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Chapter 4

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

4.1 Introduction

Microalgae are tiny organisms..

[BM for ref: At 2 hour intervals, a solenoid valve (SMC Pneumatics Pty. Ltd.) was used to stop aeration for 10 minutes. The linear increase in DO caused by these artefacts were used to calculate net photosynthesis. (reference Tamburic 2015)]

4.2 Methods

4.2.1 Data Model: Photobioreactor 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 $50 \mu\text{mol m}^{-2} \text{s}^{-1}$ of cool-white fluorescent light at a 12 hour light/12 hour dark cycle. Stock cultures were grown in f/2 saltwater medium [5] 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 photobioreactors (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 $\pm 0.2^\circ\text{C}$. 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 \pm 0.02, pH 7.00 \pm 0.02 and pH 10.00 \pm 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 $500 \mu\text{mol m}^{-2} \text{s}^{-1}$ and a temperature of 27°C. Following this acclimation period, the PBR was set to the experimental condition of $2,000 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ 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 sea-

water (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 was measured twice a day using TA titration; 0.1 M hydrochloric acid on 30 mL of *N. oceanica* media using an auto-titrator (800 Dosino; Metrohm AG).

[Chris: DIC measurement collection description]

[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. An offset term ($offset_{O_2}$) was added to the O_2 ode to account for this. The offset was assigned a normally distributed prior distribution with mean 0 and standard deviation 2. [BM:check the offset is still there and you did it this way otherwise change/remove.]

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 O₂ and pH obs?]

4.2.3 Process model: Carbon chemistry

Ideally CO2SYS [6] would have been used to calculate the carbon chemistry of the photo-bioreactor. CO2SYS is a program developed for CO₂ system calculations (CO2SYS) that calculates and returns a detailed state of the carbonate system of oceanographic water samples in seawater and freshwater [6]. Using two of the four measurable carbonate system parameters (total alkalinity, total inorganic CO₂, pH, and either fugacity fCO₂ or partial pressure of CO₂) to calculate the other two parameters at a set of input conditions (temperature and pressure).

To incorporate CO2SYS into LiBbi for solving carbon chemistry on the timescale of the microalgae model, we explicitly defined 3 iterations (Eq. 4.4 - 4.12) 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 pH₀ (Eq. 4.3) 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 4.1 and Table 4.1).

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

Total Sulfur:

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

Fluorine:

$$\begin{aligned}
 TF &= \frac{\frac{0.000067S}{18.9984}}{1.80655} \\
 KF &= e^{(-(-\frac{874.0}{T_K} - 0.111\sqrt{S} + 9.68))} \\
 SW S_{2T} &= \frac{(1 + \frac{TS}{KS})}{(1 + \frac{TS}{KS} + \frac{TF}{KF})} \\
 Free_{2T} &= 1 + \frac{TS}{KS}
 \end{aligned}$$

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:

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

Choice of carbonate dissociation constants K_1 and K_2 were Mehrbach [7] (refit by Dickson and Millero [2]) with $1.23K_1$ and $0.53K_2$ measured experiment specific adjustments:

$$K_1 = 10^{(-(\frac{3633.86}{T_K} - 61.2172 + 9.6777 \log(T_K) - 0.011555S + 0.0001152S^2))} * 1.23 \quad (4.1)$$

$$K_2 = 10^{(-(\frac{471.8}{T_K} + 25.9290 - 3.16967 \log(T_K) - 0.01781S + 0.0001122S^2))} * 0.53 \quad (4.2)$$

Approximating equation for the starting value of pH :

$$\begin{aligned} pH_0 = & 12.26 - 0.0030605DIC - 0.043752T - 0.013625S + 0.00011315TA \\ & + 1.3463e - 5DIC * T + 5.2215e - 7DIC * TA \end{aligned} \quad (4.3)$$

Newton-Raphson iterations:

$$h_n = 10^{-pH_n} \quad (4.4)$$

$$h_{n_{free}} = \frac{h_n}{Free_{2T}} \quad (4.5)$$

$$\begin{aligned} f_n = & (DIC * 1e - 6 * \frac{K_1 h_n + 2K_1 K_2}{h_n^2 + K_1 h_n + K_1 K_2} \\ & - h_{n_{free}} + \frac{KW}{h_n} - TA * 1e - 6 + \frac{TB}{1 + \frac{h_n}{KB}}) * 1e6 \end{aligned} \quad (4.6)$$

$$\begin{aligned} df_n = & (DIC * 1e - 6 * \frac{K_1 + 2K_1 K_2}{h_n^2 + K_1 h_n + K_1 K_2} \\ & - DIC * 1e - 6 * \frac{(K_1 h_n + 2K_1 K_2)}{(h_n^2 + K_1 h_n + K_1 K_2)^2} (2h_n + K_1) \\ & - TB \frac{1}{(1 + \frac{h_n}{KB})^2} / KB \\ & - \frac{KW}{h_n^2} - \frac{1}{Free_{2T}}) * 1e6 * (-\log(10) * 10^{-pH}) \end{aligned} \quad (4.7)$$

$$pH_{n+1} = pH_n - \frac{f_n}{df_n} \quad (4.8)$$

$$H_{n+1} = 10^{-pH_{n+1}} \quad (4.9)$$

$$CO_{2n+1} = \frac{H_{n+1}^2 DIC}{H_{n+1}^2 + K_1 H_{n+1} + K_1 K_2} \quad (4.10)$$

$$HCO_{3n+1} = \frac{H_{n+1} K_1 DIC}{H_{n+1}^2 + K_1 H_{n+1} + K_1 K_2} \quad (4.11)$$

$$CO_{3n+1} = \frac{K_1 K_2 DIC}{H_{n+1}^2 + K_1 H_{n+1} + K_1 K_2} \quad (4.12)$$

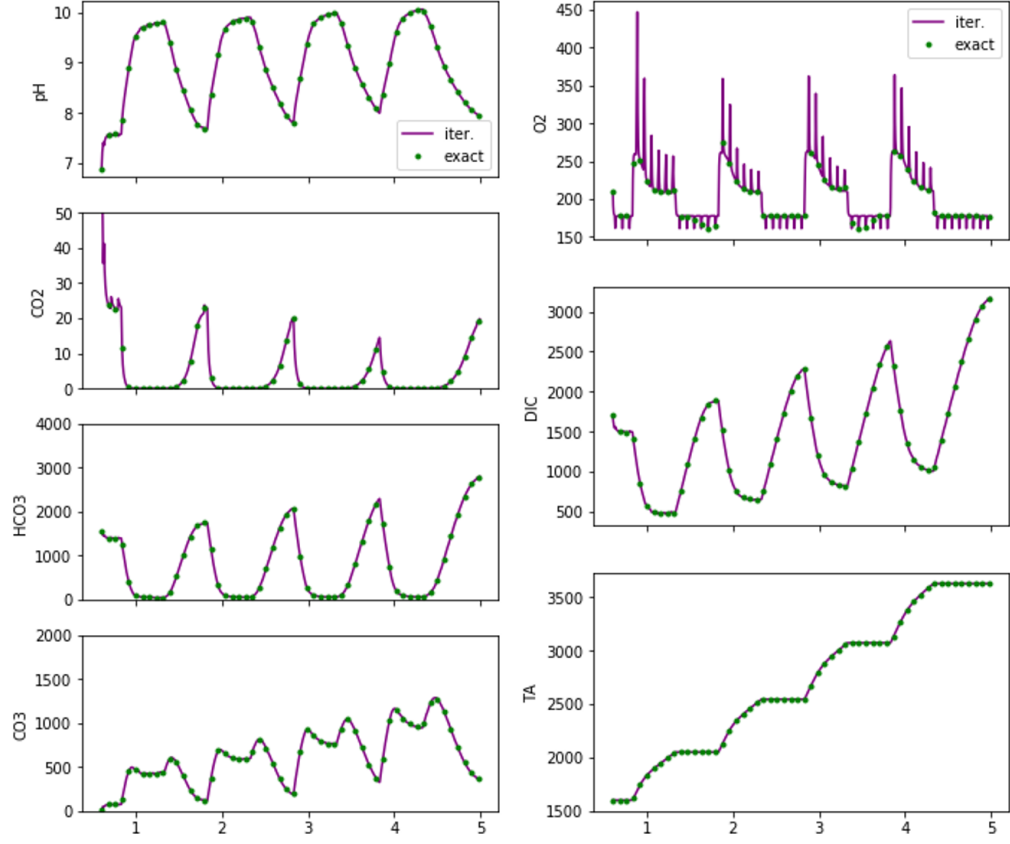


Figure 4.1 : Iterative (3rd iteration) vs exact solution for carbon chemistry CO_2 , HCO_3 , CO_3 , pH and state variables O_2 , DIC , and TA .

| 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 O₂ and CO₂

The equilibrium concentration for CO₂ solubility in water CO_{2H} (μmol/L) is calculated using Henry's law,

$$CO_{2H} = K0_{CO2} * fCO2 * 1.0220 * 1e6 \quad (4.13)$$

where fCO₂ (atm) is the fugacity or approximately the partial pressure of CO₂, 1.0220 is the density of seawater (kg/L) at salinity 34 ppt and temperature 27°C [8] [3]. 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 [9],

$$K0_{CO2} = e^{(-60.2409 + 93.4517 \frac{100}{T_K} + 23.3585 * \log(\frac{T_K}{100}) + S(0.023517 - 0.023656 \frac{T_K}{100} + 0.0047036 (\frac{T_K}{100})^2))} \quad (4.14)$$

where T_K is the temperature (K) and S is salinity (ppt). Similarly the equilibrium concentration for O₂ solubility in water O_{2H} is calculated using Henry's law,

$$O_{2H} = K0_{O2} * fO2 * 1.0220 * 1e - 6 \quad (4.15)$$

where fO₂ (atm) is the fugacity or approximately the partial pressure of O₂, 1.0220 is the density of seawater (kg/L) at salinity 34 ppt and temperature 27°C [8] [3], and K0_{O2} (mol/kg_{soln}/atm) is the solubility of oxygen in seawater with an adjusted salinity dependence [1],

$$K0_{O2} = \frac{e^{(-1282.8704 + \frac{36619.96}{T_K} + 223.1396 \log(T_K) - 0.354707 T_K + S(5.957e - 3 - \frac{3.7353}{T_K}) + 3.68e - 6 S^2)}}{0.2094e - 6} \quad (4.16)$$

where T_K is the temperature (K) and S is salinity (ppt). The equilibrium concentrations for O₂ and CO₂ are modelled together with the gas turning on and off during the experiment, as

$$Q^{air} kLa_{O_2}^{air} (O_{2H} - O_2) \quad (4.17)$$

$$Q^{air} kLa_{CO_2}^{air} (CO_{2H} - CO_2) \quad (4.18)$$

where Q^{air} is the gas state (1= on, 0= off), $kLa_{O_2}^{air}$ and $kLa_{CO_2}^{air}$ 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 [4].

4.2.5 Process model: Photosynthesis and respiration

Net photosynthesis

$$\frac{\partial DIC}{\partial t} = -(P_1 I \frac{HCO_3^-}{K_m + HCO_3^-} - R_1) \quad (4.19)$$

$$\frac{\partial O_2}{\partial t} = \frac{1}{(RQ_d I + RQ_n(1 - I))} (P_1 I \frac{HCO_3^-}{K_m + HCO_3^-} - R_1) \quad (4.20)$$

$$\frac{\partial TA}{\partial t} = R_R (P_1 I \frac{HCO_3^-}{K_m + HCO_3^-}) \quad (4.21)$$

$$\frac{\partial C_p}{\partial t} = (P_1 I \frac{HCO_3^-}{K_m + HCO_3^-} - R_1) \quad (4.22)$$

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

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

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

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.

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

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

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

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

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

4.2.7 Process model: Ordinary differential equations

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

| Rate | flux into cells | gas transfer | dilution |
|-------------------------------------|--|--|---------------------------------|
| $\frac{\partial DIC}{\partial t} =$ | $-(P_1 I \frac{HCO_3^-}{K_m + HCO_3^-} - R_1)$ | $+ \hat{Q}^{air} kLa_{CO_2}^{air} (CO_2^{air} - CO_2)$ | $+ \frac{Q^M}{V} (DIC^M - DIC)$ |
| $\frac{\partial O_2}{\partial t} =$ | $\frac{(P_1 I \frac{HCO_3^-}{K_m + HCO_3^-} - R_1)}{(RQ_d I + RQ_n(1 - I))}$ | $+ \hat{Q}^{air} kLa_{O_2}^{air} (O_2^{air} - O_2)$ | $+ \frac{Q^M}{V} (O_2^M - O_2)$ |
| $\frac{\partial TA}{\partial t} =$ | $R_R (P_1 I \frac{HCO_3^-}{K_m + HCO_3^-})$ | | $+ \frac{Q^M}{V} (TA^M - TA)$ |
| $\frac{\partial C_p}{\partial t} =$ | $(P_1 I \frac{HCO_3^-}{K_m + HCO_3^-} - R_1)$ | | $+ \frac{Q^M}{V} (\quad - C_p)$ |

(4.23)

| | Symbol | Description | Prior / Value | Unit |
|--------------------|--------------|----------------------------|--|--------------------------------|
| Initial conditions | DIC^0 | Dissolved inorganic carbon | $\text{Log}\mathcal{N}(\log(1300), 0.2)$ | $\mu\text{M L}^{-1}$ |
| | O_2^0 | Oxygen | $\text{Log}\mathcal{N}(\log(225), 0.2)$ | $\mu\text{M L}^{-1}$ |
| | TA^0 | Total alkalinity | $\text{Log}\mathcal{N}(\log(1750), 0.1)$ | $\mu\text{M L}^{-1}$ |
| | C_p^0 | | $\text{Log}\mathcal{N}(\log(300), 0.2)$ | $\mu\text{M L}^{-1}$ |
| | pH^0 | - | $\text{Log}\mathcal{N}(\log(8.5), 0.2)$ | $\log_{10}(-\text{mol/L H}^+)$ |
| | CO_2^0 | Carbon dioxide | $\text{Log}\mathcal{N}(\log(5), 0.4)$ | $\mu\text{M L}^{-1}$ |
| | HCO_3^{-0} | Bicarbonate | $\text{Log}\mathcal{N}(\log(1500), 0.3)$ | $\mu\text{M L}^{-1}$ |
| | CO_3^{2-0} | Carbonate | $\text{Log}\mathcal{N}(\log(100), 0.4)$ | $\mu\text{M L}^{-1}$ |

| | | | | |
|--------------------|--------------------|---|---------------------------|--|
| Flux into cells | P_1 | Maximum photosynthesis rate | * | $\mu\text{M L}^{-1} \text{ hour}^{-1}$ |
| | R_1 | Respiration rate | * | $\mu\text{M L}^{-1} \text{ hour}^{-1}$ |
| | K_m | Carbon restriction | * | $\mu\text{M L}^{-1}$ |
| | 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) | - |
| | $x_{CO_2}^{air}$ | Mole fraction of CO ₂ atmosphere | 400 | ppm |
| | CO_{2H} | Equilibrium CO ₂ concentration | Eq. 4.13 | $\mu\text{M L}^{-1}$ |
| | CO_2^{air} | Sat CO ₂ conc with atmosphere | $x_{CO_2}^{air} CO_{2H}$ | |
| | $kLa_{CO_2}^{air}$ | Mass transfer coefficient for CO ₂ | $0.893kLa_{O_2}^{air}$ | day^{-1} |
| | $x_{O_2}^{air}$ | Mole fraction of O ₂ atmosphere | 0.2094 | atm |
| | O_{2H} | Equilibrium O ₂ concentration | Eq. 4.15 | $\mu\text{M L}^{-1}$ |
| | O_2^{air} | Sat O ₂ conc with atmosphere | $x_{O_2}^{air} O_{2H}$ | |
| | τ | half-life of $kLa_{O_2}^{air}$ | range(2-20) | min^{-1} |
| | $kLa_{O_2}^{air}$ | 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 | $\mu\text{M L}^{-1}$ |
| | O_2^M | Media oxygen concentration | 226.65 | $\mu\text{M L}^{-1}$ |
| | TA^M | Media total alkalinity | 1797.90 | $\mu\text{M L}^{-1}$ |

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 ($P_1 = 200$, $R_1 = 30$, $kLA_{O_2}^{air} = 200$, $K_m = 150$, $RQ_d = 0.85$, $RQ_n = 0.95$, $R_R = 0.075$) and initial conditions ($O_2^0 = 225$, $DIC^0 = 1250$, $TA^0 = 1750$).

The first set of results will assimilate the simulated observations and attempt to recover the true value parameters.

[Chris: noise on simulated dataset? sigmas? can you double check what I've written here is right?]

4.2.9 Parameter Model: Priors

** include simulated data priors, and real data priors.

Decide whether the parameters vary in time or not.

| Parameter | Prior | Proposal |
|-------------------|----------------------------|---|
| S | 34 | * |
| fCO2 | 397e-6 | * |
| fO2 | 0.21 | * |
| kLA _{O2} | LogNormal(log(200.0), 0.5) | LogNormal(log(kLA _{O2}), 0.5prop _{std}) |
| K _m | LogNormal(log(200.0), 0.8) | LogNormal(log(K _m), 0.8prop _{std}) |
| R _R | Uniform(0.0001, 0.2) | TrunNormal(R _R , 0.2prop _{std} , 0.0001, 0.2) |
| R _Q | Uniform(0.66, 1) | TrunNormal(R _Q , 0.2prop _{std} , 0.66, 1.0) |
| σ _P | Normal(0.05, 0.01) | Normal(σ _P , 0.01prop _{std}) |
| σ _R | Normal(0.01, 0.001) | Normal(σ _R , 0.001prop _{std}) |

Table 4.3 : Table of Parameters, their priors and proposal distributions. * indicates the parameter was held fixed. (prop_{std} = 0.1)

4.3 Results and Discussion

4.3.1 Posteriors with simulated data

| 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.

After 50,000 samples the true values of parameters R_1 , $kLA_{O_2}^{air}$, 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 , $kLA_{O_2}^{air}$, 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 4.2, Figure 4.3).

Unobserved state variable C_p .. (Figure 4.3).

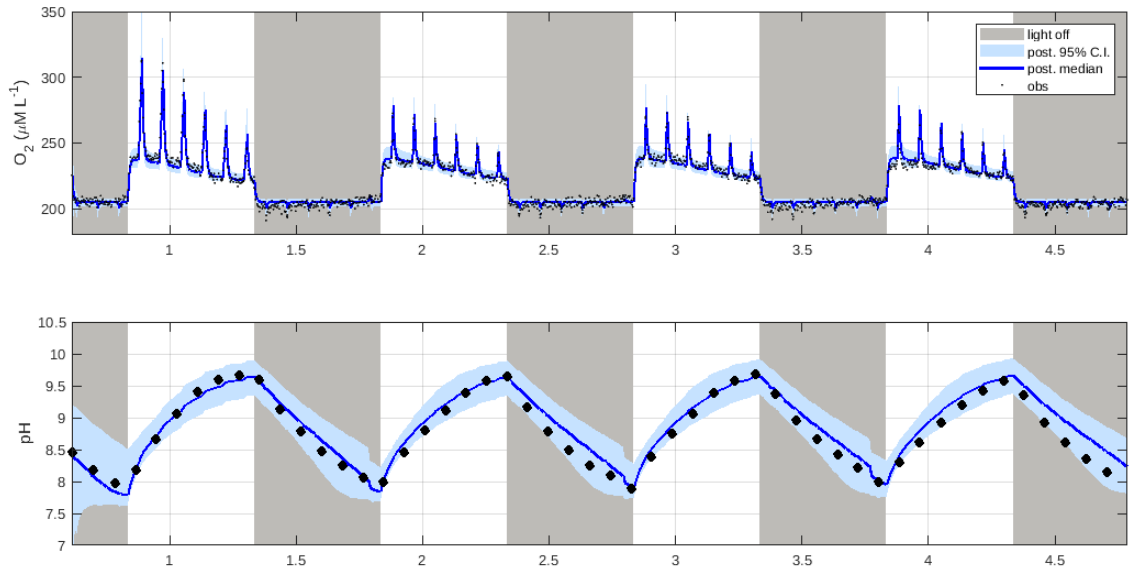


Figure 4.2 : Posterior medians (solid blue line), 95% credible intervals (shaded blue), and simulated observations (black) for O_2 and pH across 4 days.

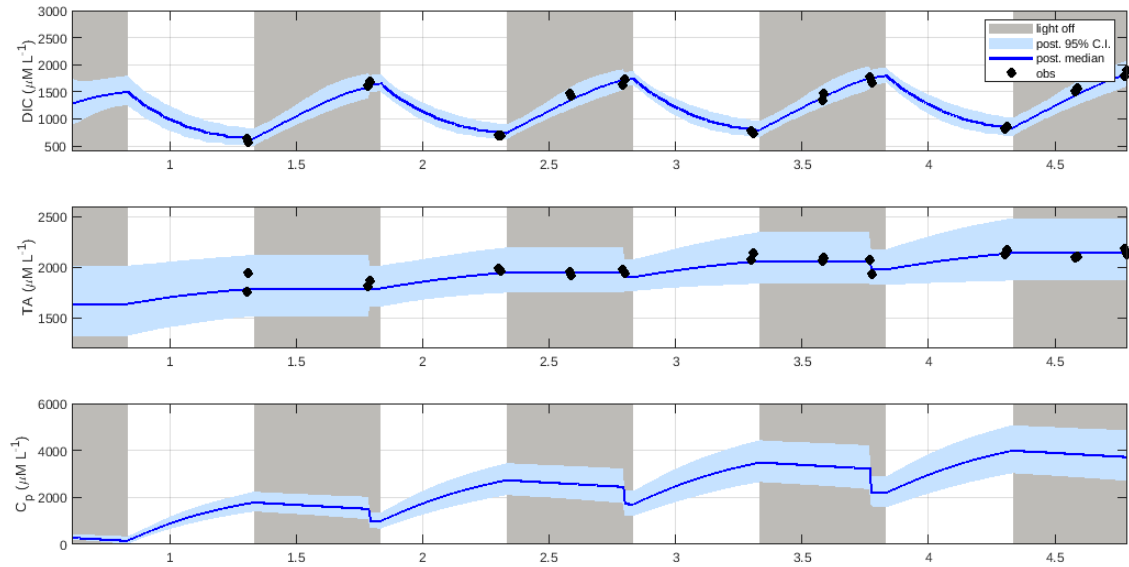


Figure 4.3 : Posterior medians (solid blue line), 95% credible intervals (shaded blue), and simulated observations (black) for DIC , TA and C_p across 4 days.

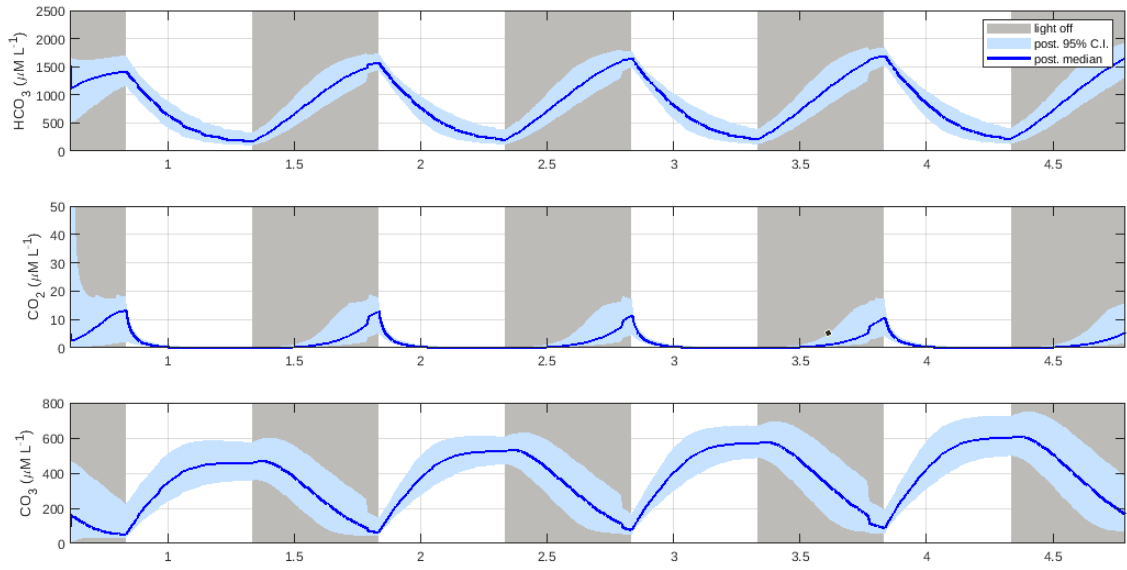


Figure 4.4 : Posterior medians (solid blue line) and 95% credible intervals (shaded blue) for HCO_3 , CO_2 and CO_3 across 4 days.

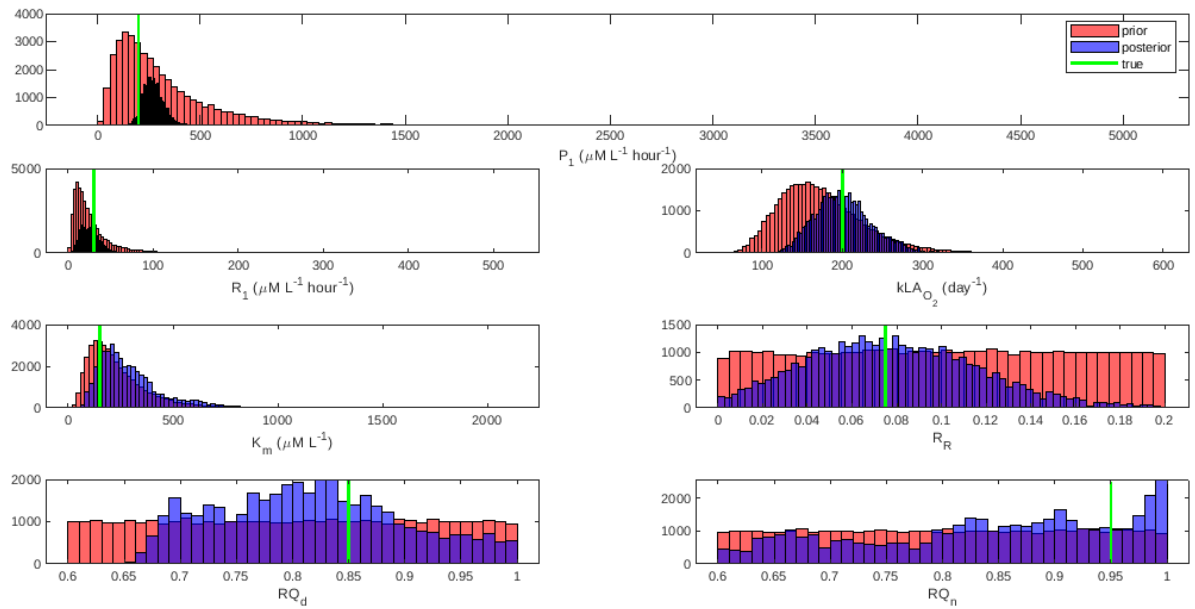


Figure 4.5 : Priors (pink), posteriors (purple) and true values (green) for model parameters.

Carbon chemistry variables HCO_3 , CO_2 , and $CO_3 \dots$ (Figure 4.4).

4.3.2 Posteriors with experimental data

[BM: Talk about the tuning of the observation error priors σ_{O_2} etc]

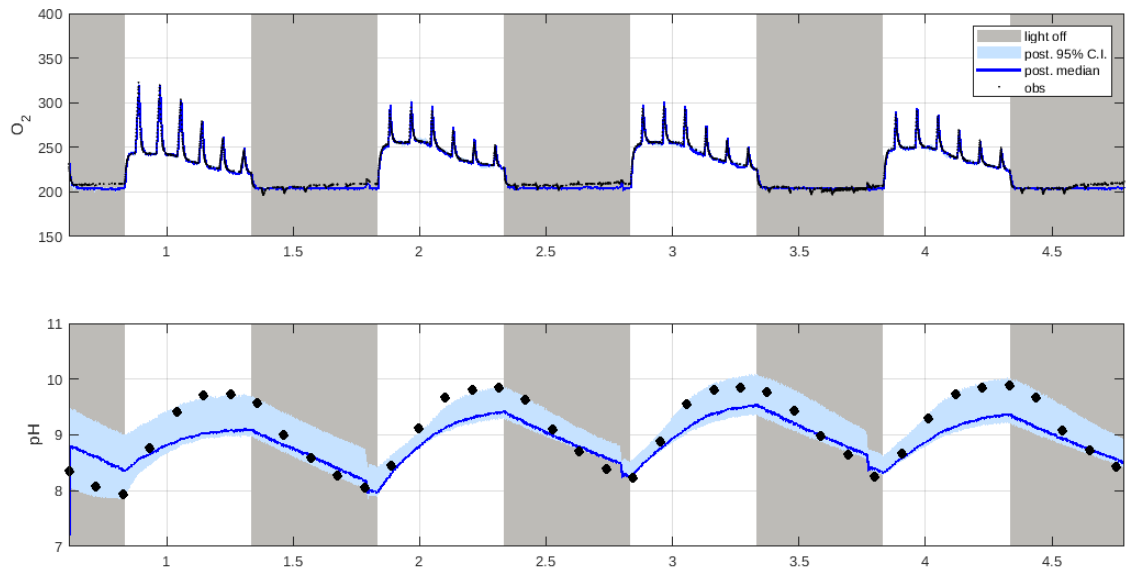


Figure 4.6 : Posteriors for O_2 and pH .

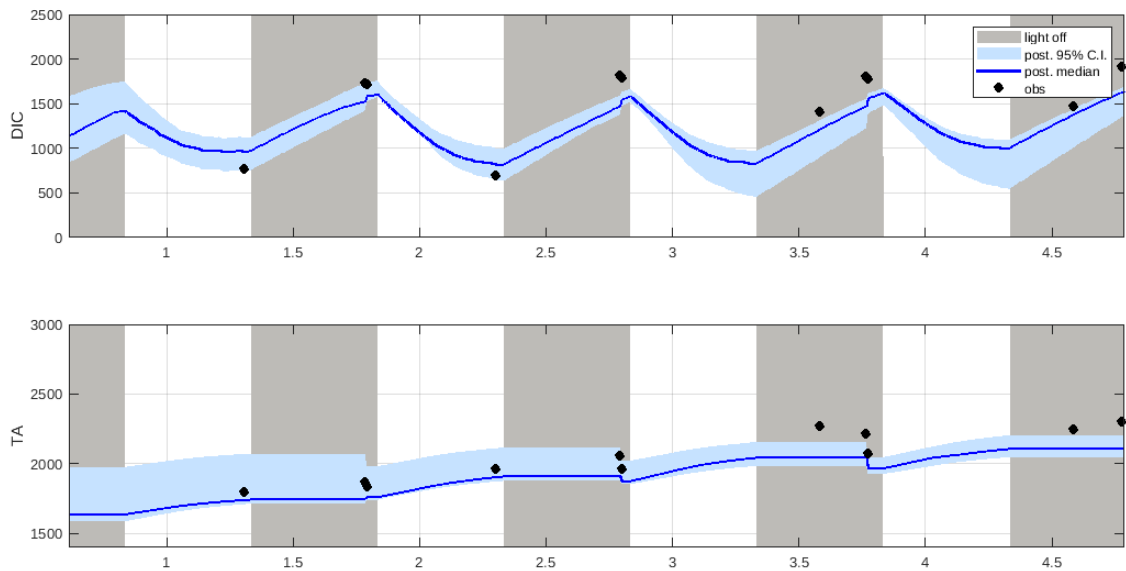


Figure 4.7 : Posteriors for DIC and TA .

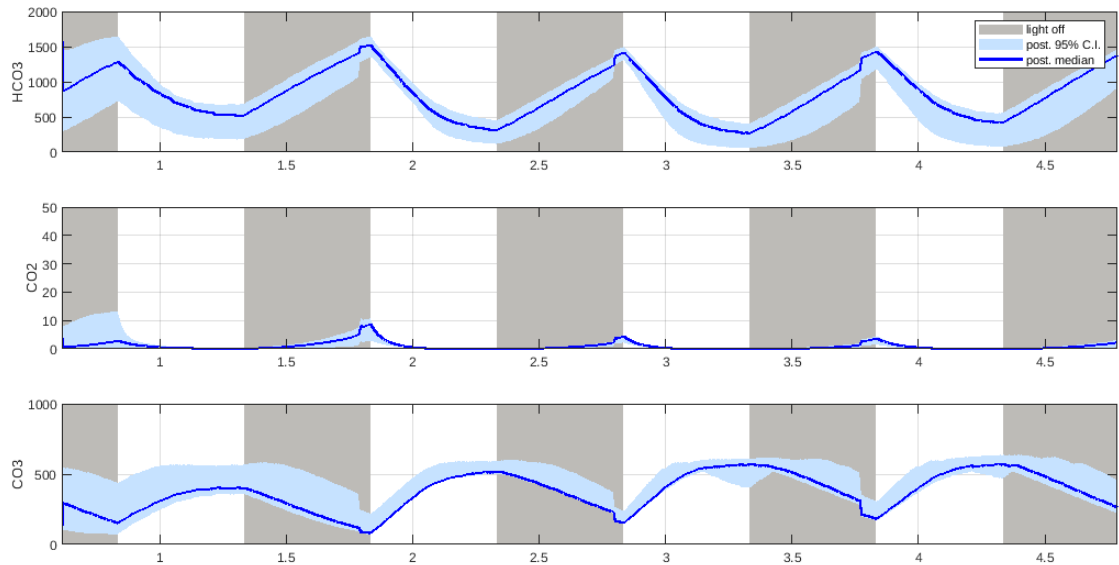


Figure 4.8 : Posteriors for carbon chem.

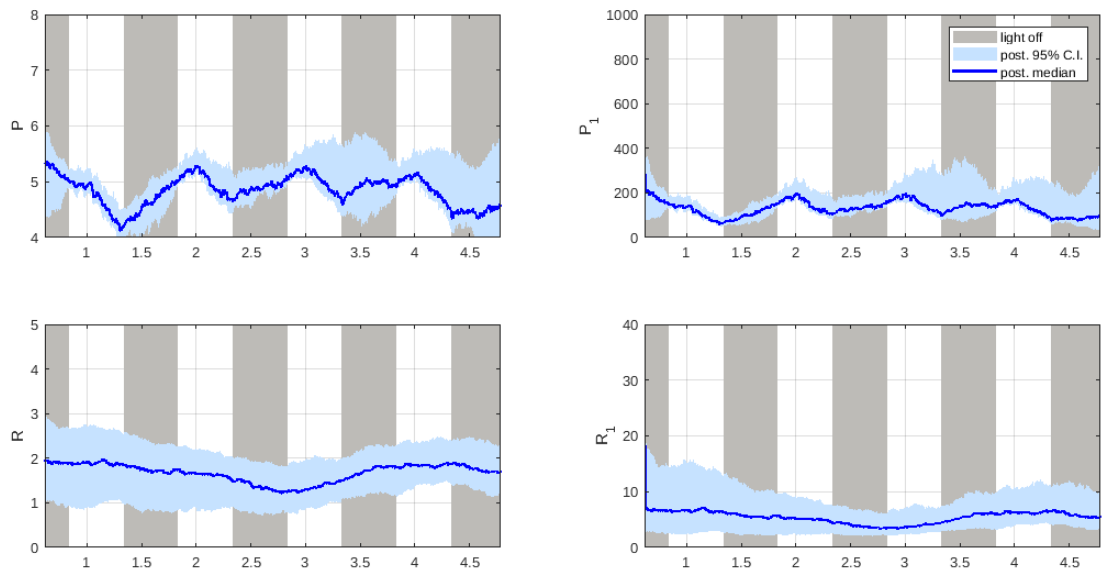


Figure 4.9 : Posteriors for photosynthesis and respiration.

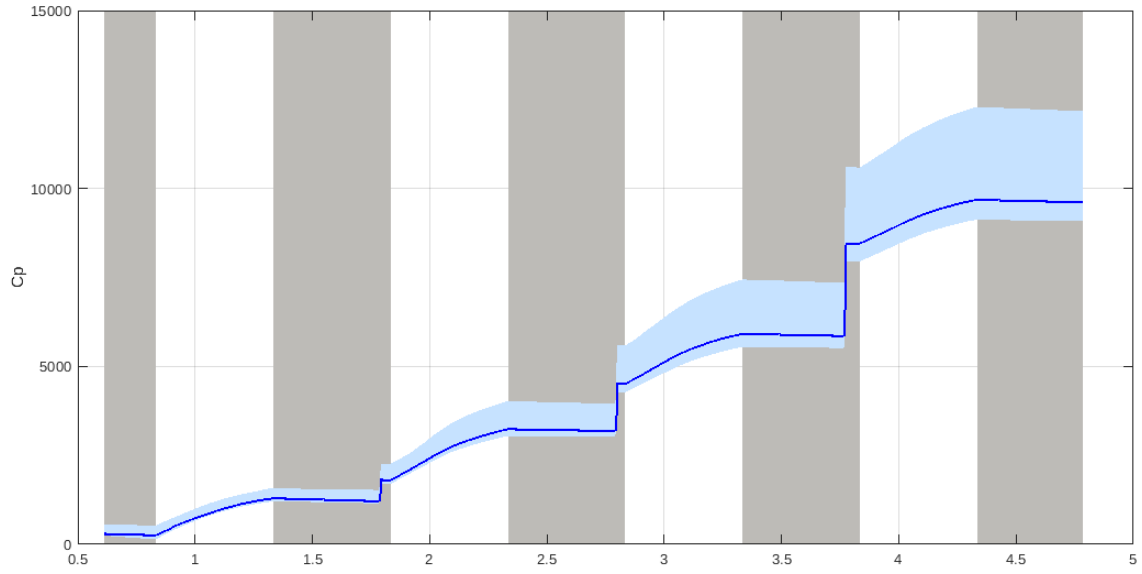
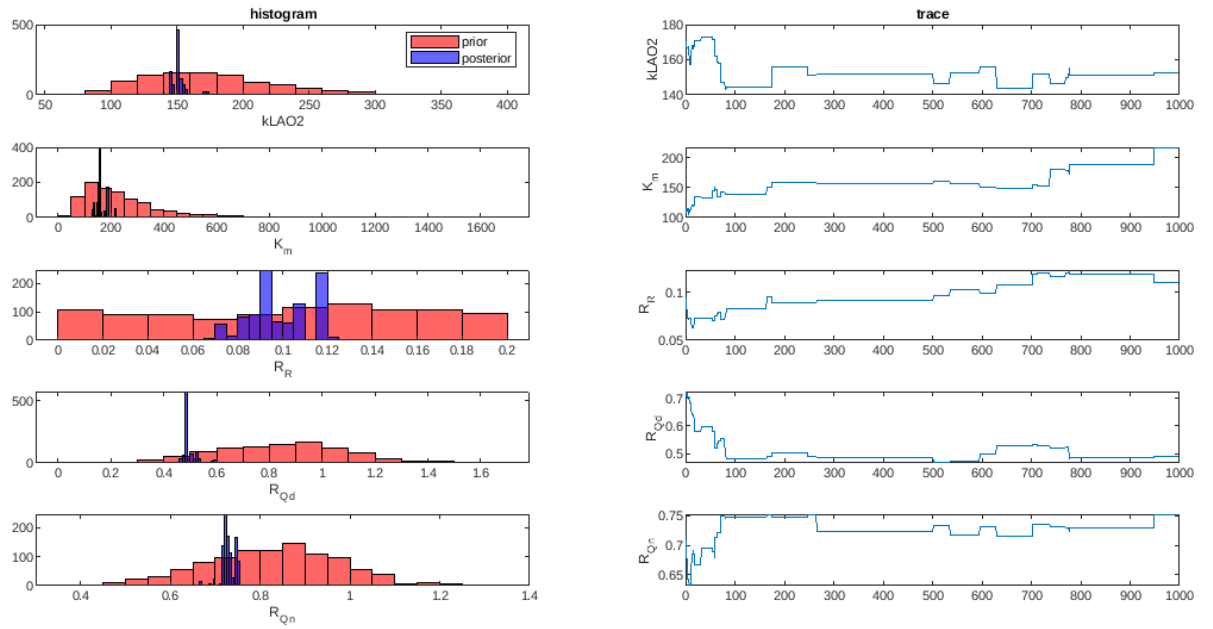
Figure 4.10 : Posterior for C_p .

Figure 4.11 : Priors, posteriors and traces for model parameters.

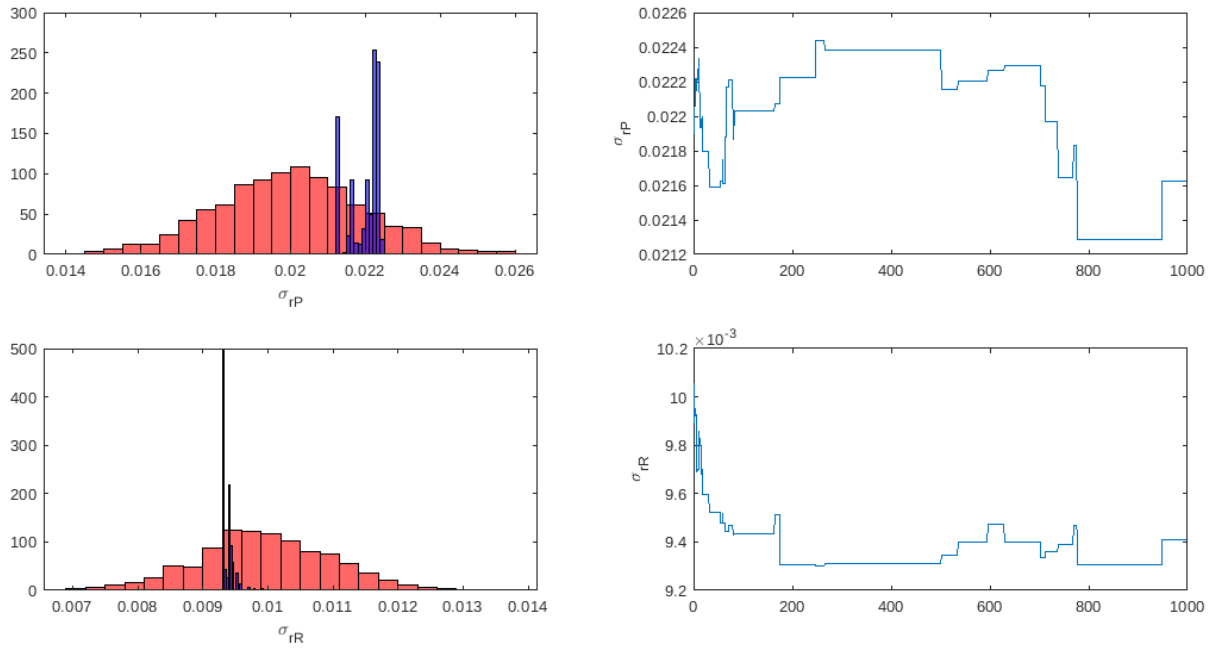


Figure 4.12 : Priors, posteriors and traces for model parameters.

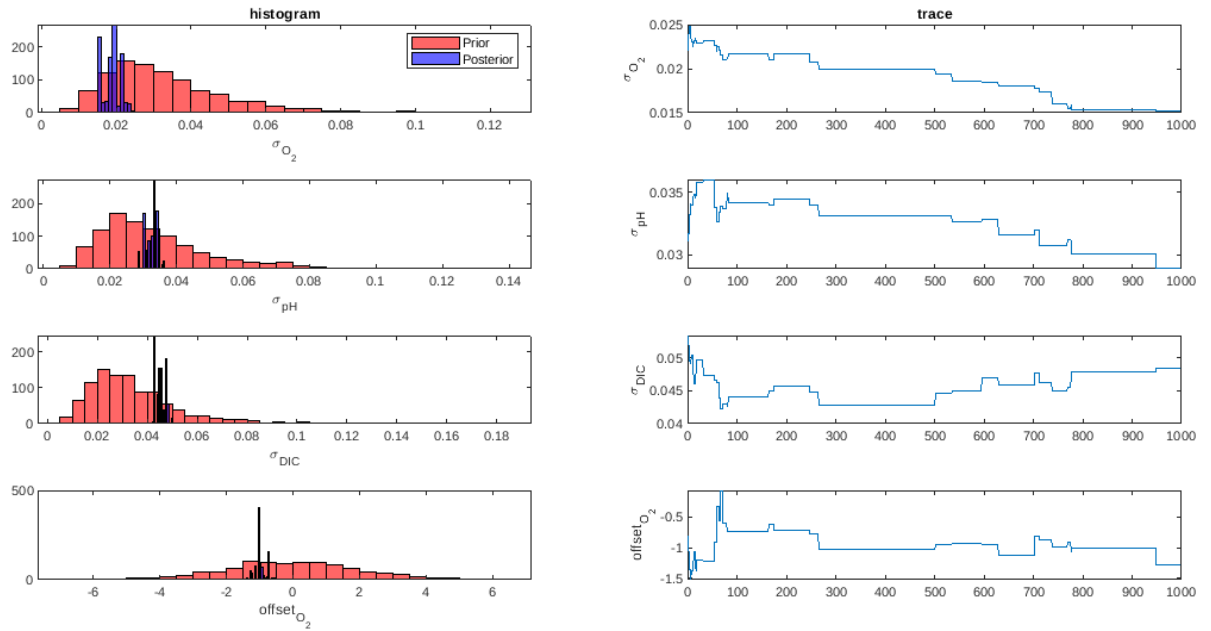


Figure 4.13 : Priors, posteriors and traces for obs. error parameters.

Appendix A

LiBbi model code

LiBbi model file: micro_iterative.bi

```

1 model micro_iterative {
2
3   const F02          = 0.2094
4   const FC02         = 397e-6
5   const S            = 34.0
6   const V            = 500.0           // volume of the
      reactor
7   const DIC_M        = 1724.20         // calculated with
      CO2SYS[ DIC_M = 1724.20, Alk = 1797.90, T = 27, S = 34]
8   const O_2_M        = 226.65
9   const alk_M         = 1797.90
10  const tau           = 6.0
11  const kLA02_m       = log(2.0)*24.0*60.0/tau
12
13  param kLA02
14  param Km
15  param RR
16  param RQ_d
17  param RQ_n
18  param sigma_O_2
19  param sigma_pH
20  param sigma_DIC

```

```
21 param offset_0_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 02_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 kLA02   ~ log_normal(log(kLA02_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_0_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_0_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 kLA02    ~ log_normal(log(kLA02), 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_0_2 ~ log_normal(log(sigma_0_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_0_2 ~ normal(offset_0_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_02*F02*1.0220*1e-6
127
128 inline PAC     = HC03          //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 dissoci
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*kLA02*(C02H - C02)          + 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*kLA02*(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 C02H_pr    <- C02H
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 }

```

251 }

LiBbi prior sampling file: prior.conf

```
1 --target prior
2 --model-file micro_iterative.bi
3 --nsamples 500
4 --start-time 0.61304
5 --end-time 4.7866
6 --noutputs 6049
7 --input-file data/input_all_2018_normalised.nc
8 --output-file results/prior_micro_iterative.nc
```

LiBbi posterior sampling file: posterior.conf

```
1 --target posterior
2 --model-file micro_iterative.bi
3 --input-file data/input_all_2018_normalised.nc
4 --obs-file data/obs_all_2018.nc
5 --nsamples 500
6 --nparticles 1024
7 --start-time 0.61304
8 --end-time 4.7866
9 --noutputs 6049
10 --output-file results/posterior_micro_iterative.nc
11 --with-transform-initial-to-param
```

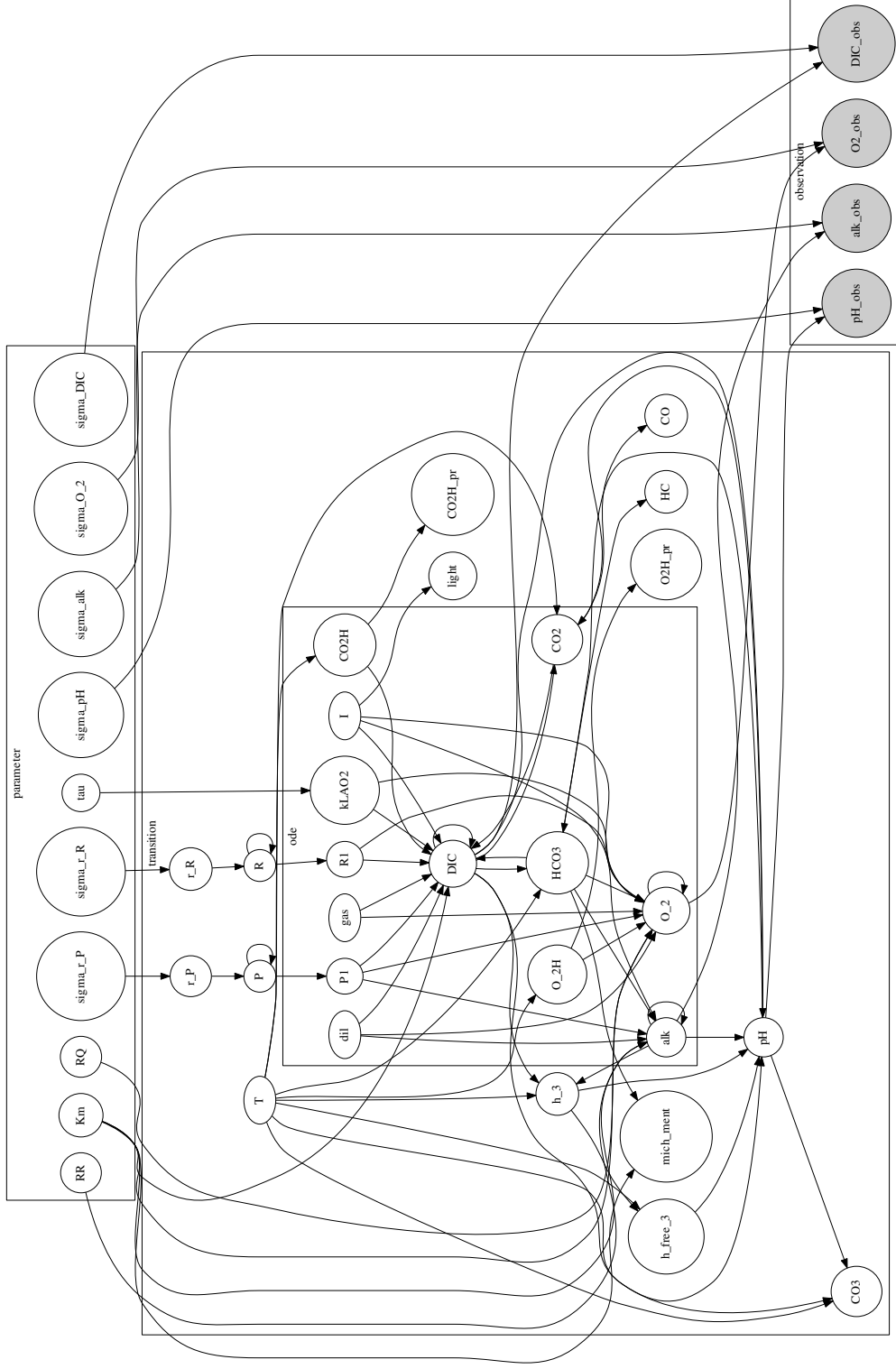


Figure A.1 : Directed Acyclic Graph of the LiBbi model file `micro_iterative.bi`

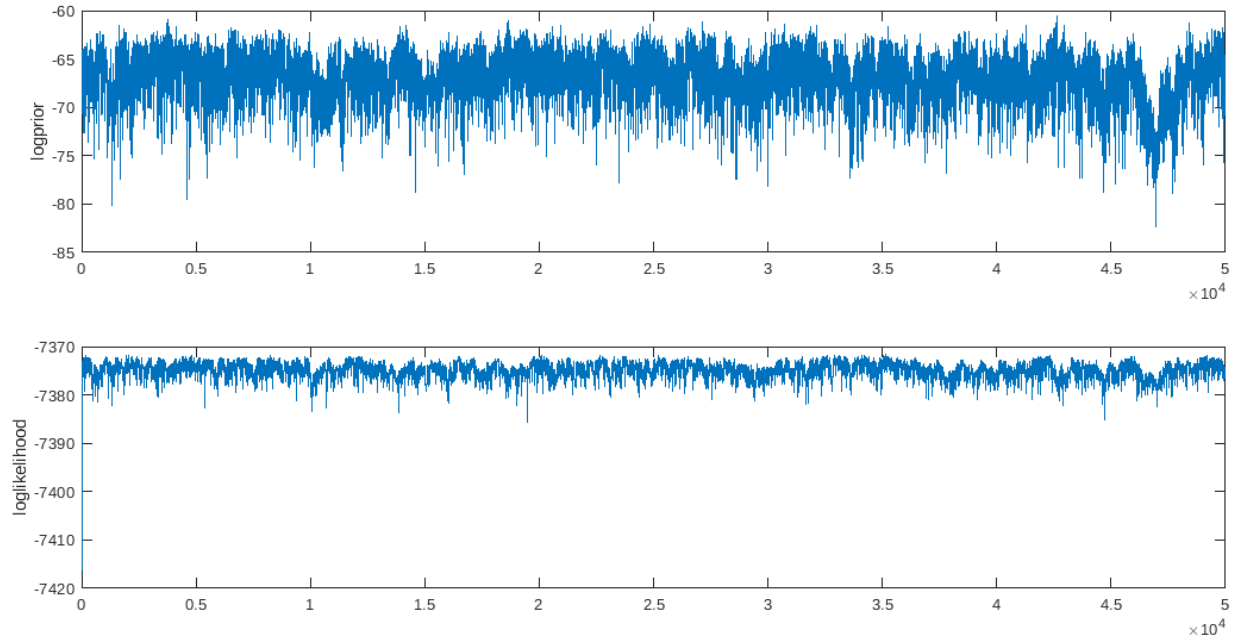


Figure A.2 : Log-prior and log-likelihood for the simulated data experiment.

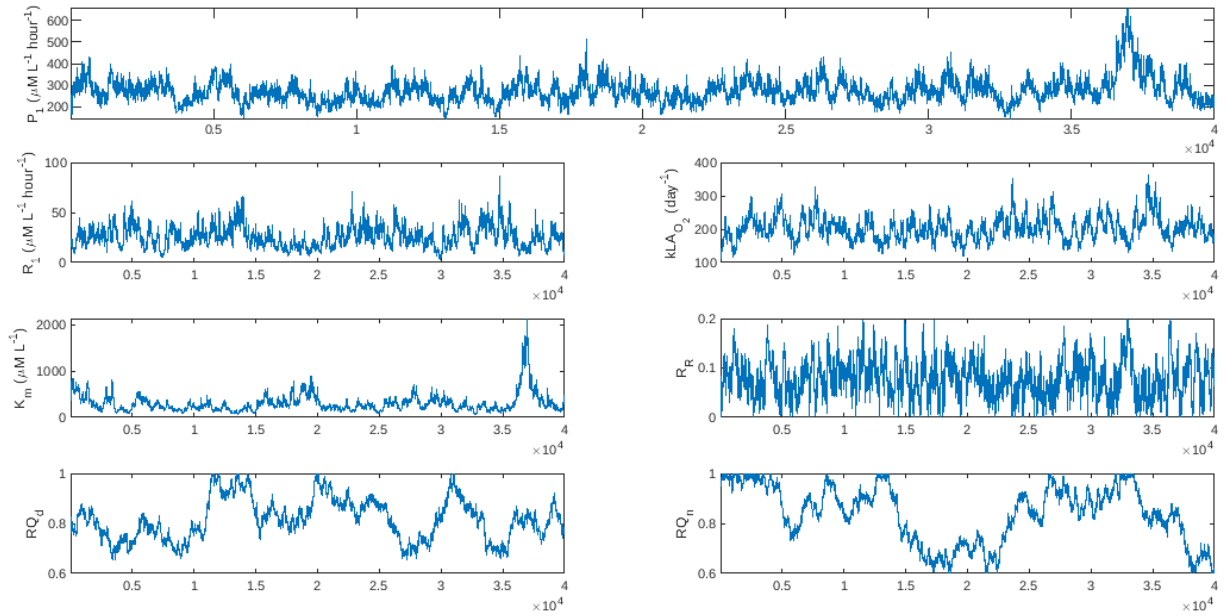


Figure A.3 : Parameter posterior traces for the simulated data experiment.

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