Large enclosure experiments (LakeLab in Lake Stechlin) to study browning, nutrient loading and deep mixing effects on plankton communities in summer 2015: Physical, chemical and biological parameters

Version 2023-10-22

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Data origin
Data were collected by IGB

Enclosure experiments: Design and Set-up
Two consecutive enclosure experiments were carried out between June and September 2015 at the LakeLab, a large enclosure facility deployed in the deep stratified clear-water Lake Stechlin, North-Eastern Germany (Fig. 1). A total of 21 enclosures were used, each ca. 20 m deep and 9 m in diameter, enclosing a water volume of ca. 1300 m^3 (Giling et al., 2017). A gradient design was chosen to maximize the number of predictor levels, instead of replication at each level (Kreyling et al., 2018; Bergström & Karlsson, 2019; Gerhard et al., 2023). This design is well suited to capture non-linear responses of phytoplankton and cyanobacteria to nutrient enrichment (Ptacnik et al., 2008; Carvalho et al., 2013).

Experiment I was designed to test for effects of a single heavy rain event by simulating one major initial pulse of nutrients and browning (Fig. 2). Seven nutrient levels were fully crossed with three browning levels. The intended concentrations of total phosphorus (TP) covered a broad gradient from oligo-meso- to eutrophic conditions including the critical threshold for cyanobacteria response to nutrient enrichment (Carvalho et al., 2013). An arithmetic progression ($a_n = 18 + n^2$) was applied to select the intended TP concentrations, starting with the lake epilimnion TP (18 µg L^{-1}). Phosphorus (P) and nitrogen (N) were added as orthophosphoric acid (H_3PO_4) and ammonium nitrate (NH_4NO_3). Nitrogen was added to ensure a ratio of bioavailable N to P as in the lake water, which was close to the Redfield ratio (7:1 by mass). Browning was achieved by adding HuminFeed (HF; HuminTech GmbH,

Grevenbroich, Germany), a highly soluble natural commercial product that has the advantage of strongly staining water without adding significant amounts of bioavailable carbon or nutrients (Scharnweber et al., 2021). Three browning levels corresponded to browning levels in natural lakes: A) low = clear or oligohumic (<5 mg Pt L^{-1}, no addition of HF); B) medium = mesohumic (67 mg Pt L^{-1}, addition of 5 mg HF L^{-1}); C) high = polyhumic (133 mg Pt L^{-1}, addition of 10 mg HF L^{-1}). Experiment I lasted 7 weeks from early June to late July 2015.

Experiment II followed directly after the end of Experiment I without changing the water in the enclosures and lasted for 7 weeks from late July to early September 2015. This experiment was designed to test for effects of repeated nutrient enrichment and browning by weekly adding nutrients and humic substances, as well as physically disrupting the thermocline by deep mixing, thus simulating a series of storm events. Nutrients and HF were added for seven weeks following the scheme shown in Table 1b. For each enclosure, the amounts of added nutrients and HF were the same each week after initially adjusting the concentrations to the intended level. The added amounts differed among the enclosures to ensure a relatively constant nutrient enrichment level for each of them. Nine of the 21 enclosures used in the experiment (three for each browning level) were weekly mixed to 14 m depth referred to as deep-mixed (dm), while avoiding sediment disturbance. Mixing of the 12 remaining enclosures, which served as controls for the deep-mixing treatments, was limited to 8 m depth referred to as shallow-mixed (sm), reflecting the epilinmion depth of Lake Stechlin between 7 and 9 m. The amounts of nutrients added were calculated to obtain the same phosphorus concentrations for both mixing treatments (15 = no addition, 20, 30 and 50 µg P L^{-1}) and the three browning levels. Target concentrations for the browning were <5 (no HF addition), 30 and 60 mg Pt L^{-1} (addition of 2.25 and 4.5 mg HF L^{-1}) for the medium and high browning levels, respectively.

***In situ* measurements, sampling and analyses**

All enclosures were equipped with automatic profilers holding multiparameter probes for continuous measurements of water temperature, pH, oxygen, turbidity and chlorophyll fluorescence (YSI Inc., Yellow-Springs, USA) and sensors to measure PAR (LI-193 Spherical Underwater Quantum Sensor, LI-COR Inc., Lincoln, NE, USA). PAR, blue, green and red light were also measured weekly with a spectroradiometer from the surface down to the euphotic depth during Experiment I. Secchi depth was recorded weekly with a 30-cm diameter white disc. Epilimnion depth in the enclosures was derived from temperature profiles in the enclosures which were the same as in the surrounding lake (Berger et al., 2006). Epilimnion depth ranged from 6.5 to 7 m. In Experiment II, the epilimnion depth in the lake increased to 7 to 9 m, and was experimentally increased weekly to 14 m in the deep-mixed enclosures.

A hose sampler was used to take weekly integrated water samples from the epilimnion. The sampling depth was adjusted weekly in line with the epilimnion depth measured on each sampling day. The water samples were filled in 20-L carboys and transported to the laboratory within 30 min after collection, where subsamples were immediately taken for analyses of water colour (absorbance at 436 nm converted to mg Pt L^{-1}), concentrations of dissolved organic carbon (DOC), total phosphorus (TP), soluble reactive phosphorus (SRP), total nitrogen (TN), ammonium (NH_4^+), nitrate (NO_3^-) and nitrite (NO_2^-) and chlorophyll *a*. Subsamples of 250 mL were preserved with acid Lugol's solution for determination of community composition and total phytoplankton biovolume. Cyanotoxins were analysed with ELISA kits on epilimnetic samples that were stored frozen (-20°C) until analysis. Mesozooplankton was sampled weekly by taking vertical hauls from 1.5 m above the sediment to the surface using a 90-µm mesh plankton net and analysed. The zooplankton:phytoplankton carbon ratio was used to indicate the level of zooplankton grazing (Jeppesen et al. 2011).

LakeLab enclosure facility

Figure 1. LakeLab enclosure facility deployed in the deep stratified clear-water Lake Stechlin, North-Eastern Germany (Photo: Peter Casper, IGB).

Figure 2. LakeLab enclosures showing brown and clear treatments during the experiments (Photo: Jens C. Nejstgaard, IGB).

Parameters

Experiment (EXP_I=Experiment I; EXP_II=Experiment II)
Date (YYYY-MM-DD), Year, Month, Day
Doy (Day of year; 2015-01-01=Doy0; 2015-08-04=Doy216)
Day_exp (Day of experiment; Day 0=2015-06-02)
HF_level (A=control, no Humin Feed; B=low Humin Feed; C=high Humin Feed)
TP_level (Total Phosphorus level 1–7=ambient-high)
Treatment_label (A=control, B=low HF, C=high HF; TP level 1–7=ambient-high)
Mixing (no=no mixing; sm=shallow mixing, dm=deep mixing)
Enclosure (Enclosure number, E01-E24)
Layer (whole water column, epilimnion)
Layer_depth_m (Water layer depth in m)
Colour_mg_Pt_L (Colour in Platin units mg Pt L^{-1})
TP_µg_L (Total Phosphorus in mg L^{-1})
SRP_µg_L (Soluble Reactive Phosphorus in µg L^{-1})
TN_µg_L (Total Nitrogen in mg L^{-1})
DIN_µg_L (Dissolved Inorganic Nitrogen in µg L^{-1})
DOC_mg_L (Dissolved Organic Carbon in mg L^{-1})
Alk_mmol_L (Alkalinity in mmol L^{-1})
Secchi_m (Secchi depth in m)
Chla_µg_L (Chlorophyll-*a* in µg L^{-1})
Kd_PAR_m (Attenuation coefficient (k_{dPAR}) of Photosynthetic Active Radiation (PAR 400-700 nm) m^{-1})
Kd_Blue_m (Attenuation coefficient (k_{dBlue}) of the Blue light spectrum (430-500 nm) m^{-1})
Kd_Green_m (Attenuation coefficient (k_{dGreen}) of the Green light spectrum (520-565 nm) m^{-1})
Kd_Red_m (Attenuation coefficient (k_{dRed}) of the Red light spectrum (625-700 nm) m^{-1})
Zeu_Zmix (Ratio of euphotic depth z_{eu} to mixing depth z_{mix})
Phyto_total_mm3_L (Total Phytoplankton biovolume in mm^3 L^{-1})
Cyanobacteria_mm3_L (Cyanobacteria biovolume in mm^3 L^{-1})
Cryptphyceae_mm3_L (Cryptphyceae biovolume in mm^3 L^{-1})
Chlorophyta_mm3_L (Chlorophyta biovolume in mm^3 L^{-1})
Chrysophyceae_mm3_L (Chrysophyceae biovolume in mm^3 L^{-1})
Dinophyceae_mm3_L (Dinophyceae biovolume in mm^3 L^{-1})
Diatom_mm3_L (Diatom biovolume in mm^3 L^{-1})
Conjugatophyceae_mm3_L (Conjugatophyceae biovolume in mm^3 L^{-1})
Haptophyta_mm3_L (Haptophyta biovolume in mm^3 L^{-1})
Euglenophyceae_mm3_L (Euglenophyceae biovolume in mm^3 L^{-1})
Z_P_ratio (Zooplankton to Phytoplankton carbon ratio)
Microcystin_µg_L (Microcystin in µg L^{-1})
Planktothrix_rub_mm3_L (Planktothrix rubescense in mm^3 L^{-1})
Daphnids_mgC_m3 (Daphnids biomass in mg C m^{-3})
Other_Zooplankton_mgC_m3 (Other Zooplankton biomass in mg C m^{-3})

Chemical analyses

Nutrients and DOC were analysed with standardised methods (Table 1). The colour was measured on filtered water (GF-75, 0.3-µm average pore size; Advantech, Tokyo, Japan) in a portable spectrophotometer (NANOCOLOR® 300D, Macherey-Nagel GmbH & Co. KG, Düren, Germany) at a wavelength of 436 nm (1-cm cuvette) and was expressed in platinum units by using the conversion factor of Cuthbert & Del Giorgio (1992).

Table 1. List of analytical methods and instruments used for dissolved and total nutrient determination, dissolved organic carbon and colour.

Parameter	**Method**	**Instrument**
Soluble reactive phosphorus (PO_4-P)	ISO 15681-1 (2003)	FIAStar 5000 (Foss, Sweden)
Total phosphorus (TP)	ISO 15681-1 (2003)	FIAStar 5000 (Foss, Sweden)
Ammonium (NH_4-N)	ISO 11732 (2005)	FIAStar 5000 (Foss, Sweden)
Nitrate+nitrite (NO_3-N+NO_2-N)	ISO 13395 (1996)	FIAStar 5000 (Foss, Sweden)
Total nitrogen (TN)	ISO 11905-1 (1997) ISO 13395 (1996)	FIAStar 5000 (Foss, Sweden)
Dissolved organic carbon (DOC)	EN 1484 (1997)	TOC-V CPH (Shimadzu, Japan)
Colour*	Absorbance at 436 nm; Cuthbert and Del Giorgio (1992)	Spectrophotometer (NANOCOLOR® 300D, Macherey-Nagel GmbH & Co. KG, Düren, Germany)

Phytoplankton analysis

Chlorophyll *a*: The chlorophyll *a* concentration of the epilimnion samples were performed by standard spectrophotometric analysis based on the ISO 10260 (1992). After filtration of 0.5 – 1 L onto GF-75 filters (Avantec, Japan), chlorophyll *a* was directly extracted with 10 ml hot 90% ethanol and exposed to sonication for 1-2 minutes before centrifugation. The clear extract was measured at absorbance at 665, 649 and 470 nm within 24 hours in a Hitachi-2900 (Japan) equipped with a 5 cm cuvette.

Phytoplankton taxonomic composition and biovolume: The phytoplankton taxonomic composition and biovolume were analysed using 250 mL of the integrated epilimnion samples fixed with acid Lugol's solution (EN 15204, 2006). Sub-samples of 10 mL were settled in Utermöhl chambers and examined with inverted microscopes (Nikon Diaphot or Leica DMI3000 B) to identify and count phytoplankton taxa. Large species were counted in half or the whole counting chamber using a 100x magnification, and smaller species were counted in two or more transects with higher magnification (200x and 400x). For each taxon a proper geometrical-shape was used to calculate biovolumes (EN 16995, 2015). The taxon-specific abundances multiplied by taxon specific cell volumes were used to provide the total phytoplankton biovolume for each algal class and the total biovolume for the whole phytoplankton community.

Cyanotoxins: Cyanotoxins were measured with ELISA tool-kits (Abraxis LLC, Warminster). All 316 samples were tested for microcystin, anatoxin, saxitoxin and cylindrospermopsin following the manufacturer's instructions. Before analysis, 40 mL of each frozen sample were thawed and frozen again three times to break the cells and release the toxins in the cells. Thus, the ELISA results do not distinguish between dissolved and cell-bound toxins.

Mesozooplankton analysis

Mesozooplankton was sampled weekly in the middle of each enclosure by vertical hauls from 1.5 m above the sediment to the surface using an Apstein Limnological standard plankton net (Model 438040, Hydrobios, Kiel) with 90 µm mesh size, 17 cm diameter opening in an Apstein cone with maximum width of 40 cm and the mesh section of the net 1 m long). Samples were preserved in 4%

formalin containing 50% sugar for later analyses of community composition and biomass. Mesozooplankton was counted and measured in Utermöhl counting chambers at 60x magnification using an inverted microscope (Leica Portugal 090-131.001). Length measurements were used to calculate the biomass for each zooplankton taxa in carbon units based on length-dry mass relationships established for Lake Stechlin (Kasprzak, 1983) and other populations (Bottrell et al., 1976), and a carbon to dry mass conversion factor of 0.5 (Winberg et al., 1971).

References

Berger, S. A., Diehl, S., Kunz, T. J., Albrecht, D., Oucible, A. M., & Ritzer, S. (2006). Light supply, plankton biomass, and seston stoichiometry in a gradient of lake mixing depths. *Limnology and Oceanography, 51*(4), 1898–1905. https://doi.org/10.4319/lo.2006.51.4.1898

Bergström, A.-K., & Karlsson, J. (2019). Light and nutrient control phytoplankton biomass responses to global change in northern lakes. *Global Change Biology, 25*(6), 2021-2029. https://doi.org/10.1111/gcb.14623

Bottrell et al. 1976. Bottrell, H.H., Duncan, A., Gliwicz, Z.M., Grygierek, E., Herzig, A., Hillbricht-Ilkowska, A., Kurasawa, H., Larsson, P., Weglenska, T., 1976. A review of some problems in zooplankton production studies. Contribution from the Plankton Ecology Group (IBP). Norw. J. Zool. 24: 419-456.

Carvalho, L., McDonald, C., de Hoyos, C., Mischke, U., Phillips, G., Borics, G., . . . Cardoso, A. C. (2013). Sustaining recreational quality of European lakes: minimizing the health risks from algal blooms through phosphorus control. *Journal of Applied Ecology, 50*(2), 315-323. https://doi.org/10.1111/1365-2664.12059

Cuthbert, I. D., and P. Del Giorgio. 1992. Toward a standard method of measuring colour in freshwater. Limnology and Oceanography 37: 1319-1326. https://doi.org/10.4319/lo.1992.37.6.1319

EN 15204, 2006. Guidance on quantification of phytoplankton in inverted microscope.

EN 16995, 2015. Guidance on estimation of biolvolume for microalgae.

Gerhard, M., Koussoroplis, A.-M., Raatz, M., Pansch, C., Fey, S. B., Vajedsamiei, J., . . . Striebel, M. (2023). Environmental variability in aquatic ecosystems: Avenues for future multifactorial experiments. *Limnology and Oceanography Letters, 8*(2), 247-266. https://doi.org/10.1002/lol2.10286

Giling, D. P., Nejstgaard, J. C., Berger, S. A., Grossart, H.-P., Kirillin, G., Penske, A., . . . Gessner, M. O. (2020). Corrigendum. *Global Change Biology, 26*(12), 7284-7287. https://doi.org/10.1111/gcb.13512

ISO 10260-1992: Water quality – Measurement of biochemical parameters – Spectrometric determination of the chlorophyll-a concentration, Int. Org. Standard., Geneva, 1st edn. 1992–07–15.

ISO 7887-2011: Water quality – Examination and determination of colour.

Kasprzak 1983. "Bestimmung des Körperkohlenstoffs von Planktoncrustaceen." Limnologica 15 (1984): 191-194.

Kreyling, J., Schweiger, A. H., Bahn, M., Ineson, P., Migliavacca, M., Morel-Journel, T., Riis Christiansen, J., Schtickzelle, N., Larsen, K. S. (2018). To replicate, or not to replicate – that is the question: how to tackle nonlinear responses in ecological experiments. *Ecology Letters, 21*(11), 1629-1638. https://doi.org/10.1111/ele.13134

Jeppesen, E., Nõges, P., Davidson, T.A. *et al.* Zooplankton as indicators in lakes: a scientific-based plea for including zooplankton in the ecological quality assessment of lakes according to the European Water Framework Directive (WFD). *Hydrobiologia* **676**, 279–297 (2011). https://doi.org/10.1007/s10750-011-0831-0

Ptacnik, R., Lepisto, L., Willen, E., Brettum, P., Andersen, T., Rekolainen, S., . . . Carvalho, L. (2008). Quantitative responses of lake phytoplankton to eutrophication in Northern Europe. *Aquatic Ecology, 42*(2), 227-236. https://doi.org/10.1007/s10452-008-9181-z

Scharnweber, K., Peura, S., Attermeyer, K., Bertilsson, S., Bolender, L., Buck, M., . . . Székely, A. (2021). Comprehensive analysis of chemical and biological problems associated with browning agents used in aquatic studies. Limnology and Oceanography Methods, 19(12), 818-835. https://doi.org/10.1002/lom3.10463

Winberg, G. (1971). *Symbols, units and conversion factors in studies of fresh water productivity*: International Biological Programme Control Office.