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Postharvest Correlation between Swordfish (*Xiphius gladius*) Size and Mercury Concentration in Edible Tissues

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ABSTRACT

Total mercury was measured via thermal decomposition amalgamation atomic absorption spectroscopy in the muscle tissue of 82 swordfish originating in the Pacific Ocean and was found to range from 228 to 2,090 ppb. The relationships between total mercury concentration and the size of the fish (i.e., length and weight) were analyzed. It was found that dressed weight (DW) was a better predictor of mercury concentration than cleithrum-to-caudal keel length in a single variable model, and DW was the only significant predictor of mercury concentration in a multivariable model. Based on these relationships, swordfish with a DW greater than 96.4 kg (213 lb; 95% confidence interval, 88 to 107 kg [195 to 235 lb]) will exceed 1,000 ppb of mercury—the action level in the United States, Canada, and Europe—and should not be sold in commercial markets. Additionally, a logistic regression model was created to illustrate the probability of a swordfish at any DW being unsafe to consume (i.e., containing more than 1,000 ppb of mercury). In this model, the probability of a swordfish being unsafe exceeds the probability of being safe at 94.6 kg (209 lb). Taken together, the models presented in this report give regulators valuable postharvest tools to use for rapid determination of the safety of swordfish intended for sale in commercial markets.

Mercury originates from natural sources and from the combustion of waste in industrial settings *(30)*. As such, it enters the environment and is recognized as a persistent contaminant *(29)*. The global cycling of mercury allows it to be taken up into the atmosphere as elemental mercury (Hg^0), slowly converted to mercuric mercury (Hg^{2+}), and then distributed throughout the world via rain water *(12)*. Once mercury reaches waterways, sulfate-reducing bacteria biotransform Hg^{2+} into methylmercury (MeHg) *(12)*. These bacteria are consumed by aquatic microorganisms, and MeHg bioaccumulates and biomagnifies as it is ingested by larger species, culminating in the largest predatory fish containing the highest MeHg levels *(28)*.

A potent neurotoxicant *(12)*, MeHg was first recognized as a threat to human health during outbreaks in Iraq *(2)* and Japan *(20)*. Based on these incidents, the symptoms of MeHg poisoning were elucidated and include ataxia, dysarthria, tremor, narrowing of the visual field (including blindness), and, in severe cases, death *(1, 7, 19, 20)*. In the aftermath of these events, the toxicokinetics of MeHg were examined, and it was found that MeHg is efficiently absorbed *(41)* but slowly excreted *(28)*, leading to in vivo half-lives of 2 to 3 months in humans *(7, 40)*. Additionally, 10% of the body burden of MeHg is in the brain *(12)*, where MeHg is demethylated to inorganic mercury *(28)*. Over time, excessive exposure to MeHg can lead to the debilitating symptoms described above, as inorganic mercury cannot cross the blood brain barrier to escape the brain *(28)* and, thus, causes neurological lesioning in the primary visual and primary motor cortices *(13–15)*.

As a result of these negative health effects and because seafood is the primary source of MeHg exposure *(12)*, regulatory agencies have set guidelines for fish sold in commercial markets. In the United States, the Food and Drug Administration has established an action level of 1,000 ppb of mercury for fish sold in commercial markets *(39)*. In both Canada *(21)* and Europe *(16)*, the maximum mercury concentration for marketable seafood is 500 ppb, with the exception of several species (including swordfish), that may contain up to 1,000 ppb of mercury. However, enforcing these limits is challenging, as evidenced by recent studies that found fish above the legal limit in commercial markets *(11, 23)*.

One species garnering a significant amount of attention due to the high levels of mercury in its tissues is swordfish, a large predatory species consumed by many people. A recent analysis by Groth *(18)* estimates that U.S. seafood consumers receive just over 5% of their total mercury intake from swordfish. Accordingly, case studies have reported detrimental effects, consistent with mercury poisoning, occurring in individuals who consume high-mercury seafood regularly *(22, 25, 35)*. In particular, these case studies show that frequent swordfish consumption (i.e., at least two meals per week) is linked to fatigue, headache, memory loss, muscle weakness, loss of coordination,

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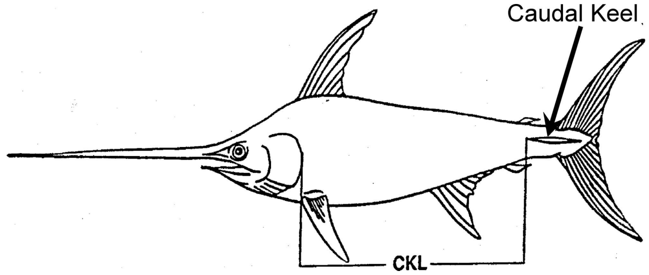

FIGURE 1. *Diagram of swordfish and cleithrum-to-caudal keel length (CKL). Dressed swordfish carcasses are received with the head, tail, fins, and guts removed (4); thus, the CKL was measured, and samples were taken from the caudal keel for analysis.*

tremors, and hearing loss *(17, 22, 35)*. Thus, it is necessary to create tools that efficiently detect swordfish containing unsafe levels of mercury (i.e., more than 1,000 ppb of mercury).

Over the past 40 years, several studies have evaluated the mercury concentrations in swordfish and modeled the change in mercury status as the fish matures. Most have studied swordfish from the Atlantic or Indian Oceans and determined significant relationships between mercury and weight, length, sex, and age *(3, 5, 8, 23, 24, 26, 27, 36, 37)*. However, the relationships between these variables and mercury concentration vary dramatically. Additionally, these studies rely on information that is not available to regulatory officials at the ports during sale. Swordfish are dressed at sea to minimize waste brought into port *(4)*, rendering studies that measure age (via otoliths) *(27)*, sex *(27)*, fork length *(8, 24)*, lower jaw-fork length *(5)*, and whole weight *(23, 24, 36)* of swordfish of great use to fishermen but of little use to regulatory officials. Thus, the current project took a postharvest approach and worked with a commercial seafood distributor, who had little knowledge of the original size of the fish. The objective of this study was to establish a correlation between the size of the fish and the mercury content in edible muscle tissue. This correlation was used to create a simple tool for regulators to use in estimating the mercury content in swordfish and determining its safety for sale in commercial markets.

MATERIALS AND METHODS

Sample collection protocol. The swordfish (*Xiphias gladius*) were caught in the Pacific Ocean from late 2013 to mid-2014 and were obtained through a commercial seafood distributor in the western United States. To ensure that the data reflected the wide range of sizes being introduced to commercial markets, swordfish samples were requested in five different weight classes, as follows: <54 kg (<120 lb), 54 to 82 kg (120 to 180 lb), 82 to 109 kg (180 to 240 lb), 109 to 136 kg (240 to 300 lb), and >136 kg (>300 lb). The seafood distributor supplied a number of samples in each weight category and provided the date of capture, the port of origin, the dressed weight (DW), and the cleithrum-to-caudal keel length (CKL) for each dressed swordfish carcass (Fig. 1). Dressed carcasses eligible for sale in U.S. markets are defined as swordfish without heads, fins (including the tail), gills, or guts *(4)*. For the current study, the distributor purchased dressed carcasses from fishermen, removed a small section (25 g) of edible muscle tissue from each fish, froze the samples, and shipped them to Purdue University for analysis. Because mercury is not evenly distributed throughout the edible portions of swordfish *(36)*, a sample of edible muscle tissue from the caudal keel of each swordfish was requested to maintain consistency in the sampling methodology and allow for a consistent comparison between samples. Upon arrival, samples were thawed at 4°C overnight; skin and excess cartilage were removed before analysis.

Mercury analysis. Total mercury concentrations were determined using a thermal decomposition (gold) amalgamation atomic absorption spectrophotometer direct mercury analyzer (DMA-80, Milestone, Inc., Sheldon, CT). The following operating conditions were used: samples were dried at 300°C for 60 s and thermally decomposed at 850°C for 180 s. Oxygen (commercial grade, 99.5% pure; Indiana Oxygen Company, Indianapolis, IN) was used to purge the system for 60 s and remove sources of interference. The amalgamator was rapidly heated to 900°C for 12 s to vaporize mercury and pass it to the detector, where the absorbance was measured at 253.65 nm for 30 s.

Calibration of the DMA-80 was achieved using previously published methods *(11)*. Briefly, a 1,000-ppm mercury standard solution (AccuStandard, Inc., New Haven, CT) was used to create three dilute mercury solutions (0.100, 1.00, and 10.0 ppm of mercury in 5% hydrochloric acid) for calibration. Calibration curves were developed for the low-range (0 to 25 ng Hg) and high-range (25 to 600 ng Hg) cells in the instrument, yielding a working range of 0.02 ng Hg (limit of detection) to 600 ng Hg. The catalyst and amalgamator were replaced once during the course of this study; the calibration curves for the first catalyst and amalgamator were $A = 4.893\text{e}{-2} \times C - 1.012\text{e}{-4} \times C^2 + 1.352\text{e}{-5} \times C^3$ (low-range cell; $r^2 = 0.9991$) and $A = 1.076\text{e}{-3} \times C - 3.1\text{e}{-7} \times C^2$ (high-range cell; $r^2 = 1.0000$), where A represents absorbance and C represents mercury concentration. The calibration curves for the second catalyst and amalgamator were $A = 4.387\text{e}{-2} \times C - 5.439\text{e}{-4} \times C^2$ (low-range cell; $r^2 = 0.9996$) and $A = 1.064\text{e}{-3} \times C - 2.7\text{e}{-7} \times C^2$ (high-range cell; $r^2 = 1.0000$).

Each sample (100 mg, wet weight) was analyzed in triplicate. At the beginning of each sample set, a blank was run, followed by 50 mg of a Standard Reference Material (TORT-2, Institute for National Measurement Standards, National Research Council of Canada, Ottawa, Canada) and 100 mg of TORT-2 to validate the performance of the full working range of the instrument. TORT-2 was reanalyzed every 18 replicates to continually monitor performance. To limit mercury carryover or memory effects in the instrument, a blank was run between each set of triplicates. Additional carryover effects have been noted in high-fat species (like swordfish) *(10, 33)*; to eliminate these effects, one boat with flour (100 mg) and one boat with 5 to 10% nitric acid (100 μl) were run every 15 replicates *(33)*. Results were rejected and the analysis repeated on all samples that had a relative standard deviation of greater than 5% between the three replicates *(6)*. Applying this criterion, 61.7% of samples failed and had to be retested. One sample had a relative standard deviation above 5%, but only four replicates were available due to the limited sample provided. The four replicates centered around the mean, and thus, this data point was included in the analysis.

Statistical analysis. Statistical analyses were performed using R statistical analysis software (version 3.1.1) and Microsoft Excel. Linear (Figs. 2 and 3) and quadratic (Fig. 4) regression models were employed to study the relationship between total mercury concentration and the size of the fish (i.e., CKL and DW).

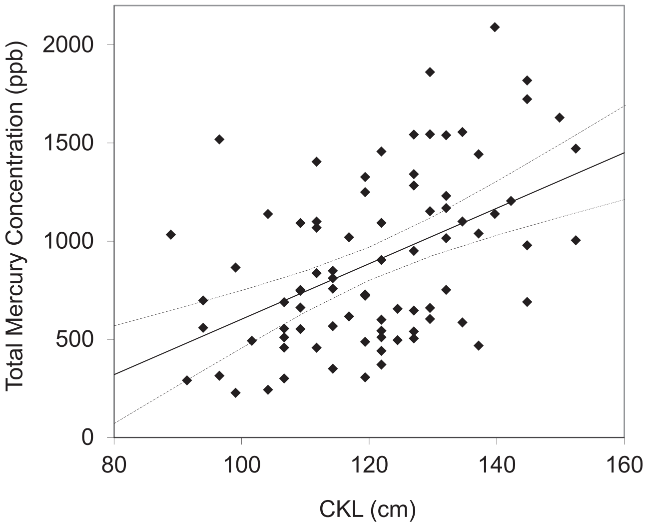

FIGURE 2. *Scatterplot of cleithrum-to-caudal keel length (CKL) versus total mercury content. The solid line represents the line of best fit* (r^2 *= 0.23), while the dashed lines illustrate the 95% confidence interval. Swordfish with a CKL above 128 cm (50 in.; 95% CI, 121 to 137 cm [48 to 54 in.]) will, on average, contain more than 1,000 ppb of mercury and so are not legally marketable in the United States (39), Canada (21), or Europe (16).*

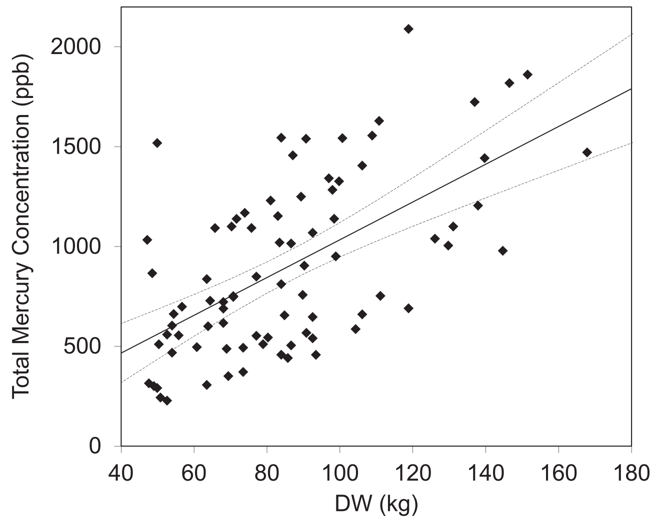

FIGURE 3. *Scatterplot of dressed weight (DW) versus total mercury content. The solid line represents the line of best fit* (r^2 *= 0.37), while the dashed lines illustrate the 95% confidence interval. Swordfish with a DW greater than 96.4 kg (213 lb; 95% CI, 88 to 107 kg [195 to 235 lb]) will, on average, contain more than 1,000 ppb of mercury.*

A logistic regression model (Fig. 5) was constructed to determine the probability that a swordfish of any DW would contain more than 1,000 ppb of mercury. In this model, all samples were assigned a value of 0 (for swordfish with less than 1,000 ppb of mercury) or 1 (for swordfish with more than 1,000 ppb of mercury). These data were used to build a model with the following equation: $\log[p/(1 - p)] = a + b \times \text{DW}$, where p represents the probability of a fish being unsafe (i.e., containing more than 1,000 ppb of mercury), $(1 - p)$ represents the probability of a fish being safe (i.e., containing less than 1,000 ppb of mercury), a represents the y intercept, and b represents the slope. Solving for p and plotting versus DW generates the logistic regression plot illustrated in Figure 5.

RESULTS

Samples from a total of 101 swordfish were collected, though 19 samples were removed from the study because they were incomplete, mislabeled, or entirely composed of cartilage (i.e., lacking edible muscle tissue). For the 82 samples included in the final analysis, total mercury concentrations ranged from 228 to 2,090 ppb, CKLs ranged from 89 to 152 cm (35 to 60 in.), and DWs ranged from 47 to 168 kg (104 to 370 lb). Furthermore, 12 samples from the lightest weight category (<54 kg [<120 lb]), 28 from the second-lightest weight category (54 to 82 kg [120 to 180 lb]), 27 from the middle weight category (82 to 109 kg [180 to 240 lb]), 9 from the second-heaviest weight category (109 to 136 kg [240 to 300 lb]), and 6 from the heaviest weight category (>136 kg [>300 lb]) were collected and analyzed. The total number of samples in each category was limited by availability and the distributor's desire to avoid marketing swordfish above a DW of 125 kg (275 lb).

Size-mercury correlations and models. All collected variables were analyzed as predictors of mercury. No significant correlations were found with either the date of capture or the port of origin; however, CKL and DW were both found to be significant predictors of mercury concentration. Many different models were tested to illustrate the relationship between total mercury concentration and swordfish size. For both CKL and DW, linear correlations exhibited the highest correlation coefficients. Both relationships were significant, indicating that the mercury concentration increases as both the CKL ($P < 0.0001$) and DW ($P < 0.0001$) of swordfish increase.

Using these correlations to determine the level of mercury in swordfish tissue, it was found that fish having a CKL above 128 cm (50 in.; 95% confidence interval [CI], 122 to 137 cm [48 to 54 in.]) will, on average, contain more than 1,000 ppb of mercury (the legal limit for commercially available swordfish in the United States *(39)*, Canada *(21)*, and Europe *(16)*) in their edible muscle tissue (Fig. 2). Likewise, fish with a DW exceeding 96.4 kg (213 lb; 95% CI, 88 to 107 kg [195 to 235 lb]) will exceed the legal limit (Fig. 3). Because the DW-mercury model explains more of the variance in the data ($r^2 = 0.37$) than the CKL-mercury model ($r^2 = 0.23$), it is more representative of the size-mercury relationship for swordfish.

Further exploration of these variables was performed by combining the DW and CKL into a single model and reanalyzing the regression model. However, in this new model, CKL is not a significant predictor of mercury ($P = 0.90$), which is unsurprising given the high correlation between the CKL and DW ($R = 0.78$). Thus, DW is the only significant predictor of mercury in this model, reinforcing the proposition that the DW-mercury correlation is most appropriate for swordfish.

Finally, a model was created to examine the averages of the DW and total mercury content. When the DWs and total mercury contents were averaged in each of the five weight

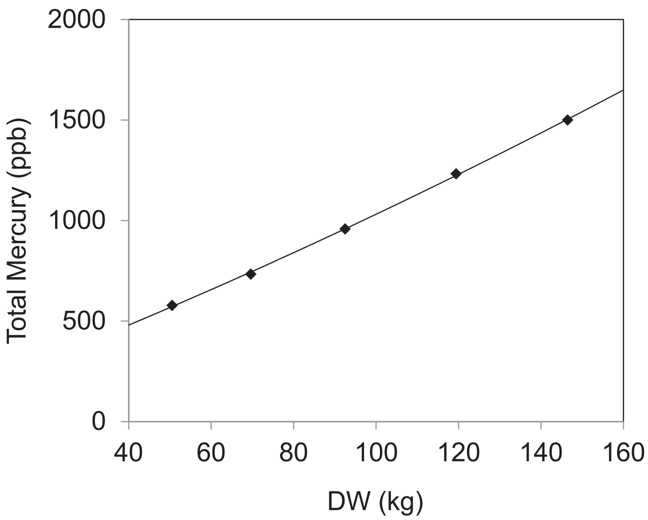

FIGURE 4. *Average total mercury concentration versus average DW for swordfish in each of the five weight classes. Samples were collected from swordfish in the following categories: <54 kg (<120 lb), 54 to 82 kg (120 to 180 lb), 82 to 109 kg (180 to 240 lb), 109 to 136 kg (240 to 300 lb), and >136 kg (>300 lb). The averages of the DWs and the total mercury concentrations for each category are illustrated here, with an excellent correlation (r^2 = 0.9996). In this model, fish above 96.7 kg (213 lb) will exceed 1,000 ppb of mercury.*

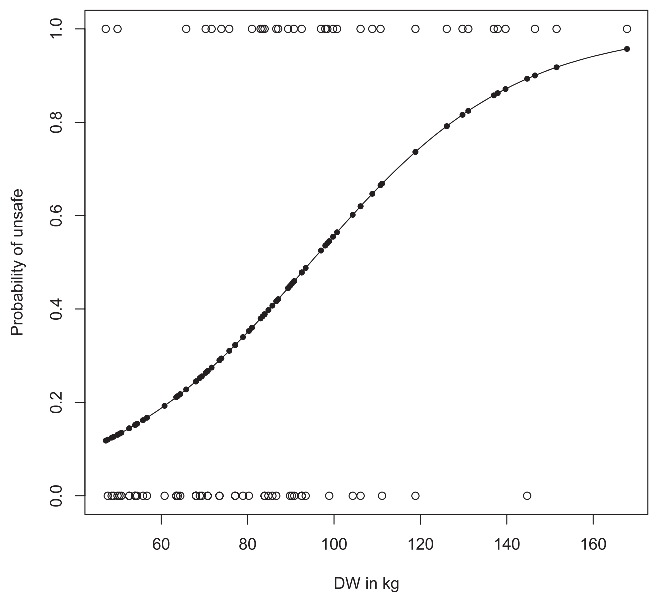

FIGURE 5. *Logistic regression plot illustrating the relationship between increasing DW and the probability of a swordfish containing more than 1,000 ppb of mercury. As the DW increases, the probability of the sample being unsafe (i.e., containing more than 1,000 ppb of mercury) increases, with the probability of the sample being unsafe exceeding the probability of the sample being safe at 94.6 kg (209 lb).*

classes, a graph with five data points was created (Fig. 4). This approach illustrates that, although the DW-mercury model for the samples taken individually has a fairly weak correlation, on average, the samples collected in this study demonstrate a remarkable trend. In this model, the five points on the graph show a near perfect correlation (r^2 = 0.9996), lending further credence to the model discussed earlier. Furthermore, this model predicts that swordfish will contain 1,000 ppb of mercury at 96.7 kg (213 lb), agreeing with the previous DW-mercury model. Although this model is not as powerful as an explanatory model, it does provide supporting evidence for the trend shown in Figure 3, and a similar model has been used elsewhere to model the average swordfish size and average mercury concentration within discrete weight categories *(23)*.

DISCUSSION

Regulatory challenges and opportunities. This study was designed as a postharvest analysis of mercury in swordfish to maximize the translatability of the results for consumer safety. Although several studies have successfully drawn correlations between the size of a fish and the total mercury content *(3, 5, 8, 23, 24, 26, 27, 36, 37)*, the authors are not aware of any studies strictly monitoring variables that can be measured after dressed carcasses arrive in port, specifically, DW. Thus, the results of this study provide not only a unique perspective on the challenge of quickly estimating the amount of mercury in commercial fish but also a new tool for regulators to use with fish arriving in ports. Such a tool represents an advancement in the regulation of large marine species like swordfish, which are known to be higher in mercury, as it allows regulators to quickly determine the amount of mercury in a fish based on the size of the fish rather than having to take a sample back to a laboratory for testing. With recent reports indicating that swordfish containing more than 1,000 ppb of mercury are regularly being sold in U.S. markets *(11, 23)*, it is evident that the current regulatory guidelines are not being enforced. Thus, a simple size-mercury correlation, like the one described here, not only improves the current regulatory system but also aids in avoiding the potential legal trouble from swordfish with excessive mercury entering commercial markets.

Additionally, this data can be used by fishermen to determine whether a fish will be allowed into commercial markets. The National Oceanic and Atmospheric Administration *(38)* has shown an excellent correlation between the eye-fork length, which measures the fork length of the fish from the tail to the posterior section of the eye socket, and the DW of swordfish (r^2 = 0.97). By using this correlation and the results of the current study, swordfish would, on average, exceed the 1,000-ppb action level when the eye-fork length is 185 cm (73 in.; 95% CI, 180 to 191 cm [71 to 75 in.]). Thus, the results of this study support the establishment of tools not only for regulators but also for fishermen.

Logistic regression model for predicting mercury in swordfish. Although these results are helpful, the authors acknowledge that the simple DW-mercury correlation does not adequately explain a significant portion of the variability between swordfish. By employing a different approach to this problem, a logistic regression model can be built to demonstrate the probability of a swordfish at any DW containing more mercury than the 1,000-ppb limit. Figure 5 illustrates a logistic regression curve, with the DW of the swordfish on the *x* axis and the probability of that fish being unsafe to consume (i.e., containing more than 1,000 ppb of mercury) on the *y* axis. From the graph, the probability of a swordfish being safe is greater than the probability of the fish being unsafe up to a DW of 94.6 kg (209 lb). Above this weight, the rising probability of the swordfish being unsafe to consume increases rapidly. This model not only supports the earlier finding that swordfish above 96.4 kg (213 lb) will contain more than 1,000 ppb of mercury in their edible muscle tissue but also gives regulatory agencies a new paradigm with which to evaluate data and set safety standards. For example, regulatory agencies may decide that the current action level is appropriate but that in order to ensure the safety of consumers and especially of sensitive populations (i.e., pregnant or nursing women and young children), a more conservative probability should be chosen. In this scenario, if regulators decide that swordfish with a greater than 40% probability of containing unsafe levels of mercury would be unacceptable, then the threshold for swordfish being sold in commercial markets would be lowered to 85.1 kg (188 lb). Conversely, the fishing industry may push for a higher limit. In either case, this logistic regression model gives more information and flexibility to regulatory agencies and provides for a fuller view of the problem: no swordfish can be deemed absolutely safe or unsafe based on size alone, but the probability of a swordfish surpassing the legal limit for mercury can be used to determine where a size limit should be established.

One caveat to using this model as currently constructed is the conservative bias that is naturally built into the data, as mercury is not evenly distributed throughout the edible muscle tissue of fish *(36)*. All samples collected in this study were taken from the caudal keel area of the fish (near the tail), which is known to be higher in mercury than other edible muscle tissue in swordfish *(36)*. Thus, it is conceivable that all samples in the current study contained higher mercury levels than would be obtained by measuring the mercury in other portions of the same fish. If this is the case, then the slope of the trend line in the resulting data would be artificially high and the 1,000-ppb mercury limit reached at a smaller swordfish size. While this biases the data in some ways, it strengthens the study in others. To keep a consistent sampling methodology and obtain maximal comparability between samples, it was necessary to compare similar sections from each fish. Additionally, the caudal keel is a small, well-defined area of a swordfish, making sample collection consistency much easier to maintain throughout the study. Because the muscle tissue from this portion of the fish is eligible for sale in commercial markets, the authors felt this was a necessary parameter for this study and gives the most conservative estimates to protect public health with the currently established action level. Follow-up studies addressing this issue would be valuable to expand the data pool and inform discussions regarding the safety of swordfish.

Study limitations. The authors recognize that this study is somewhat limited in scope and differs from the work performed by other researchers in previous studies *(26, 27)*. One major limitation is the use of a commercial seafood distributor collecting fish from one body of water (Pacific Ocean) rather than several, as some large bodies of water may contain different levels of mercury than others. While this handicaps the diversity of samples in the study and limits researcher involvement in the sample collection process, this methodology accurately models fish that would be made available in commercial markets for consumers and thus demonstrates applicability in consumer protection. Another limitation is the lack of ability to determine the age (via otolith measurements) or the sex of the fish, which have both been shown to be significant variables in determining the mercury content of swordfish *(27)*. However, the otoliths and genitalia are lost during the dressing of the fish *(4)*, making this information impossible to collect and useless to regulators determining the safety of dressed swordfish carcasses entering ports for commercial use. Finally, although not measured in this study, selenium may be a confounding factor. Selenium-mercury complexes form readily, and several recent reports suggest that the selenium present in seafood may mitigate MeHg toxicity *(32, 34)*. However, the true effect of selenium has not been definitively proven and is the subject of considerable debate within the scientific community *(9, 31)*. Thus, the current study focuses solely on mercury.

In conclusion, this study successfully created a size-mercury relationship model that offers regulators a new tool for the postharvest estimation of mercury in swordfish that can be used to prevent fish with excessive levels of mercury from entering commercial markets. The DW was found to be a better predictor of mercury than the CKL, and from this correlation, it was found that swordfish exceeding 96.4 kg (213 lb) DW will, on average, contain more than 1,000 ppb of mercury in their edible tissue and should not be allowed in commercial markets in the United States, Canada, or Europe. Finally, this study built a logistic regression model for swordfish that allows regulators to accurately determine where limits for swordfish safety should be established, based on the probability of a fish containing unsafe levels of mercury. The results of this study should be informative to many parties involved in the capture, distribution, and regulation of high-mercury fish in commercial markets. This approach should be considered for all higher-mercury marine species, including tuna.

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