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Earthy-muddy tainting of cultured barramundi linked to geosmin in tropical northern Australia

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ABSTRACT: Tainting of outdoor pond-reared barramundi *Lates calcarifer* by muddy-earthy off-flavours is frequently reported across tropical Australia. To investigate the possible causes and effects of off-flavour tainting, we analysed water samples from outdoor rearing ponds for the presence of geosmin (GSM) and 2-methylisoborneol (2-MIB), 2 microbial metabolites often associated with tainting episodes. We then conducted controlled dose–effect experiments which measured the accumulation of tainting metabolites in the flesh, and the impact tainting had on taste and flavour attributes. GSM was deemed to be the compound most likely responsible for off-flavour tainting, persisting at moderate (~1.00 µg l^{-1}) to extreme levels (~14.36 µg l^{-1}), while 2-MIB was never detected during the study. Controlled experiments revealed that the accumulation of GSM in the flesh of market-sized barramundi was directly related to GSM levels of the holding water (0 to ~4 µg l^{-1}), with higher levels resulting in significant increases in undesirable taste and flavour attributes, particularly muddy-earthy flavour and weedy aftertaste. We identified the sensory detection threshold for GSM in farmed barramundi to be ≤0.74 µg kg^{-1}, similar to estimates for GSM detection in rainbow trout *Oncorhynchus mykiss* (~0.9 µg kg^{-1}) and for 2-MIB in channel catfish *Ictalurus punctatus* (0.7 µg kg^{-1}). Quantitative estimation of flesh-bound GSM using gas chromatography-mass spectrometry (GC-MS) agreed well with human sensory assessment scores and highlights the reliability of chemical analysis of GSM in barramundi flesh while also indicating the value of GC-MS analysis in predicting the impact of GSM on the sensory properties of farmed barramundi.

KEY WORDS: Aquaculture · Geosmin · *Lates calcarifer* · Barramundi · Flavour taint

INTRODUCTION

Muddy-earthy-musty-type flavours are generally regarded as a natural characteristic of wild caught freshwater fish (Tucker 2000, Howgate 2004), although the occurrence of such flavours has also been reported for a diverse range of freshwater aquaculture species (Lovell 1983, Yamprayoon & Noonhorm 2000, Robertson et al. 2005, Petersen et al. 2011). Fish presenting with these flavour characteristics are often referred to as being 'off-flavour' or 'tainted' and are commonly considered to be spoiled or of low quality. Placing tainted fish in the marketplace typically lowers consumer confidence in the cultured product and ultimately results in significantly lower commercial returns. For example, it has been estimated that off-flavour tainting can cause a 30% reduction in the sales of cultured catfish (Engle et al. 1995).

The source of muddy-earthy-musty flavours in freshwater fish is commonly acknowledged as originating from 2 compounds, geosmin (GSM) and/or 2-methylisoborneol (2-MIB). GSM and 2-MIB are metabolites of certain groups of algae, actinomycetes and cyanobacteria (Tucker 2000) and are found in various water sources such as lakes, reservoirs and running waters (Jüttner & Watson 2007).

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These compounds are known to be particularly problematic in aquaculture systems due to persistent and elevated nutrient loading. Brief exposure to even low concentrations of GSM and/or 2-MIB are known to impart an intense muddy-earthy-type flavour to cultured fish, most notably channel catfish *Ictalurus punctatus* (Martin et al. 1988, Persson 1980) and rainbow trout *Oncorhynchus mykiss* (Robertson et al. 2006, Selli et al. 2009). Uptake of these tainting compounds is primarily via the gills (From & Hørlyck 1984) and accumulation in the flesh is influenced by the concentration of the compound(s) in the holding water, water temperature, and the physiology and lipid content of the fish (Neely 1979, Clark et al. 1990, Streit 1998, Howgate 2004).

Barramundi or Asian sea bass *Lates calcarifer* is the dominant aquaculture finfish species in tropical northern Australia with a current production of 3190 t (ABARES 2011), although production within the Asia-Pacific region is considerably higher at an estimated ~50 000 t (FAO 2010). The most common growout system in Australia is freshwater outdoor earthen ponds, with small fish typically maintained in floating cages and larger fish (>2 kg) being 'free-ranged' (Schipp 1996). In tropical north Australia, episodes of muddy-earthy tainting of freshwater outdoor pond-reared barramundi are frequently reported (Phillips 2010). Recently, this issue has been highlighted as the primary cause of an escalation in negative consumer perceptions of Australian aquacultured barramundi and a growing resistance to future purchases. Episodes of off-flavour tainting are ultimately eroding the market value of end products and returns to producers. Similar issues have previously been reported for barramundi cultured in floating cages in Lake Argyle (Percival et al. 2008). In this instance, 2-MIB was identified as the primary compound responsible for tainting. Off-flavour tainting of barramundi reared in outdoor pond-based production systems, however, has not been addressed. This lack of information is constraining efforts to understand the mechanisms of off-flavour tainting and the implementation of practices aimed at regaining consumer confidence in the quality of farmed barramundi.

The objectives of this study were to: (1) quantify levels of GSM and 2-MIB in freshwater barramundi rearing ponds, (2) determine the relationship between levels of off-flavour tainting compounds in the flesh of market-sized barramundi and levels in the rearing ponds, (3) quantify the impact of off-flavour tainting compounds on the sensory attributes of barramundi fillets, and (4) employ effective extraction and instrument analysis to resolve the relationship between levels of off-flavour taint in the flesh and the sensory properties of cultured barramundi post-harvest.

MATERIALS AND METHODS

Water sampling from rearing ponds

Three outdoor freshwater barramundi rearing ponds (~5 million litres per pond) located in North Queensland, Australia (17° 42′ 4.88″ S, 146° 2′ 3.18″ E), were sampled weekly over a period of 3 mo. Triplicate 50 ml water samples were collected from each pond, immediately placed on ice and frozen (−18°C) within 25 min of collection. Samples were collected at a fixed location in each pond (adjacent to the water outlet) from approximately 20 cm below the water surface. Samples were analysed for GSM and 2-MIB using solid phase micro extraction (SPME) and gas chromatography-mass spectrometry (GC-MS) techniques (see below).

Preparation of water samples

At the time of analysis, water samples were removed from −18°C storage and allowed to thaw at room temperature. Samples were then shaken vigorously to mix and suspend any particulates, and a 10 ml aliquot was taken and dispensed into a 20 ml headspace vial. A 10 µl aqueous solution (10 000 µg l^{-1}) of tetramethylpyrazine (TMP) was then added as an internal standard and the vial contents mixed for 20 s. Finally, 2.0 g of sodium chloride was added to the vial and the contents were mixed until dissolved. Samples were prepared in duplicate. Calibration standards were prepared using 10 ml of deionised water known to be free of GSM and 2-MIB. For each calibration level, the TMP concentration was maintained at 10 µg l^{-1}, while GSM and 2-MIB ranged from 0 to 20 µg l^{-1}.

Uptake of off-flavour tainting compounds

To produce fish with dissimilar off-flavour tainting intensities, an uptake experiment was established using 48 market-sized fish (~2.5 kg), obtained from a single rearing pond that gave no perceivable sensory indication of the presence of GSM or 2-MIB. Fish were randomly allocated across four 5000 l indoor holding tanks located on the same site and main-

tained at ambient temperature (~26°C) and photoperiod. There were no significant differences in the mean weight between the 4 groups. Four levels of tainting intensities were established (0.0 µg l^{-1}, 1.16 µg l^{-1}, 2.49 µg l^{-1}, 3.98 µg l^{-1}) across the 4 holding tanks. This was achieved by varying the proportions of taint-free bore water and water sourced directly from rearing ponds presenting with an intense level off-flavour taint. Duplicate water samples were taken from all holding tanks at the conclusion (24 h) of the uptake period and stored at −18°C until analysis. Plastic floating cages were used to establish 3 groups of 4 individuals (n = 4) within each 5000 l holding tank. Stocking holding tanks with replicate groups ensured that all individuals within a single treatment were exposed to identical holding conditions and concentrations of off-flavour compounds over the duration of the uptake period. After 24 h exposure, cages were removed from the holding tanks and fish were euthanised using standard commercial methods (ice emersion), and stored at 2°C. After 48 h storage, fish were filleted and then frozen at −18°C. One fillet from each fish was used for sensory evaluation and the other fillet consigned for the determination of GSM and 2-MIB levels using SPME and GC-MS techniques (see below).

Preparation of fish samples

Barramundi muscle (100 g) obtained from the dorsal shoulder region was minced using a blender, then 5 g was accurately weighed into each of 2 ball mill cups. A 10 ml volume with 10 µg l^{-1} TMP in water as the internal standard solution was added to each cup. The cups were then sealed and attached to the mill, which was then run at 30 cycles s^{-1} for 60 s to homogenise the fish muscle. Preparation of the calibration homogenates was identical to that of the sample homogenates except that the fish muscle was sourced from wild marine-caught barramundi known not to contain GSM or 2-MIB. Additionally, the 10 ml aliquots of the solution containing the internal standard also contained GSM and 2-MIB at concentrations ranging from 0 to 20 µg l^{-1}.

Extraction of fish homogenate

Approximately 20 g of homogenised fish muscle was transferred to a Markham still together with 10 ml of deionised water and 1 ml of 1 M NaOH. Steam was then metered into the extraction chamber of the Markham still until approximately 8 ml of condensate was collected in a 20 ml headspace vial. Deionised water was added to the vial to bring the total volume to 10 ml. Sodium chloride (2 g) was then added, the vial capped and the salt dissolved using a vortex mixer. The extracts were stored at −18°C until time of analysis.

Analysis of GSM and 2-MIB by GC-MS

At the time of analysis, water samples, fish extracts and their corresponding calibration extracts were removed from −18°C storage, thawed at room temperature and mixed thoroughly by vortex stirring. Sample analysis was undertaken by static headspace sampling of the extracts by SPME coupled with GC-MS. A 50/30 µm carboxen/divinylbenzene/polydimethylsiloxane (Car-DVB-PDMS StableFlex, Supelco) SPME fibre was used for all analyses. The GC was fitted with a 50 m capillary column and the inlet programmed to splitless injection. The mass spectrometer was set to electron ionisation mode and programmed for selective ion monitoring. The ion source was set at 70 eV and the electron multiplier at 1350 V. Identification of GSM, 2-MIB and TMP was on the basis of the correct retention times and the correct ion ratios of the selected qualifier ions for each compound. A 10 point internal standard calibration was made by the addition of GSM and 2-MIB to barramundi muscle known to be free of these compounds. The concentration of the 2 target compounds (GSM and 2-MIB) was determined using a calibration curve based on the ratios of the selected quantifying ions for the target compounds and the quantifying ion of the internal standard.

Sensory assessment of barramundi fillets

The sensory characteristics of fish from each uptake treatment were assessed using human sensory evaluation following the methods previously outlined by Percival et al. (2008). Six panellists were selected from an initial group of 22 and specifically trained in the sensory assessment of barramundi. Initial training sessions used both wild-caught and aquacultured barramundi. Sensory participants were trained to identify and describe the most significant sensory properties (flavour, odour and aftertaste) present in each sample. Following the training period, assessors then evaluated the sensory properties of randomly selected portions of fish sourced from each replicate

of each treatment group from the uptake experiment. Samples were randomly assigned to assessors and only identifiable using a blind randomly generated 3 digit code; at no time were participants aware of the specific species or research objectives of the trial. Each sensory descriptor was evaluated along a 150 mm ungraded line ranging from 0 (absent) to 150 (intense) and a percentage score was then derived. Distilled water and flat bread were used to clean the palate between samples.

Comparison of sensory and chemical analysis of barramundi

Sensory assessment scores were plotted against flesh concentrations of off-flavour tainting compounds and subjected to least squares regression analysis to resolve the correlation between sensory attributes and instrument measurement. This is seen as important in the development of a rapid quantitative method to forecast expected sensory attributes over a wide range of flesh taint concentrations.

RESULTS

GSM was consistently detected in water samples from freshwater rearing ponds (Fig. 1), being present in ~88% of all samples (n = 42). In contrast, 2-MIB was not detected at any time over the sampling period. Levels of GSM in water samples ranged from below detectable limits (<1 ng l^{-1}) to an extreme 14.37 µg l^{-1}. GSM levels differed between rearing ponds and were highly variable within individual ponds, with mean GSM levels showing coefficients of variation (CV) ranging from 74.9 to 125.2%. Despite this, GSM levels most often (~70%) ranged between 0.2 and 1.75 µg l^{-1}. Water temperatures over the sampling period averaged 27.5°C (±2.1 SD), while total solar energy averaged 20.0 MJ m^{-2} (±3.34 SD). Both failed to show any clear relationship to GSM levels in rearing ponds.

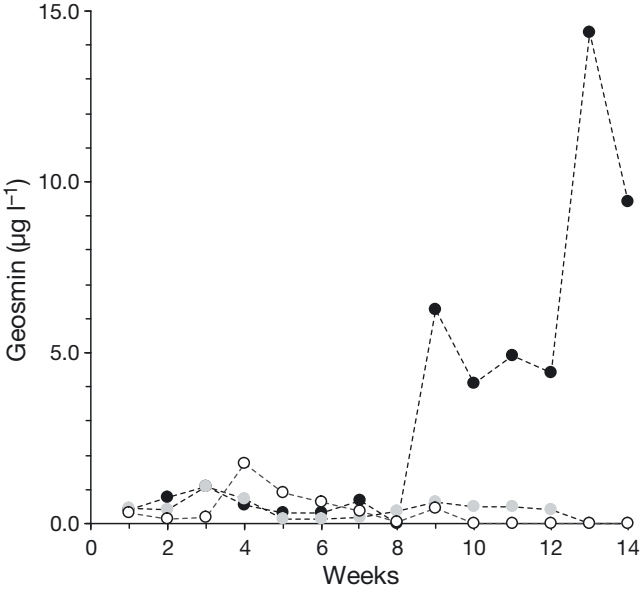

Fig. 1. Geosmin concentration in 3 freshwater barramundi rearing ponds (●, ●, ○)

Market-sized barramundi exposed to known levels of water-borne GSM for 24 h showed a strong positive correlation (r^2 = 0.97) between the level of GSM in the holding water and concentrations measured in the flesh (Fig. 2). The accumulation of GSM by barramundi is clearly dependent on the concentration of GSM in the holding water. Chemical analysis of fish held in pure bore water (0 µg l^{-1} GSM) did however show low levels (0.74 µg kg^{-1}) of GSM in the flesh after 24 h. This is most likely a result of fish having some residual GSM in the flesh when originally sourced from the rearing pond, despite water and fish having no perceivable flavour taint when originally sourced.

We observed clear differences over the 12 descriptive terms used to define the sensory attributes of barramundi fillets across the various GSM concentra-

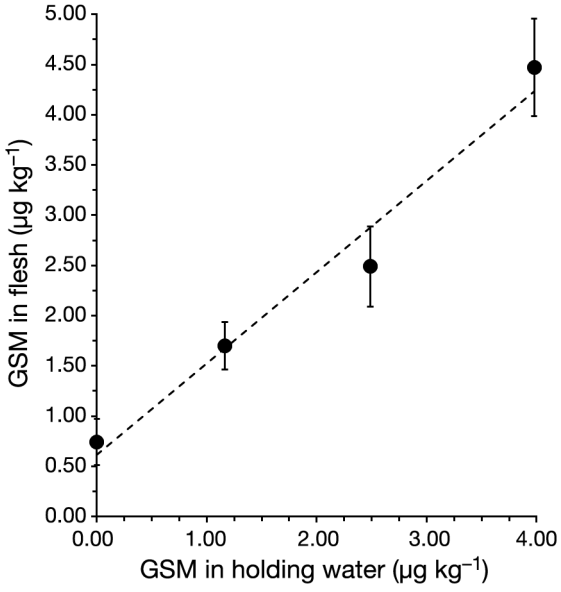

Fig. 2. *Lates calcarifer.* Relationship between concentration of geosmin (GSM) in water (0.0 µg l^{-1}, 1.16 µg l^{-1}, 2.49 µg l^{-1}, 3.98 µg l^{-1}) and flesh for 2.5 kg barramundi held in static water conditions for 24 h. Bars represent standard error of the mean. Dashed line represents least squares regression (r^2 = 0.97; $y = 0.91x + 0.62$; β = 0, t = 4.59, $p < 0.001$)

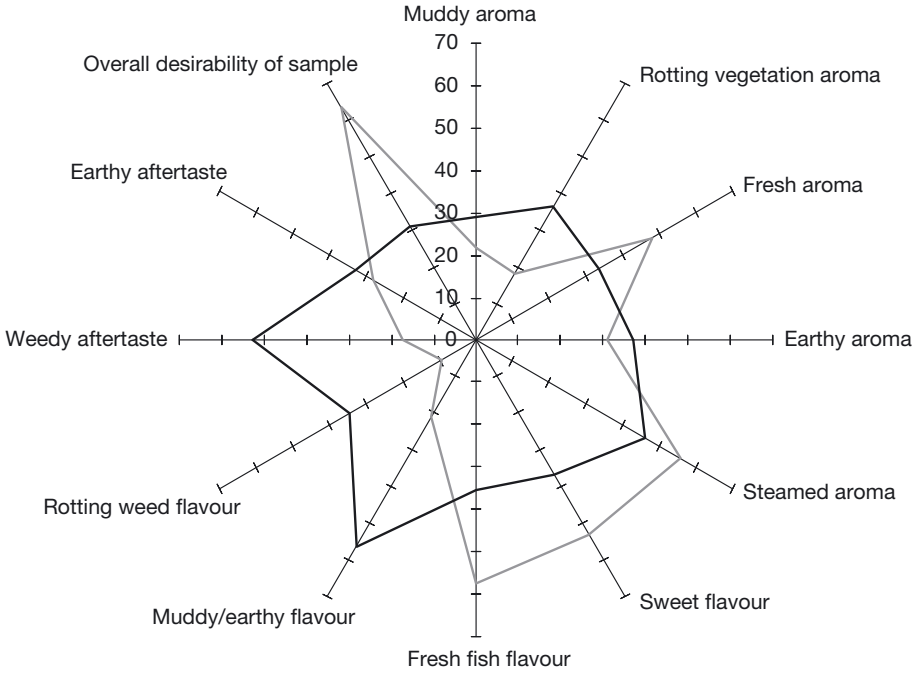

Fig. 3. *Lates calcarifer*. Sensory taste and aroma attributes (in%) of barramundi at 2 concentrations of flesh geosmin (GSM); 4.47 µg GSM kg^{-1} (black line) and 0.74 µg GSM kg^{-1} (grey line)

tions measured in the flesh. Sensory evaluation profiles (Fig. 3) revealed striking differences in several key attributes and were most divergent between the lowest (0.74 µg kg^{-1}) and highest (4.47 µg kg^{-1}) flesh GSM values tested. In general, sensory assessment scores for individual descriptive terms displayed a graded-type response, with scores strongly correlated to GSM levels in the flesh. The sensory attributes that possessed the strongest positive correlations with GSM flesh concentration (Fig. 4) were muddy/earthy flavour (r^2 = 0.99) and weedy aftertaste (r^2 = 0.94). In contrast, the strongest negative correlations with GSM flesh concentration were the sensory attributes of fresh fish flavour (r^2 = 0.98) and overall desirability (r^2 = 0.91).

Instrumental analysis of flesh GSM levels were strongly correlated with the scores obtained from the evaluation panel across a number of key sensory attributes. This confirms that members of the evaluation panel were capable of clearly differentiating between fish with differing taint intensities. This finding suggests that instrumental analysis has the potential to be employed as a forecasting tool with which to predict the impact of GSM levels on the flavour and taste attributes of pond-reared barramundi.

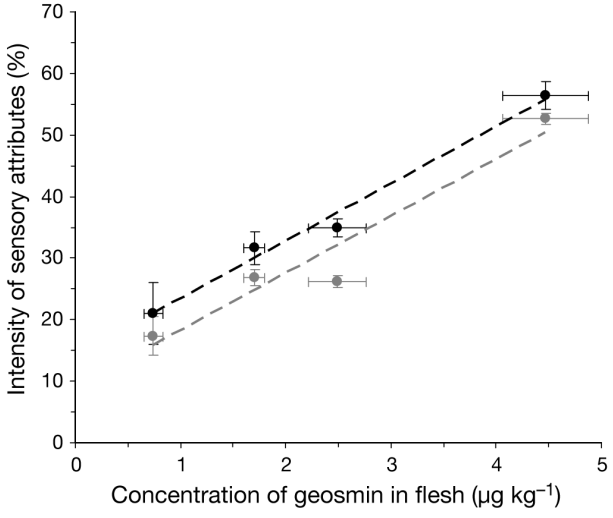

Fig. 4. *Lates calcarifer*. Relationship between the intensity of 2 negative sensory attributes, muddy/earthy flavour (●) and weedy aftertaste (●), and the concentration of geosmin as measured in barramundi flesh. Dashed lines represent least squares regression (muddy/earthy flavour, r^2 = 0.99; y = 9.32x + 14.1 and weedy aftertaste, r^2 = 0.94; y = 9.29x + 8.94). Bars represent standard error of the mean

DISCUSSION

In the present study we identified the compound GSM as the primary contributor to off-flavour tainting of tropical pond-reared barramundi. GSM was found to persist at moderate to extreme levels during the sampling period, with water-borne levels directly related to the presence and intensity of off-flavour tainting. Another compound, 2-MIB, often associated with off-flavour tainting episodes (Tucker 2000), was not detected in rearing ponds at any time. This finding is in contrast to those of Percival et al. (2008), in which 2-MIB was identified as the primary compound responsible for episodes of muddy-flavour taint of cage-reared barramundi at Lake Argyle. This production system is a vastly different from the smaller (<5000 m^2) earthen ponds most commonly used in the Australian barramundi aquaculture industry. Differences in system design may account in part for the different results observed, although further sampling is required to confirm the impacts of GSM and 2-MIB on pond-reared barramundi.

Barramundi rearing ponds showed wide variations in water-borne GSM levels. Concentrations varied within individual ponds at different sampling times as well as between different ponds. Such variations in the concentration of off-flavour compounds between rearing ponds has been well documented (Lovell 1983, Lovell et al. 1986, Martin et al. 1988, van der Ploeg & Boyd 1991, van der Ploeg et al. 1992). While GSM was undetectable in 12% of all water samples, elevated (>0.5 µg l^{-1}) GSM levels typically persisted for 2 to 4 wk. Periods of GSM levels above 4.0 µg l^{-1} were observed in Pond 1. Such high levels of off-flavour taint are generally regarded as being extreme, although similar levels have been observed in channel catfish rearing ponds (Martin et al. 1988, van der Ploeg & Boyd 1991, van der Ploeg et al. 1992).

In temperate localities, the production of off-flavour taint is somewhat seasonal, with GSM/2-MIB production often suppressed during winter periods (Lovell et al. 1986, Robertson et al. 2006, Robin et al. 2006). Lower temperatures and decreased solar radiation appear to be the cause, as the microbes responsible for GSM/2-MIB production experience suboptimal conditions. Hurlburt et al. (2009), for example, have demonstrated that soil temperature and rainfall can be risk factors promoting episodes of off-flavour tainting. Although the rearing ponds in our study were not sampled over a 12 mo period, the general persistence of GSM observed highlights that tainting episodes in the Australian barramundi industry have the potential to be severe and prolonged. This is not surprising given that tropical localities are characterised by factors that would clearly favour the growth of taint-producing microorganisms, such as high temperatures and prolonged periods of solar radiation. Nutrient availability is also known to impact on the development of taint-producing microbes. Although we did not measure nutrient levels, rearing ponds were in full production during this study and nutrient levels would not be expected to be limiting at any time. Robin et al. (2006) have shown that increased phosphorus and total suspended solids in aquaculture ponds promotes a shift in the structure of the phytoplankton community towards taint-producing cyanobacteria. Numerous other factors are also known to impact on the development of off-flavour episodes. The complexity of factors influencing the development of GSM-producing microbes may help to explain the variability in GSM levels observed in our rearing ponds.

The uptake and accumulation of GSM in the flesh by barramundi is highly dependent on water-borne GSM levels. This is in agreement with previous findings for channel catfish and rainbow trout (Johnsen & Lloyd 1992, Robertson et al. 2005, Petersen et al. 2011). Accumulation of tainting compounds in flesh is extremely rapid (Perkins & Schlenk 1997, Robertson et al. 2005), with uptake occurring passively, predominantly across the gills (Streit 1998, Howgate 2004).

Levels of GSM in barramundi fillets were only marginally higher (1.3×) than GSM levels in the holding water following 24 h of exposure under static conditions. Previous studies have demonstrated higher levels of bio-concentration in the flesh following GSM exposure, with values ranging from ~20× for Arctic charr *Salvelinus alpinus* (Houle et al. 2011), ~30× for rainbow trout (Robertson et al. 2005), and between 1 and 45× for channel catfish (Martin et al. 1988). As GSM is more soluble in lipid than water, it becomes sequestered and concentrated in the lipid of tissues (Howgate 2004). It has been proposed that, due to the relationship between GSM uptake and lipid content, the production of leaner fish could potentially lower the concentration of taint compounds in farmed fish (Johnsen & Lloyd 1992, Dionigi et al. 1998, Robertson et al. 2005). Although we did not measure lipid in the present study, total body lipid has been shown to be ~10% (wet weight) in barramundi fed artificial diets (Glencross et al. 2008) and ~24 to 44% (dry weight) in farmed channel catfish (Andrews & Stickney 1972). Farmed barramundi also show strong regionalisation of body lipid, with the lowest lipid levels (~1.3%) occurring in the anterior dorsal area and highest levels (~30%) occurring in the belly area (Percival et al. 2008). This regionalised distribution of lipid may underlie spatial differences in GSM levels throughout the fillet. This would agree with the findings of Percival et al. (2008), reporting that off-flavour caused by 2-MIB was most perceptible in the high lipid belly region. In channel catfish, 2-MIB has been shown to be related to muscle lipid; fish with >2.5% muscle lipid accumulated ~3× more 2-MIB than fish with <2% lipid (Grimm et al. 2004). In the present study, flesh samples for chemical analysis and sensory evaluation were taken from the dorsal shoulder region. Percival et al. (2008) have shown that this area is relatively low in total lipid, which may explain the low concentration of GSM observed in barramundi flesh relative to that of holding water observed in the present study. Further assessment of lipid GSM concentration in various regions of barramundi fillets is required to quantify this relationship.

Sensory levels are often categorised relative to the degree of tainting. Previous studies of rainbow trout

have categorised 'on flavour' as containing <0.25 to 1.12 µg kg^{-1} GSM and 'strongly tainted' as containing 2.05 to 4.18 µg kg^{-1}. Similar categories could easily be applied to farmed barramundi. In order to achieve this, consumer assessment would be required to determine how various levels of GSM tainting are perceived by the consumer.

Although the present study did not undertake any direct assessment of the threshold level of detection for GSM in barramundi flesh, some inferences can be made. At the lowest GSM flesh concentration of 0.74 µg kg^{-1}, muddy/earthy flavour scored 21%, while overall desirability of the sample scored 63%. This suggests that the threshold level for detection of GSM in barramundi is likely to be <0.74 µg kg^{-1}. This estimate agrees well with previous data for detection thresholds of GSM in other species. Robertson et al. (2006) determined the sensory threshold of GSM in rainbow trout flesh to be 0.9 µg kg^{-1}, Grimm et al. (2004) reported odour thresholds between 0.25 and 0.5 µg kg^{-1} for GSM in channel catfish, while Persson (1980) indicated a sensory threshold of 0.90 and 0.59 µg kg^{-1} for bream and pike, respectively. The sensory threshold of GSM will also be influenced by variations in sensory evaluation panels, the sensory characteristics of different species, and/or the presence or intensity of other flavours that may serve to mask GSM detection.

The use of instrumental analysis for quantifying GSM levels in fish flesh has become increasingly widespread (Grimm et al. 2004, Robertson et al. 2005, Petersen et al. 2011). In the present study, the results of the SPME and GC-MS analysis of GSM levels in barramundi flesh were significantly correlated with the scores obtained from the sensory evaluation panel across a number of key attributes that are often ascribed to GSM tainting. This finding agrees well with previous comparisons between human sensory scores and instrumental analysis of GSM for channel catfish (Grimm et al. 2004) and rainbow trout (Robertson et al. 2005, Petersen et al. 2011). The high correlation between these 2 methods suggests that instrumental analysis has the potential for use as a forecasting tool with which to predict changes in the flavour and taste profile of barramundi across a broad range of taint intensities. As such, instrumental analysis is highly advantageous as it offers rapid and reliable assessment of GSM levels in flesh and overcomes the major limitations of sensory evaluation panels, as it does not succumb to sensory overload (Grimm et al. 2004) and has the capacity for high sample throughput.

CONCLUSIONS AND IMPLICATIONS

GSM was identified as the primary compound associated with off-flavour tainting of pond-reared barramundi; concentrations were found to be elevated and persistent. Levels of GSM in barramundi flesh were highly dependent on levels in the holding water. Sensory evaluation of taste and flavour attributes of pond-reared barramundi clearly demonstrate that the intensity of tainting is highly correlated with the concentration of GSM present in flesh. The threshold level of detection for GSM in farmed barramundi is below 0.74 µg kg^{-1} and this is comparable with other freshwater cultured species. The findings of the present study will be used to establish protocols and practices that serve to mitigate off-flavour tainting and improve the flavour quality of pond-reared barramundi and other land-based tropical aquaculture systems in general.

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