Stable carbon and nitrogen isotope ratios and fatty acid profiles in fishes in the Kaeng Krachan Reservoir, Thailand

The name(s) of the author(s):

Ayano Medo1,\*, Nobuhito Ohte1, Keisuke Koba2, Nobuaki Arai3, Yasushi Mitsunaga4, Hideaki Nishizawa1, Manabu Kume3, Thavee Viputhanumas5, Kiattipong Kamdee6, Chakrit Saengkorakot6, Koki Ikeya7, Iroha Yamada4, Tatsuya Sugawara8, Yuki Manabe8, Akiko S. Goto2, Ayako Yokoyama8, Hiroyuki Yamane9, Hiroki Kajitani1, Daichi Kojima1, Takashi Nose1, & Hiromichi Mitamura3,8

The affiliation(s) of the author(s):

1Graduate School of Informatics, Kyoto University, Yoshida-honmachi, Sakyo, Kyoto, 606-8501 Japan

2Center for Ecological Research, Kyoto University, 2-509-3 Hirano, Otsu, Shiga, 520-2113 Japan

3Field Science Education and Research Center, Kyoto University, Kitashirakawa- Oiwake-cho, Sakyo, Kyoto, 606-8502 Japan

4Faculty of Agriculture, Kindai University, 3327-204 Nakamachi, Nara, 631-8505 Japan

5Inland Aquaculture Research and Development Division, Department of Fisheries, 50 Phahonyothin Rd., Lat Yao, Chatuchak, Bangkok, 10900 Thailand

6Thailand Institute of Nuclear Technology, 9/9 Village No. 7, Sai Mun Subdistrict, Ongkharak District, Nakhon Nayok, 26120 Thailand

7Gifu World Freshwater Aquarium, Kawashima Kasada-machi, Kakamigahara, Gifu, 5016021 Japan

8Graduate School of Agriculture, Kyoto University, Kitashirakawa- Oiwake-cho, Sakyo, Kyoto, 606-8502 Japan

9Chill Co., Ltd., 5-12-12 Sakae, Niiza, Saitama, 352-0014 Japan

\*Corresponding author:

Ayano Medo

Postal address: Graduate School of Informatics, Kyoto University, Yoshida-honmachi, Sakyo, Kyoto, 606-8501 Japan

E-mail: medo.ayano@gmail.com

Description

Sample collection

The Mekong giant catfish specimens were collected in the Kaeng Krachan Reservoir, Phetchaburi Province, western Thailand (12º54´ N, 99º36´ E) from 2017 to 2019. We collected the Mekong giant catfish of 148–230 cm in total length (TL) caught by the local fisherman during the catfish fishing season of the reservoir, from November to December in 2017 to 2019. After measuring the TL (cm) of the catfish, we collected dorsal muscles (*n* = 29) for stable isotope analyses and ventral muscles (*n* = 15) for fatty acid (FA) analyses. We could also access the three juvenile samples (35–42 cm in TL) caught accidentally during the closed fishing season, from July to August 2017, and collected the dorsal muscles for stable isotope analyses. We also collected dorsal muscles from eight fish species (*Hampala macrolepidota*, *Channa striata*, *Hemibagrus nemurus*, *Notopterus notopterus*, *Puntioplites proctozystron*, *Oreochromis niloticus*, *Hypsibarbus wetmorei*, and *Labeo rohita*) for stable isotope analysis and then collected ventral muscles from five out of the eight species (*Hampala macrolepidota*, *Hemibagrus nemurus*, *Puntioplites proctozystron*, *Oreochromis niloticus*, and *Hypsibarbus wetmorei*) for FA analysis. These fish species were selected to cover widely feeding habits (i.e., piscivore, carnivore, insectivore, and herbivore). All fish specimens were bought at local fish markets around the reservoir. To identify stable isotope values of lower trophic levels, we collected a variety of organisms and substrates, including aquatic insects (e.g., Trichoptera, Anisoptera larvae, Cymothoidae, *n* = 56), shrimp (*n* = 13), Chironomids larvae (n = 45), periphyton (*n* = 51), particulate organic matter (POM, *n* = 87), throughout three years (2017–2019). We also collected bottom sediments (*n* = 16) for one year in 2019. Periphyton was collected from the buoys, and then invertebrates (i.e., aquatic insects and shrimps) were removed from the periphyton. Shrimps were also collected near the shore area in the reservoir using a dip net (2 mm mesh size). Using a Van Dorn water sampler (water capacity, 6.0 L; Miyamoto Riken Ind. Co, Ltd., Osaka, Japan), we collected 1–2 L of water samples at eight sites at three depths (0, 5, and 10 m) to draw POM samples by suction filtration with a glass microfiber GF/F filter (Whatman, Maidstone, UK). Sediment samples were collected using an Ekman-Birge grab at a depth of 3–30 m at 12 sites. We stored all samples in a freezer at –20 ºC until further processing.

Stable isotope analysis

The sample processing for the stable isotope analysis for the catfish and the other specimens was conducted in Thailand Institute of Nuclear Technology (TINT) and Kyoto University, respectively. Biota samples (fish, periphyton, aquatic insects, and shrimp) were oven-dried at 50 ºC for 48–72 h and then were powdered by mortar and pestle. The powdered fish samples were delipidated with chloroform-methanol (2:1 v/v). Approximately 0.5 mg of the samples was packed into tin capsules for *δ*13C and *δ*15N analyses. After POM (GF/F filters) and sediment samples were freeze-dried for 24 h, we packed the GF/F filters cut into 1/16–1/8 pieces and 2.0–5.0 mg of the sediment samples into tin capsules, respectively.

*δ*13C and *δ*15N for the catfish and the other samples were measured using EA-IRMS (Delta V equipped with an elemental analyzer Flash EA 2000, Thermo Fisher Scientific, MA, USA) in TINT and Center for Ecological Research, Kyoto University (CERKU), respectively. The sediment samples were measured using the EA-IRMS modified for microanalysis in CERKU (Koba et al., 2021) due to its low total carbon and nitrogen contents. *δ*13C and *δ*15N (in ‰) were expressed as the deviation from standards according to the following equation:

*δ*X = [ (Rsample / Rstandard) - 1] × 1000

where X is 13C or 15N, Rsample is the ratio (13C/12C or 15N/14N) in the sample, and Rstandard is the ratio in the standard. The standard reference materials were PeeDee Belemnite carbonate and atmospheric N2 for the carbon and nitrogen samples, respectively. Three working standards (CERKU-02, 03, and 05) were used to calibrate *δ*13C and *δ*15N (Tayasu et al., 2011). The overall analytical precision (standard deviation, SD) for our analyses in both facilities based on three working standards was within ± 0.1‰ for *δ*13C and within ± 0.2‰ for *δ*15N.

Fatty acid analysis

We used the subadult Mekong giant catfish and five out of eight fish species for FA analysis. The sample processing for the FA analysis for the catfish and the other fish species was conducted in TINT and Kyoto University, respectively. All muscle samples of the fish were freeze-dried for 24–48 h and then were crushed by scissors. We used the samples of 100 mg in dry mass for the lipid extractions by Bligh-Dyer method (Bligh & Dyer, 1959). Then, we conducted saponification and methylation of the free-lipids by HCl–methanol (one-step method). For the catfish, we identified the structures of the individual fatty acid methyl esters (FAMEs) separately and then identified the FAME composition by a gas chromatography (GC) with a MS (GCMS-QP2020, Shimadzu Scientific Instruments, Kyoto, Japan), recording electron impact mass spectra, in Kasetsart University, Thailand. For the other fishes, the FAME composition was quantitatively analyzed by a GC coupled to a flame-ionization detector (FID; GC-14B, Shimadzu Scientific Instruments, Kyoto, Japan), and the structures of the individual FAMEs were separately identified by using GC with a mass spectrometer (MS; GCMS-QP5050, Shimadzu Scientific Instruments, Kyoto, Japan) in Kyoto University, Japan. Each GC was equipped with a Omegawax capillary column (30 m × 0.25 mm, i.d., 0.25 µm film thickness, Supelco, USA). The following temperature gradient and instrument settings were used: the GC columns initial temperature was 140 °C; this was held for 2.5 min then ramped at 4 °C/min to 240 °C and then kept for 15 min; giving a total runtime of 42.5 min. ﻿Nitrogen and helium gas was used as the carrier for FID and MS, respectively. The individual FAMEs were identified by comparing with the retention times of authentic standard mixtures, Supelco FAME mix 37 components (Sigma-Aldrich, St. Louis, MO, US), and with spectrographic patterns from mass spectral library (NIST107.LIB and NIST21.LIB). The proportion of FA (%) was determined by ratio of each FAME peak area to the total areas of all peaks from FID analysis. The FAs were abbreviated following the common nomenclature X:Yn-Z, where X is the number of carbons, Y is the number of double bonds, and Z is the position of the first double bond counted from the end of methyl group in FA (e.g. 20:5n-3). i- and a- of branched FA represented iso- and anteiso-, respectively.

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