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Using otolith ^{87}Sr:^{86}Sr as a natal chemical tag in the progeny of anadromous Baltic Sea pike (*Esox lucius*) — a pilot study

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Otolith ^{87}Sr:^{86}Sr were quantified in 54 young-of-the-year pike (*Esox lucius*) collected from five west-Estonian freshwater sites to investigate the potential use of ^{87}Sr:^{86}Sr as a natural site-specific tag. Significant differences were found among the selected study sites. Pairwise comparisons revealed that only two sites were statistically indistinguishable from one another. Our results stress the need for understanding the migratory biology of the study species if significant early-life movements (e.g. seaward migration) occur. During the chemical analysis, maternally-influenced region (i.e. the core) should also be avoided or its data excluded. Based on our results and data from other studies we suggest that otolith ^{87}Sr:^{86}Sr is a powerful, yet underused marker to study the natal origins of freshwater spawning Baltic Sea fish at local (e.g. between neighbouring rivers) and regional scale (e.g. between drainage basins).

Introduction

Different otolith microchemistry applications are constantly tested in the context of life history of fish. For example, otolith strontium-to-calcium ratio (Sr:Ca) has become a routine tool for tracking migrations between fresh- and seawater (reviewed by Elsdon *et al*. 2008). Furthermore, the so called elemental and/or isotopic fingerprints are used to study natal origins, population structure and connectivity. However, varying degrees of success are reported in such work, especially in studies using only elements (Gahagan *et al*. 2012, Veinott *et al*. 2012). There is probably also a bias towards publication of positive results over negative ones. The outcome of such studies mostly depends on the magnitude of inter-site chemical differences, but also on instrumental sensitivity, accuracy and precision (Campana *et al*. 1997, Vroon *et al*. 2008) and choice of chemical markers (i.e. trace elements or stable isotopes). While the use of laser ablation inductively coupled plasma mass spectrometry (LA-ICPMS) for quantifying elemental concentrations is relatively widespread (Miller 2007, Gahagan *et al*. 2012), the usage of multi-collector LA-ICPMS to quantify ^{87}Sr:^{86}Sr ratios (Kennedy *et al*. 2000, Martin *et al*. 2013b) is much rarer due to higher cost and slower sample throughput. Recently, however, ^{87}Sr:^{86}Sr ratios have been reported to be the most useful marker in discriminating between selected freshwater

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study sites (Walther & Thorrold 2008, Martin *et al*. 2013b).

During the calcification process, strontium partially replaces calcium in geological and biological materials. The isotope ^{87}Sr is formed by decay of ^{87}Rb (half-life of 49×10^9 years), whereas ^{86}Sr is non-radiogenic and the absolute amount does not change over time. Rocks and minerals found in bedrock vary in terms of age and composition. As a consequence, older rocks and rocks with higher Rb:Sr ratios produce higher ^{87}Sr:^{86}Sr ratios (Kennedy *et al*. 2000). While different environmental and physiological factors can alter the elemental concentrations in otoliths (e.g. Friedland *et al*. 1998, Marohn *et al*. 2011), this has no effect on relative abundance of strontium isotopes (Kennedy *et al*. 2000). Furthermore, no significant biological fractionation occurs during uptake to otoliths, resulting in the same ^{87}Sr:^{86}Sr ratio as in water and diet (Kennedy *et al*. 2000, Walther and Thorrold 2008).

Rivers within the Baltic Sea drainage basin run on rocks that vary in age and composition. A mixture of bedrocks, ranging from Mesozoic-Paleozoic sediments in the south to Proterozoic-Archean intrusives in the north, can be found. The southern rivers have high concentrations of Sr (range 100–500 μg l^{-1}) while the ^{87}Sr:^{86}Sr ratios are low (range 0.7095–0.7104) and are equal or similar to the fixed value of seawater that is ca 0.7095 in the Baltic Sea (Åberg & Wickman 1987, Löfvendahl *et al*. 1990). The northern rivers have low concentrations of Sr (range 15–50 μg l^{-1}) and high ^{87}Sr:^{86}Sr ratios (range 0.721–0.745) (Löfvendahl *et al*. 1990). However, small-scale variations in ^{87}Sr:^{86}Sr are also present (Åberg & Wickman 1987). As fish otoliths should also record those differences, ^{87}Sr:^{86}Sr could potentially serve as a valuable marker to trace the natal origins of species spawning in freshwater. There are only three studies on otolith elemental fingerprinting in the Baltic Sea. Two of them deal with a marine species, the Atlantic cod (*Gadus morhua*) (Svedäng *et al*. 2010, Heidemann *et al*. 2012) and only one was conducted in freshwater to study natal homing of the anadromous pike, *Esox lucius* (Engstedt *et al*. 2014). To our knowledge, to date otolith ^{87}Sr:^{86}Sr ratios have not been used as tracers of fish natal origin (or migration) in the Baltic Sea basin. If applicable, this marker can potentially provide means to differentiate groups of fish locally (e.g. between adjacent rivers) and regionally (e.g. between drainage basins). This kind of information would be invaluable for management and conservation because of the possibility to estimate relative recruitment from different natal areas.

The aim of this study was to investigate the feasibility of using the otolith ^{87}Sr:^{86}Sr ratio as a tracer of natal origins at a relatively local scale. Application of this method relies, however, on the presumption of inter-site chemical differences in baseline values (i.e. freshwater endmembers). To test this, we quantified otolith ^{87}Sr:^{86}Sr in young-of-the year (YOY) pike collected from five different freshwater spawning areas.

Material and methods

The study was conducted in western Estonia, in and near the Väinameri (Fig. 1). Estonia's bedrock is mainly from the early Paleozoic era, ranging from Devon in the south to Cambrium and fractions of the late Ediacaran period in the north. The Väinameri region is mainly from the Silurian period, but the rivers Taebla and Riguldi fall into the Ordovician period (http://www.egk.ee). Our study sites were selected based on the known spawning grounds of anadromous pike (*Esox lucius*), which was chosen as a model species. The pike is an ecologically, commercially and recreationally important species in the Baltic Sea region. In addition to freshwater-resident populations, anadromous and sea-living populations exist (Westin & Limburg 2002, Vetemaa *et al*. 2006, Engstedt *et al*. 2010, Rohtla *et al*. 2012). Exact sampling locations depended on the site (Table 1), but were always in freshwater. This was later confirmed by Sr:Ca line-scans (*see* below). YOY pike ($n = 54$) were collected in 2010 with beach seine or electrofishing. In Kõiguste, samples from the Maadevahe River and an unnamed ditch were pooled due to low sample size from the river. For exploratory purposes, one YOY pike and two adults were also collected from Kõiguste Bay (salinity ca. 6) using fykenets and gillnets, respectively. These fish were used

to test our ability to quantify freshwater ^{87}Sr:^{86}Sr ratios from an otolith with seawater values. Total length (TL, mm) was recorded and otoliths removed immediately after capture. Otoliths were cleaned and stored dry in microtubes. In the laboratory, YOY otoliths were mounted (sulcus side up) onto a piece of glass slide using Crystal bond thermoplastic wax. They were then ground manually on a grinding machine with sand paper (grit size P1200) until the core was visible. Final polish was done with sand paper with grit size P4000. The two otoliths of adults were fully mounted into epoxy resin. A transverse section of the otolith was subsequently ground out from the epoxy blocks. All thin sections of the otoliths were then glued onto a standard microscope slide. Before the chemical analysis, otoliths were cleaned ultrasonically for 15 minutes in NANOpure® water and dried under a class-100 laminar-flow hood.

All the chemical analyses were carried out at the Oregon State University's WM Keck Collaboratory for Plasma Spectrometry. Strontium isotopic composition was quantified using New Wave DUV193 excimer laser and a NuPlasma multicollector (MC-LA-ICPMS). A general method of Woodhead *et al*. (2005) as described in Miller and Kent (2009) was followed to correct for potential Kr and Rb interferences and monitor for Ca argide-dimer formation. The laser was set to a pulse rate of 15 Hz, with a 65 μm spot size that travelled at 10 μm s^{-1}. To maximize the material gathered from the natal region, a 200 × 200 μm raster was ablated adjacent to the core area. The resulting crater depth was 15–17 μm. By using otolith growth rate and age of fish it was estimated that the ablated area represented about a month of early life. ^{87}Sr:^{86}Sr values from that region were averaged for further analyses and presentation. In the transverse sections of adult otoliths, ablations were conducted in the compressed side of the otolith, the other side was used for Sr:Ca profiles (M. Rohtla unpubl. data). To monitor instrument accuracy and precision, the ^{87}Sr:^{86}Sr of a marine gastropod

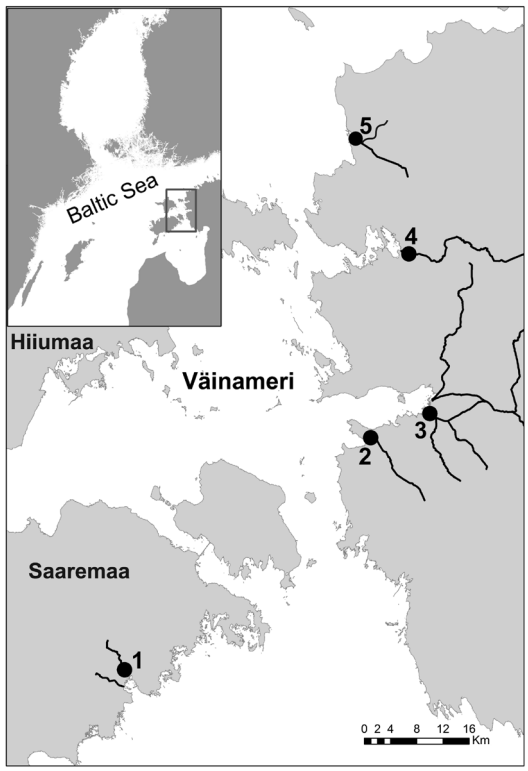

Fig. 1. Sampling sites for the freshwater collected young-of-the-year pike. 1: Kõiguste, 2: Sauemeri, 3: Kasari River, 4: Taebla River, 5: Riguldi River.

Table 1. Background information for the freshwater collected young-of-the-year pike. Sampling habitat is given in parentheses.

Location	*n*	Sampling date	Mean TL ± SD (mm)
Kõiguste* (river, ditch)	11	8 July 2010	73 ± 19
Kasari River (estuary)	12	22 July 2010	103 ± 21
Sauemeri (floodplain)	11	24 July 2010	127 ± 33
Riguldi River (main channel)	11	18 September 2010	148 ± 21
Taebla River (main channel)	9	19 September 2010	177 ± 22

* The samples from Kõiguste were collected from the Maadevahe River (n = 3) and an unnamed ditch (n = 8). Samples were pooled due to small distance (i.e. < 1 km) between the sites and low sample size from the Maadevahe River.

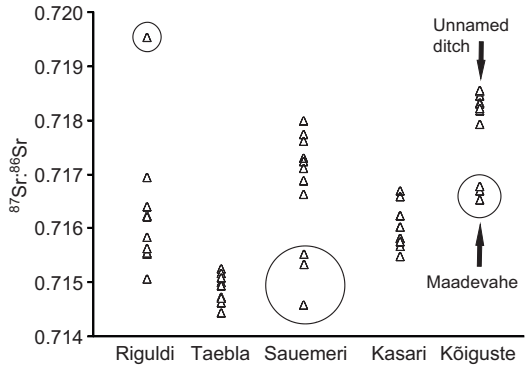

Fig. 2. Otolith strontium isotope ratios from the sampled freshwater study sites. Individuals in circles were excluded from the statistical tests to stabilize site-specific signature. Note the values from two close sites sampled in Kõiguste.

(0.70918) was used as an in-house marine carbonate standard (Miller & Kent 2009). A mean value (± 2 SE) of 0.709295 ± 0.000043 (n = 19) was consistently obtained; otolith $^{87}Sr:^{86}Sr$ were corrected for this difference.

To verify the migratory history of collected specimens, Sr:Ca profiles were also quantified (M. Rohtla unpubl. data). A freshwater Sr:Ca threshold of 0.5×10^{-3} has been set for Matsalu Bay pike (Rohtla *et al*. 2012). A VG PQ ExCell ICP-MS with a New Wave DUV193 excimer laser was used to trace line-scans across the same otoliths used in isotope analysis. The laser was set to 10 Hz with a 40 μm ablation spot size and a scan speed of 10 μm s^{-1}. Data was handled following the methods of Miller (2007) as described in Rohtla *et al*. (2014).

To stabilize the within-site signature and to facilitate statistical comparisons between sites, confirmed outliers and extremes were removed from the statistical tests (Fig. 2). The criteria for excluding individual $^{87}Sr:^{86}Sr$ values from the statistical test were as follows: (1) the values were larger or smaller than the site's mean ± 2SD, and (2) ablation of maternally influenced region or marine growth had occurred; for that Sr:Ca data and high-definition visual inspection under a microscope of the $^{87}Sr:^{86}Sr$ raster placement on the otolith were used. With regard to the first criteria we removed one specimen from Riguldi and with regard to the second criteria we removed three fish from Sauemeri (*see* Results for detailed explanations). From the Kõiguste group we removed all three Maadevahe River fish from the statistical test as the two sites differed significantly, therefore not permitting us to use a pooled sample as planned. Removal of these individuals allowed us to perform a parametric analysis of variance (ANOVA) followed by post-hoc Tukey's HSD test. The assumptions of ANOVA were met.

Results

Sr:Ca line-scans verified that all YOY pike caught from freshwater were born in freshwater (Sr:Ca < 0.5×10^{-3}) and had never entered marine waters (Fig. 3a). The $^{87}Sr:^{86}Sr$ values quantified from the otoliths of these fish varied between 0.714 and 0.719 (Fig. 2), reflecting the general south to north gradient in the Baltic Sea. The ±2SDs for these individual measurements varied between 0.00038 and 0.00084 with a mean of 0.00049.

Significant differences in otolith strontium isotope composition were found among the study sites (ANOVA; $F_{4,42} = 98$, $p < 0.001$), although considerable intra-site variability was also found for some sites (Fig. 2). Nearly all sites were significantly different from each other (Tukey HSD: $p < 0.001$ in all cases), only Kasari and Riguldi were indistinguishable ($p = 0.95$). The three fish removed from Sauemeri had $^{87}Sr:^{86}Sr$ values that were probably diluted with maternally-derived seawater values (i.e. 0.7095) as the ablations pits touched the core area which in all cases included a maternally derived Sr:Ca mother peak (Kalish 1990, Engstedt *et al*. 2010) (Fig. 3b). The specimen with the lowest $^{87}Sr:^{86}Sr$ contained also the highest Sr:Ca mother peak. We were unable to eliminate the influence of the partial ablation of a core area as low $^{87}Sr:^{86}Sr$ values persisted throughout the scan. The one fish from Riguldi was probably a drifter from an unknown source (e.g. a small brook runs into Riguldi just 1 km from the collection site).

The YOY pike caught from brackish Kõiguste Bay had $^{87}Sr:^{86}Sr$ of 0.709548, indicating marine origin. However, inspection of its Sr:Ca profile revealed that this specimen had emigrated from freshwater at an extremely early stage (Fig. 3c),

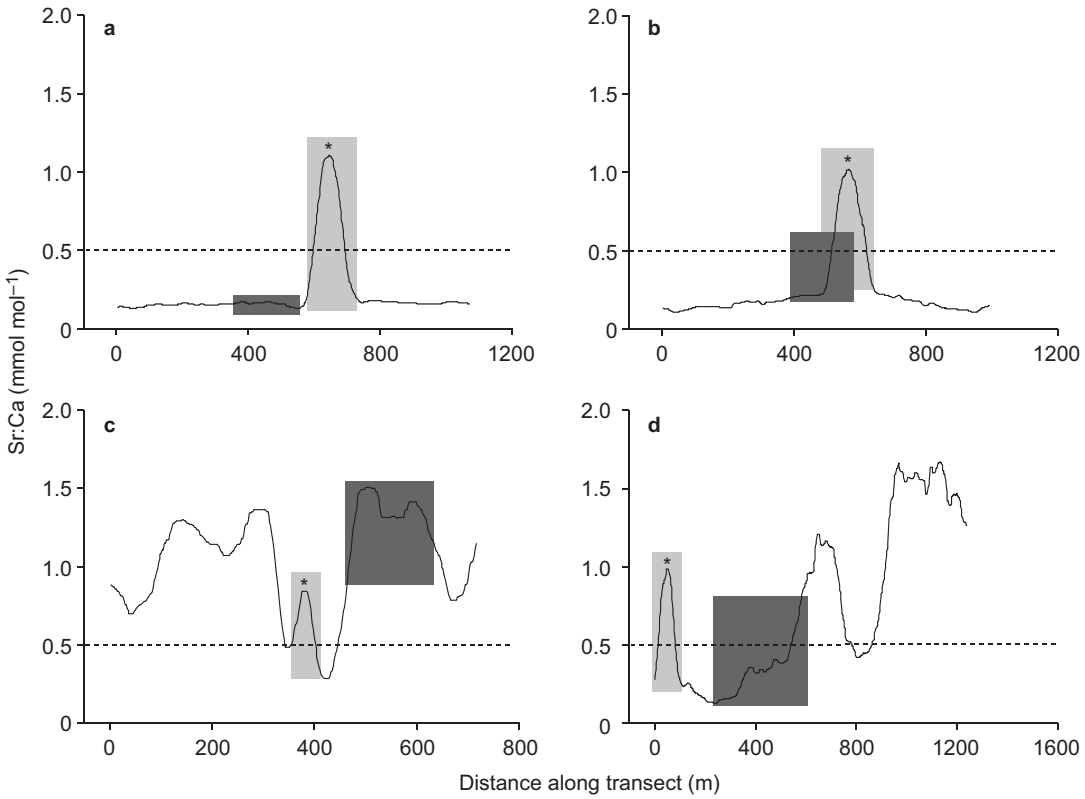

Fig. 3. Sr:Ca profiles of individuals with (**a**) representative and (**b–d**) non-representative natal $^{87}Sr:^{86}Sr$ sampling. Approximate $^{87}Sr:^{86}Sr$ sampling locations are indicated with dark-grey rectangles. Otolith core (*) and maternally-influenced region (light-grey rectangles) are also marked. Dotted lines represent the freshwater threshold of 0.5×10^{-3}. (**a**) Edge-to-edge Sr:Ca profile of YOY pike with the representative $^{87}Sr:^{86}Sr$ value. (**b**) Edge-to-edge Sr:Ca profile of YOY pike with $^{87}Sr:^{86}Sr$ ablation falling into maternally-influenced region (**c**) Edge-to-edge Sr:Ca profile of YOY pike demonstrating early emigration from freshwater and $^{87}Sr:^{86}Sr$ ablation from the marine growth region. (**d**) Core-to-edge Sr:Ca profile of adult pike with $^{87}Sr:^{86}Sr$ quantified from the other (compressed) side of the otolith; dark-grey rectangle represents the approximate area that would have been sampled on the wider side of otolith.

meaning that we ablated marine growth region of the otolith. Furthermore, ablation of the first Kõiguste Bay adult otolith resulted in $^{87}Sr:^{86}Sr$ of 0.713537. This was due to the fact that the ablation was conducted on the compressed side of the (transversally sectioned) otolith and on the fresh- and seawater transition zone (Fig. 3d). The second adult had migrated to the sea later in life and the ablation was strictly from the freshwater growth region ($^{87}Sr:^{86}Sr$ 0.717859).

Discussion

The results of the present study revealed that otolith $^{87}Sr:^{86}Sr$ is a useful chemical marker for discriminating between various freshwater spawning sites of pike around the Väinameri region. As differential fractionation of $^{87}Sr:^{86}Sr$ among species is unlikely (Blum *et al*. 2000, Bentley 2006) it can be assumed that the results of this study can be most likely applied for other fish species as well. Furthermore, in a multi-species study, Wolff *et al*. (2012) reported that in most cases otolith $^{87}Sr:^{86}Sr$ overlapped among species within one reservoir. For the Baltic Sea region, most of modern-day $^{87}Sr:^{86}Sr$ freshwater data come from the works of Åberg and Wickman (1987) and Löfvendahl *et al*. (1990) who demonstrated that freshwater $^{87}Sr:^{86}Sr$ is distributed heterogenously at various spatial scales. Although our study was conducted at a relatively local scale, these and

also the results of previous studies draw attention to the huge potential of otolith $^{87}Sr:^{86}Sr$ as a powerful marker in the Baltic Sea region. This means that otolith $^{87}Sr:^{86}Sr$ may provide a tool for determining the natal origins of relatively sedentary (semi)anadromous species (e.g. pike, burbot *Lota lota*, ide *Leuciscus idus*) locally, and also of highly migratory anadromous species (e.g. salmon *Salmo salar*, sea trout *Salmo trutta*, whitefish *Coregonus lavaretus*) regionally, e.g. between drainage basins. Discrimination power can be further improved by adding additional markers (e.g. trace elements, carbon and oxygen isotopes) to the fingerprint (Walther and Thorrold 2008, Gibson-Reinemer *et al*. 2009).

Sites within close proximity showed surprisingly strong differences in $^{87}Sr:^{86}Sr$. In Kõiguste, for example, the two freshwater sampling sites were separated by less than a kilometer and this resulted in a clearcut difference in $^{87}Sr:^{86}Sr$. Similarly, fish from the Kasari River and the Sauemeri floodplain had relatively distinct $^{87}Sr:^{86}Sr$ values, regardless of the small distance (ca. 10 km) between the sampling sites. However, it should be noted that the size of drainage areas is vastly different in the latter case. Engstedt *et al*. (2014) used trace elements and also reported distinct natal signatures for pike from four sites within 50 km. This means that fine scale discrimination of natal sites is possible even at minimal local scale (e.g. within one bay with multiple freshwater inputs). Of the two Kõiguste Bay collected adults, only one had a representative $^{87}Sr:^{86}Sr$ natal value (i.e. a true value without any confounding effects) and it closely resembled the signature of a nearby freshwater ditch. Future studies should try to determine the importance of such small spawning areas, especially in regions with limited access to freshwater (e.g. Kõiguste Bay). Increasing the sample size of freshwater YOY and adults collected from the sea would allow us to determine the relative importance of different spawning sites. Future work should also address the temporal stability of $^{87}Sr:^{86}Sr$ in freshwater as various cohorts of adults will be sampled from the sea. However, based on work done elsewhere it is known that $^{87}Sr:^{86}Sr$ values are relatively stable across years (Kennedy *et al*. 2000, Martin *et al*. 2013a).

Like in many anadromous salmonids (Kalish 1990, Volk *et al*. 2000), elevated core Sr:Ca or mother peak is also present in anadromous pike (Engstedt *et al*. 2010, Rohtla *et al*. 2012). As vitellogenesis in anadromous individuals takes mainly place in seawater, elevated Sr:Ca and also the stable value of $^{87}Sr:^{86}Sr$ (i.e. 0.7095) will be incorporated into the otolith growth zone preceding the exogenous feeding check (Kalish 1990, Miller *et al*. 2011, Courter *et al*. 2013). The natal chemical signature starts to form only after yolk absorption. In the present study, three freshwater residents from Sauemeri showed $^{87}Sr:^{86}Sr$ values that were suspiciously low. Closer inspection revealed that the traced rasters were partly placed on the otolith zone that precedes the exogenous feeding check. Thus it is highly likely that natal $^{87}Sr:^{86}Sr$ was diluted with the seawater value originating from the anadromous mother. Unfortunately, we did not quantify $^{87}Sr:^{86}Sr$ from the core region to verify that the value was indeed equal or similar to the seawater value. However, our results and results from the studies on salmonids (Kalish 1990, Courter *et al*. 2013) strongly point towards the described scenario.

Another potential pitfall arises in cases where some (Landergren 2004, Rohtla *et al*. 2012) or all YOY (Gallagher *et al*. 2012) emigrate from freshwater shortly after hatching. As the chemical saturation of otoliths takes at least two weeks or more (Miller 2011, Engstedt *et al*. 2012), true natal signature cannot be retrieved if emigration or passive drift to the sea occurs shortly after hatching. If freshwater emigration occurs later in life but still relatively early (e.g. one to five months post-hatch) one must make sure that the chemical fingerprint in sea-collected adults is indeed representative of a natal freshwater site. Basically the same situation occurs when YOY disperse to (chemically) different freshwater sites (Einum *et al*. 2012). Sr:Ca, but also $^{87}Sr:^{86}Sr$ core-to-edge line-scan is a ready solution in the former case, as it allows to determine the exact time of freshwater emigration, and therefore to pinpoint the true natal signature (Miller *et al*. 2011). In the latter case, the $^{87}Sr:^{86}Sr$ profile is the most promising option, providing that there are indeed noticeable differences among sites. Either way, it is important to understand the migratory biology of the study species if potential for early migration exists.

In conclusion, this study showed that otolith ^{87}Sr:^{86}Sr has a great potential in discriminating fish from different natal sites in western Estonia. Future studies should aim at compiling a comprehensive database of ^{87}Sr:^{86}Sr values from all (or most important) freshwater natal sites that could be producing recruits to a mixed adult population in the sea. Therefore, sampling of sea-living adult populations should also be conducted. Depending on the specific study system, it is recommended to use additional chemical markers if possible. Such information will eventually help to estimate relative recruitment from different natal areas. Ultimately, this would hopefully guide various conservational actions. Our results also indicated that when dealing with freshwater species that emigrate from their natal areas soon after hatching (e.g. early emigration to the sea) one must be aware of such movements when natal origin of adult population is quantified.

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