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Correction of metabolic parameters and unit process performance data – Part II : Comparison of analytical approaches

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ABSTRACT

Oxygen consumption and other metabolic rates for commercial systems are typically measured under flow-through conditions and therefore are biased because of hydraulic lag. To evaluate potential correction approaches, three representative (SINE, STEP, MULTI-STEP) daily metabolic responses (R_t^a) were developed. Using the values of R_t^a, the resulting concentration of dissolved oxygen (C_t^i) on a minutely basis over the day was estimated from either a hydraulic mixing model or mass balance equations. Six approaches (STEADY, FRY, NORTHBY, NIIMI, SPLINE, and POLY) were used to estimate the metabolic oxygen consumption rate (R_t) for the 20-, 40-, and 60-minute periods. Three analytical approaches were tested (MEAN, POINT, and DETAILED).

Based on all three test metabolic rates, the combination of the DETAILED analytical approach and the SPLINE correction was the most accurate. The DETAILED approach is based on estimation of the minutely oxygen consumption and computation of the average rate over a specific time period. The combination of DETAILED and NIIMI was more accurate for STEP and MULTI-STEP but was very inaccurate for the SINE response. It is not surprising that NIIMI is an excellent correction for STEP and MULTI-STEP as the derivation of this equation was based on a step change in metabolic rate. STEADY was the least accuracy for all metabolic response.

For SINE, all of the equations can be used to estimate the daily average value ($\bar{R}_{daily}$). For STEP and MULTI-STEP, STEADY is less accurate. STEADY under-estimated the upper and lower peaking factors, but the other correction equations were very accurate.

This same lag response can also bias other metabolic rates (carbon dioxide, ammonia, solids) and the computation of performance and efficiencies of unit processes such as biofilters and solids removal processes. The correction of unit process performance metrics may be further complicated by rapid changes in influent concentrations that were not considered in these correction approaches and the kinetic response of the process to changes in substrate concentrations.

1. Introduction

In the design of aquatic culture and holding systems, the metabolic characteristics of the culture animal must be known. This includes consumption of dissolved oxygen, production of carbon dioxide, total ammonia nitrogen, soluble BOD, and fecal solids as functions of temperature, life stage, biomass, feed type, feed inputs, and past feed history. For laboratory work, the preferred method for determining metabolic rates is the intermittent-flow respirometer (Steffensen, 1989). For large commercial systems, the use of intermittent-flow techniques is physically impossible because the flow cannot be stopped. For specific time intervals, the maximum error in the computed metabolic rates may range from ± 10-40%. In high intensity systems, these errors could result in lethal dissolved oxygen levels.

In high-intensity fish culture, the most common rearing containers are the circular and octagonal tanks. In Norwegian Atlantic salmon (*Salmo salar*) smolt facilities (Summerfelt et al., 2016), tank diameters ranged from 14 to 20 m (45 to 65 ft) and flows ranged from 3 to 19 m^3/min (800 to 5000 gpm). In recent commercial systems, densities typically range from 30 to 80 kg/m3 and hydraulic detention times range from 35 to 60 min. Due to economics of scale, there is a trend towards larger rearing units and higher densities.

The equations used to compute metabolic rates, performance of unit processes, and process efficiencies can be biased and under some conditions may be quite misleading (Colt and Maynard, 2019). The source of these errors in a flow-through system are due to a lag in hydraulic system response.

While a number of diverse procedures have been developed to

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Nomenclature

a_1 to a_5	Polynomial regression coefficients for C_t^{out} versus time (Eq. 6)
C_t^{in}	Influent concentration of metabolic parameter at time t (mg/L)
C_t	Effluent concentration of metabolic parameter in general (mg/L)
C_t^i	Effluent concentration of metabolic parameter on minutely interval (mg/L)
C_t^m	Mean effluent concentration of metabolic parameter over a specific interval (mg/L)
C_t^p	Point effluent concentration of metabolic parameter over a specific interval (mg/L)
C_{t-1}	Effluent concentration of metabolic parameter at time t-1 (mg/L)
C_t	Effluent concentration of metabolic parameter at time t (mg/L)
C_{t+1}	Effluent concentration of metabolic parameter at time t + 1 (mg/L)
C_t^{ave}	Average effluent concentration of metabolic parameter for two sequential time periods based on point values (mg/L)
C_1	Initial steady-state concentration prior to step change (mg/L) Eq. 10
C_2	Final steady-state concentration after step change (mg/L) Eq. 11
C_o	Initial concentration at time = 0 in mixing model (Eq. 8)
dC/dt	First derivative of C_t based on spline fit to C_t (mg/(L h))
M	Mass of animals (kg)
Q	Water flow (liters per minute)
P	Period of the cycle (24 h) in Eq. 7
R_t	Metabolic rate in general, commonly expressed in mg/kg animal h
R_t^i	Metabolic rate measured on a minutely interval (mg/kg animal h)
R_t^m	Average metabolic rate over a specific time period (mg/kg animal h)
R_t^p	Point metabolic measured at a specific time interval (mg/kg animal h)
$R_t^{a,i}$	Assumed metabolic rate on a minutely interval (mg/kg animal h)
$R_t^{a,m}$	Assumed mean metabolic rate over a specific time period (mg/kg animal h)
$R_t^{a,p}$	Assumed point metabolic rate computed at a specific time interval (mg/kg animal h)
R_1	Initial steady-state metabolic rate prior to step change (mg/kg h)
R_2	Final steady-state metabolic rate after step change (mg/kg h)
$\bar{R}_{daily}$	Computed average daily metabolic rate (mg/kg animal h)

correct these parameters for the lag times between input water and effluent water, their accuracy has not been documented. Based on the analytical approaches summarized by Colt and Maynard (2019), the objectives of this work are to evaluate their accuracy for a range of potential metabolic responses. System design based on inaccurate metabolic rates may result in system failure or over-designed and inefficient unit processes. It is hoped that this work will improve our understanding of metabolic and performance parameters and result in better system design.

This article will focus on five primary parameters: correction approaches (STEADY, FRY, NORTHBY, SPLINE, AND NIIMI), metabolic characteristics (SINE, STEP, and MULTI-STEP), amplitude of metabolic response (four levels), reporting intervals (20-, 40-, and 60-minute), and computation analysis (POINT, MEAN, and DETAILED). The development of the correction approaches was presented in Colt and Maynard (2019) and is summarized in the following background section. The metabolic characteristics, amplitude, and computational analysis approaches will be presented in the Material and Methods section. Because of the large amount of data generated, much of the detailed performance data is presented in Supplemental Files 2-4. Numerical examples of the correction approaches for relevant cases are presented in Supplemental Files 1 and 6.

2. Background

2.1. Correction approaches

Colt and Maynard (2019) presented the derivation of six correction approaches that could be used to correct metabolic data collected in a flow-through system. These correction approaches are summarized below and are based on Fry (1957), Northby (1976); Niimi (1978), and Steffensen (1989). These equations are presented in terms of point concentration values collected at defined time intervals.

2.1.1. STEADY-state

$$R_t^p = -\left[\frac{Q}{M}\right](C_t^{in} - C_t) \quad (1)$$

Eq. 1 is termed STEADY because it is only valid if R_t^p is a constant ($dC/dt = 0$). The metabolic rates computed from Eq. 1 can be quite inaccurate if the rate is changing.

2.1.2. FRY

$$R_t^p = -\left[\frac{Q}{M}\right](C_t^{in} - C^{ave}) + \left[\frac{Vol}{M}\right]\left[\frac{C_{t+1} - C_t}{\Delta t}\right] \quad (2)$$

Eq. 2 is based on a mass balance approach and assumes that dC/dt can be estimated from the forward difference between two successive pair of data points.

2.1.3. NORTHBY

$$R_t^p = -\frac{Q}{M}(C_t^{in} - C_t) + \left[\frac{Vol}{M}\right]\left[\frac{C_{t+1} - C_{t-1}}{2\Delta t}\right] \quad (3)$$

Eq. 3 is similar to Eq. 2 except that it assumes that dC/dt can be estimated from the central difference.

2.1.4. SPLINE

$$R_t^p = -\left[\frac{Q}{M}\right](C_t^{in} - C_t) + \left[\frac{Vol}{M}\right]\left[\frac{\mathrm{dC}}{\mathrm{dt}}\right] \quad (4)$$

Eq. 4 is similar to Eqs. 2 and 3, except a polynomial spline equation is fitted between two successive pair of data points. The dC/dt is computed by taking the first derivative of the polynomial equation and evaluation at a specific point.

2.1.5. NIIMI

$$R_t^p = -\frac{Q}{M}\left[\frac{C_t Exp\left(-\frac{Q\Delta t}{Vol}\right) - C_{t+1}}{1 - Exp\left(-\frac{Q\Delta t}{Vol}\right)} + C_t^{in}\right] \quad (5)$$

Eq. 5 can be derived by assuming that R_t^p is constant over the interval and integration of the basic mass balance equation. It can also be derived by assuming a step change in the metabolic rate.

2.1.6. POLY

$$R_t^p = -\frac{Q}{M}(C^{in} - \{a_5 t^5 + a_4 t^4 + a_3 t^3 + a_2 t^2 + a_1 t^1 + a_0\}) + \frac{Vol}{M}\{5a_5 t^4 + 4a_4 t^3 + 3a_3 t^2 + 2a_2 t^1 + a_1\} \quad (6)$$

Eq. 6 assumes that a single polynominal equation can be fitted to the entire metabolic response and the influent concentration is a constant. The value of C_t and dC/dt can be substituted directly into Eq. 3. For noisy dissolved oxygen data, this approach may give inaccurate results.

Where

a_1 to a_5 = Polynomial regression coefficients for C_t versus time
C_t^{in} = Influent concentration of metabolic parameter at time t (mg/L)
C_t^{in} = Effluent concentration of metabolic parameter at time t-1 (mg/L)
C_t = Effluent concentration of metabolic parameter at time t (mg/L)
C_{t+1} = Effluent concentration of metabolic parameter at time t + 1 (mg/L)
C_t^{ave} = Average effluent concentration of metabolic parameter for two sequential time periods (mg/L)
dC/dt = First derivative of C_t based on spline fit to C_t (mg/(L h))
Δt = Sampling or reporting intervals (min or h)
M = Mass of animals (kg)
Q = Water flow (liters per minute)
R_t^p = Point metabolic measured at a specific time interval (mg/kg animal h)
Vol = Effective volume of reactor corrected for volume of fish (L)

2.2. Analytical approaches

Equations 1–6 are written in terms of point concentrations (C_t^{in}, C_{t-1}, C_t, C_{t+1}) measured at t-1, t, and, t + 1 in the influent and effluent waters. As discussed in Colt and Maynard (2019), it is also possible to compute the metabolic rates based on individual minutely measurements or mean concentrations over a specific time interval. These different analytical approaches will be discussed in detail below and are summarized in Table 1.

To assess the accuracy of the different correction equations, it was necessary to develop several assumed or hypothetical metabolic rates (R_t^a). These rates were used to estimate the resulting concentration values (C_t) using either hydraulic mixing model or mass-balance relationships. The correction equations were applied to the C_t values, resulting in corrected metabolic rates (R_t). This approach allows the estimation of the error in each correction approach ($R_t - R_t^a$).

2.2.1. Minutely

Based on minutely calculated concentration values (C_t^i), the corrected R_t^i were computed. This is the simplest approach, but results in a very large amount of data that is hard to analyze. For other applications, other time frames may be appropriate (1 s and 1 min, 1 min and 60 min, etc.). The error in the corrected metabolic rate is equal to $R_t^i - R_t^{a,i}$ or a single time and the overall error can be characterized by taking the standard deviation over the entire data set.

2.2.2. Point

The point analytical approach is probably the most conventional, especially for older research using DOs based on titration or manually collected data. Based on concentrations measured at a given time interval, the corrected metabolic rates are estimated (R_t^p). The error in the corrected metabolic rate is equal to $R_t^p - R_t^{a,p}$. $R_t^{a,p}$ is the point value of the hypothetical metabolic rate based on the same time interval used for C_t^p and R_t^p. For aquaculture applications, 20-, 40-, or 60-minute time intervals are commonly used.

2.2.3. Mean

Instead of using the point values, the mean value (C_t^m) over a specific time interval is used to estimate R_t^m. The error in the corrected metabolic rate is equal to $R_t^m - R_t^{a,m}$. $R_t^{a,m}$ is the mean value of the hypothetical metabolic rate based on the same time interval used for C_t^m and R_t^m. This analytical approach is commonly used with modern logging equipment that can measure dissolved oxygen at intervals ranging from 1 s to 10 min depending on type of sensor. Thick membrane sensors may have slower response but better long-term performance with less drift.

2.2.4. Detailed

This approach uses the minutely values of C_t^i to compute the minutely metabolic rates (R_t^i). Then these rates are averaged over a specific time interval to estimate the mean metabolic rate over the time interval (R_t^d). The error in the corrected metabolic rate is equal to $R_t^d - R_t^{a,m}$. This approach eliminates some of the problems that occur when the point or mean approaches are applied to rapidly changing metabolic rates.

3. Materials and methods

3.1. Development of representative R_t^a values

To evaluate the different correction protocols, a series of representative R_t^a values were created for three assumed responses. Minutely values of $R_t^{a,i}$ were developed for 1800 min (30 h). The physical and biological characteristics of the assumed responses are summarized in Table 2.

3.1.1. SINE curve

These were based on assumed $R_t^{a,i}$ values in the form presented by Tudor, 1999:

$$R_t^{a,i} = -\left\{A_0 + A_1 Sin\left(\frac{2\pi t}{p} + \vartheta\right)\right\} \quad (7)$$

Where
$R_t^{a,i}$ = Assumed value of R_t^a from Eq. 7
A_0 = Mean value of R_t^a (mg/(kg fish h)
A_1 = Amplitude of R_t^a (mg/(kg fish h)
t = Time (h)
p = The period of the cycle (24 h)
ϑ = Phase angle (radians)
Four cases were tested:

Case	A_0	A_1	ϑ
1	−100	−20	0
2	−100	−40	0
3	−100	−60	0
4	−100	−80	0

3.1.2. STEP changes

These values were based on a step change at time = 120 min from a

Table 1
Summary of analytical approaches and concentration and rate terminology. Note that each concentration (C) or rate (R) value can be computed over 20−, 40−, and 60−minute intervals for each correction approach. The superscripts refer to the following: i: minutely, m: mean, p: point, a: assumed or theoretical values.

Approach	Concentration data	Computed rate data	Assumed rate data	Error in rate data
Minutely	C_t^i	$C_t^i \rightarrow R_t^i$	$R_t^{a,i}$	$R_t^i - R_t^{a,i}$
Mean (interval)	$C_t^i \xrightarrow{mean} C_t^m$	$C_t^m \rightarrow R_t^m$	$R_t^{a,m}$	$R_t^m - R_t^{a,m}$
Point (interval)	$C_t^i \xrightarrow{interval} C_t^p$	$C_t^p \rightarrow R_t^p$	$R_t^{a,p}$	$R_t^p - R_t^{a,p}$
Detailed	$C_t^i \rightarrow R_t^i$	$R_t^i \xrightarrow{mean} R_t^d$	$R_t^{a,m}$	$R_t^d - R_t^{a,m}$

Table 2
Physical and biological characteristics of systems test ($R_t^a$and DO are based on 20 min averages).

Response /Value	τ (min)	Vol (m^3)	Mass (kg)	Q (lpm)	C^{in} (mg/L)	Density (kg/m^3)	Loading (kg/Lpm)	R_t^a (mg/(kg h))		DO (mg/L)	
								Min	Max	Min	Max
SINE											
A_1 = -20	200	100	1340	30,000	12.00	13.4	2.68	−119	−80	6.86	8.21
A_2 = -40	200	100	1340	30,000	12.00	13.4	2.68	−140	−60	6.19	6.19
A_3 = -60	200	100	1340	30,000	12.00	13.4	2.68	−160	−40	5.52	5.52
A_4 = -80	200	100	1340	30,000	12.00	13.4	2.68	−180	−20	4.84	4.84
STEP											
Case 1	200	100	1340	30,000	12.00	13.4	2.68	−70	−50	8.87	9.77
Case 2	200	100	1340	30,000	12.00	13.4	2.68	−80	−50	8.43	9.77
Case 3	200	100	1340	30,000	12.00	13.4	2.68	−90	−50	7.98	9.77
Case 4	200	100	1340	30,000	12.00	13.4	2.68	−100	−50	7.54	9.77
MULTI-STEP											
Case 1	200	100	1340	30,000	12.00	13.4	2.68	−100	−50	7.82	9.77
Case 2	200	100	1340	30,000	12.00	13.4	2.68	−150	−50	5.88	9.77
Case 3	200	100	1340	30,000	12.00	13.4	2.68	−175	−50	4.90	9.77
Case 4	200	100	1340	30,000	12.00	13.4	2.68	−200	−50	3.93	9.77

-50 to -70 (Case 1), -50 to -80 (Case 2), -50 to -90 (Case 3), and -50 to -100 mg/(kg h) (Case 4).

3.1.3. MULTI-STEP changes

These values were based on the following R_t^a and times:

Time (h)	Case 1	Case 2	Case 3	Case 4
0	−50	−50	−50	−50
1	−60	−70	−75	−80
4	−70	−90	−100	−110
6	−80	−110	−125	−140
8	−90	−130	−150	−170
10	−100	−150	−175	−200
12	−100	−150	−175	−200
14	−90	−130	−150	−170
16	−80	−110	−125	−140
18	−70	−90	−100	−110
20	−60	−70	−75	−80
22	−50	−50	−50	−50
24	−50	−50	−50	−50

3.1.4. Computation of representative point ($R_t^{a,p}$) and mean ($R_t^{a,m}$) values

The point ($R_t^{a,p}$) and mean values ($R_t^{a,m}$) for each 20-, 40-, and 60-minute period were computed from the minutely values ($R_t^{a,i}$).

3.2. Computation of C_t from R_t^a

The resulting C_t^i values were estimated from mass balance or hydraulic mixing models on a minutely basis for 1800 min.

3.2.1. Computation of C_t from R_t - SINE curve

Assuming that the reactor was well-mixed, minutely values of C_t^i were computed from a simple mixing model:

$$t = 0 \quad C_t^i = \frac{C_0 Vol + C^{in} Q\Delta t + MR_t^a \Delta t}{Vol + Q\Delta t} \tag{8}$$

$$\text{t=1 to end} \quad C_{t+1}^i = \frac{C_t^{out} Vol + C^{in} Q\Delta t + MR_t^a \Delta t}{Vol + Q\Delta t} \tag{9}$$

Where

C_0 = Initial concentration at time = 0 in mixing model

The initial guess of C_0 (Eq. 8) was obtained from R_t^p (the first value for the second day). For the second time step (and all subsequent time steps), the value of C_0 was replaced in Eq. 9 by the R_t^a from the previous time step.

3.2.2. STEP change and multi-step

For a step change(s), the resulting C_t values could be estimated from a modified version of Eq. 4 in Colt and Maynard (2019):

$$C_1 = C_t^{in} + \frac{R_1 M}{Q} \tag{10}$$

$$C_2 = C_t^{in} + \frac{R_2 M}{Q} \tag{11}$$

$$C_t^i = C_1 + (C_2 - C_1)\left\{1 - Exp\left(\frac{-Qt}{Vol}\right)\right\} \tag{12}$$

Where

C_1 = Initial steady-state concentration prior to step change (mg/L)

C_2 = Final steady-state concentration after step change (mg/L)

R_1 = Initial steady-state metabolic rate prior to step change (mg/kg h)

R_2 = Final steady-state metabolic rate after step change (mg/kg h)

For the multi-step cases, a base value of C_t^i was computed from R_t = −50 mg/(kg h). The impact of the following 12 steps was computed individually (Eqs. 10–12) and the final C_t^i value was computed from the sum of the base value + 12 steps in R_t^a on a minutely time step.

3.2.3. Computation of point C_t^p and mean C_t^m values

The point (C_t^p) and mean values (C_t^m) for each 20-, 40-, and 60-minute period were computed from the minutely values (C_t^i) for SINE, STEP, and MULTI-STEP.

3.3. Computation of metabolic rates from computed C_t values

Based on C_t^i, C_t^p, *and* C_t^m, the corrected metabolic rates (R_t^i, R_t^p, *and* R_t^m) were computed from the six correction equations (Eqs. 1–6) for the three metabolic responses. The value of DETAILED (R_t^d) was computed differently. Instead of working with the point or mean concentrations, this method computed the individual minutely R_t^i from the minutely C_t^i and then computed the mean values directly from the R_t^i values.

Separate estimates were made for 20-, 40-, and 60-minute intervals. The values of dC/dt for the spline fit approach were computed using DERIVXY (using 4 points, ExceLab 3.0, Excel Works, Boston, MA). For the SINE curve, the polynomial correction (Eq. 6) was also computed based on C_t^i.

Because some correction equations need to use t-1 and t + 1, data was generated for time steps 1–74 (20 min), 1–50 (40 min), and 1–26 (60 min). The actual analysis was based on t = 2 to t = 73 (20 min

intervals), t = 2 to t = 49 (40 min intervals), and t = 2 to t = 25 (60 min intervals).

3.4. Comparison of computed R_t values with assumed R_t^a values

Given the way the concentrations were computed from the assumed metabolic rates, it was possible to estimate the error for each correction equation, metabolic response, and time period. For example, for the point values, the error for a time t is equal to $R_t^p - R_t^{a,p}$. The error terms for the other analysis approaches are summarized in Table 1. The 24-h means, standard deviations, and ranges were computed for each test case.

3.5. Development of general error term as a function of dC/dt

It is expected that error in computed metabolic rate will depend on the magnitude of dC/dt, the variation of C with time, and the specific correction term used. Therefore, we shall try to develop the error term in term $R_t - R_t^a$ as a function of $R_{t+1} - R_t$.

4. Results

The ability to correct for hydraulic lag impacts was developed for three assumed R_t^a responses (SINE, STEP, AND MULTI-STEP) and six correction approaches (STEADY, FRY, NORTHBY, NIIMI, SPLINE, and POLY). For each R_t^a response, there were 4 levels of change and R_t^a values were computed for 20-, 40-, and 60-minute intervals. Examples of manual computation of the correction equations are presented in Supplemental File #1 for the SINE response.

4.1. SINE response

4.1.1. Mean concentrations

The values of $R_t^{a,m}$ and the deviation between the computed and assumed values $R_t^m - R_t^{a.m}$ is presented in Table 3 for 60 min samples, A_1 = -80 mg/(kg h), or summarized in Table 4 for all the sample intervals and amplitudes. While there were differences between the means, these differences are small, and even STEADY could be used to estimate the mean daily metabolic rate ($\bar{R}_{daily}$). In terms of the deviation of the $R_t^m - R_t^{a.m}$, the ranking of the correction approaches are: SPLINE (best), NORTHBY, FRY, NIMII, AND STEADY (worst). There was little difference in performance between FRY and NIIMI. The difference between $R_t^m - R_t^{a.m}$ showed a regular variation over the day that appeared to depend on the magnitude of dC/dt (Fig. 1). While the magnitude of $R_t^m - R_t^{a.m}$ increased with the amplitude of the assumed metabolic rate, the relative rate $((R_t^m - R_t^{a.m})/(\text{Range of} R_t^{a.m})$ was relatively constant. In addition, there was little difference in $R_t^m - R_t^{a.m}$ between the 20-, 40-, and 60-minute averages.

4.1.2. Point concentrations

There was little difference between the means and any of the approaches could be used to estimate the daily metabolic rate ($\bar{R}_{daily}$). Note that the daily metabolic rate is primarily used to estimate pure oxygen usage and is misleading for system design when maximum and minimums are critical. The value of $R_t^p - R_t^{a.p}$ was slightly larger than $R_t^m - R_t^{a.m}$ for some cases (see Supplemental File #2). In terms of the deviation of the $R_t^m - R_t^{a.m}$, the ranking of the correction approaches was similar to MEAN, except that POLY had the second largest error.

4.1.3. Detailed concentrations

Any of correction approaches could be used to compute $\bar{R}_{daily}$. The values of $R_t^{a,m}$ and the deviation between the computed and assumed values $R_t^d - R_t^{a.m}$ is presented in Table 5 for 60-minute samples and A_1 = -80 mg/(kg h), or summarized in Table 6 for all the sample intervals and amplitudes. In terms of the deviation of the $R_t^m - R_t^{a.m}$, the ranking of the correction approaches are: SPLINE and NORTHBY (best), FRY and NIMII, AND STEADY (worst).

4.1.4. Comparison of analytical approaches

The DETAILED analytical approach had the smallest error, followed by MEAN and POINT. The error for MEAN and POINT ranged from 60 to 70% higher than for DETAILED.

The increase in error with amplitude and lack of differences between the different time averages are similar for all the analytical approaches (MEAN, POINT, and DETAILED); therefore, most of the following sections will focus on A_1 = -80 mg/(kg h) and 60-minute time averages. The means and deviations for all amplitudes and time averages are presented in Supplemental File #2 for the SINE response. For 60-minute averages and A_1 = - 80 mg/(kg h), the mean and standard deviation are summarized in Table 7. The combination of SPLINE and DETAILED or NORTHBY and DETAILED have the smallest deviation.

4.2. STEP response

4.2.1. Mean concentrations

For estimation of the mean daily metabolic rate $\bar{R}_{daily}$, FRY, NORTHBY, NIIMI, AND SPLINE gave acceptable results (see Supplemental File #3). STEADY was less accurate, although it may be acceptable depending the accuracy needed. In terms of the deviation of $R_t^m - R_t^{a,m}$, the ranking of the correction approaches are: SPLINE (best), NORTHBY, FRY and NIIMI, and STEADY (worst). None of the correction equations were very accurate (Table 8, Supplemental File #3).

4.2.2. Point concentrations

With the exception of STEADY, any of correction approaches could be used to compute $\bar{R}_{daily}$. In terms of the deviation of $R_t^p - R_t^{a,p}$

Table 3
Detailed performance for SINE response, MEAN concentrations. (Based on 60 min samples and A_1 = -80 mg/(kg h)).

		$R_t^m - R_t^{a,m}$ (mg/(kg h))				
Time (h)	$R_t^{a,m}$	STEADY	FRY	NORTHBY	NIIMI	SPLINE
0	−110.24					
1	−130.37	49.298	−9.349	0.532	−9.730	−0.054
2	−148.44	52.060	−7.672	0.591	−8.061	−0.004
3	−163.19	51.236	−5.473	0.610	−5.842	0.045
4	−173.64	46.891	−2.900	0.587	−3.224	0.092
5	−179.07	39.330	−0.130	0.525	−0.387	0.133
6	−179.11	29.072	2.648	0.427	2.476	0.164
7	−173.75	16.821	5.246	0.300	5.171	0.184
8	−163.36	3.415	7.487	0.153	7.513	0.192
9	−148.65	−10.231	9.216	−0.005	9.343	0.187
10	−130.63	−23.182	10.317	−0.162	10.536	0.168
11	−110.52	−34.557	10.715	−0.308	11.010	0.139
12	−89.69	−43.577	10.382	−0.433	10.733	0.100
13	−69.56	−49.628	9.341	−0.529	9.725	0.054
14	−51.51	−52.296	7.663	−0.588	8.054	0.004
15	−36.76	−51.400	5.463	−0.608	5.833	−0.046
16	−26.33	−46.999	2.890	−0.585	3.215	−0.092
17	−20.92	−39.394	0.120	−0.523	0.377	−0.133
18	−20.90	−29.103	−2.658	−0.426	−2.486	−0.164
19	−26.28	−16.828	−5.255	−0.299	−5.180	−0.184
20	−36.68	−3.406	−7.494	−0.152	−7.520	−0.192
21	−51.40	10.248	−9.221	0.006	−9.348	−0.187
22	−69.44	23.204	−10.320	0.163	−10.538	−0.168
23	−89.56	34.577	−10.715	0.309	−11.010	−0.139
24	−110.39	43.592	−10.380	0.434	−10.731	−0.099
Mean	−100.01	−0.04	0.00	0.00	0.00	0.00
STDEV	57.613	37.803	7.741	0.440	7.958	0.139
Minimum	−179.11	−52.30	−10.72	−0.61	−11.01	−0.19
Maximum	−20.90	52.06	10.72	0.61	11.01	0.19
Range	−158.20	−104.36	−21.43	−1.22	−22.02	−0.38

Table 4
Performance of correction factors for SINE response (θ_h = 200 min, M = 1340 kg, Vol = 100 m^3, C^{in} = 12.00 mg/L, MEAN concentrations). Mean and standard deviation of $R_t^m - R_t^{a,m}$ are presented for each case.

Interval (minute)	Amplitude (mg/(kg h))	Mean ($R_t^m - R_t^{a,m}$) and STDEV($R_t^m - R_t^{a,m}$) (mg/(kg h)				
		Steady-state	Fry	Northby	Niimi	Spline
20	A_1 = 20	−0.01 ± 9.34	0.00 ± 0.639	0.00 ± 0.024	0.00 ± 0.645	0.00 ± 0.021
	A_1 = 40	−0.02 ± 18.67	0.00 ± 1.279	0.00 ± 0.047	0.00 ± 1.290	0.00 ± 0.042
	A_1 = 60	−0.04 ± 28.01	0.00 ± 1.918	0.00 ± 0.071	0.00 ± 1.935	0.00 ± 0.063
	A_1 = 80	−0.05 ± 37.35	0.00 ± 2.557	0.00 ± 0.094	0.00 ± 2.580	0.00 ± 0.084
40	A_1 = 20	−0.01 ± 9.40	0.00 ± 1.280	0.00 ± 0.052	0.00 ± 1.304	0.00 ± 0.025
	A_1 = 40	−0.02 ± 18.79	0.00 ± 2.561	0.00 ± 0.104	0.00 ± 2.608	0.00 ± 0.049
	A_1 = 60	−0.03 ± 28.19	0.00 ± 3.841	0.00 ± 0.156	0.00 ± 3.913	0.00 ± 0.074
	A_1 = 80	−0.04 ± 37.58	0.00 ± 5.122	0.00 ± 0.208	0.00 ± 5.217	0.00 ± 0.099
60	A_1 = 20	−0.01 ± 9.45	0.00 ± 1.935	0.00 ± 0.110	0.00 ± 1.990	0.00 ± 0.035
	A_1 = 40	−0.02 ± 18.90	0.00 ± 3.871	0.00 ± 0.220	0.00 ± 3.979	0.00 ± 0.069
	A_1 = 60	−0.03 ± 28.35	0.00 ± 5.806	0.00 ± 0.330	0.00 ± 5.969	0.00 ± 0.104
	A_1 = 80	−0.04 ± 37.80	0.00 ± 7.741	0.00 ± 0.440	0.00 ± 7.958	0.00 ± 0.139

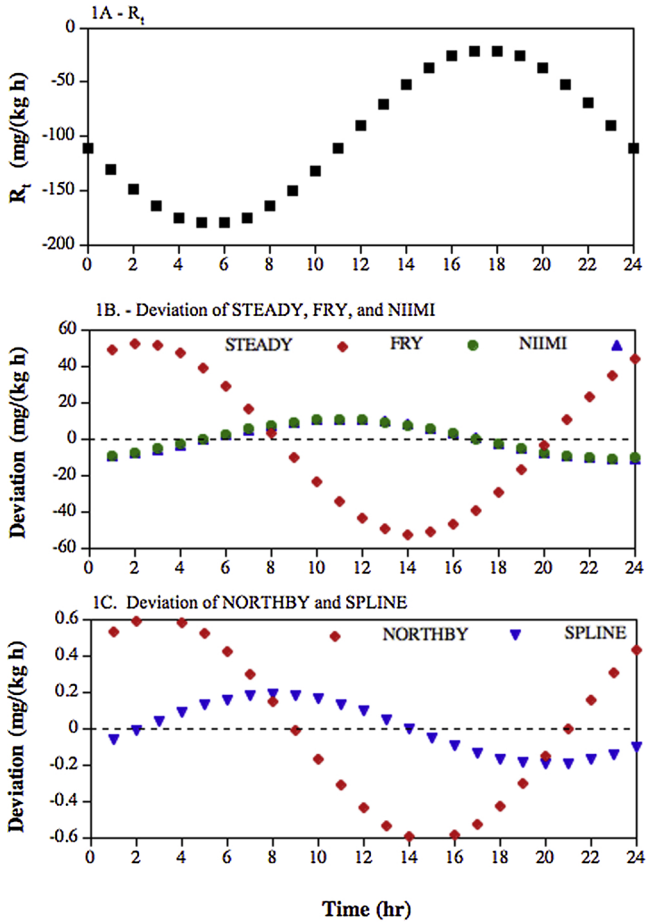

Fig. 1. A. - Variation of $R_t^{a,m}$ with time, B. Deviation ($R_t^m - R_t^{a,m}$) of STEADY, FRY, and NIIMI, C. - Deviation ($R_t^m - R_t^{a,m}$) of FRY, NORTHBY, SPLINE, and NIIMI. Note that in Fig. 1B, NIIMI is plotted under FRY symbol (SINE response, MEAN concentrations, 60 min samples, and amplitude = −80 mg/(kg h)).

(Supplemental File #3), the ranking of the correction approaches are: NIIMI (best), FRY, SPLINE, NORTHBY, POLY, and STEADY (worst). The deviation of NIIMI was in the range of 10^{-13} to 10^{-14} mg/L, which is close to a perfect correction. With the exception of NIIMI and FRY, the deviation of the others correction equations was much larger.

4.2.3. Detailed concentrations

With the exception of STEADY, all of the other correction gave

Table 5
Detailed performance for SINE response, DETAILED concentrations. (Based on 60 min samples and A_1 = -80 mg/(kg h)).

Time (h)	$R_t^{a,m}$	$R_t^d - R_t^{a,m}$ (mg/(kg h))				
		STEADY	FRY	NORTHBY	NIIMI	SPLINE
0	−110.24					
1	−130.37	49.298	−0.198	−0.037	−0.198	−0.038
2	−148.44	52.060	−0.146	−0.008	−0.146	−0.008
3	−163.19	51.236	−0.084	0.022	−0.084	0.022
4	−173.64	46.891	−0.016	0.050	−0.016	0.050
5	−179.07	39.330	0.053	0.075	0.053	0.075
6	−179.11	29.072	0.118	0.095	0.118	0.095
7	−173.75	16.821	0.175	0.108	0.175	0.108
8	−163.36	3.415	0.220	0.114	0.220	0.114
9	−148.65	−10.231	0.250	0.112	0.250	0.112
10	−130.63	−23.182	0.263	0.102	0.263	0.102
11	−110.52	−34.557	0.258	0.086	0.258	0.086
12	−89.69	−43.577	0.236	0.063	0.236	0.063
13	−69.56	−49.628	0.197	0.037	0.197	0.037
14	−51.51	−52.296	0.145	0.007	0.145	0.007
15	−36.76	−51.400	0.083	−0.023	0.083	−0.022
16	−26.33	−46.999	0.016	−0.051	0.016	−0.051
17	−20.92	−39.591	−0.098	−0.122	−0.098	−0.122
18	−20.90	−29.103	−0.118	−0.095	−0.118	−0.095
19	−26.28	−16.828	−0.175	−0.108	−0.175	−0.108
20	−36.68	−3.406	−0.220	−0.114	−0.220	−0.114
21	−51.40	10.248	−0.250	−0.112	−0.250	−0.112
22	−69.44	23.204	−0.263	−0.102	−0.263	−0.102
23	−89.56	34.577	−0.258	−0.086	−0.258	−0.086
24	−110.39	43.592	−0.236	−0.063	−0.236	−0.063
Mean	−100.01	−0.04	0.00	0.00	0.00	0.00
STDEV	57.613	37.811	0.191	0.085	0.191	0.085
Minimum	−179.11	−52.30	−0.26	−0.12	−0.26	−0.12
Maximum	−20.90	52.06	0.26	0.11	0.26	0.11
Range	−158.20	−104.36	−0.53	−0.24	−0.53	−0.24

accurate estimates of $\bar{R}_{daily}$. In terms of the deviation of $R_t^d - R_t^{a,m}$ (Supplemental File #3), the ranking of the correction approaches are: NIIMI (best), FRY, SPLINE, NORTHBY, and STEADY (worst). The deviation of NIIMI was in the range of 10^{-13} to 10^{-14} mg/(kg h) and FRY, NORTHBY, and SPLINE were in the range of 10^{-2} to 10^{-6} mg/(kg h).

4.2.4. Comparison of analytical approaches

The POINT and DETAILED analytical approaches had the smallest error; MEAN was much less accurate. The combination of NIIMI and POINT or NIIMI and DETAILED had an almost perfect fit (10^{-13} to 10^{-14} mg/(kg h) and the combination FRY and DETAILED was excellent (10^{-5}

Table 6
Performance of correction factors for SINE response (θ_h = 200 min, M = 1340 kg, Vol = 100 m^3, C^{in} = 12.00 mg/L, DETAILED concentrations). Mean and standard deviation of $R_t^d - R_t^{a,m}$ are presented for each case.

Interval (minute)	Amplitude (mg/(kg h)	Mean ($R_t^d - R_t^{a,m}$) and STDEV($R_t^d - R_t^{a,m}$) (mg/(kg h)				
		Steady-state	Fry	Northby	Niimi	Spline
20	A_1 = 20	−0.01 ± 9.34	0.00 ± 0.047	0.00 ± 0.020	0.00 ± 0.047	0.00 ± 0.020
	A_1 = 40	−0.02 ± 18.67	0.00 ± 0.094	0.00 ± 0.041	0.00 ± 0.094	0.00 ± 0.041
	A_1 = 60	−0.04 ± 28.01	0.00 ± 0.141	0.00 ± 0.061	0.00 ± 0.141	0.00 ± 0.061
	A_1 = 80	−0.05 ± 37.35	0.00 ± 0.188	0.00 ± 0.082	0.00 ± 0.188	0.00 ± 0.082
40	A_1 = 20	−0.01 ± 9.38	0.00 ± 0.074	0.00 ± 0.048	0.00 ± 0.074	0.00 ± 0.048
	A_1 = 40	−0.02 ± 18.76	0.00 ± 0.147	0.00 ± 0.096	0.00 ± 0.147	0.00 ± 0.096
	A_1 = 60	−0.03 ± 28.14	0.00 ± 0.221	0.00 ± 0.143	0.00 ± 0.221	0.00 ± 0.143
	A_1 = 80	−0.04 ± 37.52	0.00 ± 0.294	0.00 ± 0.191	0.00 ± 0.294	0.00 ± 0.191
60	A_1 = 20	−0.01 ± 9.45	0.00 ± 0.048	0.00 ± 0.021	0.00 ± 0.048	0.00 ± 0.021
	A_1 = 40	−0.02 ± 18.91	0.00 ± 0.096	0.00 ± 0.042	0.00 ± 0.096	0.00 ± 0.042
	A_1 = 60	−0.03 ± 28.36	0.00 ± 0.144	0.00 ± 0.064	0.00 ± 0.144	0.00 ± 0.064
	A_1 = 80	−0.04 ± 37.81	0.00 ± 0.191	0.00 ± 0.085	0.00 ± 0.191	0.00 ± 0.085

Table 7
Summary comparison of the mean and standard deviation of the difference between the assumed and computed value of R_t as a function correction approaches and analysis assumptions. Based on A_1 = -80 (SINE), Case 4 (STEP), Case 4 (MULTI-STEP), and 60 min averages. Complete results for all cases and time averages are presented in Supplemental Files S2 to S4). The lowest standard deviation in each row is underlined, unless none of the approaches are satisfactory.

		Mean ± Standard Deviation					
Simulation	Analysis	STEADY	FRY	NORTHBY	NIIMI	SPLINE	POLY
SINE	MEAN	−0.04 ± 37.80	0.00 ± 7.741	0.00 ± 0.440	0.00 ± 7.958	0.00 ± 0.139	N/A
	POINT	−0.04 ± 37.90	0.00 ± 7.764	0.00 ± 0.441	0.00 ± 7.981	0.00 ± 0.139	−0.32 ± 2.719
	DETAILED	−0.04 ± 37.81	0.00 ± 0.191	0.00 ± 0.085	0.00 ± 0.191	0.00 ± 0.085	N/A
STEP	MEAN	6.95 ± 11.41	−1.02 ± 5.249	0.02 ± 3.645	−1.08 ± 5.273	−0.14 ± 3.224	N/A
	POINT	8.03 ± 13.18	0.05 ± 0.085	1.09 ± 5.818	0.00 ± 0.00^0	1.09 ± 4.155	1.26 ± 7.434
	DETAILED	6.95 ± 11.41	0.00 ± 3×10^{-5}	0.02 ± 0.085	0.00 ± 2×10^{-13}	0.02 ± 0.062	N/A
MULTI-STEP	MEAN	3.48 ± 36.05	0.66 ± 9.525	0.23 ± 6.790	0.65 ± 9.673	0.21 ± 6.683	N/A
	POINT	4.02 ± 41.62	0.03 ± 0.269	−0.06 ± 11.08	0.00 ± 3×10^{-14}	−0.19 ± 8.080	−0.68 ± 12.45
	DETAILED	3.48 ± 36.05	0.00 ± 7×10^{-5}	0.00 ± 0.165	0.00 ± 2×10^{-13}	0.00 ± 0.121	N/A

to 10^{-6} mg/(kg h). For 60-minute averages and Case 4, the mean and standard error are summarized in Table 7.

4.3. MULTI-STEP response

4.3.1. Mean concentrations

For estimation of the mean daily metabolic rate $\bar{R}_{daily}$, FRY, NORTHBY, NIIMI, AND SPLINE gave acceptable results (see Supplemental File #4). STEADY was less accurate. None of the correction equations were very accurate.

4.3.2. Point concentrations

With the exception of STEADY, any of correction approaches could be used to compute $\bar{R}_{daily}$. For 20- and 60-minute averages, NIIMI resulted in an almost perfect correction (10^{-13} to 10^{-14} mg/L) (see Supplemental File #4). For 40-minute averages, NIIMI was not very accurate. FRY showed a similar response, although it was significantly less accurate than NIIMI. The reduction in accuracy of both NIIMI and FRY resulted from a large deviation at a single time (Table 9).

Two additional POINT cases were tested for the MULTI-STEP response:

(1) The time of the first step was changed from 1 h to 2 h.

(2) Same as in case 1, but 1/60 h was added to each time step

Changing the time of the first step to 2 h, resulted in the 40-minute averages that were comparable to 20- and 60-minute averages for both NIIMI and FRY (Table 9). The addition of 1/60 h to the time step, resulted in very poor accuracy. Very high deviation occurred periodically with low deviations for the remaining times. The very high deviations occur when the point values fall on either side of a step change in R_t^p. For the MULTI-STEP case, all but one of the steps (time = 1 h) occurred on even hours. For the time step at 1 h, the 20- and 60-minute averages occurred on the time step, except for the 40 min average which spanned the step. This deviation for the 40-minute average was large for the step occurring at time = 1 h.

4.3.3. Detailed concentrations

With the exception of STEADY, all of the other corrections gave accurate estimates of $\bar{R}_{daily}$. In terms of the deviation of $R_t^d - R_t^{a,m}$ (see Supplemental File #4), the ranking of the correction approaches are: NIIMI (best), FRY, SPLINE, NORTHBY, and STEADY (worst). The deviation of NIIMI was in the range of 10^{-13} mg/(kg h) and FRY were in the range of 10^{-5} mg/(kg h). The deviation of NORTHBY and SPLINE were in the range of 10^{-1} to 10^{-2}. The NIIMI approach was highly accurate for all three time averages and did not experience the same problem presented in section 4.3.2 with POINT.

4.3.4. Comparison of analytical approaches

The DETAILED analytical approaches had the smallest deviation and POINT was accurate for 20- and 60-minute averages. MEAN was much less accurate. The combination of NIIMI and DETAILED also had an almost perfect fit (10^{-13} to 10^{-14} mg/(kg h) and the combination FRY and DETAILED was excellent (10^{-5} to 10^{-6} mg/(kg h).

Table 8
Detailed performance for STEP response, MEAN concentrations. (Based on 60 min samples and Case 4).

		$R_t^m - R_t^{a,m}$ (mg/(kg h))					
Time (h)	$R_t^{a,m}$	STEADY	FRY	NORTHBY	NIIMI	SPLINE	POLY
0	−50.00						
1	−50.00	0.000	−25.664	−11.158	−25.831	−12.362	0.000
2	−100.00	43.305	0.280	13.440	0.000	9.308	43.305
3	−100.00	32.08	0.21	−0.48	0.00	−0.06	32.08
4	−100.00	23.77	0.15	−0.36	0.00	−0.05	23.77
5	−100.00	17.61	0.11	−0.27	0.00	−0.04	17.61
6	−100.00	13.04	0.08	−0.20	0.00	−0.03	13.04
7	−100.00	9.66	0.06	−0.15	0.00	−0.02	9.66
8	−100.00	7.16	0.05	−0.11	0.00	−0.01	7.16
9	−100.00	5.30	0.03	−0.08	0.00	−0.01	5.30
10	−100.00	3.93	0.03	−0.06	0.00	−0.01	3.93
11	−100.00	2.91	0.02	−0.04	0.00	−0.01	2.91
12	−100.00	2.16	0.01	−0.03	0.00	0.00	2.16
13	−100.00	1.60	0.01	−0.02	0.00	0.00	1.60
14	−100.00	1.18	0.01	−0.02	0.00	0.00	1.18
15	−100.00	0.88	0.01	−0.01	0.00	0.00	0.88
16	−100.00	0.65	0.00	−0.01	0.00	0.00	0.65
17	−100.00	0.48	0.00	−0.01	0.00	0.00	0.48
18	−100.00	0.36	0.00	−0.01	0.00	0.00	0.36
19	−100.00	0.26	0.00	0.00	0.00	0.00	0.26
20	−100.00	0.20	0.00	0.00	0.00	0.00	0.20
21	−100.00	0.14	0.00	0.00	0.00	0.00	0.14
22	−100.00	0.11	0.00	0.00	0.00	0.00	0.11
23	−100.00	0.08	0.00	0.00	0.00	0.00	0.08
24	−100.00	0.06	0.00	0.00	0.00	0.00	0.06
Mean	−97.92	6.95	−1.02	0.02	−1.08	−0.14	6.95
STDEV	10.21	11.412	5.249	3.645	5.273	3.224	11.412
Minimum	−100.00	0.000	−25.664	−11.158	−25.831	−12.362	0.000
Maximum	−50.00	43.305	43.305	43.305	43.305	43.305	43.305
Range	−50.00	−43.305	−68.969	−54.463	−69.136	−55.667	−43.305

4.4. Error response

For the sine response, it was possible to develop a relationship between dR/dt and deviation between R_t^a and R_t for a given correction approach. For this work, the value of dR/dt was represented by $(R_{t+1} - R_t)$/(Range ofR_t) and the error term by $(R_t - R_t^a)$/(Range ofR_t·sampling interval). The sampling "interval term" is needed so that the error is expressed in a consistent time basis (1/h). The error relationship for STEADY is presented in Fig. 2. The resulting curves only slightly depended on sampling intervals or amplitude, but separate curves were produced depending if the value of R_t was either increasing or decreasing. The maximum error term for STEADY was 32.9%/hour. This is an estimate of the maximum error resulting from assuming that dC/dt = 0.

A similar relationship is presented for SPLINE in Fig. 3. There is a little more scatter in Fig. 3, but it is important to note the vertical scale is 1/10 of Fig. 3. The maximum error for SPLINE is only 0.073%/hour.

4.5. Peaking factor

The upper peaking factor (maximum $R_t/\bar{R}_{daily}$) and lower peaking factor (minimum $R_t/\bar{R}_{daily}$) for values were computed as a function of correction approach (Table 10) or as a function of sampling interval (Table 11). As expected, the magnitude of peaking factors increased with the amplitude of R_t^a. Both peaking factors were very similar for FRY, NORTHBY, NIIMI, and SPLINE and were very close to the theoretical values. The STEADY approach under-estimated both the upper and lower peaking factors. For SINE, the mean STEADY upper and lower peaking factors were only 92% and 139% of the theoretical values, respectively (See Supplemental File #5). For MULTI-STEP, the mean STEADY upper and lower peaking factors were only 93% and 106% of the theoretical values, respectively. There was little difference in either peaking factor as a function of the sampling interval (Table 11).

4.6. Absolute value of R_t

Up to this point, this section has focused on the deviation between the computed and assumed value of the metabolic rate. For the SINE response (60 min averages and SPLINE), the values of R_t^m and R_t^d are very close (STDEV = 0.06), while R_t^m and R_t^p differ (STDEV = 7.41). Plotting R_t^m and R_t^p as a continuous function shows two distinct sinusoidal lines (Fig. 4) with similar amplitudes but slightly offset. $R_t^m > R_t^p$ for increasing metabolic rates and $R_t^p > R_t^m$ for decreasing rates. Since the metabolic rate is assumed a constant for each time interval, it is more accurate to plot the two curves in a step-wise fashion (Fig. 5). While there is a difference between the R_t^m and R_t^p, this difference is a direct result of how the values were computed. One was an average over the interval and the other was based on point values. For STEP and MULTI-STEP responses (60 min averages and NIIMI), R_t^p and R_t^d are very close and both are quite different from R_t^m.

5. Discussion

5.1. Accuracy of mean daily values

The accuracy of the mean daily values (mean values in Tables 7 and Supplemental Files 2–4) depends on assumed response (SINE, STEP, MULTI-STEP). For the SINE response, all of the different correction approaches give acceptable results with little real differences. For STEP and MULTI-STEP, STEADY over-estimates the mean daily value (3–7 mg/kg h for STEP and 1–4 mg/kg h for MULTI-STEP). For all of the analytical approaches, DETAILED gave the most consistent accurate mean results for all assumed responses.

Table 9
DETAILED performance for MULTI-STEP response, POINT concentrations. (Based on 40-minute samples and Case 4).

		FRY ($R_t^p - R_t^{a,p}$)			NIIMI ($R_t^p - R_t^{a,p}$)		
Time (h)	$R_t^{a,p}$	Base Case	$t_2 = 2\,h$	$t + 1/60\,h$	Base Case	$t_2 = 2\,h$	$t + 1/60\,h$
0.00	−50	0.00	0.00	0.00	0.00	0.00	0.00
0.67	−50	−15.70	0.00	0.00	−15.75	0.00	0.00
1.33	−80	0.08	0.00	0.00	0.00	0.00	0.00
2.00	−80	0.07	0.09	−29.23	0.00	0.00	−29.32
2.67	−80	0.05	0.07	0.07	0.00	0.00	0.00
3.33	−80	0.04	0.06	0.06	0.00	0.00	0.00
4.00	−110	0.13	0.14	−29.18	0.00	0.00	−29.32
4.67	−110	0.10	0.11	0.12	0.00	0.00	0.00
5.33	−110	0.09	0.09	0.09	0.00	0.00	0.00
6.00	−140	0.16	0.17	−29.15	0.00	0.00	−29.32
6.67	−140	0.13	0.14	0.14	0.00	0.00	0.00
7.33	−140	0.11	0.11	0.11	0.00	0.00	0.00
8.00	−170	0.18	0.18	−29.14	0.00	0.00	−29.32
8.67	−170	0.15	0.15	0.15	0.00	0.00	0.00
9.33	−170	0.12	0.12	0.12	0.00	0.00	0.00
10.00	−200	0.19	0.19	−29.13	0.00	0.00	−29.32
10.67	−200	0.15	0.16	0.16	0.00	0.00	0.00
11.33	−200	0.13	0.13	0.13	0.00	0.00	0.00
12.00	−200	0.10	0.10	0.11	0.00	0.00	0.00
12.67	−200	0.08	0.09	0.09	0.00	0.00	0.00
13.33	−200	0.07	0.07	0.07	0.00	0.00	0.00
14.00	−170	−0.03	−0.03	29.29	0.00	0.00	29.32
14.67	−170	−0.03	−0.03	−0.03	0.00	0.00	0.00
15.33	−170	−0.02	−0.02	−0.02	0.00	0.00	0.00
16.00	−140	−0.11	−0.11	29.21	0.00	0.00	29.32
16.67	−140	−0.09	−0.09	−0.09	0.00	0.00	0.00
17.33	−140	−0.07	−0.07	−0.07	0.00	0.00	0.00
18.00	−110	−0.15	−0.15	29.17	0.00	0.00	29.32
18.67	−110	−0.12	−0.12	−0.12	0.00	0.00	0.00
19.33	−110	−0.10	−0.10	−0.10	0.00	0.00	0.00
20.00	−80	−0.17	−0.17	29.15	0.00	0.00	29.32
20.67	−80	−0.14	−0.14	−0.14	0.00	0.00	0.00
21.33	−80	−0.12	−0.12	−0.12	0.00	0.00	0.00
22.00	−50	−0.19	−0.19	29.14	0.00	0.00	29.32
22.67	−50	−0.15	−0.15	−0.15	0.00	0.00	0.00
23.33	−50	−0.12	−0.12	−0.12	0.00	0.00	0.00
24.00	−50	−0.10	−0.10	−0.10	0.00	0.00	0.00

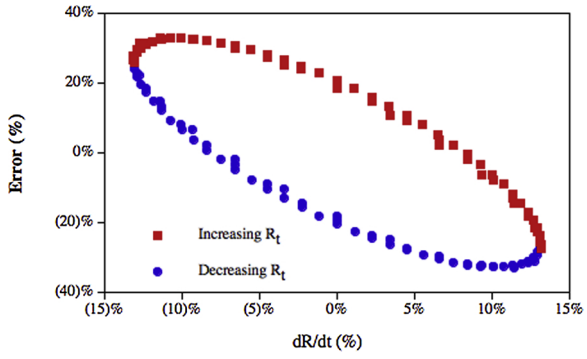

Fig. 2. Error in R_t^m as function of dR_t^m / dt for the SINE response and STEADY correction. dR_t^m / dt is expressed as$(R_{t+1}^m - R_t^m)$ /(Range of R_t^m) and the error term is expressed as $(R_t^m - R_t^{a,m})$ /(Range of $R_t^{a,m} \cdot \Delta t$) . Separate curves are plotted for both increasing and decreasing values of R_t^m .

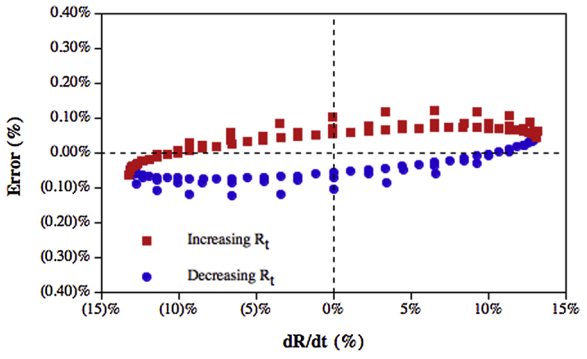

Fig. 3. Error in R_t^m as function of dR_t^m / dt for the SINE response and SPLINE correction. See Fig. 2 for description of variables.

5.2. Accuracy of daily variation (20-, 40- and 60-minute averages)

The accuracy of the daily variation (mean values in Tables 7 and Supplemental Files 2–4) depended on the assumed response and correction equations. While the deviation increased with increasing amplitude, there was minor variation between the different time averages. This is likely due to the fact that the assumed responses are clean signals with no analytical errors or random changes in metabolic rate.

Considering all the analytical approaches and correction equations, the combination of DETAILED and SPINE gave the most consistent correction (Table 7). For 60-minute averages (Table 7), POINT and NIIMI and DETAILED and NIIMI were more accurate than DETAILED and SPLINE for STEP and MULTI-STEP. This table fails to capture the huge changes in deviation resulting from small changes in timing (Table 9).

DETAILED and NIIMI is almost a perfect for STEP and MULTI-STEP, but it is very inaccurate for the SINE response. It is not surprising that NIIMI is an excellent correction for STEP and MULTI-STEP as the derivation of this equation was based on a step change. As expected, the STEADY values had the poorest fit for all cases. The POLY approach was

Table 10
Summary of upper and lower peaking factors. Based on 60-minute sampling intervals and MEAN concentrations.

R_t^a	Amplitude /Case	Actual Peaking Factor	Computed Peaking Factors (upper/lower)				
			STEADY	FRY	NORTHBY	NIIMI	SPLINE
SINE	A_1 = -20	1.20/0.80	1.15/0.85	1.20/0.80	1.20/0.80	1.20/0.80	1.20/0.80
	A_1 = -40	1.40/0.60	1.30/0.70	1.40/0.60	1.39/0.61	1.40/0.60	1.40/0.60
	A_1 =- 60	1.59/0.41	1.45/0.55	1.59/0.41	1.59/0.41	1.60/0.40	1.59/0.41
	A_1 = -80	1.79/0.21	1.60/0.40	1.79/0.21	1.79/0.21	1.79/0.21	1.79/0.21
MULTI-STEP	Case 1	1.33/0.66	1.26/0.69	1.33/0.67	1.33/0.66	1.33/0.66	1.34/0.66
	Case 2	1.49/0.50	1.39/0.53	1.49/0.50	1.49/0.49	1.49/0.50	1.49/0.50
	Case 3	1.54/0.44	1.43/0.48	1.55/0.44	1.55/0.44	1.55/0.44	1.56/0.44
	Case 4	1.58/0.40	1.46/0.44	1.59/0.40	1.59/0.39	1.59/0.40	1.60/0.40

Table 11
Comparison of peaking factors (upper/lower) as a function of sampling interval. Based on SPLINE correction and MEAN concentration.

R_t^a	Amplitude/Case	Sampling Interval (min)			
		1	20	40	60
SINE	A = -20	1.20/0.80	1.20/0.80	1.20/0.80	1.20/0.80
	A = -40	1.40/0.60	1.40/0.60	1.40/0.60	1.40/0.60
	A = -60	1.60/0.40	1.60/0.40	1.60/0.40	1.59/0.41
	A = -80	1.80/0.20	1.80/0.20	1.79/0.21	1.79/0.21
MULTI-STEP	Case 1	1.34/0.64	1.34/0.65	1.34/0.66	1.34/0.66
	Case 2	1.52/0.46	1.50/0.48	1.50/0.50	1.49/0.50
	Case 3	1.58/0.40	1.56/0.42	1.56/0.44	1.56/0.44
	Case 4	1.62/0.36	1.60/0.38	1.60/0.40	1.60/0.40

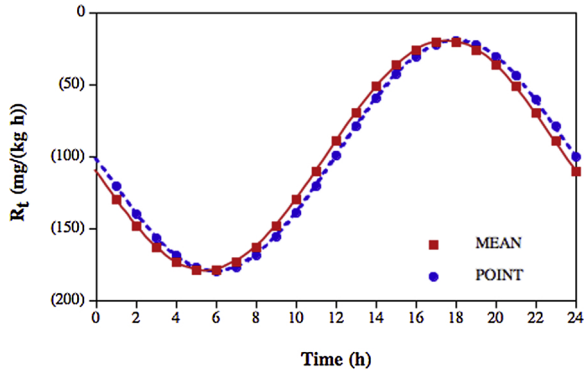

Fig. 4. R_t^m and R_t^p plotted as continuous function of time (SINE response, SPLINE correction, 60 min averages).

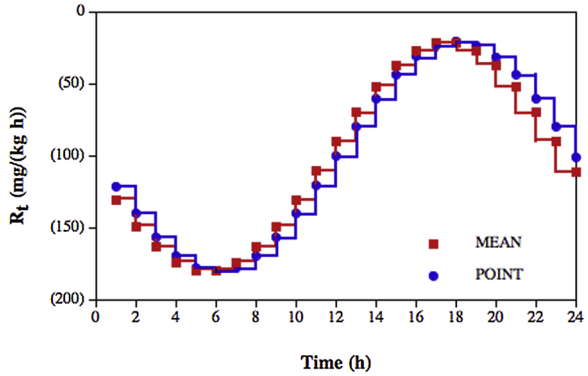

Fig. 5. R_t^m and R_t^p plotted as a constant value over each time step (same conditions as for 4).

not superior to SPLINE or NORTHBY. Because of its reduced accuracy and significantly more complex computational requirements, this approach has limited usefulness.

In terms of analytical approaches, DETAILED is superior to either MEAN or POINT. In fact, if DETAILED is selected, all of the correction equations except STEADY give acceptable results. Interestingly for the SINE response, SPINE and NORTHBY and NIIMI and FRY give almost identical results (Table 7 and Supplemental File #2).

The computed R_t were based on a relatively long detention time of 200 min that may be representative of older circular tank designs. More recent facilities for Atlantic salmon in Norway have been designed with detention time in the 35 to 50 min range (Summerfelt et al., 2016). This increased turnover rate can support higher feeding rates and results in improved water quality. Decreasing the detention, reduces the impact of hydraulic lag on dissolved oxygen concentration (see Eq. 4 in Colt and Maynard, 2019) and computed metabolic rates. For example, reducing the detention time from 200 to 30 min for SINE and MEAN while increasing M from 1340 to 9000 kg, improves the deviation from the assumed metabolic rate by a factor of 5. This change in detention time has no impact on the relative accuracy of the different correction equations. The ranking remain the same. While this change does improve the accuracy of STEADY, it is not improved enough that one would use STEADY in preference to the other correction approaches.

5.3. Accuracy of individual times

The previous section discussed the overall standard deviation of the different correction approaches. To give a better understanding of the basis of the deviation, it is necessary to explore the deviation of individual times. For the SINE response, the individual deviation does depend on dC/dt (Table 3, Figs. 2 and 3) but it is distributed over the entire data set. In contrast for STEP (Table 8), the deviation for NORTHBY and SPLINE largely occurred at t or t-1 (relative to the step in R_t^a). Manual computations for these two time steps are presented in Supplemental File #6 to demonstrate that these deviations are valid. This error is due to the fact that NORTHBY uses 3 C_t values and SPLINE uses 4 C_t values. These errors result when the correction term is based on data that spans the time where the step occurs. For MULTI-STEP, these large deviations occur on a regular basis for MEAN and POINT (Fig. 6). This error is less of a problem with the SINE response because of the gentle change in the slope and the lack of sharp discontinuities found in STEP and MULTI-STEP. The superior accuracy of DETAILED analytical approach is due to computation on minutely basis and the lack of any averaging across rapidly changing C_t values.

5.4. Accuracy of peaking factors

STEADY under estimated both the upper and lower peaking factors (Table 10). The peaking factors for the other correction equation were very accurate. While the peaking factors depended on the amplitude of

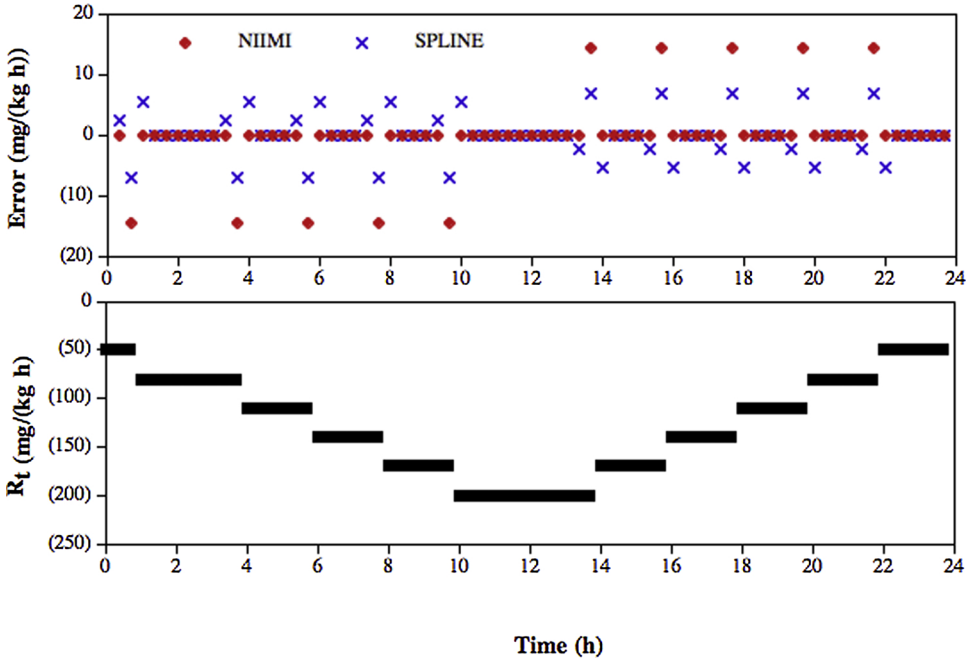

Fig. 6. Individual deviations in $R_t^m - R_t^{a,m}$ MULTI-STEP response for NIIMI and SPINE corrections (MEAN, Case 4, 20 min averages).

the daily change, it did not depend on the sampling interval (Table 11). This is likely due to the lack of any random variation inR_t^a. Real C_t data commonly shows high variability over short time intervals due to changes in swimming activity resulting from husbandry activities such as feeding, tank cleaning, or unknown causes.

The primary change in the daily dissolved oxygen concentrations is due to feeding. The other critical design factor for dissolved oxygen is the potential impact of feeding on the oxygen transfer process. Following feeding, the oxygen transfer rate may decrease by a factor of 3 in intensive aquaculture systems (Dallas Weaver, personal communications). The impact of feeds on oxygen transfer is likely to depend strongly on the type of feed, proximate composition, and physical characteristics of the feeds (Colt, 2018).

Experimentally determined peaking factors are presented in

Table 12
Characteristics of representative metabolic rates. Based on fed fish in commercial or pilot-scale facilities.

Parameter/Species	Size	τ (min)	Approach	Upper PF	Lower PF	Citation
Oxygen						
Acipenser transmontanus	510 g	62[a]	FRY	1.3	—	Thomas and Piedrahita, 1997
Dicentrarchus labrax	55 g	94	FRY	1.4	0.55	Tudor, 1999
	99 g	304	FRY	1.3	0.75	
Stizostedion vitreum	75-251 mm	—	None	1.1 - 1.3	0.59 - 0.96	Cai and Summerfelt, 1992
Stizostedion vitreum	236-425 g	95	FRY	1.18 ± 0.05	0.83 ± 0.03	Yager and Summerfelt, 1993
Tinca tinca	17 g	67	None	1.09 ± 1.44	0.74 ± 0.84	Zakes et al., 2006
Anguilla anguilla	—	49-196	None	1.2-1.4		Heinsbroek and Kamstra, 1990
Paralichthys californicus	3 g	—	FRY	1.2	—	Merino et al., 2009
	170 g	—	FRY	1.4	—	
Paralichthys californicus	3-166 g	—	FRY	1.35 ± 0.11	—	Merino et al., 2011
Salmo salar	2000 g	—	None	1.2 - 1.3	—	Forsberg, 1997
Salmo salar	> 800 g	—	None	1.2 - 2.4	—	Bergheim et al., 1993
Salmo salar	5647 g	3.44	None	1.61	0.54	Farrell, 2006
Pacific salmon	juvenile	—	NIIMI	1.20 ± 0.08	—	McLean et al., 1993
Oncorhynchus nerka	28.6 g	—	IF[c]	1.4	0.63	Brett and Zala, 1975
Carbon Dioxide						
Salmo salar	2000 g	—	None	1.2 - 1.4 [a]	—	Forsberg, 1997
Anguilla anguilla	—	49-196	None	1.4		Heinsbroek and Kamstra, 1990
TAN						
Stizostedion vitreum	75-251 mm	—	None	1.17 - 1.32	0.76 - 1.00	Cai and Summerfelt, 1992
Stizostedion vitreum	236-425 g	95	FRY	1.52 ± 0.36	—	Yager and Summerfelt, 1993
Tinca tinca	17 g	67	None	1.38 - 3.04	0.05 - 0.40	Zakes et al., 2006
Oncorhynchus nerka	28.6 g	—	IF[c]	2.4	0.57	Brett and Zala, 1975
Clarias gariepinus	1-400 g	—	None	3	≅ 0	Bovendeur et al., 1987
Anguilla anguilla	—	49-196	None	1.5-2.3		Heinsbroek and Kamstra, 1990
Anguilla anguilla	97	3-4	None	1.2-1.7	0.38-0.66[b]	Poxton & Lloyd, 1989
Suspended Solids						
Anguilla anguilla	—	49-196	None	2-5		Heinsbroek and Kamstra, 1990

[a] based on total flow.
[b] Estimated from figures; not all information presented in article.
[c] IF = Intermittent flow.

Table 12 primarily based on fed fish in commercial or pilot-scale facilities. Due to the impact of reaeration, sediment oxygen demand, or BOD uptake (Colt and Maynard, 2019) these values can be quite different from those determined in the laboratory under tightly controlled conditions. Typical values for metabolic parameters are:

Parameter	Upper Peaking Factor
Oxygen	1.2 - 1.4
Carbon dioxide	1.2 - 1.4
TAN	1.4 - 3.0
Suspended solids	2 - 5

Some of these values are based on corrected data (FRY or NIIMI), but many are uncorrected. Because carbon dioxide production is driven by oxygen consumption under aerobic conditions, it is expected that the peak will follow the oxygen consumption peak. The peaking factors for TAN and suspended solids are significantly higher than for oxygen and carbon dioxide; these are also parameters where most of the values are based on uncorrected values.

Because of the tendency of uncorrected (STEADY) values to underestimate the maximum and minimum values of R_t, peaking factors based on uncorrected values will under-estimate the true peaking factors. Because of the larger range of R_t values for TAN and suspended solids, it is expected that the uncorrected values are significantly lower than those based on the corrected values. The use of peaking factors based on uncorrected values for design may be acceptable if the new system is similar to the system where the uncorrected peaking factors were determined. If the new system is quite different, the facility may be significantly under-designed or over-designed.

5.5. What controls the accuracy of the metabolic rate correction?

The accuracy of the metabolic rate estimate can be improved by reducing the daily variation through changes in hydraulic detention time or feeding protocols or by selection of the best correction approach for a given set of R_t values.

5.5.1. dc/dt or amplitude of daily variation

The magnitude of the correction to the metabolic rate will directly depend on dC/dt, Q/M and Vol/M (Eq. 4) and can be rewritten as:

$$\frac{MR(t)}{Q} = -(C_t^{in} - C_t) + \left[\frac{Vol}{Q}\frac{dC}{dt}\right] \quad (13)$$

This equation has units of mg/L. Increasing the value of Q or decreasing the value of Vol will decrease the correction factor. For constant value of Q, M, and Vol, increasing values of dC/dt will increase the correction factor. The amplitude of the daily variation did increase the size of the correction, but errors depended strongly on the R_t^a.

For the sine response, the magnitude of the STEADY error depended on the sequential difference in R_t (proportional to dR/dt) but resulted in separate curves for increasing and decreasing values of R_t (Fig. 2). The resulting error for other correction approaches showed similar bimodal curves, but the magnitude of the error was significantly less than for SPLINE (Fig. 3). The basis for these separate curves for increasing and decreasing values of dR/dt is not fully understood at this time.

5.5.2. Discontinuities

The correction approaches worked best when the values of R_t^a vary slowly. The accuracy of the correction was significantly degraded when there were any rapid changes or discontinuities in R_t^a (Table 8 for STEP change). At this point, the dR/dt changes from zero to infinity and back to zero in a single time step. The correlation between discontinuities in R_t^a and the resulting error terms is clearly shown in Fig. 6 for the MULTI-STEP response for both NIIMI and SPLINE. Very regular errors resulted when there was a step change in R_t^a, but the error during the remaining times was very small, especially for NIIMI. These errors are accentuated for longer sampling intervals (i.e., 40 and 60 min).

In addition, magnitude of the deviation for MEAN and POINT (Table 9) depends strongly on the timing with respect to reporting time intervals (i.e., 20-, 40-, or 60-minute). These deviations do not occur for DETAILED, because the R_t^d values are computed from individual minutely concentrations (C_t^i).

5.5.3. Measurement error and random events

The assumed values of R_t^a in this article are clean signals with no measurement errors or random changes in activity. The potential impacts of this increased variability may significantly decrease the accuracy of the correction terms and peaking factors, especially for shorter sample times and POINT concentrations. Additional work is needed to document their impacts on our ability to correct flow-through data.

5.5.4. Changes in feeding protocols

Since most of the important metabolic parameters are driven by feed inputs, much research has been conducted on timing of feeding and the number of feedings per day. Probably the most common feeding schedules are feeding (a) once a day in the morning or (b) once in the morning and once in the afternoon. One advantage of this type of feeding schedule is that enough feed is provided so that all the fish are able to feed.

Many types of automatic or self-feeders are available when it is desired to feed more frequently. The ability of these types of feeders to produce good growth with acceptable size variability depends strongly on the species, social structure, size, and density. When the feeding duration is lengthened and the feeding rate (g/minute) decreased, aggressive individual fish may stake out preferential territories and prevent other fish from feeding.

It is generally assumed that increasing the number of feedings per day will reduce the peak metabolic rates (Muir, 1982; Timmons and Ebeling, 2007). For rainbow trout (*Oncorhynchus mykiss*) continuously feeding resulted in nearly constant oxygen consumption rates (Davidson et al., 2009), but the daily TAN concentration was much more variable. With juvenile walleye (*Sander vitreus*), Yager and Summerfelt (1993) found that increasing the number of feedings from 2 to 15 /day had no impact on either the mean oxygen consumption or mean TAN excretion rates. These increases in feeding frequency significantly reduced the variance in the oxygen consumption rates but not the variance in TAN excretion rates. For two feedings/day, Poxton and Lloyd (1989) found moving the time of the second feeding from 15:30 to 20:30 resulted in reduced TAN excretion by European eel (*Anguilla anguilla*). Increasing the number of feedings from 2 to 5 feedings/day or feeding continuously (8:30 to 20:30), actually increased the peak TAN concentration and the peaking factor for TAN.

Increasing the number of feedings per day may decrease the peak metabolic rate for oxygen and carbon dioxide for many aquatic animals. These changes may have less impact on the peak TAN excretion rates and for some species could result in increased peaking factors.

5.6. Implications of using point rather than mean concentrations

For the SINE case, the use of MEAN concentrations gives a slightly reduced deviation between $R_t^m - R_t^{a,m}$ compared to the use of POINT concentrations. For real data, it is expected that the use of MEAN concentration will be significantly more accurate than the use of POINT concentrations (Section 5.5.3). For some analysis, the use of MEAN concentrations may be desirable from a conceptual basis (compare Figs. 4 and 5)

5.7. Application to other metabolic parameters

Most of this article has focused on dissolved oxygen as it is one of

the most important metabolic parameters needed for system design. At this point, we shall broaden the discussion to include other metabolic or water quality parameters. As presented in the background section, R_t values computed for oxygen will be negative and for carbon dioxide and TAN will be positive.

5.7.1. In situ Parameters

Many of the important metabolic and water quality parameters must be measured *in situ*. This includes dissolved oxygen, carbon dioxide, total gas pressure, pH, salinity, temperature, and suspended solids (optical method). The primary source for measurement of these parameters is Standard Methods (2017). All of these parameters can be continuously measured and logged at specified time intervals. Some additional information will be presented on some of the parameters.

5.7.1.1. Carbon dioxide. Dissolved carbon dioxide gas is part of the overall carbonate acid-base system (Stumm and Morgan, 1981). Due to chemical reactions following carbon dioxide stripping or carbon dioxide addition, the most common measurement of carbon dioxide will give the apparent carbon dioxide removal or addition rate (Colt et al., 2012):

$$\Delta CO_2^A = \frac{C_{CO_2}^{in} - C_{CO_2}^{out}}{MW_{CO_2}} \quad (14)$$

The true carbon dioxide removal is equal to:

$$\Delta CO_2^T = C_T^{in} - C_T^{out} \quad (15)$$

Where

ΔCO_2^A = Apparent carbon dioxide removal (mmol/kg)
$C_{CO_2}^{in}$ = Influent carbon dioxide concentration (mg/L)
$C_{CO_2}^{out}$ = Effluent carbon dioxide concentration (mg/L)
MW_{CO_2} = Molecular weight of carbon dioxide (mg/mmol)
MW_{CO_2} = True carbon dioxide removal (mmol/kg)
C_T^{in} = Influent total carbonate concentration (mmol/kg)
C_T^{out} = Effluent total carbonate concentration (mmol/kg)

The use of the apparent removal rate will underestimate the true removal rate for higher alkalinities and lower influent carbon dioxide concentrations (Colt et al., 2012). For 114 g Atlantic salmon, the true rate was 16–45% higher than the apparent rate (Kvamme et al., 2018). The apparent removal rate is needed when it is necessary to know the concentration of carbon dioxide after the degassing process.

While it is important to be able to control the carbon dioxide concentration because of its potential toxicity, it is also important to understand the carbonate system is not the only acid-base present in intensive culture systems. This is more of an issue in high intensity RAS or bagged transport systems. In tight RAS, a number of organic acids can build up and the titration alkalinity can be significantly higher than the carbonate alkalinity. Because of analytical problems with a wide variety of compounds and uncertainty in pK_a values, estimation of pH following air stripping may be inaccurate under these conditions. In tilapia transport systems, ammonia may build up so that the pH is controlled by ammonia ($NH_4^+ \rightleftarrows NH_3 + H^+$) rather than the carbonate system (Colt and Kroeger, 2013).

5.7.1.2. Un-ionized ammonia. Continuous monitoring of TAN has been attempted, but none of these units are in common commercial use. If one is interested in computation of the un-ionized ammonia concentration (TAN to be covered in the next section), it is necessary to simultaneously measure TAN, temperature, salinity, and pH. Un-ionized ammonia concentration based on TANs and average temperature, salinities, and pHs can be very inaccurate.

5.7.2. Laboratory measured parameters

For aquaculture applications, TAN and suspended solids are measured in the laboratory. Information on sampling, storage, and analysis can be found in Standard Methods (2017).

5.7.2.1. TAN. Refrigerated or ice-filled automatic samplers may be

Table 13
Comparison of R_t^a and $R_t - R_t^a$ values for hourly measurements. For DETAILED, the $R_t^{a,m}$ and STEADY are idenitical to those for MEAN. (SINE response and A_1 = -80 mg/kg h).

	POINT			MEAN			DETAILED
Time	$R_t^{a,p}$	STEADY	SPLINE	$R_t^{a,m}$	STEADY	SPLINE	SPLINE
0	−100.00	39.07	#VALUE!	−110.24	43.13	#VALUE!	#VALUE!
1	−120.71	46.81	−0.08	−130.37	49.30	−0.05	−0.04
2	−140.01	51.29	−0.03	−148.44	52.06	0.00	−0.01
3	−156.58	52.24	0.02	−163.19	51.24	0.05	0.02
4	−169.29	49.60	0.07	−173.64	46.89	0.09	0.05
5	−177.28	43.55	0.11	−179.07	39.33	0.13	0.08
6	−180.00	34.51	0.15	−179.11	29.07	0.16	0.09
7	−177.26	23.11	0.18	−173.75	16.82	0.18	0.11
8	−169.26	10.12	0.19	−163.36	3.41	0.19	0.11
9	−156.53	−3.56	0.19	−148.65	−10.23	0.19	0.11
10	−139.95	−17.01	0.18	−130.63	−23.18	0.17	0.10
11	−120.64	−29.30	0.16	−110.52	−34.56	0.14	0.09
12	−99.93	−39.59	0.12	−89.69	−43.58	0.10	0.06
13	−79.22	−47.19	0.08	−69.56	−49.63	0.05	0.04
14	−59.93	−51.57	0.03	−51.51	−52.30	0.00	0.01
15	−43.37	−52.44	−0.02	−36.76	−51.40	−0.05	−0.02
16	−30.67	−49.73	−0.07	−26.33	−47.00	−0.09	−0.05
17	−22.70	−43.63	−0.11	−20.92	−39.39	−0.13	−0.12
18	−20.00	−34.56	−0.15	−20.90	−29.10	−0.16	−0.10
19	−22.76	−23.13	−0.18	−26.28	−16.83	−0.18	−0.11
20	−30.78	−10.12	−0.19	−36.68	−3.41	−0.19	−0.11
21	−43.52	3.58	−0.19	−51.40	10.25	−0.19	−0.11
22	−60.12	17.03	−0.18	−69.44	23.20	−0.17	−0.10
23	−79.43	29.32	−0.15	−89.56	34.58	−0.14	−0.09
24	−100.15	39.61	−0.12	−110.39	43.59	−0.10	−0.06
Mean	−100.00	−0.04	0.00	−100.01	−0.04	0.00	0.00
SD	57.78	37.90	0.1391	57.61	37.80	0.1387	0.0849

needed for TAN and other water quality parameters. For aquaculture applications, the salicylate method is superior to Nessler, phenate, and ion selective procedures for ammonia (Zhou and Boyd, 2016).

While much effort has been directed towards the ammonia removal characteristics of biological filters as a function of substrate concentration (Malone et al., 2006), less is known about the impact of time varying substrate concentrations on the overall daily performance. It is important to note that all of the correction procedures presented in this article assumed that the influent concentration was constant or changing slowly. The application of these equations for rapidly varying influent concentrations remains to be demonstrated.

Another issue with TAN is that a number of chemical and biological processes (nitrification, denitrification, nitrogen uptake, absorption, etc.) can change its concentration and not all these changes may occur inside the control volume. For example, measurement of the TAN oxidation in systems containing oysters is complicated by nitrifying bacteria attached to the oysters (Florence et al., 2015). In some RAS systems, a significant portion of the nitrification occurs on tank surfaces and inside pipes rather than inside the biofilter (Pfeiffer and Malone, 2006; Timmons et al., 2006). Design of a biofilter to handle the entire system TAN load would result in serious over-design and excessive capital costs on systems with significant nitrification occurring outside the biofilter.

5.7.2.2. Suspended solids. The typical concentrations of suspended solids in aquaculture systems are low and the use of conventional methods (Standard Methods, 2017) can result in high variability. For some applications, larger samples (up to 30 L) and fine screens (Pfeiffer et al., 2008) may be preferable to the use of 0.45 μm glass fiber filters and conventional sample sizes.

5.8. Application to unit process performance

The estimation of performance metrics or efficiencies for unit processes (Eqs. 2 and 3 in Colt and Maynard, 2019) is subject to the same problems with lag as dissolved oxygen. For most of the correction equations, it was assumed that was constant or slowly changing. This may be very a poor assumption for TAN that has a large daily variation (Table 12). Some biofilters have the ability to process this TAN pulse as long as the dissolved oxygen concentration is adequate.

5.8.1. Sampling interval and characterization of sample

If one has a large personnel budget, it may be possible to manually collect data every 10–60 minutes over the entire day. For sampling more than a few days long, this may prove to be unsustainable except for graduate study research. A more realistic sampling schedule might be hourly samples collected with an automatic sampler. Flows in aquaculture systems are typically relatively constant over the day, so flow-weighted sampling is not needed.

If one is interested in the mean value over the sampling interval, then a composite sample is needed. If the concentration is rapidly changing with time, grab samples may give inaccurate results. For SINE and 60-minute averages, the values of and are presented in Table 13 for POINT, MEAN, and DETAILED. For the error-free data considered, the derivation of the MEAN was slightly better than for POINT. For real data, it is expected that the MEAN results will be better than the POINT results. It is important to note that the deviation of DETAILED is only 61% of MEAN and POINT. Therefore, the accuracy of the hourly collected data will be significantly less than minutely data used to estimate the hourly values. For this example, the deviation of STEADY is 270 times larger than Mean or POINT and 450 times larger than DETAILED (Table 13). For computation of mean daily values, both composite and grab samples should give accurate results.

The common sampling schedule in aquaculture process evaluations varies from 1 grab sample/day to 1 sample/week (for example, see Christianson et al., 2015; Davidson et al., 2009; Pfeiffer et al., 2008). If the performance does not significantly change over the day, a single grab sample collected on a daily to weekly basis may give a valid estimate of the performance of the unit process. If one is interested in the average daily performance, with more detailed sampling, it may be possible to select a time period when the grab sample is close to the daily average. In many cases, one daily or weekly estimate of performance is all one can complete due to sampling or analytical constraints. Detailed information on the accuracy of sampling is presented in Standard Methods (2017). For a single daily sample, none of the correction approaches presented in this article have any use and validity of the sampling to characterize the performance of the unit process is not generally known.

5.8.2. Time to reach steady-state

Another similar question regarding sampling, is how long after a change is made does the process approach steady state. This a key question in laboratory or pilot-scale process evaluation, where key-operating variables may change on a regular basis.

5.8.2.1. Physical/chemical processes. For a pure physical unit process or chemical process with relatively fast kinetics, the impact of a single step change in the input can be computed from Eq. 4 in Colt and Maynard (2019). Typically, one should wait 3 to 4 hydraulic resident times (). For systems involving slower chemical reactions or significant changes in temperature, it may necessary to experimentally determine how long is needed to reach steady state by a comprehensive sampling plan.

5.8.2.2. Biological processes. For biological filters for nitrification or denitrification, the resident time for the water may be measured in minutes while the resident time of the bacteria may be in the range of weeks to months. The growth rates of these bacterial groups are slow, especially under cold conditions or low substrate concentrations. It is not uncommon for nitrification biofilters to take several months (or more) to reach steady state (Colt et al., 2006). The initial startup of nitrification biofilters in cold conditions ($< 5^{\circ}C$) or some type of plastic media in seawater may even be longer.

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Appendix A. Supplementary data

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