A predictive model for taste taint accumulation in Recirculating Aquaculture Systems (RAS) farmed-fish – demonstrated with geosmin (GSM) and 2-methylisoborneol (MIB)

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ABSTRACT

The accumulation of “earthy” or “muddy” off-flavours due to taste taint accumulation as geosmin (GSM) or 2-methylisoborneol (MIB) in the flesh of fish from Recirculated Aquaculture Systems (RAS) is a major concern globally. To aid RAS farm management a time dependent concentration predictive model was developed. The model is a sum of two exponential terms with time that simulate simultaneous taint uptake and elimination. Illustrative simulations for RAS barramundi (Lates calcarifer) a premium fish grown at a water temperature of 28 °C show that the threshold for consumer rejection of 0.70 μg kg⁻¹ MIB will be reached at 225 days. At a typical RAS harvest of 240 days the concentration of MIB is predicted to be four (4) times that for GSM. Because model predictions showed good agreement with independent data for both RAS barramundi and rainbow trout (Oncorhynchus mykiss) it was concluded the model was free of programming and computational errors and of a generalized form. Model simulations revealed a 5 °C variance in RAS growth temperature did not meaningfully impact taint accumulation as either GSM or MIB. A major benefit to the RAS industry is that model simulations can be used to investigate a range of growth protocols in RAS farming to limit taint. An advantage is the model can be conveniently simulated in standard spread-sheeting tools.

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1. Introduction

Fish farmed in Recirculating Aquaculture Systems (RAS) is a globally important alternative to capture of wild-fish. RAS is increasingly popular due to a higher production per unit area, less water and land usage, year-round production, and; better control of the fish-rearing environment (Ebeling and Timmons, 2012).

A drawback however is the potential for accumulation in the RAS growth water, and consequently fish-flesh, of unwanted taint as off-flavours and unpleasant odours. Of particular interest are geosmin (GSM) and 2-methylisoborneol (MIB). These semi-volatile, secondary metabolites are produced by planktonic and benthic cyanobacteria and actinomycetes (Wood et al., 2001; Guttmann and van Rijn, 2009; Tucker, 2000). The use of high nutrient load and high fish-stocking densities in RAS appear to promote these taste taint producing micro-organisms. Taint at very low concentration in RAS water, for example 0.015 μg L⁻¹ GSM or 0.018 μg L⁻¹ MIB (Persson and York, 1978; Persson, 1980), can result in, respectively, 0.056 and 0.072 μg kg⁻¹ in the fish-flesh (Howgate, 2004). Consumers can readily detect taint as “earthy” or “muddy” off-flavour and unpleasant odour at these levels and there is strong buyer resistance.

Taste taint in fish-flesh due to GSM and/or MIB in RAS has been reported for barramundi (Lates calcarifer) (Percival et al., 2008), Murray cod (Macquaria mosstella peelli) (Palmeri et al., 2008), rainbow trout (Oncorhynchus mykiss) (Robertson et al., 2006), arctic char (Salvelinus alpinus) (Houle et al., 2011), largemouth bass (Micropterus salmoides), and; white sturgeon (Acipenser transmontanus) (Schrader et al., 2005). The dynamics of the RAS growth and taint environment are complex, with the quantity of GSM/MIB in the water and fish-flesh varying with micro-organism, system location, water nutrient(s), fish species and size, and; portion of the fish which is assayed (Howgate, 2004; Percival et al., 2008).

Effective husbandry practices to control taste taint in farmed fish have not been established (Howgate, 2004). At present, purging post-growth in clean water of RAS fish prior to marketing is widely used to leach taint (Tucker and van der Ploeg, 1999); for example, seven (7) days purge for farmed barramundi (Southern Barramundi Farmer’s Association, Clarendon, Australia, pers. comm.). In addition, copper sulphate is a widely used as an algicide in RAS production tanks, but effective treatment protocols are

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not understood. This has led to excessive use of copper sulphate and ineffective application (Rimando and Schrader, 2003). Repetitive application seems to have resulted in developing copper-resistance within cyanobacteria (García-Villada et al., 2004; Shavyrina et al., 2001). Guttman and van Rijn (2009) report that aerobic bio-filters in RAS have not been successful in controlling taint. Other methods that have been tried to restrict taint, include: control of nutrients in tanks, use of organic-rich beds or absorbents (activated carbon), oxidizing agents (potassium permanganate, ozone), and; UV irradiation of the water. These have however not proved practicable (S. Poole, Department of Primary Industries, Brisbane Australia, unpublished data). Because chemical analyses of fish-flesh are costly and time-consuming, taint assessment at present is largely dependent on sensory evaluation by industry “experts” (Percival et al., 2008).

Quantitative mathematical models, widely used in the chemical engineering and process foods industries, offer the potential for insight, management, and ultimately control of taint in RAS farmed-fish. Bio-magnification, bio-concentration and food-web models have been used based on steady-state assumptions where the flux of the chemicals into and from the fish-flesh is zero (Clark et al., 1990). However, it is questionable to us whether equilibrium assumptions can be reasonably made for practical RAS systems; it is apparent there is no empirical evidence that the net chemical exchange rate is zero between the RAS water and fish-flesh phases.

Against this background, a new quantitative process model to predict concentration of taint, as either GSM or MIB, was developed to aid RAS management. The aim was to produce a quantitative guide for RAS farming practice that could be applied to minimize taint in fish-flesh.

The model is developed from a whole-of-process perspective and is based on conservation of mass and energy principles (Foust et al., 1980) and thermodynamic processes established in (bio)chemical engineering (Bailey and Ollis, 1986). It is illustrated with independent data for farmed barramundi (L. calcarifer), an important RAS fish in Australia and globally. The applicability of a generalized form of the model for prediction for other aquaculture species, in particular rainbow trout (O. mykiss), is also assessed with independent data. Model trends are shown to agree well with the (necessarily fragmented) published data from the wider literature. The benefit of a model for taint in assessing a range of farm growth protocols and the longer term need for an experimental evaluation is discussed.

2. Materials and methods

2.1. Predictive model development

The controlling route for chemical uptake into fish-flesh is widely assumed to be the gills (Persson, 1984); ingestion of taint through the skin or via feed is considered negligible (McKain et al., 1996; Nichols et al., 1996). Elimination of taint from the fish-flesh is assumed to occur through the gills and with both reduced concentration of taint molecules in the fish-flesh with fish growth plus metabolic transformations within the fish-flesh. Because RAS fish are harvested before egestion or reproduction any taint loss due to these can be reasonably ignored. Typically the RAS system is uniform in temperature. For example, for farmed barramundi in Australia, RAS water is carefully maintained at all times and seasons at a bulk temperature of 28°C through heated and insulated growth–tanks (Southern Barramundi Farmer’s Association, Clarendon, Australia, pers. comm.).

Consider the uptake and elimination of taint molecules (as GSM and MIB) in RAS farmed-fish as illustrated schematically in Fig. 1. (All symbols used are carefully defined in the Nomenclature at the beginning of this paper.)

Let \( y (\mu g \cdot kg^{-1}) \) = the concentration of taint as either GSM or MIB in the fish-flesh and \( C_W \) = the concentration (\( \mu g \cdot L^{-1} \)) of taint as either GSM or MIB in the RAS water at any time, \( t \). The rate of change of taint (\( y \)) in the fish-flesh with time (\( t \)) is therefore given by:

\[
\frac{dy}{dt} = \text{uptake rate} - \text{elimination rate} \tag{1}
\]

The uptake rate of either GSM or MIB will be a function of the rate constant for uptake (\( k_1, L \cdot kg^{-1} \cdot day^{-1} \)), 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.

<table>
<thead>
<tr>
<th>Nomenclature</th>
</tr>
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<tbody>
<tr>
<td>The equation given in parentheses after description refers to that in which the symbol is first used or defined.</td>
</tr>
<tr>
<td>( a )</td>
</tr>
<tr>
<td>( b )</td>
</tr>
<tr>
<td>( C_{OX} )</td>
</tr>
<tr>
<td>( C_W )</td>
</tr>
<tr>
<td>( d )</td>
</tr>
<tr>
<td>( dy/dt )</td>
</tr>
<tr>
<td>( e )</td>
</tr>
<tr>
<td>( E_W )</td>
</tr>
<tr>
<td>( GSM )</td>
</tr>
<tr>
<td>( G_V )</td>
</tr>
<tr>
<td>( k_1 )</td>
</tr>
<tr>
<td>( k_2 )</td>
</tr>
<tr>
<td>( k_b )</td>
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<tr>
<td>( k_G )</td>
</tr>
<tr>
<td>( k_m )</td>
</tr>
<tr>
<td>( K )</td>
</tr>
<tr>
<td>( K_{OW} )</td>
</tr>
<tr>
<td>( m_f )</td>
</tr>
<tr>
<td>( m_{f,\text{in}} )</td>
</tr>
<tr>
<td>MIB</td>
</tr>
<tr>
<td>( n )</td>
</tr>
<tr>
<td>( Q_W )</td>
</tr>
<tr>
<td>( Q_L )</td>
</tr>
<tr>
<td>( R^2 )</td>
</tr>
<tr>
<td>RAS</td>
</tr>
<tr>
<td>( T )</td>
</tr>
<tr>
<td>( t )</td>
</tr>
<tr>
<td>( t_0 )</td>
</tr>
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<td>VBGF</td>
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<td>( y )</td>
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<table>
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<th>Greek symbols</th>
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<tr>
<td>( \beta )</td>
</tr>
<tr>
<td>( \gamma )</td>
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</table>
through the gills ($k_2$, day$^{-1}$), the rate constant for growth dilution plus metabolic transformation ($k_3$, day$^{-1}$) and the instantaneous value of $y$ at time, $t$. Substitution of these parameters into Eq. (1) gives:

$$\frac{dy}{dt} = k_1 m_t C_W - (k_2 + k_g) y$$

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

The mass of fish ($m_t$) grown for harvest 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 (Glenn et al., 2007; dos Santos et al., 2008, 2013) (see below Eq. (19) for barramundi) and fish mass is therefore given by:

$$m_t = \beta e^{\alpha t}$$

Let:

$$b = \beta k_1 C_W$$

and

$$a = (k_2 + k_g)$$

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

$$\frac{dy}{dt} + ay = b e^{\alpha t}$$

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

$$y = \frac{b}{a + \gamma} \left( e^{\gamma t} - e^{\alpha t} \right)$$

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

$$y = \left( \frac{b}{a + \gamma} \right) [e^{\alpha t} - e^{\gamma t}]$$

The model for taste 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 barramundi fish) for immediate simulation of taint. For model development these rate constants need to be defined mathematically.

2.2. Rate constants $k_1$, $k_2$ 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:

$$k_1 = \frac{E_W G_V}{m_t}$$

where $E_W$ is the gill chemical uptake efficiency (dimensionless fraction) and $G_V$ is the gill ventilation rate (L day$^{-1}$). The chemical uptake efficiency was, reasonably, assumed by Gobas (1998) 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:

$$E_W = (1.85 + 155/K_{OW})^{-1}$$

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

$$G_V = \frac{1400m_t^{0.5}}{C_{OX}}$$

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

$$C_{OX} = 14.45 - 0.413T + 0.00556T^2$$

where $T$ = RAS growth water temperature in degree Celsius. The assumption of saturated growth water is justified in RAS because, in conjunction with temperature control, aerator-mixers are continuously employed to ensure optimum fish growth (Southern Barramundi Farmer’s Association, Clarendon, Australia, pers. comm.).

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:

$$\frac{1}{k_2} = \left( \frac{V_L}{Q_{OW}} \right) K_{OW} + \left( \frac{V_L}{Q_{L}} \right)$$

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

$$Q_W = 88.3m_t^{0.6 \pm 0.2}$$

Arnot and Gobas (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:

$$Q_L = 0.01 Q_W$$

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

$$V_L = e m_t$$

The rate constant for growth dilution plus metabolic transformation of the taint chemical ($k_3$) is given by:

$$k_3 = (k_C + k_m)$$

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

$$k_C = 0.00251 m_t^{-0.2}$$

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 $k_m = 0.00063$ day$^{-1}$.

Eqs. (1)–(18) plus the general value for $k_m$, defines the model for taste taint in RAS fish.

The model can be conveniently set-up and solved as a Microsoft Excel$^\text{TM}$ spreadsheet. The widespread use of these tools means that model communication can be streamlined.
2.3. Illustrative simulations for GSM in barramundi (L. calcarifer)

Barramundi (L. calcarifer) has a high demand as a premium protein in the seafood market and is becoming globally popular as an RAS-farmed fish. Interest is due to a relatively fast growth, tolerance to production handling and adaptability for high stocking densities. In Australia, RAS-farmed barramundi that are grown in 28 °C water are harvested at 0.80 kg live mass after a 240 day growth cycle, unless there is a special buyer requirement (Southern Barramundi Farmer’s Association, Clarendon, Australia, pers. comm.). A typical, detailed growth curve (n = 9) for barramundi is presented by Glenn et al. (2007) and is reproduced as Fig. 2.

The data are from pond growth systems. However in the absence of specific information for RAS, these data were correlated with growth time to give β = 0.0519 and γ = 0.0133 respectively. The high correlation coefficient (R² = 0.96) indicates a very good fit (Snedecor and Cochrán, 1989). For the purpose of model illustration it is assumed the growth of barramundi in RAS will be very similar. Therefore Eq. (3) becomes:

\[ m_t = 0.0519 \exp^{0.0133t} \]

A detailed, step-wise simulation of the model can now be made. The steps are conveniently summarized as Table 1. For example, Lines 1–6 of the table are the input parameters and corresponding check on units. Lines 7–20 are the calculations, results and the relevant equations used. Line 21 is the level of predicted taint. The particular simulations in the table, columns 4 and 5 are respectively simulation of taint as GSM and MIB, in barramundi fish-flesh at 150 day of growth in the RAS tanks.

The predicted level of taint taste in the barramundi fish-flesh at 150 days of growth is seen from the table to be for GSM, \( y = 0.0618 \mu g \frac{kg}{kg} \) and that for MIB, \( y = 0.2514 \mu g \frac{kg}{kg} \).

To complete the simulation of RAS growth of barramundi and accumulation of GSM and MIB in the fish-flesh these calculations are repeated for a range of values, 30 ≤ t ≤ 240 day. This covers the growth period from fingerling at 30 day to harvest of the fish at 240 day.

3. Results

Table 2 summarizes the model predictions for taste taint as GSM and MIB in RAS barramundi fish-flesh for the growth period 30–240 day in RAS tanks. The table shows that \( k_1 \), \( k_2 \) and \( k_3 \) values decrease with increasing growth time. For example, the level of taint as GSM ranges from 0.0115 to 0.2166 \( \mu g \frac{kg}{kg} \), respectively, with growth time, 30–240 days. The bold text in the table at 150 days of growth is the simulation illustrated in Table 1.

To visualize these taint data, a continuous plot with growth time in the RAS tanks is presented as Fig. 3. The data for MIB are superimposed and compared with those for GSM in the figure to permit a direct visual comparison of the predicted accumulation of the two taint molecules. These data highlight accumulation of taste taint in fish-flesh as an exponential pattern.

From the figure, it can be seen for example there is a predicted rapid accumulation of GSM in the barramundi fish-flesh in the period following 100 days of growth.

4. Discussion

It can be seen from Fig. 3 that the predicted level of GSM in barramundi fish-flesh is much less than that for MIB at any growth time over the period 30–240 days. Both curves are however exponential with growth time in the RAS tanks.

Generally, the reported sensory threshold for GSM in barramundi fish-flesh that can make the fish taste earthy and muddy is considered to be 0.74 \( \mu g \frac{kg}{kg} \) (Jones et al., 2013). As can be seen in Fig. 3 this concentration is predicted to be reached at about 225 days growth in the RAS tanks. At harvest at 240 days the concentration of MIB is predicted to be 0.8912 \( \mu g \frac{kg}{kg} \). Significantly, this is four (4) times the predicted concentration of GSM in the barramundi fish-flesh. This difference can be attributed to the initial lower \( C_{W1} \) for GSM (0.0004 \( \mu g \frac{L}{kg} \)) at about 1/10 that for MIB (0.003 \( \mu g \frac{L}{kg} \)) (Glencross et al., 2007) used in the simulations. Possible reasons for the difference in values for \( C_{W1} \) for the two molecules include the diversity of taint causing micro-organisms involved and the different aqueous solubility of the taint off-flavour molecules (Jütter and Watson, 2007; Pirbazari et al., 1992). In general, cyanobacteria and actinomyctes are considered to be the major contributors to taste taint and they are able to produce either GSM or MIB. Interestingly, certain species are capable of producing both molecules (Jütter and Watson, 2007). Additionally, the aqueous solubility of GSM is less than that for MIB (respectively, 150 and 194.5 mg L−1) (Pirbazari et al., 1992) that would lead to higher levels of MIB in growth water when both molecules are produced.
Although for MIB there is no reported consumer sensory threshold for barramundi fish-flesh, it can be reasonably assumed this will be about 0.70 μg kg⁻¹; this is because this is the concentration also reported for similar fish species, e.g., both channel catfish (Ictalurus punctatus) and rainbow trout (O. mykiss) (Robertson et al., 2005). It is interesting therefore that the concentration registered for off-flavour for both taint molecules in the fish-flesh is nearly identical. This may be attributed to the similar physical and chemical properties of these two molecules. For instance, both these molecules are tertiary alcohols with the octanol–water partition coefficient less than 6. The octanol–water partition coefficient is considered to be the major criterion which predominantly determines the chemical uptake route (Clark et al., 1990). Moreover, their molecular densities (~0.9) and refractive indices of reference (~1.4) are also similar (Pirbazari et al., 1992).

The simulation results have been predicted on a constant value CW for taint concentration in the growth water with growth time (t). This may not always be the case and CW may vary with growth time in the tanks (Department of Agriculture Fisheries and Forestry, Brisbane, Australia (DAFF) S. Poole, pers. comm.). The impact of changing CW with time was therefore investigated using simulations of the model.

### 4.1. Impact of varying GSM concentration in growth water (CW) on accumulation in fish-flesh

The impact of varying taint concentration in the growth water (CW) 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 CW with time in either a linear, or a moderate-exponential form.

To illustrate this for GSM, it is assumed there is a linear dependence with a reasonable estimate for 30 ≤ t ≤ 240 day given by:

\[
CW = 0.0002 + 2 \times 10^{-6} t
\]  

Eq. (20) has been fitted to predict, respectively, a mean value of CW = 0.0004, a minimum, 0.0002 and maximum, 0.0006 μg L⁻¹ at each of 30, 120 and 240 days growth. Fig. 4 summarizes the impact of varying CW. The figure reveals that the taint concentration in barramundi fish-flesh at 240 days is ~49 times greater than that for 30 days (0.0075 μg kg⁻¹) growth for GSM.

Results are compared in Fig. 4 with the predictions for constant CW = 0.0004 μg L⁻¹ GSM. It can be seen that at harvest at 240 days growth there would be expected to be a greater taint concentration

### Table 1

Model simulation procedure showing inputs, calculations and output for predicted taste taint as both GSM and MIB in barramundi fish-flesh at 150 day of growth in RAS tanks.

<table>
<thead>
<tr>
<th>Parameter and units</th>
<th>Inputs</th>
<th>Calculations</th>
<th>Output</th>
</tr>
</thead>
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<tr>
<td>Line</td>
<td>t</td>
<td>mg L⁻¹</td>
<td>y μg kg⁻¹</td>
</tr>
<tr>
<td>1</td>
<td>t</td>
<td>day</td>
<td>150</td>
</tr>
<tr>
<td>2</td>
<td>k₉₆₉₁</td>
<td>dimensionless</td>
<td>3.57</td>
</tr>
<tr>
<td>3</td>
<td>k₉₆₁</td>
<td>day⁻¹</td>
<td>0.00063</td>
</tr>
<tr>
<td>4</td>
<td>C₉₆</td>
<td>μg L⁻¹</td>
<td>0.0004</td>
</tr>
<tr>
<td>5</td>
<td>T</td>
<td>°C</td>
<td>28</td>
</tr>
<tr>
<td>6</td>
<td>e</td>
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</tr>
</tbody>
</table>

### Table 2

Predicted taste (y) as GSM and MIB in barramundi fish-flesh with growth time (t) in RAS tanks at (Glencross et al., 2007) constant CW = 0.0004 μg L⁻¹ for GSM and CW = 0.003 μg L⁻¹ for MIB. (The bold text for 150 day is the detailed illustrative simulation presented in Table 1).

<table>
<thead>
<tr>
<th>t (day)</th>
<th>m₁ (kg)</th>
<th>kₙ (day⁻¹)</th>
<th>k₁ (L kg⁻¹ day⁻¹)</th>
<th>k₂ (day⁻¹)</th>
<th>y (μg kg⁻¹)</th>
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<tbody>
<tr>
<td></td>
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<td>MIB</td>
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<td>30</td>
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</table>
in the fish-flesh of GSM for RAS growth with varying $C_W$. The ratio is 1:1.75.

The flexibility of the new model for taint accumulation also permits study of the impact of changes in the RAS growth water temperature.

4.2. Impact of varying growth water temperature ($T$) on taint accumulation in fish-flesh

In commercial RAS barramundi farming, a constant growth water temperature might not be always be maintained, due to faulty maintenance or partial power failure etc. To illustrate the impact of varying water temperature on taint taste, a $5\, ^\circ C$ decrease and increase in water temperature is assumed practical. The effect of this temperature variation on fish growth/physiology is assumed negligible and is therefore ignored.

At the resulting two different temperatures of 23 and $33\, ^\circ C$, $k_1$ values (Eqs. (9)–(12)) for GSM at, 150 day fish growth are, respectively, 131.60 and 151.14 kg $^{-1}$ day $^{-1}$. Substitution and simulation at 23 and $33\, ^\circ C$ showed, respectively, taint (y) as GSM of 0.0581 and 0.0665 g kg $^{-1}$. Repeat calculations with the model gave similar results for taint as MIB. For example, simulation results for taste taint at 23 and $33\, ^\circ C$ were, respectively, 0.2350 and 0.2687 g kg $^{-1}$.

The likely impact of a $\pm 5\, ^\circ C$ change in growth water temperature in RAS systems therefore did not meaningfully impact taint accumulation in the fish-flesh.

Repeat simulations revealed however that a RAS temperature of $18\, ^\circ C$ will give about a 16% reduction in taint accumulation for both GSM and MIB. These simulations are presented in Fig. 5 for both 18 and $28\, ^\circ C$. Seen together with Fig. 4 it is apparent that the lower the RAS water temperature, the lower the taint accumulation in the fish-flesh.

Repeat simulations for MIB revealed similar results; this might be expected given the very similar properties of the two taint molecules.

The generalized form of the model for taint accumulation in fish-flesh can be illustrated with application to other species.

4.3. Generalized model and simulations for rainbow trout (O. mykiss)

Rainbow trout is a cold-water fish of salmonidae family. Trout farming in RAS is a global practice, particularly in Europe, due to the cooler condition. Taint problems due to GSM or MIB in RAS rainbow trout have been reported in several European counties.

e.g. Denmark, UK and France (Petersen et al., 2011; Robertson et al., 2006; Zimba et al., 2012).

To illustrate the generalized form for rainbow trout, it is necessary to find a suitable growth equation for this species. A graph between the mass of the fish and the growth time ($n=11$) was obtained (Anon., 2012) and correlated to give:

$$m_t = 0.0346e^{0.0138t}$$

with $R^2 = 0.94$ (Snedecor and Cochran, 1989) indicating a very good fit. The effect of any temperature variation on fish growth/growth physiology is assumed negligible for rainbow trout also, and therefore ignored. The simulations can now be carried out using the procedure, Table 1 (with $m_1 = 0.274$ kg, $C_{OX} = 10.29$ mg L $^{-1}$ (Robertson et al., 2006), $C_{OX} = 58.62$ L day $^{-1}$, $E_W = 0.519$, $k_1 = 111.09$ kg $^{-1}$ day $^{-1}$, $e = 0.045$ (Robertson et al., 2006), $k_2 = 0.863$ day $^{-1}$, $k_0 = 0.00325$ day $^{-1}$, $k_m = 0.00063$ day $^{-1}$ (Gobas, 1993), and; $C_W = 0.011$ µg L $^{-1}$ (range 0.0–0.023 µg L $^{-1}$) for GSM in growth ponds) to give, $y = 0.5412$ µg kg $^{-1}$.

4.4. Model validation with independent data

There are no extensive independent data published on taste taint accumulation as either GSM or MIB with RAS systems.

For barramundi, limited data for taint as GSM from growth ponds were however obtained (as growth time vs y) from the Department of Agriculture Fisheries and Forestry, Brisbane, Australia (DAFF) (S. Poole, pers. comm.). Assuming a barramundi mass of about 1 kg at harvest, model simulations were carried out. A comparison between observed data and model predictions for $n = 14$ values of taste taint is presented as Fig. 6.

Given that the value of taint ranges from $\sim 2$ to 20 µg kg $^{-1}$, that is, one-order of magnitude the figure shows a moderately good fit of the model to these independent data. It is seen that there are five (5) residuals above the $Y=X$ line, five (5) below it and four (4) at (very nearly) $Y=X$.

For rainbow trout, model predictions were assessed against the independent data ($n = 15$) of Petersen et al. (2011) for growth in open recirculation aquaculture in Denmark. The growth water temperature was assumed to be $12\, ^\circ C$ for these data and the mean mass of the fish 0.30 kg. (The data were digitized from the original published plot.) A comparison of model predictions is presented in tabular form in Table 3 for values of observed taste taint as GSM from 0.183 to 2.15 µg kg $^{-1}$ that is, about a 12-fold range. The
correlation coefficient for these data was $R^2 = 0.66$ (Snedecor and Cochrane, 1989), indicating a moderately good fit.

It is clear from both Fig. 6 and Table 3 that the model predictions are on trend and give a good fit over a meaningfully large range of values of taint.

Because the predictions of the model show good agreement with these independent data for barramundi and rainbow trout, it can be concluded it is free of programming and computational errors and is of a generalized form.

### 4.5. Applying the model to investigate RAS growth protocols on accumulated taste taint

A significant advantage of the validated model is that it can be used to investigate growth protocols. For example, simulations underscore consumer acceptance of RAS barramundi at harvest of 240 day if the RAS water concentration $C_W$ of GSM and MIB is maintained at a value, respectively, less than 0.0014 and 0.0024 μg L$^{-1}$. Clearly, a range of farming practices can be investigated in this way without recourse to further unlimited experimental trials.

Admittedly, for a more thorough model validation, extensive data is needed to be collected throughout the growth period. A two-year extensive study is now underway in a commercial-scale RAS barramundi farm in South Australia. The costs can now be readily justified based on the good fit of predictions to independent data for accumulation of taste taint in fish-flesh.

Finally, during model development a widely used exponential curve for fish mass growth was used for the period to harvest of 240 day for barramundi and 270 days for rainbow trout for which independent growth data showed a very good fit for both (respectively, $R^2 = 0.96$, $n = 9$ and $R^2 = 0.94$, $n = 11$). Present model predictions are therefore adequate for these two species. More generally however, if a fish is to grow for longer (indefinite) periods then an S-curve for growth with time might be used to make the model more universal and to generalize it for a range of other fish species. The von Bertalanffy Growth Function (VBGF) for example could be used to replace the present Eq. (3). The VBGF is given by (Hopkins, 1992):

$$m_t = m_{bc0} \left(1 - \exp^{-K(t-t_b)}\right)^d$$

where $m_{bc0}$ is the mean mass the fish will grow to indefinitely, $d$ is an exponent of length-mass relationship, $K$ is a growth co-efficient, and; $t_b$, a scaling factor. It is important to note however that at maximum mass, $m_{bc}$, the rate of elimination of taint through growth dilution, $k_g$, is seen to become (Eq. (18)) constant (as it depends only on the fish mass). A consequence is that $k_g = k_c$ (Eq. (17)), simplifying the model.

### 5. Conclusions

The accumulation of taste taint as either geosmin (GSM) or 2-methylisoborneol (MIB) in the flesh of RAS fish has been simulated with a new transient-state model. The model is the sum of two exponential terms of simultaneous taint uptake and elimination with growth time. The model is generalized in form and applicable to range of species of RAS fish including both barramundi (L. calcarifer) and rainbow trout (O. mykiss).

Illustrative predictions for GSM in RAS barramundi fish-flesh revealed that at a typical harvest of 240 days the threshold for consumer rejection of 0.74 μg kg$^{-1}$ will not have been reached. However for MIB taint the threshold of (0.70 μg kg$^{-1}$) will be reached at 225 days RAS growth. Simulations revealed that a 5 °C variation in typical RAS water temperatures of 28 °C did not meaningfully impact taste taint accumulation as either GSM or MIB.

Model predictions gave good agreement with independent experimental values of taint as GSM in barramundi.

An advantage is the model can be conveniently simulated in standard spread-sheets tools for use by a range of users of different sophistication.

A major benefit of this new model when fully experimentally validated is that simulations will be used to investigate a range of growth protocols in RAS farming to minimize taste taint in fish-flesh.

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