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Off-flavors in pond-grown ictalurid catfish: Causes and management options

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Abstract

Ictalurid catfish grown in ponds often acquire undesirable off-flavors prior to harvest. Off-flavors develop when odorous, lipophilic substances in food or water are absorbed across gut or gill epithelium and concentrated in edible tissues. The most common causes of catfish off-flavors are two nontoxic secondary metabolites of planktonic cyanobacteria: geosmin (causing an earthy off-flavor) and 2-methylisoborneol (causing a musty off-flavor). Off-flavored fish are unacceptable for processing, and harvest must be postponed until the source of the odorous compound disappears and the compound purges from edible fish tissue. Harvest delays caused by episodes of off-flavor increase production time, interrupt cash flow, and increase the risk of fish loss. Catfish farmers consider off-flavor to be one of their most important production-related problems. This paper reviews the causes of off-flavor in catfish, pharmacokinetics of uptake and loss of odorous compounds in catfish, seasonality and prevalence of off-flavors, farm- and industry-level impacts, the ecology of cyanobacteria in catfish ponds, and various strategies for preventing or treating cyanobacterial (and other) off-flavors. A decision-making system based on knowledge gained from research is presented as a guide to effective use of the limited tools available to manage off-flavors.

KEYWORDS

2-methylisoborneol, blue-green algae, catfish, cyanobacteria, geosmin, off-flavor

1 | INTRODUCTION

Pond-grown ictalurid catfish¹ constitute the largest sector of United States aquaculture. Nearly all fish are sold domestically, mainly to markets in the southeastern and midwestern United States—an area roughly corresponding to the fish's native range. Within that region, wild-caught catfish were a traditional and revered food item. Since about 1980 most catfish consumed in the United States have been grown on farms in earthen ponds and consumers have expectations of certain fillet-quality attributes, especially flavor.²

Catfish grown in clean water, fed grain-based diets, and processed and handled correctly have an exceptionally mild, non-fishy flavor enjoyed by many American consumers, especially those in non-coastal areas (Drammeh, House, Sureshwaran, & Selassie, 2002; Kinnucan, Nelson, & Hiariay, 1993). But, as with all animal food products, farmed catfish occasionally develop disagreeable off-flavors that can impact consumer acceptance.

Although off-flavors can develop in any aquatic species, most information about this problem in aquaculture has been obtained from studies of pond-grown catfish in the southern United States. This review summarizes those studies. We focus our discussion on off-flavors in pond-grown catfish but believe this information will be of interest and benefit to culturists in other aquaculture situations. General reviews of off-flavors in aquaculture are available in Tucker (2000), Rimando and Schrader (2003); Howgate (2004), Smith, Boyer, and Zimba (2008), and Papp (2008).

2 | WHAT ARE OFF-FLAVORS?

Off-flavors are unpleasant, undesirable, or unexpected flavors in water, beverages, or foods (Lin, Watson, & Suffet, 2019; Mottram, 1998; Reineccius, 1991). Unusual food flavors often cause consumer complaints, probably because some off-flavors are associated with unsafe foods. However, the link between flavor and food safety is far from consistent: absence of off-flavors does not guarantee food safety and the presence of off-flavors does not necessarily mean that foods are unsafe. For example, dinoflagellate-derived toxins causing various shellfish-related poisonings impart no flavor to shellfish meat whereas the most common off-flavors in catfish are caused by odorous chemicals that are essentially nontoxic (Burgos et al., 2014; Dionigi, McFarland, & Johnsen, 1993; Nakajima et al., 1996; Watson, 2013).

Food off-flavors can be caused by many processes, which complicate efforts to identify and eliminate the source. Broadly speaking, unacceptable flavors can develop through chemical or microbial action (usually after the food is processed) or by contamination (either before, during, or after processing). Precise usage restricts the term “off-flavor” to describe unacceptable flavors caused by internal processes (e.g., spoilage) whereas “taints” refers to unacceptable flavors caused by external contamination. We will follow common practice and, for the most part, use the term “off-flavor” to describe undesirable food flavors regardless of origin.

Catfish—like all fish and shellfish—are among the more perishable foods, which increases the risk of off-flavors developing after harvest (Ólafsdóttir & Kristbergsson, 2006). The high water content of fresh seafoods predisposes products to microbial spoilage, leading to fishy, ammonia-like, or putrid off-flavors (Ólafsdóttir & Jonsdóttir, 2010). Many seafoods also have high levels of unsaturated fatty acids and are susceptible to lipid oxidation (Tao, 2015), which causes characteristic stale or rancid off-flavors when products are stored improperly or stored for long periods (Rustad, 2010). Farm-raised catfish have relatively low levels of highly unsaturated fatty acids (Li, Robinson, Tucker, Manning, & Khoo, 2009) but are nevertheless susceptible to post-harvest lipid oxidation and development of associated off-flavors (Brannan & Erickson, 1996; Eun, Boyle, & Hearnberger, 1994). We will not address post-harvest off-flavors in this review but rather focus on taints—off-flavors caused by external factors before harvest.

Taints may be caused by waterborne substances absorbed across the gills, skin, or gastrointestinal tract and deposited in the flesh or by dietary components absorbed across the gastrointestinal tract. Diet-related off-flavors are not uncommon in wild-caught fish (Tucker, 2000) but are relatively rare in aquaculture because high-quality

commercial feeds are formulated so they do not negatively affect product flavor. However, pond-grown fish occasionally eat foods other than manufactured feed, and some of those food items may impart undesirable flavors.

Most pre-harvest off-flavors are caused by odorous waterborne compounds absorbed and deposited in edible tissues. Waterborne tainting agents may come from pollutants or they may be natural products synthesized by aquatic microorganisms. Off-flavors arising from pollution are rare in aquaculture but flavor problems caused by naturally occurring microbial metabolites can be common.

The most common taints encountered in farm-raised fish are caused by chemicals that are, for practical purposes, non-toxic. Unlike other problems that farmers encounter during production—such as oxygen depletions or infectious diseases—off-flavors impact esthetics and marketability rather than causing fish to grow slowly or die. If off-flavored catfish are inadvertently harvested and marketed, the negative consumer reaction may adversely affect long-term market demand. To prevent off-flavored fish from reaching the marketplace, farmed catfish are evaluated for flavor quality before harvest and if disagreeable flavors are noted, fish will not be accepted for processing. The effect of delayed or denied harvest on farmer profits and cash flow is obvious, and catfish farmers consider pre-harvest off-flavors to be their most important production-related problem.

2.1 | Flavors, memory, and catfish marketing

Food enjoyment or displeasure is the result of sensory interactions between taste (gustation) and smell (olfaction), along with other sensory cues such as texture, temperature, and appearance. Flavor, in a narrower context than the broader hedonic experience involving all senses, is usually defined as a unified experience resulting from inputs from taste receptors on the tongue (producing sensations of bitter, salt, savory, sour, and sweet) and odor receptors in the nasal cavity. There are hundreds of specific odor receptors and the sensations may be combined to allow discrimination of many thousands—perhaps many millions—of distinct odors. For most foods and for most humans, olfaction plays the dominant role in our perception of flavor (Spence, 2015).

Olfaction, more than any other human sense, is strongly linked to memories and emotions (Herz & Engen, 1996). The relationship is so well known that it has been called “the Proust phenomenon,” based on the most famous description of odor-invoked memory in Marcel Proust's multi-volume work, *In Search of Lost Time* (Proust, 1913). The adult narrator first expresses frustration about his difficulty recalling childhood experiences:

“And so it is with our own past. It is a labor in vain to attempt to recapture it: all the efforts of our intellect must prove futile. The past is hidden....”

But then, the aroma and flavor of a small piece of sponge cake soaked in tea evokes an emotional response to long-forgotten childhood memories:

“But when from a long-distant past nothing subsists, after the people are dead, after the things are broken and scattered, ... the smell and taste of things remain poised a long time, like souls, ready to remind us, ... and bear unfaltering, in the tiny and almost impalpable drop of their essence, the vast structure of recollection.”

The same thought was expressed more succinctly by Vladimir Nabokov (1926) in his novel *Mary*,

“... nothing revives the past so completely as a smell that was once associated with it.”

The links among olfaction, memory, and emotion are more than a convenient literary device: they have a neuro-anatomic basis that was favored and conserved during animal evolution. Impulses from olfactory receptor neurons are transmitted to the olfactory bulb in the forebrain. The olfactory bulb is directly connected to the amygdala and

hippocampus brain areas, which together comprise the system responsible for memory, learning, and emotions. No other sensory system (visual, auditory, touch) has this direct connection with those brain areas.

The evolutionary significance of odor memory is obvious. Odor memory facilitates associative learning, whereby an experience—either positive or negative—is linked to an odor encountered at the same time. The odor/experience pair is then stored as a durable memory. Afterwards, if that odor is again encountered, the experience linked to the odor is recalled and the paired association either compels the animal to repeat the experience if the original experience was positive or avoid the experience if the original experience was unpleasant. Odor memory is involved in many interactions and behaviors essential for survival. Examples include maternal odor and mother–infant identification and attraction, predator detection and recognition, mate choice and attraction between mating pairs, kin and clan recognition, identification of social rank, locating food, and attraction to—or avoidance of—certain food items (Hoover, 2010; Sullivan, Wilson, Ravel, & Mouly, 2015).³

The best-studied of these behavioral phenomena is the role of odor memory in shaping food preferences, particularly the link between flavor and negative physiological responses to toxic or spoiled foods. This interaction—called *conditioned taste aversion*, or the Garcia Effect—occurs when an animal eats a food and then experiences unpleasant aftereffects, such as gastrointestinal distress, headaches, dizziness, or other toxic reactions.⁴ The animal then avoids further contact with that food. This adaptive trait is unlike other examples of associative learning in that conditioning develops rapidly (only one episode is required for the link to be formed), it is long-lasting, and conditioning occurs even when the negative physiological reaction occurs hours after the initial stimulus. The same quick and long-lasting association does not develop with other stimuli, such as sight and sound. Associative learning also occurs when a particular flavor is paired with a positive nutritive effect, such as caloric intake. This phenomenon is called *conditioned food preference* and under some circumstances the association can be as robust as conditioned aversion (Scalifani, 2013).

The role of odor and flavor memory in human food choice has changed dramatically over time. Early hominins were omnivorous and compelled to sample many foods to survive. Different foods produced either a positive, negative, or neutral reaction. Each experience was paired with an odor or flavor and stored as a long-lasting memory that shaped foraging behavior. For humans in modern developed countries, odor memory may retain some importance in food-choice decisions related to health (such as detecting, then avoiding, spoiled food—the Garcia Effect) but the day-to-day role of odor memory has shifted to that of guiding our food purchasing decisions. Simply put, if you eat something and the experience is unpleasant, the memory of that experience will make it less likely that you will purchase that food in the future—and vice versa.

As an example of the role of flavor in guiding our food preferences, look at this passage from *Goldfinger*, Ian Fleming's (1959) iconic spy novel:

"The Hôtel de la Gare was all he had expected—cheap, old-fashioned, solidly comfortable. Bond had a hot bath, went back to his car to make sure the Rolls hadn't moved, and walked into the station restaurant and ate one of his favourite meals—two oeufs cocotte à la crème, a large sole meunière (Orleans was close enough to the sea. The fish of the Loire are inclined to be muddy) and an adequate Camembert."⁵

Apparently, the novel's hero, James Bond, previously had eaten fish taken from the freshwater habitat of the River Loire, which runs through Orleans, and found them to have a muddy taste. His memory of that experience compelled him instead to order sole—a fish from the ocean—comfortable in the knowledge, based on past experience, that sole are unlikely to have the muddy flavor. Bond also seems aware that the sole was fresh and therefore unlikely to possess spoilage-related off-flavors because Orleans was "close enough to the sea" (although Secret Agent 007's idea of "close enough" is relative—Orleans is 200 km from Le Harve on the English Channel and more than 300 km from Nantes on the Bay of Biscay).

Interestingly, Bond (or rather, the author, Fleming) knew that marine fish were less likely to be “muddy” than freshwater fish. And, in fact, the microorganisms causing muddy off-flavors in fish do not thrive in full-strength saltwater—but that is a story for later.

Bond's avoidance of fish “inclined to be muddy” is a superb example of the importance of fish off-flavors and the associated memory of the off-flavor acting as a driver of food choice and marketplace decisions. The relationship between food choice and off-flavor is especially important for farm-raised catfish.

First, pond-grown catfish compete in the marketplace with other farmed freshwater fish (almost always imported), seafood from wild harvest, and with poultry and other terrestrial animal proteins. If consumers—particularly first-time buyers—encounter catfish with disagreeable flavors, they might assume the flavor is common, or even inherent (as did James Bond with fish from the River Loire), and shun future purchases in favor of competing products, many of which are less expensive as well.

Of course, marketplace competition among animal proteins is not unique to catfish. But catfish do have a singular problem not faced by most other animal food products: outside the traditional catfish-consuming region of the southern and midwestern United States, catfish are sometimes perceived as bottom-dwelling scavengers and that mistaken mental image may evoke the expectation of an unpleasant gustatory experience. And then, if disagreeable flavors are encountered when first eating catfish, the association of flavor and food will reinforce that false stereotype.

Most important, however, is the very nature of pond aquaculture, which predisposes catfish to a relatively high risk of developing pre-harvest off-flavors. An important characteristic of ponds setting them apart from other aquaculture systems is the low level of management control exerted over ecological processes. Natural processes, operating at no direct cost to the farmer, provide important resources needed for culture, such as waste treatment (Tucker & Hargreaves, 2012). With the exception of oxygen supply, which commonly is supplemented by mechanical aeration, pond water quality is difficult, impractical, or expensive to control. An unintended—but direct—consequence of the lack of ecosystem control is the dynamic, ever-changing pond microbial community, often including transient populations of odor-producing algae or bacteria. Further, ponds are “open” culture systems at higher risk to inadvertent pollution than smaller, indoor aquaculture systems. As such, it must be admitted at the outset that the risk of catfish developing off-flavors can never be reduced to zero as long as they are grown in outdoor ponds.

The challenge for catfish farmers and processors is to prevent off-flavored fish from reaching the marketplace—and this is indeed a substantial challenge. Pre-harvest off-flavors are managed through a combination of pond management practices (mostly aimed at changing the microbial community to eliminate odorous microorganisms) and pre-harvest sampling to prevent tainted fish from being harvested and marketed.

2.2 | The flavors of catfish

The discussion of odor memory in the previous section implies that odor recall, as a general phenomenon, is excellent. This is not entirely true. Odor memory is extraordinary at evoking a past experience through contact with its associated odor. However, naming an odor and recalling that name with accuracy and consistency is difficult (Herz & Engen, 1996; Schab, 1991). Compare this with vision, where a word describing an everyday object (“apple” for example) immediately evokes a mental image of the word (you imagine an apple) and, conversely, when you see the item, the name is quickly recalled.

This reciprocity does not exist with odors and flavors. That is, odors may be recognized and durably linked to an emotional experience but the sensation of an odor (the olfactory “image”) is difficult to retrieve by thinking about it. The limited ability to form odor images (e.g., the imagined smell or flavor of an apple) is linked, perhaps causally, to difficulties in tagging a smell or odor with a specific name (Stevenson, Case, & Mahmut, 2007).

The difficulty in consistently naming an odor or flavor often gives rise to the so-called “tip-of-the-nose” phenomenon, where an attempt to put a name to an odor often ends with something akin to, “I know it but I cannot recall it”

(Lawless & Engen, 1977). In some ways this is similar to the “tip-of-the-tongue” phenomenon, where a person cannot quite recall a word but can recall words with certain properties in common with the word, such as sound, form, meaning, or even the same first letter. Further, the ability to recall odor names and sensitivity to odors and flavors varies from person to person (e.g., with age and gender, for example), setting, and other factors (Kaeppeler & Mueller, 2013).

Difficulties in identifying and naming odors and flavors create a special problem for catfish off-flavor management. Most off-flavors are specific to a unique and singular source, so the first step in off-flavor management is to accurately describe the odor or flavor so the source of the problem can be identified and addressed. As a simple example, off-flavors described as “petroleum” are always caused by accidental leakage of diesel fuel or other petroleum product into ponds. Once the off-flavor is identified as “petroleum” (or similar term), the source of contamination usually can be quickly identified and addressed.

Standardization of terms (or at least some level of consistency) also is essential for communication among researchers and industry quality-control personnel. Accuracy, consistency, and sensitivity of sensory analysis can be sharply improved through training and formalization of the flavor-testing process (American Society for Testing and Materials, 1981; Codex Alimentarius Committee, 1999; Meilgaard, Civille, & Carr, 2007), and highly formalized flavor testing is routine in many food and beverage industries. To date, however, there has been little effort to standardize terminology for catfish flavors or to train flavor testers in the commercial sector.

Flavor descriptors usually are words that bring to mind a common flavor or odor because the exact chemical cause of most off-flavors is not known. For instance, a stale off-flavor sometimes found in catfish has been described as “cardboard.” The flavor is probably caused by a mixture of lipid oxidation products (Johnsen & Kelly, 1990) and is not, obviously, caused by the presence of cardboard or, for that matter, the same chemicals giving wet cardboard its “flavor.” Two important exceptions are the names given to earthy and musty off-flavors. The chemicals causing those flavors are known with certainty—geosmin and 2-methylisoborneol (2-MIB)—and the chemical names are often used to describe those specific flavors.

Because flavor names can be so subjective, it should be no surprise that several dozen terms (many of which may refer to the same flavor) have been used to describe the various off-flavors in catfish (Table 1). To date, the best effort to standardize catfish flavor terminology was that of Johnsen, Civille, and Vercelotti (1987), which was then refined by Johnsen and Kelly (1990).

Johnsen et al. (1987) empaneled 18 persons with experience with catfish farming, processing, or research. Few of the participants had any experience with formalized sensory analysis. The panel was trained in Descriptive Analysis (Civille, 1987) for 2 days. They then evaluated a series of “on-flavor” and “off-flavor” catfish samples pre-selected to represent a range of flavors and flavor intensities. Flavor descriptors were solicited from all panel members and refined by discussion and consensus to select the most appropriate terms and eliminate redundancies.

Johnsen and Kelly (1990) refined the lexicon developed by Johnsen et al. (1987) using a rigorous protocol for screening, selecting, and training a panel for descriptive flavor analysis. Sixteen panel members were selected after a screening process involving testing to assess flavor perceptiveness and a questionnaire to determine personal habits that may affect qualifications. The panel was trained for 75 hr on the analytical technique, during which they refined the list of descriptors. Flavor intensities were anchored to intensities and characteristics for other food products, in contrast to the un-referenced hedonic (degree-of-liking) scale used by flavor testers at commercial catfish processing plants. Panel sensitivity (ability to detect low levels of off-flavor and ability to discriminate minor flavor variations between samples) and reproducibility (stability of sensory evaluations over time) were considered excellent.

Another refinement in the Johnsen and Kelly (1990) study involved using of “blended individual fish samples,” or BIFS, instead of intact samples from individual fish. BIFS overcome problems of within-fish flavor variation when different parts of a fillet contain different levels of a tainting substance. Preparation of BIFS involves shredding and mixing fillets from a fish or a group of fish, sealing portions in plastic bags, freezing and storing the samples, then cooking in boiling water. Although this sample-preparation protocol is necessary for research, it is not the procedure used by flavor testers at catfish processing plants. Commercial testers taste portions from individual fish taken

TABLE 1 Terms describing flavors, tastes, and mouth-feels in fish. The Codex Alimentarius Committee (1999) list is for seafoods in general; others were developed for pond-raised catfish

Maligalig, Caul, and Tiemeier (1973)	Van der Ploeg (1991) (cont.)	Bett (1997) (cont.)
Ammoniacal	Fish oil	Decaying vegetation
Bland	Green/grassy	Eggy/sulfury
Chicken-like	Metallic	Fat complex
Earthy	Moldy	Fish oil
Fish oil	Mushroom	Gamey/fishy
Fresh fish	Musty/MIB	Gelatin/starch
Green	Nutty	Geosmin (dry musty)
Lemony	Onion	Grassy
Meaty	Paint	Green vegetables
Muddy	Pesticide	Iodine
Rubbery	Pine	MIB (wet musty)
Spinachy	Rancid	New lumber
Sweet	Rotten	Nutty
	Sewage	Painty
Lovell (1983a, 1983b)	Stale	Salty
Cardboard	Woody	Sour
Chemical		
Detritus	Chambers and Robel (1993)	Codex Alimentarius Committee (1999)
Earthy-musty	Astringent	Bilgy
Metallic	Bitter	Bitter
Moldy	Decaying vegetation	Briny
Musty-muddy	Earthy	Chalky
Petroleum	Fresh fish	Cucumber
Rancid	Metallic	Decomposed
Rooty	Nutty/buttery	Feedy
Sewage	Oily aromatic	Fecal
Stale	Salty	Fishy
Weedy	Sour	Fruity
	Sweet aromatic	Gamey
van der Ploeg (1991)	White meat	Metallic
Buttery/fat		Moldy
Camphor	Bett (1997)	Musty
Cardboard	Ammonia	Pungent
Celery	Astringent	Putrid
Chicken	Bitter	Rancid
Corn	Briny	Rotting vegetable
Crawfish	Cardboardy	Salty
Decaying vegetation	Chemical	Sour
Diesel	Chickeny	Sweet
Earthy/geosmin	Corn	Umami
Egg/sulfury	Crustacean	Watermelon
Fecal	Dairy	Yeasty

Abbreviation: MIB, 2-methylisoborneol.

TABLE 2 Descriptive terms for flavors of farm-raised catfish (modified from Johnsen & Kelly, 1990; the descriptor “petroleum” was not included in the original lexicon)

Descriptive term	Associated odor, flavor, or sensation
Aromatics	
Nutty	Fresh pecans and other hardshell tree nuts
Chickeny	Sweet cooked chicken white meat
Fat complex	Vegetable oil or cooked chicken meat
Corn	Cooked sweet corn
Green vegetable/grassy	Fresh green vegetables or grassy vegetation
Eggy/sulfury	Boiled egg
Geosmin	Old books; geosmin is the reference
MIB	Mud; 2-methylisoborneol is the reference
Decaying vegetation	Decaying pondweed or swamp grass
Cardboardy	Wet cardboard or brown paper bag; stale
Fishy	Oily marine fish; the reference is trimethylamine
Petroleum	Diesel fuel, kerosene
Tastes	
Sweet	The taste of sugar on the tongue
Salty	The taste of table salt on the tongue
Sour	The taste of citric acid on the tongue
Bitter	The taste of caffeine on the tongue
Feeling factors	
Astringent	Puckering or dry sensation; strong tea
Metallic	Flat, the sensation of iron or copper on the tongue
Peppery	Tingly on the tongue, pepper

Abbreviation: MIB, 2-methylisoborneol.

directly from the pond or transport truck. The samples may be skinned or not, different parts of edible portion may be used, and the fish usually are cooked without seasoning in a microwave oven.

The list of flavor descriptors developed by Johnsen and Kelly (1990) is presented in Table 2. The descriptor “petroleum” is added to the list in Table 2 but did not appear in the lexicons compiled by Johnsen et al. (1987) and Johnsen and Kelly (1990), despite acknowledgment by those authors that petroleum off-flavors, although extremely rare in catfish farming, do occur.

Notable in the lexicons developed by Johnsen et al. (1987) and Johnsen and Kelly (1990) are terms describing the flavor profile of “on-flavor” catfish. One of us (C.S.T.) was a member of the Johnsen et al. (1987) panel and recalls the panel’s difficulty in deriving appropriate descriptors for on-flavor catfish. The mild, non-fishy flavor results in low flavor-attribute intensities, which can make it difficult to assign a name to the flavor. The panelists agreed that the dominant attributes of on-flavor catfish were “chickeny” and “nutty/pecan.” Although these attributes were dominant, their intensity scores were low, confirming the delicate flavor of on-flavor farmed catfish (Johnsen & Kelly, 1990; Mills, Chung, & Johnsen, 1993). Other positive flavor attributes—but with lower intensity scores than chicken/nutty—were “corn”, “fat complex,” and the taste, “sweet.” It was assumed that the extremely mild flavor bouquet described by these terms represents the genetically determined flavor of catfish. Other flavors, considered off-flavors, are determined by environment or diet, and are the subject of the remainder of this review.

3 | CATFISH OFF-FLAVORS OF MICROBIAL ORIGIN

Most pre-harvest off-flavors in pond-raised catfish are caused by odorous compounds produced by microorganisms. The most common of these microbial products produce flavors described as earthy or musty—flavors described in fish as early as the mid-16th century (Gesner, 1550).

The first attempt to isolate the source of earthy/musty odors was made by Berthelot and André (1891) when they discovered that an earthy-smelling substance could be extracted from soil by using steam. Soon afterwards the focus on the source of earthy odors turned to microorganisms as techniques were developed for their culture and growth in the laboratory. In 1895, Rullman (1895) isolated a bacterium producing a strong earthy odor and later named the organism *Cladothrix odorifera*.

The first link between earthy odors in water and actinomycetes (bacteria belonging to the order Actinomycetales of the phylum Actinobacteria) appears to have been made by Adams (1929) during his studies of earthy odors emanating from the River Nile. The presence of objectionable earthy odors in aquatic systems used for drinking water led Silvey and Roach (1953) to study methods to control aquatic species of odor-producing actinomycetes, although the compound responsible for the odor was still unknown. During their attempts to isolate the earthy-odor compound produced by certain bacteria, Romano and Safferman (1963) developed distillation and extraction methods for the recovery of an odoriferous substance from cultures of *Streptomyces griseoluteus*; however, a pure compound could still not be obtained. Gaines and Collins (1963) speculated that the earthy odor from *Streptomyces odorifer* might be due to a combination of compounds such as acetic acid, acetaldehyde, ethanol, isobutanol, isobutyl acetate, or ammonia. This assumption was soon proven incorrect.

The advent of new analytical techniques and equipment, such as gas chromatography, during the latter half of the 20th century provided scientists with greater ability to identify odorous compounds produced by microorganisms. Gerber and Lechevalier (1965) were the first to identify the compound responsible for the earthy odor produced by various actinomycetes, including *Streptomyces antibioticus*, *Streptomyces fradiae*, *Streptomyces griseus*, and *Streptomyces odorifer*. Their study focused mainly on broth cultures of *S. griseus*, from which they were able to obtain milligram quantities of a compound they named “geosmin” from the Greek words “ge” for earth and “osme” for odor.

Shortly after the discovery of geosmin from actinomycete cultures, its production by certain species of cyanobacteria (blue-green algae) was confirmed. Safferman, Rosen, Mashni, and Morris (1967) reported the first example of geosmin production in cyanobacteria when they isolated the earthy compound from cultures of the filamentous cyanobacterium *Symploca muscorum*. Their research showed that microorganisms other than actinomycetes can cause earthy and musty odor problems in water supplies and other aquatic ecosystems. The following year Medsker, Jenkins, and Thomas (1968) confirmed the production of geosmin from cultures of *S. muscorum* and also reported the compound in laboratory cultures of the cyanobacterium *Oscillatoria tenuis*.

Several years after geosmin was discovered, another common odorous compound produced by actinomycetes was identified. Because the overall odor of many species of actinomycetes can be caused by a combination of volatile, odorous compounds, Medsker, Jenkins, Thomas, and Koch (1969) conducted research to isolate and identify additional odorous substances from filamentous bacteria. They discovered a “camphor-smelling” compound produced by cultures of *Streptomyces antibioticus*, *S. griseus*, and *Streptomyces praecox* and designated this compound as 2-exo-hydroxy-2-methylbornane. Interestingly, the compound had been discovered decades earlier by Zelinsky (1901), but it was not chemically identified and attributed to be a microbial product. The compound and its chemical structure were confirmed by Gerber (1969) from cultures of *Streptomyces lavendulae* and referred to as 2-methylisoborneol (often shortened to 2-MIB, or simply MIB).

3.1 | Geosmin

Geosmin is a dimethyl substituted, saturated, two-ring *tert*-alcohol with a molecular weight of 182.31 (Figure 1a). It is a colorless neutral oil with a boiling point of 270°C. The elemental composition of geosmin is 79.06% carbon, 12.16% hydrogen, and 8.78% oxygen. Water solubility is 157 mg/L at 25°C (Howard & Meylan, 1997).

Geosmin exists as four stereoisomers: *trans*-1,10-dimethyl-*trans*-9-decalol; *cis*-1,10-dimethyl-*trans*-9-decalol; *trans*-1,10-dimethyl-*cis*-9-decalol; *cis*-1,10-dimethyl-*cis*-9-decalol (Figure 1a–d, respectively). All isomers have an earthy odor although each is distinctive with additional odor qualities. The *cis*-fused isomers (Figure 1c,d) have odors similar to camphor and cedar that are less objectionable than the more pungent earthy odors produced by the *trans*-isomers (Figure 1a,b; Marshall & Hochstetler, 1968). Polak, Trotier, and Baliguet (1978) reported that humans had increasing difficulty discriminating between the odors of the four isomers as concentrations decreased, and tend to focus more on the earthy odor quality than on other odor qualities present in *trans*-1,10-dimethyl-*cis*-9-decalol (Figure 1c) when compared with the other three isomers.

The isomer of geosmin originally discovered by Gerber and Lechevalier (1965) and commonly attributed as the source of most earthy odors produced by actinomycetes and cyanobacteria is *trans*-1,10-dimethyl-*trans*-9-decalol (Figure 1a). Gerber and Lechevalier (1965) found that pure geosmin in 10% hydrochloric acid for 4 days at room temperature would yield only pure argosmin (Figure 1e) which has an approximate boiling point of 230°C. Argosmin, later confirmed by Gerber (1968) to be 1,10-dimethyl-1(9)-octalin, is odorless.

Geosmin is a potent flavor-impairing chemical in water and fish. Trained sensory panels can detect the odor in water at concentrations less than 0.02 µg/L (Buttery, Guadagni, & Ling, 1976; Persson, 1980). The sensory threshold concentration (the lowest concentration that can be tasted or smelled) for geosmin in fish ranges from <1 to about

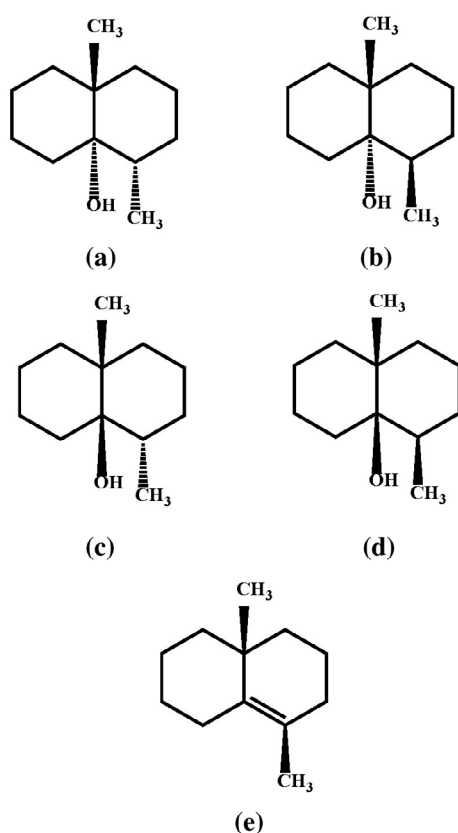


FIGURE 1 Chemical structures of the four (a–d) stereoisomers of geosmin and argosmin (e)

10 $\mu\text{g/kg}$, depending on the background flavor of the fish (e.g., degree of “fishiness”; Yurkowski & Tabachek, 1974; Persson, 1980). Farm-raised catfish have a mild flavor and, therefore, geosmin is detectable at very low concentrations. Average consumers consider catfish off-flavor when geosmin concentration exceed 0.7 $\mu\text{g/kg}$ and trained sensory panels can detect the compound at even lower concentrations (Grimm, Lloyd, & Zimba, 2004).

3.2 | 2-Methylisoborneol

2-MIB is, like geosmin, a saturated, cyclic, tertiary alcohol. Its molecular weight is 168.28 and it is a crystalline white solid at room temperature (Medsker et al., 1969). The melting point ranges of (–)-MIB and (+)-MIB are 163–165°C and 162–165°C, respectively, and the elemental analysis of (–)-MIB is 78.35% carbon, 12.07% hydrogen, and 9.58% oxygen while (+)-MIB contains 78.23% carbon, 11.94% hydrogen, and 9.83% oxygen (Tyler, Acree, & Butts, 1978). The water solubility of MIB has been reported to be within the range of 189–200 mg/L at 20°C (Pirbazari, Borow, Craig, Ravindran, & McGuire, 1992).

There are two stereoisomers of 2-methylisoborneol with the chemical names of (1-*R*-exo)-1,2,7,7-tetramethylbicyclo[2.2.1]heptan-2-ol and (1-*S*-exo)-1,2,7,7-tetramethylbicyclo[2.2.1]heptan-2-ol (Figure 2a,b, respectively). The *R*-enantiomer of 2-MIB (Figure 2a), also referred to as the (–) enantiomer, is the naturally occurring isomer and the source of off-flavors in water and fish (Wood & Snoeyink, 1977). All isomers have a camphor-like odor, which does not change over several magnitudes of concentration (Tyler et al., 1978). In dilute solutions, the odor is often described as musty, although the odor is in fact difficult to describe.

The mean threshold odor concentration of 2-methylisoborneol in water is about 0.04 $\mu\text{g/L}$ (Persson, 1979), which is slightly less odorous than for geosmin. However, the flavor imparted to fish by 2-MIB is detected by trained taste panels at concentrations similar to those for geosmin. The sensory threshold for 2-methylisoborneol in catfish is about 0.7 $\mu\text{g/kg}$ for average consumers and between 0.1 and 0.2 $\mu\text{g/kg}$ for trained sensory panels (Bett et al., 2000; Grimm et al., 2004).

3.3 | Other odorous microbial metabolites

In addition to geosmin and 2-methylisoborneol, many other compounds have been implicated as contributors to flavor problems in fish. Most of these compounds possess characteristic odors easily perceived in their pure form but research has not provided analytical confirmation (e.g., via gas chromatograph-mass spectrometer) of their presence in the flesh of the fish at the same time that the off-flavor is detected by sensory analysis. Confirmation of cause-and-effect of flavor problems in fish lags behind research on identifying compounds responsible for imparting tastes and odors to drinking water. It is likely that some of the compounds responsible for off-flavors in drinking water also cause off-flavors in fish, especially because many of the compounds are produced by cyanobacteria and eukaryotic algae common in aquaculture ponds.

2-Methyl-2-bornene (Figure 3a) and 2-methylenebornane (Figure 3b) are dehydration products of 2-methylisoborneol and have been proposed as contributors to musty off-flavor in fish after their detection in the

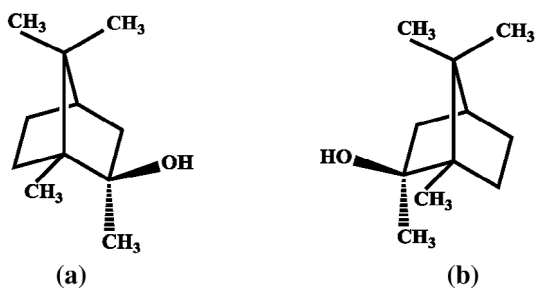


FIGURE 2 Chemical structures of the *R* (a) and *S* (b) isomers of 2-methylisoborneol

flesh of chronically off-flavored channel catfish (Martin, Bennett, & Graham, 1988; Martin, McCoy, Tucker, & Bennett, 1988). However, subsequent research showed that the two compounds are not likely to contribute to off-flavors in fish. This conclusion is based on work by Mills et al. (1993), who found that natural and synthetic forms of 2-methyl-2-bornene and 2-methylenebornane eluted from a gas chromatograph were odorless and that the compounds could be present in catfish with acceptable flavor. More recent research has determined that the major dehydration products of 2-methylisoborneol are in fact 2-methylenebornane and 1-methylcamphene (Figure 3c), with only trace amounts of 2-methyl-2-bornene produced (Schumann & Pendleton, 1997). The role, if any, of 1-methylcamphene in causing off-flavor in catfish has not been determined.

β -Cyclocitral (2,6,6-trimethylcyclohexene-1-carbaldehyde; Figure 4a) is an odorous terpenoid routinely isolated from cultures and blooms of the cyanobacterium, *Microcystis* spp. (Jüttner, 1995). Populations of *Microcystis*, especially *M. aeruginosa*, are common in summertime plankton communities of catfish ponds (Hariyadi, Tucker, Boyd, Steeby, & van der Ploeg, 1994; Tucker & Lloyd, 1984). The compound can be routinely found in catfish pond water; for example, β -cyclocitral was detected in 98% of 485 catfish ponds sampled by Zimba and Grimm (2003). By way of comparison, Zimba & Grimm found geosmin and MIB—the most common causes of catfish off-flavors—in 25% of the ponds sampled. Despite the widespread occurrence of *Microcystis* and β -cyclocitral in catfish ponds, its role as a contributor to off-flavor is uncertain.

The odor of β -cyclocitral in water changes with concentration. It has a grassy odor near its human sensory detection threshold of 0.5 $\mu\text{g/L}$, a woody or hay-like odor in the range of 2–10 $\mu\text{g/L}$, and a tobacco-like odor at concentrations greater than 20 $\mu\text{g/L}$ (Persson & Jüttner, 1983; Young, Suffet, Crozes, & Bruchet, 1999). The sensory detection threshold level for β -cyclocitral in fish is at least one order of magnitude higher than geosmin and MIB (Zimba & Grimm, 2003). The woody or hay-like odor of β -cyclocitral in water has given rise to speculation that it may be associated with the woody flavor occasionally found in pond-grown catfish. Martin and Suffet (1992) consistently isolated β -cyclocitral from water of catfish ponds with off-flavored fish, but rarely found the compound in fish. They speculated that β -cyclocitral may be metabolized to other odorous compounds in the fish or in the water (and

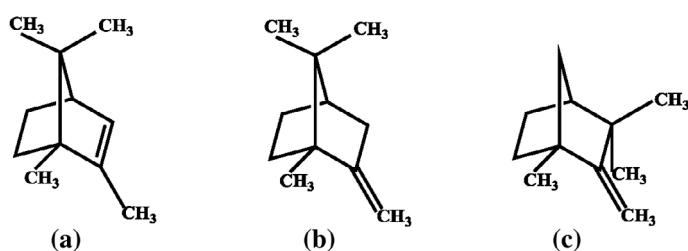


FIGURE 3 Chemical structures of 2-methyl-2-bornene (a), 2-methylenebornane (b), and 1-methylcamphene (c)

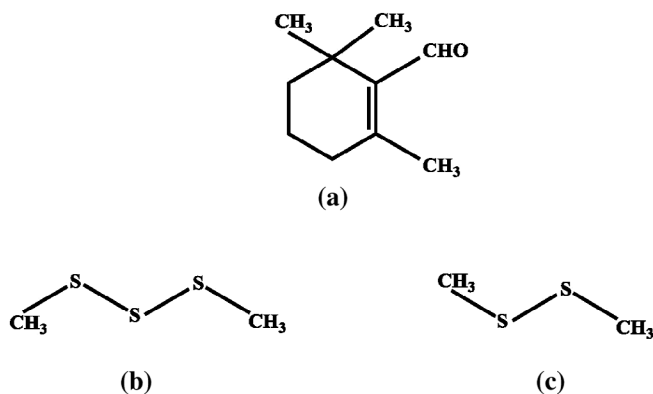


FIGURE 4 Chemical structures of β -cyclocitral (a), dimethyl trisulphide (b), and dimethyl disulfide (c)

subsequently bioconcentrated in fish). Additional research is needed to establish the role of this compound in catfish off-flavors.

Dimethyl trisulfide and dimethyl disulfide (Figure 4b,c) impart odors to drinking water described, respectively, as “decaying vegetation” and “swampy” (Khiari, Barrett, & Suffet, 1997; Suffet, Khiari, & Bruchet, 1999). These compounds are formed during the decomposition of organic matter by bacteria and are also produced in cultures of *Microcystis aeruginosa* and *Microcystis wesenbergii* (Hofbauer & Jüttner, 1988), and *Planktothrix perornata* (Tellez, Schrader, & Kobaisy, 2001). The role of these odorous compounds in off-flavor events is unknown, but “swampy” odors reminiscent of untreated sewage are often noticed in catfish ponds after massive die-offs of phytoplankton or during prolonged low dissolved oxygen conditions. Decay-type off-flavors are also common in catfish during winter although this problem, which is discussed later in the section on off-flavor incidence, is thought to be of dietary origin rather than derived from uptake of compounds dissolved in water.

Prokaryotic cyanobacteria and actinomycetes are the sources of the compounds discussed above but eukaryotic algae also produce numerous compounds contributing to taste and odor problems in drinking water. For example, a “cucumber” odor in municipal drinking water from Philadelphia, PA, was caused by *trans,cis*-2,6-nonadienal produced by eukaryotic algae (Burlingame, Muldowney, & Maddrey, 1992). Other odors and associated compounds identified from cultures of algae include 2-*trans*,4-*cis*,7-*cis*-decatrinal and isovaleric acid described as having a “fishy” and “rancid” odor, respectively (Rashash, Hoehn, Dietrich, Grizzard, & Parker, 1996). Some additional odorous compounds associated with different types of phytoplankton are listed in Table 3, with an indication as to which phytoplankton have been found in catfish ponds. However, confirmation of odor production by specific phytoplankton and subsequent off-flavor in fish is lacking.

4 | MICROBIOLOGY OF GEOSMIN AND 2-MIB

After odor-producing microorganisms were isolated and cultured in laboratories during the early part of the twentieth century, they became the primary suspects for causing odors in water and fish. Although there are other aquatic microorganisms that produce geosmin and 2-methylisoborneol, actinomycetes, and cyanobacteria have received the most attention as contributors, especially because these microorganisms are abundant in many ecosystems used for aquaculture. In addition to certain species of actinomycetes and cyanobacteria, described further below, other microorganisms can also produce geosmin and 2-methylisoborneol.

TABLE 3 Some odorous compounds associated with phytoplankton

Compound	Odor	Phytoplankton source	Reference
2t,4c,7c-Decatrienal	Fishy	<i>Dinobryon cylindricum</i> <i>Synura petersenii</i>	Rashash et al., 1996
Dimethylsulfide	Fishy	<i>Asterionella formosa</i>	Jüttner & Müller, 1979
β-Ionone	Floral	<i>Synura uvella</i>	Jüttner, 1981
Isopropylmercaptan	Onion	<i>Microcystis flos-aquae</i> ^a	Jenkins, Medsker, & Thomas, 1967
Isovaleric acid	Rancid	<i>Chlamydomonas peterfii</i>	Rashash et al., 1996
Heptanal	Rancid	<i>Cryptomonas ovata</i> ^a	Jüttner, 1983
Hexanal	Lettuce	<i>Synura uvella</i>	Jüttner, 1981
4-Methylthio-1,2-dithiolane	Pungent	<i>Chara globularis</i>	Jüttner, 1983
5-Methylthio-1,2,3-trithiane	Pungent	<i>Chara globularis</i>	Jüttner, 1983
1-Octen-3-one	Mushroom	<i>Anabaena oscillarioides</i>	Möhren and Jüttner, 1983

^aPresent in catfish pond waters in Mississippi, USA.

Myxobacteria, in the order Myxobacterales, are unique bacteria characterized by formation of multicellular fruiting bodies. Geosmin production has been confirmed in several species, including *Nannocystis exedens* (Trowitzsch, Whitte, & Reichenbach, 1981), *Myxococcus flavescens* (Yamamoto, Sakata, & Tanaka, 2000), *Myxococcus xanthus* (Dickschat, Bode, Mahmud, Müller, & Schulz, 2005), and *Chondromyces crocatus* (Schulz, Fuhlendorff, & Reichenbach, 2004). While myxobacteria can produce geosmin in three different stages of their life cycle, the vegetative cells have been found to produce much higher levels compared to the myxospores and fruiting bodies (Yamamoto et al., 2000). Myxobacteria are not considered to be contributors to geosmin-related off-flavor in aquaculture products because their presence in aquaculture systems has never been established.

Geosmin production has also been detected in certain species of fungi such as *Cyathus bulleri* (Ayer, Browne, & Fung, 1976; Ayer & Paice, 1976), *Chaetomium globosum* (Kikuchi, Kadota, Suehara, Nishi, & Tsubaki, 1981), and *Penicillium* spp. (Larsen & Frisvad, 1995; Mattheis & Roberts, 1992). The production of MIB has also been detected in *Penicillium* spp. (Larsen & Frisvad, 1995). Kikuchi et al. (1983) suggested that fungi may also be responsible for earthy and musty odors in water from public water supplies. Geosmin production in a free-living amoeba (*Vanella* species) has also been reported, although bacterial symbionts present in the cytoplasm were believed to be the actual sources of the geosmin (Hayes, Hayes, & Robinson, 1991).

The complex biosynthetic pathways leading to geosmin and 2-MIB have been conserved across a diversity of prokaryotic and eukaryotic organisms (Jüttner & Watson, 2007; Watson, Monis, Baker, & Giglio, 2016), suggesting that the compounds have some important ecophysiological function beneficial to the producing organism. Both compounds are biologically active, as evidenced by the ability of animals to detect them at vanishingly low concentrations in water—with detection thresholds in the low ng/L range. Both compounds also have antibiotic properties, reduce the growth of algae, and are toxic to larger invertebrates or fish, but only at concentrations orders of magnitude higher than those found in nature (Watson, 2007). Geosmin, 2-MIB, and similar secondary microbial byproducts probably play some beneficial role for producing organisms, particularly at small scales (such as within biofilm matrices or at cell-surface boundaries), but those functions have yet to be identified.

Of academic and practical interest, production of geosmin and 2-MIB is unknown in marine settings. There are no known marine cyanobacteria producing either compound (Jüttner & Watson, 2007) and, to our knowledge, no reports of production by actinomycetes or fungi in full-salinity seawater. Off-flavors caused by geosmin or 2-MIB have been reported only from fresh or moderately brackish waters. The practical importance of this phenomenon was shown by Lovell and Broce (1985), who reported off-flavors in pond-grown penaeid shrimp in Ecuador are highly seasonal, coinciding with the wet season when rainfall causes the salinity in the estuarine water supply to drop from 15–35‰ to <10‰.

4.1 | Actinomycetes

Actinomycetes are gram-positive, filamentous bacteria and most are aerobic, although some are facultative anaerobes or anaerobes. They include odor-producing genera such as *Micromonospora*, *Nocardia*, and *Streptomyces*. A range of odorous compounds have been identified from cultures of various species of actinomycetes (Table 4). Nevertheless, geosmin and 2-methyisoborneol are the only odorous compounds confirmed to be the cause of earthy and musty off-flavors in aquatic animals. Organisms in the genus *Streptomyces* are particularly notable as producers of odorous compounds, and geosmin and 2-methyisoborneol production are usually associated with members of that genus. Species of *Streptomyces* are generally considered to be aerobic, with some species designated as facultative anaerobes; they are spore-producing heterotrophs potentially forming aerial mycelia and producing pigments.

After the discovery of geosmin and 2-methyisoborneol, actinomycetes received more attention than cyanobacteria as the main contributors to earthy and musty off-flavors in aquatic ecosystems, including in commercial catfish ponds. One of the reasons for this focused research was due to early work by Thaysen and Pentelow (1936), who showed that odorous compounds from actinomycetes could cause off-flavor in wild fish. Actinomycetes have also been implicated as sources of objectionable odors in municipal water supply reservoirs (Jensen et al., 1994;

TABLE 4 Odorous compounds produced by various actinomycetes

Compound	Odor	Species	Reference
Cadin-4-ene-1-ol	Woody	<i>Streptomyces</i> sp.	Gerber, 1971
Furfural	Putrid	<i>Streptomyces</i> sp.	Gerber, 1979
Geosmin	Earthy	<i>Microbispora rosca</i>	Gerber, 1979
		<i>Micromonospora</i> spp.	Blevins, 1980
		<i>Nocardia</i> spp.	Gerber, 1979
		<i>Streptomyces</i> spp.	Gerber & Lechevalier, 1965
2-Isopropyl-3-methoxypyrazine	Musty	<i>Streptomyces</i> sp.	Gerber, 1977
5-Methyl-3-heptanone	Sweet	<i>Streptomyces</i> sp.	Henley, Glaze, & Silvey, 1969
2-Methylisoborneol	Musty	<i>Actinomadura</i> spp.	Gerber, 1979
		<i>Streptomyces</i> spp.	Medsker et al., 1969
Mucidone	Weak fruity	<i>Streptomyces</i> sp.	Gerber, 1983

Silvey & Roach, 1975; Weete, Blevins, Wilt, & Durham, 1977). Another reason for the early focus on actinomycetes as contributors to fish off-flavors was that isolation and culture of odor-producing actinomycetes in the laboratory was relatively easy compared with cyanobacteria. However, Johnston and Cross (1976) suggested most actinomycetes are relatively inactive in most aquatic environments and contended that off-flavor episodes from actinomycetes in ponds, lakes, and rivers were the result of the terrestrial bacteria propagules (fragmented mycelia) and spores being washed into the aquatic ecosystem from the shoreline after rainfall. Because most actinomycete propagules remain dormant and metabolically inactive in the sediments, they are unlikely to produce the high levels of off-flavor compounds encountered in certain lakes and ponds (Cross, 1981; Johnston & Cross, 1976).

Actinomycetes are, in fact, abundant in pond sediments (e.g., actinomycete propagules as high as 2.4×10^5 /g of dried sediment) while numbers are much lower (>10 propagules/mL) in pond water (Schrader, 1995). However, current belief (and in agreement with Goodfellow & Williams, 1983) is that actinomycetes in pond sediments exist as spores and resting propagules that may germinate, grow, and produce odorous metabolites under certain exceptional conditions, though rarely. Cyanobacteria are now firmly established as the major contributors to most earthy and musty off-flavor problems in aquatic ecosystems (Jüttner, 1995). In nutrient-rich environments such as catfish aquaculture ponds, certain species of planktonic cyanobacteria are the primary sources of geosmin and 2-methylisoborneol in water and, subsequently, in the cultured fish (Tucker, 2000).

4.2 | Cyanobacteria

Cyanobacteria are oxygenic photosynthetic bacteria. These prokaryotes comprise a single taxonomic and phylogenetic group and are characterized by possession of two photosystems (PSII and PSI) and the use of H_2O as the photoreductant during photosynthesis. Geosmin and 2-MIB production occurs in numerous species; Table 5 lists cyanobacteria isolated from aquaculture ponds that have been identified as producers of geosmin, 2-methylisoborneol, or both.

It should be noted that cyanobacterial taxonomy and classification seem to be in a constant state of uncertainty, making it difficult to interpret or compare various studies of taste and odor problems associated with the group. Classical or traditional classification schemes, such as those used by Geitler (1932) and Desikachary (1959), in which cyanobacteria were “botanically classified” have generally been replaced by modern approaches such as those by Anagnostidis and Komárek (1985) and Bourrelly (1985). The modern approach by Anagnostidis and Komárek (1985) appears to be gaining acceptance by many scientists because it retains smaller clusters of species within defined genera that are less ambiguous compared to the approach by Bourrelly (1985) in which many species are grouped into fewer genera. Because the taxonomy and classification of cyanobacteria is still in flux, original designations of the

TABLE 5 Freshwater cyanobacteria from fish ponds and aquaculture systems that produce geosmin or 2-methylisoborneol

Family: Species ^a	Compound produced	Origin	Reference
Nostocaceae			
<i>Anabaena spiralis</i> Thompson	Geosmin	Catfish pond	Zimba, Grimm, Dionigi, & Weirich, 2001
<i>Anabaena viguieri</i> Denis & Frémy	Geosmin	Fish pond	Wu, Ma, & Chou, 1991
<i>Aphanizomenon flos-aquae</i> Ralfs	Geosmin	Fish pond	Matsumoto & Tsuchiya, 1988
<i>Aphanizomenon</i> sp. Morren	MIB	Catfish pond	Zimba et al., 2001
Oscillatoriaceae			
<i>Lyngbya</i> cf. <i>subtilis</i> Holden [<i>Leibleinia subtilis</i>]	Geosmin	Fish pond	Schrader & Blevins, 1993
<i>Oscillatoria perornata</i> f. <i>attenuata</i> Skuja [<i>Planktothrix perornata</i> f. <i>attenuata</i>]	MIB	Catfish pond	Martin, Izaguirre, & Waterstrat, 1991
<i>Oscillatoria sancta</i> Kützting	MIB	Catfish pond	Zimba et al., 2001
<i>Pseudanabaena</i> sp. Lauterborn	MIB	RAS	Schrader, unpublished
<i>Pseudanabaena</i> sp. Lauterborn	MIB	Catfish pond	Zimba et al., 2001

Abbreviation: MIB, 2-methylisoborneol; RAS, recirculating aquaculture system.

^aTaxonomic names of cyanobacteria are those used in the original publication. Where indicated, taxonomic names within brackets are the modern taxonomic classification as indicated by Komárek, Kling, & Komárková (2003).

cyanobacteria as stated in the cited publications are used in Table 5, with modern reassignments of selected species indicated in brackets. The designation "cf." (abbreviation for the Latin "conferitum" which is defined as "compare to") is used when identification to the species level is not entirely certain.

Tabachek and Yurkowski (1976) were the first to report production of 2-methylisoborneol in cyanobacteria and establish the link between production by cyanobacteria and off-flavor in fish. During their studies, they isolated a 2-MIB-producing species of *Lyngbya cryptovaginata* from a saline lake used to grow rainbow trout (*Oncorhynchus mykiss*) in Manitoba, Canada. Brown and Boyd (1982) were the first to note the presence of certain cyanobacteria in ponds with off-flavored channel catfish. Later, Armstrong, Boyd, and Lovell (1986) established a strong correlation between earthy-muddy off-flavor episodes and cyanobacteria in Alabama catfish ponds, and van der Ploeg (1989) further established the correlation between geosmin production and cyanobacterial species in the genera *Anabaena*⁶ and *Aphanizomenon*. Although species of *Anabaena* are most often responsible for geosmin production in catfish ponds, *Aphanizomenon flos-aquae* and *Lyngbya* cf. *subtilis* have also been implicated (Schrader & Blevins, 1993; van der Ploeg, Tucker, & Boyd, 1992).

Benthic and vertically stratified mats of the macrophytic cyanobacterium *Lyngbya wollei* present along the edges of catfish ponds in eastern Alabama have been associated with the presence of geosmin in the water in part because steam distillates of "washed" mats of *L. wollei* were found to contain geosmin, though axenic isolates could not be maintained in the laboratory to confirm geosmin production (K. K. Schrader, unpublished observations). Other scientists have also associated mats of *L. wollei* to be the source of earthy odors in southeastern United States ponds (Lawrence A. Dyck, Clemson University, personal communication).

Trichomes of *Anabaena* species can be straight or coiled (Figure 5a,b) and consist mostly of vegetative cells usually described as spherical, cylindrical, or barrel-shaped. The cytoplasm of vegetative cells may appear to be mottled due to the presence of gas vacuoles. Gas vacuoles allow cyanobacteria to regulate cell buoyancy, and this physiological attribute may be important in explaining the dominance of phytoplankton communities in aquaculture ponds by cyanobacteria (Paerl & Tucker, 1995). Two types of specialized cells can also be found among the vegetative cells: heterocysts and akinetes. Heterocysts are usually larger than the vegetative cells, have thicker cell walls, and are void of any gas vacuoles. These cells can occur at intervals or at the termini of the trichome and serve as the sites of N_2 -fixation, though not all N_2 -fixing species of cyanobacteria produce them. Akinetes, sometimes referred to as resting spores, also have thicker cell walls than in vegetative cells, and they are usually larger and longer than vegetative cells.

Planktonic cyanobacteria producing 2-methylisoborneol in catfish ponds include species of *Aphanizomenon*, *Planktothrix*, and *Pseudanabaena* (Martin et al., 1991; Zimba et al., 2001), although a single species in the genus *Planktothrix* appears to be the most important producer of 2-MIB in catfish ponds in northwest Mississippi, the region with the largest concentration of catfish farms in the United States. An early study (Martin, McCoy et al., 1988) implicated *Oscillatoria agardhii* (Figure 6b; later designated as *Planktothrix agardhii*) as a producer of 2-MIB based on the observations of dense blooms of that organism in ponds with musty-flavored catfish. That association was incorrect because populations of *P. agardhii* are common, bloom-forming species in catfish ponds and often co-exist with another species—*P. perornata*—which is a prolific producer of 2-MIB but is usually less abundant and therefore less conspicuous than *P. agardhii* (Figure 6c).

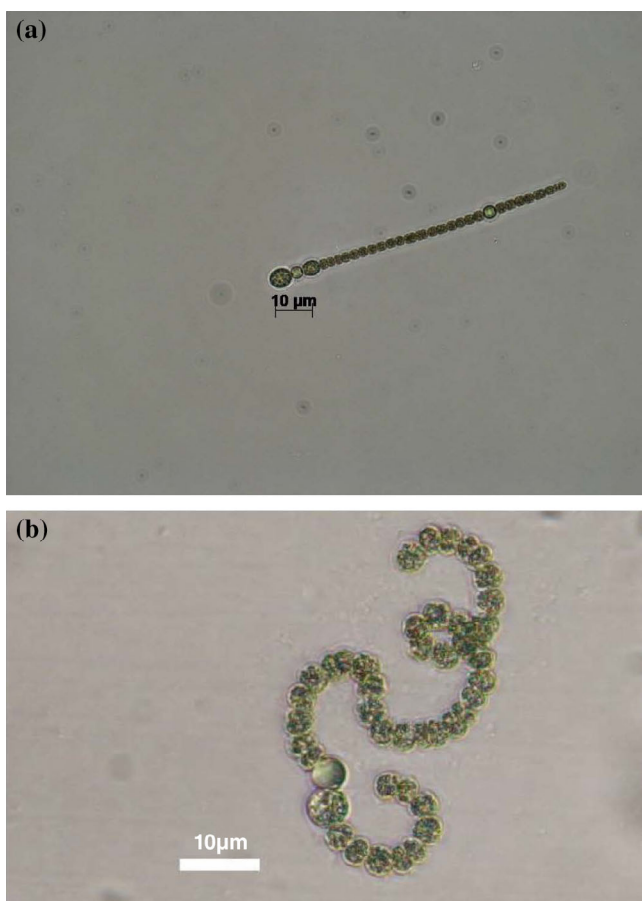


FIGURE 5 Different morphologies of the filaments of *Anabaena* species including straight trichome (*A. sphaerica* with heterocysts [clear cells]) (a) and coiled trichome (*A. spiroides* with heterocyst [clear cell]) (b)

The cyanobacterium *P. perornata* f. *attenuata* (Figure 6a) is responsible for nearly all MIB-related off-flavors in pond-grown catfish. This planktonic, filamentous, gas-vacuolated cyanobacterium has been designated under various genus or species epithets since it was first identified as the cause of musty off-flavors in catfish by Martin et al. (1991). Initially, the genus was designated as *Oscillatoria* based on the predominant classification scheme in use when it was first described from catfish ponds. Because it strongly resembled the morphology of *Oscillatoria chalybea* (Gomont, 1892) it was designated *Oscillatoria* cf. *chalybea* through much of the 1990s (e.g., van der Ploeg et al., 1992) even though that species is usually described as a benthic, rather than planktonic, cyanobacterium. In 2000, the organism was reassigned (Schrader, Dayan et al., 2000) to the more appropriate designation of *Oscillatoria perornata* f. *attenuata* using a traditional taxonomic approach (Skuja, 1949) or *P. perornata* f. *attenuata* under the modern scheme (Anagnostidis & Komárek, 1988).

Filaments of *P. perornata* isolated from west-Mississippi catfish ponds typically have curved or bent apices that are attenuated (Figure 6a,c). A slightly different morphological variation has been isolated from east Mississippi catfish ponds in which the bent end of the filament is not attenuated (Schrader & Dennis, 2005). Because *P. perornata* is consistently associated with 2-MIB off-flavors in pond-raised catfish in the southeastern United States, it has received considerable attention in research related to managing off-flavor problems in catfish aquaculture.

4.3 | Other organisms producing geosmin and 2-MIB

As mentioned earlier, microorganisms other than actinomycetes and cyanobacteria can also produce geosmin and 2-methyisoborneol. None of these are thought to contribute to flavor problems in pond-grown catfish.

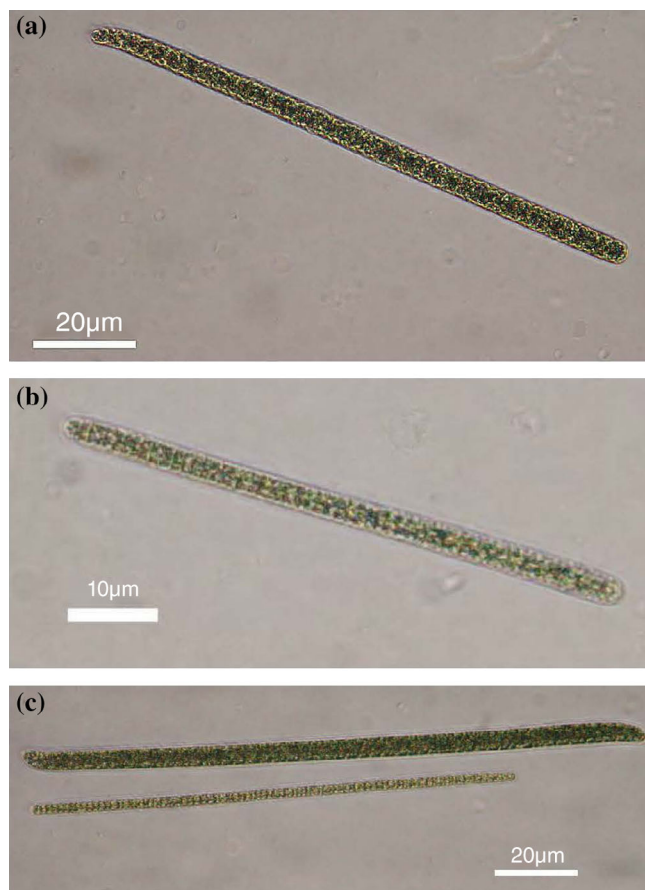


FIGURE 6 Individual filaments of *Planktothrix perornata* f. *attenuata* [Skuja] (a), *Planktothrix agardhii* [Gomont] (b), and together (larger filament is *P. perornata*) (c)

5 | CATFISH OFF-FLAVORS DERIVED FROM POLLUTION

Off-flavors caused by pollution are, regrettably, not uncommon in wild fish—although their incidence seems to be declining since the 1980s and 1990s as governments and industries become more aware of (and respond to) the widespread ecological damage done by industrial pollution and oil spills. Pollution-related off-flavors are extremely rare in fish from aquaculture because facilities should be located where they can be supplied with unpolluted water or where there is a low risk of accidental oil spills from tankers, drilling rigs, or other sources. However, some open-water culture systems, such as marine net-pens and shellfish beds, are susceptible to inadvertent and unexpected contamination (Tucker, 2000).

Most ictalurid catfish are raised in ponds supplied with groundwater. The risk of exposure to pollutants is therefore very low compared with systems immersed in, or using, surface waters. Nevertheless, that risk is not zero and there have been rare reports of flavor problems caused by accidental spills of diesel fuel from well pump engines or farm equipment.

Diesel fuel is a generic term for fuels used in compression ignition engines. Diesel fuel is refined from crude oil but may be mixed with small amounts (usually less than 5%) of other products, such as biodiesel (made from vegetable oils or recycled cooking oils and grease) or gas-to-liquid diesel (refined from “syncrude” produced from natural gas). Diesel fuel is not a precisely defined substance, but rather a complex and somewhat variable mixture of thousands of different organic compounds. The major components of diesel fuel are alkanes (acyclic saturated hydrocarbons sometimes called paraffins), naphthenes (cycloalkanes; cyclic aliphatic hydrocarbons), aromatic compounds (hydrocarbons containing one or more benzene rings), and olefins (acyclic unsaturated hydrocarbons). Most hydrocarbons in diesel fuel have between 10 and 22 carbon atoms. Smaller amounts of sulfur-, nitrogen-, and oxygen-containing organic compounds may also be present (Bacha et al., 2007).

Following a diesel spill (or a spill of any petroleum product), some components dissolve in water (the so-called water-soluble fraction) while others remain predominantly in a separate phase, such as a surface film or as minute droplets dispersed in water. The types and proportions of hydrocarbons in the water-soluble fraction of diesel fuel are distinctly different from those in the fuel itself. Compared to the product itself, the water-soluble fraction of diesel fuel consists of higher levels of polycyclic aromatic hydrocarbons compounds (e.g., various naphthalenes) and monocyclic aromatic hydrocarbons (benzene, toluene, ethylbenzene, xylenes) and lower levels of paraffins and naphthenes (Davis, Moffat, & Shepherd, 2002; Heras, Ackman, & MacPherson, 1992; Roderigues et al., 2010). The aromatic constituents of the water-soluble fraction are among the more odorous of the compounds in petroleum products and thought to be the major contributors to petroleum off-flavors (Davis et al., 2002).

Studies of petroleum taints in fish usually focus on uptake of compounds from the water-soluble fraction, based on the logic that contaminants must be solubilized before they can cross the gills into the bloodstream (Davis et al., 2002; Heras et al., 1992; Roderigues et al., 2010). However, fish held captive and fed in ponds may have additional routes of exposure. For example, aerators used to assure adequate dissolved oxygen in catfish ponds may emulsify the oil, and the emulsion may contact and adhere to the gills or skin. Emulsions may also become adsorbed onto suspended organic detritus that may be eaten directly by fish or by insects or zooplankton then eaten by fish. Also, catfish feeds are formulated as steam-extruded, floating pellets and significant quantities of oil may be consumed incidentally if fish eat pellets floating in a surface slick of diesel fuel. In any of these scenarios, fish may be exposed to higher levels of total hydrocarbons than present in the water-soluble fraction, as well as to classes of hydrocarbons not present in the soluble fraction.

Exposure to diesel fuel gives fish a flavor so characteristic that it can usually be detected and identified without difficulty. The concentration of diesel fuel in water needed to cause off-flavors in catfish has not been determined, but in laboratory studies with rainbow trout *Oncorhynchus mykiss*, 24 hr of exposure to as little as 0.08 ppm of diesel fuel induced a noticeable off-flavor (Davis, Geelhoed, MacRae, & Howgate, 1992). Assuming a similar threshold for

catfish, this equates to less than 4 L of diesel fuel spilled in a typical 4-ha, 1-m-deep pond. As discussed later, petroleum hydrocarbons are absorbed quickly from the water and stored in the fatty tissues of fish long after exposure.

Chemicals used for pest control on adjacent agricultural lands are another potential source of off-flavors in pond-grown fish. Nearly all catfish farms are located in areas with intensive row-crop agriculture, principally cotton, soybeans, corn, rice, and wheat. Some chemicals used for pest control in these crops are applied by airplane and there is a risk of inadvertent drift of the spray into ponds. There is only one report of off-flavors caused by accidental pesticide drift, and that report has technical inconsistencies making it difficult to interpret.

Martin, Bennett, and Anderson (1992) described events in the late 1980s or early 1990s (the precise date is missing in the report) where catfish from two commercial ponds were declared off-flavor in routine pre-harvest sampling at a processing plant in Mississippi. In both cases fish had a noxious odor reminiscent of the herbicide Ordram®. The active ingredient in Ordram® is molinate, *S*-ethylhexahydro-1*H*-azepine-1-carbothioate. The herbicide is used primarily for controlling grass in rice fields. In one case, the off-flavor was claimed to be the result of inadvertent drift from aerial applications on an adjacent rice field and in the other it was caused by the poorly advised use of Ordram® to control weeds on a pond levee. Fish from the second case were analyzed and found to contain molinate at the parts per billion concentration; no molinate was found in fish from the first case, presumably due to time elapsed (2 weeks) since the herbicide was applied. Laboratory studies confirmed that fish exposed to Ordram® developed off-flavors when exposed to as little as 0.5 µg/L of the herbicide.

The study is difficult to interpret because the noxious odorous compound in Ordram® is not the active ingredient, molinate, which has a mild aromatic odor, but rather a production impurity, diethyl disulfide, present in the commercial product at 1–2% by weight (National Institute for Occupational Safety and Health, 1981). Diethyl disulfide has an odor characteristic of “rotten cabbage” and is reduced under field conditions to ethyl mercaptan, which has a “skunk-like” odor detectable at extremely low concentrations in air. Although the study by Martin et al. (1992) specifically assigns the cause of off-flavor to molinate, it is unclear whether the tainting substance was actually molinate or the much more odorous contaminant (or its reduction product) in the commercial formulation.

The specific risk associated with Ordram® no longer exists because the herbicide's manufacturer voluntarily canceled registration with the U.S. Environmental Protection Agency in 2004 (Federal Register, 2004) and by 2008 the herbicide was no longer used in the United States. But the potential for accidental tainting of fish by other agricultural chemicals certainly exists, although that possibility is remote. Commercial aerial applicators and farmers in regions with catfish ponds are fully aware of the litigation risks associated with damage done by off-target pesticide drift and protocols have been developed to minimize that risk. Two simple practices—a buffer zone between crops and ponds and spraying only when the wind direction will move the drift away from ponds—can reduce opportunities for contamination to near zero. Also, modern aircraft use precision application equipment—such as global positioning systems, geographical information systems, and real-time meteorological systems—to reduce off-target drift.

6 | CATFISH OFF-FLAVORS OF DIETARY ORIGIN

Most catfish off-flavors are caused by waterborne compounds absorbed into fish across the gills or skin. There is some evidence that odorous compounds in foods eaten by pond-grown catfish can, at times, be significant sources of off-flavor.

Off-flavors of dietary origin are not uncommon in wild fish. Tucker (2000) presents several examples of fish flavors caused when compounds are absorbed from food across the lining of the gastrointestinal tract. In some instances, foods give fish undesirable flavors but they may also be responsible for desirable flavors. For example, the briny flavor of many seafoods is caused by bromophenols synthesized by certain marine algae and bioconcentrated through the food chain.

Diet-related off-flavors are rare in catfish aquaculture because high-quality commercial feeds are formulated mainly from grains that do not cause flavor problems. In fact, Johnsen and Dupree (1991) grew channel catfish on

21 experimental diets formulated from common feed ingredients at levels commonly used in commercial diets, and found little, if any, impact of any ingredient on fish flavor. Panelists reported all fish were “good-tasting farm-raised catfish.” The authors concluded that, within the limits of the study, catfish flavor is largely determined by innate biochemistry and not by the food they eat. This is important because it allows feed manufacturers to substitute common feedstuffs in commercial diets based on fish nutritional requirements, cost, palatability, and feed manufacturing consideration rather than the potential effect on ultimate product flavor.

It is clear however, that there are limits to the conclusion of Johnsen and Dupree (1991) in the previous paragraph. For example, catfish flavor can be adversely affected when fish are fed manufactured feeds containing high levels of marine fishmeal or fish oil.

Channel catfish fed commercial feeds with little or no marine fishmeal or oil have a non-fishy flavor that is favored by many consumers. However, they are also low in n-3 polyunsaturated fatty acids, which are claimed to be beneficial in preventing cardiovascular disease in human consumers. Low levels of n-3 fatty acids in catfish are the result low amounts of those fatty acids in grain-based feeds. Levels of n-3 fatty acids in catfish filets can be increased by supplementing the feed with oils derived from marine fish, such as menhaden *Brevoortia tyrannus*, that are naturally high in n-3 fatty acids. Although dietary supplementation is an effective means of increasing n-3 fatty acids in cultured catfish, levels of menhaden oil above about 2% of the diet cause a “fishy” flavor in catfish filets that many consumers find objectionable (Dupree, Gauglitz, Hall, & Houle, 1979; Lovell, 1988; Manning, Li, & Robinson, 2007).

The most common, but poorly documented, diet-related off-flavors in channel catfish develop when fish forage on food other than manufactured feeds. The clearest examples of this phenomenon come from studies using blue tilapia *Oreochromis aurea* or threadfin shad *Dorosoma petenense* co-cultured with catfish to reduce the incidence of odorous cyanobacteria. This management practice, called biomanipulation, is discussed in detail later. Threadfin shad and tilapia are not tolerant of low temperatures and will die completely (tilapia) or substantially (shad) when overwintered in catfish ponds of the southern United States. After the co-cultured fish die in winter, catfish develop obnoxious “dead fish” or “decay” off-flavors from foraging on dead fish (Mischke, Tucker, & Li, 2012; Tucker, 2000).

Decay-type off-flavors are common in catfish from commercial ponds in winter (discussed in Section 8, “Temporal Occurrence of Off-Flavors”) and are presumed to be caused by catfish foraging on dead plant or animal material (Schrader & Tucker, 2012). Catfish eat sparingly when water temperatures are less than about 10°C and farmers feed their fish infrequently, if at all, through the winter. However, weather in the southern United States is not constantly cold, but rather temperatures cycle widely as warm southerly winds precede cold fronts moving through the region. Water temperatures may vary between near freezing to 25°C in the midwinter months of December through February (Wax & Pote, 1990). Catfish actively feed at water temperatures above about 10–15°C, and often the only foods available are dead fish or decaying plant matter. Fish scavenge on these materials if not provided manufactured feed during these brief, mid-winter periods of warm weather.

7 | UPTAKE AND ELIMINATION OF ODOROUS COMPOUNDS

Rates at which fish acquire and, especially, lose off-flavors are important considerations in developing off-flavor management programs. For reasons explained later, catfish farmers seldom try to prevent off-flavors from developing in fish but rather they attempt to correct the problem after off-flavors are detected. As such, most off-flavor management practices ultimately rely on purging taints from fish. Factors affecting rates of uptake and loss therefore determine how long it will take for a population of off-flavored fish to lose the taint and become acceptable to sell. The economic implications of off-flavor pharmacokinetics for farmers are obvious.

Pharmacokinetics of off-flavor also have implications for fish processors. Relative uptake and loss rates for tainting substances vary from fish to fish, with the result that the intensity of off-flavor can vary—sometimes

greatly—within a population. Within-population variation in flavor quality has important implications for quality-control activities, as will be explained in detail in the next section.

Factors determining flavor intensity include the concentration of the odorous compound in the water, its odor intensity (that is, the human sensory detection threshold), and the relative rates of uptake and elimination. All common flavor-tainting substances are lipophilic and they move rapidly from water across gill membranes and into the bloodstream. The compounds are transported in the blood throughout the animal and initially concentrated in highly perfused tissues. Compounds may then be redistributed and stored in lipid-rich tissues. Odorous compounds are eliminated through a combination of passive diffusion back out into the water and biotransformation into polar, presumably non-odorous, compounds that are then excreted.

From a catfish farmer's perspective, the most important aspect of off-flavor pharmacokinetics is that fish acquire most off-flavors much more rapidly than they are eliminated (Persson, 1984). For example, in one of the earliest studies of catfish off-flavor, fish held in tanks containing two species of cyanobacteria associated with earthy-musty odors developed detectable taints within minutes to hours, but it took days to weeks for the catfish to lose the taint after they were transferred to clean, odor-free water (Lovell & Sackey, 1973).

Geosmin and 2-MIB are the most common catfish-tainting substances and have been the focus of most studies of the pharmacokinetics of off-flavor. This section will therefore concentrate on those compounds, although some aspects may apply to other flavor-tainting compounds.

7.1 | Uptake

Waterborne geosmin and 2-MIB are absorbed passively from the water, mostly across gill membranes (From & Hølyck, 1984). Minor amounts may be absorbed across the skin or lining of the stomach and intestines as water is swallowed incidentally while feeding. Once in the bloodstream, the compounds are eventually concentrated in lipid-rich tissues (Johnsen & Lloyd, 1992). As explained above, some off-flavors appear to be caused by fish scavenging on dead plants or fish when foraging for food during winter months when farmers do not feed fish. Although the compounds responsible for those decay-type off-flavors are unknown, they would be absorbed across the epithelium of the gastrointestinal tract rather than across the gills.

Lelana (1987) was one of the first to determine the uptake rate of geosmin in channel catfish and developed a simple model of geosmin concentration in fillet tissue as a function of time and the concentration of geosmin in water. The model is only useful for describing initial uptake rates because it never reaches steady state, where rates of uptake and elimination are equal. The model also does not account for water temperature and fillet fat content, which were later shown to be important factors.

Pharmacokinetics of 2-MIB in catfish were first studied by Martin, Bennett, et al. (1988). The study is of no practical use because the fish were very small (5 and 50 g/fish) and fish size has a marked effect on rates of uptake and elimination. Steady state was reached in less than 4 hr of exposure to 5 µg/L 2-MIB and in less than 24 hr of exposure to 50 µg/L. At steady state, concentrations of 2-MIB were about 10 times greater in fillet tissue than in water and almost 100 times greater in visceral fat than in water. Uptake and off-flavor development were extremely rapid: assuming that it is possible to obtain enough edible tissue from a 5-g fish, it would have been identified as off-flavor by consumers after only minutes of exposure to the chemical.

Market-sized (~0.5 kg) channel catfish accumulate 2-MIB more slowly than the small fish studied by Martin, Bennett, et al. (1988). When 0.5-kg channel catfish were exposed to 0.5 µg/L 2-MIB at 25°C, they became off-flavored (>0.7 µg/L MIB in fillet tissue) within 2 hr but continued to accumulate the compound until 24 hr of exposure when steady state was reached (Johnsen & Lloyd, 1992). Fatter fish (>2.5% muscle lipid) accumulated nearly three times more 2-MIB than lean fish (<2% muscle lipid).

Water temperature, through its effect on fish metabolic rate, has a large effect on uptake rate and final concentration of 2-MIB in fish. Johnsen, Lloyd, Vinyard, and Dionigi (1996) conducted trials using 0.5-kg channel catfish at water temperatures ranging from 6.5 to 34°C and developed this equation:

$$\text{MIB in fillet tissue } (\mu\text{g/kg}) = -0.61 + 4.2 [\log(t + 1)] + 0.0076(^{\circ}\text{C})(t) + 0.089(^{\circ}\text{C}),$$

where t is the duration of exposure to 2-MIB in hours and $^{\circ}\text{C}$ is the water temperature.

The equation developed by Johnsen et al. (1996) shows, for example, that catfish exposed for 24 hr to 1.0 $\mu\text{g/L}$ 2-MIB at 6.5 $^{\circ}\text{C}$ would contain about 6.4 $\mu\text{g/kg}$ of 2-methylisoborneol in fillet tissue while fish at 34 $^{\circ}\text{C}$ would contain 11.4 $\mu\text{g/kg}$ —almost twice as much. Fish would have been identified by consumers as being off-flavor (2-MIB fillet concentration of $>0.7 \mu\text{g/kg}$) after about 30 min of exposure at 6.5 $^{\circ}\text{C}$ but in <5 min at 34 $^{\circ}\text{C}$.

The 2-MIB uptake model of Johnsen et al. (1996) does not contain a term for fillet lipid constant, despite the previous work of Johnsen and Lloyd (1992) showing fattier fish bioaccumulate considerably more of the compound than lean fish. In their 1996 work, Johnsen and coworkers concluded that water temperature is more important than lipid content in predicting 2-MIB bioaccumulation in channel catfish, although they did not adequately explain the discrepancy between their finding and the earlier work of Johnsen and Lloyd (1992).

More recent uptake models (which can also be applied to loss of the compounds) are provided in the review by Howgate (2004), which provides a thorough presentation on the pharmacokinetics of flavor-tainting compounds in fish. In the one-compartment model (a fish is the single compartment consisting of a mixture of lipid and water) outlined in the review, the uptake rate factoring in the net flux of the compound into the fish tissue is described in the equation:

$$dC_F/dt = k_1 C_W - k_2 C_F,$$

where C_F and C_W are the concentrations of the compound in the fish and water, respectively, and k_1 and k_2 are uptake and elimination rate constants (Clark, Gobas, & Mackay, 1990; Gobas & Mackay, 1987; Mackay & Hughes, 1984). The rate constants are derived from the following equations:

$$k_1 = E_0 G_V / V_F,$$

and

$$k_2 = E_0 G_V / V_L K_{OW},$$

where E_0 is the gill uptake efficiency for compounds (proportion of the compound contained in the water passing over the gills at the start of uptake), G_V is the gill ventilation rate, V_F is the volume of the fish, and V_L is the volume of lipid in the fish (Gobas & Mackay, 1987; Howgate, 2004).

The model shows that main factors affecting bioconcentration of lipophilic tainting substances are the concentration of the compound in water, the tendency for the substance to move into lipids (described by the octanol-water partition coefficient, K_{OW}), ventilation rate (the relative volume of water moved across the gill surface), and the lipid content of the fish. The uptake rate constant, k_1 , is proportional to ventilation rate, which is essentially a proxy for metabolic rate. Metabolic rate (sometimes expressed as mg of oxygen consumed per kg of fish per hour) increases as temperature increases and is higher in smaller fish, explaining the trends seen in the studies described above; that is, uptake is faster in smaller fish and as water temperature increases. Smaller fish also have a greater ratio of gill area to body mass, which increases the uptake rate of waterborne lipophilic substances.

Because there are other less-studied factors potentially affecting the uptake of off-flavor compounds such as gill anatomy, gill ventilation rate, and the chemical properties of the compound, it is difficult to develop more definitive models providing specific uptake rates. For example, a study of rainbow trout exposed to different compounds found that uptake rate coefficients, k_1 , of the compounds with $\log K_{OW}$ values in the range of 1–3 were linearly related, nearly constant in the $\log K_{OW}$ values in the range of 3–6 (geosmin and 2-MIB), and decreased when $\log K_{OW} \geq 6$.

(McKim, Schmieder, & Veith, 1985). Clearly, there are multiple factors involving uptake rates for geosmin and MIB, and additional research is necessary to develop a more inclusive and predictive model of uptake and compound distribution of geosmin and 2-MIB in catfish tissues.

Martin, Bennett, et al. (1988) found highest concentrations of 2-MIB in the lipid-rich tissues of skin and visceral fat. Similarly, Martin, Plakas, Holley, Kitzman, and Guarino (1990) injected fish with 2-MIB (1 mg/kg) before depuration in clean water and reported rapid reduction of the compound in plasma after 12 hr while substantial 2-MIB accumulated in the skin, peritoneal fat, and muscle of the catfish after 96 hr. Howgate (2004) explains these results in the context of a multicompartment pharmacokinetic model wherein a compound enters the fish and rapidly partitions into one compartment before partitioning into peripheral or second compartments. In that model, lipophilic substances such as 2-MIB eventually become distributed among tissues depending on their lipid content (although loss of compounds via biotransformation may affect the ultimate concentration in some tissues, as explained in the next subsection; Schram et al., 2017).

7.2 | Elimination

Lipophilic compounds are eliminated from fish tissues by passive diffusion across the gills or skin (depuration) and by metabolism to more polar compounds which are then excreted via the kidney or in gallbladder bile (biotransformation) (Kleinow, Melancon, & Lech, 1987; Murty, 1986; Schram et al., 2017). Passive elimination across the gills occurs when the concentration of the off-flavor compound is lower in the water than in the bloodstream of fish. A net out-flow of the off-flavor compound from the fish occurs until a new equilibrium is established between concentrations in water and fish. The important point for farmers is that when concentrations in water become zero, the compound will eventually completely depurate from fish flesh. However, rates of elimination vary greatly depending on the tainting compound, water temperature, fish size, fish lipid content, and other factors.

The relatively slow rate of geosmin elimination from catfish was initially reported by Lelana (1983) in a study using market-sized channel catfish containing 90 µg/kg geosmin in their muscle tissues (a rather high level). Fish held in flowing, odor-free water required 6 days for geosmin concentrations in fish to decrease to levels where flavor was deemed to be "acceptable" by sensory analysis. Purging for 9 days was required to reduce the geosmin concentration to undetectable concentrations in fillets.

Comparison of the results of several studies (Johnsen et al., 1996; Johnsen & Lloyd, 1992; Lelana, 1983; Martin, Bennett, et al., 1988) indicates that catfish eliminate 2-MIB faster than geosmin. However, a true comparative study of geosmin and 2-MIB depuration at the same temperature and using catfish of the same size, lipid content, and tissue burden has not been conducted.

When 0.5-kg channel catfish were exposed to 1 µg/L 2-MIB in water for 24 hours and then transferred to clean, flowing water, loss of the compound from fillet tissue was described by this equation (Johnsen et al., 1996):

$$\text{MIB in fillet } (\mu\text{g/kg}) = 3.6 + 0.176(T) - 2.06[\log(h + 1)] - 0.00296(T)(h) + 0.197(\% \text{fat}),$$

where T is the water temperature (°C), h is the depuration time in hours, and % fat is the lipid content of the fillet tissue.

If the sensory threshold for 2-MIB in fillet tissue is assumed to be 0.7 µg/kg, the model shows that lean catfish (5% fillet lipid) held in warm water (24 and 34°C) will be of acceptable flavor quality in less than 3 days after transfer to clean water while fatty fish (15% fillet lipid) held in cold water (6.5°C) would require a week or longer to eliminate the off-flavor. At the same temperature, lean catfish (5% lipid) depurate 2-MIB at a faster rate than catfish with higher lipid content (15%). Therefore, it can be expected that catfish with higher lipid content in their muscle tissue will not only bioaccumulate lipophilic compounds to higher concentrations than lean catfish but also the depuration time of these compounds in fatty catfish will be longer.

The depuration equation proposed by Johnsen et al. (1996) does not account for the tissue burden (concentration) of the off-flavor compound and, obviously, depuration time will depend on initial tissue burden. This is, of course, implicit in the general model described above, $dC_F/dt = k_1C_W - k_2C_F$, with the elimination component, k_2C_F , containing the tissue concentration term, C_F . Important to catfish farmers, of course, is that intensely off-flavored fish require longer to purge than those with mild taints.

Depuration is generally assumed to be the major loss mechanism for geosmin and 2-MIB from fish, but there is indirect evidence that biotransformation may also be important. Johnsen and Lloyd (1992) believed that observed loss rates of 2-MIB from channel catfish were too fast to be accounted for by passive diffusion alone. Stronger evidence for the role of biotransformation came when Schlenk (1994) and Schlenk, Ronis, Miranda, and Buhler (1995) showed that exposure of channel catfish fish to 2-MIB induced expression of the kidney and liver cytochrome P450 monooxygenase enzyme system, which plays a role in metabolizing foreign compounds.

Even stronger evidence for biotransformation comes from the careful work of Schram et al. (2017) who showed that depuration alone did not account for loss of geosmin from rainbow trout. Specifically, when fish were first exposed to geosmin, the compound accumulated rapidly in fish livers (as would be expected in a highly perfused, lipid-rich organ) but then decreased relative to other tissues. The unexpected decrease was attributed to induction of geosmin biotransformation in the liver, which is known to be the organ responsible for initial biotransformation of most foreign organic compounds.

The practical importance of water temperature on elimination of off-flavors cannot be overstated and is, in fact, the first consideration when developing an off-flavor management plan (see Section 19). Water temperatures in the catfish ponds in the southeastern United States vary widely through the year, with summer temperature often exceeding 30°C and winter temperatures below 10°C for extended periods (Wax & Pote, 1990). Almost all cyanobacterial off-flavors develop in summer, when warm water temperatures and high nutrient loading rates favor cyanobacterial blooms (Paerl & Tucker, 1995) but fish are sold throughout the year. Fish often develop off-flavors caused by geosmin or 2-methylisoborneol late in the autumn, when temperatures are still conducive to cyanobacterial blooms but are becoming cool enough to significantly affect the rate of depuration. In fact, off-flavors of cyanobacterial origin may be detected in catfish through the winter, well after odor-producing cyanobacterial species have disappeared from the pond.

If the off-flavored fish are of market size and must be sold in cold-weather months, only two options exist: (a) move the fish to warmer water (such as a pond supplied with flowing water from a well) to accelerate purging or (b) wait until pond water temperatures rise in the spring, at which time the taint will be rapidly purged. Moving fish to warmer water is risky because the sudden temperature change can kill fish and fish may lose significant weight during the purging process. So the usual course of action is one of inaction: wait until the fish eventually purge the compounds to an acceptable level. Alternatively, the farmer can plan ahead by checking fish for off-flavor and checking water for odor-producing cyanobacteria at least a month before water temperatures are expected to start falling. In Mississippi, the best time to check fish flavor status is before October 1, while average pond water temperatures are usually above 25°C (Wax & Pote, 1990). If needed, actions can then be taken to kill the odor-producing cyanobacteria, leaving adequate time for the fish to purge the flavor in warm water before cooler weather sets in later in the autumn.

7.3 | Uptake and elimination of other tainting substances

No studies have been conducted in channel catfish to determine factors affecting uptake and elimination of tainting substances derived from petroleum products. Based on work with other species (principally trout, salmon, and marine fish), factors affecting uptake rates and final tissue burdens of the various compounds in diesel fuel are similar to those for all lipophilic tainting substances: the compound's lipophilicity and concentration in the water-soluble fraction, exposure time, rate of elimination, and fish metabolic rate (as affected by fish size, water temperature, and other factors; Heras et al., 1992; Davis et al., 2002; Roderigues et al., 2010).

Hydrocarbons in diesel fuel are highly lipophilic, so they are rapidly bioconcentrated from water and eliminated much more slowly. Diesel taints may develop within hours and persist for weeks or months. For example, Davis et al. (2002) exposed rainbow trout to diesel fuel and found a noticeable off-flavor developed within 6 hr of exposure and the taint persisted for more than 10 weeks. Depuration of petroleum taints appears to be slower under field conditions than in the laboratory, perhaps because exposure to the water-soluble fraction is not the only mechanism for uptake of hydrocarbons in the field. For example, off-flavors were detected in pen-raised Atlantic salmon exposed to an oil spill 4 months after the spill (Ritchie & O'Sullivan, 1994).

Diet-related off-flavors in catfish seem to be relatively common, particularly during the winter months when fish are not fed manufactured feed. Taints are probably caused by a variety of substances associated with decaying fish or plant matter. Despite their common occurrence and difficulties in managing these wintertime off-flavor problems, no research has been conducted to identify the tainting substances or the pharmacokinetics of uptake and elimination.

8 | TEMPORAL OCCURRENCE OF OFF-FLAVORS

Pre-harvest off-flavors in pond-grown fish are transient, unpredictable phenomena. Fish may become off-flavored when either their food or the water they live in contains an odorous substance. When the odorous substance is no longer present in diet or water, the chemical is either metabolized or depurated from the flesh and the off-flavor disappears.

Catfish feeds contain mostly oilseed and grain meals, sometimes with small amounts of animal products (fish, poultry, or porcine by-product meals). These ingredients do not cause undesirable flavors in the fish—in fact, blends of these feedstuffs contribute to the mild, non-fishy flavor that is characteristic of farm-raised catfish. Diet-related off-flavors are therefore rare during the warmer months when fish are fed manufactured feeds every day, but they can be surprisingly common in winter. But many farmers do not feed fish in winter, despite periods of warmer water temperatures when fish may actively seek food. In the absence of manufactured feed, fish will scavenge on dead plants that may contain odorous products of decomposition.

Off-flavors caused by accidental pollution are extremely rare in catfish farming. Over our combined 60 years of experience in catfish off-flavor research, we are aware of only a few off-flavor events caused by man-made substances. Problems are most likely during summer when farm equipment is frequently used around ponds, increasing the risk of accidental spills of diesel fuel, motor oil, or hydraulic fluid (Figure 7).

Cyanobacteria are the most common causes of flavor problems in pond-raised catfish—and aquaculture in general. Relatively few species of cyanobacteria produce odorous compounds and cause flavor problems in pond-grown catfish, and odor-producing populations are not always present. Most catfish off-flavors therefore coincide with the appearance and disappearance of odor-producing cyanobacterial species in the plankton community.

Each phytoplankton species, including those producing odorous metabolites, has unique environmental requirements for optimum growth. But pond environmental conditions (such as sunlight, temperature, mixing, and nutrient levels) are forever changing—on time scales ranging from minutes to months—and those changes either favor or hinder the growth of individual species. Phytoplankton communities are, therefore, in a constant state of reorganization and restructuring in response to the changing habitat. Identifying the factors responsible for shaping phytoplankton community structure has been a major focus of aquatic ecology research for more than a century. Regrettably, we have scarcely moved past the state of knowledge summarized in 1984 by the pre-eminent algal ecologist, Colin S. Reynolds (1984):

“Despite the considerable investment of resources and manpower in its investigation, it is humbling to realize that all our acquired knowledge scarcely allows us to make valid predictions about when and what species [of phytoplankton] will be abundant in given waters”

Not only do populations of odor-producing phytoplankton come and go unpredictably in individual ponds, but the continuous restructuring of phytoplankton communities is unsynchronized among ponds.⁷ As such, phytoplankton assemblages (and, therefore, fish flavor status) vary greatly among neighboring ponds at any given time (Figure 8; Millie et al., 1992; Millie, Vinyard, Baker, & Tucker, 1995; van der van der Ploeg & Tucker, 1993).

Cyanobacterial communities are most common in warm, nutrient-rich waters, so it is not surprising that algae-related off-flavors also are most common during the warmest months of the year and in ponds receiving large inputs of feed (Brown & Boyd, 1982; Armstrong et al., 1986; van der van der Ploeg & Tucker, 1993; Dionigi et al., 1998). Past that, it is impossible to be more precise in forecasting off-flavor episodes and this uncertainty has profound implications for off-flavor management in pond-grown catfish. That is, our inability to predict the occurrence of odor-producing phytoplankton populations compels catfish farmers to rely mainly on reactive management strategies rather than proactive management to prevent off-flavored fish from reaching the marketplace.

9 | PREVALENCE OF OFF-FLAVOR EPISODES

The term “prevalence,” as used here, is the percentage of all ponds sampled that contain off-flavored catfish. Depending on the study objectives, sampling may be over a period of time (a year, for example) or at a particular point in time. Off-flavor prevalence among pond populations of catfish has been estimated from catfish processing plant records, farmer surveys, and by direct sampling of ponds on farms or research facilities.

9.1 | Processing plant records

The importance of not sending off-flavored fish to the marketplace was appreciated early in development of catfish farming in the United States. By 1970—coincident with expansion of catfish markets from local to regional—processing plants required flavor testing of fish before a pond population would be accepted for processing



FIGURE 7 Farm equipment, such as this tractor-powered aerator, is common around catfish ponds and is a potential source of petroleum taints from accidental spills of fuel or oil

(Senaga, 2013). Fish found to be off-flavored were rejected until flavor improved. Quality-control records from catfish processing plants provided the first information on off-flavor prevalence among populations of pond-grown catfish.

The first estimate of off-flavor prevalence in catfish aquaculture was made by Dr. R.T. Lovell, at Auburn University, using quality-control records obtained from "several large-scale processors" in Alabama and Mississippi during autumn of 1971 (the study is described in Lovell & Sackey, 1973). Plant records showed more than 50% of fish samples submitted for flavor analysis had "... such intense off-flavor that harvesting was postponed."

About 10 years later, Lovell (1983a) obtained records from a plant in Mississippi that processed catfish from Alabama, Arkansas, and Mississippi. Over 60 days in spring and early summer, fish from 220 ponds were submitted for flavor analysis. Fish from 24 ponds were declared unmarketable due to off-flavors. This is an unusually low off-flavor prevalence (11%) compared with all other studies described here. Also notable is the low incidence of off-flavors now known to be caused by cyanobacteria, which were responsible for only four out of 24 off-flavored samples. The most common off-flavors were described as "sewage" (six out of 24) and "stale" (five out of 24). Sewage and stale off-flavors were often found together, along with a rancid off-flavor. Lovell described the flavors as redolent of "organic decomposition" and were often found in catfish sampled in cool weather (Lovell et al., 1986). The flavor suite of sewage-stale-rancid may describe the same decay-type off-flavors found by others (described below), which are presumed to be of dietary origin and caused by catfish foraging on dead plants or fish. Lovell (1983a) reported one sample with a petroleum off-flavor; this is the only published report of that rare problem.

Dionigi et al. (1998), analyzed flavor quality-control records from several commercial catfish processors in Mississippi over 2 years (1994 and 1995). Most of the fish samples in this database presumably were from farms in the Mississippi Delta.⁸ Of the 12,725 fish samples submitted by farmers and evaluated by processing plant quality-control personnel, approximately 40% were off-flavored and not suitable for processing and sale. Off-flavors were common throughout the year and there was never less than 30% of samples off-flavored in any month. Prevalence of off-flavor was highest in July, August, and September of each year, with up to 60% of samples declared unacceptable. Earthy/musty off-flavors associated with 2-MIB or geosmin were most common, particularly in summer when they accounted for up to 95% of the off-flavors.

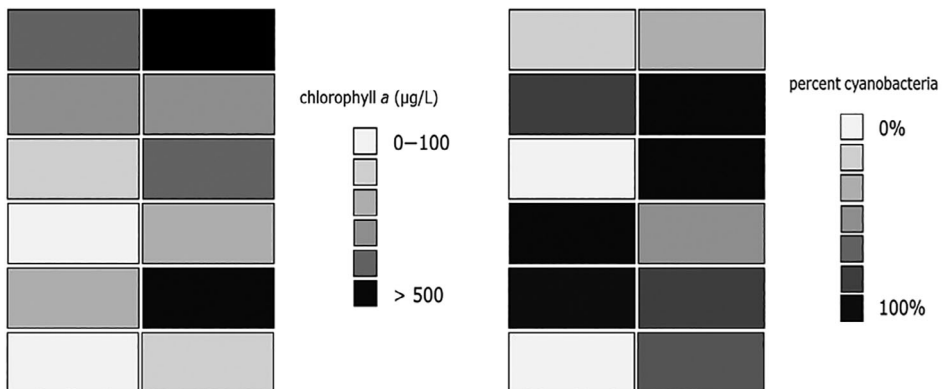


FIGURE 8 Gray-scale mapping of phytoplankton community structure in 12 commercial catfish ponds in Mississippi on May 16, 1990. The original false-color images were prepared by scaling digital images obtained by airborne, multi-spectral scanning of reflected light (nine channels covering 450–12,500 nm wavelengths) to data obtained from analysis of water samples collected concurrent with the imaging overflight. This mapping shows the range of phytoplankton biomass (using chlorophyll *a* concentration as a surrogate measure of biomass) and relative abundance of cyanobacteria (percent of phytoplankton cell biovolume represented by cyanobacteria) on the same date in ponds treated similarly with respect to fish husbandry practices. Adapted and redrawn from a larger dataset (Millie, Baker, Tucker, Vinyard, & Dionigi, 1992)

Hanson (2001) obtained flavor-assessment records for 1997–1999 from four Mississippi catfish processing plants. Again, it is assumed that most of these samples were from Mississippi Delta catfish farms. Results were similar to those of Dionigi et al. (1998). Of the 5,462 fish samples tested, about 50% were off-flavored and unacceptable for processing. Off-flavor prevalence was highest in summer (exceeding 60% in 2 years) and lowest in winter (20–30%).

Processing plant records provide large, synoptic databases but may bias assessments of off-flavor prevalence among fish populations because some populations are sampled multiple times. Catfish farmers submit samples to processors for flavor assessment when they reach sellable size (usually about 0.5 to 0.8 kg/fish). If the fish have acceptable flavor, they are scheduled for harvest, with a few additional flavor checks before harvest. If the fish are off-flavored, the farmer continues to submit samples from that pond—often many times—until the off-flavor disappears and fish are accepted for harvest and processing. On the other hand, farmers may not even submit samples from ponds with intensely off-flavored fish because characteristic odors of 2-MIB and geosmin often can be smelled downwind from ponds with odor-producing microorganisms, and there would be no purpose in sampling ponds with such obvious flavor problems.

9.2 | Farmer surveys

The United States Department of Agriculture National Animal Health Monitoring System conducted extensive surveys of fish farmers in 2002 and 2009 to obtain information on catfish production practices, including impacts and management of off-flavor in food-sized catfish (USDA/APHIS, 2003, 2010a, 2010b). Overall, farmers reported delayed harvest from approximately 50% of all ponds due to off-flavors. Delays lasted from 1 to more than 500 days, with most being between 15 and 60 days (Figure 9).

As with processing plant quality-control records, retrospective farmer surveys may provide biased estimates of off-flavor prevalence and impacts. Many farmers responding to the National Animal Health Monitoring System survey probably did not take time to review farm records—even if such records existed—and therefore relied on recollection to answer survey questions. But recollection of past events is not objective; for example, humans tend to over-emphasize recollection of negative events (Mickley & Kensinger, 2008).

9.3 | Pond sampling

Several studies of off-flavor prevalence have been conducted by sampling market-sized catfish from commercial or research ponds. Pond sampling avoids biases associated with processing plant quality-control records and farmer surveys, but the logistics of obtaining and analyzing fish samples limits the number of ponds that can be sampled at any one time. As such, results from limited sampling may not be representative of prevalence across ponds in general. Also, studies of off-flavors in fish populations usually rely on samples of a few fish from each pond to characterize population flavor quality, but, as discussed in the next section, fish flavor varies among fish within a population. Sampling a few fish from a large population (ponds may contain more than 100,000 fish) may not reliably identify populations with off-flavored fish nor provide an accurate assessment of “average” flavor intensity within the population.

Also note that the studies described below used trained sensory panels to assess flavor quality. It is possible that trained panels detected off-flavors at levels below the sensory threshold of average consumers (Grimm et al., 2004).

The first estimate of catfish off-flavor prevalence based on pond sampling was conducted by Brown and Boyd (1982) in September 1980. They sampled 23 ponds located on four farms in west Alabama and found all ponds contained off-flavored fish, although flavor intensity varied widely. The most intense off-flavors were found in fish from ponds with blooms of cyanobacteria, but the type of off-flavor was not described. In general, off-flavor intensity was greatest in ponds with the highest rates of fish feed application, although feeding rates in all but three ponds were much lower than those used in modern catfish farming.

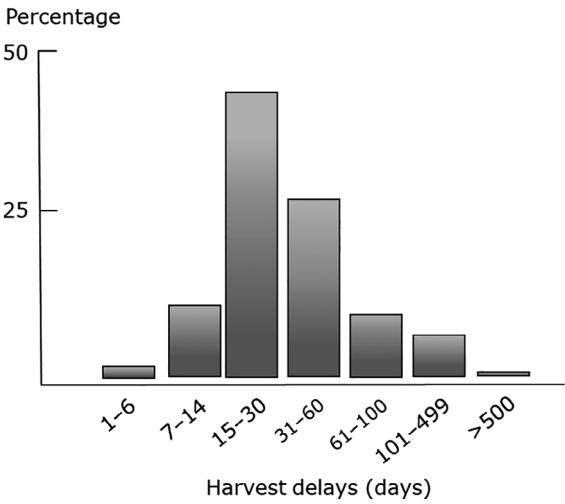


FIGURE 9 Duration of harvest delays caused by off-flavors (USDA/APHIS, 2010b)

Lovell et al. (1986) expanded on the Brown and Boyd (1982) study by sampling west-Alabama catfish ponds over time. They sampled 11 commercial catfish ponds on one farm, monthly from April through October 1983. Of the 77 samples that were collected, 31 (44%) were considered unsuitable for processing due to off-flavors (Table 6). Highest off-flavor prevalence occurred in August when eight of 11 ponds contained off-flavored fish. Earthy-musty off-flavors were responsible for 16 of 31 off-flavored samples throughout the study period, and five of eight off-flavored samples in August. Geosmin was confirmed as the source of the earthy-musty flavor in most fish, with trace amounts of 2-MIB found in three samples.

van der van der Ploeg and Tucker (1993) studied seasonal changes in off-flavor type and intensity in 10 commercial catfish ponds in the Mississippi Delta over 1 year in 1990–1991. This is the only study providing objective information on wintertime off-flavors in pond-grown catfish. Ponds were in a contiguous block of ponds on one farm and had similar fish-culture histories. All 10 ponds contained fish with detectable off-flavor at some time during the study and at no time did all ponds contain untainted fish. Two ponds (1 and 7; Table 7) contained off-flavored fish on every sampling date while others had multiple off-flavor episodes. Off-flavor prevalence and intensity were highest in summer; nine of 10 ponds had fish with 2-MIB off-flavors in September and fish in five of those ponds were distinctly off-flavored (flavor scores >2). Lowest incidence was in early winter with three of 10 pond populations containing off-flavored fish in November and four of 10 in December. Off-flavor intensity also tended to be lower in winter and dominated by mild decay and fishy off-flavors.⁹

TABLE 6 Dominant catfish off-flavors in samples from 11 commercial ponds in west Alabama in 1983 (Lovell, Lelana, Boyd, & Armstrong, 1986)

Off-flavor	April	May	June	July	August	September	October
None	10	8	7	4	3	5	9
Earthy-musty	0	0	2	4	5	4	1
Detritus	1	2	1	1	1	0	0
Sewage	0	1	1	0	2	0	1
Chemical	0	0	0	1	0	1	0
Rooty	0	0	0	1	0	0	0
Cardboard	0	0	0	0	0	1	0

Tucker, Hanson, and Kingsbury (2001) sampled fish from 18 research ponds in the Mississippi Delta to assess the effectiveness of weekly copper sulfate treatments at reducing the incidence and economic impact of off-flavors. This study, and the others below, will be described in more detail in the sections on off-flavor management. Over the 3-year study, fish in the nine untreated (control) ponds were sampled on 19 dates—all during the spring through early autumn—resulting in 171 observations. Off-flavors were detected in 69 of 171 (40%) samples. Off-flavors were most common in late summer when up to two-thirds of the ponds contained off-flavored fish. Off-flavors caused by 2-MIB were most common: 55 of the 69 off-flavored fish samples (80%) were tainted with 2-MIB. Sixteen discrete off-flavor “episodes” were identified in the untreated ponds over the study: three lasted <4 weeks, seven lasted between 4 and 8 weeks, and six lasted more than 8 weeks (1 off-flavor event persisted for 43 weeks).

Schrader, Tucker, et al. (2005) also assessed copper sulfate treatments for off-flavor management but sampled commercial catfish ponds rather than research ponds as done by Tucker et al. (2001). Twelve untreated ponds (six each on two farms in the Mississippi Delta) were sampled three times over one summer (once each in July, August, and September). Off-flavor was detected in 25 of the 36 samples (69.4%). Of the 25 off-flavored samples, 20 (80%) were caused by 2-MIB.

Tucker (2006) evaluated off-flavor prevalence in catfish ponds with or without the planktivorous fish, silver carp *Hypophthalmichthys molitrix*, co-cultured with catfish. Twenty-one research ponds¹⁰ in the Mississippi Delta were sampled on three dates in late summer and early autumn. Of 63 total samples, 35 (55%) were off-flavored. Of the 35 off-flavored samples, 25 (71%) were caused by 2-MIB and eight (23%) were caused by geosmin. Cyanobacterial off-flavors were therefore responsible for 91% of the all off-flavors in this study.

Another study assessed the impact of threadfin shad on pond phytoplankton communities and catfish off-flavor incidence (Mischke et al., 2012). Fish samples for flavor assessment were obtained from research ponds in the Mississippi Delta on two hypothetical harvest dates—one in late summer (September) and one in mid-winter (February). Of 20 ponds without shad, 15 (75%) contained off-flavored catfish in September (all caused by either 2-MIB, geosmin, or both). In winter, 11 of 20 ponds (55%) contained off-flavored catfish; of those 11 ponds, 10 (91%) were caused by 2-MIB, geosmin, or both. The high incidence of cyanobacterial off-flavors in the wintertime samples is

TABLE 7 Intensity and type of off-flavor in fish samples from 10 commercial catfish ponds in northwest Mississippi, 1990–1991

Month	Pond									
	1	2	3	4	5	6	7	8	9	10
Jul	2 m	2 m	1 m	0	0	0	1 m	4 m	1 d	0
Aug	3 m	0	2 m	0	2 m	1 m	4 m	4 m	1 d	0
Sep	4 m	1 m	3 m	1 m	1 d	2 m	3 m	3 m	1 m	1 m
Oct	3 m	1 f	2 m	0	1 m	1 m	1 m	0	2 d	1 m
Nov	4 m	0	2 m	0	0	0	1 m	1 d	0	0
Dec	4 m	0	0	0	0	1 d	1 m	1 d	1 f	0
Jan	4 m	0	0	1 d	1 d	1 d	1 f	1 m	2 d	0
Feb	4 m	0	1 d	0	1 d	0	1 d	2 d	1 d	1 d
Mar	3 m	1 d	1 d	2 d	1 d	1 d	1 d	2 m	2 d	1 d
Apr	2 m	1 d	1 d	0	0	1 f	1 d	1 d	1 d	1 f
May	3 m	0	1 d	0	1 d	0	1 d	1 d	1 d	0
Jun	3 m	0	1 m	3 m	0	3 m	2 m	1 m	1 m	0

Note: Intensity scores ranged from 0 = no off-flavor to 4 = extremely off-flavor. Flavor descriptors were m = musty (MIB), d = decay, and f = fishy. Modified from van der van der Ploeg and Tucker (1993) to simplify flavor descriptors and eliminate multiple sampling in some months.

unusual because odor-producing cyanobacteria were absent from phytoplankton communities at the time of sampling. It was proposed that the wintertime off-flavors were caused by 2-MIB or geosmin produced by cyanobacteria during the previous autumn when waters were warm, and then, as water temperatures decreased through winter, the odor-producing cyanobacterial populations disappeared, yet the tainting compounds remained in fish tissues because depuration rates are slow in cold water (Johnsen et al., 1996).

9.4 | Overall prevalence

Off-flavors are astonishingly common among populations of pond-grown catfish, with perhaps 20–40% of all ponds containing off-flavored fish in winter, increasing to 50–80% in summer. A high percentage (70–90%) of summer off-flavors are caused by the cyanobacterial products 2-MIB or geosmin, or both. Many (perhaps most) winter off-flavors are described as decay, fishy, sewage, grassy, and other terms not associated with cyanobacterial metabolites. Decay-type off-flavors seem to be caused by catfish foraging on dead organic matter. Cyanobacterial off-flavors are occasionally found in winter samples even though odor-producing cyanobacteria do not thrive in cold water. Winter cyanobacterial off-flavors are probably caused by slow purging of 2-MIB or geosmin produced by cyanobacteria in the late summer or autumn.

10 | FLAVOR VARIATION WITHIN POPULATIONS

Not all fish taste the same, even when they are caught from the same pond at the same time. Sometimes this variation is caused by the way fish are tasted. Different methods of cooking affect flavor and different parts of the fillet may vary in flavor (Bett, 1997; Bett & Johnsen, 1996). These factors can—and should—be standardized in quality-control programs. Also, as mentioned previously, people differ in their abilities to detect and describe flavors, so even if a single fish is tasted by several people, it is almost inevitable that flavor will be described differently. For that reason, many food and beverage industries go to great lengths to select and train flavor-quality assurance personnel for sensitivity and uniformity. However, even trained flavor testers suffer sensory fatigue when multiple samples are tasted over a short period of time, leading to a false impression that flavor varies when in fact it does not. For example, when trained sensory analysts taste identical off-flavored catfish samples in rapid succession, they almost always rate the second sample as “less off-flavored” than the first (Bett & Johnsen, 1996).

The impact of the factors mentioned above can be minimized by using proper sensory-analysis methods. But large variation in flavor quality may nevertheless be found among fish from the same pond even when sensory testing is rigorous. And more to the point, concentrations of odorous substances often vary over a wide range when tissues from a cohort of fish are analyzed with quantitative chemical methods.

Tissue concentrations of odorous substances depend on relative rates of uptake and depuration. Both rates are influenced by the nature of the odorous compound and by factors varying from fish to fish, such as fish metabolic rate and tissue fat content. In general, highly lipophilic compounds are taken up faster and lost more slowly than less lipophilic substances. Small fish and fish with high lipid content accumulate odorous substances faster than large fish and fish with lower lipid content; that is, small, lean fish eliminate the compounds more rapidly than large, fatty fish (Johnsen & Lloyd, 1992; Martin, Bennett, et al., 1988). Differences among fish in rates of uptake and depuration cause variation in flavor intensity among fish within a population, even when all fish are exposed to the same concentration of the substance in water.

Variation in off-flavor intensity is greatest when environmental concentrations of odorous chemicals are changing rapidly. This is clearly shown by unpublished data collected by Dr. Tom Lovell (Auburn University, unpublished) from three channel catfish culture ponds in Mississippi (Table 8). Phytoplankton communities in two of the ponds (ponds 1 and 3) had been relatively stable in the weeks before sampling: odorous cyanobacteria had been present for an extended period in pond 1 and absent from pond 3. The relative stability of those conditions is reflected in

the highly skewed distribution of flavor scores for fish sampled from the two ponds. That is, flavor quality of nearly all fish was either unacceptable (pond 1) or acceptable (pond 3). On the other hand, the phytoplankton community in pond 2 changed dramatically just before sampling. A population of odor-producing cyanobacteria was present 2 weeks before sampling but disappeared a few days before samples were collected. The changing environmental conditions—combined with differences in depuration rates among fish—resulted in substantial within-population variation in flavor quality. About half of the fish sampled from pond 2 were judged to have acceptable flavor quality while about half were unacceptable.

Similar results were obtained by Dionigi et al. (1998), who used gas chromatography to measure 2-MIB concentrations in fillets of 80 channel catfish from six catfish ponds in Louisiana. All six ponds contained some off-flavored fish based on preliminary sampling. Concentrations of 2-MIB varied among fish in all ponds. For example, concentrations ranged from <5 µg/kg to over 30 µg/kg in fish from one pond. All fish sampled in that pond would have been deemed off-flavored based on a consumer acceptance threshold for 2-MIB of 0.7 µg/kg. In one pond, 35% of the fish contained less than 0.7 µg/kg 2-MIB, while the remainder of the fish contained the compound at higher concentrations. Most of the variation in fillet 2-MIB concentrations was associated with variation in fillet fat levels among fish.

11 | HOW MANY FISH SHOULD BE SAMPLED TO PREVENT MARKETING OFF-FLAVORED FISH?

Variation in fish flavor intensity within a pond population has important practical implications. Catfish processors check fish flavor quality before fish are harvested and processed. The testing procedure differs among various processors, but farmers usually submit a fish sample to the processing plant 1–3 weeks before the desired harvest date. The sample generally consists of one, or perhaps more, fish caught by angling. The sample is washed, cooked without seasoning in a microwave oven, and then smelled and tasted. If the flavor is deemed acceptable, and the plant's processing schedule permits, the pond population of fish is provisionally approved for purchase and a harvest date is scheduled. Other samples usually are required before final acceptance, often a day or two before harvest and yet another on the day of harvest. Harvest is canceled if off-flavors are detected in any sample. A final flavor check is often made on a fish sample taken from the transport truck immediately before fish are unloaded at the plant. If that sample is found to be off-flavor, fish are rejected for processing and returned to the pond.

Some processing plants require more frequent sampling than described above and may require multiple fish samples from each pond whenever the pond is sampled. Regardless, the decision on whether to accept a fish population for processing is made by sampling relatively few fish from a large population—sometimes exceeding 100,000 fish. Depending on the distribution of flavor intensities within the population, the likelihood of detecting off-flavored fish within the population and therefore making the correct decision to not accept that population for processing may be good—or very bad—as the following three studies show.

TABLE 8 Off-flavor intensities for 100 catfish sampled from three Mississippi catfish ponds

Pond number	Mean flavor score	Number of fish		
		Score < 2	Score 2–3	Score > 3
1	4.3	1	15	84
2	2.0	54	42	4
3	1.5	82	17	1

Note: Flavors are scored on a scale of 0–5: scores <2 are acceptable; scores of 2–3 are marginally off-flavor; and scores >3 are distinctly off-flavor and unacceptable for processing. Unpublished data provided by Dr. R. T. Lovell, Auburn University.

Dionigi et al. (1998) used a simple binomial probability density function to model the probabilities of detecting off-flavored fish when either four, six, or eight fish are sampled from populations with various proportions of off-flavored individuals (Figure 10). Three important trends are apparent:

1. Sampling more fish provides a greater chance of detecting off-flavors in mixed-flavor populations. For example, if 40% of the population is off-flavored, there is ~85% chance of finding at least one off-flavored fish in a random sample of four fish, ~95% chance for a six-fish sample; and ~98% chance with an eight-fish sample.
2. When off-flavored fish constitute most of the population, there is a good chance of detecting off-flavor (and therefore making a correct decision regarding suitability for processing) with a relatively small sample size. For example, when 50% of the population is off-flavored, the probability of detecting at least one off-flavored fish in a random sample of four fish is approximately 95%. When the population consists of >70–80% off-flavored fish, it is a practical certainty that at least one off-flavored fish will be detected in a four-fish sample and the correct decision will be made regarding harvest, processing, and marketing.
3. When off-flavored fish constitute a minority of the population, there is a significant risk of not detecting an off-flavored fish during sampling and, therefore, wrongly accepting a population containing some off-flavored fish for processing. That risk is higher when fewer fish are sampled. For example, if a population contains 10% off-flavored fish, there is only a 30% chance of detecting off-flavor in a four-fish sample. In other words, if four fish from the population are tested, then off-flavor would not be detected 70% of the time and the population would be accepted for processing. This can result in a large number of off-flavored fish sent to market. Assume, as an example, that a pond contains 100,000 fish, 10% of the fish are off-flavored, and processing plant personnel make decisions based on one-time sampling of four fish from that pond. Under those conditions, off-flavor will not be detected in the sample 70% of the time and if off-flavor is not detected during sampling, the population will be accepted for processing and 10,000 off-flavored fish (10% of the population) will be sent to market.

Gautier, Boyd, and Lovell (2002) used a quality-assurance technique called “acceptance sampling for attributes” to estimate sample sizes needed to avoid sending large numbers of off-flavored fish to market. Acceptance sampling is commonly used in manufacturing to determine the number of products from a lot to be tested for defects to determine whether to reject or accept an entire lot (Schilling & Neubauer, 2017). The method is used when testing is destructive (that is, sampling permanently removes products from the lot) or when testing all products in a lot is too costly or time-consuming.

Acceptance sampling for attributes attempts to balance risks to the manufacturer (falsely rejecting good lots) and to the consumer (falsely accepting bad lots) by setting an acceptable quality limit (AQL; the highest proportion of off-flavored fish in a pond that should be acceptable for processing) and a rejectable quality level (RQL; the minimum

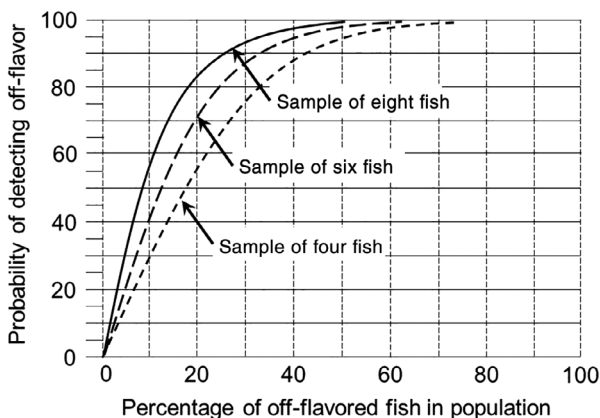


FIGURE 10 Probabilities of detecting off-flavored fish when either four, six, or eight fish are sampled from populations with various proportions of off-flavored individuals. Source: Redrawn from data in Dionigi et al., 1998

proportion of off-flavored fish in a pond that should be rejected for processing). Both limits are established to provide a low risk (probability) of making a wrong decision.

Gautier et al. (2002) sampled 100 fish from 10 ponds in west Alabama in summer and late autumn, 1999. Samples were tasted by three trained panelists and rated on a scale of 0 to 4, with 0 being acceptable and 4 being intensely off-flavored. The results were used to generate a Bayesian operating characteristic function describing the relationship between the proportion of off-flavored fish and the probability of accepting the pond population for processing (Calvin, 1984). From that function, sample sizes were calculated for various combinations of AQL and RQL.

Results of the Gautier study were complicated by using three people on each flavor-testing panel. Further complications arise because the composition of the panel varied, with the three panelists being selected from seven different people throughout the study. Although all panelists were trained in sensory analysis, flavor-sensing acuity apparently varied among panelists. For example, samples from all ponds contained off-flavored fish as detected by at least one panelist but samples from only three ponds were declared off-flavor by all three panelists. Lack of uniformity in off-flavor detection among panelists is also reflected in the overall averages for proportion of off-flavored fish in samples: ~27% of all sample fish were declared off-flavored by at least one panelist, ~5% by two panelists, and <1% by all three panelists. The authors decided to consider a fish as being off-flavored if a majority of the panel (two or three out of three) agreed on the rating. That decision resulted in low proportions of all populations being rated as unacceptable and—for the ponds used in this study—a large number of fish would therefore need to be tested to increase the probability of detecting off-flavored fish. Using the criterion of a majority decision by the panel, required sample sizes were large (25–723 fish per pond) for all combinations of AQL and RQL considered in the study. The authors suggested a “compromise” of sampling 30 fish per pond, which reduced the RQL to 20% at a probability of 0.1. In practical terms this means that sampling 30 fish per pond, under conditions of this study, would provide a 90% probability of off-flavor being detected in ponds when less than 20% of the population is off-flavored.

In practice, sampling 30 fish per pond would be an extraordinary burden on fish farmers and processing plant quality-control personnel, especially with multiple sampling of fish from the same pond before harvest. Fish samples for flavor testing are usually captured by angling and considerable time would be required to catch 30 fish from each pond population considered for harvest and sale. More important, preparing and then tasting 30 from each pond would be nearly impossible for processing plants where samples from more than 100 ponds may be submitted in 1 day. Personnel and facilities required to implement a quality-assurance program large enough to test thousands of fish each day are impractical under current conditions. Also, the study by Gautier et al. (2002) has flaws that may affect their conclusions, including pooling of data, lack of consistency among flavor-testing panelists, and the possibility that flavor-intensity distributions within a population are not normally distributed.

Zimba and Grimm (2015) noted the shortcomings of the study by Gautier et al. (2002) and proposed a non-parametric modeling approach to determine required sample sizes. They also used actual tissue concentrations of 2-MIB to avoid the subjectivity of human sensory panels. Ten groups of 30 fish each were analyzed, with mean 2-MIB tissue concentrations ranging from 1.2 to 0.043 $\mu\text{g}/\text{kg}$. They proposed two sensory thresholds for 2-MIB in catfish fillets: 0.7 $\mu\text{g}/\text{kg}$ for the average consumer and 0.2 $\mu\text{g}/\text{kg}$ for trained processing plant quality-control personnel (Grimm et al., 2004; Johnsen & Kelly, 1990). When the 0.7 $\mu\text{g}/\text{kg}$ “average consumer” threshold was used, five of the 10 sample sets were classified as 100% on-flavor, three of 10 samples sets contained some on-flavored and some off-flavored fish, and two of the 10 samples sets contained 100% off-flavored fish. When the more restrictive 0.2 $\mu\text{g}/\text{kg}$ “trained taster” threshold was used, more of the samples were classified as off-flavored: two of the 10 sample sets contained 100% on-flavor fish, four of the 10 sample sets contained some on-flavored and some off-flavored fish (mixed), and four of the 10 sample sets contained 100% off-flavored fish.

Regardless of the flavor threshold value (0.2 or 0.7 $\mu\text{g}/\text{kg}$), relatively large sample sizes were required to detect off-flavor in populations with low proportions of off-flavored fish. Sample size estimates were similar to those estimated by Gautier et al. (2002): for populations with 10% off-flavored fish, a sample of 30 fish was required to detect

off-flavor with >99% probability; for a population with 3% off-flavored fish, a sample of 40 fish was required. As expected, far smaller samples were required as the proportion of off-flavored fish increased. For example, for a population containing 50% off-flavored fish, a sample size of two fish would detect off-flavored fish 90% of the time.

The three studies described above show that a rather large number of fish must be sampled to detect off-flavor when the proportion of off-flavored fish in the population is low. In fact, when off-flavored fish represent less than 10% of the population, samples of 20–40 fish per pond are needed to reduce the risk of sending some off-flavored fish to market to near zero. Sampling all ponds at that level is not possible under current processing plant constraints of time, labor, and expense. The burden of sampling can, however, be reduced by using a tiered sampling approach wherein a small sample (two to three fish) is submitted for initial testing, with follow-up sampling depending on the outcome of the initial testing. When most fish in a pond are off-flavored, there is a high probability that off-flavor will be detected when two or three fish are sampled, and no further testing is needed to verify the presence of off-flavored fish in the population. On the other hand, if off-flavored fish are not detected in the initial sample, further testing of a larger sample size will be needed to assure that the population is suitable for processing. The required number of fish to be tested will depend on the processors willingness to accept the risk of sending some off-flavored fish to market. Further studies of the distribution of various off-flavors within populations and acceptable processor and consumer risks are badly needed.

12 | FARM-LEVEL ECONOMIC IMPACTS OF OFF-FLAVORS

Off-flavors have multiple impacts on the economics of growing catfish. Off-flavored fish populations cannot be harvested when they reach market size, so the length of time needed to sell a crop is increased and the inability to sell fish on time interrupts the orderly flow of fish from pond to processor to marketplace. Fish that are off-flavor must be held in ponds until flavor quality improves, resulting in lost income from delayed harvest (such as interest charged to the fish not sold), additional feed costs, and loss of future income because the next cropping cycle is delayed until the current crop is declared suitable for processing and harvested. Holding fish in inventory while awaiting flavor to improve also interrupts cash flow, and it may be difficult for farmers to make routine payments if the prevalence of off-flavor is high at certain times of the year. Aside from the direct economic impact of delayed harvest, off-flavor problems also reduce profits because off-flavored fish held in inventory are at risk to losses from disease, water quality deterioration, and bird depredation. These losses represent loss of investment because they are inflicted upon inventory that should have been sold were it not for off-flavor.

Most fish processors desire fish within a certain “premium” size range, usually about 0.4–1.5 kg, although the desired range may change over time. Farmers are penalized for undersized and oversized fish delivered to the processing plant (Wiese, Engle, Trimpey, Quagraine, & Green, 2006). When off-flavors occur in the warm season, farmers usually continue to feed fish while harvest is delayed because it is difficult (or impossible) to predict how long the off-flavor event may last and farmers do not want fish to lose weight by withholding feed. Also, many farmers use a cropping system in which ponds contain mixed-size fish populations. Withholding feed in ponds with mixed-size populations will slow the growth of the smaller, sub-harvest sized fish. If the harvest delay is lengthy, some proportion of the fish population may grow larger than the premium size range demanded by processing plants and farmers will receive a lower price for the fish. This problem is especially serious in ponds with fast-growing channel × blue hybrid catfish.

Off-flavors can also have economic impacts far beyond the farm. Over the long run, inconsistent product quality may adversely affect market demand and industry development, with the overall effect of reducing profits for all segments of the industry. This risk, and its associated costs, has never been quantified.

Updated studies of the economic impacts of off-flavor are badly needed. The last systematic study was conducted in 1995 (Engle, Pounds, & van der Ploeg, 1995) and costs, prices, methods of production, and the structure of the catfish industry have changed significantly since that time. Accordingly, past studies provide only a general idea

of potential impacts. Based on these old studies, the costs of off-flavor due to strictly economic considerations ranged from US\$0.04 to US\$0.26 per kg of fish produced (Coats, Dillard, & Waldrop, 1989; Engle et al., 1995; Keenum & Waldrop, 1988). At the time the studies were conducted, that range of estimated losses represented approximately 5–20% of the pond-bank value of channel catfish. Assuming the same relative economic impact, the overall cost to farmers in 2018 would range from about US\$15 to 65 million. Those impacts, which are substantial, probably underestimate overall impacts. Other aspects of the economics of off-flavor are discussed below, in sections dealing with management strategies.

13 | OFF-FLAVOR MANAGEMENT

The overall goal of off-flavor management is to prevent tainted catfish from reaching consumers and impacting market demand. This ultimately is the responsibility of processing plant quality-control personnel who assess flavor quality before fish are harvested. If the population contains off-flavored fish, the processing plant should not accept that pond population for harvest. Although the processing plant quality-control checkpoint largely prevents off-flavored fish from reaching the marketplace (but see the earlier discussion of flavor variation within fish populations), there can be other market-level impacts of off-flavor, as well as profound farm-level impacts.

When catfish are in tight supply because market demand has outpaced farm production, the additional constraint imposed by unharvestable, off-flavored fish can be a serious issue for processors and their associated marketing departments. In the modern seafood marketing world, many alternatives to domestic farm-grown catfish exist. If the supply of catfish is inadequate to meet demand and the supply shortfall is exacerbated by a bottleneck caused by a large proportion of harvest-sized fish being off-flavored, customers will purchase other white-fleshed, freshwater fish, such as pangasid catfish from Asia or imported tilapia. Recovering lost markets can be difficult, with impacts across all segment of the industry.

Although off-flavors can impact all segments of the industry, the greatest impacts occur on the farm and were summarized in the previous section. Obviously, there is considerable incentive for developing technologies to reduce off-flavor prevalence. The remainder of this review will summarize various control measures that have been proposed or studied. Some (small) measure of relief can be achieved through diligent management of fish stocks, and that will be discussed first. Most catfish off-flavors are caused by cyanobacteria, so nearly all research has focused on measures to prevent odorous cyanobacterial blooms or to kill them after they have become established. We follow our discussion of control measures for cyanobacterial off-flavors with short sections on management of pollution- and diet-related off-flavors. The penultimate section of this paper will present a decision-making scheme for off-flavor management based on our current understanding of the problem.

14 | ACCEPTING FATE: NON-ACTIVE MANAGEMENT OF OFF-FLAVORS

Off-flavor events in pond-grown catfish are episodic and fish flavor eventually improves when fish are no longer in contact with odorous compounds. Further, it is unlikely that all ponds on a farm will contain off-flavored fish at the same time, although the risk of whole-farm flavor problems is obviously greater for farms with only a few ponds. As such, some farmers make no attempt to actively manage catfish off-flavors, but rather they monitor fish flavors in all ponds with harvest-sized fish to find opportunities when flavor quality is acceptable and a crop can be harvested and sold.

Opportunities to harvest and sell fish are greatest when the farm is managed to maximize the number of ponds containing market-sized fish throughout the year. This was the incentive for developing the unique “understocking” or “multiple-batch” cropping system widely used to grow channel catfish in ponds since the 1970s (Tucker, McNevin, Torrans, & Bosworth, 2019). In the multiple-batch system, faster-growing, market-sized fish are selectively harvested using a large-mesh seine, leaving smaller fish in the pond to continue growing. Fingerlings are “understocked” to

replace the harvested fish plus any losses incurred over the production period. Size-selective harvest and understocking continues for years without draining the pond. After a few years, all ponds on the farm contain fish ranging from newly stocked fingerlings to market-sized fish. The multiple-batch system is an alternative to single-batch cropping, which is the way most other fish are grown in aquaculture. In single-batch cropping, 1 year-class cohort of fish is grown and completely harvested before starting the next cropping cycle by restocking the pond with a new batch of fingerlings.

Multiple-batch cropping was developed in the early years of catfish farming to assure a year-round supply of market-sized fish. As an ancillary benefit, multiple-batch cropping also reduces the farm-level impact of off-flavors. In Elysium—a world without catfish off-flavors—the single-batch cropping system generates greater net farm revenues than multiple-batch cropping because production tends to be greater in single-batch ponds and fish can be harvested and sold without constraint (Engle et al., 1995; Engle & Pounds, 1993). But in our real world, where unpredictable episodes of off-flavor interfere with timely harvest, farm-wide use of multiple-batch cropping is the economically favored approach. If off-flavored fish cannot be harvested from one pond because they are off-flavored, there is a greater probability of having acceptable fish to sell from another pond when ponds are managed with multiple-batch rather than single-batch cropping.

Managing around off-flavors requires frequent flavor testing to identify ponds with fish with acceptable flavor. Most farmers rely on quality-control personnel at processing plants for fish flavor testing, but this can impose a significant burden on plant personnel. To relieve some of this burden, on-farm flavor testing can be used to eliminate the most obvious problems. The on-farm flavor-testing process is described in detail by van der Ploeg (1991). Briefly, a sample of two or three fish is cooked without seasoning in a microwave oven. The cooked sample is then tasted by a person experienced in flavor testing. The person need not be formally trained in sensory analysis but, if the test is to be meaningful, the taste-tester must be consistent in identifying the flavors and in ranking the intensity of the flavor. Keeping records of changes in flavor intensity can then be used to indicate whether a flavor problem is getting better or worse in a particular pond. Ponds with off-flavored fish should be sampled weekly or at some other convenient interval to monitor changes in flavor quality. Flavor quality will eventually improve to an acceptable level. Samples from ponds judged to be free of off-flavor can then be taken to the processing plant for definitive determination of acceptability. The crop should then be harvested as soon as possible to reduce the chances that fish will not become tainted with another flavor.

Managing around off-flavor episodes in this manner is far from a desirable strategy. Harvests are still delayed (and for an indeterminate length of time) and cash-flow is hindered. Simply waiting for fish flavor to improve in one pond while harvesting on-flavor fish from other ponds still subjects the off-flavored fish to risk of loss to predation and disease. Although smaller “understocked” fish in multiple-batch ponds continue to grow more or less uninterrupted (thus reducing the impact of delayed initiation of a second crop), the larger, off-flavored fish also continue to grow while the farmer waits for flavor to improve. If the off-flavor episode is short-lived, this has little impact on production. But if the episode is protracted, two problems may develop. First, catfish convert feed to flesh less efficiently as they grow, so as time goes by and large, market-ready fish continue to eat and grow, feed conversion efficiency suffers, and production becomes less profitable. Second, processors often pay farmers less for oversized fish than for fish within the “premium” size range (usually 0.5 to about 1.8 kg). Larger fish represent considerable investment, so penalties for oversized fish can seriously impact profitability.

Problems associated with delayed harvest are particularly troublesome for farmers growing hybrid channel $\times$ blue catfish. Among the many positive production characteristics of hybrid catfish, they are easier to capture by seining than channel catfish. However, hybrids do not size-grade efficiently through standard netting. They have a smaller head and deeper body than channel catfish, which causes many intermediate-sized fish to be “gilled” during grading, resulting in fish death and increased labor to remove fish stuck in the net mesh during harvest. To avoid issues with passive size grading, which is necessary for multiple-batch cropping, most hybrid farmers use the single-batch cropping system. Prolonged harvest delays due to off-flavor can therefore have more severe

consequences when growing hybrid catfish. Initiation of the subsequent crop is delayed and hybrid catfish, which grow extremely fast, can rapidly exceed the desirable premium size range.

Although it is possible to reduce the impacts of off-flavor through careful fish-stock management, the economic costs of off-flavor remain. Accordingly, most farmers seek ways to either prevent off-flavors from developing or to correct problems after they develop. Active off-flavor management will be the focus of the next sections.

15 | PREVENTING CYANOBACTERIAL OFF-FLAVORS

Cyanobacteria are ancient and extraordinarily successful organisms inhabiting an amazing variety of aquatic, marine, and terrestrial ecosystems. As a group they provide valuable ecosystem services, including fixation of inorganic carbon and atmospheric nitrogen gas into organic compounds, thereby making those elements available to support growth of organisms at higher trophic levels. However, a few species in a handful of genera cause serious problems, including toxin production, taste and odor problems in drinking water and fish, and a tendency to become overabundant and dominate aquatic plant communities.

Problematic cyanobacteria—including the common odor-producing species in catfish ponds—come from the genera *Anabaena*, *Aphanizomenon*, *Cylindrospermopsis*, *Microcystis*, *Nodularia*, *Planktothrix*, and *Trichodesmium*. Nuisance species within those genera are relatively large, multicellular colonies or filaments, and can regulate cell buoyancy (and position in the water column) through production of carbohydrate ballast and the collapse and reformation of intracellular gas vesicles. They tend to be relatively slow growers compared with most eukaryotic phytoplankton, but they compete well for limited resources and can dominate certain ecosystems. Some species in these genera are common and abundant organisms in catfish pond summertime phytoplankton communities.

The ability of bloom-forming cyanobacteria to outcompete faster-growing eukaryotic phytoplankton has been ascribed to a variety of physiological attributes, including (a) some species fix atmospheric nitrogen and thereby thrive in habitats with low supplies of combined inorganic nitrogen, (b) they have efficient inorganic carbon-concentrating mechanisms that provide an advantage in habitats with low supplies of carbon dioxide, (c) the large colonies and filaments resist grazing losses by zooplankton, (d) they possess a combination of primary and accessory photosynthetic pigments allowing them to use light in wavelengths not used by other phytoplankton, and (e) they can change cell density and control their position in the water column, allowing cells to avoid light limitation in turbid, poorly mixed waters and avoid carbon limitation by accessing atmospheric carbon dioxide near the water's surface.

The ability of some cyanobacteria to fix atmospheric nitrogen is not an important advantage in catfish ponds because large feed inputs result in substantial loadings of ammonium—usually well in excess of that needed to limit phytoplankton productivity. The most important factor contributing to the success of cyanobacteria in warm, nutrient-rich waters is their ability to compete effectively for limited light supplies (Paerl & Tucker, 1995). In short, as total phytoplankton biomass increases in response to high nutrient loading, algal cells absorb more and more light, to the point where light availability becomes a strong species-selection pressure. High algal turbidity and low rates of water-column vertical mixing (conditions typical of catfish ponds in the summer) strongly select for buoyancy-regulating cyanobacteria, including the common odor-producing species found in catfish ponds.

15.1 | Catfish ponds as habitats for cyanobacteria

Catfish ponds in the southern United States are extreme examples of environments selecting for phytoplankton communities dominated by cyanobacteria. In the warm season from March through October, large amounts of manufactured catfish feed are added daily to promote rapid fish growth. Most of the feed nutrient content enters the pond in fish metabolic waste products because only a minority of the feed nutrient content is retained in fish (Boyd & Tucker, 2014). Waste loadings vary depending on feed quality, fish size, and other factors, but typically about 60–80% of the nitrogen and phosphorus in catfish feeds is lost to the pond.

Nitrogen and phosphorus loadings in catfish ponds are extreme. At a modest summertime feeding rate of 110 kg of feed/ha per day, direct loading of ammonium-N excreted by fish exceeds 0.4 g N/m² per day and direct loading of phosphorus exceeds 0.04 mg P/m² per day. Using the Redfield mass ratio of 42:7:1 for the proportions of C, N, and P in algal tissue, addition of nitrogen and phosphorus at those rates should support gross carbon fixation rates of about 2 g/m² per day—a high productivity rate for phytoplankton in unmixed water bodies and a productivity rate indicative of hypereutrophic conditions (Boyd, 1985; Likens, 1973; Smith, 1979).

The calculation in the preceding paragraph underestimates nutrient loadings in most catfish ponds because summertime feeding rates often exceed 110 kg/ha—sometimes by factors of 2 or 3. Further, direct nutrient loadings from feed wastes, regardless of feeding rate, underestimate total nutrient availability for phytoplankton growth. Nutrients are also made available when organic matter from dead phytoplankton (and other organisms) is mineralized during bacterial decomposition. The process of nutrient assimilation-death-decomposition-nutrient re-assimilation is called internal nutrient recycling, and it significantly increases nutrient availability for phytoplankton growth in shallow lakes and ponds (Boyd & Tucker, 1998; Hargreaves, 1997, 1998; Ibelings et al., 2007; Schindler, 2012).

The overall result of high summertime nutrient loading in catfish ponds is the development of extraordinarily abundant phytoplankton communities whose growth is generally not limited by nutrient availability but rather by light. It is difficult to define precisely the point where phytoplankton growth becomes light-limited because rates of nutrient recycling, mixing, and other processes vary among ecosystems. Sas (1989) proposed that phytoplankton growth in shallow, eutrophic lakes in western Europe is nutrient limited when dissolved inorganic nitrogen concentrations are below 0.1 mg N/L and soluble reactive phosphorus concentrations are below 0.01 mg P/L. Based on those guidelines, Tucker and van der Ploeg (1993) concluded that summertime phytoplankton growth was not nutrient limited in catfish ponds at feed inputs rates of 100 kg/ha per day. More recently, Schindler (2012) suggested concentrations exceeding 100 µg/L of soluble reactive phosphorus, dissolved inorganic nitrogen, and chlorophyll *a* as indicators of nutrient-saturated conditions in shallow lakes. Concentrations in catfish ponds typically far exceed those values (Tucker, 1996).

The ratio of the depth of the euphotic zone (Z_{eu}) to the mixed depth (Z_{mix}) is an index used to explain the effect of underwater light climate on the type of phytoplankton community developing in lakes.¹¹ Low $Z_{eu}:Z_{mix}$ ratios tend to favor shade-adapted or buoyancy-regulating phytoplankton (Reynolds, 1984). For example, in a study of 28 eutrophic lakes, prevalence of *Planktothrix agardhii* (a common cyanobacteria in catfish ponds and a close relative of the odor-producer, *P. perornata*) increases rapidly as the ratio of euphotic depth to mixed depth ($Z_{eu}:Z_{mix}$) falls below 1.62 (Bonilla et al., 2012).

The euphotic zone depth in catfish ponds can be estimated from the relationship between chlorophyll *a* concentration (a common index of phytoplankton biomass) and Secchi-disc transparencies (Portielje & Van der Molen, 1999) and the rule of thumb that Z_{eu} is about twice the Secchi disc transparency (Reynolds, 1984). Chlorophyll *a* concentrations in catfish ponds range from near 0 to over 800 µg/L, but are usually 400 to 700 µg/L during the summer (Tucker & van der Ploeg, 1993). Values in that range correspond to the maximum sustainable phytoplankton standing crops in shallow (<2 m) water bodies (Wetzel, 2001). Based on an average chlorophyll *a* concentration of 500 µg/L, catfish ponds have summertime euphotic depths of less than 0.25 m. Summer days are often calm, with little wind-induced mixing, but ponds are shallow, do not permanently stratify, and are mixed to some degree each day, so the mixed depth can be assumed equal to the pond depth (~1.5 m). The resulting $Z_{eu}:Z_{mix}$ of less than 0.2 is far below the threshold value of 1.62 found in the Bonilla et al. (2012) study, and supports the common observation that shade-tolerant cyanobacteria dominate summertime phytoplankton communities in catfish ponds.

15.2 | Cyanobacterial blooms as stable-state ecosystems

Factors affecting plant community structure and succession are complex, but a comprehensive and qualitatively simple theory—alternative stable states—has been developed to describe broadscale outcomes of those interacting factors. The theory has been applied to many ecosystem types (Beisner, Haydon, & Cuddington, 2003) and has been

particularly useful in aquatic ecology (Moss, 1990; Scheffer et al., 2003; Scheffer, Hosper, Meijer, Moss, & Jeppesen, 1993; Scheffer & van Nes, 2007). As developed in its simplest form for aquatic plant communities, the theory postulates that plant communities in ponds and shallow lakes exist in one of several alternative stable states, depending on the interactions of various ecological forcing factors. Stable states usually are defined broadly rather than in fine detail; for example, communities may be dominated by submerged angiosperms, by free-floating plants such as duckweeds, by phytoplankton, or by other broad ecological groups. Community types are, for practical purposes, discrete and do not coexist to a large degree except when one type of community transitions to another type (called a state shift). As the theory's name suggests, ecosystems resist changes among states.

Early development of stable state theory for aquatic ecosystems considered only the effects of dissolved nutrient levels and turbidity on competition between submersed plants and phytoplankton in shallow lakes (Scheffer et al., 1993). The idea was extended to factors leading to the existence of other plant communities, including cyanobacterial dominance of phytoplankton communities in certain waters (Scheffer, Rinaldi, Gragnani, Mur, & van Nes, 1997; Scheffer & van Nes, 2007). In fact, Scheffer et al. (1997) used stable state theory explicitly to explain persistent blooms of *Planktothrix* in shallow, nutrient-rich lakes—very much like catfish ponds. Based on the model they developed, cyanobacterial dominance is a stable state in warm, shallow, nutrient-enriched waters mainly based on the ability of that group to outcompete eukaryotic phytoplankton for light (although resistance to grazing by zooplankton may also play a role).¹²

Alternative stable state theory explains failures that are often encountered in managing aquatic plant communities. If conditions favor one type of plant community, that community will become the preferred state and it will be difficult to force development of another community type—usually one that is more desirable from an anthropocentric perspective. Practical experiences in lake and pond management confirm that inducing a state shift almost always requires powerful actions that may be expensive or need to be repeated routinely to be effective—otherwise the system will return to its original state (Scheffer & van Nes, 2007; van Nes, Scheffer, Van den Berg, & Coops, 2002).

The implication of stable state theory for off-flavor management is clear and, regrettably, discouraging—or at least challenging. Most catfish off-flavors are caused by cyanobacteria; the summertime catfish pond environment strongly selects for a stable state of cyanobacterial dominance; and inducing a persistent shift to another type of community is difficult. This situation is not unique to catfish ponds, although catfish ponds represent an extreme example of an environment selecting for cyanobacteria. Harmful cyanobacterial blooms are increasing throughout the world and there has been enormous investment in research to determine causes and management strategies (see reviews in Jančula & Mařálek, 2011; Paerl & Otten, 2013; Rastogi, Madamwar, & Incharoensakdi, 2015; Stroom & Kardinaal, 2016; Paerl, 2017; Huisman et al., 2018). Despite the best efforts of science, a consistently successful approach to cyanobacterial bloom management has yet to be developed—convincing evidence of the stability of cyanobacterial communities when conditions favor those organisms.

15.3 | Nutrient-input reduction

Reducing nutrient inputs is the most common strategy for controlling nuisance cyanobacterial blooms in lakes and reservoirs (Huisman et al., 2018; Paerl, 2017; Reynolds, 1997; Stroom & Kardinaal, 2016). The strategy is based on what appears to be straightforward logic: high nutrient inputs (usually phosphorus or nitrogen, or both) cause dense phytoplankton blooms that restrict light penetration, thereby giving bloom-forming cyanobacteria a competitive advantage over eukaryotic algae. The deduction is that reducing nutrient inputs will lead to clearer water, thereby eliminating conditions that favor cyanobacteria. The logic is sound but putting logic into practice can be a daunting task. Reducing nutrient loading to the point where conditions no longer favor the cyanobacterial “stable state” may require heroic effort and results are far from consistent (Ibelings et al., 2007; Moss, 1990; Osgood, 2017; Schindler, 2012; Van Liere & Gulati, 1992).

Manufactured feeds represent more than 95% of the direct loadings of phosphorus and nitrogen to catfish ponds and feeds are the origin of essentially all nutrients available through internal recycling. Obviously, the only way to

appreciably reduce inputs is to decrease the amount of fish feed applied to ponds. And, more to the point, one of the early studies of catfish off-flavors (Brown & Boyd, 1982) showed the relationship between feeding rate, phytoplankton abundance, and intensity of catfish off-flavors—just as one might predict based on the relationship described in the preceding paragraph. In that study, ponds with “low” feeding rates (<40 kg of feed/ha per day) had low phytoplankton standing crops and better tasting fish than ponds with “high” feeding rates (>50 kg/ha per day).

Brown and Boyd (1982) clearly showed the possibility of reducing the impacts of off-flavor by feeding less feed and producing less fish. However, limiting maximum daily feeding rates to less than 40 kg/ha per day limits annual fish production to less than about 2,500 kg/ha, and this is not profitable under current economic conditions (Johnson, Engle, & Wagner, 2014). In effect, economic incentives to increase farm productivity require higher feed inputs to support high levels of fish production and have led to chronic problems with cyanobacterial off-flavors.

Rather than reducing overall feeding rates, another approach to reducing waste loading to catfish ponds is to improve feed-nutrient retention by fish. In other words, if more of the feed nutrient content is retained by fish, less is excreted into water as waste. Considerable research has been conducted on this subject with the goal of improving the economic efficiency of feed use, which is itself a worthwhile objective. Most of the effort has concentrated on improving phosphorus use.

The economic benefits of improving feed phosphorus use and retention by fish are obvious. Also, reduced waste phosphorus production is an important regulatory consideration when fish are grown in raceways or net pens discharging directly to the environment (Tucker & Hargreaves, 2008). However, improvements in feed phosphorus use through quantitative and qualitative modifications of dietary phosphorus do not reduce phytoplankton standing crops in catfish ponds (Li et al., 2019; Tucker, Hargreaves, & Kingsbury, 2005).

Catfish ponds receive large feed additions daily, and the high baseline nutrient loading of phosphorus contained in practical feed ingredients combined with high rates of internal phosphorus recycling within ponds overwhelm the effect of modest reductions in phosphorus loading associated with diet modifications. Lack of effect on overall phytoplankton abundance strongly suggests that diet modifications alone will not impact cyanobacterial dominance of plankton communities or reduce off-flavor incidence in ponds with high feeding rates.

15.4 | Nutrient removal

Phosphorus reacts with aluminum, iron, and calcium to form compounds of low solubility, so additions of those cations have been used to sequester phosphorus and reduce the incidence of cyanobacterial blooms in eutrophic lakes (Jančula & Maršálek, 2011; Stroom & Kardinaal, 2016). Salts of aluminum have an additional effect because they form flocs of aluminum hydroxide when added to water. The heavy flocs of aluminum hydroxide co-precipitate phytoplankton (and other particles) as they settle through the water.

Masuda and Boyd (1994) showed that treatments with aluminum sulfate, or alum, reduces soluble reactive phosphorus and total phosphorus concentrations in catfish ponds. They did not evaluate the effects on cyanobacterial abundance or off-flavor incidence but studies in other water bodies (De Julio, Fioravante, De Julio, Oroski, & Graham, 2010; Lam & Prepas, 1997), including fishponds in the Czech Republic (Lelekova et al., 2008), show that addition of aluminum salts can remove cyanobacterial cells from water, implying that production of odorous compounds may be interrupted, at least temporarily. And that is the catch—addition of aluminum salts to water does not cause a long-term increase in Al^{3+} concentrations because aluminum ions are quickly converted to insoluble aluminum hydroxides that precipitate to the bottom. Because there is little residual effect, conditions soon return to those existing before treatment unless direct inputs of phosphorus are concurrently and permanently reduced (Lelekova et al., 2008). The lack of long-lasting effects of aluminum salts will be especially noticeable in catfish ponds where phosphorus concentrations increase quickly in response to large, daily inputs of phosphorus from feed.

Overall, it does not appear that control of cyanobacterial with chemical coagulants is a practical possibility in catfish ponds. Treatment effects will be short-lived and continuous nutrient inputs from fish feeds will quickly stimulate

a return to pre-treatment conditions. Long-lasting effects will depend on frequent treatments, which are impractical and costly.

15.5 | Manipulating nutrient ratios

Past studies of eutrophic lakes suggested that the ratio of total nitrogen to total phosphorus (N:P) in water is a determinant of phytoplankton community structure (Schindler, 1977; Smith, 1983). Specifically, low N:P ratios favor cyanobacteria over other types of phytoplankton. For example, Smith (1983) concluded cyanobacteria were rare or absent when the N:P ratio exceeded 29. This conclusion seemed to be consistent with the facts that cyanobacteria generally compete well for limited nitrogen supplies and some species are capable of fixing atmospheric dinitrogen gas—traits that confer an advantage over other phytoplankton when N:P ratios are low due to nitrogen deficiency. These observations stimulated interest in modifying N:P ratios to control cyanobacterial blooms; specifically, the focus on N:P ratios reinforced the notion that the best way to reduce the incidence and severity of nuisance blooms is to decrease phosphorus inputs (Schindler et al., 2008).

Mathematically, of course, there are two approaches to increasing N:P ratios (and therefore, as the theory implies, to discourage cyanobacteria). Most efforts focus on reducing phosphorus inputs or removing phosphorus from the water. Of course, this approach works if phosphorus levels are reduced to levels low enough to limit overall phytoplankton growth, although this is often difficult in large watersheds. As discussed in the preceding two subsections, it does not appear possible to reduce phosphorus loading to catfish ponds to the point where it will reduce phytoplankton biomass or change community structure.

The other approach to increasing N:P ratios is to add nitrogen. This approach seems counterintuitive (and does not work, as explained below) but it is a logical interpretation of the N:P ratio hypothesis. Several small- and large-scale studies were, in fact, conducted to determine if nitrogen additions could shift plankton communities away from cyanobacterial dominance. One of the most interesting studies was conducted in Mississippi catfish ponds (Earnheart, 1991). Ammonium nitrate was added weekly to ponds with blooms of *Anabaena spiroides*, a nitrogen-fixing cyanobacterium. Nitrogen fertilization did cause a shift in phytoplankton community structure, but rather than eliminating cyanobacteria, the communities changed from the nitrogen-fixing cyanobacterial species to dense blooms of the non-nitrogen-fixing cyanobacterium, *Oscillatoria* (now *Planktothrix*) *agardhii*. These results, where there is simply a shift in the type of cyanobacteria rather than elimination of cyanobacteria, are similar to results in other ecosystems (Barica, King, & Gibson, 1980; Donald, Bogard, Finlay, & Leavitt, 2011; Huisman et al., 2018; Lathrop, 1988).

Simple nutrient ratios show only the relative availability of resources and not absolute quantities. Low N:P ratios in many systems do not indicate extreme nitrogen-limiting conditions for growth (which would favor nitrogen-fixing cyanobacteria) but rather are caused by high phosphorus concentrations. Catfish ponds are excellent examples. Average summertime N:P ratios in Mississippi catfish ponds range between 4 and 20 (Tucker & van der Ploeg, 1993), well within the range of ratios postulated by Forsberg, Ryding, and Dugdale (1978) to be indicative of nitrogen-limiting conditions favoring the presence of cyanobacteria. This is demonstrably true: most catfish ponds sustain abundant cyanobacteria. However, low N:P ratios in catfish ponds are not due to low total nitrogen levels (they are very high: 4–10 mg N/L), but to extraordinarily high concentrations of total phosphorus (0.5–1 mg P/L). So, cyanobacterial dominance in catfish ponds is not caused by conditions favoring nitrogen-fixing species but rather because phytoplankton grow luxuriantly in response to abundance of all nutrients and the resulting light-limited conditions favor cyanobacteria.

15.6 | Off-flavor management through nutrient manipulation: Summary

Nutrient manipulation holds little, if any, promise for managing nuisance cyanobacterial blooms or off-flavor in catfish ponds. Large, continuous feed additions throughout the summer provide phosphorus, nitrogen, and other nutrients at rates well in excess of that required to nutrient-saturate the system and promote lush phytoplankton blooms

dominated by cyanobacteria—some of which produce odorous compounds. Economic considerations preclude reducing feeding rates to the point where cyanobacteria no longer dominate phytoplankton communities and affecting a positive change on community structure is not possible through diet modifications to reduce nutrient loadings or by manipulating nutrient ratios.

15.7 | Mixing

Bloom-forming cyanobacteria, including species producing odorous compounds in catfish ponds, have a competitive advantage over most eukaryotic phytoplankton in turbid, poorly mixed waters. Under those conditions, buoyancy-regulating cyanobacteria can stay in the upper, illuminated layer of water whereas other phytoplankton may eventually sink into deeper, poorly lit waters and die. Artificial mixing eliminates conditions giving cyanobacteria an advantage. In theory, complete, continuous turbulent mixing of the water column allows all phytoplankton to stay in suspension and all cells are periodically exposed to adequate light for growth by continuously circulating algal cells from deeper water into the euphotic zone. Mixing increases overall photosynthetic rates and theoretically favors eukaryotic algae that grow faster than the larger, bloom-forming cyanobacteria.

Mixing has an additional effect on phytoplankton in some waters by reducing rates of internal phosphorus recycling from sediments. Nutrients initially assimilated by phytoplankton are distributed throughout the plankton food chain and when plankton die, they decompose and release the nutrients back to the water in the process of mineralization. Those nutrients are then available for re-assimilation by phytoplankton. Most decomposition and mineralization occurs near or in the surface sediment layers. Recycled nutrients add to direct nutrient inputs from fish wastes and are a significant source of nutrients for phytoplankton in most lakes and ponds.

Nitrogen is more or less freely recycled to and from waters and sediments because the nitrogenous product of organic matter decomposition, ammonium (NH_4^+), as well as major products of ammonium transformations, nitrite (NO_2^-) and nitrate (NO_3^-), do not form sparingly soluble compounds with other common ions. The situation is different with phosphorus. Orthophosphate ions (H_2PO_4^- , HPO_4^{2-}) released during organic matter mineralization form poorly soluble compounds with calcium (Ca^{2+}), ferric iron (Fe^{3+}), and aluminum (Al^{3+}). The importance of the various cations in determining the fate of phosphorus depends on water and bottom soil chemistry, but sediment-associated iron plays a critical role in determining phosphorus availability in many, if not most, freshwater lakes and ponds (Boyd & Tucker, 1998).

In aerobic sediments, substantial phosphorus becomes associated with amorphous ferric oxyhydroxide gels or as phosphorus co-precipitated in coatings of ferric oxide surrounding silt or clay particles. Under reducing conditions, insoluble ferric iron (Fe^{3+}) is reduced to soluble ferrous iron (Fe^{2+}) and reducible iron-bound phosphorus becomes soluble. Decomposition of organic matter at the sediment–water interface and respiration in the water column can reduce dissolved oxygen concentration near the pond bottom, resulting in loss of the oxidized barrier to diffusion from anaerobic sediments at the sediment–water interface. In the absence of an oxidized barrier to diffusion, phosphorus can diffuse from sediment porewaters into the overlying water in response to a concentration gradient. The upshot is that when the sediment surface is aerobic, bottom muds become a strong sink for phosphorus and there is net movement of phosphorus from water to sediments. When surficial sediments are anaerobic, bottom muds can become a source of phosphorus, with net movement from sediments to water. The role of mixing and aeration on phosphorus uptake and release from fishpond sediments was shown in a study by Masuda and Boyd (1994). In that study, soluble reactive phosphorus concentrations in pond water were higher in unaerated ponds than in aerated, completely mixed ponds.

Artificial mixing has, therefore, two potential impacts on phytoplankton communities: (a) turbulence may offset the light-harvesting advantage of buoyancy-regulating cyanobacteria and (b) mixing may help sequester phosphorus in bottom muds, reducing phosphorus available to support phytoplankton growth. Accordingly, artificial mixing using diffused aeration (bubblers) or impellor pumps is a common recommendation for managing cyanobacterial blooms in

eutrophic lakes (Huisman et al., 2018; Paerl, 2017; Rastogi et al., 2015; Reynolds, 1997; Stroom & Kardinaal, 2016). Boyd and Tucker (2014) review some of the devices used to mix lakes and ponds.

Although mixing is often recommended to control cyanobacterial blooms, results are inconsistent. Mixing works best in deep lakes and when mixing is complete on both vertical and horizontal scales—that is, the whole water body is mixed (Visser, Ibelings, Bormans, & Huisman, 2016). Success is greatly improved when external phosphorus loading rates are concurrently reduced so as not to negate the effects of mixing on internal phosphorus recycling. Water depth appears to be especially critical to success. Reynolds (1997) implies that the lake should be deeper than 6 m for mixing to be an effective bloom-management tool; others feel that water should be at least 15 m deep (Visser et al., 2016).

Mixing is most successful at reducing abundance of strongly buoyant, scum-forming species such as certain species of *Microcystis* and *Anabaena*, but often fails to impact abundance of *Planktothrix agardhii*, a species seldom producing surface scums (Reynolds, Wiseman, Godfrey, & Butterwick, 1983; Steinberg, 1983; Steinberg & Tille-Backhaus, 1990). In fact, the lack of impact on *P. agardhii* blooms prompted Visser et al. (2016) to label that species as “turbulence tolerant.” This observation may have significance for managing catfish off-flavors because *P. agardhii* is perhaps the most common phytoplankton species in catfish ponds. Although *P. agardhii* does not appear to produce odorous compounds, the closely related species, *P. perornata*, does.

Based on the criteria for ecosystems likely to respond to mixing (Reynolds, 1997; Visser et al., 2016), the strategy appears to have limited, if any, usefulness for reducing cyanobacterial abundance or off-flavors in catfish ponds. Catfish ponds are very shallow (<2 m), daily nutrient inputs are high, and blooms often consist of *Planktothrix* species. Only one study (discussed in the following paragraphs) has been conducted to examine the effects of vigorous pond mixing on catfish off-flavors, and the results tend to support this pessimistic conclusion—although not entirely.

In 2002, E. L. Torrans and C. S. Tucker (National Warmwater Aquaculture Center, Stoneville, MS, unpublished) used 15, 0.4-ha catfish ponds to evaluate the impacts of continuous mixing on water quality. Complete water-column mixing was induced in five ponds with underwater air diffusers and 10 ponds were managed traditionally, without continuous artificial mixing.¹³ On a hypothetical fish-harvest date in mid-October, fish were collected from each pond and evaluated by a trained, 3-person sensory-analysis panel. Off-flavors were rated on a hedonic scale of 0–5, where 0 = no detectable off-flavor, 1 = slight off-flavor, increasing to 5 = intensely off-flavored. Each panelist also provided a qualitative descriptor of the flavor. Pond water samples were also collected from each pond and examined microscopically to determine phytoplankton community structure.

On the hypothetical harvest date, two of the five mixed ponds contained off-flavored fish that would not be acceptable for processing (average off-flavor scores ≥ 1). The off-flavor in fish from one pond was described as musty, or 2-MIB (average flavor score = 1.3) and the pond contained a sparse population of *P. perornata*; the off-flavor in fish from the other mixed pond was described as earthy, or geosmin (average flavor score = 2.7), although no known geosmin-producing cyanobacteria were observed microscopically. Five of the 10 unmixed ponds contained off-flavored fish. Fish in four ponds had off-flavors described as 2-MIB (average flavor scores = 2.3, 3.3, 4.0, and 4.0) and fish in one pond had geosmin off-flavor (average flavor score = 3.3). All ponds with fish tainted by 2-MIB had sparse to abundant populations of *P. perornata* and the one pond with fish tainted by geosmin contained a species of *Anabaena*.

Primarily of academic interest (because the species does not produce odorous compounds), eight of 10 unmixed ponds had phytoplankton communities dominated by abundant populations of *P. agardhii*. The community in one unmixed pond was dominated by a population of *Microcystis aeruginosa* and the other was a mixed community of eukaryotic phytoplankton (centric diatoms and green algae). All five mixed ponds contained abundant populations of *P. agardhii*, confirming the characterization of that species by Visser et al. (2016) as “turbulence tolerant.”

Off-flavor incidence and intensity vary greatly over time and among like-treated ponds, so it is difficult to interpret the results of this study. Clearly, continuous mixing did not prevent cyanobacterial off-flavors—two of the five mixed ponds (40%) contained off-flavored fish on the target harvest date. This is slightly lower than the incidence in unmixed ponds (five of 10, or 50%), although the incidence of off-flavored fish populations in both sets of ponds is

typical of commercial ponds in summer and early autumn. Off-flavors appeared to be less intense in mixed ponds: on the hypothetical harvest date, the overall average off-flavor scores for the two mixed ponds was 2.0 and for the five unmixed ponds the overall average score was 3.1. Although there are some interesting trends in the results of this study, they do not strongly support further research on mixing as a management strategy for cyanobacterial off-flavors in catfish ponds.

15.8 | Pond dyes

Pond dyes are non-phytotoxic alternatives to traditional herbicides for preventing growth of submersed aquatic plants. One popular pond dye is a mixture of two food colorants absorbing light in wavelengths near 410 nm and between approximately 550 and 680 nm, with peak absorbance at 630 nm. Light absorbance by the dye roughly corresponds to wavelengths absorbed by chlorophyll *a*, the major receptor of light energy in plants.

Communities of rooted submersed plants are favored over other plants in shallow ponds and lakes with low dissolved nutrient concentrations. Such waters are usually clear because they support sparse phytoplankton communities. Under those conditions, light penetrates to the bottom in shallow areas where submersed plants start growing. Rooted plants can thrive even though the water is nutrient-poor because they can use nutrients in bottom muds for growth. Dyes reduce penetration of light through water, thereby depriving submersed plants of adequate light energy for growth.

Although pond dyes were developed to control underwater angiosperms and filamentous macroalgae, there has been interest in using them to reduce phytoplankton growth. Results have been mixed. Spencer (1984) found that a popular commercial pond dye decreased photosynthesis in laboratory cultures of green algae (Chlorophyta) and two species of *Anabaena* when used at label-recommended rates (0.5–2 ppm). However, application of the same commercial product at label-recommended rates in two field studies resulted in higher phytoplankton standing crops than in untreated ponds (Boyd & Noor, 1982; Ludwig, Perschbacher, & Edziyie, 2010).

Tucker and Mischke (2019) conducted the only study to evaluate the effects of a commercial pond dye on the incidence of off-flavor in pond-grown catfish. The dye was the same commercial product used in the studies described in the previous paragraph. They made four monthly dye applications of 1 ppm to six earthen ponds stocked with food-sized catfish; six additional ponds were treated identically, but were not treated with the dye. Dye application had no effect on overall phytoplankton biomass, incidence of cyanobacteria known to produce odorous metabolites, or incidence of off-flavor in catfish.

Failure of the dye to prevent blooms of odorous cyanobacteria was hardly surprising. The common odor-producing cyanobacteria in catfish ponds have non-chlorophyll accessory pigments allowing them to harvest sunlight at wavelengths outside that absorbed by the dye and they can change cell density to regulate vertical position in the water column. These physiological attributes allow them to thrive in waters with strong vertical light gradients, such as waters turbid with abundant phytoplankton or otherwise have restricted light penetration (as in dye-treated ponds).

15.9 | Biological control with planktivorous fish

Phytoplankton community productivity and organization are influenced by resource availability and predation from higher trophic levels (Carpenter, Kitchell, & Hodgson, 1985; McQueen, Johannes, Post, Stewart, & Lean, 1989). The influence of resource availability is called *bottom-up* control and describes how nutrients and light affect phytoplankton communities.¹⁴ The impact of predation is called *top-down* control and describes how feeding activities at higher trophic levels affect the structure and biomass of communities at lower trophic levels.

Phytoplankton community structure, including the presence of bloom-forming cyanobacteria, can therefore be manipulated either from the bottom up or the top down. The potential for bottom-up control of odorous cyanobacteria was described in previous sections. Mixing and dyes attempt to influence community structure by changing

light availability. Nutrient-input manipulation obviously tries to control relative and absolute availability of essential plant nutrients. It should be obvious from previous discussions that bottom-up control of phytoplankton is difficult in catfish ponds. Meaningful long-term reduction of nutrient inputs can be accomplished only by dramatically decreasing feed inputs, which is economically unsound. Top-down control—sometimes called *biomanipulation*—is therefore an attractive alternative.

The most common application of biomanipulation for control of cyanobacterial blooms in non-aquaculture settings involves reducing the abundance of fish feeding on large zooplankton. Large-bodied zooplankton, such as *Daphnia*, are important consumers of large phytoplankton, so increasing their numbers will, in theory, lead to greater grazing pressure on colonial and filamentous cyanobacteria. In many lakes, the primary predators on large zooplankton are sight-feeding, young-of-the-year fish and their removal often consists of adding piscivorous fish to eat the juvenile fish. The “top-down” nature of the process can be visualized as (a) big fish eat small planktivorous fish; (b) fewer planktivorous fish leads to more large-bodied zooplankton; and (c) more large-bodied zooplankton eat more colonial cyanobacteria. Biomanipulation is an important technique for improving water quality in eutrophic lakes and reservoirs, although success is spotty and varies depending on lake size (it works best in smaller water bodies), water depth (it works best in shallow lakes), climate (it works best in cool-water, temperate lakes), nutrient loading (it is often only successful when accompanied by concurrent nutrient-load reduction), and other factors (Benndorf, 1987; Benndorf, Böing, Koop, & Neubauer, 2002; Jeppesen et al., 2007; Kasprzak, Benndorf, Mehner, & Koschel, 2002).

Biomanipulation as used in catfish pond aquaculture involves adding planktivorous fish feeding directly on colonial or filamentous cyanobacteria. At first glance this appears to contradict the approach described in the previous paragraph where the goal was to remove, rather than add, planktivorous fish. The difference lies in the prey-size selectivity of the planktivorous fish involved in the biomanipulation. Lake restoration efforts usually attempts to remove sight-feeding juveniles (young-of-the-year bluegill *Lepomis macrochirus*, as an example from North America) that eat large zooplankton but do not feed directly on phytoplankton. Biomanipulation in catfish ponds involves adding filter-feeding species that eat smaller prey items—phytoplankton as well as zooplankton. Planktivorous fish that have been used for biological control of phytoplankton in fishponds include blue tilapia *Oreochromis aureus*, Nile tilapia *Oreochromis niloticus*, silver carp *Hypophthalmichthys molitrix*, and threadfin shad *Dorosoma petenense*.

Torrans and Lowell (1987) were the first to evaluate the effects of co-culturing planktivorous fish on the incidence of catfish off-flavors. They added blue tilapia to six catfish ponds either as young-of-the-year fish or as 1-year-old breeding pairs that would reproduce and populate ponds with juvenile fish through the summer. Three ponds had no tilapia. Catfish were partially harvested from all ponds five times in the late summer and autumn to remove larger fish. At each partial harvest, catfish were sampled for flavor quality. Tilapia had no effect on overall phytoplankton abundance, which was low in all ponds (averaging $<50 \mu\text{g/L}$ chlorophyll *a*). However, tilapia had a noticeable impact on off-flavor incidence. More than 60% of the catfish samples from ponds without tilapia had unacceptable flavor quality; fewer than 10% of the catfish samples from ponds with tilapia were off-flavored. Off-flavor incidence was reduced whether tilapia were added as juveniles or as sexually mature breeding pairs. Off-flavors were not described, and no attempt was made to determine phytoplankton community structure.

In contrast to the Torrans and Lowell (1987) study, Tucker & Martin (1991) failed to demonstrate a benefit of tilapia-catfish polyculture on summertime flavor quality of catfish. Channel catfish were stocked into 14 ponds, with tilapia added to seven ponds as mature breeding pairs. On a projected harvest date in late October, four of seven ponds in both treatments contained off-flavored fish. Three ponds in each treatment had fish with an off-flavor described as musty (2-MIB); off-flavor in one pond with tilapia was described as “decaying plants” and in one pond without tilapia the off-flavor was described as “tobacco-like.” Tilapia did not appear to affect the incidence of the cyanobacterium *Planktothrix perornata*, which was present in all ponds with fish off-flavored by 2-MIB. Moreover, all tilapia died with the onset of cold temperatures in the winter, and catfish in all ponds with tilapia developed severe “rotten-fish” off-flavors that appeared to be related to feeding on the dead and decaying tilapia.

Considerable research has been conducted on the impacts of silver carp and bighead carp *Aristichthys nobilis* on phytoplankton communities when co-cultured with other fish (summarized in Boyd & Tucker, 1998; and see the

useful, although dated, review by Smith, 1988). Both fish are non-native filter feeders. Adult silver carp are highly efficient filter feeders, feeding mostly on phytoplankton down to 10 μm in size; bighead carp are not as effective at retaining small particles and feed mostly on larger zooplankton.

Several catfish farmers in Mississippi and Arkansas co-cultured silver or bighead carp in catfish ponds in the 1980s and 1990s to supply specialty markets for the fish in the northern United States. Anecdotally it appeared that co-culturing carp, especially silver carp, reduced catfish off-flavor incidence. In a short-term outdoor study in 500-L tanks, Perschbacher (2018) showed that silver carp quickly eliminated the odor-producing cyanobacteria *P. perornata* and *Anabaena circinalis*, confirming the potential for silver carp to reduce cyanobacterial off-flavors.¹⁵ In his study, Perschbacher noted that threadfin shad, blue tilapia, and Nile tilapia were also effective at removing odorous cyanobacteria. Bighead carp were considerably less effective.

Although silver carp have potential as a biological control agent for odorous cyanobacteria, the fish has serious drawbacks for routine use in catfish ponds. Silver carp grow quickly in eutrophic catfish ponds and may exceed 5 kg in their second year. They are strong and active fish, and farm workers have been injured by large fish thrashing about and leaping over seines during harvest activities. Catfish farmers also report that silver carp tend to dominate the area near mechanical aerators when dissolved oxygen concentrations are low, forcing catfish to inhabit areas with lower oxygen availability. Silver carp also are the least-valued of the major Asian carps and few, if any, markets currently exist. Because silver carp have little commercial value in the United States, catfish farmers are penalized when they are mixed with catfish delivered to processing plants. Perhaps most important, silver carp are not native to North America and are considered a nuisance species in many states.

If silver carp are to be useful for plankton management in catfish ponds, they must either be confined (Laws & Weisburd, 1990; Smith, 1985, 1994) or carp stocking rates must be relatively low to reduce problems associated with free-roaming fish. Catfish farmers familiar with silver carp indicate that carp densities greater than about 250/ha would be undesirable. That information set the upper boundary for a study evaluating the impacts of silver carp co-cultured with catfish on phytoplankton communities and cyanobacterial off-flavors (Tucker, 2006). Carp were stocked at densities of 0, 75, or 250 fish/ha in seven replicate catfish ponds. Silver carp had no effect on phytoplankton standing crops and did not eliminate odor-producing cyanobacteria from phytoplankton communities. Throughout early autumn, catfish in three to five ponds in each treatment were tainted with either musty (2-MIB) or earthy (geosmin) off-flavors. Silver carp also had no effect on off-flavor intensity. The impact of planktivorous fish is known to depend on fish biomass (Lazzaro, Drenner, Stein, & Smith, 1992) and the feeding activities of silver carp at densities acceptable for commercial catfish production appear inadequate to offset the strong, bottom-up influences on phytoplankton community organization in hypertrophic catfish ponds.

Threadfin shad functionally are a better planktivore choice than silver carp for co-culture with catfish. The range of prey items captured by filter-feeding threadfin shad more or less overlaps that for silver carp, but they are small fish that do not interfere with normal fish cultural activities. Threadfin shad also are native to North America.

Green, Perschbacher, Ludwig, and Duke (2010) showed clear impacts of threadfin shad on catfish pond plankton communities. Although shad had no effect on total phytoplankton biomass, they decreased abundance of all taxonomic groups of zooplankton and altered phytoplankton community structure. Phytoplankton in ponds without shad consisted mainly (53–60%) of bloom-forming cyanobacteria in the genera *Planktothrix*, *Anabaena*, *Aphanizomenon*, and *Microcystis*. Cyanobacteria constituted only 2–3% of the phytoplankton in ponds with shad; communities consisted mainly of diatoms, green algae, and other eukaryotic phytoplankton. Notably, *P. perornata*—the most common producer of 2-methylisoborneol in catfish ponds—was present in several ponds without shad but was not observed in ponds with shad.

Mischke et al. (2012) confirmed the general findings of Green et al. (2010) and added an important new observation. Mischke et al. (2012) found, as expected, that size-selective filter-feeding by shad reduced cyanobacterial abundance and increased relative abundance of smaller eukaryotic phytoplankton and small zooplankton (rotifers). On a target harvest date in late September, seven of 10 catfish-only ponds had populations of odorous cyanobacteria (*P. perornata* and *Anabaena* spp.). Only two of 10 ponds with shad contained odorous cyanobacteria. Corresponding

to the reduced incidence of odor-producing cyanobacteria, fish from seven of 10 catfish-only ponds were off-flavored (all caused by either 2-MIB or geosmin) whereas fish from three of 10 ponds with shad were off-flavored (catfish in two ponds had mixed off-flavors caused by 2-MIB and geosmin; fish in one pond had an off-flavor described as “stale”). Off-flavors were also noticeably less intense in fish from ponds with shad.

Impacts of threadfin shad on summertime phytoplankton communities were similar in both studies (Green et al., 2010; Mischke et al., 2012) and the results are functionally supported by short-term studies conducted by Perschbacher (2018), who showed threadfin shad can rapidly reduce populations of the odorous cyanobacteria *P. perornata* and *A. circinalis*. This indicates—rather strongly—that threadfin shad co-culture can be an effective off-flavor management tool in catfish ponds. However, Mischke et al. (2012) continued their study through the winter and found catfish in five of 10 ponds with shad developed noticeable “fishy” off-flavors in February. Threadfin shad are known to be cold-intolerant and cold temperatures killed shad in all ponds. The noxious catfish off-flavor that developed in winter appeared to be caused by catfish foraging on dead, decaying shad.

Biomanipulation of the plankton community is the most promising of all currently available tools for reducing the incidence of cyanobacterial off-flavors. Specifically, co-culturing certain planktivorous fish with catfish can dramatically reduce incidence and abundance of odor-producing cyanobacteria, with concomitant reduction in the incidence and intensity of catfish off-flavors. However, catfish markets demand fish year-round, with peak demand in February and March, coinciding with the Lenten season (Hargreaves & Tucker, 2004). The three most promising candidates for biological control—blue tilapia, Nile tilapia, and threadfin shad—do not tolerate cold water temperatures. Although catfish are grown primarily in the deep South, the normally mild winters are interrupted by strong cold fronts that may drop water temperatures below 10°C for many days. Tilapia may survive winters in parts of the southern-most United States, but they never survive in ponds through the winter in the major catfish-producing regions of Mississippi, Alabama, and Arkansas. Although threadfin shad are more cold-tolerant than tilapia, many die during winter. Using tilapia or threadfin shad for biological control of phytoplankton presents a dilemma for catfish farmers: they reduce catfish off-flavor incidence in warm months but cause off-flavors in the colder months.

15.10 | Bioaugmentation

Bioaugmentation is an environmental remediation technology in which concentrated suspensions of non-photosynthetic bacteria are added to soil or water to carry out a beneficial activity. It is based on the idea that rates of a microbe-mediated process might be limited by low numbers of the microorganisms carrying out that process and the process rate can be increased by adding more of the microorganism. Another technology used in remediation is *biostimulation*, which involves improving environmental conditions to stimulate the growth and activity of naturally occurring microorganisms. For example, if a goal is to increase rates of organic matter decomposition in an aquaculture system, two approaches might be to (a) add more bacteria (bioaugmentation) or (b) provide more oxygen to increase decomposition rates (biostimulation).

Microorganisms used in bioaugmentation usually are isolated from nature using enrichment techniques and then grown in mass laboratory cultures. Many commercial products consist of various *Bacillus* species—common, spore-forming heterotrophic bacteria that can metabolize a wide variety of organic compounds. Products may also contain other bacteria for specific purposes.

Bioaugmentation is most commonly used in wastewater treatment and to remediate soils contaminated with hazardous organic pollutants such as crude oil, petroleum products, or pesticides. Bioaugmentation has also been used in lake and pond management. Treatment success has been variable regardless of the application, particularly when bioaugmentation is used alone, without concurrent biostimulation.

Dozens of bioaugmentation products have been marketed to aquaculturists. Common claims include improved rates of organic matter decomposition, better dissolved oxygen concentrations, and lower concentrations of ammonia—a potentially toxic product of protein metabolism excreted into water by fish. One proposed mode of action is that introduced bacteria compete with phytoplankton for dissolved inorganic nitrogen (including ammonia)

and phosphorus, thereby reducing water-borne concentrations of those nutrients. Because microbial products are not registered as pesticides, label claims cannot include specific reference to reduced algal growth, although this is an implied outcome of reduced nutrient availability. In fact, bioaugmentation products have been used by lake and pond managers attempting to control phytoplankton (Duvall & Anderson, 2001; Duvall, Anderson, & Goldman, 2001) and claims have been made that microbial bioaugmentation can reduce the incidence of cyanobacterial off-flavors.

Assertions that heterotrophic bacteria can outcompete phytoplankton for inorganic nutrients are true—sometimes. Bacteria have a high ratio of surface area to volume and, therefore, they have the capacity for high nutrient-uptake rates (Bradley et al., 2010). When inorganic nutrients are in low supply, as in oligotrophic waters, assimilation by bacteria may reduce nutrient availability for phytoplankton and reduce algal productivity and biomass (Grover, 2000; Joint et al., 2002). However, the relative role of bacteria is much reduced in ecosystems with high external nutrient loading rates (Biddanda, Ogdahl, & Cotner, 2001; Cotner & Biddanda, 2002; Danger, Leflaive, Oumarou, Ten-Hage, & Lacroix, 2007). In waters with high nutrient loadings, growth of heterotrophic bacteria may be limited by supply of organic carbon rather than the supply of inorganic nutrients. Heterotrophic bacteria in these systems will not assimilate excess nitrogen or phosphorus until a labile carbon source is added (Grover, 2000; Joint et al., 2002; Le Chevanton et al., 2016). This phenomenon is well known to aquaculturists as the basis for adding molasses, cane sugar, or other simple carbon sources to heterotrophic biofloc aquaculture systems to stimulate bacterial uptake of excess ammonia (Avnimelech, 2014).

Simply adding bacteria to a complex aquatic ecosystem and expecting them to flourish and make long-lasting changes in environmental conditions also runs counter to the ecological principle that “the population reflects the habitat.” That is, the physical, chemical, and biological characteristics of the environment define microbial community organization and simply adding more of a particular type of microorganism will have little long-term impact on community structure (Alexander, 1977). This explains why bioaugmentation alone has such an uneven record of success unless accompanied by concurrent efforts to change the environment to favor the growth and activities of the new microorganism.

There is, therefore, little theoretical support for simply adding bacteria to nutrient-rich catfish ponds with the intent of altering phytoplankton community organization. Nevertheless, these products are aggressively marketed and there has been considerable research on bioaugmentation of catfish ponds (Boyd, Hollerman, Plumb, & Saeed, 1984; Chiayuvareesajja & Boyd, 1993; Quieroz & Boyd, 1998; Tucker, Kingsbury, & Mischke, 2009; Tucker & Lloyd, 1985). None of these published studies showed meaningful changes in water quality, although the effects of bioaugmentation on incidence of off-flavors was not evaluated.

Between 1983 and 1998, one of us (C. S. Tucker, unpublished) conducted nine trials of four commercial bioaugmentation products on catfish farms in Mississippi. The products were used according to label directions or instructions provided by the manufacturer. All studies were either double-blinded so neither the applicator or analyst knew the identity of treated ponds or single-blinded so the analyst did not know which ponds were treated. Response variables were selected by the manufacturer: all manufacturers claimed their product would reduce phytoplankton biomass (among other claims) and two manufacturers claimed their product would reduce off-flavor incidence.

One product with claims of reducing off-flavor incidence was a liquid suspension of *Bacillus* sp., *Enterobacter* sp., *Pseudomonas* sp., *Cellulomonas* sp., *Nitrobacter* sp., and *Rhodopseudomonas* sp. The product was applied weekly to 12, large (3–7 ha) commercial catfish ponds for 1 year. Twelve ponds were untreated, serving as controls. A representative of the manufacturer applied the product and the study was single-blinded so the person collecting and analyzing samples was unaware which ponds were treated and which were untreated. Fish samples were collected monthly from June through October for sensory analysis by processing plant quality control personnel. Bioaugmentation did not reduce phytoplankton biomass and had no effect on incidence or severity of off-flavors.

The other product with claims of off-flavor control was a powdered formulation of three species of *Bacillus*. The product was incubated in a liquid nutrient solution for 3 hr before application to allow sporulation and increase bacterial numbers. Eight, large (4–7 ha) commercial catfish ponds were treated monthly; eight ponds were untreated

controls. Again, a representative of the manufacturer applied the product and the study was single-blinded to the analyst. The study began in March and ended in October. Fish samples were collected monthly and flavors were assessed by processing plant quality control personnel. Overall, bioaugmentation did not reduce phytoplankton biomass and had no effect on incidence or severity of off-flavors.

Bioaugmentation of catfish ponds is one of the most intensely studied water-quality management techniques, yet results are consistently neutral. The lack of effect is consistent with modern understanding of microbial ecology and, specifically, the competition between heterotrophic bacteria and phytoplankton for nutrients in eutrophic ecosystems. Further studies do not appear justified.

15.11 | Biological control with microbial agents

Cyanobacteria, in common with all organisms, have a variety of natural microbial antagonists that are lethal or growth-suppressing. These antagonists include cyanophages (cyanobacteria-infecting viruses), fungal parasites, protozoal grazers, and lytic bacteria (Sigee et al., 1999; Van Wichelen, Vanormelingen, Codd, & Vyverman, 2016). Hundreds of natural microbial enemies have been isolated from nature and their role in shaping plankton communities has been suggested since their first discovery. Many are extraordinarily effective in suppressing cyanobacterial populations under controlled laboratory conditions, suggesting, of course, they might be useful as agents for biological control of natural cyanobacterial blooms.

Despite their promise based on laboratory studies and suggestions that biological control using microorganisms might be the least expensive, most specific, and most environmentally sound strategy for managing cyanobacterial blooms, successful large-scale application of the strategy has yet to occur. The complexity of the natural systems, high degree of host specificity, rapid evolution of resistant strains, and difficulties in producing inoculum in large, commercial-scale amounts contribute to persistent failures of biocontrol using microbial antagonists of cyanobacteria (Lürling, Waajen, & de Senerpont Domis, 2016; Ndela, Oberholster, Van Wyk, & Cheng, 2018; Sigee et al., 1999; Van Wichelen et al., 2016).

Although biological control of cyanobacteria with microbial antagonists has not been successfully applied at large scale, there is an intrinsic appeal to the strategy and some interesting work has been conducted using lytic bacteria to manage undesirable cyanobacteria in catfish ponds. Walker and Higginbotham (2000) isolated a bacterium (originally designated SG-3) from catfish pond water in Louisiana, USA, that was capable of lysing cells of some species of *Anabaena* and *Oscillatoria* (now *Planktothrix*) but had no effect on eukaryotic green algae (Chlorophyta) in laboratory studies. Subsequent studies (Walker, 2003) were conducted using water from commercial catfish ponds with blooms of *P. perornata*. About 570-L of pond water was added to polypropylene tanks and the bacterium SG-3 was added at 2×10^9 PFU/mL (PFU = plaque forming unit, which provides a count of SG-3 bacteria cells). Each tank also contained 10 channel catfish fingerlings. The large inoculum of SG-3 bacteria caused rapid lysis of *P. perornata*: the cyanobacterium was microscopically unobservable 2 days after introducing SG-3 to the tanks. The abundance of the SG-3 rapidly increased in the pond water, indicating that it reproduced readily after initial inoculation. Catfish appeared unharmed by the presence of high numbers of the bacterium SG-3. This approach to biological control has been patented in the United States (Walker & Higginbotham, 2001, 2002, 2006).

The bacterium SG-3 was later designated as *Lysobacter* cf. *brunescens* and found to be effective in lysing cells of the cyanobacteria *Cylindrospermopsis raciborskii* (two isolates) and *Pseudanabaena limnetica* in laboratory studies (Flaherty, Walker, Britton, & Lembi, 2007). The researchers also concluded that *L. cf. brunescens* at high concentrations could be used to eliminate blooms of *C. raciborskii* while lower concentrations could be used to act as an algistatic control to prevent bloom development. The lytic activity of *L. cf. brunescens* is likely due to the production of a protease as has been described for another species of *Lysobacter* (Mitsutani & Takesue, 1993).

We (K. K. Schrader, H. L. Walker, and C. S. Tucker, unpublished) conducted studies using 5.5 m³ limnocorrals¹⁶ placed in catfish ponds to evaluate the efficacy of *L. cf. brunescens* in eliminating *Planktothrix perornata* from pond water. Our results were inconsistent: *P. perornata* was eliminated in only one of the three efficacy trials. Reasons for

the inconsistent elimination of *P. perornata* in our studies are unknown but may involve trial-to-trial differences in the preparation of the *L. cf. brunescens* inocula, water temperature and other water quality differences among trials, and lack of adequate inoculum.

The lytic bacterium *Lysobacter cf. brunescens* is the only microbial biocontrol agent studied for managing off-flavor cyanobacteria in catfish ponds. Despite biocontrol's non-existent record of success at large scale, we believe additional research should be pursued to isolate other lytic or pathogenic bacteria and perhaps other biocontrol microorganisms (cyanophages, for example). Catfish ponds are relatively small compared to other aquatic ecosystems, which would require a smaller inoculum and may make it easier to control environmental conditions to favor proliferation of the biocontrol agent. Other advantages to the strategy—if it works—are the selectivity of the approach and potential cost-effectiveness (especially because it may be less expensive to obtain government approval compared to the expensive approval process for new chemical control agents).

15.12 | Suppressing cyanobacteria with long-term use of algicides

Odor-producing cyanobacteria are essentially a “weed problem,” and management often is approached in the same manner as weed control in terrestrial agriculture—using phytotoxic chemicals to kill undesirable plants and allow desirable plants to grow. The ecological soundness of using chemicals to control cyanobacterial blooms can (and will) be debated endlessly (Jančula & Maršálek, 2011; Matthijs, Jančula, Visser, & Maršálek, 2016; Stroom & Kardinaal, 2016) but there is no doubt that chemical control is the approach most favored by commercial catfish farmers, many of whom were indoctrinated into chemical weed control as row-crop farmers before becoming catfish farmers.

Eliminating odor-producing cyanobacteria is essential for off-flavor management. Methods based on reducing concentrations of geosmin or 2-MIB in the water, such as using absorption agents (powdered or granular activated carbon or hydrophobic substances; Kelly, Holmes & Schultz, 2006) will invariably fail because they do not address the source of the tainting compound. Unless the responsible organisms eradicated, odorous compounds will continue to be produced and absorbed by fish, regardless of the rate they are removed from water.

In the next section we summarize the use of algicides (sometimes spelled *algacides* or *algaecides*) to solve existing off-flavors problems. Here we discuss algicide use to prevent off-flavors. The two strategies overlap to a degree but differ in that the former consists of one (or a few) applications of a chemical to ponds shortly before fish harvest with the intention of killing the odor-producing cyanobacterial population whereas the latter consists of applying a chemical throughout the production cycle (or at least throughout the summer) to prevent odorous blooms from becoming established.

Long-term suppression of cyanobacterial population with chemicals is fraught with problems. First, phytoplankton is an essential component of pond ecosystems. Phytoplankton produce oxygen and assimilate inorganic nutrients that would otherwise accumulate in the water (Boyd & Tucker, 1998, 2014). Only a few species of phytoplankton, most of which are cyanobacteria, are undesirable. The goal, therefore, is to remove or inhibit the growth of noxious species while allowing the rest of the community to perform essential ecosystem functions necessary for successful pond aquaculture. That goal is difficult to achieve with current technology. Second, catfish are off-flavored only when tasted, and for nearly all of the fish-production cycle it does not matter if they taste bad. As such, long-term suppression of odorous cyanobacteria must be inexpensive, logistically simple, and harmless to fish if it is to be a worthwhile strategy. Third, algicidal chemicals may cause unintended, non-target toxicity problems in the pond or outside the pond if exported in pond effluents. This is especially problematic for the copper-containing algicides—the most commonly used class of chemicals to control cyanobacteria—which persist indefinitely in the environment. And fourth, long-term repeated use of algicides may induce development of resistant strains of cyanobacteria, rendering future control more difficult (Price & Morel, 1994; Rouco et al., 2014).

The active agent of copper-based products is the cupric ion, Cu^{2+} , which is transported into cells where it disrupts a variety of cellular functions, including photosynthesis, respiration, chlorophyll synthesis, and cell division. Cyanobacteria appear to be relatively sensitive to copper (Matthijs et al., 2016), but it is difficult to take advantage

of differential toxicity under field conditions because the difference in copper tolerance among groups is not great (Mastin & Rodgers, 2000; Schrader, De Regt, Tidwell, Tucker, & Duke, 1998a) and copper tolerance varies significantly among strains within species (Twiss, Welbourn, & Schwartzel, 1993; Wu, Gaojie, Xiao, Lin, & Ming, 2017).

Despite shortcomings as selective cyanobacteriocides, copper compounds are the most common off-flavor control measure used by catfish farmers, and a procedure was developed empirically by catfish farmers to use repeated low doses of copper sulfate pentahydrate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) to prevent cyanobacterial off-flavors. The procedure was evaluated in two large studies (Schrader, Tucker, et al., 2005; Tucker et al., 2001).

The first study (Tucker et al., 2001) was conducted in 0.4-ha experimental ponds. Copper sulfate was applied at weekly intervals through the spring and summer when water temperatures increased above 20°C and discontinued each fall when water temperatures declined below 20°C. Nine ponds were treated weekly in the mid-morning of sunny days at 0.12 mg Cu/L (0.5 mg/L copper sulfate pentahydrate). Treatments were made by placing the required amount of copper sulfate crystals in a double-layered burlap bag suspended about 7 m in front of a paddlewheel aerator for 2–3 hr to fully dissolve and distribute the chemical throughout the pond.

Over 3 years, copper sulfate treatment reduced the incidence of ponds with off-flavored fish in late summer by about 80%. Treatment also reduced the duration of off-flavor episodes by 85% and no episodes occurred when fish were scheduled for harvest. Fish yield from untreated ponds was 9% less than that from treated ponds. The reduction in fish yield was caused by fish lost to infectious disease outbreaks in a few ponds each year where harvest was delayed by off-flavor. Enterprise budgets showed average net returns above variable costs were 43% higher for ponds treated with copper sulfate. Variation in net returns was twice as great for control ponds as for treated ponds, indicating increased stability in production and economic returns when off-flavors were managed using copper sulfate. High variation in annual economic performance on control ponds resulted from one or more ponds having high net returns while one or more ponds had extremely poor returns due to protracted episodes of off-flavor.

Ponds treated with copper sulfate required 20% more aeration than control ponds during the summer, indicating that copper treatment had a significant adverse effect on oxygen production by phytoplankton. In addition, ammonia and nitrite concentrations were often greater in copper-treated ponds than in control ponds during the summer. After 3 years of treatments, total copper concentrations in treated pond sediments (173 mg/kg) was much greater than in control pond sediments (36 mg/kg) (Han, Hargreaves, Kingery, Hugget, & Schlenk, 2001). Sediment copper in treated ponds was mainly present in carbonate and organic fractions. Most of the sediment copper in control ponds was firmly bound in stable fractions. Although soil analysis indicated potentially greater bioavailability of copper in treated pond soils, toxicity tests with amphipods and cattail roots showed no toxicity related to copper.

The second study (Schrader, Tucker, et al., 2005) was conducted over 3 years in large (3.2–8.4 ha) ponds on two catfish farms. Copper sulfate pentahydrate was applied weekly to obtain 0.12 mg/L Cu beginning in the spring and continuing through early autumn until water temperatures dropped below 20°C. Treatment reduced the abundance of *Planktothrix perornata* and increased the abundance of eukaryotic phytoplankton. Overall prevalence of all types of off-flavor was 50% lower in treated ponds than in control ponds and treatment reduced potential harvest delays by nearly half and reduced costs associated with off-flavor by 35%.

Although weekly low dose applications of copper sulfate reduced catfish off-flavor incidence and improved farm economic performance in both studies, catfish farmers do not currently use this strategy, probably because it is labor-intensive. We also cannot endorse the long-term use of this practice because copper from copper-based algicides never dissipates. Although sediment toxicity tests were negative after 3 years of copper sulfate application (Han et al., 2001), those results cannot be used to predict consequences from longer-term use.

16 | TREATING EXISTING CYANOBACTERIAL OFF-FLAVORS

The previous section reviewed strategies for preventing—or at least mitigating—cyanobacterial off-flavors. Most of those strategies attempt to change the environment so that eukaryotic phytoplankton communities are favored over

odorous cyanobacteria. We hope it was clear from those discussions that it is difficult to permanently change phytoplankton community structure in catfish ponds. The only strategy based on sound principles is biomanipulation to increase grazing losses of large colonial or filamentous cyanobacteria.

Difficulties in preventing cyanobacterial blooms in catfish ponds are consistent with failures and inconsistent results encountered in managing cyanobacteria in other aquatic ecosystems. The only proven strategy for controlling cyanobacteria in non-aquaculture settings is dramatically reducing inputs of phosphorus, nitrogen, or both. Otherwise, as stated by Stroom and Kardinaal (2016), "... the number of proven options [for managing cyanobacteria] is limited."

Lack of success in preventing blooms of odorous cyanobacteria compels catfish farmers to take a different approach: allow uncontrolled development of phytoplankton blooms and, if fish are off-flavored when they reach marketable size, resolve the problem at that time. This "reactive" approach to off-flavor management has three logistical advantages. First, as stated above, a safe, consistently successful strategy for changing the cyanobacterial stable state has yet to be developed. Second, although most ponds have summertime cyanobacterial communities, not all those communities contain species producing odorous compounds. At any one time during the summer, perhaps half of all catfish ponds contain fish tainted with odorous cyanobacterial metabolites, meaning half of all ponds have fish with acceptable flavor. Unless the control strategy is relatively inexpensive and causes no ill effects on fish, treating or managing *all* ponds may be economically unsound because only *half* may eventually benefit from treatment. And third, fish flavor is unimportant until they are sold, processed, and eaten. No one cares about fish flavor while they are simply growing in a pond (that is the reason no one attempts to manage off-flavors in populations of fingerling catfish). So again, treating ponds throughout the production cycle—during most of which flavor is unimportant—may be unsound unless the treatment is inexpensive and harmless to fish.

The most common strategy for managing cyanobacterial off-flavors is therefore to disregard phytoplankton community composition and fish flavor status until fish reach a marketable size.¹⁷ At that point, flavor is assessed and if the flavor is acceptable, fish are harvested and sold without the expense of treatment. If fish are off-flavored, an attempt is then made to rid fish of the tainting compound.

All off-flavors will eventually disappear when fish are no longer exposed to the odorous chemical. Purging taints from fish can be accomplished in two ways: (a) physically move the fish to clean water or (b) kill the odor-producing cyanobacterial population and allow time for odorous compounds to be lost from the water and purged from fish.

16.1 | Moving fish to clean water

Moving off-flavored catfish to clean water is the most dependable way of improving flavor quality: it always works. The process involves harvesting off-flavored fish from the growout pond, moving them to another water body with clean water, allowing adequate time for the taint to be purged, and then harvesting fish a second time for transport to the processing plant.

The kinetics of purging taints from fish was discussed earlier in Section 7. Off-flavors caused by geosmin and 2-MIB are purged relatively quickly from fish in warm water. Depending on fish size, fattiness, and initial concentration of geosmin and 2-MIB, the flavors can be reduced below detectable levels within a few days to perhaps 2 weeks in clean, warm (>25°C) water. Purging time may be much longer for other taints and for large, fatty fish in cold water.

Farmers sometimes simply move off-flavored fish to an adjacent foodfish growout pond. Off-flavored fish in one pond are seined, concentrated into a small area, and scooped into a loading net attached to a boom truck crane. The loading net is swung over the levee and the fish are emptied into the adjacent pond. The only advantage to using an adjacent growout pond to purge off-flavored fish is that the process of moving fish can be accomplished quickly, which reduces fish stress (and perhaps subsequent losses to stress-induced infectious diseases). If an existing growout pond is used to purge fish, it should have a low standing crop of fish so the total weight of fish in the pond does not become excessive after adding the off-flavor fish. Obviously, it is important that existing fish in the purging

pond are not off-flavored. A serious disadvantage to using another growout pond to purge fish is that the resulting fish population then has mixed flavor quality, which complicates sampling to determine if the entire population is suitable for processing (see Section 10). Another problem is the risk of the “purging pond” developing a population of odor-producing cyanobacteria. There is no way to predict whether this will happen and no sure way to prevent it unless algicides are used as a preventive measure. The probability of fish acquiring a new off-flavor after being moved can be reduced by making sure the existing off-flavor in the fish being moved will be rapidly purged.

A better approach is to use a pond freshly filled with clean water, preferably from a well. This is preferred because a newly filled pond does not have a preexisting fish population and is less likely to develop populations of odor-producing cyanobacteria soon after fish are added. In the 1980s, several catfish farmers in Mississippi used small (0.5–1 ha) ponds to purge off-flavors. A 0.5-ha pond can safely hold about 10,000 kg of fish for a week in summer if adequate mechanical aeration is available and aeration is used continuously. Fish can be held longer if water is exchanged with clean well water. Off-flavored fish from different ponds should not be mixed into one group for purging. Purging rates vary for fish with different off-flavors and some fish may remain off-flavor well after most of the fish have purged and are acceptable for processing.

Moving fish to another pond to purge taints is justified only when the off-flavor can be purged in a reasonable amount of time. Taste-testing alone cannot predict whether a flavor will be quickly purged. Although geosmin and 2-MIB are lost from tissues relatively rapidly and have characteristic flavors, the flavors are intense and can mask other off-flavors that may be more difficult to eliminate. The only way to be relatively certain that purging will eliminate off-flavors in a reasonable length of time is to conduct a small-scale test, such as putting a few fish in an aerated tank with clean, flowing water. If fish flavor improves within a week or so, chances of successful treatment are greatly improved (van der Ploeg, 1991).

Despite the certainty that the process will work, few farmers currently move fish to another pond for purging. The process is laborious and involves the added expense of paying a seining crew to move fish twice (once to the purging pond and then again to the processing plant). Seining and transport stresses fish regardless of how carefully they are carried out, especially in hot weather when cyanobacterial off-flavors are most prevalent. Stress and mechanical abrasions associated with harvest can predispose fish to infectious diseases and death during the purging process, particularly if purging is protracted in warm water. Further, fish held at high densities for purging usually are not fed during the purging process and can lose considerable weight. Lovell (1983b) determined weight loss for fish held in clean water during off-flavor purging and found that losses ranged from about 5% of initial weight for fish held at 15°C for 3 days to 18% for fish held at 26°C for 15 days.

One of us (C.S. Tucker, unpublished) conducted numerous trials of off-flavor purging in 1988–1992. The trials were conducted in a small, 0.04-ha earthen with abundant aeration. Off-flavored fish were obtained from commercial ponds, 800 kg of fish were held in the pond, and a few fish were sampled for flavor quality every 2–3 days. Trials were conducted in summer and water temperatures were 30 to 35°C. Some qualitative findings were (a) fish from commercial ponds with severe off-flavors caused by 2-MIB could be purged of the taint within 7 days; (b) fish from a commercial pond with a mild off-flavor described as “woody”¹⁸ could not be purged of the taint within 14 days; (c) fish from a commercial pond with a severe off-flavor described as “petroleum” could not be purged of the taint within 21 days; and (d) weight loss for fish harvested, transported, and then held for a week without feeding averaged about 15%.

16.2 | Using algicides to treat existing flavor problems

Using algicides to treat existing cyanobacterial off-flavors is simply another approach to purging. Rather than moving fish to odor-free water, the pond in which fish are living is made odor-free by killing the odor-producing cyanobacterium. After the algicide kills the cyanobacterium, geosmin or 2-MIB in the water naturally dissipates and the compounds purge from fish without having to move them to another pond. Under the right circumstances this can be reasonably successful—and is by far the most common strategy used by catfish farmers.

A key to successful use of algicides to treat existing off-flavors is to confirm that cyanobacteria are, in fact, causing the problem. This important point is thoroughly discussed in Section 19 of this paper. Another key is to recognize that phytoplankton are essential components of the pond ecosystem and killing all or part of that community will have consequences that must be managed. Most important, death and oxygen-consuming decomposition of phytoplankton cells after algicide application will upset the pond's precariously balanced dissolved oxygen budget. Reduction in oxygen produced during photosynthesis and the additional oxygen demand created by decomposing phytoplankton will cause a significant dissolved oxygen deficit that must be offset by vigorous mechanical aeration (Boyd, Torrans, & Tucker, 2018). Water quality deterioration following algicide use is, of course, greater for non-selective algicides that kill a greater proportion (perhaps all) of the community. Failure to account for changes in water quality after using algicides can lead to oxygen depletion and fish loss.

Copper-based products are the most commonly used algicides to treat cyanobacterial off-flavors in catfish ponds and will be discussed first. The synthetic, photosynthesis-inhibiting herbicide diuron [3-(3,4-dichlorophenyl)-1,1-dimethylurea] is also commonly used and our summary of that chemical follows that of copper-based products. Copper products and diuron are, admittedly, blunt instruments for managing cyanobacterial blooms in catfish ponds but are the only legally available chemicals routinely used for that purpose. Shortcomings with those algicides prompted considerable research in search of alternatives, including other synthetic algicides, peroxides, and natural-based chemicals. These alternatives (almost none of which can be used legally) will be discussed last.

16.3 | Copper-based algicides

Herbicides and algicides with cupric ion as the active ingredient are commonly used in aquatic habitats to control a variety of vascular plants, algae, and cyanobacteria. Copper herbicides are formulated as (a) copper sulfate pentahydrate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$), sometimes called bluestone or abbreviated CSP; (b) acidic solutions of copper; or (c) organically chelated copper.

Copper sulfate algicides are formulated as powders or crystals. Treatment rates range from about 0.06 mg/L as copper (0.25 mg/L as $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) to over 0.5 mg/L as copper (2 mg/L as $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) depending on the type of algae to be controlled and water chemistry. Copper tends to be more toxic to cyanobacteria than to eukaryotic phytoplankton (Matthijs et al., 2016) but, in practice, it is difficult to obtain significant or useful selectivity.

Copper toxicity to phytoplankton (and fish) is affected by water quality variables that reduce the amount of Cu^{2+} in water or reduce uptake of Cu^{2+} by the organism. In general, copper toxicity decreases as pH, alkalinity, calcium hardness, and dissolved and particulate organic matter concentrations increase. These interactions are extraordinarily complex and are discussed elsewhere (Boyd & Tucker, 1998; Snoeyink & Jenkins, 1980; United States Environmental Protection Agency, 2007). To simplify calculations, suggested treatment rates are sometimes based only on water hardness or alkalinity. One suggested rate is based on total alkalinity:

$$\text{Copper sulfate pentahydrate (mg/L)} = \text{total alkalinity (mg/L as CaCO}_3) \div 100$$

This equation is widely used but it is based on practical experience rather than chemical principles: it ignores the effect of organic matter and assumes that alkalinity, pH, and calcium hardness vary together—which they do in many, but not all, waters (Boyd, Tucker, & Somridhivej, 2016). Precipitation as copper oxides and assimilation of cupric ion by algae rapidly reduce waterborne copper levels after treatment (Haughey, Anderson, Whitney, Taylor, & Losee, 2002; McNevin & Boyd, 2004). Most of the chemical is rendered inactive within a few hours of application to most waters.¹⁹ Essentially all the copper applied to ponds eventually ends up in pond bottom muds (Han et al., 2001).

Copper sulfate is applied by dissolving the powder or crystals in water and distributing or spraying the concentrated solution over the pond. It is essential that the powder or crystals be dissolved before application. Simply broadcasting dry copper sulfate over the water is not efficient because much of the chemical will sink to the bottom before it dissolves and be lost in reactions with various substances in bottom muds.

Some copper-based algicides are formulated as solutions of copper sulfate (or other copper salts) dissolved in acid. Because copper is already in solution as Cu^{2+} , using this formulation reduces the amount of copper lost as undissolved crystals during application. The acidic solution is applied directly to ponds or diluted with water. Formulation as an acidic solution does not enhance algicidal activity because the acid carrier is neutralized by bases of the pond's alkalinity system as soon as the product is applied to the pond, leaving Cu^{2+} —the same species initially present when solid copper sulfate dissolves in water. Toxicity of acidic copper solutions is therefore affected by the same water quality variables affecting copper added to water as copper sulfate pentahydrate.

Chelated copper herbicides use organic compounds (such as ethanolamines) to bind Cu^{2+} so that copper does not rapidly precipitate out of solution in waters of high pH. Label instructions for the use of chelated copper algicides call for applications rates of 0.2–0.5 mg/L as copper depending on the type of plant to be controlled. Chelated products are claimed to be more effective than copper sulfate in water of high pH, but they are seldom used to treat cyanobacterial off-flavors because of expense. In addition, they lack significant selectivity against undesirable cyanobacteria compared to desirable eukaryotic phytoplankton (Schrader et al., 1998a).

Regardless of formulation, all copper algicides are used similarly to treat existing cyanobacterial off-flavors. The process is described later, in Section 19.

16.4 | Diuron

Diuron is one of the oldest synthetic herbicides and has been in use since 1954. The active ingredient, 3-(3,4-dichlorophenyl)-1,1-dimethylurea, or DCMU, is a specific inhibitor of photosynthesis. It is used for selective control of broadleaf weeds and some annual grasses in agricultural (mainly citrus, cotton, berries, asparagus, and pineapples) and non-agricultural settings (railroad rights-of-way, commercial and industrial areas) (United States Environmental Protection Agency, 2003).

Diuron is approved for use as an algicide in certain catfish-producing states under a Section 24(c) Special Local Needs exemption granted by the U.S. Environmental Protection Agency. The label specifically states that the chemical is to be used only "For control of the algae responsible for production of the off-flavor compound 2-methylisoborneol...." Diuron is algicidal at low concentrations (0.01 mg/L active ingredient). Initial data submitted to the USEPA for registration review indicated that *Planktothrix perornata*, the common producer of 2-MIB in catfish ponds, was somewhat more sensitive to diuron than other phytoplankton species tested (C. S. Tucker, unpublished). However, other data on selectivity of diuron toward *P. perornata* are equivocal (Schrader et al., 1998a).

Diuron is applied at 0.01 mg/L at intervals no less than 7 days for a maximum of nine treatments in the same pond each year. The typical use pattern (discussed more fully in the last section of this paper) is to apply the chemical weekly until the odorous cyanobacterial population is eradicated. When used according to the label directions, diuron appears to eliminate odorous cyanobacteria with few water quality problems (Zimba, Tucker, Mischke, & Grimm, 2002). The treatment protocol of weekly, low-dose applications was developed to avoid exceeding a 2 mg/kg tolerance level for diuron residues in catfish filets, although one study (Tucker, Kingsbury, & Ingram, 2003) shows that diuron accumulates in catfish filets to a considerably lesser extent than previous models predicted.

16.5 | Hydrogen peroxide

Hydrogen peroxide (H_2O_2) is a strong oxidizing agent formulated as a solution in water. Hydrogen peroxide was proposed for control of cyanobacterial blooms by Barroin and Feuillade (1986). Subsequent studies confirmed its potential as a relatively selective algicide in wastewater lagoons (Barrington & Ghadouani, 2008; Barrington, Ghadouani, & Ivey, 2011; Barrington, Reichwaldt, & Ghadouani, 2013), lakes (Matthijs et al., 2012) and aquaculture ponds (Yang et al., 2018). Reviews of hydrogen peroxide as a cyanobactericide are available in Jančula and Maršálek (2011), Matthijs et al. (2016), and Stroom and Kardinaal (2016).

Hydrogen peroxide acts by producing highly reactive hydroxyl radicals ($^{\circ}\text{OH}$) that destroy cell membranes (Huo, Chang, Tseng, Burch, & Lin, 2015; Mikula, Zezulka, Jančula, & Maršálek, 2012). Certain cyanobacteria are significantly more sensitive to hydrogen peroxide than eukaryotic phytoplankton (Drábková, Admiraal, Marsalek, 2007; Drábková, Matthijs, Admiraal & Marsalek, 2007). The compound decomposes to oxygen and water and is therefore a perfect herbicide with respect to persistence and residue issues.

Yang et al. (2018) examined the effectiveness of hydrogen peroxide as a selective algicide in a fishpond at Auburn, AL. Results were encouraging, with a single treatment of 6.7 mg/L hydrogen peroxide quickly reducing abundance of *Planktothrix* and *Microcystis*, with slight increases in abundance of eukaryotic phytoplankton. The treatment rate found effective by Yang et al. (2018) is in the general range of rates used to control cyanobacteria in lakes in The Netherlands (2–5 mg/L; Matthijs et al., 2016).

Hydrogen peroxide is a promising tool for managing odor-producing cyanobacteria in catfish ponds. However, there are significant issues that need to be resolved before the chemical is widely used in commercial catfish ponds.

First, hydrogen peroxide reacts with any easily oxidized material in pond water, such as dissolved and particulate organic matter, including the phytoplankton themselves. These reactions consume hydrogen peroxide, expressing a “peroxide demand” that rapidly reduces the effective concentration of the chemical in water. Concentrations of dissolved and particulate organic matter vary widely in catfish ponds (Tucker, 1984) and the “peroxide demand” will likewise vary, similar to chlorine demand (Snoeyink & Jenkins, 1980) and permanganate demand (Tucker, 1984). The variable “peroxide demand” of different waters probably explains the extremely wide range of hydrogen peroxide concentrations needed to control blooms. Hydrogen peroxide has been used at concentrations ranging from less than 5 mg/L in some lakes (Barroin & Feuillade, 1986) to 100 mg/L in organically loaded wastewater lagoons (Barrington & Ghadouani, 2008; Matthijs et al., 2012). Extremely high doses may affect non-target organisms, and, for that reason, some lakes are considered unsuitable for treatment because high rates of hydrogen peroxide loss will necessitate dangerously high treatment rates (Matthijs et al., 2016).

Barrington et al. (2011) attempted to compensate for the “peroxide demand” of water by basing treatment rates on algal biomass (using chlorophyll *a* concentration as a measure of biomass). They proposed a rate equaling 0.00011 g H_2O_2 per μg of chlorophyll *a*. Assuming a typical chlorophyll *a* concentration in catfish ponds of 400 $\mu\text{g}/\text{L}$ (Tucker, 1996), this would result in treatment with almost 45 mg/L H_2O_2 —a very high and potentially dangerous rate. Using chlorophyll *a* as an index of “peroxide demand” also ignores the large but highly variable concentration of easily oxidized substances in the dissolved fraction of catfish pond waters (Tucker, 1984).

Rapid loss of hydrogen peroxide also means there will be little residual herbicidal activity. Most of the chemical is lost within hours after application and completely within a day or two (Drábková, Admiraal, et al., 2007; Matthijs et al., 2012; Weenink et al., 2015). As such, treatments must be made quickly and evenly throughout the water body (Yang et al., 2018). More important, lack of residual activity means that treatments may need to be repeated for long-term control (Jančula & Maršálek, 2011).

Hydrogen peroxide may become a valuable tool for catfish farmers but to date (2019) only one study (Yang et al., 2018) has been conducted to evaluate its effectiveness as a cyanobactericide and no studies have been conducted to evaluate its usefulness to manage catfish off-flavors. Also, hydrogen peroxide in concentrated solutions (>10%) is an unpleasant chemical to handle and is not currently registered by the USEPA as an algicide for use in foodfish ponds.

16.6 | Sodium carbonate peroxyhydrate

Sodium carbonate peroxyhydrate ($2\text{Na}_2\text{CO}_3 \cdot 3\text{H}_2\text{O}_2$; abbreviated SCP) is a dry, granular material that releases hydrogen peroxide when added to water. It is 27% hydrogen peroxide by weight. The mode of action is identical to liquid hydrogen peroxide and it has the same desirable characteristic of degrading to harmless substances—in this case, sodium, bicarbonate, water, and oxygen. The stable, dry formulation of SCP overcomes some handling difficulties

associated with liquid hydrogen peroxide. Also, some SCP products are approved by the USEPA for use in catfish ponds as algicides.

In 2005, we conducted preliminary tests of the effects of SCP on an odor-producing cyanobacterium in catfish pond water (K. K. Schrader and C. S. Tucker, unpublished). Five trials were conducted in 5.5 m³ limnocorrals placed in catfish ponds with populations of *Planktothrix perornata*, a known producer of 2-MIB. A commercial formulation of SCP was added to duplicate limnocorrals at concentrations of 0, 2.5, 5, and 10 mg/L (equivalent to 0, 0.68, 1.35, and 2.70 mg/L H₂O₂). The SCP concentrations we used were within the range of concentrations suggested by the manufacturer for phytoplankton control. We also chose 10 mg/L as the highest treatment rate based on our assumption it would be near the highest concentration a catfish farmer would be realistically willing to apply (10 mg/L = 750 kg in a typical 5-ha, 1.5 m-deep pond). We measured chlorophyll *a*, abundance of *P. perornata*, and concentrations of 2-MIB in water for 5 days after treatment.

Results were extraordinarily variable. In the trial that showed the best effectiveness, 10 mg/L SCP reduced total phytoplankton biomass by 90% and reduced abundance of *P. perornata* by 93% on the second day after treatment. Treatment did not eliminate *P. perornata* and by the fifth day after treatment, abundance of total phytoplankton and *P. perornata* began to increase. Concentrations of 2-MIB remained high (>450 ng/L) in all treatments through the second day of treatment but fell to less than 10% of concentrations in the untreated control limnocorrals by the fifth day after treatment. The study was terminated after 5 days but the rebounding population of *P. perornata* in the highest SCP treatment suggests that concentrations of 2-MIB would have increased in the following days.

In a trial at the other end of the effectiveness spectrum, abundance of *P. perornata* and concentrations of 2-MIB increased in limnocorrals at all SCP treatment rates. For example, in the highest SCP treatment (10 mg/L), 2-MIB concentrations averaged 5,462 ng/L before treatment and 17,209 ng/L 5 days after treatment. In that same treatment group, numbers of *P. perornata* filaments increased from 5,060/mL before treatment to 8,050/mL 5 days after treatment.

Overall, *P. perornata* was not eliminated at any SCP treatment rate in any of the five trials, although there were temporary reductions in *P. perornata* abundance in two trials at SCP treatment rates of 5 and 10 mg/L. Concentrations of 2-MIB also were reduced by treatment with 5 and 10 mg/L SCP in those two trials. In the two trials where populations of *P. perornata* were reduced 2 days after treatment, there was evidence of regrowth by the fifth day. To be an effective treatment for cyanobacterial off-flavors, merely reducing abundance of odor-producing cyanobacteria is not enough. These organisms are such prolific producers of 2-MIB or geosmin that they must be eradicated completely to prevent continued production of odorous compound, thereby allowing fish to purge the taints.

Clearly the SCP concentrations used in our trials were too low for effective control of cyanobacterial populations. Sinha, Eggleton, and Lochmann (2018) conducted a larger, longer study to evaluate the effects of higher SCP treatment rates on phytoplankton communities in fertilized fishponds in Arkansas. They initially conducted range-finding tests in small (75-L) limnocorrals treated with 5.6, 7.4, 9.3, 11.1, 13.0, 14.8, 18.5, and 29.6 mg/L SCP (1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 5.0 and 8.0 mg/L H₂O₂, respectively). Treatment with 9.3–14.8 mg/L SCP dramatically reduced (but did not eliminate) a species of *Planktothrix*²⁰ without major impacts on eukaryotic phytoplankton.

Based on their range-finding tests, Sinha et al. (2018) then conducted a 6-week study in 0.04-ha fertilized ponds using a single application of either 9.3 or 14.8 mg/L SCP. Treatment at both SCP concentrations quickly reduced (but did not eliminate) *Planktothrix* sp. populations, which remained suppressed for 5 weeks, at which time populations started to regrow. Abundance of eukaryotic phytoplankton increased in the ponds treated with 9.3 mg/L but was reduced in ponds treated with 14.8 mg/L.

Sinha et al. (2018) did not measure concentrations of odorous cyanobacterial metabolites or the specific effect on populations of known odor-producing cyanobacteria, so the usefulness of SCP for off-flavor management cannot be assessed directly. Nevertheless, the results are encouraging, and further study is warranted. There are, however, some puzzling aspects of the study.

First, Sinha et al. (2018) reported hydrogen peroxide from SCP treatment persisted for 3–5 days after treatment. Although the authors considered this to be “rapidly degraded,” it is longer than other reports (Drábková, Admiraal,

et al., 2007; Matthijs et al., 2012; Weenink et al., 2015), especially considering the extraordinarily high phytoplankton biomass (>1,000 µg/L chlorophyll *a*) and the corresponding high “peroxide demand” associated with algal biomass that great.

Second, treatment with 9.3 and 14.8 mg/L SCP suppressed *Planktothrix* sp. populations for more than 5 weeks, which is a long period for residual activity considering the active ingredient was “rapidly degraded” within 3–5 days. They invoke a mechanism involving “... catalytic products of H₂O₂ (i.e., hydroxyl and hydroperoxyl radicals) persist [ing] for a longer period of time in the water column (even after H₂O₂ disappearance). ...” to explain the long-lasting effects of SCP treatment. It is difficult, however, to envision persistence of those highly reactive oxidizers in pond water with such large amounts of easily oxidized substances. Also note that the SCP concentrations (9.3 and 14.8 mg/L) used by Sinha et al. (2018) in their 6-week pond study bracket the highest concentration (10 mg/L) we used in our studies, where we failed to see lasting suppression of *P. perornata* populations even over the short, 5-day study duration. We cannot explain this discrepancy.

In summary, SCP offers a safer and more convenient alternative to liquid hydrogen peroxide—a chemical known to have selective activity against cyanobacteria. However, before it can be recommended as a tool of off-flavor management in commercial catfish ponds, more research is needed to identify factors affecting efficacy and persistence and to develop effective treatment rates that account for the variable “peroxide demand” of catfish pond waters.

16.7 | Other synthetic herbicides

Diuron and copper-based products are the only algicides commonly used in catfish ponds to manage cyanobacterial off-flavors. Although they can be effective when used in a focused management program (see Section 19), both chemicals have serious deficiencies related to lack of selectivity or environmental persistence. The limited options and their shortcomings stimulated efforts to identify potential alternatives from the array of other commercially available synthetic herbicides.

This algicide discovery program was based on a rapid 96-well microplate bioassay developed by Schrader, de Regt, Tucker, and Duke (1997). The goal of this bioassay was (a) identify herbicides nearly as toxic or possessing greater toxicity against *Planktothrix perornata* compared to copper-based algicides and diuron and (b) identify herbicides possessing greater selective toxicity than copper-based algicides and diuron against *P. perornata*. This reliable and reproducible in vitro bioassay was used to evaluate more than 20 common herbicides with different modes of action. The herbicides were selected from those that were frequently used in terrestrial agriculture (e.g., row crops, fruit orchards) and for aquatic weed control in ponds and lakes (Schrader et al., 1998a).

Diquat (6,7-dihydrodipyrido[1,2-*α*:2', 1'-*c*]pyrazinediium ion) and paraquat (1,1'-dimethyl-4,4'-bipyridinium ion) were very toxic to *P. perornata* based on growth inhibition (lowest complete inhibition concentration [LCIC] of 0.1 µM). This was lower (i.e., more toxic) than diuron, with a LCIC of 1.0 µM, and a commercial chelated-copper product, with a LCIC of 10.0 µM. Both herbicides were also selectively toxic to the cyanobacterium, with at least two orders of magnitude more toxicity against *P. perornata* compared to *Selenastrum capricornutum* (a common species of eukaryotic phytoplankton found in catfish ponds), with a LCIC of 10.0 µM for diquat and paraquat.

Atrazine [6-chloro-*N*-ethyl-*N'*-(1-methylethyl)-1,3,5-triazine-2,4-diamine], a member of the triazine chemical family that inhibits photosynthesis, was relatively less toxic to *P. perornata* (LCIC = 10.0 µM) and was non-selective, with a LCIC of 10.0 µM for *S. capricornutum*. At one time, simazine—another triazine herbicide—was commonly recommended for broad-spectrum (non-selective) phytoplankton control in catfish ponds (Tucker & Boyd, 1977, 1978), but it is no longer labeled for aquatic use in the United States.

The toxicity of diquat and paraquat against photosynthetic organisms is due to the formation of reactive oxygen species (ROS; superoxide anion radical, hydrogen peroxide, and hydroxyl radical). Initially, formation of superoxide radicals occurs as these herbicides accept electrons from photosystem I. Superoxide then forms hydrogen peroxide in the presence of superoxide dismutase which will react with remaining superoxide to generate hydroxide radicals. These reactive oxygen species are highly disruptive to cells, especially hydroxyl radicals which cause lipid oxidation

and subsequently destroy cell membranes and chlorophyll. The discovery that these two herbicides possess such high levels of toxicity and selectivity against *P. perornata* initiated the search for other compounds, mostly natural (discussed in more detail in the next section), with a similar mode of action due to the apparent susceptibility of *P. perornata* to ROS (Schrader, de Regt, Tidwell, Tucker, & Duke, 1998b).

Paraquat is not approved by the USEPA as an aquatic herbicide and both it and diquat are highly toxic to mammals (Ahrens, 1994). Diquat is approved for use in the management of aquatic weeds and macrophytic filamentous algae in ponds and lakes, but it is not currently approved for the management of microalgae in ponds used to grow fish for human consumption. To further evaluate the potential of diquat in the selective management of *P. perornata*, we performed an efficacy study by applying diquat to a small (1,160 m³) catfish pond (Schrader & Tucker, 2003). Two applications (3 days apart) of diquat at the label-recommended rate for aquatic weed control (equivalent to 10.7 µM of active ingredient) were hand sprayed over the pond surface in the morning on sunny days. The pond was not mixed until several hours after application to prevent generating additional turbidity from clay particles suspended from bottom muds by the action of mechanical aeration (suspended clay particles inactivate diquat by binding the diquat cation to clay cation exchange sites).

Overall, diquat applications did not reduce the abundance of *P. perornata* or concentrations of 2-MIB in the pond water. The lack of efficacy was attributed to suspended matter (clay particles, detritus, and phytoplankton cells) which was high in the pond, but typical of most catfish ponds. Binding of the diquat ion to clay and organic detritus and uptake by other phytoplankton likely lead to reduced availability of diquat. In addition, low light conditions in the pond water (due to dense phytoplankton blooms) likely decreased the toxicity of diquat because lower light intensity reduces the rate of electron transfer from photosystem I to diquat, with subsequent decreases in lipid peroxidation and destruction of cellular membranes (Devine, Duke, & Fedkte, 1993). Our conclusion from this preliminary study was that diquat is not an effective management approach as a selective cyanobactericide in catfish ponds.

16.8 | Natural compounds as selective algicides

All organisms have evolved in a complex environment full of pathogens, antagonists, and competitors. Part of the evolutionary process allowing organisms to live in a world teeming with pests is the production of secondary metabolites that kill or suppress the antagonistic organisms. An important part of herbicide discovery is to evaluate these natural, biologically active products for desirable activity (Duke et al., 2002). Some natural products can be used directly but more often they give insight into new modes of action that can be exploited through development of manufactured chemicals based on the natural product. Some natural compounds may have fewer adverse environmental impacts than synthetic compounds, which is advantageous in itself but may also lead to quicker, less expensive approval by regulatory agencies.

16.8.1 | Fatty acids

Before the mid-1990s, limited research had been performed to discover and evaluate natural and/or natural-based compounds for their activities against undesirable species of cyanobacteria, especially *P. perornata*, present in southeastern United States catfish ponds. Natural compounds from plants and other organisms (e.g., marine organisms) were relatively unexplored sources for potential selective algicides. An early discovery of antialgal compounds from plants against noxious pond species of cyanobacteria was made by van Aller and Pessoney (1982, 1983). They identified several oxygenated fatty acids with antialgal properties from extracts of the aquatic plant *Eleocharis microcarpa*. More important, this class of allelopathic chemicals inhibited the growth of cyanobacteria to a greater extent than eukaryotic algae (van Aller & Pessoney, 1982; van Aller, Pessoney, Rogers, Watkins, & Leggett, 1985). Subsequently, the commercial product Solricin 135[®] (Caschem, Inc., Bayonne, NJ) was developed from their patent (van Aller & Pessoney, 1983).

Solricin 135[®] was an aqueous solution of potassium hydroxide-saponified castor oil containing about 32% (by weight) of the potassium salts of fatty acids and 3% glycerol. About 90% of the fatty acid content of castor oil is ricinoleic acid (12-hydroxyoleic acid), which is structurally similar to certain naturally occurring alleopathic compounds isolated from *E. microcarpa*. Solricin 135[®] was approved in 1985 by USEPA for use in catfish ponds as a selective algicide. However, in subsequent studies (Scott, Bayne, & Boyd, 1989; Tucker & Lloyd, 1987) the product failed to reduce the abundance of cyanobacteria or prevent off-flavor problems in catfish. Tucker and Lloyd (1987) attributed the failure of Solricin 135[®] to suppress cyanobacteria to rapid biodegradation of the compound and formation of sparingly soluble calcium salts of the fatty acid in the hard waters of ponds in their study (100–250 mg/L as CaCO₃; Tucker, Francis-Floyd, & Bealeau, 1986). However, the ponds used by Scott et al. (1989) contained relatively soft water (20–30 mg/L as CaCO₃). Application of the product to commercial catfish ponds also failed to show consistent results (C. S. Tucker, unpublished) and USEPA approval for the use of the product was discontinued in 1989.

Laboratory studies evaluating over 20 fatty acids and water-soluble salts of fatty acids in a rapid bioassay determined that erucic acid and linoleic acid were the most active against *P. perornata* (Schrader, 2003). However, salts of fatty acids formed to make them water-soluble and more likely to be efficacious in catfish ponds may not be effective in reducing the abundance of cyanobacteria in ponds because of the potential for the water-soluble forms to precipitate as sparingly soluble salts in water of hardness of ≥ 100 mg/L as CaCO₃.

16.8.2 | Barley straw

Studies conducted in the United Kingdom in the 1990s reported decomposing barley straw was effective in reducing the abundance of phytoplankton in freshwater systems (Barrett, Curnow, & Littlejohn, 1996; Overall & Lees, 1996; Newman & Barrett, 1993). The antialgal properties of the decomposing barley straw were believed to be caused by the release of biologically active compounds, possibly quinones, during aerobic decomposition of lignin and the oxidation of phenolic compounds (Pillinger, Cooper, & Ridge, 1994). However, efficacy studies performed in Mississippi catfish ponds showed no control of odor-producing cyanobacteria and no difference in the occurrence of musty off-flavor between catfish from ponds treated with decomposing barley straw and catfish from untreated ponds (Wills, Tucker, & Jones, 1999). The lack of efficacy was thought to be caused by anaerobic decomposition within the large masses of barley straw, which was not resulting in the proper release of antialgal compounds.

Additional research using aerobic “digesters” to permit aerobic decomposition of the barley straw also did not result in a reduction in musty off-flavors in catfish (Gene Wills, Mississippi State University, personal communication). In laboratory studies, several commercial barley straw extract products were evaluated in a microplate bioassay and determined to not be effective in killing *P. perornata* and two other species of filamentous cyanobacteria at concentrations orders of magnitude (e.g., >100 times) higher than the label-recommended application rate (Schrader, 2005). Use of barley straw and/or commercial products to effectively manage musty off-flavor problems in pond-cultured catfish and in reducing the abundance of species of undesirable cyanobacteria in catfish ponds has yet to be demonstrated as an effective management approach.

16.8.3 | Quinones

The algicidal activity of certain quinones against bloom-forming species of cyanobacteria was initially reported by Fitzgerald, Gerloff, and Skoog (1952). Among the compounds they evaluated, 2,3-dichloronaphthoquinone and phenanthraquinone were the most toxic against the common bloom-forming, toxin-producing *Microcystis aeruginosa*. Laboratory studies evaluated various quinones for their selective toxicity against *P. perornata* (Schrader, 2003; Schrader et al., 1998b; Schrader & Harries, 2001). A particular quinone—9,10-anthraquinone—was found to be one of the most selectively toxic, with complete inhibition of *P. perornata* growth at 0.1 μ M (approximately 21 μ g/L) while a concentration of 100.0 μ M was not toxic against the eukaryotic green alga *Selenastrum capricornutum* (Schrader

et al., 1998b). Subsequent studies on the mode of action of 9,10-anthraquinone against *P. perornata* revealed that it inhibits photosynthetic electron transport, possibly at photosystem II, which causes growth inhibition (Schrader, Dayan, et al., 2000). Fitzgerald et al. (1952) attributed the toxicity of quinones against cyanobacteria to their redox character and possibly another property due to the large degree of selective toxicity toward cyanobacteria.

The quinone-based commercial biocide SeaKleen®—a water-soluble derivative of menadione (also known as vitamin K₃ or 2-methyl-1,4-naphthoquinone)—was also found to be effective against *P. perornata* in laboratory and pond studies (Schrader, Rimando, et al., 2004). However, higher concentrations of SeaKleen (1.3 mg/L) were required to reduce abundance of *P. perornata* compared to 9,10-anthraquinone. Subsequently, 9,10-anthraquinone was selected for additional studies in the laboratory and for efficacy studies due to its high degree of selective toxicity against *P. perornata*. These studies are discussed next.

16.8.4 | 9,10-Anthraquinone-based compounds

One of the most important and obvious considerations for using natural or natural-based compounds as a selective algicide in catfish aquaculture ponds is water solubility. Because 9,10-anthraquinone is poorly soluble in water, preliminary field efficacy testing (Schrader, Tucker, de Regt, & Kingsbury, 2000) used 9,10-anthraquinone dissolved in ethanol, which was then applied to catfish pond water contained within limnocorrals (Schrader et al., 2003). Results from those studies found no reduction in the abundance of *P. perornata* and MIB concentrations in the pond water, possibly due to 9,10-anthraquinone's lack of water solubility. Additional efficacy testing using different formulations of 9,10-anthraquinone (e.g., emulsions with canola oil and Tween 80) to maintain effective concentrations of 9,10-anthraquinone in the water did not provide positive results (Schrader et al., 2003). Finally, a different approach was undertaken in which water-soluble derivatives of 9,10-anthraquinone were synthesized in the laboratory. Two of these natural-based derivatives of 9,10-anthraquinone were found to be effective in selectively reducing the abundance of *P. perornata* and MIB concentrations in pond water, and these compounds were subsequently patented (Schrader et al., 2003; Schrader & Nanayakkara, 2005).

Among the anthraquinone derivatives, the most active and promising compound was 2-[methylamino-N-(1'-methylethyl)]-9,10-anthraquinone monophosphate (designated as anthraquinone-59) which was selectively toxic against *P. perornata* compared to eukaryotic green algae and significantly more selective than diuron or copper-based products. Therefore, anthraquinone-59 was the focus for further research and development.

Another important consideration in the discovery and use of natural or natural-based compounds for use as selective algicides in catfish aquaculture ponds is the potential toxicological effects on non-target organisms such as catfish. Anthraquinone-59 was evaluated and determined to be non-lethal to channel catfish at an application rate below 1.0 mg/L, with a 96-hour 50% lethal concentration (LC50) of 2.06 ± 0.31 mg/L for channel catfish compared to an effective application rate of 0.125 mg/L for control of *P. perornata* in catfish ponds (Schrader, Foran, et al., 2004; Schrader, Tucker, & Mischke, 2007). Anthraquinone-59 was also determined to have a half-life of approximately 19 hours in pond water (Schrader et al., 2003) while diuron can persist for weeks and copper from copper sulfate applications can accumulate in the pond sediments indefinitely and may adversely impact microbial activity (Han et al., 2001). Compounds must persist in pond water for an adequate amount of time to provide algicidal properties. For example, ferulic acid was found to have algistatic properties against *P. perornata* in the laboratory (Schrader et al., 1998a), but the lack of efficacy when applied to catfish ponds was attributed to its relatively short half-life of 2 hr in the pond water (Schrader, Duke, et al., 2000).

Additional studies on anthraquinone-59 determined the mode of action was related in part to the generation of reactive oxygen species (ROS) by anthraquinone-59 in *P. perornata* (Schrader, Dayan, & Nanayakkara, 2005). Also, the deficiency of antioxidant enzyme activities within *P. perornata* compared to certain eukaryotic algae (e.g., *S. capricornutum*) to deal with ROS-generating compounds is thought to be a contributing factor in the selective toxicity of anthraquinone-59 (Schrader & Dayan, 2009). Other natural compounds inducing ROS formation would

also be potential leads for evaluation in the management of *P. perornata* and perhaps other undesirable species of cyanobacteria found in catfish ponds.

The discovery that derivatives of 9,10-anthraquinone are selective, highly active algicides against a known producer of 2-methylisoborneol was a significant advance in efforts to identify strategies to control cyanobacterial off-flavors in catfish. Nevertheless, the compound has not been commercialized and is not approved for use by the USEPA. There are many evaluations and tests required during the processes of herbicide development and obtaining approval from USEPA for a novel compound, whether it is a natural or natural-based compound or not. For example, the environmental fate and potential uptake and absorption of the compound by catfish into their flesh are other important factors that must be determined. The high costs required to develop a novel algicidal compound can delay or hamper the process of providing new algicides for aquaculturists. Also, the potential competition against existing commercially available products can be prohibitive in developing new commercial products for use in the United States catfish industry. And this is what happened to anthraquinone-59: it currently sits as a compound with technical promise but with little hope it will be commercialized any time soon.

16.9 | Other chemicals for suppressing cyanobacterial blooms

In addition to the substances described above, dozens of other chemicals have been proposed or tested for controlling cyanobacteria. Some are obvious (existing agricultural herbicides), some are odd (low levels of potassium chloride), and some are mystical (various root extracts). Most are illegal to use in catfish ponds for phytoplankton control and none of them have enough promise to merit further consideration. Many of these substances are reviewed by Jančula and Maršálek (2011) and Matthijs et al. (2016).

17 | MANAGING PETROLEUM OFF-FLAVORS

Petroleum off-flavors in pond-grown fish develop only after contamination of the water, as might occur when diesel fuel leaks from the fuel tank of a tractor or other equipment. Prevention is the only logical management strategy, because petroleum off-flavors can be extremely disagreeable and are difficult to purge from fish once they develop. Fuel and oil storage facilities should be located away from ponds and care should be exercised when refueling vehicles or equipment, or when handling petroleum products near ponds. Every employee on the farm should be made aware that even small spills of diesel fuel and other petroleum products are enough to cause noticeable off-flavors in fish.

If fish develop petroleum off-flavors, the only recourse is to let the flavor purge over time. Depending on the size of the spill, purging will be quicker if fish are moved to another pond, away from contaminated water and soils. Fish with petroleum taints should not be mixed with other fish in the “purging pond” because it may take months for the taint to completely leave the fish. Once the off-flavored fish are removed, the petroleum-contaminated pond should be drained and allowed to air-dry for as long as possible before refilling. This may provide a good opportunity to renovate the pond bottom and levees, so some benefit derives from the process. Air-drying and reworking the bottom and levees will promote volatilization and weathering of hydrocarbon residues, which reduces the possibility that the subsequent fish crop will develop off-flavors from residual contamination.

18 | MANAGING DECAY/FISHY OFF-FLAVORS

Some—maybe most—flavors in this category develop when fish scavenge on dead fish or other decaying organic matter when fish are not being fed. Because catfish are fed daily in warm weather, these off-flavors are rare in the summer. But feeding activity slows considerably in cold water, so many farmers feed their fish infrequently, if at all, through the winter. However, water temperatures vary widely over winter in the southern United States, with

periods of cool (rather than cold) water when catfish will actively seek food. Hungry catfish deprived of manufactured feed will scavenge for food, and often the only foods available are dead fish or decaying plant matter, which will give fish undesirable “fishy” or “decay” flavors when eaten.

A good example of off-flavors in this category is provided in a study where threadfin shad were added to catfish ponds as a biological control measure to manage cyanobacterial off-flavors (Mischke et al., 2012). Shad reduced the incidence and intensity of cyanobacterial off-flavors in summer but caused offensive “fishy/decay” off-flavors in winter as catfish ate shad killed by low water temperatures.

It may be possible to diminish the likelihood that decay/fishy flavor problems will develop by assuring manufactured feed is offered whenever water temperatures rise to the point where fish become active.²¹ It may also be possible to reduce decay/rotten off-flavors by removing dead fish from the pond when they first appear, although this is a formidable task on large commercial catfish farms.

All wintertime off-flavors are more difficult to manage than summer problems because, regardless of the cause, taints purge slowly in cold water. Wintertime off-flavors caused by scavenging also present special problems for processors because the intensity of off-flavor often varies greatly among fish in the same pond. Some fish will taste fine while others are badly off-flavored. This is most likely caused by appetite differences among fish—some fish are scavenging for food while others are not. Wide variation in flavor among fish in the same pond makes it difficult to determine whether a pond can be harvested and processed without many off-flavored fish reaching the marketplace. Normal pre-harvest sampling for flavor acceptability may not be adequate to detect off-flavors in the population when only a small percentage of the population tastes bad. Processors should consider increasing the number of fish sampled in winter quality-control testing to account for increased fish-to-fish variation in flavor quality.

19 | A DECISION-MAKING TOOL FOR OFF-FLAVOR MANAGEMENT

The preceding sections of this paper point out difficulties in managing off-flavors in pond-grown catfish. Most summertime flavor problems are caused by secondary metabolites produced by certain cyanobacteria. The odor-producing species—and bloom-forming cyanobacteria in general—are well-adapted to warm, eutrophic environments, and altering the environment to favor eukaryotic phytoplankton is difficult or expensive. The only preventive measure based on sound principles and with some record of success is biomanipulation to increase grazing losses of large filamentous cyanobacteria. But even that approach is fraught with problems: the best candidate fish for biomanipulation are either non-native (silver carp), cold-intolerant (threadfin shad), or both (certain tilapias).

Lack of consistently successful, logistically workable strategies for preventing off-flavors compels catfish farmers to take a reactive approach to off-flavor management. Fish flavor is assessed only when fish are near harvest-size. If fish are found to be off-flavored, either a copper-containing product or diuron (or rarely another legal algicide) is used to kill the odor-producing species, allowing fish to purge the taint over time. Regrettably, copper products and diuron are blunt instruments for this task, with significant deficiencies. And, just as important, not all off-flavors are caused by cyanobacteria.

But even in the face of the difficulties summarized above, it is possible to manage off-flavors with some success. The general facts in the preceding two paragraphs, together with specific information derived from research, can be pieced together into a system to guide decision-making when fish are near harvest-size and found to be off-flavored.

Here are the key facts to consider when making off-flavor management decisions (details are provided in previous sections):

1. Summertime cyanobacterial blooms are difficult to prevent, so reactive management is the most dependable strategy;
2. Copper products, diuron, and certain peroxides are currently the only algicides legal to use in catfish ponds;
3. Algicides only work when the off-flavor is caused by “algae” (cyanobacteria in this case);

4. Synthesis of 2-MIB and geosmin by cyanobacteria is temperature-dependent and essentially stops at temperatures below about 15°C;
5. Only a few cyanobacterial species produce 2-MIB and geosmin in catfish ponds. In Mississippi, western Alabama, and eastern Arkansas, 2-MIB is produced by *Planktothrix perornata*; and geosmin is usually produced by certain species of *Anabaena*;
6. The cyanobacteria producing 2-MIB and geosmin are distinctive and easily identified using an inexpensive bright-field microscope;
7. After the odor-producing cyanobacteria are killed, 2-MIB and geosmin production stops and the odorous compounds are purged from fish. Purging rates for both compounds are temperature-dependent: taints are purged within days in warm water but much more slowly (weeks to perhaps months) in cold water; and
8. Other off-flavors are slowly purged from fish regardless of water temperature.

These facts can be used to develop a simple decision-support tool for managing off-flavors in pond-grown catfish in the southern United States. Listing the current status of algicide registration in the United States (number 2, above) may seem to be an odd part of decision making but it is, in fact, central to the careful approach formulated below. The tools available to kill odorous cyanobacteria in catfish ponds are few and crude, and it is unlikely additional chemicals will be available any time soon. As such, farmers must make best use of the chemicals available and use them judiciously and only when they will actually help.

The decision-support scheme in Figure 11 is based on knowledge of only three factors—water temperature, the type of off-flavor, and whether odor-producing cyanobacteria are present. The scheme is functionally similar to the qualitative “decision-tree” developed by Reynolds (1992, 1997) to provide the “probable best approach” for managing cyanobacteria as part of lake-restoration programs. In effect the scheme in Figure 11 provides guidance on which off-flavors will respond to treatment and which ones will not. By treating only problems that will respond, time and money will not be wasted on treatments with no hope of working.

19.1 | Water temperature

Water temperature is the first critical factor because it affects growth of odor-producing cyanobacteria, synthesis of odorous compounds, and purging rate. Odorous cyanobacteria do not thrive or synthesize odorous metabolites in cold water. If fish are off-flavored in cold water, the odorous compounds were either produced by cyanobacteria during a previous period of warm water or the off-flavor was not produced by phytoplankton (such as fishy/decay off-flavors derived from foods consumed during scavenging). In either case, it is pointless to use algicides because there are no odor-producing cyanobacteria to treat. If fish are off-flavored in cold water, the only option is resampling fish in a couple of weeks to see if flavor quality has improved. Sampling more frequently is seldom productive because off-flavors purge slowly in cold water.

19.2 | Taste-testing

When fish are off-flavored in warm water, the first step is to determine the type of off-flavor. This is critical because only musty-earthy off-flavors produced by cyanobacteria are treatable using algicides. Determining the off-flavor type is simple. Cook a small, unseasoned piece of fish fillet in a microwave oven. Smell the fillet immediately after cooking and then taste a portion. The musty-camphorous flavor and aroma of 2-MIB is difficult to describe, yet is distinctive even at low concentrations and easily remembered. Geosmin gives fish an earthy-moldy flavor that is somewhat reminiscent of the odor of freshly plowed soil or a damp basement.

If the off-flavor is not in the musty-earthy category, it will not respond to algicide treatment and the proper decision is to wait a week or two and taste the fish again. If a musty-earthy off-flavor is detected, the next step is to

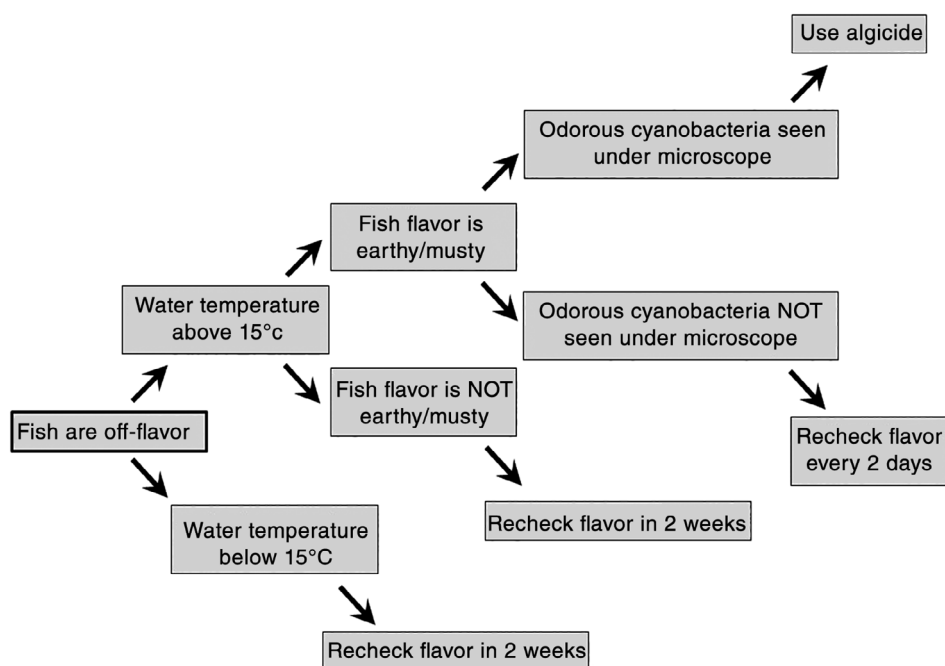


FIGURE 11 A guide to decision-making when managing off-flavors in pond-grown catfish

determine whether the odorous compound is being actively produced in the pond or is a remnant of a past episode. This bit of evidence is obtained by examining the pond water for odor-producing cyanobacteria.

19.3 | Microscopic examination

Catfish pond phytoplankton blooms continuously change. Populations of odor-producing cyanobacteria seem to appear from nowhere, persist for days or months, and then disappear. Odorous cyanobacteria may be seldom found in some ponds while occurring several times every year in other ponds. It is impossible to predict the development of odor-producing populations, and the only way to know when they are present is to examine a sample of pond water under a microscope.

Common odor-producing cyanobacteria in west Mississippi, east Arkansas, and west Alabama are easy to identify microscopically (Figures 5 and 6a). If fish in those regions have musty-earthy off-flavors and either *Planktothrix perornata* or *Anabaena* spp. are seen under the microscope—even in very low numbers—then musty-earthy compounds are being actively produced in the pond. Treating the pond with the proper algicide will kill the odorous cyanobacterium and allow fish to purge the off-flavor.

If fish have musty-earthy off-flavors but odor-producing algae are not present, the most probable explanation is that the cyanobacteria that produced 2-methylisoborneol or geosmin have recently disappeared from the pond as part of the natural cycle of phytoplankton community dynamics. In most cases, the off-flavor will rapidly disappear from fish without needing to treat the pond with algaecides. Fish should be sampled for flavor quality daily because musty-earthy off-flavors usually disappear from fish rapidly in warm water.

19.4 | Using the decision-support scheme

Note that only one path in Figure 11 leads to a decision of using an algicide: water is warm; the flavor is earthy or musty, and cyanobacteria known to produce odorous substances are present. This will be the decision pathway for

most off-flavor encountered during warm-weather months when cyanobacterial off-flavors are common. All other pathways lead to decisions that algicides should not be used. Those pathways are more common in cold-weather months.

If the decision pathway leads to algicide treatment, the effectiveness of treatment must be evaluated after the algicide is applied to determine if all odor-producing cyanobacteria were killed. It is not enough to reduce their abundance: they must be eradicated. The cyanobacteria *P. perornata* and species of *Anabaena* are such prolific producers of 2-MIB and geosmin that even sparse population will synthesize enough of the odorous substances to prevent fish from purging the taints to undetectable levels. A pond water sample should be examined 3–5 days after algicide use. If odor-producing species have been eradicated, fish are then given time to purge the off-flavors without further treatment. If, however, the initial algicide treatment did not completely eradicate the odorous cyanobacterium, another application must be made.

Successful on-farm use of the decision-support scheme in Figure 11 depends on allowing adequate time for the algicide to kill the odor-producing species, time for 2-MIB or geosmin to dissipate from water after cyanobacteria are eradicated, and time for fish to purge the taint. The best one can hope for is that initial algicide treatment eliminates odor-producing cyanobacteria and fish rapidly purge the taint in warm water. In our experience, this process requires a minimum 7–10 days after algicide treatment—and that is an optimistic time frame. If the algicide treatment must be repeated and fish purge slowly in cooler water, several weeks may be required. The bottom line is that farmers should plan ahead and initiate flavor assessment and treatment planning well ahead of the intended harvest date.

Planning ahead is especially important when harvest is planned for the winter. All odorous compounds are eliminated slowly in cold water, so if fish are off-flavored during cold-weather months, there is little that can be done to accelerate the rate of purging. Farmers should check fish for off-flavor and examine water for odor-producing cyanobacteria at least a month before average water temperatures are expected to start falling below 20°C. If fish are off-flavored and odor-producing cyanobacteria are present, algicides can be used to kill the cyanobacteria, leaving enough time for the odorous substance to purge in warm water before cooler weather sets in later in the autumn.

19.5 | An example from a commercial catfish farm

One of us (C. S. Tucker, unpublished) evaluated the usefulness of the decision tree in Figure 11 on three large catfish farms in Mississippi in 2006–2007. Results from one trial conducted in August 2006 are presented in Table 9. All episodes involved musty or earthy off-flavors, as would be expected in mid-summer. Of the seven off-flavor episodes, odorous cyanobacteria were seen microscopically in four of the pond water samples. In two cases (ponds 297 and 300), one treatment with copper sulfate pentahydrate eradicated odor-producing cyanobacteria and fish purged the taints in 10–17 days without further treatment. In two other cases (ponds 305 and 307), multiple treatments with copper sulfate pentahydrate were needed because post-treatment microscopic examination of water showed the initial treatment did not eradicate the odorous cyanobacterium. In one instance (pond 305), three treatments with copper sulfate were needed to rid the pond of odor-producing cyanobacteria.

Most notable, however, are three ponds (298, 306, and 309) with fish having musty off-flavors, yet odor-producing cyanobacteria were not observed in water samples. The ponds most likely contained an odorous cyanobacterial population in the preceding weeks, but the population disappeared as part of the natural wax and wane of individual populations within the community. Although 2-MIB was no longer being produced, the taint had not yet cleared from fish. The decision in those cases was to not treat with an algicide (because there was nothing to treat) but rather to wait for the flavor to purge. These three cases show the value of microscopic examination of water as part of the decision-making process. If decisions were based solely on the presence of musty off-flavors, then all three ponds would have been treated with an algicide and treatment would have appeared successful (fish flavor quality rapidly improved). Instead, fish in all three of those ponds were suitable for processing within 7–14 days without the expense and risk of algicide treatment.

TABLE 9 Decision outcomes for off-flavor episodes in seven commercial catfish ponds in northwest Mississippi, August 2006

Pond	Flavor	Odorous cyanobacterium	Decision	Outcome
297	Musty	<i>Planktothrix perornata</i>	Algicide	One CSP; fish sold in 17 days
298	Musty	None	Recheck	Fish sold in 7 days
300	Musty	<i>P. perornata</i>	Algicide	One CSP; fish sold in 10 days
305	Musty	<i>P. perornata</i>	Algicide	One CSP; fish sold in 21 days
306	Musty	None	Recheck	Fish sold in 14 days
307	Earthy	<i>Anabaena</i> sp.	Algicide	Two CSP; fish sold in 17 days
309	Musty	None	Recheck	Fish sold in 7 days

Note: Treatment decision were guided by the decision-making scheme in Figure 11.
Abbreviation: CSP, treatment with copper sulfate pentahydrate.

20 | CONCLUDING THOUGHTS

Off-flavors and their adverse role in shaping human food choices are not new problems. Fish have been an important source of human food for at least 40,000 years (Hu et al., 2009), and probably much longer than that. Somewhere in that distant past, an early human surely noticed that fish tasted different, or perhaps unpleasant, when taken from certain waters or at certain times of the year. The first written account of fish off-flavors was published nearly 500 years ago when Conrad Gesner (1550) noted that tench *Tinca tinca* (a hardy cyprinid from Eurasia) sometimes acquired an “unappealing” (“unlieblichen”) muddy flavor. Two centuries later, Bloch (1783) reported pond-grown carp *Cyprinus carpio* may also develop a muddy flavor that could be removed by moving fish to clean water. Muddy off-flavors in tench and carp are mentioned in several 18th and 19th century books on cooking (Hunter, 1804), natural history (Boreman, 1782; Goldsmith, 1779) and sport fishing (Cholmondeley-Pennell, 1884; Hughes, 1842; Watson, 1890)—often referencing Gesner’s work three centuries prior.

Anton Chekov (1899) used a comment about fish off-flavor as a foil to develop a critical turning point in his acclaimed short story *The Lady with the Dog*. The story concerns two strangers who meet while vacationing without their spouses. After a quick affair, they part, expecting to quickly forget each other. But the main character, Dmitri Gurov, is haunted by his memory of Anna Sergeyevna, and he desperately wants to pour out his soul to someone about his secret passion. One night after dinner with a friend, he brings up the subject of the “charming woman I met in Yalta.” His dining partner wants nothing to do with a deeply personal conversation and instead remarks that the sturgeon served for dinner “was a little off.” The comment provokes Gurov’s cynical reflections on the paradox of his living with hidden, deeply felt emotions while the world around him moves on, concerned only with impersonal issues such as the flavor of a fish served for dinner. The first non-fictional account of consumer dissatisfaction with off-favored fish was in 1909, when a newspaper reported a customer sued a restaurant owner for serving muddy flavored fish (Persson, 1995).

We do not know when off-flavors were first noticed in catfish, but if the first catfish grown in a pond was not off-flavored, then the second or third probably was. Early on, people living in the southeastern United States probably accepted occasional earthy-musty flavors in pond-cultured catfish as being typical of wild-caught “river fish,” which sometimes have those flavors as part of their natural flavor profile. Catfish off-flavors became a significant issue in the 1960s when farmers in the southeastern United States started selling catfish to a wider market and the new catfish consumers had different preferences and expectations for fish flavor. Nowadays, catfish farmers and processors want to sell fish with a consistently mild, sweet flavor that will appeal to consumers across the country. This has been a challenge because the pond aquaculture environment predisposes fish to off-flavors acquired before

harvest. The high incidence of off-flavored fish and associated economic costs have made it the one of the most important production-related problems in the catfish industry.

Research on catfish off-flavors began in the early 1970s. Most of the early work was descriptive but since about 1980 significant progress has been made to understand the chemical causes of off-flavors, their sources, their behavior in fish and water, and the occurrence of off-flavors over time and within populations. Dozens of studies have been conducted to evaluate various management strategies, mostly aimed at preventing or eliminating odor-producing cyanobacterial populations.

We believe it is important to put research on catfish off-flavors into a much larger perspective. Research on catfish off-flavors (and fish off-flavors in general) represents a small subset of research on aquatic off-flavors, most of which focuses on understanding and managing tastes and odors in drinking water (Mallevalle & Suffet, 1987; Suffet, Khiari, & Mallevalle, 1996; Lin et al., 2019). Objectionable tastes and odors in drinking water are a global problem, with persistent problems affecting water supplies for many of the world's largest cities. The most common odor problems in drinking water are caused by the same microorganisms and the same odorous substances that cause off-flavors in catfish.

People in developed countries do not tolerate unusual tastes and odors in drinking water because of a presumed (and sometimes true) connection with safety (McGuire, 1995). If unfamiliar flavors are detected in their drinking water, consumers demand the problem be fixed. In 1987, Mallevalle and Suffet (1987) summarized the state of knowledge as follows:

"Despite the water supply industry's efforts to control taste and odors, the problems persist throughout the developed world."

Thirty-two years later, Lin et al. (2019) provided this disheartening update:

"There has been considerable improvement in our ability to deliver safe drinking water in many areas of the world, yet despite this progress, poor taste and odor in source and finished water supplies continues to be a major issue, costing the drinking water and other associated industries (aquaculture, tourism, recreation, fishing, food and beverage production) millions of dollars annually...."

Most catfish off-flavors are caused by cyanobacteria, so off-flavor management effectively becomes cyanobacteria management. Cyanobacteria cause problems much more serious than making catfish off-flavored—they produce potent toxins, for example—and finding ways to prevent or manage nuisance cyanobacteria has been the subject of a worldwide research effort for many years. Yet harmful cyanobacterial blooms are more common now than ever (Huisman et al., 2018).

Put simply, cyanobacteria blooms and off-flavors in aquatic environments are complex problems not amenable to simple solutions.

All off-flavors can be purged from fish by moving them to clean water, but this is not a preferred approach because of uncertainties about the time required to purge various taints, the added cost of harvesting and transporting fish twice before processing, weight loss during purging process, and stress-related fish mortality. Aside from purging, it will be impossible to develop a single strategy to prevent or eliminate *all* off-flavors in pond-grown fish because taints can be caused by a variety of substances, each with a different origin and unique pharmacokinetic properties. Further, the complexity of pond microbial communities and obstacles to commercialization of new algicides make it unlikely that simple solutions to prevent the most common off-flavors—those caused by cyanobacteria—will be available to farmers any time soon. Nevertheless, research has provided adequate information for farmers to make rational decisions on off-flavor management, greatly increasing the probability of selling flavorful, market-ready fish without delays caused by impaired flavor. Future discovery and development of novel

management strategies improving on current approaches will provide farmers with additional tools that can be integrated into the decision-support scheme described in this paper.

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ENDNOTES

- ¹ The two common farmed North American catfishes (Ictaluridae) are the channel catfish *Ictalurus punctatus* and the hybrid ♀ channel catfish × ♂ blue catfish *Ictalurus furcatus*. Throughout this paper, the term “catfish” will refer to these two fishes grown in ponds in the southern United States.
- ² Karen Senaga (2013) provides a detailed history of the role of catfish in the food culture of the southeastern United States and the socioeconomics of catfish off-flavors. She interweaves those stories with an account of how southern Land-Grant Universities—mainly Auburn and Mississippi State University—responded to the challenge of improving catfish product quality as farmers expanded from local to regional and national markets. This interesting and worthwhile article is difficult to access and must be ordered as paper copy from the Mississippi Department of Archives and History at <http://www.mdah.ms.gov/new/interact/subscribe/journal-of-mississippi-history/>
- ³ Associative olfactory learning as a factor in food choice has been conserved across an amazingly diverse phylogenetic range of animals, from nematodes to humans (Bernstein, 1999; Zhang, Lu, & Bargmann, 2005).
- ⁴ The Garcia Effect is named after John Garcia, a scientist at the U.S. Naval Radiological Defense Lab, who first reported the relationship from studies of rats that were given sweetened water and then irradiated. Rats that became sick from the radiation then avoided sweetened water after only one bad experience (Garcia, Kimeldorf, & Koelling, 1955).
- ⁵ Although James Bond seems to have disobeyed a dogma of Italian cooking—never mix seafood and cheese—the worldly spy was aware that sole cooked in brown butter pairs wonderfully with the salty, mushroomy flavor of Camembert cheese. Ironically, one of the compounds that often causes the undesirable muddy flavor of fish (as from the River Loire) is 2-methylisoborneol, which is also an important contributor to the characteristic—and desirable—musty flavor of Camembert (Karahadrian, Josephson, & Lindsay, 1985).
- ⁶ Genetic analysis of the genus *Anabaena* shows that planktonic species with gas vesicles form a distinct cluster. Wacklin, Hoffman, and Komárek (2009) propose a separate genus, *Dolichospermum*, for the planktonic species, including the common odor-producing species found in catfish ponds. The proposed taxonomic change is slowly being adopted but we will retain the older genus epithet, *Anabaena*, for continuity with past studies and ease of communication.
- ⁷ A somewhat predictable and synchronized cycle in phytoplankton community structure occurs with seasonal changes in water temperature, day length, nutrient loading, wind, and rainfall. These changes collectively cause total phytoplankton biomass and relative abundance of cyanobacteria in catfish ponds to increase through the warmer months and decrease through the autumn and winter (Tucker, 1996; Tucker & Lloyd, 1984; Tucker & van der Ploeg, 1993).
- ⁸ The Mississippi “Delta” is not a true river delta in the geological sense, but rather a unique geographic and socioeconomic region of northwest Mississippi. The Delta is a remarkably flat, lens-shaped floodplain lying between Memphis, TN, southward 320 km to Vicksburg, MS, and between the Mississippi River on the west and a loess-covered escarpment on the east. Most farmed catfish in the United States are grown in the Delta.
- ⁹ Off-flavors described as “woody” were common in wintertime samples collected by van der Ploeg and Tucker (1993) and in processing plant records summarized by Dionigi et al. (1998). The “woody” off-flavor is enigmatic and has been ascribed to several possible sources, including β -cyclocitral and metabolites of 2-methylisoborneol (Tucker, 2000). Based on high variation of woody off-flavors within fish populations and the association of that flavor with decay and fishy off-flavors, van der Ploeg and Tucker (1993) suggested that the flavor is not derived from the water but rather from decay processes within the pond, presumably via a dietary route.
- ¹⁰ This study consisted of three treatments (0, 75, or 250 silver carp/ha) with seven replicates for each treatment. Presence of silver carp did not affect off-flavor incidence so data for all 21 ponds are combined here.

- ¹¹ The euphotic depth is deepest depth at which net photosynthesis is possible. It corresponds roughly to the depth at which 1% of incident photosynthetically active solar radiation penetrates. The mixed depth is an ill-defined variable that attempt to define the maximum depth at which turbulent mixing can prevent phytoplankton cells from sinking permanently below the euphotic zone. Mixed depth is influenced by lake morphometry, depth, wind, and daily and seasonal changes in air temperature. In deep temperate lakes, mixed depth in summer corresponds to the water depth down to the lake's thermocline.
- ¹² Stable state theory as applied to catfish ponds does not preclude the possibility of communities dominated by eukaryotic algae. In fact, communities consisting solely, or predominantly, of eukaryotic algae are common in catfish ponds, particularly in winter and early spring (Tucker & Lloyd, 1984; Schrader, Tucker, Brown, Torrans, & Whitis, 2016). Non-cyanobacterial communities develop when conditions no longer give cyanobacteria an advantage. For example, cool, windy weather over the wintertime in the southern United States favors communities of eukaryotic algae because growth rate of large, bloom-forming cyanobacteria decreases rapidly with temperature and wind-induced water-column mixing eliminates the advantage of buoyancy regulation (Reynolds, 1984). These factors have been invoked to explain seasonal cycling between cyanobacteria (in summer) and eukaryotic phytoplankton (in winter) in lakes in The Netherlands, as well as the presence of mixed, transitional communities during state shifts in autumn and spring (Scheffer et al., 1997).
- ¹³ Each of the five "mixed" ponds were equipped with an evenly spaced array of five deep-water, U-tube diffusers (Boyd, 1995) with air delivered to diffusers from an air blower on the pond bank. Diffusers operated all day throughout the study. The "unmixed" ponds were managed traditionally, with paddlewheel aeration provided at night when dissolved oxygen concentrations decreased to predetermined levels considered stressful to fish. Otherwise, all ponds were managed identically with respect to fish culture practices.
- ¹⁴ In the broadest sense, bottom-up control describes the impacts of resource availability throughout the aquatic community. Availability of plant nutrients and sunlight affects each trophic level of the food web sequentially from primary producers (plants) to consumers (zooplankton and other invertebrates, and, ultimately, fish). But here we are concerned only with the effects on phytoplankton.
- ¹⁵ Although *Anabaena circinalis* has previously been confirmed as a source of geosmin in other aquatic systems (Rosen, MacLeod, & Simpson, 1992), it has yet to be isolated from catfish ponds and confirmed to produce geosmin. For now, we presume it to be a source of geosmin in catfish ponds in the southern United States.
- ¹⁶ "Limnocorrals" are enclosures that are deployed in lakes or ponds to conduct replicated, mesocosm-scale (1–500 m³) studies of aquatic ecology. The enclosures are usually tubes or rings, open at the top and bottom, and made of plastic or glass fiber. The bottom is anchored in the pond mud, isolating a volume of water that can be manipulated and studied. Limnocorrals provide a means of conducting highly replicated studies with much less expense than using multiple earthen ponds. They also offer the advantage of having identical conditions within each replicate limnocorral when a study is initiated (Schrader et al., 2000).
- ¹⁷ Bloom-forming cyanobacteria are undesirable in catfish ponds for reasons other than causing off-flavors in fish: they are poor oxygenators of pond waters and some species can produce toxins that can kill fish (Paerl & Tucker, 1995). These problems may argue for general elimination of cyanobacteria from catfish ponds but here we limit our discussion only to problems caused by odor-producing species.
- ¹⁸ As noted in previous sections, the "woody" off-flavor in catfish has been used to describe several different taints, including β -cyclocitral, metabolites of 2-methylisoborneol, decay products in food eaten by scavenging catfish, or others. The cause of the woody flavor described in fish from this trial is unknown.
- ¹⁹ Copper remains in solution as cupric ion at higher concentrations and for a longer time in acidic waters (Snoeyink & Jenkins, 1980). However, nearly all catfish ponds waters have moderate to high levels of hardness and alkalinity and abundant dissolved and particulate organic matter that quickly inactivate copper.
- ²⁰ It is likely that the species was *Planktothrix agardhii*, the most common cyanobacterium in catfish ponds in Mississippi and Arkansas. The authors describe the population as constituting 95% of the phytoplankton community, which is common for *P. agardhii*. The odor-producing cyanobacterium *P. perornata* is never the predominant population in catfish pond phytoplankton communities.
- ²¹ Robinson, Manning, and Li (2004) recommend feeding food-sized catfish once a week when water temperatures are 10–15°C and every other day when water temperatures are 15–20°C.

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