Contents

Chapter 4

A data assimilating state-space model for algal growth under controlled conditions within a photo-bioreactor

4.1 Introduction

Microalgae has long been viewed as a potential platform for bioengineering. During the 1970's and 2000's much of the research focussed on biofuel production, while recently the potential for pharmaceuticals and other high-value chemicals has been explored. Whether the desired product is a primary metabolite (eg lipids in biofuel production) or secondary metabolite (eg astaxanthin as high-value dye) it is important to be able to measure processes such as photosynthesis and respiration that place in the cells.

- More understanding of processes in high temporal resolution, non-destructive.
- 'Fuse' high resolution measurements with more direct measure
- Incorporate prior information and constraints

4.1.1 Overview

At the broadest scale / biggest picture the growth of microalgae can be expressed as the following chemical reaction in which C is taken from the atmosphere as CO_2, along with aqueous nutrients N and P, to produce biomass

$$106\,CO_2(g) + 122\,H_2O + 16\,NO_3{}^-(aq) + PO_4{}^{3-}(aq) + 19\,H^+ \xrightarrow[[10^3 - 10^4]\,\gamma]{} C_{106}\,H_{263}\,O_{110}\,N_{16}\,P_1 + 138\,O_2\,(g) \tag{4.1}$$

This equation captures a number of features of interest. The total biomass is determined by the concentrations of N and P. A typical growth media for microalgae is Guillard's Marine Enriched Seawater (F/2), which has

species	**conc** / μM
N	880
P	36
TA	2300

- **Biomass**: Taking the limiting nutrient to be Nitrogen, the maximum biomass of algae is $200 mgL^{-1}$, of which about $70 mgL^{-1}$ is Carbon. This is equivalent $0.12L$ of pure CO_2 At Standard Temperature and Pressure, which around to $325L$ of air for each litre of media.

- **Change in alkalinity**: Alkalinity is central concept in seawater chemistry that allows one to calculate how much Carbon can be dissolved in solution, and what form it takes (Dissolved Inorganic Carbon exists as CO_2, $HCO_3{}^-$ and $CO_3{}^{2-}$). Alkalinity measures the charge imbalance between strongly and weakly dissociating ions in solution. As the charged nutrients are removed from the solution during growth the alkalinity increases and more Carbon is able to be dissolved in the media. For PBR F/2 media the initial TA is 2300

4.1.2 Growing microalgae in a PBR

microalgae grown in open system constanct exh In laboratory photbioreactor small production system ponds.

Look at the fluxes into and out the system, like respiromtery.

Carbon in seawater

Of

Carbon and light availability are two of the most common limiting factors of biomass productivity [**?**].

4.2 Methods

4.2.1 Data Model: Photo-bioreactor setup, experimental design and data collection methods

All data collection methods for this chapter were carried out by Peter Wood as part of a collection of PhD experiments (Peter Wood 2019 UTS PhD).

Microalgal culture *Nannochloropsis oceanica* (Droop) Green (strain CS-179) obtained from the Australian National Algae Culture Collection was cultured in 200 mL conical flasks; maintained in an incubator (Labec Pty Ltd) at 20°C, under an irradiance of 50 μmol m^{-2} s^{-1} of cool-white fluorescent light at a 12 hour light/12 hour dark cycle. Stock cultures were grown in f/2 saltwater medium [6] and diluted 5 days prior to the start of experiments to ensure that *N. oceanica* was in the exponential growth phase and not nutrient deprived. f/2 was sparged prior to stock culture dilutions to maximise carbon and oxygen content.

N. oceanica was cultured in four, 500 mL environmental photo-bioreactors (ePBRs, Phenometrics Inc) with a 10% v/v inoculation of stock culture. Top-side illumination over a path length of 25 cm was provided by a cool-white light LED,

whilst temperature was maintained at 27°C using a Peltier heater-cooler connected to a water jacket. In-built thermocouples, calibrated against external temperature sensors attached to the Firesting module (TeX4; PyroScience GmbH), measured every 5 minutes were used to control the Peltier heater-cooler jacket through a feedback loop to an accuracy of ± 0.2°C. pH was also measured in 5 min intervals by in-built pH electrodes (Van London Inc); controlled by periodic CO2 (5%) injections using valves in the ePBRs. pH was 3-point calibrated using pH buffer solutions at pH 4.00 ± 0.02, pH 7.00 ± 0.02 and pH 10.00 ± 0.02. PBR mixing was controlled by magnetic stirring bars at 110 rpm. All four ePBRs were aerated with filtered/humidified air through a 1.2 mm needle valve (Terumo Co).

A period of 2 days was allowed for *N. occulata* to acclimate to the ePBRs at an irradiance of 500 μmol m^{-2} s^{-1} and a temperature of 27°C. Following this acclimation period, the PBR was set to the experimental condition of 2,000 μmol photons m^{-2} s^{-1} for another 2 days and a 12 hour light/12 hour dark cycle with a temperature of 27°C. ePBRs were maintained at an optical density (OD) of 0.4 using manual dilutions, creating a semi-batch culturing system. Dilutions occurred once per day (one hour before the light cycle), using aerated f/2 media. The experiment was conducted over a period of 4 days, samples were extracted post and prior dilution, as well as half way through the light cycle. 50 mL was extracted to examine total alkalinity and dissolved inorganic carbon. Dissolved oxygen (DO) was measured using a 3 mm robust optical probe (OXROB10-OI; PyroScience GmbH) attached to a FireStingO2 logger (PyroScience GmbH). DO measurements were taken every 60 seconds and temperature-corrected using a temperature extension module (TeX4; PyroScience GmbH). DO was two-point calibrated using air-saturated seawater (100% saturation) and sodium sulfate-saturated water (0% saturation). At 2 hour intervals, a solenoid valve (SMC Pneumatics Pty. Ltd.) was used to stop aeration for 10 minutes to allow for observations of net photosynthesis.

Alkalinity and DIC was measured twice a day closely following the Standard Operating Procedures (SOP) outline in [3]. Approximately 30 mL of *N. oceanica* media was titrated agains 0.1 M hydrochloric acid on an auto-titrator (800 Dosino; Metrohm AG) [SOP3b Open-cell tritration].

[Chris: DIC measurement collection description] The Total Alkalinity and DIC were calculated from the output of the auto-titrator (volume of HCl delivered, pH) by calculating the the pH as a function of the volume of the acid delivered (see SOP3a Annexe 1). Bayesian inference on TA, DIC, voffset, fK1, fK2, pHoff, pHslope.

table here for priors

link to code.

[BM: confirm that reference with PW]

4.2.2 Data model: Data treatment, distributions and measurement error

Valve, temperature, light (normalised to 0/1) and dilution rates were used to force the model. Dissolved oxygen, pH, dissolved inorganic carbon and total alkalinity observations for 4 days post acclimation were assimilated. While pH observations were calibrated and corrected, it was visible that O_2 observations were not completely calibrated and experienced some sensor drift during the experiment.

The data model assigned log normally distributed observation errors for each instrument; $O_{2_{obs}} \sim \text{Log}\mathcal{N}(\log(O_2),\ \sigma_{O_2})$, $pH_{obs} \sim \text{Log}\mathcal{N}(\log(pH),\ \sigma_{pH})$, $DIC_{obs} \sim \text{Log}\mathcal{N}(\log(DIC),\ \sigma_{DIC})$, $TA_{obs} \sim \text{Log}\mathcal{N}(\log(TA),\ \sigma_{DIC})$, where the standard deviations (σ_{O_2}, σ_{pH}, σ_{DIC}) were unknown parameters to be estimated as part of the assimilating model. Dissolved inorganic carbon and total alkalinity measurements were obtained from the same instrument thus the error is shared between these states. Initial observation error priors started at $\sigma_{O_2} \sim \text{Log}\mathcal{N}(\log(0.1),\ 0.5)$ and

then were adjusted during the PMMH tuning phase.

[Chris/SW: should I talk about the thinning out of O_2 and pH obs?] [Chris, if you can be bothered Yes, you might even expand this a bit by showing the a couple of runs with differing density. This issue of how to combine fuse different measurements with huge differences in sampling density is an interesting one)

4.2.3 Process model: Carbon chemistry

[Chris : Carbon in seawater summary] main equations and rate constants role of TA This consists of 4(?) equations with (4?) uknowns

When carbon dioxide dissolves in water it undergoes a series of reactions which results in three species that exist in equilibrium, CO_2, HCO_3^- and CO_3^{-2}, accompanied the release of hydrogen ions. This chemical system as been the focus of extensive research as it central role in understanding the effect of anthropogenic carbon emissions and is generally referred to as "Ocean Acidification". The dependence of these equilibrium constants on temperature and salinity has been experimentally determined and functional forms have been fitted. This has been integrated into CO2SYS, a program developed for CO_2 system calculations that calculates and returns a detailed state of the carbonate system of oceanographic water samples in seawater and freshwater [7].research. A thorough explanation of CO2SYS, and seawater carbon chemistry in general, can be found in Zeebe and Wolf-gladrow [12]. A concise summary of the equations can be found in SOP 3a, Annexe 1 of [3].

It is recognised as the defacto standard for carbon system calculations and is widely used in oceanographic and marine biology. Given two of the four measurable carbonate system parameters (total alkalinity, total inorganic CO_2, pH, and either fugacity fCO_2 or partial pressure of CO_2) the other two parameters can be calculated for a set of input conditions (temperature and pressure). In the model the state variables TA and DIC are given and the pH and pCO_2 are calculated.

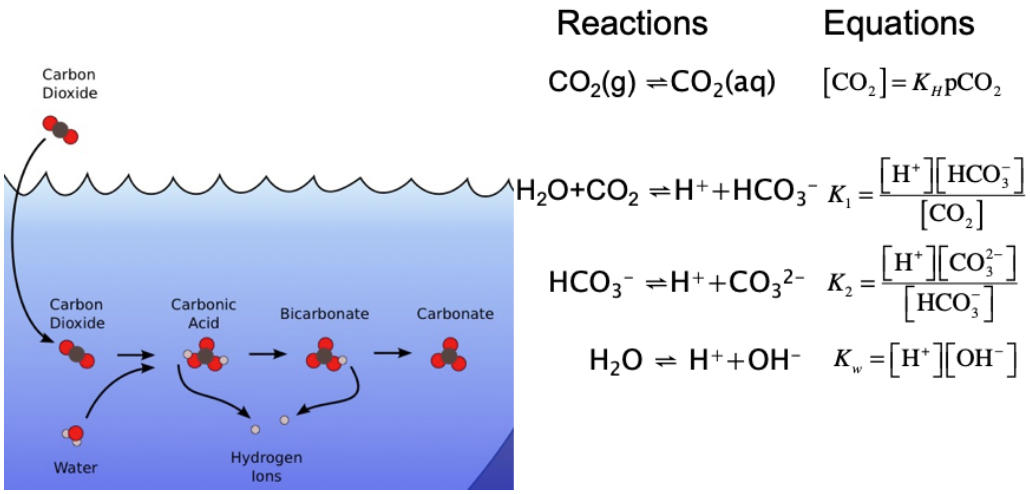

Figure 4.1 : CO2sys

Ideally CO2SYS [7] would have been used to calculate the carbon chemistry of the photo-bioreactor. However, it is not possible to directly implement CO2sys in LibBi due the constraints imposed by the GP-GPU computing model. CO2SYS iteratively solves the set of chemical equilibrium equations which requires a for / while loop and if constructs which are not available in LibBI. A number of approaches were explored to circumvent this restriction:

1. Creating functional approximation of the solutions from CO2sys
2. Recasting the steady state equilibrium as a set of coupled ODE's
3. Manually 'unrolling' the while loop as fixed number of iterations.

In addition to being tedious, it was not possible to achieve the accuracy needed via functional approximation. The set of ODE's the describe the chemical reactions are notoriously stiff [11] which resulted in a prohibitively small step size. Coding a fixed number of iterations also presented issues as if the number of iterations is too low the solution is inaccurate while too many iterations is inefficient and may be limited by memory available to the process on the GPU.

CO2SYS was successfully incorporated into LiBbi by a combination of the first and third approaches. The functional approximation from step 1 was used to precondition the solution and 3 iterations were explicitly coded. (Eq. 4.5 - 4.13) of the Newton-Raphson method for finding approximations to roots of real valued functions. The Newton-Raphson method is an iterative process $x_{n+1} = x_n - \frac{f(x_n)}{f'(x_n)}$ considering a function $f(x_n)$, its derivative $f'(x_n)$ and an initial starting value x_0. The approximate root x_{n+1} converges to the exact solution very quickly if a close initial starting value is picked. To ensure the quick convergence of the Newton-Raphson method, an approximating equation for pH_0 (Eq. 4.4) was obtained by fitting a stepwise regression with interactions to a range of simulated CO2SYS input parameters (temperature: 20-30, salinity: 30-40, DIC: 200-2500, and alkalinity: 1500-3000). A range of initial conditions and parameter values were tested, and each converged with RMSE < 0.01 across pH, HCO_3^-, CO_2, and CO_3, DIC, O_2, TA by the 3rd iteration (Figure 4.2 and Table 4.1).

CO2SYS constants and iterative solution for pH, HCO_3^-, CO_2, and CO_3

Total Sulfur:

$$
\begin{aligned}
TS &= \frac{0.14}{96.062} * \frac{S}{1.8065} \\
IS &= 19.924 \frac{S}{(1000 - 1.005S)} \\
KS_{int} &= -\frac{4276.1}{T_K} + 141.328 - 23.093 log(T_K) + (-\frac{13856.0}{T_K} + 324.57 \\
&\quad - 47.986 log(T_K))\sqrt{IS} + (\frac{35474}{T_K} - 771.54 + 114.723 log(T_K))IS \\
&\quad - \frac{2698}{T_K} IS^{1.5} + \frac{1776}{T_K} IS^2 \\
KS &= (1 - 0.001005S) e^{(KS_{int})}
\end{aligned}
$$

Fluorine:

$$TF = \frac{\frac{0.000067S}{18.9984}}{1.80655}$$
$$KF = e^{(-(-\frac{874.0}{T_K} - 0.111\sqrt{S} + 9.68))}$$
$$SWS_{2_T} = \frac{(1 + \frac{TS}{KS})}{(1 + \frac{TS}{KS} + \frac{TF}{KF})}$$
$$Free_{2_T} = 1 + \frac{TS}{KS}$$

H2O dissoc:

$$KW = e^{(148.9802 - \frac{13847.26}{T_K} - 23.6521 log(T_K) + (\frac{118.67}{T_K} - 5.977 + 1.0495 log(T_K))\sqrt{S} - 0.01615S)}$$

Boron:

$$KB = exp(\frac{(-8966.90 - 2890.53\sqrt{S} - 77.942S + 1.728S\sqrt{S} - 0.0996S^2)}{T_K}$$
$$+ 148.0248 + 137.1942\sqrt{S} + 1.62142S$$
$$- (24.4344 + 25.085\sqrt{S} + 0.2474S) log(T_K) + 0.053105 T_K \sqrt{S})$$
$$TB = 0.0004326 \frac{S}{35}$$

Choice of carbonate dissociation constants K_1 and K_2 were Mehrbach [8] (refit by Dickson and Millero [2]) with $1.23K_1$ and $0.53K_2$ measured experiment specific adjustments:

$$K_1 = 10^{(-(\frac{3633.86}{T_K} - 61.2172 + 9.6777 log(T_K) - 0.011555S + 0.0001152S^2))} * 1.23 \quad (4.2)$$
$$K_2 = 10^{(-(\frac{471.8}{T_K} + 25.9290 - 3.16967 log(T_K) - 0.01781S + 0.0001122S^2))} * 0.53 \quad (4.3)$$

Approximating equation for the starting value of pH:

$$pH_0 = 12.26 - 0.0030605DIC - 0.043752T - 0.013625S + 0.00011315TA$$
$$+ 1.3463e-5DIC * T + 5.2215e-7DIC * TA \quad (4.4)$$

Newton-Raphson iterations:

$$h_n = 10^{-pH_n} \tag{4.5}$$

$$h_{n_{free}} = \frac{h_n}{Free_{2_T}} \tag{4.6}$$

$$\begin{aligned} f_n = (DIC * 1e-6 * \frac{K_1 h_n + 2K_1K_2}{h_n^2 + K_1 h_n + K_1K_2} \\ - h_{n_{free}} + \frac{KW}{h_n} - TA * 1e-6 + \frac{TB}{1 + \frac{h_n}{KB}}) * 1e6 \end{aligned} \tag{4.7}$$

$$\begin{aligned} df_n = (DIC * 1e-6 * \frac{K_1 + 2K_1K_2}{h_n^2 + K_1 h_n + K_1K_2} \\ - DIC * 1e-6 * \frac{(K_1 h_n + 2K_1K_2)}{(h_n^2 + K_1 h_n + K_1K_2)^2}(2h_n + K_1) \\ - TB\frac{1}{(1 + \frac{h_n}{KB})^2}/KB \\ - \frac{KW}{h_n^2} - \frac{1}{Free_{2_T}}) * 1e6 * (-log(10) * 10^{-pH}) \end{aligned} \tag{4.8}$$

$$pH_{n+1} = pH_n - \frac{f_n}{df_n} \tag{4.9}$$

$$H_{n+1} = 10^{-pH_{n+1}} \tag{4.10}$$

$$CO_{2_{n+1}} = \frac{H_{n+1}^2 DIC}{H_{n+1}^2 + K_1 H_{n+1} + K_1K_2} \tag{4.11}$$

$$HCO_{3_{n+1}} = \frac{H_{n+1} K_1 DIC}{H_{n+1}^2 + K_1 H_{n+1} + K_1K_2} \tag{4.12}$$

$$CO_{3_{n+1}} = \frac{K_1K_2 DIC}{H_{n+1}^2 + K_1 H_{n+1} + K_1K_2} \tag{4.13}$$

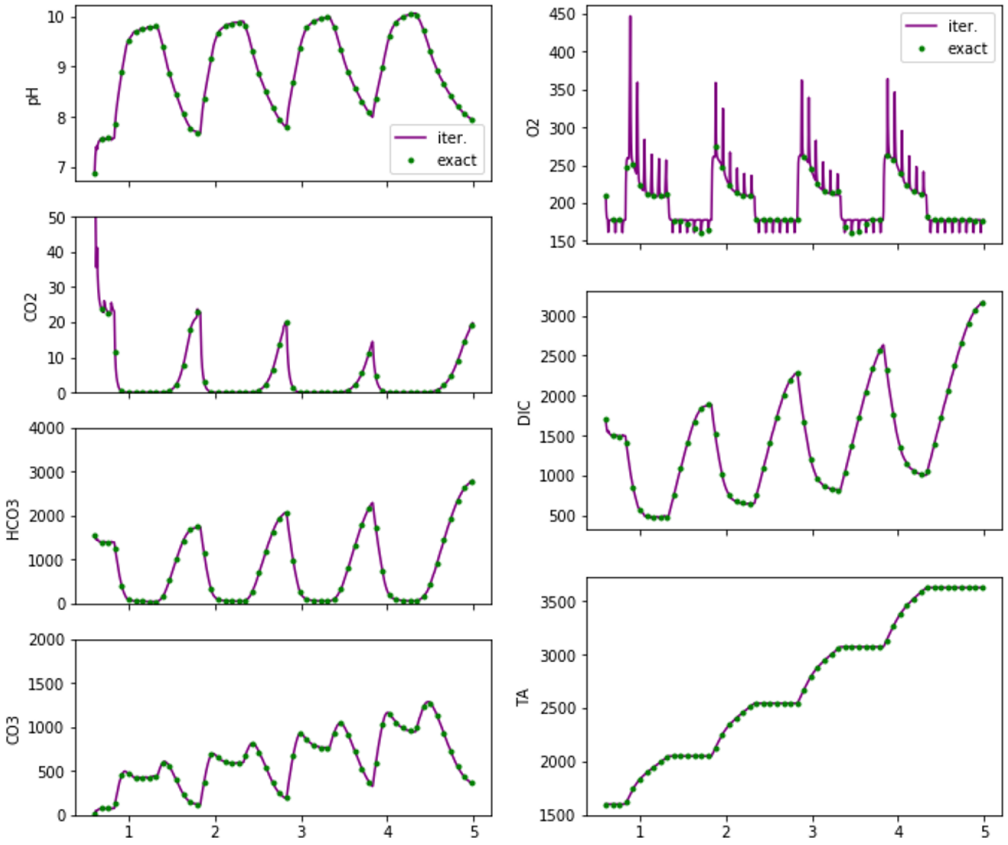

Figure 4.2 : Iterative (3rd iteration) vs exact solution for carbon chemistry CO_2, HCO_3, CO_3, pH and state variables O_2, DIC, and TA.

Variable	**Iter. 1**	**Iter. 2**	**Iter. 3**	**Iter. 4**	**Iter. 5**
pH	0.036092734	0.002355758	1.41E-05	6.93E-06	6.93E-06
CO_2	2.109401968	0.145719349	0.001222812	0.000866728	0.000866727
HCO_3	19.81869214	1.21021115	0.008016765	0.001025002	0.001025139
CO_3	20.89660704	1.307061652	0.00867642	0.001102278	0.001102434
DIC	16.78775711	0.958511825	0.005229318	0.002305054	0.002333411
O_2	0.308389964	0.016044284	4.18E-05	6.89E-05	7.59E-05
TA	2.607767674	0.160897272	0.000688102	0.001257725	0.001218981

Table 4.1 : RMSE for 5 iterations of the Newton-raphson carbon chemistry iterative solution.

4.2.4 Process model: Gas transfer equilibrium concentrations for O_2 and CO_2

The equilibrium concentration for CO_2 solubility in water CO_{2H} (μmol/L) is calculated using Henry's law,

$$CO_{2H} = K0_{CO2} * fCO2 * 1.0220 * 1e6 \tag{4.14}$$

where fCO2 (atm) is the fugacity or approximately the partial pressure of CO_2, 1.0220 is the density of seawater (kg/L) at salinity 34 ppt and temperature 27°C [9] [4]. $K0_{CO2}$ (mol/kg_{soln}/atm) is the solubility of gas in seawater [BM: ask Chris: solubility of gas? is this right] and is calculated from the fitted van't Hoff equation and the logarithmic Setchenow salinity dependence [10],

$$K0_{CO2} = e^{(-60.2409+93.4517\frac{100}{T_K}+23.3585*log(\frac{T_K}{100})+S(0.023517-0.023656\frac{T_K}{100}+0.0047036(\frac{T_K}{100})^2))} \tag{4.15}$$

where T_K is the temperature (K) and S is salinity (ppt). Similarly the equilibrium concentration for O_2 solubility in water O_{2H} is calculated using Henry's law,

$$O_{2H} = K0_{O2} * fO2 * 1.0220 * 1e-6 \tag{4.16}$$

where fO2 (atm) is the fugacity or approximately the partial pressure of O_2, 1.0220 is the density of seawater (kg/L) at salinity 34 ppt and temperature 27°C [9] [4], and $K0_{O2}$ (mol/kg_{soln}/atm) is the solubility of oxygen in seawater with an adjusted salinity dependence [1],

$$K0_{O2} = \frac{e^{(-1282.8704+\frac{36619.96}{T_K}+223.1396log(T_K)-0.354707T_K+S(5.957e-3-\frac{3.7353}{T_K})+3.68e-6S^2)}}{0.2094e-6} \tag{4.17}$$

where T_K is the temperature (K) and S is salinity (ppt). The equilibrium concentrations for O_2 and CO_2 are modelled together with the gas turning on and off during the experiment, as

$$Q^{air} kLa^{air}_{O_2}(O_{2H} - O_2) \tag{4.18}$$

$$Q^{air} k La^{air}_{CO_2} (CO_{2H} - CO_2) \tag{4.19}$$

where Q^{air} is the gas state (1= on, 0= off), $kLa^{air}_{O_2}$ and $kLa^{air}_{CO_2}$ are the mass transfer coefficients for air (d^{-1}), and 0.893 is the ratio between measured O_2 and CO_2 mass transfer constants [5].

4.2.5 Process model: Photosynthesis and respiration

Net photosynthesis

$$\frac{\partial DIC}{dt} = -(P_1 I \frac{HCO_3^-}{K_m + HCO_3^-} - R_1) \tag{4.20}$$

$$\frac{\partial O_2}{dt} = \frac{1}{(RQ_d I + RQ_n(1-I))}(P_1 I \frac{HCO_3^-}{K_m + HCO_3^-} - R_1) \tag{4.21}$$

$$\frac{\partial TA}{\partial t} = R_R(P_1 I \frac{HCO_3^-}{K_m + HCO_3^-}) \tag{4.22}$$

$$\frac{\partial C_p}{\partial t} = (P_1 I \frac{HCO_3^-}{K_m + HCO_3^-} - R_1) \tag{4.23}$$

RQ_d and RQ_n are the day and night respiratory quotients, the ratio of CO_2 produced and O_2 consumed by a cell.

The michaelis menton term $\frac{HCO_3^-}{K_m + HCO_3^-}$ represents the photosynthetically active carbon that the microalgae use for photosynthesis.

4.2.6 Process model: Dilution

$\frac{Q^M}{V}(DIC^M - DIC)$

$\frac{Q^M}{V}(\ O_2^M - \quad O_2)$

$\frac{Q^M}{V}(\ TA^M - \ TA)$

$\frac{Q^M}{V}(\qquad - \quad C_p)$

4.2.7 Process model: Ordinary differential equations

A summary of the ode's that make up the process model:

$$
\begin{array}{lrlll}
\text{Rate} & & \text{flux into cells} & \text{gas transfer} & \text{dilution} \\
\frac{\partial DIC}{\partial t} = & -(P_1 I \frac{HCO_3^-}{K_m + HCO_3^-} - R_1) & +\hat{Q}^{air} kLa^{air}_{CO_2}(CO_2^{air} - CO_2) & +\frac{Q^M}{V}(DIC^M - DIC) \\
\frac{\partial O_2}{\partial t} = & \frac{(P_1 I \frac{HCO_3^-}{K_m + HCO_3^-} - R_1)}{(RQ_d I + RQ_n(1-I))} & +\hat{Q}^{air} kLa^{air}_{O_2}(\ O_2^{air} - \ O_2) & +\frac{Q^M}{V}(\ O_2^M - \quad O_2) \\
\frac{\partial TA}{\partial t} = & R_R(P_1 I \frac{HCO_3^-}{K_m + HCO_3^-} \quad) & & +\frac{Q^M}{V}(\ TA^M - \ TA) \\
\frac{\partial C_p}{\partial t} = & (P_1 I \frac{HCO_3^-}{K_m + HCO_3^-} - R_1) & & +\frac{Q^M}{V}(\quad - \ C_p)
\end{array}
\tag{4.24}
$$

	Symbol	Description	Prior / Value	Unit
Initial conditions	DIC^0	Dissolved inorganic carbon	Log$\mathcal{N}$(log(1300), 0.2)	μM L^{-1}
	O_2^0	Oxygen	Log$\mathcal{N}$(log(225), 0.2)	μM L^{-1}
	TA^0	Total alkalinity	Log$\mathcal{N}$(log(1750), 0.1)	μM L^{-1}
	C_p^0		Log$\mathcal{N}$(log(300), 0.2)	μM L^{-1}
	pH^0	-	Log$\mathcal{N}$(log(8.5), 0.2)	log$_{10}$(-mol/L H+)
	${CO_2}^0$	Carbon dioxide	Log$\mathcal{N}$(log(5), 0.4)	μM L^{-1}
	${HCO_3^-}^0$	Bicarbonate	Log$\mathcal{N}$(log(1500), 0.3)	μM L^{-1}
	${CO_3^{2-}}^0$	Carbonate	Log$\mathcal{N}$(log(100), 0.4)	μM L^{-1}
Flux into cells	P_1	Maximum photosynthesis rate	*	μM L^{-1} hour^{-1}
	R_1	Respiration rate	*	μM L^{-1} hour^{-1}
	K_m	Carbon restriction	*	μM L^{-1}
	RQ_d	Daytime respiratory quotient	*	-
	RQ_n	Night respiratory quotient	*	-
	R_R	Redfield ratio	*	-
	I	Light indicator	forcing (0/1)	-

Gas transfer terms	$\hat{Q}^{air}$	Indicator for flow in air line	forcing (0/1)	-
	$x^{air}_{CO_2}$	Mole fraction of CO_2 atmosphere	400	ppm
	CO_{2H}	Equilibrium CO_2 concentration	Eq. 4.14	μM L^{-1}
	CO^{air}_2	Sat CO_2 conc with atmosphere	$x^{air}_{CO_2} CO_{2H}$	
	$kLa^{air}_{CO_2}$	Mass transfer coefficient for CO_2	$0.893 kLa^{air}_{O_2}$	day^{-1}
	$x^{air}_{O_2}$	Mole fraction of O_2 atmosphere	0.2094	atm
	O_{2H}	Equilibrium O_2 concentration	Eq. 4.16	μM L^{-1}
	O^{air}_2	Sat O_2 conc with atmosphere	$x^{air}_{O_2} O_{2H}$	
	τ	half-life of $kLa^{air}_{O_2}$	range(2-20)	min^{-1}
	$kLa^{air}_{O_2}$	Mass transfer coefficient for O_2	$\ln(2) * 24 * 60/\tau$	day^{-1}
Dilution terms	Q^M	Dilution rate	forcing	ml day^{-1}
	V	Volume of the reactor	500	ml
	DIC^M	Media dissolved inorganic carbon	1724.20	μM L^{-1}
	O^M_2	Media oxygen concentration	226.65	μM L^{-1}
	TA^M	Media total alkalinity	1797.90	μM L^{-1}

Table 4.2 : State variable, parameter and forcing definitions with units, and their assignments: either fixed values, priors on initial condition or priors on parameters (*) defined later in Section 4.2.9.

4.2.8 Design and setup of data assimilation model with both simulated and experimental data

A simulated dataset was created by running the process model described in Section 4.2.7 with a fixed set of parameters ($P_1 = 200$, $R_1 = 30$, $kLA^{air}_{O_2} = 200$, $K_m = 150$, $RQ_d = 0.85$, $RQ_n = 0.95$, $R_R = 0.075$) and initial conditions ($O^0_2 = 225$, $DIC^0 = 1250$, $TA^0 = 1750$).

The first set of results assimilated the simulated observations and attempted to

recover the true value parameters.

[Chris: noise on simulated dataset? sigmas?] Normally distributed errors with std devs of

σ^2_{DA}	50.
$\sigma^2_{O_2}$	2.
σ^2_{pH}	0.01

4.2.9 Parameter Model: Priors

[BM: I've put these in at the beginning of each results section for now and described how each run is different in the **In this run:** parts, so that they're clear when we're discussing the results. Will move it here after our meeting.]

4.3 Results and Discussion

4.3.1 Posteriors with simulated data (photosynthesis and respiration as parameters)

State posteriors are visualised by plotting the median and shading 95% credible intervals, while parameter priors and posteriors are displayed by histograms.

In this run:

P_1, R_1, kLA_{O_2}, K_m, R_R, RQ_d, and RQ_n and all treated as parameters constant through time but unknown. The data model assigned log normally distributed observation errors for each instrument;

$O_{2_{obs}} \sim \text{Log}\mathcal{N}(\log(O_2), \sigma_{O_2})$

$pH_{obs} \sim \text{Log}\mathcal{N}(\log(pH), \sigma_{pH})$

$DIC_{obs} \sim \text{Log}\mathcal{N}(\log(DIC), \sigma_{DIC})$

$TA_{obs} \sim \text{Log}\mathcal{N}(\log(TA), \sigma_{DIC})$

Parameter	**Prior**	**Proposal**
P_1	Log$\mathcal{N}$(log(250.0), 0.8)	Log$\mathcal{N}$(log(P_1), 0.08)
R_1	Log$\mathcal{N}$(log(20.0), 0.8)	Log$\mathcal{N}$(log(R_1), 0.08)
kLA_{O2}	Log$\mathcal{N}$(log(200.0), 0.3)	Log$\mathcal{N}$(log(kLA_{O2}), 0.03)
K_m	Log$\mathcal{N}$(log(200.0), 0.6)	Log$\mathcal{N}$(log(K_m), 0.06)
R_R	Uniform(0, 0.2)	Trun$\mathcal{N}$(R_R, 0.01, lower = 0, upper = 0.2)
RQ_d	Uniform(0.6, 1)	Trun$\mathcal{N}$(RQ_d, 0.005, lower = 0.6, upper = 1.0)
RQ_n	Uniform(0.6, 1)	Trun$\mathcal{N}$(RQ_n, 0.005, lower = 0.6, upper = 1.0)
σ_{O_2}	0.3	*
σ_{pH}	0.3	*
σ_{DIC}	0.3	*

Table 4.3 : Table of Parameters, their priors and proposal distributions. * indicates the parameter was held fixed.

Parameter	**Quantiles (25%, 75%)**	**Quantiles (5%, 95%)**	**True value**
P_1	(237.2167, 302.0565)	(200.8971, 365.1751)	200
R_1	(17.5370, 32.3501)	(10.6537, 45.3096)	30
$kLA_{O_2}^{air}$	(177.1383, 222.9641)	(148.2838, 267.5934)	200
K_m	(182.6643, 362.6714)	(118.4125, 643.8518)	150
R_R	(0.0504, 0.1041)	(0.0196, 0.1458)	0.075
RQ_d	(0.7529, 0.8718)	(0.6894, 0.9666)	0.85
RQ_n	(0.7469, 0.9338)	(0.6400, 0.9920)	0.95

Table 4.4 : True values for parameters used to create the simulated observations, and posterior (25%, 75%), (5%, 95%) quantiles for parameters after assimilating observations.

Results:

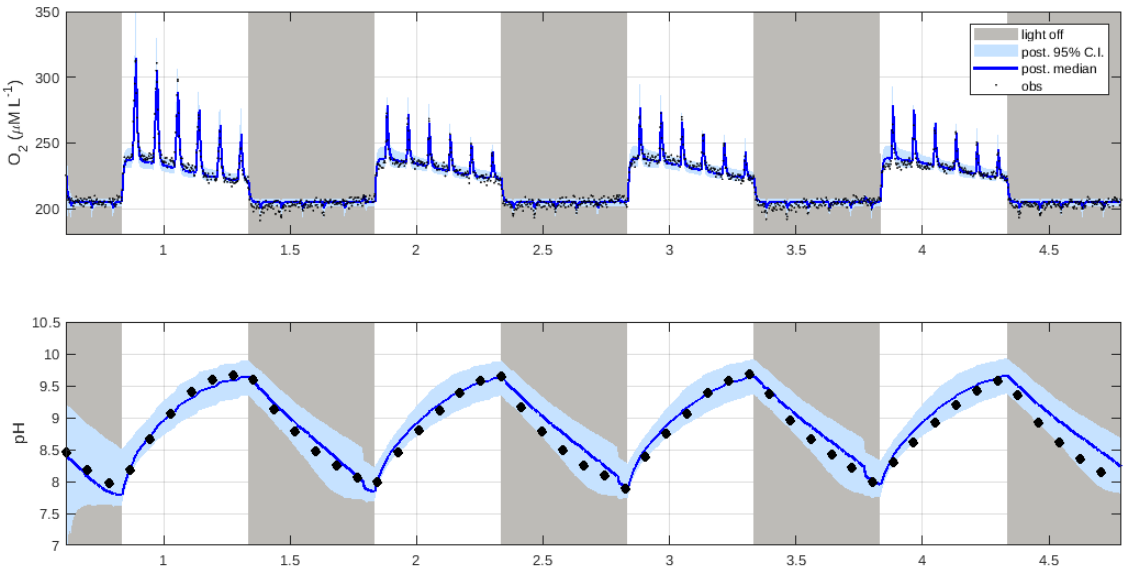

Figure 4.3 : Posterior medians (solid blue line), 95% credible intervals (shaded blue), and simulated observations (black) for O_2 and pH across 4 days.

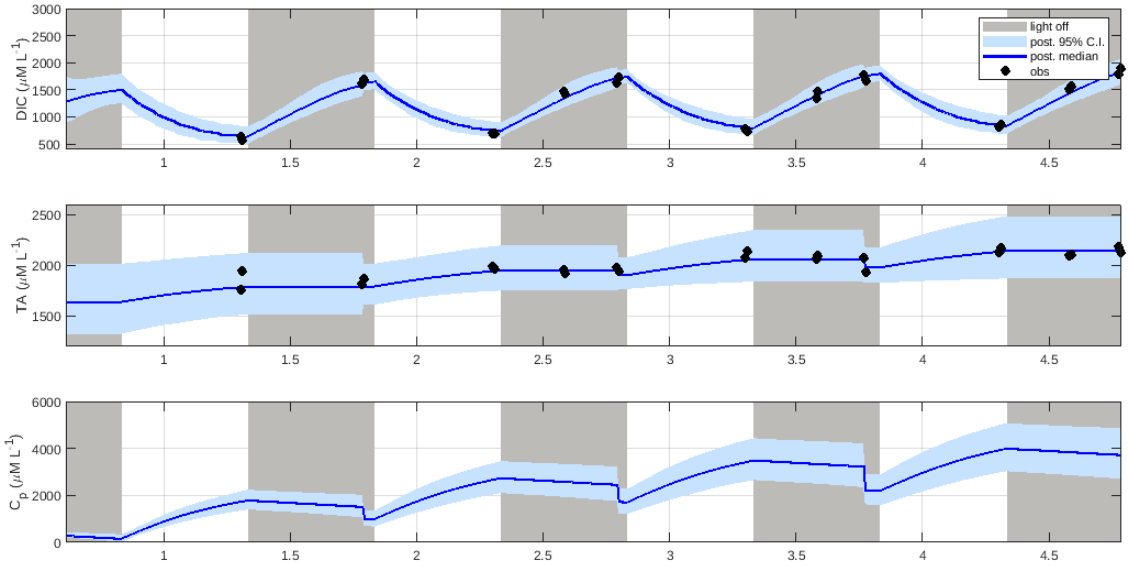

Figure 4.4 : Posterior medians (solid blue line), 95% credible intervals (shaded blue), and simulated observations (black) for DIC, TA and C_p across 4 days.

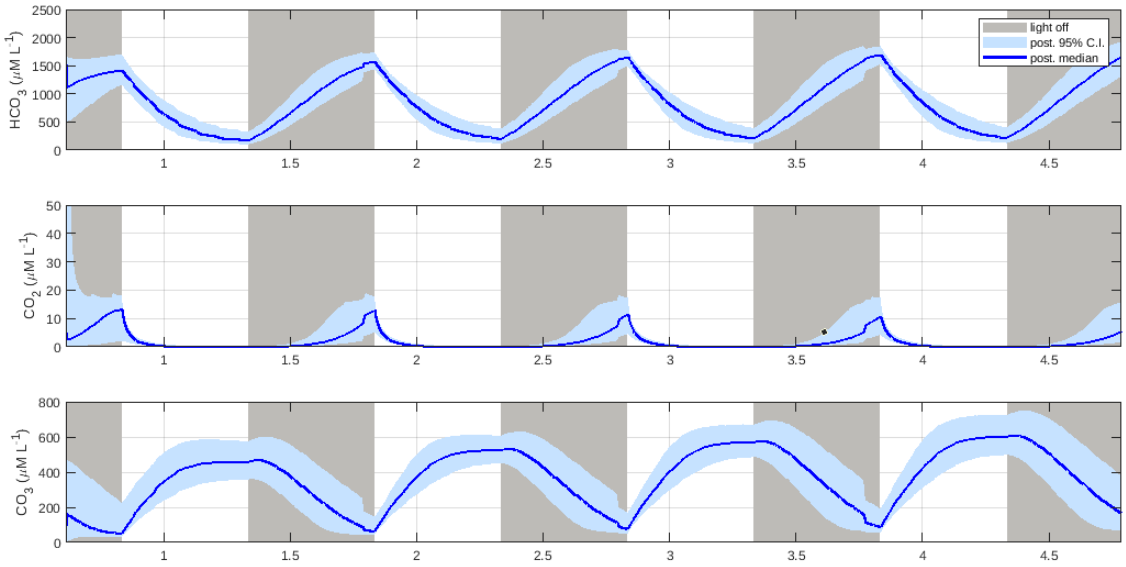

Figure 4.5 : Posterior medians (solid blue line) and 95% credible intervals (shaded blue) for HCO_3, CO_2 and CO_3 across 4 days.

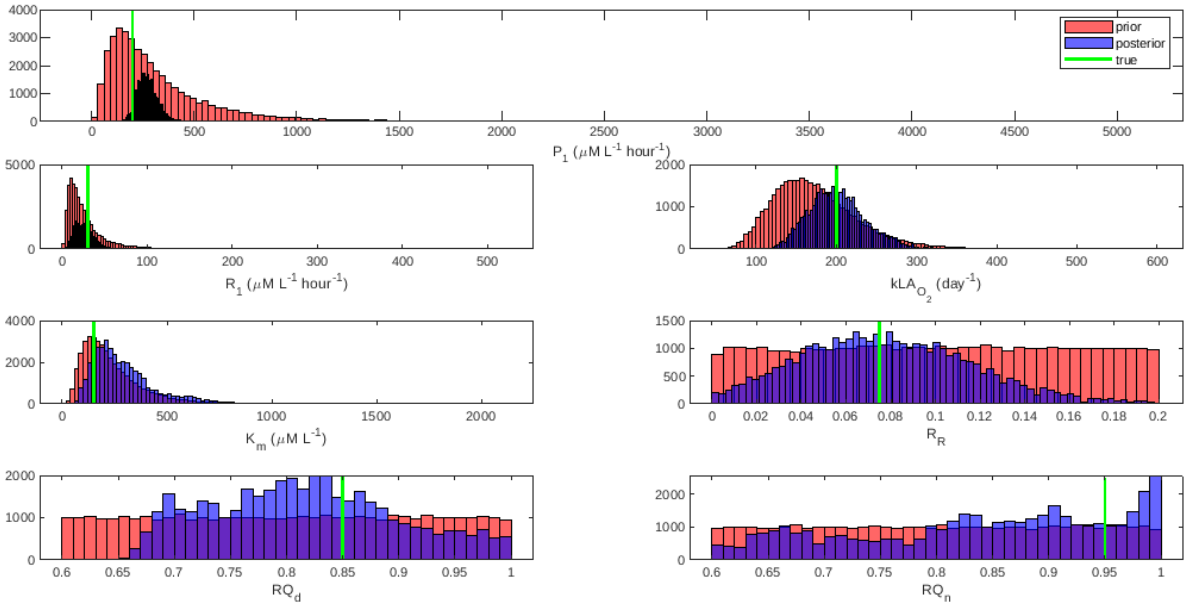

Figure 4.6 : Priors (pink), posteriors (purple) and true values (green) for model parameters.

After 50,000 samples the true values of parameters R_1, $kLA^{air}_{O_2}$, R_R, and RQ_d were recovered to the 25th and 75th percentile (Table 4.4). While the true values of K_m and RQ_n did not lie within the 25th and 75th quantile, they were captured by the 5th and 95th percentiles (Table 4.4). P_1 was the only parameter whose true value lied on the cusp of the 5th percentile. Parameters P_1, R_1, $kLA^{air}_{O_2}$, and R_R mixed well, while parameters K_m, RQ_d, and RQ_n did not mix as well, some autocorrelation between samples was present (Figure A.3).

Log-likelihood stopped rising (Figure A.2). Acceptance rate was XX.

Observed state variables O_2, DIC, TA, and observed pH posteriors were in excellent agreement with observations, with all observations fitting within the 95% credible interval posteriors (Figure 4.3, Figure 4.4).

Unobserved state variable C_p .. (Figure 4.4).

Carbon chemistry variables HCO_3, CO_2, and CO_3 ... (Figure 4.5).

4.3.2 Posteriors with simulated data (photosynthesis and respiration modelled as random walks)

State posteriors are visualised by plotting the median and shading 95% credible intervals, while parameter priors and posteriors are displayed by histograms.

In this run:

kLA_{O_2}, K_m, R_R, RQ_d, and RQ_n and all treated as parameters constant through time but unknown. Photosynthesis (P_1) and respiration (R_1) are both modelled as random walks, by taking P and R, previously constant parameters, and replacing them by $P_1(t)$ and $R_1(t)$. Here, we take $P_1(t)$ and $R_1(t)$ to be such that

$$P_1(t + \Delta t) = P(t) + r_P$$

$$R_1(t + \Delta t) = R(t) + r_R$$

where $r_P \sim N(0, \sigma_{r_P})$, $r_R \sim N(0, \sigma_{r_R})$, and Δt is the length of discrete time-step. For the purpose of the Bayesian analysis here, σ_{r_P} and σ_{r_R} are treated as parameters

to be inferred.

The data model assigned log normally distributed observation errors for each instrument;

$O_{2_{obs}} \sim \text{Log}\mathcal{N}(\log(O_2),\, \sigma_{O_2})$

$pH_{obs} \sim \text{Log}\mathcal{N}(\log(pH),\, \sigma_{pH})$

$DIC_{obs} \sim \text{Log}\mathcal{N}(\log(DIC),\, \sigma_{DIC})$

$TA_{obs} \sim \text{Log}\mathcal{N}(\log(TA),\, \sigma_{DIC})$

Parameter	**Prior**	**Proposal**
kLA_{O2}	$\text{Log}\mathcal{N}(\log(200.0),\, 0.3)$	$\text{Log}\mathcal{N}(\log(kLA_{O2}),\, 0.03)$
K_m	$\text{Log}\mathcal{N}(\log(200.0),\, 0.6)$	$\text{Log}\mathcal{N}(\log(K_m),\, 0.06)$
R_R	Uniform(0, 0.2)	$\text{Trun}\mathcal{N}(R_R,\, 0.01,\, \text{lower} = 0,\, \text{upper} = 0.2)$
RQ_d	Uniform(0.6, 1)	$\text{Trun}\mathcal{N}(RQ_d,\, 0.005,\, \text{lower} = 0.6,\, \text{upper} = 1.0)$
RQ_n	Uniform(0.6, 1)	$\text{Trun}\mathcal{N}(RQ_n,\, 0.005,\, \text{lower} = 0.6,\, \text{upper} = 1.0)$
σ_{r_P}	$\mathcal{N}(0.01,\, 0.001)$	$\mathcal{N}(\sigma_{r_P},\, 0.0001)$
σ_{r_R}	$\mathcal{N}(0.01,\, 0.001)$	$\mathcal{N}(\sigma_{r_R},\, 0.0001)$
σ_{O_2}	0.3	*
σ_{pH}	0.3	*
σ_{DIC}	0.3	*

Table 4.5 : Table of Parameters, their priors and proposal distributions. * indicates the parameter was held fixed.

Parameter	**Quantiles (25%, 75%)**	**Quantiles (5%, 95%)**	**True value**
$kLA_{O_2}^{air}$	(170.9854, 216.0578)	(145.6899, 253.4652)	200
K_m	(187.3586, 386.4103)	(93.9670, 641.1432)	150
R_R	(0.0521, 0.1075)	(0.0192, 0.1544)	0.075
RQ_d	(0.7906, 0.8965)	(0.6833, 0.9711)	0.85

RQ_n	(0.6654, 0.8334)	(0.6164, 0.9739)	0.95

Table 4.6 : True values for parameters used to create the simulated observations, and posterior (25%, 75%), (5%, 95%) quantiles for parameters after assimilating observations.

Results:

After 50,000 samples the true values of parameters $kLA^{air}_{O_2}$, R_R, and RQ_d were recovered to the 25th and 75th percentile (Table 4.6). While the true values of K_m and RQ_n did not lie within the 25th and 75th quantile, they were captured by the 5th and 95th percentiles (Table 4.6). Parameters σ_{rP}, σ_{rR}, $kLA^{air}_{O_2}$, and R_R mixed well, while parameters K_m, RQ_d, and RQ_n did not mix as well, some autocorrelation between samples was present (Figure 4.12). The true values of P_1 (200) and R_1 (30) lie within the posterior 95% credible intervals (Figure 4.11).

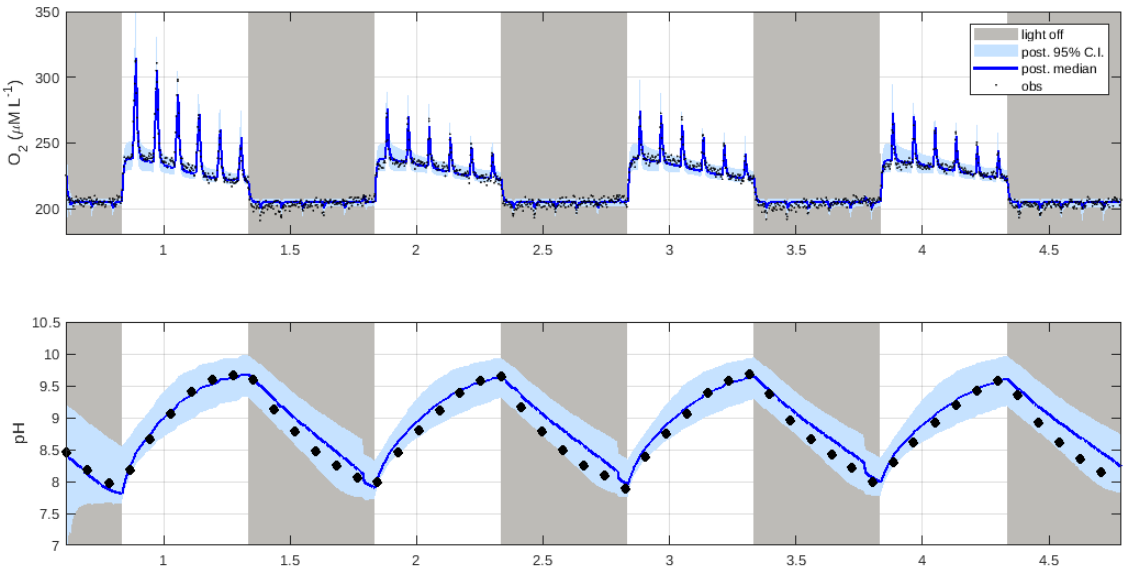

Figure 4.7 : Posterior medians (solid blue line), 95% credible intervals (shaded blue), and simulated observations (black) for O_2 and pH across 4 days.

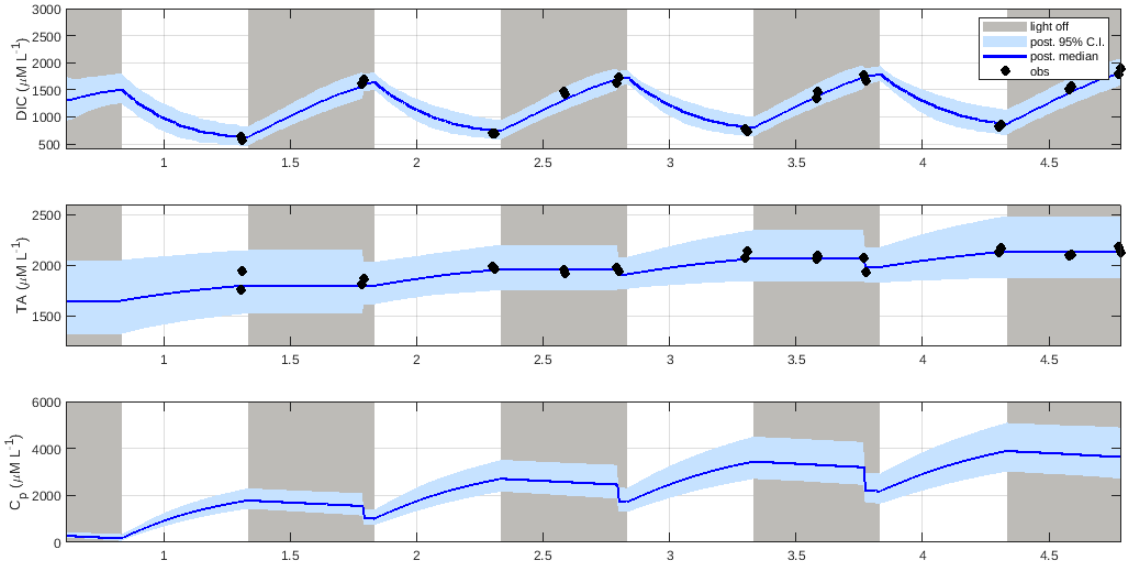

Figure 4.8 : Posterior medians (solid blue line), 95% credible intervals (shaded blue), and simulated observations (black) for DIC, TA and C_p across 4 days.

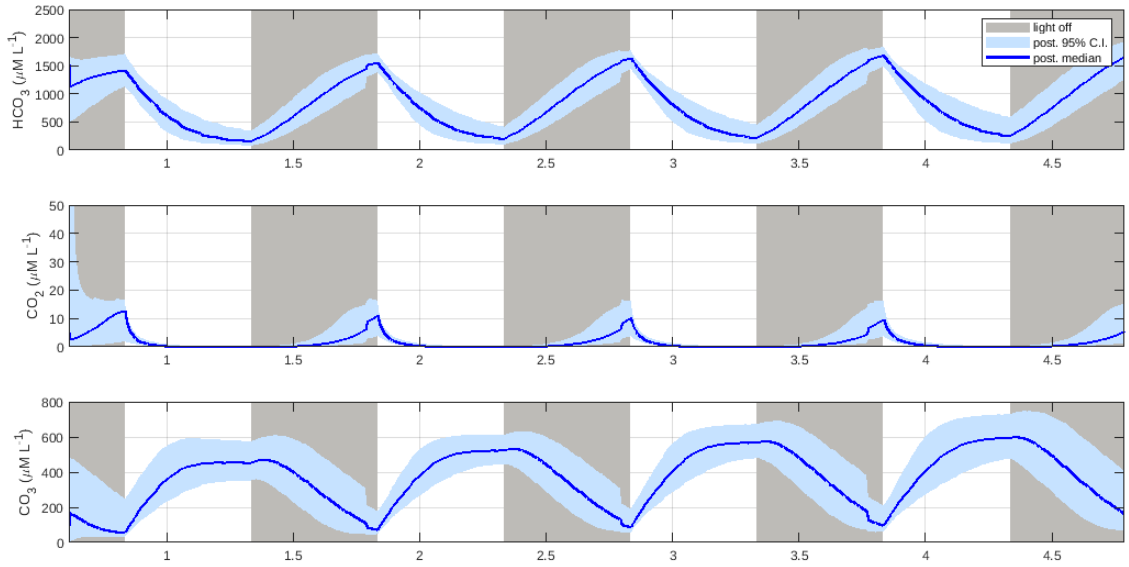

Figure 4.9 : Posterior medians (solid blue line) and 95% credible intervals (shaded blue) for HCO_3, CO_2 and CO_3 across 4 days.

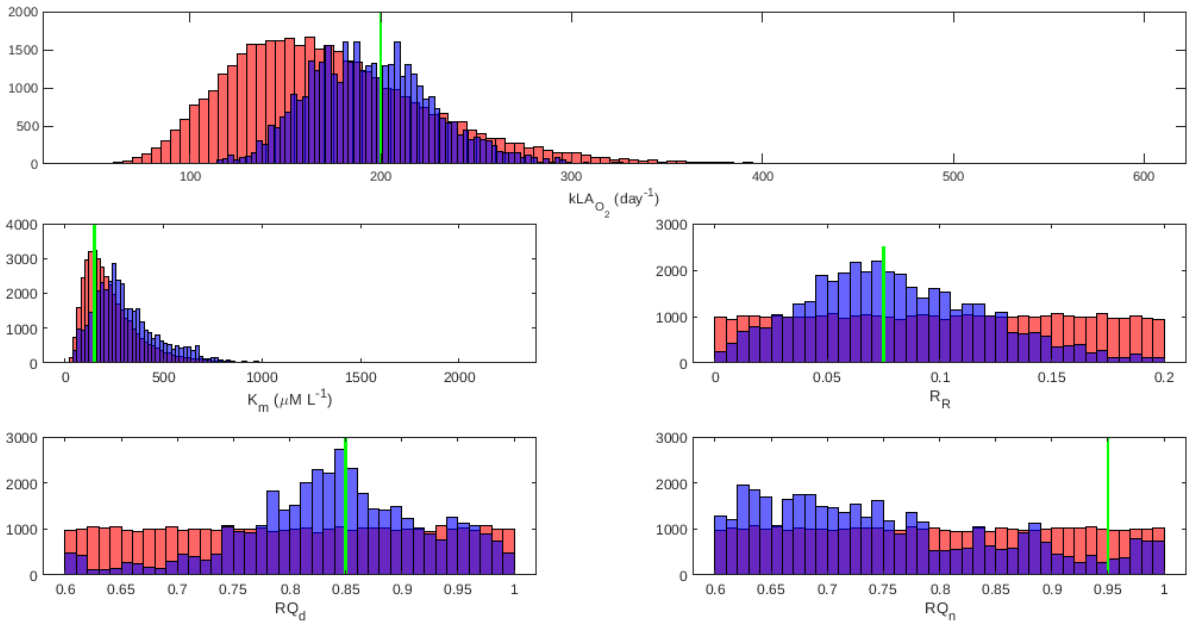

Figure 4.10 : Priors (pink), posteriors (purple) and true values (green) for model parameters.

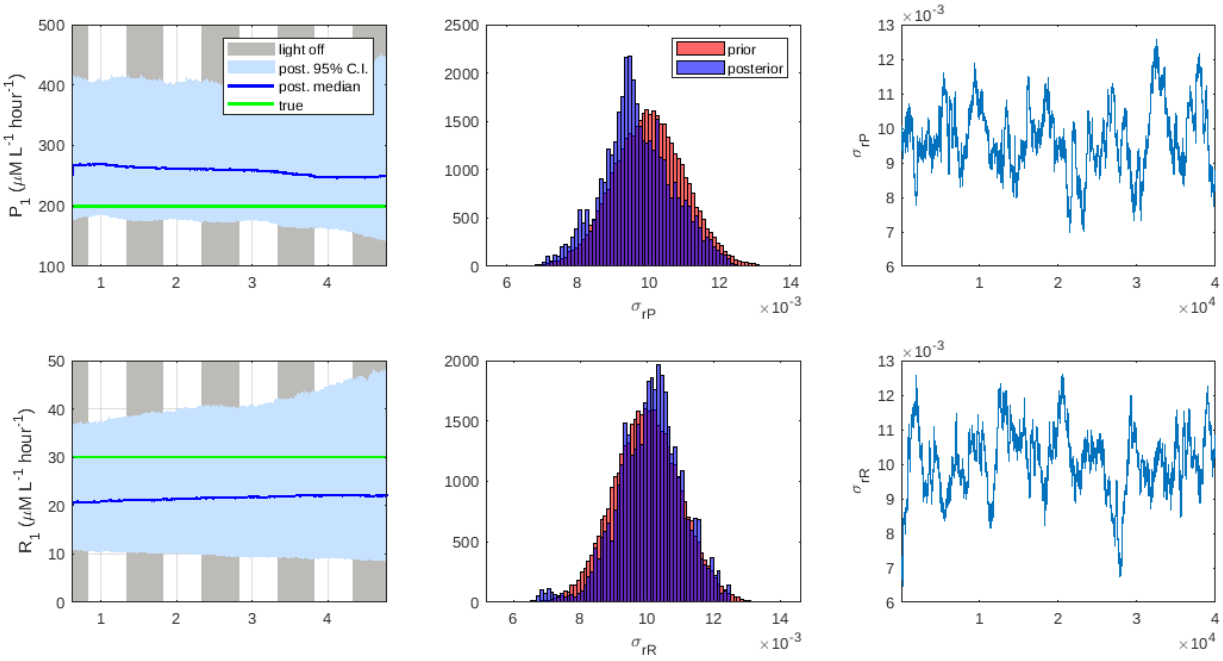

Figure 4.11 : Random walk posteriors P_1 and R_1 medians (solid blue), 95% credible intervals (shaded blue), and true values (green). σ_{rP} and σ_{rR} priors (pink), posteriors (purple), true values (green) and traces.

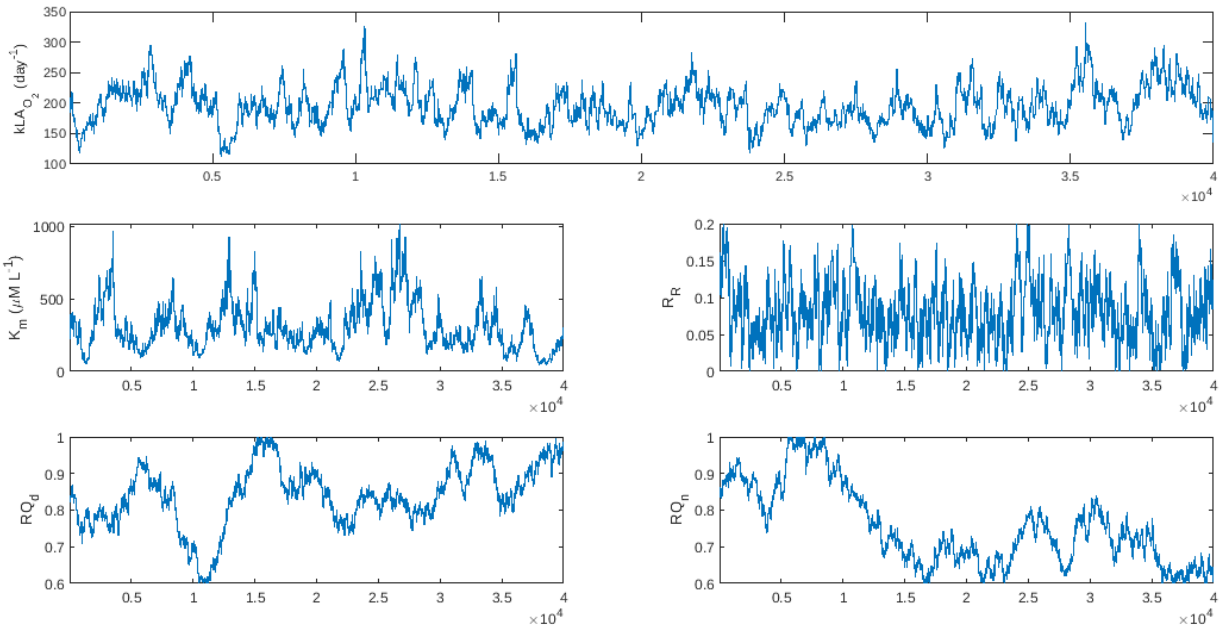

Figure 4.12 : Traces for model parameters.

4.3.3 Posteriors with experimental data (photosynthesis, respiration and respiratory quotients are random walks)

State posteriors are visualised by plotting the median and shading 95% credible intervals, while parameter priors and posteriors are displayed by histograms.

In this run:

Photosynthesis (P_1) and respiration (R_1) are both modelled as random walks, by taking P and R, previously constant parameters, and replacing them by $P_1(t)$ and $R_1(t)$. Here, we take $P_1(t)$ and $R_1(t)$ to be such that

$$P_1(t + \Delta t) = P(t) + r_P$$

$$R_1(t + \Delta t) = R(t) + r_R$$

where $r_P \sim N(0, \sigma_{r_P})$, $r_R \sim N(0, \sigma_{r_R})$, and Δt is the length of discrete time-step. For the purpose of the Bayesian analysis here, σ_{r_P} and σ_{r_R} are treated as parameters to be inferred. The respiratory quotients RQ_d and RQ_n were also treated as random walks with rP and rR as wiener processes. kLA_{O_2}, K_m, R_R, σ_{rP}, σ_{rR} are all treated as parameters constant through time but unknown. The data model assigned log normally distributed observation errors for each instrument;

$O_{2_{obs}} \sim \text{Log}\mathcal{N}(\log(O_2), \sigma_{O_2})$

$pH_{obs} \sim \text{Log}\mathcal{N}(\log(pH), \sigma_{pH})$

$DIC_{obs} \sim \text{Log}\mathcal{N}(\log(DIC), \sigma_{DIC})$

$TA_{obs} \sim \text{Log}\mathcal{N}(\log(TA), \sigma_{DIC})$

Parameter	Prior	Proposal
kLA_{O2}	Log$\mathcal{N}$(log(200.0), 0.3)	Log$\mathcal{N}$(log(kLA_{O2}), 0.03)
K_m	Log$\mathcal{N}$(log(200.0), 0.6)	Log$\mathcal{N}$(log(K_m), 0.06)
R_R	Uniform(0, 0.2)	Trun$\mathcal{N}$(R_R, 0.005, lower = 0, upper = 0.2)
σ_{r_P}	$\mathcal{N}$(0.02, 0.002)	$\mathcal{N}$(σ_{r_P}, 0.0002)
σ_{r_R}	$\mathcal{N}$(0.01, 0.001)	$\mathcal{N}$(σ_{r_R}, 0.0001)
σ_{O_2}	0.3	*
σ_{pH}	0.3	*
σ_{DIC}	0.5	*

Table 4.7 : Table of Parameters, their priors and proposal distributions. * indicates the parameter was held fixed.

Parameter	Quantiles (25%, 75%)	Quantiles (5%, 95%)
$kLA_{O_2}^{air}$	(139.5979, 170.8102)	(120.9171, 204.5474)
K_m	(168.1931, 378.8001)	(104.2598, 599.6881)
R_R	(0.0815, 0.1512)	(0.0284, 0.1844)
σ_{r_P}	(0.0178, 0.0202)	(0.0159, 0.0222)
σ_{r_R}	(0.0094, 0.0113)	(0.0081, 0.0121)

Table 4.8 : Posterior (25%, 75%), (5%, 95%) quantiles for parameters after assimilating observations.

Results:

40,000 samples run with 1024 particles (40,000 was all that could complete in the maximum time allocation on the hpc cluster). 10,000 discarded as burn-in, acceptance rate was XX.

Observed state variables DIC and TA posteriors perform well with all observations lying within the 95% credible intervals (Figure 4.14). O_2 posteriors tracked the

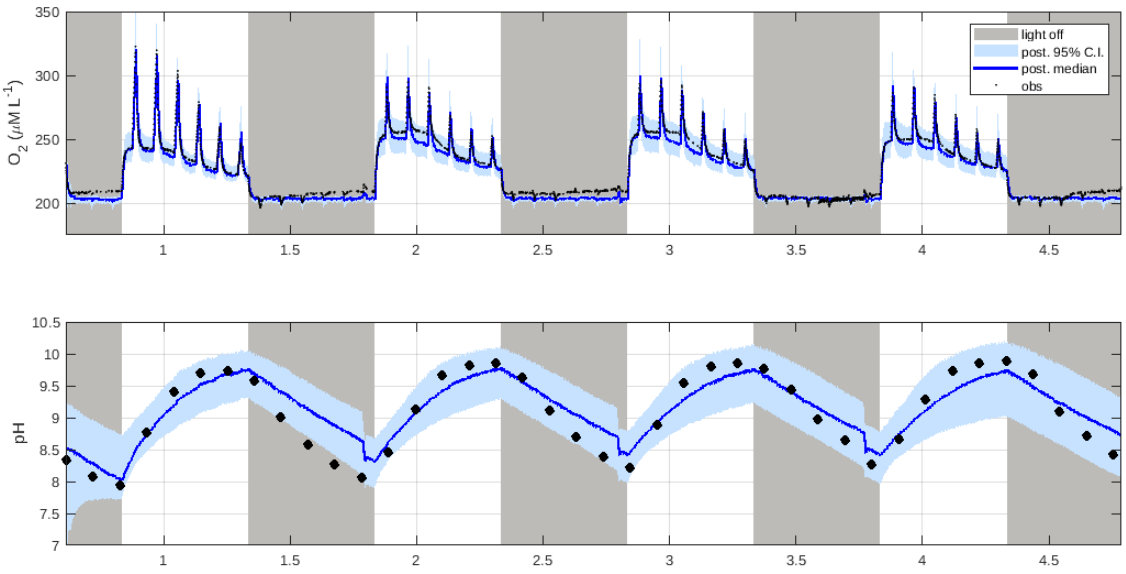

Figure 4.13 : Posterior medians (solid blue line), 95% credible intervals (shaded blue), and simulated observations (black) for O_2 and pH across 4 days.

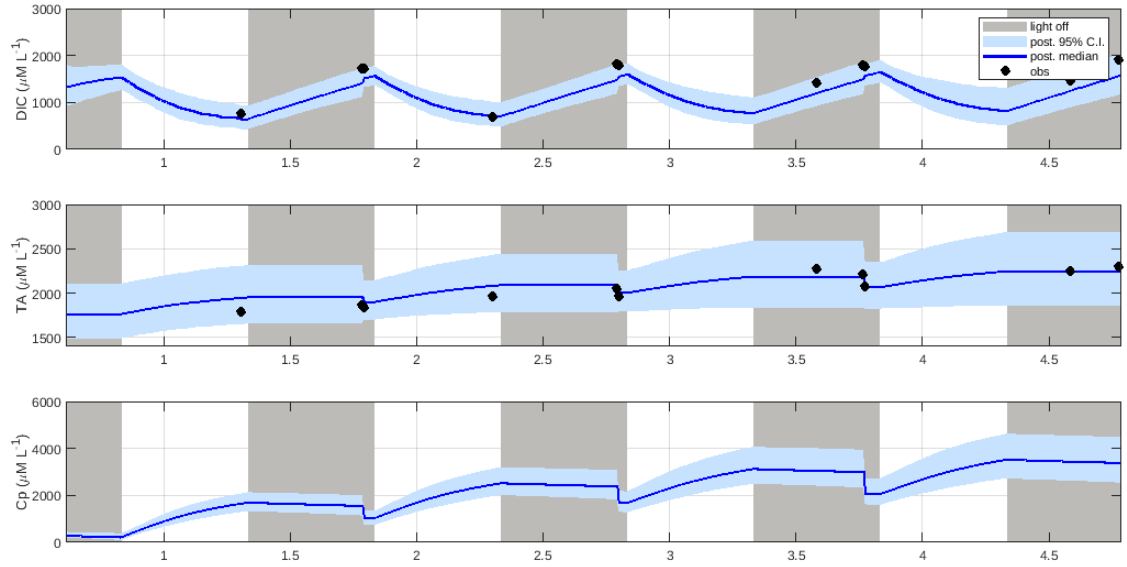

Figure 4.14 : Posterior medians (solid blue line), 95% credible intervals (shaded blue), and simulated observations (black) for DIC, TA and C_p across 4 days.

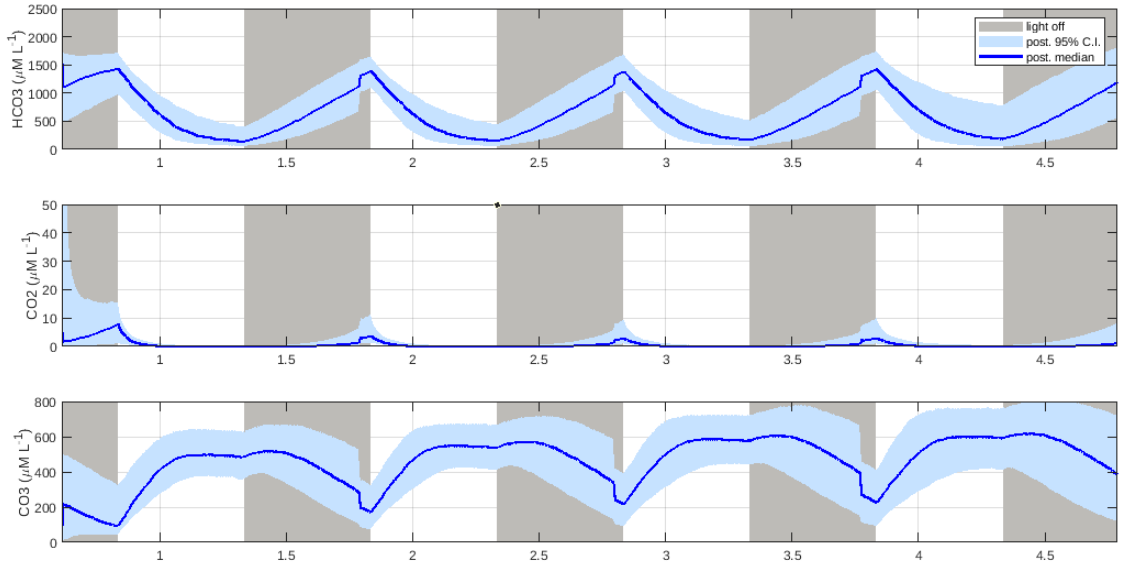

Figure 4.15 : Posterior medians (solid blue line) and 95% credible intervals (shaded blue) for HCO_3, CO_2 and CO_3 across 4 days.

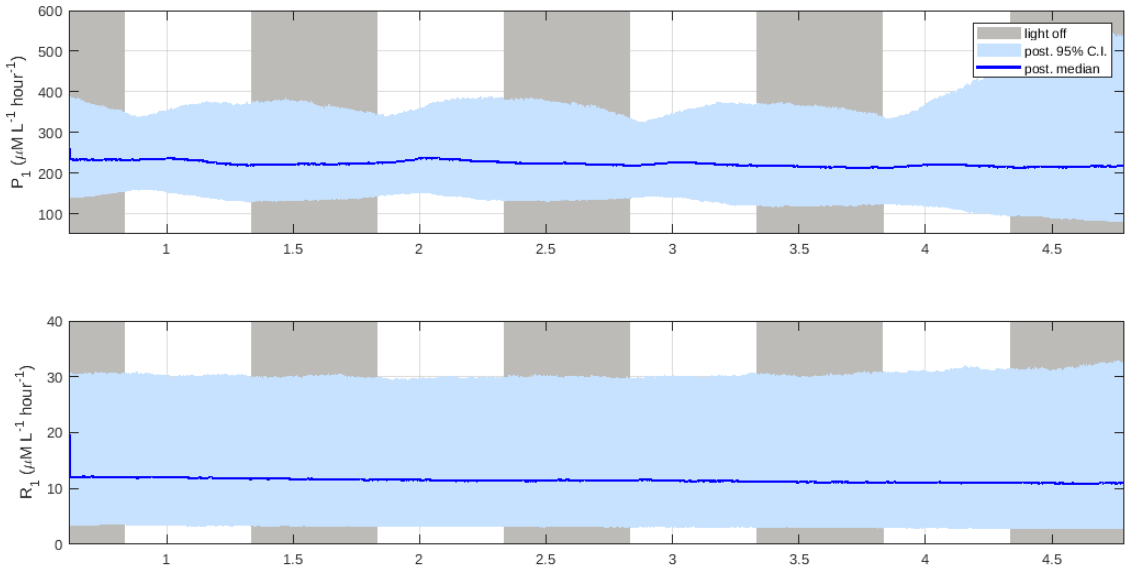

Figure 4.16 : Posterior medians (solid blue line) and 95% credible intervals (shaded blue) for photosynthesis P_1 and respiration R_1.

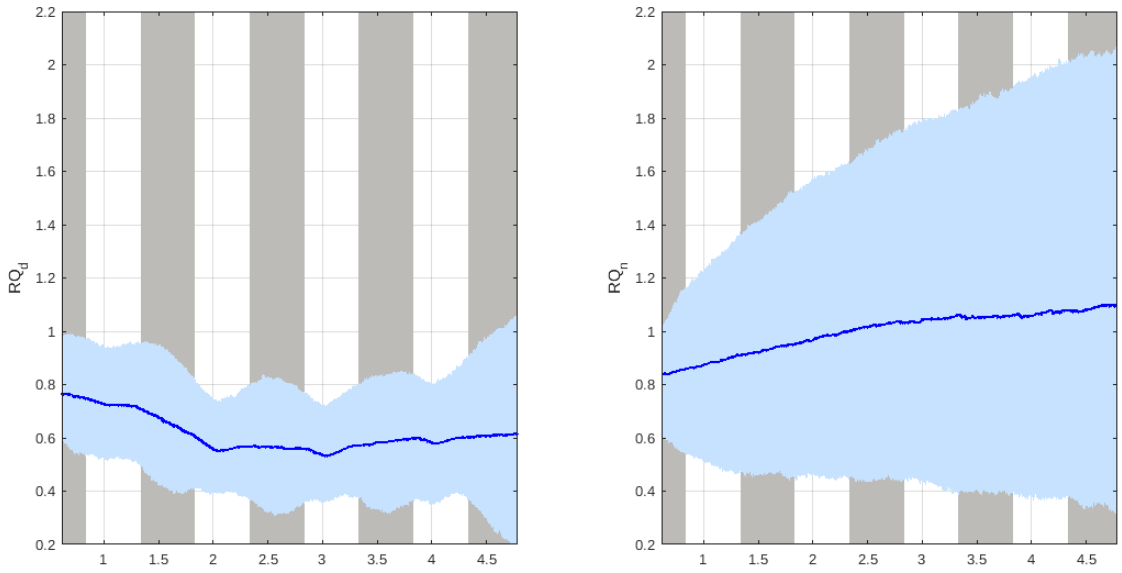

Figure 4.17 : Posterior medians (solid blue line) and 95% credible intervals (shaded blue) for RQ_d and RQ_n.

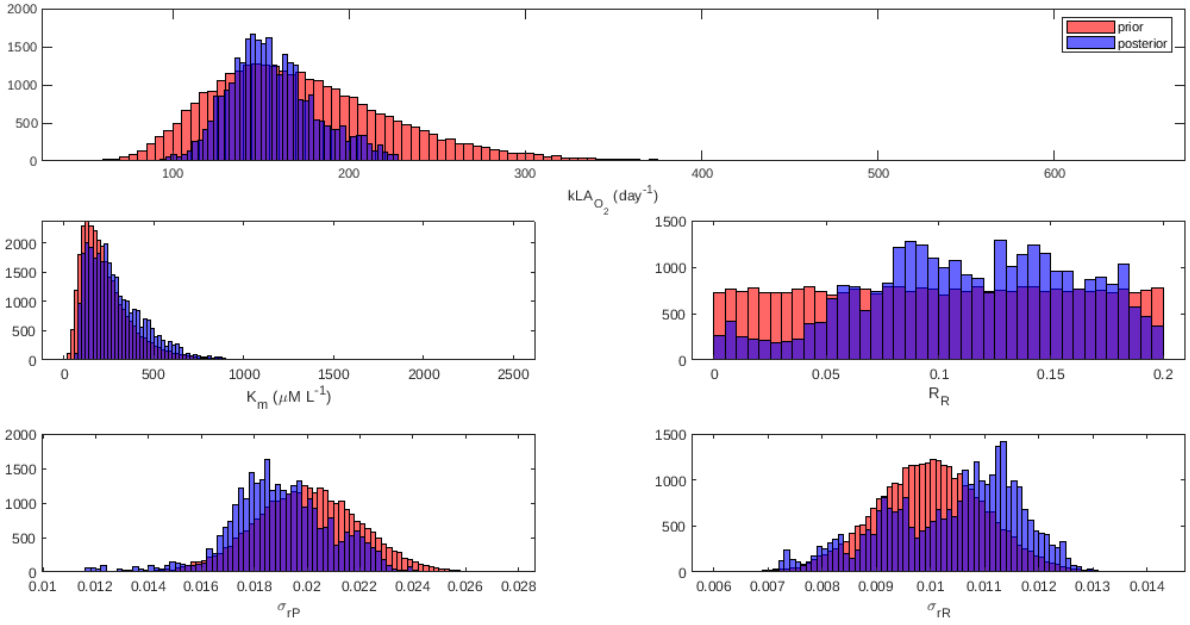

Figure 4.18 : Priors (pink) and posteriors (purple) for model parameters.

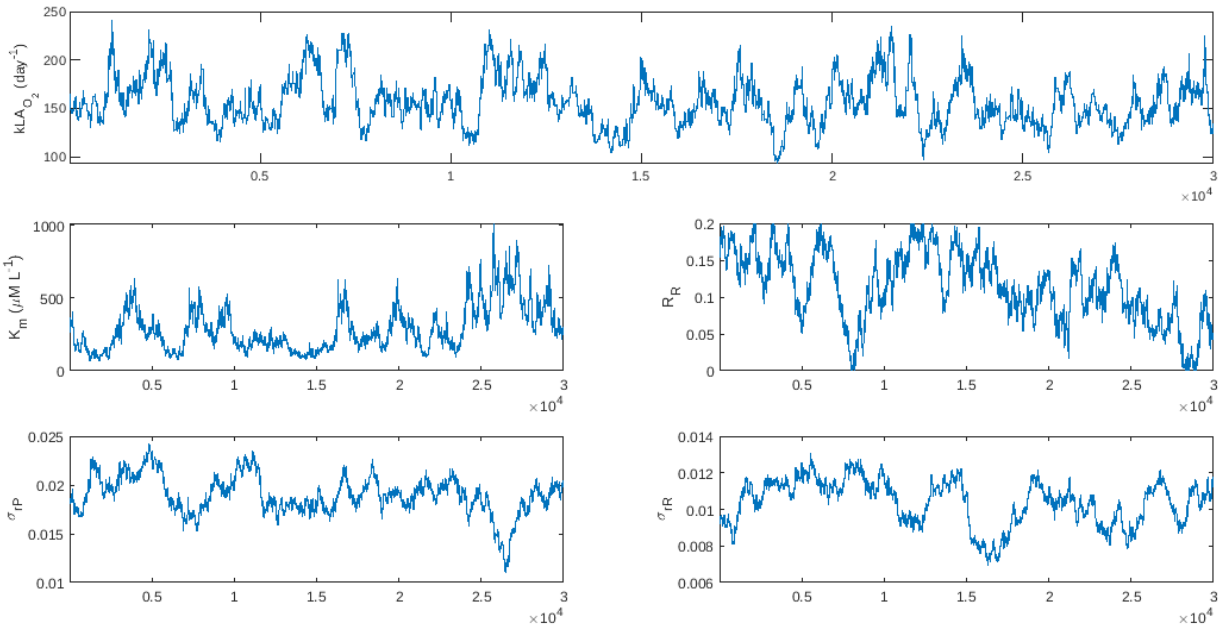

Figure 4.19 : Traces for model parameters.

observations well while the light was on, with all observations falling inside tightly constrained 95% credible intervals. During times when there was no light, most days the posteriors would fit the observations well to start and then potentially there was a sensor drift causing increasing observations that the model was not accounting for (Figure 4.13). pH captured most observations within the 95% credible intervals except for 2 points on the 1st day while the light was off (Figure 4.13).

P_1 R_1 (Figure 4.16).

The day-time respiratory quotient RQ_d centred around 0.75 during the first day, dropped to 0.6 during the 2nd, 3rd and 4th days. The night-time respiratory quotient RQ_n centred around 0.85 and slowly rose to 1.1 by the end of the experiment. [Values >1 are not really realistic but this can be a compensation for not having an offset on the O_2 obs]

4.3.4 Posteriors with experimental data (photosynthesis and respiration are random walks and the respiratory quotients are noisy states)

State posteriors are visualised by plotting the median and shading 95% credible intervals, while parameter priors and posteriors are displayed by histograms.

In this run:

Photosynthesis (P_1) and respiration (R_1) are both modelled as random walks, by taking P and R, previously constant parameters, and replacing them by $P_1(t)$ and $R_1(t)$. Here, we take $P_1(t)$ and $R_1(t)$ to be such that

$$P_1(t + \Delta t) = P(t) + r_P$$

$$R_1(t + \Delta t) = R(t) + r_R$$

where $r_P \sim N(0, \sigma_{r_P})$, $r_R \sim N(0, \sigma_{r_R})$, and Δt is the length of discrete time-step. For the purpose of the Bayesian analysis here, σ_{r_P} and σ_{r_R} are treated as parameters to be inferred. The respiratory quotients (RQ_d and RQ_n) are treated as normally distributed noisy states, truncated between 0.6 and 1, where the mean and standard deviation are unknown parameters to be estimated.

$RQ_d \sim truncated\mathcal{N}(\mu_{RQ_d}, \sigma_{RQ_d}, lower = 0.6, upper = 1.0)$

$RQ_n \sim truncated\mathcal{N}(\mu_{RQ_n}, \sigma_{RQ_n}, lower = 0.6, upper = 1.0)$

kLA_{O_2}, K_m, R_R, σ_{rP}, σ_{rR}, μ_{RQ_d}, σ_{RQ_d}, μ_{RQ_n}, σ_{RQ_n} are all treated as parameters constant through time but unknown.

Parameter	**Prior**	**Proposal**
kLA_{O2}	Log$\mathcal{N}$(log(200.0), 0.3)	Log$\mathcal{N}$(log(kLA_{O2}), 0.03)
K_m	Log$\mathcal{N}$(log(200.0), 0.6)	Log$\mathcal{N}$(log(K_m), 0.06)
R_R	Uniform(0, 0.2)	Trun$\mathcal{N}$(R_R, 0.01, lower = 0, upper = 0.2)
μ_{RQ_d}	Uniform(0.6, 1)	$\mathcal{N}$(μ_{RQ_d}, 0.01)
μ_{RQ_n}	Uniform(0.6, 1)	$\mathcal{N}$(μ_{RQ_n}, 0.01)
σ_{RQ_d}	Uniform(0, 0.5)	$\mathcal{N}$(σ_{RQ_d}, 0.01)
σ_{RQ_n}	Uniform(0, 0.5)	$\mathcal{N}$(σ_{RQ_n}, 0.01)
σ_{r_P}	$\mathcal{N}$(0.01, 0.001)	$\mathcal{N}$(σ_{r_P}, 0.0001)
σ_{r_R}	$\mathcal{N}$(0.01, 0.001)	$\mathcal{N}$(σ_{r_R}, 0.0001)
σ_{O_2}	0.4	*
σ_{pH}	0.4	*
σ_{DIC}	0.4	*

Table 4.9 : Table of Parameters, their priors and proposal distributions. * indicates the parameter was held fixed.

Results:

10,000 samples run (no burn-in was discarded) with 1024 particles.

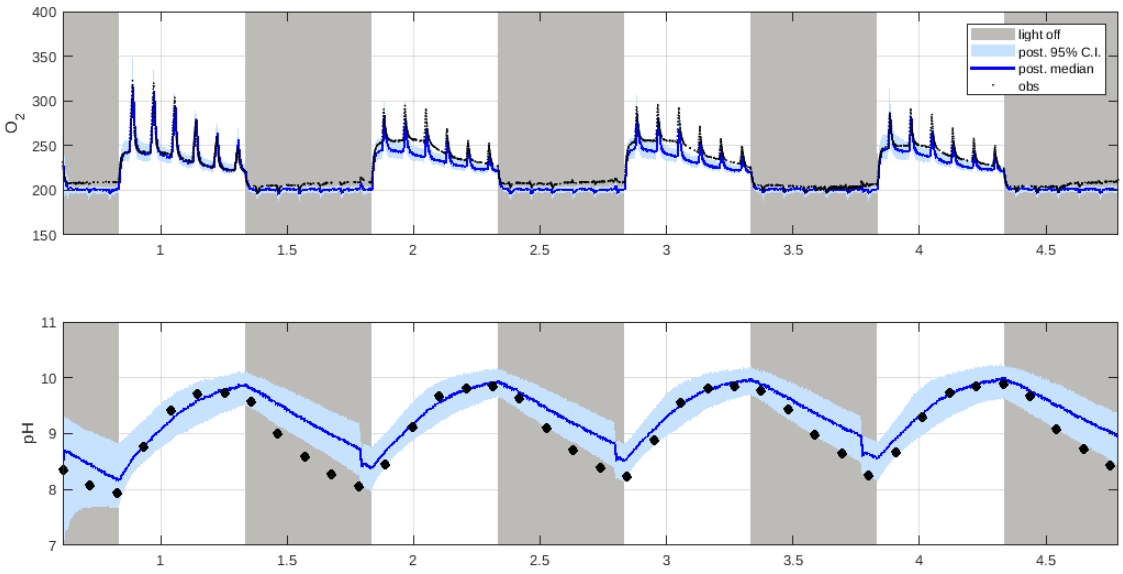

Figure 4.20 : Posterior medians (solid blue line), 95% credible intervals (shaded blue), and simulated observations (black) for O_2 and pH across 4 days.

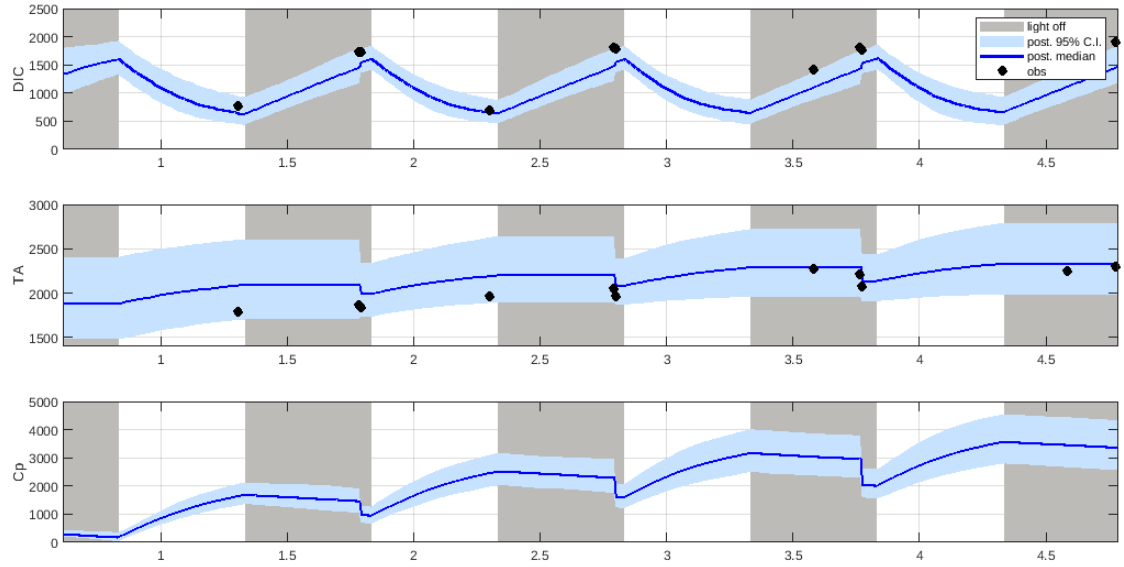

Figure 4.21 : Posterior medians (solid blue line), 95% credible intervals (shaded blue), and simulated observations (black) for DIC, TA and C_p across 4 days.

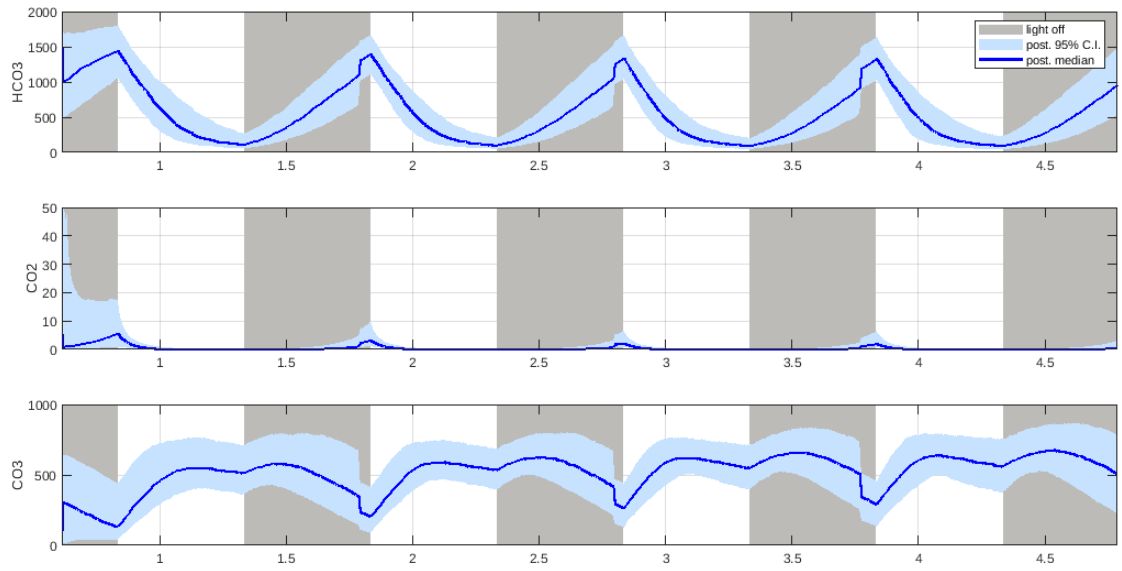

Figure 4.22 : Posterior medians (solid blue line) and 95% credible intervals (shaded blue) for HCO_3, CO_2 and CO_3 across 4 days.

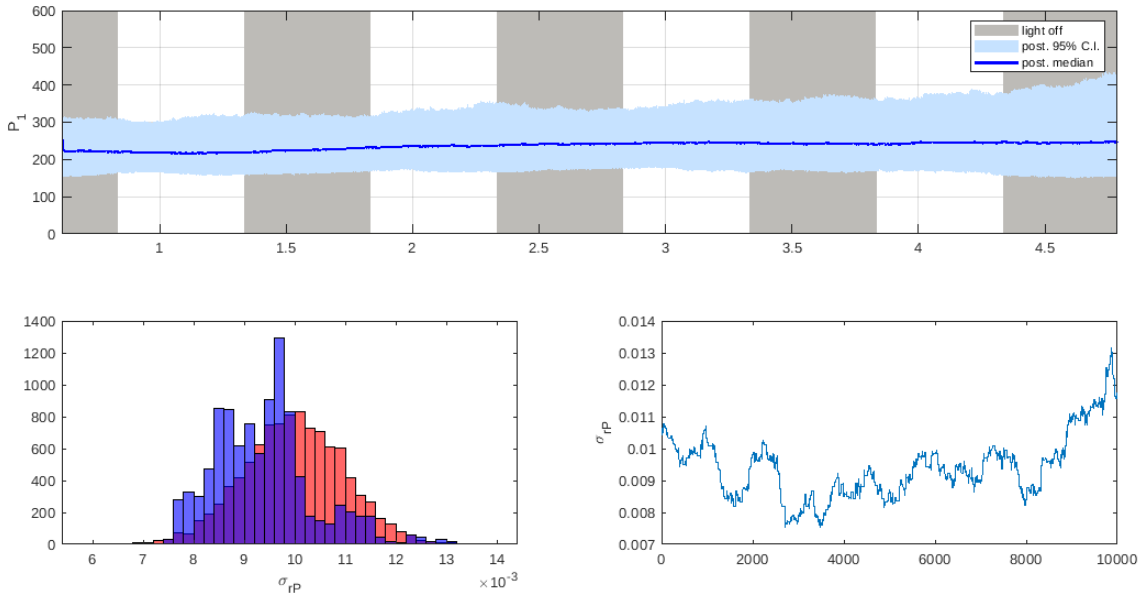

Figure 4.23 : Posterior medians (solid blue line) and 95% credible intervals (shaded blue) for photosynthesis P_1.

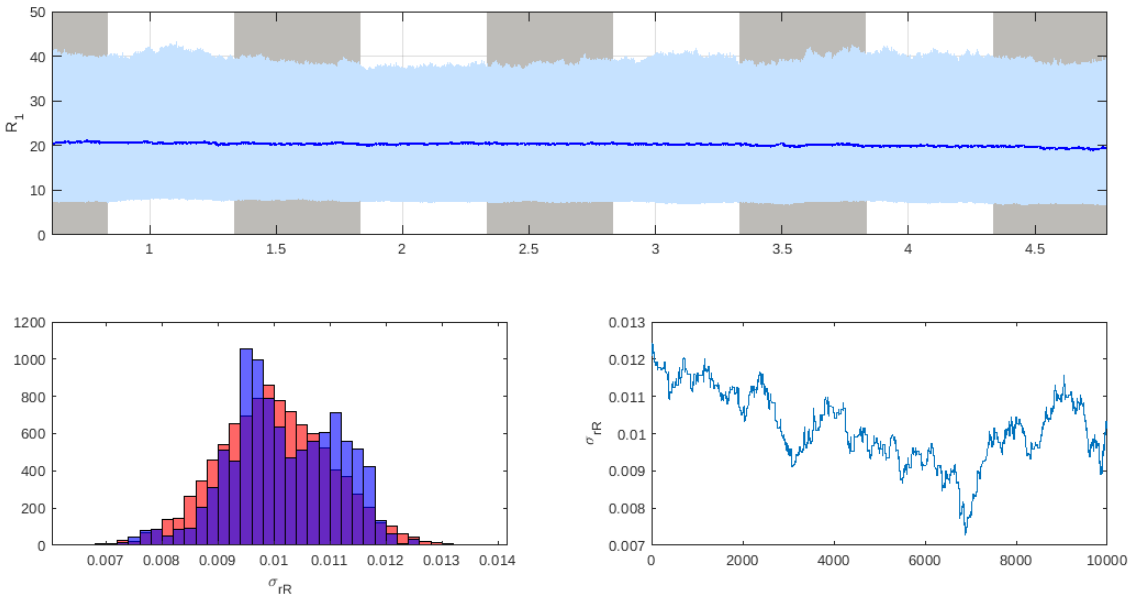

Figure 4.24 : Posterior medians (solid blue line) and 95% credible intervals (shaded blue) for respiration R_1.

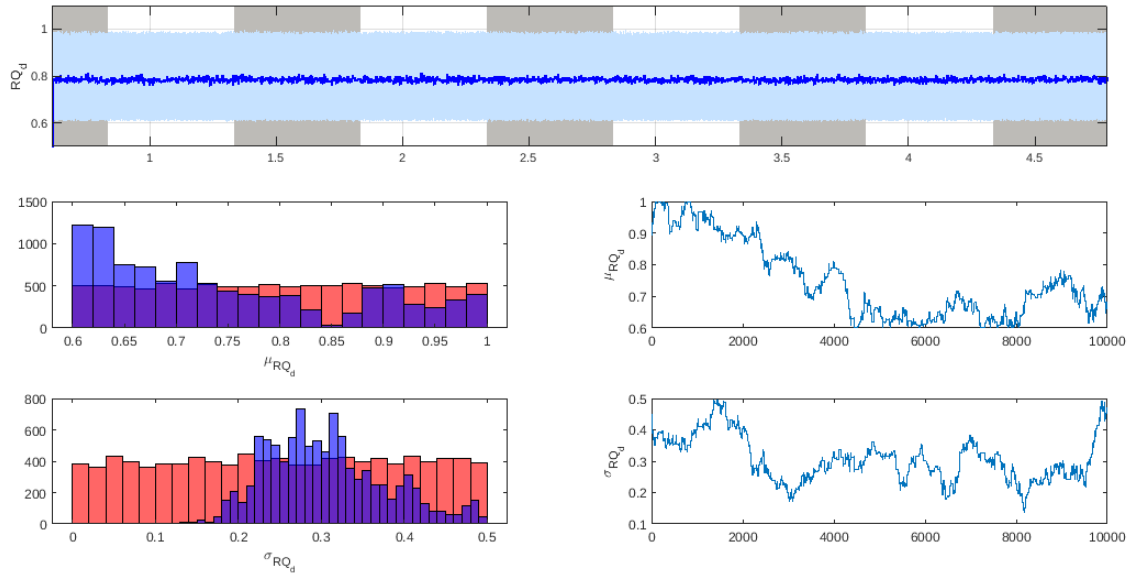

Figure 4.25 : Posterior medians (solid blue line) and 95% credible intervals (shaded blue) for RQ_d.

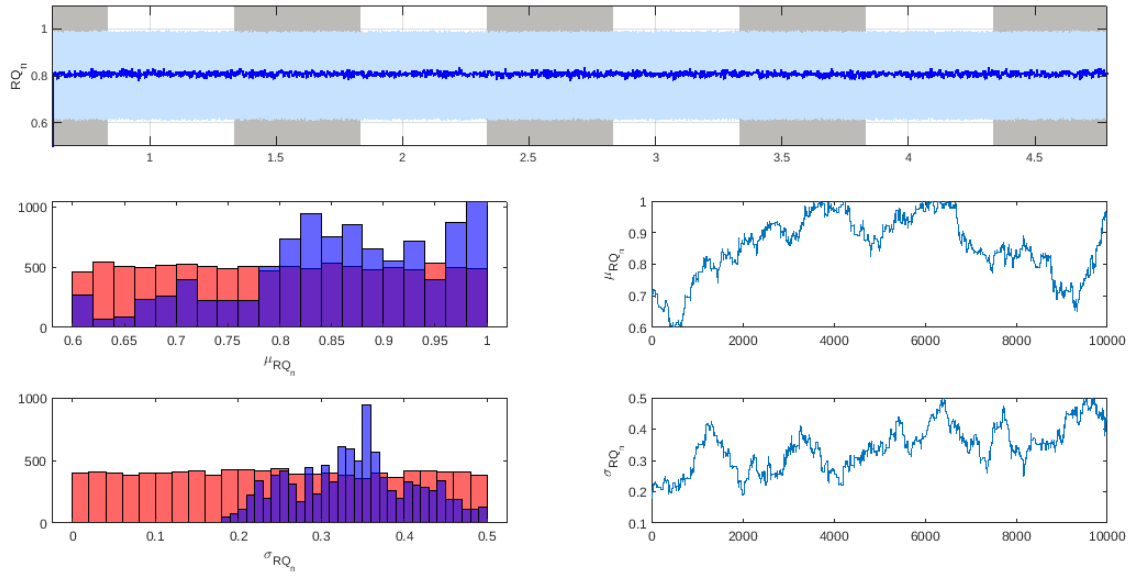

Figure 4.26 : Posterior medians (solid blue line) and 95% credible intervals (shaded blue) for RQ_n.

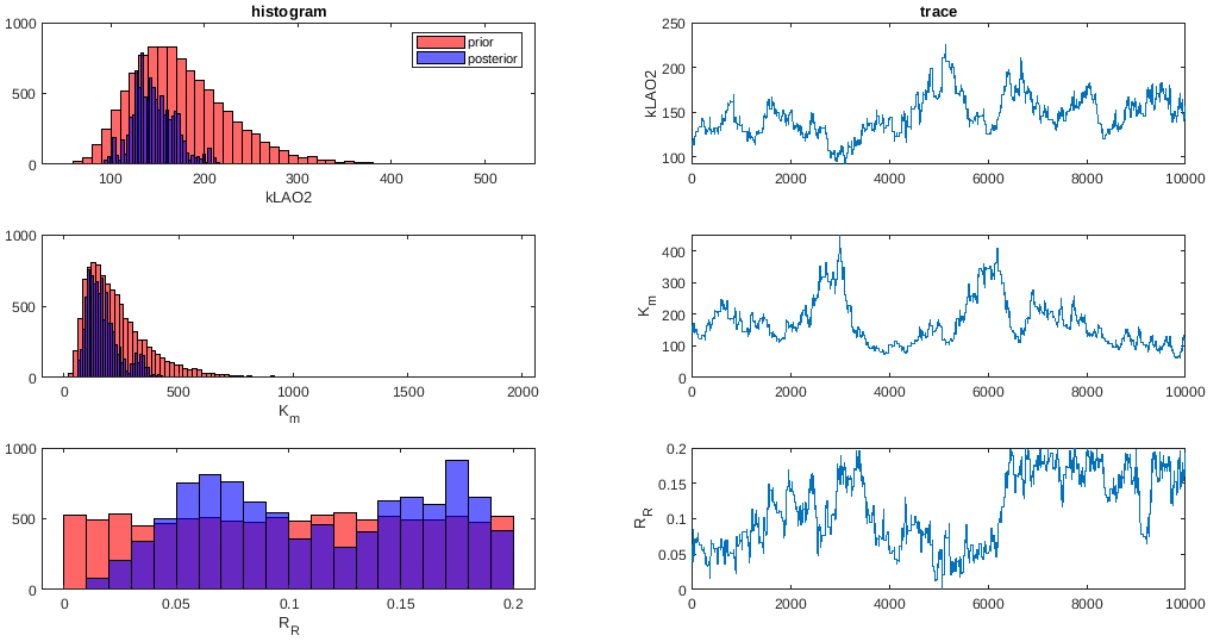

Figure 4.27 : Priors (pink) and posteriors (purple) for model parameters.

4.3.5 Posteriors with experimental data (estimating obs error)

State posteriors are visualised by plotting the median and shading 95% credible intervals, while parameter priors and posteriors are displayed by histograms.

In this run:

P_1 and R_1 are random walks with rP and rR as wiener processes. kLA_{O_2}, K_m, R_R, RQ_d, RQ_n, σ_{O_2}, σ_{pH}, and σ_{DIC} are all treated as parameters constant through time but unknown. The data model in this run is:

$O2_{obs} \sim normal(O_2, \sigma_{O_2})$

$pH_{obs} \sim normal(pH, \sigma_{pH})$

$DIC_{obs} \sim normal(DIC, \sigma_{DIC})$

$TA_{obs} \sim normal(TA, \sigma_{DIC})$

Parameter	**Prior**	**Proposal**
kLA_{O2}	Log$\mathcal{N}$(log(200.0), 0.3)	Log$\mathcal{N}$(log(kLA_{O2}), 0.03)
K_m	Log$\mathcal{N}$(log(200.0), 0.6)	Log$\mathcal{N}$(log(K_m), 0.06)
R_R	Uniform(0, 0.2)	Trun$\mathcal{N}$(R_R, 0.01, lower = 0, upper = 0.2)
RQ_d	Uniform(0.6, 1)	Trun$\mathcal{N}$(RQ_d, 0.01, lower = 0.6, upper = 1.0)
RQ_n	Uniform(0.6, 1)	Trun$\mathcal{N}$(RQ_n, 0.01, lower = 0.6, upper = 1.0)
σ_{O_2}	Uniform(0, 20)	Trun$\mathcal{N}$(σ_{O_2}, 2, lower = 0, upper = 20)
σ_{pH}	Uniform(0, 1)	Trun$\mathcal{N}$(σ_{pH}, 0.1, lower = 0, upper = 1)
σ_{DIC}	Uniform(0, 150)	Trun$\mathcal{N}$(σ_{DIC}, 15, lower = 0, upper = 150)

Table 4.10 : Table of Parameters, their priors and proposal distributions. * indicates the parameter was held fixed.

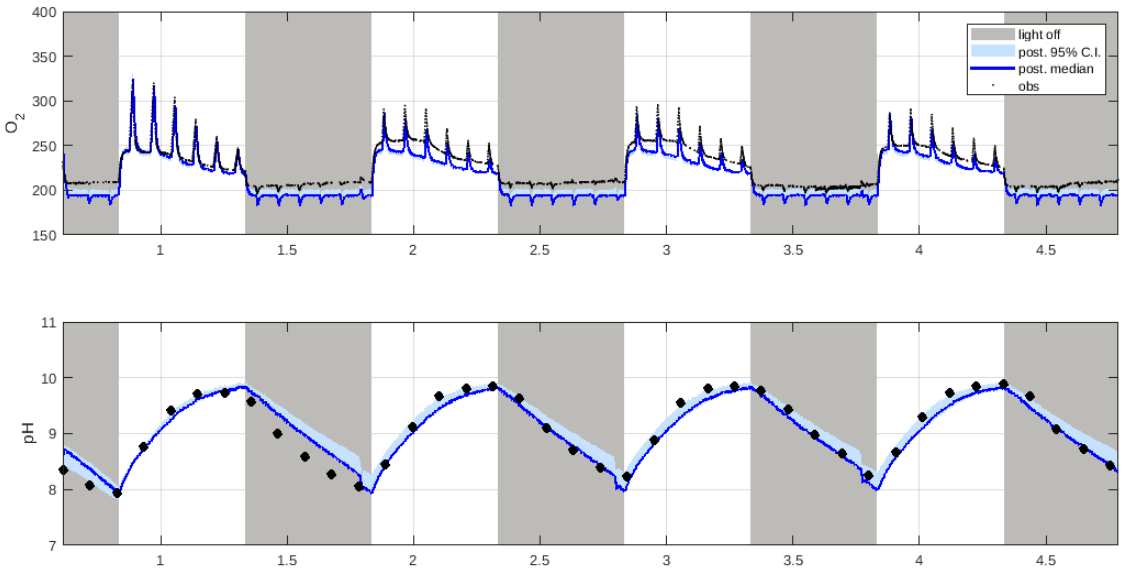

Figure 4.28 : Posterior medians (solid blue line), 95% credible intervals (shaded blue), and simulated observations (black) for O_2 and pH across 4 days.

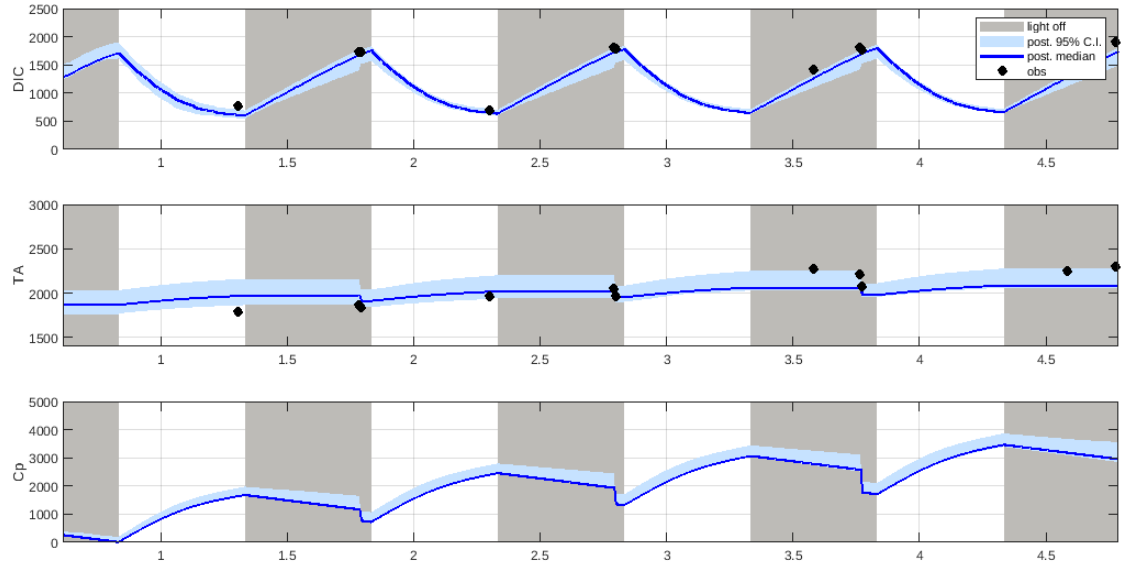

Figure 4.29 : Posterior medians (solid blue line), 95% credible intervals (shaded blue), and simulated observations (black) for DIC, TA and C_p across 4 days.

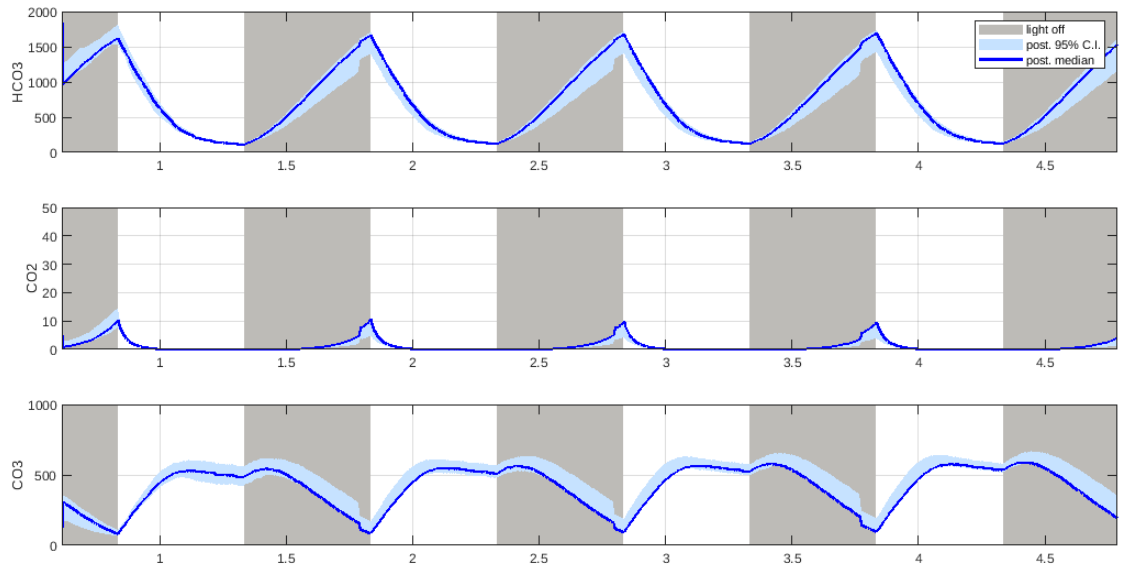

Figure 4.30 : Posterior medians (solid blue line) and 95% credible intervals (shaded blue) for HCO_3, CO_2 and CO_3 across 4 days.

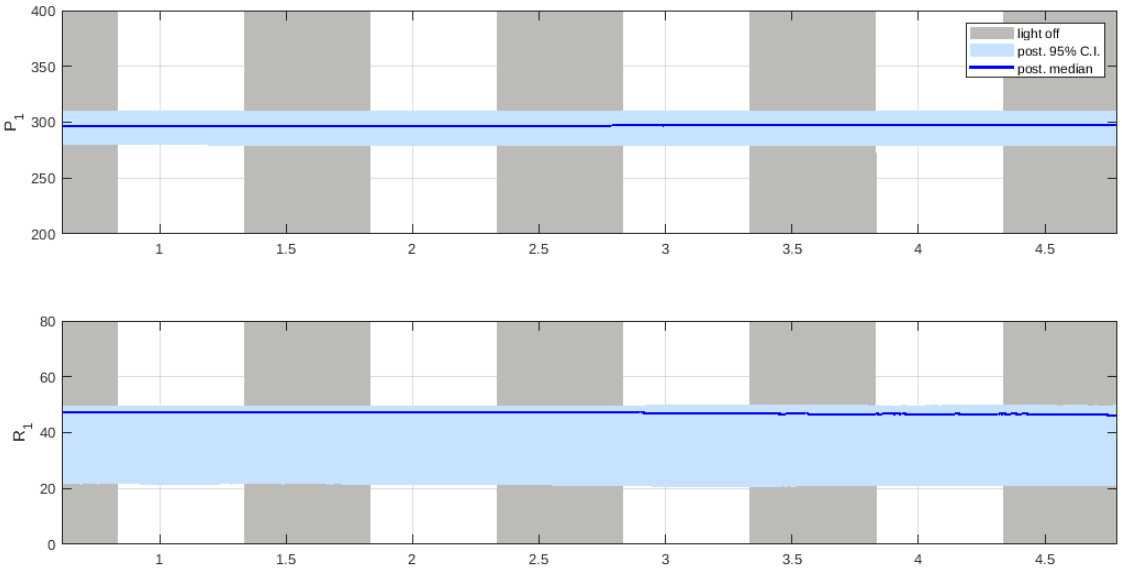

Figure 4.31 : Posterior medians (solid blue line) and 95% credible intervals (shaded blue) for photosynthesis P_1.

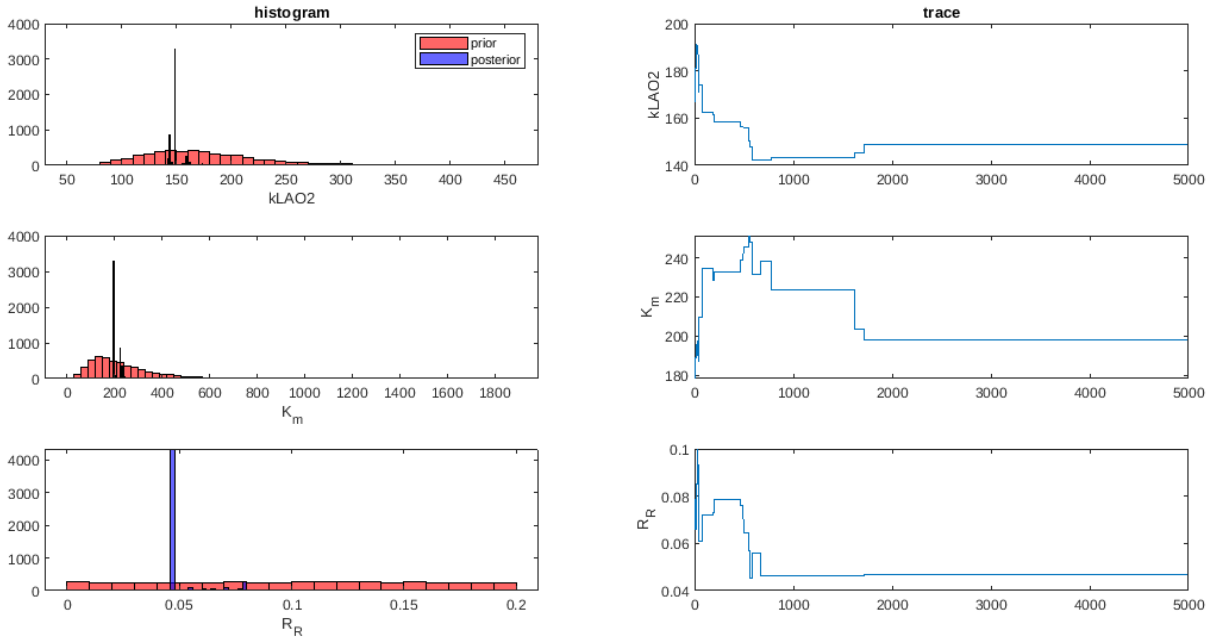

Figure 4.32 : Priors (pink) and posteriors (purple) for model parameters.

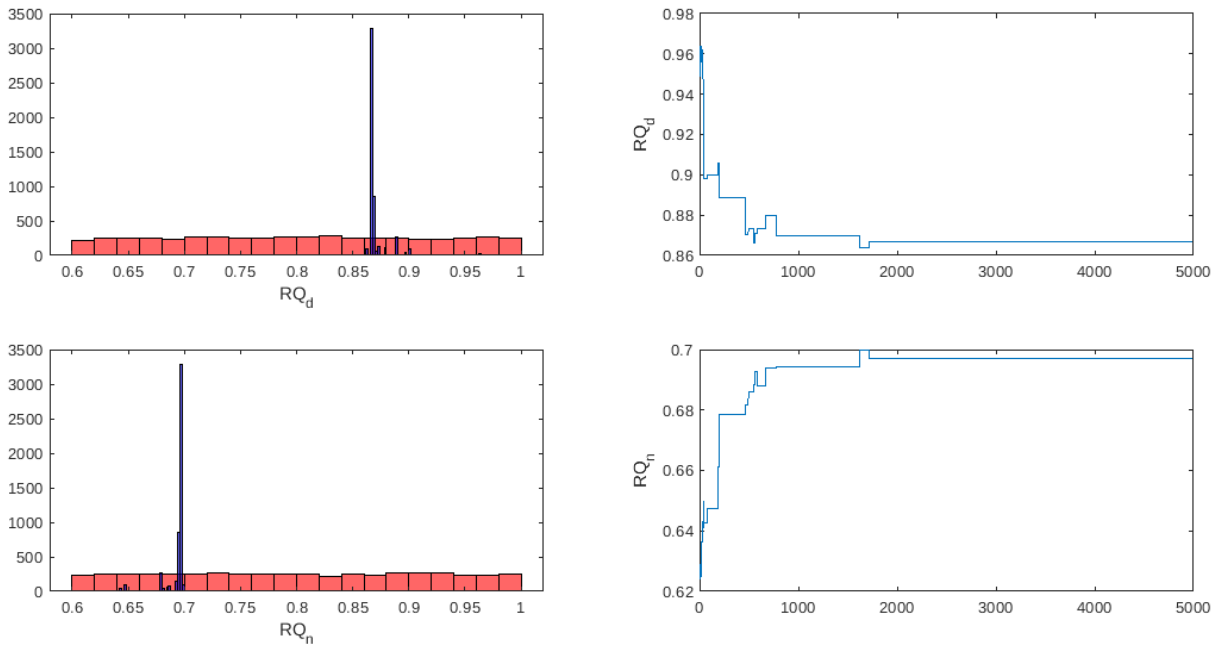

Figure 4.33 : Priors (pink) and posteriors (purple) for model parameters.

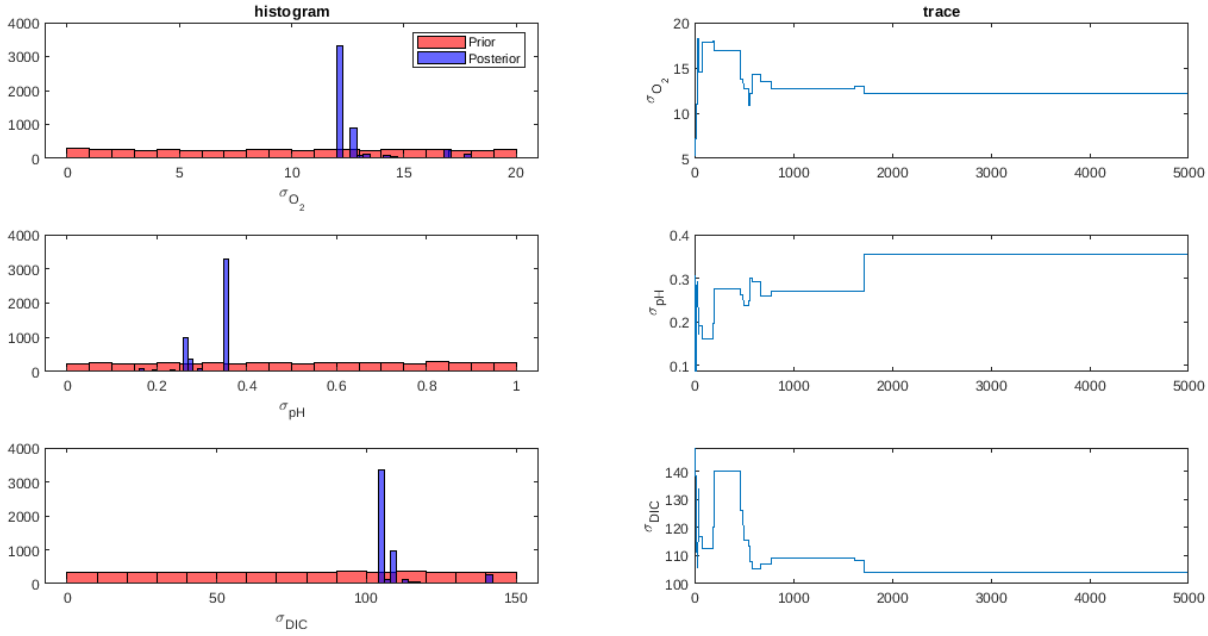

Figure 4.34 : Priors (pink) and posteriors (purple) for model parameters.

Appendix A

LiBbi model code

LiBbi model file: micro_iterative.bi

```
model micro_iterative {

const FO2       = 0.2094
const FCO2      = 397e-6
const S         = 34.0
const V         = 500.0                 // volume of the
   reactor
const DIC_M     = 1724.20               // calculated with
   CO2SYS[DIC_M = 1724.20, Alk = 1797.90, T = 27, S = 34]
const O_2_M     = 226.65
const alk_M     = 1797.90
const tau       = 6.0
const kLAO2_m   = log(2.0)*24.0*60.0/tau

param kLAO2
param Km
param RR
param RQ_d
param RQ_n
param sigma_O_2
param sigma_pH
param sigma_DIC
```

```
21 param offset_O_2
22
23 input I                  // light intensity
24 input T                  // temperature (C)
25 input gas                // gas on/off
26 input dil                // dilution rate
27
28 state DIC // state variables
29 state O_2
30 state pH
31 state Cp
32 state mich_ment
33 state O2H_pr
34 state CO2H_pr
35 state R
36 state R1
37 state P
38 state P1
39 state alk
40 state CO2
41 state HCO3
42 state CO3
43 state O_2H
44 state CO2H
45 state h_3
46 state h_free_3
47
48 noise r_R
49 noise r_P
```

```
50
51 /* random walk parameter */
52 param sigma_r_R
53 param sigma_r_P
54
55 obs O2_obs
56 obs pH_obs
57 obs DIC_obs
58 obs alk_obs
59
60 sub parameter {/* prior distribution over parameters */
61 Km      ~ log_normal(log(100.0), 0.5)
62 kLAO2   ~ log_normal(log(kLAO2_m), 0.3)
63 RR      ~ uniform(0.0001, 0.2)
64 RQ_d    ~ uniform(0.66, 1.0)
65 RQ_n    ~ uniform(0.66, 1.0)
66
67 sigma_O_2 ~ log_normal(log(0.03), 0.5)
68 sigma_pH  ~ log_normal(log(0.03), 0.5)
69 sigma_DIC ~ log_normal(log(0.03), 0.5)
70
71 offset_O_2 ~ normal(0, 2.0)
72
73 sigma_r_R       ~ normal(0.01, 0.001)
74 sigma_r_P       ~ normal(0.05, 0.01)
75 }
76
77 const prop_std = 0.1;
78 sub proposal_parameter {
```

```
79 Km      ~ log_normal(log(Km), 0.5*prop_std)
80 kLAO2   ~ log_normal(log(kLAO2), 0.3*prop_std)
81 RR      ~ truncated_normal(RR, 0.2*prop_std, lower = 0.0001,
      upper = 0.2)
82 RQ_d    ~ truncated_normal(RQ_d, 0.2*prop_std, lower = 0.66,
      upper = 1.0)
83 RQ_n    ~ truncated_normal(RQ_n, 0.2*prop_std, lower = 0.66,
      upper = 1.0)
84
85
86 sigma_O_2       ~ log_normal(log(sigma_O_2), 0.5*prop_std)
87 sigma_pH        ~ log_normal(log(sigma_pH), 0.5*prop_std)
88 sigma_DIC       ~ log_normal(log(sigma_DIC), 0.5*prop_std)
89
90 offset_O_2      ~ normal(offset_O_2, 2.0*prop_std)
91
92 sigma_r_R       ~ normal(sigma_r_R, 0.001*prop_std)
93 sigma_r_P       ~ normal(sigma_r_P, 0.01*prop_std)
94 }
95
96 sub initial {/* prior distribution over initial conditions,
      given parameters */
97 // specify the initial condition model
98 R       ~ normal(log(20.0), 0.4)
99 R1      ~ log_normal(log(20.0), 0.4)
100 P       ~ normal(log(200.0), 0.4)
101 P1      ~ log_normal(log(200.0), 0.4)
102
103 Cp      ~ log_normal(log(300.0), 0.2)
```

```
104 alk      ~ log_normal(log(1750.0), 0.1)
105 DIC      ~ log_normal(log(1300.0), 0.2)
106 O_2      ~ log_normal(log(225.0), 0.2)
107 pH       ~ log_normal(log(8.5), 0.2)
108 CO2      ~ log_normal(log(3.0), 0.4)
109 HCO3     ~ log_normal(log(1000.0), 0.3)
110 CO3      ~ log_normal(log(300.0), 0.4)
111 O_2H     ~ log_normal(log(200.0), 0.2)
112 CO2H     ~ log_normal(log(10.0), 0.2)
113 }
114
115
116 //sub transition(delta = 0.0023) { // obs are in days ie
       delta=1.0 for daily solving. delta=0.00069 for solving
       every minute, 0.0014 for every 2 mins, 0.0021 for 3 mins,
       0.0028 for 4mins, delta=0.000011574 for solving every
       second
117 sub transition(delta = 0.0021) {
118
119 /* processes */
120
121 inline TK     = T + 273.15              // temp in kelvin
122 inline K0_CO2 = exp(-60.2409 + 93.4517*(100.0/TK) + 23.3585*
       log(TK/100.0)+ S*(0.023517 - 0.023656*(TK/100) +
       0.0047036*(TK/100.0)*(TK/100.0)))
123 CO2H          <- K0_CO2*FCO2*1.0220*1e6
124
125 inline K0_O2  =  (exp(-1282.8704 + 36619.96/TK + 223.1396*log
       (TK) -0.354707*TK + S*(5.957e-3 -3.7353/TK) + 3.68e-6*S*S)
```

```
    )/(0.2094e-06)
126 O_2H          <- K0_O2*FO2*1.0220*1e-6
127
128 inline PAC    = HCO3            //PAC=photosynthetically
    active carbon. if the phyto are just using CO2 to
    photosynthesise then PAC=CO2
129 inline mm     = PAC/(Km + PAC)
130
131 // CO2SYS iterative solution
132 // set up all the constants
133
134 inline logTK  = log(TK)
135 inline S2     = S*S
136 inline sqrtS  = sqrt(S)
137
138 // total sulphur
139
140 inline TS     = (0.14/96.062)*(S/1.80655)
141 inline IS     = 19.924*S/(1000.0 - 1.005*S)
142
143 inline KS_int = -4276.1/TK + 141.328 - 23.093*logTK +
    (-13856.0/TK + 324.57  -  47.986*logTK)*sqrt(IS) + (
    35474.0/TK - 771.54  + 114.723*logTK)*IS - 2698.0/TK*IS
    **1.5 + 1776.0/TK*IS**2
144 inline KS     = exp(KS_int)*(1 - 0.001005*S)
145
146 // Fluorine
147
148 inline TF       = 0.000067*S/18.9984/1.80655
```

```
149 inline KF       = exp(-(-874.0/TK - 0.111*sqrtS + 9.68))
150 inline SWS_2_T  = (1.0 + TS/KS)/(1.0 + TS/KS + TF/KF)
151 inline Free_2_T = 1.0 + TS/KS
152
153 // H2O dissoc
154
155 inline KW = exp(148.9802 - 13847.26/TK  - 23.6521*logTK +
       (118.67/TK - 5.977 + 1.0495*logTK)*sqrtS - 0.01615*S)
156
157 // Boron
158
159 inline KB = exp((-8966.90 - 2890.53*sqrtS - 77.942*S + 1.728*
       S*sqrtS - 0.0996*S2)/TK + 148.0248 + 137.1942*sqrtS +
       1.62142*S - (24.4344 + 25.085*sqrtS + 0.2474*S)*logTK +
       0.053105*sqrtS*TK)
160 inline TB = 0.0004326*S/35.0
161
162 // Carbon eq constants
163
164 inline K1 = 10**(-(3633.86/TK - 61.2172 + 9.6777 *logTK -
       0.011555*S + 0.0001152*S**2))*1.23    //1.23 experiment
       specific and measured
165 inline K2 = 10**(-( 471.8/TK + 25.9290 - 3.16967*logTK -
       0.01781*S + 0.0001122*S**2))*0.53      //0.53 experiment
       specific and measured
166
167 // end all the constants
168
169 // intial guess at the pH (use the approximating equation)
```

```
170
171 inline pH_init = 12.26 -0.0030605*DIC -0.043752*T -0.013625*S
       + 0.00011315*alk + 1.3463e-05*DIC*T + 5.2215e-07*DIC*alk
172
173 // iteration 1
174
175 inline h_1      = 10.0**(-pH_init)
176 inline h_free_1 = h_1/Free_2_T
177 inline f0_1     = (DIC*1e-6*(K1*h_1 + 2.0*K1*K2)/(h_1*h_1 +
       K1*h_1 + K1*K2) - h_free_1 + KW/h_1 - alk*1e-6 + TB/(1.0 +
        h_1/KB))*1e6
178 inline df0_1    = (DIC*1e-6*(K1 + 2.0*K1*K2)/(h_1**2.0 + K1*
       h_1 + K1*K2) - DIC*1e-6*(K1*h_1 + 2.0*K1*K2)/(h_1**2.0 +
       K1*h_1 + K1*K2)**2.0*(2.0*h_1 + K1) - TB*1.0/(1.0 + h_1/KB
       )**2.0/KB - KW/h_1**2.0 - 1.0/Free_2_T)*1e6*(-log(10.0)
       *10.0**(-pH_init))
179 inline pH_1     = pH_init - f0_1/df0_1
180
181 // iteration 2
182
183 inline h_2      = 10.0**(-pH_1)
184 inline h_free_2 = h_2/Free_2_T
185 inline f0_2     = (DIC*1e-6*(K1*h_2 + 2.0*K1*K2)/(h_2*h_2 +
       K1*h_2 + K1*K2) - h_free_2 + KW/h_2 - alk*1e-6 + TB/(1.0 +
        h_2/KB))*1e6
186 inline df0_2    = (DIC*1e-6*(K1 + 2.0*K1*K2)/(h_2**2.0 + K1*
       h_2 + K1*K2) - DIC*1e-6*(K1*h_2 + 2.0*K1*K2)/(h_2**2.0 +
       K1*h_2 + K1*K2)**2.0*(2.0*h_2 + K1) - TB*1.0/(1.0 + h_2/KB
       )**2.0/KB - KW/h_2**2.0 - 1.0/Free_2_T)*1e6*(-log(10.0)
```

```
      *10.0**(-pH_1))
187 inline pH_2     = pH_1 - f0_2/df0_2
188
189 // iteration 3
190
191 h_3             <- 10.0**(-pH_2)
192 h_free_3        <- h_3/Free_2_T
193 inline f0_3     = (DIC*1e-6*(K1*h_3 + 2.0*K1*K2)/(h_3*h_3 +
      K1*h_3 + K1*K2) - h_free_3 + KW/h_3 - alk*1e-6 + TB/(1.0 +
       h_3/KB))*1e6
194 inline df0_3    = (DIC*1e-6*(K1 + 2.0*K1*K2)/(h_3**2.0 + K1*
      h_3 + K1*K2) - DIC*1e-6*(K1*h_3 + 2.0*K1*K2)/(h_3**2.0 +
      K1*h_3 + K1*K2)**2.0*(2.0*h_3 + K1) - TB*1.0/(1.0 + h_3/KB
      )**2.0/KB - KW/h_3**2.0 - 1.0/Free_2_T)*1e6*(-log(10.0)
      *10.0**(-pH_2))
195 pH              <- pH_2 - f0_3/df0_3
196
197 // iteration 4
198
199 //      inline h_4      = 10.0**(-pH_3)
200 //      inline h_free_4 = h_4/Free_2_T
201 //      inline f0_4     = (DIC*1e-6*(K1*h_4 + 2.0*K1*K2)/(h_4
      *h_4 + K1*h_4 + K1*K2) - h_free_4 + KW/h_4 - alk*1e-6 + TB
      /(1.0 + h_4/KB))*1e6
202 //      inline df0_4    = (DIC*1e-6*(K1 + 2.0*K1*K2)/(h_4
      **2.0 + K1*h_4 + K1*K2) - DIC*1e-6*(K1*h_4 + 2.0*K1*K2)/(
      h_4**2.0 + K1*h_4 + K1*K2)**2.0*(2.0*h_4 + K1) - TB
      *1.0/(1.0 + h_4/KB)**2.0/KB - KW/h_4**2.0 - 1.0/Free_2_T)
      *1e6*(-log(10.0)*10.0**(-pH_3))
```

```
203 //      inline pH_4     = pH_3 - f0_4/df0_4
204
205 //      pH              <- pH_4
206
207 // calculate the final concentrations
208
209 inline H        = 10.0**(-pH)
210 inline H2       = H*H
211 inline denom    = (H2 + K1*H + K1*K2)
212 CO2             <- DIC*H2/denom
213 HCO3            <- DIC*H*K1/denom
214 CO3             <- DIC*K1*K2/denom
215
216 // end CO2SYS iterative solution
217
218
219 /* R and P as random walks */
220
221 r_R     ~ normal(0.0, sigma_r_R)
222 R       <- R + r_R
223 R1      <- exp(R)
224
225 r_P ~ normal(0.0, sigma_r_P)
226 P       <- P + r_P
227 P1      <- exp(P)
228
229 ode(h = 0.1, atoler = 1.0e-6, rtoler = 1.0e-6, alg = 'RK4(3)
    '){
```

```
230 dDIC/dt  =  -P1*24.0*I*mm + R1*24.0
                + gas*0.893*kLAO2*(CO2H - CO2)          + dil/V*(
    DIC_M - DIC)
231 dO_2/dt  =  (P1*24.0*I*mm - R1*24.0)/(RQ_d*I + RQ_n*(1.0-I))
          + gas*kLAO2*(O_2H - O_2)                 + dil/V*(O_2_M
    - O_2)   + offset_O_2
232 dalk/dt  =   RR*P1*24.0*I*mm
                                                   + dil/V*(
    alk_M - alk)
233 dCp/dt   =   (P1*24.0*I*mm - R1*24.0)
                                                   + dil/V*(
    Cp)
234
235 }
236
237 mich_ment <- mm
238 O2H_pr    <- O_2H
239 CO2H_pr   <- CO2H
240
241 }
242
243
244 sub observation {
245
246 O2_obs  ~ log_normal(log(O_2), sigma_O_2)
247 pH_obs  ~ log_normal(log(pH),  sigma_pH)
248 DIC_obs ~ log_normal(log(DIC), sigma_DIC)
249 alk_obs ~ log_normal(log(alk), sigma_DIC)
250 }
```

```
}
```

LiBbi prior sampling file: prior.conf

```
--target prior
--model-file micro_iterative.bi
--nsamples 500
--start-time 0.61304
--end-time 4.7866
--noutputs 6049
--input-file data/input_all_2018_normalised.nc
--output-file results/prior_micro_iterative.nc
```

LiBbi posterior sampling file: posterior.conf

```
--target posterior
--model-file micro_iterative.bi
--input-file data/input_all_2018_normalised.nc
--obs-file data/obs_all_2018.nc
--nsamples 500
--nparticles 1024
--start-time 0.61304
--end-time 4.7866
--noutputs 6049
--output-file results/posterior_micro_iterative.nc
--with-transform-initial-to-param
```

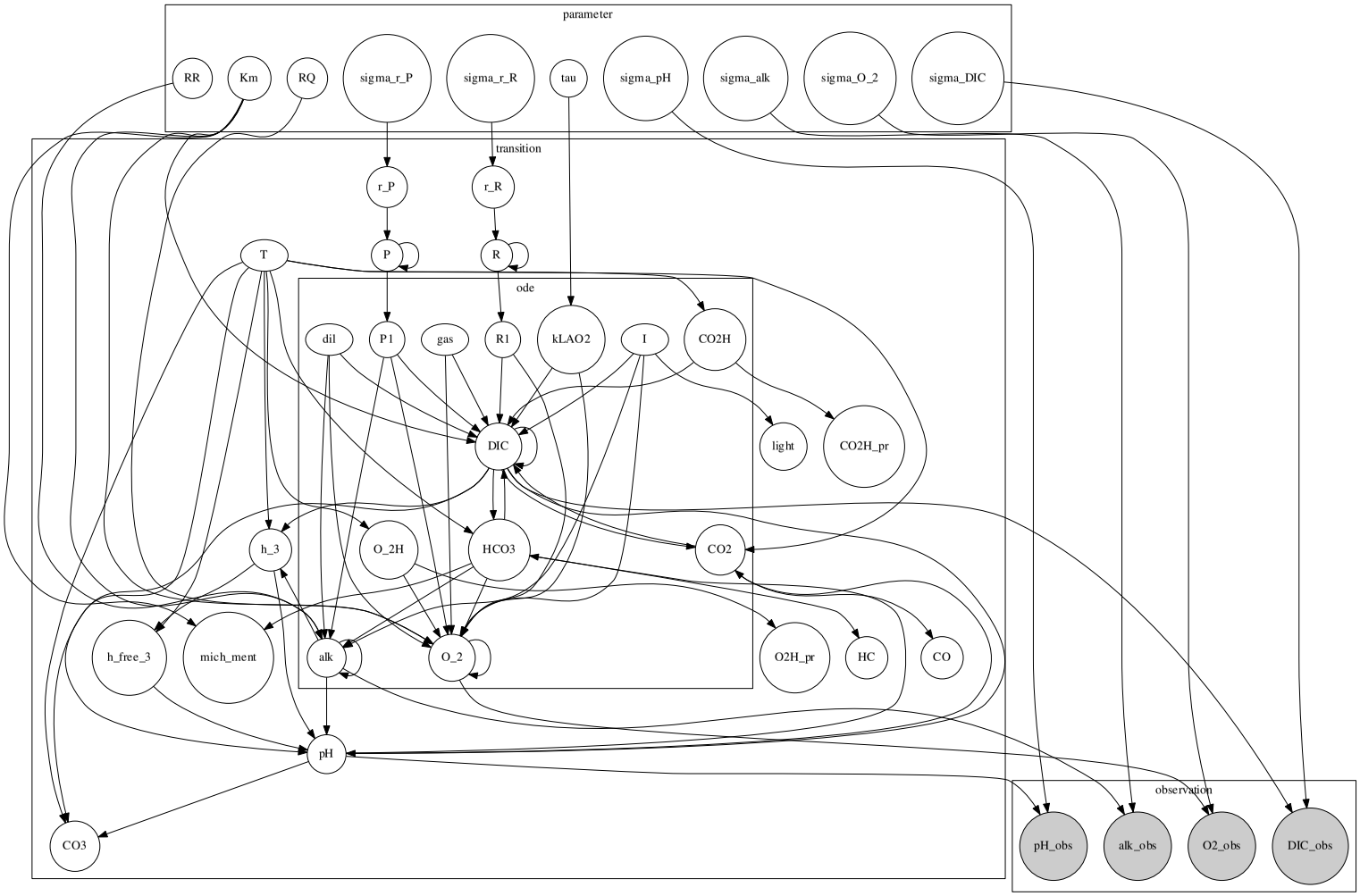

Figure A.1 : Directed Acyclic Graph of the LiBbi model file micro_iterative.bi

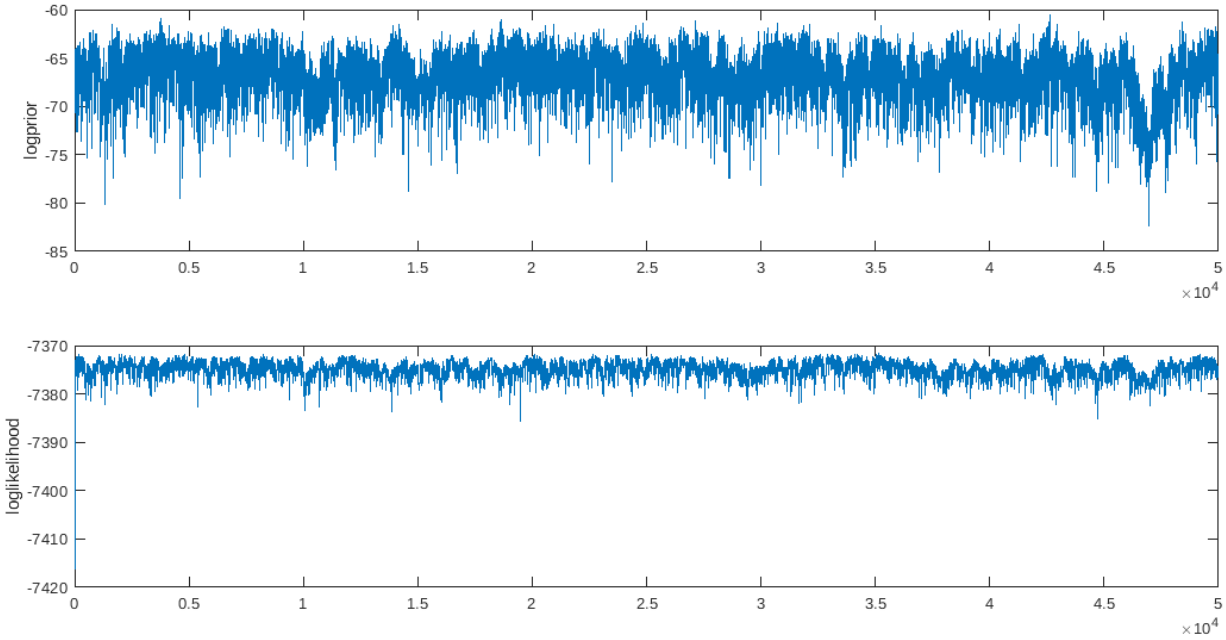

Figure A.2 : Log-prior and log-likelihood for the simulated data experiment.

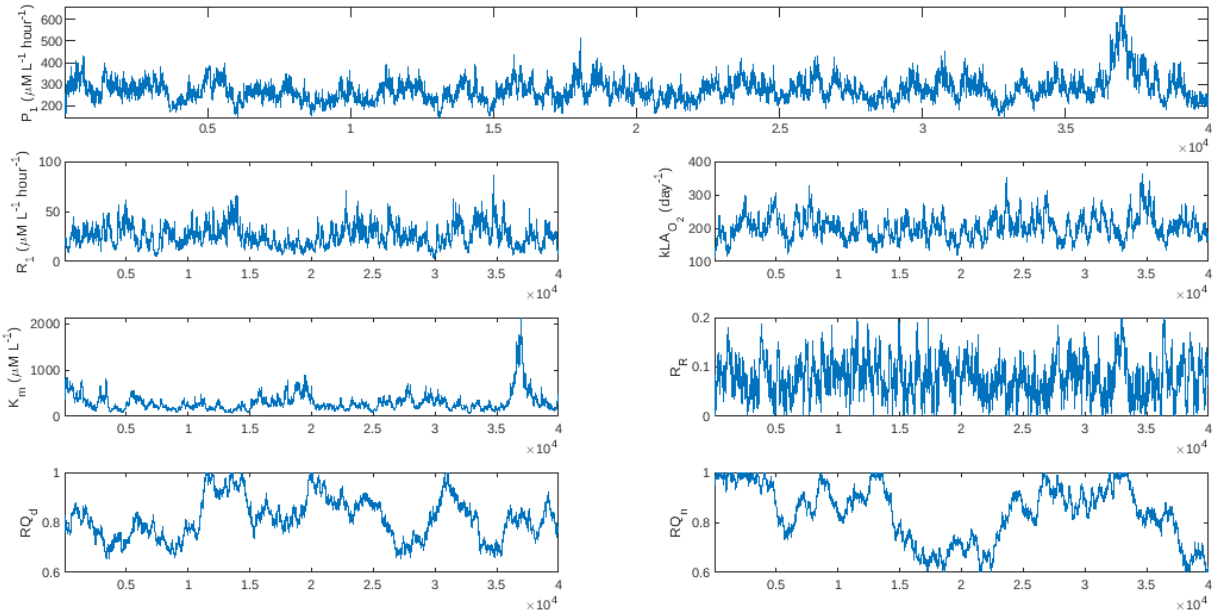

Figure A.3 : Parameter posterior traces for the simulated data experiment.

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