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Fatty acids in thirteen Wisconsin sport fish species

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ABSTRACT

Fish are an easily-obtainable source of low-fat protein and fatty acids (FAs), particularly beneficial omega-3 and omega-6 polyunsaturated fatty acids (PUFAs). While data concerning FAs in marine and/or farmed fish are readily available, data regarding spatial variation in FA concentrations in wild, freshwater sport fish species are lacking. To begin addressing this data gap and to provide the general public with more comprehensive consumption advice, we analyzed 13 sport fish species from several of Wisconsin's inland and Great Lakes waters for 16 FA analytes. FA concentrations were compared between species, trophic levels, and with research previously published on freshwater species in our study. We found that fish length was positively correlated with total FA content ($r = 0.617$, $P < 0.0001$) for the whole dataset, but not for any individual species. Salmonids generally contained the highest total FAs while percids and centrarchids contained the lowest concentrations. However, diet was a better predictor of FA concentration than taxonomic family. Species that were completely or partly piscivorous contained higher PUFAs ($P \leq 0.001$) than those that consumed primarily invertebrates. We also found that Wisconsin sport fish generally contained lower concentrations of monounsaturated and saturated FAs than those found in reference studies, whereas omega-3:omega-6 FA ratios and concentrations of omega-6 FAs were largely similar. Incorporating beneficial FA data into existing fish consumption advice is a challenge at this time, and it is recommended that additional FA information be obtained with the goal of quantitatively incorporating benefits into risk assessments and advisory protocols.

Introduction

Fish has long been recognized as a source of low-fat protein and essential fatty acids (FAs), particularly omega-3 and omega-6 polyunsaturated fats (PUFAs) such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Previous research has demonstrated that PUFAs can act as a preventative measure against cardiovascular problems (Kris-Etherton et al., 2002) and hypertension (Xun et al., 2011) in adults and are vital in fetal brain development (Innis, 2000). FA signatures are also used to assess a wide array of trophic interactions (Daly et al., 2010; Iverson et al., 2002; Makhutova et al., 2011). Data concerning FAs in marine and/or farmed fish are readily available to both fish consumers and members of the scientific community (Ackman, 1990; US EPA and FDA, 2004), but data regarding spatial variation in FA concentrations in wild, freshwater sport fish species are lacking.

To begin addressing this data gap, we analyzed 13 sport fish species from many of Wisconsin's inland and Great Lakes waters for 16 FA analytes. The research presented here represents the first step in our efforts to quantify the beneficial components of a wide variety of sport fish species from many locations. This data will not only contribute to the body of knowledge regarding freshwater fish characteristics, but it will eventually be used to provide the general public with more comprehensive fish consumption advice.

Advisories are of particular importance in Wisconsin and other Great Lakes states where consumption of fish from local waters is high (Imm et al., 2005). Wisconsin's recreational anglers have well-established traditions of sharing their catch. The Friday fish fry is an institution in many Wisconsin families, and the diets of immigrant communities like Hmong often include fish from Wisconsin's sport fishery (Hutchison and Kraft, 1994; Schantz et al., 2010). It is for these and other reasons that the Wisconsin Departments of Natural Resources (DNR) and Health Services (DHS) have been testing fish for contaminants and issuing consumption advice since the 1970s (Schrank, 2011). Primary concerns are PCBs and mercury in the Great Lakes (Veith, 1969) and in inland waters (Monson et al., 2011), respectively.

In accordance with our eventual goal of synthesizing FA data with contaminant data using a risk–benefit model (Ginsberg and Toal, 2009), this initial study surveys species covering a broad range of

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geographic locations within Wisconsin (inland waters, streams/rivers, multiple great lakes), containing a range of contaminant levels, and spanning multiple trophic positions (benthivores to top predators). We also compare FA profiles in Wisconsin fish to those in fish from other locations analyzed as part of previous research to further assess spatiotemporal variability.

Methods

Sample preparation and analysis

During 2010 and 2011, 97 scaled skin-on fillets from fish collected as part of WI DNR's annual contaminant survey were selected for FA analysis. These fillets represent 13 species (Table 1). Species selected for analysis were chosen to cover a broad range of trophic positions, geographic locations, water body types, and severity of contamination (Anderson and Geis, 2012). Fish were packaged, labeled, and frozen immediately after collection, and stored at −15 °C until processing by the Wisconsin State Laboratory of Hygiene (WSLH). Before analysis, each sample was thawed, weighed, scaled or skinned (depending on species), and filleted. Fillets were then homogenized using a meat grinder and frozen at −15 °C until analysis. Fish tissues were analyzed for 16 FAs (4 saturated, 2 monounsaturated, 10 polyunsaturated; Table 2) by the Organic Chemistry Section of the WSLH. Fatty acid concentrations (wet weight) reported using this method, detailed below, were not significantly different (ANOVA, $P = 0.995$) from concentrations reported by Clarkson University or the Minnesota Department of Agriculture in an interlaboratory comparison study (Crimmins et al., 2013).

Derivatization of fatty acid methyl esters (FAMEs) occurred as follows: 1 ml extracted sample, 200 μl octadecanoic acid (C18:0-d5, an extraction efficiency surrogate), and 2 ml BF_3 were added to a 15 ml conical tube and purged with N_2. Vials were placed in 60 °C oven for 10 min, cooled to room temperature, and 1 ml Milli-Q water was added to quench the reaction. Phases were separated by adding 1 ml of HPLC-grade hexane to each vial and removing the organic layer. The organic phase was quantitatively transferred to a clean gas chromatograph (GC) autosampler vial.

FAMEs were quantified using an Agilent 7890 gas chromatograph with a flame ionization detector. Separation of FAMEs occurred in an Agilent HP-88 capillary column (100 m length × 0.25 mm internal diameter × 0.2 μm film thickness). The oven temperature was held at 80 °C for 1 min, then increased to 10 °C/min to 175 °C and held for 10 min. The carrier gas was helium with a constant flow of 2 ml/min, 1:50 split injection, and the injection volume was 1 μl. The injection port and detector were kept at 240 °C with hydrogen, air, and helium used as detector gases (40 ml, 450 ml, and 30 ml/min, respectively). Nu-Chek Prep® Component FAME Mixture was analyzed in order to determine response factors relative to the internal standard C18:0-d5 and analysis began only if the calibration curve $r^2 \geq 0.990$. Supelco® 37 Component Fatty Acid Mixture was used as a secondary source confirmation standard.

Duplicates were analyzed with every ten sample batches. Additionally, Nu-Chek Prep® standards C17:1 and C23:1 were analyzed in order to ensure that FA recoveries were within accepted limits (±20% for duplicates, ±30% for standards). Detection limits for this method were 0.40 mg FA/kg tissue (Steve Geis, WSLH Organic Chemistry Supervisor, personal communication, 16 April 2013).

Percent lipid (wet weight) was determined by extracting ground tissue with dichloromethane. The extract was transferred to a tared aluminum weighing pan where the dichloromethane was allowed to evaporate until a constant weight was achieved. Percent lipid was calculated by dividing the final wet weight by the initial wet mass of tissue.

Data analysis

Statistical analysis was conducted using SAS Enterprise Guide (SAS Institute Inc. 2010, Cary, NC). All concentrations of individual fatty acids and FA groups (i.e., saturated: SFA, monounsaturated: MUFA, ω−3 and ω−6) were log-transformed to approximate normality. If sample size was large enough to permit analysis, intra- and interspecific differences in concentrations were determined using ANOVA with post-hoc Tukey HSD test if significant differences were detected. Further

Table 1
Summary information for species sampled.

Species	N	Year(s) sampled	Location(s) sampled (county)	Mean length (min, max)	g Lipid/100 g fish (Mean ± SE)	g FA/100 g fish (Mean ± SE)
Black crappie	5	2010	Bear Lake (Forest)	10.1 (9.1, 11.4)	Not measured	0.47 ± 0.04
P. nigromaculatus		2011	Peshtigo River (Marinette)			
Brown trout	5	2010	Lake Michigan (Racine)	23.1 (23.0, 23.5)	9.5 ± 0.7	6.32 ± 0.34
S. trutta						
Chinook salmon*	5	2010	Lake Michigan (Racine)	31.7 (25.7, 35.0)	4.7 ± 2.1	3.57 ± 1.60
O. tshawytscha	3	2011	Lake Superior (Bayfield)	31.6 (27.9, 33.6)	3.3 ± 1.9	1.22 ± 0.70
Coho salmon	5	2010	Lake Michigan (Racine)	24.7 (20.2, 27.0)	4.8 ± 0.8	3.0 ± 0.58
O. kisutch						
Lake sturgeon	3	2011	Menominee River (Marinette)	50.9 (50.5, 51.6)	5.4 ± 1.3	3.76 ± 1.12
A. fulvescens						
Lean lake trout*	7	2010	Lake Michigan (Racine), Trout Lake (Vilas)	25.7 (21.6, 29.5)	8.0 ± 3.6	6.58 ± 2.45
S. namaycush	5	2011	Lake Superior (Bayfield)	24 (21.2, 26.9)	9.5 ± 1.4	1.66 ± 0.74
Lake whitefish	5	2011	Lake Superior (Ashland)	19.3 (17.2, 21.1)	5.9 ± 2.9	1.74 ± 0.38
C. clupeaformis						
Largemouth bass	5	2010	Bear Lake (Forest)	14.4 (12.9, 26.3)	Not measured	0.35 ± 0.05
M. salmoides		2011	Anderson Lake (Bayfield)			
Northern pike	5	2010	Bear Lake (Forest)	21.8 (16.3, 28.7)	Not measured	0.39 ± 0.05
E. lucius		2011	Flambeau River (Price)			
Rainbow trout	5	2010	Lake Michigan (Racine)	27.6 (23.0, 29.8)	3.3 ± 1.1	1.53 ± 0.57
O. mykiss						
Siscowet Lake trout	3	2011	Lake Superior (Ashland)	26.2 (23.9, 28.3)	12.7 ± 1.2	2.26 ± 0.77
S. namaycush						
Walleye*	4	2010	Spillerberg Lake (Ashland), Trout Lake (Vilas)	19.5 (18, 20.9)	2.1 ± 1.5	0.86 ± 0.61
S. vitreus	8	2011	Green Bay (Brown), Peshtigo River (Marinette)	19.2 (15.0, 23.2)	0.8 ± 0.4	0.53 ± 0.27
Yellow Perch*	11	2010	Bear Lake (Forest), Lake Michigan (Racine), Spillerberg Lake (Ashland)	9.2 (6.9, 11.4)	1.0 ± 0.4	0.75 ± 0.34
P. flavescens	13	2011	Lake Superior (Ashland), Peshtigo River (Marinette)	9.5 (7.7, 15)	0.4 ± 0.1	0.38 ± 0.13

* Separate data are reported for each sampling year because [lipids] or [FA] was significantly different between years/locations.

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