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Climate change effects on shallow lakes: design and preliminary results of a cross-European climate gradient mesocosm experiment

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Abstract. Climate change is expected to profoundly affect both temperature and net precipitation, with implications for lake water level. We describe the design of a harmonized, simultaneous, cross-European mesocosm experiment to elucidate the effects of climate change on community structure, functioning, and metabolism in shallow lakes at low and high nutrient levels with contrasting depths (1 and 2 m). We used cylindrical (D = 1.2 m) tanks that were either 1.2 or 2.2 m high, each having a 10-cm sediment layer. We inoculated the mesocosms with a mixed sample of sediment and plankton from lakes with contrasting nutrient concentrations and added macrophytes and planktivorous fish. Sediment was pre-equilibrated to the required experimental nutrient concentration. During the experiment the water level decreased with increasing temperature (up to 90 cm in the Mediterranean mesocosms) while conductivity increased. The average chlorophyll *a* concentration increased with temperature in the deep mesocosms but was more variable in the shallow mesocosms. Macrophyte

abundance increased with temperature, while the oxygen data suggest that net primary production peaked at intermediate temperatures. We conclude that our experimental design has the potential for tracking the interacting effects of global warming and eutrophication in shallow lakes.

Key words: climate change, REFRESH project, macrophytes, nutrient enrichment, metabolism, water level effects, macroecology.

INTRODUCTION

Climate change is expected to significantly change temperature regimes and precipitation patterns across the world (IPCC, 2007; Bates et al., 2008), with implications for the nutrient status of lakes. In the northern temperate zone, loadings of phosphorus and nitrogen are expected to increase due to elevated precipitation and soil decomposition levels, leading to higher nutrient loss from land to lakes (Schindler and Vallentyne, 2008; Adrian et al., 2009; Jeppesen et al., 2009, 2011). Moreover, internal nutrient loading tends to increase in eutrophic lakes due to higher temperature and increased mineralization and as an indirect warming effect of prolonged or temporary thermal stratification (Jensen and Andersen, 1992; Søndergaard et al., 2003; Mooij et al., 2005; Wilhelm and Adrian, 2008). These changes favour the outbreak of algal blooms, often of toxic cyanobacteria (Huisman et al., 2004; Wagner and Adrian, 2009). However, there are also examples of shallow lakes showing lower nutrient concentrations in warm years due to higher water levels. For example, in Estonian lakes higher winter North Atlantic Oscillation (NAO) years, which are characterized by warmer and wetter winters, are associated with higher water temperatures but also with higher lake water levels (Nõges, 2004). In shallow Lake Võrtsjärv, high water level years are characterized by a lower P concentration due to weaker resuspension and leakage from bottom sediments, while the N concentration is higher as a result of lower denitrification rates in the deeper water column, leading to a higher N : P ratio and less favourable conditions for N-fixing cyanobacteria (Nõges et al., 2003).

In Europe global climate change is predicted to result in an approximately 25–30% decrease in precipitation and enhanced evaporation in the Mediterranean region. Pronounced interannual variation can be expected due to increasing frequencies and magnitudes of extreme drought events (Giorgi, 2006; Giorgi and Lionello, 2008). Droughts may reduce runoff, cause lower external nutrient loading, and potentially increase water clarity. However, such effects may be context dependent. For instance, in eutrophic lakes, higher evaporation, higher internal nutrient loading, and, possibly, reduced nitrification under low-oxygen conditions can lead to higher nutrient concentrations and lower water clarity (Jeppesen et al., 2009, 2011; Özen et al., 2010; Papastergiadou et al., 2010).

Climate warming may also affect trophic structure and dynamics. In warm lakes, top-down control by fish is strong due to the dominance of small and abundant planktivorous and benthivorous fish (Jeppesen et al., 2010a, 2012), enhancing zooplankton predator control and reducing grazing on phytoplankton (Gyllström et al., 2005; Meerhoff et al., 2007; Stefanidis and Papastergiadou, 2010).

Such changes may have an adverse influence on submerged macrophytes due to the deteriorating light conditions. Conversely, a lower water level can improve the light climate for macrophyte growth (Blindow, 1992; Nõges and Nõges, 1999; Coops et al., 2003; Beklioğlu et al., 2006), depending on lake morphology (Beklioğlu et al., 2006). A mesocosm study in a Turkish shallow lake showed that macrophytes resist increased nutrient loading, perhaps as a result of evaporation-triggered water level reduction, overriding the deleterious effect of periphyton- and phytoplankton-induced turbidity (Özkan et al., 2010). A similar outcome was observed in a recent experiment where water level and the presence or absence of fish were manipulated in a warm eutrophic shallow lake (Central Anatolia, Ankara) (Bucak et al., 2012). Results from both studies suggest that increased evaporation during summer in this region may help maintain the growth of submerged macrophytes in eutrophic shallow lakes despite the reduction in water clarity caused by fish predation. Bucak et al. (2012) concluded that the adverse effects of climate-driven eutrophication on water clarity may be counteracted by reduced water levels, provided that physical disturbance is not severe in the shallow margins of the lake. Climate change induced alterations in water levels may therefore be critical, both in shallow lakes in the Mediterranean and the continental part of the north temperate region where summer may become drier in the future. Consequently, water level effects need to be considered more explicitly in the analyses of climate change impacts on shallow lakes.

Space-for-time substitution is one of the most widely used approaches in ecological and climate change research. It accounts for key differences between ecosystems resulting from their location along broad latitudinal and altitudinal gradients and therefore allows assessment of how factors influencing ecosystem structure and function vary along these gradients (Meerhoff et al., 2012; Jeppesen et al., 2014). This approach has been used to analyse a series of lake surveys in Europe (Moss et al., 2003; Declerck et al., 2005; Gyllström et al., 2005; De Meester et al., 2006; Brucet et al., 2012), South America (Kosten et al., 2009, 2011), and in cross-continental studies (Jeppesen et al., 2007; Meerhoff et al., 2007; Kosten et al., 2012). The strength of this approach is that the biological assemblages *per se* have had time to evolve and adapt to the climate in which they live. However, it does have weaknesses in that biogeographical issues may be of importance and that correlative studies do not necessarily provide causal relationships (Jeppesen et al., 2014). Furthermore, the influence of co-variates of temperature and latitudinal variation on other climate-related variables such as seasonality and the length of the growing season may go undetected, and confounding factors that cannot be controlled may swamp the climate signal. Thus, alternative approaches are also needed to elucidate climate change effects more mechanistically, despite the challenging nature of the macroecological scales of interest.

A useful alternative is to combine correlative space-for-time substitution approaches with controlled experiments. A cross-European mesocosm experiment conducted in 1998 and 1999 (under the auspices of the SWALE project) in six lakes provides an example of such an approach. In this experiment, lakes were distributed from Finland to southern Spain, and the effects of fish addition and

nutrient loading on shallow lakes were studied using a common protocol (Moss et al., 2004; Stephen et al., 2004). In these experiments, and contrary to the findings from latitude gradient studies, no differences in the degree of top–down control along the latitudinal gradient were observed, perhaps because of fixed fish densities. However, the variability of the experimental outcome from one year to the next increased with latitude, reflecting the greater variation in weather at the onset of the experiments (Moss et al., 2004). The SWALE study was restricted to a single depth and differed in the starting level of nutrient loading. Moreover, lake metabolism was not included.

This paper describes a refined design of a cross-European mesocosm experiment that accounts for the importance of water level fluctuation in driving ecological processes in lakes following global change. It examines the overarching effects of climate change on trophic structure and dynamics, as well as lake metabolism, at two nutrient levels and water depths (1 and 2 m) along a climatic gradient in six countries from Sweden to Turkey. The aim is to describe the highly standardized experimental design and sampling procedures, a key objective in terms of optimizing the comparison of results and reducing the risk of bias. We present the design of the experiment and some physico-chemical and biological data before discussing the strengths and weaknesses of the approach. This paper will be followed by a number of more detailed papers on the various physico-chemical variables studied.

METHODS

Experimental lakes and study period

For the experiment we selected a shallow, alkaline, clear freshwater lake in each of the six participating countries (Table 1). The lakes had: (i) a mean depth ≤4 m,

Table 1. Summary information on the study sites. Precipitation and air temperature cover the period 1 May to 1 November 2011

Experimental site	Coordinates	Climate	Altitude, m a.s.l.	Total precipitation, mm	Air temperature, °C
Sweden – Erken	59°49′59″N 18°33′55″E	Boreal	11	271	14.6
Estonia – Võrtsjärv	58°12′17″N 26°06′16″E	Boreal	35	298	14.9
Germany – Müggelsee	52°26′0″N 13°39′0″E	Transient maritime/continental	32	431	16.9
Czech Republic – Vodňany	49°09′14″N 14°10′11″E	Transient maritime/continental	395	401	15.3
Turkey – ODTÜ-DSİ Gölet	39°52′38″N 32°46′32″E	Transient/continental Mediterranean	998	223	18.7
Greece – Lysimachia	38°33′40″N 21°22′10″E	Mediterranean	16	252	23.4

allowing us to cover the natural patterns of temperature seasonality that characterize shallow lakes, (ii) low nutrient concentrations as we wanted to use natural lake water (where possible) and to run the experiment at both low and high nutrient concentrations, (iii) total alkalinity between 1 and 4 meq L^{-1}, (iv) low salinity (<1‰) and colour (<20 mg Pt L^{-1}). These conditions reflect natural abiotic characteristics of lakes across Europe, increasing the general relevance of our study. The experiments ran for six months in 2011 from spring (May) to the end of autumn (October/November), thus avoiding the ice-covered period at the northern sites. In all countries the experiments started on 9 May (day 1), enabling a synchronized sampling protocol to be employed.

Experimental set-up

The experimental set-up in each country encompassed two nutrient levels both with two different water levels, each represented by four replicates (16 mesocosms in total). A pontoon bridge with eight mesocosms (Figs 1 and 2) arranged in two rows divided by a boardwalk for ease of access was established in each lake. The treatment position was organized randomly but followed the same protocol in all countries (Fig. 1). The pontoon bridges were constructed from wooden or plastic boards and floating devices consisting of 32 plastic barrels, each 120 L. At one site the bridge was anchored at the bottom (the Czech Republic). At one end of the pontoon bridge a small working platform was established. For the free-floating systems the pontoon was anchored only at the platform end, which, irrespective of wind direction, functioned as a wave breaker and landing point for boats. To prevent birds from landing on or foraging in the mesocosms, fruit nets (thin net with, for example, 5 cm × 5 cm or 10 cm × 10 cm mesh sizes) were fixed above the mesocosms with a minimum height of 50 cm.

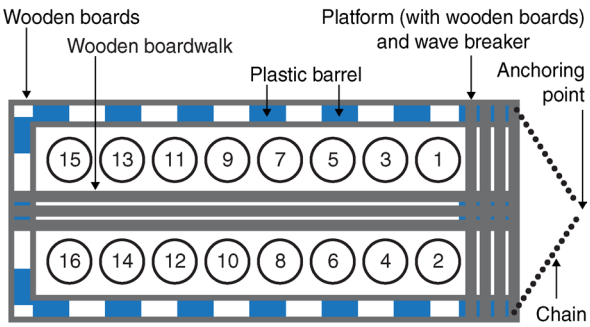

Fig. 1. Schematic representation of the floating pontoon bridge established at sites with prevailing wind exposure. A platform, functioning as a landing platform and wave breaker, is placed on the right side, which also serves as the anchoring end of the bridge. Randomly selected enclosure numbers are ascribed.

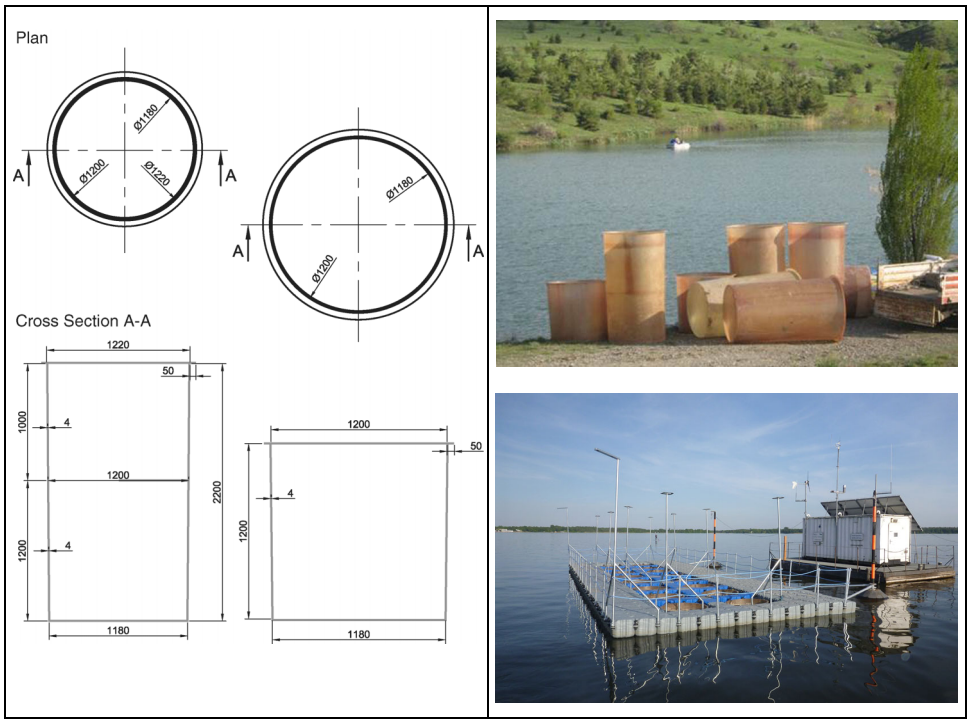

Fig. 2. Dimensions and photographs of the enclosures produced by Armaplast Composites & Plastics in Turkey and of the experimental set-up in Germany at Lake Müggelsee (photo: Thomas Hintze).

All mesocosms were constructed by the same manufacturer (Fig. 2) and consisted of cylindrical (D = 1.2 m) fibreglass (4 mm) tanks of two heights, 1.2 and 2.2 m. The robustness of the experimental set-up minimized the risk of loosing sites or treatments and offered a potential for reuse and prevented the diffusion of O_2 and CO_2, thereby avoiding artefacts in the calculation of mass balances on oxygen and carbon. The upper edges of all mesocosms were attached to the pontoon bridges 20 cm above the water surface to avoid incursion of water during windy periods. The water level in the 1.2 m deep mesocosms was 1 m and in the 2.2 m deep mesocosms, 2 m.

Once established, a 10 cm deep sediment layer was added to each mesocosm. The sediment contained 90% (by volume) washed sand (grain size <1 mm) and 10% sediment from a mesotrophic lake situated close to the experimental site in each country. Large particles (e.g. plant fragments, mussels, stones, debris, etc.) were removed by sieving through a 10 mm mesh. Before the sediment was added, it was equilibrated to the two experimental total phosphorus (TP) treatment levels (25 and 200 µg TP L^{-1}). This avoids transient states in sediment equilibration (resulting from different nutrient loading patterns in the original lakes) during the experiment observed in earlier experiments (E. Jeppesen and M. Søndergaard, personal observations). The pre-equilibration (done country-wise) commenced

during late autumn and winter of the previous year and ran until the start of the experiment. Two tanks were filled with a 10 cm sediment layer (ca 0.2 m^3 sediment in each tank), and 20–50 cm water with low (25 µg L^{-1}) or high (200 µg L^{-1}) TP concentrations, respectively. This sediment : water ratio ensured proper exchange of nutrients between the water and the sediment. Following water addition, the sediment and water were mixed with a rake. The TP concentration was measured periodically (at least biweekly) and the water was replaced with fresh nutrient-rich or nutrient-poor water whenever the TP differed from the initial concentrations of 25 and 200 µg TP L^{-1}, respectively. This procedure was repeated until the TP concentrations in the water were at the desired levels (±25%).

Following the addition of sediment, the mesocosms were filled with 500 µm filtered nutrient-poor water from neighbouring lakes with P concentrations <25 µg TP L^{-1}. If this was not possible, water from another oligotrophic lake was transported to the enclosures or, less desirable, tap water was used (e.g. in Germany and the Czech Republic). The mesocosms were left undisturbed for four days to allow the suspended matters to settle.

To simulate a shallow, fully mixed lake, the water in the mesocosms was continuously circulated during the experiment using water pumps. Standard 2 to 5 W aquarium pumps were adequate to pump ca 300 L h^{-1}. To establish comparable mixing between deep and shallow mesocosms, pumps running at half power were used for the shallow (1 m) mesocosms. The pumps were placed with their inlets (via a PVC tube) in the middle of the mesocosm, ca 10 cm above the sediment, and their outlets 10 cm below the surface. Power for the pumps was obtained either by (i) a power cord from the shore (Czech Republic, Estonia, Sweden), (ii) a solar panel (placed horizontally to ensure similar irradiance irrespective of the direction of the floating pontoon bridge) in Greece (240 W, in combination with 12 V 250 Ah batteries) or solar panels from a nearby lake station (Germany), or (iii) a power supply with six parallel connected 120 Ah batteries changed three times a week (Turkey).

Inoculations

To standardize initial conditions and enable the potential for developing a diverse flora and fauna, the mesocosms were inoculated with a mixed sample of sediment and plankton from five local lakes in each country covering a nutrient gradient of 25–200 µg TP L^{-1}. The inoculum was added on day 4 after setting up the experiment and when the initial disturbance effect of adding water and sediment had subsided. For the inocula of plankton, five vertical net hauls (using a plankton net with a diameter of 20 cm and 50 µm mesh), covering the entire water column (bottom to the surface) without disturbing the sediment, were taken in each of the five selected lakes and pooled (per lake). The five samples were kept separately in 5 L barrels, which were filled with lake water from the sample lake. Plankton samples were kept cool prior to addition and were not stored longer than 24 h. The contents of the five 5 L barrels from the five lakes were carefully mixed and a 1 L subsample was added to each mesocosm.

Five litres of sediment was also collected from each of the five lakes to add biota and/or resting stages of biota. The sediment was collected at a depth approximating the mean depth of the lake. To avoid introducing fish and large mussels (e.g. *Anadonta* spp. and *Dreissena* spp.), the sediment was sieved through a 10 mm mesh. Care was also taken to remove any fish eggs. The inoculum sediment from the five lakes was mixed thoroughly, and 1 L was added on day 4 to each enclosure, dispersed evenly on top of the TP equilibrated sediment–sand layer.

Submerged macrophytes and fish were added to the mesocosms three days after the sediment and plankton additions (day 7). Eight 5–10 cm long apical shoots of water-milfoil (*Myriophyllum spicatum*) collected from the experimental lake (or another lake in the region) were planted (evenly distributed) into each mesocosm. Water-milfoil was selected as it was present in lakes in all countries. In Germany the plants were collected in autumn of the previous year to ensure availability at the start of the experiment. These were kept in aquaria in the laboratory until use. Before introducing plants to the mesocosms, the plants were placed in soda mineral water (carbonated) for 15 min to remove snails and invertebrates. To ensure that shoots would sink into and remain in the sediment until root development (approx. 2 weeks), ca 5 g pebble stones were attached to the shoots by duct tape.

The original intention was to stock a fish species present in all countries and three-spined stickleback (*Gasterosteus aculeatus* L.), size 2–4 cm, was selected. However, it was not possible to catch sticklebacks in Greece, Turkey, and Sweden prior to the experiment, either due to low population numbers or ethical issues (legislation prohibiting the transfer of populations between sites). Thus, sticklebacks were imported from Germany and acclimatized for the Turkish experiment. Underyearling roach (*Rutilus rutilus* L.) and western mosquito fish (*Gambusia affinis* Baird and Girard) obtained from the study lakes were used in Sweden and Greece, respectively. Between 4 and 20 g of fish biomass (equating to six sticklebacks or mosquito fish or two roach) was added to each enclosure irrespective of nutrient level. We sought to attain a male : female ratio of 1 : 1, allowing breeding during the experiment. Before addition, the fish were measured (mm). Fish were caught at least 1–2 weeks before the experiment to ensure that they would survive handling. Length–weight relationships were established from the remaining pool (20 fish used). Any dead fish were replaced from a stock of reserve fish when necessary. Fish density, length, and weight were determined at the end of the experiment.

Nutrient loading

The experiment included two levels of nutrient concentrations representing mesotrophic and eutrophic conditions with four replicates at each depth. To adjust and maintain the concentrations, P and N were added to all mesocosms (Table 2) using Na_2HPO_4 and $Ca(NO_3)_2$ as the P and N source, respectively. The ratio of P and N added was 1 : 20 (by molecular weight). The initial nutrient addition was conducted on day 4 after the addition of the sediment and plankton inoculum. At this stage

Table 2. Nutrient dosage to the mesocosms four days after establishment (initial) and thereafter monthly. The ratio between total phosphorus (TP) and total nitrogen (TN) addition is 1 : 20 (by molecular weight)

Mesocosm type	Initial P, mg mesocosm^{-1}	Monthly P, mg mesocosm^{-1}	Initial N, mg N mesocosm^{-1}	Monthly N, mg N mesocosm^{-1}
Shallow (1 m), low NP	0	5.1	0	102
Shallow (1 m), high NP	179*	81.6	1575***	1632
Deep (2 m), low NP	0	10.8	0	216
Deep (2 m), high NP	376**	172	3225****	3440

* 1020 L × 175 µg P L^{-1}; ** 2150 L × 175 µg P L^{-1}; *** 1020 L × 1.5 mg N L^{-1}; **** 2150 L × 1.5 mg N L^{-1}.

only high nutrient mesocosms were dosed with the objective of reaching the high nutrient concentrations of 200 µg P L^{-1} and 2 mg N L^{-1}. Thereafter, all mesocosms received monthly dosing of N and P (Table 2). The dosing levels and ratios followed those used in previous experiments (Gonzales Sagrario et al., 2005; Jeppesen et al., 2007). In addition, the tanks received input via precipitation; in the Czech Republic this was, on average, 3.4 mg TP and 76 mg total nitrogen (TN) per mesocosm per month, which are low compared to the added TP and TN in the high nutrient mesocosms (Table 2), but as much as 68% and 75% of the added TP and TN, respectively, in the low-dosed shallow mesocosms (Table 2). The addition of nutrients took place after sampling and ecosystem metabolism measurements had been concluded.

Sampling procedures

Sampling was initiated on day 7 following the addition of fish and macrophytes. Thereafter, samples were taken at monthly intervals in all countries. The entire water column, from the surface to approximately 5 cm above the sediment, was sampled randomly with a tube sampler (diameter 7 cm) at 10, 30, and 60 cm intervals from the mesocosm wall and pooled. For phytoplankton, chlorophyll *a*, and water chemistry analyses, the sample was taken outside the macrophyte stands. For zooplankton, samples were collected irrespective of whether plants were present or not at the sampling point as zooplankton tend to hide in the daytime among plants (Timms and Moss, 1984; Burks et al., 2002). If water depth in the enclosure was low, three to six extra samples were taken (10, 30, and 60 cm from the enclosure wall, at different points than the first sample) to obtain sufficient water. For chemical analyses a 500 mL sample was taken and kept dark and cool (5 °C) until reaching the laboratory where a 100 mL subsample was filtered. The filtered and unfiltered samples were stored frozen until analysis.

For phytoplankton, 50 mL glass bottles were filled with unfiltered water, 0.5 mL Lugol's solution was added, and the bottles were kept in dark until analysis. For zooplankton, 5 L of well-mixed pooled water was filtered through a 20 µm mesh,

poured into a 50 mL bottle, and preserved with 2.5 mL Lugol's solution. After sampling, the remaining water was returned to the mesocosm.

Salinity, conductivity, temperature, dissolved oxygen, and pH were measured in situ in the centre of the mesocosms. A multi-parameter probe was used and water depth was averaged from measurements at four points.

On each sampling date and for each mesocosm, a species list of dominant and subdominant submerged macrophytes (not including filamentous algae) was produced. Percent Plant Volume Inhabited (PVI%) was calculated each month by visually estimating the percentage coverage and measuring average plant height of macrophytes using the formula:

$$\mathrm{PVI\%} = \frac{\%\mathrm{coverage} \times \mathrm{average\ height}}{\mathrm{water\ depth}}.$$

To determine macrophyte coverage, the enclosures were divided into quarters to allow estimation of area. If present, filamentous algae were included in the estimate of total macrophyte coverage. Following the final PVI% estimate and at the end of the experiment, the macrophytes were harvested by cutting the stands close to the sediment surface. Surplus water was removed from the harvested material and wet and dry weights (drying at 60 °C for 24 h) were measured. Benthic invertebrates were also sampled at this point by taking three separate Kajak cores (Plexiglass cores, ∅ = 52 mm and core length = approx. 10 cm) per mesocosm or an Ekman grab. Samples were pooled, rinsed, and filtered through a 500 µm mesh in the field before being preserved in 96% ethanol in a 500 mL plastic beaker with a wide opening (one beaker per mesocosm).

From 15 July to 15 August a periphyton growth experiment was undertaken in all deep mesocosms. Artificial transparent polypropylene strips (21 mm × 297 mm each) with a slightly textured surface (IBICO®, Germany) were placed 30 cm from the mesocosm wall, 50 cm below the water surface (Köhler et al., 2010). A small weight was attached to the middle of each strip to ensure that they remained fixed at the required depth, even during a drop in the water level. After the strips were removed in August, they were kept cool and dark in a plastic container and frozen in the laboratory until analysis.

Processing of water chemistry and chlorophyll *a* samples

Total phosphorus, soluble reactive phosphorus, total nitrogen, ammonia, nitrate + nitrite, alkalinity, and chlorophyll *a* were determined using comparable standard procedures in the different laboratories.

Processing of plankton samples

At least two phytoplankton and two zooplankton samples from each mesocosm were counted: one sample representing the starting conditions and one sample

representing an integrated sample of the whole experimental period. The latter consisted of a mixed sample of subsamples (25% of the original sample volumes) from each of the monthly samples. The remaining 75% of the original sample volume was stored separately for subsequent analysis of seasonal dynamics.

Ecosystem metabolism

Metabolism in each mesocosm was estimated on a monthly basis. Oxygen, pH, and temperature were measured (upper 20 cm of the fully mixed water column) at least every second hour for a 24-hour period using a multi-parameter probe following the same mesocosm order each time. Light attenuation was measured once a month at 1 p.m. (every 10 cm down to <1% of the surface light) using a light meter. At the same time samples for alkalinity were taken. The gas exchange coefficient was estimated in late October or early November when temperatures, and thus respiration, were low. The oxygen concentration was lowered to ca 30% of saturation in the late evening by adding N_2. Oxygen recovery was then tracked during the night and the exchange rate calculated, taking respiration into account (for details about the method see Liboriussen et al., 2011).

Statistical analysis

In this paper we use averages over the entire duration of the experiment, which covered the period from May to October for all variables except for submerged macrophyte PVI% covering the period from July to October. We analysed these data with analysis of covariance with nutrient dosing and depth as fixed factors and water temperature as the covariate variable using the GLM procedure SAS (SAS Institute Inc., 2008). All variables except temperature were log-transformed.

RESULTS

Average water temperature during the experiment varied between 15.5 and 25.1 °C (Fig. 3) and air temperature between 14.6 and 23.4 °C, but these did not faithfully follow the latitude gradient (Table 1). While limited water level changes occurred between Sweden to Czech Republic (ranging from –3 cm to +12 cm), profound reductions were observed in Turkey and Greece (accumulating to 46 and 84 cm, respectively by the end of the experiment) (Fig. 3 shows a significant temperature effect, but no significant depth effect (Table 3)). This coincided with the high summer temperatures and low precipitation (223 and 252 mm, respectively, compared to 271–431 mm further north), reflecting the typically low summer water levels found in the Mediterranean region. Conductivity tended to be higher in the mesocosms at these two southern sites, but also in the mesocosms in Germany, probably due to the use of tap water. The analysis of covariance showed a significant temperature effect (Table 3).

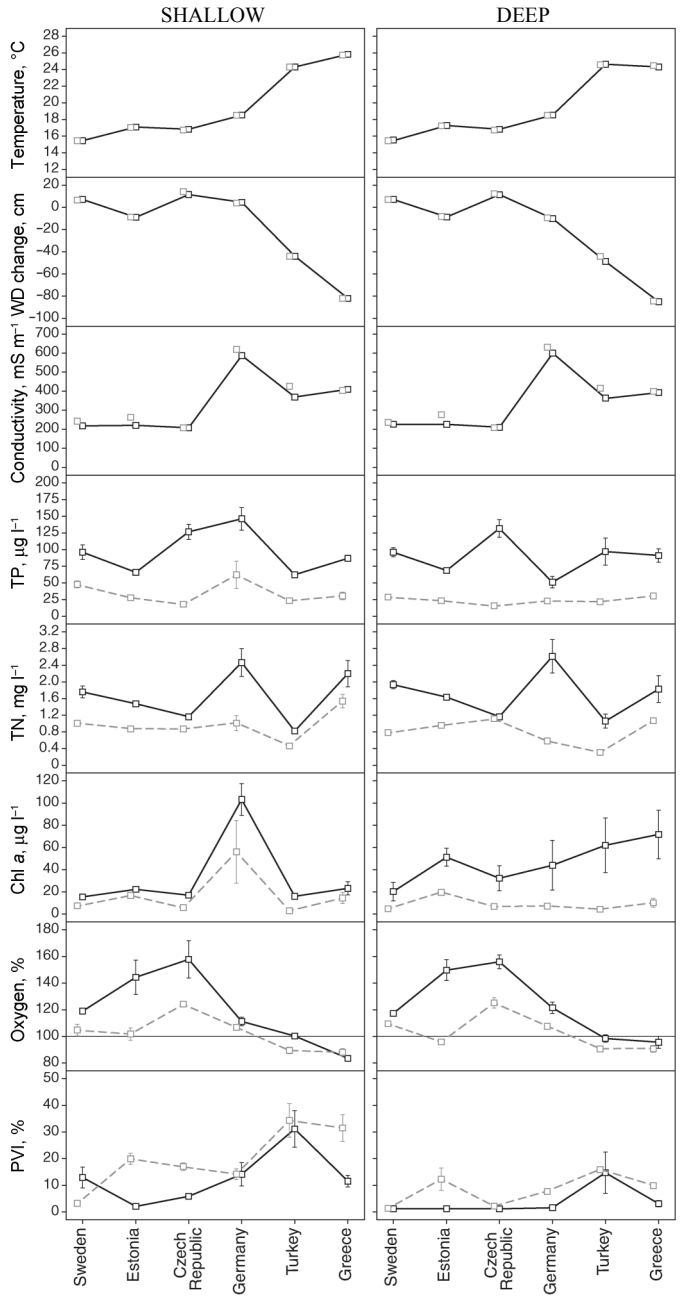

Fig. 3. Selected physical and chemical variables ordered country-wise according to increasing mean water temperature during the experiment, i.e. May–October 2011. Means (SE, standard error) of water temperature, maximum change in water depth (WD), conductivity, total phosphorus (TP), total nitrogen (TN), chlorophyll *a* (Chl *a*), mean diurnal oxygen saturation during the experiment in the high nutrient (full line) and the low nutrient mesocosms (dashed line). Also shown is plant volume inhabited (PVI%) for the period July–October. For the upper three panels SEs are not included as they are so low that they would be largely hidden in the symbols.

Table 3. Analysis of covariance tests of significance using mean values for the study period for each mesocosm. Significant variables are in bold. Marginally significant values are in parentheses

	Water level change	Total nitrogen	Total phosphorus	Chloro-phyll *a*	PVI% macrophytes	Conduct-ivity	O_2
Depth	0.84	0.25	**(0.055)**	0.18	**(0.056)**	0.81	0.74
Nutrients	0.99	0.27	**0.0012**	0.49	0.59	0.65	**0.0001**
Depth × Nutrients	0.99	0.52	0.37	0.27	0.43	0.94	0.83
Temp	**<0.0001**	**0.03**	0.35	0.97	**<0.0001**	**<0.0001**	**0.0001**
Temp × Depth	0.90	0.16	0.10	0.17	0.60	0.87	0.80
Temp × Nutrients	0.97	0.73	0.64	**0.040**	0.64	0.86	**0.003**
Temp × Depth × Nutrients	0.99	0.38	0.57	0.15	0.67	0.96	0.95

Low and high nutrient levels were maintained throughout the experiment (data not shown). Average TP in the shallow and deep low nutrient mesocosms was 35 and 24 µg P L^{-1}, respectively, and 103 and 90 µg P L^{-1} in the shallow and deep high nutrient mesocosms, respectively. Nutrient dosing and depth, but not temperature, had a significant effect for TP in the analysis of covariance (Table 3). Total nitrogen averaged 0.96 and 0.81 mg N L^{-1} in the shallow and deep mesocosms with low nutrient addition, respectively. The corresponding figures were 1.65 (shallow) and 1.70 (deep) mg L^{-1} in the high nutrient mesocosms. The analysis of covariance showed a temperature effect, but no effect of dosing for TN (Table 3).

Chlorophyll *a* averaged 17.3 (shallow) and 8.8 (deep) µg L^{-1} in the low nutrient mesocosms and 33 (shallow) and 47 (deep) µg L^{-1} in the high nutrient mesocosms (Fig. 3). While the concentration in the deep mesocosms demonstrated an increasing trend from cold to warm lakes as expected, chlorophyll *a* values were more variable in the shallow mesocosms. The analysis of covariance showed that the interaction between temperature and nutrients was significant, but no depth effect was detected (Table 3).

Submerged macrophyte PVI% (average July to October) showed an increasing trend with temperature, particularly in the shallow mesocosms. The analysis of covariance revealed temperature to be significant and depth to be marginally significant, whereas no nutrient effect was observed (Fig. 3, Table 3).

The northern mesocosms were generally supersaturated with oxygen during the study period and saturation tended to peak at intermediate temperatures during the experimental period, while saturation in the mesocosms in Greece and Turkey was below 100% (Fig. 3). The mixed effect model showed saturation to be significantly affected by depth, temperature, and interactions between nutrients and temperature (Table 3).

DISCUSSION

Our experiment illustrates how careful design, preparation, and sampling procedures can be used to optimize the results from cross-national mesocosm experiments. The more detailed results from the experiment are yet to be published, but some overall conclusions can already be drawn. To avoid compromizing these publications, in this overview we have restricted our analysis to averages for the study period, which potentially can hide important interactions. As expected, we observed a strong gradient in temperature (air temperature 14.6–24.3 °C, water temperature 15.5–25.1 °C) as an average for the experimental period and an accompanying large change in water level due to variation in net precipitation. While no significant difference was found in the water level in the mesocosms from Sweden to the Czech Republic, it fell by 46 and 84 cm in Turkey and Greece, respectively, with major implications for the shallow mesocosms. The temperature gradient did not follow latitude. The Czech and Turkish sites were located at higher altitude and were therefore exposed to cooler climatic conditions relative to their latitudinal counterparts (Germany, Greece).

As expected, the phosphorus levels differed significantly among high and low nutrient treatments. We attribute this to: (i) the thorough pre-equilibration of the sediment to the experimental P concentrations over a period of several months prior to the start of the experiment using a standardized protocol, and (ii) standardization of the sediment composition (1 : 10 ratio of sediment to sand). Although some minor variation occurred, our procedure ensured that relatively similar P concentrations were maintained across sites. Experience from a Danish long-term mesocosm study has shown that the same sand–sediment combination as used in the present study (but without pre-equilibration) results in prolonged high internal loading (particularly of phosphorus) in the systems under low nutrient concentrations but in P retention under high nutrient concentrations (M. Søndergaard et al., unpublished results). Ideally, the same type of sediment should have been used in all countries, but this was not possible for practical reasons.

Nitrogen also tended to be higher in the mesocosms receiving high nutrient doses. Nitrogen concentration increased with temperature, possibly resulting from reduced nitrogen retention under warmer conditions as carbon can be limiting under high assimilation rates (Kosten et al., 2012).

Since our primary goal was to produce similar nutrient concentrations at contrasting depths, nutrients were dosed per volume (Table 2). While this provides good opportunities for comparing shallow lakes with similar N and P concentrations but contrasting depths, the drawback is that the biota has more total nutrients available in the deep tanks, which makes the comparison, including the nutrient balances, of deep and shallow tanks difficult.

For chlorophyll *a* we found the interaction between nutrients and temperature to be significant, with nutrients enhancing the effect of temperature. This concurs with several recent studies based on space-for-time analysis of large data sets (Jeppesen et al., 2007, 2010b; Moss et al., 2011), time series (Wilhelm and Adrian,

2008; Wagner and Adrian, 2009), experiments (see review in Stewart et al., 2013) and modelling (Mooij et al., 2005; Trolle et al., 2011); for a review, see Jeppesen et al. (2014).

The overall PVI% of macrophytes increased with increasing temperature, particularly in the shallow mesocosms. Concurrently, Bucak et al. (2012) found in a shallow mesocosm experiment in Turkey that water level reductions lead to faster growth of macrophytes due to improved light conditions for the plants. The water level reduction in the deep mesocosm experiments of Bucak et al. (2012) was, however, not accompanied by increasing macrophyte coverage. Macrophyte growth and colonization depth are, in general, positively affected by temperature (Rooney and Kalff, 2000), but whether this growth potential is realized depends on a number of factors, including the trophic structure, periphyton and plant grazer abundance, and winter climate (Jeppesen et al., 2014), which were largely standardized in our study.

We also sought to describe system production and respiration from diurnal variation in oxygen and CO_2 exchange based on alkalinity measurements and diurnal variation in pH. Generally, there is a good relationship between oxygen saturation and net production in fully mixed lakes and mesocosms (Nielsen et al., 2013). Our results indicate higher net production for the study period in the mesocosms in the northernmost four countries, peaking in Central Europe, than in Greece and Turkey. This concurs with other findings, suggesting that net production decreases from cold to warm lakes (Kosten et al., 2010).

In conclusion, the experimental design described is suitable for studying how changes in nutrients affect the biota and metabolism in shallow lakes at contrasting water levels and in different climate zones. A considerable effort was required to standardize the experiments and to ensure low risk of failure. During the course of a year we discussed and prepared a detailed sampling protocol and considered the types of mesocosms to be used. Detailed protocols are particularly necessary when groups with very different backgrounds and experience are running a joint experiment. Our design also has some drawbacks. (1) Due to budget constraints our mesocosms were relatively small, but scale is of considerable importance (Schindler, 1998). We had a simple fish community structure (1 species), with no piscivores. At such a small scale, adequate conditions for piscivory cannot be created. It is evident from a number of recent studies that the proportion of piscivores decreases with decreasing latitude (Jeppesen et al., 2010a, 2010b; Meerhoff et al., 2012), and the experiment cannot account for this. In larger (and more costly) systems, such problems could be minimized and stocking of piscivorous fish would be possible, enabling natural development of planktivorous fish. By ensuring full mixing of the water column as in natural shallow lakes that are typically polymictic, scale was, in part, compensated for. (2) We lost several mesocosms, either because they sank during heavy storm events or leaked water due to damage. In total, 6 of the 96 mesocosms were lost. The storm effect could have been avoided by running the experiment on land (perhaps in buried tanks), but such tanks tend to get warmer than those placed in water.

Despite these drawbacks we conclude that the system presented here offers great potential to study the effects of global warming and eutrophication on in-lake processes and dynamics in shallow lakes. The thorough preparatory phase encompassing the design of the experiment and writing of protocols has been essential for the success of this multi-national, multi-cultural experiment.

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