Use of Fatty Acid Profiles to Distinguish Cultured from Wild Fish: A Possible Law Enforcement Tool[1]

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Abstract: Fatty acid profiles of cultured hybrid striped bass and red drum were compared to their diets. Correlation coefficients were 0.94 and 0.98, respectively. Of the fatty acids examined, linoleic acid (18:2n6) levels were particularly high in cultured fish due to various dietary sources and extremely low in wild fishes. Such differences may be suitable to distinguish cultured from wild fish and may become another biochemical tool for use by law enforcement agencies involved in the protection and conservation of natural resources.

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Since 1980, annual seafood consumption by American consumers has increased 38% but there has not been a concomitant increase in production of domestic fishery products (Anon. 1988). Consequently, the United States now imports approximately 63% of its seafood from foreign countries. In1987, imports of edible fishery products cost $5.7 billion and represented over 20% of all U.S. food imports (Anon. 1987). Seafood is a low fat, high quality food and is being promoted to a health conscious nation. This fact, coupled with the realization that landings from U.S. fisheries are not going to substantially increase, means that additional seafood products will have to be imported and/or domestically produced through aquaculture to meet increased demand.

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Cognizant of the role that seafood plays in human nutrition, food imports, trade balance, employment, etc., the federal government has attempted to encourage aquaculture development through various research and demonstration activities. Further, a revision of the National Aquaculture Plan (Joint Subcommittee on Aquaculture 1983) which discusses the biological and economic potential as well as constraints for various aquaculture candidates is currently underway.

In the United States, there is a diversity of organisms being cultured including molluscs, crustaceans and fishes (McVey 1983, Dupree and Huner 1984, Huner and Brown 1985). However, by far, the largest volume and value product is the channel catfish, *Ictalurus punctatus*. In 1987, approximate production was 347.6 million pounds, with a value of $245.3 million. The leading producers of these fish were Mississippi, Arkansas and Alabama (U. S. Dep. Agric. 1988).

In recent years, considerable interest has focused on commercially farming several species which are now listed as game species in many states (Parker 1988). This desire for multiple use of certain species, in particular marine and anadromous finfishes, has often resulted in conflicts between the recreational and commercial sectors. Such conflicts are usually based on lack of awareness of aquaculture techniques and objectives, and/or philosophical differences concerning commercial use of natural resources also utilized by recreational fishermen. In certain instances, there have also been conflicts between aquaculturists and commercial fishermen, with the latter group fearing possible competition and/or loss of employment.

In spite of these conflicts, aquaculture of certain game species will undoubtedly increase. Further, large quantities of recreational species are already being produced for both private and public programs and such activities will probably be expanded (Stroud 1986).

Striped bass and or its hybrids are a focus of commercial aquaculture in many states (Smith 1988). As such, many states from Massachusetts through Texas either have or are considering legal provisions for the commercial growing of these fish (Sharpe and Moore 1987, Parker and Miller 1988). In certain states, such as South Carolina, these game fish support substantial recreational activities. Consequently, major concern has been voiced by the recreational and law enforcement sectors dealing with illegal taking and trafficking of these fish. This concern over poaching has also been echoed by the developing commercial industry which feels that such illegal activities could seriously impact legal sales of cultured fish and cause economic hardship to aquaculture operations.

To address this concern over poaching, the South Carolina Wildlife and Marine Resources Department (SCWMRD) and the National Marine Fisheries Service, Charleston Laboratory (NMFS) initiated a cooperative program focused on the identification of biochemical techniques to differentiate wild from cultured fish. The question examined is whether or not wild and cultured fish can be readily distinguished based on differences in their fatty acid profiles. It is hoped that such data, when combined with solid law enforcement investigative techniques will become a useful tool to deter and apprehend natural resource violators.

Methods

Cultured fish were pond-reared using commercial techniques at the SCWMRD's Waddell Mariculture Center, Bluffton, South Carolina. These fish were hatched in captivity and fed a commercially available trout ration for about 20 months. Hybrid striped bass (white bass, *M. chrysops* x striped bass, *Morone saxatilis* cross) and red drum (*Sciaenops ocellatus*) were used as cultured fish because both are listed as game species and can be produced and marketed by aquaculture operations in South Carolina and in other states. Fish size at harvest was about 0.5–1.5 kg, which is typical of a farmed, market-sized fish. For comparative purposes, samples of wild striped bass and hybrids from 5 different major lake/river systems in the state were obtained from SCWMRD and the Department of Health and Environmental Control. These samples consisted of 1 to several fish in the range (0.5–7.0 kg in weight). Approximately 2 kg of fillets were homogenized in a commercial food processor. Fillets from 5 fish were composited, for large fish (7.0 kg), a single fish was utilized. The homogenized tissues were placed in air tight containers maintained at $-20°$ C until analyzed. All sample preparation and analyses were performed at the NMFS Charleston Laboratory. Just prior to analysis, the frozen, homogenized tissues were allowed to warm to room temperature. Lipids were extracted from duplicate 5-g portions of each sample using a chloroform-methanol extraction method (Smith et al. 1964).

Fatty acid methyl esters were prepared by saponification of the extracted lipids followed by esterification (Metcalfe and Schmitz 1961, Metcalfe et al. 1966). The esters were analyzed by gas-liquid chromatography (GLC) utilizing either a Hewlett Packard (H-P) 5840 or H-P 5890 GLC, both were equipped with flame ionization detector and electronic integrater. During the course of the study, 2 wall-coated open tubular columns were used, a 60 m $\times$ 0.2 mm ID Carbowax 20M (Chrompack) and a 30 m $\times$ 0.25 mm I DB225 (J and W Scientific). The separation characteristics of both columns were similar and analytical results were comparable. Helium was the carrier gas at a flow rate of about 1 ml/minute. Nitrogen was used as make-up gas. Runs were temperature programmed from 170° to 225° C at 1°/minute. Injections were in the split mode with a split ratio of 1:100.

Fatty acids were tentatively identified by comparison of their equivalent chain length values, calculated from isothermal runs, with those of primary and secondary standards, by GLC analysis of hydrogenated samples, and by argentation thin layer chromatography followed by GLC (Ackman 1984).

Correlation coefficients of the fatty acid profiles of cultured hybrid striped bass and red drum to their commercial diets were determined from a composite sample of 6 fillets analyzed in duplicate.

Results

Fatty acid profiles of the cultured fish reflect the fatty acids contained in the diets (Tables 1,2). Correlation coefficients of the fatty acid profiles of the cultured

Table 1. Major fatty acids of cultured hybrid striped bass and its pelleted feed. Data are expressed as weight percent of total fatty acids.

Fatty acid	Cultured hybrid	Dry diet
14:0	2.7	3.4
16:0	20.2	18.5
18:0	4.6	5.5
16:1n7	5.2	4.3
18:1n7	2.5	2.3
18:1n9	28.8	20.3
20:1n9	2.2	2.8
18:2n6[a]	13.0	17.9
18:3n3	1.0	1.6
20:4n6	0.6	0.4
20:5n3	4.3	5.2
22:5n3	0.9	—
22:6n3	5.5	4.5

[a]Linoleic acid.

hybrid striped bass and red drum to those of their pelleted rations were 0.94 and 0.98, respectively. An examination of the fatty acid profiles of the cultured hybrids and red drum indicates that these fish contained 72.6% and 88.6%, respectively, of the linoleic acid (18:2n6) concentration of their diets (Tables 1,2).

Linoleic acid concentration in cultured fish was several times higher than in the wild fish. This is exemplified in Table 3 which compares wild hybrids and/or striped bass taken from 5 major lakes/river systems in South Carolina to the pond-cultured hybrids.

Discussion

General Considerations

Although found in natural foods, linoleic acid is especially high in commercial fish feeds because soybean meal is often used as a major ingredient in fish feeds and soybean oil contains approximately 64% linoleic acid (Haard 1976). Similarly, cottonseed, corn, wheat, and sunflower oils are often used in the production of commercial rations and linoleic acid comprises about 45%–57% of the fatty acids in these oils (Haard 1976).

Biological Considerations

Our data strongly suggest that use of fatty acid profiles, especially linoleic acid concentration, may become an important law enforcement tool for distinguishing cultured from wild-caught fish. Of the 40 finfish species examined by Gooch et al. (1987), all species exhibited typical low concentrations of linoleic acid. In contrast, the levels in cultured fish were much greater and reflect the higher concentrations in

Table 2. Major fatty acids profiles of cultured red drum and its pelleted feed. Data are expressed as weight percent of total fatty acids.

Fatty acid	Cultured red drum	Dry diet
14.0	1.3	3.7
16:0	22.8	23.6
18:0	6.7	7.5
16:1n7	4.5	5.3
18:1n7	2.6	2.6
18:1n9	19.0	21.5
20:1n9	1.2	0.9
18:2n6[a]	20.2	22.8
18:3n3	1.0	2.4
20:4n6	1.3	0.3
20:5n3	4.4	3.6
22:5n3	1.7	
22:6n3	6.9	2.4

[a]Linoleic acid.

manufactured fish feeds (Tables 1, 2, 3). A similar relationship between linoleic acid levels in cultured catfish (*I. punctatus*) and cultured marine shrimp to their diet was also demonstrated by Chanmugam et al. (1986).

Based on our preliminary findings, additional research is planned in South Carolina to verify and document the fatty acid profiles based on fish size, sex, physiological condition, season, and site of capture for various cultured and wild fishes to verify the validity of using fatty acid profiles as a law enforcement tool.

Law Enforcement Considerations

Collection and storage of samples is simple and results of the analysis can be obtained within a day. Approximately 50 g of fresh or frozen tissue is needed for the fatty acid analysis. The relatively simple and rapid procedures involve extracting the lipids from the fish tissue in a mixture of chloroform and methanol. Fatty acid methyl esters are then prepared from the extracted lipids and analyzed on a gas chromograph.

The extraction and methylation of the fatty acids are the most time consuming steps in the procedure. A technician can learn the procedure within a few weeks and become proficient in preparing approximately 6 samples in an 8-hour day. GLC analysis automated for overnight operations would require about 1 hour per sample. Finally, interpretation of results would be completed in 1–2 hours after GLC analysis. For the actual analysis and interpretation, it is suggested that an experienced chemist be utilized who can serve as an "expert witness" in the courtroom. A sample can be completely processed and the results interpreted in about 18 hours.

Table 3. Major fatty acids of cultured and wild hybrids, and wild striped bass. Data are expressed as weight percent of total fatty acids.

Fatty acids	Cultured hybrid striped bass	Lake Hartwell hybrid	Lake Hartwell striped bass	Lake Murray striped bass	Santee River striped bass	Lake Greenwood striped bass	Savannah River striped bass
14:0	3.5	2.9	3.2	3.3	4.0	3.8	3.1
16:0	18.7	17.2	17.5	17.7	17.2	18.6	19.6
18:0	3.8	3.4	3.2	3.4	3.4	3.4	2.7
16:1n7	6.3	6.6	8.3	6.5	12.6	7.9	10.2
18:1n7	2.5	3.6	3.6	3.2	4.2	3.3	3.9
18:1n9v11	26.1	22.6	19.3	16.3	17.0	17.0	24.8
20:1n9	2.3	1.8	2.4	1.6	2.0	2.3	3.7
18:2n6[a]	11.7	3.0	2.8	3.7	2.7	3.7	1.1
18:3n3/19:0	1.3	3.1	2.9	4.2	4.9	7.0	0.9
20:4n6	0.5	5.2	5.1	5.1	2.5	2.7	2.4
20:5n3	6.4	4.1	4.5	4.1	4.3	4.3	7.1
22:5n3	0.3	0.1	1.5	0.2	1.5	1.2	0.3
22:5n3	5.9	11.3	11.7	12.3	5.8	6.5	8.0

[a]Linoleic acid.

Conclusions

Linoleic acid content appears to be a reliable method for distinguishing wild from cultured fish. Data are currently being analyzed to determine the fatty acid profiles of cultured hybrids which were fed a 40% fish meal diet and to determine the seasonal variation in fatty acid profiles of wild striped bass and its hybrids collected from various South Carolina water systems. These efforts will be expanded to include analyses of cultured and wild fish from various regions of the country as well as a variety of commercially utilized diets. Once such information is compiled, law enforcement agencies should have a new tool for use in protection and conservation of our natural resources.

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