Aquat Ecol
https://doi.org/10.1007/s10452-021-09907-9

Effects of short-term decomposition on isotope values of fish tissues under natural conditions

Kathryn S. Peiman · Hsien-Yung Lin · Michael Power · Scott G. Hinch · David A. Patterson · Steven J. Cooke

Received: 3 March 2021 / Accepted: 14 September 2021

Abstract Dead animals may be an important or the only source of tissues to analyze for stable isotopes, with the goal of making inferences about an animal's past ecological history. However, in nature, stable isotope values may be affected by myriad decomposition processes, such as abiotic environmental conditions and bacterial and fungal decay, potentially reducing the accuracy of derived ecological conclusions. We used Pacific salmon carcasses left in air or submerged under water to test whether stable isotope values of metabolically active (skin, muscle, and adipose fin) and inactive (scale) tissues changed over time. We found that the $\delta^{13}C$ values of active tissues were all affected by decomposition but not in a predictable direction, and hard scale tissue was not. In contrast, there was no effect on $\delta^{15}N$ values for any tissue up to 4 days under water or 8 days in air. This suggests that tissues can be analyzed for nitrogen stable isotope values even after several days in water and even if covered in fungus on the surface. For populations within which dead animals are frequently encountered, e.g., salmonid carcass surveys, hard tissues of fish (scales, otoliths) and other animals (baleen, bone, hair, feathers) will likely yield relevant biological information even if soft tissue is rotting, providing

Handling Editor: Télesphore Sime-Ngando.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10452-021-09907-9.

K. S. Peiman (✉) · H.-Y. Lin · S. J. Cooke
Fish Ecology and Conservation Physiology Laboratory, Department of Biology and Institute of Environmental and Interdisciplinary Science, Carleton University, 1125 Colonel By Drive, Ottawa, ON K1S 5B6, Canada
e-mail: kathryn.peiman@carleton.ca

M. Power
Department of Biology, University of Waterloo, 200 University Avenue West, Waterloo, ON N2L 3G1, Canada

S. G. Hinch
Pacific Salmon Ecology and Conservation Laboratory, Department of Forest and Conservation Sciences, University of British Columbia, 2424 Main Mall, Vancouver, BC V6T 1Z4, Canada

D. A. Patterson
Fisheries and Oceans Canada, Cooperative Resource Management Institute, School of Resource and Environmental Management, Simon Fraser University, Burnaby, BC V5A 1S6, Canada

Published online: 05 October 2021

another avenue to collect important data about the life history of those animals.

Keywords Decomposition · Pink salmon · Scale · Stable isotope · Tissue

Introduction

Stable isotope analysis is a frequently used method in ecological studies to identify aspects of an individual's resource and habitat use (e.g., Rubenstein and Hobson 2004; DeNiro and Epstein 1978; Layman et al., 2012). Stable carbon ($\delta^{13}C$ values) and nitrogen ($\delta^{15}N$ values) isotope ratios are the most commonly used isotopes by ecologists and can be used to inform population-level differences and individual specialization in resource and habitat use (Bolnick et al. 2003; Matthews and Mazumder 2004; Newsome et al. 2012). The tissues used to analyze isotopes range from those that are relatively inert (metabolically inactive once formed, e.g., scales, otolith, feathers, hair, baleen, bone) to ones that continually renew (e.g., muscle, skin, fins, mucous, blood), and while these tissues provide useful ecological information about different time points in an organisms' life, they may also be more or less susceptible to degradation after death. Although samples are usually obtained from live or recently euthanized animals, in some field ecology cases, unpreserved tissues from animals that have been dead for several days are often the only samples available for use, especially for rare or hard to sample animals (e.g., Wild et al. 2018; Arthur et al. 2008). The impact of decomposition in metabolic active vs inactive tissues at variable postmortem times is still widely unknown, reducing our ability to make inferences about the animal's past history based on those values.

Tissue decomposition by bacterial and fungal species may affect the accuracy of stable isotope values. Nitrogen in protein is broken down by microbes during proteolysis into amino acids, and the microbes more readily uptake lighter N14 compared to heavier N15 (Balzer et al. 1997), while additionally the production of ammonia during degradation further expels more lighter N14 (Keenan and DeBruyn 2019), both of which result in source tissues becoming enriched in $\delta^{15}N$. However, for carbon, some bacteria selectively break down isotopically heavier amino acids which results in depleted $\delta^{13}C$, while light isotopes are expelled more than heavy isotopes during production of carbon dioxide (CO_2) leaving $\delta^{13}C$ enriched (Dent et al. 2004; Balzer et al. 1997). These processes result in a consistent direction of effect for nitrogen, but conflicting possible directions for changes in carbon isotope values.

Studies on the effects of decomposition on the stability of stable isotope ratios were initially focused on plants (e.g., Fenton and Ritz 1988; Fernandez et al. 2003; Schweizer et al. 1999). More recently, the effect of decomposition on animal tissues has been investigated (Burrows et al. 2014, Yurkowski et al. 2017, Payo Payo et al. 2013, Perkins et al. 2018, Keenan and DeBruyn 2019) building off Ponsard and Amlou's (1999) work using fruit flies. However, most studies have been conducted under laboratory conditions, whereas animals die and decay in natural environments. Many of these studies also froze tissues prior to decomposition, which causes changes in microbial abundance (Davis 1976), a switch from aerobic to anaerobic decomposition (Micozzi 1986; Sulakova et al. 2014), and physical damage to cells during freezing (Dannheim et al. 2007). Previous studies also found inconsistent changes in $\delta^{13}C$ (increase, decrease, or no change) and $\delta^{15}N$ (increase or no change). These differences in methodologies and inconsistencies in results have led to an incomplete understanding of the effects of decomposition, especially as it relates to interpreting isotope signatures obtained from different tissue samples in field studies.

We used Pacific salmon carcasses from spawning grounds as a case study to assess if tissues collected for stable isotope analysis would provide useful information about the past state of these fish. Stable isotopes have been effectively used in many ecological studies of Pacific salmonids (e.g., Johnson and Schindler 2009, 2012; Qin and Kaeriyama 2016) but many salmon populations are in decline, making it undesirable/difficult to sacrifice live individuals. The ability to use carcasses from the spawning grounds would enable further insights into their ocean ecology, such as comparisons of spawned vs non-spawned individuals. We asked whether the tissues of fish carcasses found in natural situations (either on land or under water) could still provide accurate information for stable isotope analysis as decomposition progresses. We predicted that both $\delta^{13}C$ and $\delta^{15}N$ would increase

as time progressed due to preferential uptake of lighter isotopes by microbes; that soft tissues (skin, muscle, and adipose fin) would change, whereas hard scales would not; and that carcasses under water would have larger changes due to more extensive growth of fungus and microbes.

Methods

On September 27–29, 2017, male Pink Salmon (*Oncorhynchus gorbuscha*) were located within 24 h of natural death on their spawning grounds near Lillooet, British Columbia. Once located, each fish was tagged with a dart tag for individual identification and then sampled (day 0). Each sample consisted of removing four tissues: one inactive tissue (scales), and three metabolically active tissues (adipose fin, muscle, and skin). Scales were scraped from the lateral part of the fish posterior to the dorsal fin. Adipose fin was sampled by cutting a piece from the posterior part of the lobe. A plug of muscle with attached skin was cut from the lateral part of the fish ventral to the adipose fin, and subsequent samples were cut in the anterior direction (Fig. 1). Once sampled, fish were alternatively assigned to either air or water treatment, for a final sample size of six fish per treatment. Air treatment fish were placed on their side on a grate under insect netting to prevent insects laying eggs or consuming the fish, but were otherwise exposed to ambient weather conditions. Air temperature ranged from 1.2 to 26.3 °C during the experiment (www.climate.weather.gc.ca), and though rainfall was not measured, the nearest weather station indicates precipitation during this period was minimal (~ 8 mm). Water treatment fish were placed together in a single black bag with mesh ends that blocked light but allowed for continuous water flow, and was kept fully submerged, in an off-channel of Gate's Creek. Water temperature varied between 13 and 14.5 °C. Both treatments were sampled on day 0, 1, 2, 3, and 4 (Fig. 1a,b), but only samples from day 0 and 4 were analyzed for isotopes as that was the maximum duration of both treatments. As effects were small even after four days, we chose not to run the isotopes of the intermediate days. Air treatment fish were also sampled on day 8, but water treatment fish were too decayed to sample.

Fig. 1 **a** A male pink salmon in the air treatment after 5 days of sampling. **b** A male pink salmon in the water treatment after 5 days of sampling; note the extensive fungus covering the underwater fish

Samples were kept frozen on site (− 18 °C) then stored at − 80 °C until analyzed. All samples had external fungus removed under a microscope, were washed in distilled water, and then were dried for 48 h (except scales, which were dried for 24 h) at 60 °C. Tissues were analyzed for $\delta^{13}C$ and $\delta^{15}N$ at the Environmental Isotope Laboratory at the University of Waterloo using a Delta Plus Continuous Flow Stable Isotope Ratio Mass Spectrometer (Thermo Finnigan, Bremen, Germany) coupled to a Carlo Erba elemental analyzer (CHNS-O EA1108, Carlo Erba, Milan, Italy). Precision was determined through repeat analysis of internal laboratory standards cross-calibrated against certified international reference materials (i.e., IAEA-N1 + N2, IAEA-CH3 + CH6). No less than 20% of samples in any given run were comprised of internal standards or reference materials, with measurements used to assess linearity and or mass spectrometer drift throughout the duration of the analytical run. Results (precision ± 0.2‰) for $\delta^{13}C$ are reported in per mil (‰) units, against the primary reference scale of Vienna Pee Dee Belemnite (VPDB). The results for $\delta^{15}N$ (precision ± 0.3‰) are similarly reported but against the primary reference scale of Atmospheric Air.

We used a mixed effects ANOVA to examine the effect of time (day 0 vs 4), treatment (air vs water), and their interaction on $\delta^{13}C$ and $\delta^{15}N$ for each tissue separately, with fish as the random factor to account for multiple measurements on individuals. If the interaction was not significant, we removed that effect

and tested main effects only (time and treatment). Data passed normality tests, except $\delta^{15}N$ in scales, where we used permutation tests. We also used a permutation paired t-test (due to a small sample size of $n = 6$ samples per treatment) to examine the effect of day (day 0 vs 8) for the air treatment on $\delta^{13}C$ and $\delta^{15}N$ for each tissue separately. Statistical analyses were conducted in R 4.0.3 (R Core Team, 2020) using packages rstatix 0.6.0 (Kassambara, 2020), permuco 1.1.0 (Frossard and Renaud, 2019), and coin 1.3-1 (Hothorn et al 2006, 2008).

Results

Treatment (air vs water) and time (day 0 vs 4)

There was no effect of either treatment, time, or their interaction on $\delta^{13}C$ for scales (time x treatment: $p = 0.237$, time: $p = 0.239$, treatment: $p = 0.998$). The effect of treatment depended on time for skin (treatment x time: $F_{1,10} = 6.87$, $p = 0.026$; Fig. 2), with the water treatment resulting in $\delta^{13}C$ enrichment by 0.85‰ at day 4 compared to day 0 ($F_{1,5} = 20.2$, $p = 0.012$) whereas the air treatment showed no change ($p = 0.446$). Adipose fin tissue was enriched in $\delta^{13}C$ by 0.86‰ at day 4 compared to day 0 (time: $F_{1,9} = 12.96$, $p = 0.006$; Fig. 3), but there was no effect of treatment ($p = 0.424$) or their interaction (time x treatment: $p = 0.340$). Muscle tissues were depleted in $\delta^{13}C$ by 0.35‰ at day 4 compared to day 0 (time: $F_{1,10} = 9.68$, $p = 0.011$; Fig. 4), but there was no effect of treatment ($p = 0.462$) or their interaction (time x treatment: $p = 0.666$).

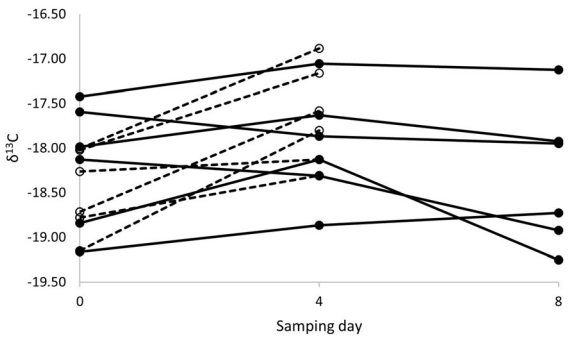

Fig. 2 Skin carbon values. Air treatment is solid circles and lines; water is open circles and dashed lines. Circles linked by a trend line indicate repeated measures over time within an individual fish. $n = 6$ samples per treatment for each time point, with no water samples at day 8

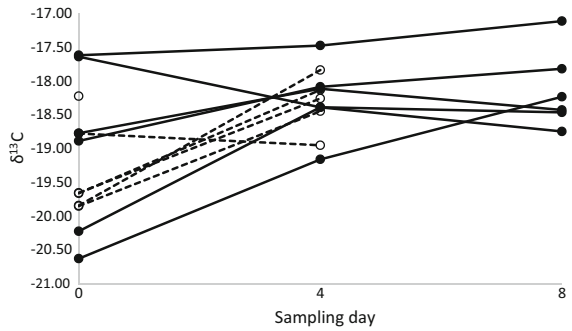

Fig. 3 Adipose carbon values. Air treatment is solid circles and lines; water is open circles and dashed lines. Circles linked by a trend line indicate repeated measures over time within an individual fish. $n = 6$ samples per treatment for each time point, except one water carbon value was not available at day 4; no water samples at day 8

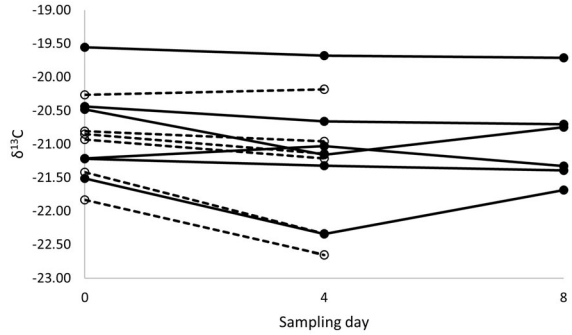

Fig. 4 Muscle carbon values. Air treatment is solid circles and lines; water is open circles and dashed lines. Circles linked by a trend line indicate repeated measures over time within an individual fish. $n = 6$ samples per treatment for each time point, with no water samples at day 8

There was no effect of either treatment, time, or their interaction on $\delta^{15}N$ for any tissue (scale: time x treatment $p = 0.426$, time $p = 0.914$, treatment $p = 0.381$; skin: time x treatment $p = 0.293$, time $p = 0.512$, treatment $p = 0.412$; adipose fin: time x treatment: $p = 0.361$, time $p = 0.147$, treatment $p = 0.388$; muscle: time x treatment: $p = 0.832$; time $p = 0.413$, treatment $p = 0.482$).

Time (day 0 vs 8), air treatment only

There was no effect of time on $\delta^{13}C$ for scales ($p = 0.26$), skin ($p = 0.484$; Fig. 2), or adipose fin ($p = 0.144$; Fig. 3). Muscle $\delta^{13}C$ was depleted by

0.19‰ at day 8 compared to day 0 ($Z = 2.355$, $p = 0.018$; Fig. 4). There was no effect of time on $\delta^{15}N$ for any tissue (scale: $p = 0.887$; skin: $p = 0.824$; adipose fin: $p = 0.410$; muscle: $p = 0.609$).

Discussion

We examined if $\delta^{15}N$ and $\delta^{13}C$ stable isotopes within four types of salmon tissues changed over the short term under natural environmental conditions. We found that $\delta^{15}N$ did not change under water (over four days) or in air (eight days) within soft or hard tissues, indicating the utility of salmon carcasses for this source of ecological data. For $\delta^{13}C$ in both air and water, we found that change did not occur in hard tissue (scales), and relatively small (< 1 ppt) changes were observed in soft tissues (skin, adipose fin, and muscle). Therefore, over the short term (eight days in air and four days under water), our study indicates it is valid to utilize tissues of salmon carcasses for $\delta^{15}N$ and $\delta^{13}C$ stable isotope data, though $\delta^{13}C$ data based on soft tissues should be treated with suitable caution.

It is well known that chemical (Kelly et al. 2006) and nonchemical preservation techniques can affect stable isotope values. For example, fermentation, baking, and drying approaches do not (Bostic et al 2015; Hobson et al. 1997; Bugoni et al. 2008; Barrow et al. 2008; Xu et al. 2011), but freezing sometimes does due to cell damage, loss of compounds, and disruption of proteins (Kim et al. 2016; Dannheim et al. 2007; Wolf et al. 2016; Feuchtmayr and Grey 2003; Bosley and Wainwright 1999; Barrow et al. 2008). The effects of decomposition within a natural, live environment, inclusive of live microorganisms and variable climate, are less clear. Microorganisms preferentially uptake the lighter isotopes from tissue as decomposition progresses and discriminate against the heavier isotopes thereby depleting microbial $\delta^{13}C$ but concurrently enriching substrate $\delta^{13}C$ (McGoldrick et al. 2008). Microorganisms can also selectively break down isotopically heavier amino acids thereby decreasing the substrate ^{13}C:^{12}C ratio and depleting its $\delta^{13}C$ (e.g., Macko et al. 1994). However, light isotopes are expelled more than heavy isotopes during the production of gases such as carbon dioxide, leaving $\delta^{13}C$ enriched (Dent et al. 2004; Balzer et al. 1997). Our two tissues that were exposed (skin and adipose fin) were enriched by 0.85‰ and 0.86‰, respectively, after 4 days, whereas the tissue encased in the body (muscle) was depleted by 0.35‰ at 4 days and 0.19‰ at 8 days, which we infer means that more carbon dioxide may have been produced on surface tissues under aerobic conditions, both in air and under water, as described for human decomposition by Dent et al. (2004). While samples in water were too degraded to allow for stable isotope analysis at 8 days, we were able to test samples in air. We observed that effects of degradation upon $\delta^{13}C$ were actually less at eight days compared to four days. (Adipose and skin were no longer significantly enriched at eight days, and depletion in muscle was reduced at eight compared to four days.) These results may be explained by differences in statistical power; at four days we had a larger data set including water-based samples and the water samples showed stronger effects than air-based tissues, likely driving the stronger effects observed at day four. Alternatively, even on short timescales bacterial composition and activity may have changed, resulting in different patterns of isotopic changes. The latter explanation may be why patterns of change in $\delta^{13}C$ across studies are not consistent. Similar to our results, $\delta^{13}C$ was depleted in lipid extracted ringed seal muscle after 8 days by 0.4‰ and lipid extracted lake trout (by 0.3‰) and Greenland shark (by 0.8‰) muscle in open vials after 256 days (Yurkowski et al. 2017), fruit flies by 0.81‰ after 10 days (Ponsard and Amlou 1999), lipid extracted whale blubber by ~ 0.3‰ after 14 days (Burrows et al. 2014), and non-lipid extracted bivalves by ~ 0.25‰ and mantis shrimp by ~ 1.2‰ after 5 days (Perkins et al. 2018). In contrast, $\delta^{13}C$ was enriched in lipid extracted whale skin after 3 days by 0.15‰ (Burrows et al. 2014), non-lipid extracted rabbitfish after 5 days by ~ 0.35‰ (Perkins et al. 2018), and lipid extracted ringed seal in closed (by 0.7‰) and open (by 0.3‰) vials and lake trout in closed vials (by 0.4‰) after 256 days (Yurkowski et al. 2017). However, there was no change in non-lipid extracted $\delta^{13}C$ of crab, grouper, and gastropods after 5 days (Perkins et al. 2018), lipid extracted Greenland shark and lake trout muscle after 8 days and lipid extracted Greenland shark muscle in closed vials after 256 days (Yurkowski et al. 2017), rotting beaver tissues (fat, gut, heart, liver, lungs, muscle) during active decomposition (263–326 accumulated degree days) (Keenan and Debruyn 2019) nor in lipid extracted striped dolphin or loggerhead sea turtle skin or muscle after 62 days (Payo and Payo

2013). Changes over shorter timescales may be opposite to those over longer timescales. For example, lipid extracted ringed seal muscle was depleted after eight days but was enriched by 256 days (Yurkowski et al. 2017). Thus, the subtleties of changes in $\delta^{13}C$ may depend more heavily on the bacterial composition of the tissues, which may differ depending on sample preparation (tissues frozen before starting decomposition in some studies), surrounding environment (whole bodies outside vs separate tissues in laboratory culture), moisture (wet tissues promote faster bacterial growth), and bacteria type (aerobic vs anaerobic digestion), and analytically on whether lipids were extracted prior to analysis (e.g., Perkins et al. 2018). Among-study variation in the observed decomposition effect may also be related to differences in temporal sampling with respect to steady-state growth, with heterotrophic bacteria having higher discrimination rates against heavier isotopes during their exponential growth phase (McGoldrick et al. 2008).

When there is a decomposition effect on $\delta^{15}N$, it is always enriched (not depleted), but there is still considerable variation across studies in terms of whether any enrichment is detected statistically, with some significant differences in other studies within the range of our nonsignificant differences. One possible explanation for the discrepancy in $\delta^{15}N$ among studies is that decomposition fluids appear to be enriched in $\delta^{15}N$ but not $\delta^{13}C$ compared to tissues (Wheeler and Kavanaugh 2017), and studies vary in whether those fluids are included in the isotope analysis, and whether the samples are kept wet or dry. However, Yurkowski et al. (2017) also examined this effect and found that for two out of three species, tissues in closed vials (which kept the fluids with the tissue) were enriched in both $\delta^{13}C$ and $\delta^{15}N$ compared to the same tissues in open vials (which allowed fluids to evaporate) up to 256 days ($\delta^{15}N$: on average, 1.5‰ for ringed seal and 0.9‰ for Greenland shark, while lake trout at 0.5‰ was not significant; $\delta^{13}C$: on average, 0.4‰ for ringed seal and 0.7‰ for lake trout, while Greenland shark at 0.9‰ was not significant), though enrichment for the first 8 days was not significant (except for open vials of lake trout with a 0.4‰ increase, though it decreased to within 0.1‰ of the starting value at the next sampling time point of 16 days). Our study found no effect on $\delta^{15}N$ up to 4 days (average tissue change on land ranged from -0.33 to 0.12‰ and average tissue change in water ranged from -0.09 to 0.21‰) and 8 days (average tissue change on land ranged from -0.08 to 0.09‰). However, this differs from other studies that found enriched $\delta^{15}N$: Burrows et al. (2014) found increases in whale skin and blubber including decomposition fluids in vials after 3 days (skin by $\sim$ 0.15‰, approximately equal to measurement error at 20 °C; blubber at 4 °C by $\sim$ 0.45‰), Ponsard and Amlou (1999) found fruit flies including decomposition fluids increased 0.42‰ in vials after 10 days, and Perkins et al. (2018) found increases in fish (grouper $\sim$ 0.6‰; rabbitfish $\sim$ 1%) and crustacean muscle (mantis shrimp $\sim$ 1.0‰; crab: $\sim$ 0.8‰) but not molluscs in open laboratory trays after 5 days. Additionally, Keenan and Debruyn (2019) found on average $\sim$ 3‰ (range 2.4 to 4.4‰) enrichment in rotting beavers at active decomposition (263–326 accumulated degree days), but not during bloat (101–138 accumulated degree days), and Payo and Payo (2013) found no effect in striped dolphin or loggerhead sea turtle skin or muscle after 62 days. Similar to $\delta^{13}C$, there has yet to emerge a consistent pattern on when $\delta^{15}N$ will be enriched, and the same effects of variation in initial microbial communities may have a role in explaining these disparate results.

If the goal of a study is to inform whether stable isotopes measured from tissues collected in the field from decomposing specimens are accurate, then whole unfrozen specimens in the field are needed to answer this question. Freezing causes changes in microbial abundance (Davis 1976) as well as a switch from aerobic (decay from the outside-in) to anaerobic (putrefaction from the inside-out) decomposition (Micozzi 1986; Sulakova et al. 2014). Thus, the results should be interpreted with caution when samples are first frozen prior to decomposition (e.g., Burrows et al 2014; Yurokowski et al. 2017; Payo Payo et al. 2013; Keenan and DeBruyn 2019) and when samples are held separately from the rest of the microbiome of the animal (e.g., Burrows et al. 2014; Yurokowski et al. 2017) as they may not represent what would happen under natural conditions. A caveat to our study is that we held water treatment fish in a dark flow-through bag which resulted in carcasses not being exposed to solar radiation. In a more natural setting carcasses would be exposed to light which may influence microbial composition and action, and decomposition processes. However, salmon carcasses remain submerged for 1–3 days at warmer temperatures and 12–18 days at colder temperatures

(Patterson et al. 2007); our temperatures would result in carcasses floating in 4–5 days, making our 4-day submerged water sampling a relevant time scale for carcasses under natural conditions, even though they received less light. The lack of light does not invalidate our findings but is important context and something that should be explored in future studies.

The effect of decomposition on $\delta^{15}N$ and $\delta^{13}C$ values also depends on temperature. Lower temperatures generally reduce or eliminate decomposition effects. For example, keeping samples on ice eliminated almost all changes in $\delta^{13}C$ and $\delta^{15}N$ observed at 21 °C (Perkins et al. 2018). Similarly, skin and blubber had changes in stable isotopes at 20 °C but not at 4 °C or − 20 °C for up to 14 days, but surprisingly blubber $\delta^{15}N$ was enriched at 4 °C whereas at − 20 °C and + 20 °C it showed inconsistent changes (Burrows et al. 2014). In our study, although minimum and maximum air temperature varied across 25 degrees, average daily temperatures encompassed a smaller range (10.0–16.9 °C) that bracketed water temperatures (13–14.5 °C). It is possible, therefore, that the effect of time on skin tissue in water but not air may be due to water itself rather than temperature differences between mediums. Due to the small absolute levels of these isotope changes, it is unclear whether some of these changes are true effects or reflect random sample variation due to low sample size—e.g., Burrows et al. (2014) had only three samples per time point, a caveat which applies to our study as well (six samples per time point).

Although group mean values are often the primary summary statistics of interest, in some areas researchers make conclusions based on individual isotope values. Our study shows that the sampling location on the fish and/or individual variation in accumulation of fungal/bacterial species will affect these interpretations, as within-individual changes between sampling time points were not consistent in direction (Figs. 2, 3 and 4). Similarly, in other studies, not only did homogenous samples from single individuals also show variation in stable isotope values, but decomposing tissues showed seemingly random differences at some sampling days that are hard to explain as a real decomposition effect (e.g., Burrows et al. 2014: whale blubber at 20 °C had an increase in $\delta^{15}N$ of 0.5‰ at day 1 but had decreased back to the starting value at day 3, which was attributed to variation within tissue or nitrogen extraction). These statistical differences may not have biological importance, as some were within the measurement error for the mass spectrometer, typically ± 0.3‰. However, these results still imply that although population-level conclusions may still be made using decomposed tissues within appropriate timeframes, inferences that require the rank order of individuals to be maintained should not be made. For example, Perkins et al. (2018) found individual variability up to 1.4‰ for $\delta^{15}N$. It is unclear if changes between sampling time points are transient and real, due to microbial growth and turnover, or due to heterogeneous tissue isotope values even from adjacent sampling locations. The former issue applies to decomposed tissue, while the latter applies more broadly to all stable isotope studies. Here, the temporal variation in isotopic values for muscle tissue known to have low isotopic variability (Pinnegar and Polunin 1999) points to the effects of microbial growth and turnover. Also, possibly due to small variation among tissue samples in microbial communities at the start, there was more variation in stable isotope values after longer timeframes (Yurkowski et al. 2017).

Pacific salmon carcasses provide usable tissues for stable isotope analysis of $\delta^{15}N$ up to 4 days, thus still providing useful information about the past state of these fish. Tissues that differ in their metabolic activity provide different timescales of past ecological information (Willis et al. 2013; Vander Zanden et al. 2015), and so tissues collected from dead salmon can add to the growing literature about Pacific salmonid ocean ecology (e.g., Johnson and Schindler 2009, 2012; Qin and Kaeriyama 2016), and help to elucidate differences in nutritional status between spawned and non-spawned individuals. However, more caution is required before making inferences based on $\delta^{13}C$ values in soft tissues, as these seem to change fairly rapidly; in our study we found depletion up to 0.35‰ and enrichment up to 0.86‰ after 4 days. Hard tissues appear to be more resistant to changes due to microbial and autolytic processes. Bone and hair of beaver did not change in either isotope value over 326.9 accumulated degree days (Keenan and DeBruyn 2019), and we found no changes in scales over eight days implying that the collagen matrix of scales had not been degraded by bacterial collagenases. Though preliminary, this suggests that collagen fibers are more resistant or are a less preferred substrate for

microbes. Future studies should investigate the isotopic consistency of hard tissues that are left in the natural environment, as hard tissues (otoliths, baleen, bone, hair, feathers, and scales) may be the only source of biological information left of an animal after a long period of time or after carcasses have been scavenged. Historic or archived hard tissues are already being used for retrospective isotope analysis, which assume that no degradation has occurred in these tissues (e.g., Guiry et al. 2016; Espinasse et al. 2019; Blight et al. 2015). For populations within which dead animals are frequently encountered, e.g., salmonid carcass surveys, hard tissues (scales, otoliths) will likely yield relevant biological information even if soft tissue is rotting providing another avenue to collect important data about the life history of those fish.

Acknowledgements We thank Robert de Bruijn, Andrew Lotto, Rebecca Riley, and Steve Hall for field and logistic support. We also thank the St'at'imc First Nation, in particular Bonnie Adolph and St'at'imc Eco Resources (SER), for allowing access to the study site and for assisting with research coordination.

Supplementary Information Data are included in supplementary material.

Funding Funding was provided through BCHydro and SER to SGH, the Natural Sciences and Engineering Research Council of Canada Discovery Grants to SGH, SJC and MP, the Canada Research Chairs program for SJC, and the DFO Environmental Watch Program to DAP.

Declarations

Conflict of interest The authors declare no conflicts of interest/competing interests.

References

Arthur KE, Boyle MC, Limpus CJ (2008) Ontogenetic changes in diet and habitat use in green sea turtle (Chelonia mydas) life history. Marine Ecol Prog Series 362:303–311

Balzer A, Gleixner G, Grupe G, Schmidt HL, Schramm S, Turban-Just S (1997) In vitro decomposition of bone collagen by soil bacteria: the implications for stable isotope analysis in archaeometry. Archaeometry 39(2):415–429

Barrow LM, Bjorndal KA, Reich KJ (2008) Effects of preservation method on stable carbon and nitrogen isotope values. Physiol Biochem Zool 81(5):688–693

Blight LK, Hobson KA, Kyser TK, Arcese P (2015) Changing gull diet in a changing world: a 150-year stable isotope (δ13C, δ15N) record from feathers collected in the Pacific Northwest of North America. Global Change Biol 21(4):1497–1507

Bolnick DI, Svanbäck R, Fordyce JA, Yang LH, Davis JM, Hulsey CD, Forister ML (2003) The ecology of individuals: incidence and implications of individual specialization. Am Nat 161(1):1–28

Bosley KL, Wainright SC (1999) Effects of preservatives and acidification on the stable isotope ratios (15N: 14N, 13C: 12C) of two species of marine animals. Can J Fish Aquat Sci 56(11):2181–2185

Bostic JN, Palafox SJ, Rottmueller ME, Jahren AH (2015) Effect of baking and fermentation on the stable carbon and nitrogen isotope ratios of grain-based food. Rapid Commun Mass Sp 29(10):937–947

Bugoni L, McGill RA, Furness RW (2008) Effects of preservation methods on stable isotope signatures in bird tissues. Rapid CommunMass Sp 22(16):2457–2462

Burrows DG, Reichert WL, Bradley Hanson M (2014) Effects of decomposition and storage conditions on the δ13C and δ15N isotope values of killer whale (*Orcinus orca*) skin and blubber tissues. Marine Mammal Sci 30(2):747–762

Dannheim J, Struck U, Brey T (2007) Does sample bulk freezing affect stable isotope ratios of infaunal macrozoobenthos? J Exp Mar Biol Ecol 351(1–2):37–41

Davis CP (1976) Preservation of gastrointestinal bacteria and their microenvironmental associations in rats by freezing. Appl Environ Microbiol 31(2):304–312

DeNiro MJ, Epstein S (1978) Influence of diet on the distribution of carbon isotopes in animals. Geochim Cosmochim Acta 42:495

Dent BB, Forbes SL, Stuart BH (2004) Review of human decomposition processes in soil. Environ Geol 45(4):576–585

Espinasse B, Hunt BP, Coll YD, Pakhomov EA (2019) Investigating high seas foraging conditions for salmon in the North Pacific: Insights from a 100-year scale archive for Rivers Inlet sockeye salmon. Can J Fish Aquati Sci 76(6):918–927

Fenton GE, Ritz DA (1988) Changes in carbon and hydrogen stable isotope ratios of macroalgae and seagrass during decomposition. Estuar Coast Shelf Sci 26(4):429–436

Fernandez I, Mahieu N, Cadisch G (2003) Carbon isotopic fractionation during decomposition of plant materials of different quality. Glob Biogeochem Cycles. https://doi.org/10.1029/2001GB001834

Feuchtmayr H, Grey J (2003) Effect of preparation and preservation procedures on carbon and nitrogen stable isotope determinations from zooplankton. Rapid Commun Mass Sp 17(23):2605–2610

Frossard J, Renaud O (2019) permuco: permutation tests for regression, (Repeated Measures) ANOVA/ANCOVA and comparison of signals. R package version 1.1.0. https://CRAN.R-project.org/package=permuco

Guiry EJ, Needs-Howarth S, Friedland KD, Hawkins AL, Szpak P, Macdonald R, Courtemanche M, Holm E, Richards MP (2016) Lake Ontario salmon (*Salmo salar*) were not migratory: a long-standing historical debate solved through stable isotope analysis. Sci Rep 6(1):1–7

Hobson KA, Gloutney ML, Gibbs HL (1997) Preservation of blood and tissue samples for stable-carbon and stable-nitrogen isotope analysis. Can J Zool 75(10):1720–1723

Hothorn T, Hornik K, van de Wiel MA, Zeileis A (2006) A Lego system for conditional inference. The Am Stat 60(3):257–263

Hothorn T, Hornik K, van de Wiel MA, Zeileis A (2008) Implementing a class of permutation tests: the coin package. J Stat Softw 28(8):1–23

Johnson SP, Schindler DE (2009) Trophic ecology of Pacific salmon (Oncorhynchus spp.) in the ocean: a synthesis of stable isotope research. Ecol Res 24(4):855–863

Johnson SP, Schindler DE (2012) Four decades of foraging history: stock-specific variation in the carbon and nitrogen stable isotope signatures of Alaskan sockeye salmon. Marine Ecol Prog Series 460:155–167

Kassambara A (2020) Rstatix: pipe-friendly framework for basic statistical tests. R package version 0.6.0. https://CRAN.R-project.org/package=rstatix

Keenan SW, DeBruyn JM (2019) Changes to vertebrate tissue stable isotope (δ 15 N) composition during decomposition. Sci Rep 9(1):1–12

Kelly B, Dempson JB, Power M (2006) The effects of preservation on fish tissue stable isotope signatures. J Fish Biol 69(6):1595–1611

Kim MS, Lee WS, Suresh Kumar K, Shin KH, Robarge W, Kim M, Lee SR (2016) Effects of HCl pretreatment, drying, and storage on the stable isotope ratios of soil and sediment samples. Rapid Commun Mass Sp 30(13):1567–1575

Layman CA, Araujo MS, Boucek R, Hammerschlag-Peyer CM, Harrison E, Jud ZR, Matich P, Rosenblatt AE, Vaudo JJ, Yeager LA, Post DM (2012) Applying stable isotopes to examine food-web structure: an overview of analytical tools. Biol Rev 87(3):545–562

Macko SA, Engel MH, Qian Y (1994) Early diagenesis and organic matter preservation—a molecular stable carbon isotope perspective. Chem Geol 114(3–4):365–379

Matthews B, Mazumder A (2004) A critical evaluation of intrapopulation variation of δ 13 C and isotopic evidence of individual specialization. Oecologia 140(2):361–371

McGoldrick DJ, Barton DR, Power M, Scott RW, Butler BJ (2008) Dynamics of bacteria–substrate stable isotope separation: dependence on substrate availability and implications for aquatic food web studies. Can J Fish Aquat Sci 65:1983–1990

Micozzi MS (1986) Experimental study of postmortem change under field conditions: effects of freezing, thawing, and mechanical injury. J Forensic Sci 31(3):953–961

Newsome SD, Yeakel JD, Wheatley PV, Tinker MT (2012) Tools for quantifying isotopic niche space and diet variation at the individual and population level. J Mammal 93(2):329–341

Patterson DA, Skibo KM, Barnes DP, Hills JA, Macdonald JS (2007) The influence of water temperature on time to surface for adult sockeye salmon carcasses and the limitations in estimating salmon carcasses in the Fraser River, British Columbia. North Ame J Fish Manag 27(3):878–884

Payo-Payo A, Ruiz B, Cardona L, Borrell A (2013) Effect of tissue decomposition on stable isotope signatures of striped dolphins *Stenella coeruleoalba* and loggerhead sea turtles *Caretta caretta*. Aquat Biol 18(2):141–147

Perkins MJ, Mak YK, Tao LS, Wong AT, Yau JK, Baker DM, Leung KM (2018) Short-term tissue decomposition alters stable isotope values and C: N ratio, but does not change relationships between lipid content, C: N ratio, and Δδ13C in marine animals. PloS one 13(7):e0199680

Pinnegar JK, Polunin NVC (1999) Differential fractionation of δ^{13}C and δ^{15}N among fish tissues: implications for the study of trophic interactions. Funct Ecol 13:225–231

Ponsard S, Amlou M (1999) Effects of several preservation methods on the isotopic content of *Drosophila* samples. C R Acad Sci III 322:35

Qin Y, Kaeriyama M (2016) Feeding habits and trophic levels of Pacific salmon (*Oncorhynchus* spp.) in the North Pacific Ocean. N Pac Anadromous Fish Com Bul 6:469–481

R Core Team (2020) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Rubenstein DR, Hobson KA (2004) From birds to butterflies: animal movement patterns and stable isotopes. TREE 19(5):256–263

Schweizer M, Fear J, Cadisch G (1999) Isotopic (13C) fractionation during plant residue decomposition and its implications for soil organic matter studies. Rapid Commun Mass Sp 13(13):1284–1290

Šuláková H, Harakalová L, Barták M (2014) Effect of freezing on the initial colonization of the carcass with necrophagous organisms. Acta Musei Silesiae Sci Nat 63(1):29–37

Vander Zanden MJ, Clayton MK, Moody EK, Solomon CT, Weidel BC (2015) Stable isotope turnover and half-life in animal tissues a literature synthesis. PloS one 10(1):e0116182

Wheeler TA, Kavanagh KL (2017) Soil biogeochemical responses to the deposition of anadromous fish carcasses in inland riparian forests of the Pacific Northwest, USA. Can J Forest Res 47:1506–1516

Wild LA, Chenoweth EM, Mueter FJ, Straley JM (2018) Evidence for dietary time series in layers of cetacean skin using stable carbon and nitrogen isotope ratios. Rapid Commun Mass Sp 32(16):1425–1438

Willis TJ, Sweeting CJ, Bury SJ, Handley SJ, Brown JCS, Freeman DJ, Cairney DG, Page MJ (2013) Matching and mismatching stable isotope (δ 13 C and δ 15 N) ratios in fin and muscle tissue among fish species: a critical review. Marine Biol 160(7):1633–1644

Wolf JM, Johnson B, Silver D, Pate W, Christianson K (2016) Freezing and fractionation: effects of preservation on carbon and nitrogen stable isotope ratios of some limnetic organisms. Rapid Commun Mass Sp 30(5):562–568

Xu J, Yang Q, Zhang M, Zhang M, Xie P, Hansson LA (2011) Preservation effects on stable isotope ratios and consequences for the reconstruction of energetic pathways. Aquat Ecol 45(4):483–492

Yurkowski DJ, Hussey AJ, Hussey NE, Fisk AT (2017) Effects of decomposition on carbon and nitrogen stable isotope values of muscle tissue of varying lipid content from three aquatic vertebrate species. Rapid Commun Mass Sp 31(4):389–395