1 **Probing the Living Ocean With Ecogenomic Sensors**

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9 **Abstract**

10 This review discusses the role of ecogenomic sensors in biological oceanography.
11 Ecogenomic sensors are instruments that can autonomously collect biological samples
12 and perform molecular analyses. This technology reduces logistical constraints on the
13 length and duration of biological data collection. Autonomous, robotic performance of
14 molecular assays shows particular promise in the field of public health. Recent
15 applications include simultaneous detection of harmful algal species and fecal markers
16 paired with same-day remote reporting of test results. These instruments are also
17 showing promise for molecular ecological studies. Autonomous collection and
18 preservation of biological samples is facilitating high-resolution ecological studies that
19 are expanding our understanding of marine microbial ecology and dynamics. This
20 review discusses recent applications of these instruments and makes recommendations
21 for future developments.

29 **Introduction**

30 Microorganisms, including bacteria, archaea, microeukaryotes, and viruses, dominate and
31 drive marine biogeochemical cycles. Recent years have seen a number of advances in the

field of microbial oceanography, particularly in the area of ‘omics—genomics, transcriptomics, and proteomics—technologies. These technologies are improving our understanding of how microbes interact with their environment and how the environment determines microbial community structure and function. However, the microbial ocean remains grossly undersampled in both the temporal and spatial domains.

The ocean is a highly dynamic and heterogeneous environment. Episodic, mesoscale upwelling events have been found to strongly impact nutrient and carbon cycles even in the deep ocean [1-5]. Similarly, studies of marine microbes suggest a surprising degree of patchiness in the abundance and activities on microbes, even at scales of meters to kilometers [6,7] and temporal scales of hours to days [8*,9]. A view of microbial activity as spatially and temporally heterogeneous suggests a need for microbial sampling and monitoring across a wide variety of spatial and temporal scales.

Advances in remote instrumentation have made high-frequency, long-term data collection possible for a wide variety of physical (meterological and current-based measurements), chemical (salinity, oxygen concentrations, etc) and optical (turbidity, chlorophyll, etc) parameters. However, it has proven more difficult to automate biological and microbiological data collection, which typically require substantial manual laboratory processing. As a result, logistical considerations constrain the spatial and temporal scales at which microbiological studies are practical, with most ship-based expeditions constrained to a few weeks of operation. A few long-term time series, most famously the Hawaii Ocean Time Series (HOTS) and Bermuda Atlantic Time Series (BATS) [10,11]

55 have continued for a decade or more with regular observation of a single location, but
56 ship-based biological observations for these time series are limited to monthly sample
57 collections. This approach has a low probability of capturing mesoscale and episodic
58 oceanographic features, and can therefore fail to capture the full dynamic range of
59 biological activity at a given location. Automated sampling technologies can facilitate
60 long-term and high-frequency sampling regimes and therefore fill in these gaps in the
61 oceanographic record (Fig. 1).

62
63 This review will discuss currently available technology for automated sampling and
64 monitoring of marine microbes. In addition, we will address recent application of these
65 instruments for collection and automation of water quality monitoring efforts, capture of
66 transient, aperiodic oceanographic features, and collection of molecular ecological time
67 series.

68 69 **Ecogenomic Sensors**

70 Ecogenomic sensors have been defined as instruments that are capable of utilizing
71 molecular analytical techniques to autonomously detect and/or quantify specific
72 organisms of interest in the field [12]. In general, molecular analytical techniques are
73 challenging to implement in the context of field-portable, remote instrumentation.
74 Biological materials are highly labile, with changes in unpreserved cellular and DNA
75 templates occurring on time scales of hours to days, and RNA and protein abundances
76 capable of changes on time scales of minutes. In addition, most molecular analytical
77 techniques require multiple, wet-chemistry processing steps, including concentration,

78 extraction, and purification of the desired target (DNA, RNA, protein, or bulk cells)
79 followed by hybridization or amplification of molecular targets using specific probes. As
80 a result, ecogenomic sensors typically require implementation of relatively complex fluid
81 handling and sample manipulation approaches. Table 1 lists examples of ecogenomic
82 sensors and other devices for automated collection and preservation of biological
83 samples.

84
85 One of the most versatile ecogenomic platforms is the Environmental Sample Processor,
86 or ESP. The ESP can be equipped with environmental sensors, and serves as an
87 autonomous in situ liquid handling laboratory. It has been deployed in dockside [13**],
88 moored [14*], free-drifting [15**], and deep-water configurations [16]. Published
89 capabilities include on-board detection of harmful algal species [17-19], prokaryotes [20],
90 and invertebrate larvae [21,22] via nucleic acid probe hybridization arrays, on-board
91 detection of algal toxins [23], quantitative PCR [14*], and collection and fixation of
92 archival samples for on-shore genomic and transcriptomic analyses [8*]. As an example,
93 data from a free-drifting deployment in the North Pacific is shown in Figure 2. Fixation
94 methods for proteomic analysis are currently under development [24].

95
96 Another ecogenomic sensor under development is the Autonomous Microbial
97 Genosensor (AMG) [25,26]. This sensor utilizes isothermal RNA amplification
98 (NASBA, or nucleic acid sequence based amplification [27]) to detect and quantify
99 transcriptionally active target populations. Sequence-specific RNA based detection
100 offers the ability to distinguish between active and dead/dying target cells. In addition,

101 this technology could allow scientists to detect whether specific metabolic pathways of
102 interest are active at a given time.

103
104 Other instrument platforms focus primarily on remote collection and fixation of
105 biological samples for laboratory-based analyses. One such instrument is the Microbial
106 Sampler-Submersible Incubation Device (MS-SID), which is based on the Submersible
107 Incubation Device, a robotic instrument originally developed to carry out in situ
108 incubation experiments [28] and the Fixation Filter system [29]. By pairing incubation
109 capabilities with sample collection and fixation capabilities, this instrument promises to
110 allow an examination of microbial gene expression profiles and/or community
111 compositions associated with specific metabolic activities [30,31]. Another is the
112 Biological OsmoSampling System (BOSS), which is designed to continuously collect and
113 preserve fluid samples from aquatic environments, and has been shown capable of
114 preserving biological samples for long periods of time in the deep sea [32]. As an
115 unpowered instrument, the BOSS shows promise for long-term deployments in
116 inaccessible areas, although its sampling capabilities (0.1-5 mL per day) may ultimately
117 limit the number and types of analyses that can be performed on returned samples.

118
119 Other developments that show promise but have not yet been incorporated into
120 autonomous instrumentation include the *in situ* Filtration and Fixation Sampler [33], and
121 the Automatic Flow Injection Sampler [34]. A number of other devices have utilized
122 whole water collection and preservation systems for microscopy [35], chemical analysis
123 [36], or detection of toxins [37] but have not yet been validated for general-purpose

molecular analysis. Systems that allow remote collection of whole seawater without preservation, including AUV-based whole water collection systems [38-40] and the Autonomous Microbial Sampler [41], are not suitable for transcriptional analysis given the rapidity with which transcriptional profiles can change, but may be suitable for collection of samples for genomic analysis under conditions where the interval between sample collection and analysis is less than the doubling time of the microorganisms being studied.

Ecogenomic Platforms and Public Health.

One of the major drivers of ecogenomic sensor development has traditionally been the field of public health. The ability to cheaply and autonomously monitor environmental conditions, quantify organisms of interest, and rapidly report the results of these analyses has clear advantages over traditional laboratory approaches, which all too often require extensive sample handling and result in delayed reporting of public health threats. In particular, initial configurations of both the ESP and the AMG focused on detection of harmful algal species. Currently, assays for the AMG include monitoring of *Karenia brevis* [25], while ESP assays targeting both *K. brevis* [17] and *Pseudo-nitzschia australis* [19] have been developed. These capabilities are continuously expanding, with a recent deployment of the ESP demonstrating 45-day, autonomous monitoring of fecal indicator bacteria (*Enterococci* spp.), a human-specific microbial source tracking marker (*Bacteroidales* spp.), and harmful algal species (*Pseudo-nitzschia* spp.) simultaneously [13**]. Results were reported wirelessly and were available within hours of sample collection, suggesting that this method could be used to facilitate same-day public

notification of water quality conditions. This type of long-term, lightly supervised monitoring shows promise for automation of routine water quality measurements.

Adaptive Sampling for Capture of Oceanographic Features

Robotic sampling also shows promise for capturing transient, sporadic events that can impact ecosystem function. As an example, Herfort et al [42**] integrated an ESP into the Science and Technology University Research Network (SATURN; <http://www.stccmop.org/saturn>) observational network [43]. Using this system, they developed adaptive sampling scripts that allowed them to predict and target transient Estuarine Turbidity Maxima (ETM) and dissolved oxygen minima for archival sample collection. ETM features are associated with changes in microbial activity and community structure [44,45]. However, ETM are highly mobile and transient, and capturing these events typically requires high-intensity spatial and/or temporal sampling, with accompanying logistical and practical limitations. The authors developed a protocol by which they provided a sampling plan with pre-determined environmental parameters (as measured by SATURN stations) for triggering ESP-based sampling and fixation, allowing for autonomous sampling of ETM features upon occurrence near the sample inlet. This integration between an observational network incorporating a wide array of sensors, computational modeling, and an autonomous sampling device represents a promising paradigm for future ecogenomic studies.

Filling in the Gaps: Time Series Collection using Ecogenomic Sensors

Currently, one of the areas in which ecogenomic sensors are facilitating rapid new advances is in the area of marine microbial ecology. In particular, the ability to collect high-resolution time series is providing new insight into a variety of microbial groups and processes. The most widely-used sampling platform, the ESP can hold a maximum of 132 sample pucks and typically requires 40 min-1 hr to filter a 1-2 L seawater sample. Maximum deployment duration depends on the power source, reagent stability, and deployment method, with published deployments lasting 30-45 days.

Early ESP-facilitated time series involved the use of on-board qPCR to enumerate *Synechococcus* and *Thaumarchaeota* populations at daily resolution [46*]. This study supplemented existing monthly time series of these groups, providing new insight into their ecology [47-49]. In particular, this study found periods of unexpectedly high Thaumarchaeal abundances in surface waters during the stratified fall period, which had been missed during previous, monthly time series [49].

A second study using the ESP utilized a powerful combination of on-board and laboratory-based analyses [50**]. In this study, the authors examined regulation of dimethylsulfoniopropionate (DMSP) degradation using the ESP, pairing on-board monitoring of the abundance a DMSP-degrading clade of bacteria (*Roseobacter* sp. HTCC2255) with fixation of samples for laboratory-based transcriptional analyses. This approach allowed automated collection of 15 samples for on-board DNA extraction and qPCR as well as 19 archival samples for laboratory analysis over the course of a month. The laboratory-based transcriptional analyses were then used to generate insight into

192 differential regulation of DMSP demethylation vs. DMSP cleavage pathways, with the
193 authors finding that expression of the demethylation pathway was associated with a
194 bloom of DMSP-producing dinoflagellates, while expression of the cleavage pathway
195 was associated with a community featuring both diatoms and dinoflagellates. The
196 authors also found that catalase was upregulated during periods of high demethylase
197 expression, suggesting a linkage between this pathway and oxidative stress.

199 **Automated Collection of Lagrangian Time Series**

200 One of the analytical challenges facing marine time series collection is the difficulty of
201 repeatedly sampling within a single water mass. A number of recent studies have
202 attempted to circumvent this by deploying the ESP in a free-drifting context, facilitating
203 Lagrangian sample collection. An example of this is a study by Robidart et. al [9], who
204 used an ESP suspended below a free-drifting buoy to collect a Lagrangian time series in
205 the North Pacific Ocean. The authors quantified the abundance of two groups of
206 nitrogen-fixing organisms using targeted on-board qPCR assays conducted at 16-hour
207 intervals for 10 days (Fig. 2). One of the interesting components of this study was that
208 these measurements were paired with satellite sensing data, ship- and ESP-based
209 biogeochemical measurements, ship-based flow cytometric community analyses, and *in*
210 *situ* measurements of nitrogen fixation utilizing the SID [51]. The ESP reported large
211 variations in the abundance of nitrogen fixers over the 10-day time series, potentially
212 linked to the presence of a localized current of low-salinity, high-phosphate water [9].
213 The SID also reported substantial variability in measured nitrogen fixation rates [51].

While *in situ* molecular analyses provide a real-time view of the environmental conditions, the required processing time can limit sampling frequency. Several studies have taken advantage of the ESP's sample collection and archival capabilities to facilitate very high-resolution transcriptional surveys. In particular, the combination of simultaneous monitoring of transcriptional activity across a microbial community using RNASeq techniques with high-resolution Lagrangian sampling shows promise for providing insight into microbial interactions and microbe-environment interactions. Three such surveys have been published thus far [15**,52**,53]. The first, conducted using samples collected off the California coast, identified diel rhythms in transcription among phototrophs and synchronous, non-rhythmic transcriptional changes amongst multiple groups of heterotrophic bacteria, suggesting either cross-species interactions or simultaneous responses to environmental signals [15**]. The second, conducted in open waters in the North Pacific, identified diel rhythms in transcription among both phototrophic and several heterotrophic microbial groups, suggesting that heterotrophic microbes were either responding directly to light or indirectly to diel cycles due to light- or phototroph-driven environmental signals [53]. This survey occurred simultaneously with the SID and ESP-based explorations of nitrogen fixation described above [9,51], suggesting the potential for integration of data from many sources and approaches (Fig. 2). The third drift again took place off the Pacific coast, and identified conserved transcriptional patterns common to both that and the North Pacific dataset, both within and across microbial species [52**]. Together, these studies show the promise of ultra high-resolution time series for providing new insight into marine microbial behavior.

Conclusions and Thoughts for the Future

While they are increasingly contributing to the fields of public health and marine ecology, ecogenomic sensors remain a relatively young technology. The majority of the instruments listed in Table 1 remain in the prototype stage and have to date been deployed by only a single research group. To our knowledge only the ESP is currently available commercially. This instrument is beginning to see wider use, with instruments held and deployed not only by the Monterey Bay Aquarium Research Institute, where it was developed, but also the Center for Coastal Margin Observation and Prediction at Oregon Health and Science University, Woods Hole Oceanographic Institution, and the United States National Oceanographic and Atmospheric Administration. As a result, we expect to see increasing contributions from these instruments in the next few years. In addition, a next-generation ESP is currently under development, with plans to implement this technology in a miniaturized format that can be deployed in an Autonomous Underwater Vehicle. The later would greatly expand the utility of the ESP, facilitating tracking of mobile oceanographic features and studies in locations that are not amenable to the establishment of moorings or the use of (unpowered) free-drifting platforms.

The development of sophisticated robotic instruments for in situ molecular analyses suggests a future in which it is possible to remotely monitor the abundance and activities of key microbial taxa. This type of study is likely to be a useful complement to existing oceanographic monitoring packages. The ability to autonomously perform and remotely report on water quality has clear implications for public health, including the possibility of same-day public notification of hazardous conditions. In addition, carefully placed

261 monitoring stations paired with ocean models may allow advance warning of hazardous
262 conditions.

263
264 Recent applications have focused on automated sample collection and preservation for
265 ecological studies. Daily-resolution time series are filling in gaps in long-term
266 monitoring records and providing insight into how episodic oceanographic features
267 impact microbial community structure and function. In addition, hourly-resolution time
268 series collected over periods of days are providing insight into microbial transcriptional
269 networks and diel rhythms in microbial gene expression. The success of these studies
270 suggests that there is a role for simplified autonomous instruments specialized for robotic
271 collection and preservation of biological samples. Such instruments are likely to represent
272 useful complements to long-term monitoring stations and ship-based sample collection.

273
274 Overall, ecogenomic sensors show promise as a complement to existing oceanographic
275 monitoring efforts, allowing us to more effectively capture and examine microbial
276 responses to transient and episodic oceanographic features, such as mesoscale eddies and
277 upwelling events. Together, these efforts will allow us to build a more complete picture
278 of how these features shape overall biogeochemical fluxes, and improve our ability to
279 build predictive models of marine microbial contributions to global biogeochemical
280 cycles.

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References

1. McGillicuddy DJ, Anderson LA, Bates NR, Bibby T, Buesseler KO, Carlson CA, Davis CS, Ewart C, Falkowski PG, Goldthwait SA, et al.: **Eddy/wind interactions stimulate extraordinary mid-ocean plankton blooms.** *Science* 2007, **316**:1021-1026.
 2. McGillicuddy DJ, Robinson AR, Siegel DA, Jannasch HW, Johnson R, Dickey T, McNeil J, Michaels AF, Knap AH: **Influence of mesoscale eddies on new production in the Sargasso Sea.** *Nature* 1998, **394**:263-266.
 3. Letelier RM, Karl DM, Abbott MR, Flament P, Freilich M, Lukas R, Strub T: **Role of late winter mesoscale events in the biogeochemical variability of the upper water column of the North Pacific Subtropical Gyre.** *J Geophys Res-Oceans* 2000, **105**:28723-28739.
 4. Sakamoto CM, Karl DM, Jannasch HW, Bidigare RR, Letelier RM, Walz PM, Ryan JP, Polito PS, Johnson KS: **Influence of Rossby waves on nutrient dynamics and the plankton community structure in the North Pacific subtropical gyre.** *J Geophys Res-Oceans* 2004, **109**:C05032.
 5. Falkowski PG, Ziemann D, Kolber Z, Bienfang PK: **Role of eddy pumping in enhancing primary production in the ocean.** *Nature* 1991, **352**:55-58.
 6. Martin AP, Zubkov MV, Burkill PH, Holland RJ: **Extreme spatial variability in marine picoplankton and its consequences for interpreting Eulerian time-series.** *Biol Lett* 2005, **1**:366-369.
 7. O'Mullan GD, Ward BB: **Relationship of temporal and spatial variabilities of ammonia-oxidizing bacteria to nitrification rates in Monterey Bay, California.** *Appl Environ Microbiol* 2005, **71**:697-705.
 - *8. Ottesen EA, Marin R, 3rd, Preston CM, Young CR, Ryan JP, Scholin CA, DeLong EF: **Metatranscriptomic analysis of autonomously collected and preserved marine bacterioplankton.** *ISME J* 2011, **5**:1881-1895.
- The first use of the ESP for collection and preservation of samples for transcriptional analysis.
9. Robidart JC, Church MJ, Ryan JP, Ascani F, Wilson ST, Bombar D, Marin R, Richards KJ, Karl DM, Scholin CA, et al.: **Ecogenomic sensor reveals controls on N₂-fixing microorganisms in the North Pacific Ocean.** *ISME J* 2014, **8**:1175-1185.
 10. Fasham MJR, Baliño BM, Bowles MC, Anderson R, Archer D, Bathmann U, Boyd P, Buesseler K, Burkill P, Bychkov A, et al.: **A new vision of ocean biogeochemistry after a decade of the Joint Global Ocean Flux Study (JGOFS).** *Ambio* 2001:4-31.

11. Karl DM, Dore JE, Lukas R, Michaels AF, Bates NR, Knap AH: **Building the long-term picture: The U.S. JGOFS time-series programs.** *Oceanography* 2001, **14**:6-17.
12. Scholin CA: **What are "ecogenomic sensors?" A review and thoughts for the future.** *Ocean Sci* 2010, **6**:51-60.
- **13. Yamahara KM, Demir-Hilton E, Preston CM, Marin III R, Pargett D, Roman B, Jensen S, Birch JM, Boehm AB, Scholin CA: **Simultaneous monitoring of faecal indicators and harmful algae using an *in-situ* autonomous sensor.** *Lett Appl Microbiol* 2015, **61**:130-138.
- Demonstrates the use of the ESP for same-day reporting of water quality data and simultaneous tracking of fecal indicator bacteria, microbial source markers, and harmful algae.
- *14. Preston CM, Harris A, Ryan JP, Roman B, Marin R, Jensen S, Everlove C, Birch J, Dzenitis JM, Pargett D, et al.: **Underwater application of quantitative PCR on an ocean mooring.** *Plos One* 2011, **6**: e22522.
- Implementation of quantitative PCR on an autonomous sampler.
- **15. Ottesen EA, Young CR, Eppley JM, Ryan JP, Chavez FP, Scholin CA, DeLong EF: **Pattern and synchrony of gene expression among sympatric marine microbial populations.** *Proc Natl Acad Sci USA* 2013, **110**:E488-497.
- The first use of a free-drifting ESP to collect a Lagrangian time series
16. Ussler W, Preston C, Tavormina P, Pargett D, Jensen S, Roman B, Marin R, Shah SR, Girguis PR, Birch JM, et al.: **Autonomous application of quantitative PCR in the deep sea: *in situ* surveys of aerobic methanotrophs using the Deep-Sea Environmental Sample Processor.** *Environ Sci Technol* 2013, **47**:9339-9346.
17. Haywood AJ, Scholin CA, Marin R, Steidinger KA, Heil C, Ray J: **Molecular detection of the brevetoxin-producing dinoflagellate *Karenia brevis* and closely related species using rRNA-targeted probes and a semiautomated sandwich hybridization assay.** *J Phycol* 2007, **43**:1271-1286.
18. Greenfield DI, Marin III R, Doucette G, Mikulski C, Jones K, Jensen S, Roman B, Alvarado N, Feldman J, Scholin C: **Field applications of the second-generation Environmental Sample Processor (ESP) for remote detection of harmful algae: 2006-2007.** *Limnol Oceanog Methods* 2008, **6**:667-679.
19. Greenfield DI, Marin R, Jensen S, Massion E, Roman B, Feldman J, Scholin CA: **Application of environmental sample processor (ESP) methodology for quantifying *Pseudo-nitzschia australis* using ribosomal RNA-targeted probes in sandwich and fluorescent *in situ* hybridization formats.** *Limnol Oceanog Methods* 2006, **4**:426-435.
20. Preston CM, Marin R, 3rd, Jensen SD, Feldman J, Birch JM, Massion EI, Delong EF, Suzuki M, Wheeler K, Scholin CA: **Near real-time, autonomous detection of marine bacterioplankton on a coastal mooring in Monterey Bay, California, using rRNA-targeted DNA probes.** *Environ Microbiol* 2009, **11**:1168-1180.
21. Jones WJ, Preston CM, Marin R, Scholin CA, Vrijenhoek RC: **A robotic molecular method for *in situ* detection of marine invertebrate larvae.** *Mol Ecol Resour* 2008, **8**:540-550.
22. Goffredi SK, Jones WJ, Scholin CA, Marin R, 3rd, Vrijenhoek RC: **Molecular detection of marine invertebrate larvae.** *Mar Biotechnol (NY)* 2006, **8**:149-160.

23. Doucette GJ, Mikulski CM, Jones KL, King KL, Greenfield DI, Marin R, Jensen S, Roman B, Elliott CT, Scholin CA: **Remote, subsurface detection of the algal toxin domoic acid onboard the Environmental Sample Processor: Assay development and field trials.** *Harmful Algae* 2009, **8**:880-888.
24. Saito MA, Bulygin VV, Moran DM, Taylor C, Scholin C: **Examination of microbial proteome preservation techniques applicable to autonomous environmental sample collection.** *Frontiers in microbiology* 2011, **2**:215.
25. Paul J, Scholin C, Van Den Engh G, Perry MJ: **In situ instrumentation.** *Oceanography* 2007, **20**:70-78.
26. Fries D, Paul J, Smith M, Farmer A, Casper E, Wilson J: **The Autonomous Microbial Genosensor, an in situ sensor for marine microbe detection.** *Microscopy and Microanalysis* 2007, **13**:514-515.
27. Guatelli JC, Whitfield KM, Kwoh DY, Barringer KJ, Richman DD, Gingeras TR: **Isothermal, in vitro amplification of nucleic acids by a multienzyme reaction modeled after retroviral replication.** *Proceedings of the National Academy of Sciences of the United States of America* 1990, **87**:1874-1878.
28. Taylor CD, Doherty KW: **Submersible Incubation Device (SID), autonomous instrumentation for the in situ measurement of primary production and other microbial rate processes.** *Deep Sea Research Part A Oceanographic Research Papers* 1990, **37**:343-358.
29. Taylor CD, Edgcomb VP, Doherty KW, Engstrom I, Shanahan T, Pachiadaki MG, Molyneaux SJ, Honjo S: **Fixation filter, device for the rapid in situ preservation of particulate samples.** *Deep-Sea Res Pt I* 2015, **96**:69-79.
30. Edgcomb VP, Taylor C, Pachiadaki MG, Honjo S, Engstrom I, Yakimov M: **Comparison of Niskin vs. in situ approaches for analysis of gene expression in deep Mediterranean Sea water samples.** *Deep Sea Research Part II: Topical Studies in Oceanography*:Epub. 14 Nov 2014.
31. Pachiadaki MG, Taylor C, Oikonomou A, Yakimov MM, Stoeck T, Edgcomb V: **In situ grazing experiments apply new technology to gain insights into deep-sea microbial food webs.** *Deep Sea Research Part II: Topical Studies in Oceanography*:Epub. 9 Dec 2014.
32. Robidart J, Callister SJ, Song PF, Nicora CD, Wheat CG, Girguis PR: **Characterizing microbial community and geochemical dynamics at hydrothermal vents using osmotically driven continuous fluid samplers.** *Environ Sci Technol* 2013, **47**:4399-4407.
33. Wurzbacher C, Salka I, Grossart HP: **Environmental actinorhodopsin expression revealed by a new in situ filtration and fixation sampler.** *Env Microbiol Rep* 2012, **4**:491-497.
34. Feike J, Jürgens K, Hollibaugh JT, Krüger S, Jost G, Labrenz M: **Measuring unbiased metatranscriptomics in suboxic waters of the central Baltic Sea using a new in situ fixation system.** *ISME J* 2012, **6**:46*1-470.
35. Cunningham A, McKee D, Craig S, Tarran G, Widdicombe C: **Fine-scale variability in phytoplankton community structure and inherent optical properties measured from an autonomous underwater vehicle.** *J Marine Syst* 2003, **43**:51-59.

36. Honda MC, Watanabe S: **Utility of an automatic water sampler to observe seasonal variability in nutrients and DIC in the Northwestern North Pacific.** *J Oceanogr* 2007, **63**:349-362.
37. Eberhart BTL, Bill BD, Trainer VL: **Remote sampling of harmful algal blooms: A case study on the Washington State coast.** *Harmful Algae* 2012, **19**:39-45.
38. Dodd PA, Price MR, Heywood KJ, Pebody M: **Collection of water samples from an autonomous underwater vehicle for tracer analysis.** *J Atmos Ocean Tech* 2006, **23**:1759-1767.
39. Bird LE, Sherman A, Ryan J: **Development of an active, large volume, discrete seawater sampler for autonomous underwater vehicles.** *2007 Oceans, Vols 1-5* 2007:1249-1253.
40. Zhang YW, McEwen RS, Ryan JP, Bellingham JG: **Design and tests of an adaptive triggering method for capturing peak samples in a thin phytoplankton layer by an autonomous underwater vehicle.** *Ieee J Oceanic Eng* 2010, **35**:785-796.
41. Taylor CD, Doherty KW, Molyneaux SJ, Morrison AT, Billings JD, Engstrom IB, Pfitsch DW, Honjo S: **Autonomous Microbial Sampler (AMS), a device for the uncontaminated collection of multiple microbial samples from submarine hydrothermal vents and other aquatic environments.** *Deep-Sea Res Pt I* 2006, **53**:894-916.
- **42. Herfort L, Seaton C, Wilkin M, Roman B, Preston CM, Marin III R, Seitz K, Smith MW, Haynes V, Scholin CA, et al.: **Use of continuous, real-time observations and model simulations to achieve autonomous, adaptive sampling of microbial processes with a robotic sampler.** *Limnol Oceanog Methods* 2015:50-67.
- Demonstrates combined use of oceanographic data, models, and adaptive sampling scripts to autonomously trigger sampling of transient oceanographic features.
43. Baptista A, Howe B, Freire J, Maier D, Silva CT: **Scientific exploration in the era of ocean observatories.** *Comput Sci Eng* 2008, **10**:53-58.
44. Crump BC, Baross JA: **Particle-attached bacteria and heterotrophic plankton associated with the Columbia River estuarine turbidity maxima.** *Mar Ecol Prog Ser* 1996, **138**:265-273.
45. Crump BC, Armbrust EV, Baross JA: **Phylogenetic analysis of particle-attached and free-living bacterial communities in the Columbia river, its estuary, and the adjacent coastal ocean.** *Appl Environ Microb* 1999, **65**:3192-3204.
- *46. Robidart JC, Preston CM, Paerl RW, Turk KA, Mosier AC, Francis CA, Scholin CA, Zehr JP: **Seasonal *Synechococcus* and *Thaumarchaeal* population dynamics examined with high resolution with remote *in situ* instrumentation.** *ISME J* 2012, **6**:513-523.
- Demonstrates the use of daily-resolution autonomous molecular analyses to gain insight into microbial population dynamics.
47. Mincer TJ, Church MJ, Taylor LT, Preston C, Karl DM, DeLong EF: **Quantitative distribution of presumptive archaeal and bacterial nitrifiers in Monterey Bay and the North Pacific Subtropical Gyre.** *Environ Microbiol* 2007, **9**:1162-1175.
48. Paerl RW, Turk KA, Beinart RA, Chavez FP, Zehr JP: **Seasonal change in the abundance of *Synechococcus* and multiple distinct phylotypes in Monterey**

- 459 **Bay determined by *rbcl* and *narB* quantitative PCR.** *Environ Microbiol* 2012,
460 **14:580-593.**
- 461 49. Murray AE, Blakis A, Massana R, Strawzewski S, Passow U, Alldredge A, DeLong
462 EF: **A time series assessment of planktonic archaeal variability in the Santa**
463 **Barbara Channel.** *Aquat Microb Ecol* 1999, **20:129-145.**
- 464 **50. Varaljay VA, Robidart J, Preston CM, Gifford SM, Durham BP, Burns AS, Ryan
465 JP, Marin R, Kiene RP, Zehr JP, et al.: **Single-taxon field measurements of**
466 **bacterial gene regulation controlling DMSP fate.** *ISME J* 2015, **9:1677-1686.**
467 Demonstrates combined use of *in situ* molecular analyses and laboratory analysis of
468 autonomously collected and preserved samples to gain insight into microbial gene
469 expression dynamics.
- 470 51. Bombar D, Taylor CD, Wilson ST, Robidart JC, Rabines A, Turk-Kubo KA, Kemp
471 JN, Karl DM, Zehr JP: **Measurements of nitrogen fixation in the oligotrophic**
472 **North Pacific Subtropical Gyre using a free-drifting submersible incubation**
473 **device.** *J Plankton Res* 2015, **37:727-739.**
- 474 **52. Aylward FO, Eppley JM, Smith JM, Chavez FP, Scholin CA, DeLong EF:
475 **Microbial community transcriptional networks are conserved in three**
476 **domains at ocean basin scales.** *Proc Natl Acad Sci U S A* 2015, **112:5443-5448.**
477 A synthesis of data from multiple Lagrangian time series collected using the ESP to gain
478 insight into microbial transcriptional networks.
- 479 53. Ottesen EA, Young CR, Gifford SM, Eppley JM, Marin R, 3rd, Schuster SC, Scholin
480 CA, DeLong EF: **Multispecies diel transcriptional oscillations in open ocean**
481 **heterotrophic bacterial assemblages.** *Science* 2014, **345:207-212.**
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Figure Captions

Figure 1. Sampling capabilities of ecogenomic sensors. This figure illustrates sample frequency and duration for various ecogenomic sensors. Instruments shown include the Environmental Sample Processor (ESP), the Autonomous Microbial Genosensor (AMG), the Microbial Sampler and Submersible Incubation Device (MS-SID), and the Biological OsmoSampling System (BOSS).

Figure 2. Data from a free-drifting ESP deployment. This figure illustrates data collection during an ESP deployment in the North Pacific. The top two graphs show contextual data collected at high resolution by on-board sensors, including temperature, salinity, chlorophyll concentrations, and percent light transmission. The third graph shows results from on-board quantitative PCR analyses examining gene copies per liter seawater of *nifH* nitrogen fixation genes from *Trichodesmium* and *Candidatus Atelocyanobacterium* as reported by Robidart et al [9]. The third shows transcript abundance (normalized to mean abundance of each transcript) for *Prochlorococcus* photosystem genes as detected during a high-resolution metatranscriptomic time series conducted during the first portion of the deployment [53]. Dashed lines mark the start and end of the metatranscriptomic time series.

Figure 1

1 **Table 1.** Ecogenomic sensors and automated sampling technologies.

Instrument	Applications	References
Environmental Sample Processor (ESP)	Nucleic acid probe hybridization for detection of harmful algae, invertebrate larvae, bacteria, and archaea	[17,19-22]
	Enzyme-linked immunosorbent assays for detection of algal toxins	[23]
	Quantitative PCR for quantification of microbial groups and metabolic genes	[9,14*,16,46*,50**]
	Particulate/cell collection and preservation for transcriptional analysis	[8*,15**,52**,53]
Autonomous Microbial Genosensor (AMG)	Nucleic acid sequence-based amplification for detection of harmful algae	[25,26]
Microbial Sampler and Submersible Incubation Device (MS-SID)	Particulate/cell collection and preservation paired with <i>in situ</i> incubations for measurements of primary production, grazing and nitrogen fixation	[29-31]
Biological OsmoSampling System (BOSS)	Continuous, unpowered collection and preservation of fluid samples for biological analysis	[32]
<i>In situ</i> Filtration and Fixation Sampler	Particulate/cell collection and preservation	[33]
Automatic Flow Injection Sampler	Whole water collection and preservation	[34]

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