

# A NEW TRANSIENT PREDICTIVE MODEL TO QUANTIFY TAIN T AS EITHER GEOSMIN (GSM) OR 2-METHYLISOBORNEOL (MIB) IN RAINBOW TROUT (*ONCORHYNCHUS MYKISS*) FARMED IN RECIRCULATING AQUACULTURE SYSTEMS (RAS)

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## ABSTRACT

Taint, as off-flavour, accumulation in fish as either geosmin (**GSM**) or 2-methylisoborneol (**MIB**) is a major concern globally in fish farmed in Recirculated Aquaculture Systems (RAS), especially in European farmed rainbow trout (*Oncorhynchus mykiss*). To aid farm management in RAS growth protocols a new transient (unsteady-state) predictive model for taint was developed. The approach is based on conservation of mass and energy and established thermodynamics. The model is the sum of two exponential terms with growth-time that simulate both taint uptake (through gills) and elimination (through gills, growth dilution, and metabolic-transformations). The model was simulated with growth water concentrations of **GSM** and **MIB** for the typical growth period of 9 months and gave good agreement with independent data. It is concluded the model is therefore free of computational and programming errors. It is planned the model be used to develop specific growth protocols to minimize taint development in fish in RAS systems.

Keywords: taint, geosmin (**GSM**), 2-methylisoborneol (**MIB**), Recirculated Aquaculture Systems (RAS)

## 1. INTRODUCTION

Farming of rainbow trout (*Oncorhynchus mykiss*) in Recirculating Aquaculture systems (RAS) is becoming increasingly popular. However, taint as ‘earthy’ or ‘muddy’ off flavors can accumulate in the fish flesh. This is true also of other species in RAS such as barramundi (*Lates calcarifer*) (Percival et al. 2008), arctic charr (*Salvelinus alpinus*) (Houle et al. 2011), largemouth bass (*Micropterus salmoides*), and; white sturgeon (*Acipenser transmontanus*) (Schrader et al. 2005).

Two major taint molecules have been identified; these are Geosmin (**GSM**), and; 2-methylisoborneol (**MIB**). These are produced by benthic and planktonic cyanobacteria, some fungi and actinomycetes species as byproducts (Wood et al. 2001). Taint due to **GSM** and **MIB** has been a common feature in conventional aquaculture systems e.g. ponds, but in RAS, reduced water consumption, higher nutrient(s) load, and higher

stocking densities have acerbated the problem. These two taint compounds impart earthy or muddy taste off flavors and odor in the growth water which is then taken into the fish flesh via gills or skin (Howgate 2004). The result is strong buyer resistance.

Effective control measures to minimize taint are yet to be established. However, leaching of fish in clean water prior to market is widely used (Tucker and van der ploeg 1999). A drawback is that this can lead to a loss of body mass of the fish. Also, the time taken for leaching may be several days depending on the intensity of the taint (Tucker 2000). During RAS growth, copper sulfate is sometimes used as an algacide to control the growth of taint producing bacteria. There are however some concerns over effectiveness of this treatment (Rimando and Schrader 2003).

### 1.1 This research

Against this background, a new transient-state quantitative model was developed to predict taint as either **GSM** or **MIB** in European farmed rainbow trout. Published mechanistic models have been based on steady-state conditions where chemical uptake into and from the fish is assumed to be zero (e.g. Arnot and Gobas 2004; Gobas 1993). However, RAS is dynamic and its growth conditions continuously change with time. Therefore, it is questionable as to whether steady-state condition can be used when developing predictive models for fish grown in RAS.

Here the approach used is to refine the generalized model for taint accumulation of Hathurusingha and Davey (2013; 2014) for both constant and varying concentration of **GSM** and **MIB** in the RAS growth water. This is then used to simulate taint accumulation up to the consumer rejection threshold. Predictions are compared with best available independent data. The model can be conveniently set-up and solved as a Microsoft Excel™ spread sheet. The widespread use of these tools means that communication of results can be streamlined. It is planned the validated model will be used to investigate a number of practical RAS growth protocols to minimize taint. A typical growth-period for rainbow trout in RAS is 270 days (9 months) (Petersen et al. 2011).

## 2. MATERIALS AND METHODS

### 2.1 Model development

The model of Hathurusingha and Davey is a sum of two exponential terms with growth-time: one for taint uptake (through the gills) and one for taint elimination (through gills plus growth dilution and metabolic-transformations). The model can be conveniently summarized as shown schematically in Figure 1. All the symbols used are carefully defined in the Nomenclature.

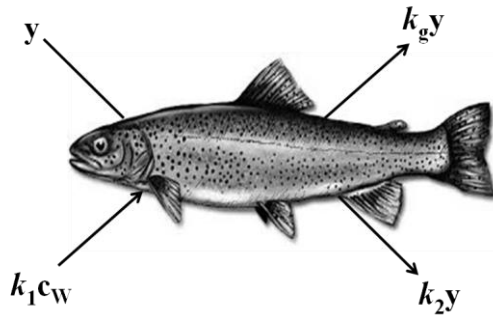


Figure 1: Schematic of rainbow trout fish with uptake ( $k_1 C_w$ ) and elimination ( $k_2 y + k_g y$ ) of taint molecules (as GSM or MIB)

It is assumed that the taint concentration, as either **GSM** or **MIB** in the fish-flesh, is 'y' ( $\mu\text{g kg}^{-1}$ ). The concentration of the taint compound in the RAS growth water at any time ( $t$ ) is  $C_w$  ( $\mu\text{g L}^{-1}$ ). Therefore, the rate of change of taint ( $y$ ) in the fish-flesh with time ( $t$ ) can be expressed by:

$$\frac{dy}{dt} = \text{uptake rate} - \text{elimination rate} \quad (1)$$

The uptake rate of **GSM** or **MIB** will be a function of the rate constant for uptake ( $k_1$ ,  $\text{L kg}^{-1} \text{day}^{-1}$ ), the fish mass ( $m_f$ , kg) and the taint concentration in the RAS water ( $C_w$ ). The elimination rate of **GSM** or **MIB** will be a function of the rate constant for elimination through the gills ( $k_2$ ,  $\text{day}^{-1}$ ), the rate constant for bio-transformation and growth dilution ( $k_g$ ,  $\text{day}^{-1}$ ) and the instantaneous value of  $y$  at time,  $t$ . Substitution of these parameters into Eq. (1) gives:

$$\frac{dy}{dt} = k_1 m_f C_w - (k_2 + k_g) y \quad (2)$$

where  $\left(\frac{dy}{dt}\right)$  is the rate of change of taint in fish-flesh ( $\mu\text{g kg}^{-1} \text{day}^{-1}$ ).

The mass of the fish ( $m_f$ ) is known to be a function of growth-time in the RAS tank. Published growth data as mass of fish (kg) vs growth-time (day) is generally highly exponentially correlated (*see* below Eq. (19) for rainbow trout) and fish mass is therefore given by:

$$m_f = \beta \exp^{\gamma t} \quad (3)$$

Let:

$$b = \beta k_1 C_w \quad (4)$$

and;

$$a = (k_2 + k_g) \quad (5)$$

Substitution for  $b$  and  $a$  into Eq. (2) and rearranging gives:

$$\frac{dy}{dt} + ay = b \exp^{\gamma t} \quad (6)$$

Eq. (6) can be integrated by parts (Evans 2010) to give:

$$y = \frac{b \exp^{\gamma t}}{(a + \gamma)} - \frac{b}{(a + \gamma) \exp^{at}} \quad (7)$$

Eq. (7) can be rearranged to conveniently give:

$$y = \left(\frac{b}{a + \gamma}\right) \left[ \exp^{\gamma t} - \exp^{-at} \right] \quad (8)$$

The model for taint in fish-flesh of Eq. (8) shows that the predicted level is the sum of two exponential terms with time in the RAS growth tanks, namely, uptake and elimination.

There are generally however no published data for  $k_1$  and  $k_2$  or  $k_g$  in the refereed literature (for example for rainbow trout fish) for immediate simulation of taint in the model. For model development these rate constants need to be defined mathematically.

### 2.2 Rate constants $k_b$ , $k_2$ and $k_g$

Arnot and Gobas (2004) found the gill uptake rate constant ( $k_1$ ) is a combination of two processes: gill ventilation, and chemical uptake efficiency across the gills. Accordingly, the chemical uptake rate was expressed as:

$$k_1 = \frac{E_w G_v}{m_f} \quad (9)$$

where  $E_w$  is the gill chemical uptake efficiency (dimensionless fraction) and  $G_v$  is the gill ventilation rate ( $\text{L day}^{-1}$ ). The chemical uptake efficiency was, reasonably, assumed by Arnot and Gobas (2004) to be a function of an octanol-water partition coefficient ( $K_{ow}$ ) of the chemical of interest that can be expressed through the following relationship:

$$E_w = (1.85 + 155 / K_{ow})^{-1} \quad (10)$$

A relationship between gill ventilation rate and oxygen consumption rate of the fish species based on empirical data is given by Aront and Gobas (2004), namely:

$$G_v = \frac{1400 m_f^{0.65}}{C_{ox}} \quad (11)$$

where  $C_{ox}$  is the concentration of dissolved oxygen ( $\text{mg L}^{-1}$ ). This is considered to be a function of temperature and can be computed from the equation given by Neely (1979):

$$C_{ox} = 14.45 - 0.413T + 0.00556T^2 \quad (12)$$

where  $T$  = RAS growth water temperature in degree Celsius.

The chemical elimination rate from the fish gills to the water ( $k_2$ ) is correlated with the chemical transport rate in aqueous and lipid phases of the fish, lipid content of the fish and octanol water partition coefficient of the taint chemical. The relationship can be expressed (Gobas 1993) as:

$$\frac{1}{k_2} = \left(\frac{V_L}{Q_W}\right)K_{OW} + \left(\frac{V_L}{Q_L}\right) \quad (13)$$

where  $Q_W$  is rate of chemical transport in the aqueous phase ( $L \text{ day}^{-1}$ ),  $Q_L$  is the rate of chemical transport in the lipid phase ( $L \text{ day}^{-1}$ ) and  $V_L$  is lipid weight (mass). Gobas and Mackay (1987) derived a relationship between  $Q_W$  and  $m_f$  using experimental data to give:

$$Q_W = 88.3m_f^{0.6(\pm 0.2)} \quad (14)$$

Gobas and Mackay (1987) reported that the chemical transport rate in the aqueous phase is ~100 times higher than in the lipid phase; therefore it can be assumed that:

$$Q_L = 0.01Q_W \quad (15)$$

Lipid mass ( $V_L$ ) of the fish is correlated to the lipid mass ratio ( $e$ ) (dimensionless) and can be conveniently expressed as:

$$V_L = em_f \quad (16)$$

The rate constant for combined metabolic transformation of the taint chemical and growth dilution rate of the taint chemical ( $k_g$ ) is given by:

$$k_g = (k_G + k_m) \quad (17)$$

The rate constant for growth dilution ( $k_G$ ) can be computed using the equation given in Thomann et al. (1992) as:

$$k_G = 0.000502m_f^{-0.2} \quad (18)$$

The rate constant for metabolic transformation of the taint chemical ( $k_m$ ) is available in the refereed literature (Gobas, 1993) to cover a range of fish species of  $k_m = 0.00063 \text{ day}^{-1}$ .

Equations (1) through (18) plus the general value for  $k_m$ , defines the model for taint in RAS farmed fish-flesh.

### 2.3 Simulations for European farmed rainbow trout (*Oncorhynchus mykiss*)

To illustrate the generalized form for rainbow trout (*Oncorhynchus mykiss*), it is necessary to find a suitable growth equation for this species.

A graph of the mass of the fish and the growth-time was obtained (Anon 2010) and correlated (Figure 2) to give:

$$m_f = 0.0346 \exp^{0.0138t} \quad (19)$$

with  $R^2 = 0.94$  (Snedecor and Cochran 1989) indicating a very good fit.

The model simulations can now be carried out using the procedure (Table 1) as follows:

At a mid-range of 150 days RAS growth, from Eq. (21),  $m_f = 0.274 \text{ kg}$ . The average growth water

temperature  $14 \text{ }^\circ\text{C}$  (Petersen et al. 2011) is substituted into Eq. (12) to give  $C_{OX} = 9.75 \text{ mg L}^{-1}$ . Substitution for  $m_f$  into Eq. (11) gives  $G_V = 61.68 \text{ L day}^{-1}$ . Substituting for  $\log_{10}(K_{OW}) = 3.57$  (dimensionless) (Howgate 2004) into Eq. (10) gives  $E_W = 0.528$  (dimensionless).

The uptake rate constant ( $k_1$ ) for **GSM** now can be calculated by substituting the  $E_W$ ,  $G_V$  and  $m_f$  values into Eq. (9) to give  $k_1 = 119.29 \text{ L kg}^{-1} \text{ day}^{-1}$ .

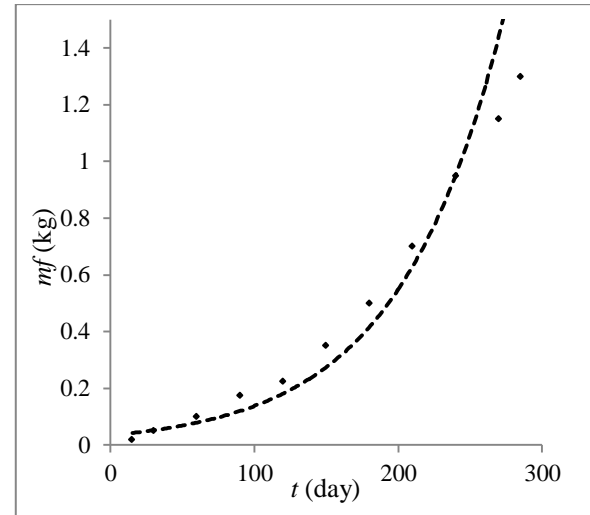


Figure 2: Fit of growth data (◆) for rainbow trout fish farmed in ponds at a water temperature  $15 \text{ }^\circ\text{C}$  (Anon. 2010)  $m_f = 0.0346 \exp^{0.0138t}$ ,  $R^2 = 0.94$

Substitution for  $m_f = 0.274 \text{ kg}$  at a mid-range growth of 150 days into Eq. (14) gives  $Q_W = 40.62 \text{ L day}^{-1}$  which when substituted into Eq. (15) gives  $Q_L = 0.406 \text{ L day}^{-1}$ . A lipid to mass ratio of rainbow trout is assumed to be 0.045 (Robertson et al. 2006) and hence from Eq. (16), lipid weight (mass)  $V_L = 0.0123 \text{ kg}$ . The taint elimination rate  $k_2$  now can be determined by substituting values for  $V_L$ ,  $Q_L$ ,  $Q_W$  and  $\log_{10}(K_{OW})$  into Eq. (13) to give  $k_2 = 0.863 \text{ day}^{-1}$ .

Substitution for  $m_f = 0.274 \text{ kg}$  into Eq. (18) gives  $k_G = 0.00065 \text{ day}^{-1}$ . The published value for  $k_m = 0.00063 \text{ day}^{-1}$  (Gobas 1993). From Eq. (17) the sum of  $k_G$  and  $k_m$  gives  $k_g = 0.00128 \text{ day}^{-1}$ .

A typical  $C_W$  value for **GSM** in growth ponds is reported to be  $0.0066 \text{ } \mu\text{g L}^{-1}$  (Petersen et al. 2011). Substitution for  $C_W = 0.0066 \text{ } \mu\text{g L}^{-1}$  into Eq. (4) gives **GSM** taint uptake in the rainbow trout,  $b = 0.04094 \text{ } \mu\text{g kg}^{-1} \text{ day}^{-1}$  at 150 day in the RAS growth cycle. The level of taint as **GSM** in the rainbow trout fish-flesh at 150 day of growth can now be obtained from Eq. (8) such that  $y = 0.3573 \text{ } \mu\text{g kg}^{-1}$ .

Similar calculations can be readily carried out for **MIB** accumulation in the RAS rainbow trout fish-flesh. A typical value for  $C_W$  for **MIB** is  $0.0032 \text{ } \mu\text{g L}^{-1}$  (Petersen et al. 2011). Substitution for  $\log_{10}(K_{OW}) = 3.31$  (dimensionless) (Howgate 2004) into Eq. (10) gives  $E_W = 0.519$ . Parameters  $k_g$ ,  $C_{OX}$ ,  $G_V$ ,  $Q_W$ ,  $Q_L$  and  $V_L$  are the same as that for **GSM** (as they are dependent on the mass of the fish only). Accordingly, the

calculated values for  $k_1$  and  $k_2$  for **MIB** are, respectively,  $117.17 \text{ L kg}^{-1}\text{day}^{-1}$  and  $1.537 \text{ day}^{-1}$ , and that for taint as **MIB** in the fish-flesh,  $y = 0.099 \mu\text{g kg}^{-1}$ .

### 3. RESULTS

#### 3.1 Predicted GSM levels

The (generic) procedure for model simulation is summarized in Table 1 and is illustrated with **GSM**. The procedure is such that the computations can be read conveniently down column 4 of the table. The value of **GSM** taint at 150 days RAS growth with constant  $C_w = 0.0066 \mu\text{g L}^{-1}$  (Petersen et al. 2011) is shown in the table to be  $y = 0.3573 \mu\text{g kg}^{-1}$ .

Table 2 summarizes the model predictions for taint as **GSM** in RAS rainbow trout fish-flesh for the growth period 30 to 270 days in RAS tanks. The table shows that  $k_1$ ,  $k_2$  and  $k_g$  values decrease with increasing growth-time. The level of predicted **GSM** taint ranges from 0.0508 to  $2.0219 \mu\text{g kg}^{-1}$ , respectively, with growth time, 30 to 270, days. The bold text in Table 2 at 150 days of growth is the detailed simulation illustrated in Table 1. The mass of the fish over the period increases some 34 times from 0.042 to 1.436, kg.

Table 1: Generic simulation procedure showing the model inputs, calculations and outputs. The particular illustration is for **GSM** accumulation in rainbow trout fish-flesh at 150 days of growth in RAS tanks

Inputs			
line			
1	$t$	day	150
2	$k_{ow}$	dimensionless	3.57
3	$k_m$	$\text{day}^{-1}$	0.00063
4	$C_w$	$\mu\text{g L}^{-1}$	0.0066
5	$T$	$^{\circ}\text{C}$	14
6	$e$	dimensionless	0.045
Calculations			
7	$C_{ox}$	$\text{mg L}^{-1}$	9.75, (Eq.12)
8	$G_v$	$\text{L day}^{-1}$	61.88, (Eq.11)
9	$E_w$	dimensionless	0.528, (Eq.10)
10	$k_1$	$\text{L kg}^{-1}\text{day}^{-1}$	119.29, (Eq.9)
11	$Q_w$	$\text{day}^{-1}$	40.62, (Eq.14)
12	$Q_L$	$\text{day}^{-1}$	0.406, (Eq.15)
13	$V_L$	kg	0.012, (Eq.16)
14	$k_2$	$\text{day}^{-1}$	0.863, (Eq.13)
15	$k_G$	$\text{day}^{-1}$	0.00065, (Eq.18)
16	$k_g$	$\text{day}^{-1}$	0.00128, (Eq.17)
17	$m_f$	kg	0.274, (Eq.19)
18	$a$	$\text{day}^{-1}$	0.8668, (Eq.5)
19	$b$	$\mu\text{g kg}^{-1}\text{day}^{-1}$	0.04094, (Eq.4)
20	$y$	dimensionless	0.0138, (Eq.19)
Output			
21	$y$	$\mu\text{g kg}^{-1}$	<b>0.3573</b> , (Eq.8)

#### 3.2 Predicted MIB

Table 3 presents a summary of the model predictions for taint as **MIB** in RAS rainbow trout fish-flesh for the growth period 30 to 270 day. The value of taint in the table is seen to range from 0.0140 to  $0.5621 \mu\text{g kg}^{-1}$ .

#### 3.3 Accumulation of GSM and MIB

To visualise clearly the accumulating taint as **GSM** and **MIB** the data of Tables 2 and 3 are presented as Figure 3 together with the consumer rejection threshold

for off-flavour for both taint molecules. From the figure, it can be seen there is a rapid accumulation of **GSM** and **MIB** in the rainbow trout fish-flesh in the period following 100 days of growth. Viewed this way, the data highlight that accumulation of taint as **GSM** and **MIB** in the fish-flesh has an exponential pattern. The consumer threshold for **GSM** is  $0.9 \mu\text{g L}^{-1}$  and for **MIB** is  $0.7 \mu\text{g L}^{-1}$  (Robertson et al. 2005).

Table 2: Predicted taint ( $y$ ) as **GSM** in rainbow trout fish-flesh with growth-time ( $t$ ) in RAS tanks with  $T = 14 \text{ }^{\circ}\text{C}$  and  $C_w = 0.0066 \mu\text{g L}^{-1}$  (Petersen et al. 2011). (The **bold text** at 150 days is the detailed illustrative simulation presented in Table 1)

$t$ (day)	$m_f$ (kg)	$k_1$ ( $\text{L kg}^{-1}\text{day}^{-1}$ )	$k_2$ ( $\text{day}^{-1}$ )	$k_g$ ( $\text{day}^{-1}$ )	$y$ ( $\mu\text{g kg}^{-1}$ )
15	0.042	228.98	1.818	0.0016	0.0508
30	0.052	212.98	1.673	0.0015	0.0631
60	0.079	184.25	1.418	0.0015	0.0973
90	0.119	159.40	1.201	0.0014	0.1502
120	0.181	137.89	1.018	0.0013	0.2317
<b>150</b>	<b>0.274</b>	<b>119.29</b>	<b>0.862</b>	<b>0.0013</b>	<b>0.3573</b>
180	0.414	103.20	0.731	0.0012	0.5512
210	0.627	89.28	0.619	0.0012	0.8502
240	0.949	77.24	0.525	0.0011	1.3112
270	1.436	66.82	0.444	0.0010	2.0219

Table 3: Predicted taint ( $y$ ) as **MIB** in rainbow trout fish-flesh with growth-time ( $t$ ) in RAS tanks at  $T = 14 \text{ }^{\circ}\text{C}$  and  $C_w = 0.0032 \mu\text{g L}^{-1}$  (Petersen et al. 2011)

$t$ (day)	$m_f$ (kg)	$k_1$ ( $\text{L kg}^{-1}\text{day}^{-1}$ )	$k_2$ ( $\text{day}^{-1}$ )	$k_g$ ( $\text{day}^{-1}$ )	$y$ ( $\mu\text{g kg}^{-1}$ )
15	0.042	224.92	3.238	0.0016	0.0140
30	0.052	209.20	2.981	0.0015	0.0174
60	0.079	180.98	2.526	0.0015	0.0269
90	0.119	156.56	2.140	0.0014	0.0415
120	0.181	135.44	1.814	0.0013	0.0641
<b>150</b>	<b>0.274</b>	<b>117.17</b>	<b>1.537</b>	<b>0.0013</b>	<b>0.099</b>
180	0.414	101.37	1.302	0.0012	0.1528
210	0.627	87.69	1.103	0.0012	0.2359
240	0.949	75.86	0.935	0.0011	0.3641
270	1.436	65.63	0.792	0.0010	0.5621

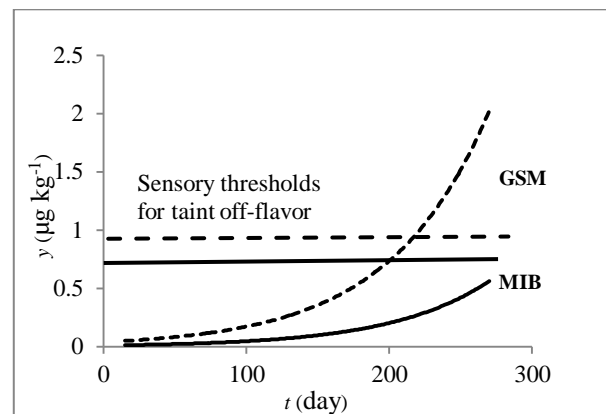


Figure 3: Predicted taint as **GSM** (---) and **MIB** (—) in rainbow trout with RAS growth-time at growth water temperature  $T = 14 \text{ }^{\circ}\text{C}$  with  $C_w$ , respectively, 0.0066 and 0.0032,  $\mu\text{g L}^{-1}$ . The threshold for **GSM** is 0.9, and for **MIB** is  $0.7 \mu\text{g L}^{-1}$  (Robertson et al. 2005)

#### 4. DISCUSSION

It can be seen clearly from the continuous plot of data as Figure 3 that the predicted concentration of **MIB** in rainbow trout fish-flesh is less than that for **GSM** at any growth-time over the period 30 to 270 days. Both curves are however exponential with growth time in the RAS tanks.

The reported sensory threshold for **GSM** in rainbow trout fish-flesh is  $0.9 \mu\text{g kg}^{-1}$  (Robertson et al. 2005). As seen from Figure 3 this threshold is predicted to be reached at about 220 days growth in the RAS tanks.

At harvest (270 days) it is seen from Figure 3 the predicted concentration of **MIB** is  $0.5621 \mu\text{g kg}^{-1}$  (see also Table 2). Given the threshold reported for **MIB** is  $0.7 \mu\text{g kg}^{-1}$  (Robertson et al. 2005) it is concluded that this value will not be reached in a typical RAS growth.

Significantly, **GSM** concentration in the harvested fish is around 3.5 times that predicted for **MIB**. This difference can be attributed to the initial higher  $C_w = 0.0066 \mu\text{g L}^{-1}$  for **GSM** compared with  $C_w = 0.0032 \mu\text{g L}^{-1}$  for **MIB** used in the simulations. Possible reasons for the reported difference in these  $C_w$  values (Petersen et al. 2011) for the two molecules include the diversity of taint causing micro-organisms involved and the different aqueous solubility of the molecules (Jüttner and Watson 2007, Pirbazari et al. 1992). In general, cyanobacteria and actinomycetes are considered to be the major contributors to taint and they are able to produce either **GSM** or **MIB**. Interestingly, certain species are capable of producing both molecules (Jüttner and Watson 2007).

Given the reported consumer sensory threshold for **MIB** in rainbow trout fish-flesh is  $0.70 \mu\text{g kg}^{-1}$  (Robertson et al. 2005) this threshold is not exceeded with typical growth water concentration of  $C_w = 0.0032 \mu\text{g L}^{-1}$ .

The model simulations have been predicted on a constant value of  $C_w$  in the growth water with growth-time ( $t$ ). This may not always be the case and  $C_w$  may vary with growth-time in the tanks due to various factors that include environmental (light, growth water temperature), nutrient(s) load, change(s) of water volume, and the (complex) dynamic nature of the taint causing bacteria. The impact of changing  $C_w$  with time was therefore investigated using simulations of the model.

##### 4.1. Impact of $C_w$ on GSM accumulation in fish-flesh

The impact of varying taint concentration in the growth water ( $C_w$ ) on accumulation in the fish-flesh for both taint molecules can readily be modelled as a function of growth-time,  $t$ , in the tanks.

A suitable form is likely to be increasing  $C_w$  with time in either a linear, or, a moderately-exponential form.

To illustrate this for **GSM**, it is assumed there is a linear dependence with a reasonable estimate for  $30 \leq t \leq 270$  day given by:

$$C_w = 0.0052 + 0.0001t \quad (20)$$

Eq. (20) has been synthesized to predict, respectively, a mean value of  $C_w = 0.0213$ , a minimum, 0.0066 and maximum, 0.0361,  $\mu\text{g L}^{-1}$  at each of 15, 135 and 270 days growth.

Repeat simulations of the model (Table 1) using Eq. (20) to vary  $C_w$  were carried out, and; the predicted results for **GSM** in the flesh of the rainbow trout are presented in Table 4.

Table 4: Predicted taint ( $y$ ) as **GSM** in rainbow trout fish-flesh at varying concentration of  $C_w$  (Eq. (20)) with growth-time ( $t$ ) in RAS tanks

$t$ (day)	$m_f$ (kg)	$C_w$ ( $\mu\text{g L}^{-1}$ )	$y$ ( $\mu\text{g kg}^{-1}$ )
15	0.042	0.0067	0.0515
30	0.052	0.0082	0.0776
60	0.079	0.0112	0.1604
90	0.119	0.0142	0.3078
120	0.181	0.0172	0.5638
150	0.274	0.0202	1.0009
180	0.414	0.0232	1.7369
210	0.627	0.0262	2.9627
240	0.949	0.0292	4.9853
270	1.436	0.0322	8.2970

Figure 4 compares and summarizes the impact of assumptions regarding  $C_w$  on the accumulation of **GSM** in the fish-flesh. It is clearly seen in the figure that at harvest taint is 4.1 times if  $C_w$  is assumed to vary with growth-time in the RAS tanks according to Eq. (20). The exact dependence is not known at present. The model simulations have demonstrated a practical utility (and flexibility) in illustrating the impact however.

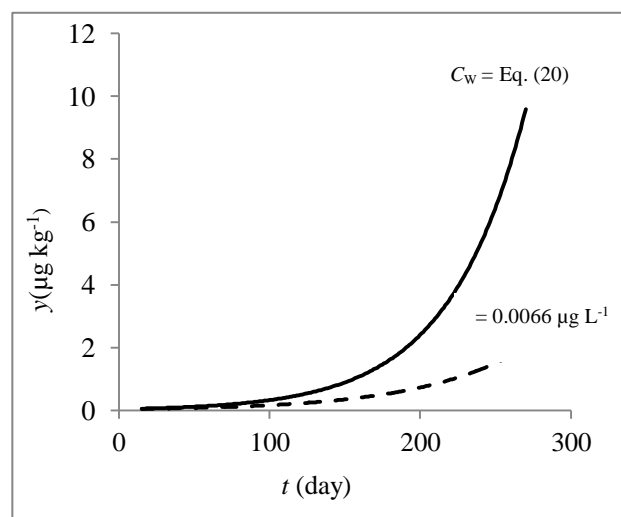


Figure 4: Predicted taint ( $y$ ) as **GSM** in rainbow trout fish-flesh with  $C_w$  constant ( $= 0.0066 \mu\text{g L}^{-1}$ ) and  $C_w$  varying (Eq. (20)), with growth-time ( $t$ )

##### 4.2 Model validation

A model validation was carried out using the independent experimental data of Petersen et al. (2011)

for rainbow trout farmed in open recirculation aquaculture in Denmark.

The mean growth water temperature was 12 °C and the mean mass of the fish at harvest was 0.30 kg. Model inputs and rate constants were computed as described in Section 2.

A comparison of the experimental data ( $n = 14$ ) and model predictions is summarized as Figure 5.

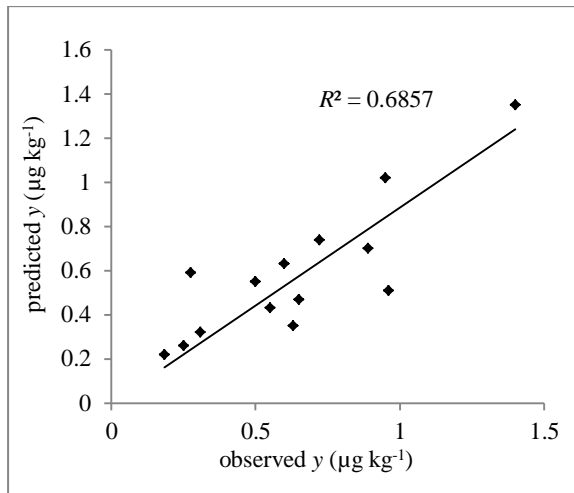


Figure 5: Relationship between observed and predicted **GSM** ( $y$ ) in rainbow trout

The figure shows a good fit to these independent data with  $R^2 = 0.68$  (Snedecor and Cochran 1989). Strictly, the (open) tank system of Petersen et al. (2011) would not be as easily controlled as RAS. The overall good fit suggests the model simulation is free of (gross) computational and programing errors.

#### 4.3 Applying the model to RAS growth protocols to minimise taint taste

A significant advantage of the validated model for taint taste is that it can be used to investigate growth protocols to minimize taint. For example, simulations underscore consumer acceptance of RAS rainbow trout at harvest of 270 day if the RAS water concentration  $C_w$  for **GSM** and **MIB** is maintained at a value, respectively, less than 0.0028, and 0.004  $\mu\text{g L}^{-1}$ .

Clearly, a range of farming practices can be investigated in this way without recourse to further unlimited experimental trials.

#### 4.4 Benefits of the model to the supply chain

Analyses of taint molecules as **GSM** and **MIB** in fish-flesh is difficult, expensive and time consuming and few research institutes have the necessary facilities.

Judicial use of the validated model however can assist famers however to develop growth protocols to result in fish at harvest that have taint at levels lower than the consumer threshold without recourse to extensive analytical analyses. It is clear a key parameter in impacting taint taste is the value of  $C_w$ .

Significantly, the quantitative nature of the model suggests it could be applied through the application of automatic growth controls.

#### 4.5 Experimental studies

Indeed at present, extensive experimental studies are underway by the authors using automatic-controlled dosing of hydrogen peroxide (*ProMinent* Pty Ltd) as an environmental benign treatment to regulate  $C_w$  of the growth-water (*Global Pumps* Pty Ltd) in a commercial farm in Australia (Hathurusingha and Davey 2013).

Although the particular fish is barramundi (*Lates calcarifer*), a premium export protein from Australia, the generalized form of the model will mean findings will be applicable to a range of species.

It is planned the model will be widely used to provide insight into establishing effective growth protocols on resulting taint taste in fish-flesh and result in the elimination of algaecides such as copper sulphate, and periods of purging post-harvest (Tucker 2000). A significant reduction in production costs is expected, together with enhanced consumer acceptance.

### 5. CONCLUSIONS

A generalized, new transient model for predicting taint taste accumulation in flesh of farmed fish has shown a key parameter is the concentration of taint molecule (as either **GSM** or **MIB**) in the growth-water.

For rainbow trout (*Oncorhynchus mykiss*), which is typically harvested at 270 days, the consumer threshold for **GSM** of 0.9  $\mu\text{g kg}^{-1}$  will have been exceeded at 220 days growth with usual commercial practices. For **MIB** the threshold (0.70  $\mu\text{g kg}^{-1}$ ) will not be reached prior to typical harvest.

Model validation against independent (limited  $n = 14$ ) data suggests that accumulation of taint as **GSM** and **MIB** in fish-flesh can be reliably modelled as the sum of an exponential uptake and exponential elimination from the fish-flesh.

Importantly, the generalized form of the model means it can be applied to a range of fish species to investigate a range of growth farming protocols, and; that it could be applied to commercial practice using automatic controls.

The model can be set-up in standard spreadsheeting to facilitate ready communication with a range of users of varying sophistication.

#### NOMENCLATURE

The equation given in parentheses after description refers to that in which the symbol is first used or defined

- $a$  taint elimination coeff.,  $\text{day}^{-1}$  Eq. (5)
- $b$  taint accumulation coeff.,  $\mu\text{g kg}^{-1} \text{day}^{-1}$  Eq. (4)
- $C_{\text{ox}}$  dissolved oxygen,  $\text{mg L}^{-1}$  Eq. (11)
- $C_w$  taint (as **GSM** or **MIB**) in water,  $\mu\text{g L}^{-1}$
- $\frac{dy}{dt}$  rate of change of taint,  $\mu\text{g kg}^{-1} \text{day}^{-1}$  Eq. (1)
- $e$  lipid mass ratio, dimensionless Eq. (16)

$E_w$  chemical uptake efficiency across gill, dimensionless Eq. (9)

**GSM** geosmin

$G_v$  gill ventilation rate, L day<sup>-1</sup> Eq. (9)

$m_f$  mass of the fish, kg Eq. (2)

$k_1$  taint accumulation gills, L kg<sup>-1</sup> day<sup>-1</sup> Eq. (2)

$k_2$  taint elimination gills, day<sup>-1</sup> Eq. (2)

$k_g$  elimination through gills and metabolic transformation, day<sup>-1</sup> Eq. (2)

$k_G$  rate const. growth dilution, day<sup>-1</sup> Eq. (17)

$k_m$  rate const. metabolic transformation, day<sup>-1</sup> Eq. (17)

$K_{OW}$  octanol-water partition coefficient, dimensionless Eq. (10)

**MIB** 2-methylisoborneol

$Q_w$  chemical transport aq. phase, L day<sup>-1</sup> Eq. (13)

$Q_L$  chemical transport lipid phase, L day<sup>-1</sup> Eq. (13)

$R^2$  correlation coefficient

**RAS** Recirculating Aquaculture System

$T$  temperature, °C Eq. (12)

$t$  time, day (Eq. (1))

$V_L$  lipid mass, kg Eq. (13)

$y$  taint (as **GSM** or **MIB**), µg kg<sup>-1</sup> Eq. (1)

#### Greek symbols

$\beta$  pre-exponential constant Eq. (3), Eq. (22)

$\gamma$  exponential constant Eq. (3), Eq. (22)

$e$  lipid mass ratio, dimensionless Eq. (16)

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