

Video Article

In Situ Chemotaxis Assay to Examine Microbial Behavior in Aquatic Ecosystems

Estelle E. Clerc¹, Jean-Baptiste Raina², Bennett S. Lambert³, Justin Seymour², Roman Stocker¹

¹Institute of Environmental Engineering, Department of Civil, Environmental and Geomatic Engineering, ETH Zürich

²Climate Change Cluster, University of Technology Sydney

³School of Oceanography, University of Washington

Correspondence to: Jean-Baptiste Raina at Jean-Baptiste.Raina@uts.edu.au, Roman Stocker at romanstocker@ethz.ch

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Abstract

Microbial behaviors, such as motility and chemotaxis (the ability of a cell to alter its movement in response to a chemical gradient), are widespread across the bacterial and archaeal domains. Chemotaxis can result in substantial resource acquisition advantages in heterogeneous environments. It also plays a crucial role in symbiotic interactions, disease, and global processes, such as biogeochemical cycling. However, current techniques restrict chemotaxis research to the laboratory and are not easily applicable in the field. Presented here is a step-by-step protocol for the deployment of the in situ chemotaxis assay (ISCA), a device that enables robust interrogation of microbial chemotaxis directly in the natural environment. The ISCA is a microfluidic device consisting of a 20 well array, in which chemicals of interest can be loaded. Once deployed in aqueous environments, chemicals diffuse out of the wells, creating concentration gradients that microbes sense and respond to by swimming into the wells via chemotaxis. The well contents can then be sampled and used to (1) quantify strength of the chemotactic responses to specific compounds through flow cytometry, (2) isolate and culture responsive microorganisms, and (3) characterize the identity and genomic potential of the responding populations through molecular techniques. The ISCA is a flexible platform that can be deployed in any system with an aqueous phase, including marine, freshwater, and soil environments.

Introduction

Diverse microorganisms use motility and chemotaxis to exploit patchy nutrient environments, find hosts, or avoid deleterious conditions^{1,2,3}. These microbial behaviours can in turn influence rates of chemical transformation⁴ and promote symbiotic partnerships across terrestrial, freshwater, and marine ecosystems^{2,5}.

Chemotaxis has been extensively studied under laboratory conditions for the past 60 years⁶. The first quantitative method to study chemotaxis, the capillary assay, involves a capillary tube filled with a putative chemoattractant immersed in a suspension of bacteria⁶. Diffusion of the chemical out of the tube creates a chemical gradient, and chemotactic bacteria respond to this gradient by migrating into the tube⁷. Since the development of the capillary assay, still widely used today, many other techniques have been developed to study chemotaxis under increasingly controlled physical/chemical conditions, with the most recent involving the use of microfluidics^{8,9,10}.

Microfluidics, together with high-speed video microscopy, enables tracking of the behavior of single cells in response to carefully controlled gradients. Although these techniques have vastly improved our understanding of chemotaxis, they have been restricted to laboratory use and do not translate easily to field deployment in environmental systems. As a consequence, the capacity of natural communities of bacteria to use chemotaxis within natural ecosystems has not been examined; thus, current understanding of the potential ecological importance of chemotaxis is biased toward artificial laboratory conditions and a limited number of laboratory-cultured bacterial isolates. The recently developed ISCA overcomes these limitations¹¹.

The ISCA builds on the general principle of the capillary assay; however, it makes use of modern microfabrication techniques to deliver a highly replicated, easily deployable experimental platform for the quantification of chemotaxis toward compounds of interest in the natural environment. It also allows identification and characterization of chemotactic microorganisms by direct isolation or molecular techniques. While the first working device was self-fabricated and constructed of glass and PDMS¹¹, the latest injection-molded version is composed of polycarbonate, using a highly standardized fabrication procedure (for interest in the latest version of the device, the corresponding authors can be contacted).

The ISCA is credit card-sized and consists of 20 wells distributed in a 5 x 4 well array, each linked to the external aquatic environment by a small port (800 µm in diameter; **Figure 1**). Putative chemoattractants loaded into the wells diffuse into the environment via the port, and chemotactic microbes respond by swimming through the port into the well. As many factors can influence the outcome of an ISCA experiment in the natural environment, this step-by-step protocol will help new users overcome potential hurdles and facilitate effective deployments.

Protocol

We recommend executing section 1 prior to field experiments to optimize results.

1. Laboratory optimization

NOTE: The volumes described in the optimization procedure are sufficient for a single ISCA (composed of 20 wells).

1. Preparation of the chemical of interest

NOTE: The optimal concentration for each chemoattractant often needs to be determined under laboratory conditions prior to field deployments. The chemical concentration field will decrease in magnitude with distance from the source (ISCA well), which means that the concentration experienced by microorganisms in the environment will be lower than that present inside the device. The optimal concentration to use in the ISCA well depends on the diffusion rate of the chemoattractant. If the concentration in the well is too low (close to the detection limit of the microbes), no positive chemotaxis will be observed. Conversely, if the concentration in the well is too high, the concentration will also be high in the immediate environment and microbial accumulation will occur above the ISCA wells rather than inside. Therefore, dilution series should be carried out in laboratory conditions for each compound in order to determine the optimal concentration for field deployments. Ideally, a concentration range should also be tested in the field to confirm laboratory results.

1. Prepare 2.5 L of a suitable medium containing the appropriate salt concentration (e.g., phosphate-buffered saline [PBS] or artificial seawater). Filter the medium with a 0.2 μm filter using a peristaltic or vacuum pump and autoclave.
2. Prepare a 10 mM solution of chemoattractant in 1 mL of the sterile medium. Filter the chemoattractant solution with a 0.2 μm syringe filter to remove particles and potential contaminants.

NOTE: Ideally, no organic compounds other than the chemoattractant should be present in the final solution.

2. Concentration range by dilution series

1. Dilute the filtered chemoattractant stock solution in series, ranging (for example) from 10 mM to 100 μM .

NOTE: At least a 0.7 mL final volume is needed per concentration tested.

3. Loading the ISCA

1. Fill a 1 mL syringe with the filtered chemoattractant and connect it to a 27 G syringe. Holding the ISCA with the port facing upward, slowly inject the substance until a small droplet appears on top of the port.

NOTE: 1) Each dilution or substance must be injected with a separate syringe and needle to prevent cross-contamination. 2) This small droplet is important because it ensures that no air bubble is trapped within the port, which could impair the ability of microbes to migrate through the port. 3) It is recommended to fill an entire row per substance or concentration (five wells) to provide an adequate minimal volume for further analyses. 4) One row per ISCA should act as negative control and should be filled with the filtered medium in which the microbes will be suspended. This treatment accounts for the effect of random motility by microbes into the ISCA wells and should be used to normalize the treatments containing a chemoattractant.

4. Deployment in the laboratory

1. Overnight, incubate a 5 mL culture enriched with 1% marine broth (for marine bacteria) or 1% lysogeny broth (LB, for fresh water bacteria).
- NOTE: Motile bacterial isolates or natural bacterial communities can be used for laboratory deployments.
2. Incubate the culture for 12 h at room temperature (RT) and 180 rpm. After 12 h, ensure that the microbial communities are motile by direct observation under a microscope.
3. Spin down the culture at 1,500 $\times g$ for 10 min and resuspend 1/100 in 150 mL of appropriate medium (e.g., filtered seawater, filtered freshwater).
4. Place two small pieces of double-sided adhesive tape on the flat surface of a 200 mL capacity tray (the lids of 1 mL tip boxes have the ideal dimensions for this purpose and can easily be autoclaved). Place one ISCA on top, ensuring that it attaches securely to the tape. Slowly fill the deployment tray with the bacterial solution using a 50 mL serological pipette.
- NOTE: Fill the tray until the ISCA is submerged under approximately 1–2 cm of liquid. If using multiple trays, use the same volume across all.
5. Leave the ISCA to incubate for 1 h to allow bacterial chemotaxis. After 1 h, remove the medium very gently with a 50 mL serological pipette to minimize turbulent flow.
6. Retrieve the ISCA from the deployment tray without touching the upper surface. Use a pipette and disposable wipers to remove remaining liquid on the ISCA surface.
- NOTE: It is important to avoid touching the ports during this process, as the resulting changes in pressure can remove or add bacteria from the outside environment into the well and thereby bias the bacterial density and composition inside the well.

5. Retrieval of the samples

1. Holding the ISCA with the port facing downward, retrieve the volume of the wells using a sterile 27 G syringe needle attached to a 1 mL syringe.
- NOTE: Each row (if containing the same substance) can be pooled to provide a working sample of approximately 550 μL . This sample can subsequently be aliquoted into different tubes depending on the required downstream applications.
2. Determine the number of bacteria attracted to each chemoattractant concentration by analyzing the samples with flow cytometry¹². Choose the concentration of chemoattractant that maximizes chemotaxis for subsequent field deployments.

2. Preparation for field deployment

NOTE: Preparation of material and construction of the flow-damping enclosure (section 2) must be conducted prior to deployment.

1. Preparation of materials
 1. Prepare all materials listed in **Table 1**.
NOTE: Material quantities are provided for one ISCA.
2. Construction and preparation of the flow-damping enclosure
NOTE: The flow-damping enclosure minimizes unwanted turbulences that otherwise prevents the establishment of chemical gradients emanating from the ISCA.
 1. Cut the pieces for the deployment enclosure with a laser cutter from a 3 mm acrylic sheet.
NOTE: The file for the pieces can be found using the following link: <https://figshare.com/articles/Flow_damping_enclosure_for_ISCA_deployments/10630220>.
 2. Assemble the laser-cut pieces as demonstrated in **Figure 2** using acrylic glue.
NOTE: Assemble the pieces with care. Holes or misalignment can create leaks upon deployment, which directly impacts data quality.
 3. Leave the assembled enclosure to dry overnight.
 4. Wash the enclosure with deionized water.
 5. Identify potential leakage by pouring deionized water into the enclosure. Fix any potential leaking joints by adding more acrylic glue, then repeat steps 2.2.3–2.2.5.
 6. Cut the screw threads into the acrylic piece that will be used to secure the ISCA. This can be achieved using a tap with a diameter and pitch matching the mounting screws.
 1. First, affix the tap into a tap wrench, then secure the acrylic piece to be tapped in a benchtop vice. For the best results, make sure the acrylic piece is as level as possible. Make sure that the tap is perpendicular to the acrylic piece and start turning the tap wrench (clockwise), applying light pressure to the tap.
 2. After several full revolutions in the acrylic piece, reverse the rotation of the tap (counterclockwise) for one-quarter of a rotation to clear acrylic from the tap. Repeat the process until the entire depth of the acrylic piece is tapped.
 3. Finally, remove the tap (turning counterclockwise) and test the threads using a screw.

3. Procedure in the field

1. Water filtration
 1. Collect water from the field site when ready to start the experiment. Filter 5 mL of water per ISCA through a 0.2 μ m syringe filter (with a 50 mL syringe) into a 50 mL conical centrifuge tube.
NOTE: Approximately 3 mL of filtered water are required to fill all the wells of an ISCA; however, it is recommended to 1) filter 5 mL per device to account for losses during the quadruple filtration process, and 2) preserve aliquots of the filtrates as negative controls for both flow cytometry and molecular procedures.
 2. Filter the filtrate twice through a 0.2 μ m hydrophilic GP filter cartridge (using the same one, both times) with a new 50 mL syringe into a new 50 mL conical centrifuge tube. Filter the filtrate through a 0.02 μ m syringe filter (with a new 50 mL syringe) into a new 50 mL conical tube.
NOTE: This quadruple filtration should remove nearly all microorganisms and particles from the water. Keep the final filtrate away from any source of heat until use. This water will be used to resuspend all chemicals used in the ISCA, and it should be maintained at the same temperature as the water at the deployment site. Convective flows triggered by differences in temperature between the ISCA wells and outside environment may otherwise occur.
 3. Use aliquots of the filtrate to resuspend all chemoattractants of interest (typically dry) to the desired concentrations in 15 mL conical centrifuge tubes.
 4. Filter the resuspended chemoattractants through a 0.2 μ m syringe filter with a 10 mL syringe into sterile 15 mL conical centrifuge tubes to remove unwanted particles or water-insoluble compounds (if using extracts).
NOTE: Filter gently to prevent particles from passing through the filter. It is important to resuspend the chemoattractants in the ultrafiltered water from the field site and not solubilize them into other solutions. Using water from the field site is necessary to (1) obtain the same salt concentration inside the wells as that in the bulk environmental water to prevent density-driven flow, and (2) guarantee that background nutrient levels are equal inside and outside of the well.
2. ISCA filling
 1. Perform section 1.3 to fill the ISCA.
NOTE: It is recommended to fill one row (five wells) per substance (i.e., three different substances per ISCA and one ultrafiltered seawater control).
3. Deployment in the field
 1. Screw the ISCA (**Figure 3A**) to piece 9 of the enclosure (**Figure 2K** and **Figure 3B**).
NOTE: The flow-damping enclosure outlined above can contain two ISCA's side-by-side or one ISCA placed at its center.
 2. Close the enclosure (**Figure 3C**) and seal it with adhesive tape (**Figure 3D**).
NOTE: Wrinkles must be avoided to ensure a perfect seal. Seal all sides first, then (in a second step) seal the side holes, which will be used to drain water from the enclosure at the end of sampling. Do not seal the top and bottom holes. Do not place the ISCA upside down, as density-driven flow can occur in wells containing chemoattractants, which will bias the number of cells in the wells.
 3. Because the enclosure must remain steady during deployment, it is recommended to attach it to manmade structures (e.g., pontoon, ladder) using bungee cords.

NOTE: The enclosure can be attached to a deployment arm (here, a modified clamp with a perpendicular platform) using bungee cords before immersion in the water. Alternatively, the enclosure can be filled and secured with a small weight on shallow substrates. If deployments are intended in the pelagic ocean, the enclosure can be attached to a net with a buoy on one side and dive weight on the other.

4. Submerge the enclosure completely to start filling. While filling, hold the enclosure firmly to prevent excessive water movement inside. Once the level of the water reaches the top of the enclosure, make sure that no air is trapped inside.
NOTE: In case some air bubbles are trapped, tilt the enclosure gently with the vent hole facing upward, which will enable the bubbles to escape.
5. Once completely full, seal the bottom and top holes with two plugs, which can be made out of silicon or rubber or by sealing the extremities of 20 μ L pipette tips (**Figure 4**).
NOTE: This step prevents flow inside the enclosure during sampling.
6. Leave the ISCA in place for sampling for 1–3 h.
NOTE: The ideal deployment time is primarily dictated by the temperature of the water and doubling time of the bacterial community. When the water temperature is above 20 $^{\circ}$ C, it is not recommended to deploy the ISCA for more than 1 h, because cell division can occur in the wells containing chemoattractants after 1.5–2.0 h. However, optimal deployment time can be tested prior to the ISCA experiment by amending natural communities with the loaded chemicals and quantifying the number of cells through time.
7. Remove the enclosure from the water. Place it over a container enabling the water to be drained from the enclosure.
8. Remove the upper part of the adhesive tape from the front holes very gently.
NOTE: The flow of the water leaving the enclosure must be at a dripping speed. Proceed one hole at a time, from the top of the enclosure to the bottom. It should take approximately 10–15 min to drain the enclosure completely.
9. Once the waterline passes below the top of the ISCA, remove the bottom plug, and drain the rest of the water.
10. While the ISCA is still attached to the enclosure, carefully remove the water trapped on top of the ISCA with a 1 mL pipette.
11. Remove the ISCA without touching the upper surface and use a disposable wipe to remove any remaining liquid on the surface.
NOTE: It is important not to touch the ports during this process, as the resulting changes in pressure can remove or add bulk bacteria into the well and bias the bacterial counts.
12. Retrieve the samples from the ISCA by repeating step 1.5.1.

4. Downstream applications

NOTE: Volumes are given based on a 550 μ L sample (one row of an ISCA).

1. Fix 100 μ L of well contents with glutaraldehyde (2% final concentration) for flow cytometry to quantify chemotaxis to each attractant.
NOTE: Store on ice (or at 4 $^{\circ}$ C) and analyze the samples on the same day. Alternatively, samples can be flash frozen in liquid nitrogen following fixation if analysis is not feasible on the same day. Flow cytometry is the recommended method to quantify the number of cells in the ISCA wells, as it is straightforward, fast, and accurate¹².
2. Snap freeze 300 μ L of well content in liquid nitrogen for subsequent DNA extraction and analysis¹¹.
NOTE: Store the samples at -80 $^{\circ}$ C until analysis.
3. Add 90 μ L of well contents to 10 μ L of TE-glycerol buffer and snap-freeze the samples for single-cell genomics¹³.
4. Spread 10–20 μ L on agar plates containing the desired medium for bacterial isolation.

Representative Results

This section presents laboratory results using the ISCA to test the chemotactic response of marine microbes to a concentration range of glutamine, an amino acid known to attract soil bacteria¹⁴. The concentration of glutamine that elicited the strongest chemotactic response in the laboratory tests was used to perform a chemotaxis assay in the marine environment.

To perform the laboratory tests, seawater communities sampled from coastal water in Sydney, Australia, were enriched for motile cells through a simple nutrient amendment⁴, as described in step 1.4. Glutamine was serially diluted in ultrafiltered seawater to obtain final concentrations ranging from 10 mM to 100 μ M. Five ISCA replicates were deployed simultaneously for this experiment, and each contained three different glutamine concentrations (one concentration per row) as well as a filtered seawater control row. After a 1 h deployment, the contents of each ISCA row (containing the same glutamine concentration) were pooled to provide working samples of approximately 550 μ L. This volume was fixed in glutaraldehyde (2% final concentration), and the number of responding bacteria quantified via flow cytometry.

Briefly, bacterial abundance was quantified by 1) staining the cells with a green fluorescent DNA dye and 2) analysis using a flow cytometer with ultrafiltered deionised water as the sheath fluid. For each sample, forward scatter (FSC), side scatter (SSC), and green fluorescence were recorded. The samples were analysed at a flow rate of 25 μ L/min, with bacterial cells discriminated according to SSC and green fluorescence. The chemotactic index (I_c) was determined by dividing the bacterial counts present in each sample by the averaged bacterial counts in the filtered seawater control wells (FSW).

Results showed that 1 mM was the optimal glutamine deployment concentration, as it induced a significant chemotactic response that was 18-fold higher than the filtered seawater control (t -test, $p < 0.001$) (**Figure 5A**). Higher or lower concentrations of glutamine induced significant but weaker chemotactic responses ($I_c = 5.43$ for 100 μ M, $p < 0.001$; $I_c = 7.34$ for 10 μ M, $p < 0.001$). If the chemoattractant concentration added to an ISCA well is too high, chemotaxis into the well can be reduced, because (1) bacteria will not be able to detect a gradient in the port section and may aggregate above the well, or (2) the pH or osmolarity of the well may be affected.

The optimal glutamine concentration was subsequently used for field deployment. Five ISCA replicates filled with 1 mM glutamine were deployed for 1 h at a coastal site near Sydney, Australia (33.91 $^{\circ}$ S, 151.26 $^{\circ}$ E). Glutamine attracted 2.98 times more bacteria than the control wells filled

with filtered seawater from the deployment site (**Figure 5B**). The chemotactic response in this field experiment was significantly different from the controls (t -test, $p < 0.001$) and constituted a strong response for coastal seawater¹¹.

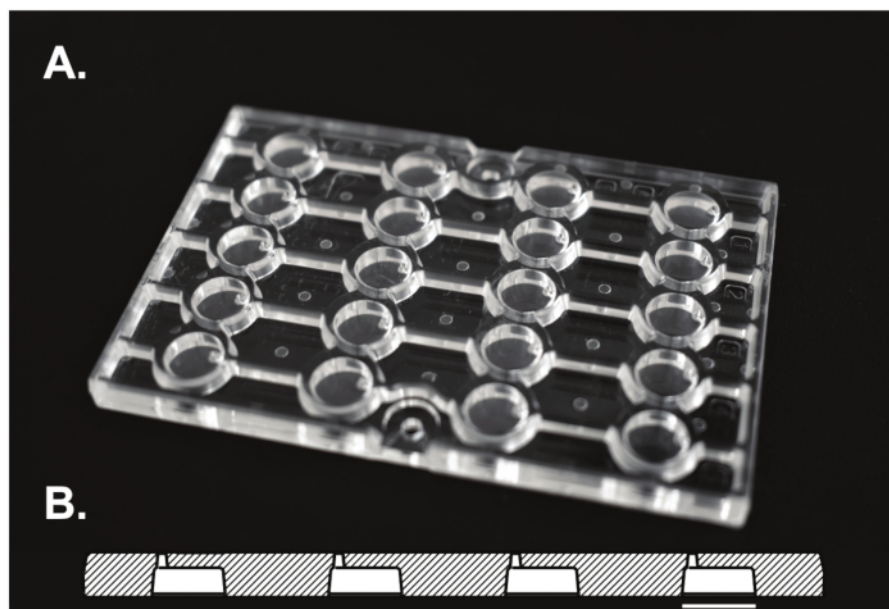


Figure 1: Detailed views of the in situ chemotaxis assay (ISCA). (A) The latest injection-molded ISCA. (B) Schematic of an ISCA well. Scale bar = 7.463 mm. [Please click here to view a larger version of this figure.](#)

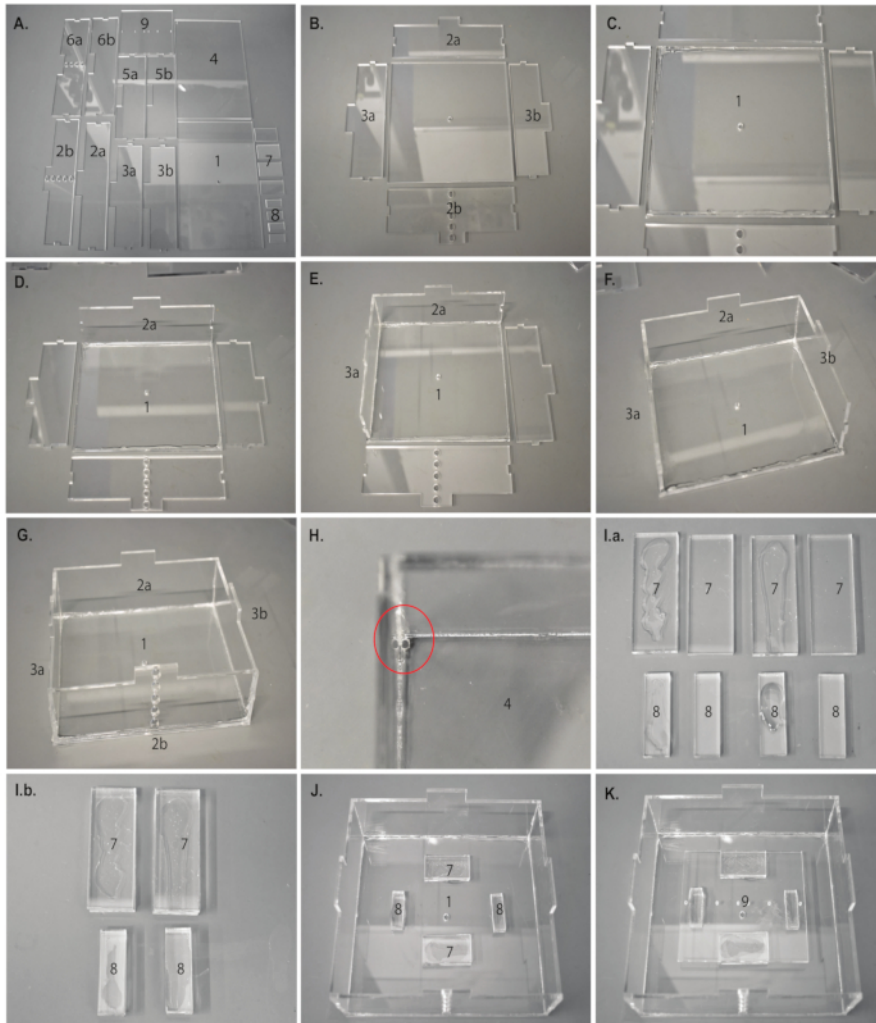


Figure 2: Assembly of the flow-damping enclosure. (A) The pieces required for assembly of the deployment enclosure. During fabrication, edges should be smooth. (B) Place pieces 2a, 2b, 3a, and 3b around piece 1 (lower surface). (C) Assemble the lower part of the enclosure by putting a thin layer of acrylic glue around the first edge of the lower surface (1). (D) Place the first longer sidewall piece (2a) vertically onto the glue and hold it in place. The glue requires ~1 min to solidify and allows piece 2a to support itself while placing the next element. (E) Apply a thin layer of acrylic glue to the lower surface on a shorter side of piece 1. Place the short sidewall piece (3a) onto the acrylic glue and lock it into the previously placed sidewall. Hold the two pieces for approximately 1 min. (F) Place the other short sidewall piece (3b) onto the opposite side of the lower surface (1). Again, lock it into the connecting piece (2a) and hold it for approximately 1 min. (G) If needed, reapply acrylic glue to the remaining long side of the lower surface (1). Place the last long sidewall piece (2b) and connect it into the two adjacent pieces (3a and 3b). Make sure that all the pieces are properly aligned with the lower piece (1) and that no signs of misalignment or gaps between sidewalls or the lower surface are present. Repeat these steps to assemble the complementary upper portion of the enclosure (4, 5a, 5b, 6a, and 6b). (H) Make sure that the hole in the corner of piece 4 is not obstructed during assembly, otherwise punch the opening with a sharp object such as a needle. The holes of the enclosure play a critical role in the deployment process and allow water to drain in a slow and controlled manner. Their diameter has been optimized to reduce turbulent flow inside the enclosure, which prevents disturbance of the fluid surrounding ISCA ports upon retrieval. (I.a,b) Glue together two large rectangles (7) and separately glue two smaller ones (8). Repeat once for each. (J) Glue the four assembled rectangles in the center of the enclosure's lower surface (1). (K) Glue the upper deck (9) on top of the rectangles (7 and 8). Make sure that the side holes of the piece are on the external side of the rectangles. [Please click here to view a larger version of this figure.](#)

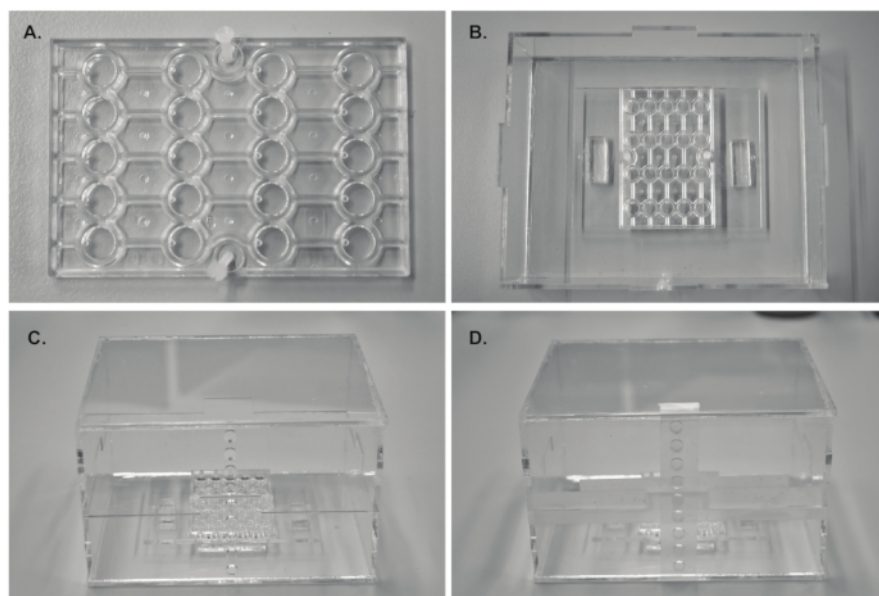


Figure 3: Placement of ISCA in the enclosure and sealing by taping. (A) Place the mounting screws in the ISCA. (B) ISCA placement in the deployment enclosure. Place the ISCA in the middle of the deployment enclosure and attach it with the specified screws. The lower drain hole of the enclosure must be sealed with a modified 20 μ L tip (**Figure 4**) once the enclosure is filled with water. This helps to avoid generation of turbulent flows that can affect the stability of the chemical field and effectiveness of chemotaxis. (C) The upper and lower parts are assembled together. (D) Sealing of the enclosure using adhesive tape. Wrinkles must be avoided to prevent leaks. [Please click here to view a larger version of this figure.](#)



Figure 4: Plug for sealing the flow-damping enclosure. The plug can be made by sealing a 20 μ L pipette tip with heat. [Please click here to view a larger version of this figure.](#)

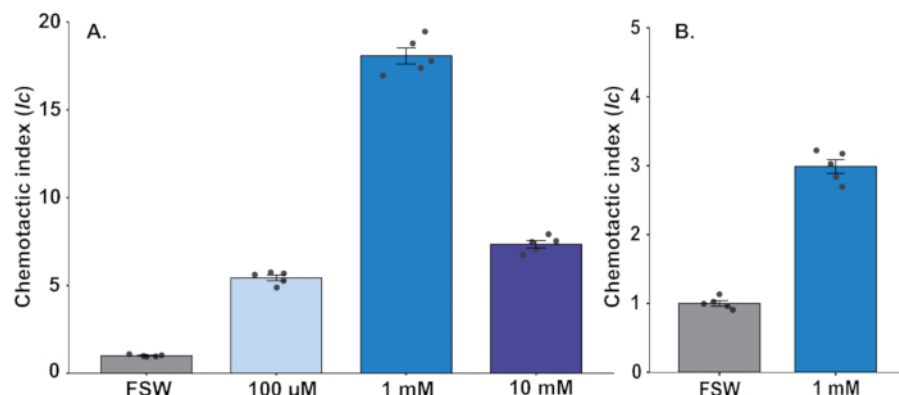


Figure 5: Chemotaxis assays using the ISCA toward glutamine of an enriched motile community in the laboratory and natural microbial population in the field. Chemotaxis index I_c (representing the concentration of cells within ISCA wells) normalized by the mean concentration of cells within the wells containing the filtered seawater (FSW) after 60 min of deployment. Each concentration was tested in five ISCA replicates. Bacterial cells were quantified by flow cytometry (A): FSW = $4.46 \pm 0.25 \times 10^3$; 100 μ M = $2.43 \pm 0.16 \times 10^4$; 1 mM = $8.07 \pm 0.45 \times 10^4$; 10 mM = $3.28 \pm 0.20 \times 10^4$; (B): FSW = $1.26 \pm 0.11 \times 10^4$; 1 mM = $3.76 \pm 0.28 \times 10^4$ cells/mL). All concentrations of glutamine tested in the (A) laboratory and (B) field induced a chemotactic response significantly higher than the filtered seawater (FSW) controls. In all pairwise comparisons: (A) Tukey HSD, $n = 5$, $p < 0.005$; (B) Tukey HSD, $n = 5$, $p < 0.005$. Error bars represent SEM. [Please click here to view a larger version of this figure.](#)

Field material	Quantity
Water filtration	
Tube rack - 50 mL	1
Hydrophilic GP filter cartridge - 0.2 µm	1
Syringe filter - 0.2 µm	1
Syringe filter - 0.02 µm	1
Syringes - 50 mL	4
Conical centrifuge tube - 50 mL	5
Chemical resuspension	
Conical centrifuge tube - 15 mL	4
Chemoattractants	
Syringes - 10 mL	4
Syringe filter - 0.2 µm	4
Tube rack - 15 mL	1
ISCA filling	
Syringe needle 27G	4
Syringe - 1 mL	4
ISCA	1
Deployment	
Deployment enclosure	1
Nylon slotted flat head screws	2
Deployment arm	1
Bungee cord	2
Adhesive tape	1
Deployment enclosure plug	1
Samples retrieval	
Syringe needle 27G	4
Syringes - 1 mL	4
Centrifuge tube - 2 mL	12
Disposable wipers	1 box
Glutaraldehyde 25%	10 mL
Other material	
Pipettes set	1
Pipette tips 200 µL	1 box
Pipette tips 20 µL	1 box
Pipette tips 1 mL	1 box

Table 1: Materials necessary for field deployment.

Discussion

At the scale of aquatic microorganisms, the environment is far from homogenous and is often characterized by physical/chemical gradients that structure microbial communities^{1,15}. The capacity of motile microorganisms to use behavior (i.e., chemotaxis) facilitates foraging within these heterogeneous microenvironments¹. Studying chemotaxis directly in the environment has the potential to identify important interspecific interactions and chemical preferences, and this can help untangle the contributions of specific microbes to biogeochemical processes. The presented protocol deploys the ISCA in the environment¹¹ to facilitate the acquisition of reproducible research on chemotaxis in situ.

Using the ISCA, it is shown that glutamine elicits a positive chemotactic response both in laboratory conditions and in the field. The ISCA deployment of glutamine in the field yields a lower chemotactic response than in the laboratory (**Figure 5**). Similar patterns between laboratory

and field experiments have been observed previously¹¹. These can be explained by the lower proportion of motile cells in the environment compared to the enriched communities or single motile isolate used in laboratory assays.

The importance of preliminary laboratory-based experiments should not be underestimated, as they permit determination of optimal chemoattractant concentrations to use in field deployments. The optimal concentration is specific to each chemoattractant and influenced by its molecular weight, solubility, and diffusivity from the wells. In the case of deployment of multiple distinct substances, each should be tested individually across a concentration range. If no chemotaxis is detected in the field after 1 h, longer deployments can be carried out. However, the length of the deployment is strongly constrained by bacterial growth and should always be shorter than the division rate of bacteria in the targeted environment. This helps to avoid population growth within the ISCA.

The ISCA is sensitive to water turbulence and care should be taken when filling and emptying the flow-damping enclosure. These steps must be performed slowly, because flows resulting from rapid filling can flush or dilute the contents of the wells. As a result, this removes or prevents chemoattractants from diffusing properly or introducing bacteria from the surrounding environment, ultimately biasing cell counts. Fully filling the enclosure with water while venting all air, then closing it completely, ensures that turbulence will not interfere with the deployment. Collecting metadata at the deployment site (i.e., temperature, salinity, chlorophyll/nutrient concentration) is also a critical step to interpret results, as these factors can influence chemotaxis.

The ISCA is an accessible, user-friendly device that provides new insights into the role and prevalence of chemotaxis in the environment. It enables interrogation of chemotaxis in any system containing a liquid phase (e.g., marine, freshwater, soil, wastewater systems). Finally, it can be used for targeted studies on pathogens and antibiotic resistance in the environment, isolation of key microbes for bioprospecting, and bioremediation of specific pollutants and microplastics.

Disclosures

The authors declare no conflict of interest.

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