The Effect of Organic Matter Supply and Lake Sediment History on Freshwater Lakes Methanogenesis

BIOS 35502: Practicum in Environmental Field Biology

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Abstract

Freshwater lakes are major contributors to global methane emissions through biological methanogenesis. Previous research has shown that primary productivity and organic matter supply may be linked to methane emissions. However, there has not been much research that focuses on the relationship between a freshwater lake's trophic status and methane production. This studies primary focus was to establish if the historical state of lake sediments influenced the response to algal additions. Using anoxic lake sediments from two lakes of varying trophic statuses on the border of Wisconsin and Michigan, we set up 7 different algal addition treatments in a regression design with three replicates for each lake. The treatments were incubated at 4°C in the dark for 25 days and 10mL of the headspace was sampled and analyzed via gas chromatography every 4 days. Using a linear regression we documented a positive correlation between algal additions and carbon dioxide production in both lakes, production in the mesotrophic lake had a greater response to algal additions. In addition, we documented a positive relationship between methane production and algal additions in the oligotrophic lake, but not in the mesotrophic lake. The oligotrophic sediments response to algal additions was more extreme than that of the mesotrophic sediments. The response of the oligotrophic sediments suggests that lake methane concentrations and emissions will increasingly contribute to global atmospheric methane concentrations in the face of anthropogenically induced lake eutrophication. Factors that contribute to freshwater lake methanogenesis are complex and further experimentation is necessary to understand the role the historical state of lake sediments plays on methane production.

Keywords: anaerobic, methane (CH_4), hydrogenotrophic methanogens, homoacetogenic bacteria, carbon dioxide (CO_2), oligotrophic, mesotrophic

Introduction

Methane that is emitted to the atmosphere is a major greenhouse gas that is capable of affecting Earth's temperature and climate systems (Liu & Whitman, 2008; EPA, 2016). Greenhouse gases, such as methane, affect the Earth's climate and temperature by trapping heat in the atmosphere and altering Earth's overall energy balance (Coalbed, 2005; Shine, 2007). In addition, methane is one of the most potent greenhouse gases and it is estimated to have 25 times the direct global warming effect when compared to that of carbon dioxide (CO_2) (Borrel, 2011). The effects of this surface and atmosphere warming have been shown to have implications for rainfall levels, glacial retreats, sea levels and seasonal patterns across the globe (Ramanathan, 2009). Approximately three times the amount of methane is currently being emitted into the atmosphere when compared to rates 200 years ago (Liu & Whitman, 2008). Although methane emissions have some natural background in production such as within soils, wetlands, and lakes (Shine, 2007; Liu & Whitman, 2008). It is predicted that in the face of anthropogenic change even the natural production of methane will increase as a result of overexploitation.

It is estimated that 74% of global methane production is produced through biological methanogenesis (Liu & Whitman, 2008). Freshwater lakes are major contributors both to biological methanogenesis, through microbial processes in lake sediments, and the emission of atmospheric methane, contributing between 6-16% of the current global methane emissions (Liu & Whitman, 2008; West et. al, 2012). Methanogens are anaerobic archaea that produce methane as an end-product of respiration (Liu & Whitman, 2008). Other anaerobic bacteria consume organic matter in lakes and produce acetate, fatty acids, and H_2 and CO_2 as byproducts of their metabolism (Ferry, 1993; West et. al, 2016). These compounds are then utilized by methanogens and converted to CH_4 in environments where oxygen is depleted, such as within anaerobic lake sediments (CH_4) (Ferry, 1993; Liu & Whitman, 2008; Kammann, 2012)

Methane production from lake sediments occurs naturally as a result of carbon cycling in freshwater systems, and in return influences a variety of in lake processes (Bertolet, 2019). Recent studies have shown that methane production in freshwater lakes varies between ecosystems and have suggested that the rate of organic matter supplied to sediments may be a driving force of methane production. Organic matter supplied to lake sediments is linked to the trophic state, which are sensitive to changes in nutrient availability (West et. al 2015). The shift in the naturally occurring nutrient regime, such as in eutrophied systems, can result in increased phytoplankton productivity and this, in turn, increases organic matter supplied to lake sediments. Recent studies have suggested that it is possible that the primary driving factor in lake methane production is substrate supply, which may be further influenced by the ratio of organic matter supply to lake sediments (West et. al 2015; Bertolet, 2019). There has not been much research that focuses on the correlation between freshwater lakes trophic status and the effects that this may have on overall methane production. However, it is possible that the historical state of the lake sediments may influence the response to algal additions.

The purpose of this study was to quantify the relationship between organic matter supply and methane production in two lake ecosystems that vary in historical primary productivity. In addition, this study aimed to determine whether a lakes trophic status acts as a regulating factor of methane production in lake sediments. In recent studies, it has been shown that increased nutrient content within freshwater systems can result in an increase in substrate quantity, which has been shown to increase methanogenesis (West et. al, 2015). This led us to hypothesize that organic matter supply is positively related to lake methane production rates and that this relationship is linear. We also hypothesized that the slope of the relationship would differ between the mesotrophic and oligotrophic lake. In mesotrophic lakes there is already an adequate amount of organic matter supply, resulting from the lakes high primary productivity. Therefore, it is predicated that the response of mesotrophic sediments to the additions of algal material may not be as extreme when compared to the response of oligotrophic sediments. The research proposed here should help in understanding the sources of variation in methane production across different ecosystems and also aid in the prediction of the role that freshwater lakes will play in global carbon cycling in the face of pervasive environmental change.

Methods

Sampling Sites

Sediment retrieval occured at the University of Notre Dame Environmental Research Center (UNDERC) near Land O' Lakes, Wisconsin (46° 13'N, 89° 32' W), in June of 2019. Lake sediments were retrieved from two lakes of varying trophic status, one oligotrophic lake and one Mesotrophic. We Sampled two lakes, Morris and Crampton, and a limnological profile of both lakes, showing lake characteristics ensuring anoxic conditions, is depicted in Figure 1. The thermocline for Morris and Crampton was 2 and 3 meters, respectively (Figure 1).

Experimental Design

On June 13, 2019, sediment was collected using the ekman sampler at the deepest locations of lakes Morris and Crampton. In addition, hypolimnetic water was collected from the middle of the hypolimnion with a van dorn water sampler. After sediment retrieval, 25mL of sediment and 25mL of hypolimnetic water was placed in 125mL serum bottles. Seven treatments, with three replicates per treatment, were run for each of the two lakes that were sampled: no algal addition, .01g algal addition, .02g algal addition, .03g algal addition, 04g algal addition, .05g algal addition, .06g algal addition. The serum bottles were capped with a rubber septa and aluminium crimp seal, and the remaining headspace was purged with N_2 gas to simulate and maintain anoxic conditions as well as promote methane production. The slurries were incubated at 4°C in the dark for 25 days. Every 4 days, 10mL of slurry headspace was extracted and placed into gas chromatography vials for methane quantification and 10mL of N_2 gas was inserted into the serum bottles to maintain anoxic conditions.

Phytoplankton Growth

Phytoplankton strain *Scenedesmus obliquus* was used for the algal additions in this study and was cultured in 50% Bold Basal Medium (BBM) for 7 days. Phytoplankton biomass was placed in 50mL conical tubes and centrifuged for 10 minutes at 2000rpm. After centrifugation the sediments were dried for 48h in a 60 °C drying oven. The phytoplankton was then transferred into a vial and homogenized with a previously grown culture of algae.

Quantification of methanogenesis rates

To estimate methanogenesis rates in the incubations, methane concentration in the headspace of the incubations were measured repeatedly every 4 days for 25 days using gas chromatography as described in West et. al. 2015. Rates of methanogenesis was inferred from the slope of the linear regression of the time series.

Statistical methods

To determine the shape of the relationship between organic matter supply and methanogenesis rates, we fit linear models to the data on algal additions and methanogenesis rates. Linear models were also used to determine the relationship between carbon dioxide production and algal additions. To determine whether the effect of organic matter supply differed between the oligotrophic and mesotrophic lake, we compared the independent measures of the slope of the linear regressions.

Results

Rates of CO_2 *production*

Rates of CO_2 production were positively correlated with algal additions in both Crampton ($R^2 = 0.44$, $p < 0.05$) and Morris ($R^2 = 0.58$, $p < 0.05$) lakes. CO_2 production in the mesotrophic Lake Morris had a greater response to algal additions with a slope of 463, compared to Crampton which had a slope of 227.

Rates of CH_4 *production*

Rates of CH_4 production were also positively correlated with algal additions in relation to the oligotrophic lake, Crampton ($R^2 = 0.25$, $p = <.05$), but not in the mesotrophic lake Morris ($R^2 = 0.05$, $p = 0.33$). Crampton has a greater response to the algal additions with a slope of 322, compared to Morris, which did not exhibit a positive relationship. Despite the positive relationship in Crampton and lack of significance in Morris, CH_4 production was maximized at the third algal additions in both lakes.

Discussion

It was predicted that methane production in relation to organic matter supply would be positively correlated, and that the slope of this relationship would differ between the mesotrophic and oligotrophic lake. In addition, we also predicted that the response of the oligotrophic lake sediments would be greater when compared to the mesotrophic lake sediments as a result of the mesotrophic lake already having an adequate amount of organic matter supply. Our results showed a positive correlation between algal additions and CO_2 production in both Morris and Crampton (Figure 2). Our results supported our hypothesis that the oligotrophic lake sediments would experience a more extreme response to algal additions (Figure 3). However, we were unable to compare the slopes of the relationships for CH_4 production between the two lakes as a result of a lack of statistical significance for Morris.

The CO_2 production rates in response to increasing levels of algae (Figure 2) indicates that the microbes are immediately starting to decompose the algae and release carbon CO_2. Although CO_2 production was positively correlated for both Morris and Crampton, the incubations from the mesotrophic lake had the highest level of CO_2 production (Figure 2 & Figure 4). It was established that Morris had a greater response to the algal additions with a slope of 463, compared to Crampton that had a slope of 227. The higher CO_2 production observed in Morris, in comparison to Crampton, could be due to differences in the microbial community between lakes, but further research is needed to confirm this.

Similar to CO_2 production, CH_4 was also positively correlated in relation to the algal additions in Crampton (Figure 3). Our statistical analysis of the relationship between algal additions and CH_4 production in Crampton proved to be significant ($R^2 = 0.25$, $p = < .05$) with a slope of 322. The results shown in Crampton is supported by similar research that has shown that an increase in overall substrate supply in return increases methanogenesis rates within lake sediments (Schwarz et. al 2008; West et. al 2012; West et. al, 2016). Although there was a positive correlation between the algal additions and CH_4 production in Crampton, it would be unreasonable to assume that CH_4 production rates would increase indefinitely as shown in Figure 3. A more realistic model would show an increase to a point and then we would see the CH_4 production level off or decrease. However, in order to confirm this future experiments with treatments containing higher levels of algal additions would need to be performed, as we do not have data from this experiment to support this hypothesis. In Morris there was no statistically significant relationship between the algal additions and CH_4 production ($R^2 = 0.05$, $p = 0.33$) (Figure 3). The lack of CH_4 production in Morris could be a result of the high levels of CO_2 production (Figure 2).

Carbon Dioxide is a substrate used by hydrogenotrophic methanogens and is converted into CH_4 (Liu & Whitman, 2008). However, when supplied in excess CO_2 can overwhelm hydrogenotrophic methanogens and cause them to be outcompeted by homoacetogenic bacteria. Similar to hydrogenotrophic methanogens, homoacetogenic bacteria also participate in the uptake of CO_2, however they differ in their environmental preferences. Homoacetogenic bacteria prefer and tolerate high levels of CO_2 and low pH, while the inverse is true for methanogenic archaea. Instead of producing CH_4, like methanogenic archaea, homoacetogenic bacteria produce acetate (Liu & Whitman, 2008). The high CO_2 production associated with Morris in Figure 2 may have resulted in a lowering of pH, which has been associated with high levels of CO_2. The low pH and high CO_2 levels would have created conditions that were more favorable for the homoacetogenic bacteria resulting in acetate production rather than methane. Testing the pH levels in the slurries could confirm these conditions and determine if this is why we did not see higher levels of CH_4 production.

Although the results in this study proved statistically significant, with the exception of CH_4 production in Morris, there were still limitations that in future studies could be amended to further insight and ensure confidence. A limitation of this study was the inability to measure acetate concentrations within incubations due the lack of an Ion Chromatograph (IC) in the lab. We did measure CO_2 concentrations and while this is an important aspect of the anaerobic food chains, two-thirds of biologically generated methane, that is produced within lake sediments, is derived from the substrate acetate (Liu & Whitman, 2008). In addition, Human induced error may have resulted in the low CH_4 production associated with Morris. If bottles are not purged properly, then CH_4 production could be inhibited by the presence of O_2. This could potentially explain the variance demonstrated in the T_2 bottles in Figure 5 from Morris.

The oligotrophic lake sediments response to algal additions was more extreme than that of the eutrophic lake sediments (Figure 3). This response suggests that lake CH_4 concentrations and emissions will increasingly contribute to global atmospheric CH_4 concentrations in the face of anthropogenically induced lake eutrophication. In order to quantify the effects of eutrophication on both current and future CH_4 emissions from freshwater lakes, future data collection and analysis could be used in the continuation of this research. Future studies could potentially amend some of the limitations outlined in this research as well as provide further insight on the effects of lake eutrophication. First, more treatments that include higher levels of algal additions might prove to show a more of an applicable model for the relationship between algal additions and methane concentrations. Second, a gradient of lakes that have different trophic levels could be utilized, outlining the severity that methane concentrations increase in relation to primary productivity levels. Third, the manipulation of nutrient level concentrations, such as nitrogen and phosphorus, could potentially show if a variance in CH_4 production occurs depending on the levels of nitrogen and phosphorus present in proportion to each other in lakes of varying trophic status.

Figures

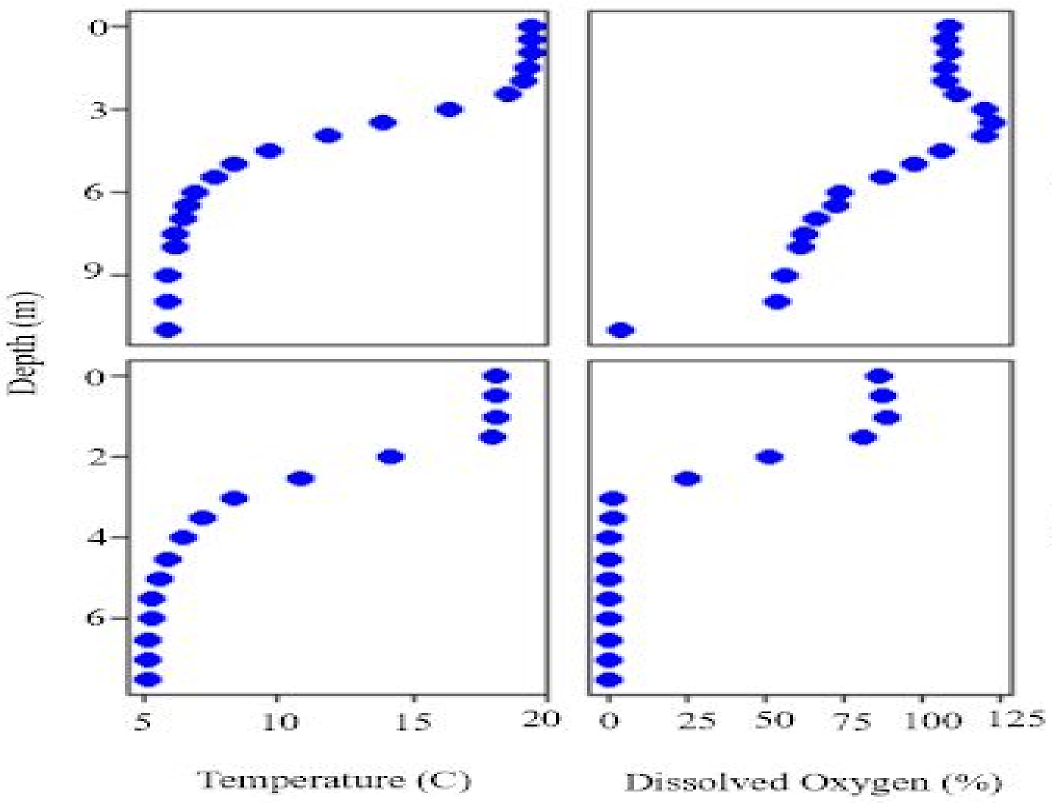

Figure 1. Limnology profiles of lakes Morris and Crampton. The two lakes differ in trophic status and

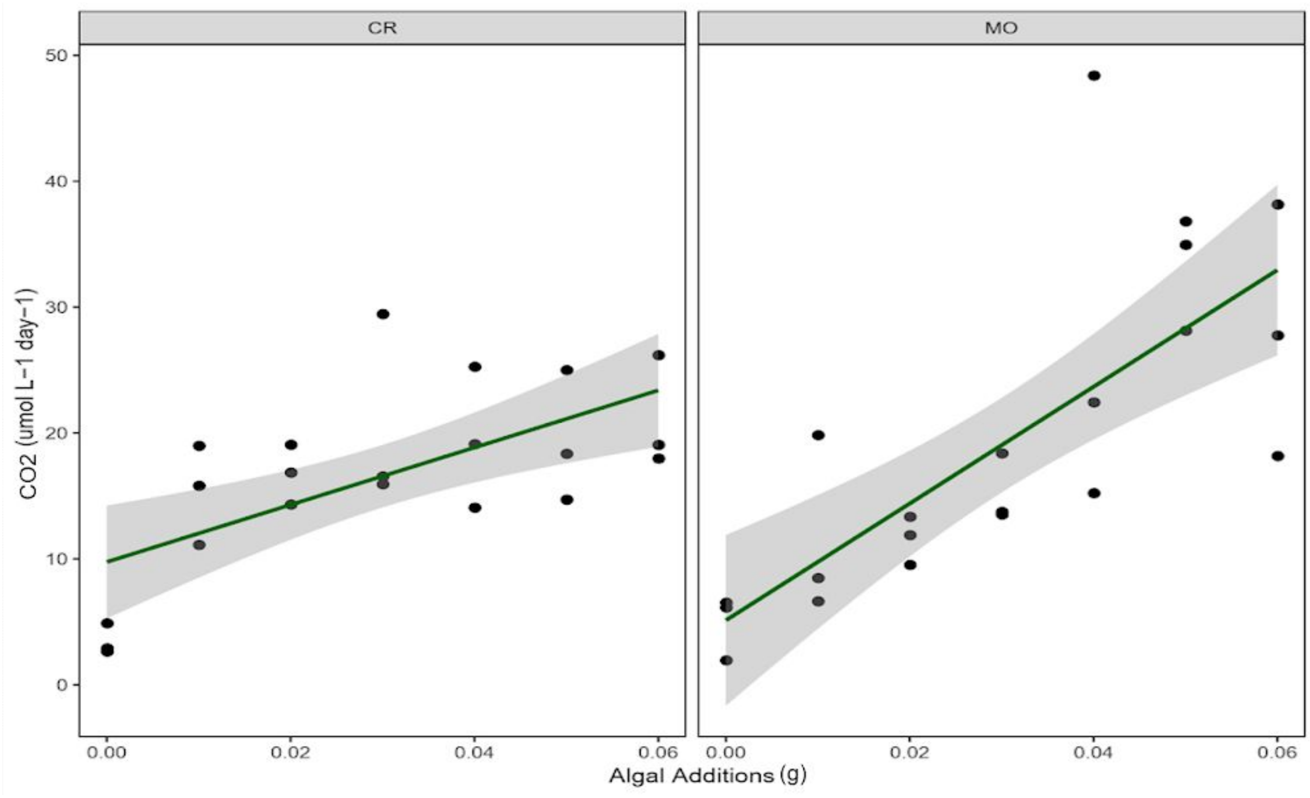

Figure 2. A linear model showing the relationship between algal additions and Carbon Dioxide production (CO_2) in both Crampton (CR) and Morris (MO) lake sediments. CO_2 production was positively correlated with algal additions in Crampton ($R^2 = 0.44$, $p < 0.05$) and Morris ($R^2 = 0.58$, $p < .05$).

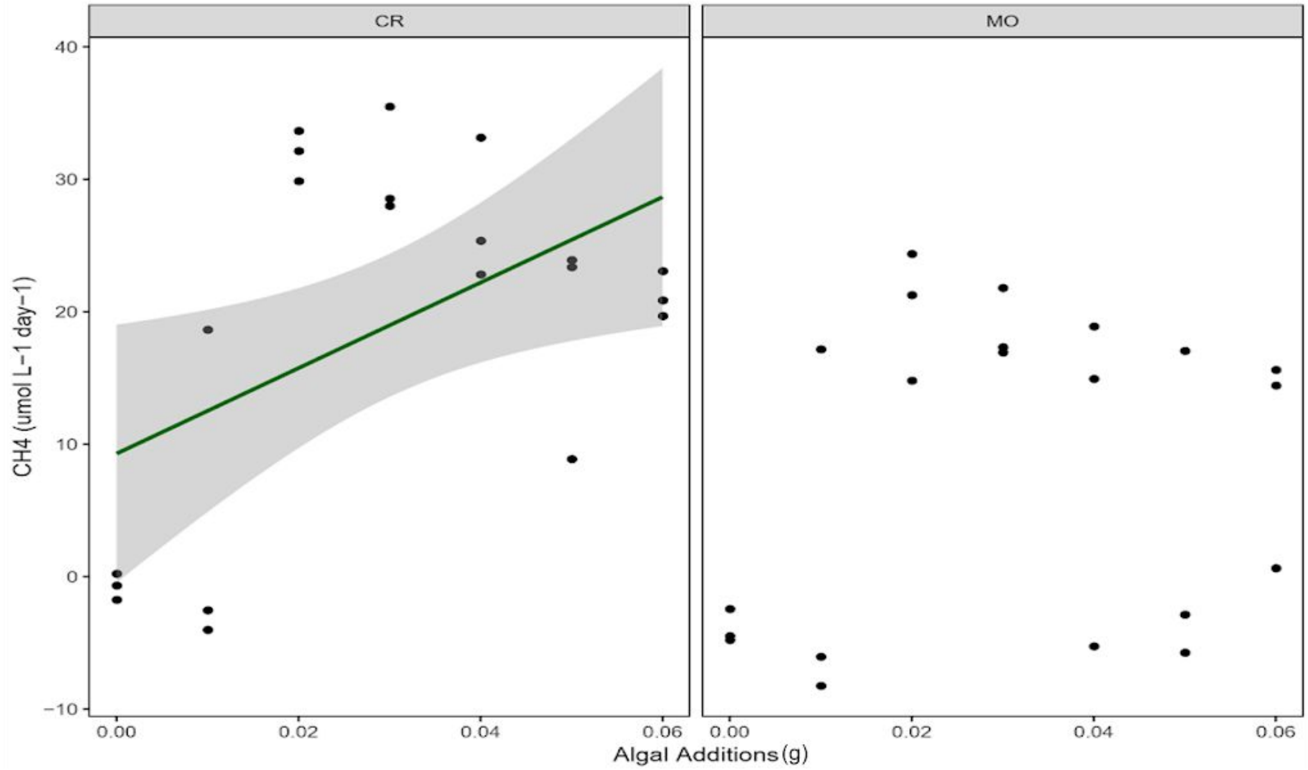

Figure 3. A linear model showing the relationship between methane (CH_4)production in relation to algal additions for both Crampton (CR) and Morris (MO). The two lakes differ in trophic status with Crampton being Oligotrophic and Morris Mesotrophic. Methane production was significantly related to algal additions in Crampton, but not in Morris. CH_4 production was positively correlated for Crampton ($R^2 = 0.25$, p = <.05), but not for Morris ($R^2 = 0.05$, p = 0.33).

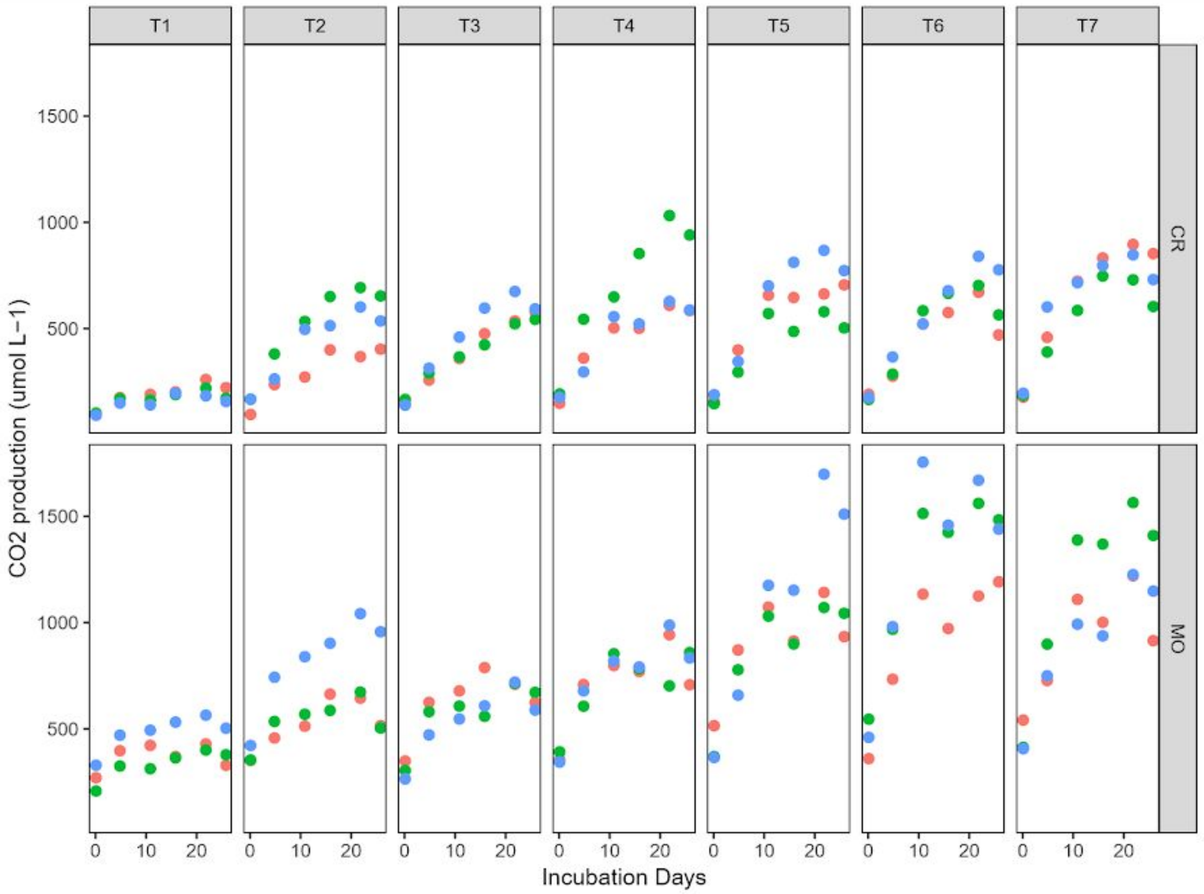

Figure 4. Time series of CO_2 concentration in the headspace of experimental incubations.

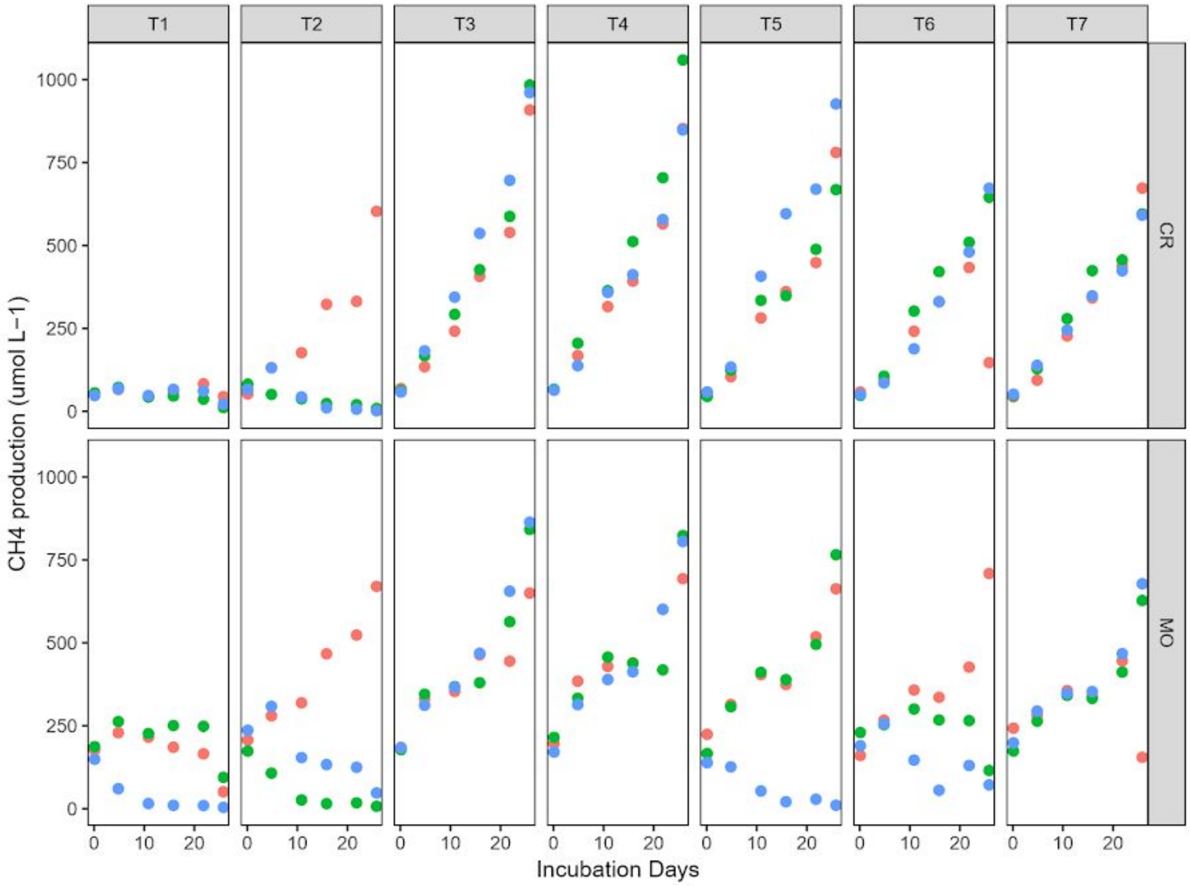

Figure 5. Time series of CH_4 concentration in the headspace of experimental incubations.

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Appendix

lakeID	**depth**	**temp_C**	**DO_mgL**	**DO_perc**
CR	0	19.4	9.52	109.6
CR	0.5	19.4	9.36	108.3
CR	1	19.4	9.46	109.7
CR	1.5	19.3	9.39	108.2
CR	2	19.1	9.34	107.5
CR	2.5	18.6	9.7	111.2
CR	3	16.4	11.05	120.2
CR	3.5	13.9	11.83	122.9
CR	4	11.8	12.23	120.1
CR	4.5	9.6	11.52	107.2
CR	5	8.4	10.76	97.6
CR	5.5	7.6	9.87	88
CR	6	6.9	8.55	74.3
CR	6.5	6.6	8.31	72.4
CR	7	6.4	7.62	66
CR	7.5	6.2	7.3	63.2
CR	8	6.1	7.18	61.7
CR	9	5.9	6.63	56.6
CR	10	5.8	6.35	54.3

CR	11	5.8	0.38	3.2
MO	0	18.1	7.8	86.4
MO	0.5	18.1	7.84	88
MO	1	18.1	7.96	89
MO	1.5	18	7.28	81
MO	2	14.2	5.04	52
MO	2.5	10.8	2.61	25
MO	3	8.4	0.1	1
MO	3.5	7.2	0.08	0.7
MO	4	6.4	0.07	0.6
MO	4.5	5.9	0.07	0.6
MO	5	5.5	0.05	0.4
MO	5.5	5.3	0.06	0.5
MO	6	5.2	0.05	0.5
MO	6.5	5.1	0.05	0.5
MO	7	5.1	0.06	0.5
MO	7.5	5.1	0.05	0.4

lakeID	**treatment**	**algal**	**replicate**	**CH4Prod_umolLday**	**CH4intercept_umolL**	**CO2Prod_umolLday**	**CO2intercept_umolL**
MO	T1	0	1	-4.49786	229.3831	1.935399	344.0502
MO	T1	0	2	-2.45182	243.6799	6.128279	250.7416
MO	T1	0	3	-4.78701	104.0426	6.517516	396.4627

MO	T2	0.01	1	17.15635	186.0493	8.47059	412.8167
MO	T2	0.01	2	-6.05712	137.1933	6.625872	449.9188
MO	T2	0.01	3	-8.2501	275.3891	19.82012	557.5507
MO	T3	0.02	1	14.78943	210.6475	9.517419	504.1809
MO	T3	0.02	2	21.25465	167.2083	11.88057	416.9368
MO	T3	0.02	3	24.35332	155.4516	13.33643	358.259
MO	T4	0.03	1	16.92498	235.2335	13.51463	536.1387
MO	T4	0.03	2	17.31443	220.5252	13.72552	519.3553
MO	T4	0.03	3	21.78859	163.1343	18.37363	501.3668
MO	T5	0.04	1	14.92454	220.5751	15.21404	708.3373
MO	T5	0.04	2	18.87972	174.8021	22.42546	571.1845
MO	T5	0.04	3	-5.26591	131.8783	48.37885	458.939
MO	T6	0.05	1	17.0369	152.5991	28.11758	550.5563
MO	T6	0.05	2	-2.87275	276.1076	34.93728	791.2267
MO	T6	0.0	3	-5.74547	216.8825	36.79717	811.2842

		5					
MO	T7	0.06	1	0.629452	295.0715	18.16118	680.7638
MO	T7	0.06	2	14.42022	169.3763	38.14313	673.6532
MO	T7	0.06	3	15.59986	185.2598	27.74311	546.0665
CR	T1	0	1	0.212026	55.29284	4.878697	127.5927
CR	T1	0	2	-1.75537	67.23667	2.891094	132.2192
CR	T1	0	3	-0.68078	61.01867	2.628092	119.0394
CR	T2	0.01	1	18.63243	25.46555	11.10711	150.4951
CR	T2	0.01	2	-2.54708	70.67625	18.97267	264.7405
CR	T2	0.01	3	-4.02626	95.99866	15.80216	222.8077
CR	T3	0.02	1	29.85333	-8.09505	16.84388	171.7919
CR	T3	0.02	2	32.12681	-1.03821	14.30923	198.2362
CR	T3	0.02	3	33.64422	21.95829	19.05771	213.2609
CR	T4	0.03	1	27.98475	26.14538	15.9245	242.7452
CR	T4	0.03	2	35.4797	20.0832	29.43417	315.9681
CR	T4	0.03	3	28.52795	25.77095	16.54422	244.3001

CR	T5	0.04	1	25.35068	6.370294	19.10521	288.1229
CR	T5	0.04	2	22.80677	35.80401	14.05966	246.1864
CR	T5	0.04	3	33.13863	30.83216	25.25462	283.6579
CR	T6	0.05	1	8.868569	101.2163	14.7015	258.3854
CR	T6	0.05	2	23.36163	32.49118	18.34278	254.7547
CR	T6	0.05	3	23.89388	-11.8553	24.99129	232.4868
CR	T7	0.06	1	23.06069	0.593195	26.17404	313.6496
CR	T7	0.06	2	20.85172	48.49746	17.96521	305.1104
CR	T7	0.06	3	19.67575	41.86123	19.05018	398.4493

lakeID	**treatment**	**sampleTimes**	**replicate**	**incub_days**	**CH4adj_umolL**	**CO2adj_umolL**
MO	T1	6/16/2019 20:00	1	0	176.86	269.9556
MO	T1	6/21/2019 13:30	1	4.729167	228.9221	397.0485
MO	T1	6/27/2019 13:30	1	10.72917	215.5374	422.315
MO	T1	7/2/2019	1	15.72917	185.0105	369.6495

		13:30				
MO	T1	7/8/2019 13:30	1	21.72917	165.2549	428.9978
MO	T1	7/12/2019 13:30	1	25.72917	50.97571	328.5458
MO	T1	6/16/2019 20:00	2	0	186.8093	207.3701
MO	T1	6/21/2019 13:30	2	4.729167	262.6283	324.9736
MO	T1	6/27/2019 13:30	2	10.72917	226.3954	312.2434
MO	T1	7/2/2019 13:30	2	15.72917	250.4934	363.491
MO	T1	7/8/2019 13:30	2	21.72917	248.5816	400.3621
MO	T1	7/12/2019 13:30	2	25.72917	94.34574	377.9732
MO	T1	6/16/2019 20:00	3	0	148.9693	328.9131
MO	T1	6/21/2019 13:30	3	4.729167	60.10672	470.0744
MO	T1	6/27/2019 13:30	3	10.72917	15.39469	493.6139
MO	T1	7/2/2019 13:30	3	15.72917	10.0933	531.6187
MO	T1	7/8/2019 13:30	3	21.72917	9.316755	564.2469
MO	T1	7/12/2019 13:30	3	25.72917	3.896999	502.8848

MO	T2	6/16/2019 20:00	1	0	206.7816	351.2946
MO	T2	6/21/2019 13:30	1	4.729167	279.8655	457.1661
MO	T2	6/27/2019 13:30	1	10.72917	319.1239	512.4713
MO	T2	7/2/2019 13:30	1	15.72917	466.6676	663.1948
MO	T2	7/8/2019 13:30	1	21.72917	523.2477	644.3249
MO	T2	7/12/2019 13:30	1	25.72917	669.8846	514.6249
MO	T2	6/16/2019 20:00	2	0	173.5765	353.8063
MO	T2	6/21/2019 13:30	2	4.729167	107.2645	534.353
MO	T2	6/27/2019 13:30	2	10.72917	26.26714	568.6123
MO	T2	7/2/2019 13:30	2	15.72917	15.11828	586.9418
MO	T2	7/8/2019 13:30	2	21.72917	17.56066	672.8246
MO	T2	7/12/2019 13:30	2	25.72917	7.004977	504.0723
MO	T2	6/16/2019 20:00	3	0	236.3599	421.6035
MO	T2	6/21/2019 13:30	3	4.729167	308.3728	742.7155

MO	T2	6/27/2019 13:30	3	10.72917	153.8595	838.9711
MO	T2	7/2/2019 13:30	3	15.72917	132.9278	902.512
MO	T2	7/8/2019 13:30	3	21.72917	124.7041	1041.764
MO	T2	7/12/2019 13:30	3	25.72917	47.27501	956.5083
MO	T3	6/16/2019 20:00	1	0	184.35	348.6817
MO	T3	6/21/2019 13:30	1	4.729167	331.1239	624.4224
MO	T3	6/27/2019 13:30	1	10.72917	353.3142	678.484
MO	T3	7/2/2019 13:30	1	15.72917	463.7867	788.1554
MO	T3	7/8/2019 13:30	1	21.72917	444.6128	709.8411
MO	T3	7/12/2019 13:30	1	25.72917	649.8239	624.0059
MO	T3	6/16/2019 20:00	2	0	177.8354	304.17
MO	T3	6/21/2019 13:30	2	4.729167	345.0631	580.4546
MO	T3	6/27/2019 13:30	2	10.72917	367.6689	607.069
MO	T3	7/2/2019 13:30	2	15.72917	379.4591	559.1339

MO	T3	7/8/2019 13:30	2	21.72917	563.281	713.6426
MO	T3	7/12/2019 13:30	2	25.72917	841.5319	671.5084
MO	T3	6/16/2019 20:00	3	0	183.4838	264.398
MO	T3	6/21/2019 13:30	3	4.729167	311.7644	471.4428
MO	T3	6/27/2019 13:30	3	10.72917	365.6319	546.3308
MO	T3	7/2/2019 13:30	3	15.72917	468.3916	608.2868
MO	T3	7/8/2019 13:30	3	21.72917	655.2806	719.3656
MO	T3	7/12/2019 13:30	3	25.72917	863.4445	588.5847
MO	T4	6/16/2019 20:00	1	0	196.4285	354.3215
MO	T4	6/21/2019 13:30	1	4.729167	384.319	708.1221
MO	T4	6/27/2019 13:30	1	10.72917	428.7648	797.7934
MO	T4	7/2/2019 13:30	1	15.72917	439.3925	769.5038
MO	T4	7/8/2019 13:30	1	21.72917	600.4862	942.5007
MO	T4	7/12/2019 13:30	1	25.72917	693.089	707.4599

MO	T4	6/16/2019 20:00	2	0	215.1068	391.7253
MO	T4	6/21/2019 13:30	2	4.729167	332.8617	606.4879
MO	T4	6/27/2019 13:30	2	10.72917	456.8543	853.2816
MO	T4	7/2/2019 13:30	2	15.72917	438.3827	783.1778
MO	T4	7/8/2019 13:30	2	21.72917	418.3347	702.0711
MO	T4	7/12/2019 13:30	2	25.72917	823.3186	858.8431
MO	T4	6/16/2019 20:00	3	0	170.6329	343.5763
MO	T4	6/21/2019 13:30	3	4.729167	313.5511	678.7233
MO	T4	6/27/2019 13:30	3	10.72917	389.3318	818.9487
MO	T4	7/2/2019 13:30	3	15.72917	412.6483	790.7547
MO	T4	7/8/2019 13:30	3	21.72917	601.0454	987.9803
MO	T4	7/12/2019 13:30	3	25.72917	805.1776	833.2269
MO	T5	6/16/2019 20:00	1	0	224.2134	514.5373
MO	T5	6/21/2019 13:30	1	4.729167	313.8582	871.0781

MO	T5	6/27/2019 13:30	1	10.72917	403.7231	1072.53
MO	T5	7/2/2019 13:30	1	15.72917	374.4576	913.1093
MO	T5	7/8/2019 13:30	1	21.72917	518.1283	1141.584
MO	T5	7/12/2019 13:30	1	25.72917	662.8232	933.7055
MO	T5	6/16/2019 20:00	2	0	166.343	369.6064
MO	T5	6/21/2019 13:30	2	4.729167	307.1453	777.9468
MO	T5	6/27/2019 13:30	2	10.72917	410.8229	1029.856
MO	T5	7/2/2019 13:30	2	15.72917	388.8936	899.0982
MO	T5	7/8/2019 13:30	2	21.72917	495.1139	1070.666
MO	T5	7/12/2019 13:30	2	25.72917	765.3057	1043.603
MO	T5	6/16/2019 20:00	3	0	138.5716	365.1474
MO	T5	6/21/2019 13:30	3	4.729167	125.9786	658.4724
MO	T5	6/27/2019 13:30	3	10.72917	53.29613	1175.001
MO	T5	7/2/2019 13:30	3	15.72917	20.69074	1152.227

MO	T5	7/8/2019 13:30	3	21.72917	28.34263	1697.51
MO	T5	7/12/2019 13:30	3	25.72917	10.24818	1510.071
MO	T6	6/16/2019 20:00	1	0	160.2188	359.8334
MO	T6	6/21/2019 13:30	1	4.729167	266.7444	733.3614
MO	T6	6/27/2019 13:30	1	10.72917	357.5696	1133.472
MO	T6	7/2/2019 13:30	1	15.72917	335.682	971.7428
MO	T6	7/8/2019 13:30	1	21.72917	426.5792	1124.753
MO	T6	7/12/2019 13:30	1	25.72917	708.682	1191.506
MO	T6	6/16/2019 20:00	2	0	229.4917	545.556
MO	T6	6/21/2019 13:30	2	4.729167	253.1176	967.9853
MO	T6	6/27/2019 13:30	2	10.72917	300.3644	1512.716
MO	T6	7/2/2019 13:30	2	15.72917	267.2981	1424.488
MO	T6	7/8/2019 13:30	2	21.72917	265.4176	1560.831
MO	T6	7/12/2019 13:30	2	25.72917	115.0263	1483.455

MO	T6	6/16/2019 20:00	3	0	189.5612	459.8593
MO	T6	6/21/2019 13:30	3	4.729167	256.0714	980.5345
MO	T6	6/27/2019 13:30	3	10.72917	146.3791	1754.624
MO	T6	7/2/2019 13:30	3	15.72917	55.49811	1458.148
MO	T6	7/8/2019 13:30	3	21.72917	130.1404	1669.165
MO	T6	7/12/2019 13:30	3	25.72917	71.78768	1439.319
MO	T7	6/16/2019 20:00	1	0	242.4798	541.1695
MO	T7	6/21/2019 13:30	1	4.729167	281.1657	727.0772
MO	T7	6/27/2019 13:30	1	10.72917	355.9197	1108.977
MO	T7	7/2/2019 13:30	1	15.72917	339.8559	1000.831
MO	T7	7/8/2019 13:30	1	21.72917	445.6028	1219.953
MO	T7	7/12/2019 13:30	1	25.72917	154.9087	914.8769
MO	T7	6/16/2019 20:00	2	0	173.6599	413.0847
MO	T7	6/21/2019 13:30	2	4.729167	263.7645	898.1394

MO	T7	6/27/2019 13:30	2	10.72917	341.5143	1388.226
MO	T7	7/2/2019 13:30	2	15.72917	332.4268	1368.805
MO	T7	7/8/2019 13:30	2	21.72917	411.7416	1564.032
MO	T7	7/12/2019 13:30	2	25.72917	627.2412	1409.43
MO	T7	6/16/2019 20:00	3	0	198.5213	406.4539
MO	T7	6/21/2019 13:30	3	4.729167	294.1784	749.3758
MO	T7	6/27/2019 13:30	3	10.72917	347.6617	992.1632
MO	T7	7/2/2019 13:30	3	15.72917	353.0172	937.0355
MO	T7	7/8/2019 13:30	3	21.72917	467.1039	1225.247
MO	T7	7/12/2019 13:30	3	25.72917	677.9401	1148.003
CR	T1	6/16/2019 20:00	1	0	51.54525	97.81215
CR	T1	6/21/2019 13:30	1	4.729167	66.07242	175.2133
CR	T1	6/27/2019 13:30	1	10.72917	43.09451	190.7388
CR	T1	7/2/2019 13:30	1	15.72917	60.26534	202.2761

CR	T1	7/8/2019 13:30	1	21.72917	82.85781	260.9109
CR	T1	7/12/2019 13:30	1	25.72917	44.59672	222.2943
CR	T1	6/16/2019 20:00	2	0	55.5729	102.7675
CR	T1	6/21/2019 13:30	2	4.729167	72.23561	170.9759
CR	T1	6/27/2019 13:30	2	10.72917	43.3572	163.82
CR	T1	7/2/2019 13:30	2	15.72917	46.2691	189.8132
CR	T1	7/8/2019 13:30	2	21.72917	36.60643	220.3102
CR	T1	7/12/2019 13:30	2	25.72917	11.32638	173.0011
CR	T1	6/16/2019 20:00	3	0	47.77078	92.37581
CR	T1	6/21/2019 13:30	3	4.729167	67.84586	149.8834
CR	T1	6/27/2019 13:30	3	10.72917	47.59756	141.0503
CR	T1	7/2/2019 13:30	3	15.72917	66.40513	196.5025
CR	T1	7/8/2019 13:30	3	21.72917	61.3973	183.8587
CR	T1	7/12/2019 13:30	3	25.72917	21.55513	157.2542

CR	T2	6/16/2019 20:00	1	0	52.61934	96.27494
CR	T2	6/21/2019 13:30	1	4.729167	131.9078	236.5227
CR	T2	6/27/2019 13:30	1	10.72917	176.7636	272.0755
CR	T2	7/2/2019 13:30	1	15.72917	322.7089	400.1759
CR	T2	7/8/2019 13:30	1	21.72917	331.3271	368.2497
CR	T2	7/12/2019 13:30	1	25.72917	602.8293	403.1997
CR	T2	6/16/2019 20:00	2	0	81.92188	168.2637
CR	T2	6/21/2019 13:30	2	4.729167	50.98416	381.011
CR	T2	6/27/2019 13:30	2	10.72917	37.94089	534.0215
CR	T2	7/2/2019 13:30	2	15.72917	23.83333	650.607
CR	T2	7/8/2019 13:30	2	21.72917	20.36588	693.148
CR	T2	7/12/2019 13:30	2	25.72917	8.694349	653.5133
CR	T2	6/16/2019 20:00	3	0	66.24241	167.701
CR	T2	6/21/2019 13:30	3	4.729167	131.0514	263.3892

CR	T2	6/27/2019 13:30	3	10.72917	43.45392	496.706
CR	T2	7/2/2019 13:30	3	15.72917	10.50667	514.1056
CR	T2	7/8/2019 13:30	3	21.72917	6.375653	601.7376
CR	T2	7/12/2019 13:30	3	25.72917	1.713647	535.9813
CR	T3	6/16/2019 20:00	1	0	68.87259	147.3971
CR	T3	6/21/2019 13:30	1	4.729167	134.5355	257.9066
CR	T3	6/27/2019 13:30	1	10.72917	241.6727	359.4387
CR	T3	7/2/2019 13:30	1	15.72917	406.8079	476.3426
CR	T3	7/8/2019 13:30	1	21.72917	539.0699	535.8108
CR	T3	7/12/2019 13:30	1	25.72917	908.3109	578.5567
CR	T3	6/16/2019 20:00	2	0	62.49673	167.2377
CR	T3	6/21/2019 13:30	2	4.729167	167.4572	289.9405
CR	T3	6/27/2019 13:30	2	10.72917	292.2358	367.1741
CR	T3	7/2/2019 13:30	2	15.72917	426.9394	423.9996

CR	T3	7/8/2019 13:30	2	21.72917	587.6232	522.858
CR	T3	7/12/2019 13:30	2	25.72917	983.6581	543.5687
CR	T3	6/16/2019 20:00	3	0	58.18931	139.9773
CR	T3	6/21/2019 13:30	3	4.729167	182.1681	314.1491
CR	T3	6/27/2019 13:30	3	10.72917	344.2067	460.0726
CR	T3	7/2/2019 13:30	3	15.72917	536.4499	596.529
CR	T3	7/8/2019 13:30	3	21.72917	696.1745	674.7212
CR	T3	7/12/2019 13:30	3	25.72917	960.5389	592.9261
CR	T4	6/16/2019 20:00	1	0	64.16931	148.7494
CR	T4	6/21/2019 13:30	1	4.729167	167.9184	361.4593
CR	T4	6/27/2019 13:30	1	10.72917	315.6737	503.2079
CR	T4	7/2/2019 13:30	1	15.72917	392.5131	501.2465
CR	T4	7/8/2019 13:30	1	21.72917	564.8907	609.4286
CR	T4	7/12/2019 13:30	1	25.72917	852.5912	584.7753

CR	T4	6/16/2019 20:00	2	0	66.69733	192.5455
CR	T4	6/21/2019 13:30	2	4.729167	205.3117	544.124
CR	T4	6/27/2019 13:30	2	10.72917	363.8096	649.3802
CR	T4	7/2/2019 13:30	2	15.72917	511.7522	852.8003
CR	T4	7/8/2019 13:30	2	21.72917	704.1596	1031.741
CR	T4	7/12/2019 13:30	2	25.72917	1059.1	940.0919
CR	T4	6/16/2019 20:00	3	0	64.11661	176.5943
CR	T4	6/21/2019 13:30	3	4.729167	137.4887	296.3104
CR	T4	6/27/2019 13:30	3	10.72917	358.3972	556.3503
CR	T4	7/2/2019 13:30	3	15.72917	411.6315	522.2167
CR	T4	7/8/2019 13:30	3	21.72917	578.393	628.2374
CR	T4	7/12/2019 13:30	3	25.72917	848.2029	587.2257
CR	T5	6/16/2019 20:00	1	0	56.27769	158.9497
CR	T5	6/21/2019 13:30	1	4.729167	104.7551	399.6079

CR	T5	6/27/2019 13:30	1	10.72917	281.4305	657.2009
CR	T5	7/2/2019 13:30	1	15.72917	360.8286	646.4738
CR	T5	7/8/2019 13:30	1	21.72917	448.3004	663.2318
CR	T5	7/12/2019 13:30	1	25.72917	780.355	705.8184
CR	T5	6/16/2019 20:00	2	0	44.98607	147.0709
CR	T5	6/21/2019 13:30	2	4.729167	124.1233	295.3678
CR	T5	6/27/2019 13:30	2	10.72917	334.5248	570.9984
CR	T5	7/2/2019 13:30	2	15.72917	348.0917	486.6287
CR	T5	7/8/2019 13:30	2	21.72917	488.4099	579.502
CR	T5	7/12/2019 13:30	2	25.72917	668.3455	503.2845
CR	T5	6/16/2019 20:00	3	0	58.51412	189.2674
CR	T5	6/21/2019 13:30	3	4.729167	133.4251	344.9295
CR	T5	6/27/2019 13:30	3	10.72917	407.2542	701.0758
CR	T5	7/2/2019 13:30	3	15.72917	595.6848	811.9687

CR	T5	7/8/2019 13:30	3	21.72917	669.7103	868.0107
CR	T5	7/12/2019 13:30	3	25.72917	926.6193	772.8656
CR	T6	6/16/2019 20:00	1	0	58.25236	191.1173
CR	T6	6/21/2019 13:30	1	4.729167	94.2629	276.376
CR	T6	6/27/2019 13:30	1	10.72917	241.4724	521.3576
CR	T6	7/2/2019 13:30	1	15.72917	330.4472	575.7568
CR	T6	7/8/2019 13:30	1	21.72917	433.496	671.1224
CR	T6	7/12/2019 13:30	1	25.72917	146.8428	470.7941
CR	T6	6/16/2019 20:00	2	0	48.12734	167.0957
CR	T6	6/21/2019 13:30	2	4.729167	106.0213	286.1471
CR	T6	6/27/2019 13:30	2	10.72917	302.1675	584.1915
CR	T6	7/2/2019 13:30	2	15.72917	420.9621	666.3014
CR	T6	7/8/2019 13:30	2	21.72917	509.6599	702.8292
CR	T6	7/12/2019 13:30	2	25.72917	645.3035	564.5462

CR	T6	6/16/2019 20:00	3	0	50.77019	176.3073
CR	T6	6/21/2019 13:30	3	4.729167	85.61318	366.802
CR	T6	6/27/2019 13:30	3	10.72917	188.4508	521.8679
CR	T6	7/2/2019 13:30	3	15.72917	330.4326	678.5437
CR	T6	7/8/2019 13:30	3	21.72917	480.1078	840.1401
CR	T6	7/12/2019 13:30	3	25.72917	672.6474	776.7209
CR	T7	6/16/2019 20:00	1	0	43.84853	177.4936
CR	T7	6/21/2019 13:30	1	4.729167	93.95099	458.8757
CR	T7	6/27/2019 13:30	1	10.72917	227.2144	722.9418
CR	T7	7/2/2019 13:30	1	15.72917	341.8854	832.4401
CR	T7	7/8/2019 13:30	1	21.72917	437.4632	896.1134
CR	T7	7/12/2019 13:30	1	25.72917	672.8239	852.512
CR	T7	6/16/2019 20:00	2	0	48.08431	186.6181
CR	T7	6/21/2019 13:30	2	4.729167	128.5387	389.9721

CR	T7	6/27/2019 13:30	2	10.72917	279.2669	585.6224
CR	T7	7/2/2019 13:30	2	15.72917	424.2564	747.3899
CR	T7	7/8/2019 13:30	2	21.72917	455.8614	729.8206
CR	T7	7/12/2019 13:30	2	25.72917	594.8777	604.1285
CR	T7	6/16/2019 20:00	3	0	51.65481	196.0478
CR	T7	6/21/2019 13:30	3	4.729167	138.6556	601.9854
CR	T7	6/27/2019 13:30	3	10.72917	245.1973	716.1671
CR	T7	7/2/2019 13:30	3	15.72917	348.2449	796.7298
CR	T7	7/8/2019 13:30	3	21.72917	423.6249	847.1528
CR	T7	7/12/2019 13:30	3	25.72917	591.2055	730.8301