# Template for manuscript

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12	MATERIALS AND METHODS						
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20	AUTHOR CONTRIBUTIONS						
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## 30 CONFLICTS OF INTEREST

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## 59 APPENDIX

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# 0.1 Study design and setup

In 2019, 12 glacial lakes (N = 12) were chosen for an experiment near Narsarsuaq, Greenland (61.1567°N, -45.4254°E). These glacial lakes vary in area (0.21 to 1.82 hectares) and maximum depth (2 to 8 m), but 62 are all clustered within a few kilometers of each other. All lakes were fishless at the beginning of the 63 experiment. Six lakes were subsequently introduced with three-spined sticklebacks (Gasterosteus aculeatus) from nearby lakes. Lake B1P1, B2P2, and B3P3 were introduced with Gasterosteus aculeatus from a single 65 population (lake L26, 61.253333°N, -45.529141°E), while lake B2P3, B3P1, and B3P2 were introduced with Gasterosteus aculeatus from two populations (lake L26, 61.253333°N, -45.529141°E and lake ERL33, 67 61.118369°N, -45.580845°E). The remaining six lakes B1P4, B2P4, B3P0, ERL85, ERL122, and ERL152 68 were used as fishless control. For the purpose of this study, the origin of the introduced Gasterosteus aculeatus 69 is of minor importance, as they all originate from the same area (Supplementary Table S1). 70

In 2021, 2022, and 2023, all 12 lakes were monitored over several days. For that purpose, EXO2 multiparameter sondes were installed (YSI, Yellow Springs, OH, USA), tracking ecosystem parameters with high frequency (2-minute intervals in 2021 and 2022, 5-minute intervals in 2023 with the exception of ERL122, which was monitored in 15-minute intervals). For the purpose of this study, only dissolved oxygen (hereafter DO) and temperature measurements yielded from these sondes are relevant. The sensors were situated at a water depth of approximately 1-1.5 m in each lake. All optical sensors were wiped clean before every measurement with a built-in wiper. The monitoring period was 16 September-24 September in 2021, 22 June-3 July in 2022, and 22 June-17 July in 2023.

**Supplementary Table S1.** Lakes included in the experiment, along with treatment and general characteristics.

Lake	Treatment	Latitude (°N)	Longitude (°E)	Altitude (m)	Area (hectare)	Maximum Depth (m)
B1P1	Fish	61.15338	-45.57081	272	0.21	4.00
B2P2	Fish	61.12299	-45.55988	255	0.50	3.00
B3P3	Fish	61.13385	-45.57556	258	0.30	5.00
B2P3	Fish	61.12275	-45.55696	261	0.41	4.25
B3P1	Fish	61.13130	-45.51195	180	0.40	2.00
B3P2	Fish	61.12788	-45.51031	201	0.53	4.50
B1P4	No Fish	61.16552	-45.56801	304	0.44	2.20
B2P4	No Fish	61.12192	-45.55497	261	0.51	7.00
B3P0	No Fish	61.13210	-45.51416	177	0.81	4.00
ERL85	No Fish	61.14171	-45.59328	120	1.82	8.00
ERL122	No Fish	61.14182	-45.53623	111	1.10	5.00
ERL152	No Fish	61.14646	-45.59235	156	0.73	4.50

## 9 0.2 Data sources

BOO  $(mgL^{-1})$  and water temperature (°C) measurements were yielded from EXO2 multiparameter sondes, as described in section Study design and setup. For the purpose of estimating ecosystem metabolism, wind and irradiation data were yielded from the Programme for Monitoring of the Greenland Ice Sheet (PROMICE), providing automatic weather station data (Fausto et al., 2021). The dataset named QAS\_L\_hour.csv originating from the QAS\_L automated weather station near Narsarsuaq was downloaded from a public database provided by PROMICE (How et al., 2022). Wind data was provided as average wind speed in hourly intervals  $(ms^{-1})$ , while irradiation was provided as downwelling shortwave irradiation additionally corrected for the tilt of the sensor  $(Wm^{-2})$ .

## 8 0.3 Data analysis pipeline

The data preparation and analysis for estimating lake metabolism was done separately for each monitoring period (16 September-24 September 2021, 22 June-3 July 2022, and 22 June-17 July 2023).

First, the raw sonde data from each lake were imported and merged into one dataset for a given monitoring period. The variables required for lake metabolism estimation were water temperature and DO. Next, an outlier analysis was performed by removing values higher than three times the median absolute deviation of all values in a sliding window of one day window size (Lürig et al., 2021). After outlier removal, the time series of water temperature and DO were plotted and subsequently investigated for larger data gaps due to potential lack of sensor measurements over certain periods.

To ensure overlapping estimation of lake metabolism, the data was subsequently cut to achieve equal starting and ending time points for all lakes during a given monitoring period. Due to large data gaps during the period of 2022, we chose to keep as much complete days as possible in order to estimate the maximum amount of days, rather than selecting the longest common monitoring period for all lakes.

After cutting the sonde data, they were subsequently merged with the weather data. Since the weather data were collected in hourly intervals, they were inflated to match the measurement intervals of the sonde data (see section Study design and setup).

After merging sonde and weather data, potential missing values due to outlier removal were imputed by a weighted moving average, where the weights of observations around the central value to be imputed decrease exponentially (Moritz and Bartz-Beielstein, 2017). For larger data gaps spanning over several hours (due to sonde malfunction rather than outlier removal), we chose to not estimate lake metabolism for the affected days.

All data preparation and analysis was done with the R programming language (R Core Team, 2024). To estimate lake metabolism, the package LakeMetabolizer was used (Winslow et al., 2016). Specifically, a Bayesian framework to estimate whole lake metabolism from free-water DO by using the function metab.bayesian was chosen for this analysis (Holtgrieve et al., 2010). In order to be able to fit the model, a minimum of 6 variables are needed as input:

- 1. doobs: DO concentration measurements  $(mgL^{-1})$
- 115 2. do.sat: Equilibrium DO concentration for specific temperature ( $mgL^{-1}$ , hereafter do.sat)
- 3. k.gas: Gas and temperature specific gas transfer coefficient  $(m^{-1}, \text{ hereafter k.gas})$
- 4. par: Photosynthetically active radiation ( $\mu mol \, m^{-2} \, s^{-1}$ , hereafter par)
- 5. z.mix: Actively mixed layer depth (*m*, hereafter z.mix)
  - 6. wtr: Water temperature ( ${}^{\circ}C$ )

Some of the variables listed above have to be derived from other time series data prior to estimating lake metabolism, namely do.sat, k.gas, par, and z.mix. do.sat was derived by using the function o2.at.sat, which calculates the equilibrium DO concentration of oxygen in water at supplied conditions. To estimate k.gas, a gas transfer coefficient model must be fit. The LakeMetabolizer package offers different methods, and we chose an empirical wind-based gas exchange model, introduced by Vachon and Prairie in 2013 (Vachon and Prairie, 2013). This model not only takes wind into consideration, but also lake area. All models of gas exchange return a  $k_{600}$  value, a gas exchange normalized to a Schmidt number (Sc) of 600, or  $O_2$  at 17.5 °C. After estimating  $k_{600}$ , it must be transformed to k.gas by using the function  $k_{600}.2.k_{GAS}$  provided by the LakeMetabolizer package. par was derived from shortwave irradiation, which we had available (see section Data sources). To achieve this transformation, we used the function sw.to.par, which uses the simple empirical transformation  $par = 2.114 \times sw$  (Britton and Dodd, 1976). To estimate z.mix, one normally needs a time series profile of water temperature or water density. Since we did not have such data available, we decided to simulate actively mixed layer depth by drawing from a uniform distribution with limits 0 and maximum depth of the lake. The reasoning behind this simulation is that, although the

simulated time series of z.mix likely differing substantially from the actual, a possible treatment effect of fish versus no fish should still be consistent, given the simulation is performed equally in all lakes.

After performing the data preparation and the derivation for certain model inputs, the Bayesian lake metabolism model was fitted for each lake and monitoring period. The Bayesian model is special, as it considers 2 types of errors: Differences between the true data generating process and the process defined in the model (process error) and errors resulting from inaccuracies of DO measurements (observation error). Reflecting this philosophy, the Bayesian model distinguishes between 3 categories of DO values: y represents the DO measurements,  $\alpha$  the true but unknown DO values, and  $\alpha^*$  the model's estimates of the true value. The observed DO measurements y are modeled as random deviations from the true value  $\alpha$ :

$$y_t \sim N(\alpha_t, \tau_v),$$
 (1)

where  $\tau_v$  is the precision of the observation error (precision is the reciprocal of variance). Note, that the Bayesian model fits parameter values by making a comparison between observed and theoretical values of DO. This comparison is made in equation 2, where we define the true value of DO at time t,  $\alpha_t$ , as being normally distributed with mean  $\alpha_t^*$  and process precision  $\tau_w$ :

$$\alpha_t \sim N(\alpha_t^*, \tau_w),$$
 (2)

where  $\tau_w$  is the precision of the process error. The important distinction of process and observation error is that process error affects the state of the system at time t + k(k = 0, 1, ..., T - t), because the state evolves dynamically, whereas observation error only affects the state of the system at time t.

Both  $\tau_v$  and  $\tau_w$  are given minimally informative priors following a gamma distribution with shape and rate parameters of 0.001.

The Bayesian model can be described by the following equations:

$$x^2$$
 (3)

$$x^3$$
 (4)