AOAC Southern Section: Fish Species Identification a Method Conundrum

The analytical community, seemingly more often, is faced with a need to update traditional methods of analysis that have become antiquated by modern technology. The protein method used for fish species identification is a primary example. *Official Method*SM **980.16** has been used since the 1980s, but has some shortcomings. First, some species cannot be differentiated using this method, and, second there is a susceptibility of the proteins to degradation due to temperature changes [Lundstrom, R. (1980) *JAOAC* **63,** 69-73]. DNA methods for species identification, such as the one used by the Consortium for the Barcode of Life (CBOL), are modern analytical methods that demonstrate better accuracy and ruggedness than the standard method.

Fish species substitution is primarily an economic fraud issue but can be a health issue if a species such as puffer fish or escarole is substituted for an edible species. Puffer fish are toxic and much of the world's human population is susceptible to gastric distress from eating escolar. Escolar is sometimes called "white tuna" in sushi restaurants even though it is not a tuna.

With these factors in mind, the AOAC Southern Section held a session during this year's meeting on April 21-22, 2008, that aimed to address the best way to approach establishing DNA-based methods for fish species identification. One of the barriers to the implementation of new methodology is that this analysis was traditionally done in regulatory laboratories by analytical chemists, who today are not familiar with DNA methodologies.

The first speaker, Marc Engel of the Florida Department of Agriculture and Consumer Services, discussed the experiences of a regulatory laboratory. One analytical challenge is establishing a cache of authenticated fish, which is difficult both from the perspective of obtaining authentics and having them validated. Deciding on which method(s) to use can be an issue too. The method traditionally used in their laboratory is *Official Method*SM **980.16,** which is an isoelectric focusing gel analysis of sarcoplasmic proteins. Although the DNA methodology is more reliable, using a validated and published AOAC *Official Method*SM provides credibility and defensibility. Engel also showed data demonstrating that red snapper, Lutjanus *campechanus,* is substituted in about 42% of the samples tested in a targeted analysis. Additionally, he discussed the difficulties of analyzing generically labeled samples for authentication. For example, if a sample is labeled grouper, one has to consider that there are 15 genera considered to be grouper and the laboratory may only have a few authentics representing the grouper species.

The second speaker, David Price of Fish DNA ID, focused on two main areas: by-catch and contamination of sample DNA by parasites or symbionts. Since much of today's commercial fishing is done on large catch and process boats, there are going to be accidental misbrandings due to on board sorting errors. Obviously, this would not be the case if a freshwater aquaculture fish like Tra is mixed in with a case of grouper. However, it may very well be the scenario when other reef fish are mislabeled grouper. Most seemed to think a 2-5% by-catch error is to be expected. Price also noted that, especially in shellfish, but also possible in finfish, that symbionts or parasites or other foreign DNA could be amplified and a false result could be obtained.

Peter Marko of the University of Clemson discussed fisheries and how their declines and the misconception of healthy fisheries add to the problem of species fraud. He also said that as communities become smaller from overfishing, hybridization between species or introgression is more likely to occur. This could, at some point, confuse results when mitochondrial genes are used for species identification analysis. He also discussed expected divergence of sequences within a species. It is gene dependent and he noted several citations.

The remainder of Marko's talk was focused on using phylogenies for species identification. He said that a single gene may not be able to distinguish two very closely related species like the red snapper and the silk snapper. It has been noted by others that the various species of tuna are very difficult to differentiate.

Marko and others agreed that identification at the genus level can be done confidently using modern phylogenic tools. The end of his talk focused on the use of public databases as tools for species identification and building phylogenic trees.

The AOAC Southern Section meeting also featured a panel discussion, in which the three speakers mentioned above were joined by Selester Bennett of Applied Food Technologies. The panelists discussed questions such as do amplicons need to be sequenced? Is purchasing a sequencer or sending the amplicons for sequencing more cost effective? At what level do species or genus need to be regulated? Are nuclear genes or mitochondrial best for speciation? How often would one have to drill down further than the CO1 gene? Is it best to use Fish Bar Code of Life Initiative (FISHBOL) methodology? How many laboratories would be interested in participating in a validation study? In response, most agreed that the process is best served by following the FISHBOL protocols while using the CBOL database to identify the DNA sequences. In addition, at least eight organizations in attendance representing state, federal, or private laboratories expressed interest in participating in a method validation project. Next steps include organizing a small ring study. ■

— Marc Engel
Contributing Writer
engelm@doacs.state.fl.us