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Statistical Approaches to Optimize Detection of MIB Off-Flavor in Aquaculture Raised Channel Catfish

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Abstract

The catfish industry prides itself on preventing inadvertent sale of off-flavor fish. Typically, several fish are taste tested over several weeks before pond harvest to confirm good fish flavor quality. We collected several data sets of analytically measured off-flavor concentrations in catfish to assess the type of distribution (parametric/non-normal). Coincident measures of fat content were made on three subsections of each fillet. These data were then used to model the number of fish required to detect off-flavor in mixed populations containing on and off flavor fish. In fish collected from the same pond, off-flavor concentrations typically were not normally distributed, thereby requiring specialized statistical procedures. Even with log transformation, data still violated assumptions of normality. We used a non-parametric approach, using fish samples that were ordered, and then randomly sampled 1000 times, to determine the number of fish necessary to detect off-flavor. A sample of 40 fish was required to detect off-flavor when the population was nearly all on-flavor (97%) and <11 fish when populations contain >20% off-flavor fish. A sample size of six fish in a mixed population was effective in identifying off-flavor occurrence in 60% of ponds having off-flavor present. Sampling more fish fewer times can more accurately identify ponds containing mixed flavor fish populations than the current sampling procedure.

Keywords: Channel catfish; *Ictalurus punctatus;* 2-methylisoborneol; Off-flavor; Randomization statistics; Sample size

Introduction

During the past 35 years, the United States channel catfish industry has achieved remarkable growth. According to the last aquaculture survey by the USDA National Agricultural Statistics Service, catfish aquaculture in the United States accounts for over 67% of all freshwater food fish sales within the United States [1]. Genetic improvements, improved feed formulations, and pond management practices have improved farm efficiency and profitability [2].

One growth impediment to the industry is the occurrence of off-flavor fish. Fish deemed off-flavor are unsuitable for human consumption. Fish must be held until on-flavor, thus increasing costs associated with feeding and maintenance, as well as the potential loss from disease/toxicity events, disrupted pond restocking/turnover, and reduced profit from having fish larger than desired sizes for the processing plants.

Recognizing the specific off-flavor compound responsible for imparting odor/taste issues is critical Martin and Suffet [3] identified three odorants in channel catfish production ponds-B-cyclocitral, geosmin, and 2-methylisoborneol (MIB). Cyanoprokarotic algae appear to be the primary source of these secondary metabolites in production ponds given the high correlation between algal biomass and off-flavor occurrence [4,5]. It is unlikely these compounds serve as a chemical signal in the planktonic environment [6]. Odor thresholds for two of these most common compounds MIB (1-R-exo-1,2,7,7-tetramethyl bicyclo-[2-2-1]-heptan-2-ol and geosmin (trans-1,10-dimethyl-trans—9-decalol) range from 9-40 parts per trillion. The widespread occurrence of MIB and geosmin in production ponds and rapid uptake of these compounds in fish tissue [7] provides clear evidence of the importance associated with identification, detection, and prevention of off-flavored products from entering the marketplace. Recognizing pond populations of fish having mixed on and off-flavor fish is critical to prevent negative consumer response to substandard products reaching the marketplace.

Most processing facilities use human flavor checkers to assess fish flavor quality and to assure high quality fish fillets reach the marketplace. These professional testers typically are able to identify off-flavors present objectively (R=0.9) when compared to analytical methods [8,9]. The overall goal of these testing efforts is to provide a consistent flavored product for consumers, as these compounds have been shown to be non-carcinogenic at ambient concentrations by Ames test [10]. The sensitivity of flavor checkers to off-flavor appears to be at least two-fold greater than that reported previously as the human threshold.

Standard practice for processing plants is to require pre-harvest fish flavor check(s) to assess pond level off-flavor. Typically, from one to two fish are tested weekly for three to four weeks prior to harvest. This repetitive sampling is one means of naturally depurating fish, as each taste-test event must be on-flavor for harvesting [11] and processing to occur. An alternative sampling strategy recommended sampling an increased number of fish just before pond harvest. Their rationale is a larger number of fish sampled just before harvest would provide a more accurate estimate of fish quality using Bayesian analysis [12]. Gautier et al. recommended around 30 fish to be sampled from ponds to detect off-flavor fish. Several inherent study design and sampling issues are present in this approach by [13] : 1) the use of categorical data to test for significance in within and between pond variation, 2) apparently

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data was pooled for all ponds sampled with no separation of on-flavor and (partially) off-flavor ponds, and 3) it is likely if fish were measured analytically, the frequency distributions of fish collected from the same pond are not normally distributed. In Dionigi et al. [14] Figures 1 and 2 Pond 112 in these two figures is skewed to the right, whereas Pond 217 has a right skew in Figure 1 and has kurtosis and right skew issues in Figure 2 which directly violates normality assumptions of parametric procedures. For these reasons an alternative statistical approach was investigated.

One approach used in epidemiological studies of fish disease for sample size determinations involves the establishment of confidence intervals [15]. An expanded version of this work with additional confidence intervals [16] provides better insight applicable to off-flavor analyses. To detect disease (or possibly off-flavor) occurrence in 0.1% in a population of 1,000,000 fish with 95% confidence, nearly 3,000 fish would be sampled. Clearly this approach is outside of reasonable flavor assessment and profitability. Additionally, it would be expected that fish diseases are more non-uniformly distributed within ponds relative to volatile aromatics dissolved in water.

To assess this distributional question, our first approach was to assess the distribution of off-flavor, mixed, and on-flavor samples. We used fish from a single pond to reduce variation in off-flavor content. We used resampling statistical (RS) methods, combined with nonparametric frequency distributions and Monte Carlo simulation, well established procedures [17] as an alternative to Bayesian or normal distribution dependent statistics. RS methods have no assumptions of normalcy and are particularly useful in estimating population response based on small sample sizes [18]. We used a bootstrapping approach, coupled with sampling with replacement, to develop a population (n=1000) for subsampling, and then randomly sampled this population to determine the fish number required to identify the presence of off-flavor fish.

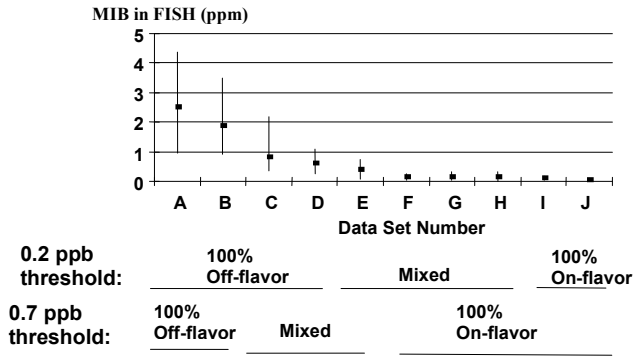

Figure 1: Mean and range of MIB concentration in fish samples analyzed.

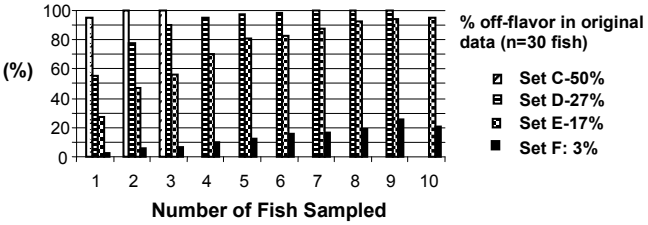

Figure 2a: Cumulative detection percentage for detecting off-flavor fish using 0.7 ppb threshold at varying number of fish sampled.

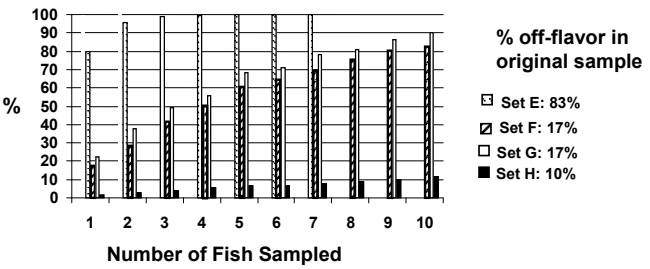

Figure 2b: Cumulative detection percentage of detecting off-flavor fish using 0.2 ppb threshold at varying number of fish sampled.

Methods

Off-flavor study design

Data were derived from 10 dates of sampling depurated fish. Pond-raised catfish that were off-flavor were collected from a single pond at the completion of a study described in Zimba et al. [19]. Fish (average weight=400 g) from this off-flavor pond were transported in one truckload and randomly stocked in three flow-through raceways supplied with Mississippi aquifer water (flow-rate 48 L min-1, temperature 15°C, n=150 fish per raceway). Ten fish were randomly sampled from the three raceways (n=30) on days 0, 0.16, 1, 2 (n=29 fish), 3, 4, and 5.

Fish processing and data analyses

In all cases fish were stunned, fillets were removed by hand filleting, skinned, and frozen at –80°C. Fillet subsamples were processed as described in [20] : For off-flavor concentration using microwave SPME gas chromatography.

Data from each sampling date were analyzed for normalcy using the SAS program PROC univariate before further analyses were considered. Three essential components of a normally distributed population are small variance:mean ratios, and low skewness and kurtosis in distribution using the Shapiro-Wilk W statistic and the Kolmogrov-Smirnoff statistics. As there was no raceway effect (data not presented) data were pooled for analysis using the SAS PROC mixed procedure.

Non-parametric data analyses first required ordering fish samples from lowest to highest MIB concentration within each date. A population of 1000 MIB values was then made by random sampling with replacement from each 30 fish data set. Using the processor MIB detection limit of 0.2 ppm for considering fish fillets off-flavor the percentage of fish passing through the sensory evaluation that were off-flavor was determined as a frequency distribution.

An alternative Monte Carlo method was also used to confirm fish sample size required. The original data set was sampled with replacement 1000 times; then estimates of mean values based upon different fish sample sizes was made three times. These three values were averaged and standard deviation was calculated. As the standard deviation of MIB concentration (e.g. the tails within the range of values measured) is an indicator of tailing-a plot of standard deviation versus fish number was made. PROC NONLIN in SAS was used to non-linear curve fit data to determine breakpoints in this exponential curve.

Results and Discussion

Three factors affecting off-flavor detection in fish are the sensitivity and precision of the analytical method used to identify and quantify

these off-flavor compounds, the uniformity of off-flavor distribution within fish tissue, and the uniformity of fish (i.e. size and conditioning factors affecting body fat) harvested. We have not investigated fish size and conditioning (other than fat content) as a factor in off-flavor content of fish in this study-other studies suggests a strong linkage. Other factors such as water temperature and water flow rates can significantly affect fish metabolism and depuration Accuracy and precision of the GC/MS method has been solidly established, with threshold levels in the parts per trillion level. Typical coefficients of variation are around 5% for water samples; for tissues coefficients of variation from the SPME/GC/MS method are less than 20% (Grimm, unpublished).

The distribution of off-flavor is not uniform with fillet tissue (Table 1). High concentrations of MIB are typically found nearest the head. Uptake of MIB is known to occur through gill tissue, so a gradient from this region to the tail might result. Fat content is strongly correlated to weight and fat content (Table 2) as previous shown [8,21].

Two sensory thresholds have been suggested for detecting off-flavors-0.2 and 0.7 ppb MIB concentration. Data sets encompassed the gamut of potential samples-from fully off-flavor to fully on-flavor, as well as mixed populations (Figure 1). Using the 0.2 ppb threshold criterion, we would consider four data sets to represent ponds having all off-flavor fish (data sets A, B, C, and D) whereas two datasets (I, and J) contained all on-flavor fish. The four data sets having a mixed assemblage of on- and off-flavor fish contained 3-27 fish (of 30 total) being below sensory detection levels. If a sensory threshold of 0.7 ppb is used, three data sets contain samples of mixed on- and off-flavor fish (C, D, E), while five datasets would be regarded as on-flavor. This higher sensory threshold would result in nearly double the number of fish considered on-flavor.

Analyte	Fillet Location		
	Head	Middle	Tail
2-methylisoborneol concentration (ppb)	0.254	0.0177	0.0167
Geosmin (ppb)	0.173	0.1067	0.051

Table 1: Off-flavor in fish fillet portions (n=30 fish). Significant differences indicated by broken underline.

	MIB	Fat	Weight
MIB	1	0.147	0.044
		(-0.0112)	(-0.4469)
Fat		1	0.434
			(<0.001)

Table 2: Pearson correlations (probability in parentheses) for fish weight, fat content, and MIB concentration in purged fish (n=297).

Data generally did not fit a normal distribution, with extreme skew or kurtosis evident in most data sets (Table 3). Log transformation of 2-MIB concentration did not result in a normal distribution. This is not surprising as you cannot have values below 0 for MIB concentration-therefore populations typically will have a right skewed population.

We approached these data sets as representative of field populations composed of fully off-flavor to fully on-flavor fish. Both sensory threshold levels (0.7 and 0.2 ppb) were evaluated (Figure 2 and 2a respectively) using RS approaches. For ponds initially having 17% on-flavor fish, it would require sampling 7 fish to assure near 100% detection of off-flavor fish within the population using the 0.7 ppb detection criteria. In ponds with 83% on-flavor fish, randomly sampling 10 fish would detect off-flavor in 82.5% of fish and over 40 fish would be required to assure no off-flavor fish were processed. A similar result was obtained from the third mixed data set that contained 80% on-flavor fish. Slightly less than 40 fish would be required to identify the presence of off-flavor fish when 80% of fish were below 0.7 ppm MIB. When fish populations contained 90% on-flavor fish, sampling 10 fish identified 90.3% occurrence of off-flavor and a sample of 30 fish would be required to assure no off-flavor fish were processed from this pond. This inconsistency is the result of the variance associated with the initial fish samples from sets G and H where the standard deviation was 50% or higher of the mean MIB level (Table 3) and can be explained by differences in MIB concentration within fillets and between fish.

Using the average human threshold for MIB of 0.7 ppb versus the flavor checker value of 0.2, two of the data sets previously considered mixed (off and on-) flavor fish would now be considered on-flavor using RS methods (Figure 2b), resulting in twice the number of fish available for sale given this threshold level. While this safety margin may prevent acutely sensitive people from tasting off-flavor fish, it is also clear a significant portion of available fish would not be processed. With the current practice of sampling two fish, up to 20% of off-flavor fish would not be identified.

An alternative approach to test the sample size required to have minimal variation in standard deviation of measured MIB concentration estimates as fish number was varied (Table 4). From 1-10 fish were used for these analyses. Significant linear relationships were obtained, suggesting an optimal reduction of between fish variation when sampling less than 10 fish. These results appeared to be less sensitive to defining sample size required as compared to the frequency assessment (Figure 2a), however both methods differed by less than a factor of three. Combining results for detection of off-flavor fish (Table

Pond ID	Mean MIB	Std dev	Skewness, kurtosis	S-W[1]	P.	Mean Log MIB	Std dev of Log MIB	Log Skewness, kurtosis	Log S-W[1]	P.
A	1.228	0.247	-0.120, -0.296	0.967	0.467	0.184	0.214	-0.676, 0.528	0.945	0.124
B	1.027	0.234	0.408, -0.723	0.959	0.316	0.001	0.228	0.020, -0.775	0.973	0.655
C	0.574	0.185	1.262, 2.099	0.913	0.017	-0.6	0.299	0.419, 0.066	0.983	0.899
D	0.447	0.155	0.417, -0.826	0.939	0.086	-0.865	0.356	-0.167, -0.755	0.957	0.253
E	0.307	0.144	1.270, 2.934	0.917	0.022	-1.286	0.49	-0.691, 1.997	0.957	0.259
F	0.135	0.048	0.071, -0.671	0.971	0.563	-2.073	0.397	-0.728, 0.180	0.944	0.115
G	0.123	0.063	0.619, -0.551	0.938	0.081	-2.181	0.517	-0.164, -0.889	0.968	0.487
H	0.123	0.069	0.513, 0.108	0.951	0.179	-2.301	0.748	-1.278, 2.163	0.901	0.009
I	0.089	0.036	0.575, -1.049	0.893	0.006	0.043	0.02	0.978, 1.103	0.924	0.033
J	0.043	0.02	0.978, 1.103	0.924	0.034	0.041	0.019	0.925, 0.916	0.928	0.045
[1]Shaprio Wilk's test										

Table 3: Univariate test results for 2-methylisobroneol concentration in fish samples from ten data sets (n=30 for all except for D, n=29).

Data Set	Number of Fish	R^2
A	1	-
B	1	-
C	2	-
D	3	0.89
E	4	0.89
F	5	0.86
G	5	0.91
H	6	0.9
I	7	0.81
J	10	0.83

Table 4: Nonlinear breakpoints for fish sample number and significance of the regression relationships. In all cases $P \leq 0.05$.

Beginning on-flavor %	# fish sampled	Probability of off-flavor detection
17	2	95
	4	>99.9
50	2	90
	3	>99.9
73	5	90
	7	>99.9
83	10	92
	9	81
	10	90
90	10	90
	30	99.5
97	10	83
	40	99.5

Table 5: Probability of detecting off-flavor fish in mixed off-flavor populations.

5) provides a method for predictive assessment of preventing off-flavor fish from being processed.

Differences in channel catfish MIB concentrations have been demonstrated from field as well as laboratory dosed fish samples. Fat content is one factor influencing uptake and depuration of off-flavor Several important other sources of variation in off-flavor content from this study would include position within the fillet that is sampled , and within fish variation in pond populations.

Farmers can assess pond conditions using repeated flavor checks to determine if off-flavor is present, and if so, the degree of fish retention of off-flavor. By using these sampling relationships, it is possible to estimate the efficiency of the farmer's management efforts to remove off-flavor, and allow processing plants to identify off-flavor in mixed populations. By combining ponds evaluated by 0.7 and 0.2 ppb thresholds (Table 5), we can estimate probability of correct identification of off-flavor occurrence. Regardless of the sensitivity threshold selected, it is clear that increasing the number of fish tested for off-flavor would enhance detection of off-flavor in mixed populations of on and off-flavor fish. One option might be to use a tiered approach, whereby a single is tested from a pond for 1-2 weeks before the planned harvest, and 3-7 fish sampled on the final flavor check. This approach would allow depuration to proceed naturally if fish are slightly off-flavor or extremely off-flavor, while providing greater confidence in the results obtained just prior to harvest and processing.

Conclusions

Testing 5-7 fish on one occasion will prevent >60% of off-flavor fish from being harvested as confirmed by multiple non-linear and Bayesian statistical approaches.

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