A microfluidics-based *in situ* chemotaxis assay to study the behavior of aquatic microbial communities

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ABSTRACT

Microbial interactions influence the productivity and biogeochemistry of the ocean, yet they occur in miniscule volumes that cannot be sampled by traditional oceanographic techniques. To investigate behaviors of marine microorganisms at spatially relevant scales, we engineered an *in situ* chemotaxis assay (ISCA) based on microfluidic technology. Here we describe fabrication, testing, and first field results of the ISCA, demonstrating its value in accessing the microbial behaviors that shape marine ecosystems.

MAIN TEXT

Planktonic microorganisms control the biogeochemistry and productivity of marine ecosystems[1]. This global-scale influence is governed by the rate at which individual microbes access organic substrates from the water column, which in turn is dependent upon the spatial distribution of substrates and the capacity of cells to exploit microscale nutrient hotspots[2]. Seawater is surprisingly heterogeneous at the scale of individual microbes[3], with nutrient hotspots on and around organic particles[4], phytoplankton cells[5] and zooplankton fecal pellets[6]. These microscale features of the water column represent important biogeochemical microenvironments where microbial activity and transformation rates considerably exceed background levels[5,7]. Consequently, the microbial behaviors involved in accessing, and maintaining contact with, these microenvironments can have profound implications for basin-scale chemical cycling, but have remained largely inaccessible by traditional oceanographic sampling approaches.

Chemotaxis – the ability of microbes to move in response to chemical gradients – is a pervasive microbial phenotype that allows them to exploit heterogeneous chemical landscapes. The first quantitative measurements of chemotaxis were carried out fifty years ago using the capillary assay[8], which relies on the molecular diffusion of a chemical cue from the tip of a capillary tube to attract bacteria. Many alternative techniques have since been developed[9], including the recent application of microfluidics to establish controlled chemical gradients and quantify microbial responses[10]. Although chemotaxis is conventionally studied in the context of physically

57 structured microenvironments such as biofilms, or in association with animal or plant hosts[11],
58 there is growing evidence (albeit mostly limited to laboratory-based assays[12]) that indicates its
59 ubiquity among copiotrophic planktonic marine microbes[3,5], which likely use it to access fleeting
60 microscale nutrient patches in the water column[1,3]. In order to quantify and understand the
61 importance of chemotaxis in the ocean, we need to measure this behavior *in situ*. Here we
62 leverage the control afforded by modern microfabrication methods to design an *in situ*
63 chemotaxis assay (ISCA) that enables measurement of marine microbial behavior under natural
64 conditions.
65
66 The ISCA is a robust, rapidly-producible device that bridges the gap between lab-based
67 microfluidics and traditional oceanographic methods, by providing an *in situ* system to
68 interrogate microbial behavior (**Fig. 1**). The ISCA consists of a scalable array of 110 μL wells
69 embedded in a polydimethylsiloxane (PDMS) slab, with each well connected to the outside
70 seawater by an port (0.8 mm diameter, 1.6 mm depth) (**Fig. 1a** and **Supplementary Fig. 1**; see
71 Methods). The device is made of inert materials, is single-use, and is fabricated using a standard
72 soft lithography workflow, based on a mold created using 3D printing. Each well is filled with a
73 chemoattractant, which diffuses out of the inlet port and into the surrounding seawater during
74 deployment, resulting in a chemical microplume extending 1-2 mm above each well
75 (**Supplementary Video 1**). As in the capillary assay, microbes can respond to a specific cue by
76 using chemotaxis to swim into the well. Following deployment, cells can be enumerated with
77 flow cytometry to quantify the strength of chemotactic accumulation within each well and DNA
78 sequencing approaches applied to identify responding microbial populations.
79
80 Prior to field deployment, we thoroughly tested the ISCA in the laboratory by employing several
81 chemotactic bacterial isolates, in order to assess the potentially confounding roles of random
82 motility, ambient flow, and Brownian motion. To measure the chemotactic accumulation
83 dynamics of bacteria within individual wells, a small ISCA (2×2 array of wells) was embedded
84 within a microcosm placed on the stage of an inverted microscope (**Supplementary Fig. 1**). The
85 microcosm contained fluorescently labeled *Vibrio coralliilyticus* (YB2), a copiotrophic marine
86 bacterium and coral pathogen[13], suspended in 0.2-μm-filtered artificial seawater (Instant Ocean;
87 Spectrum Brands, Inc.). Bacterial fluorescence was used to identify cells throughout the well

88 depth by epifluorescence microscopy. One ISCA well was filled with a broad-spectrum
89 chemoattractant (Marine Broth, Becton Dickinson, USA) and another, serving as negative
90 control, with filtered artificial seawater (see Methods; **Supplementary Fig. 2**). We used 10%
91 Marine Broth inside the ISCA in order to generate signals at ecologically relevant concentrations
92 (**Supplementary Note 2**). We imaged a predetermined location within each well, scanned over
93 the entire well depth every minute for 60 minutes, and used image analysis to obtain cell counts
94 as a function of time (see Methods).
95
96 The ISCA captured the characteristically strong chemotactic response of *V. coralliilyticus*[13]. An
97 intense accumulation of cells occurred within wells containing Marine Broth (56.1 ± 10.4 % of
98 the microcosm cell density; $n = 3$), whereas the accumulation was negligible in the control wells
99 (4.1 ± 2.4 % of the microcosm cell density; $n = 3$). The ratio of the cell concentrations in
100 treatment and control wells – the chemotactic index (I_c; ratio of cells in treatment to cells in
101 control) – was 14, denoting a strong response to the chemoattractant (**Fig. 1b** and
102 **Supplementary Figs. 3,4**). These results confirm that the ISCA generated robust chemical
103 signals and effectively attracted and retained chemotactic bacteria in the wells (**Fig. 1c**). The
104 high value of I_c also indicates that random motility – which results in bacteria entering wells
105 even in the absence of chemical gradients – is negligible compared to chemotaxis. Furthermore,
106 experiments with a non-motile bacterium, *Marinobacter adhaerens* (HP15 ΔfliC), did not result
107 in significant cell accumulation relative to the surrounding medium (0.09 ± 0.03 % of the
108 microcosm cell density; $n = 3$; **Fig. 1a** and **Supplementary Fig. 5**), implying that environmental
109 factors such as fluid flow do not contribute significantly to the accumulation of cells in the wells
110 under laboratory conditions.
111
112 Imaging-based laboratory experiments revealed three phases in the chemotactic accumulation: (*i*)
113 an initial lag phase lasting ~5 min where cells swam into the inlet port but had not yet entered the
114 main cavity of the well; (*ii*) a sharp increase in bacterial abundance in the well as bacteria began
115 to enter, lasting until ~25 min; and (*iii*) a slowdown in the accumulation (at 30–40 min) as the
116 outward diffusive flux of cells began to balance the inward chemotactic flux (**Fig. 1b**). In the
117 second phase, the spreading of cells from the top of the well downwards (**Fig. 1d** and

118 **Supplementary Figs. 3,6**) provides direct visual evidence of the chemotactic dynamics and
119 helps rule out confounding factors such as residual fluid flow in the wells.
120
121 To further validate the ISCA, we compared the observed accumulation dynamics with those
122 predicted by a mathematical model of chemotaxis (Methods; **Supplementary File 2,**
123 **Supplementary Videos 1,2** and **Supplementary Figs. 6–9**). Chemotactic transport models are
124 only well-developed for a few specific model organisms such as *Escherichia coli*[14], so the model
125 was parameterized for *E. coli* responding to the non-metabolizable chemoattractant α-
126 methylaspartate, a pairing studied extensively in chemotaxis literature[15]. To allow direct
127 comparison, a laboratory ISCA experiment was carried out with a fluorescent strain of *E. coli*
128 (AW405) and α-methylaspartate as the chemoattractant (see Methods; **Supplementary Fig. 6**).
129 In this experiment, image stacks over the entire well's depth were acquired over time at two
130 different locations, one close to the inlet port (1.5 mm from the edge of the inlet) and the second
131 further away (2.5 mm), so that the progression of chemotactic bacteria within the well could be
132 followed. The comparison of experimental results with model predictions demonstrated highly
133 consistent timescales and magnitudes of accumulation, with cells first appearing in the field of
134 view at ~10 min (1.5 mm location) and ~20 min (2.5 mm location) (**Supplementary Fig. 9**).
135 Furthermore, the chemotactic drift velocity inferred from the time-lag between the appearance of
136 bacteria at the two imaging locations (~1.1–1.85 μm/s; $n = 3$) is consistent with values reported
137 in the literature for *E. coli*[16], further supporting the absence of residual flows within the device.
138 This set of experiments in conjunction with the numerical model confirms that chemotaxis is the
139 primary mode of accumulation in the ISCA.
140
141 With experimental results providing confidence in the ISCA's operation, the device was next
142 tested *in situ*. We deployed four replicate ISCAs (each consisting of a 5 × 5 array of wells) for 1
143 h (a duration well below typical doubling times of marine microbes[17]; **Supplementary Note 3**)
144 in surface waters (1 m depth) at a coastal marine site in Sydney, Australia (Clovelly Beach;
145 33.91°S, 151.26°E). To retain consistency with the laboratory-based experiments, we compared
146 the chemotactic response of microbes to wells filled with Marine Broth and to control wells
147 filled with filtered seawater (0.02 μm; see Methods) derived from the deployment site. To

148 prevent density differences between well contents and bulk seawater, which could lead to
149 density-driven flows, this filtered seawater was also used to dilute the Marine Broth.
150
151 Although fluid flow into the wells was ruled out in the laboratory experiments, *in situ* conditions
152 are far more dynamic and may potentially disrupt the chemical microplumes emanating from the
153 wells. To prevent this, each ISCA was mounted within a transparent 1.9-liter volume, laser-cut
154 acrylic enclosure (**Supplementary Fig. 10**). The enclosure was soaked for 48 hr in ddH_2O to
155 remove labile DOM from the acrylic surface. The stable environment provided by the enclosure
156 also ensured that bacteria were not swept past the wells at a speed that overpowered their
157 swimming ability. This replicates the physical environment that cells encounter at the
158 microscale, where bacteria and nutrient hotspots are essentially transported together and
159 movement of one relative to the other is primarily achieved through motility[18]. After securing the
160 ISCA, the acrylic enclosure was slowly filled with seawater while being submerged and once at
161 its final depth was completely sealed using a plug and left *in situ* for 1 hour (**Supplementary**
162 **Fig. 11**). Strong chemotaxis was observed, with the mean number of bacteria in Marine Broth
163 wells 3.6 ± 0.2 times greater than in negative control wells (t-test, $n = 4$, $p < 0.005$; **Fig. 2**).
164 These results constitute *in situ* confirmation that pelagic marine bacteria exhibit strong
165 chemotaxis, which not only supports results of previous laboratory-based studies[3,5,19], but also
166 long-standing hypotheses about the occurrence of this behavior in the pelagic environment[1,2,20].
167
168 Chemotactic populations were identified by their 16S rRNA sequences in shotgun metagenomic
169 data obtained using a low DNA-input library preparation procedure[21]. Whereas the taxonomic
170 profile of bulk seawater at the sampling site was characteristic of a typical coastal marine
171 community, including a high proportion of non-motile Pelagibacteraceae (**Fig. 2**), the ISCA
172 wells containing Marine Broth were highly enriched with motile copiotrophs, including
173 Vibrionales, and Alteromonadales (**Supplementary Fig. 12**). These groups are well known for
174 their chemotactic capabilities[5] and metabolic versatility[22], which enable them to respond to and
175 use a large number of organic compounds, such as those present in Marine Broth. These results
176 therefore provide *in situ* confirmation that copiotrophic microbes of this type can rapidly respond
177 to microscale nutrient patches[19]. In addition, less-studied motile taxa, such as Flammeovirgaceae,
178 were also enriched in the wells containing Marine Broth (**Supplementary Fig. 12**). The latter

179 finding reveals the power of the ISCA to identify cryptic chemotactic taxa and highlights its
180 potential use in prospecting for taxa capable of responding to specific chemicals, such as
181 pollutants.
182
183 We believe that the ISCA represents a significant advance in microbial oceanography, enabling
184 the interrogation of the chemically-mediated interactions that support the base of the food web
185 and drive biogeochemical cycles at scales relevant to microorganisms in their natural
186 environment. Here we capitalized on rapid advances made in the fields of microfluidics and low-
187 input DNA sequencing[21] to couple micro-engineering with genomic analyses. The ISCA is not
188 only deployable in aquatic environments, but also in any system containing a liquid phase (eg.
189 soil and sediment matrices or wastewater systems). Future use of the device will enable high-
190 throughput testing of environmentally-derived chemicals, and when coupled with genomic and
191 metabolomic analyses, will generate rich datasets allowing the behavior and chemical ecology of
192 microbes to be dissected within their natural habitats.
193
194 **METHODS**
195
196 **ISCA design and assembly**
197
198 A 3D printed mold was designed using SolidWorks 2015 (Dassault Systemes SOLIDWORKS
199 Corp., Concord, USA) and made out of the polymer VeroGrey on an Objet30 3D printer
200 (Stratasys Ltd., Eden Prairie, USA; **Supplementary File 1**). In the implementation used for the
201 field deployments, each ISCA consisted of an array of 5 × 5 wells. Each well was 6.8 mm in
202 diameter and 3 mm in depth, which corresponds to approximately 110 µL of fluid
203 (**Supplementary Fig. 1**), a volume selected based on the estimated lower limit of input required
204 for DNA extraction and sequencing. The wells are spaced 17 mm apart between rows (center to
205 center) and 9 mm within a row (**Supplementary Fig. 1**). The port of each well was offset from
206 the center of the well to allow release of air bubbles during filling of the well (**Supplementary**
207 **Fig. 1**). Considering a representative diffusion coefficient (D) of 1×10^{-9} m s^{-1}, the estimated
208 distance L a solute travels in a time t = 1 h is $L = \sqrt{4Dt}$ = 3.8 mm. The spacing used therefore
209 ensures no well-to-well interaction of the diffusion plumes within typical deployment times of 1

210 h. The port diameter was 800 µm with a depth of 1.6 mm (**Supplementary Fig. 1**). A high aspect
211 ratio for the inlet port was selected to diminish the effects of cavity flow on device performance
212 (see **Supplementary Note 1**).
213
214 Each mold was filled with 26 g of polydimethylsiloxane (PDMS; 10:1 PDMS base to curing
215 agent, wt/wt; Sylgard 184, Dow Corning Corp., Midland, USA). Curing was carried out
216 overnight at 40°C (the deflection point of VeroGrey is 48°C). The cured PDMS slab (95 mm ×
217 65 mm × 4.6 mm) was cut using a razor blade and carefully peeled from the mold. The PDMS
218 blocks were inspected and any port obstructions cleared using a biopsy punch of the appropriate
219 diameter (ProSciTech, Townsville, Australia). Finally, the devices were UV-sterilised and
220 plasma-bonded to sterile glass microscope slides (100 mm × 76 mm × 1 mm, VWR,
221 Pennsylvania, USA) by exposing both to oxygen plasma for 5 min using a Plasma
222 Cleaner/Sterilizer (Harrick Scientific, New York, USA). Following bonding, the ISCA was
223 heated at 90°C for 10 min to accelerate the formation of covalent bonds and then stored at room
224 temperature until use.
225
226 **Laboratory experiments**
227
228 ***Bacterial cultures***. The marine bacterium *Vibrio coralliilyticus* (YB2) was used as a model
229 organism for laboratory experiments due to its high levels of motility and strong chemotactic
230 response[13]. A glycerol stock of *V. coralliilyticus* was streaked onto Difco 2216 Marine Broth
231 (BD Biosciences, San Jose, USA) agar plates containing 50 µg/ml kanamycin (Axonlab AG,
232 Baden, Switzerland). Individual colonies were then removed and grown in 1% (v/v) 2216 Marine
233 Broth in 0.22 µm filtered artificial seawater (Instant Ocean; Spectrum Brands, Inc., Madison,
234 USA) for 22 h[13]. The cultures were then diluted 1/20 (v/v) in 0.22-µm filtered Instant Ocean to
235 obtain the bacterial suspension used in the experiments. All cells were grown at 30°C and 180
236 rpm.
237
238 To ensure that bacteria found in the ISCA well had entered them via chemotaxis and not by
239 flushing due to fluid flow or Brownian motion, we carried out an experiment with non-motile
240 bacteria. We used *Marinobacter adhaerens* (HP15 ΔfliC), which is a non-motile mutant lacking

241 fliC, a gene necessary for flagellar synthesis[23]. A glycerol stock of *M. adhaerens* HP15 ΔfliC
242 was streaked onto Difco 2216 Marine Broth agar plates containing 50 µg/ml ampicillin (Axonlab
243 AG, Baden, Switzerland). Individual colonies were then suspended in 10% 2216 Marine Broth
244 for 1 d. The cells were then washed three times in Instant Ocean before being diluted 1/10 (v/v)
245 in Instant Ocean to obtain the bacterial suspension used in the experiments. All cells were grown
246 at 30°C and 180 rpm.
247
248 To directly compare experimental results to results from the mathematical model of chemotaxis,
249 which was implemented based on parameters for *Escherichia coli* chemotaxis (due to the lack of
250 information on parameters for marine bacterial chemotaxis), we also performed laboratory
251 experiments with *E. coli* (AW405). In order to directly visualize the bacteria in the ISCA wells,
252 *E. coli* were transformed to carry a plasmid constitutively expressing a red fluorescent protein
253 (pFM210)[24,25]. Cells in log phase (OD = 0.3) were washed and resuspended in 50 mM calcium
254 chloride, heat-shocked in a solution containing plasmid DNA, and spread on tryptone broth
255 plates containing 50 µg/ml kanamycin. Resistant colonies were regrown in tryptone broth
256 containing 50 µg/ml kanamycin and screened for fluorescence. Glycerol stocks (15% v/v) of
257 colonies displaying strong fluorescent signal were made and stored at -80°C. The transformed *E.*
258 *coli* were streaked onto tryptone broth agar plates containing 50 µg/ml kanamycin. Individual
259 colonies were then removed and grown overnight in tryptone broth containing 50 µg/ml
260 kanamycin. These cells were then diluted 1:100 in tryptone broth and incubated until they
261 reached mid-exponential phase (OD = 0.3). Cells were then diluted 1/20 (v/v) in motility
262 medium (10 mM potassium phosphate, 0.1 mM EDTA, 1 µM methionine, 10 mM lactic acid, pH
263 = 7) to obtain the suspension used in the experiments. All cells were grown at 30°C and 180 rpm.
264
265 ***Assembly of the integrated ISCA for laboratory experiments and chemotaxis experiments with***
266 ***isolates***. To visualize chemotactic accumulations in the ISCA wells without interference by
267 external fluid flows, a modified version of the ISCA was integrated into a microcosm designed
268 for use on an inverted microscope. To achieve this, an ISCA was prepared in the same manner as
269 described above, but only four of the wells were excised from the PDMS block to reduce the
270 footprint of the device (**Supplementary Fig. 1**). The four wells were plasma-bonded to the
271 center of a microscope slide (75 mm × 50 mm × 1 mm) and a four-walled PDMS enclosure was

272 plasma bonded around the wells (**Supplementary Fig. 1**). The design of the mold used to
273 generate the enclosure is provided in **Supplementary File 1**. This ISCA-containing microcosm
274 was placed onto the stage of an inverted microscope (Eclipse Ti-E, Nikon, Tokyo, Japan). A well
275 of the integrated ISCA was filled with the prepared bacterial suspension and three stacks of
276 images (80 µm step size, 2960 µm total) were acquired within a well containing the bulk
277 suspension to calculate the bacterial concentration throughout the microcosm. This bacterial
278 concentration (C_0) was subsequently employed to normalize the concentration measured in
279 individual treatments, so that chemotactic accumulations were quantified relative to the absolute
280 concentration of bacteria present in each experiment. The device was then removed from the
281 microscope stage and one well of the device was filled with a chemoattractant (10% Marine
282 Broth for experiments with *V. coralliilyticus*; 100 µM α-methylaspartate for experiments with *E.*
283 *coli*; **Supplementary Figs. 3,6**), while a second well was filled with Filtered Artificial seawater
284 (FASW) for experiments *with M. adhaerens and V. coralliilyticus* or with Motility Media for
285 experiments with *E. coli*, both serving as negative controls (**Supplementary Figs. 4,7**). After
286 placing the microcosm back on the microscope stage, an initial image stack was acquired (80 µm
287 step size, 2960 µm total; time $t = 0$). The microcosm was then filled with the bacterial suspension
288 and one image stack was acquired every minute for 60 min (**Supplementary Fig**. **2**). Image
289 stacks acquired within the wells consisted of 1 image every 80 µm of depth, throughout the depth
290 (2960 µm) of the well. Image acquisition was carried out with a 20× objective in all cases using
291 Nikon Elements software (Nikon, Tokyo, Japan) and a Zyla 4.2 PLUS sCMOS camera (Andor,
292 Belfast, Ireland).
293
294 **Image analysis**
295
296 To quantify cells in our laboratory experiments it was necessary to accurately identify cells in
297 each image, while excluding out-of-focus cells and debris. To accomplish this, image processing
298 and analysis was carried out in MATLAB 2015a (The MathWorks, Natick, USA) using the
299 Image Processing Toolbox. The image stacks obtained in Nikon Elements were first filtered with
300 a bandpass Gaussian filter to reduce high-frequency noise and remove larger objects due to
301 uneven illumination or out-of-focus halos. These processed images were then segmented to
302 identify candidate bacteria. These candidates were subsequently gated based on average pixel

303 intensity and object area to enumerate the cells in each image. The resulting cell counts were
304 manually verified for a subset of images in each image stack. Videos displaying the success of
305 image analysis in identifying cells can be found in the supplementary material (**Supplementary**
306 **Videos 5-7**). The analysis was successful in identifying the majority of cells while excluding out-
307 of-focus cells.
308
309 To determine absolute cell concentrations it was necessary to quantify the depth of field for each
310 of the three strains of bacteria used, so that cell concentration could be computed from the
311 number of cells and the imaging volume. The detection of cells described above depends on the
312 choice of gating parameters and the fluorescent intensity of the cells themselves. The depth of
313 field was determined empirically by the analysis of vertical image stacks of fluorescently labeled
314 cells on a horizontal surface. The image processing described above was carried out and the
315 depth of field was defined as twice the height at which the number of detected in-focus cells was
316 $<10\%$ of the value at the surface. This depth of field was then used consistently throughout the
317 analysis to determine cell concentrations. The cell concentration in each well, C, was normalized
318 by the cell concentration in the initial bacterial suspension, C_0, measured in a well filled with that
319 suspension.
320
321 **Mathematical model of chemotaxis into ISCA wells**
322
323 We compared our laboratory observations with the predictions from a mathematical model,
324 implemented in COMSOL Multiphysics 5.1 (COMSOL Inc., USA) using the 'Transport of
325 Diluted Species' and 'Coefficient Form PDE' modules. The model domain consisted of a single
326 three-dimensional ISCA well and the fluid (1 ml) outside the inlet port (**Supplementary Fig. 8**).
327 The model, based on coupled partial differential equations, solves for the spatiotemporal
328 distribution of the chemoattractant (**Supplementary Video 1**), which is then used as input into a
329 transport equation for populations of chemotactic or non-chemotactic bacteria, modeled as a
330 concentration of cells[26] (**Supplementary Video 2** and **Supplementary Fig. 9**). The mesh
331 consisted of two domains (port and well; **Supplementary Fig. 8**) and boundary layers were
332 enabled, with edges trimmed. The chemical field and bacterial field were initialized as opposing
333 smoothed step functions, with the transition zone determined by the penetration depth of a cavity

334 flow (**Supplementary Note 1**). Because chemotactic transport models are only well-developed
335 for specific model organisms, and in particular *Escherichia coli*[14], the model was parameterized
336 for *E. coli* responding to the non-metabolizable chemoattractant α-methylaspartate, based on a
337 model described previously[16]. The model was initialized with the bulk fluid containing 1×10^5
338 cells ml^{-1} and the well contained 100 μM α-methylaspartate. The model then ran in two stages:
339 an initial, 1-s long stage with 0.1-s time-steps and a subsequent, 1-h long stage with 10-s time-
340 steps. This two-stage method saves on computation time while allowing sufficient temporal
341 resolution early in time, when very steep gradients occur. The output of the model consisted in
342 the concentration of chemoattractant and bacteria over the full computational domain and over
343 time, which was then used to compare with experimental observations. The COMSOL model
344 file (**Supplementary File 2**) can be found in the supplementary materials.
345
346 **Field deployments**
347
348 Field deployments occurred in April 2016 at Clovelly Beach (33.91° S, 151.26° E), situated on
349 the eastern coast of Australia. To ensure the initial absence of microbial cells in the seawater
350 used as both a negative control and as a base for chemoattractant suspensions, seawater from the
351 site was subjected to a triple-filtration process: 50 ml was collected and first filtered through a
352 0.2-μm Sterivex filter (Millipore, USA), followed by a 0.2-μm Millex FG (Millipore, USA) and
353 finally through a 0.02-μm Anotop filter (Whatman, England). Five 80-μl samples of this ultra-
354 filtered seawater were fixed into 2% glutaraldehyde for subsequent flow cytometry analysis,
355 which confirmed the effectiveness of this filtration protocol in removing all bacterial cells.
356
357 For initial testing of the ISCA in the field we used a broad-spectrum chemoattractant, Marine
358 Broth 2216 (Beckton Dickson, NJ, USA), a common growth medium for marine microbes,
359 which contains peptone (5 g/l) and yeast extract (1 g/l). A 10% final concentration of Marine
360 Broth was resuspended in freshly ultra-filtered seawater from the deployment site immediately
361 before loading in the ISCA wells. The ISCA wells were filled with the port facing up: sterile 1-
362 ml syringes and 27G needles (Terumo, Japan) were used to load samples into the wells. The
363 diameter of the needle being smaller than the port allows air to escape as the well is filled. This

364 procedure was found to be optimal in ensuring complete filling and no residual air bubbles in the
365 wells.
366
367 Across each ISCA, treatments (filtered seawater or Marine Broth) were randomly allocated to an
368 ISCA row (consisting of five wells). All wells in a row acted as technical replicates and four
369 different ISCAs were deployed in parallel to act as biological replicates. Each ISCA was secured
370 inside a deployment enclosure (**Supplementary Fig. 10**), which was sealed by pressure-sensitive
371 tape (Scotch, 3M, USA) and an enclosure plug to seal the bottom drain (**Supplementary Fig.
372 11**). Each enclosure was then attached to a modified vice enabling the deployed ISCA to be
373 firmly secured to a rigid structure (e.g., pontoon, ladder, pole) to minimize enclosure movement
374 and standardize the deployment depth at 1 m. As the enclosures were submerged, they slowly
375 filled with seawater through the enclosure inlets. Once completely filled with seawater, the
376 enclosure inlets were plugged (to seal the enclosure) and the ISCAs were left *in situ* for 1 h.
377
378 Upon retrieval of the enclosures, the side outlets were unsealed progressively to slowly drain the
379 seawater. Once the enclosure were completely empty, the contents of ISCA wells were then
380 collected using 1-ml syringes and 27G needles (Terumo, Japan), with the ports facing down. For
381 each ISCA, the liquid in the wells acting as technical replicates was pooled (five wells per
382 treatment) in order to increase the amount of DNA collected per sample. The total volume of
383 each pooled sample was 550 μL, out of which 80 μL was fixed with filtered glutaraldehyde (2%
384 final concentration) for flow cytometry analysis (conducted on fresh samples the same day) and
385 470 μL was snap-frozen immediately in liquid nitrogen for subsequent DNA extraction and
386 sequencing. In addition to the ISCA samples, bulk seawater samples (n = 4) were also collected
387 for both flow cytometry and DNA sequencing. Water temperature and salinity were recorded
388 with a multiprobe meter (WTW Multiparameter Meter, WTW, Germany).
389 Although the deployment enclosure was required to generate a microenvironment that is
390 analogous to that experienced by planktonic bacteria interacting with chemical hotspots in the
391 water column, the manner in which the ISCA is housed is entirely dependent on the scientific
392 question investigated. One may wish to study the interaction of bacteria with cues released from
393 surfaces, such as in the benthic environment. In this case, the surface is stationary but the

394 bacteria are advected by the flow, a scenario that is best replicated by deploying the ISCA
395 without an enclosure.
396
397 ***Flow cytometry analysis***. Samples for flow cytometry were transferred into sterile Titertube
398 micro test tubes (Bio-Rad, CA, USA), stained with SYBR-Green I (Thermo-Fisher, MA, USA),
399 incubated for 15 min in the dark, and analyzed on a BD Accuri C6 flow cytometer (Becton
400 Dickinson, NJ, USA) with filtered MilliQ water as the sheath fluid. For each sample, forward
401 scatter (FSC), side scatter (SSC), green (SYBR-green), and red (chlorophyll) fluorescence were
402 recorded. The samples were analyzed at a flow rate of 35 μl min^{-1}. Microbial populations were
403 characterized according to SSC and SYBR Green fluorescence[27] and cell abundances were
404 calculated by running a standardized volume of sample (50 μl). To quantify the strength of
405 chemotaxis, the Chemotactic Index (I_C) was calculated by dividing the number of cells present in
406 the chemoattractant treatment by the number of cells present in the filtered seawater (FSW)
407 negative control.
408
409 **Molecular analysis**
410
411 ***DNA extraction, library preparation, sequencing and bioinformatics***. DNA extraction from
412 seawater samples was performed using the UltraClean® Tissue & Cells DNA Isolation Kit,
413 following the manufacturer's instructions with minor modifications described previously[21].
414 Libraries for shotgun metagenomic sequencing were prepared using the Nextera XT DNA
415 Sample Preparation Kit (Illumina Inc., San Diego, CA, USA) following a previously described
416 modified protocol designed for generating low DNA input libraries[21]. All libraries were
417 sequenced with an Illumina NextSeq 500 platform 2× with 150 bp High Output v.1 run
418 chemistry. Libraries were pooled on an indexed shared sequencing run, resulting in 1/37 of a run
419 or ~3.2 Gbp per sample. The raw fastq read files were deposited on the Microscale Ocean
420 webpage (http://microscaleocean.org/data/category/12-in-situ-chemotaxis-assay).
421
422 To characterize the composition of bacterial communities, 16S rRNA gene-based taxonomic
423 profiles of the samples were generated with GraftM (http://geronimp.github.io/graftM) using the
424 16S rRNA package (4.39.2013_08_greengenes_97_otus.better_tree.gpkg). The pipeline was

425 designed to identify reads encoding 16S rRNA genes based on HMMs and to assign taxonomic
426 classifications by comparison against a reference taxonomy. A detailed feature description, user
427 manual, and example runs are available on the GitHub wiki
428 (https://github.com/geronimp/graftM/wiki). For the heatmap, the GraftM output was manually
429 curated, whereby mitochondrial and chloroplast sequences were removed. Relative abundances
430 were calculated and trimmed (>5% max) in the software environment R (www.r-project.org),
431 and the data was displayed as a heat-map (pheatmap). Differences in 16S rRNA gene abundance
432 between samples (n = 4) were compared via ANOVA, using the Statistical Analysis of
433 Metagenomic Profiles (STAMP) software[28].
434
435 In addition, a reference genome based taxonomic classification approach was employed using
436 the bacterial genome collection in GTDB (gtdb.ecogenomic.org). Reads were aligned back with
437 Bowtie2[29], BAM files were created and reads counted with SAMtools[30]. Taxonomy parsing and
438 cross-reference of sequences was performed using MGKit (bitbucket.org/setsuna80/mgkit) and
439 analysis performed in Python (www.python.org) and Pandas (pandas.pydata.org/) environments.
440 Counts were grouped to the class level and relative abundances calculated. Taxonomic
441 assignments that accounted for less than 5% of the total reads were filtered and a clustered
442 heatmap produced with Seaborn (http://seaborn.pydata.org/index.html).
443

444 **DATA AVAILABILITY**

445

446 The raw fastq read files are available on the Microscale Ocean webpage
447 (http://microscaleocean.org/data/category/12-in-situ-chemotaxis-assay). The experimental image
448 data are available from the corresponding author upon request, due to large file size.
449

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511

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513

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519

520

521 **AUTHOR CONTRIBUTIONS**

522

523 B.L., J-B.R., J.R.S., and R.S. designed the experiments. B.L., J-B.R., and N.S. performed the
524 experiments. B.L., J-B.R., V.F., F.R. and C.R. analyzed the results. B.L., J-B.R., J.R.S., and R.S.
525 wrote the manuscript. All authors edited the manuscript before submission.

526

527 **FIGURES**

528

529 **Figure 1 | Fabrication of the *in situ* chemotaxis assay (ISCA) and laboratory tests.** (**a**)
530 Polydimethylsiloxane (PDMS) is cast onto a 3D-printed mold and cured overnight. The solid
531 PDMS, containing multiple wells, is then excised and plasma-bonded onto a glass slide (100 mm
532 × 76 mm × 1 mm). Each well has an independent connection to the external environment via a
533 port, through which chemicals can diffuse and microbes enter. Upon deployment, the ISCA
534 produces chemical microplumes that mimic transient nutrient patches. Chemotactic bacteria
535 respond by swimming into the wells of the device, and after collection can be enumerated by

536 flow cytometry and identified by sequencing. Computer-aided-design files can be found in
537 Supplementary File 1. (**b**) Accumulation of fluorescently labeled marine isolates within ISCA
538 wells quantified through video microscopy. The solid line represents the mean cell concentration
539 ($n=3$) over the imaging volume normalized to that in the surrounding medium and the shaded
540 area is one standard deviation around the mean. Red: *V. coralliilyticus* swimming into a well
541 initially filled with 10% Marine Broth. Black: *V. coralliilyticus* and FASW (filtered artificial
542 seawater; control). Purple: *M. adhaerens* ΔfliC and FASW (non-motile control; almost
543 indistinguishable from zero). The triangle on the right-hand axis indicates the chemotactic index,
544 I_C, for *V. coralliilyticus* after 60 min, calculated as the ratio of the number of cells responding to
545 the chemoattractant and to the FASW. (**c**) Representative images taken at mid-depth of the well
546 after 60 min. MB: 10% Marine Broth, FSW: Filtered seawater control, ΔfliC: Non-motile mutant
547 control. Scale bar = 100 µm; n = 3. Note the near absence of cells from the controls (FSW and
548 ΔfliC). (**d**) Average accumulation through well depth and time of fluorescently labeled *V.*
549 *coralliilyticus* in response to 10% Marine Broth (left, $n = 3$); and in response to FASW (right, n
550 $= 3$). Minor accumulation shows that random motility does not contribute significantly to the
551 final concentration of cells in each well. The color bar applies to both panels and indicates the
552 concentration of cells, C, in each image normalized to that in the surrounding medium, C_0. The
553 resolution is 80 µm in the depth and 1 min in time.
554
555 **Figure 2 | Field tests of the ISCA.** (**a**) Chemotactic index I_C, denoting the concentration of cells
556 within ISCA wells, normalized by the mean concentration of cells (n = 4) within wells
557 containing filtered seawater (FSW), after a 60 min field deployment. Cells were enumerated by
558 flow cytometry (MB: $9.4 \pm 0.4 \times 10^3$, FSW: $2.6 \pm 0.3 \times 10^3$; cell/mL). Note that wells containing
559 10% Marine Broth had significantly more bacteria than the FSW control (t-test, $n = 4$, $p <$
560 0.005). Error bars represent standard errors. (**b**) Principal component analysis comparing family-
561 level taxonomic profiles of bulk seawater from the sampling site with the contents sampled from
562 the ISCA wells containing Marine Broth. (**c**) Taxa differing significantly between bulk seawater
563 and ISCA wells containing Marine Broth (n = 4). The p-values were corrected for multiple
564 hypothesis-testing using the Bonferroni correction (95% confidence intervals).
565