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CURRENT EVIDENCE

Salty sensors, fresh ideas: The use of molecular and imaging sensors in understanding plankton dynamics across marine and freshwater ecosystems

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Scientific Significance Statement

In situ molecular and imaging instrumentation development has advanced our knowledge of plankton processes in aquatic ecosystems. However, these sensors have only begun to be used in freshwater ecosystems. Through a combination of literature review and interviews with instrument developers, we found that there is little technological barrier to transferring marine in situ molecular and imaging technology to freshwater ecosystems. Identified barriers are largely related to infrastructure and funding. These sensors have the ability to inform fundamental and applied plankton research in all types of aquatic systems.

Abstract

Understanding plankton dynamics in marine ecosystems has been advanced using in situ molecular and imaging instrumentation. A range of research objectives have been addressed through high-resolution autonomous sampling, from food web characterization to harmful algal bloom dynamics. When used together, molecular and imaging sensors can cover the full-size range, genetic identity, and life stages of plankton. Here, we briefly review a selection of in situ instrumentation developed for the collection of molecular and imaging information on plankton communities in marine ecosystems. In addition, we interviewed a selection of instrumentation developers to determine if the transfer of sensor technology from marine to freshwater ecosystems is feasible and to describe the process of creating in situ sensors. Finally, we discuss the status of in situ molecular and imaging sensors in freshwater ecosystems and how some of the reviewed sensors could be used to address basic and applied research questions.

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Scientific interest in the "patchy" distribution of plankton has been ongoing for more than a century; it has been over 50 yr since Hutchinson (1961) posited the "Paradox of the Plankton" based on evidence from lake studies. Since that pioneering research, the heterogeneous nature of zooplankton and phytoplankton distributions at the macro scale (submeter to kilometer) has been well accepted for both lakes and ocean basins (Wiebe and Benfield 2003). It has taken longer to appreciate the heterogeneous distribution of smaller organisms like bacterioplankton (Stocker 2012). Such distributions are not random, but are the result of underlying chemical, physical, and biological interactions, which can be heterogeneous even at micrometer scales. Over the last two decades, both the oceanographic and limnological communities have explored these distributions and their underlying mechanisms as well as their effects through the food chain (e.g., Brentnall et al. 2003; Franks 2005; Blukacz et al. 2009; McGillicuddy and Franks 2019).

Historically, resolving plankton distributions and abundances within lakes was relatively easier than in oceans due to their smaller size. Ocean basins require a much greater expenditure of resources to gather equivalent samples. In situ molecular and imaging sensors are arguably at the forefront of such efforts in oceanographic studies, because of the capability of these instruments to resolve plankton distributions at small spatial scales over large areas (up to several kilometers) and across wide depth ranges (hundreds of meters vertically) alongside ancillary measurements (e.g., temperature, salinity, dissolved oxygen, carbonate chemistry, pH, photosynthetic active radiation, fluorescence). Advances in the high throughput sampling capabilities of these instruments has dramatically decreased the per sample cost for oceanographic research campaigns and has allowed for unique and highly targeted sampling of communities of plankton and their processes.

Most of the research that has made use of in situ molecular and imaging sensors has taken place in marine ecosystems (Table 1). The goals of these campaigns have ranged from fundamental research, such as investigating predator-prey dynamics (Brownlee et al. 2016), to applied research objectives, such as early harmful algal bloom (HAB) detection (Greenfield et al. 2008; Ryan et al. 2011; Caron et al. 2017). A few in situ molecular and imaging instruments have been applied in limnological studies of plankton (e.g., in situ filtration and fixation sampler [IFFS], Wurzbacher et al. 2012; laser optical plankton counter [LOPC], Yurista et al. 2009; Yurista et al. 2012; Table 1); however, an abundance of lake-based research questions could benefit from their expanded use in freshwater ecosystems. Freshwater and saltwater habitats exhibit overlap in multiple environmental issues, including proliferation of algal blooms, biodiversity loss due to climate change, invasive species, overfishing, and changes in biogeochemical cycling due to eutrophication and hypoxia. Much has already been learned from the adoption of in situ molecular and imagining sensors, and the continued transfer of technology between the marine and freshwater sciences will further develop our knowledge of plankton dynamics in rapidly changing aquatic ecosystems.

Molecular and imaging sensors designed for marine environments have already been used to address a range of research questions related to plankton dynamics. Results from this research has led to advancements in the study of gene expression, microbial responses (Edgcomb et al. 2016; Ottesen 2016), and bloom dynamics (Robidart et al. 2012; Brosnahan et al. 2015; Hunter-Cevera et al. 2016). There are similar needs to better understand plankton dynamics in freshwater ecosystems. For instance, one of the most pressing areas of research in all aquatic sciences is the detection and mitigation of HABs (Anderson et al. 2012; Paerl et al. 2016, 2018). Eutrophication of aquatic environments is predicted to get worse as a result of climate change-induced increases in precipitation, which deliver nutrients from the surrounding landscape (Sinha et al. 2017). Remote sensing has been useful in tracking HABs at the macroscale (Clark et al. 2017) while in situ molecular sensors have been useful in characterizing and predicting HABs in coastal waters (Babin et al. 2005; Ryan et al. 2011). Furthermore, molecular in situ sensors have been proposed as a method for detecting toxic bloom development in the Laurentian Great Lakes (Bullerjahn et al. 2016). HABs are one issue that illustrates the need to apply marine molecular and imaging sensors to freshwater environments. However, there is a whole range of freshwater plankton research (i.e., biogeochemical cycling, food web dynamics, invasive species, community reorganization, climate change, etc.) that could benefit with the implementation of marine molecular and imaging in situ sensors in freshwater ecosystems, problems and paradigms shared by both limnology and oceanography sciences (Downing 2014).

In this current evidence article, we identify and briefly review in situ oceanographic instruments developed for the collection of molecular and imaging data, and we outline how these sensors can be applied to environmental issues and research areas in freshwater ecosystems. Molecular and imaging sensors are complementary systems that provide the ability to assess community composition and molecular scale processes of the plankton at relatively low cost per sample, allowing for wide coverage in time and space. To determine the suitability of transferring molecular and imaging sensor technology from marine to freshwater, we interviewed a selection of developers of these instruments. In doing so, we show both the challenges in the development of the sensors and the variety of applications of the sensors. Finally, we discuss some of the successes in the pioneering use of imaging and molecular in situ sensors in freshwater systems. We conclude with the ways that these sensors could be employed to address basic and applied research questions in lake ecosystems, thereby, using technology transfer to bridge marine and freshwater ecosystem sciences.

Two categories of in situ sensors for plankton

One of the major challenges in studying plankton is making accurate measurements of community composition while

Table 1. Referenced in situ molecular and imaging systems and their characteristics.

System	Volume capacity	Mode(s) of deployment	Biological target	Size range of organisms*	Habitat and sampling deployment notes	References
Imaging sensors						
ISIIS	108–168 L s^{-1}	Towed (undulated and depth keeping)	Large protozoans, phytoplankton chains, mesozooplankton, ichthyoplankton	150 μm–14 cm	Marine and offshore river plumes. 0–150 m depth—hundreds of km horizontal, up to 48 h. deployments	Cowen and Guigand (2008); Greer et al. (2016)
VPR	216 mL s^{-1}	Towed (undulated)	Mesozooplankton	100 μm–1 cm	Marine; 0–350 m depth; thousands of km horizontal.	Davis et al. (2005)
LOPC	1.2–196 L s^{-1} (depending on tunnel and platform configuration)	Towed (free falling vertical profiling)	Mesozooplankton	100 μm–35 cm	Marine and freshwater. 0–300 m depth—Days to weeks	Herman et al. (2004)
SOLOPC	0.7–1.2 L s^{-1}	Vertical profiling (free falling–autonomous; Lagrangian)	Phytoplankton to mesozooplankton	100 μm–1 cm	Marine. 0–100 m depth—Up to 4 d per deployment cycle	Checkley et al. (2008)
UVP	8–20 L s^{-1}	Profiler (mounted on CTD) or towed (attached to AUV or remotely operated vehicle (ROV) platforms)	Large protozoans, mesozooplankton	105 μm–2.66 mm	Marine. 0–3000 m depth	Picheral et al. (2010)
IFCB	5 mL every 20 min	Moored; Bench Top (underway sampling)	Microplankton and phytoplankton	10–150 μm	Marine and freshwater. Days, months, years	Olson and Sosik (2007)
FCB	Adjustable: 12.5–50 μL min^{-1}	Moored	Picoplankton and nanoplankton	0.5–10 μm	Marine; days, months, years	Olson et al. (2003)
ZOOVIS	~240 mL per frame	Towed	Mesozooplankton	20 μm to few cm	Marine and estuarine. 0–250 m depth	Bi et al. (2015)
LOKI	2.6 mL per frame	Vertical profiler with attached plankton net	Mesozooplankton	0.9–13 mm	Marine. 0–500 m depth	Schulz et al. (2010); Schmid et al. (2016)
SPC	3 mL per frame	Moored	Phytoplankton and Mesozooplankton	100 μm–2.5 cm	Marine and freshwater. Moored. Years	http://spc.ucsd.edu/
Zoocam	250 mL per frame	Mounted on a Zooglider	Protists to mesozooplankton	0.45 mm–4.95 cm	Marine. 0–400 m depth; up to 50 d per mission	Ohman et al. (2019)
Molecular sensors						
AFIS	2.7 L	Mounted on CTD	Microbial community metatransciptome	>0.2 μm	Marine (surface—~100 m depth)	Fieke et al. (2012)
AFISsys	250 mL	Moored	Microbial community metatransciptome	>0.2 μm	Brackish (surface); deployed for several days	Charvet et al. (2019)

(Continues)

Table 1. Continued

System	Volume capacity	Mode(s) of deployment	Biological target	Size range of organisms*	Habitat and sampling deployment notes	References
AMS	50 mL	ROV, AUV	Microbial community composition	>0.45 μm	Marine (deep sea, hydrothermal vents)	Taylor et al. (2006)
AMG	30–50 mL	Moored	Targeted nucleic acid sequences (amplification-based)	>0.45 μm	Marine (surface and subsurface); deployed for several days	Paul et al. (2007); Fries et al. (2007)
BOSS	0.1–5.0 mL d^{-1} (depends on flow rate and length of deployment)	Moored; can be deployed by ROV	DNA and protein	>0.1 μm	Marine (deep sea, hydrothermal vents); deployed for >1 yr.	Robidart et al. (2013)
ESP (ESP; D-ESP; 3G-ESP)	ESP and D-ESP: 2 L 3G-ESP: 1 L h^{-1} (depending on water turbidity)	ESP and D-ESP: Moored; ROV; cabled; drifter 3G-ESP: mobile (LRAUV), benchtop	ESP & D-ESP: Targeted hybridization assays; metatranscriptomics; quantitative PCR; proteomics 3G-ESP: Archival of filtered water samples, lysis for sample homogenization (in development)	Filter size dependent	ESP and D-ESP: Marine (surface and deep sea, hydrothermal vents, cold seeps) 3G-ESP: Marine (coastal and open ocean), stream, lake	Birch et al. (2018); Ottesen et al. (2011); Preston et al. (2009); Preston et al. (2011); Saito et al. (2011); Scholin et al. (2006); Scholin et al. (2017) (and references within)
IS-ABS	2 L	Modular; moored or integrated onto other instrument (such as an AUV)	Microbial community composition	>0.2 μm	Marine (surface—150 m); deployed for variable time/space resolution	Ribeiro et al. (2019)
IFFS	100–900 mL	Ship-deployed	Transcriptomics; reverse-transcription quantitative PCR	0.2–5.0 μm	Freshwater (surface—60 m).	Wurzbacher et al. (2012)
MS-SID	Up to 4 L	Moored, free-drifting, or cabled	Metatranscriptomics	>0.2 μm	Marine; deployed in deep-water extreme habitats	Edgcomb et al. (2016)
SUPR/ SUPR-V2/ SUPR-REMUS	SUPR: 30–100 L SUPR-V2: Up to 2 L SUPR-REMUS: Up to 66 L	Moored, mounted to CTD, or ROV	Microbial or mesozooplankton community composition	SUPR: >1 μm SUPR-V2: >0.2 μm SUPR-REMUS: >200 μm	Marine SUPR and SUPR-V2: Hydrothermal vent plume waters SUPR-REMUS: Up to 600 m	Breier et al. (2009); Breier et al. (2014); Govindarajan et al. (2015); Sheik et al. (2015)

*In most cases for the molecular sensors, the size range of organisms depends on the pore size of a filter, which is often a modular component and can be easily changed. The size range listed in the table is the size range applied in referenced studies.

simultaneously measuring activity or turnover processes. Across almost all size classes of plankton, molecular and imaging techniques are being used for in situ observation and classification. For nano-, pico-, and microplankton, sized 0.2–200 μm, molecular sensors and flow cytometry-based imaging solutions exist which can differentiate taxa and functional processes. For mesoplankton, macroplankton, and megaplankton (sized 200 μm–20 mm, 2–20 cm, and > 20 cm, respectively), imaging solutions play a critical role in differentiating plankton by size, morphology and behavior, and recent advances in environmental DNA (eDNA) analyses have opened the door to in situ genomic observations of species in these size ranges and even larger (Govindarajan et al. 2015; Djurhuus et al. 2018).

Molecular sensors

Recent advances in molecular in situ instrumentation resolve problems of collecting samples from heterogeneous communities in difficult-to-reach locations, while capturing true variability of the plankton. These instruments use samples of DNA, RNA, or other cellular products and perform some processing or preservation step in situ, allowing for their molecular characterization. They remove potential artifacts due to classical sampling methods, such as CTD Niskin bottles (Suter et al. 2017), or from delays in sample processing during transport of samples back to a ship or lab (Feike et al. 2012). Recent reviews discuss the breadth of "ecogenomic" sensors and their abilities to solve these problems (e.g., Ottesen 2016; McQuillan and Robidart 2017). Here, we highlight general capabilities within classes of instruments and how they have increased our ability to understand ecological phenomena of the plankton.

Molecular sensors differ in their ability to be deployed for varying periods of time, their ability to analyze samples in real time vs. postdeployment, their capability to preserve or conduct molecular tasks such as polymerase chain reaction (PCR) or incubations, their ability to conduct adaptive sampling, and their mobility to sample different environments (Table 1). Some classes of instruments collect and preserve filtered particulate samples in situ during short deployments (hours to days) until instrument recovery (e.g., the automatic flow injection sampler [AFIS], Feike et al. 2012; the autonomous in situ fixation multisampler, AFISsys, Charvet et al. 2019; the autonomous microbial sampler [AMS], Taylor et al. 2006; the in situ autonomous biosampler [IS-ABS], Ribeiro et al. 2019; the IFFS, Wurzbacher et al. 2012; the suspended particulate rosette sampler [SUPR], Breier et al. 2014, and the microbial sampler submersible incubation device [MS-SID], Edgcomb et al. 2016). Similar instruments, such as the Biological OsmoSampling System (BOSS), can preserve samples for longer deployments over days to months (Robidart et al. 2013); however, they have lower volume capacity per sample. Other instruments are able to collect, preserve, and analyze molecular samples in situ in order to detect gene targets or other cellular products such as HAB-produced toxins in near real-time (e.g., the autonomous microbial genosensor [AMG], Fries et al. 2007 and the environmental sample processor [ESP], reviewed in Scholin et al. 2017). This capability facilitates long-term deployments (days to weeks), and the collection of samples over wide environmental gradients. Some instruments also allow for in situ tracer incubations, and thus the determination of rate measurements concomitant with collection of molecular samples (e.g., MS-SID; Taylor and Doherty 1990; Taylor and Howes 1994; Taylor et al. 2015; Edgcomb et al. 2016; Pachiadaki et al. 2016; Medina et al. 2017). Deployments of many of these instruments can be adapted to allow for short, high intensity or longer-term time series sampling regimes in order to capture different modes of variability. Furthermore, investigators have developed novel capabilities for adaptive sampling in several of these instruments including, for example, transmission of biogeochemical real-time data to the user, which allows for triggered sampling under specific environmental conditions: (MS-SID, Fig. 1a, Edgcomb et al. 2016; moored ESP and 3G [3rd generation] ESP, Herfort et al. 2016). Others have been specifically adapted for extreme environments: for example, the AMS and SUPR are capable of collection from hydrothermal plume waters while the Vent-SID (currently in development) will allow for incubation studies of hydrothermal vent fluids in situ at vent fluid temperatures up to ~ 70°C (C. Taylor et al. pers. comm.).

Compatibility between molecular sensors and autonomous underwater vehicles (AUVs) or long-range autonomous underwater vehicles (LRAUVs) has resulted in incredible advances in mobility and targeted sampling with some sensors, such as the MS-SID and 3G ESP (Birch et al. 2018). Similarly, the SUPR-REMUS, a cousin of the SUPR, was recently incorporated into the AUV, REMUS 600, and deployed to detect larval distributions by genetic markers in a coastal bay (Govindarajan et al. 2015). Clio, a molecular sensing AUV that is under development will be capable of reaching depths of 6000 m and collecting molecular samples at preset depth intervals (Jakuba et al. 2018). These advances allow the survey of aquatic populations without the expense and burden of shipboard operations, allowing for the increased frequency and flexibility in the environmental sampling of populations of plankton. Using the same techniques, a new generation of molecular sensor technologies is evolving; genetic techniques traditionally applied to microbial life are now being adapted to the study of larger organisms through eDNA analyses (reviewed in Deiner et al. 2017). This allows for molecular sensors to be used to study larger size classes such as meso-, macro-, and megaplankton. All the above-described capabilities are atypical of traditional shipboard sampling, and thus emphasize the utility of in situ collection of molecular samples for the community composition of plankton and their activity, making them increasingly popular for oceanographic studies around the world (e.g., Fig. 2a).

Imaging sensors

Imaging sensors are another rapidly developing and powerful method to study plankton dynamics. While molecular sensors

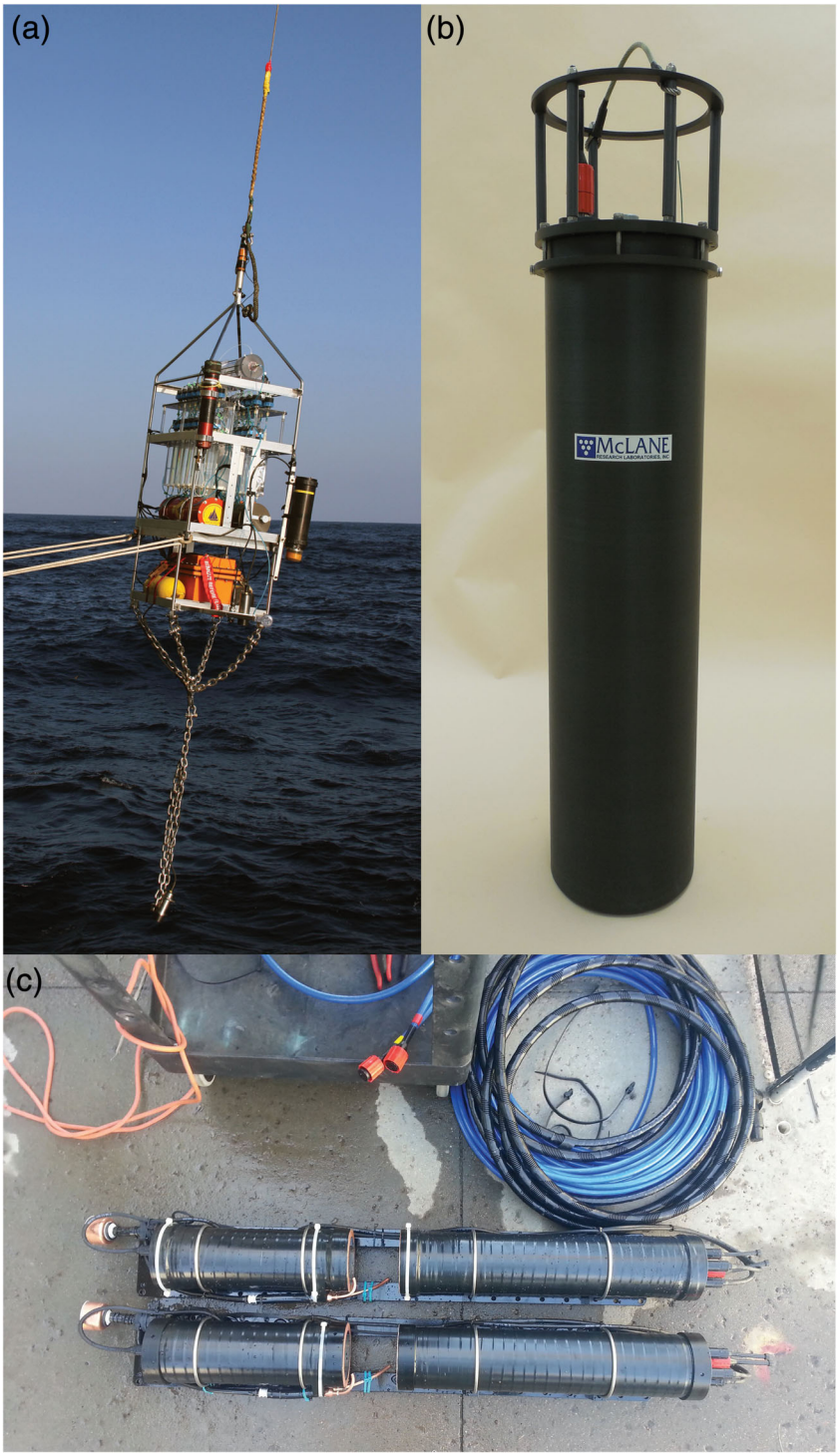

Fig. 1. Images of molecular and imaging in situ sensors. (**a**) MS-SID image. (**b**) Imaging Flow Cytobot (IFCB) image. Credit: McLane Research Laboratories. (**c**) SPC image. Credit: Jaffe Lab for Underwater Imaging, Scripps Institution of Oceanography.

rely on the cellular products of organisms for identification and study, imaging sensors allow for direct observation, granting additional types of information often not possible to infer from molecular data. These data can include cell size, shape, life cycle stage, behavioral patterns, and colocalization of other organisms such as symbionts or parasites. Some basic information such as in situ physical morphology may never have been known previously due to organismal fragility (e.g., cnidarians) or

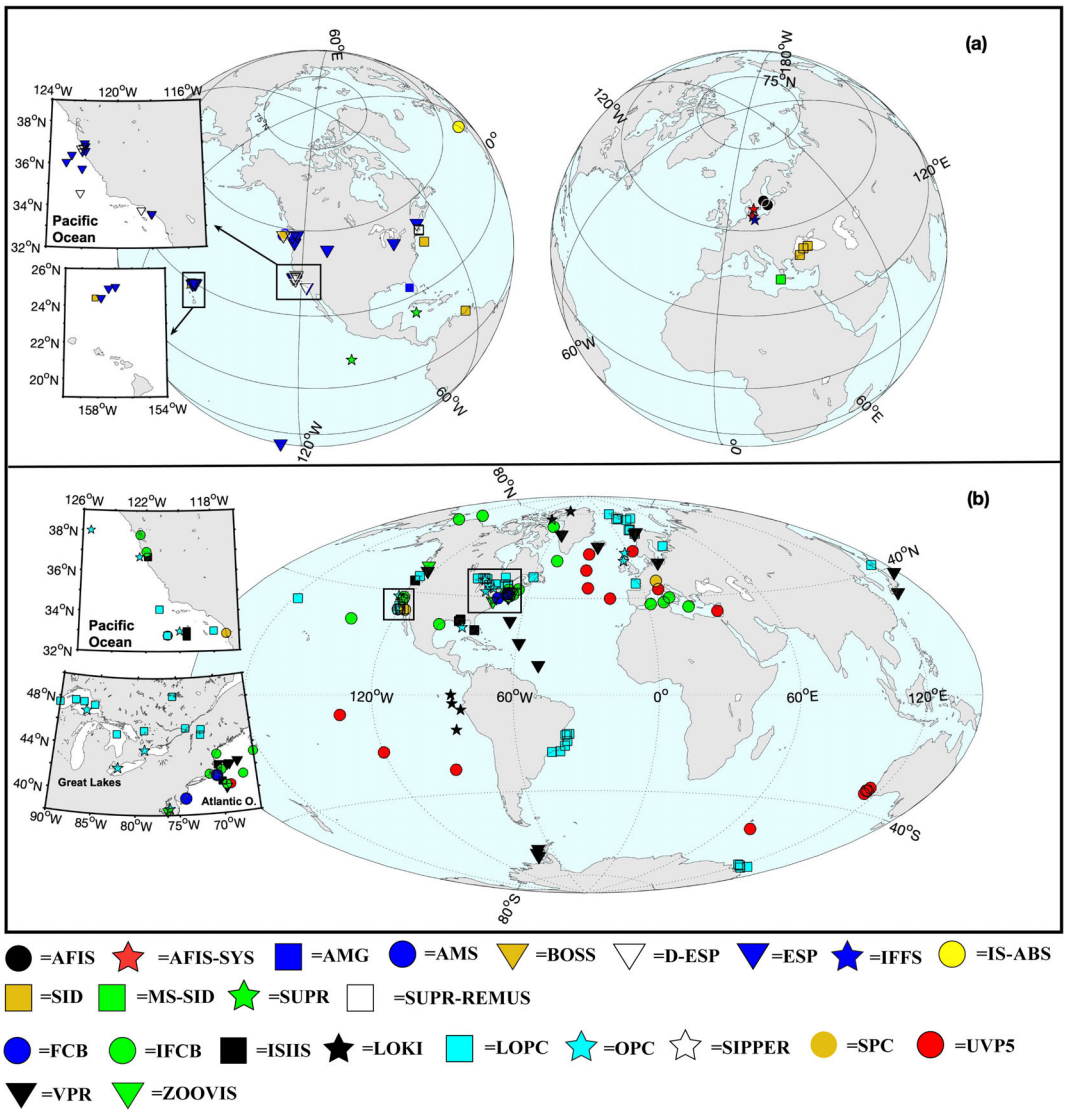

Fig. 2. Approximate sampling deployment locations of in situ sensors. (**a**) Molecular sensors; black circles: AFIS; red star: autonomous in situ fixation multisampler (AFIS-SYS); blue squares: AMG; blue circles: AMS; dark yellow inverted triangles: BOSS; open inverted triangles: Deep-Sea ESP (D-ESP); blue inverted triangles: ESP; blue star: IFFS; yellow circle: IS-ABS; dark yellow squares: SID; green squares: MS-SID; green star: SUPR; open square: Suspended Particulate Rosette Sampler-Remote Environmental Monitoring UnitS (SUPR-REMUS). (**b**) Imaging systems. Blue circles: FCB; green circles: IFCB; black squares: ISIIS; black stars: Lightframe On-sight Keyspecies Investigation (LOKI) system; cyan squares: LOPC (including the SOLOPC); cyan stars: optical plankton counter (OPC); open stars: shadowed image particle profiling and evaluation recorder (SIPPER); dark yellow circles: SPC; red circles: underwater vision profiler (UVP); black inverted triangles: VPR; green inverted triangles: ZOOplankton Visualization System (ZOOVIS). Methods on location deployment data mining and respective references available on Dryad along with the data set (Briseño-Avena 2019).

extremeness of the environment (e.g., the deep sea). Since some of the earliest experiments in the 1950s with underwater camera and television systems, to mounting cameras onto nets in the 1970s, imaging devices for studying plankton in situ have advanced considerably (reviewed in Wiebe and Benfield 2003), and recently several new modern systems have emerged. No single system was designed with the same questions in mind; instruments vary in the size class of organisms they can detect, their mode of deployment, the volume imaged, the duration of deployment, and the image resolution (ultimately determining the taxonomic resolution of the system). We summarize the characteristics of the major imaging systems currently in use globally for studying each size class of plankton and their distributions and processes (Table 1).

Similar to the challenges in understanding microbial and phytoplankton life in the oceans, investigating the dynamics of mesoplankton to megaplankton (which includes ichthyoplankton) also requires sampling at spatial and temporal scales that often are not possible through traditional means. Nets, for example, integrate samples over large spatial scales

both vertically (tens to hundreds of meters) and horizontally (several meters to kilometers). However, cost and time constraints of traditional shipboard net sampling limit the spatial and temporal availability of environmental samples. Furthermore, many organisms are too fragile to be sampled with nets and are thus missed with traditional sampling (Remsen et al. 2004). A new set of in situ imaging sensors can avoid many of these constraints and have opened new avenues of scientific inquiry (Fig. 2b; Table 1). The video plankton recorder (VPR), which was one of the earliest imaging systems, was designed to be towed for kilometers horizontally and profile hundreds of meters vertically while continuously imaging mesozooplankton, such as copepods, euphausiids, and small gelatinous organisms (e.g., Benfield et al. 1996; Ashjian et al. 2008). The extensive use of the VPR has led to the understanding of physical and biological interactions such as micropatchiness and turbulence (Ross 2014), predator-induced diel vertical migration in *Calanus finmarchicus* (Baumgartner et al. 2011), and copepod-marine snow associations (Möller et al. 2012; Nishibe et al. 2015). These studies illuminated processes affecting carbon export to the deep ocean. The in situ ichthyoplankton imaging system (ISIIS; Cowen and Guigand 2008) was designed to image a large volume of water (70 L s^{-1}) in order to capture images of less abundant organisms, such as fish larvae and large gelatinous organisms, while still encompassing images of phytoplankton and mesozooplankton. To our knowledge, the ISIIS is the only imaging system that can quantitatively resolve fish larvae distributions with respect to environmental parameters and prey fields (i.e., phytoplankton and zooplankton). More recently, the Scripps Plankton Camera (SPC; Roberts et al. 2014; http://spc.ucsd.edu/), a system consisting of two cameras: one designed for microplankton and phytoplankton, and a second one for mesozooplankton, aims to collect rapid time series data with a resolution of 1 frame s^{-1} (Fig. 1c). The SPC, while in its early stages, has already proven its usefulness by revealing a time-sensitive cryptic phenomenon not observed previously. Using a subset of the SPC images, Briseño-Avena (Briseño-Avena unpubl.) observed the external parasitic expression (a phase that lasts only a few minutes) of the *Paradium*-like parasite attached to the urosome of the copepod *Oithona similis*.

Other imaging sensors have been developed to detect picoplankton, nanoplankton, and microplankton (0.2–2 μm, 2–20 μm, and 20–200 μm, respectively). The Imaging FlowCytobot (IFCB; Olson and Sosik 2007), for example, is a moored system designed to image microplankton (< 10–150 μm) over time scales from minutes to years. An earlier instrument, the FlowCytobot (FCB; Olson et al. 2003), can detect picoplankton and nanoplankton (Table 1) and has been collecting data since 2006 at Martha's Vineyard Observatory (Fig. 1b). Both instruments adapted flow cytometry methods to a mooring system that allows for high-frequency sampling over long time periods. Time series data generated from both the IFCB and FCB have allowed ecologists to understand phytoplankton dynamics underlying bloom initiation and evolution (hours to days), species successions (seasons), and regime shifts (multiple years) (Sosik and Olson 2008; Anglès et al. 2015; Henrichs et al. 2015; Hunter-Cevera et al. 2016). The IFCB has also been used to study ciliates and other microzooplankton, as well as parasitic infections of diatoms (Peacock et al. 2014; Brownlee et al. 2016). Ecosystem factors have largely determined the locations of deployment of in situ imaging systems. Most studies in marine ecosystems have occurred in high latitudes where plankton diversity is low (Fig. 2b). The few studies in lower latitudes have been focused in environments with near oligotrophic conditions where imaging conditions are ideal due to lower particle loads (Fig. 2b). Furthermore, few oceanic deployments have occurred in nearshore areas (hundreds of meters from shore), with the exception of the IFCB, FCB, and SPC systems (Fig. 2b). Turbidity has been a challenge for underwater imaging, where light is already a limiting factor due to attenuation. Highly productive waters with high plankton concentrations are also challenging since image volume must be adapted to avoid overlap of imaged particles and plankton on each image frame. However, within the last decade, attempts have been successful in applying in situ underwater imaging systems in low-visibility waters. For example, Bi et al. (2013, 2015) successfully deployed the ZOOVIS in the turbid waters of an estuary in the Chesapeake Bay to study gelatinous organisms. The LOPC with its most recent modifications has increased its operational capacity in waters with particle concentrations of up to 10^3 particles L^{-1} (Herman et al. 2004). In a similar fashion, the ISIIS has been deployed within the turbid waters of the Mississippi River plume with positive results (Greer et al. 2016).

The other challenge posed by turbid waters is data processing; countless particles are imaged, and manual annotation of these images becomes a near-impossible task. Automated processing is being tested by some major research groups, and thus this major roadblock is diminishing (Benfield et al. 2007; Sosik and Olson 2007; Schmid et al. 2016; Orenstein and Beijbom 2017; Robinson et al. 2017; Luo et al. 2018). Very recently two new in situ imaging sensors became available, the Zoocam (Ohman et al. 2019), which is attached to the Zooglider, and the Continuous Particle Image Classification System (CPIC; www.coastaloceanvision.com), which can be mounted on a CTD frame. The latter incorporates onboard image segmentation and an automated classification system.

While in the past decade underwater imaging systems have been gaining traction within the scientific community, they have had limited deployments in freshwater systems (*see* "From intellection to instrumentation: How in situ ocean technology becomes a reality" section). The Great Lakes, for example, share some similar environmental problems with coastal marine regions such as HABs, invasive species, and waterborne pathogens of humans and native organisms, among other issues. Moreover, while the marine science community has gained much understanding of ecological phenomena such as bloom initiation due in part to imaging

systems such as the FCB (see Hunter-Cevera et al. 2016 as a recent example), there are fewer systematic efforts to deploy imaging sensors in freshwater systems. One major exception is the LOPC (Herman et al. 2004), which was deployed in the Great Lakes (Fig. 2b) with the objective to compare net and imaging system biomass estimates (Yurista et al. 2009). Such an effort was recently conducted over the global ocean using the Underwater Vision Profiler (UVP5; Biard et al. 2016) from data collected on cruises from 2008 to 2013; unlike the LOPC estimates, however, the latter estimates were based on conversion factors from the literature, and not compared directly to biological samples. More recently, the SPC was tested in Lake Zürich, Switzerland in order to compare image-based density estimates of phytoplankton against laboratory microscopy counts using water samples (Reyes et al. 2017). However, as mentioned above, one imaging system alone cannot be used to address every phenomenon, as each imaging system focuses on a different size class of organisms. Since underwater imaging sensors can be used in freshwater systems (a less corrosive environment than saltwater), there are great opportunities for gains in knowledge through the application of multiple imaging technologies in freshwater systems.

From intellection to instrumentation: How in situ ocean technology becomes a reality

To understand what is required for the development of in situ instrumentation, and the challenges faced in bringing an idea into a tangible reality, we spoke to four investigators with experience in the development and implementation of these types of technologies in their research: Virginia Edgcomb, Jules Jaffe, Heidi Sosik, and Craig Taylor. Each investigator took part in the development of in situ instruments including the Scripps Plankton (and Phytoplankton) Cameras (SPC), the IFCB and the (microbial sampler) SIDs, among others. In each case, these oceanographic instruments were built with broad scientific needs in mind: to increase sample throughput while minimizing artifacts associated with shipboard measurements and to study the organisms at biologically relevant spatiotemporal scales. These interviews illustrated several themes common across the researcher's experience: the importance of institutional benefits, such as local engineering expertise and the support of high-risk projects; the importance of collaboration, which insures instrument relevance; and finally, that novel instrument creation is a lengthy process that requires multiple changing sources of funding and may dominate the careers of the primary investigator during its development.

Edgcomb, Jaffe, Sosik, and Taylor work at institutions in the United States with significant institutional benefits including internal grant programs, an aspect that greatly enhances technology development. In each case, initial pilot studies were run with small institutional grants in order to develop a proof-of-concept instrument. Sosik emphasized the importance of these small grants for high-risk projects such as instrument prototype development, which are not typically funded by federal agencies. Taylor also emphasized that these small grants can be used to develop a novel aspect of a larger instrument. Critical institutional support also included technical staff and machine shop facilities, which aided in the design and construction of novel instruments. Both Scripps Institution of Oceanography (SIO) and the Woods Hole Oceanographic Institution (WHOI) employ staff that can build most of the electrical or mechanical components of a larger instrument. Instrument development requires many experts and multiple sources of funding over a sustained period. Therefore, different features of a single instrument may be designed with support from several different agencies over the duration of its development. Once initial proof-of-concept aspects were developed, results from these small institutional grants were used as critical preliminary evidence in larger grant proposals to federal organizations such as the Ocean Technology and Interdisciplinary Coordination (OTIC) program at the National Science Foundation (NSF), the National Oceanographic Partnership Program (NOPP), and programs at the Department of Energy (DOE), and the Office of Naval Research (ONR).

Collaborate across scientific disciplines is among the most important activity to take part in during the development of new technologies. Each of our interviewees has had long-standing research relationships with other scientist(s) with skills that complement their own. Sosik also argues strongly for interdisciplinary collaboration among different lab groups early on in technology development. In this way, the instrument developers are forced to adapt the instrument to be more user-friendly and flexible in order to answer other scientific questions, promoting broad applicability and commercialization. Edgcomb stressed that making the instrument user-friendly should be the ultimate goal, and that federal funding agencies prioritize this aspect in proposals. Early collaboration can also help in the acceptance of the instrument's usefulness and validity of results within the researcher's field. In general, acceptance occurs over years and with sufficient data collection. An instrument that has multiple users across many subdisciplines has a greater chance of becoming widely accepted by the field. Once the in situ instrument is developed and successfully implemented, its design may be purchased by a company that can increase the production of the instrument, and further refine user-friendliness. Several such ocean instrumentation companies exist, such as McLane Research Laboratories and Bellamare, which helped manufacture the ESP, IFCB, SID, and ISIIS instruments. Edgcomb and especially Taylor have had a long-standing relationship with McLane, for example, and frequently discuss scientific needs and collaborate with engineers at the company. Many employees in such companies were trained in academic, federally funded labs, and so there is a close relationship between the research and development process and the commercialization process. Additionally, the home institution itself may be

interested in patenting the design of the instrument. In either case, the principal investigators involved in instrument design are not responsible for mass production or customer service. Despite this, Sosik described the commercialization process as nerve-racking due to a sense of responsibility in the instrument's success even outside of her own research interests.

While each of the scientists we spoke with has had great success with design and application of in situ instruments, they also outlined several challenges. The development of a new instrument can have an "infinite gestation period," as Jaffe put it, but in general, each of these projects took 6–12 yr from conception to full application in the environment. Furthermore, while there were a core group of 2–3 scientists working on the project, a total of 4–12 people were required for full design, including engineers, technicians, and students. An instrument design is not static; these instruments are still constantly being upgraded or modified in response to new scientific questions or improving ease-of-use. In many cases, the evolution of these technologies included many "cousins" of the same instrument. For example, there are several versions of the SID which have each been adapted to sampling in particular environments, such as high temperatures hydrothermal vent systems or oxygen minimum zones. The IFCB was developed based in part on the questions left unanswered by its older cousin, the FCB. During the development period, Sosik emphasized the importance of continuing to pursue scientific questions and generating interesting data with the instrument. This allows for continual assessment of what the instrument can do and what practical limitations should be addressed in the next development stage. Meanwhile, publications and conference presentations are a good way to verify the instrument is successful and to get other groups interested in adopting the technology.

Most of these projects were started several years or even decades ago, when the interviewees noted that funding for instrumentation was easier to obtain. Taking on technology development is also a long and risky endeavor, particularly for an early career scientist who may have fewer publications as a result. Therefore, it was suggested that successful instrument design should be considered in promotion assessments for tenure. Furthermore, a common problem we heard was that there are few options for completion of an instrument once a prototype is developed; while institutions typically fund the initial proof-of-concept instrument and a federal organization typically funds the development and application of a prototype, many of the projects required a second round of engineering to realize the full capabilities of the instrument and ease the transfer of technology to other groups. Funding for these issues is hard to come by, however, Sosik suggests that continually modifying the instrument so that it answers novel scientific enquiries with each additional engineering capability is a good way to continue to fund an instrument's development.

Despite the aforementioned challenges, each of the instruments we discussed during these interviews is available to the scientific community either as commercial products or through open collaboration with the developers. System design poses a nontrivial constraint that might prevent the instrument from being widely adopted in freshwater sciences. While in development mode, most instruments are typically bulky, requiring large, ocean-going ships that can support deployment. It is not until miniaturization takes place that instruments can move into smaller bodies of water or dockside deployment. However, each interviewee emphasized that there would be no major roadblocks to use of the instrument in freshwater and that they are willing to work alongside freshwater scientists in developing the instruments further for freshwater use. In fact, some of the instruments have already been applied in lakes or rivers (Table 1), but broad adoption in limnological studies is still on the horizon. Collaboration and communication between limnologists and oceanographers are key to this crossover process.

Fresh ideas: Opportunities to forward the use of in situ sensors in freshwater research

Lakes provide abundant ecosystem services from vital habitat for aquatic organisms to drinking water supply and recreation. Plankton are the foundation of aquatic food webs, can indicate trophic state, and blooms of certain species can negatively affect the environment. Therefore, understanding plankton community dynamics is essential to preserving ecosystem health and sustainability. In situ instruments in lake settings are powerful tools for gathering vast amounts of data on biological communities and the changing conditions of lakes (Hampton 2013). To date, much of this effort has focused on chemical and fluorescence sensors. For instance, water quality has been tracked using fluorescence sensors to detect dissolved organic matter in a shallow eutrophic lake (Niu et al. 2014). Some in situ instruments have readily been adopted in freshwater systems, for example, the Sequoia Scientific's Laser In Situ Scattering Transmissometry (LISST) instrument (e.g., Serra et al. 2001). Even further, comprehensive data sets on water quality have proved especially useful when comparing multiple lakes to generate an understanding about how freshwater ecosystems respond to environmental change. The Global Lake Ecological Observatory Network (GLEON) addresses this through a network of high frequency in situ observatories managed collaboratively by members from over 40 countries (gleon.org; Rose et al. 2016). Access to aggregated data from multiple lakes has allowed for an improved understanding of regional and global patterns. For example, Brentrup et al. (2016) found that profiling buoys that collect high-frequency chlorophyll fluorescence out-performed conventional sampling when identifying subsurface chlorophyll maxima, which helped to clarify food web dynamics and carbon cycling.

The application of in situ water quality sensors in lake environments has led to several important discoveries and interesting observations. For instance, a global data set of summer

water surface temperatures gathered from in situ sensors and/or satellite measurements revealed a rapid warming trend in lakes over the last two decades (O'Reilly et al. 2015). Observations like these are essential for tracking environmental change. In situ molecular and imaging sensors can further this effort by obtaining a more refined understanding of plankton dynamics in freshwater, thereby enhancing our knowledge of food web interactions, trophic state, HABs, and more. However, the optical and molecular sensors we reviewed here are just beginning to be used in freshwater systems, and these deployments often are not yet reflected in the peer-reviewed literature. Few and often negligible engineering barriers exist for moving these instruments from a saltwater to a freshwater environment (*see* "From intellection to instrumentation: How in situ ocean technology becomes a reality" section). Instead, barriers to transference may be infrastructural. Many instruments require specific technical equipment and specialized teams for deployment and retrieval that may be available on ocean-going vessels but are not currently widely available in lakes (a notable exception are large vessels on the Laurentian Great Lakes operated by NOAA and the EPA). Some of the most compelling freshwater environments for transferal of this technology are relatively large bodies of water, such as the Great Lakes in the United States or Lake Baikal in Russia. These large lakes share many of the same challenges to sampling as ocean environments and pose similar ecological questions regarding species distributions (e.g., Yurista et al. 2009), harmful algae (e.g., Brooks et al. 2016), and the roles of planktonic organisms in biogeochemical cycling (e.g., Wurzbacher et al. 2012). However, continuous presence and the generation of high-resolution long-term data sets, such as those created by the IFCB, would also be valuable in small bodies of water (such as lake or stream systems) to resolve questions of trophic interactions or bloom progression.

When in situ molecular and imaging sensors have been used in lake environments, they have most commonly been applied to large lake systems. For instance, the LOPC was used in Lake Superior to assess zooplankton abundance and size (Yurista et al. 2009). In situ instruments for detecting toxins are of particular interest due to the widespread issues of HABs in freshwater systems (Brooks et al. 2016). In 2016, the first deployment of an ESP occurred in Lake Erie and had the capability to detect microcystin, a toxin produced by cyanobacteria that threatens drinking water supplies and other benefits from lakes (http://www.fondriest.com/news/espniagara-tracks-algal-toxins-lake-erie-protects-drinking-water.htm, 25 June 2018). The SID has also been deployed in the Great Lakes for educational purposes (C. Taylor pers. comm.). Another technology developed by MBARI, the LRAUV Tethys, was first deployed in the Great Lakes in 2016 to test its capability to be used in collaborative ship-LRAUV deployments. An MBARI LRAUV has recently returned to the Great Lakes in 2018 with the 3G ESP module installed, illustrating how in situ instrumentation that can be miniaturized and adapted to mobile platforms can be more widely used. These recent steps are encouraging and demonstrate the capability to transfer technology from marine to freshwater ecosystems, and that their deployments can address both basic and applied questions in freshwater systems. These new avenues of research are especially needed in the Great Lakes, since those ecosystems are changing rapidly and have experienced large economic and human health impacts from the increasing threat of HABs (Brooks et al. 2016; Carmichael and Boyer 2016).

Research on gene expression is one example of in situ molecular sensor technology being used in both marine and freshwater systems to address similar types of questions. Using the ESP, coordinated regulation of gene expression was observed for a multispecies complex marine microbial community, suggesting synchrony among unrelated taxa in response to environmental change (Ottesen et al. 2013). In a similar but targeted gene expression study in a freshwater ecosystem using the IFFS, Wurzbacher et al. (2012) followed the expression of an unknown Actinobacterial rhodopsin gene. The function of this gene, although very abundant, was unknown, but their results allowed for the authors to hypothesize its function based on diurnal activity. This example highlights a major finding in a freshwater system that resulted from in situ molecular sensor technology. Instruments such as the 3G ESP which can be used to detect gene expression on broad scales and in high resolution would more than likely bring many more of these discoveries to the forefront for lake researchers.

Another benefit from a wider application of in situ sensor technology to freshwater systems would be the creation of long-term time series of high-frequency sampling of plankton assemblages. Long deployments of imaging sensors (such as the IFCB, FCB, and SPC) have generated data that have advanced our understanding of interannual and seasonal variation in plankton assemblage composition (e.g., Sosik et al. 2003), as well as how relatively cryptic phenomena (such as parasitic infections) may be shaping seasonal dynamics (e.g., Peacock et al. 2014). Although lake systems may be more accessible to sampling than marine environments, the benefit of automated high-frequency observations is still large. Often, it is only through using such datasets that we can detect the importance of episodic events that may remain unobserved through less frequent sampling (such as storm events which may introduce an influx of nutrients to a lake).

The potential applications of in situ molecular and imaging sensors are very broad, from population dynamics that occur over short periods to community and ecosystem processes that are adapting to environmental change over longer time scales. In situ technology can also help inform applied research in the areas of HABs and the detection and monitoring of invasive species. For instance, instruments like the ESP are useful for monitoring real time dispersal of invasive or harmful species while instruments like the IFCB and ISIIS are suitable for assessing food web dynamics before, during, and after HABs, and visualizing organisms that may be more difficult to detect or quantify genetically. These potential

applications do not come without some obstacles. However, through collaboration and ingenuity, the transfer of technology between freshwater and marine systems is feasible and the promise of scientific advancement is high, a goal shared historically by both disciplines and highlighted by Downing (2014), where he rightly points out that there is "a major convergence between limnology and oceanography in paradigms as global change advances."

Conclusion

Molecular and imagining in situ sensors have revolutionized sampling of plankton populations and communities, from the nano- to the macroscale. These sensors link population processes to physical and geochemical dynamics at varying spatiotemporal scales, which has been vital to understanding the ecology of plankton. Detailed knowledge of plankton is essential as they form the base of the food web and are responsible for a large portion of carbon cycling. The term "plankton" covers a diverse array of organisms and is reflected in the breadth of technologies that have been applied to their study. It is perhaps only through the application of multiple technologies that we gain a fuller picture of the complexity of interactions of plankton and their important effects on both ecosystem biodiversity and human interests.

Molecular and imaging in situ sensors take effort and time to develop. Once developed, they can be applied, through collaboration or commercialization, in a variety of aquatic ecosystems. Current applications of in situ molecular and imaging sensors are just beginning to be explored in freshwater ecosystems. There is great potential to look at issues such as the threat of HABs and invasive species, and the effects of changing climate on freshwater systems with these instruments. Overall, as is apparent from recent freshwater deployments of oceanographic sensors and our discussions with instrument developers, few technological barriers exist and there is a lot to be gained from the transferal of technology from ocean basins and coastal ecosystems to freshwater systems. It is an exciting time to have these expanded capabilities as we enter an age of high environmental variability.

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