

Do Eurasian minnows (*Phoxinus phoxinus* L.) inhabiting brackish water enter fresh water to reproduce: Evidence from a study on otolith microchemistry

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Abstract

Dynamics of coastal fish assemblages in the Baltic Sea are still rather poorly understood. In particular, little information is available on migrations and movements of the small-bodied littoral fish species like Eurasian minnow (*Phoxinus phoxinus* L.). Minnow is considered typically as a river and lake species, but it also inhabits brackish coastal waters of the Baltic Sea. In this study, we investigated movement patterns of brackish water inhabiting minnows using otolith microchemistry. Fish were collected from four different sites (from two bays and two stream mouths) around Saaremaa Island. The results indicated that at least three distinct migration patterns exist: (i) seawater residency, (ii) fast springtime migrations to fresh water or to bays with lower salinities (areas near stream mouths) and (iii) prolonged migrations to fresh water (some cases overwintering in streams). Migration patterns listed above were not evenly distributed among sites, and some individuals did not migrate to fresh water in every year. None of the analysed fish were freshwater residents. Additionally, potentially ontogenetic effects on Mn and Sr concentrations were observed in the otolith core regions, which may have important implications for the interpretation of otolith chemistry data. The overall findings of this study demonstrate that much more complex migration patterns exists in brackish water inhabiting minnow populations than previously thought and at least some individuals do enter freshwater to reproduce.

KEYWORDS

Sr:Ca, Mn:Ca, Baltic sea, migrations, cyprinids, small-sized fish

1 | INTRODUCTION

The Baltic Sea is one of the largest brackish water bodies in the world. It has a relatively dynamic salinity regime that is mainly influenced by freshwater run-off from rivers and water exchange with the North Sea. The salinity decreases from the Kattegat (surface salinity 12–30 psu) towards the northernmost and easternmost parts of the sea (less than 2–3 psu). This north–south oriented salinity gradient allows the Baltic Sea to be regarded as a vast estuary. Because of its salinity regime and relatively young age, the species richness of the sea is comparatively

low and there are no endemic fish species. The Baltic Sea fish community is composed of species with marine and freshwater origin. Due to its brackish and estuarine character, both vertical and horizontal variations of environmental conditions in the Baltic Sea are typically much greater than in seas with constant salinity regimes (Ojaveer & Pihu, 2003).

Coastal areas of the Baltic Sea are typically dominated by freshwater fish species. Although a number of those species have populations which are able to successfully propagate in brackish water (Jorgensen et al., 2010; Svirgsden et al., 2015; Tibblin, Koch-Schmidt, Larsson, &

Stenroth, 2012), a noticeable proportion of them are still dependent on freshwater for spawning (Engstedt, Stenroth, Larsson, Ljunggren, & Elfman, 2010; Lappalainen, Dörner, & Wysujack, 2003; Rohtla et al., 2014) and often undertake extensive spawning migrations from the brackish foraging grounds to freshwater spawning sites (Müller & Berg, 1982). In general, dynamics of coastal fish assemblages in the Baltic Sea are still rather poorly understood (Mustamäki, Jokinen, Scheinin, Bonsdorff, & Mattila, 2015). Furthermore, the majority of research has been focused on commercially important species like pike *Esox lucius* L. and perch *Perca fluviatilis* L. Very little information is available on migrations and movements of the small-bodied littoral fish species. Although commercially not valuable, they are ecologically important links in food webs as important prey items for many economically valuable predatory species (Mustamäki, Cederberg, & Mattila, 2014; Taal, Saks et al., 2014).

Eurasian minnow *Phoxinus phoxinus* L. (hereafter referred as minnow) is a small slender schooling freshwater fish, belonging to the Cyprinidae family. Its native range encompasses almost the whole of Europe and northern Asia (Mills, 1988). Typically minnows inhabit rivers, streams and clean lakes with bottoms of stones and gravel (Frost, 1943). This short-lived species produces successive batches of eggs during an extended spawning season, which usually lasts from May to July (Mills & Eloranta, 1985; Saat, Järvekülg, & Turovski, 2003). Spawning season is shorter, and the number of egg batches is smaller in northern regions than in southern ones (Mills, 1988). In Estonia, it is one of the most widespread species in streams, but it is also common in the brackish coastal waters of the Baltic Sea (Saat et al., 2003). Furthermore, minnows inhabiting the coastal sea of West Estonian archipelago (they are absent from streams of those islands themselves) are so far considered as a separate ecomorph that resides solely in brackish waters and do not enter freshwater streams and rivers (Järvekülg, 2001).

In recent decades, otolith microchemistry has emerged as an efficient tool for studying the movements of fish between environments with different water chemistry (Campana, 1999; Elsdon et al., 2008; Gillanders, 2005; Walther & Limburg, 2012). A variety of trace elements and stable isotope markers have been used. To successfully use these markers, they must comply with two key assumptions: ambient dissolved concentrations of the selected markers in water must vary among environments investigated, and they must be incorporated into otoliths in proportion with their ambient values with minimal interferences from confounding environmental and physiological factors (Elsdon et al., 2008; Walther & Limburg, 2012). Strontium-to-calcium ratio (Sr:Ca) has proven to be the most efficient marker for studying fish migrations when movements occur between habitats with different salinity, for example in a river-sea system (Gillanders, 2005; Rohtla, Vetemaa, Urtson, & Soesoo, 2012; Secor & Rooker, 2000). Strontium-to-calcium ratio in the water is generally positively correlated with salinity (Limburg, 2004; Zimmerman, 2005), and those ambient Sr:Ca values are reflected in the otoliths (Bath et al., 2000; Kraus & Secor, 2004). Several previous studies have successfully used otolith microchemistry (Sr:Ca as a chemical tracer) for studying fish movements in the Baltic Sea, although, yet again, research has

been focused on economically valuable species (Limburg, Landergren, Westin, Elfman, & Kristiansson, 2001; Limburg, Wickström, Svedäng, Elfman, & Kristiansson, 2003; Rohtla et al., 2015; Shiao, Ložys, Iizuka, & Tzeng, 2006; Taal, Kesler et al., 2014).

The objective of this study was to use otolith Sr:Ca concentrations and age data to investigate Eurasian minnow's movements in the brackish coastal areas of the Baltic Sea. Specifically, whether minnows inhabiting the coastal sea of the Western Estonian archipelago undertake spawning migrations into fresh water. So far those fish have been considered as a separate ecomorph that inhabit only the brackish environments.

2 | MATERIALS AND METHODS

2.1 | Study sites

Fish were collected from four sites around Saaremaa Island: Kõiguste Bay, Uudepanga Bay, Ligeoja Brook and Jämaja Brook (Figure 1). Kõiguste Bay is situated in the northern part of the Gulf of Riga, Baltic Sea. It is a shallow semi-enclosed bay with a dominant sediment type of sandy clay mixed with pebbles, gravel and boulders (Lauringson & Kotta, 2006). The area is affected by a diffuse nutrient load from the moderately eutrophicated Gulf of Riga (Astok, Otsmann, & Suursaar, 1999). The bay is divided into two relatively distinct subunits (inner and outer bay) by a peninsula and a belt of small islets. The inner bay is shallower (up to 2 m) and slightly less saline (3–5 psu) compared to the outer bay (up to 6 m and 4–6 psu). A number of seasonal ditches and one small river (Maadevahe River) drain into the inner bay, and only a few ditches drain into the outer bay. The sampling site was situated on the northern shore of the outer bay.

Uudepanga Bay is situated in the eastern part of the Northern Baltic Proper. It is a small open bay with depths reaching up to 20 m. The dominating substrate in shallower areas is sand, except in the eastern coast, where a limestone bottom with patches of rocks and gravel dominates. Surface water salinity ranges between 5 and 7 psu. The bay receives marginal freshwater inflows from a few seasonal brooks (most notable are the Tüdrü and Urva brooks). The sampling site was situated on the eastern shore of the bay.

Ligeoja Brook is situated in the north-western part of Saaremaa Island. This perennial spring-fed stream is only 1.3 km long with a catchment area of 3.3 km². The prevailing sediment type is sand with only limited patches of gravel. Dominant fish species are brown trout *Salmo trutta* L. and ninespine stickleback *Pungitius pungitius* L. (Järvekülg, 2001). The brook drains into Tagalaht Bay. The mouth is situated 200 m north from the mouth of Pidula Brook, which is significantly larger than Ligeoja Brook. The sampling site was situated approximately 150 m upstream from the brook mouth.

Jämaja Brook is located on the Sörve Peninsula in the south-western part of Saaremaa Island. This perennial brook is 8.6 km long and has a catchment area of 25.3 km². Rapids alternate with deeper water sections with a sandy bottom. Dominant fish species is brown trout. The brook drains into the Northern Baltic Proper, and the mouth

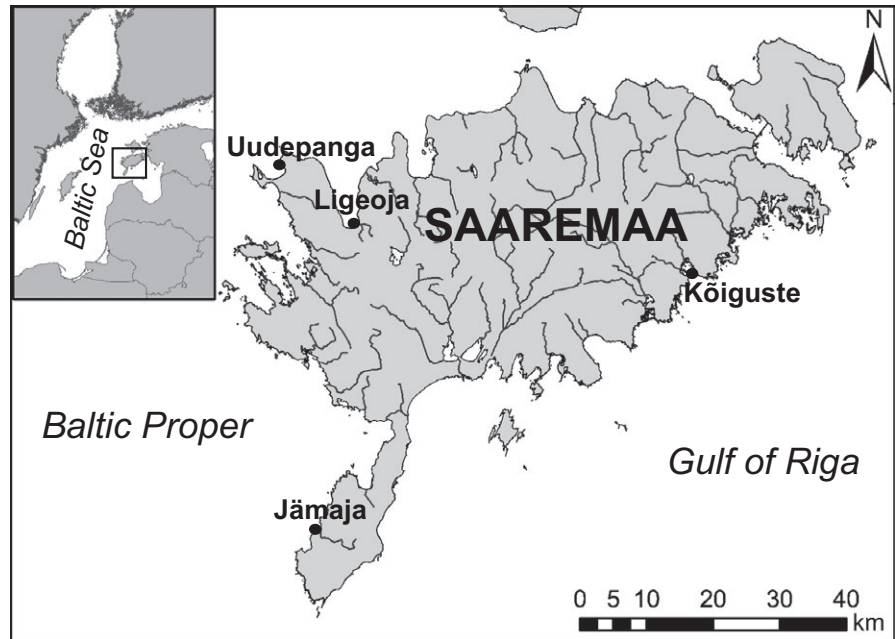


FIGURE 1 Location map of the study sites

is fully exposed to the open sea. There are no other significant freshwater streams nearby. The sampling site was located in the stream mouth, approximately 80 m from the sea.

2.2 | Fish sampling and processing

In Kõiguste and Uudepanga bays, the fish were caught by beach seine; in Ligeoja and Jämaja brooks fish were collected by electrofishing. In Kõiguste and Uudepanga bays, sampling was carried out in 2010 on 3 July at Kõiguste ($N = 36$) and on 29 May and 14 June at Uudepanga ($N = 22$ and 19 respectively). In Ligeoja Brook and Jämaja Brook, fish collection was carried out in 2011 on 28 September at Ligeoja ($N = 11$) and on 29 October at Jämaja ($N = 36$). The fish were killed immediately after capture by severing the central nervous system. Total length (TL, to the nearest 1 mm) and weight (TW, to the nearest 0.1 g) were recorded in the laboratory. Sex was determined by visual examination of the gonad. Otoliths (lapilli) were extracted, cleaned from adhering tissue and stored dry in microtubes.

2.3 | Otolith preparation and ageing

Each otolith was individually embedded into a mixture of epoxy resin and hardener (Epofix, Stuers ApS, Radovre, Denmark) so that a frontal section could be ground out. To expose the core area, the mount was ground manually with a grinding machine (Metaserv 250, Buehler Ltd., IL, USA) from both sides using abrasive papers with grit sizes P400 and P2500. Final polishing was carried out with P4000-grit sanding paper. Prepared otoliths were mounted on a petrographic slide. Individual ages were determined by counting the narrow winter growth zones from unstained and stained (1% neutral red and 0.5% acetic acid) otoliths under a stereomicroscope against dark and light backgrounds. Otoliths were carefully checked for any vaterite intrusions before and after the staining.

2.4 | LA-ICPMS and water analysis

The elemental composition of otoliths was quantified with laser ablation inductively coupled plasma mass spectrometry (LA-ICPMS) in Oregon State University's WM Keck Collaboratory of Plasma Spectrometry, Corvallis, OR. Before the analysis, otoliths were sonicated in ultrapure water for 10 min and dried under a laminar flow fume hood. A VG PQ ExCell ICPMS (Thermo Scientific, Waltham, MA, USA) with a New Wave DUV193 excimer laser (New Wave Research, Fremont, CA, USA) was used for quantifying the concentrations of ^{43}Ca , ^{86}Sr and ^{55}Mn . The laser was operated at 70% output power with a 10-Hz pulse rate and a 40- μm ablation spot size, scanning speed was 5 $\mu\text{m s}^{-1}$. A carrier gas mixture of He and Ar was used to introduce ablated material into the inductively coupled Ar plasma. A continuous line scan was traced along the longest axis from the otolith core to the edge. Data reduction was carried out by following the methods of Miller (2007). Background levels of ^{43}Ca , ^{86}Sr and ^{55}Mn were measured for 33 s prior to otolith ablation and subtracted from those measured during otolith ablation. Count rates for ^{86}Sr and ^{55}Mn were normalised to ^{43}Ca to account for variations in instrument sensitivity and ablation rate (Campana et al., 1997). The ion ratios were converted to elemental concentrations using measurements of NIST-612 glass standard. Elemental ratios were further converted to molar ratios based on the molar mass of Sr, Mn and Ca and presented as mmol mol^{-1} . Extreme outliers were removed following the methods of Veinott and Porter (2005). An eight-point moving mean was used to reduce the noise and smooth the data.

Water samples from the relevant sites were collected in 2012 (Table 1). From every site a, single sample of 20 ml was filtered through nylon filter (0.45 μm) and acidified to pH 2 with nitric acid. Acidified samples were analysed using X Series II ICPMS coupled with a CETAC ASX100 autosampler (CETAC Technologies Inc., Omaha, NE, USA).

Site (sampling date)	Salinity	Sr (ppm)	Ca (ppm)	Sr:Ca (mmol mol ⁻¹)
<i>Kõiguste (08.11.2012)</i>				
<i>Maadevahe River</i>	<0.5	0.102	105.6	0.4
<i>Kõiguste Bay</i>	4.7	1.186	102.9	5.3
<i>Uudepanga (17.11.2012)</i>				
<i>Tüdru Brook</i>	<0.5	0.079	61.5	0.6
<i>Urva Brook</i>	<0.5	0.087	63.2	0.6
<i>Uudepanga Bay</i>	6.5	1.541	119.5	5.9
<i>Ligeoja (11.11.2012)</i>				
<i>Ligeoja Brook</i>	<0.5	0.100	71.7	0.6
<i>Pidula Brook</i>	<0.5	0.070	57.7	0.5
<i>Tagalaht Bay</i>	6.6	1.551	138.1	5.1
<i>Jämaja (08.11.2012)</i>				
<i>Jämaja Brook</i>	<0.5	0.116	105.0	0.5
<i>200 m from the mouth of the Jämaja Brook</i>	1.8	0.573	107.6	2.4
<i>Baltic Proper near Jämaja</i>	6.2	1.498	117.0	5.9

TABLE 1 Water sample data for the fish collecting sites and adjacent locations (in *italic*)

External calibration with Claritas PPT multielement standards CLMS-1 and CLMS-2 (SPEX CertiPrep Inc., Metuchen, NJ, USA) were used to quantify Sr and Ca in the water. NIST standard reference material 1643e was used for quality control.

3 | RESULTS

Strontium concentrations and Sr:Ca values in the water showed a strong positive relationship with salinity (Table 1). Otolith Sr:Ca revealed that at least three different migratory patterns exist: (i) seawater residency, (ii) fast springtime migrations to fresh water or to bays with lower salinities (e.g. areas near stream mouths) and (iii) prolonged migrations to fresh water (Figure 2). Those patterns were not evenly distributed among the sites (Table 2; Figure 3). No freshwater resident fish were observed among the sampled fish.

About half of the fish in Kõiguste Bay did not show any clearly distinguishable Sr:Ca oscillations (Figure 2a). The other half showed small-scale fluctuations in their Sr:Ca profile that were often located on the annulus, indicating that those changes occurred in springtime (Figure 2b). Small part (17%) of the fish in Kõiguste showed limited occasional increases or decreases in their Sr:Ca values after which those new values were stable for a period of time (Figure 2c). The majority of fish in Uudepanga Bay showed just one moderate nadir in their Sr:Ca profile which coincided with an annulus (Figure 2d). This indicates that in springtime, fish experienced fresh water or water with lower salinity compared to the ambient sea water. Some fish exhibited deeper nadirs (Figure 2e) indicating temporally longer stays in fresh water or water with lower salinity. In both cases, the nadir usually occurred only once which means that fish did not migrate to fresh water in every year. As in Kõiguste, some fish in Uudepanga did not show any notable Sr:Ca oscillations (Figure 2f).

Sr:Ca profiles in Ligeoja and Jämaja brooks were more complex than in the bays. All fish in Ligeoja Brook showed fluctuations in their Sr:Ca profiles. A majority of the profiles had relatively deep nadirs which usually concurred with annulus (Figure 2g). Some fish had modest nadirs indicating shorter stays in fresh water (Figure 2h). In some cases, the nadirs did not occur every year. All fish from the Ligeoja showed a decrease at the end of their Sr:Ca profile; in three cases, this decrease was quite prominent (Figure 2i). About half of the fish in the Jämaja Brook showed moderate oscillations in their Sr:Ca profiles (Figure 2j). Most of the other profiles exhibited deeper nadirs (Figure 2k). In the majority of the cases, nadirs did not occur in every year. Three individuals did not display any fluctuations in their profiles (Figure 2l). Two specimens in the Jämaja had very low core values (Sr:Ca <0.5 mmol mol⁻¹) indicating that those fish were presumably hatched and reared for some time in fresh water (Figure 2m). Forty-seven per cent of the fish in the Jämaja showed a decrease at the end of their Sr:Ca profiles. A new maximum age for minnows in Estonia was recorded in the Jämaja (7 years; Table 2; Figure 2j).

Reduced Sr:Ca core values (≥ 0.3 mmol mol⁻¹ difference between core and adjacent zone) were observed in the otoliths of most fish, which in the majority of cases concurred with a Mn peak (Mn:Ca peak ≥ 0.005 mmol mol⁻¹) (Figure 2a–d,f–i,k–n). Diminished Sr values in the core were also apparent in freshwater resident minnows from the Umbusi River in Central Estonia (58°35.37'N 26°6.15'E) (Figure 2n). Elevated Mn in the core was positively associated with the occurrence of decreased Sr levels in the core (chi-squared test, $\chi^2=40.31$, 1 df, $p < .001$; Table 3).

There was a significant relationship between sex and migratory behaviour (chi-squared test, $\chi^2 = 5.58$, 1 df, $p = .018$). Males tended to migrate to fresh(er) water more than expected by chance, whereas females tended to be more likely seawater residents (Table 4).

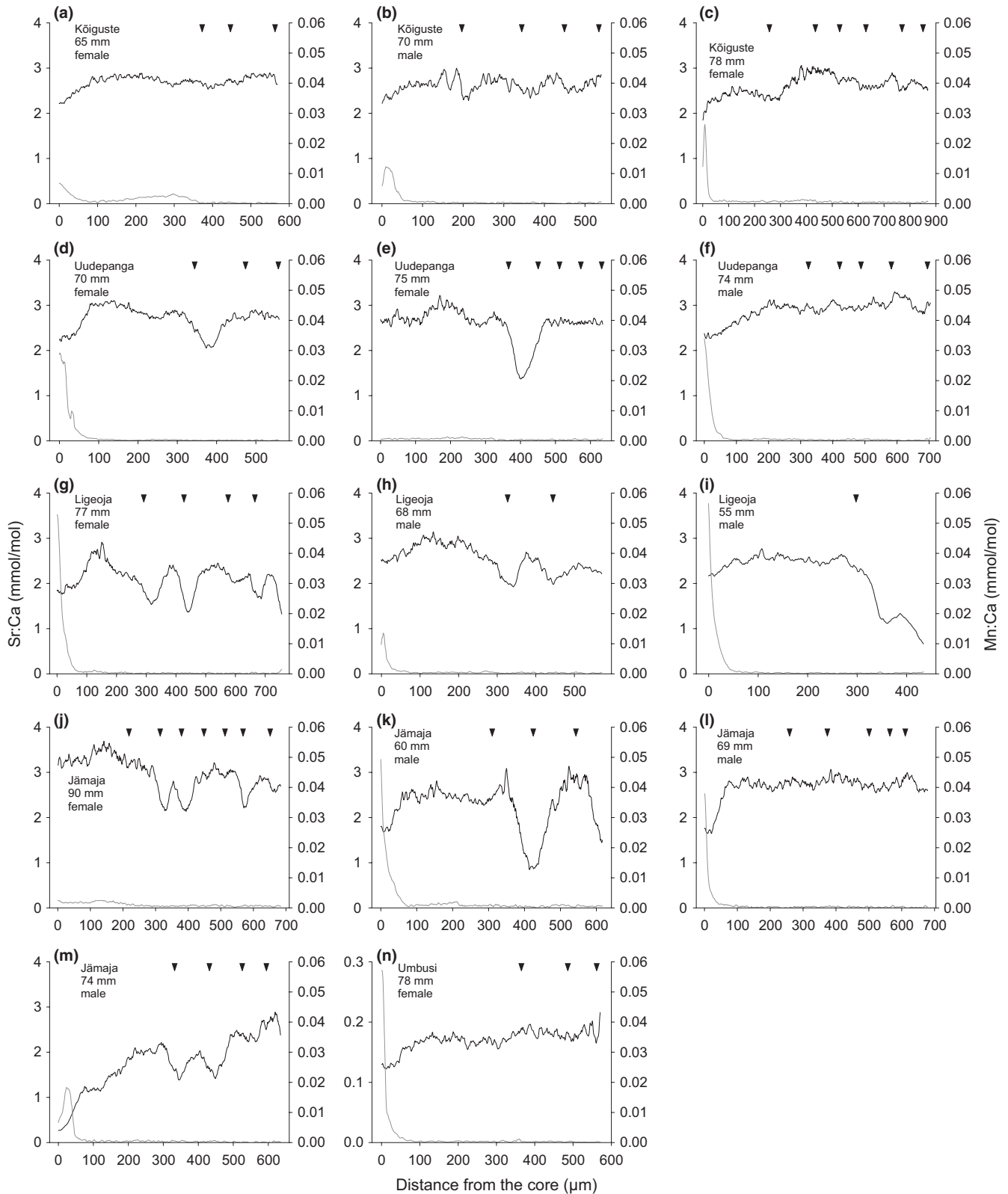


FIGURE 2 Representative otolith Sr:Ca (black line) and Mn:Ca (grey line) profiles of Eurasian minnow: (a) Kõiguste Bay, demonstrating seawater residency (migration type I); (b) Kõiguste Bay, showing short/fast migrations to fresh water (migration type II); (c) Kõiguste Bay (type II); (d) Uudepanga Bay (type II); (e) Uudepanga Bay, showing prolonged migration to fresh water (migration type III); (f) Uudepanga Bay (type I); (g) Ligeoja Brook (type III); (h) Ligeoja Brook (type II); (i) Ligeoja Brook showing prominent decrease at the end of profile (type III); (j) Jämaja Brook with record age (type II); (k) Jämaja Brook (type III); (l) Jämaja Brook (type I); (m) Jämaja Brook with freshwater Sr:Ca natal value (type III); (n) Umbusi River from Central Estonia showing freshwater residency (added for comparative purposes; note the different scale of Sr:Ca). Note that Mn:Ca peak coincides with Sr:Ca nadir in the core regions (even in the case of freshwater resident [n]). Triangles denote the annuli

Collection site	N	Seawater residency	Fast/short migrations to fresh water	Prolonged migrations to fresh water	Total length (mm)	Age (years)
Kõiguste Bay	36	56%	44%	0%	69 ± 5 60–80	4.0 ± 0.9 2–6
Uudepanga Bay	41	20%	68%	12%	70 ± 3 66–76	3.9 ± 0.6 3–5
Ligeoja Brook	11	0%	27%	73%	66 ± 9 55–81	2.5 ± 1.0 1–4
Jämaja Brook	36	8%	53%	39%	76 ± 9 60–90	4.4 ± 1.0 3–7

TABLE 2 Distribution of migration patterns among different sites. Fish length and age are given as mean ± SD and range. Note that fish from different sites were caught at different seasons: Uudepanga in late spring, Kõiguste in early summer, Jämaja and Ligeoja in autumn

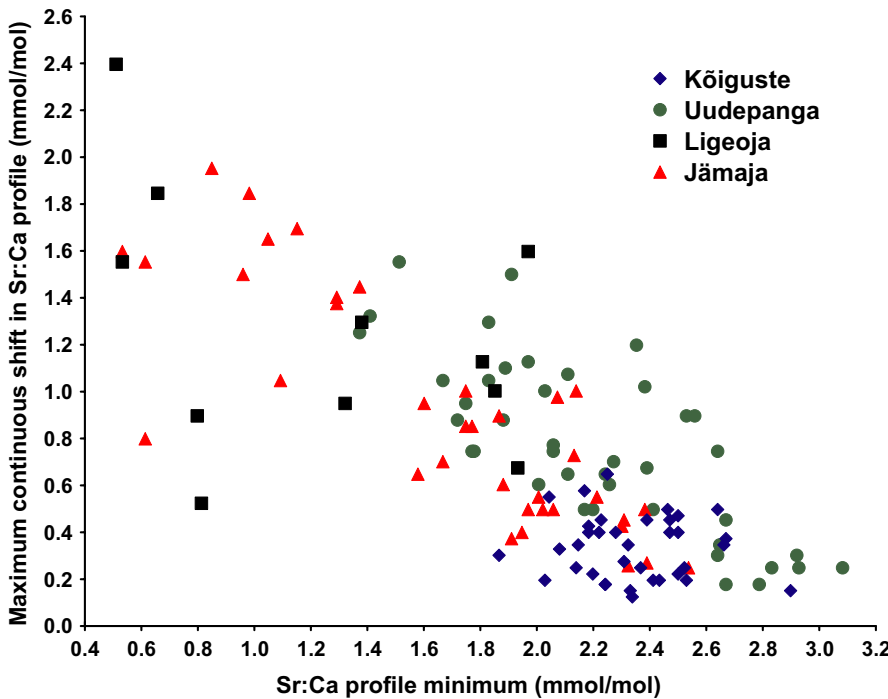


FIGURE 3 Distribution of migration patterns among different sites. Fish in the upper left quarter represent prolonged migration pattern, fish in the lower right quarter represent seawater residency and all other are classified as fast migrants

TABLE 3 Association between Sr and Mn concentrations in the otolith core

	Decreased Sr in the core	
	Evident	Absent
Mn peak in the core		
Evident	98 (79%)	5 (4%)
Absent	9 (7%)	12 (10%)

TABLE 4 Association between sex and migratory behaviour

Sex	Migratory behaviour	
	Seawater resident	Migrant
Male	6 (5%)	40 (32%)
Female	25 (20%)	53 (43%)

4 | DISCUSSION

In this study we found that several different migratory patterns exist for brackish water inhabiting Eurasian minnow. To our knowledge, this is the first study quantifying movement patterns of Eurasian minnow between environments with different salinity regimes. Broadly speaking those patterns can be categorised into three different types: (i) seawater residency, (ii) fast springtime migrations to fresh water or to bays with lower salinities (e.g. areas near stream mouths) and (iii) prolonged migrations to fresh water. Our study refutes the conception that minnows inhabiting the coastal waters of the West Estonian archipelago are a separate ecomorph that resides solely in brackish water (Järvekülg, 2001). So far this opinion was supported by the fact that no minnows have ever been caught in streams of Saaremaa Island during fish surveys, although isolated fish have been recorded near the stream mouths (Estonian Marine Institute, unpublished data). The fact that two of our sampling sites were situated in freshwater does not itself give enough reason to believe that fish caught from those

sites make extensive use of those habitats, because those sites were situated very close to the stream mouths and minnows could be there by chance. However, otolith microchemistry revealed that those fish did indeed use freshwater habitats regularly. Considering that we did not identify any freshwater residents and the fact that no minnows have ever been recorded in inland stretches of Saaremaa streams, it seems that a solely freshwater inhabiting minnow ecomorph is indeed absent on Saaremaa Island.

Different migration patterns were not evenly distributed among sites. Minnows from the bays tended to be more likely seawater residents. This was especially true in Kõiguste Bay. It can be explained by the fact that only a few small freshwater streams drain into Kõiguste and Uudepanga bays and most of those streams are seasonal with limited suitable spawning areas. The smallest Sr:Ca oscillations were observed in Kõiguste, and it is quite possible that all the fish from that bay were actually seawater residents and the small-scale fluctuations in their Sr:Ca profiles were caused by snowmelt in spring which temporarily reduced the water salinity in the shallow sheltered bay. Migration patterns in Ligeoja and Jämaja brooks were more diversified and exhibited more prominent nadirs in their profiles. This could be explained by the fact that those brooks are perennial with plenty of suitable spawning habitats available. Majority of nadirs in Sr:Ca profiles coincided with annuli which indicate that migrations took place in springtime. This demonstrates that at least a portion of brackish water living minnows readily exploit freshwater spawning grounds. First migration to fresh water usually occurred at age one which indicates that the majority of minnows reached maturity at that age. This is in accordance with previous studies that show early maturity of this species (Mills, 1987; Zivkov & Trichkova, 2006). A substantial proportion of minnows did not migrate to fresh water in every year. This could be the result of skipped spawning (Rideout & Tomkiewicz, 2011) or more likely that springtime conditions in freshwater streams were suboptimal in certain years (i.e. low water volume). Skipped migrations were most prominent in Uudepanga Bay where there are only a few seasonal streams available for freshwater spawning. Those streams are heavily influenced by springtime snowmelt and therefore in years with low snow cover have low water levels which may prevent minnow's springtime spawning migrations. As we observed large numbers of seawater residents and also migrants with skipped migrations in the same population, it seems that minnows can freely switch between freshwater and brackish water reproduction. This switching between spawning biotopes is further supported by the fact that some individuals from brackish Uudepanga Bay were caught with running gametes (indicating brackish water spawning), but their Sr:Ca profiles displayed clear nadirs during the previous breeding season (indicating freshwater spawning).

Some of the fish in Ligeoja and Jämaja brooks entered the streams in autumn or even in summer and overwintered there. This overwintering and also springtime spawning migration patterns, whereby only a fraction of the minnow population migrates, can be regarded as partial migration (Chapman, Hulthén et al., 2012; Chapman et al., 2012). We did not identify any winter migrants in Kõiguste and Uudepanga bays. The reason behind this is probably the lack of suitable streams for overwintering in those areas. Previous tagging studies have shown

partial winter migration in many species of cyprinids in lake-stream systems (Brodersen et al., 2012; Jepsen & Berg, 2002; Skov, Brodersen, Nilsson, Hansson, & Brönmark, 2008). It has been shown that predator avoidance may be the main driving factor behind cyprinids winter migration (Bronmark, Skov, Brodersen, Nilsson, & Hansson, 2008; Skov et al., 2011). Also, fish condition (Brodersen, Nilsson, Hansson, Skov, & Brönmark, 2008) and an individual's personality traits (Chapman et al., 2011) can influence migratory propensity. Predator avoidance is the most plausible explanation for minnow's winter migration observed in our study, because in winter, Ligeoja and Jämaja brooks are relatively devoid of predators compared to the coastal waters. On the other hand, the sea has a more stable environment in winter and provides more food resources than the streams. Bronmark et al. (2008) concluded that individual winter migration decisions are based on trade-offs between predator avoidance and growth, and this might also be the case for the coastal minnows. Aforementioned studies have shown that partial winter migration is a common phenomenon in lake-dwelling cyprinids; we demonstrate that it is also common in minnows inhabiting a brackish coastal sea.

The majority of analysed fish showed elevated Mn:Ca values in otolith core regions. Markedly high Mn concentrations in otolith cores have been observed in a wide range of fish species (Brophy, Jeffries, & Danilowicz, 2004; Miller & Kent, 2009; Ruttenberg et al., 2005). Those elevated Mn:Ca levels are seemingly not related to dissolved Mn concentrations in the ambient environments, and exact mechanisms behind high Mn values in otolith cores remain obscure (Bouchard, Thorrold, & Fortier, 2015; Brophy et al., 2004). Several studies have used increased Mn as a clear marker for identification of the otolith primordia (Macdonald, Shelley, & Crook, 2008; Miller & Kent, 2009; Veinott, Westley, Purchase, & Warner, 2014), and it performed satisfactorily in the current study as well. Our failure to identify the distinct Mn peak in some individuals was probably caused either by misplacement of laser transect or over/under grinding of the otolith core. To our knowledge, Eurasian minnow is the only cyprinid species besides common carp *Cyprinus carpio* L. (Blair & Hicks 2012) with a reported distinct Mn peak in the otolith core. Besides elevated Mn levels, we observed decreased Sr values in the core. It has been shown that maternal associations with fresh- and/or seawater may affect offspring's otolith core Sr concentrations in some fish species (Kalish, 1990; Rohla et al., 2012; Volk, Blakley, Schroder, & Kuehner, 2000). Usually this manifests as a Sr peak (mother peak) in otolith cores of offspring with anadromous maternal origin, but we observed the opposite of that: a nadir in the core. Our data suggest that those diminished Sr levels in the cores of the minnow's otoliths have ontogenetic origin and are not associated with the mother's life history. This is because those nadirs were evident in the majority of the individuals regardless of their migration patterns (even for the freshwater residents [Figure 2n]). Also, most of the fishes without a Sr nadir in the core did not exhibit a Mn peak which indicates that we did not ablate the otolith core and thus missed the Sr nadir. This unusual pattern of Sr concentrations in the core has not been previously reported. This has important implications for interpreting of otolith Sr:Ca profiles, as it may mask or distort a natal freshwater signal.

Considering the aforementioned ontogenetic effect on the core Sr:Ca values, we cannot assign analysed minnows hatching habitat for certain, except for two specimens from the Jämaja Brook, which showed very low natal Sr values (Sr:Ca <0.5 mmol mol⁻¹). Water chemistry data and previous studies (Rohtla et al., 2012, 2014, 2015) suggest that Sr:Ca natal values ≤0.6 mmol mol⁻¹ should be considered as fresh water. This indicates that those two individuals were hatched in fresh water and had freshwater natal rearing habitat. For the rest of the fishes, one of two scenarios may apply: a) fish were hatched and reared in the brackish coastal sea or b) fish were hatched in the fresh water, but shortly drifted or emigrated to brackish water. In the case of the latter scenario, the brief freshwater signal in otolith Sr:Ca profile could have been masked by aforementioned Sr phenomenon in the core.

In the current study, we observed fish up to seven years (eight summers) old. This is a new maximum age for minnows in Estonia, as the previous reported age was four summers (Saat et al., 2003). In Kõiguste, Uudepanga and Jämaja, we observed a number of fish older than four years, which means that those age groups are not uncommon in those populations. The minnow displays a notable variability in its life-history traits, including lifespan, over its wide geographical range. In warmer southern regions, its age does not usually exceed three years (Mills, 1987), but in northern areas, ages up to 13 years have been observed (Mills, 1988). Our reported seven years fit well into the before mentioned south-north gradient. It is worth mentioning that age reading from the otoliths was occasionally quite complicated because in many cases annuli in otoliths were not clearly recognisable. Problems with age determination of the minnow have been reported in previous studies as well (Frost, 1943; Mills, 1987).

In this study we demonstrated, for the first time, movement patterns of Eurasian minnow between brackish and freshwater environments using otolith microchemistry. It was revealed that much more complex migration patterns exist in brackish water inhabiting minnow populations than previously thought. Furthermore, we observed potentially ontogenetically affected Mn and Sr concentrations in the otolith core regions that may aid (Mn peak) and/or complicate (Sr nadir) the interpretation of otolith element:Ca data. The answer to the titular question "Do Eurasian minnows (*Phoxinus phoxinus*) inhabiting brackish water enter fresh water to reproduce?" is No (in the case of Kõiguste), Maybe (in the case of Uudepanga) and Yes (in the cases of Ligeoja and Jämaja).

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