1 **Chemotaxis shapes the microscale organisation of the ocean's microbiome**

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Abstract

The capacity of planktonic marine microorganisms to actively seek out and exploit microscale chemical hotspots has been widely theorised to impact ocean-basin scale biogeochemistry[1-3], but has never been comprehensively examined *in situ* among natural microbial communities. Using a field-based microfluidic platform to quantify the behavioural responses of marine bacteria and archaea, we observed significant levels of chemotaxis towards microscale hotspots of phytoplankton-derived dissolved organic matter (DOM) at a coastal field site across multiple deployments, spanning several months. Microscale metagenomics revealed that a wide diversity of marine prokaryotes, spanning 27 bacterial and 2 archaeal phyla, displayed chemotaxis towards microscale patches of DOM derived from ten globally distributed phytoplankton species. The distinct DOM composition of each phytoplankton species attracted phylogenetically and functionally discrete populations of bacteria and archaea, with 54% of chemotactic prokaryotes displaying highly specific responses to the DOM derived from only one or two phytoplankton species. Prokaryotes exhibiting chemotaxis towards phytoplankton-derived compounds were significantly enriched in capacity to transport and metabolise specific phytoplankton-derived chemicals, while also displaying enrichment in functions conducive to symbiotic relationships, including genes involved in the production of siderophores, B-vitamins and growth-promoting hormones. Our findings demonstrate that the swimming behaviour of natural prokaryotic assemblages is governed by specific chemical cues, which dictate important biogeochemical transformation processes and the establishment of ecological interactions that structure the base of the marine food-web.

Main text

Understanding how organisms forage within a heterogeneous resource landscape is a fundamental goal of ecology[4]. Within the ocean, populations of microorganisms govern marine productivity and biogeochemical cycling over vast, ocean basin scales[5, 6]. However, from the perspective of an individual planktonic microbe, important ecological processes including resource acquisition[7], predation and symbiosis, occur over microscopic scales, often within a surprisingly heterogeneous seascape shaped by microscale gradients of chemical resources and foraging cues[3, 8]. Evidence from theoretical and laboratory-based studies indicate that some marine microbes are highly adept at foraging within patchy environments using chemotactic behaviour[9-11] – the capacity to migrate up or down chemical

67 gradients – and that these behaviours may have important ecological and biogeochemical
68 implications[12-14].
69
70 Microbial chemotaxis has primarily been studied in highly structured
71 microenvironments such as biofilms, soil or host tissues[15]. However, this behaviour might
72 also play important roles in the ocean water column, allowing bacteria to exploit localized
73 nutrient hotspots[16], colonise particles[14] or establish spatial associations with other
74 microorganisms[17], such as phytoplankton[18]. For instance, it has been proposed that
75 chemotaxis allows bacteria to colonise the microenvironment surrounding individual
76 phytoplankton cells, called the phycosphere[19], which is characterised by pronounced
77 gradients of dissolved organic matter (DOM)[18, 20]. Chemotaxis may be critical for microbes to
78 establish and maintain the close spatial association required for reciprocal chemical
79 exchanges to occur in the phycosphere, which can enhance the growth of both the bacterial
80 and phytoplankton partners[21] and ultimately influence the productivity of marine
81 ecosystems[18]. Although the role of chemotaxis in marine systems has been explored
82 extensively in model systems within laboratory settings, there is currently little evidence that
83 natural communities of marine bacteria and archaea use these behaviours *in situ* and our
84 understanding of which chemical currencies drive these behaviours in the environment is
85 limited.
86
87 To determine whether natural assemblages of marine microbes can use chemotaxis to
88 exploit a patchy chemical seascape and to examine the behavioural, chemical and genomic
89 features regulating interactions between phytoplankton and prokaryotes, we used the *in situ*
90 chemotaxis assay (ISCA)[22]. This microfluidic platform is comprised of a parallelized array of
91 micro-wells, each connected to the outside seawater by a port[22]. When deployed in the ocean,
92 chemoattractants pre-loaded within each well diffuse into the surrounding seawater, creating
93 microscale chemical plumes analogous to those resulting from diffusing hotspots, such as the
94 phycosphere (Fig. 1a). Chemotactic microorganisms in the surrounding water column migrate
95 up the chemical gradients towards the source of the plume and become trapped within the
96 well. We used the ISCA to simulate phycospheres and measure the behavioural responses of
97 planktonic prokaryotes to phytoplankton-derived DOM hotspots, and then characterised the
98 genomic and biochemical basis for these responses by analysing the identity and metabolic
99 capacity of microorganisms trapped in the wells. Using this *in situ* approach, we tested the
100 hypotheses that i) chemotaxis is pervasive among natural assemblages of marine bacteria and

101 archaea, enabling them to exploit localised chemical hotspots such as the phycosphere; and ii)
102 differences in chemical composition between phytoplankton-derived DOM underpin
103 selectivity in behavioural responses, leading to taxonomic and functional partitioning of
104 prokaryotic communities at the ocean's microscale.
105
106 DOM was harvested from a total of 14 marine phytoplankton species, spanning
107 globally abundant and ecologically significant groups (diatoms, dinoflagellates, haptophytes,
108 cryptophytes, chlorophytes, and cyanobacteria), which occur in the coastal surface waters of
109 eastern Australia (Table S1). ISCA wells were loaded with phytoplankton-derived DOM or
110 filtered seawater from the deployment site (which acted as a control) and were deployed for 1
111 h in surface waters (1 m depth) in 12 independent experiments, performed over two years at a
112 coastal site near Sydney, Australia. Each treatment was replicated across four different ISCAs
113 that were deployed simultaneously ($n = 4$). Following deployment, the contents of the ISCA
114 wells were retrieved and flow cytometry was used to enumerate microbial cells, allowing
115 quantification of the strength of chemotaxis towards the DOM of each phytoplankton species,
116 which was defined by the chemotactic index (*Ic*), equivalent to the number of cells in a
117 treatment divided by the average number of cells in the control wells[22].
118
119 ## *In situ* chemotaxis assays
120 ISCA experiments revealed that natural populations of marine prokaryotes exhibited
121 strong chemotaxis towards the chemicals produced by phytoplankton (ANOVA, $p<0.05$, Fig.
122 1a-c, Extended Data Fig. 1, Table S3). Chemotactic strength varied considerably between
123 ISCA deployments (Fig. 1b, Extended Data Fig. 1) and exhibited a significant and positive
124 correlation with water temperature (Fig. 1d, Pearson's R=0.75, $p<0.01$, Extended Data Fig.
125 2). Chemotaxis was not detected during the three ISCA deployments carried out during
126 austral winter months, potentially due to the lower numbers of motile cells in winter[8], slower
127 swimming speed in colder water[23], or other biological factors (e.g. protozoan grazing, viral
128 lysis). In summer and autumn, DOM from the dinoflagellate *Amphidinium* elicited significant
129 chemotactic responses in eight out of nine (88.8%) ISCA deployments, with *Ic* values up to
130 4.6 ± 0.9 (which correspond to 4.6 times more cells than in the controls; Extended Data Fig.
131 1, Table S3), while the diatoms *Ditylum* and *Thalassiosira*, as well as the chlorophyte
132 *Dunaliella*, elicited significant chemotactic responses in seven out of nine (77.7%)
133 deployments. These results deliver the first *in situ* evidence that natural assemblages of

marine prokaryotes have the capacity to sense and respond to microscale patches of phytoplankton-derived DOM in the water column.

The strongest chemotactic responses were recorded in February 2018, and these samples were selected for detailed analysis using metabolomics and microscale metagenomics[24]. During this deployment, the DOM of 8 of the 10 phytoplankton species promoted positive chemotaxis (ANOVA, $p<0.05$, Fig. 1b, Table S3). The strongest chemotactic responses were recorded for DOM derived from the dinoflagellate *Alexandrium minutum* and the haptophyte *Prymnesium parvum*, with I_c values of 4.8 ± 0.8 and 3.6 ± 0.9, respectively. DOM derived from *Synechococcus*, *Dunaliella*, *Chaetoceros*, *Thalassiosira* and *Amphidinium* elicited I_c values between 2.3 - 2.7. The DOM derived from each phytoplankton species displayed distinct chemical fingerprints (Fig. 1, Extended Data Fig. 3, Table S4), which is consistent with previous reports showing that marine phytoplankton can release characteristic suites of organic material[25]. A total of 111 phytoplankton-derived compounds were detected from the water-soluble fraction of the harvested DOM, and consisted primarily of amino acids, amines, sugars, organic acids, fatty acids and other metabolic intermediates (Fig. 2a, Extended Data Fig. 3). These results indicate that variability in the extent of chemotactic responses was governed by the chemical composition of the different phytoplankton-derived DOM.

Identity of attracted prokaryotes

Microscale metagenomic analysis[24] revealed that phytoplankton-DOM treatments attracted specific microbial communities that were significantly different from both those present in the filtered seawater control and those present in the surrounding seawater at the deployment site (PERMANOVA, $p<0.05$, Table S5, Fig. 2b, Extended Data Fig. 4). In addition, the microbial communities present in the ten phytoplankton-DOM treatments were significantly different to each other (PERMANOVA, $p<0.05$). We identified 163 taxa belonging to 27 bacterial and 2 archaeal phyla, which were significantly enriched (F-tests, $p<0.05$, Table S6) in ISCA wells containing phytoplankton-derived DOM relative to the control. The most highly enriched species belonged to the Vibrionaceae, Alteromonadaceae and Pseudoalteromonadaceae, with the absolute abundance of these highly motile and chemotactic bacterial families[20] up to 60-fold higher in DOM derived from the dinoflagellate *Alexandrium tamarense* compared to the control (Fig. 2c). Approximately half (46%) of the significantly enriched taxa responded to DOM from three or more phytoplankton species,

168 indicating a generalist response to phytoplankton-derived DOM (Fig. 2d, Extended Data Fig.
169 5, Table S6). Among the generalist taxa, populations of the Gammaproteobacteria genera
170 *Pseudoalteromonas*, *Thalassomonas* and *Vibrio* were significantly enriched in 9 of 10
171 phytoplankton-derived DOM (Extended Data Fig. 5). The other half (54%) of the
172 significantly enriched taxa responded to DOM from only one (29%) or two (25%)
173 phytoplankton species, indicating a more specialist chemotactic response (Fig. 2d, Extended
174 Data Fig. 5, Table S6). These specialist groups included the Alphaproteobacteria genera
175 *Novispirillum*, *Dinoroseobacter* and *Octadecabacter*, and the Bacteroidia genera *Maribacter*
176 and *Aquimarina* (Extended Data Fig. 5). These results suggest that these specialist
177 chemotactic responses might promote the establishment of specific associations between
178 phytoplankton and prokaryotes, ultimately driving microscale partitioning in the composition
179 of marine microbial assemblages.
180
181 **Functions of attracted prokaryotes**
182 In addition to being taxonomically different, the bacterial and archaeal populations
183 that exhibited chemotaxis to phytoplankton-derived DOM were also functionally distinct
184 from those in the filtered seawater controls (which represent prokaryote populations that enter
185 the ISCA wells via random motility without using chemotaxis) and surrounding seawater
186 (PERMANOVA, $p<0.05$; Table S7). As expected, all ISCA treatments, including the filtered
187 seawater controls, were significantly enriched in genes involved in motility and chemotaxis
188 (e.g. *che*, *flg*, and *fli* genes, F-tests, $p<0.05$, Extended Data Fig. 6), but also in genes
189 mediating surface attachment (e.g. *cpa*, and *pil* genes, F-tests, $p<0.05$). Notably, several
190 functions that promote beneficial interactions with phytoplankton, including production of
191 siderophores, plant growth-promoting hormones and vitamins, as well as quorum sensing and
192 secretion systems, were enriched in prokaryotes responding to phytoplankton-derived DOM
193 (Fig. 3).
194
195 Functional orthologs involved in the production of siderophores, including
196 Mycobactin and Vibriobactin, were significantly enriched in prokaryotes responding to
197 phytoplankton-DOM compared to controls (F-tests, $p<0.05$, Table S8). Siderophores are
198 small compounds excreted by bacteria and archaea that have high affinity for iron and
199 increase its solubility and bioavailability[26]. Iron limits primary production in vast areas of the
200 world ocean[27], but phytoplankton can gain access to this essential micronutrient through
201 bacterial siderophores[28], which have been proposed to promote phytoplankton-bacteria

202 mutualism[29]. The observed enrichment of siderophore-related genes in responding
203 prokaryotic populations raises the intriguing prospect that some phytoplankton may release
204 specific chemical cues to attract chemotactic siderophore-producing bacteria into their
205 phycosphere.
206
207 Genes required for the biosynthesis of the plant growth-promoting hormones indole-
208 3-acetic acid, jasmonate, ethylene, 2,3-butanediol, as well as multiple B-vitamins (thiamine
209 (vitamin B_1); cobalamin (vitamin B_{12})) were significantly enriched in prokaryotes responding
210 to phytoplankton-derived DOM (F-tests, $p<0.05$, Table S8). For example, orthologs involved
211 in the production and transport of thiamine were on average 2.3 ± 0.5 times more abundant in
212 communities exhibiting chemotaxis towards phytoplankton-derived DOM compared to
213 controls (Fig. 3). Most phytoplankton are auxotrophs for essential cofactors, such as B-
214 vitamins, and are completely reliant on prokaryotic production to fulfil their needs[30, 31]. In
215 addition, prokaryotic production of growth-promoting hormones is common among soil
216 bacteria associated with the rhizosphere of terrestrial plants[32] and has been observed in
217 bacteria within diatom cultures, where these molecules have been proposed to affect
218 phytoplankton cell division and productivity[21]. B-vitamins and growth-promoting hormones
219 have therefore been predicted to be key currencies of chemical transactions between
220 phytoplankton and bacteria[18] and the enrichment of genes involved in their production in
221 phytoplankton-DOM containing ISCA wells provides evidence that chemotactic prokaryotes
222 that colonise phycospheres might play a critical role in supporting phytoplankton growth and
223 metabolism.
224
225 Genes encoding type III and IV secretion systems were on average 50.1 ± 5.1 and 2.5
226 $\pm$ 0.7 times more abundant in phytoplankton-DOM treatments compared to controls,
227 respectively (F-tests, $p<0.05$, Table S8). These large protein complexes enable bacterial
228 translocation of molecules and have been primarily studied in the context of pathogenic
229 interactions[33, 34], but bacterial symbionts also use these molecular mechanisms to efficiently
230 transfer specific compounds to their hosts[35]. For example, type IV secretion systems are
231 present in the genomes of nearly half of the most common phytoplankton-associated
232 *Roseobacters*[36]. The abundance of secretion system genes in prokaryotes attracted by
233 phytoplankton-derived DOM suggests that phycosphere microbes may use them to facilitate
234 the exchange of metabolites between cells. Finally, genes required to produce quorum
235 sensing molecules were also significantly enriched in phytoplankton-DOM treatments (F-

236 tests, p<0.05, Table S8). Quorum sensing has been predicted to regulate phytoplankton-
237 bacteria interactions by mediating surface attachment[37], and the colonisation of the
238 phycosphere by prokaryotes[38]. The observed prevalence of genes encoding siderophores,
239 growth-promoting hormones, quorum sensing and vitamins reveal that the prokaryotic
240 populations responding to phytoplankton-DOM treatments were considerably enriched in
241 functions that can mediate mutualistic interactions with phytoplankton cells. Our results
242 therefore suggest that chemotaxis plays an initial filtering role in the establishment of these
243 important marine symbioses.
244
245 The prokaryotic communities exhibiting chemotaxis to each of the 10 phytoplankton
246 DOM treatments displayed significantly different functional profiles to one another
247 (PERMANOVA, p<0.05; Table S7), indicating functional partitioning of the microbial
248 assemblages responding to different simulated phycospheres. Among the microbial
249 communities exhibiting chemotaxis towards phytoplankton DOM, there was a significant
250 enrichment in a suite of genes involved in the metabolism of phytoplankton-derived
251 compounds[18], such as the osmolyte dimethylsulfoniopropionate (DMSP), the sulfonate 2,3-
252 dihydroxypropane-1-sulfonate (DHPS) and the polyamine putrescine (F-tests, p<0.05,
253 Extended Data Fig. 7, Table S8). Genes involved in the uptake of other exogenous substrates
254 (e.g. sugars, sugar alcohols, amino acids) were also enriched in these treatments (F-tests,
255 p<0.05, Extended Data Fig. 7, Table S8), although future research is needed to confirm the
256 directionality and substrate specificity of these transporters. Such patterns reveal that marine
257 prokaryotes display chemotactic responses to phytoplankton DOM that are potentially linked
258 to their ability to uptake and metabolise specific DOM components.
259

260 **Identification of chemoattractants**

261 To further identify specific chemical compounds underpinning chemotactic responses,
262 we examined correlations in the relative abundance of each chemical identified in a
263 phytoplankton-derived DOM with the relative abundance of each responding prokaryotic
264 taxon in the corresponding ISCA wells. We identified 131 significant positive correlations
265 (Pearson's correlation, p<0.05, Fig. 4a) linked to 46 compounds, some of which are known to
266 play important roles in the metabolic relationships between bacteria and phytoplankton, such
267 as xylose, putrescine, and glutamate[39]. Some prokaryotic taxa were significantly correlated to
268 more than one compound. Among them, members of the *Agarivorans* genus
269 (Celerinatantimonadaceae) exhibited the strongest correlations with ethanolamine, putrescine,

glycerol, and erythritol, while *Photobacterium* (Vibrionaceae) were highly correlated with gluconate, digalactosylglycerol, galactose, and succinate. In addition, members of the *Maribacter* (Flavobacteriaceae) and Poseidoniales (Marine Group II), the most abundant archaeal group in the surface ocean[40], were highly correlated with xylitol, citric acid, proline, ornithine, lysine, 3-aminopiperidin-2-one, and three unknown compounds.

To validate the importance of these specific compounds in driving chemotactic behaviour, we performed additional laboratory-based chemotaxis assays, whereby the chemotactic response of the marine isolates *Agarivorans albus*, *Photobacterium* sp., and *Maribacter dokdonensis* were measured towards the compounds with which they were significantly correlated. These experiments revealed that 18 out of 22 compounds tested (81.8%) significantly attracted these isolates (ANOVA, $p<0.05$, Fig. 4b). Some compounds not previously implicated in phytoplankton-prokaryote interactions were identified as chemoattractants, such as the sugar alcohol erythritol (mostly found in the DOM from the green algae *Dunaliella*), or 3-aminopiperidin-2-one (which was particularly abundant in *Phaeodactylum*-derived DOM; Fig. 4b). Our results therefore strongly suggest that these compounds constitute previously unrecognised chemotactic cues and chemical currencies in the marine food web. To assess whether these compounds are metabolised by responding bacteria, in addition to acting as chemoattractants, we performed growth assays on each of the 18 identified chemoattractants. These experiments showed that 12 of these 18 compounds (66.6%) were also used by the isolates to support growth (Fig. 4b, Extended Data Fig. 8), revealing a direct link between chemotactic behaviour and the ability of these microorganisms to metabolise specific molecules. The remaining 6 compounds (33.3%) therefore acted as non-metabolisable signals that may be used solely to locate specific microenvironments, illustrating the different ecological functions underpinning chemotaxis.

Marine microbial processes are generally examined at the community level across large spatiotemporal scales, in contrast here we used *in situ* microfluidics to interrogate microbial behaviours at the microscale. Our sampling strategy captured seasonal changes in the extent of chemotaxis at a single site, but we acknowledge that spatial variability (i.e. across different environments and depths) in chemotaxis strength is also likely to occur according to local physicochemical conditions (including microscale gradients in other signals such as viscosity, pH, or dissolved gases), and that this warrants future research. Furthermore, the chemotactic behaviour observed here will likely not be restricted to

304 prokaryotes, with some marine protists also capable of foraging responses to microscale
305 chemical hotspots[41], but longer deployment times of the ISCA would be required to attract a
306 sufficient number of eukaryotic cells.
307
308 **Conclusion**
309 Metabolic responses of marine prokaryotes to microscale chemical heterogeneity in
310 the water column have been predicted to generate activity hotspots that influence all major
311 pathways of elemental flux in the ocean[1, 12]. Our *in situ* experiments demonstrate that DOM
312 derived from phytoplankton induces specific chemotactic responses across a broad range of
313 prokaryotes in natural marine assemblages. We identified generalist and specialist
314 chemotactic responses by diverse groups of bacteria and archaea, the molecules involved in
315 these responses, and evidence that the prokaryotic taxa attracted to phytoplankton DOM were
316 enriched in functions conducive to symbiotic relationships. Together, these observations
317 provide the first *in situ* evidence that chemotactic behaviour promotes the selective
318 recruitment of specific marine prokaryotes and leads to microscale partitioning of
319 biogeochemical transformation processes in the ocean. By unveiling this rich tapestry of
320 microbial interactions through *in situ* microscale observations, these results provide the basis
321 for quantifying the role of chemotaxis in accessing microscale hotspots in marine systems,
322 and an opportunity to scale up the impact of these processes on the ocean's biogeochemistry.
323
324 **Data availability**
325 The raw metabolome data files were deposited in MetaboLights (accession number:
326 MTBLS1980). The raw metagenome fastq files were deposited in Sequence Read Archive
327 (SRA; accession number: PRJNA639602). The raw amplicon fastq files were deposited in
328 SRA (accession number: PRJNA707306). The 16S rRNA gene sequences of the three
329 isolates were deposited in GenBank (accession numbers: MT826233-MT826234 and
330 MZ373175). All other raw data are available as source data.
331
332 **Code availability**
333 All custom analysis scripts are available on GitHub (https://github.com/JB-Raina-
334 codes/ISCA-paper).
335
336 **Acknowledgements**

337 The authors thank Glynn Gorick for his work on Figure 1a, Lucia Bennar, Gurjeet Kholi,
338 Michele Fabris, Nine Le Reun, Marco Giardina, and David Hughes for laboratory assistance
339 and Emmanuelle Botté for her support throughout this project. This work was supported by
340 the Gordon and Betty Moore Foundation through a grant to J.R.S., G.T., P.H. and R.S.
341 (GBMF3801) and two Investigator Awards to R.S. (GBMF3783 and GBMF9197;
342 https://doi.org/10.37807/GBMF9197), and through Australian Research Council grants
343 DP110103091 to J.R.S. G.W.T. and R.S and DP180100838 to J.R.S., R.S and J.B.R. B.S.L.
344 was supported by the Simons Foundation (Award 594111). G.W.T. was supported by an
345 Australian Research Council Future Fellowship (FT170100070), P.H. was supported by
346 Australian Research Council Laureate Fellowship (FL150100038). C.R. was supported by an
347 Australian Research Council Future Fellowship (FT170100213). J.B.R was supported by an
348 Australian Research Council Fellowship (DE160100636).

349

350 **Author contributions**

351 J-B.R., B.S.L., C.R., R.S., P.H., G.W.T., and J.R.S. designed all the experiments. J-B.R.,
352 C.R., A.B., and N.S. performed all the experiments. J-B.R., A.L. and H.M. generated and
353 analysed the metabolomic data. J-B.R., C.R., F.R., B.S.L., and D.P. generated and analysed
354 the metagenomic data. J-B.R., M.O., B.S.L., A.B., V.I.F., and B.S. generated and analysed
355 the amplicon data and performed the network and correlative analyses. J-B.R., B.S.L., R.S.
356 and J.R.S. wrote the manuscript. All authors edited the manuscript before submission.

357

358 **Competing interests**

359 The authors declare no competing interests.

360

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451

452 **Figures**

453

454 **Figure 1: Use of the *in situ* chemotaxis assay (ISCA) to probe for chemotaxis towards**
455 **phytoplankton-derived DOM in the natural environment.** (**a**) Phytoplankton-derived
456 DOM is loaded in ISCA wells prior to deployments in the ocean. Each well is independently
457 connected to the external environment via a port, through which chemicals can diffuse and
458 microorganisms can enter. Upon deployment, the ISCA produces chemical microplumes that
459 mimic microscale nutrient hotspots. Chemotactic prokaryotes respond by swimming into the
460 ISCA wells and can then be enumerated by flow cytometry and characterised by microscale
461 metagenomics. Scale bar: 7.5 mm. (**b**) Average chemotactic index (*Ic*) elicited by the
462 phytoplankton-derived DOM over a two year period at Clovelly Beach (33.91°S, 151.26°E).
463 The *Ic* denotes the concentration of cells within ISCA wells, normalized by the mean
464 concentration of cells within wells containing filtered seawater (FSW; $n = 10$). The full
465 dataset is presented in Figure S1. The blue bar represents the ISCA experiment (February
466 2018) that was further analysed using metagenomics and metabolomics. Data are presented
467 as mean values ± SEM. (**c**) Details of the *Ic* across the 10 different phytoplankton-derived
468 DOM treatments in February 2018 (normalized by the mean concentration of cells within
469 wells containing FSW), after 60 min field deployment. Full bars are significantly different
470 from wells containing FSW (ANOVA (one-sided), $n = 4$, $p < 0.05$, all *p*-values are reported

471 in Table S3). Each treatment was replicated across four different ISCAs (n = 4). Data are
472 presented as mean values ± SEM. (**d**) Significant correlation between chemotactic index
473 (presented in panel b) and temperature (Pearson's correlation (two-sided), p = 0.0053). The
474 95% confidence interval of the correlation coefficient is displayed. Artwork: Glynn Gorick.
475
476 **Figure 2: 'Generalist' and 'specialist' prokaryotic taxa responding to phytoplankton-**
477 **DOM.** (**a**) Principal component analysis (PCA) of the chemical composition of
478 phytoplankton-derived DOM. The first two components account for 52.3% of the total
479 variance. (**b**) PCA of prokaryotic taxonomic composition (16S rRNA gene) of the bulk
480 seawater (dark grey), FSW controls (grey) and phytoplankton-derived DOM. The first two
481 components account for 40.2% of the total variance. (**c**) Absolute abundance of selected
482 "generalists" (responding significantly to more than two different phytoplankton-derived
483 DOM) and "specialists" (significantly enriched in only one or two phytoplankton-derived
484 DOM) in ISCA wells, grouped at the family level. Full bars are significantly different from
485 wells containing FSW (ANOVA (one-sided), n = 4, p < 0.05, all p-values are reported in
486 Table S6). For the full list of significantly enriched taxa see Table S6. Data are presented as
487 mean values ± SEM. (**d**) Network analysis showing the differentiation between "generalist"
488 and "specialist" taxa. Chemotactic prokaryotic taxa (small circles; nodes) are linked to the
489 phytoplankton-DOM treatments they significantly responded to (large circles) by lines
490 (edges) coloured according to each treatment. The colour of each node corresponds to the
491 number of phytoplankton treatments they are significantly enriched in (see Extended Data
492 Fig. 5, Table S6). If a prokaryotic taxon responded only to one or two treatments, its
493 corresponding node appears in grey at the periphery of the network. If a prokaryotic taxon
494 responded to three or more treatments, its corresponding node appears towards the centre in a
495 colour ranging from blue to brown.
496
497 **Figure 3: Enrichment of prokaryotic genes involved in phytoplankton-bacteria**
498 **interactions.** Genes involved in B-vitamin, siderophore and phytohormone biosynthesis, as
499 well as quorum sensing, type III and IV secretion system assembly were significantly
500 enriched in the prokaryotic communities responding to phytoplankton-derived DOM. Data
501 shown as log-transformed relative abundance (generalized logarithm; n = 4) for each ISCA
502 treatment. The full list of genes significantly enriched in phytoplankton-DOM can be found
503 in Table S8.
504

505

506 **Figure 4: Specific associations between prokaryotes and phytoplankton-derived
507 metabolites.** (**a**) Network analysis showing the positive correlation between specific
508 compounds and prokaryotic taxa. Chemotactic prokaryotic taxa (circles; nodes) are linked to
509 the chemicals they responded to (grey octagons) by lines (proportional in colour and
510 thickness to the strength of the correlation, ranging from 0.7 to 0.9). From 112,110 possible
511 correlations (111 compounds and 1,010 taxa), only 131 were significant (and are all
512 displayed here) and all of them were positive. Because of this conservative approach, many
513 potential links between taxa and compounds were excluded. (**b**) To test the validity of this
514 correlative approach, laboratory-based chemotaxis assays were carried out on isolates of three
515 of the most prevalent prokaryotic nodes from the network (presented in panel a). 1:
516 *Agarivorans albus*, 2: *Photobacterium* sp., and 3: *Maribacter dokdonensis*. Full bars are
517 significantly different from wells containing FSW (ANOVA (one-sided), $n = 4$, $p < 0.05$, all
518 p-values are reported in Table S9). Error bars: standard errors. Eico. acid: Eicosanoic acid;
519 DGDG: Digalactosyldiacylglycerol; 3-aminopip: 3-aminopiperidin-2-one. In total, 18 out of
520 22 compounds tested (81.8%) attracted the isolates. The donut charts indicate the proportion
521 of chemoattractant also used as carbon source by each isolate, in total 12 out of 18
522 chemoattractants (66.6%) were also used as a carbon source (see Figure S10 for more
523 details).

524

525 **Methods**

526 Phytoplankton cultures

527 Fourteen phytoplankton cultures were selected, each based on their cosmopolitan distribution,
528 occurrence in the coastal waters of Sydney, Australia (Table S1), and taxonomic diversity.
529 Cultures were grown at their optimal light, temperature and nutrient conditions (see Table
530 S10 for the full list of phytoplankton taxa and culture conditions). The growth dynamic of
531 each phytoplankton culture was predetermined by conducting growth curves using flow
532 cytometry (see below). To grow cells for chemotaxis assays, four flasks per species
533 containing 250 mL of cultures were monitored twice a day for photophysiology parameters
534 (photosynthetic efficiency (F_V/F_M) and chlorophyll) using Fast-Repetition Rate Fluorometry
535 (FRRf; FastOcean MKIII, Chelsea Technologies Group, UK) coupled to a FastAct laboratory
536 system (Chelsea Technologies Group, UK). The system was programmed to deliver single
537 turnover saturation of photosystem II from a succession of 100 flashlets (1 µs pulse with a 2
538 µs interval between flashes), followed by a relaxation phase of 40 flashlets (1 µs pulse with a

539 50 µs interval between flashes). A total of 40 sequences were performed per acquisition, with
540 an interval of 150 ms between sequences[42]. Algal cells were harvested when their F_V/F_M was
541 at its maximal value and chlorophyll was exponentially increasing (mid-exponential phase).
542
543 Metabolomics – sample preparation
544 The content of each algal culture flask was split as follows: (i) 70 mL were allocated for
545 metabolomics, (ii) the remaining 180 mL were allocated for ISCA deployments. Samples
546 were centrifuged at low speed (1,500 g for 10 min, room temperature), supernatant was
547 removed, and the algal pellet was snap frozen in liquid nitrogen and kept at -80°C until
548 required. Chemical extractions for metabolomics were carried out as follows: frozen algal
549 pellets were freeze-dried overnight and extracted using 450 µl of HPLC-grade methanol
550 (containing internal standards (ITSD) at a 0.5% final concentration; $^{13}C_6$-Sorbitol; ^{13}C-^{15}N -
551 Valine, penta-fluorobenzoic acid and 2-aminoanthracene). Samples were mixed briefly, and
552 the extract and cell slurry were transferred into a 2 mL Eppendorf tube. The process was
553 repeated with an extra 450 µl of HPLC-grade methanol (with ITSD), ensuring that no
554 phytoplankton material remained in the freeze-dried tube. The Eppendorf tubes were mixed
555 by vortexing, then sonicated for 10 min on ice to rupture the cells, before being incubated for
556 30 min in a thermomixer at 1000 rpm (room temperature). Tubes were then centrifuged at
557 13,000 rpm for 10 min (room temperature), and supernatant was transferred into new
558 Eppendorf tubes. The remaining cell debris was resuspended in 900 µl of 50% HPLC-grade
559 methanol (without ITSD), incubated for 30 min in a thermomixer at 1000 rpm and then
560 centrifuged at 13,000 rpm for 10 min (room temperature). The supernatants were combined,
561 mixed and centrifuged at 13,000 rpm for 10 min at 4°C. The final supernatant was transferred
562 into a new tube, dried using a vacuum concentrator (Vacufuge, Eppendorf, Germany) and
563 analysed for metabolomics. The same extraction protocol was followed for the samples
564 allocated to ISCA deployments, with the exception that no internal standards were added to
565 the methanol. At the end of the extraction protocol, each extract was aliquoted, dried using a
566 vacuum concentrator and the resulting dry weight was quantified.
567
568 Metabolomics: sample derivatisation
569 Dried samples for untargeted analysis were prepared by adding 20 µL of Methoxyamine
570 Hydrochloride (30 mg/mL in Pyridine) followed by shaking at 37°C for 2h. Samples were
571 then derivatised with 20 µL of *N,O-bis* (Trimethylsilyl)trifluoroacetamide with
572 Trimethylchlorosilane (BSTFA with 1% TMCS, Thermo Scientific, USA) for 30 minutes at

573 37°C. The sample was left for 1 h before 1 µL was injected into the GC column using a hot
574 needle technique. Splitless and split (1:20) injections were done for each sample.
575
576 Metabolomics: analytical instrumentation
577 The GC-MS system used to characterise phytoplankton DOM composition consisted of a
578 Gerstel 2.5.2 autosampler, a 7890A Agilent gas chromatograph and a 5975C Agilent
579 quadrupole mass spectrometer (Agilent, Santa Clara, USA). The mass spectrometer was
580 tuned according to the manufacturer's recommendations using tris-(perfluorobutyl)-amine
581 (CF43). GC-MS was performed on a 30m Agilent J & W VF-5MS column with 0.25µm film
582 thickness and 0.25mm internal diameter with a 10 m Integra guard column. The injection
583 temperature (Inlet) was set at 250°C, the MS transfer line at 280°C, the ion source adjusted to
584 230°C and the quadrupole at 150ºC. Helium was used as the carrier gas at a flow rate of 1
585 mL/min. The analysis of TMS samples was performed under the following temperature
586 program; start at injection 70°C, a hold for 1 minute, followed by a 7°C min^{-1} oven
587 temperature ramp to 325°C and a final 6 minute heating at 325°C. Mass spectra were
588 recorded at 2.66 scans s^{-1} with an *m/z* 50-600 scanning range. Both chromatograms and mass
589 spectra were evaluated using the Agilent MassHunter Qualitative and Quantitative Analysis
590 version B.08.00 software and AMDIS software. Mass spectra of eluting TMS compounds
591 were identified using the commercial mass spectra library NIST (http://www.nist.gov), the
592 public domain mass spectra library of Max-Planck-Institute for Plant Physiology, Golm,
593 Germany (http://csbdb.mpimp-golm.mpg.de/csbdb/dbma/msri.html) and the *in-house*
594 Metabolomics Australia mass spectral library (containing chemical reference standards). This
595 approach is classified as Level 1-2[43] or level C according to the proposed reporting standards
596 by the Metabolite Identification Task Group of the Metabolomics Society
597 (http://metabolomicssociety.org/board/scientific-task-groups/metabolite-identification-task-
598 group). Resulting area responses were normalized to the ITSD $^{13}C_6$ Sorbitol area response
599 and to the dry weight of the extracts. The raw data files were deposited in MetaboLights
600 (accession number: MTBLS1980).
601
602 Metabolomics: data analysis
603 Metabolomic data were analysed using MetaboAnalyst 4.0[44, 45]. Normalised data were log-
604 transformed (glog), mean-centred and displayed as a heatmap and principal component
605 analysis (PCA) in Figure 1c and Figure S3. To determine if statistical differences existed
606 between phytoplankton-derived DOM treatments, a Bray-Curtis similarity matrix was

607 generated on the normalised data. A permutational multivariate analyses of variance
608 (PERMANOVA) was carried out using PRIMER (v6), with 999 unrestricted permutations.
609
610 ISCA design and assembly
611 ISCA moulds were 3D-printed out of the polymer VeroGrey on an Objet30 3D printer
612 (Stratasys, USA), using previously described designs and protocols[22, 46]. Each ISCA consisted
613 of an array of 5×5 wells, linked to the outside environment by an 800-μm-diameter port,
614 emitting chemical gradients that are analogous to those around large phytoplankton cells or
615 marine aggregates[20, 22]. Each mould was filled with 25 g of polydimethylsiloxane (PDMS;
616 10:1 PDMS base to curing agent, wt/wt; Sylgard 184, Dow Corning, USA). Curing was
617 carried out overnight at 40°C. The cured PDMS slab (95 mm × 65 mm × 4.6 mm) was cut
618 using a razor blade and carefully peeled from the mould. The PDMS blocks were inspected
619 and any port obstructions were cleared using a biopsy punch (ProSciTech, Australia). Finally,
620 the devices were UV-sterilized and plasma-bonded to sterile glass microscope slides (100
621 mm × 76 mm × 1 mm, VWR, USA) by exposing both to oxygen plasma for 5 min using a
622 plasma cleaner/sterilizer (Harrick Scientific, USA). Following bonding, the ISCA was heated
623 at 90°C for 10 min to accelerate the formation of covalent bonds and then stored at room
624 temperature, covered with a protective film, until use.
625
626 Field deployments
627 Field deployments were carried out between April 2016 and March 2018 at Clovelly Beach
628 (33.91°S, 151.26°E), a coastal location near Sydney, on the eastern coast of Australia. We
629 used seawater freshly collected from the field site and applied an ultra-filtration protocol to
630 ensure the complete removal of microbial cells[22, 46]. This ultrafiltered seawater acted as a
631 control in the ISCA and was also used to resuspend all dried phytoplankton-derived DOM
632 treatments, maintaining the same water chemistry as the surrounding seawater. Specifically,
633 60 mL were collected from the field site and filtered first through a 0.2 μm Millex FG (Merck
634 Millipore, USA); followed by two successive filtrations through a 0.22 μm Sterivex filter
635 (Merk Millipore, USA), and finally through a 0.02 μm Anotop filter (Whatman, UK). Four
636 replicate samples (80 μL) of this ultrafiltered seawater were fixed in 2% glutaraldehyde
637 (prefiltered at 0.2 μm) for subsequent flow cytometry analysis, which confirmed the
638 effectiveness of this filtration protocol in removing bacterial cells from seawater. In addition,
639 ultrafiltered seawater samples were collected as blanks for subsequent DNA extractions and
640 sequencing.

641
642 We used DOM derived from 10-12 different phytoplankton as chemoattractants in each
643 deployment. Each treatment was resuspended with ultrafiltered seawater to a final
644 concentration of 1 mg mL^{-1}. Notably, chemical concentrations decay exponentially with
645 distance away from the ISCA port, meaning that concentrations experienced by prokaryotes
646 in the surrounding seawater will be substantially lower, see Supplementary Note 2 in Lambert
647 et al.[22]). Treatments (filtered seawater and each 10 phytoplankton DOM) were randomly
648 allocated to an ISCA row (consisting of five wells). All wells in a row contained the same
649 treatment, with each treatment was replicated on four discrete ISCAs, which were deployed
650 in parallel to act as biological replicates[22]. As previously described each ISCA was secured
651 inside a transparent flow-damping enclosure, which prevents the disruption or interaction of
652 the chemical microplumes emanating from the ports[22]. The enclosure was completely sealed
653 *in situ* and deployed at 1 m depth for 1 h.
654
655 Upon retrieval, the contents of ISCA wells were then collected using 1 mL syringes and 27G
656 needles (Terumo, Japan). For each ISCA, the liquid from each row (five wells containing the
657 same treatment) was pooled to increase the volume collected per sample for downstream
658 analyses. The total volume of each pooled sample recovered was approximately 500 µL, of
659 which 100 µL was fixed with filtered glutaraldehyde (2% final concentration) for flow
660 cytometry analysis (conducted the same day) and 400 µL was snap-frozen immediately in
661 liquid nitrogen for subsequent DNA extraction and sequencing. In addition to the ISCA
662 samples (n = 4), bulk seawater samples (500 µL, n = 4) were also collected for both flow
663 cytometry and DNA sequencing.
664
665 This sampling strategy was used to capture seasonal changes in the extent of chemotaxis at a
666 single site, but we acknowledge that spatial (i.e. inter-environment) variability in chemotaxis
667 strength is also likely to occur according to local physicochemical conditions (including
668 microscale gradients in other signals such as viscosity, pH, or dissolved gases), and that this
669 warrants future research.
670
671 Environmental Data
672 Water temperature, salinity, pH and oxygen levels were recorded during the ISCA
673 deployments using a multiprobe meter (WTW Multiparameter Meter, WTW, Germany).
674 Seawater samples were collected in triplicate for inorganic nutrient analyses: 50 mL per

675 sample was filtered through 0.45 µm pore size and frozen at -20°C until analysis. Nitrite
676 (NO_2^-), nitrate (NO_3^-), ammonia (NH_3) and phosphate (PO_4^{3-}) were then quantified on an
677 Aquakem analyser (Thermo Scientific) using standard colorimetric techniques (APHA NO_2^-
678 B, APHA 4500-NH_3 F, APHA 4500 P E, practical quantitation limit: 0.005 mg L^{-1}). Seawater
679 samples were also collected in triplicate for chlorophyll concentrations, 200 mL per sample
680 were filtered through 0.7 µm glass fibre filters (GF/F, Whatman, UK), which were snap-
681 frozen in liquid nitrogen and stored at -80°C until required. Samples were extracted with ice
682 cold ethanol, cells were lysed by sonication into an ice bath (10 min), and incubated
683 overnight at -20°C. The next day samples were vortexed, centrifuged (4°C, 5 min, 1000g)
684 and absorption was immediately recorded at 629, 649, 665 and 696 nm using a FLUOstar
685 Omega microplate reader (BMG Labtech, Germany). Chlorophyll a content was calculated
686 following established protocols[47]. All environmental metadata are reported in Table S2. To
687 ensure that the low-volume bulk seawater samples (500 µL, $n = 4$) were representative of the
688 bacterial communities at the site, triplicate 10 L samples were collected simultaneously,
689 transported to the laboratory, and filtered upon arrival through 0.2 µm Sterivex cartridges
690 (Millipore, USA). All cartridges were sealed with parafilm and were preserved at -80°C for
691 further processing.
692
693 Flow cytometry
694 Samples for flow cytometry were transferred into sterile Titertube micro test tubes (Bio-Rad),
695 stained with SYBR Green (1:10,000 final dilution; ThermoFisher, USA), incubated for 15
696 min in the dark and analysed on a CytoFLEX S flow cytometer, using CytExpert version 2.4
697 (Beckman Coulter, USA) with filtered MilliQ water as sheath fluid. For each sample, forward
698 scatter (FSC), side scatter (SSC), and green (SYBR) fluorescence were recorded. The
699 samples were analysed at a flow rate of 25 µL min^{-1}. Microbial populations were
700 characterized according to SSC and SYBR Green fluorescence[48] (Fig. S1) and cell
701 abundances were calculated by running a standardized volume of sample (50 µl). To quantify
702 the strength of chemotaxis, the chemotactic index *Ic* was calculated by dividing the number
703 of cells present in the phytoplankton-DOM treatment by the average number of cells present
704 in the filtered seawater control[22].
705
706 DNA extraction
707 DNA extraction from all ISCA samples was performed under a UV cleaner hood (UVC/T-M-
708 AR, Biosan, Latvia) using a recently developed physical lysis extraction designed for

709 microvolume samples[49]. All tubes and reagents (except Ethanol and magnetic beads) were
710 UV-sterilised for 1 hour in a UV-crosslinker (CL-1000 Ultraviolet Crosslinker, UVP, USA).
711 Briefly, 300 µl of sample was mixed with 162.5 µL of lysis buffer (made by mixing 700 µl of
712 KOH (0.0215 g mL^{-1}), 430 µL of DTT (0.008 g/mL^{-1}) and 520 µL of UV-treated Ultrapure
713 water; pH 12) and incubated for 10 min at room temperature. Samples were then frozen at -
714 80°C for 15 min, followed by an incubation on a heat bock at 55°C for 5 min. Following this
715 freeze-thaw cycle, 162.5 µl of STOP buffer (Tris-HCl 0.4 g mL^{-1}; pH 5) was added and
716 mixed to bring the pH of the solution to 8. AMPure beads (1,250 µL; Beckman Coulter,
717 USA) were added to each sample to capture the DNA, then mixed and incubated for 15 min
718 at room temperature, the sample tubes were then placed on a magnetic stand for 10 min. The
719 supernatant was removed and the beads were washed twice with 80% Ethanol (molecular
720 biology grade), all residual ethanol was removed and the beads were left to air-dry for 15
721 min. Tubes were removed from the magnetic stand and 20 µL of elution buffer (10 mM Tris-
722 HCl) was added, mixed by pipetting and the solution incubated for 5 min at room
723 temperature. Finally, tubes were placed on a magnetic stand and 18 µL of bead-free liquid
724 was transferred to a new tube. Samples were stored at -20°C until library preparation.
725
726 Library preparation and sequencing
727 Libraries for shotgun metagenomic sequencing were prepared using the Nextera XT DNA
728 Sample Preparation Kit (Illumina, USA) following a previously described modified protocol
729 designed for generating low-input DNA libraries[24], and ISCA treatments were all processed
730 the same way. All libraries were sequenced on the Illumina NextSeq 500 platform 2 × with
731 150 bp High Output v.2 run chemistry, with the analysis including a total of 69 samples: 44
732 ISCA samples, 7 bulk seawater samples and 18 controls (2 mock communities, 3 DNA
733 extraction controls, 2 library prep controls, and 11 undeployed ISCA controls). These
734 additional controls were used to identify and remove potential reagent contaminants.
735 Libraries were pooled on an indexed shared sequencing run, resulting in ~3 Gbp per sample.
736 The raw fastq read files were deposited in Sequence Read Archive (SRA) (accession number:
737 PRJNA639602).
738
739 Metagenomics: quality control of the reads
740 Reads were processed using Trimmomatic v0.36[50] to remove adapters, filter leading or
741 trailing bases with a quality score <3, clip reads when the average 4-base window had a
742 quality score <15, and discard reads <50 bp in length after applying the previous QC steps.

Read pairs passing QC (87.6% ± 0.42 on average, excluding controls), and ranging from 11,082,294 to 35,206,174, were further processed to remove potential human contamination or contamination from the ten phytoplankton species used to produce DOM. Specifically, paired reads were mapped to reference genomes or available transcriptomic data (Table S11) using the MEM mapping method of BWA v 0.7.12-r1039[51] and pairs were removed from further consideration if either read had a percent identity ≥95% and percent alignment length ≥95% to any reference sequence. QC results are available in Table S12.

Metagenomics: taxonomic profiles

The 16S rRNA gene-based taxonomic profiles of the samples were generated with GraftM v0.11.1[52] using the 7.05.2013_08_greengenes_97_otus.gpkg reference package. GraftM identifies reads encoding 16S rRNA genes using HMMs and assigns taxonomic classifications to these reads by placing them into an annotated reference tree. The GraftM output was manually curated to removed reads classified as mitochondrial or chloroplast sequences. Relative abundances were calculated in the R software environment (www.r-project.org) and all taxa present in blanks at > 0.5% relative abundance were removed prior to subsequent analyses. Surprisingly, members of Desulfobacteraceae, a bacterial family thought to be primarily anaerobic, were present in some ISCA treatments. As sequences originating from this family were completely absent from our blank samples (un-deployed filtered seawater and DNA extraction blanks), we are confident that they are not a contaminant. Members of the Desulfobacteraceae are known to be highly motile[53], and have therefore the capacity to migrate into the ISCA wells. In addition, recent evidence has shown that prokaryotes that are thought to be primarily anaerobic can inhabit oxygenated pelagic water columns, potentially within anoxic microenvironments associated with particles[54, 55]. Note: the taxonomy presented in the main text is compatible with the Genome Taxonomy Database (GTDB)[56].

Metagenomics: functional profiles

A reference database was constructed from all UniRef100[57] proteins available on 6th March, 2018 which had a KEGG Orthology (KO) annotation in the KEGG database[58]. Quality-controlled reads were compared to this reference database using the BLASTX option of DIAMOND v0.9.22[59]. A read was assigned to a UniRef protein if the top hit had an E-value $<1e^{-3}$, a percent identity >30%, an alignment covering >70% of the read, and the UniRef100 protein was annotated as being bacterial or archaeal. Otherwise, the read was considered

777 unclassified. Assigned reads were mapped to KO IDs using UniProt ID mapping files. In a
778 small number of cases, a read was assigned to multiple KOs (<0.2% across all ISCA
779 samples). Hits to each KO were summed across all assigned reads to produce a KO count
780 table for each sample.
781

782 Metagenomics: statistical analysis

783 Normalised sequence counts were generated using variance-stabilizing normalisation on the
784 raw counts[60], using the R package *metagenomeSeq* version 1.26.3 (functions
785 cumNormStatFast and cumNorm)[60]. This normalisation method corrects for biases associated
786 with uneven sequencing depth[60, 61]. We then employed a zero-inflated Gaussian mixture
787 model[60] to determine if the abundance of prokaryotic taxa and functional genes were
788 significantly different between treatments. To determine if statistical differences existed at
789 the taxonomic and functional levels between treatments, Bray-Curtis similarity matrices were
790 generated on the relative abundances of normalised reads. PERMANOVA were carried out
791 using PRIMER (v6), with 999 unrestricted permutations.
792

793 Phytoplankton-derived metabolomes were correlated with taxonomic profiles (all data were
794 log transformed) using Pearson's correlation with adjusted *p* values (using the Holm-
795 Bonferroni method). Data handling and production of graphics was performed using the
796 following R packages: *tidyr*, *dplyr*, *tibble*, *pheatmap*, *psych*, *ggplot2*, *metagenomeSeq*,
797 *metaboanalyst*, *mixomics*. Networks were produced using the R package *tidyverse* and edited
798 using Gephi. All analysis scripts are available on GitHub (https://github.com/JB-Raina-
799 codes/ISCA-paper).
800

801 Laboratory-based chemotaxis assays

802 Cultured strains of the *Agarivorans*, *Photobacterium* and *Maribacter* genera, isolated from
803 phytoplankton species used in this experiment (*Maribacter*: *Thalassiosira pseudonana*;
804 *Photobacterium*: *Thalassiosira pseudonana*; *Agarivorans*: *Chaetoceros muelleri*) were used
805 in subsequent laboratory assays. Bacterial strains were grown for 4 hours in 1% Marine Broth
806 (BD Difco, USA), washed with artificial seawater and resuspended in artificial seawater at a
807 concentration of 10^6 cells mL^{-1}. The compounds tested were added to four ISCA replicates (n
808 = 4) at a concentration of 1 mM and incubated for 1 hour in individual trays. Chemotactic
809 cells in each treatment were enumerated by flow cytometry, as previously described. Note:

810 Digalactosyldiacylglycerol, the acylated form of Digalactosylglycerol, was tested for
811 chemotaxis due to unavailability of a commercial Digalactosylglycerol standard. The 16S
812 rRNA gene sequences of the three isolates were deposited in GenBank (accession numbers:
813 MT826233-MT826234 and MZ373175).
814
815 <u>Metabolism of chemoattractants</u>
816 Each compound that was identified as a chemoattractant in the previous set of experiments
817 was also individually tested for its potential to support the growth of the isolates. Bacterial
818 strains were grown overnight in 1% Marine Broth (BD Difco, USA), supplemented with
819 0.2% casamino acids, washed with artificial seawater and inoculated at a concentration of
820 106 cells mL-1 into a minimal medium consisting of: artificial seawater[62] supplemented with
821 0.2% casamino acids, and 1 mM of chemoattractant (n = 4 for each chemoattractant).
822 Bacterial growth was monitored over two days using optical density (OD600; two of the
823 bacterial strains (*Agarivorans* and *Maribacter*) formed aggregates at high densities and their
824 density could not be accurately quantified using flow cytometry) and was compared against
825 controls containing only 0.2% casamino acids.
826
827 <u>Control tests for ISCA deployment times</u>
828 To ensure that the *in situ* incubation length (1 hour) did not elicit prokaryotic growth in the
829 ISCA wells, which could conceivably lead to increases in cell number (affecting *Ic* levels)
830 and shifts in prokaryote community composition, we carried out control incubations of the
831 bulk seawater from the Clovelly Beach field site with the 10 phytoplankton-derived DOM
832 used in the ISCA experiments. To mimic the conditions occurring during the ISCA
833 experiments, samples were added to ISCA wells and incubated at 23°C (same as *in situ*
834 conditions). Samples were taken before incubation (T0), after one hour (T1) and after five
835 hours (T5) in the ISCA wells (*n* = 3). Samples were then divided as previously described and
836 either: (i) fixed with filtered glutaraldehyde (2% final concentration) to enumerate cells via
837 flow cytometry analysis (conducted the same day, same method as above); or (ii) snap-frozen
838 immediately in liquid nitrogen for subsequent DNA extraction (same method as above).
839
840 To characterise bacterial community composition at each time-point of the incubation, the
841 27F and 519R primers[63], which specifically target the V1-V3 region of the bacterial 16S
842 rRNA gene, were used for PCR amplification of extracted DNA. The PCR reactions included
843 2.5 µmol of each deoxyribonucleotide triphosphate (Bioline, USA), 6 µl of template, 1 µl of

844 UltraPure Bovine Serum Albumin (Thermo Fisher, USA), 0.25 µl of Velocity DNA
845 polymerase and 5 × PCR buffer (Bioline, USA), 10 pmol of each primer (resuspended in UV-
846 sterilised water) with the following adaptors: 5'-
847 TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG-27F-3'; and 5'-
848 GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG-519R-3'). The reaction conditions
849 were as follow: 98°C for 2 minutes; followed by 30 cycles of 98°C for 30 seconds, 30
850 seconds of annealing (46°C for 3 cycles, 48°C for 3 cycles and 50°C for 24 cycles), 72°C for
851 30 seconds; and then a final extension of 72°C for 10 minutes. PCR clean-up, indexing and
852 sequencing (on an Illumina MiSeq (2 × 300bp)) were performed at the Australian Genome
853 Research Facility (AGRF), Australia.
854
855 Paired end R1 and R2 reads were processed using the DADA2 pipeline (version 1.22.0)[64].
856 Reads with any 'N' bases were removed, together with primers using cutadapt. R1 and R2
857 were trimmed to remove low quality terminal ends (trunc(R1= 260; R2= 255)), in order to
858 produce the highest number of merged reads after learning error rate and removing chimera
859 sequences. Amplicon sequence variants (ASVs) were then annotated using SILVA (release
860 138)[65], using a 50% probability cut-off. The quality ASV table was secondarily filtered to
861 remove ASVs not annotated to kingdom Bacteria, as well as any annotated as chloroplast or
862 mitochondria. We processed and sequenced 2 extraction blanks and 2 PCR sequencing
863 blanks, which revealed that 10 ASVs overlapped between the samples and the blanks. After
864 removing these 10 ASVs from the dataset, the samples were rarefied to 25,000 reads using
865 the vegan package[66] (rrarefy function). The rarefied reads were then filtered to remove
866 singletons. The raw fastq read files were deposited in Sequence Read Archive (SRA)
867 (accession number: PRJNA707306).
868
869 These control tests revealed that the cell densities and community compositions did not
870 change significantly during a one-hour incubation (Extended Data Fig. 9-10). Therefore,
871 these results confirm that the cell abundances and community profiles observed within the
872 ISCA wells occurred as a result of chemotactic migration, not cell growth.
873
874 Identification of phytoplankton taxa at coastal sites
875 To confirm the presence of the phytoplankton genera used for our chemotaxis assay in coastal
876 water of Sydney, Australia, we used publicly available datasets derived from the Australian
877 Microbiome Initiative (https://data.bioplatforms.com/organization/about/australian-

878 microbiome). We focused on three coastal sites (Cobblers Beach, Salmon Haul, Taren Point),
879 raw data were processed through the DADA2 pipeline (version 1.22.0)[64], annotated using
880 SILVA[65] (release 138), with a taxonomic assignment to >50% bootstrap level.

881

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945

946 **Extended Data**

947

948 **Extended Data Figure 1: ISCA deployments through a two years period at Clovelly**
949 **Beach (33.91°S, 151.26°E).** Chemotactic index *Ic*, denoting the concentration of cells within
950 ISCA wells, normalized by the mean concentration of cells within wells containing filtered
951 seawater (FSW), after 60 min field deployment. Full bars are significantly different from
952 wells containing FSW (ANOVA (one-sided), $p < 0.05$, all p-values are reported in Table S3).
953 Each treatment was replicated across four different ISCAs ($n = 4$), except between April and
954 August 2016 ($n = 3$). Data are presented as mean values ± SEM. FSW: filtered seawater,
955 Syne: *Synechococcus*, Proch: *Prochlorococcus*, Duna: *Dunaliella*, Rhodo; *Rhodomonas*,
956 Phaeo: *Phaeocystis*, Ehux: *Emiliania*, Prym: *Prymnesium*, Chae: *Chaetoceros*, Dityl:
957 *Ditylum*, Phae: *Phaeodactylum*, Thala: *Thalassiosira*, Durus: *Durusdinium*, Alex:
958 *Alexandrium*, Amphi: *Amphidinium*.

959

960 **Extended Data Figure 2: Environmental variables influencing the strength of**
961 **chemotaxis.** (**a**) Average chemotactic index (*Ic*) elicited by the phytoplankton-derived DOM
962 for each of the 12 ISCA deployments described in this study at Clovelly Beach (33.91°S,
963 151.26°E). Error bars: standard errors. (**b**) Correlogram of the metadata measured during
964 each deployment (the size and colour of each bubble is proportional to the strength of the
965 correlation). Only statistically significant correlations are not crossed (Pearson's correlation

966 (two-sided), $p < 0.01$). (**c**) Significant correlation between chemotactic index and temperature
967 (Pearson's correlation (two-sided), $p < 0.01$).
968
969 **Extended Data Figure 3: Differences in chemical composition between the**
970 **phytoplankton-derived DOM.** (**a**) Heatmap of the 111 compounds identified between the
971 different phytoplankton species. An interactive version of this figure is available (Fig. S2).
972 (**b**) Principal component analysis (PCA) of chemical composition of the phytoplankton-
973 derived DOM: displaying the top three components (explaining 64.7% of the variance).
974
975 **Extended Data Figure 4: Relative abundance of the prokaryotic families present in the**
976 **bulk seawater, the FSW controls, and in the different phytoplankton-derived DOM.**
977 Only taxa representing more than 2% of the communities are displayed in colours, those
978 representing less than 2% and grouped in "Other". ND: taxonomy not determined at the
979 family level.
980
981 **Extended Data Figure 5: Prokaryotic taxa significantly enriched the phytoplankton-**
982 **derived DOM treatments.** (**a**) Number of prokaryotic taxa enriched in each phytoplankton-
983 DOM treatment (compared to filtered seawater controls). The full list of taxa significantly
984 enriched in phytoplankton-derived DOM treatments can be found in Table S6. (**b**) Network
985 analysis showing the differentiation between "generalist" and "specialist" families at the
986 taxonomic level. This network has the same topology than the Figure 2b. Chemotactic
987 prokaryotic taxa (small circles; nodes) are linked to the treatments they responded to (large
988 circles) by lines with colours corresponding to each treatment. Each node is colour coded
989 based on its taxonomy. (**c**) Number of prokaryote taxa significantly enriched in one or more
990 phytoplankton-derived DOM treatments (compared to filtered seawater controls). Another
991 graphical representation of this data can be found in Figure 2b.
992
993 **Extended Data Figure 6: Genes involved in motility, chemotaxis and surface-attachment**
994 **were significantly enriched in the ISCA treatments compared to the bulk seawater.** Data
995 shown as log-transformed relative abundance ($n = 4$) for each ISCA treatment. Asterisks
996 highlight significant enrichment compared to the bulk seawater (F-tests (one-sided), $p <$
997 0.05).
998

999 **Extended Data Figure 7: Genes involved in the uptake and degradation of**
1000 **phytoplankton-derived molecules (selected from the literature)[39, 67-70], as well as in the**
1001 **transport of a range of labile substrates, were significantly enriched in the prokaryotic**
1002 **communities responding to phytoplankton-derived DOM.** Data shown as log-transformed
1003 relative abundance ($n = 4$) for each ISCA treatment. Asterisks highlight significant
1004 enrichment compared to the FSW treatment (F-tests (one-sided), $p < 0.05$, all p-values are
1005 reported in Table S8). DMSP: dimethylsulfoniopropionate; DHPS: 2,3-dihydroxypropane-1-
1006 sulfonate; GBT: Glycine betaine.
1007
1008 **Extended Data Figure 8: Assay testing the ability of the bacterial isolates to catabolize**
1009 **the validated chemoattractants (Figure 4b).** Each chemoattractant was inoculated at a
1010 concentration of 1 mM ($n = 4$) in an artificial seawater medium supplemented with 0.2% of
1011 casamino acids. After 48 hours, the optical density (OD600) of each culture was compared to
1012 controls only containing casamino acids. DGDG: Digalactosyldiacylglycerol; 3-aminopip: 3-
1013 aminopiperidin-2-one. Full bars are significantly different from wells containing FSW
1014 (ANOVA (one-sided), $p < 0.05$, all p-values are reported in Table S9). Data are presented as
1015 mean values ± SEM.
1016
1017 **Extended Data Figure 9: Control for bacterial growth during the ISCA deployment**
1018 **time.** (**a**) Comparison of prokaryotic cell counts before, and then 1 hour and 5 hours after
1019 post incubation with phytoplankton-derived DOM (1 mg mL^{-1}). The number of prokaryotic
1020 cells were not statistically different between pre-incubation and one hour of incubation
1021 (ANOVA (one-sided), $n = 3$, $p = 0.8026$). Data are presented as mean values ± SEM. (**b**)
1022 Principal component analysis (PCA) of bacterial community composition resulting from
1023 incubations (explaining 80.4% of the variance), revealing the overlap between bacterial
1024 community compositions pre-incubation and those after 1 hour of incubation. An analysis of
1025 similarities confirmed that community compositions were not significantly different pre-
1026 incubation and after one hour of incubation (ANOSIM; 99,999 permutations; $n = 33$; R =
1027 0.108; $p = 0.2$), but significant differences were observed after five hours (R = 0.602; p =
1028 0.001).
1029
1030 **Extended Data Figure 10: Control for shifts in bacterial composition due to growth**
1031 **during the ISCA deployment time.** Relative abundance of the bacterial communities (at the

1032 ASV level) before, 1 hour and 5 hours of incubation with phytoplankton-derived DOM (1 mg
1033 mL^{-1}). The legend only shows the 30 most abundant ASVs.