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Effect of season and trophic level on fatty acid composition and content of four commercial fish species from Krasnoyarsk Reservoir (Siberia, Russia)

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ABSTRACT

Two groups of factors, phylogenetic and ecological, are presently regarded as controlling fatty acid composition of fish, including essential eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids. Environmental effects, e.g., trophic position, temperature and/or seasonality, were previously studied using sums of fatty acids or only their level data. We tested the hypothesis that differences in trophic levels of piscivorous (pike and perch) and omnivorous (roach and bream) fish from a mesotrophic reservoir allow discriminating levels and contents of individual fatty acids, especially EPA and DHA. The more established measurements, i.e., stomach contents and carbon and nitrogen stable isotopes in fish muscles, were also carried out to provide linkages between the different ecological tracers, fatty acids versus stable isotopes, and matching the methods for long-term food sources (fatty acids and stable isotopes) and recent foraging (stomach content analysis). We also studied a putative influence of seasonality. Similar to other studies, there were seasonal changes in fatty acid composition and contents of two fish, perch and roach, due to direct and indirect effects of water temperature. Meanwhile, the piscivorous and omnivorous species captured in the same month, were explicitly differentiated on a base of stable isotopes and fatty acids. Significantly higher percentages and contents of DHA in piscivorous fish, perch and pike, relatively to those in roach and bream, likely indicated a higher trophic transfer efficiency for this essential fatty acid. All the fishes have commercial importance for regional fishery and are harvested from the studied reservoir for human nutrition. Regarding content of EPA + DHA ($mg\,g^{-1}$ fish) as the indicator of nutritive value for humans, pike had the highest nutritive value, roach and perch had intermediate overlapped values, and bream was of the least benefit.

1. Introduction

Consumption of fish is an important part of human diet, accounting for about 17 percent of the global population's intake of animal protein (FAO, 2016). In addition to protein, wild fish are unique and rich sources of such essential compounds as polyunsaturated fatty acids (PUFA), eicosapentaenoic acid (20:5n-3, EPA) and docosahexaenoic acid (22:6n-3, DHA), in human western diets (Robert 2006; Gladyshev et al., 2013, 2015b). EPA and DHA are biochemical precursors of important signaling molecules (prostaglandins, thromboxanes, leukotrienes, neuroprotectins) and on the base of over 30 years of human clinical trials and epidemiological surveys have been specifically recommended for prevention of cardiovascular diseases, psychiatric disorders and some other illnesses (Hibbeln et al., 2006; Plourde and Cunnane 2007; Bazan 2009; De Caterina 2011; Casula et al., 2013). Mechanisms underlying the cardioprotective effects of EPA and DHA as the signaling molecule (endogenous mediators) precursors, include arrhythmia prevention, vascular relaxation improvement, antiinflammatory responses, platelet aggregation inhibition and enhancement of plaque stability (e.g., Adkins and Kelley, 2010). To reduce the risk of morbidity and mortality from cardiovascular disease, a number of international and national health organizations recommend personal intake of 0.5–1.0 g of EPA + DHA per day (Kris-Etherton et al., 2009; Adkins and Kelley 2010).

The main indicator of nutritive value of fish for humans, content of EPA + DHA ($mg\,g^{-1}$ of wet weight) in edible part, muscle tissues (filets), can vary among species and habitats by more than

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two orders of magnitude (Gladyshev et al., 2013). Exact causes of such great variations are unknown yet. Phylogenetic (species identity) factor may be of importance for fatty acid composition and content, i.e., some species contain extremely small amounts of EPA and DHA in their flesh (e.g., Kwetegyeka et al., 2008; Vasconi et al., 2015). In addition to phylogeny, fatty acid composition and PUFA supplies in fish may vary within a given species due to various physiological and ecological factors (e.g., Ahlgren et al., 2009; Lau et al., 2012; Vasconi et al., 2015).

Main ecological factors are believed to be food and water temperature, which are determined by type of habitat and season (e.g., Ahlgren et al., 1996, 2009; Sushchik et al., 2006; Czesny et al., 2011; Guler et al., 2011; Vasconi et al., 2015). In addition, seasonal changes of reproductive phases, e.g. ripening, spawning and regeneration, which lead to the mobilisation and re-allocation of endogenous reserves, also affect fatty acid composition both in reserve somatic tissues, muscle and liver, and in gonads of fish (Mairesse et al., 2006; Perez et al., 2007; Sushchik et al., 2007; Rojbek et al., 2014). A relative importance of the above ecological factor is still unknown; moreover, results of experimental and field studies often are controversial (Gribble et al., 2016). Recently, trophic position of fish, e.g., herbivorous, omnivorous (invertivorous) or piscivorous, was shown to determine their FA composition (e.g., Ahlgren et al., 2009; Czesny et al., 2011; Vasconi et al., 2015). For instance, in two freshwater studies species that occupied higher trophic position, i.e. whose diet were part or all fish, contained higher proportion of PUFA of *n*-3 and *n*-6 families (Williams et al., 2014; Vasconi et al., 2015). The cited authors indicated that trophic position (food habits) was illuminating in characterization of fatty acid composition compared to phylogenetic factor (taxonomic family).

As found recently, trophic transfer efficiency (TTE), measured as the ratio between production of a trophic level and that of the previous level, was two-fold higher for long-chain PUFA than for total organic carbon and short-chain PUFA (Gladyshev et al., 2011). The higher TTE results in higher proportions (% of total fatty acids) and/or contents (mg/g tissue) in upper levels of trophic chains: phytoplankton (seston) – zooplankton (e.g., Kainz et al., 2004), phytobenthos – zoobenthos (Gladyshev et al., 2009b), fish – bird (Gladyshev et al., 2010a) and plankton – fish (Strandberg et al., 2015). For fish of different trophic levels, there are pioneer data of Ahlgren et al. (1996) on FA content of omnivorous and carnivorous species. However, in the cited work sums of FAs of certain structural groups (saturated, mono- and polyunsaturated, EPA + DHA) were compared. Meanwhile, as demonstrated by Strandberg et al. (2015), trophic transfer patterns of *n*-3 PUFA, including EPA and DHA, from food to fish related to their molecular structure. Thus, comparison of individual fatty acids, rather their sums, in biomass of fish of different trophic levels appears to be important.

In our work, we aimed to compare fatty acid composition and content using individual FA of two piscivorous and two planktivorous-benthivorous fish species from a large mesotrophic water body, Krasnoyarsk Reservoir, which is located in Central Siberia, Russia. The reservoir is one of the largest regional freshwater bodies and provides total amount of caught fish of nearly 1500 metric tons per year (Analytic Reports, 2016). The main commercial fish of interest are Eurasian perch (*Perca fluviatilis*), roach (*Rutilus rutilus*), bream (*Abramis brama*), and pike (*Esox lucius*), which average yearly harvests are of 940, 210, 170, and 15 metric tons, respectively. Most part of the caught fish is sold fresh, and some salted or dried. To take into account putative influence of seasonality, which may confound the comparison of trophic levels, we also studied seasonal dynamics of FA composition and content in two fish species, perch and roach, available during whole period of the study.

2. Methods

2.1. Study site

Krasnoyarsk Reservoir is a large water body that was created in the upper part of the Yenisei River during electric power station building (Fig. 1). It has previously been described in detail (Gladyshev et al., 1993; Ageev et al., 2008). The reservoir is deep (up to 110 m) and thermally stratified, and surface water temperature (0–10 m) varied from near zero (0.8 °C) under ice cover in March to 13 °C–22 °C in June–August (Dubovskaya et al., 2004; Ageev et al., 2008). It is partly eutrophicated, and blooms of nuisance cyanobacteria species regularly occur in bays and stretches. Zooplankton comprises mostly copepods, cladocerans and rotifers. The samples were taken in Ubei Bay, which is situated in the middle part of the reservoir (55°06′59″N, 91°37′44″E).

2.2. Sampling

Four fish species were caught in Ubei Bay of Krasnoyarsk Reservoir (Fig. 1) in spring and summer months of 2014 and 2015 (Table 1). During the summer, the fish were caught using gill nets. Nets were set at a distance of 5–50 m from the shore, at a depth of 3–15 m. In March, perch was caught from under the ice using a hook fishing gear. Weather and variable catch rates resulted in incomplete sampling among the fish species and across each month and year (Table 1). All caught fish were sexually mature. The ratio of males and females was approximately 1:1.

Fish were immediately brought to the nearby laboratory at the Biological station, School of Fundamental Biology and Biotechnology (Siberian Federal University, Krasnoyarsk, Russia). In the laboratory, fish were measured and weighed. Additionally, digestive tracts were removed for analysis of diet composition. For biochemical analyses, samples of the muscle tissues, weighing approximately 2–3 g, were taken from the dorsal side of fish individuals, 1–2 cm below the dorsal fin. When cutting the muscle samples, we avoided red muscles, skin and bones. The samples were divided into two subsamples: for fatty acid and stable isotope analyses. Stable isotope subsamples were additionally used for moisture measurements. For fatty acid analyses, ca. 1 g of muscle tissues were placed into chloroform: methanol mixture (2:1, volume/volume) and kept until further analysis at −20 °C. To measure moisture and stable isotope analyses, subsamples of ca. 1–2 g of wet weight were weighed, dried at 75 °C until constant weight, and weighed dry. Then, they were kept in a desiccator for further stable isotope elemental analysis.

2.3. Diet composition analysis

To characterize the diet of fish species, digestive tracts were removed through longitudinal cuts in the abdomen using a scissor, scalpel and tweezers. For Cyprinidae species (roach and bream), only content of the first 1/3 of intestine was analyzed, due to a high degree of digestion in the final part. For piscivorous fish (perch and pike), the stomach contents were used to analyze the diet composition. Food items were identified to the lowest practical taxonomic level (order or class) and sorted under optical and stereoscopic microscopes. To tentatively quantify the diet composition, we counted items, summed, and visually estimated their approximate volumetric portion of the total content in each digestive tract analyzed. Then, the diet items were divided into three groups based on their approximate percentage of the total stomach volume (range of 30–60%, 10–30%, 1–10% of the total, respectively) for a given species in a given month.