Received: 23 May 2025 | Accepted: 30 April 2026

DOI: 10.1002/2688-8319.70277

Ecological Solutions and Evidence

RESEARCH ARTICLE

A framework for using tissue chemistry and biochronologies to reconstruct fish habitat use and growth

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Funding information
Natural Environment Research Council, Grant/Award Number: NE/S007334/1; UK Research and Innovation, Grant/Award Number: MR/V023578/1

Handling Editor: Michael James Roast

Abstract

1. Understanding fish habitat use, migration patterns and growth are fundamental for effective fisheries management and conservation, yet reconstructing individual life histories in variable environments remains challenging.
2. Recent advances in tissue chemical tracers have provided novel tools to retrospectively ascertain habitat use; however, these approaches are often deemed inaccessible to individuals without chemistry backgrounds or applied inconsistently between studies, often without clear rationale. Here, we present a framework for using chemical tracers from archival tissues to reconstruct the lifetime habitat use and growth histories of fish.
3. We demonstrate the utility of the framework by applying it to brown trout (*Salmo trutta*) in a Norwegian system of interconnected lake, river and marine habitats. We describe the extent to which adult trout used two freshwater lakes as juveniles and explore variation in outmigration timing and growth rates between anadromous and resident forms. We illustrate that chemical tracers can provide novel and valuable information when applied correctly.
4. *Practical implication*. This framework offers a clear, transferable tool for using chemical tracers to reconstruct habitat use and growth across diverse fish species. This is of use to both researchers and managers to deliver key insights into lifetime habitat use and growth in both freshwater and marine environments.

KEYWORDS
archival tissues, biochronologies, eye lens, framework, growth rate, otolith chemistry, Sclerochronology, stable isotopes

1 | INTRODUCTION

The use of natural chemical tracers recorded in organism tissues has increased markedly over recent decades, particularly in the fields of behavioural ecology, food traceability and archaeology (Krahn et al., 2007; Lichtfouse, 2000). Most of these applications rely on the principle that geographic variation in local environmental chemistry and diet will generate chemical differences in the tissues of organisms living there, mediated by factors such as temperature, trophic level and physiology (Hüssy et al., 2021;

Trueman & St John Glew, 2019). Given the challenges and costs associated with handling and tagging aquatic animals or performing laboratory experiments, natural chemical tracers such as tissue isotope ratios and element concentrations are increasingly used to reconstruct fish habitat use (Tabouret et al., 2010), ontogenetic niche shifts (McMahon et al., 2011), metabolic phenotypes (Trueman et al., 2023) and thermal histories (Holden et al., 2022). These studies often utilise tissues that grow incrementally through the lifetime of the animal—so-called archival tissues—such as otoliths (earstones), eye lenses, vertebrae, fin rays and scales (Campana, 1999; Tzadik et al., 2017). These techniques can be used in combination to identify natal habitats contributing most to mixed populations and habitats supporting higher growth, allowing targeted restoration or protection of those habitats that contribute most to supporting a population.

However, despite widespread applicability, often low-cost relative to telemetry, and unique suitability to smaller-bodied life stages and species, the use of chemical tracers to track fish movements is rarely utilised by managers and not consistently applied between studies (Benjamin et al., 2014; Munroe et al., 2018). Studies using chemical tracers often fail to follow a clear and logical approach, which is key to reproducibility and transferring best practices across species, studies and systems. As the complexity of research questions increases, more decisions and assumptions are required to answer them, and a transparent and consistent methodological framework becomes increasingly important (Elsdon et al., 2008). As such, we have developed a logical framework for managers and researchers to follow when considering and applying chemical tracers to reconstruct fish habitat use in their system. This framework will help to select the appropriate tool(s) for a given study system, species or question (or to confirm if none are available). First, we outline each step (Figure 1) along with their justification and context, providing additional detail for associated statistical and chemical analyses, which tend to be the most complex and daunting for new practitioners entering this field. Second, we use brown trout (*Salmo trutta*) as a model species to apply the framework to, given its diversity in movement behaviour and growth (Jonsson & Jonsson, 2011). We focus on a trout population inhabiting a Norwegian coastal catchment where both anadromous 'sea trout' and freshwater 'residents' coexist. In the Methods, we apply the framework to address

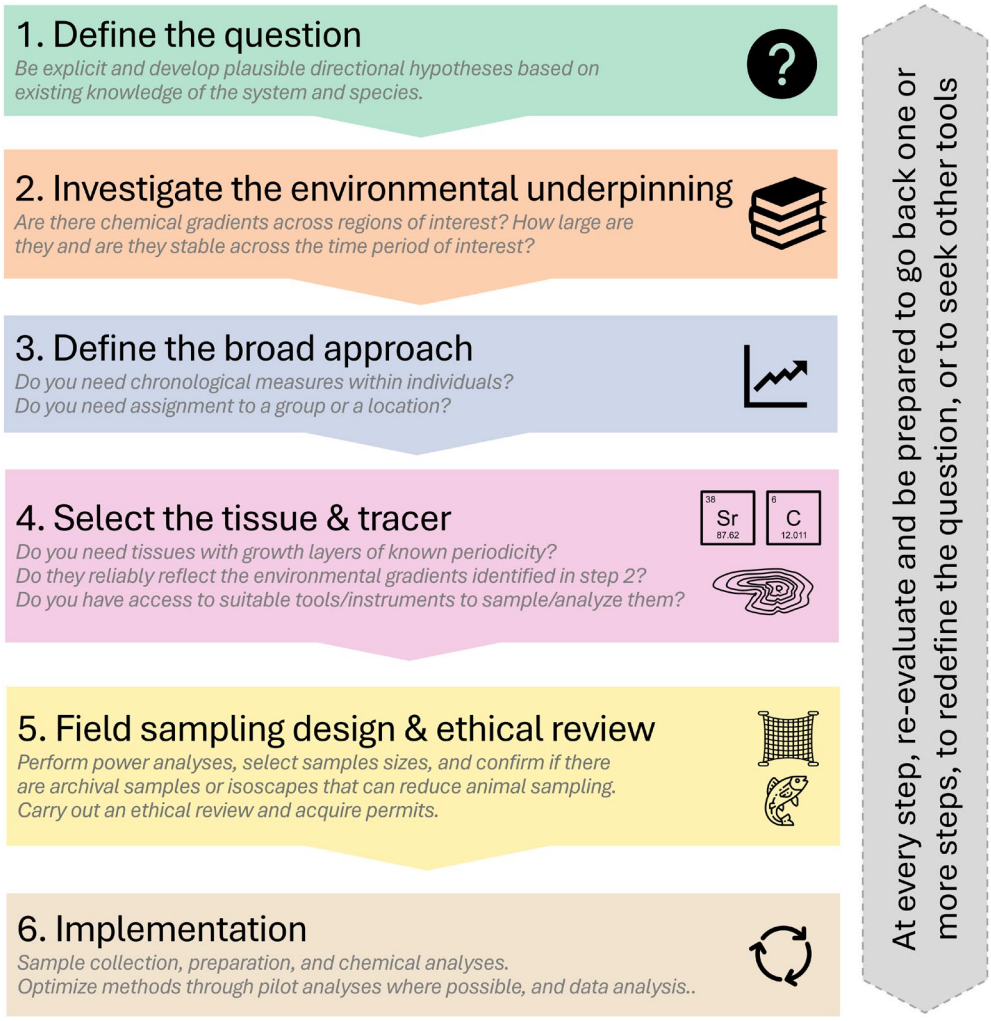

FIGURE 1 A framework for using chemical tracers in fish tissues for addressing ecological questions, with a primary focus on habitat use and movement reconstruction.

two key questions around variation in (1) freshwater nursery habitat use and (2) growth rates among migratory forms, highlighting the cost-benefits of our approach and ways we attempted to reduce uncertainty along the pipeline. In the Results and Discussion, we use the outputs to discuss what we observed and the advantages and disadvantages of different techniques and potential refinements that could be considered.

1.1 | A framework for using tissue chemical tracers to infer fish habitat use

1.1.1 | Step 1: Define the question

Be explicit about the spatiotemporal scales of interest. Identify whether it is important to resolve to site or region level, or whether geographic location is less important (e.g. if interested in habitat type or stock membership). The temporal scale could be for a single time point (e.g. recent, natal, juvenile; Barnett-Johnson et al., 2008) or span a time series of individual movements (e.g. sequential geolocations (Tanaka et al., 2010)).

1.1.2 | Step 2: Investigate the environmental underpinning

Review existing literature, maps, and datasets to assess if environmental gradients are likley to occur across the region of interest. Tracers may vary as a function of salinity, temperature, geology, pollution, local biogeochemical processes such as hypoxia, or physicochemical features such as ocean fronts (Sturrock et al., 2012). Environmental gradients can also be created by differences in local food webs relating to energy sources and habitat types that might be inferred from stomach contents and stable isotope data, which are often presented and analysed in the form of 'isoscapes' (Trueman & St John Glew, 2019). Consider whether spatial gradients remain stable over the study period, whether their magnitude outweighs seasonal or interannual variability, or whether global change may modify them (e.g. the Suess effect causing a global shift in $\delta^{13}C$ (Keeling, 1979)).

1.1.3 | Step 3: Define the broad approach

Decide between a single time point or repeated measures and select a statistical approach. Four main approaches include

a. Continuous isoscapes (isotopic maps): Interpolate isotope values and environmental covariates to predict origin based on tissue isotopic measurements. This requires known tracer-tissue relationships, with most applications using organic $\delta^{13}C$ and $\delta^{15}N$ (Trueman et al., 2017), otolith $\delta^{18}O$ (Sakamoto, 2024) or otolith $^{87}Sr/^{86}Sr$ (Brennan et al., 2019). Given the complex structure of dendritic riverscapes, water $^{87}Sr/^{86}Sr$ isoscapes require more complex modelling techniques such as Spatial Stream Network Modelling (Brennan et al., 2016).
b. Nominal assignment: Classifies individuals into predefined groups using models like discriminant function analysis or random forest (Wang et al., 2018). Does not require tracer-tissue relationships but needs extensive, tissue- and context-matched reference libraries, hampering transferability.
c. Unsupervised clustering: Groups individuals by similar chemical signatures (e.g. stocks or migratory types) using methods such as Bayesian mixture models. A disadvantage is that geographic resolution is often unclear (Hobbs et al., 2019; Wang et al., 2018)
d. Movement reconstruction: Typically uses time series chemical tracer data recorded in sequential growth bands of archival tissues to infer individual movements via zoning, change point analysis or recursive partitioning (Hedger et al., 2008; Vignon, 2015).

1.1.4 | Step 4: Select the tissue and tracer

This step is the most complex, with multiple tissues from a single individual and/or multiple tracers in the same tissue potentially required to answer a given question. See SI Text 1 for additional details.

Tissue: In teleost fish, archival tissues such as otoliths and eye lenses are well-suited to movement reconstruction as they are metabolically inert, and therefore tracers should be retained in growth layers permanently; however, they can only be lethally sampled (Campana & Thorrold, 2001; Quaeck-Davies et al., 2018). Non-lethally sampled tissues such as fin clips (single time point), scales or fin rays (chronological) may be suitable, depending on the question, tracer type, movement timing and tissue turnover rate (Jovičić et al., 2023), but post-depositional chemical alterations need to be carefully considered (Tray et al., 2022).

Tracer: Variations in water chemistry are generally better reflected in inorganic matrices (e.g. otolith calcium carbonate). Conversely, dietary differences generally manifest in protein-rich tissues (e.g. muscle, liver, eye lenses), although organic material can be isolated from calcified structures (McMahon et al., 2011). Common tracers in fish movement research include:

a. Elemental concentrations in otoliths and other calcified structures are widely used due to the ease of measuring multiple elements at fine temporal resolution via Laser Ablation Inductively Coupled Plasma Mass Spectrometry (LA-ICP-MS). While some elements (e.g. Sr, Ba, Mn) reflect environmental conditions, most have complex or weak relationships with ambient concentrations (Sturrock, Hunter, et al., 2015; Sturrock, Wikert, et al., 2015). Beyond salinity-driven movement (inferred via Sr, Ba), elemental data are most often used for nominal assignment, where physiology enhances population differences (Sturrock et al., 2012). Elements like Zn, Mg and P are also used for age estimation and to create chemical chronologies for movement reconstructions (Hüssy et al., 2021).

b. Stable isotope analysis (SIA) of $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ in organic tissues (e.g. muscle, fin, eye lens) via Isotope Ratio Mass Spectrometry (IRMS) is often used to infer food web dynamics and past habitat use (Bell-Tilcock et al., 2021; Post, 2002); however, temporal resolution is heavily influenced by soft tissue turnover rates (relatively short for blood and liver, longer for muscle and fins/skin). Tissue $\delta^{13}C$ and $\delta^{34}S$ values of low trophic level prey items also vary with salinity, serving as tracers of marine-freshwater movement and estuarine residency (Moore et al., 2016). Compound-specific SIA offers finer resolution for movement but is costly and time-intensive (Harada et al., 2022).
c. Isotope ratios in the inorganic phase of calcified structures: Otolith $\delta^{18}O$ and $^{87}Sr/^{86}Sr$ broadly reflect geographic variation in water $\delta^{18}O$ (and salinity), temperature and bedrock geology, aiding movement reconstruction in aquatic systems (Brennan et al., 2015; Sakamoto, 2024). A key challenge is being able to sample the otolith at fine enough temporal resolution, although secondary ion mass spectrometry and multi-collector LA-ICP-MS can help to address this (Hanson et al., 2010). While used less for movement reconstruction, otolith $\delta^{18}C$ is often measured alongside carbonate $\delta^{18}O$, providing additional information about individual metabolic rate (Chung et al., 2019).

1.1.5 | Step 5: Field sampling design and ethical review

Where possible, a power analysis should be conducted using appropriate pilot data or data from the literature, ideally for the same species, tissue(s) and system (Kemal, 2020). See Sequeira et al. (2019) for advice on predicting optimal sample sizes in different contexts. Where a multivariate approach is planned, such as here, a multivariate power analysis using a simulation approach (e.g. using the ecopower R package) is ideal (Maslen et al., 2023). However, if appropriate multivariate data are unavailable, such as for this study—a simple univariate power analysis may also be used. Here, using a univariate approach based on prey $\delta^{15}N$ suggested a sample size of 24, but we assumed that with additional markers and tissues the true sample size requirements would be lower.

The results should be used to inform a sampling plan that will give sufficient power for the proposed statistical approach. An ethical review should then be conducted that considers sample sizes relative to the population size, and how to minimise lethal take or animal suffering (e.g. using non-animal alternatives or existing tissue archives), and how to minimise potential habitat damage caused by the sampling (Bennett et al., 2016). Finally, sampling permits, and local permissions should be obtained.

1.1.6 | Step 6: Implementation

Perform pilot analyses to check that the planned instrument and tissue sampling methods can measure the chosen tracer(s) at the required temporal resolution and detection levels. If required, perform method development to optimise the approach. Fieldwork, sampling and tissue analyses should then be carried out and the statistical approaches identified in Step 3 used to analyse the data and answer the research question. Throughout this framework, it is important to constantly re-evaluate and revisit previous steps or even stop altogether and explore other tools if the selected tracer does not vary as expected or is below the instrument's detection limits in specific tissues.

2 | METHODS: NORWEGIAN TROUT CASE STUDY

In 2021, we sampled 53 trout from two lakes in Trondheim, Norway, with the goal of answering the following questions: (1) What fraction of brown trout used each of the two lakes along the river network as nursery habitat? and (2) At what age do sea trout first leave freshwater, and do their growth rates differ to resident trout before and/or after their first seaward migration? We used the framework to design our methods, as outlined below and in Table 1.

The two freshwater lakes, Storvatnet (upstream) and Litjvatnet (downstream; Figure 2) are 2.92km^2 and 0.47km^2, respectively, and known from tagging studies and fish surveys to be frequently used by juvenile trout. To ensure good representation of all lake habitats and a wide spread of size classes, trout were sampled by gill nets using a broad range of mesh sizes (5–55 mm) that were left overnight across a range of habitats. Total sample sizes for each lake were 10 reference fish (mean FL = 167 mm, range = 104–241 mm) and 17 adults (mean FL = 496 mm, range = 450–562 mm) for Litjvatnet, and 19 reference fish (mean FL = 181 mm, range = 108–234 mm) and 7 adults (mean FL = 482 mm, range = 403–576 mm) from Storvatnet. Based on the timings and sizes of tagged trout that performed marine migrations (Paterson et al., 2021), we were confident that our immature reference fish (fork lengths (FL) <250 mm) had never been to sea and were thus used as lake and freshwater 'reference samples' (Table 1). Conversely, the adults (>400 mm FL) had unknown migration histories. Sampling was authorised by the County Governor of Trøndelag (Statsforvalteren i Trøndelag; permit no. 2021/1934).

Fish were euthanised and their eye lenses and sagittal otoliths extracted. Eye lenses were stored frozen and otoliths stored dry. To investigate each fish's trophic history (and thus habitat use), the lenses were thawed then sequential laminae dissected, air-dried then encapsulated in tin cups using the methods of Bell-Tilcock et al. (2021). Lens laminae were then analysed for bulk $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ (excluding the core ~600 μm diameter, as it was below the instrument's minimum mass requirements). Using Elemental Analyzer-Isotope Ratio Mass Spectrometry via a vario PYRO cube elemental analyser (CN mode) coupled with visION isotope ratio mass spectrometer at SEAPORT laboratories, UK. Both fish muscle standard ERM-BB422 (sample no. 0487 certified reference material fish muscle, European Commission, JRC, Geel, Belgium) and Sulfanilamide were used as standards. Isotope ratios are reported in delta (δ) notation relative

TABLE 1 Applied examples of each step taken using our framework.

Step	Example 1	Example 2	Possible improvements
1. Define the question	'What fraction of brown trout used each of the two lakes along the river network as nursery habitat?'	'At what age do sea trout first leave freshwater, and do their growth rates differ to resident trout before and/or after their first seaward migration?'	—
2. Investigate environmental underpinning	Differences in nitrogen inputs from agriculture to the two lakes deemed likely. Differences in $\delta^{15}N$ observed in invertebrate prey and trout muscle $\delta^{15}N$ (Grönberg et al., 2024; Lægran, 2022). Differences in geology also observed (granodiorite vs granite); (Norwegian Geological Survey, 2020) that may influence water element concentrations and $\delta^{34}S$ (Frengstad et al., 2000; Kabalika et al., 2020). 1.3°C difference in temperature had been observed in previous sampling	Many studies show that water Sr concentrations, $\delta^{34}S$, and $\delta^{13}C$ values tend to exhibit positive relationships with salinity, while water Ba concentrations tend to be negatively correlated (Hale & Swearer, 2008; Limburg, 1995; Raoult et al., 2024). However, on rare occasions water Sr can be higher in freshwater than seawater (Walther & Limburg, 2012)	Q1: Analyse elemental and isotopic markers in water and prey samples (including prey $\delta^{34}S$) from each lake or use caged fish to confirm chemical differences Q2: Confirm spatial patterns in salinity and its relationships with water Sr:Ca, Ba:Ca and prey $\delta^{13}C$ and $\delta^{34}S$. (Walther & Limburg, 2012)
3. Define the broad approach	To differentiate between the two lakes, we used a nominal assignment approach, trained on reference fish too young to have gone to sea that were caught from each lake (i.e. assuming their outermost otolith and eye lens growth layers were formed while inhabiting said lake)	To differentiate between marine and freshwater, we used a threshold approach using salinity-correlated markers, whereby values above or below said threshold would be classified as marine or freshwater, allowing us to identify multiple migrations to and from the sea in the same individual. We defined the threshold based on observed values, supported by literature searches	Q1 & 2: Reference samples should be obtained for all possible nursery habitats, e.g. also tributaries and mainstem rivers Q2: Validate the threshold value using otoliths from tagged adults with known migration histories, and cohort-match individuals for growth rate comparisons
4. Select the tissue and tracer	Given the potential tracer differences identified in Step 1, eye lens stable isotopes ($\delta^{15}N$, $\delta^{34}S$, $\delta^{13}C$) and a suite of otolith element tracers (7Li, 23Na, 24 Mg, 31P, 39 K, 43Ca, 52Cr, 55Mn, 56Fe, 59Co, 60Ni, 63Cu, 66Zn, 85Rb, 88Sr, 107Ag, 111Cd, 138Ba, 208Pb) were selected for analysis. Given the lack of water chemistry data we selected a broad element list, including all markers that were deemed most likely to vary as a function of geology, temperature, and pollution that might be detectable in otoliths (Campana, 1999). Eye lenses and sagittal otoliths were selected as archival tissues. They both grow allometrically with fish length, allowing us to isolate the same size range (presumed to reflect a similar age) in reference and adult samples (Quaeck-Davies et al., 2018; Trueman et al., 2012). For Q2, we focused on the subset most likely to covary with salinity (lens $\delta^{13}C$, $\delta^{34}S$, otolith Sr:Ca, Ba:Ca) to address migratory strategy and otolith Mg:Ca and Zn:Ca for chemical ageing and growth		Q1: Ideally target a narrow size and age range in reference samples from each nursery area to avoid noise introduced by ontogenetic changes in physiology and diet. However additional immature samples from each lake could not be obtained in our case Q2. Chemical ageing would ideally be validated for this species in this system (e.g. using tagged fish and otolith marking)

(Continues)

TABLE 1 (Continued)

Step	Example 1	Example 2	Possible improvements
5. Field sampling design and ethical review	To determine the number of reference fish required to build the baseline, we conducted a power analysis using the *pwr* package (Champely et al., 2020) assuming a 17% overlap in trout muscle $\delta^{15}N$ between lakes (Grönberg et al., 2024) equivalent to an effect size of 0.83. This indicated an optimal sample size of 24 fish per lake. In the field, fish were euthanised by percussive stunning in accordance with European codes of practice	To determine the number of fish required to resolve growth rate differences among anadromous and resident trout, a power analysis was carried out using the differences in length-at-age reported by Jonsson (1981), which suggested a large effect size of 15.3, requiring just two fish per phenotype. To identify the otolith and eye lens chemical thresholds we would use to determine anadromy (i.e. marine–freshwater transition), we used the 'lake reference samples' from Q1 to characterise the freshwater range (supported by the values used in the literature)	Q1: Ideally more reference samples would have been collected but in practice we could not sample the number of juveniles identified by the power analysis Q1 & 2: Collect reference samples over multiple years to test for interannual variation in lake baselines/freshwater threshold value. Ideally, we would have also cohort-matched reference and adult samples Q2: Relationships between tissue tracers and salinity could be characterized via laboratory or enclosure experiments, ideally testing for potentially confounding effects of age and size (Walther & Limburg, 2012)

Note: The 'possible improvements' column outlines additional or alternative methodological steps that could enhance the inferences made.

to VPDB (C), AIR (N) and VCDT (S), and analytical precision based on repeated measurements of internal standards was ±0.06‰ for $\delta^{13}C$, ±0.09‰ for $\delta^{15}N$, and ±0.33‰ for $\delta^{34}S$ (1 SD). Otoliths were ground and polished on the sagittal plane following (Sturrock, Hunter, et al., 2015; Sturrock, Wikert, et al., 2015), then analysed for element concentrations (^{7}Li, ^{23}Na, ^{24}Mg, ^{31}P, ^{39}K, ^{43}Ca, ^{52}Cr, ^{55}Mn, ^{56}Fe, ^{59}Co, ^{60}Ni, ^{63}Cu, ^{66}Zn, ^{85}Rb, ^{88}Sr, ^{107}Ag, ^{111}Cd, ^{138}Ba and ^{208}Pb) from the core to the dorsal edge using LA-ICP-MS via an ESI NWR193 laser ablation unit coupled to an Agilent 7900 ICP-MS at the University of Essex, UK (see SI Text S2 for full details). Prior to finalising the laser ablation settings, we performed a series of trials to identify the optimal settings and beam diameter that would allow us to meet detection limits for low level elements for among-lake habitat discrimination while maximising temporal resolution to determine brief marine excursions. Details of these trials and final instrument conditions for analyses are outlined in SI Text S2, Figure S1, and Table S1. Otolith element concentrations were then despiked and smoothed (detailed in SI Text S3) and elements with >50% of measurements below detection limits or exhibiting high RSD values were excluded (Li, P, Cr, Fe, Co, Ni, Cu, Rb, Ag, Cd, Pb; Table S2).

2.1 | Question 1: What fraction of brown trout used each of the two lakes along the river network as nursery habitat?

To characterise each lake's signature, we used the outermost material from the eye lenses (outer ~243 ± 43 μm of the eye lens, diameter range = 1396-3182 μm) and otoliths (final 20 μm of each line scan; radius range = 540–1094 μm) from the immature trout, representing the most recent growth. We then targeted the same approximate size and age period in the adult otoliths as the immature reference trout, taking the ten measurements either side of the mean otolith radius observed in the reference trout (radius range = 708–736 μm). Eye lens isotope data were not used for the adults for this question, as discussed in the results. Chemical differences between lakes were characterised using the reference samples and Wilcoxon tests as the data did not meet assumptions required for parametric tests, for these tests the element tracers were natural log transformed. Random forest models (from the *caret* R package (Kuhn et al., 2023)) were then trained on 70% or 100% of the reference samples, with their accuracy tested using the remaining 30% (out-of-sample; OOS) or out-of-bag (OOB) approaches, respectively. The model with the highest accuracy was then used to analyse the equivalent size range in the adult fish tissues and assigned each individual to a nursery habitat. To generate 95% confidence intervals (CI) for our predictions, the model was trained on all training data 100 times, and each output model used to predict the adult assignments, with the mean probability used to predict final assignment to either lake. We classified these assignments as 'high confidence' if their 95% CI did not overlap the 0.5 chance of being assigned to either lake cf. 'low confidence' if they did.

2.2 | Question 2: At what age do sea trout first leave freshwater, and do their growth rates differ to resident trout before and/or after their first seaward migration?

The lifetime eye lens $\delta^{13}C$ and $\delta^{34}S$ and otolith Sr:Ca and Ba:Ca chronologies were used to reconstruct marine migrations in the adult trout. The four putative salinity markers showed changes suggestive

FIGURE 2 Map of the Fremstad catchment in central Norway, with the inset map showing the two focus lakes and their connection to the Trondheim fjord and sea.

of movement across the salinity gradient in the predicted directions (Figure 3a–c). However, the lenses could not be sampled at sufficient temporal resolution to detect sub-annual migrations and meet IRMS mass requirements, while otolith Ba:Ca tended to be noisier than otolith Sr:Ca and less effective for identifying freshwater-marine migrations later in life (Figure 3). Therefore, otolith Sr:Ca was selected for detecting anadromy, using a threshold value of 2.42 mmol/mol (equivalent to 2052 ppm) based on the maximum value observed in the freshwater reference samples after excluding the core material (specifically, the first 250 μm from the core), given that this would exhibit elevated Sr:Ca if the mother was anadromous (Barnett-Johnson et al., 2008). This threshold was similar to those used in other salmonid-focused studies (Hart et al., 2015; Mainguy et al., 2023).

Otoliths were both visually and chemically aged, the latter using the minima from Zn:Ca and Mg:Ca cycles drawing confidence among the two tracers when one was noisier. A second reader aged a subset ($n = 10$), visually and ($n = 8$), chemically for comparison (Figure 3d–f). As among-reader variation was higher for visual reads than chemical reads (Figure S2), chemical ages were selected to estimate fish age

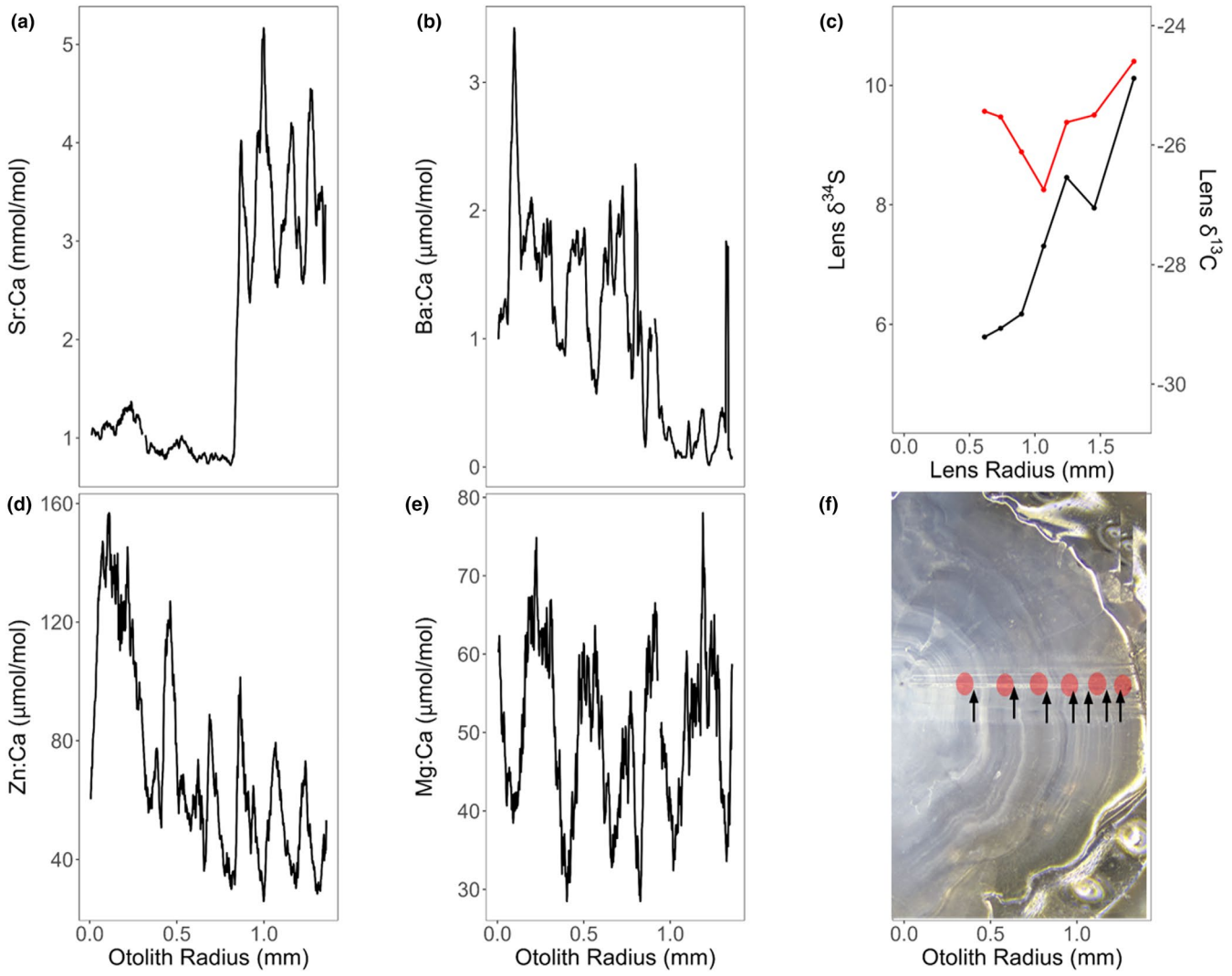

FIGURE 3 Example tracer profiles against otolith or eye lens radius from a single adult trout. Top row shows putative salinity markers, otolith (a) Sr:Ca and (b) Ba:Ca, and (c) eye lens $\delta^{34}S$ (black) and $\delta^{13}C$ (red). Bottom row shows putative age markers, otolith (d) Zn:Ca and (e) Mg:Ca, and (f) a photograph of the sagittal otolith on the dorsal axis under reflected light. Red dots in (d) to (f) denote estimated annuli based on chemical ageing. The blue dot in (e) denotes an annulus that was placed based on otolith Zn:Ca cycles despite lack of a clear pattern in otolith Mg:Ca. Visually identified growth bands in (f) are denoted by black arrows, which were generally deemed more difficult to read due to false annuli, here resulting in an additional year of growth compared to chemical ageing.

and growth rate using annuli widths. Incomplete annuli at the edge were excluded.

Given ontogenetic trends in growth rate, a generalised additive mixed model (GAMM) was fitted in the *mgcv* R package relating annulus width to age, excluding ages >7 to ensure good representation across fish and phenotypes (Ashworth et al., 2017; Vigliola & Meekan, 2009). The GAMM included an interaction between migratory phenotype and age, so that differences in growth rate at different ages could be tested for using GAM difference in the *gratia* package (Simpson, 2019). The model also included a categorical variable for phenotype (anadromous vs. resident) and habitat (freshwater if the annulus was formed wholly in freshwater vs. marine if the annulus was at least partially formed while the fish was at sea). Finally, the model included a random effect of fish ID to allow the intercept to vary by fish and allow for individual variation in growth.

3 | RESULTS AND DISCUSSION

3.1 | Question 1: What fraction of brown trout used each of the two lakes along the river network as nursery habitat?

Of all chemical tracers examined, only eye lens $\delta^{15}N$ values and ln (otolith Mn:Ca) exhibited significant differences between the lakes (Wilcoxon: $W=148$, $p=0.014$ and $W=145$, $p=0.021$, respectively; Table S3, Figure 4), though markers such as ln (otolith Sr:Ca) and ln (otolith Ba:Ca) did show sufficient difference to contribute meaningfully to differentiating between lakes in the multivariate classifier (Figure S3b). Many tracers showed a large spread of values and in some cases, for example ln (otolith Mg:Ca), there was negligible variation among lakes ($\eta^2=0.0003$), indicating that only 0.3% of the

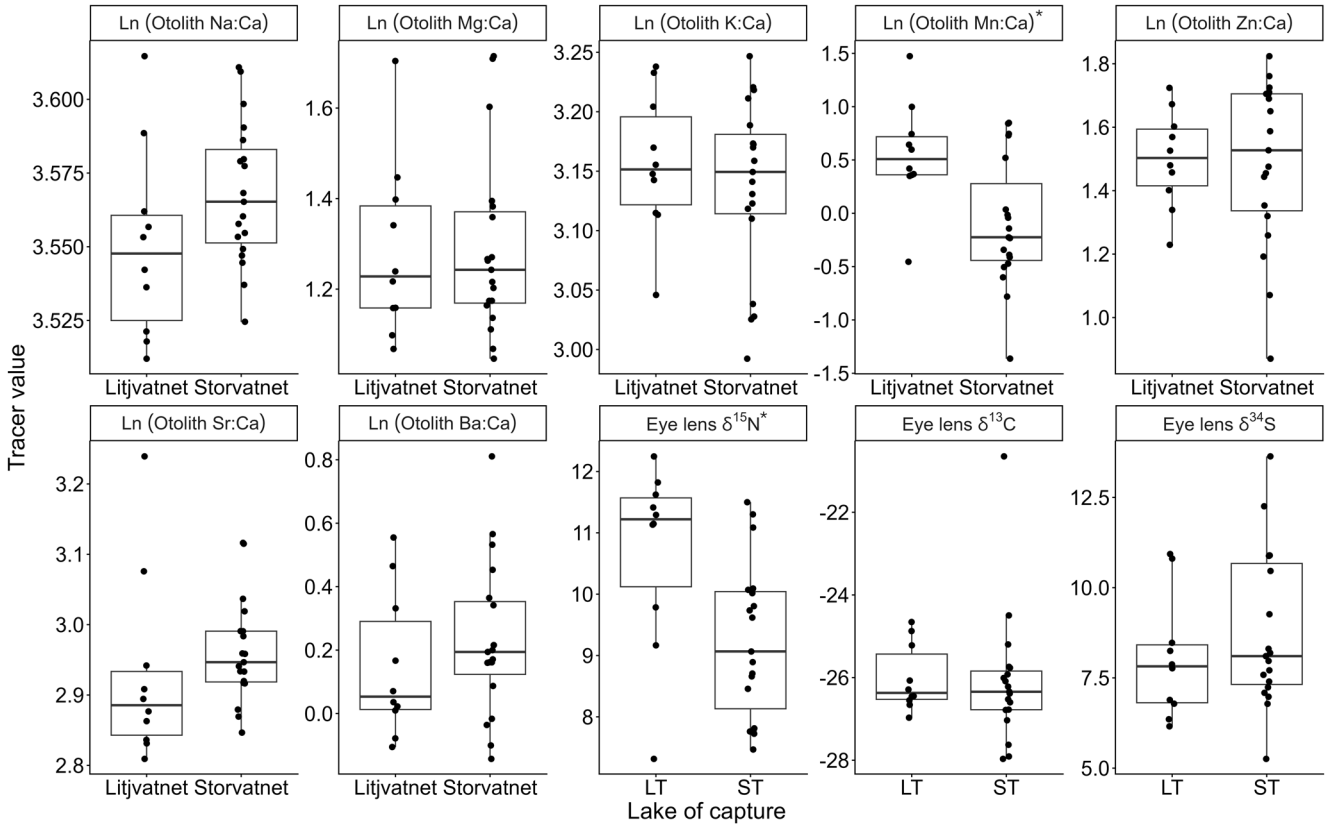

FIGURE 4 Differences in tracer values measured in the outer eye lens lamina (measured δ-isotope values) and at the otolith edge (elemental concentrations expressed as natural log mmol/mol) of immature ('freshwater reference') brown trout captured in Litjvatnet and Storvatnet lakes in Trøndelag, Norway. Asterisks denote significant relationships.

TABLE 2 Prediction accuracy of random forest iterations, trained on otolith element concentrations, eye lens stable isotopes, and both combined.

Model type	Dataset	Prediction type	Accuracy	Kappa
Otolith only model ln (Na:Ca, Mg:Ca, K:Ca, Mn:Ca, Zn:Ca, Sr:Ca, Ba:Ca)	Test data (30% of all data, $n=3$ & 5 per lake)	OOS	0.75	0.38
	All data	OOB	0.70	0.32
Lens-only model ($\delta^{13}C$, $\delta^{34}S$, $\delta^{15}N$)	Test data (30% of all data, $n=3$ & 5 per lake)	OOS	0.75	0.53
	All data	OOB	0.64	0.15
Otolith and lens combined model	Test data (30% of all data, $n=3$ & 5 per lake)	OOS	0.75	0.38
	All data	OOB	0.66	0.22

Note: The datasets were trained with either 70% of the data and tested on the remaining 30% or trained on 100% of the data with prediction accuracy estimated from out-of-bag sampling.

variation was explained by lake identity (Figure S3). This between-individual variation is likely due to differences in physiology, growth rate and diet (see Hüssy et al., 2021; Sturrock, Hunter, et al., 2015; Sturrock, Wikert, et al., 2015).

The random forest (RF) models trained on (a) lens isotopes, (b) log-transformed otolith element concentrations or (c) both, exhibited prediction accuracies ranging from 64% to 70% (OOB) and 75% for all three (OOS; Table 2). Given the small dataset (resulting in very small test datasets for OOS, Table 2), we decided to include all training data to predict the rearing lake used by the adults and thus used the model with the highest OOB accuracy (i.e. the model trained only on otolith elements). This model was driven primarily by among-lake variation in otolith Mn:Ca, with the higher concentrations observed in fish caught from the lower lake (Litjvatnet) characterised

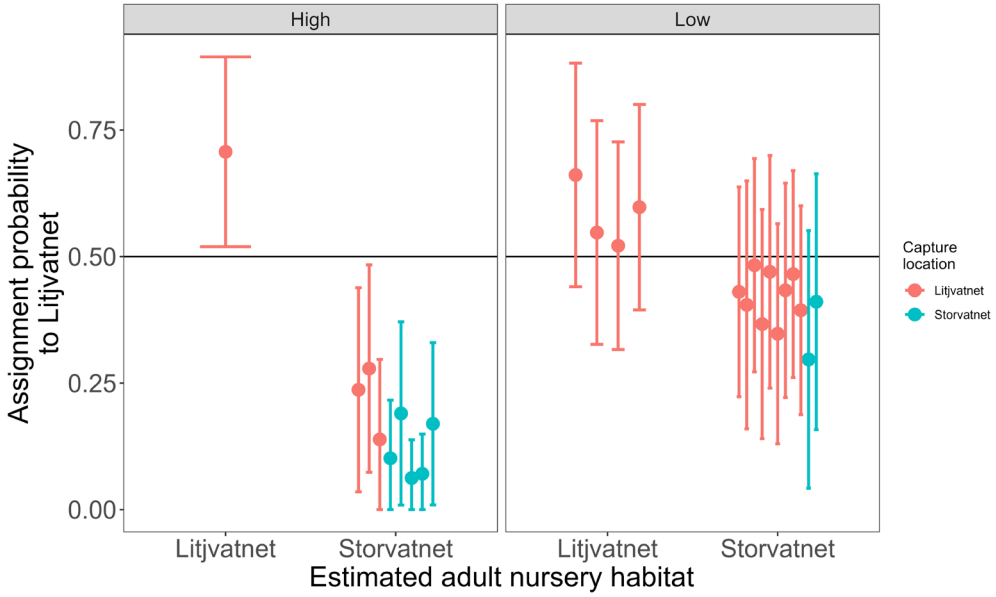

FIGURE 5 Mean assignment probability for unknown origin adults to Litjvatnet (assignment probability to Storvatnet is the inverse) using the random forest model trained on otolith elements ±95% CI based on 100 repeat models. Individuals with mean probabilities >0.5 were assigned to Litjvatnet and <0.5 to Storvatnet. The adults are separated between high and low confidence predictions based on their 95% CI overlapping or not overlapping an assignment probability of 0.5, respectively. Colour indicates the lake in which the adult was captured.

by higher levels of agriculture, potentially reflecting periods of hypoxia (Figure S3b) (Limburg et al., 2015). Surprisingly, the model trained on both otolith and eye lens markers exhibited lower OOB accuracy (66%), despite also including lens $\delta^{15}N$ values, which drove discriminatory power in the lens-only model (Figure S3c).

Our model predicted that 21% of the lake-sampled adults had used the lower lake, Litjvatnet, as nursery habitat (4% at high confidence and 17% at low confidence) (Figure 5). Conversely, 79% were predicted to have reared in the upstream lake, Storvatnet (33% at high confidence and 46% at low confidence) (Figure 5). This was expected given that Storvatnet is a larger, less human-impacted lake, fed by multiple spawning streams (i.e. the first large non-natal habitat experienced by downstream migrating fry). The failure to assign many adult fish to one of the two lakes with high confidence may be due to rearing in areas excluded from our baseline (e.g. upstream spawning streams). However previous electrofishing efforts indicated few two year old (2+) trout in nearby spawning streams, and the otoliths of adults were sampled at a radius greater than the mean radius of a 2+ fish (614 μm) (R. Paterson, personal communication, March 06, 2025), suggesting that these populations reside mainly in the lakes prior to onset of sexual maturity and potential seaward migration. It may also be due to regular movement of immature fish between the lakes, having lower numbers of reference samples than suggested by our power analysis, and/or interannual variability in water or diet chemistry (Jonsson, 1985). Future studies should consider sampling upstream tributaries and connecting rivers, and cohort-matching reference and adult samples (Table 1). However, of the adults assigned to either lake with high confidence, 67% had developed in the same lake that they were captured in, suggesting high site fidelity (Figure 5).

3.2 | Question 2: At what age do sea trout first leave freshwater, and do their growth rates differ to resident trout before and/or after their first seaward migration?

Among the 24 adult trout sampled, 70.8% ($n = 17$) were identified as anadromous, 12.5% ($n = 3$) as freshwater residents, and 16.7% ($n = 4$) as having an uncertain migratory history, given otolith Sr:Ca concentrations that increased, but to levels only just above or below the threshold value (Figure 6). Many individuals appeared to undertake repeated migrations, and four exhibited a distinct Sr:Ca spike at the core greater than the marine threshold value, suggesting that their mothers were anadromous and feeding at sea during vitellogenesis (Barnett-Johnson et al., 2008; Volk et al., 2000). Multiple other individuals also exhibited elevated core Sr:Ca concentrations, though below our putative marine threshold. Given difficulties in sampling the absolute core, variation in core Sr values depending on the timing of vitellogenesis and maternal migration patterns (Volk et al., 2000), and thus uncertainty in the appropriate threshold to indicate maternal phenotype, we did not include maternal strategy as one of our research questions. However, it is interesting that many of the anadromous adults exhibited completely flat Sr:Ca concentrations at the core, suggesting that they were the offspring of resident mothers and evidencing transgenerational phenotype-switching, a phenomenon that has been confirmed previously for trout using tagging (Berejikian et al., 2014; Dębowski & Dobosz, 2016), and highlighting the life history diversity of this highly plastic species. In fully marine fish, variation in otolith Sr:Ca is largely explained by physiology (particularly growth rate), potentially with some

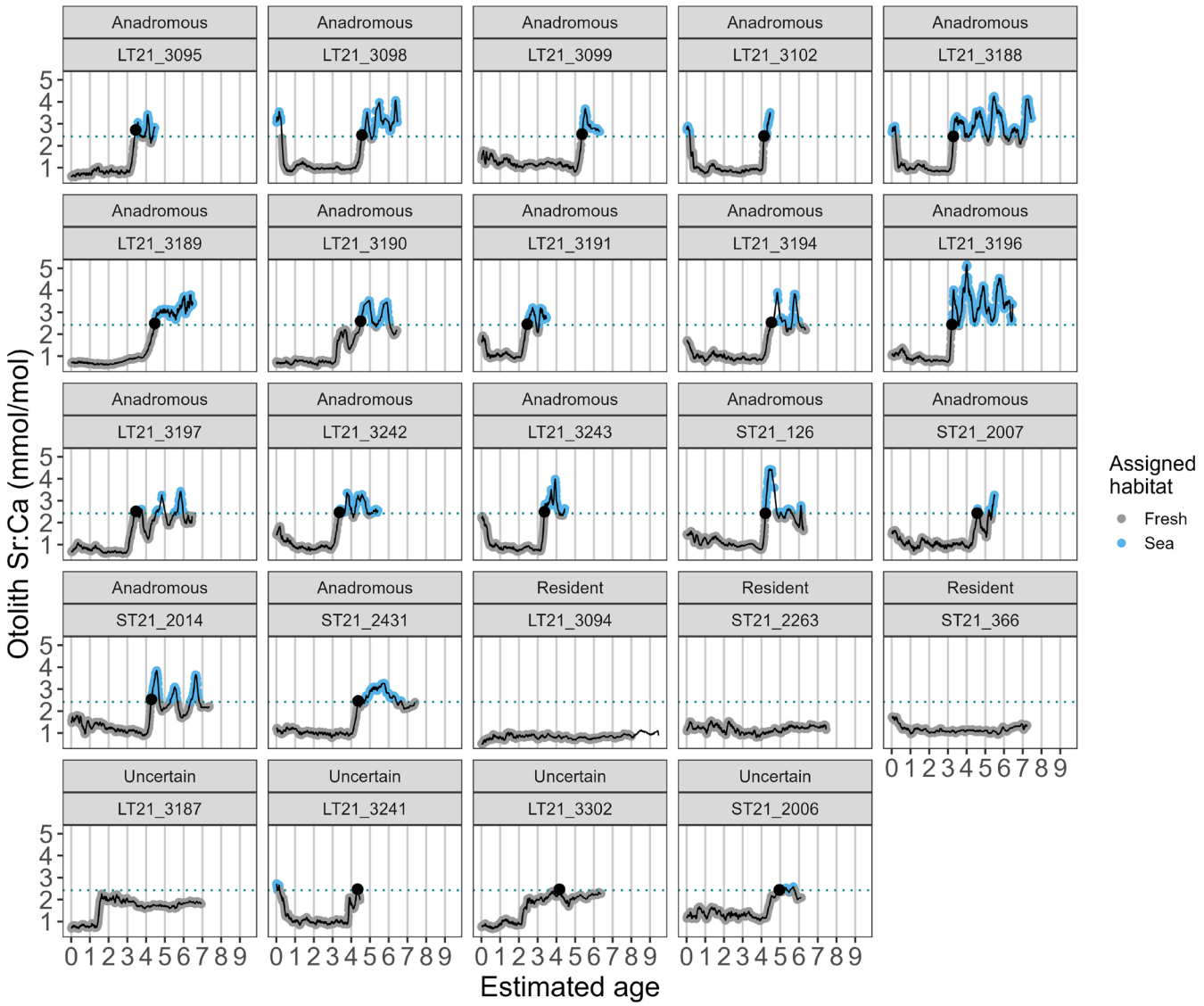

FIGURE 6 Otolith Sr:Ca profiles for 24 adult trout, separated into migratory phenotypes identified by otolith Sr concentrations: Anadromous, resident, or uncertain given otolith Sr values just above or below the threshold value of 2.42 mmol/mol (dotted line). Periods interpreted as freshwater or sea residence are indicated in grey and blue, respectively, and age at first outmigration (based purely on the first exceedance of our threshold value outside of the core) is indicated by a black circle. Note that elevated Sr concentrations in the otolith core suggest an anadromous mother, however, difficulties in accurately sampling the core and uncertainty in an appropriate threshold meant that maternal strategy was not reported in this study.

influence of temperature (Hüssy et al., 2021; Sturrock et al., 2012; Sturrock, Hunter, et al., 2015; Sturrock, Wikert, et al., 2015). For trout, however, a lot of the among-individual variation in otolith Sr peak values is also likely explained by the interaction between otolith growth rate, time spent in marine or freshwater habitats, and the laser beam diameter (Miller, 2011; Sturrock et al., 2012; Vignon et al., 2023). For example, a slow growing fish at sea for a relatively short period (say 1 month) would likely exhibit a lower peak otolith Sr concentration than a fast-growing fish at sea for the same length of time, as the laser beam is overlapping more otolith material deposited in freshwater before and after the excursion in the former. While annual and seasonal changes in water chemistry may influence the strontium concentrations in water, and while some freshwater systems exhibit water Sr concentrations higher than marine, the mixing curves between water Sr:Ca and salinity tend to be logarithmic, with most of the change in Sr:Ca occurring between 0 and 10 ppt (Brown & Severin, 2009; Hicks et al., 2010; Walther & Limburg, 2012). Thus, we are confident that if a trout had migrated from fully fresh to fully marine water, it would exhibit large increases in otolith Sr:Ca which would well exceed our threshold, independent of the year, as observed in all anadromous-assigned fish (Figure 6). All fish were captured in freshwater, but not all individuals exhibited 'freshwater' Sr:Ca values at the otolith edge. This was likely in part due to the lags and signal averaging mentioned above, but also due to challenges associated with sampling the outer otolith margin, where drops in

calcium counts often result in the loss of chemical data from the last few days to weeks of life.

The individuals identified as having an 'uncertain' migratory phenotype exhibited lens $\delta^{34}S$ values in their outer laminae that tended to be higher than in resident fish (Figure S4). Taken with the intermediate otolith Sr:Ca values, this suggests frequent movements across the salinity gradient or foraging in brackish habitats, and thus these fish were assumed most likely to be anadromous. This highlights the disadvantage of using a static threshold for defining marine migration, as opposed to methods such as zoning algorithms or change point analysis (Walther et al., 2011). Overall, the results suggest a higher proportion of sea trout in this system compared to other Norwegian populations (Jonsson, 1985) and what had been expected based on tagging studies carried out in this system (Paterson et al., 2021). This may be due to the majority of the adults (67%) being sampled from the lower lake, Litjvatnet, which is closer to the sea and smaller in area than Storvatnet, potentially increasing our observed rates of anadromy. It is also likely that our size-based selection criteria to define adult fish (>400 mm) biased the sample towards larger, anadromous individuals.

Among the individuals we confidently assigned as anadromous (Figure 6), age at first marine migration occurred between 3 and 7 years old (mean = 3.99 ± 0.73 years SD), similar to ages observed in other Norwegian systems, with smolt ages ranging between 2 and 7 years (Økland et al., 1993). Here, the frequency of return migrations from river to sea varied among individuals, but typically occurred annually, suggesting sub-annual periods at sea (Figure 6). Similarly, in other systems, tagging data have shown many trout overwintering in freshwater and only spending a few months per year at sea (Eldøy et al., 2015; Paterson et al., 2021; Thorstad et al., 2016).

The GAMM explained about 70% of the variance in otolith annulus, with most of this explained by a strong effect of ontogeny ($p < 0.001$ for both phenotypes), with growth rate generally decreasing with age (Figure 7; Table S4). No overall difference in growth rate was observed among phenotypes ($p = 0.168$), although the low sample size of resident fish ($n = 3$) would have greatly reduced our ability to resolve any differences. There was also no effect of within-year 'habitat' use on growth rate ($p = 0.175$). However, the age-phenotype interaction (Figure 7b) suggests that growth rates were significantly higher in anadromous fish in the first year, i.e. before any of them have gone to sea (Figure S5). This was consistent whether we assumed the 'uncertain' fish were anadromous (Figure 7b) or excluded them from the analysis (Figure S5).

We posit three potential reasons for the lack of differences in growth among migratory phenotypes when sea trout had been to sea vs. remained in freshwater. First, our sample of freshwater residents was very small ($n = 3$) and likely biased towards faster growing fish, given the size threshold of 400 mm used to define 'adults'. Second, due to the high productivity of these coastal lakes, residents may actually grow at similar rates to their anadromous counterparts (Ulsund, 2013; Winnem, 2010). Third, reduced marine growth may occur in this system due to sea lice infestations (*Lepeophtheirus salmonis*) associated with the intensive salmon farming in this region, which can reduce the health and growth of sea trout and lead to premature freshwater return (Bolstad et al., 2025; Gjelland et al., 2014; Thorstad et al., 2015). Sea trout are particularly vulnerable to sea lice infection (compared to other salmonids) due to their marine migrations often concentrating in nearshore areas used for salmon farming (Eldøy et al., 2015).

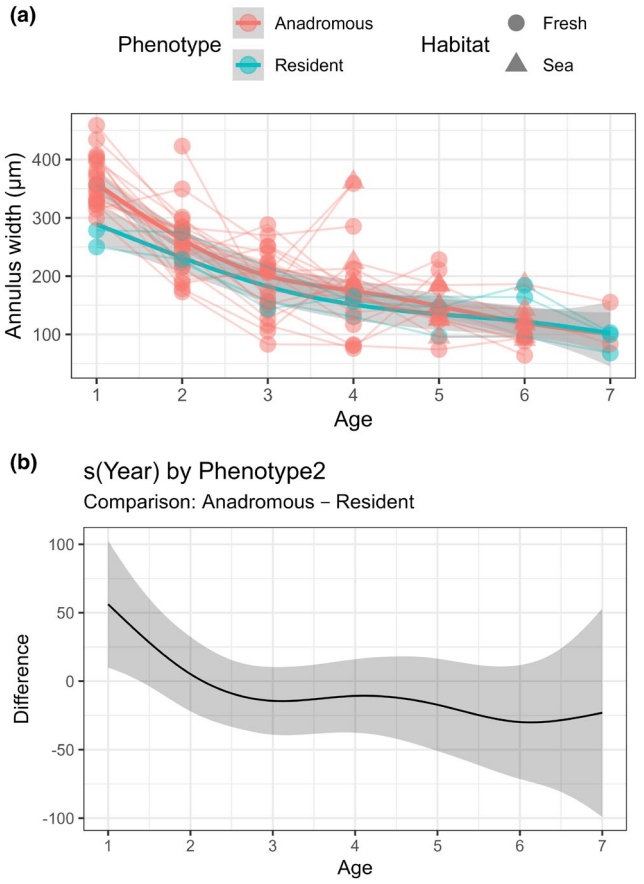

FIGURE 7 Growth rates of anadromous (red) vs. resident (teal) adult trout in this study ($n = 24$), with (a) showing the raw annulus width data and whether the annulus was deposited entirely in freshwater (circle) or at least part was formed while the fish was at sea (triangle). Individual growth trajectories are linked by thin lines in (a), and GAM smooths were fitted to each phenotype. Plot (b) shows the difference between the GAM smooths for the relationships between otolith annulus width and age, by phenotype, suggesting significant differences when confidence intervals do not overlap 0.

4 | CONCLUSION

Developing and applying explicit frameworks is vital to ensure reproducible science and to guide new users in the use of emergent methods. We developed such a framework for using tissue chemistry and biochronologies to reconstruct trout habitat use and growth, demonstrating its application in both marine and freshwater

settings to investigate fish nursery areas and to explore variation in migratory behaviours and growth rates. Our results suggest that Storvatnet, the upstream lake, plays a disproportionate role in supporting the growth and survival of juvenile trout in this system. Future steps could include additional tagging experiments and targeted fish sampling to better understand the underpinning mechanisms. If the results reflect lower survival rates in the lower lake (cf. simply lower movement rates into it), this could guide management actions aiming to increase trout productivity in this system. Our study also supports previous studies illustrating the plasticity of migration patterns exhibited by anadromous trout, an adaptation that has—at least historically—increased their resilience to disturbances across marine and freshwater realms. The results suggest that early life growth rates may influence migratory tendency, and—unlike previous studies—that growth rates were similar among resident vs. anadromous forms. Future work should confirm this result with larger sample sizes and validate otolith reconstructions using tagged fish with known migration histories. One could also compare results from contemporary vs. historical otolith samples (e.g. pre-aquaculture or even pre-humans) to explore the influence of anthropogenic influences on trout behaviour and growth. Overall, by using a methodical and transparent approach, we demonstrated how the framework could be used to help researchers and managers decide whether chemical tracers are the best tool for their question, species and system, and how to apply them to answer different questions about fish movement and growth.

AUTHOR CONTRIBUTIONS

Fieldwork and sample collection were conducted by Rachel Paterson, Tor Næsje, and Astrid Tonstad. Sample processing and analysis were conducted by Peter Betts, Astrid Tonstad, Coralie Moccetti, and Anna Sturrock. Peter Betts analysed the results and led the writing of the manuscript with support from Anna Sturrock, Eoin O'Gorman, Tor Næsje, and Rachel Paterson.

ACKNOWLEDGEMENTS

We thank all persons involved in gathering the samples, alongside Statsforvalteren for providing fishing permission and the Norwegian Environment Agency for providing funding for sample collections and the Norwegian Institute for Nature Research for funding the sample processing. We also thank Joe Dawson for assisting with the ICP-MS analyses. This work was supported by the ARIES Doctoral Training Partnership (Grant Number: [NE/S007334/1]), and the Atlantic Salmon Trust. Funding for A.S. was provided by UKRI [MR/V023578/1].

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflict of interest.

PEER REVIEW

The peer review history for this article is available at https://www.webofscience.com/api/gateway/wos/peer-review/10.1002/2688-8319.70277.

DATA AVAILABILITY STATEMENT

All data and code (Betts et al., 2026) are available at the University of Essex's Research Data Repository: https://doi.org/10.5526/ERDR-00000250.

STATEMENT OF INCLUSION

This study includes authors from a range of institutes and countries including researchers based in Norway where the samples were collected and who oversaw and directed the sampling program.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Text S1. Additional information to support new users working through Step 4: Selecting the tissue(s) and tracer(s) for your question.

Text S2. Laser protocol.

Text S3. Outlier removal and smoothing protocol.

Table S1. Operating conditions of the Agilent 7900 and ESI NWR 193 nm Laser.

Table S2. Mean limits of detection (LOD), percentage of measurements below detection, range of these elements ppm, range of despiked and smoothed values, and per cent relative standard deviation (RSD) based on background counts and repeat measures of NIST 610 and 612 certified reference material, respectively, averaged between the runs.

Table S3. Outputs of Wilcoxon tests comparing the outermost measurements of log otolith elemental concentrations and eye lens stable isotope ratios between immature reference trout sampled from Litjvatnet vs. Storvatnet Lake (Figure 1).

Table S4. GAM outputs.

Figure S1. Otolith Sr:Ca profile from primordia to the dorsal edge of the otolith from an adult anadromous trout, with inferred habitat shown by colour, with grey denoting freshwater and blue denoting marine. For each plot, laser power was at 60%, frequency was 20 Hz while beam diameter and speed are as labelled with the top line denoting beam diameter and the bottom-line representing scan speed.

Figure S2. Boxplots of differences in assigned age between the primary and secondary reader for chemical ageing vs. visual ageing.

Figure S3. Showing the Gini-based importance of each variable in the assignment of reference fish to one of the two lakes via a random forest model trained on (a) eye lens stable isotope ratios, (b) otolith element concentrations and (c) both eye lens stable isotopes and otolith element concentrations. Note that while many of these variables were not significantly different among lakes individually, they can still help to discriminate among lakes in a multivariate framework.

Figure S4. $\delta^{34}S$ profile from core to edge for each adult trout, with phenotype denoting fish identified in Figure S6 as anadromous, resident or 'uncertain' based on otolith Sr concentrations. Fish LT21_3187 (dashed line) exhibited otolith Sr concentrations just below the threshold value, but lens $\delta^{34}S$ values in outer layers >10 and was thus deemed most likely to be anadromous, along with all the other 'uncertain' fish.

Figure S5. Shows the difference between the GAM smooths for the relationships between otolith annulus width and age, by phenotype, with the four 'uncertain' fish excluded. Significant differences are suggested when confidence intervals do not overlap zero.

How to cite this article: Betts, P., Næsje, T. F., O'Gorman, E. J., Paterson, R. A., Tonstad, A. M., Moccetti, C., & Sturrock, A. M. (2026). A framework for using tissue chemistry and biochronologies to reconstruct fish habitat use and growth. *Ecological Solutions and Evidence*, *7*, e70277. https://doi.org/10.1002/2688-8319.70277