

## SITES AquaNet: An open infrastructure for mesocosm experiments with high frequency sensor monitoring across lakes

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### Abstract

For aquatic scientists mesocosm experiments are important tools for hypothesis testing as they offer a compromise between experimental control and realism. Here we present a new mesocosm infrastructure—SITES AquaNET—located in five lakes connected to field stations in Sweden that cover a ~760 km latitudinal gradient. SITES AquaNet overcomes major hindrances in aquatic experimental research through: (i) openness to the scientific community, (ii) the potential to implement coordinated experiments across sites and time, and (iii) high-frequency measurements (temperature, photosynthetic photon flux density, turbidity and dissolved oxygen, chlorophyll a and phycocyanin concentrations) with an autonomous sensor system. Moreover, the infrastructure provides operational guidance and sensor expertise from technical staff, and connections to a multi-layered monitoring programme (“SITES Water”) for each lake. This enables ecological observations from whole lake ecosystems to be compared with experimental studies aiming at disentangling major drivers and mechanisms underlying observed changes. Here we describe the technical properties of the infrastructure along with possibilities for experimental manipulations to tackle pressing issues in aquatic ecology and global change science. As a proof of concept, we also present a first mesocosm experiment across all five field sites with a cross-factorial design to evaluate responses of the sensor measurements to press/bottom-up (constant light reduction) and pulse/top-down (temporary fish predation) disturbances. This demonstrates the suitability of the infrastructure and autonomous sensor system to host modularized experiments and exemplifies the power and advantages of the approach to integrate a network of mesocosm facilities with manageable costs across large geographic areas.

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The key ecosystem services provided by many lakes worldwide are increasingly being constrained by the multifaceted impacts of global change driven by human activities (Millennium Ecosystem Assessment 2005). In addition to direct in-lake anthropogenic-induced changes in the biotic (species extinction rates and food web structure) and abiotic environment (hydrological, hydrothermal and biogeochemical cycles), lake ecosystems are particularly susceptible to ongoing changes in climate that affect both internal processes and

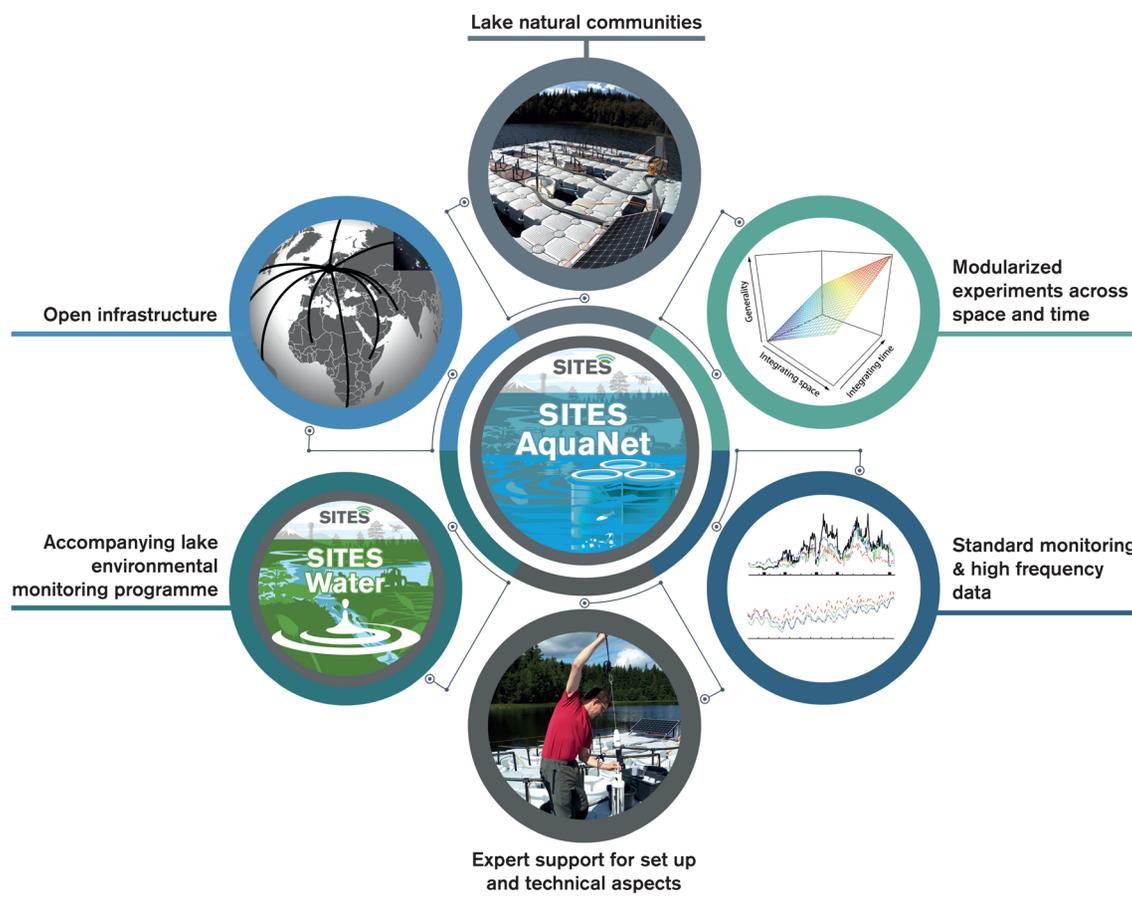
catchment-derived exports from their surrounding landscape (Adrian et al. 2009; Heino et al. 2020). As a result, there is growing interest from scientists, politicians and water managers to understand and disentangle the mechanisms underpinning the overall stability of lake communities and ecosystem functions in response to multiple environmental disturbances (Donohue et al. 2016).

Assessing effects of environmental change on lakes at the ecosystem level is challenging. Various inferential approaches are available for elucidating effects of global change on lakes with high ecological realism (space-for-time substitution, time series or paleoecology; see e.g., Kosten et al. 2009, 2012; Sorvari et al. 2002; Smol et al. 2005, Jeppesen et al. 2009; Urrutia-Cordero et al. 2016a). However, these are often constrained by confounding factors that limit understanding of causal relationships, especially when environmental gradients span broad spatial areas with distinct biogeographical, climatological and other macroecological features (Jeppesen et al. 2014). Experimental approaches hold the potential to overcome these limitations. Manipulation experiments fundamentally pursue specific testing of hypotheses and can better elucidate the processes underlying observed changes. These experiments comprise different approaches that vary in their degree of controlled settings and ecological reality (De Boeck et al. 2015). Notably, mesocosm facilities are being increasingly used to address global change impacts on lakes (e.g., see Landkildehus et al. 2014; Li et al. 2016; Urrutia-Cordero et al. 2017; Velthuis et al. 2017; Wilken et al. 2017; Verbeek et al. 2018), because they provide a relevant degree of biological complexity compared to laboratory settings, and a sufficient level of replication compared to whole-system manipulations, to enable rational hypothesis testing (Stewart et al. 2013). Here we present a new and open modular mesocosm infrastructure—SITES AquaNet—located in lakes at five field sites spanning wide geographic and environmental gradients in Sweden. SITES AquaNet tackles several major hindrances in aquatic experimental research: (i) reliance of results on the specific context of each study (i.e., lack of coordinated experiments across space and time), (ii) knowledge gaps related to insufficient temporal resolution in data collection, (iii) costly and limited access to mesocosm facilities for the scientific community.

Global change experiments aim to generate understanding of ecological phenomena by extrapolation from case-studies to wider contexts and across space and time (De Boeck et al. 2015). Yet results from ecological experiments are highly influenced by the local setting of each study, often leading to contrasting responses to common drivers of environmental change (Stephen et al. 2004; Mahdy et al. 2015). While meta-analyses have become a crucial tool to move beyond contingencies of individual systems to find common trends and differences across case studies (Gurevitch et al. 1992; Hillebrand 2008; Hillebrand and Cardinale 2010), dissimilarity between studies in terms of design, methods and location can, on occasions, set limits to their applicability (Hillebrand et al. 2014). An even more powerful tool, therefore, is to combine

multiple experiments with the same methods and treatments across different sites and environmental gradients (De Boeck et al. 2015). Two successful experimental examples are the Nutrient Network ([www.nutnet.org](http://www.nutnet.org)) and Zostera Experimental Network (<http://zenscience.org/>) for manipulations in grasslands and seagrass meadows, respectively. Results from such networks allow both, examination of specific results from single-site studies and of generalized patterns across geographically distant field sites (Adler et al. 2011; Duffy et al. 2015). Similar projects have also been implemented in lake ecosystems based on collaborative research projects (Stephen et al. 2004; Mahdy et al. 2015). SITES AquaNet allows researchers to use this collaborative experimental approach across several modules (i.e., field sites) to improve the potential for extrapolation of findings by providing open access to a network of standardized mesocosm facilities (Figs. 1 and S1). In practice, this is achieved by combining experiments across five lakes in order to determine how local differences between important variables across sites (e.g., temperature, humic content, nutrients, food web structure) mediate ecological outcomes of experimental manipulations. In addition to spatial influences, it is also important to address how temporal differences in environmental conditions and community composition within systems affect the outcome of experiments with standardized treatments. Examples from “single system experiments” replicated in time could, for example, show strong “year effects” and “season effects” in response to the same manipulation (Beisner and Peres-Neto 2009; Werner et al. 2020). SITES AquaNet consists of five permanent field stations which are an integral part of SITES - Swedish Infrastructure for Ecosystem Science (<http://www.fieldsites.se/en-GB>). The infrastructure is located at four core SITES stations (Asa, Erken, Skogaryd, and Svartberget) and one associated station (Bolmen) and is jointly funded by the Swedish Research Council, the Swedish Agricultural University, Uppsala University, Gothenburg University and Southern Sweden Water Supply/Sweden Water Research AB. Within this framework SITES AquaNet offers open access to and technical support at each mesocosm facility. More details, including contact information, additional facilities at the stations and the application procedure can be found on the SITES AquaNet website (<https://www.fieldsites.se/en-GB/sites-thematic-programs/sites-aquanet-32634394>). SITES has a clear vision to promote and support long-term observation and experimental research, and by that provides a persistent infrastructure that allows replication of standardized experiments in time. SITES AquaNet therefore expands the endeavors of previous studies considerably, as it allows for replicate experiments across both space and time (Fig. 1).

Another important constraint that ecologists experience when performing mesocosm experiments are the limitations imposed by tedious and costly standard sampling methods, which can rarely be implemented at higher than weekly temporal resolution. Reconstructing response variables from less frequent discrete monitoring does not only risk distorting the original signal which may be of continuous nature



**Fig 1.** Conceptual presentation of SITES AquaNet showing the main deliverables of the infrastructure to the scientific community.

(cf. “Nyquist–Shannon sampling theorem” or “temporal aliasing”; Shannon 1948; Marce et al. 2016), but also limits the number of ecological questions that can be addressed. With the advent of high-frequency sensor monitoring of the abiotic and biotic environment, an improved understanding of responses to environmental perturbations over a range of time scales is therefore becoming a reality (see review by Marce et al. 2016 for advantages and limitations). In addition to avoid generating biased results from infrequent monitoring and failure to capture short-lived episodic events, autonomous sensors can at high resolution describe the temporal trajectories that communities and biogeochemical processes take in response to perturbations as well as the pace of recovery from such disturbances (Donohue et al. 2016). High-frequency real-time data can therefore be beneficial for detection of early warning signals preceding critical transitions (“tipping points”) that can occur in nature (Pace et al. 2016; Wilkinson et al. 2018). They can also be beneficial for ecosystem metabolism estimates from real time measurements of oxygen concentrations which can greatly improve our understanding of whether aquatic systems are sinks or sources of atmospheric carbon dioxide (Staeher et al. 2010). Several indoor and

outdoor mesocosm facilities have demonstrated the benefits of using sensor systems either to measure simulated environmental conditions of global change (Gall et al. 2017; Li et al. 2017; Velthuis et al. 2017) or monitor response variables (Giling et al. 2016; Aben et al. 2017). SITES AquaNet provides researchers the possibility to use a standardized autonomous sensor monitoring system across field stations and is therefore the first network that allows high frequency monitoring of experiments across multiple sites (Fig. 1).

Mesocosm infrastructures can be costly to implement and maintain. SITES AquaNet supports researchers by providing onsite technical and scientific advice for conducting single-site and modularized experiments. This includes open access to full mesocosm facility instrumentation, operational network guidance and sensor expertise from technical staff, so that researchers only have to cover expenses associated with running their specific projects (Fig. 1). Connected to this, another major advantage is that the mesocosm infrastructure is embedded in a multi-layered programme (“SITES Water”) that monitors various physical, chemical and biological variables as well as greenhouse gas fluxes (Fig. 1). Thus, researchers have the possibility to combine ecological observations from the local

lakes with experimental studies aiming at disentangling major drivers and mechanisms behind patterns observed in nature (e.g., see Hansson et al. 2014; Urrutia-Cordero et al. 2015, 2016b).

Here, we describe the technical properties of the infrastructure along with possibilities for experimental manipulations. As a proof of concept, we also present a first mesocosm experiment carried out across all five sites in the AquaNet network. Natural plankton from each local lake was inoculated in a standardized setup of mesocosms and their temporal dynamics were evaluated in response to a press bottom-up disturbance (constant light reduction) and pulse top-down effects (temporary fish predation) in a cross-factorial designed experiment. Our investigation therefore tested the suitability of the infrastructure and autonomous sensor system to host modularized experiments. We also demonstrate the power of the approach to address ecological questions across large geographic areas with manageable costs by using a larger network of mesocosms facilities.

## Materials and procedures

### Infrastructure sites and construction

SITES-AquaNet is composed of five different experimental facilities (Asa, Bolmen, Erken Skogaryd and Svartberget), located along a ~760 km latitudinal gradient in Sweden (from Bolmen lat. 56° 57'N to Svartberget lat. 64° 16'N) (Fig. S1). The experimental facilities are located in lakes (Feresjön, Bolmen, Erken, Erssjön and Stortjärn) that vary considerably in local environmental conditions, such as trophic status, temperature, and humic substances (Table 1). The experimental facilities consist of 144 floating connected blocks (50 × 100 × 40 cm) (Jetfloat Inc., Canada) at each site. For the standard arrangement, the platform is organized to form five rectangular openings (size 1 × 7 m) where the mesocosms are located (Fig. S2). The mesocosms are UV-resistant translucent cylindrical polyethylene containers (1.5 m deep, 0.8 m diameter) with a total volume of 700 L (CPX Container 700 L cylindrical, Cipax AB, Sweden). In total, 20 mesocosms are available at each site, of which at least 16 can be equipped with an autonomous sensor system (see below). They have welded polyethylene extensions at

the top and bottom on all four opposing directions. At the bottom, as a ballast, a heavy iron chain is attached, to compensate for the buoyancy of the mesocosms and increase stability in the water column. At the top, shackles are attached to the extensions, and straps are threaded through the shackles and attached to the platform to hold the mesocosms in place. In principle the platform is flexible and can accommodate a larger number of mesocosms or fewer larger mesocosms than described below, including the option to use polyethylene bags.

Several water quality variables (light, turbidity, temperature and oxygen, chlorophyll-a and phycocyanin concentrations) can be continuously monitored in each mesocosm. For this, sensors are mounted on a UV-resistant polyethylene tube that is vertically deployed in the center of each enclosure (see below) and attached to an arc consisting of a tube of the same material that is fixed to the platform diagonally over the mesocosm (Fig. 2). The entire sensor system can be lifted up and cleaned with a brush to reduce biofouling as needed. All sensors are wired to a common cabinet where data is recorded using a Campbell Scientific CR1000 datalogger and an AM16/32 multiplexer (Campbell Sci, Utah, USA). Variables measured are:

1. Photosynthetically Active Radiation (PAR), expressed as Photosynthetic Photon Flux Density (PPFD): An Apogee SQ-500 sensor (Apogee Instruments Inc., USA) is mounted at 40 cm depth on a stainless steel plate with a distance from the pole of 25 cm to the south. Due to the very humic water of Lake Stortjärn (Svartberget site) and the consequently strong light attenuation, the sensors at this site are placed at 20 cm depth.
2. Dissolved oxygen saturation (DO<sub>sat</sub>) and water temperature: An Oxygen optode 4531 sensor (Aanderaa Data Instruments AS, Norway) is mounted at 60 cm depth.
3. Chlorophyll *a* fluorescence, turbidity and phycocyanin fluorescence: A TriLux sensor (Chelsea Technologies Group, UK) is mounted at 60 cm depth. To minimize the effects of biofouling, the sensors were cleaned manually in conjunction with the sampling occasions, that is, at most 7 d apart, but at some sites cleaning was also done more frequently in between.

**Table 1.** Description of SITES AquaNet sites, lakes, and running period during the test experiment in 2017

Site	Lake	Location	Trophic status	Mean depth (m)	Experimental period
Asa	Feresjön	57°11'N, 14°48'E	Oligotrophic	4	August 14–September 11
Bolmen	Bolmen	56°57'N, 13°38'E	Oligotrophic	5	August 22–September 19
Erken	Erken	59°50'N, 18°38'E	Mesotrophic/eutrophic	9	August 14–September 11
Skogaryd	Erssjön	58°22'N, 12°10'E	Humic	2	August 28–September 25
Svartberget	Stortjärn	64°16'N, 19°46'N	Humic	3	August 28–September 25

Note: For more information on the lake's characteristics see Urrutia-Cordero et al. 2021.

The analog signals from the Apogee SQ-500 and Oxygen optode 4531 sensors are read by the datalogger through the multiplexer, while the TriLux sensors are read digitally directly by the datalogger with the SDI-12 protocol. At the Skogaryd station, Oxygen optode 4531 sensors are read digitally instead.

Besides the sensors in the mesocosms, one Oxygen optode 4531, one TriLux and two Apogee SQ-500 sensors are also mounted outside the platform, to monitor each lake in parallel to the mesocosm measurements. The two Apogee SQ-500 sensors are mounted at 5 and 40 cm depth (at Svartberget at 5 and 20 cm), respectively, while the Oxygen optode 4531 and TriLux sensors are mounted at 60 cm depth. These sensors are connected directly to the datalogger and they are read at the same intervals as the sensors in the mesocosms. The Apogee SQ-500 sensors in the lake are placed at two different depths in order to calculate the diffuse attenuation coefficient of light in the lake.

Sensor data are stored in the datalogger in separate tables at temporal resolutions of one and 10 min, respectively. In addition, aggregated data of 10 min, hourly, and daily mean values are stored in different tables. The dataloggers are connected to the internet, either through direct cable or wireless connection or via a mobile internet router. Data from all dataloggers at the SITES AquaNet platforms are collected hourly by a centrally located server and immediately put in an SQL (Structured Query Language) database. A website graphically shows the data stored in the database from all sites and all sensors for the whole period of an experiment. The main purpose of the website is for surveillance of the experiments without the need to visit the platforms or to connect to each datalogger separately.

A surveillance camera is also installed on some of the platforms to monitor the mesocosms and document undesired activities of birds or other animals. The camera model is a Mobotix M25 (Mobotix AG, Germany) with a wide-angle lens seeing 90° horizontally, providing oversight of the whole platform from one corner.

In principle, it is possible that researchers add their own instruments and equipment to the infrastructure, such as additional sensors, multiplexers, dataloggers, and wipers to suit specific needs of their projects. However, this requires careful planning together with SITES AquaNET, to ensure that the adjustments are feasible and do not interfere with the short- and long-term operation of the standard infrastructure, including the comparability between sites and has to be done at the researchers own cost.

#### Manual sensor measurements and other equipment and facilities

Water samples from the mesocosms and the lake can be taken at a frequency that is needed to answer the scientific question of choice. For our experimental tests (see “Assessment”), integrated water samples (around 10 L of water pooled in a bucket) were taken with a Ruttner sampler (0.4 m height), two from both the upper (0–40 cm depth) and lower (40–

80 cm depth) part of the water column, respectively. From the pooled water sample, sub-samples were taken at the platform or in the nearby lab at each station’s facility for various analyses, where filtration equipment and freezers and cooling rooms for sample storage are available (see Supporting Information).

Included in the infrastructure is the possibility to make manual assessments with handheld sensors. A handheld Apogee MQ-500 sensor can be used for more detailed vertical measurements of PPF, for example, for estimating the diffuse attenuation coefficient of light. An AquaRead Ap-2000 multi-probe with sensors for measuring pH, conductivity, dissolved oxygen saturation and concentration, and temperature is also available for measurements in the mesocosms and in the lake. The Ruttner sampler and sensors were rinsed in the lake between sampling of different mesocosms.

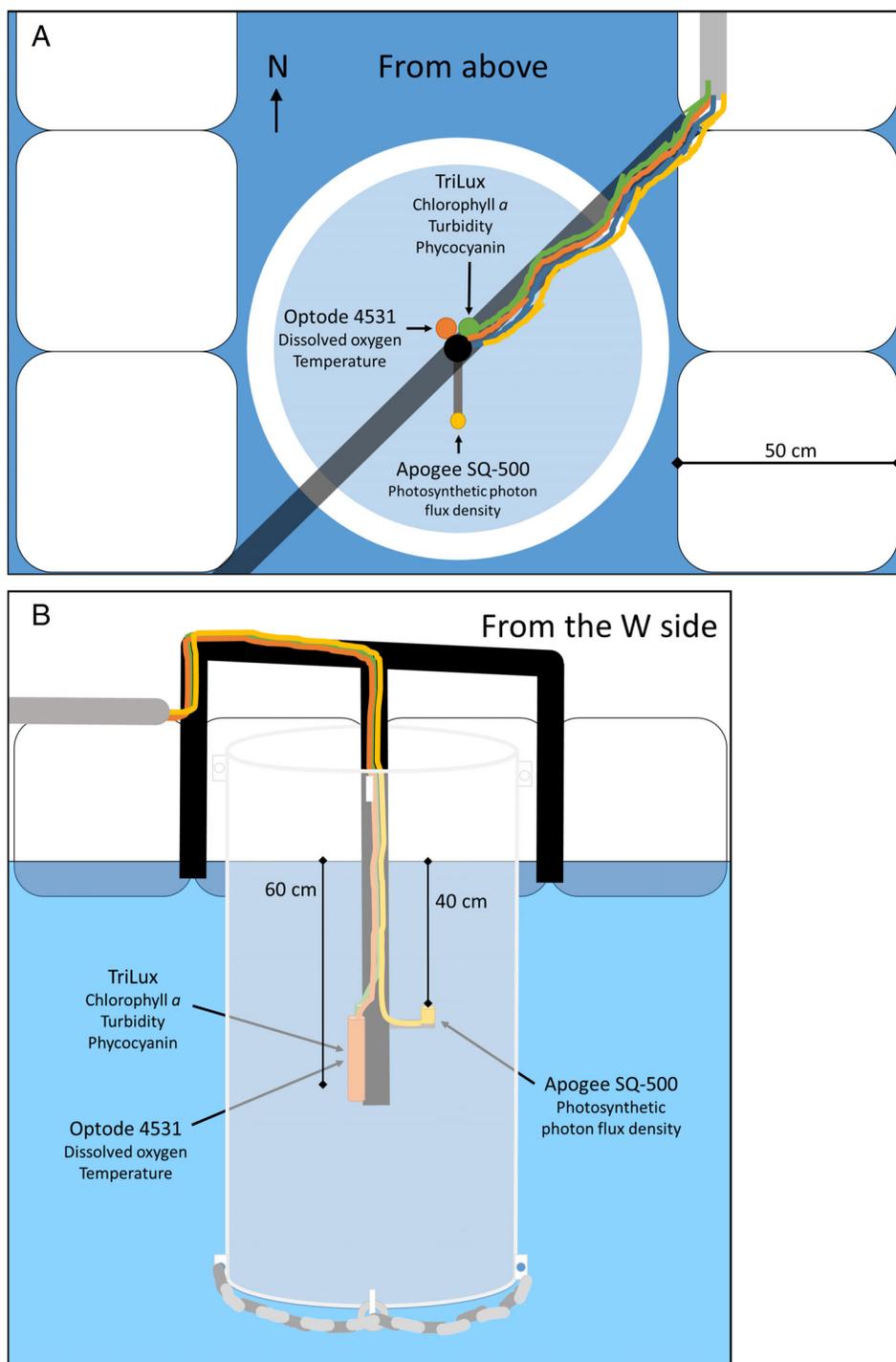
## Assessment

### Set up of experimental test

We conducted a modularized experiment in summer 2017 to test the infrastructure, and sensor system. Identical experiments at five sites (lakes) constituted experimental modules to which four treatments (see below) with four replicates were applied, resulting in 16 mesocosms within each experimental module. The mesocosms were cleaned and filled with lake water during the week preceding the start of the experimental period. The filling was performed with a gasoline-driven water pump (Meec tools 735-018, JULA AB, Sweden), and water intake was placed outside the platform at a depth of approximately 50 cm. All mesocosms were filled with approximately 550 L of water. After filling and stabilizing the mesocosms, the sensors were deployed.

Four treatments in each of the four rectangular openings in the jetfloat were used: (i) Control (C): no manipulation was applied. (ii) Fish (F): a pulse disturbance where two small crucian carp (*Carassius carassius*, Linnaeus) were introduced into the mesocosms at experimental day 0 (i.e., start day) and removed at day 7. (iii) Shading (S): a press disturbance where the available light was reduced by placing a dark mesh on top of the mesocosms. The mesh was permeable for rain water and gas exchange between the air inside the mesocosm and the open air. The mesh was mounted on the mesocosm at day 0 and remained in place until the last experimental day (day 28). (iv) Fish + Shading (FS): both pulse and press disturbances combined. The four treatments were replicated four times in a perfect Latin square design (Fig. S2). This was done to reduce the possibility of having biased errors between treatments, induced by local variation due to the internal placement of mesocosms within each pool. The study was approved by the Uppsala animal ethics committee in permission number 5.8.18-03672/2017.

We sampled the mesocosms six times, at days 0 (just before the experimental treatments were applied), 3, 7, 9, 14, and



**Fig 2.** Sensor installations within the mesocosms. **(A)** View of an experimental unit (mesocosm) from above. The cross black tube is used to sustain the three sensors inside of the mesocosm, with the fluorometer (TriLux) and optode (Aanderaa) facing north and the PPF sensor facing south. **(B)** View from the west side showing the depth at which the sensors are deployed. In addition, the black tubing (which is possible to separate from the jetfloat) allows for the sensors to be pulled out of the mesocosms for maintenance and cleaning procedures.

28 (i.e., the last experimental day). We took integrated water samples with a Ruttner sampler (Supporting Information 1) from each mesocosm and from the lake (from the

southernmost part of the jetfloat), and subsequently collected subsamples for the following analyses: concentrations of total nitrogen, phosphorus and carbon, phosphate, nitrate, nitrite,

ammonium, dissolved organic carbon and chlorophyll *a*; bacterial abundance, zooplankton biomass and composition, as well as 16S and 18S rRNA gene sequencing for bacterial and microeukaryote community composition (for details see Supporting Information).

Manual assessments with the handheld Apogee MQ-500 sensor and the AquaRead multiprobe were made during sampling days. The PPFD measurements were taken at a 5, 40, and 80 cm depth (except at 5, 20, and 40 cm for Svartberget), but only if weather conditions were stable. Conductivity, pH, dissolved oxygen saturation and concentration, and temperature were measured with the AquaRead multiprobe at a 40 cm depth on all sampling days.

Polyethylene ribbons of the same material as the enclosures were mounted from the surface to the bottom of each mesocosm throughout the experimental period, to monitor for periphytic algal growth.

### Sensor data management

During the experiment performed in 2017, PPFD, dissolved oxygen saturation, and water temperature readings were collected every minute, and chlorophyll *a* fluorescence, turbidity, and phycocyanin fluorescence readings were collected every 10 min, since the TriLux sensors need a delay of 30 s between activation and response. Due to communication issues, the TriLux sensors did not always reply and, hence, the total datasets feature readings that are less frequent than the anticipated 10 min. The TriLux datasets also contained some drift, mainly due to biofouling, which was not possible to compensate for due to the low frequency of collected data.

Continuous sensor data from the dataloggers were entered into the AQUARIUS Time-series Software (Aquarius Informatics Inc., Vancouver, BC, Canada), for quality check and cleaning procedures. The variables PPFD, dissolved oxygen saturation, water temperature, and chlorophyll *a* concentration, collected from all 16 mesocosms, were then processed by removing obvious outliers, applying offsets or drift correction (except for biofouling issues). Special focus was placed on erroneous sensor readings due to the up-lifting of the sensors from the water during the sensor-cleaning process. After cleaning, the datasets were gap-filled to get complete time series for the whole experiment period or, in some cases, for the period covered by the automatic data acquisition system (which started a bit later than day 0 at some field sites). For gap-filling, we used a linear filling in cases where the size of the gap was very limited. In a special case, the DO saturation and temperature data from the optodes showed an unnatural offset to the expected values during a period of up to 24 h. This occurred 1–2 times during the experiment at individual times for each sensor. In most cases, the data during these periods could be adjusted by applying an offset and linear gap fills at the start and end of the periods. In some cases, however, the data of the specific sensor were instead replaced with data from a sensor in another mesocosm of the same treatment, to achieve gap-free

data with the same diurnal pattern as expected by the parameter. The data to be copied was assured to fit both sensors well during the periods before and after the gap and, if necessary, an offset or drift component was applied to the copied data so that any difference between the two sensors data would remain intact during the gap period. Hence, this procedure should neither cause lower variance in the data within treatments, nor cause higher variance, which could happen if gap filling procedures that are not based on natural data were applied, for example, linear fill over extended periods on data with diurnal patterns.

Several stations made individual sensor calibration measurements of dissolved oxygen saturation and PPFD, which was then applied to the quality-checked and gap-filled data. For PPFD data, an immersion coefficient of 1.32 was also applied, as the PPFD sensors were calibrated in the air instead of in the water, as recommended by Apogee (<https://www.apogeeinstruments.com/underwater-par-measurements/>).

Dissolved oxygen concentration ( $DO_{conc}$ ) was calculated from measurements of oxygen saturation by the Aquarius software, using the formulas below (Garcia and Gordon 1992).

$$DO_{conc} = DO_{sat}/100 \times DO_0$$

where

$$DO_0 = 1.42905 \times \exp(2.00907 + 3.22014T_s + 4.05010T_s^2 + 4.94457T_s^3 - 0.256847T_s^4 + 3.88767T_s^5)$$

where

$$T_s = \ln([298.15 - t]/[273.15 + t])$$

where  $DO_{sat}$  is the dissolved oxygen saturation in % and  $t$  is the water temperature in degrees Celsius.

### Statistical analyses

The main purpose of the experimental assessment of the infrastructure was to evaluate the suitability of the five facilities to run nearly simultaneous mesocosm experiments which included high frequency sensor measurements, and followed the same protocols thus demonstrating the possibility to investigate ecological responses to environmental disturbances across multiple sites, as well as to capture ecological phenomena across multiple temporal scales. For this purpose, we focus on presenting data from the high frequency sensors (PPFD, DO concentration, temperature, and chlorophyll *a* concentration) at hourly and daily scales, showing changes in both the abiotic and biotic environment at different time scales. Since the purpose here is to introduce the infrastructure and its potential to monitor biological and physical processes using high-frequency data, we refrain from presenting further analyses of data collected from mesocosm samplings of the water column and manual sensor assessments, but present those

results in a separate paper (Urrutia-Cordero et al. 2021). All data is publicly available for download and use at <https://meta.fieldsites.se/collections/AYswWim7bx-O3t1CUUIgQDNR>

Here, we demonstrate two levels of analyses, involving (i) general abiotic and biotic responses to the experimental treatments across the five lakes, and (ii) site-specific responses. First, we ran Linear Mixed Models (LMMs) for each response variable (PPFD, DO concentration, temperature, and chlorophyll *a* concentration), and then included in the model the experimental treatments (fish presence and shading), time, and their interactions as explanatory variables and lake and mesocosm ID (nested within lake) as random explanatory variables. For site-specific analyses, we ran a LMM for each response variable and experiment with fish presence, shading, time, and their interactions as fixed explanatory variables, and mesocosm ID as random explanatory variable. For each model we evaluated whether the residuals of the models were normally distributed with q-q plots. If the distribution of the residuals deviated from normality, the response variable was log- or sqrt-transformed. For these statistical analyses, we centered on analyzing daily average data. However, we also discuss below the suitability of time series analysis using hourly data for capturing other potential ecological patterns at finer temporal scales with data from two example lakes. All statistical analyses were run with the software R-4.0.0, whereas the plots were created with the OriginPro 2017 (OriginLab Co, Northampton, MA) software.

## Experimental test: Results and conclusions

### *Photosynthetically active radiation dynamics*

Photosynthetically Active Radiation, expressed as Photosynthetic Photon Flux Density (PPFD), significantly decreased over time across all sites (Table 2 significant time effects). These temporal changes in PPFD were particularly strong in the southernmost, oligotrophic site (Asa, Figs. 3 and S3). We found very strong shading effects across all lakes (Table 2 significant shading effects), with a 40–60% daily reduction in PPFD for shaded mesocosms relative to non-shaded mesocosms most of the days in all lakes (Figs. 3, S3, and Table S1). This corroborates the effectiveness of the experimental manipulations in reducing the availability of light in the mesocosms, and the reproducibility of the shading protocol when applied to the different sites in the study.

The high frequency sensor data captured the very strong diurnal variation in PPFD that occurs during the day and between days, as an effect of the variation in cloud cover and solar angle (Fig. 4).

### *Temperature dynamics*

We also found very strong temporal changes in temperature across all sites (Table 2 significant time effects). We found temperature in the mesocosms to decrease gradually over time in all lakes (Figs. 3, S4, and Table S1). In addition,

the temperature in shaded mesocosms was significantly higher than in non-shaded mesocosms (Table 2 significant shading effects), a treatment effect that was consistently found for all sites (Figs. 3, S4 and Table S1). These effects could be the result of a reduction in heat dissipation overnight due to the presence of the shading cloth over the mesocosms. However, the increase in temperature from the shading manipulation was minor and did not exceed more than 0.18°C on average across sites.

As an example, hourly sensor measurements at Asa and Erken sites captured very strong fluctuations in temperature between day and night, with the highest peaks during midday, and sometimes displaying differences between day and night of about 2°C (Fig. 4). Thus, similar to the PPFD measurements, these results demonstrate the potential of the sensors to capture fine scale changes in the abiotic environment at sub-daily scales.

### *Dissolved oxygen dynamics*

Dissolved oxygen (DO) concentrations were significantly affected by the time course of the experiment (Table 2, significant time effects). Fish presence did not significantly affect the DO concentrations across sites (Table 2, no significant fish effects), largely due to variable response patterns for the different sites. Specifically, we found statistically clear positive effects of fish presence on DO concentrations in Bolmen and Skogaryd, but negative effects in Svartberget (Figs. 3, S5, and Table S1). DO concentrations also increased relative to control conditions during the first days with fish present at the Erken site, but this pattern was reversed towards the end of the experiment (Figs. 3, S5, and Table S1, fish × time interaction). In addition, we found contrasting recovery responses after fish were removed. In Bolmen and Svartberget, the positive and negative effects of fish on the DO concentrations, respectively, diminished over time and tended to approach control conditions towards the end of the experiment (Figs. 3, S5, and Table S1, fish × time interaction). In contrast, we found that the positive effects of fish presence on DO concentrations in Skogaryd became slightly stronger over time, thus indicating an absence of recovery (Figs. 3, S5, and Table S1, fish × time interaction).

Shading had a significant overall negative effect on DO concentrations across sites (Table 2, significant shading effects). Specifically, we found statistically clear negative effects of shading on DO concentrations in Bolmen, Skogaryd, Erken, and Svartberget, although the relative change with respect to control conditions varied notably among sites (Figs. 3, S5, and Table S1, fish × time interaction). We also found variation in the temporal trends in DO concentrations among sites in response to shading, with shading effects either increasing and prevailing over time (e.g., in Svartberget), or tending to converge with control conditions towards the end of the experiment (e.g., in Bolmen). We only found significant or marginally significant interactive effects between fish presence, shading and time in Svartberget and Skogaryd (Figs. 3,

**Table 2.** Results from linear mixed models evaluating changes in photosynthetic photon flux density (PPFD), temperature, dissolved oxygen (DO) concentration and chlorophyll *a* concentration (Chla) to the experimental treatments over time across all experiments

Response variable	m-R <sup>2</sup>	c-R <sup>2</sup>	Explanatory variable	Df/Df.Res	F-statistic	p-value
PPFD	0.187	0.837	F	1/72	0.624	.432
			S	1/72	445.443	<.001
			T	1/2117	414.736	<.001
			S × T	1/72	0.707	.403
			F × T	1/2132	2.339	.126
			F × S	1/2132	2.603	.107
			F × S × T	1/2132	0.024	.876
Temperature	0.168	0.976	F	1/71	0.019	.891
			S	1/71	83.097	<.001
			T	1/2125	15,311.942	<.001
			F × S	1/71	0.037	.849
			F × T	1/2173	0.010	.921
			S × T	1/2173	0.006	.936
			F × S × T	1/2173	0.002	.969
DO	0.376	0.792	F	1/72	1.788	.185
			S	1/72	33.285	<.001
			T	1/2127	2836.716	<.001
			F × S	1/72	0.129	.721
			F × T	1/2127	1.371	.242
			S × T	1/2127	56.787	<.001
			F × S × T	1/2127	0.019	.892
Chlorophyll <i>a</i>	0.037	0.778	F	1/72	22.697	<.001
			S	1/72	1.069	.305
			T	1/2117	83.011	<.001
			F × S	1/72	0.672	.415
			F × T	1/2132	4.542	.033
			S × T	1/2132	0.020	.888
			F × S × T	1/2132	3.269	(.071)

Notes: For each response variable, the LMMs included fish predation (F), shading (S), time (T), and their interactions as fixed explanatory variables, and lake and mesocosm ID (nested within lake) as random explanatory variables. The degree of variation (df) explained by the fixed effects is indicated by the m-R<sup>2</sup>, whereas c-R<sup>2</sup> stands for the total variation explained including both fixed and random effects. Black-bolded *p*-values and black-bolded *p*-values in brackets denote significant effects of the explanatory variables at  $\alpha = 0.05$  and  $\alpha = 0.1$ , respectively.

S5, and Table S1, fish × shading × time interaction), indicating quantitatively different temporal patterns emerging from the interaction between the presence of fish and shading.

Altogether, these results demonstrate that ecological responses to both top-down and bottom-up experimental manipulations can be highly influenced by the local environment. In addition, our measurements of hourly dynamics in DO concentrations revealed very strong fluctuations over day and night (Fig. 4), which likely responded to changes in ecosystem metabolic processes (photosynthesis and respiration), as also shown in other mesocosm experiments and observational studies (Giling et al. 2016).

#### Chlorophyll *a* concentration dynamics

Overall, chlorophyll *a* concentration fluctuated considerably over time (Table 2, significant time effects), especially in

Bolmen, Erken, and Skogaryd (Figs. 3 and S6), where we found statistically clear increases in chlorophyll *a* concentration (Figs. 3, S6, and Table S1) and significant fish effects (Table 2). However, there was also substantial response variability among lakes. In most lakes, we found that chlorophyll *a* concentration in response to the presence of fish tended to return to control levels towards the end of the experiment, but in Erken the initial positive effects of fish presence reversed and became negative towards the end of the experiment (Figs. 3, S6, and Table S1, significant fish × time interaction).

Shading did not affect chlorophyll *a* concentration across sites (Table 2, no significant shading effects), but when the responses were analyzed within each site, we identified a significant reduction in chlorophyll *a* concentration relative to control conditions in Skogaryd (Figs. 3, S6, and Table S1, significant shading effects). Our analyses also revealed marginally

**Table 3.** Examples of disturbances and environmental change factors as well as other treatment factors that could be manipulated using the SITES AquaNet infrastructure

Factor	Manipulation	Effect	Example references for lakes
Fertilization	Nutrient additions	Change in resource availability either as pulse or continuous changes	Cottingham et al. 1997, Feuchtmayr et al. 2009
Light reduction/changes in light composition	Shading blends	Press or pulse change in resource availability	Urabe et al. 2002
Osmotic stress	Acid/salt addition	Press or pulse change in pH, press change in salinity	Hintz et al. 2017, Lind et al. 2018, Sinclair and Arnott 2017
Browning	Addition of humic matter extract	Press change resulting in increasing water color > reduced light conditions and potential subsidy effect on bacteria	Lebret et al. 2018, Nydahl et al. 2019, Rivera Vasconcelos et al. 2018
Micropollutants (e.g., personal care products, microplastics, nanoparticles)*	Addition of substance	Press change in pollutant concentration	Baho et al. 2019, Aljabachi et al. 2020, Ward et al. 2019
Food web manipulations*	Addition or removal of organisms at higher trophic levels	Press or pulse change in biomass at manipulated trophic level	Forrest and Arnott 2007, Loewen and Vinebrooke. 2016, Zöllner et al. 2003, Beisner and Peres-Neto 2009
Dispersal/connectivity*	Addition of species from other locations/habitats	Changes in diversity and composition	Loewen and Vinebrooke 2016, Sinclair and Arnott 2017, Declerck et al. 2013, Howeth and Leibold 2008
Diversity, species density *	Addition of species	Potential increase in species richness or biomass of specific species leading to changes in community composition, richness and functioning	Carey et al. 2017, Thompson and Shurin 2012, Barros-Setubal 2020, Lennon et al. 2003
Priority effects*	Arrival order of species	Different initial community composition	Viana et al. 2016
Sediment resuspension	Artificial mixing	Increasing nutrient concentration and turbidity in water	Blottiere et al. 2019

Notes: Manipulations of different factors can also be combined. See Supporting Information, Supplementary text 2 for a full list of references included in the table. Factors marked with an \* indicate manipulations for which ethical permit and/or permits from local environmental authorities might be needed.

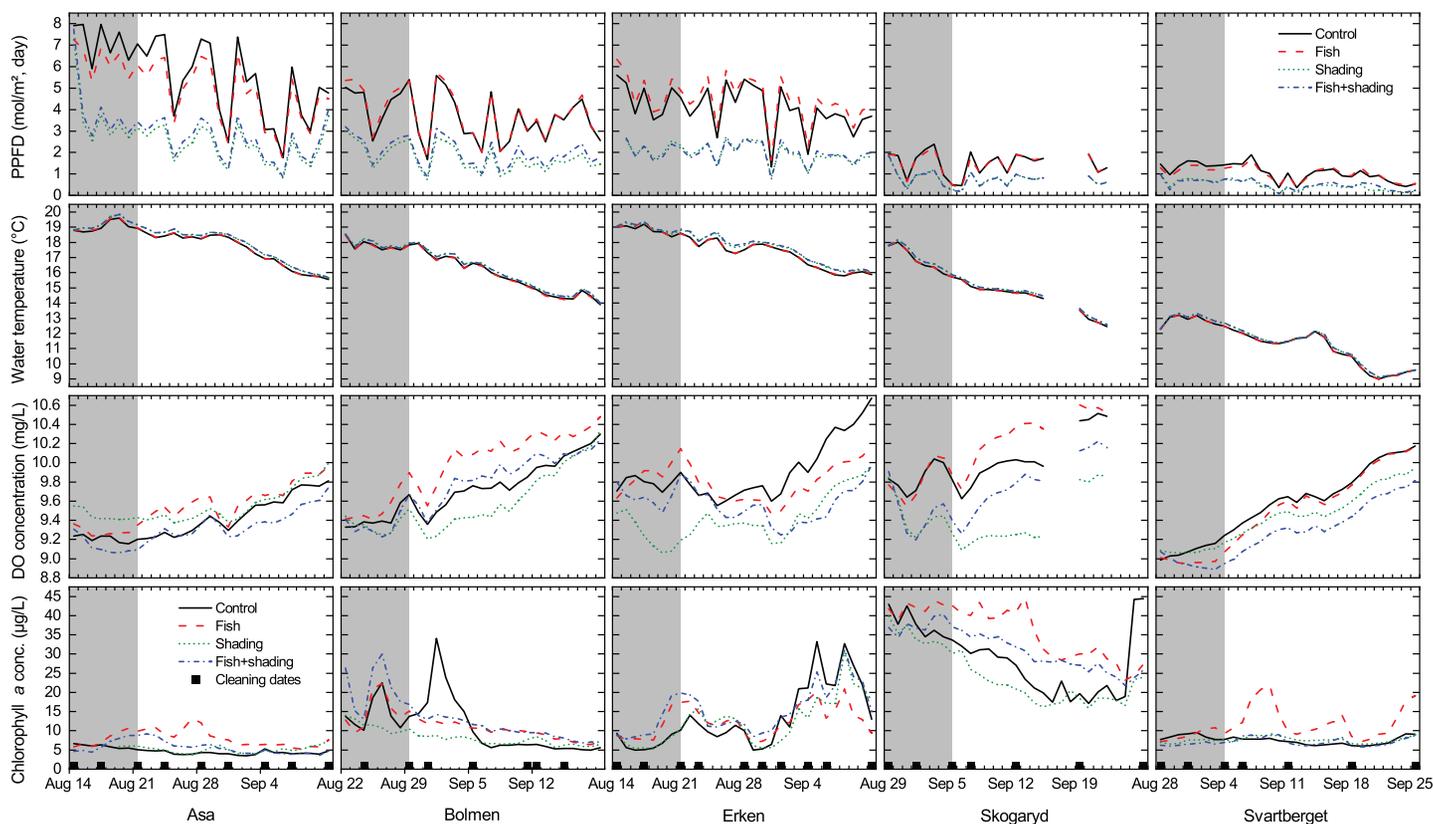
significant interactive effects between fish presence, shading and time across sites on chlorophyll *a* concentration (Table 2), which were the result of quantitatively different temporal patterns emerging from the interaction between fish and shading in all lakes except in Skogaryd (Figs. 3, S6, and Table S1, significant fish × shading × time interaction).

The overall positive response trends in chlorophyll *a* concentration to the presence of fish are in accordance with food web theory, whereby fish predation reduces top-down pressure by zooplankton on lower trophic levels, and subsequently increases total phytoplankton biomass (Carpenter et al. 1985; Carpenter and Kitchell 1993). In addition, the negative chlorophyll *a* concentration responses to shading at site Skogaryd are likely the consequence of reduced photosynthesis

and growth in waters that were already highly attenuating, thus leading to a suppression of the total phytoplankton biomass (Karlsson et al. 2009). Hence, these results do not only demonstrate the capacity of the sensor system to capture meaningful changes in the biotic environment of the mesocosms, but also that controlling the biofouling of the sensors on a weekly basis was likely sufficient to capture a strong statistical signature of the effects of the experimental treatments.

## Discussion

The SITES AquaNet mesocosm program was designed to allow testing of general hypothesis in experimental aquatic research. This is allowed by examination of how differences



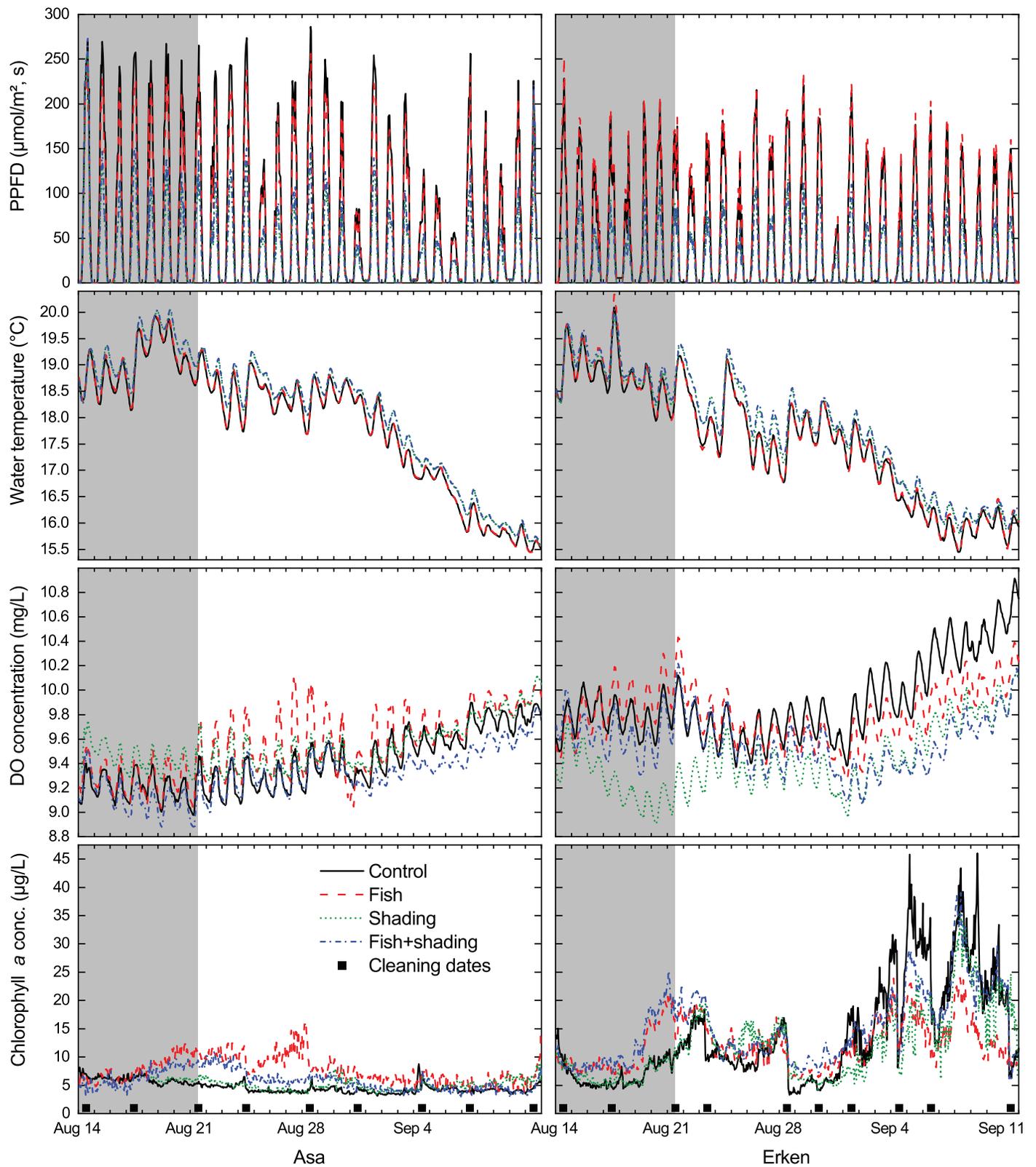
**Fig 3.** Aggregated (daily) data for the four treatments applied to the experiment, for the parameters total photosynthetic photon flux density (PPFD), average water temperature, average dissolved oxygen (DO) concentration and average chlorophyll *a* concentration during the experiment, for all five sites. Gray areas indicate the period when fish was present in the mesocosms. See also Figs. S3–S6 that include standard errors.

and similarities between important variables across sites (e.g., temperature, nutrients) mediate the outcomes of experimental manipulations, enabling the explanatory predictors for these phenomena to be revealed. Here we discuss the novelty and main advantages for using the infrastructure in future experiments.

The steeply rising number of ecological studies using mesocosm experiments over the last decades is associated with a significant amount of single-site facilities, many of which differ considerably in design and ecosystem properties. Many of these facilities are part of a virtual network and together they provide the possibilities for researchers to tackle a diverse number of scientific questions (Stewart et al. 2013, <http://mesocosm.org/>). There are, however, two major drawbacks that have emerged from the present development of single-site meocosm facilities. The first one is that many of the mesocosm facilities are established and operated by individual researchers using project funds that are often limited and have a short term. This expense subsequently constrains the possibility to spend funds on other relevant aspects of projects. The second is that researchers are forced to synthesize knowledge from uncoordinated, context-specific studies performed in very different experimental facilities (Benton et al. 2007). Over recent

years, there has been a shift towards experimental standardization and replicability, including equipment (e.g., mesocosm type), experimental set-up and sampling procedures across multiple sites, to achieve generality (e.g., Kemp et al. 2009; Borer et al. 2014; Duffy et al. 2015). However, bottom-up consortia of already established experimental facilities linked to individual research units has clearly dominated this development. There is a strong need for open access research facilities that allow researchers across the globe to use mesocosm facilities at low cost, as well as participate in multi-site networks addressing research questions of regional and global relevance. SITES AquaNet promotes both open access to researchers worldwide and the possibility to conduct modularized experiments integrating variability across space and time (Fig. 1).

Our experimental test illustrates the need for conducting modularized experiments that transcend single site studies. First, we found that most variability in our LMM models evaluating abiotic and biotic responses across sites was explained by the random structure of the model (Table 3). Second, our results also demonstrate that response differences of abiotic and biotic variables to either bottom-up or top-down disturbances largely varied across sites (Table S1). Because our experimental design included pulse disturbances with and without



**Fig 4.** Hourly averaged data, aggregated for the four treatments applied to the experiment, for the parameters photosynthetic photon flux density (PPFD) water temperature, dissolved oxygen (DO) concentration and chlorophyll *a* concentration during the experiment, for the sites Asa (left panels) and Erken (right panels), respectively. Gray areas indicate the period when fish was present in the mesocosms.

the concomitant action of press disturbances, we could also observe that response variability did not only pertain to how ecosystems respond to environmental change, but also how they recover from it. These are critical ecological aspects in current biodiversity-stability research (Donohue et al. 2016, Kéfi et al. 2019).

An important, additional asset of the SITES AquaNet infrastructure is the possibility to evaluate abiotic and biotic variables at fine temporal scales with the automatic sensor system (Fig. 1). Our modularized experimental test demonstrates the possibility to capture changes across multiple temporal scales, at temporal resolution of days, hours and even minutes. These data do not only improve mechanistic understanding of ecological phenomena, but also amplify the potential of the infrastructure to answer critical research questions. Short-term physiological and functional responses of plankton can be linked to changes in lake metabolism (Giling et al. 2017) or day-night variation in chlorophyll *a* concentration (Maxwell and Johnson 2000) calculated using the high-frequency data delivered by the automatic sensor system. Unfortunately, in the test experiment reported here, we found a draw-back in not having frequent enough chlorophyll *a* concentration data, to capture day-night variations, nor was it possible to compensate for biofouling. Consequently, the infrastructure has now been improved such that chlorophyll *a* fluorescence, turbidity and phycocyanin fluorescence readings can be performed at 1-min intervals. The higher frequency of readings makes it then possible to apply a correction for drift in data due to biofouling in the periods between sensor cleaning operations. In addition, high frequency data can better capture statistical signatures preceding transitions among alternative ecosystem states (i.e., early warning signals) compared to traditional monitoring (Pace et al. 2013). With regards to manual sampling, we took integrated water samples from the mesocosms six times in our modularized experiment for the determination of total and dissolved nutrients and carbon, chlorophyll *a* concentration, bacterial abundance, zooplankton biomass, zooplankton composition and bacterial and phytoplankton composition (16 and 18S rRNA gene sequencing, respectively) (see Supporting Information for details). However, other variables, such as greenhouse gas emissions (e.g., Grasset et al. 2020), emergence of insects (Scharnweber et al. 2020), stoichiometric shifts (Welti et al. 2017), specific functions (e.g., primary production, bacterial extracellular enzyme activities) (Urrutia-Cordero et al. 2017), dissolved organic matter composition and diversity (Limberger et al. 2019), and many other features could be investigated in future experiments in response to the specific interest of research groups using the infrastructure. In this way, it would be possible to link short term variations in specific processes as well as population dynamics to system level responses, which are particularly relevant for organisms with short generation times, including bacterioplankton and microbial eukaryotes. This also opens the possibility for coordinated experiments where multiple research groups with different objectives come together

to share experiments that are being conducted. This would not only be resource-effective, but also enable interesting new comparisons and unanticipated ecological linkages to be revealed.

The SITES AquaNet infrastructure is focused on providing a flexible, outdoor mesocosm facility, where researchers can work with natural lake communities to evaluate research questions related to disturbance ecology or other experimental manipulations (Fig. 1). In the test experiment, we investigated interactive effects of a top-down pulse disturbance (presence of fish) and bottom-up press disturbance (light reduction) in a factorial design (see Urrutia-Cordero et al. 2021 for further details on functional and compositional dynamics across trophic levels). Similar experiments can also be done with other disturbances or global environmental change factors (see Table 3 for some examples). In particular, more complex experimental designs that include incremental changes of press disturbances and/or manipulations of the number, frequency and chronology of pulse disturbances to identify tipping points and detect early warning signals in response to those manipulations are promising avenues for future experiments. Further, experiments with gradient designs that address changes in plankton composition and functioning in response to increasing intensities of environmental change could also be implemented. For example, three of the SITES AquaNet sites participated in the GLEON (Global Lake Ecological Observatory Network) Global Salt experiment, addressing effects of increasing salt concentrations on zooplankton using mesocosm experiments that included a total of 16 sites in North America and Europe. Other possibilities for future experiments include manipulations of dispersal of species from external sources, species arrival orders, connectivity among mesocosms and local (and regional) diversity to investigate how these factors influence community assembly and ecosystem functioning (Table 3). Manipulations may require ethical permits and approval from local environmental authorities to ensure no adverse effects on the lake biota (Table 3). Here, SITES AquaNET can help researchers to investigate the possibility with specific manipulations and to obtain permits.

The standard SITES AquaNet set-up uses closed hard-plastic polyethylene containers, however, in principle, plastic bags can easily be deployed in the floating platform as well. This could also include bags that are open to the sediments, allowing researchers to probe interactions between planktonic and benthic processes and communities (e.g., Bartels et al. 2012; Urrutia-Cordero et al. 2020).

In summary, we have now a fully functioning mesocosm infrastructure that offers the opportunity to perform coordinated experiments across space and time with the collection of temporally highly resolved data of several key variables. The infrastructure offers great opportunities for scientists to conduct future collaborative experiments across sites to depict general responses to experimental manipulations as well as to

gain a better understanding of the factors that regulate site-specific responses. Statistical modeling approaches, such as Structural Equation Modeling, are a good candidate to help disentangling the direct and indirect influence of key abiotic and biotic variables that differ across sites on ecological responses (see e.g., Grace et al. 2016). Conducting mesocosm experiments across a larger array of sites should also increase environmental heterogeneity, which may in turn strengthen our ability to disentangle effects of drivers that may co-vary across different sites. SITES-AquaNet demonstrates the potential to integrate a large network of mesocosm facilities across broad geographic areas to move beyond regional scale studies, and thereby the network offers an excellent platform to initiate and foster larger collaborative efforts across the globe. Both humanity and the natural world as we know it are currently threatened by an array of environmental stressors of global concern. This poses a pressing need to understand the impacts of environmental drivers on biodiversity, ecosystem functioning and services from local and regional to global scales. Several initiatives in which SITES AquaNet is part of (e.g., AQUACOSM-Plus, [www.mesocosm.eu](http://www.mesocosm.eu)) are currently paving the way for such endeavors.

## References

- Aben, R. C. H., B. Nathan, E. van Donk, and others. 2017. Cross continental increase in methane ebullition under climate change. *Nat. Commun.* **8**: 1682. doi:[10.1038/s41467-017-01535-y](https://doi.org/10.1038/s41467-017-01535-y)
- Adrian, R., C. M. O'Reilly, H. Zagarese, and others. 2009. Lakes as sentinels of climate change. *Limnol. Oceanogr.* **54**: 2283–2297. doi:[10.4319/lo.2009.54.6\\_part\\_2.2283](https://doi.org/10.4319/lo.2009.54.6_part_2.2283)
- Adler, P. B., E. W. Seabloom, E. T. Borer, and others. 2011. Productivity is a poor predictor of plant species richness. *Science* **333**: 1750–1753. doi:[10.1126/science.1204498](https://doi.org/10.1126/science.1204498)
- Bartels, P., J. Cucherousset, C. Gudasz, and others. 2012. Terrestrial subsidies to lake food webs: An experimental approach. *Oecologia* **168**: 807–818. doi:[10.1007/s00442-011-2141-7](https://doi.org/10.1007/s00442-011-2141-7)
- Beisner, B. E., and P. R. Peres-Neto. 2009. Seasonal trophic dynamics affect zooplankton community variability. *Freshw. Biol.* **54**: 2351–2363. doi:[10.1111/j.1365-2427.2009.02266.x](https://doi.org/10.1111/j.1365-2427.2009.02266.x)
- Benton, T. G., M. Solan, J. M. J. Travis, and S. M. Sait. 2007. Microcosm experiments can inform global ecological problems. *Trends Ecol. Evol.* **22**: 516–521. doi:[10.1016/j.tree.2007.08.003](https://doi.org/10.1016/j.tree.2007.08.003)
- Borer, E. T., W. S. Harpole, P. B. Adler, E. M. Lind, J. L. Orrock, E. W. Seabloom, and M. D. Smith. 2014. Finding generality in ecology: A model for globally distributed experiments. *Methods Ecol. Evol.* **5**: 65–73. doi:[10.1111/2041-210X.12125](https://doi.org/10.1111/2041-210X.12125)
- Carpenter, S. R., J. F. Kitchell, and J. R. Hodgson. 1985. Cascading trophic interactions and lake productivity. *Bioscience* **35**: 634–639. doi:[10.2307/1309989](https://doi.org/10.2307/1309989)
- Carpenter, S. R., and J. F. Kitchell. 1993. The trophic cascade in lakes. New York: Cambridge Univ. Press.
- De Boeck, H. J., S. Vicca, J. Roy, and others. 2015. Global change experiments: Challenges and opportunities. *Bioscience* **65**: 922–931. doi:[10.1093/biosci/biv099](https://doi.org/10.1093/biosci/biv099)
- Donohue, I., H. Hillebrand, J. M. Montoya, and others. 2016. Navigating the complexity of ecological stability. *Ecol. Lett.* **19**: 1172–1185. doi:[10.1111/ele.12648](https://doi.org/10.1111/ele.12648)
- Duffy, J. E., P. L. Reynolds, C. Boström, and others. 2015. Biodiversity mediates top-down control in eelgrass ecosystems: A global comparative-experimental approach. *Ecol. Lett.* **18**: 696–705. doi:[10.1111/ele.12448](https://doi.org/10.1111/ele.12448)
- Gall A., U. Uebel, U. Ebensen, and others. 2017. Planktotrons: A novel indoor mesocosm facility for aquatic biodiversity and food web research. *Limnol. Oceanogr.: Methods* **15**: 663–677.
- Garcia, H. E., and L. I. Gordon. 1992. Oxygen solubility in seawater—Better fitting equations. *Limnol. Oceanogr.* **37**: 1307–1312. doi:[10.4319/lo.1992.37.6.1307](https://doi.org/10.4319/lo.1992.37.6.1307)
- Giling, D. P., J. C. Nejtgaard, S. A. Berger, and others. 2016. Thermocline deepening boosts ecosystem metabolism: Evidence from a large-scale lake enclosure experiment simulating a summer storm. *Glob. Chang. Biol.* **23**: 1448–1462. doi:[10.1111/gcb.13512](https://doi.org/10.1111/gcb.13512)
- Giling, D. P., J. C. Nejtgaard, S. A. Berger, and others. 2017. Thermocline deepening boosts ecosystem metabolism: Evidence from a large-scale lake enclosure experiment simulating a summer storm. *Global Change Biology* **23**: 1448–1462.
- Grace, J. B., T. M. Anderson, E. W. Seabloom, E. T. Borer, P. B. Adler, W. S. Harpole, et al. 2016. Integrative modelling reveals mechanisms linking productivity and plant species richness. *Nature* **529**: 390–393. doi:[10.1038/nature16524](https://doi.org/10.1038/nature16524)
- Grasset, C., S. Sobek, K. Scharnweber, and others. 2020. The CO<sub>2</sub>-equivalent balance of freshwater ecosystems is nonlinearly related to productivity. *Glob. Chang. Biol.* **26**: 5705–5715. doi:[10.1111/gcb.15284](https://doi.org/10.1111/gcb.15284)
- Gurevitch, J., L. L. Morrow, W. Alison, and J. S. Walsh. 1992. A meta-analysis of competition in field experiments. *Am. Nat.* **140**: 539–572.
- Hansson, L.-A., M. K. Ekvall, M. K. Ekvall, J. Ahlgren, W. S. Holm, L. Dessborn, and C. Brönmark. 2014. Experimental evidence for a mismatch between insect emergence and waterfowl hatching under increased spring temperatures. *Ecosphere* **5**: 1–9. doi:[10.1890/ES14-00133.1](https://doi.org/10.1890/ES14-00133.1)
- Heino, J., J. Alahuhta, L. M. Bini, Y. Cai, A.-S. Heiskanen, S. Hellsten, et al. 2020. Lakes in the era of global change: Moving beyond single-lake thinking in maintaining biodiversity and ecosystem services. *Biol. Rev.* **96**: 89–106. doi:[10.1111/brv.12647](https://doi.org/10.1111/brv.12647)
- Hillebrand, H. 2008. Meta-analysis in ecology. John Wiley & Sons.
- Hillebrand, H., and B. J. Cardinale. 2010. A critique for meta-analyses and the productivity-diversity relationship. *Ecology* **91**: 2545–2549. doi:[10.1890/09-0070.1](https://doi.org/10.1890/09-0070.1)

- Hillebrand, H., and J. Gurevitch. 2014. Meta-analysis results are unlikely to be biased by differences in variance and replication between ecological lab and field studies. *Oikos* **123**: 794–799. doi:[10.1111/oik.01288](https://doi.org/10.1111/oik.01288)
- Jeppesen, E., B. Kronvang, M. Meerhoff, and others. 2009. Climate change effects on runoff, catchment phosphorus loading and lake ecological state, and potential adaptations. *J. Environ. Qual.* **38**: 1930–1941. doi:[10.2134/jeq2008.0113](https://doi.org/10.2134/jeq2008.0113)
- Jeppesen, E., M. Meerhoff, T. A. Davidson, and others. 2014. Climate change impacts on lakes: an integrated ecological perspective based on a multi-faceted approach, with special focus on shallow lakes. *Journal of Limnology* **73**: 84–104.
- Karlsson, J., P. Bystrom, J. Ask, P. Ask, L. Persson, and M. Jansson. 2009. Light limitation of nutrient-poor lake ecosystems. *Nature* **460**: 506–509. doi:[10.1038/nature08179](https://doi.org/10.1038/nature08179)
- Kéfi, S., V. Domínguez-García, I. Donohue, C. Fontaine, E. Thébault, and V. Dakos. 2019. Advancing our understanding of ecological stability. *Ecol. Lett.* **22**: 1349–1356. doi:[10.1111/ele.13340](https://doi.org/10.1111/ele.13340)
- Kemp, W. M., J. E. Petersen, E. D. Houde, C.-C. Chen, J. C. Cornwell, and E. T. Porter. 2009. Spatial and temporal scaling. In J. E. Peterson, V. S. Kennedy, W. C. Dennison, and W. M. Kemp [eds.], *Enclosed experimental ecosystems and scale. Tools for understanding and managing coastal ecosystems*. New York, NY: Springer-Verlag.
- Kosten, S., G. Lacerot, E. Jeppesen, D. M. Marques, E. H. van Nes, N. Mazzeo, and M. Scheffer. 2009. Effects of submerged vegetation on water clarity across climates. *Ecosystems* **12**: 1117–1129. doi:[10.1007/s10021-009-9277-x](https://doi.org/10.1007/s10021-009-9277-x)
- Kosten, S., V. L. M. Huszar, E. Bécares, and others. 2012. Warmer climate boosts cyanobacterial dominance in lakes. *Glob. Chang. Biol.* **18**: 118–126. doi:[10.1111/j.1365-2486.2011.02488.x](https://doi.org/10.1111/j.1365-2486.2011.02488.x)
- Landkildehus, F., M. Søndergaard, M. Beklioglu, and others. 2014. Climate change effects on shallow lakes: Design and preliminary results of a cross-European climate gradient mesocosm experiment. *Estonian J. Ecol.* **63**: 71–89. doi:[10.3176/eco.2014.2.02](https://doi.org/10.3176/eco.2014.2.02)
- Li, Z., L. He, H. Zhang, P. Urrutia-Cordero, M. K. Ekvall, J. Hollander, and L.-A. Hansson. 2016. Climate warming and heat waves affect reproductive strategies and interactions between submerged macrophytes. *Glob. Chang. Biol.* **23**: 108–116. doi:[10.1111/gcb.13405](https://doi.org/10.1111/gcb.13405)
- Limberger, R., J. Birtel, H. Peter, N. Catalan, D. D. Farias, R. J. Best, J. Brodersen, H. Burgmann, and B. Matthews. 2019. Predator-induced changes in dissolved organic carbon dynamics. *Oikos* **128**: 430–440. doi:[10.1111/oik.05673](https://doi.org/10.1111/oik.05673)
- Marce, R., G. George, P. Buscarinu, M. Deidda, D. E. Dunalska, and E., and Jennings, E. 2016. Automatic high frequency monitoring for improved lake and reservoir management. *Environ. Sci. Technol.* **50**: 10780–10794. doi:[10.1021/acs.est.6b01604](https://doi.org/10.1021/acs.est.6b01604)
- Maxwell, K., and G. N. Johnson. 2000. Chlorophyll fluorescence—A practical guide. *J. Exp. Bot.* **51**: 659–668. doi:[10.1093/jexbot/51.345.659](https://doi.org/10.1093/jexbot/51.345.659)
- Millennium Ecosystem Assessment. 2005. *Ecosystems & human well-being: Wetlands and water synthesis*. Washington, DC: World Resources Institute.
- Pace, M. L., R. N. Batt, C. D. Buelo, S. R. Carpenter, J. J. Cole, J. T. Kurtzweil, and G. M. Wilkinson. 2016. Reversal of a cyanobacterial bloom in response to early warnings. *P. Natl. Acad. Sci. USA* **115**: 352–357. doi:[10.1073/pnas.1612424114](https://doi.org/10.1073/pnas.1612424114)
- Scharnweber, K., F. Chaguaceda, E. Dalman, L. Tranvik, and P. Eklöv. 2020. The emergence of fatty acids—Aquatic insects as vectors along a productivity gradient. *Freshw. Biol.* **65**: 565–578. doi:[10.1111/fwb.13454](https://doi.org/10.1111/fwb.13454)
- Shannon, C. E. 1948. A mathematical theory of communication. *Bell Syst. Tech.* **27**: 379–423. doi:[10.1002/j.1538-7305.1948.tb01338](https://doi.org/10.1002/j.1538-7305.1948.tb01338)
- Smol, J. P., A. P. Wolfe, H. J. B. Birks, and others. 2005. Climate-driven regime shifts in the biological communities of arctic lakes. *P. Natl. Acad. Sci. USA* **102**: 4397–4402. doi:[10.1073/pnas.0500245102](https://doi.org/10.1073/pnas.0500245102)
- Mahdy, A., S. Hilt, N. Filiz, and others. 2015. Effects of water temperature on summer periphyton biomass in shallow lakes: A pan-European mesocosm experiment. *Aquat. Sci.* **77**: 499–510. doi:[10.1007/s00027-015-0394-7](https://doi.org/10.1007/s00027-015-0394-7)
- Sorvari, S., A. Korhola, and R. Thompson. 2002. Lake diatom response to recent Arctic warming in Finnish Lapland. *Glob. Chang. Biol.* **8**: 171–181. doi:[10.1046/j.1365-2486.2002.00463.x](https://doi.org/10.1046/j.1365-2486.2002.00463.x)
- Staehr, P. A., D. Bade, M. C. Van de Bogert, and others. 2010. Lake metabolism and the diel oxygen technique: State of the science. *Limnol. Oceanogr-Meth.* **8**: 628–644. doi:[10.4319/lom.2010.8.0628](https://doi.org/10.4319/lom.2010.8.0628)
- Stephen, D., D. M. Balayla, E. Becares, and others. 2004. Continental-scale patterns of nutrient and fish effects on shallow lakes: Introduction to a pan-European mesocosm experiment. *Freshw. Biol.* **49**: 1517–1524. doi:[10.1111/j.1365-2427.2004.01302.x](https://doi.org/10.1111/j.1365-2427.2004.01302.x)
- Stewart, R. I. A., M. Dossena, D. A. Bohan, and others. 2013. Mesocosm experiments as a tool for ecological climate change research. *Adv. Ecol. Res.* **48**: 71–181. doi:[10.1016/B978-0-12-417199-2.00002-1](https://doi.org/10.1016/B978-0-12-417199-2.00002-1)
- Urrutia-Cordero, P., M. K. Ekvall, and L.-A. Hansson. 2015. Responses of cyanobacteria to herbivorous zooplankton across predator regimes: Who mows the bloom? *Freshw. Biol.* **60**: 960–972. doi:[10.1111/fwb.12555](https://doi.org/10.1111/fwb.12555)
- Urrutia-Cordero, P., M. K. Ekvall, and L.-A. Hansson. 2016a. Local food web management increases resilience and buffers against global change effects on freshwaters. *Sci. Rep.* **6**: 29542. doi:[10.1038/srep29542](https://doi.org/10.1038/srep29542)
- Urrutia-Cordero, P., M. K. Ekvall, and L.-A. Hansson. 2016b. Controlling harmful cyanobacteria: Taxa-specific responses of cyanobacteria to grazing by large-bodied *Daphnia* in a biomanipulation scenario. *PLOS ONE*. **11**: e0153032. doi:[10.1371/journal.pone.0153032](https://doi.org/10.1371/journal.pone.0153032)
- Urrutia-Cordero, P., M. K. Ekvall, J. Ratcovich, M. Soares, S. Wilken, H. Zhang, and L.-A. Hansson. 2017. Phytoplankton

- diversity loss along a gradient of future warming and brownification in freshwater mesocosms. *Freshw. Biol.* **62**: 1869–1878. doi:[10.1111/fwb.13027](https://doi.org/10.1111/fwb.13027)
- Urrutia-Cordero, P., H. Zhang, F. Chaguaceda, H. Geng, and L.-A. Hansson. 2020. Climate warming and heat waves alter harmful cyanobacterial blooms along the benthic pelagic interface. *Ecology* **101**: e03025. doi:[10.1002/ecy.3025](https://doi.org/10.1002/ecy.3025)
- Urrutia-Cordero, P., S. Langenheder, M. Striebel, et al. 2021. Functionally reversible impacts of disturbances on lake food webs linked to spatial and seasonal dependencies. *Ecology*, **102**: e03283. doi:[10.1002/ecy.3283](https://doi.org/10.1002/ecy.3283)
- Velthuis, M., L. N. De Senerpont Domis, T. Frenken, and others. 2017. Warming advances top-down control and reduces producer biomass in a freshwater plankton community. *Ecosphere* **8**: e01651. doi:[10.1002/ecs2.1651](https://doi.org/10.1002/ecs2.1651)
- Verbeek, L., A. Gall, H. Hillebrand, and M. Striebel. 2018. Warming and oligotrophication cause shifts in freshwater phytoplankton communities. *Glob. Chang. Biol.* **24**: 4532–4543. doi:[10.1111/gcb.14337](https://doi.org/10.1111/gcb.14337)
- Werner, C. M., K. L. Stuble, A. M. Groves, and T. P. Young. 2020. Year effects: Interannual variation as a driver of community assembly dynamics. *Ecology* **101**: e03104. doi:[10.1002/ecy.3104](https://doi.org/10.1002/ecy.3104)
- Welti, N., M. Striebel, A. J. Ulseth, and others. 2017. Bridging food webs, ecosystem metabolism, and biogeochemistry using ecological stoichiometry theory. *Front. Microbiol.* **8**: 1–14. doi:[10.3389/fmicb.2017.01298](https://doi.org/10.3389/fmicb.2017.01298)
- Wilken, S., M. Soares, P. Urrutia-Cordero, J. Ratcovich, M. K. Ekvall, E. Van Donk, and L.-A. Hansson. 2017. Primary producers or consumers? Increasing phytoplankton bacterivory along a gradient of lake warming and browning. *Limnol. Oceanogr.* **63**: S142–S155. doi:[10.1002/lno.10728](https://doi.org/10.1002/lno.10728)
- Wilkinson, G. M., S. R. Carpenter, J. J. Cole, M. L. Pace, R. D. Batt, C. D. Buelo, and J. T. Kurtzweil. 2018. Early warning signals precede cyanobacterial blooms in multiple whole-lake experiments. *Ecol. Appl.* **88**: 188–203. doi:[10.1002/ecm.1286](https://doi.org/10.1002/ecm.1286)

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### Conflict of interest

The authors declare that there are no conflicts of interest.

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