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Article in *Isotopes in Environmental and Health Studies* · March 2016

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Turnover of hydrogen isotopes in lake sturgeon blood: implications for tracking movements of wild populations

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ABSTRACT

Naturally occurring deuterium (^2H) in biota can be used to trace movement, migration and geographic origin of a range of organisms. However, to evaluate movements of animals using $\delta^2\text{H}$ measurements of tissues, it is necessary to establish the turnover time of ^2H in the tissues and the extent of isotopic discrimination from different environmental ^2H sources to those tissues. We investigated the turnover of ^2H in lake sturgeon (*Acipenser fulvescens*) blood by manipulating both environmental water $\delta^2\text{H}$ and diet $\delta^2\text{H}$ over a four-month period. The half-life of deuterium in lake sturgeon blood was 37.9 days after an increase in the environmental water $\delta^2\text{H}$ of +714 ‰. However, no clear turnover in blood ^2H occurred over the same period in a separate trial following a change of -63.8 ‰ or +94.2 ‰ in diet. These findings suggest that environmental water ^2H exchanges much faster with blood than diets and that blood $\delta^2\text{H}$ values can be used to trace movements of sturgeon and other fish moving among isotopically distinct waters.

ARTICLE HISTORY

Received 14 April 2015

Accepted 28 November 2015

KEYWORDS

Blood; fish; hydrogen-2; isotope ecology; lake sturgeon; migration; stable isotope tracer technique; tissue turnover

1. Introduction

Naturally occurring stable isotopes of several elements have been used to infer movements of a number of organisms.[1] The basic premise of this approach is that isotopic patterns in the environment often show spatial structure as 'isoscapes' and animals can acquire isotopic signals related to position based on their movements across and within such isoscapes. In terrestrial systems, stable hydrogen isotope analysis ($\delta^2\text{H}$) has been used to elucidate movements of a wide range of organisms from insects to humans.[2-7] This approach is based on the fact that $\delta^2\text{H}$ values of precipitation are influenced primarily by ambient temperature and distance from source [8] resulting in continent-wide spatial patterns or isoscapes.[9] These isotopic patterns in local waters are incorporated into locally formed animal tissues providing an isotopic

signal of origin. This signal is detectable in the tissues for a period that depends on the turnover rate of the tissue in question and the movement of the animal.[1,3] However, the environmental isotopic signal is complicated by the fact that organism tissue $\delta^2\text{H}$ is influenced by both the assimilated food and ambient water and, in addition, the isotopic composition of diet can vary depending on feeding habits of each species.[10,11] Assigning fish to prior locations using $\delta^2\text{H}$ measurements thus requires an understanding of tissue- and species-specific isotopic discrimination and turnover information.

The metabolic rate of a given tissue and the metabolic pathways linking that tissue to a dietary or environmental source govern the rate and extent to which a new isotopic dietary composition is incorporated into a particular tissue type.[12] For example, metabolically active splanchnic tissues such as blood plasma and liver have a much faster tissue turnover rate than relatively metabolically inactive structural tissues such as bone or hair. [12–15] Despite some studies into the turnover rate of stable isotopes in blood of non-piscine species [16,17] and some in fish tissues,[14,18,19] few have focused on turnover of H isotopes in fish.[20,21]

Lake sturgeon (*Acipenser fulvescens* Rafinesque 1817) is an endemic North American benthic species ranging from Central USA, through to the Great Lakes and Hudson Bay drainages of Canada.[22] However, a sustained decline in the species' range and abundance [23] has led to the listing of lake sturgeon populations as either endangered or of special concern by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC).[24] Lake sturgeon decline has been linked to the species' long lifespan, late age to reproductive maturity and protracted spawning frequency.[25] which has resulted in poor recovery from historic overfishing.[26] However, the most pressing factor limiting sturgeon recovery is current habitat loss and degradation.[23,26–29]

In Western Canada, the most abundant population of lake sturgeon occurs in the Saskatchewan River basin.[29] However, this population is still considered endangered by the COSEWIC and is currently under consideration for status under the Species at Risk Act.[30] Of particular concern are the impoundments in the basin that interrupt the species' ability to migrate.[31] Therefore, an understanding of how the Saskatchewan River basin is used by lake sturgeon is essential in future management planning for this species. To assess sturgeon movement, a multidisciplinary approach is currently being undertaken by the Water Security Agency, Saskatchewan. Radio tracking data indicate that sturgeon move along all arms of the Saskatchewan River system.[31,32] However, the relative importance of each tributary remains unclear. Further, the high cost and potential influence on sturgeon behaviour of exogenous markers [33] led to the ongoing investigation of intrinsic markers for determining migration movements.

As part of a larger programme to use stable isotope measurements to examine movement patterns in sturgeon that perform seasonal migrations among waters that differ in $\delta^2\text{H}$ values, our objective was to experimentally establish baseline turnover and discrimination of deuterium in sturgeon blood when exposed to changes in $\delta^2\text{H}$ values in environmental waters and diet using stable isotope tracer technique. Estimates of those parameters would then inform spatial assignment of wild sturgeon to tributary of recent origin.

2. Materials and methods

2.1. Experimental design

We obtained sturgeon during the summer of 2012 from Genoa National Fish Hatchery, WI, USA. The fry were spawned from a Wolf River, Richmond, WI, USA, population of lake sturgeon. At the start of the experiment, sturgeon were approximately 2 years old (23 ± 1.6 cm standard length). We raised the sturgeon on blood worms (Chironomidae, San Francisco Bay Brand, Inc.) as fry before switching them to a commercial diet of Aqua Pride Trout 42:15 3 mm sinking pellets (ProForm Aquaculture Feed). The mean $\delta^2\text{H}$ of the long-term pelleted diet was -122.7 ± 20 ‰ ($n = 10$). This diet remained unchanged for fish in experiment 1. This pelleted diet consisted of approximately 42 % crude protein, 15 % crude fat and 5 % crude fibre.

2.2. Experiment 1: changing environmental water $\delta^2\text{H}$

We used 10 sturgeon during experiment 1; 5 control and 5 treatment fish. Each fish was housed in an individual 40 L aquarium. Two 100 L tanks were used as head tanks for the control and treatment water sources. All water for tank renewal and top-ups in the experimental tanks was taken from these head tanks. We changed the aquarium water weekly and collected water samples for $\delta^2\text{H}$ analysis from every tank after renewal. Fish were raised in the control water until the start of the experiment. Fish were approximately 2 years of age at the start of the experiment and are expected to be at equilibrium with the pellet food and water after this time. We spiked the local water in the 100 L head treatment tank using 20 mL of 99.9 atom % deuterium oxide (Sigma-Aldrich) to achieve a $\delta^2\text{H}$ value of approximately 500 ‰.

2.3. Experiment 2: changing diet $\delta^2\text{H}$

Due to the unexpected death of some fish during experiment 1, we increased the sample size for experiment 2. A total of 20 sturgeon were used during this experiment; 10 control and 10 treatment fish. Each fish was housed in an individual 40 L aquarium. A continuous-flow system with an approximate flow rate of 15 L/h was connected to each tank to provide continual water renewal. Water temperature remained between 13 and 14 °C throughout the experiment, and water quality was tested regularly for the pH, nitrate and chlorine levels using 6-in-1 aquarium test strips (Tetra).

All experimental sturgeon were switched from the long-term pelleted food they were raised on to treatment food 1 on day one of the experiment. Crayfish from isotopically distinct waters were selected as the diet of choice for this study to minimize any variation in the partitioning of protein to lipid content in the diet. Each crayfish diet was treated identically. Treatment food 1 consisted of locally caught crayfish (*Oroconectes virilis* Hagen 1870) collected from the Saskatchewan River and Qu'Appelle River basins, Saskatchewan. Whole frozen crayfish were homogenized using a Kenwood food processor, the mean $\delta^2\text{H}$ of this homogenate was -186.5 ± 17 ‰ ($n = 10$). After eight weeks, experimental sturgeon were switched to treatment food 2 with a higher $\delta^2\text{H}$ diet consisting of crayfish (*Procambarus clarkii* Girard 1852) from the Louisiana Crawfish Company (Natchitoches, LA, USA). Whole frozen treatment food 2 crayfish were homogenized in the same manner as the

treatment food 1 crayfish, and the mean $\delta^2\text{H}$ of the resulting homogenate was -92.3 ± 8 ‰ ($n = 10$). Sturgeon were fed daily ad libitum, as determined by excess food in the tanks after feeding. We collected water samples from all tanks five times throughout the experiment at days 0, 28, 55, 84 and 112 to ensure a relatively constant water $\delta^2\text{H}$. We weighed and measured each fish on days 0, 55 and 112 (corresponding to diet shifts) to test for effects on $\delta^2\text{H}$ of any growth and size differences among sturgeon. Measurements included girth (behind the pectoral fins), total length (head to tip of tail), fork length (head to beginning of fork) and standard length (head to beginning of tail fin).

2.4. Sampling and sample treatment

We took blood samples from each sturgeon individual weekly or bi-weekly using a 29 G 1/2^l Covidien Monojet 3/10 mL insulin syringe. Approximately 4 μL of blood was taken from the caudal vein between the caudal and tail fin. Sampling was reduced to bi-weekly during experiment 1 as turnover in deuterium was slower than anticipated and this sampling regime still enabled the turnover to be observed while minimizing stress to the fish. We stored all blood samples in 10 mL vacutainer's (BD Franklin Lakes, NJ, USA) and froze them until preparation. Blood samples were dried at 60 °C for 48 h, and approximately 0.35 mg was weighed into silver capsules for analysis. All diet samples were dried at 60 °C for 44 h and lipid extracted by soaking and rinsing in a solution of 2:1 v/v chloroform–methanol. Samples were then ground using a mortar and pestle, and 0.35 mg was weighed into silver capsules for analysis.

2.5. Stable isotope analyses

Stable hydrogen isotope analysis was conducted at the Stable Isotope Laboratory, Environment Canada, Saskatoon, Saskatchewan. Dried blood samples were pyrolysed at 1350 °C using a Eurovector 3000TM elemental analyser, and the H_2 produced was analysed using a continuous-flow isotope-ratio mass spectrometer (Micromass IsoprimeTM with an electrostatic analyser). We used the Environment Canada keratin standards CBS (-197 ‰) and KHS (-54.1 ‰) to obtain estimates of the non-exchangeable $\delta^2\text{H}$ values of samples according to the comparative equilibration approach.[34] Within-run error of standards was ± 2 ‰ (standard deviations, $n = 5$). Water samples were analysed using a DLT-100 v.1 Liquid Water Isotope Analyzer (Los Gatos Research). Precision of $\delta^2\text{H}$ in standard waters is ± 1 ‰ and ± 5 ‰ in enriched samples.

Results are expressed in delta notation (δ), in units of per mil (‰). We normalized all isotope values reported to the Vienna Standard Mean Ocean Water – Standard Light Antarctic Precipitation standard scale (VSMOW-SLAP).

2.6. Data analyses

Tissue turnover was modelled using the exponential model $\delta^2\text{H} = a + b \cdot \exp^{ct}$ as outlined by Hobson and Clark [16] where the blood $\delta^2\text{H}$ was a function of the initial and asymptotic conditions (a and b), the turnover rate (c) and the time since the deuterium switch (t). The half-life of deuterium in the sturgeon blood was defined as $\ln(0.5)/c$. Significance tests were conducted using R version 3.0.3 (R Foundation for Statistical Computing).

Exponential models were estimated using the non-linear regression function in XLSTAT version 2014.2.06 (Addinsoft™) with the input Function as $y = pr1 \cdot \exp(pr2 \cdot X1) + pr3$, where $pr1$, $pr2$ and $pr3$ are the parameters estimated by the model. Error estimates of the individual model parameters are given as standard error. The overall error estimate for turnover time in days was calculated by combining the percentage error at all three parameters to find a total percentage error for the model. All errors reported for mean values are presented as standard deviations of the mean.

3. Results

3.1. Experiment 1: changing water $\delta^2\text{H}$

Of the 10 experimental fish, 2 control fish and 1 treatment fish died during the experiment. The loss of fish occurred in different weeks and did not coincide with untoward water quality or obvious disease in the tanks and is thought not to have influenced the health and results of the remaining experimental fish.

Mean (\pm SD) $\delta^2\text{H}$ of control water was -130.8 ± 5.4 ‰ ($n = 5$) with mean $\delta^2\text{H}$ of control fish blood of -164.8 ± 6.4 ‰ ($n = 5$) across the trial. The $\delta^2\text{H}$ of control fish blood remained constant throughout the experiment (Figure 1). Mean $\delta^2\text{H}$ of treatment water was $+445.4 \pm 55$ ‰ ($n = 5$). At the commencement of the experiment, mean $\delta^2\text{H}$ of lake sturgeon blood was -151.3 ± 7.6 ‰ ($n = 5$) and at the end of the experiment on day 161 mean $\delta^2\text{H}$ of sturgeon blood was -32.5 ± 25.5 ‰ ($n = 4$).

The pooled half-life of deuterium in sturgeon blood with changing environmental water deuterium was 37.9 ± 33 days, with an exponential growth model of $\delta^2\text{H} = -141 \exp^{-0.018t} - 17.2$ (Figure 1). The half-life of deuterium in the blood of individual fish varied, with the fastest turnover time being 28 days for Fish 2 and the longest half-life

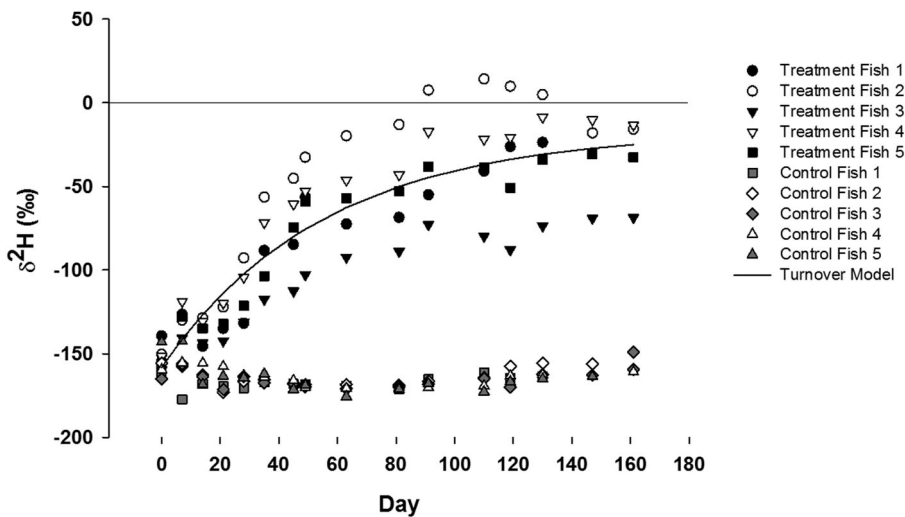


Figure 1. Change in lake sturgeon blood deuterium composition over time after a shift in environmental water $\delta^2\text{H}$ values at day 0. Initial sample size of both treatment and control fish was 5. Tissue turnover was modelled as $\delta^2\text{H} = -140.805e^{-0.018t} - 17.226$, where t is the number of days since the deuterium shift and the half-life of deuterium in lake sturgeon blood is 37.9 days.

Table 1. Tissue turnover half-lives for lake sturgeon blood $\delta^2\text{H}$ modelled as $\delta^2\text{H} = a + b \cdot \exp^{ct}$, where the blood $\delta^2\text{H}$ was a function of the initial and asymptotic conditions (a and b), the turnover rate (c) and the time since the deuterium switch (t).

Fish	A	b	c	R^2	Half-Life (days)
Fish 1	40.5 ± 88	-189 ± 82	-0.008 ± 0.006	0.90	85.08
Fish 2	8.94 ± 10	-175 ± 13.1	-0.025 ± 0.005	0.93	27.96
Fish 3	-60.5 ± 7.8	-99.8 ± 7.0	-0.016 ± 0.003	0.96	44.48
Fish 4	0.123 ± 9.1	-154 ± 9.0	-0.019 ± 0.003	0.96	37.46
Fish 5	-22.4 ± 10	-137 ± 10	-0.019 ± 0.004	0.94	37.30
Overall	-17.2 ± 10	-141 ± 10	-0.018 ± 0.004	0.76	37.85

Notes: The half-life is calculated as $\ln(0.5)/c$. Error is reported as the standard error for each parameter. Fish 1 did not survive the duration of the experiment.

determined for Fish 1, which did not survive the duration of the experiment, at 85 days (Table 1).

3.2. Experiment 2: changing diet $\delta^2\text{H}$

Despite a similar starting mass, changes in body mass across the experiment were inconsistent between the control and treatment sturgeon (Table 2). However, the majority of experimental fish lost mass throughout the experiment. Mean mass of control (89.4 ± 21.7 g; $n = 10$) and treatment sturgeon (102.3 ± 19.9 g; $n = 10$) were not significantly different (t -test, $t = -1.38$, $df = 17.9$, $p = .184$) at the start of the experiment. For control fish, the mean mass change was -11.2 ± 5.5 % of their initial body mass (range of -5.0 to -23.8 %). For treatment fish, mean mass change was -1.4 ± 10.2 % (range of +8.4 to -28 %). Despite the loss of mass, a slight increase in standard length was observed for

Table 2. Stable hydrogen isotope values ($\delta^2\text{H}$), mass (g) and standard length (cm) measurements of control lake sturgeon (CF1–CF 10) and treatment lake sturgeon (TF1–TF10) throughout a diet switch experiment.

	$\delta^2\text{H}$			Mass (g)			Standard length (cm)		
	Day 0	Day 55	Day 105	Day 0	Day 55	Day 105	Day 0	Day 55	Day 105
CF1	-178.9	-171.2	-172.0	82.0	82.5	75.0	22.5	23.8	23.6
CF2	-176.0	-178.5	-171.8	118.8	112.0	107.5	25.5	26.5	28.4
CF3	-175.0	-167.9	-175.5	104.6	100.8	96.2	23.0	25.4	25.0
CF4	-169.8	-174.3	-175.8	101.4	94.4	92.9	23.0	24.5	24.0
CF5	-175.2	-168.3	-177.7	78.1	76.6	67.7	22.0	23.5	23.6
CF6	-185.9	-177.9	-166.2	91.8	85.8	78.7	23.0	23.7	23.5
CF7	-166.3	-172.9	-173.3	50.4	47.7	38.4	19.5	20.1	20.8
CF8	-176.0	-179.4	-169.5	103.7	103.7	98.5	23.0	24.0	24.0
CF9	-179.9	-164.0	-170.1	59.6	59.3	50.7	20.6	20.5	20.5
CF10	-174.7	-176.9	-151.1	103.6	100.4	96.8	23.3	24.5	24.7
TF1	-174.0	-174.1	-164.4	111.4	108.5	106.1	23.3	25.5	24.5
TF2	-188.7	-176.6	-161.1	105.5	93.6	75.9	24.2	25.0	25.0
TF3	-164.3	-170.9	-163.7	70.6	67.9	71.1	21.0	22.0	22.0
TF4	-183.4	-170.7	-162.0	88.5	84.4	90.6	22.7	24.0	23.7
TF5	-192.9	-169.9	-161.2	112.9	111.0	110.2	24.7	25.0	25.5
TF6	-170.5	-169.5	-164.2	71.8	69.4	77.8	21.2	22.5	22.0
TF7	-167.6	-175.1	-160.4	121.5	119.3	123.2	23.4	24.4	23.9
TF8	-203.1	-177.6	-162.7	95.3	90.5	102.4	23.1	25.5	24.5
TF9	-170.1	-181.9	-164.1	123.6	118.8	120.8	25.0	26.0	26.0
TF10	-168.1	-172.2	-186.3	121.7	122.2	125.3	25.5	26.5	26.6

Notes: At day 0 all fish were switched to a diet with a $\delta^2\text{H}$ of -186.5 ± 17 ‰. At day 55 only treatment sturgeon were switched to a diet with a $\delta^2\text{H}$ of -92.3 ± 7.5 ‰. The experiment ceased on day 105.

both control and treatment fish (Table 2). Mean increase in standard length for control fish was 1.3 ± 0.8 cm (range 0–2.9 cm). Mean increase in standard length for treatment fish was 1.0 ± 0.3 cm (range 0.5–1.4 cm). This equated to a 5.5 ± 3.2 % increase in standard length for control fish and a 4.1 ± 1.1 % increase for treatment fish.

Despite isotopic difference in diets, no clear tissue turnover was observed in lake sturgeon blood with changing dietary $\delta^2\text{H}$ during either diet switch. At the start of the experiment treatment and control fish had a mean blood $\delta^2\text{H}$ of -178.3 ± 13 ‰ and -175.7 ± 5.7 ‰, respectively. At the end of the experiment treatment and control fish had a mean blood $\delta^2\text{H}$ of -165.0 ± 7.6 ‰ and -171.9 ± 9.0 ‰, respectively. If we consider treatment fish in isolation, at the start of the second diet shift (day 55) the mean blood $\delta^2\text{H}$ was $-183. \pm 4.2$ ‰, which increased to a mean blood $\delta^2\text{H}$ of -165.0 ± 7.6 ‰ by the end of the experiment. A change in mean $\delta^2\text{H}$ in control fish occurred on the same scale observed in treatment fish during this time with the mean $\delta^2\text{H}$ increasing from -191 ± 13 ‰ on day 63 to -170.3 ± 7.6 ‰ on day 105 despite a constant deuterium depleted diet for control fish. This change in sturgeon $\delta^2\text{H}$ coincided with a mean change in water $\delta^2\text{H}$ for all tanks of -136.8 ± 1.5 ‰ to -132.9 ± 3.7 ‰. However, over the duration of the experiment mean $\delta^2\text{H}$ of tank water was -135.3 ± 3.5 ‰ ($n = 10$) for control tanks and -136.1 ± 2.7 ‰ ($n = 10$) for treatment tanks.

4. Discussion

Our findings highlight how the hydrogen isotopic content of environmental water is incorporated into sturgeon blood, and this has important ramifications for tracing movements of sturgeon among isotopically distinct waters. Our finding for turnover times related to diet changes was more equivocal due to an experimental design which may not have allowed adequate time on experimental diets. However, unlike patterns found for terrestrial homeotherms,[35,36] our study indicates that for blood tissue of lake sturgeon, diet may play a less significant role in the short-term turnover of blood hydrogen. For the control fish that were only subjected to a single diet shift, no turnover in the tissues was observed despite twice the equilibration time (i.e. 16 weeks) to the diet than in the treatment sturgeon. For treatment sturgeon that underwent a shift to a more depleted deuterium source, followed by a shift to a more enriched source, opposing direction of the isotopic shift in diet clearly complicates interpretation. Nonetheless, the lack of a clear short-term change in blood $\delta^2\text{H}$ with changing $\delta^2\text{H}$ in dietary sources in light of a relatively rapid change due to changes in ambient water $\delta^2\text{H}$ has implications for the future study of migratory fish. For example, it may facilitate the use of isotope analysis for mapping migratory routes of fasting individuals that move between isotopically distinct waters. Future studies examining tissue turnover in sturgeon due to changes in dietary $\delta^2\text{H}$ will need to consider an experimental design involving many months and a substantial unidirectional change in dietary isotope values.

Our research has implications for the use of two-source mixing models when considering $\delta^2\text{H}$ in blood of sturgeon and potentially other aquatic organisms. For fish that have recently migrated, the use of two-source mixing models based on consumer blood to account for both a dietary source and an environmental water source, such as those described previously,[35–37] may be problematic if not all tissues are in equilibrium

with their dietary and environmental water $\delta^2\text{H}$. However, care in extrapolating these findings across different aquatic organisms, different tissue types or different time scales must be taken. For example, Solomon et al. [37] demonstrated that for salmonid muscle tissue, $\delta^2\text{H}$ was derived predominantly from the dietary source and not from the environmental water. However, in that study, the fish were at equilibrium with both their environmental water and their diet, and turnover of H in tissues was not considered. Similar findings of diet and water deuterium contributions have been found in other fish species as well.[11] Therefore, while the findings of Solomon et al. [37] do indicate an importance of dietary $\delta^2\text{H}$ to muscle tissue $\delta^2\text{H}$ over longer periods of equilibration, such conditions may be rare in nature where fish may perform short-term movements among isotopically different waters.

Our study suggests that it is a slow process for fish-like sturgeon to reach equilibration between diet and blood. In a diet switch experiment using growing broad whitefish (*Coregonus nasus*), Hesslein et al. [18] noted that turnover in sulphur, nitrogen and carbon was largely a result of growth and not metabolic turnover even in the liver, a highly vascularized organ. They concluded that for slower growing populations this would result in a very slow turnover to a new diet of possibly multiple years. A similar finding was reported for rainbow trout where growth influenced the turnover rate of nitrogen in different tissues more than metabolic replacement did.[14] As the fish in this study showed no mass gain and minimal growth, it is plausible that they were only undergoing maintenance metabolism. Thus, suggesting that growth plays an important factor in the assimilation of deuterium for lake sturgeon. However, it is important to note that the lack of turnover between the diet switches may also be a result of similar isotopic ratios in the assimilated portion of the diet; as dietary $\delta^2\text{H}$ values were reported for total diet and not for diet components individually. To minimize problems with variation in the composition of the chosen diets, both diets consisted of homogenized whole crayfish sourced from isotopically distinct environments. The selection of a single species diet ensured similar physiologies and biochemical processes regarding $\delta^2\text{H}$ in both diets. As the $\delta^2\text{H}$ of invertebrates (including crayfish) is consistent with their environmental waters,[38] we therefore believe that the lack of turnover as a result of dietary $\delta^2\text{H}$ changes is unlikely to reflect similar dietary $\delta^2\text{H}$ of the assimilated portion.

We found a strong response in turnover of ^2H for lake sturgeon blood following our experimental change in the environmental water. Although individual variation in turnover time among fish was observed, a similar trend in turnover was demonstrated for all fish, and an estimated turnover time of 37.9 ± 33 days established. We proposed that the slight variation in tissue turnover rates among surviving fish may be attributed to fish size and differential growth, although no biometrics of fish from experiment 1 were obtained to confirm this. For example, fish with a faster growth rate have faster tissue turnover rates.[39] Further, absolute fish size can influence the turnover rate of individuals as it can impact on the relative contribution of the dietary protein and lipid $\delta^2\text{H}$ in fish tissues. This may be due to differential accumulation of lipid-based food components in fish of differing size.[11] Lastly, the surface-to-volume ratio in fish is inversely proportional to size which alters the relative metabolic water versus environmental water contribution in fish of different sizes.[11] A combination of fish size and differential growth may therefore account for the variation observed in tissue turnover and equilibrium $\delta^2\text{H}$ values of the fish in experiment 1.

Fish body water equilibrates within hours with their environmental water, it then takes longer for this water to be incorporated into protein bound tissues.[20] For example, in

European sea bass (*Dicentrarchus labrax*), plasma body water $\delta^2\text{H}$ equilibrated with environmental water after only 6 h, with clear turnover already evident in blood samples after 1 h following introduction of the fish to deuterium-enriched environmental tank water.[21] This may explain not only the observed turnover in $\delta^2\text{H}$ for experiment 1, but also the observed slight increase in blood $\delta^2\text{H}$ for both control and treatment sturgeon in experiment 2 when an increase in the tank water $\delta^2\text{H}$ was observed. The rapid exchange of hydrogen between body waters and environmental waters may thus account for the observed turnover in lake sturgeon blood in response to changing water $\delta^2\text{H}$. As the diets used in this study were dried, and all available dietary $\delta^2\text{H}$ was protein bound and not from dietary water, it is likely that the lack of turnover we observed in blood, related to dietary isotopic change, resulted from a similar reliance on growth for turnover as described above. Lake sturgeon are slow growing fish,[25] and because individuals in our experiment showed no mass gain over the duration of the experiment, we hypothesize that this resulted in tissue turnover being undetectable using our experimental protocol. To elucidate the diet-to-blood deuterium relationship in lake sturgeon further, future research studying a stock population over the duration of multiple months to years is necessary. A long-scale study may demonstrate a slow but measurable turnover in response to dietary changes in $\delta^2\text{H}$.

The strong association of the environmental water $\delta^2\text{H}$ with that of lake sturgeon blood means that movement of fish between isotopically distinct water bodies can be traced relatively quickly even when fish fast throughout the migration. This information has direct management implications by providing a tracking tool to monitor movements of sturgeon in systems where sturgeon may move among isotopically different tributaries and river stretches. For example, it can help provide managers of water basins with a better estimate of time since migration of a given fish, and thus a better understanding of how the species uses the water system. Heady and Moore [14] have demonstrated the potential of such migratory clocks to estimate movement or diet shifts in rainbow trout. Given the half-life of blood in the lake sturgeon in our study was 37.9 ± 33 days, the timing of movement in sturgeon could be estimated for up to 75 days (i.e. two half-lives) after movement providing those fish were moving between waters that differed enough isotopically.

Migration and movement analysis can be greatly facilitated with blood hydrogen isotope analysis, as only a single sampling event is required to understand movement behaviour. We believe that hydrogen isotope measurements can make significant contributions to our understanding of fish movement patterns, and as a result contribute to the preservation of sturgeon and other endangered fish species.

Acknowledgments

We thank D. Phillips, C. Phillips, E. McVittie and W. Schenn for assistance in collecting crayfish in Saskatchewan, and A. Matichuk from the Saskatchewan Wildlife Federation for administering the project. We also thank A. Schmidt and Geoff Koehler for assistance with laboratory preparation and analysis. David Soto made critical comments on the earlier draft of this paper. We also thank the anonymous reviewers for their invaluable comments to improved the paper.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This research was funded by grants from SaskPower and the Saskatchewan Fish and Wildlife Development Fund to TRoutreach Saskatchewan. An NSERC PGS-D scholarship and Fish and Wildlife Development Fund Scholarship to IDP helped in the development and initiation of this project.

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