dt, using a diffusion constant of D = 1400 µm^2/s for fluorescein, showing an expected distance of 15 mm to be crossed in 45 min, see Figure S1(a). When analysing the observed data, significantly faster transport of fluorescein was observed in the chamber, Figure S1(b), suggesting its transport is the result of diffusion and convection.

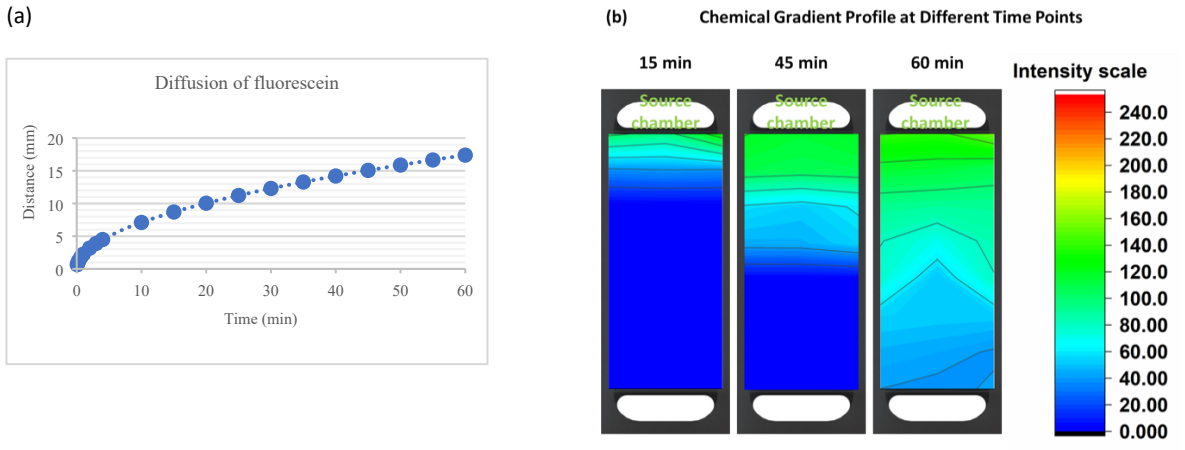

Figure S1 (a) Diffusion of fluorescein and (b) Colour contour plot representing the progression of fluorescein in the observation chamber at three different time points - 15, 45, and 60 minutes, respectively representing the starting point, middle, and end point of the fluorescein study.

As shown in Figure S1(b), it took approximately 15 min for the fluorescein cross the 300 µm wide membrane and become visible in observation chamber. This was slower than anticipated based on the diffusion data (less than a minute to cross 300 µm). The different surface to volume ratio in the pores and the longer pathway through the interconnected globular network in the membrane as well as the poor sensitivity in detecting the yellow colour may all have contributed to this delay.

From its initial detection in the chamber, the fluorescein front reached the centre of the study chamber (20 mm) in 30 minutes, filling the remaining 20 mm of the study chamber in an additional 15 minutes. This high transport rate suggests fluorescein transport was driven by convection, probably driven by differences in density between the fluorescein (or nutrient) solutions and the spring water in the chamber and evaporation [9].

While practically, sources of convection could be minimized, this was not attempted as the study involves free-swimming animals (zebrafish embryos and planaria) that will enhance convection analogous to the open flumes and aquatic systems environments [3, 5, 6, 11]. It was therefore decided to experimentally define the operational window, with the highly reproducible results obtained both studying the fluorescein transport and during the behavioural studies confirming the validity of this approach.

Statical Analysis of Chemoattractant Behavioural Response Results

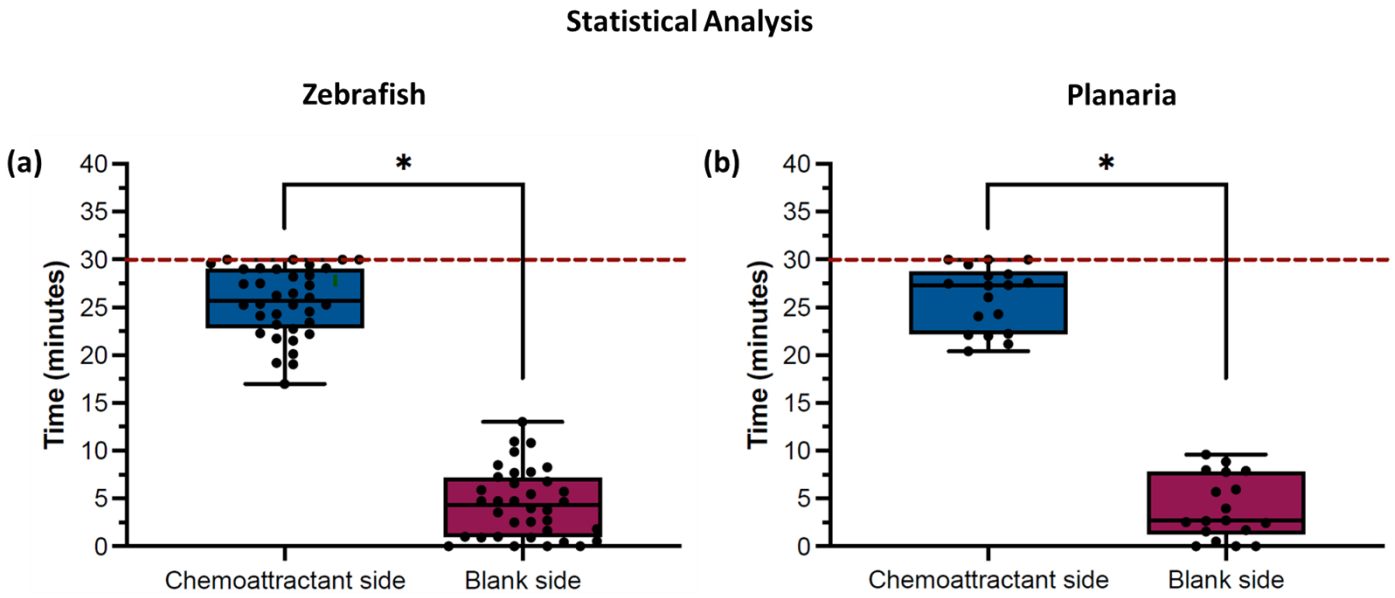

Figure S1: Dashed line represents total experimental time. Asterisk (*) denote significant differences between time spent in chemoattractant side vs blank side (two-way ANOVA, tukey's multiple comparisons test) in each device. (a) Combined results from each device demonstrating that zebrafish embryos spent a significantly greater amount of time in the chemoattractant side of the devices (25.53 ± 3.58 min) compared to the blank side (4.47 ± 3.58 min). (b) Combined results from each device demonstrating that planaria spent a significantly greater amount of time in the chemoattractant side of the devices (26.01 ± 3.31 min) compared to the blank side (3.99 ± 3.31 min)

Control Experiment Results

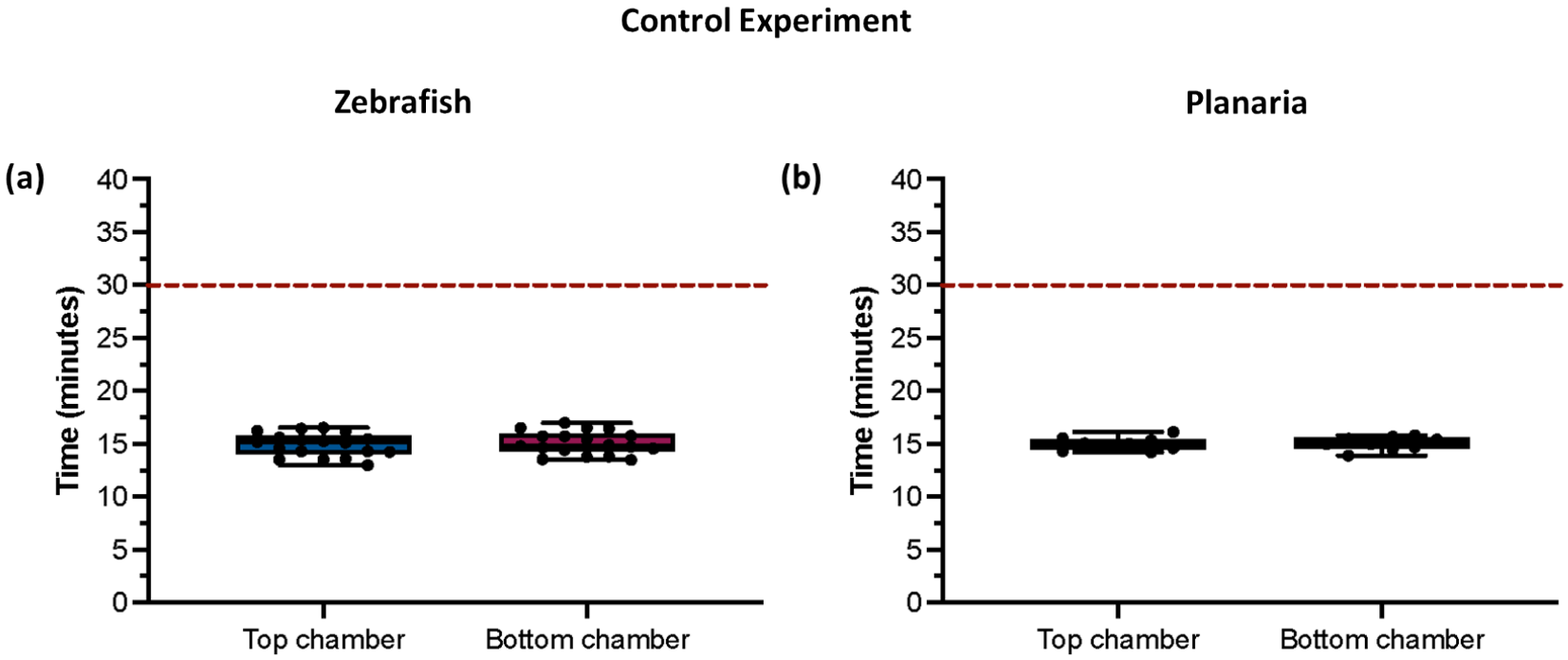

Figure S2: (a) Figure denotes the control experiment behavioural response for zebrafish embryos. (b)) Figure denotes the control experiment behavioural response for Planarians