Maejo Int. J. Sci. Technol. **2013**, *7*(Special Issue), 1-13

Maejo International Journal of Science and Technology

ISSN 1905-7873
Available online at www.mijst.mju.ac.th

Full Paper

Off-flavour in Nile tilapia (*Oreochromis niloticus*) cultured in an integrated pond-cage culture system

Redel Gutierrez[1,2], Niwooti Whangchai[1,*], Udomluk Sompong[1], Wiwat Prarom[3], Norio Iwami[4], Tomoaki Itayama[5], Nakao Nomura[6] and Norio Sugiura[6]

[1]Faculty of Fisheries Technology and Aquatic Resources, Maejo University, Sansai, Chiangmai 50290, Thailand
[2] College of Arts and Sciences, Central Luzon State University, Science City of Munoz, Nueva Ecija, 3120, Philippines
[3] Phayao Inland Fisheries Research and Development Center, Muang, Phayao 56000, Thailand
[4] Graduate School of Science and Engineering, Meisei University, 2-1-1 Hodokubo, Hino, Tokyo 191-8506, Japan
[5] Graduate School of Engineering, Nagasaki University, 1-14 Bunkyou-machi, Nagasaki 852-8521, Japan
[6] Graduate School of Life and Environmental Sciences, University of Tsukuba, 1-1-1 Tennodai, Tsukuba 305-0008, Japan
* Corresponding author, e-mail: niwooti@hotmail.co.th

Received: 6 March 2013 / Accepted: 18 March 2013 / Published: 19 March 2013

Abstract: Thai farmers are shifting to cage farming in ponds due to drastic fluctuations in production in river cages resulting mainly from deteriorating water quality and diseases. Cage culture of tilapia in ponds has its merits but it is not immune to off-flavour problems. This study compares the levels of off-flavour compounds: geosmin and 2-methylisoborneol (MIB), in Nile tilapia (*Oreochromis niloticus*) cultured alone in ponds (T1), alone in pond cages (T2), in ponds with caged catfish (T3), and in pond cages with catfish in ponds (T4). Off-flavour levels of caged tilapia in the Ping River were similarly determined for comparison. Analysis of geosmin and MIB by HS-SPME-GC/MS revealed that fish in all pond treatments were found to be off-flavoured. At harvest, Nile tilapia in pond cages had significantly higher geosmin and MIB levels ($P<0.05$) compared to tilapia from the Ping River cages. No significant differences were observed in both geosmin and MIB levels among the treatments for Nile tilapia and hybrid catfish. The levels of MIB were generally higher than those of geosmin in fish, pond water and sediment. The prevalence of MIB could be partly due to the presence of MIB-producing actinomycetes in the ponds as revealed from their isolation in tilapia's stomach from T4 case. *Microcystis aeruginosa* and known off-flavour producers, *Anabaena* spp. *and Oscillatoria* spp., colonised the ponds,

Maejo Int. J. Sci. Technol. **2013**, *7*(Special Issue), 1-13

indicating that the latter two cyanobacteria might have caused off-flavour in Nile tilapia raised in integrated pond-cage culture system.

Keywords: Nile tilapia, hybrid catfish, pond-cage culture, geosmin, 2-methylisoborneol, cyanobacteria

---

INTRODUCTION

Tilapia cage culture has gained great popularity in certain parts of Thailand as evidenced by the growing number of tilapia fish cage farmers in the country. Relatively low investment cost is one of the major reasons why more marginal Thai fish farmers are engaging in this livelihood. In 2005, Thailand's production of caged tilapia exceeded 22,000 tons and was valued at about US$ 27 million [1]. Although cage culture takes place in various places such as rivers, reservoirs, irrigation canals and large ponds, most occurs in flowing waters. However, more farmers are now shifting to cage farming in ponds due to drastic fluctuations in production in river cages resulting mainly from deteriorating water quality and diseases. Raising caged tilapia in ponds has its advantages and merits but it is not immune to problems regarding the accumulation of off-flavour in fish flesh. Off-flavour, caused by the presence of trace organic compounds, geosmin and 2-methylisoborneol (MIB) produced by actinomycetes and cyanobacteria (blue-green algae) [2, 3] in water and soil, is currently the most serious economic problem faced by the tilapia export industry.

The development of environment-related off-flavour is an important aspect of water quality management. Off-flavour does not pose a direct threat to fish health but rather affects the acceptability of fish for processing, which eventually causes delays in harvesting [4]. It increases the cost of production and exposes fish to additional risk of loss due to diseases or predators. One possible way of dealing with water quality problems in tilapia pond culture, and possibly with off-flavour, is to integrate hybrid catfish with tilapia in a pond-cage system. This integration allows phytoplankton to grow by utilising nutrients in the waste from intensive hybrid catfish pond or culture cages, which support tilapia culture in the same pond [5, 6]. Tilapia reared in cages, feeding on phytoplankton in intensive channel catfish ponds, have been shown to improve pond water quality as well as produce an extra crop [7]. Since tilapia feed on cyanobacteria and also forage on the sediment surface where actinomycetes grow, it is possible that raising tilapia in integrated pond-cage culture with hybrid catfish could be effective in reducing off-flavour in the fish and at the same time reduce effluent effects from catfish culture, gain extra fish production at low cost, and provide a significant economic benefit to farmers [6, 8]. Torrans and Lowell [9] also demonstrated the benefits of tilapia-catfish polyculture, using blue tilapia (*Oreochromis aureus*) to reduce the incidence of off-flavour in channel catfish.

Since many Thai farmers are now shifting to pond cage farming, it is imperative to assess (and compare) the levels of geosmin and MIB in caged tilapia reared in rivers and ponds to gain an insight in the development of effective culture protocols for minimising off-flavour. To our knowledge, a comparative assessment of off-flavour levels in tilapia reared in river cages and cages in earthen ponds has not been investigated and the effectiveness of integrated pond-cage culture scheme in reducing off-flavour in fish has not been fully explored. This study thus aims to evaluate the practical significance of pond-cage culture of Nile tilapia (*Oreochromis niloticus*) and hybrid catfish (*Clarias macrocephalus x C. gariepinus*) in terms of its effect on the incidence of off-flavour in fish and other economic benefits.

Maejo Int. J. Sci. Technol. **2013**, *7*(Special Issue), 1-13

MATERIALS AND METHODS

Experimental Set-up

The study was carried out during a 5-month period at the Faculty of Fisheries Technology and Aquatic Resources, Maejo University. Sixteen experimental ponds, each with a 100-m^2 area and 1.5 m depth, were used. Four treatments with 4 replicates in a completely randomised design were performed as follows: T1 – Nile tilapia in ponds at 5000 fish ha^{-1}; T2 - Nile tilapia in cages at 5000 fish ha^{-1}; T3 – Nile tilapia in ponds (at 4000 fish ha^{-1}) with caged hybrid catfish (at 1,000 fish ha^{-1}); and T4 – hybrid catfish in ponds (at 4000 fish ha^{-1}) with caged Nile tilapia (at 1,000 fish ha^{-1}).

Nile tilapia and hybrid catfish fry with overall average initial weights of 66.8 g and 57.5 g respectively were purchased from private hatcheries. The fry were initially placed in a separate 5-m^2 cage (hapa) for 24 hr prior to stocking in the experimental ponds and cages (Table 1).

Table 1. Description of experimental treatments (T1- Nile tilapia alone in ponds; T2 – Nile tilapia alone in pond cages; T3 – Nile tilapia in ponds with caged hybrid catfish; and T4 – Nile tilapia in pond cages with hybrid catfish in ponds)

	Treatment			
	T1	**T2**	**T3**	**T4**
Pond area (m^2)	100	100	100	100
Cage dimension (m^3)	-	4 × 6 × 2	2 × 3 × 2	4 × 5 × 2
Cage depth (m)	-	1.4	1.4	1.4
No. of Nile tilapia stocked	313	313	250	250
No. of hybrid catfish stocked	-	-	63	63
Nile tilapia average weight (g)	69.8	67.9	61.2	68.2
Hybrid catfish average weight (g)	-	-	57.6	57.4
Feeding rate	full satiation			

Sampling

Samples for physico-chemical (water) and off-flavour (water, sediment and fish) analyses were collected between 3-4 pm throughout the duration of the experiment. Composite samples of water (2 L) and sediment (500 g) were collected monthly, along with samples of Nile tilapia and hybrid catfish from each pond and cage. Samples of Nile tilapia (n=18) from Ping River were collected from cages situated downstream and upstream of Chiang Mai city.

Analysis of Geosmin and MIB in Water, Sediment and Fish

Off-flavour analyses were conducted by headspace solid phase micro-extraction (HS-SPME) and gas chromatography–mass spectrometry (GC/MS) [10]. A 50/30 µm divinylbenzene/carboxen/ polydimethylsiloxane SPME fibre (SUPELCO, USA) was extended into the headspace of sample [10 mL unfiltered water (frozen and then thawed); 5 g sediment with 10 mL MilliQ (Millipore, UK) water; or 5 g minced fish with 10 mL methanol] placed in a 20-mL straight-sided vial, added with sodium chloride (1.9 g) and a polytetrafluoroethylene (PTFE)-coated stirring bar and sealed with an aluminum crimp cap fitted with a pre-pierced PTFE-faced silicone septum. The sample was then heated to 65^oC on a hotplate-stirrer and exposed to the SPME fibre for a 12-min. adsorption period while undergoing

vigorous agitation. After 12 min., the fibre was withdrawn from the sample and desorbed under a splitless mode at 230°C for 5 min. in the injection port of an HP 6890 N Network gas chromatograph equipped with a 5973 mass selective detector (Agilent Technologies, USA) operated in scanning mode. A Durabond HP-5 capillary column of 30-m length, 0.32-mm i.d. and 0.25-µm film thickness was used with helium carrier gas operated at a rate of 2.5 mL min^{-1}. The oven temperature was programmed at 60°C for 1 min., then increased to 220°C with a rate of 15°C $min.^{-1}$ and maintained at 220°C for 8 min. All analyses were performed as single measurements. Standard geosmin and MIB from Sigma were used for calibration.

Water Quality and Nutrient Analysis

Physico-chemical parameters (pH, temperature and dissolved oxygen) were measured *in situ* using a multimeter (TOA DKK WQC-22A Model, Japan). Laboratory analysis of total ammonia-nitrogen was determined by indophenol method [11], nitrate-nitrogen by cadmium reduction method [11], nitrite-nitrogen by diasotising colourimetric method [11] and phosphate-phosphorus by stannous chloride method [11]. All water quality parameters were generally within the acceptable ranges for fish culture and were not significantly different among all treatments.

Hydro-biological Analysis

Chlorophyll *a* was measured in the laboratory following a method adapted from Wongrat and Boonyapiwat [12]. Phytoplankton was sampled every 30 days by filtration of pond water with a net of 10-µm mesh. Samples were concentrated in a 30-mL bottle and preserved with Lugol's solution. Species and count of phytoplankton were determined as described by Wongrat and Boonyapiwat [12].

Isolation and Screening of Actinomycetes in Fish

Samples of residues from the stomach of five tilapia samples from T4 treatment cages (tilapia in pond cages with catfish in ponds) were cultured for actinomycetes using starch-casein agar [13]. Nystatin (25 µg mL^{-1}), cycloheximide and nalidixic acid (10 µg mL^{-1}) were added into the medium as antibacterial and antifungal agents. Isolates of actinomycetes were purified by streak-plate technique. Pure cultures of selected isolates were streaked on yeast extract-malt extract agar [14] with 20% glycerol added and stored at -20°C [15]. Isolates were transferred to Hickey-Tresner medium, to cultivate geosmin and MIB-producing actinomycetes for GC/MS qualitative testing and verification.

Data Analysis

Analysis of variance (ANOVA) was used to test for difference between means of observed parameters and each treatment. Duncan Multiple Range Test (DMRT) at 95% confidence level was used for treatment comparison. T-test was used to compare means of two groups.

RESULTS AND DISCUSSION

Geosmin and MIB were detected in all Nile tilapia sampled throughout the experimental period (Figure 1). No significant differences in both geosmin and MIB levels were observed among treatments for Nile tilapia. Apparently, MIB levels were generally higher than geosmin in the experimental ponds between June-November, with highest MIB concentration (7.25 ± 3.18 ug L^{-1}) recorded for T1 (tilapia pond monoculture) in October. The presence of these odorous terpenoids in the fish persisted until the end of the experiment, thereby rendering the fish off-flavoured and temporarily unmarketable. Similarly, geosmin and MIB were detected in hybrid catfish in T3 and T4 (Figure 2),

Maejo Int. J. Sci. Technol. **2013**, *7*(Special Issue), 1-13

where tilapia culture was integrated. Hybrid catfish in T3 and T4 were strongly off-flavoured and at the end of the experiment (November) had very high MIB concentrations at 9.82±2.22 and 9.48±2.72 µg kg^{-1} respectively. Likewise, the prevalence of MIB over geosmin in the treatments throughout the experiment was observed in hybrid catfish.

The levels of both geosmin and MIB in tilapia and hybrid catfish flesh were above the sensory threshold concentrations (THC) except for geosmin level detected in T4 hybrid catfish in November. The THCs in fish are 0.9 µg kg^{-1} for geosmin [16] and 0.6 µg kg^{-1} for MIB [17]. The THC (the lowest concentration that can be detected by humans) used were for rainbow trout since there is none yet established for Nile tilapia and hybrid catfish. High incidence of off-flavour in tilapia was observed in integrated pond-cage cultures, comparable to that of pond (T1) and cage (T2) tilapia monocultures. Geosmin levels of hybrid catfish in T3 and T4 were below the THC but still considered to be strongly off-flavoured due to very high MIB content observed in the two treatments. However, the above results may suggest that the filtering of algae by tilapia in T3 and T4 significantly reduced the level of geosmin in hybrid catfish but had little effect on the concentration of MIB. Off-flavour concentrations in tilapia measured in the present study were lower than in previous experiments on red tilapia, but were relatively higher than in rainbow trout and channel catfish (Table 2). This could be due to differences in culture pattern, pond bottom structure, management practice and off-flavour sources. Fish can acquire off-flavours directly from contaminated water by osmosis through their skin and gills and/or from the gut by ingesting food contaminated with the substances which are later on accumulated in their flesh [20, 21]. However, in the cases of geosmin and MIB, uptake is overwhelmingly through the gills based on their octanol/water partition coefficients (Kow) [21]. Thus, the magnitude of contamination in fish may depend on the concentration of geosmin and MIB excreted into water by off-flavour sources (cyanobacteria and actinomycetes). Yamprayoon and Noomhorm [22] reported that among various tissues of Nile tilapia, the intestines contained the highest concentration of geosmin, followed in descending order by abdominal, skin and muscle tissues (flesh).

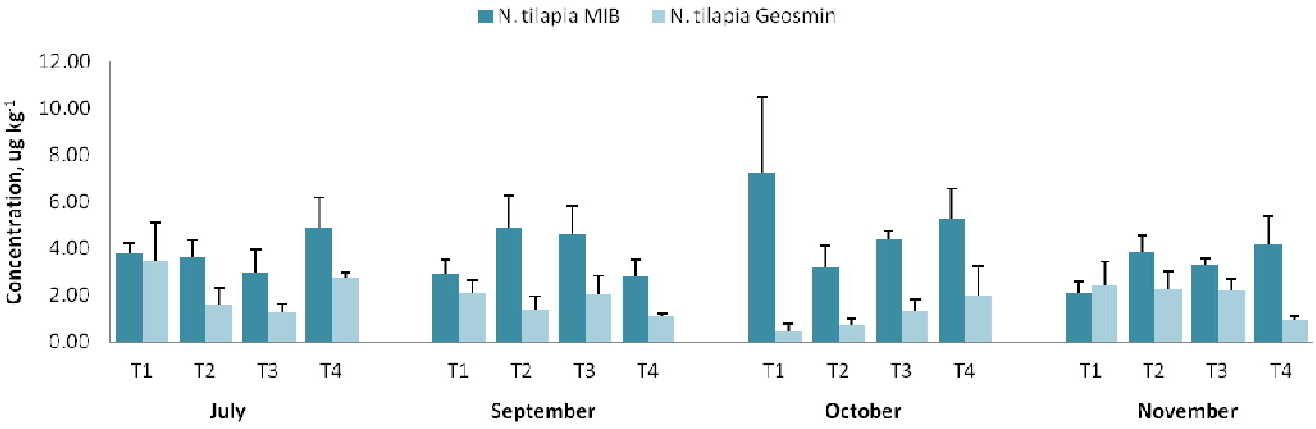

Figure 1. Concentrations of geosmin and MIB in Nile tilapia in the 4 treatments, sampled between July – November 2010. Error bars represent the standard error of 3 replicates (T1- Nile tilapia alone in ponds; T2 – Nile tilapia alone in pond cages; T3 – Nile tilapia in ponds with caged hybrid catfish; and, T4 – Nile tilapia in pond cages with hybrid catfish in ponds)

Maejo Int. J. Sci. Technol. **2013**, *7*(Special Issue), 1-13

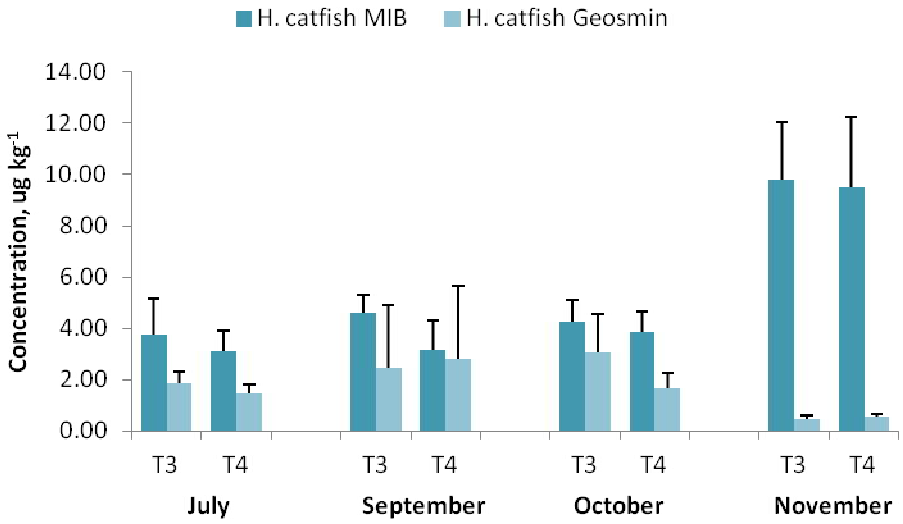

Figure 2. Concentrations of geosmin and MIB in hybrid catfish in T3 and T4, sampled between July – November 2010. Error bars represent the standard error of 3 replicates (T3 – Nile tilapia in ponds with caged hybrid catfish; and, T4 – Nile tilapia in pond cages with hybrid catfish in ponds)

Pond water and sediment contained significant levels of geosmin and MIB in all treatments throughout the experimental period (Figure 3). Geosmin concentrations in water (0.41 - 2.33 µg L^{-1}) were generally higher than in sediment (0.49 -1.70 µg kg^{-1}). Conversely, MIB levels in sediment (3.90 - 9.46 µg kg^{-1}) were higher than in water (0.81 - 6.77 µg kg^{-1}). MIB concentrations in both pond water and sediment in all sampling months were higher than geosmin, especially in the latter where it was overwhelmingly prevalent. This is probably because the majority of benthic cyanobacteria or actinomycetes thriving in the sediment were MIB producers. Actinomycetes are common sediment microorganisms in most aquatic environments [23]. The amount of MIB in sediment was notably higher than in fish samples, averaging about 7 times above the amount of geosmin in the four treatments at the end of the experiment (November). Yamprayoon and Noomhorm [22] reported that the concentration of geosmin in water directly affects the rate of geosmin absorption by tilapia, which is in agreement with the observations of Lelana [24]. In this study, however, there was no clear indication or trend that the concentration of geosmin or MIB in pond water and sediment directly influenced the levels detected in Nile tilapia and hybrid catfish. It could also be asserted that MIB is more stable and persists longer in the pond than geosmin. Lawton et al. [25] claimed that geosmin is biodegraded within 3 days and MIB in 5–14 days, hence the occurrence of MIB at higher concentrations than geosmin.

Off-flavour concentrations in cage-reared Nile tilapia in experimental ponds, T2 and T4, were compared with those in the fish reared in river cages. Nile tilapia from the Ping River, both from upstream and downstream cages (Table 3), had significantly ($P < 0.05$) much lower geosmin and MIB contents in their flesh (highest recorded value = 0.23 µg kg^{-1}) than their pond counterparts (Table 4). Moreover, all river-cage Nile tilapia samples (n=18) had off-flavour concentrations below the sensory threshold levels [16, 17]; percentage detection of geosmin and MIB in the fish samples was very low at 11.1% and 22.2% respectively. Therefore, the levels of geosmin and MIB in tilapia from Ping River were negligible in terms of their potential to cause taint. This result validates claims that river fishes reared in cages have much lower or no off-flavours.

Maejo Int. J. Sci. Technol. **2013**, *7*(Special Issue), 1-13

Table 2. Geosmin and MIB levels in shrimp and various kinds of finfish

Fish Species	Geosmin (µg kg^{-1})	MIB (µg kg^{-1})	Reference
Rainbow trout	-	0.055	[18]
Shrimp	78	-	[32]
Channel catfish	0.7	-	[20]
Channel catfish	0.25-0.5	0.1-0.2	[11]
Red tilapia	4.6-41.0	10.0-74.0	[19]
Nile tilapia			
Ponds	0.49-3.51	2.11-7.25	In this study
River cage	ND - 0.23	ND - 0.23	
Hybrid catfish	0.48-3.05	3.16-9.82	

Note: ND = not detected

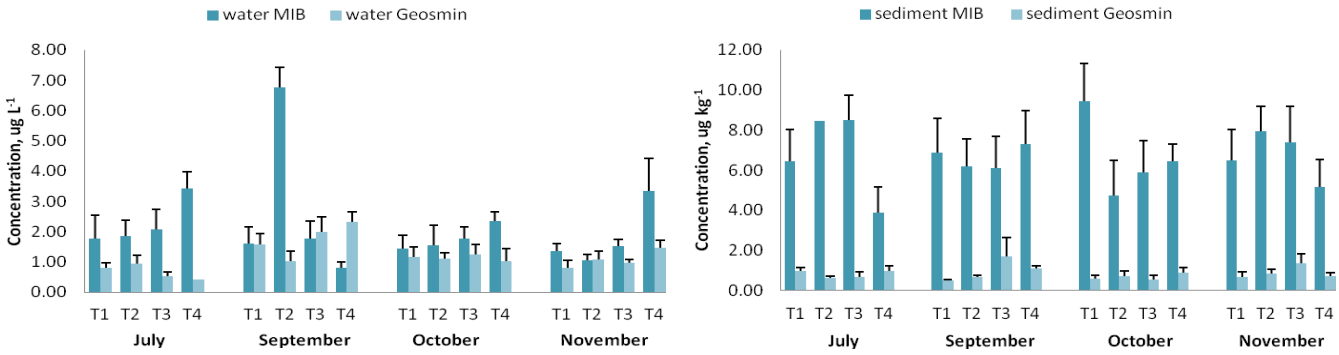

Figure 3. Concentrations of geosmin and MIB in pond water and sediment in the 4 treatments sampled between July – November 2010. Error bars represent the standard error of 3 replicates (T1- Nile tilapia alone in ponds; T2 – Nile tilapia alone in pond cages; T3 – Nile tilapia in ponds with caged hybrid catfish; and, T4 – Nile tilapia in pond cages with hybrid catfish in ponds)

Table 3. Geosmin and MIB levels in Nile tilapia from Ping River (n=18)

Sampling area		Geosmin (µg kg^{-1})	MIB (µg kg^{-1})	Sampling area		Geosmin (µg kg^{-1})	MIB (µg kg^{-1})
	1	ND	ND		10	ND	ND
	2	ND	ND		11	ND	0.22
	3	ND	ND		12	ND	ND
City	4	ND	ND	City	13	ND	ND
downstream	5	0.21	0.16	upstream	14	ND	ND
	6	ND	ND		15	ND	ND
	7	ND	ND		16	ND	ND
	8	0.23	0.23		17	ND	0.14
	9	ND	ND		18	ND	ND

Note: ND = not detected

Maejo Int. J. Sci. Technol. **2013**, *7*(Special Issue), 1-13

Table 4. Comparison of off-flavour levels (±SE) in Nile tilapia from Ping River and pond cages (T2 and T4) at harvest

Off-flavour	River cages ($\mu g\ kg^{-1}$)	Pond cages ($\mu g\ kg^{-1}$)	Mean difference ($\mu g\ kg^{-1}$)	t-Value
Geosmin	0.03±0.03	1.60±0.44	1.58*	3.60
MIB	0.05±0.03	4.02±0.65	3.97*	6.12

*Significant at $P < 0.05$

Cyanobacteria were recorded to be present in high densities (measured as colonies mL^{-1}), with sharp increase from the second to fourth month and slight decrease towards the end of the experimental period (Figure 4). The observed increase in cyanobacterial population coincided with the highest recorded pond temperatures (Figure 5) exceeding 32°C during the above-mentioned months, as well as with recorded increased phosphates in the water of all treatments (Figure 6). It seems that high temperature, along with nutrient enrichment in the experimental ponds, has significantly contributed to the observed increase in cyanobacterial density, especially those genera that secrete off-flavours in water.

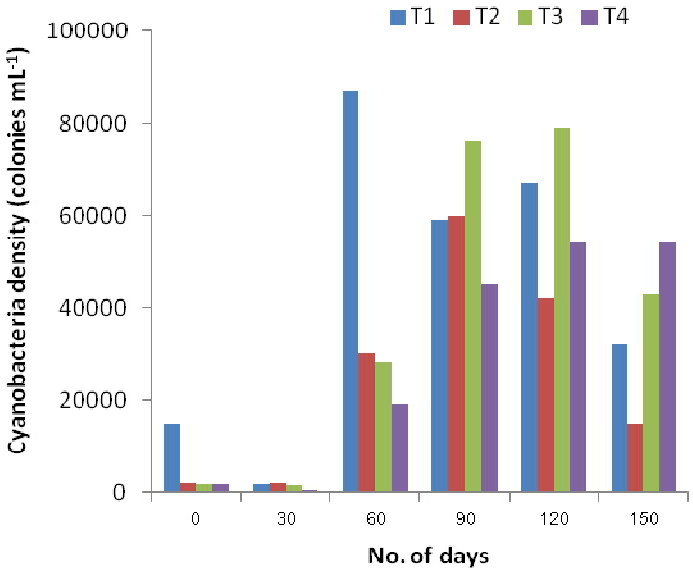

Figure 4. Average monthly cyanobacterial density in experimental ponds

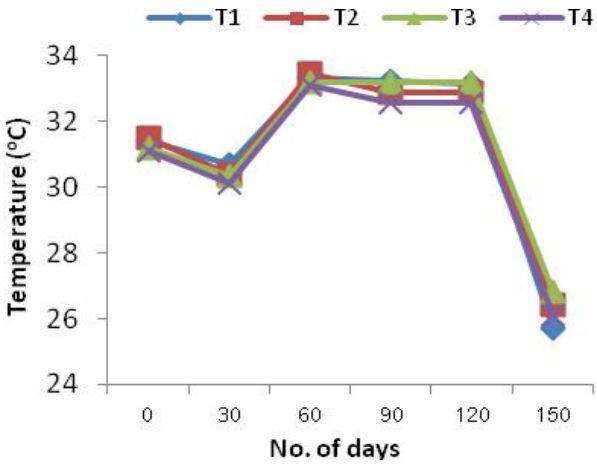

Figure 5. Monthly temperature variations over the 150-day experimental period

A total of 26 different genera from 6 divisions of phytoplankton were identified in the experimental ponds (Table 5). Division Chlorophyta (11 genera) was most abundant followed by Cyanophyta (8 genera), Euglenophyta (3 genera), Baccillariophyta (2 genera), Cryptophyta (1 genus) and Pyrrhophyta (1 genus). This confirms the results obtained by Wirasith and Traichaiyaporn [26], Sen and Sonmez [27], and Chowdhury and Mamun [29]. These phytoplanktonic divisions commonly occur in fish production ponds with cyanobacteria (Cyanophyta) dominating the waters [28]. Among the cyanobacteria identified in this study, *Microcystis aeruginosa, Anabaena* spp. and *Oscillatoria* spp. were dominant in the treatment ponds (Table 5). *Microcystis aeruginosa,* which co-colonised the water of T2, is not known as geosmin- or MIB-producer. On the other hand, *Anabaena* spp., *Oscillatoria* spp. and *Phormidium* spp., which were also identified in pond waters, are known producers of geosmin and MIB [29] and could be responsible for off-flavour in Nile tilapia and hybrid catfish through

Maejo Int. J. Sci. Technol. **2013**, *7*(Special Issue), 1-13

excretion of volatile terpenoids into the water. Some studies reported that *Anabaena* [30, 31] are responsible for geosmin while *Oscillatoria* [2] are responsible for MIB production. *Phormidium*, on the other hand, produce both geosmin and MIB, according to another report cited in Juttner and Watson [3]. Consequently, these findings partially support the results in this study wherein a higher MIB concentration was observed, compared to geosmin, in caged tilapia, pond water and sediment from T4 and T2 (co-colonised with *Microcystis aeruginosa*), where the MIB-producers *Oscillatoria* dominate. However, in T1 and T3, MIB was the more prevalent terpenoid, despite dominance of geosmin-producing *Anabaena* spp. It was possible that other MIB-producing cyanobacteria or MIB-secreting actinomycetes were present, which contributed to higher MIB concentrations in fish samples in both treatments.

Table 5. Average densities of phytoplankton genera over the 150-day experimental period (T1- Nile tilapia alone in ponds; T2 – Nile tilapia alone in pond cages; T3 – Nile tilapia in ponds with caged hybrid catfish; and, T4 – Nile tilapia in pond cages with hybrid catfish in ponds)

Phytoplankton genera	**Phytoplankton density (x 10^3 cell or colonies mL^{-1})**			
	T1	**T2**	**T3**	**T4**
Cylindrospermopsis spp.	0.10	0.06	0.44	0.21
Anabaena spp.	39.9	5.34	34.80	12.81
Oscillatoria spp.	2.06	20.47	3.52	15.06
Spirulina spp.	0.73	0.13	0.08	0.05
Phormidium spp.	0.98	0.56	0.35	0.22
Merismopedia spp.	2.36	1.08	0.81	0.62
Chroococcus spp.	2.97	0.71	3.29	2.07
Microcystis aeruginosa	0.65	34.74	3.85	8.88
Pediastrum simplex	0.33	0.23	0.53	0.28
Scenedesmus spp.	0.50	0.18	0.35	0.26
Staurastrum spp.	0.12	0.18	0.09	0.18
Pediastrum duplex	0.23	0.14	1.24	0.38
Actinastrum spp.	0.32	0.10	0.21	0.05
Tetraedron spp.	0.05	0.09	0.04	0.06
Tetrastrum spp.	0.28	0.05	0.09	0.13
Coelastrum spp.	0.11	0.06	0.17	0.13
Eudorina spp.	1.38	2.01	1.51	1.36
Pandorina spp.	1.18	1.18	0.42	0.18
Kirchneriella spp.	0.15	0.05	0.13	0.03
Cryptomonas spp.	0.21	0.31	0.16	0.66
Euglena spp.	0.45	0.42	0.26	0.51
Phacus spp.	0.28	0.28	0.14	0.11
Trachelomonas spp.	0.34	0.67	0.21	0.35
Nitzschia spp.	1.14	0.29	0.88	0.32
Aulacoseira spp.	0.38	0.10	0.47	0.23
Peridinium spp.	0.04	0.10	0.15	0.11

Maejo Int. J. Sci. Technol. **2013**, *7*(Special Issue), 1-13

No significant variation in chlorophyll *a* content was observed during the five-month period except in T1 (tilapia pond monoculture), which showed a sharp increase in the first month (Figure 7). Chlorophyll *a* value for T1 did not vary much until the third month but remained highest among the treatments. A decrease and increase trend was observed from the third to the fifth month in all the treatments except for T4. Chlorophyll *a* levels were similar to those reported by Yi *et al.* [8] in a non-integrated treatment, which was higher than in pen-cum-pond integrated culture systems using both natural and artificial water circulation.

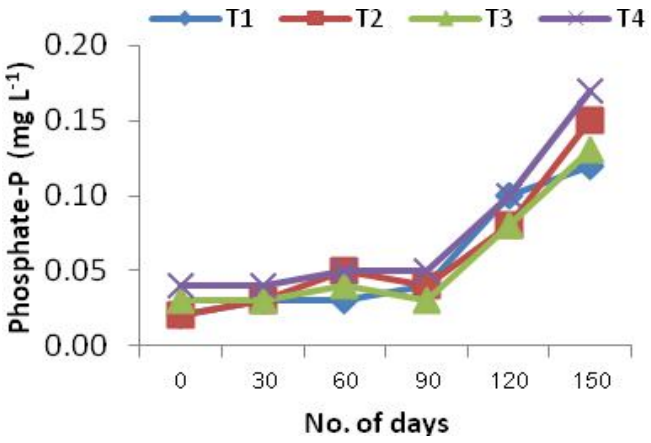

Figure 6. Monthly variations in phosphate-P over the 150-day experimental period

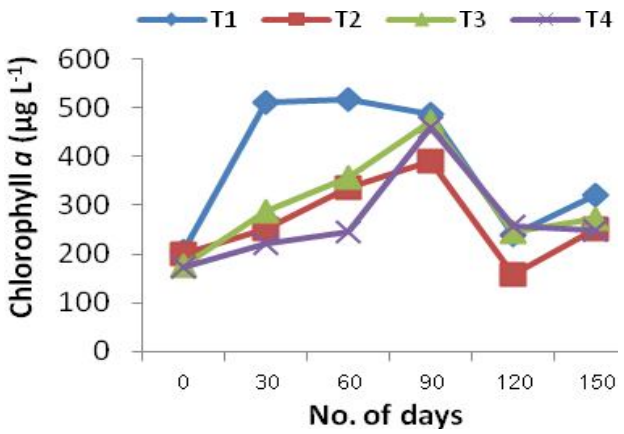

Figure 7. Monthly variations in chlorophyll *a* over the 150-day experimental period

Actinomycetes were isolated from tilapia (stomach) sampled from T4 cages at harvest (November) and were qualitatively tested for off-flavour production. Analytical screening of the bacterial isolates by HS-SPME-GC/MS for geosmin and MIB shows that all were MIB-producers (Table 6) and only 50% of the isolates produced geosmin. As a forage feeder, tilapia consumes and digests benthic cyanobacteria living on the side-net and bottom of the cage. Jüttner and Watson [3] reported that off-flavour-causing *Oscillatoria spendida*, *O. brevis*, *O. tenuis*, *Lyngbia subtilis* and *L. allogei* can grow in benthic form, thriving on the surface of the side-net and bottom parts of the cage. It is possible in this study that actinomycetes also inhabited the cage bottom, thriving on decomposing feeds and settled particulate materials, which were then eaten by tilapia, hence the presence of actinomycetes in tilapia's stomach. Actinomycetes and other benthic cyanobacteria thus consumed by tilapia could have contaminated the fish in T4 with off-flavours since the lining of the gastrointestinal tract is another possible route of uptake of off-flavour compounds [32]. It was not clear in this study, however, whether the presence of actinomycetes in the stomach of tilapia could have significantly contributed to the prevalence of MIB in the flesh.

Maejo Int. J. Sci. Technol. **2013**, *7*(Special Issue), 1-13

Table 6. Off-flavour screening of actinomycetes isolates from caged Nile tilapia (T4) with GC/MS

Actinomycete isolate	Off-flavour compound	
	Geosmin	MIB
AIN-01	+	+
AIN-02	+	+
AIN-03	-	+
AIN-04	-	+

Note: (+) = detected, (-) = not detected

CONCLUSIONS

Off-flavour incidence and water quality in a tilapia pond-cage culture system with hybrid catfish are generally comparable with tilapia monoculture systems (pond or cage). Detected levels of geosmin and MIB in fish are above the sensory threshold levels, thus causing off-flavour. Conversely, off-flavour concentrations in tilapia from the river are considered negligible in terms of its potential to cause taint. Geosmin- and MIB-producing cyanobacteria are generally present in both integrated (pond-cage) and monoculture (pond or cage) systems with *Anabaena* spp. and *Oscillatoria* spp. dominating the community. Their presence, along with actinomycetes, might have contributed to the observed off-flavour in tilapia and hybrid catfish. Actinomycetes isolated from caged tilapia are mostly MIB-producers, leading to the prevalence of MIB over geosmin in this study. Therefore, management strategies need to be developed or refined for integrated pond-cage culture to mitigate these common off-flavour problems.

ACKNOWLEDGEMENTS

The authors thank the National Research Council of Thailand for providing a research grant, and Maejo University for the laboratory instruments and research facilities.

REFERENCES

1. Fisheries Information Technology Center, "Fishery Statistics of Thailand 2005", Department of Fisheries, Ministry of Agriculture and Cooperatives, Bangkok, **2007**, p.91.
2. G. Izaguirre, C. J. Hwang, S. W. Krasner and M. J. McGuire, "Geosmin and 2-methylisoborneol from cyanobacteria in three water supply systems", *App. Envron. Microbiol*., **1982**, *43,* 708-714.
3. F. Juttner and S. B. Watson, "Biochemical and ecological control of geosmin and 2-methylisoborneol in source waters", *Appl. Environ. Microbiol*., **2007**, *73,* 4395-4406.
4. C. S. Tucker, T. R. Hanson and S. K. Kingsbury, "Management of off-flavors in pond-cultured channel catfish with weekly applications of copper sulfate", *N. Am. J. Aquacult.,* **2001***, 63***,** 118-130.
5. C. K. Lin, K. Jaijen and V. Muthuwana, "Integration of intensive and semi-intensive aquaculture: Concept and example", *Thai Fisheries Gazette*, **1990**, *43*, 425-430.

6. C. K. Lin and J. S. Diana, "Co-culture of catfish (*Clarias macrocephalus* x *C. gariepinus*) and tilapia (*Oreochromis niloticus*) in ponds", *Aquat. Living Resour.*, **1995**, *8*, 449-454.
7. J. W. Andrews, T. Murai and G. Gibbons, "The influence of dissolved oxygen on the growth of channel catfish", *Trans. Am. Fish. Soc.*, **1973**, *102,* 835-838.
8. Y. Yi, C. K. Lin and J. S. Diana, "Hybrid catfish (*Clarias macrocephalus x C. gariepinus*) and Nile tilapia (*Oreochromis niloticus*) culture in an integrated pen-cum-pond system: Growth performance and nutrient budgets", *Aquaculture*, **2003**, *217*, 395-408.
9. L. Torrans and F. Lowell, "Effects of blue tilapia/channel catfish polyculture on production, food conversion, water quality and channel catfish off-flavor", *Proc. Arkansas Acad. Sci.*, **1987**, *41*, 82-86.
10. C. C. Grimm, S. W. Lloyd and P. V. Zimba, "Instrumental versus sensory detection of off-flavors in farm-raised channel catfish", *Aquaculture*, **2004**, *236,* 309-319.
11. C. E. Boyd and C. S. Tucker, "Water Quality Pond and Soil Analyses for Aquaculture", Alabama Agriculture Experiment Station, Auburn University, Auburn (AL), **1992**, pp.73-135.
12. L. Wongrat and S. Boonyapiwat, "Manual of Sampling and Analytical Methods of Plankton", Kasetsart University Press, Bangkok, **2003** (in Thai).
13. E. Küster and S. T. Williams, "Selection of media for isolation of streptomycetes", *Nature,* **1964**, *202*, 928-929.
14. E. B. Shirling and D. Gottlieb, "Methods for characterization of *Streptomyces* species", *Int. J. Syst. Evol. Microbiol.*, **1966**, *16*, 313-340.
15. E. M. H. Wellington and S. T. Williams, "Preservation of actinomycete inoculum in frozen glycerol", *Microbiol. Letters*, **1977**, *6*, 151-157.
16. R. F. Robertson, A. Hammond, K. Jauncey, M. C. M. Beveridge and L. A. Lawton, "An investigation into the occurrence of geosmin responsible for earthy-musty taints in UK farmed rainbow trout, *Onchorhynchus mykiss*", *Aquaculture,* **2006**, *259*, 153-163.
17. P. E. Persson, "Sensory properties and analysis of two muddy odour compounds, geosmin and 2-methylisoborneol, in water and fish", *Water Res.*, **1980**, *14*, 1113-1118.
18. N. Whangchai, K. Kannika, S. Deejing, T. Itayama, N. Iwami, T. Kuwabara and Y. Peerapornpisal, "Growth performance and accumulation of off flavor in red tilapia, *Oreochromis niloticus x Oreochromis mosambicus*, cultured by green water system using chicken manure", *Asian Environ. Res.*, **2008**, *1*, 8-16.
19. C. P. Dionigi, P. B. Johnsen and B. T. Vinyard, "The recovery of flavor quality by channel catfish", *N. Am. J. Aquacult.*, **2000**, *62,* 189-194
20. U. Tanchotikul, "Studies on important volatile flavor components in Louisiana Rangia clam (*Rangia cuneata*)", *Doctoral Dissertation*, **1990**, Louisiana State University, USA, p.96.
21. K. E. Clark, F. A. P. C. Gobas and D. Mackay, "Model of organic chemical uptake and clearance by fish from food and water", *Environ. Sci. Technol.*, **1990**, *24*, 1203-1210.
22. J. Yamprayoon and A. Noomhorm, "Geosmin and off-flavor in Nile tilapia (*Oreochromis niloticus*)", *J. Aquat. Prod. Technol.*, **2000**, *9,* 29-41.
23. C. Klausen, M. H. Nicolaisen, B. W. Strobel, F. Warnecke, J. L. Nielsen and N. O. Jorgensen, "Abundance of actinobacteria and production of geosmin and 2-methylisoborneol in Danish streams and fish ponds", *FEMS Microbiol. Ecol.*, **2005**, *52*, 265-278.
24. I. Y. B. Lelana, "Geosmin and Off-Flavor in Channel Catfish", *PhD Thesis*, **1987**, Auburn University, USA.

Maejo Int. J. Sci. Technol. **2013**, *7*(Special Issue), 1-13

25. L. A. Lawton, P. K. J. Robertson, R. F. Robertson, F. G. Bruce, "The destruction of 2-methylisoborneol and geosmin using titanium dioxide photocatalysis", *Appl. Catal. B. Environ*.**,** **2003**, *44*, 9-13.
26. C. Wirasith and S. Traichaiyaporn, "Water quality variation and algal succession in commercial hybrid catfish production ponds", *Maejo Int. J. Sci. Technol*., **2012**, *6*, 105-118.
27. B. Sen and F. Sonmez, "A study on the algae in fish ponds and their seasonal variations", *Int. J. Sci. Technol.*, **2006**, *1*, 25-33.
28. A. H. Chowdhury and A. A. Mamun, "Physio-chemical conditions and plankton population of two fishponds in Khulna", *Univ. J. Zool. Rajshahi Univ.*, **2006**, *25*, 41-44.
29. S. B. Watson, "Cyanobacterial and eukaryotic algal odour compounds: Signals or by-products? A review of their biological activity", *Phycol.*, **2003**, *42*, 332-350.
30. P. E. Persson, "Odorous algal cultures in culture collections", *Water Sci. Technol*., **1988**, *20*, 211-213.
31. R. T. Lovell and D. Broce, "Cause of musty flavor in pond-cultured penaeid shrimp", *Aquaculture*, **1985**, *50*, 169-174.
32. J. From and V. Horlyck, "Sites of uptake of geosmin, a cause of earthy-flavor, in rainbow trout (*Salmo gaidneri*)", *Can. J. Fish. Aquat. Sci.*, **1984**, *41*, 1224-1226.