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Molecular-biological sensing in aquatic environments: recent developments and emerging capabilities

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Aquatic microbial communities are central to biogeochemical processes that maintain Earth's habitability. However, there is a significant paucity of data collected from these species in their natural environment. To address this, a suite of ocean-deployable sampling and sensing instrumentation has been developed to retrieve, archive and analyse water samples and their microbial fraction using state of the art genetic assays. Recent deployments have shed new light onto the role microbes play in essential ocean processes and highlight the risks they may pose to coastal populations. Although current designs are generally too large, complex and expensive for widespread use, a host of emerging bio-analytical technologies have the potential to revolutionise this field and open new possibilities in aquatic microbial metrology.

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Background

Marine microorganisms are central to our relationship with the sea. In coastal regions, changes in precipitation, sewage treatment and agricultural practices fuel harmful algal blooms and the dispersal of pathogenic microorganisms, with direct impacts on food biosecurity and human health [1]. In contrast, microbes have the ability to consume anthropogenic pollutants and thereby ameliorate the global human footprint [2], as well as sustain processes that are essential to earth's habitability. In each case there is a definite and immediate need to increase the resolution of sampling and analytics in order to accurately determine factors impacting microbial communities, their distributions and the threats they may pose. With this in mind, the Global Ocean Observing System (GOOS) has prioritised zooplankton diversity, phytoplankton, microbes and harmful algal blooms as well as fish and apex predators among their list of 'Essential Ocean Variables' or EOVs, for the development of marine sensing platforms.

The advent of molecular bio-analytical methods including genetic sequence amplification and quantification has revolutionised the study of ecology by providing an accurate and sensitive means of identifying and enumerating organisms, based on their unique genetic (DNA or RNA) signatures ('molecular ecology') [3]. Genetic assays are able to distinguish species with no phenotypic differences in complex mixed species samples [4] and are ideally suited to automation. Achieving the goal of routine, autonomous molecular-biological sensing in aquatic habitats would represent a step-change in our capacity to measure the majority of biological EOVs by increasing spatiotemporal sampling using the most state of the art scientific methods. This review highlights the most recent developments in this field and emerging capabilities for *in situ* genetic analysis, which will influence the future development of aquatic microbial sensors.

In situ microbial sampling and sensing instrumentation

The available suite of ocean-deployable instrumentation for molecular ecology is summarised in Table 1. Photographs of the apparatus are shown in Figure 1. Most of these devices are Samplers, which collect water and/or filter retentate (cells and suspended particles), with or without preservation (archival) of the material for lab-based analysis upon retrieval. Microbiological samplers such as the PhytoPlankton Sampler (PPS), Remote Access Sampler (RAS) and Water and Microplankton Sampler (WaMS) are designed to collect samples amenable to lab-based DNA measurements following deployments, quantifying changes in key phylogenetic or functional genes (indicative of key populations) over space and time. Samplers that preserve cells *in situ* allow subsequent, lab-based quantification of the activities of key functional clades via transcript and/or protein quantification. The provision of RNA and protein preservation greatly enhances the level of analytics possible post sampling. Preservation-type samplers include the Biological OsmoSampling System (BOSS), the Suspended Particulate Rosette (SPR), the Microbial Sampler-*in situ* Incubation Device (MS-SID) and the Environmental Sample Processor (ESP). While the BOSS is small and robust, low sample volumes (typically < 5 mL seawater per time point) restrict the types of analytics possible.

Table 1

Sensor and sampler technologies and their specifications. WHOI: Woods Hole Oceanographic Institute; MBARI: Monterey Bay Aquarium Research Institute; instruments manufactured at McLane Research Laboratories are commercially available. Filter pore sizes $\leq$ 0.22 μm include prokaryotes. Max volume depends on cell concentration sampled. Instrument sizes are (S)mall (<5 L), (M)id-sized (5–100 L) and (L)arge (>100 L). M: Mooring, P: Pier, FB: Ferry Box, ROV: Remotely Operated Vehicle, Df: Drifter, AUV: Autonomous Underwater Vehicle. * The 3rd Generation ESP (in development) is small and deployable on AUV platforms. ** WaMS typical sample volume is 150 mL and it is being developed to preserve cells with Lugols Solution after sample collection

Instrument	Manufacturer or developer	Method	Organisms measured	Max volume per sample	Size	Platforms	Samples collected per deployment	References
DNA detection: population change								
Autonomous Microbial Sampler (AMS)	WHOI	Filtration (Supor)	≥0.45 μm	150 mL	S	ROV, SRV	6	Taylor et al. (2006)
Phytoplankton Sampler (PPS)	McLane Research Laboratories	Filtration (GFF)	≥0.8 μm	10 L	S	M, P	24	Honda and Watanabe (2007) and Winslow et al. (2014)
Remote Access Sampler (RAS)	McLane Research Laboratories	Seawater collection	No filtration	500 mL	M	M, P	48	McKinney et al. (1997) and Winslow et al. (2014)
Large Volume Water Transfer System (WTS LV)	McLane Research Laboratories	Filtration (GFF)	≥0.8 μm	5,000 L	S	ROV, CTD, Ship	1	Beaulieu et al. (2009)
Suspended Particulate Rosette (SUPR)	WHOI	Filtration (polycarbonate)	≥0.2 μm	30–100 L	S	ROV, CTD	24	Breier et al. (2009)
Water and Microplankton Sampler (WaMS)	SAFOS	Seawater collection	No filtration	150 mL	M	FB	10	Stern et al. (2015)
DNA, RNA & protein detection: changes in populations and activities								
In situ Filtration and Fixation Sampler (IFFS)	Leibniz Institute of Freshwater Ecology and Inland Fisheries	Filtration (Sterivex) & preservation	≥0.2 μm	900 mL	M	Ship	1	Wurzbacher et al. (2012)
Automatic Flow Injection Sampler (AFIS)	Leibniz Institute for Baltic Sea Research	Seawater preservation prior to filtration (custom)	≥0.22 μm	2.7 L	M	CTD	1	Feike et al. (2012)
Biological OsmoSampling System (BOSS)	MBARI & Harvard University	Seawater preservation	≥0.1 μm	flow rate: < 5 mL/day	S	M	5	Jannasch et al. (2004) and Robidart et al. (2013)
Suspended Particulate Rosette Version 2 (SUPR/SUPR-REMUS)	WHOI	Filtration (Supor) & preservation	≥0.22 μm	2 L	M	ROV, AUV	14	Breier et al., (2014) and Govindarajan et al. (2015)
Microbial Sampler-Submersible Incubation Device (MS-SID)	WHOI	Filtration (Supor) & preservation; biogeochemical rates	0.22 μm	4L	M	M.Df	48	Taylor and Doherty (1990), Bombar et al. (2015) and Edgcomb et al. (2016)
Autonomous Microbial Genosensor (AMG)	University of South Florida	Filtration (custom) & transcript quantification	≥0.45 μm	50 mL	S	M	12	Fries et al. (2007) and Paul et al. (2007)
Environmental Sample Processor (ESP)	MBARI & McLane Research Laboratories	Filtration (Supor) & rRNA hybridization; protein hybridization; qene quantification	≥0.22 μm	2 L	L*	M, P, ROV, DP	132 (sampler); 32 (sensor)	Scholin et al. (2009), Preston et al. (2011) and Ottesen et al. (2011)