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Off-Flavor Characterization in High-Nutrient-Load Tilapia Ponds in Northern Thailand

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Abstract

The purpose of this research was to determine the levels of odorous compounds (geosmin or 2-methylisoborneol (MIB) that potentially cause off-flavor problems in fish reared in integrated, high-nutrient-load ponds in northern Thailand. Water samples were collected from threetilapia ponds located in Chiangrai province, every month from May 2013 to May 2014. Geosmin and MIB were analyzed by gas chromatography-mass spectrometry. The physico-chemical parameters were determined using standard techniques. The concentration of geosmin in water samples ranged between 0.01 and 0.06 µg L^{-1}, while that of MIB ranged between 0.09 and 0.11 µg L^{-1}. The concentration of geosmin was positively correlated ($r=0.84$; $P<0.01$) with the density of cyanobacteria and chlorophyll *a* ($r=0.57$; $P<0.01$) in the water. The highest concentration of MIB (0.11 µg L^{-1}) was observed in October, and over all samples, concentrations were positively correlated ($r=0.56$; $P<0.01$) with culture period but negatively correlated with light intensity ($r=-0.39$; $P<0.05$). Seven genera of cyanobacteria were most frequently encountered, namely: *Anabaena* sp., *Oscillatoria* sp., *Pseudanabaena* sp., *Lyngbya* sp., *Phormidium* sp., *Planktolyngbya* sp. and *Synchococcus* sp. The detailed patterns of association found suggest that *Anabaena* sp. and *Oscillatoria* sp. were the biological origins of geosmin, whereas the source of MIB was *Pseudanabaena* sp.These findings are an important step towards the prediction, control and management of the off-flavor problem in tilapia culture in high-nutrient-load ponds.

Keywords: Earthy-musty off-flavor, tilapia pond, cyanobacteria, geosmin, MIB.

Introduction

Earthy-musty off-flavors are one of the most serious problems affecting commercial freshwater aquaculture since consumers are strongly averse to such flavors in fish products (Robertson *et al*., 2006; Robin *et al*., 2006; Gutierrez *et al*., 2011). The costs associated with management off-flavor include holding and feeding fish until off-flavors decrease, delays in restocking ponds until fish are acceptable for sale/harvest, as well as fish mortality resulting from predation, diseases, or toxic events in ponds (Engle *et al*., 1995; Hurlburt *et al*., 2009) are troublesome. The most common off-flavor compounds, geosmin and 2-methylisoborneol (MIB), are produced and released from cyanobacteria species, particularly filamentous forms such as *Anabaena circinalis* Kütz, *Lyngbya cryptovaginata*, *Oscillatoria* sp., *Phormidium* sp., and *Pseudanabaena* sp. into the water (Smith *et al*., 2008). The uptake by fish occurs mainly through the gills or skin and leads to an earthy and musty smell and taste. A number of commercial important species such as Atlantic salmon, *Salmo salar* (Farmer *et al*., 1995), rainbow trout, *Salmo gairdneri* (From and Horlyck, 1984), catfish species (Lovell *et al*., 1986; Martin *et al*., 1987; Zimba and Grimm, 2003; Hurlburt *et al*., 2009) including Nile tilapia (*Oreochromis niloticus*) (Yamprayoon and Noomhorm, 2000; Gutierrez *et al*., 2011; Gutierrez *et al*., 2013) have been reported to be affected by off-flavor problems.

Nile tilapia has been an economically important commercial fish in Thailand since 1965, with an estimated 182,841 tons produced in 2013 and the value of exports approximately 4.9 million USD in 2012 (Information Technology Center, 2013). In northern Thailand, tilapia fish culture in earthen ponds can be divided into three types: commercial,

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subsistence, and integrated farming (Pimolrat *et al*., 2013). Integrated farms culture tilapia together with chickens or pigs. Under this system, nutrients from uneaten feed, manure and other wastes from pig or chicken cages over-hanging the pond fertilize pond waters to produce natural food for the fish (Tunkthongpairoj, 1993; Pant *et al*., 2004), and thus reduce commercial feed cost. However, without proper management of fish and animal numbers, as well as good water exchange, water quality can deteriorate and result in eutrophication which promotes cyanobacterial blooms that can harmfully affect water quality and produce earthy-musty compounds (Alamri and Mohamed, 2013; Gutierrez *et al*., 2011).

In order to control and reduce this problem, it would be helpful to identify the sources and main factors influencing the production of off-flavors in fish ponds. Therefore, this study monitored the levels of geosmin and MIB in water that potentially cause off-flavor problems in earthen ponds used to rear tilapia under integrated, high nutrient load, culture system in northern Thailand. In addition, the species of phytoplankton responsible for these undesirably odorous compounds were investigated and the correlation between these compounds and their potential sources was explored.

Materials and Methods

Study Site

This study was carried out in 3 integrated tilapia ponds CR1, CR2 and CR3 located on same farm in Chiangrai province of northern Thailand. Basic characteristics of the culture system used in ponds studied are summarized in Table 1.

Water and Phytoplankton Sampling

Samples were collected from each sampling site every month (usually between 8:^{00} a.m. to 10:^{00} a.m.) from May 2013 to May 2014. All samples for physico-chemical (1.5 L) and earthy-musty odor analyses (35 ml) were collected 40-cm below the surface using a modified water sampler and subsequently transferred to plastic containers. Phytoplankton was sampled by filtering 5 L of pond water through plankton net with 25 µm mesh. Samples were transferred into a 30 ml plastic bottle and preserved with 3 drops of Lugol’s solution. Samples of water and phytoplankton was immediately placed on ice in sample coolers and transported to the laboratory within 24 hours.

Analysis of Geosmin and MIB

Geosmin and MIB were extracted from water samples using solid phase microextraction (SPME) and quantified with gas chromatography-mass spectrometry (GC/MS) according to Gutierrez *et al.* (2013).

Water Quality and Nutrient Analysis

Physicochemical parameters (pH, temperature, dissolved oxygen, turbidity, and conductivity) were measured in situ using a multimeter (TOA-DKK WQC-22A Model, Japan). Standard methods (APHA, 1980) were used for the analysis of total ammonia-nitrogen (TAN), nitrate-nitrogen, orthophosphate-phosphorus and total suspended solids (TSS) in the laboratory.

Chlorophylla and Algal Analysis

Chlorophyll *a* in the water samples was measured, after filtered (Whatman GF/C) extraction with 10 mL hot methanol (60°C in water bath), with spectrophotometric detection of chlorophyll *a* (APHA, 1980). Chlorophyll*a* concentration in the extract was calculated, as described previously (Wintermans and de Mots, 1965). Phytoplankton species and numbers were determined using a light microscope (Olympus BH2, Japan).

Statistical Analysis

Relationships between water quality variables and earthy-musty odor compounds were analyzed using pairwise Pearson correlation analysis. A correlation was assumed significant when P<0.05.

Table 1. Key features of ponds monitored in the study. Mean±SD

Pond feature	CR1	CR2	CR3
Elevation (masl)	379	385	390
Pond area (m^2)	2870	2728	4606
Pond depth (m)	1.05±12.93	1.22±10.79	1.07±36.08
Stocking rate (fish m^{-2})	2-3	2-3	2-3
Culture period (month)	7.75±1.06	7.75±1.06	6.67±0.58
Feed type	Pellet feed and natural food	Pellet feed and natural food	Pellet feed and natural food
Source of water	Irrigation canal	Irrigation canal	Irrigation canal
Water renewal rate	20-50% per crop	30-50% per crop	50% per crop
Source of nutrients	Pig manure	Pig manure	Pig manure
Production	All harvest sold	All harvest sold	All harvest sold

Results and Discussion

Earthy-Musty Odor in High-Nutrient-load Ponds

Geosmin and MIB concentrations measured in the water samples collected from three high-nutrient-load, integrated, ponds are shown in Figure 1. The concentration of geosmin in fish pond water samples ranged between 0.01 and 0.06 µg L^{-1}, while that of MIB ranged between 0.09 and 0.11 µg L^{-1}. Geosmin was mainly responsible for the off-flavor episodes in the hot season (February-May). The highest concentration of MIB (0.11 µg L^{-1}) was observed in October. In the present study, MIB concentrations were detected in samples from ponds in which culture period was longer than 180 days especially in October and February for CR2. However, the observation was not consistent with CR1 and CR3, where significant MIB concentration was detected only in October. Consequently, geosmin is probably the main cause of off-flavor during the study period since the detection of geosmin in the ponds was prevalent over MIB (12 out of 16 samples).Moreover, we found that culture time affected geosmin concentration as expected Geosmin was normally detectable in the later part of the culture (after 120 days) but not earlier (Figure 1).

At present, the acceptable threshold levels for geosmin is 0.9 µg kg^{-1}(Robertson *et al.*, 2005) and 0.6 µg kg^{-1} for MIB (Persson, 1980) in fish. In particular, the compounds 2-methylisoborneol (MIB) and geosmin can be detected by consumers as earthy odor at levels as low as 10 ng L^{-1} in drinking water (Cook *et al.*, 2001). The study by Yamprayoon and Noomhorm (2000) showed that geosmin and MIB could get into fish within a short period of time (hours), but required a much longer period, even weeks, in order to be eliminated. Changing culture-water and/ or storing the fish in the clean water before harvesting has been shown to help reduce off-flavor contamination (Robertson *et al.*, 2006; Gutierrez *et al.*, 2011). In our study, we found that water exchange did not consistently reduce off-flavor in the high-nutrient-load ponds. Geosmin and MIB were still detected in all ponds at some point despite water exchange was applied.

Planktonic and benthic cyanobacteria are known to produce geosmin and MIB. Both compounds are rapidly absorbed from water into the lipid tissue of

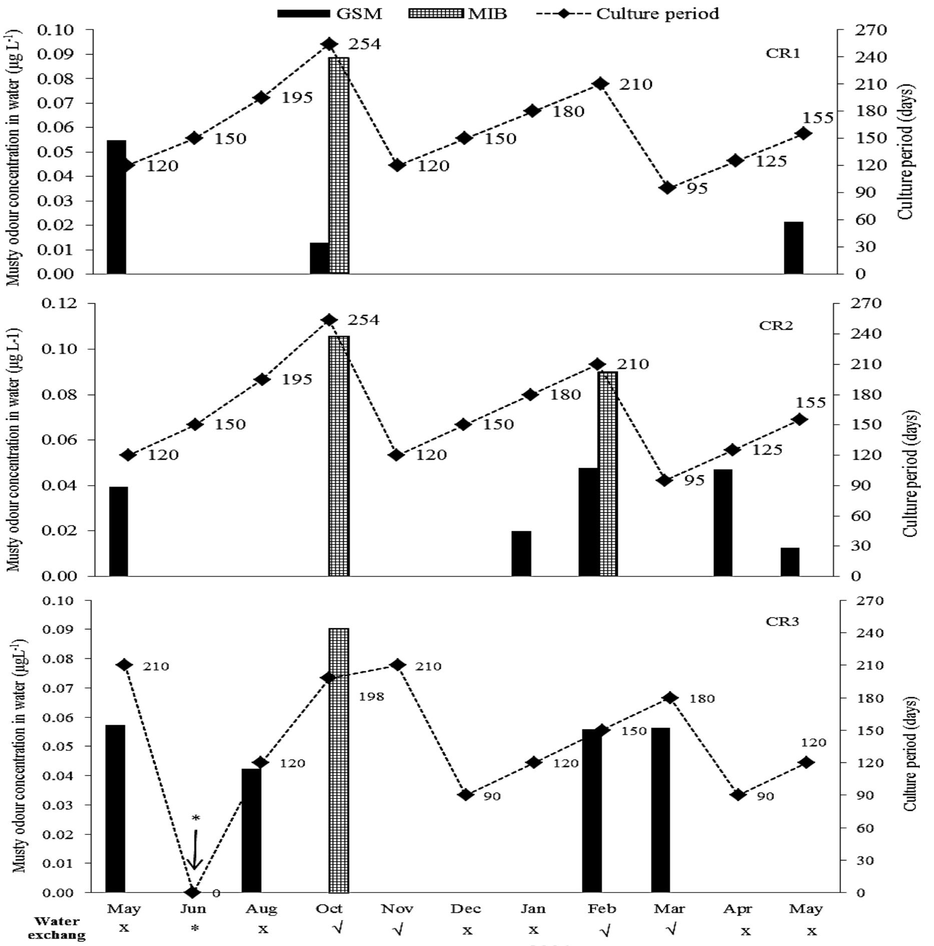

Figure 1. Earthy-musty odor levels in water and culture period (days) in three high-nutrient-load ponds (CR1, CR2 and CR3) at different times of the year (*Harvested pond; X= no water exchange;√= exchanged water).

fish and other aquatic organisms (Lloyd and Grimm, 1998). In this study, the densities of cyanobacteria in three high-nutrient-load ponds are shown in Figure 2. The amount of geosmin (r=0.714, P<0.01) and MIB (r=0.391, P<0.05) in water were significantly correlated (P<0.05) with the density of cyanobacteria and chlorophyll *a* concentration. These relationships suggest that chlorophyll *a* levels may indicate increasing cyanobacterial abundance, which may result in increased geosmin or MIB concentrations.

Physicochemical Parameters

The variations in the physicochemical parameters (temperature, light intensity, TSS, chlorophyll a, nitrate and orthophosphate concentrations) in three high-nutrient-load ponds are shown in Figure 3. The highest water temperature values around 31-32°C were observed in April, while the lowest water temperature values were observed in December, around 22 °C. The lowest light intensity

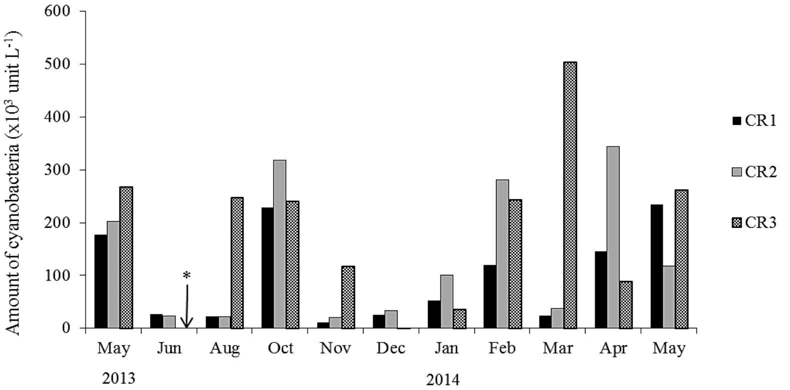

Figure 2. Amount of cyanobacteria in water samples taken from three high-nutrient-load ponds at different times of the year (*Harvested pond).

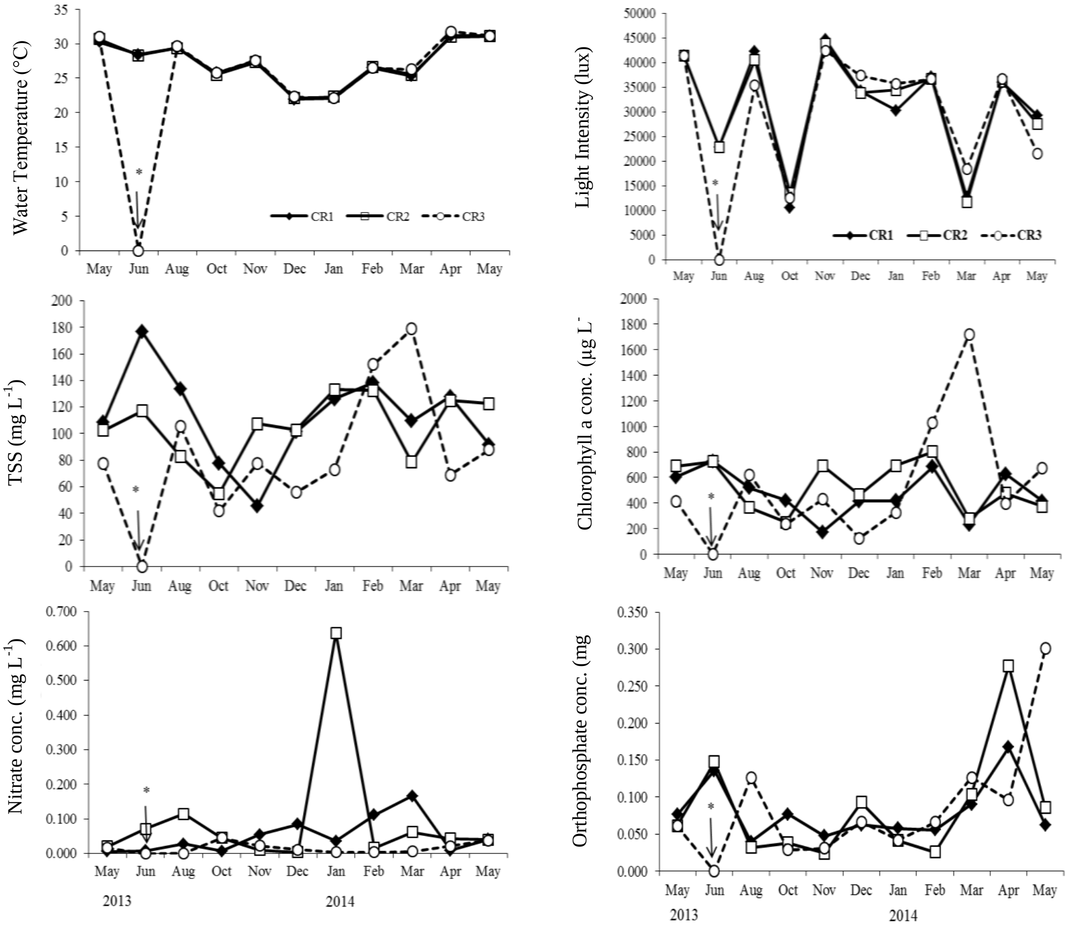

Figure 3. Physicochemical parameters (temperature, light intensity, TSS, chlorophyll a, nitrate and orthophosphate concentrations) of water samples taken from high-nutrient-load pondsat different times of the year (*Harvested pond).

values for the three ponds were observed in October, which coincided with the time of *Pseudanabaena* sp. bloom and also when detected the highest concentration of MIB (Figure 1). The highest chlorophyll *a* concentration (1,720 µg L^{-1}), TSS (179.00 mg L^{-1}) and total cyanobacteria (504x10^{3} unit L^{-1}, see Figure 2) were observed in March for pond 3 (CR3), which coincided with the time of highest *Anabaena* sp. bloom and also detected the highest values of geosmin. The nitrate concentrations generally ranged between 0.004 and 0.64 mg L^{-1}, while the amount of orthophosphate in tilapia ponds ranged between 0.024 and 0.300 mg L^{-1}.

Physical, chemical and biological water quality in fish pond ecosystem influences growth and survival rate as well as reproductive and likelihood of disease infection (Andrews *et al*., 1973; Kunlasak *et al*., 2013). The suitable temperature for tilapia is 29-31°C, while stress-associated mortality possibly occur when the temperature is higher than 37-38°C (Lim and Webster, 2006; Rodrigo and Whangchai, 2009). In the present study, the unsuitable cold temperatures for tilapia culture were observed in December and January which also affected cyanobacteria growth.

Chlorophylla and Cyanobacterial Abundance

The concentrations of chlorophyll *a* and density of cyanobacteria are shown in Figure 4. No significant variation in chlorophyll *a* content was observed during the eleven month period. The variability of chlorophyll *a* values most related to the abundance of cyanobacteria. The lowest chlorophyll *a* concentrations (123.33 µg L^{-1}) and total cyanobacteria (3.15 x 10^{3} unit L^{-1}) for pond 3 (CR3) were observed in December when the water temperature was the coldest.

Cyanobacteria were observed frequently in the hot season (February- May) and hardly found in the dry season (November 2013-January 2014). Seven genera of cyanobacteria were most frequently encountered, namely: *Anabaena* sp., *Oscillatoria* sp., *Pseudanabaena* sp., *Lyngbya* sp., *Phormidium* sp., *Planktolyngbya* sp. and *Synchococcus* sp. Furthermore, the three cyanobacterial genera (*Anabaena* sp., *Oscillatoria* sp. and *Pseudanabaena* sp.) were most dominant; these species are well known as producers of odorous compounds *(Smith et al*., 2008). Species and biomass of cyanobacteria are known to be important factors influencing MIB and geosmin concentrations in water (van der Ploeg and Boyd, 1991).*Anabaena* sp.and *Oscillatoria*sp. areknown to be a common planktonic bloom-forming cyanobacterium that produces geosmin (Jüttner and Watson, 2007; Smith *et al*., 2008; Zhang *et al*., 2010). In the present study, *Anabaena* sp. (254.80 x 10^{3} unit L^{-1}) and *Oscillatoria*sp. (24.85 x 10^{3} unit L^{-1}) reached a maximum in March for pond 3 (CR3) (Figure 5A, B), which coincided with the highest geosmin levels in water and also shown positive correlation (r=0.533;

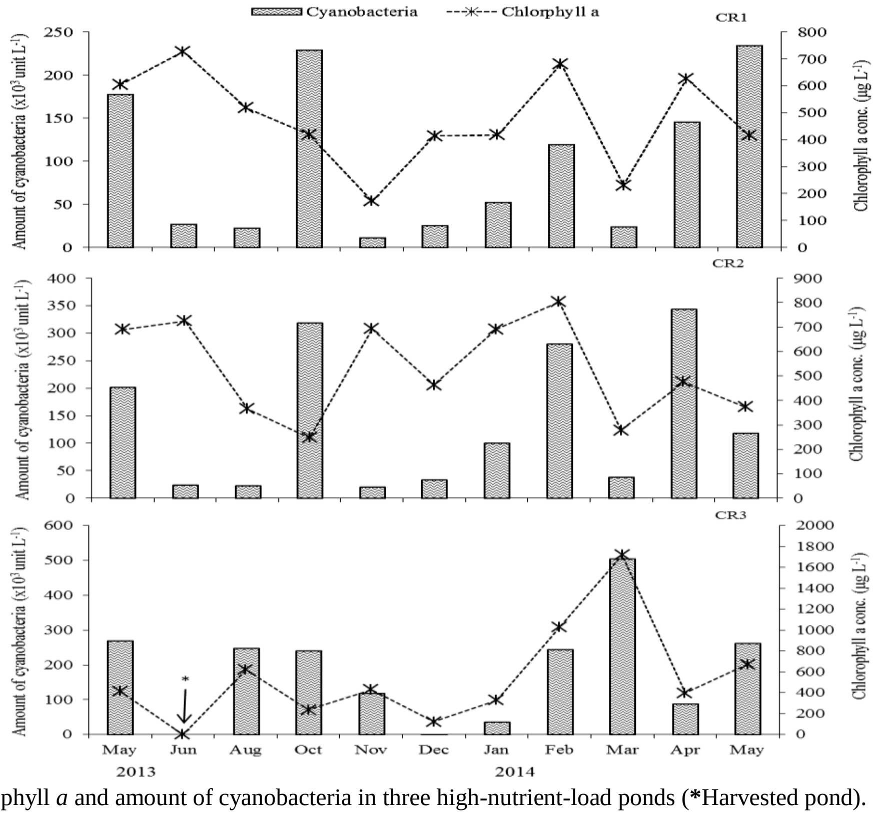

Figure 4. Chlorophyll *a* and amount of cyanobacteria in three high-nutrient-load ponds (*Harvested pond).

P<0.01) with chlorophyll *a* concentration and orthophosphates (r=0.403; P<0.05) in the water (Table 2). Therefore, the results suggest that *Anabaena* sp. and *Oscillatoria* sp. might be the biological origins of geosmin in high-nutrient-load tilapia ponds. While, *Pseudanabaena* sp. was positively correlated (r=0.487; P<0.01)) with the culture period and reached a maximum in October and February, which coincided with the highest MIB levels in water. It was possible that *Pseudanabaena* sp. might be the biological origin of MIB in these sample ponds (Figure 5C).

Correlation Between Odorous Compounds, Cyanobacteria and Physico-Chemical Parameters

Pearson correlations between the odorous compounds, cyanobacteria and physico-chemical parameters are shown in Table 3. The concentration of geosmin was positively correlated with the density of cyanobacteria and chlorophyll *a* in the water. The concentration of MIB was positively correlated with culture period and density of cyanobacteria, but negatively correlated with light intensity.

In this study, the concentration of geosmin was strongly positively correlated with the genera of cyanobacteria including *Anabaena* sp., *Lyngbya* sp., *Oscillatoria* sp. and *Phormidium* sp., while MIB was positively correlated only with *Pseudanabaena* sp. On the basis of this relationship between *Pseudanabaena* sp. and MIB, we suggested that the cyanobacteria *Pseudanabaena* sp. was the source of MIB in high-nutrient-load tilapia ponds. Accordingly, *Pseudanabaena* has been implicated in MIB problem in five reservoirs in California (Taylor *et al*., 2006) and the Arizona Canal (Baker *et al*., 2006). *Pseudanabaena*, which was known in Japan under a different name, *Phormidium tenue*, was also long implicated in MIB problems in Lake Biwa and Lake Kasumiguara (Sugiura *et al*., 1986; Yagi *et al*., 1983; Zhang *et al*., 2010).

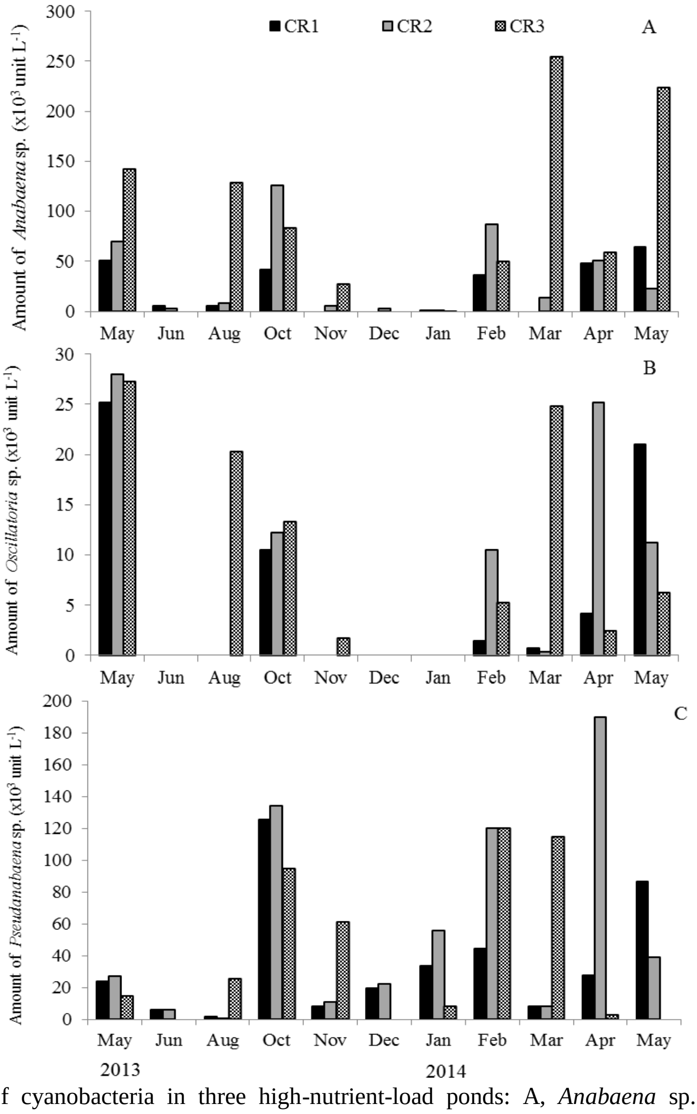

Figure 5. The amount of cyanobacteria in three high-nutrient-load ponds: A, *Anabaena* sp.; B, *Oscillatoria* sp.; C, *Pseudanabaena* sp.

Table 2. Correlations (Pearson) between physicochemical parameters and cyanobacteria in three high-nutrient-load tilapia ponds (n=33)

Physicochemical parameters	Cyanobacteria (unit L^{-1})	*Anabaena* sp. (unit L^{-1})	*Lyngbya* sp. (unit L^{-1})	*Oscillatoria* sp. (unit L^{-1})	*Pseudanabaena* sp. (unit L^{-1})
Nitrate (mg L^{-1})	-0.141	-0.205	-0.206	-0.222	-0.006
Phosphate (mg L^{-1})	0.329	0.403*	0.135	0.224	0.125
Culture period (days)	0.392*	0.228	0.260	0.162	0.487**
Temperature (°C)	0.333	0.307	0.230	0.387*	0.126
Light intensity (lux)	-0.116	-0.156	0.008	0.062	-0.157
TSS (mg L^{-1})	0.247	0.134	0.210	0.112	0.208
Chlorophyll *a* (mg L^{-1})	0.550**	0.533**	0.526**	0.313	0.303

*Significant correlation at the level of P<0.05.

** Significant correlation P<0.01.

Table 3. Correlations (Pearson) between physicochemical and cyanobacteria parameters and concentrations oftwo odorous compoundsin three high-nutrient-load tilapia ponds (n=33)

Physico-chemical and cyanobacteria Parameters	Geosmin (µg L^{-1})	MIB (µg L^{-1})
Nitrate (mg L^{-1})	-0.072	-0.084
Phosphate (mg L^{-1})	0.134	-0.235
Culture period (days)	0.117	0.564**
Temperature (°C)	0.254	-0.038
Light intensity (lux)	0.234	-0.390*
TSS (mg L^{-1})	0.381*	-0.243
Chlorophyll *a* (mg L^{-1})	0.567**	-0.120
Cyanobacteria (unit L^{-1})	0.714**	0.391*
Anabaena sp. (unit L^{-1})	0.510**	0.223
Lyngbya sp. (unit L^{-1})	0.800**	0.124
Oscillatoria sp. (unit L^{-1})	0.779**	0.153
Pseudanabaena sp.(unit L^{-1})	0.495**	0.565**
Phormidium sp. (unit L^{-1})	0.559**	0.001

*Significant correlation at the level of P<0.05.

** Significant correlation P<0.01.

Conclusions

This study clearly demonstrated that both geosmin and MIB are present in high-nutrient-load tilapia ponds. The concentration of geosmin was significantly associated with the abundance of *Anabaena* sp. and *Oscillatoria* sp., while that of MIB was significantly associated with the amount of *Pseudanabaena* sp. and culture period. This suggests that*Anabaena* sp. and *Oscillatoria* sp. were the biological origins of geosmin, whereas the source of MIB was *Pseudanabaena* sp.in high-nutrient-load tilapia ponds in northern Thailand.The improved understanding of the biological origins of off-flavor from this study is important for the prediction, control and management of off-flavor problem in tilapia pond culture.

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