AARHUS UNIVERSITY

Coversheet

This is the accepted manuscript (post-print version) of the article.

Contentwise, the accepted manuscript version is identical to the final published version, but there may be differences in typography and layout.

How to cite this publication

Please cite the final published version:

Audet J, Neif EM, Cao Y, et al. Heat-wave effects on greenhouse gas emissions from shallow lake mesocosms. *Freshwater Biol.* 2017;62:1130–1142. <https://doi.org/10.1111/fwb.12930>

Publication metadata

Title:	Heat-wave effects on greenhouse gas emissions from shallow lake mesocosms
Author(s):	Joachim Audet, Érika M. Neif, Yu Cao, Carl C. Hoffmann, Torben L. Lauridsen, Søren E. Larsen, Martin Søndergaard, Erik Jeppesen, Thomas A. Davidson
Journal:	Freshwater Biology
DOI/Link:	10.1111/fwb.12930
Document version:	Accepted manuscript (post-print)

This is the peer reviewed version of the following article: [Audet J, Neif EM, Cao Y, et al. Heat-wave effects on greenhouse gas emissions from shallow lake mesocosms. *Freshwater Biol.* 2017;62:1130–1142. <https://doi.org/10.1111/fwb.12930>], which has been published in final form at [\[https://doi.org/10.1111/fwb.12930\]](https://doi.org/10.1111/fwb.12930). This article may be used for non-commercial purposes in accordance with Wiley Terms and Conditions for Use of Self-Archived Versions.

Supporting information can be found at <https://doi.org/10.1111/fwb.12930>

General Rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognize and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

If the document is published under a Creative Commons license, this applies instead of the general rights.

Heat wave effects on greenhouse gas emissions from shallow lake mesocosms

Joachim Audet^{1,2}, Érika M. Neif^{1,3}, Yu Cao^{1,4}, Carl C. Hoffmann¹, Torben L. Lauridsen^{1,5,6}, Søren E. Larsen¹, Martin Søndergaard^{1,5,6}, Erik Jeppesen^{1,5,6}, Thomas A. Davidson^{1,7}

¹Aarhus University, Department of Bioscience, Vejlshøvej 25, DK-8600 Silkeborg, Denmark

²Department of Aquatic Sciences and Assessment, Swedish University of Agricultural Sciences, P.O. Box 7050, 75007 Uppsala, Sweden

³Departamento de Biologia, Núcleo de Pesquisas em Limnologia, Ictiologia e Aquicultura – Nupélia, Universidade Estadual de Maringá – UEM, Av. Colombo, 5790, CEP 87020-900, Maringá, PR, Brazil

⁴Key Laboratory of Aquatic Botany and Watershed Ecology, Wuhan Botanical Garden, Chinese Academy of Sciences, 430074 Wuhan, China

⁵Sino-Danish Centre for Education and Research (SDC), Beijing, China

⁶Aarhus University, Arctic Research Centre (ARC), C. F. Møllers Allé 8, DK-8000 Aarhus, Denmark

⁷Aarhus University, Department of Bioscience, Section for Ecoinformatics and Biodiversity, Ny Munkegade 114, DK-8000 Aarhus, Denmark

Corresponding author: Joachim Audet,

Email: joachim.audet@slu.se, Telephone: +46 1867 2233

Keywords: Greenhouse gases; Shallow lakes; Eutrophication; Global warming; Trophic interactions.

Abstract

1. Shallow lakes are a key component of the global carbon cycle. It is, therefore, important to know how shallow lake ecosystems will respond to the current climate change. Global warming affects not only average temperatures but also the frequency of heat waves. The impact of extreme events on ecosystems processes, particularly greenhouse gas (GHG) emissions, is uncertain.

2. Using the world's longest-running shallow lake experiment, we studied the effects of a simulated summer heat wave on the fluxes of carbon dioxide (CO₂), methane (CH₄) and nitrous oxide (N₂O). The experimental mesocosms had been exposed to different temperature treatments and nutrient loading for 11 years prior to the artificial heat wave.

3. In general, there was an increase in total GHG emissions during the one-month artificial heat wave, with a significant increase in CO₂, CH₄ and N₂O being observed in the shallow lake mesocosms. No significant effect of the heat wave on CO₂ emissions could be traced, though, in the mesocosms with high nutrient levels. Furthermore, the data suggested that in addition to the direct effect of increased temperature on metabolic processes during the heat wave, biotic interactions exerted a significant control of GHG emissions. For example, at low nutrient levels, increased CO₂ emissions were associated with low macrophyte abundance, whereas at high nutrient levels, decreased phytoplankton abundance was linked to increased emissions of CO₂ and CH₄.

4. In contrast to the observable heat wave effect, no clear general effect of the long-term temperature treatments could be discerned over the summer, likely because the potential effects of the moderate temperature increase applied as a press disturbance were overridden by biotic interactions. This study underpins that the role of biotic interactions needs to be considered within the context of global warming on ecosystems processes.

Introduction

Lakes cover about 3.7% of the Earth's continental land surface (Verpoorter *et al.*, 2014). Despite their limited spatial extent, they are of great importance in regulating global biogeochemical processes, in particular the cycling of carbon (Tranvik *et al.*, 2009). Thus, lakes are sites of storage, transport and transformation of carbon and other nutrients derived from terrestrial ecosystems (Tranvik *et al.*, 2009). A large part of the dissolved and particulate organic carbon reaching lakes is mineralised to carbon dioxide (CO₂) and methane (CH₄) (Molot & Dillon, 1996; Tranvik *et al.*, 2009), and lakes consequently function as a significant source of greenhouse gas (GHG), although a part of the CH₄ produced in lakes can be oxidised by methanotrophic bacteria (Bastviken *et al.*, 2008; Bastviken *et al.*, 2011; Matveev *et al.*, 2016).

A temperature increase will likely alter GHG emissions from lakes (Walter *et al.*, 2006; Marotta *et al.*, 2014), but the precise nature of such an alteration remains unclear. Global warming not only increases average temperatures but also changes the occurrence frequency of extreme events such as heat waves (Meehl & Tebaldi, 2004; IPCC, 2014; Christidis, Jones & Stott, 2015). For example, Christidis *et al.* (2015) found that heat wave events occurring only twice a century in the early 2000s now take place twice a decade. Therefore, the likelihood of extremely hot summers has increased over the last decades. Heat waves can severely affect the thermal regime of lakes; by way of example, the 2003 summer heat wave in Europe resulted in strong oxygen depletion of the hypolimnion of two deep lakes in Switzerland (Jankowski *et al.*, 2006). In addition, heat waves can promote blooms of cyanobacteria (Jöhnk *et al.*, 2008). Despite these adverse trends, the effect of such potentially stressful climatic events on GHG emissions is largely unknown (Bartosiewicz *et al.*, 2016). Shallow lakes may be particularly sensitive to heat waves as their average water temperature will increase more quickly than in deeper water bodies as a result of their close link to air temperature (Mooij, De Senerpont Domis & Hülsmann, 2008). It can therefore be presumed that heat waves have an impact on the GHG emissions from shallow lakes. This is of global importance

for the carbon cycling as shallow lakes are the most abundant lake type (Downing *et al.*, 2006) and therefore contribute about 6-16%, a large part, to the total natural CH₄ emissions (Bastviken *et al.*, 2004; Bastviken *et al.*, 2011; Holgerson & Raymond, 2016). The emission of GHGs from lakes is likely to be affected by the heat wave-induced higher water temperatures due to the constraints of temperature on metabolic processes (Allen, Gillooly & Brown, 2005; Yvon-Durocher *et al.*, 2010). Laboratory studies have demonstrated a clear increase in CO₂ and CH₄ production in lake sediments with enhanced temperatures (Liikanen *et al.*, 2002; Marotta *et al.*, 2014; Gudas *et al.*, 2015). Warming can also increase denitrification (Veraart, de Klein & Scheffer, 2011) and therefore potentially alter the subsequent emission of nitrous oxide (N₂O), a strong GHG with a warming potential 298 times greater than CO₂ (IPCC, 2013).

Heat waves are likely to affect primary production. For example, warmer temperatures can contribute to a decrease in the abundance of submerged macrophytes, a key structuring element in shallow lakes, because of stronger competition from floating plants or phytoplankton (Moss *et al.*, 2011); such an effect is not always seen, however (Kosten *et al.*, 2011; Cao *et al.*, 2015). Warming can also favour the establishment of invasive plant species (McKee *et al.*, 2002). Apart from warming, the abundance of submerged macrophytes is also largely controlled by the nutrient status of the lake; consequently, nutrient-poor lakes are generally dominated by submerged macrophytes, whereas nutrient-rich lakes are often dominated by phytoplankton (Moss, 1990; Scheffer *et al.*, 1993). Warmer temperatures may reinforce the detrimental effects of eutrophication such as deoxygenation of the hypolimnion, disappearance of oxidised microzones in the upper sediment, dominance of cyanobacteria and reduction of macrophyte cover (Jeppesen *et al.*, 2009; Moss *et al.*, 2011; Kosten *et al.*, 2012). Previous studies have shown that eutrophication might not increase but perhaps reduce CO₂ emissions, augmenting, instead, CH₄ and N₂O emissions (Huttunen *et al.*, 2003; Sobek *et al.*, 2009; Davidson *et al.*, 2015). Hence, under natural conditions, the levels of nutrient enrichment and the intensity of biotic interactions may complicate the seemingly straightforward

relationships between temperature and GHG emissions from shallow lakes (Davidson *et al.*, 2015; Attermeyer *et al.*, 2016; Bartosiewicz *et al.*, 2016).

Here, we investigated the effect of a simulated summer heat wave on GHG fluxes from shallow lakes. We used the longest-running mesocosm experimental facility exploring climate effects on shallow lakes (Liboriussen *et al.*, 2005) and simulated the effect of a heat wave on the mesocosms which prior to our experiment have undergone 11 years of acclimation to different temperature and nutrient treatments. We hypothesised that 1) a heat wave would increase the emission of GHG because of the differential effect of temperature on the rates of metabolic processes. The emission may, however, also be influenced by related changes in submerged macrophytes and phytoplankton biomass that may, also, affect nutrient availability and the subsequent GHG processes; 2) nutrient-rich mesocosms would emit less CO₂ but more CH₄ and N₂O than low-nutrient mesocosms during the heat wave because of increased CO₂ uptake and higher nutrient availability.

Material and methods

The experiment was conducted in lake mesocosms located in Central Jutland, Denmark (56°2448N, 9°5298E). These mesocosms are part of a lake experiment initiated in August 2003 with the aim to investigate the effects of climate change (see details in Liboriussen *et al.* (2005)). Briefly, the experimental facility includes 24 outdoor and uncovered flow-through mesocosms (diameter 1.9 m, water depth 1 m, retention time ~ 2.5 months). At the start of the experiment in 2003, a 0.1 m layer of washed sand was placed at the bottom of each mesocosm with a 0.1 m layer of nutrient-rich sediment from a nearby freshwater pond on top (Liboriussen *et al.*, 2005). The mesocosms are exposed to three temperature treatments: unheated (AMB), treatment A2 (A2) according to the IPCC-predicted temperature increase (Houghton *et al.*, 2001) and treatment A2+50% (A2+). The A2 and A2+ mesocosms are warmed up using heating elements placed about 10 cm above the sediment. To avoid water stratification, the water column in the mesocosms is automatically fully mixed by paddles (Liboriussen *et al.*, 2005). The water temperature in the unheated mesocosms fluctuates with the air temperature, while the water temperature in the two heated treatments is continuously adjusted to be 2-4°C (A2) and 4-6°C (A2+) warmer than the AMB mesocosms. The seasonal differences between the temperature treatments are based on future climatic projections for Denmark downscaled to monthly resolution using 1961-1990 as reference period (Houghton *et al.*, 2001). The three temperature treatments are crossed with two nutrient treatments (low and high), yielding six treatments with four replicates. In the low-nutrient treatment, no nutrients are added, i.e. the level is that of the inflowing water, while in the high-nutrient treatment, nutrients are added at concentrations of 7 mg P m⁻² d⁻¹ and 27.1 mg N m⁻² d⁻¹. Consequently, in the low-nutrient mesocosms nutrient levels are 11±9 µg TP L⁻¹ (mean ± standard deviation) and 0.28±0.29 mg TN L⁻¹ and 40±24 µg TP L⁻¹ and 2.61±1.44 mg TN L⁻¹ in the high-nutrient mesocosms. The purpose of these two nutrient states is to mimic a clear water shallow lake and a turbid water shallow lake (Scheffer *et al.*, 1993). Submerged macrophytes generally dominate in the low-nutrient mesocosms, whereas

the high-nutrient mesocosms are more turbid, often with abundant phytoplankton. Probes in the mesocosms record water temperature, oxygen and pH every 30 minutes (Liboriussen *et al.*, 2005). Our measurements took place in summer 2014, i.e. 11 years after the initiation of the mesocosms experimental setup. During the whole month of July 2014, a heat wave was simulated in treatments A2 and A2+ by increasing the water temperature by about 5°C, while the AMB mesocosms remained unheated. Hence, during the heat wave temperatures in the heated mesocosms were about 7-9°C (A2) and 9-12°C (A2+) warmer than in the ambient treatment. Similar to the long-term temperature treatments, the water temperature in A2 and A2+ was continuously adjusted during the heat wave to follow the temperature variation in AMB. The mesocosms were sampled 8 times between 19-06-2014 and 01-09-2014. All samplings were carried out at the same time, between 7 a.m. and 9 a.m.

On every sampling occasion, water samples were collected to determine the aqueous concentrations of N₂O and CH₄. Using a syringe, 9 ml water samples were taken 5 cm below the surface of the water and injected into 12 mL vials containing 6 mL helium and 0.2 mL 50% ZnCl to prevent bacterial activity. After transportation to the laboratory, the vials were kept at room temperature in the dark until analysis within one week. Nitrous oxide and CH₄ were determined on a dual-inlet Agilent 7890 GC system interfaced with a CTC CombiPal autosampler (Agilent, Nærum, Denmark) (Petersen *et al.*, 2012). The aqueous concentrations of N₂O and CH₄ were calculated from the headspace gas concentrations according to Henry's law and using Henry's constant corrected for temperature and salinity (Wiesenburg & Guinasso, 1979; Weiss & Price, 1980). Carbon dioxide concentrations were calculated from acid neutralizing capacity (ANC) and pH was measured on every sampling occasion following the approach described in Cole *et al.* (1994). The estimated CO₂ concentrations agreed relatively well with previous direct CO₂ measurements ($R^2=0.88$, $n=330$ in Cole *et al.* (1994); $R^2=0.60$, $n=452$ in Raymond, Caraco and Cole (1997)), although calculated CO₂ concentrations may occasionally be overestimated, particularly at low ANC.

The fluxes of N₂O, CH₄ and CO₂ between the water and the overlying atmosphere were estimated as:

$$f_g = k_g \beta_g (C_{wat,g} - C_{eq,g})$$

where f_g (g m⁻² h⁻¹) is the flux of a specific gas g , k_g (m h⁻¹) is the gas transfer velocity, β_g is the chemical enhancement factor and $C_{wat,g} - C_{eq,g}$ (g m⁻³) is the gradient of concentration between the concentration of gas dissolved in the water ($C_{wat,g}$) and the concentration of gas that the water would have at equilibrium with the atmosphere ($C_{eq,g}$), assuming concentrations of 390, 1.8 and 0.325 ppm for CO₂, CH₄ and N₂O, respectively. Gas transfer velocities for the different gases were calculated based on direct measurements of the gas transfer velocity for O₂ in three mesocosms in December 2009 (Liboriussen *et al.*, 2011) and corrected for the water temperature (Jähne *et al.*, 1987). We found little variation in the k_{O_2} estimates based on the recovery of artificial reduction in O₂ in three of the mesocosms (0.0080, 0.0086 and 0.0100 m h⁻¹ at 20°C) (Liboriussen *et al.*, 2011). Therefore, the same gas transfer velocity was applied to all mesocosms ($k_{O_2} = 0.0088$ m h⁻¹ at 20°C). Macrophyte abundance may affect water stratification and turbulence at the interface between water and atmosphere and thus alter the gas transfer velocity. However, because the mesocosms are all constantly mixed with automatic paddles, we assumed that between-mesocosm differences in gas transfer velocity were minor.

$$k_g = k_{O_2} \left(\frac{Sc_g}{Sc_{O_2}} \right)^x$$

Sc_g is the Schmidt number (Wanninkhof, 1992). We chose $x = -2/3$ as this factor is used for smooth liquid surface (Deacon, 1981). Wind speed alters piston velocity; however, at low wind speed (<3 m s⁻¹) the effect is negligible (Cole & Caraco, 1998), which was the case in our study where the mesocosms were relatively well sheltered from the wind. At high pH and low wind speeds, the transfer of CO₂ from the atmosphere to the water can be enhanced because of the chemical reaction between CO₂ and OH⁻. This effect was taken into account and chemical enhancement β_{CO_2}

was estimated as a function of pH following the model of Wanninkhof and Knox (1996). For N₂O and CH₄, β was set to 1 (i.e. no chemical enhancement). Total greenhouse gas flux was calculated as the sum of CO₂, CH₄ and N₂O after conversion to CO₂ equivalents, assuming a global warming potential (GWP) of 25 for CH₄ and 298 for N₂O (IPCC, 2013).

Gross primary production calculation

Gross primary production was estimated using the 30 min interval records of DO, light intensity and water temperature according to the methods described in Erlandsen and Thyssen (1983) and Kelly, Thyssen and Moeslund (1983) (see Jeppesen *et al.* (2012b) for details):

$$\left[\frac{dDO}{dt}\right] = [K_2 \cdot 1.0241^{(T_W-20)} \cdot (DO_{sat} - DO_t)] - [\rho_{20} \cdot 1.07^{(T_W-20)}] + \left[\pi_{20} \cdot 1.035^{(T_W-20)} \cdot \frac{I_t}{\eta + I_t}\right]$$

$$[Oxygen\ change] = [Air\ water\ exchange] - \left[\frac{Temperature\ dependent}{respiration}\right] + \left[\frac{Light\ and\ temperature\ dependent}{production}\right]$$

where

T_W : water temperature °C

DO_{sat} : dissolved oxygen saturation concentration mg L⁻¹

DO : dissolved oxygen concentration mg L⁻¹

I_t : light intensity – Photosynthetically Active Radiation (PAR) mol m⁻² 30 min⁻¹.

ρ_{20} , π_{20} and η were estimated using the secant non-linear method, PROC NLIN, in the statistical software SAS 9.3. DO_{sat} was represented as the dissolved oxygen percentage saturation (DO%), calculated as a function of T_W (Lin, 2001):

$$DO_{sat} = 14.652 - 0.41022 \cdot T_W^1 + 0.0079910 \cdot T_W^2 - 0.000077774 \cdot T_W^3$$

203

204 Macrophyte and phytoplankton abundance estimation

205 Water samples to be analysed for chlorophyll-a (Chl-*a*) were collected on every sampling occasion.

206 To measure Chl-*a*, 100-1000 mL of water was filtered through GFC filters and the Chl-*a*

207 concentration determined on a spectrophotometer after extraction with ethanol (Jespersen &

208 Christoffersen, 1987). Macrophyte abundance was quantified as per cent volume inhabited (PVI) as

209 described in Davidson *et al.* (2015). Only two species of macrophytes were present in the

210 mesocosms, *Elodea canadensis* and *Potamogeton crispus*.

211

212 Statistical methods

213 The statistical analysis was performed using the R software (version 3.1.1) (R Development Core

214 Team, 2014) and using the R package “nlme” and the function “lme” (Pinheiro *et al.*, 2012). Linear

215 mixed effect models were used to explore linkages between gas fluxes and treatments and/or

216 environmental variables. Linear mixed effect models are particularly suitable to analyse patterns in

217 time series data sets with crossed treatments (Zuur *et al.*, 2009).

218 In a first analysis, the whole data set, two nutrient treatments and three temperature treatments (of

219 which two included a heat wave), each with four replicates and sampled eight times (n=192), was

220 tested for significant treatment effects (temperature, nutrient, and heat wave treatments (HW)) for

221 each of the individual gas fluxes (i.e. CO₂, CH₄ and N₂O). Treatment effects were included in the

222 model as fixed effects and mesocosms as a random effect (Zuur *et al.*, 2009). A variance structure

223 was added in the CO₂ and N₂O models (varFixed and varPower, respectively) to deal with the

224 heterogeneity of the variance (Pinheiro & Bates, 2000). The long-term temperature and nutrient

225 treatments had been applied to the mesocosms for 11 years prior to our heat wave experiment,

226 whereas the heat wave was applied only for one month and only in the heated treatments (i.e. A2

and A2+). The non-heated treatment (AMB) followed ambient temperatures, both during the 11 years prior to the heat wave and during the simulated heat wave. Hence, due to the overlap of the heat wave with the temperature treatments, we did not have true controls to test the particular effects of the heat wave. In the models, HW had three levels: HW₀, the pre- and post-heat wave period (i.e. June and August in AMB, A2 and A2+); HW₁, the heat wave period (July in A2 and A2+); HW₂, the “ambient summer” period (July in the AMB treatment) (Fig. 1). When HW was found to be significant in the mixed models, the variations among the different levels of the heat wave treatments were tested using Tukey’s *post-hoc* test.

Starting with a full model including treatments and environmental variables, model selection was made in a backward stepwise fashion using the likelihood ratio involving removal of the term of least significance until all remaining covariables were significant (Zuur *et al.*, 2009). All mixed models were checked for normality and homogeneity of variance by visual inspection of plots of residuals against fitted values. The significance of the models was assessed by comparison with a null-model using likelihood ratio. The covariables were checked for collinearity using variance inflation factor (VIF) values (VIF<5). To reduce variance heterogeneity in the data and to meet the assumptions of linear mixed effect models, the fluxes of CO₂, CH₄ and N₂O were transformed using natural logarithm (+0.4 g C m⁻² d⁻¹, +25 µg N m⁻² d⁻¹ for CO₂ and N₂O, respectively) prior to analysis. Thereafter, the data set was split based on nutrient treatment (low and high) to explore treatment effects potentially masked by nutrient effects. Hence, in this second step, only temperature and heat wave treatment effects on individual gas fluxes were tested.

To test for the importance of biotic interactions relative to heat wave effects, a second analysis was conducted using the high-nutrient and low-nutrient data sets separately and adding the following environmental variables to the models: primary production (as the mean of the 5 days prior to the gas sampling), macrophyte abundance (PVI) and phytoplankton biomass (Chl-*a*), change in macrophyte abundance (as ΔPVI) and change in phytoplankton biomass (as ΔChl-*a*). ΔPVI and ΔChl-*a*

252 represented the rate of change in PVI and Chl-*a* between two consecutive samplings and were
253 defined as:

254
$$\Delta PVI_{ti,ti+1} = \frac{PVI_{ti+1} - PVI_{ti}}{t_{i+1} - t_i},$$

255 and
$$\Delta Chl-a_{ti,ti+1} = \frac{Chl-a_{ti+1} - Chl-a_{ti}}{t_{i+1} - t_i}$$

256 Negative Δ indicates a decrease in the abundance of macrophytes or phytoplankton between two
257 sampling occasions.

258

Results

During the heat wave, the maximum water temperatures recorded in the heated mesocosms were 30.2 and 31.5°C for the temperature treatments A2 and A2+, respectively, and 22.8°C for the ambient treatment (Fig. 1a). The lowest water temperatures recorded during the sampling period (19-06-2014 to 01-09-2014) were measured on 25 August with 14.2, 17.9 and 19.7°C in AMB, A2 and A2+, respectively. The artificial heat wave coincided with a period of increased air temperature (mean 19.1°C) compared with the rest of the study period (14.8°C). There was a sudden drop in water temperature around 13 July as a result of a rainy and cold period in an otherwise sunny and warm July (Fig. 1b).

Submerged macrophytes were present in the low-nutrient mesocosms in all temperature treatments, whereas at high nutrient levels only the mesocosms with ambient temperature held submerged macrophytes; their volume gradually decreasing, though, until complete disappearance by the end of July (Fig. 2a,b). The abundance of submerged macrophytes generally decreased over the summer and there was no clear effect of the heat wave on macrophyte abundance. The Chl-*a* concentration was $13 \pm 27 \mu\text{g L}^{-1}$ (mean $\pm$ SD) in the low-nutrient mesocosms and much higher ($255 \pm 219 \mu\text{g L}^{-1}$) in the high-nutrient mesocosms (Fig. 2c,d). However, there were no clear differences between the temperature treatments. In the high-nutrient treatment, the Chl-*a* concentrations peaked a few days after the start of the heat wave ($573 \pm 280 \mu\text{g L}^{-1}$ in A2, $219 \pm 166 \mu\text{g L}^{-1}$ in A2+); however, a concomitant increase was observed in the mesocosms with ambient temperature ($474 \pm 410 \mu\text{g L}^{-1}$). The water pH was 8.1 ± 0.4 in the low-nutrient mesocosms and higher and more variable (8.7 ± 0.6) in the high-nutrient mesocosms, particularly during the heat wave (Fig. 2e,f). Dissolved oxygen (a mean of the half hourly measurements for the 5 days preceding each gas sampling) was more variable in the high-nutrient mesocosms ($9.4 \pm 3.0 \text{ mg L}^{-1}$) than in the low-nutrient mesocosms ($8.1 \pm 1.6 \text{ mg L}^{-1}$) (Fig. 2g,h), and primary production was higher and more variable in the high-nutrient mesocosms ($0.24 \pm 0.06 \text{ mg O}_2 \text{ L}^{-1} \text{ d}^{-1}$) than in the low-nutrient

mesocosms ($0.14 \pm 0.04 \text{ mg O}_2 \text{ L}^{-1} \text{ d}^{-1}$). Dissolved oxygen and primary production were slightly lower in mid-July, coinciding with a short rainy and cloudy period.

Carbon dioxide fluxes ranged between -0.30 and $2.2 \text{ g C m}^{-2} \text{ d}^{-1}$, negative fluxes indicating that the mesocosms sometimes acted as sinks for CO_2 (Fig. 3a,b). The mesocosms were sources of CH_4 with fluxes ranging between 0.10 and $2.81 \text{ mg C m}^{-2} \text{ d}^{-1}$ (Fig. 3c,d). In the low-nutrient mesocosms, emissions of CH_4 increased shortly after the start of the heat wave. Nitrous oxide fluxes were generally close to zero except in a few instances where emission peaks up to $1048 \text{ } \mu\text{g N m}^{-2} \text{ d}^{-1}$ were recorded (Fig. 3e,f). These peaks in N_2O emissions generally occurred when dissolved oxygen dropped below 3 mg L^{-1} . When converting the fluxes into CO_2 equivalents, CO_2 dominated the greenhouse gas budget and contributed, on average, 67% of the total fluxes (Table 1). However, there were large differences across the treatments; for example, at high nutrient levels N_2O emissions (in CO_2eq) were greater than CO_2 emissions in the AMB mesocosms (Table 1). The mean daily GHG fluxes in the heated treatment were higher during the heat wave than during the pre/post heat wave period at both low and high nutrient levels, with an average increase of $0.31 \text{ g CO}_2 \text{ eq m}^{-2} \text{ d}^{-1}$. However, while the AMB mesocosms at low-nutrient concentrations had relatively stable fluxes during the study period (0.14 ± 0.29 vs $0.19 \pm 0.28 \text{ g CO}_2 \text{ eq m}^{-2} \text{ d}^{-1}$ during and outside the heat wave period, respectively), the AMB mesocosms at high-nutrient levels were greater during the heat wave period than in the pre/post heat wave (0.43 ± 1.55 vs $-0.14 \pm 0.39 \text{ g CO}_2 \text{ eq m}^{-2} \text{ d}^{-1}$) (Table 1).

According to the mixed models, the heat wave had a positive effect on the CO_2 emission in comparison with the pre/post heat wave period, but this effect was significant only at low nutrient levels. CO_2 emission was significantly reduced at high nutrient levels compared with low nutrient levels, but no effect of the long-term temperature treatments could be discerned (Table 2). The CH_4 emission was significantly greater during the heat wave but was not affected by the nutrient treatments or by the long-term temperature treatments. Nevertheless, comparison of the CH_4 emission in the heated mesocosms (A2 and A2+) with the ambient temperature mesocosms (AMB)

during the heat wave period (i.e. July) revealed that CH₄ emission only differed significantly at low nutrient levels. This means that for CH₄ at high nutrient levels, the heat wave-induced increase in the A2 and A2+ mesocosms was not significantly different from the increase observed also in the AMB mesocosms in July. Specifically, during the heat wave the mean CH₄ emission increased by 0.37 mg C m⁻² d⁻¹ in the heated treatments for all nutrient treatments. Focusing on the low-nutrient mesocosms, the mean increase was 0.42 mg C m⁻² d⁻¹ (Supplementary table 1). N₂O emission did not seem affected by the nutrient levels or the long-term temperature treatments but increased significantly (+33 µg N m⁻² d⁻¹) during the heat wave. However, the enhanced N₂O emission observed in the A2 and A2+ mesocosms did not differ significantly from the increase in the ambient temperature mesocosms although the difference was marginally significant at low nutrient levels (+54 µg N m⁻² d⁻¹, $p=0.064$).

The significant effect of the heat wave could still be traced after including primary production, PVI, ΔPVI, Chl-*a* and ΔChl-*a* in the models. When significant, decreases in primary production, PVI, Chl-*a* and ΔChl-*a* seemed to trigger an increase in GHG emissions. The effect on GHGs differed between treatments depending on the dominant primary producer; accordingly, at low nutrient levels CO₂ emissions increased with decreasing primary production and declining PVI, whereas at high nutrient levels increasing CO₂ emissions were connected with decreasing Chl-*a* levels (ΔChl-*a*). Similarly, decreasing Chl-*a* levels seemed associated to increased CH₄ emissions in the high-nutrient mesocosms, whereas at low nutrient levels the heat wave was the only significant variable for CH₄ emissions. Emissions of N₂O seemed affected by primary production at low nutrient concentrations and by Chl-*a* at high levels. Primary production was also linked to N₂O emissions at low nutrient levels. Hence, a decrease in primary producers seemed associated with an increase in GHG emissions (Table 3).

Discussion

Our study demonstrated that a simulated one-month summer heat wave coincided with a significant increase in the emissions of CO₂, CH₄ and N₂O from the experimental shallow lake mesocosms; thus, the average daily GHG emission increased by 0.31 g CO₂ eq m⁻² d⁻¹. However, in the case of the CO₂ flux, the heat wave effects were overridden by those of eutrophication, the heat wave having no significant effect on CO₂ fluxes in the nutrient-enriched mesocosms. Notwithstanding the impact of eutrophication on the CO₂ flux, the increased probability of climate change-induced heat waves may result in higher GHG emissions from shallow lakes via increased CH₄ and N₂O emissions. This effect is in apparent agreement with the metabolic theory that links higher temperature with an increase in the rates of processes responsible for the emission of GHG (Allen *et al.*, 2005; Yvon-Durocher *et al.*, 2010). The relatively clear heat wave effect contrasts sharply with the lack of a detectable effect of the long-term temperature treatments (AMB, A2, A2+) as previously recorded in the same system (Davidson *et al.*, 2015). This suggests that a sudden increase in temperature, rather than an increase in average temperature, may have a strong, albeit perhaps short term, effect on GHG emissions, at least in summer. The fact that previous studies have shown decreased CO₂ emissions as a result of higher temperatures (Davidson *et al.*, 2015), contrasting with our finding of heat-induced increased CO₂ emissions, indicates unpredictable outcomes of extreme events. Such events can lead to a step change in ecosystem structure and function – such as loss of submerged plants or a sharp decline in Chl-*a* biomass. It also highlights the importance of investigating not only increases in average temperatures but also the impact of extreme events, such as heat waves.

The apparent contradiction between the notable effect of the heat wave and the lack of significance of the long-term temperature treatments is intriguing. It might be at least partially explained by the effect of changes in biotic interactions related to warming overriding the direct metabolism-related temperature effect on GHGs, at least for a moderate increase in temperature. The prevalent role of biotic interactions compared with that of temperature has also been observed for C cycling and

plant production in forest ecosystems (Fernández-Martínez *et al.*, 2014; Michaletz *et al.*, 2014) and for GHG processes in shallow lakes (Davidson *et al.*, 2015; Bartosiewicz *et al.*, 2016). Indeed, in the present study, primary production, macrophyte abundance or phytoplankton biomass (Chl-*a*) were significant in the models explaining the GHG flux. Submerged macrophytes, in particular, appear to be an important controller of CO₂ and CH₄ emissions. Hence, at low nutrient levels lower macrophyte abundance (PVI) was linked to higher CO₂ emissions. Also a previous study found that macrophyte abundance can be connected with GHG emissions (Davidson *et al.*, 2015). Specifically, higher macrophyte abundance was related to lower CH₄ emissions (Davidson *et al.*, 2015). A possible explanation may be that macrophytes alter oxygen dynamics and provide habitats to CH₄-oxidizing bacteria, thereby reducing the emissions of CH₄ (Yoshida *et al.*, 2014; Davidson *et al.*, 2015). Furthermore, in our study, decreasing phytoplankton abundance was coupled with increased emissions of CO₂ and CH₄ at high nutrient levels. Overall, it appears that the metabolic response of GHG processes is affected, at least to some extent, by complex biotic interactions.

Nutrient treatment significantly affected the fluxes of CO₂ over the summer and affected the response of the shallow lake mesocosms to the heat wave. At high nutrient levels, the insignificance of the heat wave on CO₂ emissions might be related to the absent effect of the heat wave on phytoplankton abundance (i.e. Chl-*a*) which, in turn, may reflect an increase in zooplankton biomass relative to the ambient treatment during the heat wave (Uğur Işkın, unpublished results). But contrary to our hypothesis, the nutrient treatments did not affect the emissions of CH₄ and N₂O. Overall, the balance over the whole study period showed that, the high-nutrient mesocosms were lower emitters of CO₂ than the low-nutrient mesocosms, consequently confirming that shallow lake trophic status is strongly linked to CO₂ emissions (Provoost *et al.*, 2010; Trolle *et al.*, 2012; Jeppesen *et al.*, 2016). The decreased CO₂ emissions at high nutrient concentrations were not counterbalanced by increased N₂O and CH₄ emissions – at any rate not over the duration of our study. This suggests that GHG emissions decrease with eutrophication, at least the diffusive fluxes in summer. Our study showed that heat waves have the potential to increase GHG emissions from

shallow lakes, especially at low nutrient levels where the effect of the heat wave was clearer. The fluxes of N₂O did not differ significantly between the two nutrient levels. This lack of significance is surprising considering that other studies have found a link between the nitrogen load and N₂O emissions in lakes (Huttunen *et al.*, 2003; Hongjun *et al.*, 2006). The authors of these works highlighted the importance of lake littoral zones as N₂O emission hotspots because they often receive a higher nitrogen loading. Davidson *et al.* (2015) recorded CH₄ and N₂O fluxes in the same mesocosms used in our study over a year and found a significant effect of N and P enrichment on CH₄ and N₂O fluxes. That nutrient treatment had no significance for CH₄ and N₂O fluxes in our study is likely due to the large variability in gas fluxes observed during the study period and the fact that our focus was on summer rather than a full year. Therefore, although diffusive CH₄ fluxes do not appear to increase with eutrophication, care should be taken when drawing conclusions about N₂O fluxes –these are difficult to measure since they often exhibit large spatial and temporal variability (Groffman *et al.*, 2009).

The heat wave experiment conducted here lacks a true control due to the limited number of mesocosms. As a consequence, our best test is to compare the heat wave data with the data from the periods before and after (Table 2). As two treatments were artificially heated (A2 and A2+) and one was not (AMB), therefore, we compared the gas fluxes in July – when the artificial heat wave was applied in A2 and A2+ but not in AMB – with those in June and August. As in A2 and A2+, an increase in GHG emissions occurred also in AMB mesocosms despite that they were not subjected to a heat wave. Nevertheless, the air temperature was considerably warmer in July than in June and August (Fig. 1). Interestingly, the increase in GHG emissions in the AMB mesocosms during the heat wave was also linked to a decline in submerged macrophyte cover, supporting the idea that aquatic vegetation is a key factor controlling GHG emissions. Our results suggest that low-nutrient, plant-dominated systems are more likely to be affected by heat waves, creating increased emission of CO₂ and CH₄. In contrast, at high nutrient levels, the effects of eutrophication are already so profound that the heat wave impact will be negligible, or undetectable.

410 As the mesocosm system had already been in operation for 11 years prior to this experiment, we
411 assume that the different compartments (i.e. sediment, water and biota) had reached an
412 equilibrium, allowing reliable comparison with natural systems. However, disentanglement of the
413 effects of the heat wave from those of seasonality is a challenging task. The heat wave was applied
414 only to mesocosms that were already warmer than ambient temperature, implying that these might
415 be pre-adapted to warmer temperature and exhibit different responses to a heat wave than those
416 that have not been subjected to a temperature treatment. There is some evidence that aquatic
417 species can develop higher tolerance to increased temperatures (Geerts *et al.*, 2015) but are still
418 affected by heat waves in complex ways (Cao *et al.*, 2015).

419 A major uncertainty of this study concerns CH₄ fluxes. Methane produced in lakes is transferred to
420 the atmosphere through diffusion, ebullition or plant-mediated fluxes (Bastviken *et al.*, 2004). Only
421 diffusive fluxes were recorded in our study. However, ebullition might account for as much as 40-
422 60% of the total CH₄ emission (Bastviken *et al.*, 2004), which leads to underestimation of CH₄
423 emission. Furthermore, heat waves might alter CH₄ emission pathways as was observed in a
424 Canadian shallow lake where the dominant pathway of CH₄ emission shifted from ebullition to
425 diffusion during a summer heat wave (Bartosiewicz *et al.*, 2016). Plant-mediated fluxes across the
426 water-air interface did not occur in our study as only submerged plants were present in the
427 mesocosms. N₂O and CH₄ fluxes might be affected by diel dynamics in light, pH (related to changes
428 in primary production) and temperature (Käki, Ojala & Kankaala, 2001). Although diel variation in gas
429 fluxes is probably of significance, we assumed that this would bear no impact on our conclusions
430 since we aimed at comparing fluxes across the different treatments and did not have focus on
431 absolute fluxes. Heat waves can promote water stratification in lakes (Jankowski *et al.*, 2006;
432 Jeppesen *et al.*, 2012a) and a study of a Canadian shallow lake found that GHGs produced in the
433 sediment were trapped in the colder water of the hypolimnion (Bartosiewicz *et al.*, 2016).
434 Consequently, when shallow lakes stratify, GHG might be released only when the water column
435 becomes fully mixed again (Bartosiewicz *et al.*, 2016). However, this was not the case in our

experimental setup where the water was continuously mixed. Consequently, our results cannot be transferred to shallow lakes that become temporarily or permanently stratified in summer as a consequence of warming. Indeed, it has been suggested that stratification of shallow lakes may become the norm under warmer temperatures and some studies have shown that reduction in water transparency, related to eutrophication or brownification, can promote stratification of the water (Kirillin & Shatwell, 2016). Our results are, however, still of relevance as an analysis of the thermal structure of 300 lakes worldwide suggested that most lakes with a mean depth <3 m will remain polymictic during the summer (Kirillin *et al.*, 2016).

In a broader perspective, our results demonstrate that when mimicking the effect of a potentially stressful event (e.g. global warming), differences may occur between press (e.g. increase in seasonal/average temperature) and pulse (e.g. heat wave) disturbances. Hence we observed that temperature had a significant effect on GHG emissions only when considering the heat wave event. Accordingly, our results illustrate the importance of pulse events in ecosystems, which is a circumstance that should be considered when attempting to simulate the effect of a disturbance on an ecosystem. Finally, this study also highlighted the importance of biotic interactions when assessing the effect of global warming on ecosystems processes.

Acknowledgements

This work was supported by CLEAR (a Villum Kann Rasmussen Centre of Excellence project on lake restoration), the Danish Council for Strategic Research (Centre for Regional Change in the Earth System – CRES, contract no: DSF-EnMi 09-066868) and MARS (Managing Aquatic ecosystems and water Resources under multiple Stress), EU 7th Framework Programme, contract No.: 603378. JA was supported by the Carlsberg Foundation and FORMAS (grant 2015-1559). TD and EJ were supported by CIRCE, an AU ideas programme. We are very grateful for the technical assistance by the staff at Aarhus University, Silkeborg. We thank A.M. Poulsen for manuscript editing.

References

- Allen A.P., Gillooly J.F. & Brown J.H. (2005) Linking the global carbon cycle to individual metabolism. *Functional Ecology*, **19**, 202-213.
- Attermeyer K., Flury S., Jayakumar R., Fiener P., Steger K., Arya V., Wilken F., van Geldern R. & Premke K. (2016) Invasive floating macrophytes reduce greenhouse gas emissions from a small tropical lake. *Scientific Reports*, **6**, 20424.
- Bartosiewicz M., Laurion I., Clayer F. & Maranger R. (2016) Heat-Wave Effects on Oxygen, Nutrients, and Phytoplankton Can Alter Global Warming Potential of Gases Emitted from a Small Shallow Lake. *Environmental Science & Technology*, **50**, 6267-6275.
- Bastviken D., Cole J., Pace M. & Tranvik L. (2004) Methane emissions from lakes: Dependence of lake characteristics, two regional assessments, and a global estimate. *Global Biogeochemical Cycles*, **18**, GB4009.
- Bastviken D., Cole J.J., Pace M.L. & Van de Bogert M.C. (2008) Fates of methane from different lake habitats: Connecting whole-lake budgets and CH₄ emissions. *Journal of Geophysical Research: Biogeosciences*, **113**, G02024.
- Bastviken D., Tranvik L.J., Downing J.A., Crill P.M. & Enrich-Prast A. (2011) Freshwater Methane Emissions Offset the Continental Carbon Sink. *Science*, **331**, 50.
- Cao Y., Neif É., Li W., Coppens J., Filiz N., Lauridsen T., Davidson T., Søndergaard M. & Jeppesen E. (2015) Heat wave effects on biomass and vegetative growth of macrophytes after long-term adaptation to different temperatures: a mesocosm study. *Climate Research*, **66**, 265-274.
- Christidis N., Jones G.S. & Stott P.A. (2015) Dramatically increasing chance of extremely hot summers since the 2003 European heatwave. *Nature Climate Change*, **5**, 46-50.
- Cole J.J. & Caraco N.F. (1998) Atmospheric exchange of carbon dioxide in a low-wind oligotrophic lake measured by the addition of SF₆. *Limnology and Oceanography*, **43**, 647-656.
- Cole J.J., Caraco N.F., Kling G.W. & Kratz T.K. (1994) Carbon Dioxide Supersaturation in the Surface Waters of Lakes. *Science*, **265**, 1568-1570.
- Davidson T.A., Audet J., Svenning J.-C., Lauridsen T.L., Søndergaard M., Landkildehus F., Larsen S.E. & Jeppesen E. (2015) Eutrophication effects on greenhouse gas fluxes from shallow-lake mesocosms override those of climate warming. *Global Change Biology*, **21**, 4449-4463.
- Deacon E.L. (1981) Sea-air gas transfer: The wind speed dependence. *Boundary-Layer Meteorology*, **21**, 31-37.
- Downing J.A., Prairie Y.T., Cole J.J., Duarte C.M., Tranvik L.J., Striegl R.G., McDowell W.H., Kortelainen P., Caraco N.F., Melack J.M. & Middelburg J.J. (2006) The global abundance and size distribution of lakes, ponds, and impoundments. *Limnology and Oceanography*, **51**, 2388-2397.
- Erlandsen M. & Thyssen N. (1983) Modelling the community oxygen production in low-land streams dominated by submerged macrophytes. In: *Analysis of ecological systems: State of the art in*

- ecological modelling. (Eds W.K. Lauenroth & G.V. Skogerboe & M. Flug), pp. 855-860. Elsevier, Amsterdam.
- Fernández-Martínez M., Vicca S., Janssens I.A., Sardans J., Luyssaert S., Campioli M., Chapin Iii F.S., Ciais P., Malhi Y., Obersteiner M., Papale D., Piao S.L., Reichstein M., Roda F. & Penuelas J. (2014) Nutrient availability as the key regulator of global forest carbon balance. *Nature Climate Change*, **4**, 471-476.
- Geerts A.N., Vanoverbeke J., Vanschoenwinkel B., Van Doorslaer W., Feuchtmayr H., Atkinson D., Moss B., Davidson T.A., Sayer C.D. & De Meester L. (2015) Rapid evolution of thermal tolerance in the water flea *Daphnia*. *Nature Climate Change*, **5**, 665-+.
- Groffman P., Butterbach-Bahl K., Fulweiler R., Gold A., Morse J., Stander E., Tague C., Tonitto C. & Vidon P. (2009) Challenges to incorporating spatially and temporally explicit phenomena (hotspots and hot moments) in denitrification models. *Biogeochemistry*, **93**, 49-77.
- Gudas C., Sobek S., Bastviken D., Koehler B. & Tranvik L.J. (2015) Temperature sensitivity of organic carbon mineralization in contrasting lake sediments. *Journal of Geophysical Research: Biogeosciences*, **120**, 1215-1225.
- Holgerson M.A. & Raymond P.A. (2016) Large contribution to inland water CO₂ and CH₄ emissions from very small ponds. *Nature Geosci*, **9**, 222-226.
- Hongjun W., Wang W., Yin C., Wang Y. & Lu J. (2006) Littoral zones as the “hotspots” of nitrous oxide (N₂O) emission in a hyper-eutrophic lake in China. *Atmospheric Environment*, **40**, 5522-5527.
- Houghton J., Ding D., Griggs M., Noguer P., Dai X., Maskell K. & Johnson C. (2001) Climate Change 2001: The scientific basis. Contribution of working group I to the third assessment report of the intergovernmental panel on climate change., Cambridge.
- Huttunen J.T., Alm J., Liikanen A., Juutinen S., Larmola T., Hammar T., Silvola J. & Martikainen P.J. (2003) Fluxes of methane, carbon dioxide and nitrous oxide in boreal lakes and potential anthropogenic effects on the aquatic greenhouse gas emissions. *Chemosphere*, **52**, 609-621.
- IPCC. (2013) *Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change.*, Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.
- IPCC. (2014) *Climate Change 2014–Impacts, Adaptation and Vulnerability: Regional Aspects*, Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.
- Jankowski T., Livingstone D.M., Bührer H., Forster R. & Niederhauser P. (2006) Consequences of the 2003 European heat wave for lake temperature profiles, thermal stability, and hypolimnetic oxygen depletion: Implications for a warmer world. *Limnology and Oceanography*, **51**, 815-819.
- Jeppesen E., Kronvang B., Meerhoff M., Sondergaard M., Hansen K.M., Andersen H.E., Lauridsen T.L., Liboriussen L., Beklioglu M., Ozen A. & Olesen J.E. (2009) Climate change effects on runoff, catchment phosphorus loading and lake ecological state, and potential adaptations. *Journal of Environmental Quality*, **38**, 1930-1941.
- Jeppesen E., Sondergaard M., Lauridsen T., Liboriussen L., Bjerring R., Johansson L., Landkildehus F., Kronvang B., Andersen H. & Trolle D. (2012a) Recent climate induced changes in freshwaters

539 in Denmark. *Climatic change and global warming of inland waters: impacts and mitigation*
540 *for ecosystems and societies. John Wiley & Son Ltd, 156-171.*

541 Jeppesen E., Søndergaard M., Lauridsen T.L., Davidson T.A., Liu Z., Mazzeo N., Trochine C., Özkan K.,
542 Jensen H.S. & Trolle D. (2012b) Biomanipulation as a restoration tool to combat
543 eutrophication: recent advances and future challenges. *Advances in Ecological Research*,
544 411-487.

545 Jeppesen E., Trolle D., Davidson T.A., Bjerring R., Søndergaard M., Johansson L.S., Lauridsen T.L.,
546 Nielsen A., Larsen S.E. & Meerhoff M. (2016) Major changes in CO₂ efflux when shallow
547 lakes shift from a turbid to a clear water state. *Hydrobiologia*, **778**, 33-44.

548 Jespersen A.-M. & Christoffersen K. (1987) Measurements of chlorophyll—a from phytoplankton
549 using ethanol as extraction solvent. *Archiv Fur Hydrobiologie*, **109**, 445-454.

550 Jähne B., Münnich K.O., Börsinger R., Dutzi A., Huber W. & Libner P. (1987) On the parameters
551 influencing air-water gas exchange. *Journal of Geophysical Research: Oceans*, **92**, 1937-1949.

552 Jöhnk K.D., Huisman J.E.F., Sharples J., Sommeijer B.E.N., Visser P.M. & Stroom J.M. (2008) Summer
553 heatwaves promote blooms of harmful cyanobacteria. *Global Change Biology*, **14**, 495-512.

554 Kelly M.G., Thyssen N. & Moeslund B. (1983) Light and the annual variation of oxygen- and carbon-
555 based measurements of productivity in a macrophyte-dominated river. *Limnology and*
556 *Oceanography*, **28**, 503-515.

557 Kirillin G. & Shatwell T. (2016) Generalized scaling of seasonal thermal stratification in lakes. *Earth-*
558 *Science Reviews*, **161**, 179-190.

559 Kosten S., Huszar V.L.M., Bécares E., Costa L.S., van Donk E., Hansson L.-A., Jeppesen E., Kruk C.,
560 Lacerot G., Mazzeo N., De Meester L., Moss B., Lürling M., Nöges T., Romo S. & Scheffer M.
561 (2012) Warmer climates boost cyanobacterial dominance in shallow lakes. *Global Change*
562 *Biology*, **18**, 118-126.

563 Kosten S., Jeppesen E., Huszar V.L.M., Mazzeo N., Van Nes E.H., Peeters E.T.H.M. & Scheffer M.
564 (2011) Ambiguous climate impacts on competition between submerged macrophytes and
565 phytoplankton in shallow lakes. *Freshwater Biology*, **56**, 1540-1553.

566 Käki T., Ojala A. & Kankaala P. (2001) Diel variation in methane emissions from stands of *Phragmites*
567 *australis* (Cav.) Trin. ex Steud. and *Typha latifolia* L. in a boreal lake. *Aquatic Botany*, **71**, 259-
568 271.

569 Liboriussen L., Landkildehus F., Meerhoff M., Bamm M.E., Søndergaard M., Christoffersen K.,
570 Richardson K., Lauridsen T.L. & Jeppesen E. (2005) Global warming: Design of a flow-through
571 shallow lake mesocosm climate experiment. *Limnology and Oceanography-Methods*, **3**, 1-9.

572 Liboriussen L., Lauridsen T.L., Søndergaard M., Landkildehus F., Søndergaard M., Larsen S.E. &
573 Jeppesen E. (2011) Effects of warming and nutrients on sediment community respiration in
574 shallow lakes: an outdoor mesocosm experiment. *Freshwater Biology*, **56**, 437-447.

575 Liikanen A., Murtoniemi T., Tanskanen H., Vaisanen T. & Martikainen P.J. (2002) Effects of
576 temperature and oxygen availability on greenhouse gas and nutrient dynamics in sediment
577 of a eutrophic mid-boreal lake. *Biogeochemistry*, **59**, 269-286.

578 Lin S. (2001) *Water and Wastewater Calculations Manual.*, McGraw-Hill, USA.

579 Marotta H., Pinho L., Gudas C., Bastviken D., Tranvik L.J. & Enrich-Prast A. (2014) Greenhouse gas
580 production in low-latitude lake sediments responds strongly to warming. *Nature Climate*
581 *Change*, **4**, 467-470.

582 Matveev A., Laurion I., Deshpande B.N., Bhiry N. & Vincent W.F. (2016) High methane emissions
583 from thermokarst lakes in subarctic peatlands. *Limnology and Oceanography*, **61**, S150-S164.

584 McKee D., Hatton K., Eaton J.W., Atkinson D., Atherton A., Harvey I. & Moss B. (2002) Effects of
585 simulated climate warming on macrophytes in freshwater microcosm communities. *Aquatic*
586 *Botany*, **74**, 71-83.

587 Meehl G.A. & Tebaldi C. (2004) More Intense, More Frequent, and Longer Lasting Heat Waves in the
588 21st Century. *Science*, **305**, 994-997.

589 Michaletz S.T., Cheng D., Kerkhoff A.J. & Enquist B.J. (2014) Convergence of terrestrial plant
590 production across global climate gradients. *Nature*, **512**, 39-43.

591 Molot L.A. & Dillon P.J. (1996) Storage of terrestrial carbon in boreal lake sediments and evasion to
592 the atmosphere. *Global Biogeochemical Cycles*, **10**, 483-492.

593 Mooij W.M., De Senerpont Domis L.N. & Hülsmann S. (2008) The impact of climate warming on
594 water temperature, timing of hatching and young-of-the-year growth of fish in shallow lakes
595 in the Netherlands. *Journal of Sea Research*, **60**, 32-43.

596 Moss B. (1990) Engineering and biological approaches to the restoration from eutrophication of
597 shallow lakes in which aquatic plant communities are important components. In:
598 *Biomanipulation Tool for Water Management*. (Eds R. Gulati & E.R.R. Lammens & M.-L.
599 Meijer & E. Van Donk), pp. 367-377. Developments in Hydrobiology. Springer Netherlands.

600 Moss B., Kosten S., Meerhof M., Battarbee R., Jeppesen E., Mazzeo N., Havens K., Lacerot G., Liu Z. &
601 De Meester L. (2011) Allied attack: climate change and eutrophication. *Inland waters*, **1**, 101-
602 105.

603 Petersen S.O., Hoffmann C.C., Schäfer C.M., Blicher-Mathiesen G., Elsgaard L., Kristensen K., Larsen
604 S.E., Torp S.B. & Greve M.H. (2012) Annual emissions of CH₄ and N₂O, and ecosystem
605 respiration, from eight organic soils in Western Denmark managed by agriculture.
606 *Biogeosciences*, **9**, 403-422.

607 Pinheiro J., Bates D., DebRoy S., Sarkar D. & R Development Core Team. (2012) nlme: Linear and
608 Nonlinear Mixed Effects Models. R package.

609 Pinheiro J. & Bates D.M. (2000) *Mixed-Effects Models in S and S-PLUS*, Springer, New-York.

610 Provoost P., van Heuven S., Soetaert K., Laane R.W.P.M. & Middelburg J.J. (2010) Seasonal and long-
611 term changes in pH in the Dutch coastal zone. *Biogeosciences*, **7**, 3869-3878.

612 R Development Core Team. (2014) R: A language and environment for statistical computing. R
613 Foundation for Statistical Computing, Vienna, Austria.

614 Raymond P.A., Caraco N.F. & Cole J.J. (1997) Carbon dioxide concentration and atmospheric flux in
615 the Hudson River. *Estuaries*, **20**, 381-390.

616 Scheffer M., Hosper S.H., Meijer M.L., Moss B. & Jeppesen E. (1993) Alternative equilibria in shallow
617 lakes. *Trends in Ecology & Evolution*, **8**, 275-279.

618 Sobek S., Durisch-Kaiser E., Zurbrügg R., Wongfun N., Wessels M., Pasche N. & Wehrli B. (2009)
619 Organic carbon burial efficiency in lake sediments controlled by oxygen exposure time and
620 sediment source. *Limnology and Oceanography*, **54**, 2243-2254.

621 Tranvik L.J., Downing J.A., Cotner J.B., Loiselle S.A., Striegl R.G., Ballatore T.J., Dillon P., Finlay K.,
622 Fortino K., Knoll L.B., Kortelainen P.L., Kutser T., Larsen S., Laurion I., Leech D.M., McCallister
623 S.L., McKnight D.M., Melack J.M., Overholt E., Porter J.A., Prairie Y., Renwick W.H., Roland F.,
624 Sherman B.S., Schindler D.W., Sobek S., Tremblay A., Vanni M.J., Verschoor A.M., von
625 Wachenfeldt E. & Weyhenmeyer G.A. (2009) Lakes and reservoirs as regulators of carbon
626 cycling and climate. *Limnology and Oceanography*, **54**, 2298-2314.

627 Trolle D., Staehr P., Davidson T., Bjerring R., Lauridsen T., Søndergaard M. & Jeppesen E. (2012)
628 Seasonal Dynamics of CO₂ Flux Across the Surface of Shallow Temperate Lakes. *Ecosystems*,
629 **15**, 336-347.

630 Walter K.M., Zimov S.A., Chanton J.P., Verbyla D. & Chapin F.S. (2006) Methane bubbling from
631 Siberian thaw lakes as a positive feedback to climate warming. *Nature*, **443**, 71-75.

632 Wanninkhof R. (1992) Relationship between Wind-Speed and Gas-Exchange over the Ocean. *Journal*
633 *of Geophysical Research-Oceans*, **97**, 7373-7382.

634 Wanninkhof R. & Knox M. (1996) Chemical enhancement of CO₂ exchange in natural waters.
635 *Limnology and Oceanography*, **41**, 689-697.

636 Weiss R.F. & Price B.A. (1980) Nitrous oxide solubility in water and seawater. *Marine Chemistry*, **8**,
637 347-359.

638 Veraart A.J., de Klein J.J.M. & Scheffer M. (2011) Warming Can Boost Denitrification
639 Disproportionately Due to Altered Oxygen Dynamics. *PLoS ONE*, **6**, e18508.

640 Verpoorter C., Kutser T., Seekell D.A. & Tranvik L.J. (2014) A global inventory of lakes based on high-
641 resolution satellite imagery. *Geophysical Research Letters*, **41**, 2014GL060641.

642 Wiesenburg D.A. & Guinasso N.L. (1979) Equilibrium solubilities of methane, carbon monoxide, and
643 hydrogen in water and sea water. *Journal of Chemical & Engineering Data*, **24**, 356-360.

644 Yoshida N., Iguchi H., Yurimoto H., Murakami A. & Sakai Y. (2014) Aquatic plant surface as a niche for
645 methanotrophs. *Frontiers in Microbiology*, **5**, 30.

646 Yvon-Durocher G., Jones J.I., Trimmer M., Woodward G. & Montoya J.M. (2010) Warming alters the
647 metabolic balance of ecosystems. *Philosophical Transactions of the Royal Society of London*
648 *B: Biological Sciences*, **365**, 2117-2126.

649 Zuur A.F., Ieno E.N., Walker N., Saveliev A.A. & Smith G.M. (2009) *Mixed Effects Models and*
650 *Extensions in Ecology with R*, Springer, New York.

651

652

Table 1. Time-interpolated greenhouse gas fluxes in shallow lake mesocosms over the study period (19-06-2014 to 01-09-2014). All fluxes are expressed as the carbon equivalent, g CO₂ eq m⁻². Total fluxes during the heat wave are also shown (01-07-2014 to 31-07-2014). Results are presented as mean ± standard deviation.

Nutrient	Temperature	g CO ₂ eq m ⁻²								Mean daily flux, pre/post heat wave period	Mean daily flux, heat wave period
		CH ₄	N ₂ O	CO ₂	Total flux whole period				g CO ₂ eq m ⁻² d ⁻¹		
Low	AMB	1.5 ±0.3	2.1 ±1.4	9.4 ±20.0	13.0 ±21.2				0.19 ±0.28		0.14 ±0.29
	A2	1.9 ±0.6	2.1 ±0.5	9.3 ±25.4	11.9 ±25.1				0.02 ±0.31		0.37 ±0.36
	A2+	2.0 ±0.3	2.2 ±1.0	26.5 ±34.2	30.7 ±35.4				0.26 ±0.42		0.62 ±0.55
High	AMB	1.5 ±0.7	2.5 ±1.5	1.9 ±54.9	6.0 ±55.4				-0.14 ±0.39		0.43 ±1.55
	A2	1.4 ±0.4	3.4 ±3.1	-13.1 ±3.5	-8.2 ±4.7				-0.24 ±0.14		0.11 ±0.22
	A2+	2.8 ±0.7	3.3 ±2.4	12.0 ±45.2	18.1 ±46.0				0.17 ±0.57		0.36 ±1.13

Table 2. Results from the linear mixed effect models used to test the effect of nutrient, temperature and heat wave treatments (HW) on GHG emissions – ns, not significant, **, p<0.01; ***, p<0.001. Significant HW indicates that the heat wave treatment (HW1, i.e. July in A2 and A2+) differed significantly from the period outside the heat wave (i.e. HW0: AMB, A2 and A2+ in June and August). The sign between brackets indicates the effect (positive or negative) of the heat wave treatment on the gas flux. “1” indicates that the heat wave treatment (HW1) differed significantly from the treatment without heat wave (HW2, i.e. AMB in July). “0” means that the HW1 treatment did not differ significantly from the HW2 treatment, i.e. the effect of the heat wave did not differ from the effect of the ambient summer temperature (see ‘Material and methods’ and Fig. 1).

Data set	Treatment	CO ₂	CH ₄	N ₂ O
Full	Nutrient	**(-)	ns	ns
	Temperature	ns	ns	ns
	HW	***(+) ⁰	***(+) ¹	***(+) ¹
Low nutrient	Temperature	ns	ns	ns
	HW	***(+) ¹	***(+) ¹	***(+) ⁰
High nutrient	Temperature	ns	ns	ns
	HW	ns	***(+) ⁰	***(+) ⁰

Table 3. Results from the linear mixed models testing the effect of: temperature treatments (A2 and A2+), heat wave (HW), primary production (PP), PVI, Δ PVI, Chl-*a* and Δ Chl-*a* on GHG fluxes. HW1: heat wave period (i.e. July in A2 and A2+; HW2: July in AMB, i.e. no artificial heat wave. The model tested if these treatment levels results differed significantly from those from the period outside the heat wave period (i.e. HW0: AMB, A2 and A2+ in June and August) (see ‘Material and methods’ and Fig. 1).

Data set	Gas	Variables	Slope	Standard error	Df	t-value	p-value
Low-nutrient	CO ₂	(Intercept)	-0.438	0.099	80	-4.43	0.000
		PVI	-0.006	0.001	80	-8.25	0.000
		HW ₁	0.375	0.034	80	11.15	0.000
		HW ₂	0.002	0.046	80	0.03	0.973
		PP	-2.873	0.713	80	-4.03	0.000
	CH ₄	(Intercept)	-0.664	0.073	75	-9.12	0.000
		HW ₁	0.570	0.096	75	5.92	0.000
		HW ₂	0.173	0.134	75	1.28	0.203
	N ₂ O	(Intercept)	4.600	0.221	79	20.77	0.000
		A2	0.229	0.131	9	1.74	0.115
		A2+	-0.145	0.106	9	-1.36	0.206
		HW ₁	0.719	0.110	79	6.56	0.000
		HW ₂	0.186	0.150	79	1.24	0.218
		PP	-4.842	1.661	79	-2.91	0.005
High-nutrient	CO ₂	(Intercept)	-1.268	0.070	81	-18.14	0.000
		Δ Chl- <i>a</i>	-0.006	0.002	81	-3.26	0.002
		HW ₁	0.126	0.111	81	1.14	0.258
		HW ₂	0.388	0.156	81	2.49	0.014
	CH ₄	(Intercept)	-0.791	0.121	79	-6.54	0.000
		Δ Chl- <i>a</i>	-0.007	0.003	79	-2.70	0.009
		HW ₁	0.541	0.138	79	3.92	0.000
		HW ₂	0.348	0.184	79	1.88	0.063
	N ₂ O	(Intercept)	4.184	0.117	79	35.76	0.000
		Chl- <i>a</i>	-0.001	0.000	79	-2.13	0.036
		HW ₁	0.565	0.124	79	4.56	0.000
		HW ₂	0.431	0.169	79	2.54	0.013