Seasonal methane dynamics in high-elevation lakes in the Sierra Nevada California: the role of elevation, temperature, organic matter, and inorganic nutrients

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Abstract

Freshwater lakes are important but poorly constrained sources of methane (CH_4) to the atmosphere due to high, but variable, rates of CH_4 production, as well as limited and inconsistent measurements worldwide. High-elevation lakes have been particularly overlooked—despite their large numbers in mountain ranges around the world, and despite CH_4 dynamics at high elevations may be altered by rapid increases in temperature due to climate change. We examined variations in surface CH_4 concentrations and diffusive fluxes, temperature, dissolved organic matter (DOC), and inorganic nutrients in five montane lakes spanning multiple elevations in the Sierra Nevada of California. Over two years, we found strong and consistent seasonality in CH_4 concentrations in lakes; higher concentrations were typically observed in the warmest months and lower concentrations in fall. Changes in CH_4 concentrations were significantly related to temperature in the majority of the individual lakes ($r^2 = 0.43$-0.81) and related to elevation ($r^2 = 0.39$) and DOC ($r^2 = 0.30$) across lakes. Methane concentrations in lakes at elevations <3000 m were strongly related to temperature, nitrite concentrations and elevation ($r^2 = 0.90$), whereas at elevations >3000 m, CH_4 correlated with dissolved inorganic nitrogen to dissolved inorganic phosphorus ratios and elevation ($r^2 = $

0.48). Our results expand on our understanding of temporal variations in CH$_4$ and demonstrate substantial seasonality in CH$_4$ concentrations and diffusive fluxes in freshwater lakes—suggesting that temporal variation should be considered in large-scale estimates, and may be a predictable function of elevation, temperature, organic matter, and nutrients.

Keywords: high-elevation lakes; lake biogeochemistry; methane concentrations; methane diffusive fluxes; montane lakes; seasonality

Introduction

Methane (CH$_4$) is a potent greenhouse gas with great influence on the world's climate (Hoegh-Guldberg et al. 2018; Reay et al. 2018). While anthropogenic emissions have increased atmospheric CH$_4$ concentrations over the last century, an important baseline contribution of CH$_4$ to the atmosphere comes from natural ecosystems (Conrad 2009; Kirschke et al. 2013). Methane emissions from freshwater lakes are a particularly significant, but poorly constrained, component of natural CH$_4$ emissions. For example, the contribution of freshwater ecosystems (lakes and rivers) to the global CH$_4$ budget is estimated to range from 12% to 32% (with uncertainties ranging from 2% to 47%) of natural emissions (Saunois et al. 2016). More specifically, current estimates of the global CH$_4$ total emissions from freshwater lakes and impoundments range an order of magnitude, from 69 to 204 Tg CH$_4$-C yr^{-1} (DelSontro et al. 2018). These uncertainties stem partly from the substantial spatial and temporal variability in CH$_4$ cycling across and within freshwater lakes, as well as significant under-sampling of this variability. For instance, only a few hundred lakes have been sampled for CH$_4$ emissions out an estimated total of 304 million lakes globally (Downing 2009; Bastviken et al. 2011), and only a handful of studies have measured CH$_4$ variation over time (Xing et al. 2005;

Casper et al. 2009; Palma-Silva et al. 2013; Natchimuthu et al. 2014; Martinez-Cruz et al. 2015). Clearly, additional studies are needed of the spatial and temporal heterogeneity in CH_4 emissions from these ecosystems, as well as their potential controls (Tranvik et al. 2009; Bastviken et al. 2011; Reay et al. 2018).

Variations in CH_4 concentrations in lake water columns are driven by microbial CH_4 production (predominantly via methanogenesis in sediments) and consumption via microbial CH_4 oxidation in surface sediments and the water column (Bastviken et al. 2004). Both of these processes can be altered by changes in environmental conditions and, in general, temperature seems to have a strong positive effect on both of them (Zeikus and Winfrey 1976; Duc et al. 2010; Palma-Silva et al. 2013; Lofton et al. 2014; Marotta et al. 2014; Sepulveda-Jauregui et al. 2018). Moreover, ecosystem-level analyses seem to indicate that as temperatures rise, CH_4 lake concentrations and fluxes will also increase (Natchimuthu et al. 2014; Yvon-Durocher et al. 2014; Rasilo et al. 2015). However, much of our understanding of temperature regulation of CH_4 concentrations in freshwater is derived from laboratory- or field-based experimental temperature manipulations. While these studies are extremely useful for isolating the effects of temperature from other variables, *in situ* temperature may vary in concert with other seasonally changing environmental properties, such as lake organic carbon (C) inputs and nutrients concentrations. For example, the quantities and types of organic C present in lakes during the year influence CH_4 production (Conrad 1999), and this in turn is regulated by nutrient availability (Sepulveda-Jauregui et al. 2018). For CH_4 consumption, on the other hand, temperature may only play a role when neither CH_4 nor DO concentrations are limiting (Harrits and Hanson 1980; Liikanen et al. 2002; Martinez-Cruz et al. 2015). Collectively, these environmental factors may interact to

influence CH_4 production, consumption, and emissions. The degree to which temperature—versus other environmental factors—affects CH_4 emissions from lakes remains largely unknown. Further characterization of this response is necessary as it could result in a positive climate feedback as global temperatures increase.

High-elevation regions are predicted to experience increased air temperatures, reduced lake ice cover, reduced snow-albedo, and changes in cloud cover as a result of climate change (Mountain Research Initiative EDW Working Group et al. 2015; O'Reilly et al. 2015; Sadro et al. 2019). Temperate montane lakes therefore could be disproportionately affected by climate change—including both increases in average temperatures, as well as changes in seasonal variability (e.g., reduced ice cover and a longer growing season). Importantly, high-elevation lakes can exhibit high CH_4 concentrations and fluxes (McCrackin and Elser 2011), but CH_4 cycling is rarely characterized in these lakes. Their contribution to the CH_4 budget may be underrepresented in comparison to tropical or boreal lakes due to a lack of measurements (Saunois et al. 2016), even though they represent around 10% of lakes globally (Verpoorter et al. 2014). The Sierra Nevada of California is home to thousands of such lakes (Melack and Stoddard 1991; Sickman et al. 2003), and this region already displays a long-term warming trend (Sadro et al. 2019). Snow-albedo feedbacks and changes in the type of precipitation will likely further increase warming and reduce snow pack melt in the decades to come (Walton et al. 2016; Sun et al. 2019).

Further reduction in snowpack melt and rain could also affect high-elevation lakes by altering nutrient inputs into freshwater lakes from snowmelt (Williams et al. 2001; Sickman et al. 2003). At the same time, increased nutrient loading from atmospheric

deposition has been shown to alter algal communities and trophic dynamics in high-elevation ecosystems (Baron et al. 2000; Elser et al. 2007; Elser et al. 2009). Atmospheric deposition is a significant source of nutrients to the Sierra Nevada (Aciego et al. 2017) , where changes in nutrient limitation may already be occurring due to human activity in the adjacent San Joaquin Valley (Sickman et al. 2003). Such changes could consequently impact CH_4 cycling in lakes by altering organic C sources and quantities to these ecosystems (Tranvik et al. 2009; West et al. 2016; Reay et al. 2018; Moser et al. 2019). Finally, recent research indicates that the combination of increased temperature and nutrient concentrations can strongly enhance lake CH_4 production and ebullition (Davidson et al. 2018; Sepulveda-Jauregui et al. 2018). An improved understanding of the effects of environmental variation on CH_4 emissions in this ecosystem will help us account for future changes in CH_4 fluxes and better predict long-term climate trends.

We used large natural variations in temperature over time and with increasing elevation to determine the potential importance of temperature controls on CH_4 emissions from montane lakes. High-elevation lakes in the Sierra Nevada represent an ideal experimental system for examining temperature effects on CH_4 emissions within natural freshwater ecosystems because of these strong natural variations. Over two years, we quantified CH_4 concentrations and diffusive emissions in five lakes spanning an elevation gradient in the Sierra Nevada, California (Fig. 1). In addition to temperature variation, we measured two main categories of environmental variation that are likely to affect CH_4 emissions: nutrient (ammonium, nitrite, nitrate, phosphate) concentrations and availability and organic C production and properties (dissolved organic C and specific UV absorbance at 254 nm). Our aim was to answer the following research

questions: 1) To what degree do CH_4 concentrations vary spatially over an elevation gradient and temporally over the seasons in high-elevation lakes, and how does this compare with other lake types? and 2) how much of the variation in CH_4 concentrations within and among lakes can be explained by seasonal fluctuations in environmental parameters (independently or collectively) such as temperature, nutrients and organic C concentrations, as well as lake intrinsic characteristics (e.g., elevation)? We hypothesized that temperature and nutrient concentrations would have the greatest effect on CH_4 concentrations in surface lake water, especially at higher elevations where temperatures are rapidly increasing, and landscapes characteristics contribute less allochthonous nutrient sources to lakes.

Materials and methods

Study site and sample collection

Five high-elevation lakes in the Sierra Nevada of California were selected based on preliminary data showing high microbial production and consumption rates and methanogen and CH_4-oxidizer abundances (Hayden and Beman 2016). Lukens, Lower Cathedral, Upper Cathedral, Lower Gaylor, and Upper Gaylor Lakes have an elevation range of 2489 to 3185 m, and mean summer surface water temperature range of 12.8 to 17.2 ˚C (Fig. 1). Water samples were collected every ~2-3 weeks in the littoral and limnetic zones from July to November of 2016 and 2017 (sampling season) due to field site inaccessibility from December to June. Samples were collected at 0.1 m depth with a previously acid washed plastic or glass containers to measure: CH_4, nitrite (NO_2^-), nitrate (NO_3^-), ammonium (NH_4^+) and phosphate (PO_4^{3-}) concentrations; and dissolved organic carbon (DOC). Temperature (T) and dissolved oxygen (DO) were measured on site using a ProODO YSI probe (YSI Inc., Yellow Springs, OH, USA).

Methane measurements

Methane concentrations were measured via headspace equilibration and gas chromatography. In brief, triplicate water samples were collected directly into 170 ml glass vials, capped with halogenated butyl stoppers, and crimped with aluminum seals to avoid gas loss. Twenty-five ml of water was then replaced with 25 ml of air collected on site, while air samples were collected into 12-mL Labco Exetainer vials (Labco Ltd., Lampeter, Ceredigion, UK). One hundred seventy mL glass vials containing water and headspace were shaken for 2 minutes to reach equilibration, and the headspace was collected with a gas-tight syringe; headspace gas samples were immediately transferred into 12-mL Exetainer vials. Triplicate samples were later analyzed using a Shimadzu GC-2014 gas chromatograph with flame ionization detection (FID) for CH_4 (Weiss 1981). Samples were analyzed within 2-3 months of collection (a time period of up to 15 weeks has been tested and found to result in no significant change in CH_4 concentrations, with 2% or less decrease in 5 ppm CH_4 concentration from storage). Methane standards (Air Liquide, Houston, Texas USA) ranged from 1.5 to 333 ppm and bracketed every 15 samples; standard curve r^2 values ranged from 0.997 - 0.999 across different runs. Headspace CH_4 concentration measurements were then used to calculate CH_4 concentration in lake water based on Henry's law of equilibrium (Yamamoto et al. 1976).

Diffusive methane fluxes

Diffusive CH4 fluxes were calculated with the following equation:

$$F = k \ (C_w - C_a),$$

where F is the flux, k is the gas transfer coefficient, C_w is the dissolved CH4 concentration in the water, and C_a is the concentration of CH4 in the air. The parameter k was estimated from wind-speed data collected from meteorological stations (White Wolf, Tuolumne Meadows, and Tioga Pass stations, each selected for their close proximity to Lukens, Cathedral, and Gaylor Lakes respectively) in Yosemite National Park maintained by the California Department of Water Resources (http://cdec.water.ca.gov) and the relationship developed by Cole and Caraco (1998) for low-wind speeds:

$$k_{600} = 2.07 + \ 0.215U_{10}^{1.7},$$

where k_{600} is the gas coefficient normalized to Schmidt number (Sc) 600, and U_{10} is the wind speed adjusted to 10 m following Amorocho and DeVries (1980).

The k_{600} values were converted to k values using the equation from Bartosiewicz et al. (2015):

$$k = k_{600}(Sc/600)^c,$$

where k is the gas transfer coefficient, c equals -0.67 according to Guérin et al. (2007), and Sc is calculated following Wanninkhof (2014). While there are acknowledged uncertainties associated with calculating k from wind-speed models, we aimed to provide an estimate of diffusive emissions from high-elevation lakes that is lacking in

the literature. However, these estimated diffusive fluxes were not included in further statistical analyses.

Nutrients and organic matter

For nutrient measurements, water collected in the lakes was filtered (0.22 µm) and analyzed for NH_4^+, NO_2^-, NO_3^-, and PO_4^{3-}. Ammonium and NO_2^- were analyzed using a Trilogy Laboratory Fluorometer (Turner Designs; San Jose, CA, USA) with NH_4^+ and NO_2^- modules using the fluorescent method of Holmes et al. (1999) and the sulfanilamide coloration method (APHA 1998), respectively. Samples were frozen at -20 °C after collection and stored for no longer than two months (Chapman and Mostert 1990). Standards ranged from 31 to 186 nM for NH_4^+ and 0 to 10,000 nM for NO_2^-, and standard curve r^2 values ranged from 0.997 to 0.999 for different runs. Nitrate and phosphate were analyzed using flow injection analysis on a QuikChem 8000 (Zellweger Analytics; Concord, ON, CA) at the University of California, Santa Barbara, Marine Sciences Institute Analytical Laboratory (standard curves $r^2 = 0.996$ and $r^2 = 0.997$, respectively).

Samples for DOC were only collected in 2017. Water was filtered through a GFF Whatman filter and collected in glass vials what had been combusted previously in a muffle furnace. Samples for DOC were acidified to pH 2 with 2 M HCl and analyzed in a TOC analyzer (Shimadzu TOC-Vcsh Total Organic Carbon Analyzer, Kyoto, Japan) at the Environmental Analytical Laboratory at University of California, Merced; standards ranged from 0 to 25 mg L^{-1}, with a standard curve $r^2 = 0.999$. Samples for dissolved organic matter (DOM) absorbance were kept in the dark at 4 °C for no longer than 5 days until analyzed in a Genesys 10 UV spectrophotometer for absorption at 254

nm (Thermo Scientific; Madison, WI, USA). The specific UV absorbance at 254 nm

($SUVA_{254nm}$) was calculated by dividing measured 254 nm absorbance per m of path

length values by the DOC concentration of each sample. These $SUVA_{254nm}$ values have

been used as indices of DOC aromaticity (e.g.,Weishaar et al. 2003).

Apparent oxygen utilization (AOU) was calculated from the following equation:

$$AOU = O_{2,sat} - O_2,$$

where $O_{2,sat}$ is the saturation oxygen concentration dependent on temperature and

salinity and O_2 is the observed oxygen concentration.

Statistical analyses

We tested relationships between CH_4 concentrations in lake surface water and the

following individual environmental variables using linear regression: T, DO, NO_2^-,

NO_3^-, NH_4^+, PO_4^{3-}, ratios of dissolved inorganic nitrogen to dissolved inorganic

phosphorus (DIN:DIP), DOC, $SUVA_{254nm}$, and elevation. We also used these same

variables in standard multiple-linear regression analyses to predict CH_4 concentrations.

Predictor variables and multi-linear models were selected based upon adjusted R^2

values, Aikake Information Criteria (AIC) of goodness of fit, and model significance. A

priori significance level was defined as $\alpha<0.05$. We ran multi-linear regressions for the

pooled data as well as for mid-elevation (Lukens, Upper and Lower Cathedral Lakes)

and upper-elevation (Lower and Upper Gaylor Lakes) lakes separately. Data were

assessed to ensure they met the assumptions of regression (independence of

observations, homoscedasticity, and normality of residuals). Methane concentrations

were natural-log-transformed due to non-normality of residuals and heteroscedasticity,

but the predictor variables met all linear regression assumptions previously mentioned.

All statistical analyses were performed using IBM SPSS Statistics for Macintosh, Version 25.0. Graphs were developed with Plotly Technologies Inc. Chart Studio (Cambridge, MA, USA) and SPSS (Chicago, IL, USA).

Results

Methane concentrations and diffusive fluxes within and across lakes

Methane concentrations and fluxes in lakes are often measured at single time points, even though they may have a highly dynamic nature. In our study, CH_4 concentrations showed large variations across lakes and over time, ranging from 16 to 3679 nM (Fig. 2a). All of these values were supersaturated, indicating that all lakes were net sources of CH_4 to the atmosphere at all times (Table 1). Within individual lakes, the highest and most variable CH_4 concentrations were found in Lukens and Lower Gaylor Lakes. Lukens is located at the lowest elevation and generally showed the highest CH_4 concentrations—although the single highest value was observed in Lower Gaylor, the majority of concentrations >500 nM occurred in Lukens Lake (Fig. 2a). Methane concentrations were highest in Lukens Lake in August in both years. In 2016, CH_4 concentrations showed a clear peak, while in 2017, CH_4 ranged from 1000 to1500 nM before declining later in September to a November minimum. Lower Gaylor had highly variable CH_4 concentrations in 2017, with an abnormally high value of 3679 nM in July 2017. Unlike the rest of the lakes, Lower Gaylor CH_4 concentrations were also significantly elevated at the end of the sampling season in late September and early October 2017, when temperatures were lowest. However, during 2016, CH_4 concentrations and patterns in Lower Gaylor Lake were more similar to the other lakes—values were <500 nM, with the highest values in summer. Methane

concentrations were typically confined to a narrow range within both Cathedral Lakes, ranging from 132 to 354 nM in Lower Cathedral and from 129 to 356 nM in Upper Cathedral, with the highest concentrations occurring in July through August for both lakes. Upper Gaylor is located at the highest elevation and presented the lowest surface CH_4 concentrations, ranging from 16 to 280 nM. In both 2016 and 2017, CH_4 concentrations were the highest earlier on the sampling season. Similar to the other lakes, CH_4 concentrations reached a minimum at the end of the sampling season. All lakes therefore showed significant temporal variation in dissolved CH_4 concentrations.

Diffusive CH_4 fluxes ranged from 0.007 to 2.3 mmol m^{-2} day^{-1} (Fig. 2b, Table 1). Lukens Lake exhibited higher diffusive fluxes during 2016 than 2017 (Fig. 2b, Table 1), and diffusive fluxes were generally higher in August through September (with the exception of July 2017), while Cathedral and Upper Gaylor lakes observed fluxes of >0.2 mmol m^{-2} day^{-1} during the whole sampling season for both 2016 and 2017. Lower Gaylor diffusive fluxes were low overall in 2016 (0.03-0.3 mmol m^{-2} day^{-1}), but higher and more variable in 2017, when we observed the highest diffusive flux in July (2.3 mmol m^{-2} day^{-1}) followed by the lowest in August (0.08 mmol m^{-2} day^{-1}).

Seasonal and elevational variation in methane and temperature

Along with variation in CH_4, temperature showed expected seasonal and elevational variation within and across lakes. We observed similar seasonal trends in all lakes, where peak temperatures occurred in late July and early August, and temperatures were lowest at the end of the sampling season in October through November for both 2016 and 2017 (Fig. 2c). Within individual lakes, Lukens Lake is located at the lowest elevation (2489 m) and experienced the highest temperatures, ranging from 11.6 ˚C to 22.4 ˚C. At higher elevations, Lower Cathedral reached the highest temperature on July

2016 at 21.0˚C, and the lowest on October 2017 at 10.9 ˚C. Upper Cathedral had a similar temperature range (11.6-20.5 ˚C) and timing of peak values. The two lakes at the highest elevations presented the lowest temperatures at the beginning of the sampling season during ice thaw, and again at the end of sampling season in October, when temperatures declined to 7.9 ˚C in Lower Gaylor and 7.7 ˚C in Upper Gaylor. Highest temperatures in these lakes occurred in July 2016: 18.8 ˚C for Lower Gaylor and 15.5 ˚C for Upper Gaylor. Temperature seasonal trends were consistent for both 2016 and 2017; these temporal variations emphasize the large temperature range that high-elevation lakes display and the effect of elevation on their average temperatures.

Given coincident seasonal variations in CH_4 concentrations and temperature, consistent differences between lakes at different elevations, and previous work demonstrating temperature effects on CH_4 emissions, we analyzed potential relationships between temperature and CH_4 concentrations (Fig. 3a). Within individual lakes, CH_4 concentrations correlated with seasonal temperature variations in lake water in Lukens ($r^2 = 0.65$, $p<0.005$, n = 12), Lower Cathedral ($r^2 = 0.53$, $p<0.05$, n = 8) and Upper Cathedral Lake ($r^2 = 0.81$, $p<0.005$, n = 8). Methane concentrations were not significantly related to temperature in Upper Gaylor Lake, and were inversely related to temperature in Lower Gaylor Lake ($r^2 = 0.43$, $p<0.05$, n = 11). Data pooled across all lakes showed no significant correlation between CH_4 concentrations and temperature, likely due to the different patterns observed in the individual lakes. In particular, lower elevation lakes with more substantial temperature variation showed stronger correspondence between temperature and CH_4. We therefore analyzed relationships with elevation and found that elevation was the most significant factor determining CH_4 concentrations in the water—overall, the lower the elevation, the higher the CH_4

concentration in the lake ($r^2 = 0.39$, $p<0.005$, n = 49). Temporal variations within individual lakes are superimposed on this overall pattern.

Organic matter production and composition

To examine potential relationships between CH_4 concentrations and organic C dynamics, we measured DOC and SUVA in 2017, and DO in 2016 and 2017. DOC followed an elevational trend (Table 1); Lukens Lake presented the highest DOC values (2.54 - 3.74 mg L^{-1}), followed by Lower and Upper Cathedral Lakes (1.36 – 2.78 mg L^{-1} and 1.65 – 1.97 mg L^{-1}, respectively), and Lower Gaylor presented the lowest values most of the season (1.29 – 2.58 mg L^{-1}). We observed a DOC maximum in August and a minimum in October for all lakes in 2017. Higher CH_4 concentrations correlated with higher DOC concentrations in lake water for the measurements taken from all lakes ($r^2 = 0.30$, $p<0.05$, n = 16; Supplemental Fig. 2a). Methane concentrations were also significantly correlated with $SUVA_{254nm}$ ($r^2 = 0.23$, $p<0.05$, n = 16; Supplemental Fig. 2b).

We also measured DO, as it (1) is affected by changes in temperature, (2) can integrate changes in production and consumption of organic matter, and (3) affects the redox favorability of CH_4 production and oxidation in sediments and water column. During both 2016 and 2017, DO increased over the summer and fall with the lowest values in July and the highest in October (Fig. 4a). Dissolved oxygen ranged from 6.0 to 8.0 mg L^{-1} in Lukens Lake, 6.0 to 7.8 mg L^{-1} in Lower Cathedral Lake, and 5.0 to 7.7 mg L^{-1} in Upper Cathedral Lake. Gaylor Lakes, located at the highest elevation, presented the highest DO, with Lower Gaylor ranging from 5.0 to 8.7 mg L^{-1} and Upper Gaylor ranging from 6.0 to 8.9 mg L^{-1}. Methane concentrations were inversely correlated with

DO in Lukens Lake ($r^2 = 0.69$, $p<0.005$, n = 11), while correlations for the other lakes individually or using pooled data across all five lakes were not significant. Given that variations in temperature likely affect observed DO concentrations, we calculated apparent oxygen utilization (AOU) from the difference between DO values expected in equilibrium with the atmosphere at different temperatures versus those observed. Apparent oxygen utilization showed consistent seasonal trends in most of the lakes (Figure 4b). However, CH_4 concentrations were not significantly related to AOU.

Inorganic nutrients dynamics

We measured three forms of dissolved inorganic nitrogen (DIN; ammonium, nitrite, and nitrate), as well as dissolved inorganic phosphorus (DIP; phosphate). High-elevation lakes are typically nutrient depleted (Sickman et al. 2003; Moser et al. 2019), and the lakes in this study are no exception, with low concentrations of all measured inorganic nutrients (Fig. 5).

Ammonium concentrations were low and variable (0.18 to 2.83 µM, with the majority of NH_4^+ concentrations <1.5 µM), but in contrast to CH_4, NH_4^+ concentrations did not display a seasonal trend and CH_4 was not significantly related to NH_4^+ (Fig. 5a). Both NO_2^- and NO_3^- concentrations were also low over the period studied, typically ranging from 0 to 1 µM (Fig. 5b and 5c). Nitrite trends in the lakes differed from year to year. In 2016, overall variation was higher, and there were no discernible seasonal trends; in 2017, all NO_2^- concentrations were uniformly <0.3 µM, and we observed a seasonal trend for Lukens, Lower and Upper Cathedral Lakes. Methane concentrations were statistically significantly correlated to NO_2^- in lake water in Lukens ($r^2 = 0.41$ $p<0.05$, n = 12) and Upper Cathedral ($r^2 = 0.52$, $p<0.05$, n= 8). Methane concentrations in Lower

and Upper Cathedral Lakes were also significantly related to NO_3^- in the water ($r^2 = 0.64$, $p<0.05$, n = 7 for Lower Cathedral and $r^2 = 0.67$, $p<0.05$, n = 8 for Upper Cathedral). For both NO_2^- and NO_3^-, there were no other significant relationships for individual lakes or the pooled lake data. Phosphate concentrations remained below 0.8 µM in these oligotrophic mountain lakes (Fig. 5d). Phosphate concentrations were more variable in 2016 (0.1 to 0.8 µM) compared to 2017 (0.0 to 0.2 µM), especially for Lukens and Lower and Upper Gaylor Lakes. However, none of the lakes showed discernible seasonal trends over the summer (although PO_4^{3-} tended to be lowest at the end of the sampling season in October), and CH_4 was not significantly related to PO_4^{3-}.

The DIN:DIP ratios were typically low, ranging from 3 to 18 for 2016 and 2 to 25 in 2017 (Fig. 5e). Low DIN:DIP ratios indicate that the lakes studied are mainly N limited (DIN:DIP < 10), with fewer cases of DIN:DIP ratios indicative of co-limitation (10 - 17), or P limitation (DIN:DIP > 17; Morris and Lewis 1988; Nürnberg and Shaw 1998). Upper Cathedral CH_4 concentrations showed a negative correlation with DIN:DIP ratio ($r^2 = 0.64$, $p<0.05$, n = 8), while the other lakes individually and overall nutrient dataset were not significantly related to CH_4 concentrations.

Multi-linear model for CH_4 concentrations in high-elevation lakes

Given the seasonal and elevation-related patterns observed in high elevation lakes, we tested for statistically significant relationships between CH_4 and potential explanatory variables using multiple linear regression. Pooling all lakes together showed that the only significant predictor variable across all lakes was elevation (Table 2); however, individual lakes displayed strong temperature responses, especially at lower elevations. As a result, we classified lakes into two elevational bands (mid- and upper elevation) to

better understand relationships at different elevations (Table 2). The multilinear

regression in the mid-elevation band showed that CH_4 concentrations in lake water were

significantly correlated to temperature, elevation, and NO_2^-; in the upper-elevation lake

band, CH_4 concentrations were significantly related to elevation and DIN:DIP ratios.

Methane concentration in individual lake surface water and in the pooled lake data was

consistently related to elevation, as well as in the elevation band regressions. Contrary

to our hypothesis, CH_4 concentrations in mid-elevation lakes, but not upper-elevation

lakes, were positively correlated to temperature over the growing season.

Discussion

Seasonal variation in methane

Our findings have multiple implications for our understanding of CH_4 biogeochemistry

in freshwater ecosystems. First and most fundamentally, significant seasonal variability

was clearly evident in CH_4 concentrations in Sierra Nevada lakes, as highest CH_4

concentrations were observed in the warmest months, and lowest CH_4 concentrations

were typically observed at the end of the sampling season before winter. Field site

accessibility prevented taking measurements during the winter and spring seasons, but

Greene et al. (2014) and Jammet et al. (2015) showed that there can be significant CH_4

release during ice-off due to the build-up of CH_4 under ice throughout winter and

spring. While this phenomenon was not captured here and could be significant, it

underlines our over-arching finding that dissolved CH_4 can be highly variable over time.

Second, our data indicate that single time point measurements of CH_4 concentrations in

lakes do not adequately reflect overall seasonal CH_4 diffusive flux, as CH_4

concentrations varied 3- to 73-fold over time in our study. Previous seasonal CH_4 measurements have been conducted in only a handful of lakes worldwide and also show significant variation. For example, Casper et al. (2009) observed a seasonal cycle in a single lake in Germany, where CH_4 lake concentrations and fluxes increased in the summer and decreased by winter. Similarly, Palma-Silva et al. (2013) detected higher CH_4 concentrations in one oligotrophic and one eutrophic lake in Brazil when higher temperatures were observed—a finding shared for a single shallow pond in Sweden (Natchimuthu et al. 2014), and for a single subtropical lake in China (Xing et al. 2005). Contrarily, Martinez-Cruz et al. (2015) found the opposite pattern for thirty Alaskan Lakes, where CH_4 concentrations were on average lower during the summer and higher during the wintertime due to changes in lake ice cover. Taken together, these limited data indicate that seasonal CH_4 variations can be significant, and our results provide additional context from five contrasting, high-elevation lakes in the Sierra Nevada.

Third, changes in the length of the growing season will likely increase overall CH_4 diffusive flux if periods of high CH_4 concentrations in lake water expand in time. This may be especially relevant for high-elevation lakes, where warmer air temperatures will increase the lake ice-free period, increase water temperature, and potentially increase organic matter and nutrient inputs from the surrounding watershed—at least in the short-term (Moser et al. 2019; Sadro et al. 2019). In line with this idea, our results showed that CH_4 concentrations in high-elevation lakes were most strongly correlated with elevation, with higher average CH_4 concentrations at lower elevations. Elevation can be a proxy for temperature, as lower elevations showed the lowest mean temperatures (see below). If high-elevation lakes shift to resemble lower-elevation lakes, the strong correlation with elevation suggests increased CH_4 concentrations.

However, both organic C concentrations and composition, as well as nutrient concentrations, may also vary with elevation. In general, lakes at higher elevation tend to be more oligotrophic, as allochthonous sources of C and nutrients become more scarce in alpine and sub-alpine regions (Urmy and Warren 2019). We therefore evaluated organic matter and nutrients as potentially relevant factors for CH_4 cycling in lakes that are also elevation dependent.

Relationships between methane, organic matter production and composition, and dissolved nutrients

The quality and quantity of organic matter can affect methanogenesis in lakes because organic matter provides substrates for CH_4 production, and affects oxygen availability in sediments due to heterotrophic aerobic respiration (Tranvik et al. 2009; Grasset et al. 2018; Sepulveda-Jauregui et al. 2018). We found that patterns in DOC and $SUVA_{254nm}$ were consistent with lake elevation and watershed characteristics (Table 1). In particular, Lukens Lake is located at the lowest elevation with a surrounding meadow, and likely has a larger input of allochthonous C, while Lower and Upper Cathedral Lakes are at higher elevation where allochthonous C would be relatively lower. Lower and Upper Gaylor Lakes are located above the tree line where DOC levels tend to be lower (Moser et al. 2019). Consistent with this, DOC concentrations were the highest at the lowest elevation and decreased with increases in elevation (Table 1). The specific UV absorbance at 254 nm is a useful measure to perceive changes in organic C over the growing season. Low $SUVA_{254nm}$ is indicative of overall low molecular weight (Chowdhury 2013) and low percent aromaticity (Weishaar et al. 2003). Allochthonous sources of organic C are often complex molecules (aliphatic polymers, humic substances) that are mainly degraded under aerobic conditions, whereas autochthonous

sources can be mineralized under both aerobic and anaerobic conditions (Zehnder and Svensson 1986; Hulthe et al. 1998; Bastviken et al. 2004). In our study, the inverse correlation between CH_4 concentrations and $SUVA_{254nm}$ values (Supplemental Fig. 2) suggests that simple C molecules favor enhanced CH_4 production.

High-elevation lakes are also often oligotrophic, such that changes in nutrient concentrations can affect the overall ecology of the lake. Higher nutrient input to lakes can alter lake community structure and enhance primary productivity and CH_4 production (Tranvik et al. 2009; West et al. 2016; Reay et al. 2018). Overall, we found low concentrations of all dissolved inorganic nutrients—consistent with oligotrophic conditions prevalent in high-elevation lakes. Although both nitrogen (N) and phosphorus (P) may be limiting nutrients in freshwater ecosystems (Elser et al. 2007), P availability is particularly relevant to the CH_4 paradox (i.e., the observation of consistent supersaturation of CH_4 in freshwater and marine oxic surface waters; Karl et al. 2008; Tang et al. 2014). Several studies have proposed that biological mechanisms other than traditional methanogenesis produce CH_4 (Grossart et al. 2011; Bogard et al. 2014; Tang et al. 2016; Bižić et al. 2020) and one mechanism that may be particularly important in oligotrophic ecosystems is the demethylation of methyl phosphonate. This can be performed by multiple groups of bacteria using C-P lyase genes during the degradation of DOM (Repeta et al. 2016; Yao et al. 2016; Wang et al. 2017), and has been observed in both freshwater (Yao et al. 2016; Wang et al. 2017) and marine (Karl et al. 2008; Metcalf et al. 2012; Carini et al. 2014; Repeta et al. 2016) ecosystems.

In our study, we found low but detectable PO_4^{3-} concentrations through most of the ice-free season in most lakes. This result is consistent with a long-term study of Emerald

Lake in the Sierra Nevada (Sickman et al. 2003). We focused on inorganic nutrient concentrations because previous work has shown that ecological changes associated with nutrient enrichment can affect CH_4 fluxes, and changes in bioavailable P could affect CH_4 production via methyl phosphonate breakdown. In particular, we might expect that low P availability overall, or in comparison to N, might result in increased microbial methylphosphonate breakdown, and therefore increased CH_4 production. However, we did not observe significant relationships between CH_4 concentrations and DIP.

Instead, CH_4 was related to dissolved nitrite, nitrate, and DIN:DIP ratios within some individual lakes and in multiple linear regression (Supplemental Fig. 1, Table 2). While CH_4 concentrations were not as consistently related to inorganic nutrients as they were to temperature and elevation, they still displayed significant correlations in some of the high-elevations lakes studied here (Supplemental Fig. 1). Higher elevation lakes exhibit lower nutrient and DOC concentrations than mid-elevations lakes (Moser et al. 2019; Urmy and Warren 2019); in this area, microbial activities may be constrained by nutrient concentrations. Previous studies have found that alpine ecosystems are highly sensitive to modest N deposition (Baron et al. 2000; Wolfe et al. 2003; Vinebrooke et al. 2014). Consistently, lakes in this study were likely N-depleted for the majority of the season, which may explain why CH_4 concentrations were predicted to be higher when N was more available either in the NO_2^- form or as a higher DIN:DIP ratio.

Methane dynamics in relation to elevation and temperature

We found significant relationships between CH_4 concentrations and elevation and temperature. Highest CH_4 concentrations were observed at the lowest elevation lake and

decreased with elevation. While associated with elevation, temperature was also correlated with CH_4 concentrations measured over time in the mid-elevation lakes (<3000 m, Lukens, Upper and Lower Cathedral Lakes). In our study, lakes located at different elevations with different mean temperatures showed distinct seasonal patterns in temperature and CH_4 concentrations. Likewise, lakes sampled in earlier work also varied in the strength of CH_4-temperature relationships (Xing et al. 2005; Casper et al. 2009; Palma-Silva et al. 2013; Natchimuthu et al. 2014), but showed that temperature increases have overall a corresponding positive response on lake CH_4 concentrations (Natchimuthu et al. 2014; Marotta et al. 2014; Yvon-Durocher et al. 2014; Rasilo et al. 2015). Our data provide additional evidence of positive temperature-CH_4 relationships in several lakes.

Collectively, these observations lend support to the hypothesis that CH_4 concentrations are typically closely related to temperature, but other factors that vary seasonally or with elevation may also be important in regulating CH_4 concentrations in lakes. In particular, CH_4 concentrations were correlated with temperature, elevation, and NO_2^- in mid-elevation lakes. In contrast, the lack of a temperature response in the upper-elevation lakes indicates that CH_4 concentrations may be affected by other factors. For example, nutrient availability regulates lake productivity, and nutrient increases have been correlated with enhanced CH_4 emissions (Palma-Silva et al. 2013; West et al. 2016). Disentangling the relative influence of temperature, nutrients, and C in high elevation lakes may be achieved through additional experimental work, as our measurements indicate that all of these factors can be significantly related to CH_4 concentrations.

Methane ebullitive fluxes were not measured in this study but can be a major contribution to total lake CH_4 emissions, especially in shallow lakes (Bastviken et al. 2004; DelSontro et al. 2016). Lake CH_4 ebullitive emissions have been found to be significantly sensitive to increases in temperature and changes in nutrient concentrations (DelSontro et al. 2016; Aben et al. 2017; Davidson et al. 2018) and deserve further research. Combining these data from five Sierran lakes with additional observations (including ebullitive fluxes) from other temperate montane lakes may result in the development of robust, multivariate predictive models to accurately predict CH_4 concentrations and emissions over space and time as the climate continues to warm. Concentrations of CH_4 in high elevation lakes are notably high and variable, and are indicative of dynamic CH_4 cycling. Our study provides additional fundamental information on freshwater CH_4 biogeochemistry in montane lakes; these data should be useful in the development of predictive models of CH_4 fluxes from freshwater ecosystems under this current period of rapid global change.

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Tables and figures

Table 1. Summary of descriptive statistics of environmental parameters measured from early July to late October 2016 and 2017 for five temperate montane lakes in the Sierra Nevada, California, USA.

	All				Lukens				Lower Cathedral				Upper Cathedral				Lower Gaylor				Upper Gaylor			
	N	Min	Max	Mean	N	Min	Max	Mean	N	Min	Max	Mean	N	Min	Max	Mean	N	Min	Max	Mean	N	Min	Max	Mean
Methane (nM)	49	16	3679	553	12	562	2839	1226	8	132	354	216	8	129	356	251	12	50	3679	652	9	16	280	93
Methane (% saturation)	49	461	104,951	17,758	12	16,800	93,800	41,300	8	4000	11,300	7000	8	4000	11,300	8400	12	1600	104,951	18,800	9	461	8500	2800
Diffusive methane flux (mmol m^{-2} day^{-1})	47	0.007	2.3	0.3	12	0.1	1.6	0.66	8	0.05	0.2	0.11	8	0.06	0.2	0.13	11	0.03	2.3	0.4	8	0.007	0.2	0.05
Temperature (°C)	47	7.7	22.4	15.6	12	11.2	22.4	17.2	8	10.9	21	16.7	8	11.6	20.5	17.4	11	7.9	18.8	13.8	8	7.7	15.5	12.8
Dissolved oxygen (mgL^{-1})	43	5	8.9	7.3	11	6	7.9	7.2	8	6	7.8	7	7	5	7.7	6.9	10	5	8.7	7.6	7	6	8.9	7.7
Dissolved oxygen (% saturation)	42	60	81.5	74.6	11	65	81.5	75.3	8	70	80	74.8	7	60	76.6	71.3	9	60	78.1	76.4	6	70	80	74
Apparent oxygen utilization (mgL^{-1})	43	-1.43	1.37	-0.3	11	-0.76	0.99	-0.04	8	-0.49	0.26	-0.15	7	-0.73	1.37	-0.13	10	-1.43	1.3	-0.48	7	-1.17	0.93	-0.47
Nitrite (µM)	48	0	0.7	0.2	12	0	0.7	0.2	7	0.0	0.44	0.2	8	0	0.3	0.2	12	0	0.4	0.2	9	0	0.4	0.2
Nitrate (µM)	46	0	1.9	0.4	12	0	0.8	0.3	7	0	1.34	0.5	8	0.1	0.4	0.3	12	0	1.9	0.6	7	0.2	0.9	0.4
Ammonium (µM)	48	0.3	2.8	0.9	12	0.5	1.9	0.7	7	0.3	2.4	0.8	8	0.3	1.2	0.7	12	0.5	2.8	1.3	9	0.3	1.9	0.8
Phosphate (µM)	46	0	0.8	0.2	12	0.1	0.4	0.2	7	0.1	0.2	0.1	8	0	0.2	0.1	12	0.1	0.8	0.3	7	0.1	0.4	0.2
Dissolved	46	2.5	25.4	8.9	12	2.5	17.7	6.7	7	4.2	24.7	10.1	8	6.4	20.3	10.9	12	3.5	25.4	9.5	7	3.9	13.4	7.8

inorganic nitrogen :dissolved inorganic phosphorus																							
Dissolved organic carbon (mgL^{-1})	16	1	10.7	2.5	4	2.5	10.7	5	4	1.4	2.78	1.9	3	1.65	1.97	1.8	4	1.3	2.6	1.7	1		1
Specific UV absorbance at 254 nm (L mg–C^{-1} m^{-1})	16	0.5	2.7	1.6	4	0.5	1.9	1.5	4	1.2	2.36	1.9	3	1.8	2.3	2.3	4	0.9	1.3	1.1	1		1.5
Elevation (m)	49	2489	3185	2891.3			2489			2815			2905			3115							3185
Maximum depth sampled (m)	49	3	13	8.2			5			11			3			13							8

Table 2. Multi-linear regression models for methane (CH_4) surface concentrations as a function of environmental parameters measured in five Sierra Nevada montane lakes. Abbreviations: NO_2^- = nitrate and DIN:DIP = dissolved inorganic nitrogen to dissolved inorganic phosphorus ratio.

Lakes	Equation	R^2 adjusted	*P*-value	n
All Lakes	Predicted $\ln(CH_4)$ = 5.650 - 0.003(Elevation)	0.39	<0.005	49
Mid-elevation cluster (>3000 m) (Lukens, Lower and Upper Cathedral)	Predicted $\ln(CH_4)$ = 6.079 +0.082(Temperature) - 0.004(Elevation)+1.268(NO_2^-)	0.90	<0.0005	27
Upper-elevation cluster (<3000 m) (Lower and Upper Gaylor)	Predicted $\ln(CH_4)$ = 5.128 - 0.022(Elevation) + 0.092 (DIN:DIP)	0.48	<0.005	19

Lukens Lake	Lower Cathedral	Upper Cathedral	Lower Gaylor	Upper Gaylor
Elevation: 2489 m Latitude: 37.8598 Longitude: 119.6160 Mean Temperature: 17.2 °C Mean CH_4 concentration: 1226 nM Depth: 5 m	Elevation: 2815 m Latitude: 37.8450 Longitude: 119.4241 Mean Temperature: 16.7 °C Mean CH_4 concentration: 216 nM Depth: 11 m	Elevation: 2905 m Latitude: 37.8395 Longitude: 119.4154 Mean Temperature: 17.4 °C Mean CH_4 concentration: 251 nM Depth: 3 m	Elevation: 3115 m Latitude: 37.9093 Longitude: 119.2862 Mean Temperature: 13.8 °C Mean CH_4 concentration: 652 nM Depth: 13 m	Elevation: 3185 m Latitude: 37.9223 Longitude: 119.2673 Mean Temperature: 12.8 °C Mean CH_4 concentration: 93 nM Depth: 8 m

Figure 1. Morphology and environmental characteristics of five temperate montane lakes in the Sierra Nevada, California, USA. Depth represents the maximum depth observed in each lake. Mean surface temperature and methane concentration were calculated over the sampling period July-October 2016 and July-October 2017.
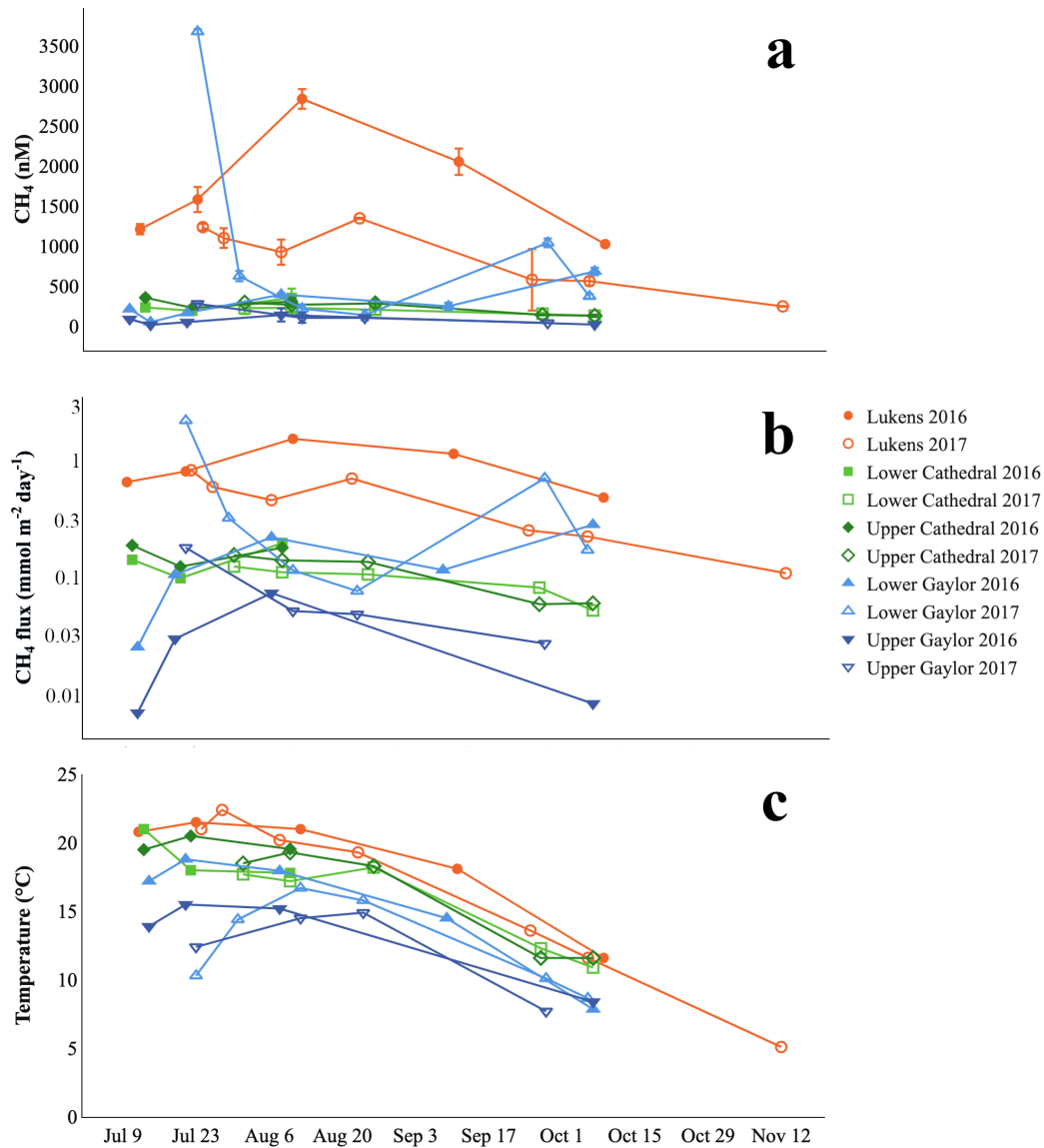
Figure 2. a) Dissolved methane concentrations (error bars represent standard deviation of triplicate water samples), b) methane effluxes (natural log transformed CH_4 diffusive flux), and c) water temperature in five temperate montane lakes in the Sierra Nevada, California, USA. Colored symbols denote different lakes sampled in 2016 (open symbols) and 2017 (closed symbols), with the date of sampling along the horizontal axis
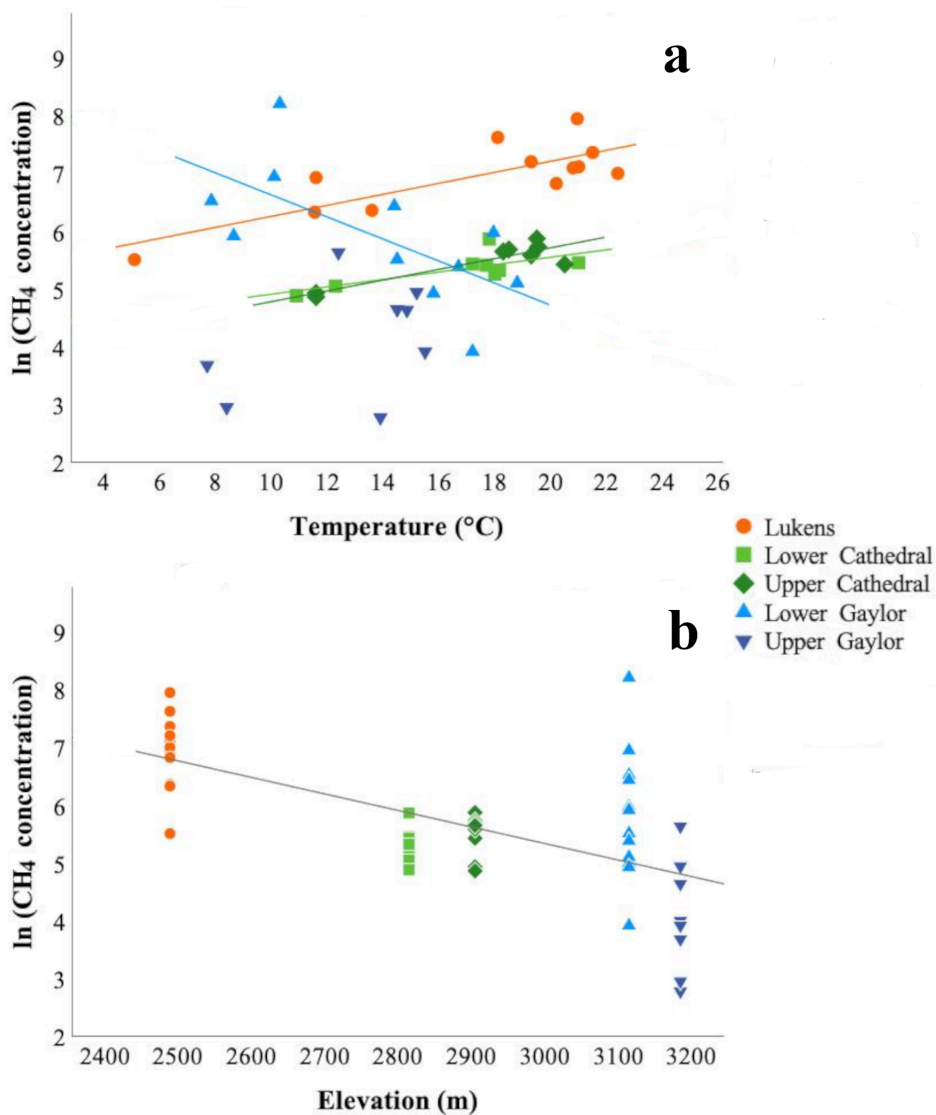
Figure 3. Dissolved methane concentrations in surface water (natural log transformed CH_4 concentrations) as function of a) temperature, and b) elevation for the five temperate montane lakes sampled in the Sierra Nevada, California, USA. Colored symbols denote different lakes sampled. Colored lines represent significant linear relationships for individual lakes (Lukens: ln (CH_4) = 5.31 + 0.1*Temperature; Lower Cathedral: ln (CH_4) = 4.27 +0.06*Temperature, Upper Cathedral: ln (CH_4) = 3.85 +0.09*Temperature; and Lower Gaylor: ln (CH_4) = 8.54 – 0.19*Temperature). Black line represents a significant linear relationship across all lakes (ln (CH_4) = 5.650 - 0.003*Elevation)
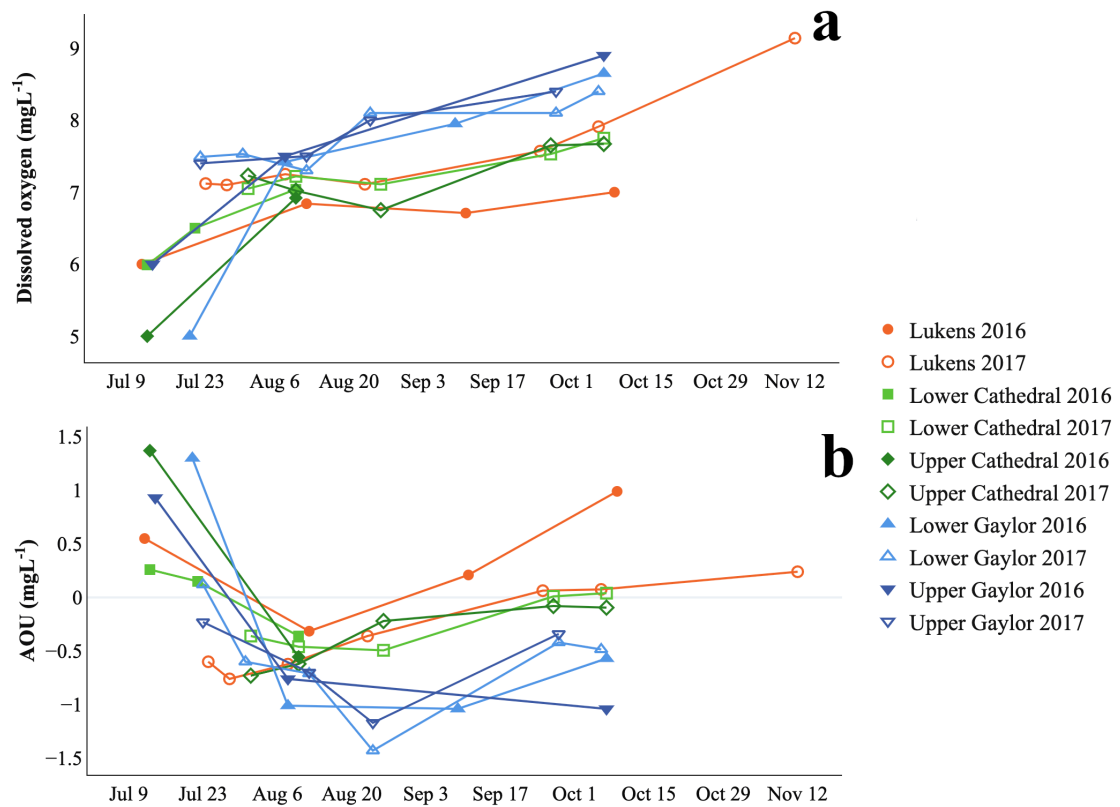
Figure 4. a) Dissolved oxygen concentrations and b) Apparent Oxygen Utilization (AOU) of five temperate montane lakes in the Sierra Nevada, California, USA. Colored symbols denote different lakes sampled in 2016 (open symbols) and 2017 (closed symbols), with the date of sampling along the horizontal axis
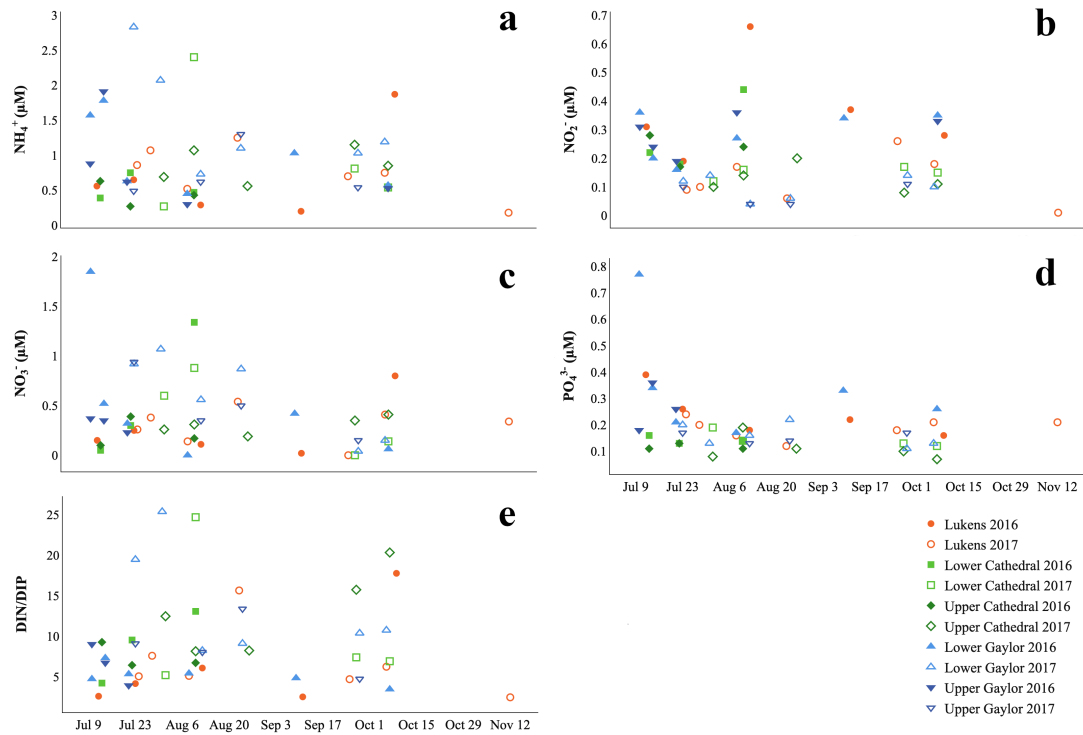
Figure 5. Inorganic nutrients concentrations: a) Ammonium, b) Nitrite, c) Nitrate, d) Phosphate, and e) Dissolved inorganic nitrogen : dissolved inorganic phosphorus ratios for five temperate montane lakes in the Sierra Nevada, California, USA. Colored symbols denote different lakes sampled in 2016 (open symbols) and 2017 (closed symbols), with the date of sampling along the horizontal axis.