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Research Article

Migration history of North Sea houting (*Coregonus oxyrinchus* L.) caught in Lake IJsselmeer (The Netherlands) inferred from scale transects of ^{88}Sr:^{44}Ca ratios

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Abstract. North Sea houting, *Coregonus oxyrinchus*, became extinct in the River Rhine in the 1940 s and was reintroduced in the 1990 s. To study the migration history of individuals, the ^{88}Sr:^{44}Ca ratio of scales of 39 houting (10 – 44 cm TL) caught in Lake IJsselmeer was analysed using laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS). Scales of houting inhabiting freshwater ponds and two Danish rivers containing the last original populations in the North Sea basin were used as controls. Fish that lived exclusively in freshwater had ^{88}Sr:^{44}Ca ratios of around 0.2 from the nucleus to the edge; 29 of the analysed houting from Lake IJsselmeer were of this type. Most of these were small, but some were mature and up to 42 cm in length. Seven houting had ^{88}Sr:^{44}Ca ratios over 0.27 from the nucleus to the scale maximum values, indicating migration to the sea at early life stages. Three houting with low ^{88}Sr:^{44}Ca ratios at the scale nucleus and increased ^{88}Sr:^{44}Ca ratios towards the scale edge probably lived in freshwater for a longer period after hatching and then moved to brackish/marine environments. The scale analysis indicates different migration patterns for houting in Lake IJsselmeer and provides evidence that this species (1) is sometimes able to pass the migratory barriers between the Wadden Sea and Lake IJsselmeer, and (2) does not need to migrate to sea to reach maturity.

Key words. *Coregonus oxyrinchus*; diadromous fish; nonlethal method; scale microchemistry; strontium; River Rhine.

Introduction

The life history of anadromous fish depends on both the quality of different habitats and the connectivity between these habitats that make them suitable for migration during the different life stages. During the last few centuries, most of these habitats have been severely impacted by humans, which resulted in a massive decline of all anadromous fish species in the River Rhine (De Groot, 2002) and in many other European rivers. Starting in the 19th century, major dam building activities for hydro-electric power began to disconnect the different habitats within the River Rhine system. After the Second World War, connec-

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tions of the freshwater reaches of the River Rhine and its estuaries in the Netherlands were blocked by massive dams and locks. In 1932, a large dam (the Afsluitdijk) was constructed that closed off the former northern estuary of the branch of the River Rhine known as the River IJssel. This dam resulted in the formation of a large shallow eutrophic freshwater lake (Lake IJsselmeer, Fig. 1) and an abrupt saline gradient. Excess freshwater is discharged into the Wadden Sea through two sluices. These sluices hamper upstream migration, even though some opportunities for upstream passage of fish are provided as part of sluice management (De Leeuw et al., 2005). Today, the only unblocked waterway in the Rhine delta is the Nieuwe Waterweg, a man-made canal connecting the harbours of Rotterdam with the North Sea (Breukelaar et al., 1998; De Leeuw et al., 2005).

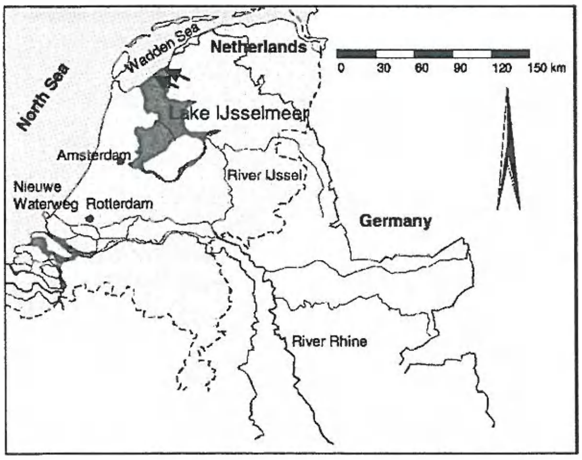

Figure 1. Catch locations (arrows) of North Sea houting in Lake IJsselmeer at the Afsluitdijk.

One anadromous species, the North Sea houting, *Coregonus oxyrinchus*, used to be common in the River Rhine as well as in the whole Wadden Sea area (Fig. 1). Until the beginning of the 20th century, commercial fisheries in the Dutch reaches of the lower Rhine caught up to 15 tonnes per year (De Groot, 1990). These catches declined sharply during the period from 1916 to 1940. As a consequence, houting has been considered extinct in the River Rhine (Kranenbarg et al., 2002) as well as in nearly the whole North Sea basin since the 1940 s (Lelek, 1987). In the 1980 s, the last known reproducing population of houting in the Danish River Vidå was used to establish a brood-stock in Denmark, whose descendants served as the basis for the reintroduction of houting in Germany (Jäger, 1999). Since 1996, houting have been stocked in the Lower Rhine regularly and in increasing numbers (Borcherding et al., 2006). Catches of houting in Lake IJsselmeer (The Netherlands, Fig. 1) are believed to originate from stocks in the lower Rhine (De Groot, 2002; Kranenbarg et al., 2002; De Leeuw et al., 2005).

North Sea houting, when they reach maturity at a size of about 35–45 cm, migrate from the North Sea to their spawning grounds in the potamal reaches of rivers in November/December. As this species can reach sizes of up to 60 cm and a maximum age of up to 10 years, individuals can return several times to their spawning grounds, which are characterized by moderate current over a gravel or sandy bottom. The fry hatch in February/March, and immediately start to drift / migrate downstream. When the fish reaches 30–40 mm in length, its physiology changes so it can withstand the move from fresh to salt water (Jensen et al., 2003). With respect to the above-mentioned dams and locks in the Rhine delta, the question arises of whether juvenile and adult houting can migrate from the river to the North Sea and from there back to the spawning grounds in the rivers.

The Sr:Ca ratio variability in the hard structures of fish has been widely applied as a tracer to describe the migration of fish between freshwater and marine environments, with the assumption that low salinity habitats correspond to lower ratios of Sr:Ca (Kalish, 1989; Kraus and Secor, 2004). Strontium is substituted for Ca and is deposited in fish scales, fin rays and otoliths in proportion to the Sr:Ca ratio of the water in which the fish lived (Campana et al., 1997; Veinott et al., 1999). Laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) is considered to be one of the most appropriate methods to use in such analyses (Campana et al., 1997; Campana, 1999; Veinott et al., 1999). In order to study the environmental history of Norwegian Atlantic salmon stocks, Flem et al. (2005) determined the trace element concentrations of their scales by LA-ICP-MS. The differences in elemental composition in the scales reflect geological differences in the bedrock at freshwater locations experienced by the salmon during the pre-smolt stage. Recently, element distribution and strontium isotope ratios in otoliths have been studied by microlocal analysis using LA-ICP-MS (Woodhead et al., 2005)

The objectives of the present study were to determine (1) whether houting caught in Lake IJsselmeer had at any time been at sea, (2) the size at which they migrate to the North Sea and back to freshwater reaches, and (3) if sexual maturation depends on whether the fish had spent a certain period in the marine environment. Houting caught in freely accessible Danish rivers served as controls for the migration patterns to be normally expected in anadromous houting. In contrast, individuals reared in freshwater ponds were used as a freshwater control under the assumption that the incorporation of strontium is similar in all freshwater environments.

Material and methods

Sampling of fish

For this study, 39 houting caught in Lake IJsselmeer during 2001–2002 were used. Total length (TL), weight, sex and maturation were recorded and about five scales from the dorsal side of the flank were sampled. All the houting were caught at the freshwater side of the dam (the Afsluitdijk) as part of a monitoring programme with large fyke nets (De Leeuw et al., 2005). In addition to the scales from houting from Lake IJsselmeer, (1) scales of four fish from two Danish rivers draining to the Wadden Sea (Varde Å; Hjortvad Å, a tributary of the Ribe Å, cf. Jensen et al., 2003), and (2) scales of three fish that were reared in fish ponds in Lohmar (Borcherding et al., 2006) were analysed and served as controls. The houting had been introduced into the fish ponds as small juveniles (2–3 cm TL) in May 2004. When recaptured in March 2005 they had grown to sizes of up to 20 cm TL. The houting from these fish ponds only lived in freshwater and thus no changes in the low ^{88}Sr:^{44}Ca ratio typical of freshwater fish were expected in the scale analysis. In contrast, houting from the Danish rivers should represent ratios of populations that can migrate freely between the marine environment and the freshwater reaches because these rivers do not have any migratory barriers. The ^{88}Sr:^{44}Ca ratios of these fish should thus be significantly higher than the values measured in freshwater fish, indicating a marine environment.

Sample preparation

For investigations by LA-ICP-MS, the houting scales were fixed on sticky high-purity carbon ribbon that was placed on a plastic sample holder. Samples were analysed directly by LA-ICP-MS without any additional sample preparation steps.

Instrumentation for LA-ICP-MS measurements

Inductively coupled plasma mass spectrometry (ICP-MS) and laser ablation ICP-MS (LA-ICP-MS) are now the most frequently used inorganic mass spectrometric techniques for a fast and sensitive multi-element determination at the trace and ultratrace concentration levels as well as for precise and accurate isotope ratio measurements (Becker, 2002a; 2002b; 2005). In LA-ICP-MS, the sample material is ablated by a focused laser beam. The ablated material is transported by argon as a carrier gas into the inductively coupled plasma where the molecules of ablated sample material are dissociated and ionized. The ions formed in the ICP are extracted in the mass spectrometer and separated according to their mass-to-charge ratios. The separated ion beams are detected electrically, mostly by secondary electron multiplier. In the present study, a commercial laser ablation (LA) system (CETAC LSX 200, Cetac Technologies, Omaha, NE, USA) connected to the inductively coupled plasma ion source of a quadrupole mass spectrometer (ICP-QMS, ELAN 6000, Perkin Elmer, SCIEX Corp., Norwalk, CT, USA) was used for measurements of the ^{88}Sr:^{44}Ca ratio in fish scales. A detailed figure of the experimental arrangement is given elsewhere (Pickhardt et al., 2005).

Sample material was ablated by the UV wavelength beam of a Nd-YAG laser in single line modus (point by point) from the scale nucleus to the edge of the scale, whereby 10 to 100 spots (depending on the diameter of the scale) were analysed with the 50 μm diameter of the laser beam (see Table 1 for technical details). Measurements were performed in such a way that the spots did not overlap and therefore the distance from the middle of one spot to the neighbouring spot was about 63 μm. Each spot was ablated by 120 shots of the laser beam focused on the sample surface. The ablated material was carried into the inductively coupled plasma (ICP) of the quadrupole-based mass spectrometer by argon gas. Isotopic data were acquired in the form of counts per second for each isotope selected for this analysis ($^{43}Ca^+$, $^{44}Ca^+$, $^{46}Ca^+$, $^{48}Ca^+$, $^{84}Sr^+$, $^{86}Sr^+$, $^{87}Sr^+$, $^{88}Sr^+$). The ICP-MS was

Table 1. Experimental parameters for LA-ICP-MS.

ICP-MS Elan 6000	
Rf power	1300 W
Coolant gas flow rate	14 L min^{-1}
Auxiliary gas flow rate	0.8 L min^{-1}
Carrier gas flow rate	0.78 L min^{-1}
Mass resolution m/Δm	300
Acquisition mode	peak hopping
Points/mass	1
Detector mode	pulse
Auto lens	on
Dwell time	10 ms
No. of sweeps	10
No. of readings	1
No. of replicates	500 -1000
Sampling cone	nickel with a 1.1 mm orifice
Skimmer cone	nickel with a 0.9 mm orifice
Laser ablation system CETAC LSX 200	
Laser type	Nd:YAG laser
Wavelength	266 nm (4th harmonic)
Pulse duration	5 ns
Repetition frequency	20 Hz
Pulse energy	4 mJ
Laser power density	$1.1x10^9$ W cm^{-2}
Spot diameter	50 μm

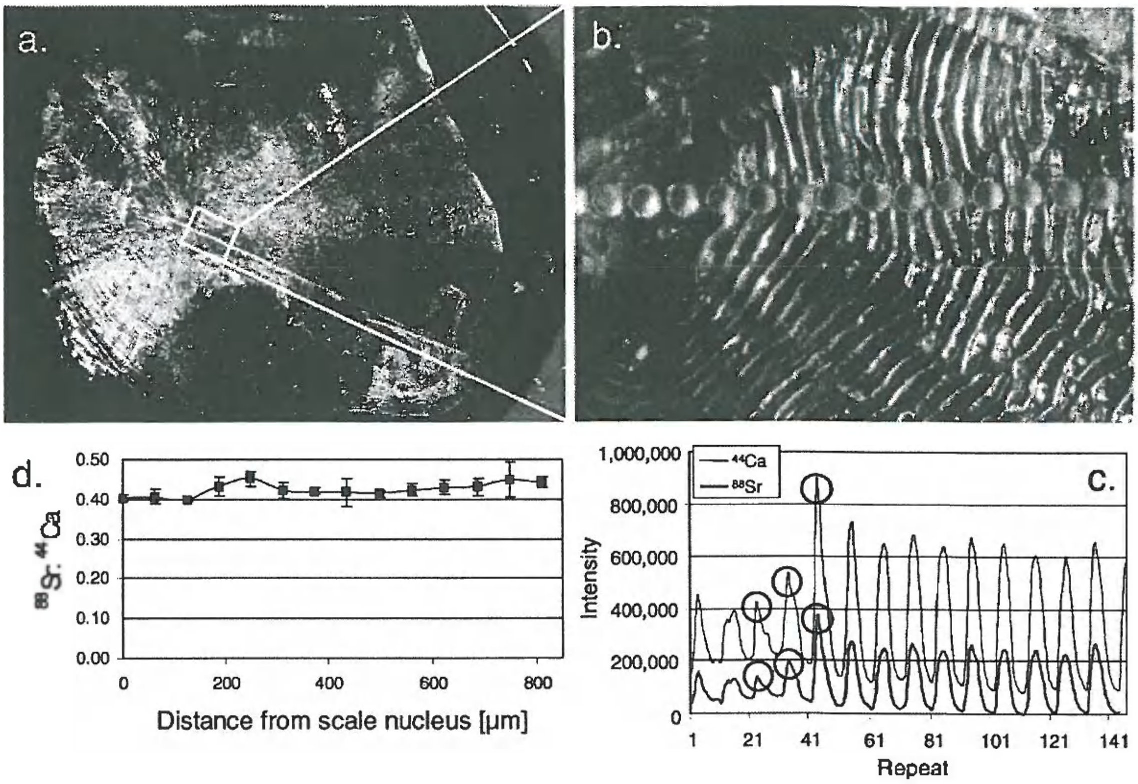

Figure 2. Steps in scale analysis by laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS). (a) Photograph of a scale of North Sea houting (Lake IJsselmeer, 6/19/02, male, 43.3 cm TL) with the line of laser dots; (b) Detailed picture of the same scale with the laser dots; (c) Results of LA-ICP-MS for ^{44}Ca and ^{88}Sr; (d) ^{88}Sr:^{44}Ca ratio calculated as the mean of three measurements for each intensity peak (indicated by the circles in 1c.) relative to the distance from the scale nucleus; error bars = standard deviation.

operated in peak hopping mode with the number of replicates set between 500 and 1000 (depending on the diameter of the scale) in order to obtain a total time for the ICP-MS measurement that was longer than the sampling time in laser ablation. By this means, gas blank data for blank correction were obtained before and after laser ablation of the sample and subtracted from the measured ion intensities during laser ablation of the sample. Optimization of ICP-MS parameters was performed daily using NIST glass standard reference material (National Institute of Standards and Technology, Gaithersburg, MD) to the maximum ion intensity of ^{88}Sr^{+}. The mass spectrometric measurements were performed at low mass resolution ($m/\Delta m = 300$). To calculate the ^{88}Sr:^{44}Ca ratio (see recommendations in Campana et al., 1997), the three central values of the peaks were averaged for each laser spot, thereby also providing a relative standard deviation (RSD, see Fig. 2).

Analysis of ^{88}Sr:^{44}Ca ratios of houting from Lake IJsselmeer

The ^{88}Sr:^{44}Ca ratio at the nucleus can be used to reveal where the fish lived when the scales began to grow. Scales in houting begin to grow at a size of about 35–40 mm TL (Borcherding, unpublished results). The ^{88}Sr:^{44}Ca ratio near the nucleus should correlate to the environment of the juvenile at a size of approximately 40–50 mm TL. The difference between the maximum ^{88}Sr:^{44}Ca ratio and the ^{88}Sr:^{44}Ca ratio at the nucleus provides information about whether there was an increase in the ^{88}Sr:^{44}Ca ratio or whether it stayed more or less constant from the juvenile stage onwards.

In a first step, the ^{88}Sr:^{44}Ca ratios near the nucleus of all 39 houting from Lake IJsselmeer were plotted against the differences between the maximum ^{88}Sr:^{44}Ca ratio and the ^{88}Sr:^{44}Ca ratio at the nucleus. Several studies have demonstrated at least a 50 % increase in Sr:Ca ratios from freshwater to brackish water, and up to a 4-fold increase in the marine environment (e.g. Kalish, 1989; Secor et al., 1995; Veinott et al., 1999; Arai et al., 2002). Therefore, the measured ^{88}Sr:^{44}Ca ratios of the houting from the freshwater ponds were used to define two thresholds. All individuals with ^{88}Sr:^{44}Ca ratios at the nucleus higher than 150 % of the maximum values of the houting from the freshwater ponds were assumed to be in brackish or marine environments at the time that the scales began to develop (40–50 mm TL). The remaining individuals, which apparently remained in freshwater environments when their scales began to grow, were then further subdivided by the second threshold indicating a migration to brackish or marine environments at life stages after the scales started to grow. For this, an increase of the differences between the maximum ^{88}Sr:^{44}Ca ratio and the ^{88}Sr:^{44}Ca ratio at the nucleus above 50 % of the maximum values of the houting from the freshwater ponds was used as a second threshold. Finally, (1) the ^{88}Sr:^{44}Ca ratios at the nucleus, (2) the maximum ^{88}Sr:^{44}Ca ratios, (3) the difference between the maximum ^{88}Sr:^{44}Ca ratio and the ^{88}Sr:^{44}Ca ratio at the nucleus, and (4) the ^{88}Sr:^{44}Ca ratios at the edge of the scale for the different types of houting from Lake IJsselmeer and the Danish houting were compared with non-parametric statistics (Kruskal-Wallis, Mann-Whitney U-test) using WinSTAT® for Microsoft®-Excel.

Results

Precision of LA-ICP-MS measurements

The mean RSD for the 120 laser shots in a single point of all the fish was 9.3 %, which can be taken as the overall variability of the LA-ICP-MS used in this study. This variability becomes obvious from the analysis of four different scales, with one scale measured three times, of one individual from Lake IJsselmeer (Fig. 3). The curves show exactly the same tendency for all six scale analyses, providing the reliability needed for the following scale measurements in which only one scale per fish was analysed.

Houting from fish ponds

The scales of three individuals (sizes 17.5, 19, 19 cm TL) showed ^{88}Sr:^{44}Ca ratios that were relatively constant through time and always less than 0.2 and with mean values between 0.14 ± 0.02 and 0.16 ± 0.03 (Fig. 4). The maximum ^{88}Sr:^{44}Ca ratio of these scales (~0.18) was used to define the threshold to separate different migration types of houting from Lake IJsselmeer.

Houting from Denmark

Analysis of the scales of four houting from two Danish rivers and four different years yielded ^{88}Sr:^{44}Ca ratios ranging from 0.17 to about 0.7 (Fig. 5). Starting with a ^{88}Sr:^{44}Ca ratio of about 0.45–0.5 at the nucleus, a slow increase up to the maximum value is visible in all individuals. The ^{88}Sr:^{44}Ca ratios near the edge of the scales are somewhat different for the individual fish. While for the female from Hjortvad Å (Den-3, Fig. 5a), a clear drop to a ^{88}Sr:^{44}Ca ratio of less than 0.2 is obvious, all other houting from Denmark had ^{88}Sr:^{44}Ca ratios of around 0.5 at the edge of the scales. In the 45-cm male from Varde Å (Den-4, Fig 5b), a clear minimum at 4.8 mm was followed by another maximum at a distance about 5 mm from the nucleus, thus more than 600 µm from the edge of the scale. Similar but not so clear tendencies were also found for the other houting from Denmark.

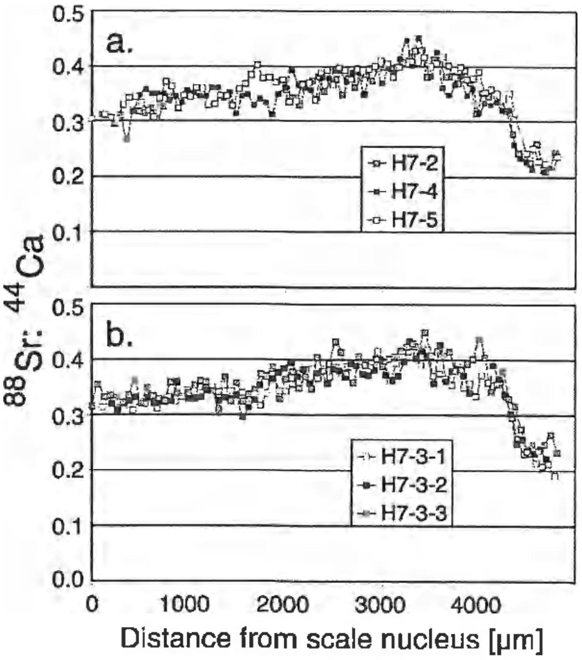

Figure 3. ^{88}Sr:^{44}Ca ratio for a houting (7/8/02 female, 40.7 cm TL) caught in Lake IJsselmeer. (a) 3 different scales, (b) a fourth scale of this fish that was measured three times.

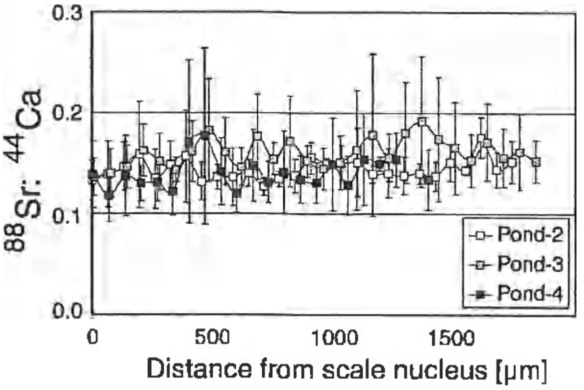

Figure 4. ^{88}Sr:^{44}Ca ratio of three houting from a freshwater fish pond in Lohmar (Germany); error bars = standard deviation of each laser spot measurement (cf. Fig. 2d).

Houting from Lake IJsselmeer

All specimens larger than 35 cm TL were mature. The scale size (y = distance from the nucleus to the edge)

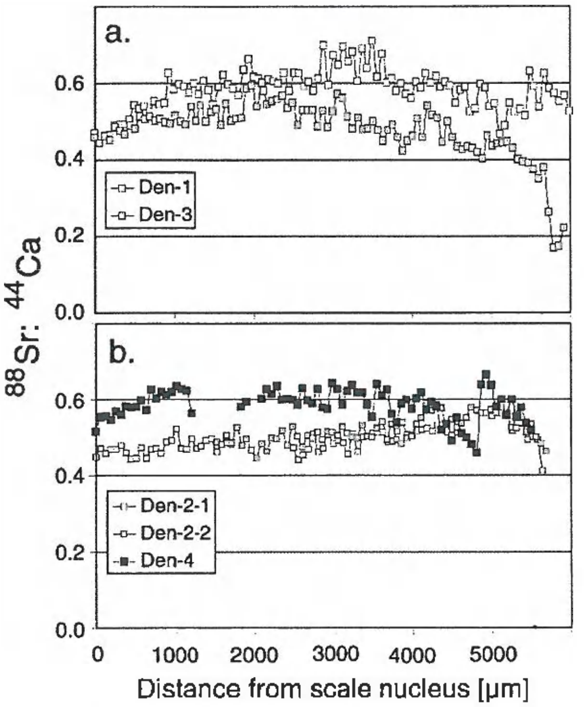

Figure 5. ^{88}Sr:^{44}Ca ratios of four Danish North Sea houting from scale nucleus to scale edge. (a) Den-1: Varde Å, 12/4/02, male 56 cm TL, Den-3: Hjortvad A, 11/23/98, female 55 cm TL; (b) Den-2: Hjortvad Å, 12/5/92, female 56 cm TL; Den-4: Varde Å, 11/27/03, male 45 cm TL. The values for Den-2 depend on two different measurements that were performed to resolve some measuring problems.

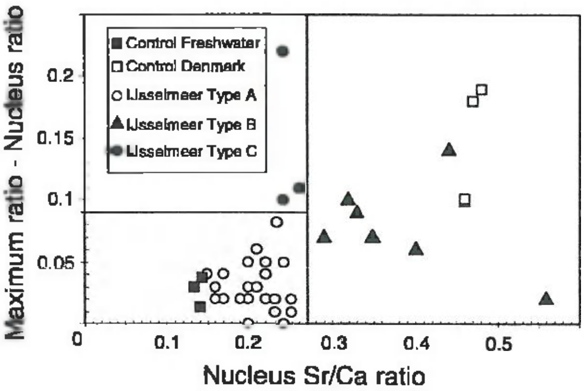

Figure 6. Difference between the maximum ^{88}Sr:^{44}Ca ratio and the ^{88}Sr:^{44}Ca ratio at the nucleus relative to the ^{88}Sr:^{44}Ca ratios at the nucleus for the 39 houting from Lake IJsselmeer, the houting from Denmark and the freshwater ponds. The vertical line (150 % of the maximum ^{88}Sr:^{44}Ca ratios of the houting from the freshwater ponds) and the horizontal line (differences between the maximum ^{88}Sr:^{44}Ca ratio and the ^{88}Sr:^{44}Ca ratio at the nucleus plus 50 % of the maximum values of the houting from the freshwater ponds) were used to divide the different migration types of houting from Lake IJsselmeer. Type A: fish that only lived in freshwater; Type B: fish that obviously moved quickly to sea water after hatching (period from hatching in freshwater until arrival at sea was too short to produce a ^{88}Sr:^{44}Ca ratio that normally indicates freshwater) and returned to freshwater before they were caught; Type C: fish that lived in freshwater for a longer period, then moved to sea water and returned to freshwater where they were caught.

was significantly related to the size of the fish ($y=16.62\ TL[cm]^{1.535}$, $r^2=0.976$), with almost 6 mm distance from the nucleus to the edge for fish of about 45 cm TL. The smallest fish analysed were about 10 cm TL and had scales with a distance of about 0.6 mm from the nucleus to the edge. Considering the distance applied of about 63 µm from the middle of one laser spot to the next, it was possible to analyse a range from about 10 to 100 laser spots from the nucleus to the edge of the scale in relation to the size of the fish.

In total, the scales of 39 houting caught in Lake IJsselmeer showed ^{88}Sr:^{44}Ca ratios between 0.16 and 0.6. By plotting the ^{88}Sr:^{44}Ca ratios at the nucleus against the differences between the maximum ^{88}Sr:^{44}Ca ratio and the ^{88}Sr:^{44}Ca ratio at the nucleus, and using the two threshold values derived from the pond houting, a distinction of three 'types' of migration patterns was obtained (Fig. 6). Fish that remained in freshwater were characterized by relatively low ^{88}Sr:^{44}Ca ratios around 0.2 from the nucleus to the edge (Fig. 7, Type A). If the ^{88}Sr:^{44}Ca ratio at the nucleus was above 0.27 (i.e., 150 % of the maximum ^{88}Sr:^{44}Ca ratio of the houting from the freshwater ponds), the period from hatching in freshwater until arrival at sea was too short to produce a ^{88}Sr:^{44}Ca ratio that normally indicates freshwater. For these fish, no distinct further increase in the ^{88}Sr:^{44}Ca ratio occurred (in contrast to the ^{88}Sr:^{44}Ca ratios of the Danish houting). A clear decrease in the ^{88}Sr:^{44}Ca ratio was measured near the edge of the scales from these fish, indicating their return to freshwater where they were caught (Fig. 7, Type B). Fish that had low ^{88}Sr:^{44}Ca ratios around 0.2 at the scale nucleus, followed by increasing ^{88}Sr:^{44}Ca ratios of at least 0.09 (i.e., 50 % of the maximum ^{88}Sr:^{44}Ca ratio of the houting from the freshwater ponds), remained for a longer period in freshwater after hatching, then moved to brackish or saltwater, and then returned to freshwater where they were caught (Fig. 7, Type C).

While the ^{88}Sr:^{44}Ca ratios at the nucleus of Type A and Type C were near to 0.2, Type B had significantly higher values (median 0.35), which were still significantly lower than those from the Danish houting (median 0.46, Kruskal-Wallis, df = 3; $p<0.0001$). For the maximum ^{88}Sr:^{44}Ca ratio of each scale, significant differences were obvious between all three groups, with the lowest ratios for Type A (median 0.24) up to a median maximum ^{88}Sr:^{44}Ca ratio of 0.61 for the Danish houting (Kruskal-Wallis, df = 3; $p<0.0001$).

The difference between the maximum ^{88}Sr:^{44}Ca ratio and the ^{88}Sr:^{44}Ca ratio at the nucleus provides

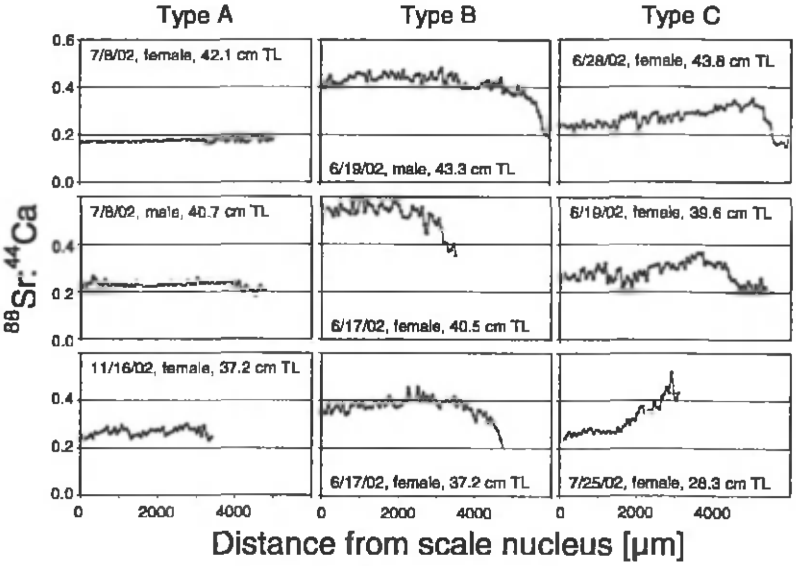

Figure 7. ^{88}Sr:^{44}Ca ratio for 9 houting caught in Lake IJsselmeer in 2002 (for classification into different migration types, see Fig. 6).

information about whether there was an increase in the ^{88}Sr:^{44}Ca ratio or whether it stayed more or less constant from the juvenile stage onwards. For Type A the median of the difference between maximum and nucleus ^{88}Sr:^{44}Ca ratio was 0.03, thus supporting the hypothesis that these fish never moved between environments of different salinities. In contrast, fish of Type C (median 0.11) showed a significant increase in the ^{88}Sr:^{44}Ca ratio from the nucleus to the maximum values of the scales (Mann-Whitney U-test, $z = 2.853$; $p = 0.0043$).

The ^{88}Sr:^{44}Ca ratio at the edge of a scale should be related to the environment where the fish was caught. It must be considered, however, that also the length of the period is important, as the fish must remain in this environment long enough to produce a signal in the scale. Considering that a houting of about 40 cm TL is about two or three years of age, a rough estimation revealed that there is about a 12-16-day period between two laser spots. No differences were found in the median ^{88}Sr:^{44}Ca ratio at the edge of the scales for all groups from Lake IJsselmeer (medians slightly above 0.2). For the Danish houting, the median ^{88}Sr:^{44}Ca ratio at the edge was higher, and only one fish had a ^{88}Sr:^{44}Ca ratio at the edge of the scale of less than 0.2 (Den-3, Fig 5a).

Most of the houting analysed from Lake IJsselmeer belonged to Type A ($n = 29$, 74 %). The majority of these fish were small in size, but the largest was a female of 42.1 cm TL that had ripe gonads. The group of Type B fish consisted of seven individuals (18 %) and their sizes ranged between 25.7 and 44.7 cm TL. The remaining 3 houting belonged to Type C (8 %), of which the smallest measured 28.3 cm TL and the largest 43.8 cm TL.

Discussion

Precision and accuracy of ^{88}Sr:^{44}Ca ratios of scales measured by LA-ICP-MS

Campana et al. (1997) published the results of the *"International Otolith Composition Experiment"* in which LA-ICP-MS was evaluated as a method with high levels of precision and accuracy for the trace element strontium. The authors concluded that the relative concentration of most detectable elements was accurately estimated and recommended standardization to the calcium concentration in the sample to reduce variance (Campana et al., 1997). We used two approaches to test the reliability of the method applied. On the one hand, we found a mean RSD of less than 10 % for the ^{88}Sr:^{44}Ca ratios of all fish. On the other hand, the six measurements of four scales from the same fish (Fig. 3, see also Fig. 5b, the two lines of houting Den-2) clearly proved the same tendency and thus provided evidence that only one measurement of one scale from each fish should provide a reliable result.

In order to obtain detailed information on the migratory behaviour of diadromous fish, in most of the studies the strontium concentration or the Sr:Ca ratio was measured in otoliths (e.g. Kalish, 1989; Rieman et al., 1994; Secor et al., 1995; Howland et al., 2001). It was demonstrated, however, that other calcified structures such as vertebrae or scales can provide comparable results (Campana and Thorrold, 2001; Wells et al., 2003; Courtemanche et al., 2005). Wells et al. (2003) showed that otolith and scale chemistry were linearly related to the Sr:Ca ratio in water. With respect to the low Sr concentrations in some freshwater fish scales, the authors recommended that future researchers should quantify the more abundant isotope, ^{88}Sr, which is the isotope used for analysis in the present study. Using this method, Wells et al. (2003) concluded that the microchemistry of the scales can be applied to describe the movements between freshwater and the marine environment, and that it should be possible to evaluate the relative contributions of resident and migratory fish within one river system.

Besides otoliths and scales, even analyses of pectoral fin rays have been used to study the migratory history of species of sturgeon (Veinott et al., 1999; Arai et al., 2002). The use of scales or fin rays has one important advantage over the use of otoliths; it is a nonlethal sampling alternative that should be preferred, or is in fact mandatory, for rare or endangered species (Veinott et al., 1999; Wells et al., 2003; Courtemanche et al., 2005). Another advantage is the low cost of sample preparation if scales are analysed with LA-ICP-MS.

^{88}Sr:^{44}Ca ratios of scales indicate salinity changes

^{88}Sr:^{44}Ca ratios of around 0.2 were found to be indicative of freshwater, as was confirmed by the scale analyses of houting that only lived in freshwater (Fig. 4). With respect to the low level of methodological variability in the second control group, i.e. on scales from houting caught in freely accessible Danish rivers (Fig. 5), ^{88}Sr:^{44}Ca ratios above 0.27 were assumed to originate from periods when the fish lived in brackish water or seawater. This is in accordance with many studies that have demonstrated at least a 1.5-fold increase in Sr:Ca ratios from freshwater to brackish water, and up to a 4-fold increase in the marine environment (e.g., Kalish, 1989; Secor et al., 1995; Veinott et al., 1999; Arai et al., 2002). Changes in otolith Sr:Ca ratios could be caused not only by salinity but also, for example, by thermal effects (Radtke and Shafer, 1992), or some physiological factors such as growth and maturation (Kalish, 1989; Campana, 1999). Comparable effects cannot be excluded for scales, although we assume that salinity changes are the dominant factor causing changes in the Sr:Ca ratios in scales (Wells et al., 2003), as has been proven for otoliths (e.g., Kalish, 1989; Secor et al., 1995, cf. also Campana, 1999).

Migration patterns of houting from Lake IJsselmeer

The migratory patterns of houting re-introduced into the River Rhine basin were not known prior to this study. Therefore, determining their migratory history was based on a comparison with two control groups, i.e. the scales from "landlocked" freshwater pond houting and houting from different Danish rivers that are known to migrate to the marine environment at early life stages (Jensen et al., 2003).

The scale analysis of the Danish river houting suggested that the juveniles migrated from the rivers to the brackish/marine environment before or at the time when the scales began to grow (from a size of about 35–40 mm TL.). This conclusion is based on the fact that the ^{88}Sr:^{44}Ca ratio at the nucleus of all scales was in the range of 0.47, and thus clearly greater than the estimated ratio of 0.27, above which the brackish/marine environment is indicated. In addition, the scales from the Danish houting also suggest that the estimated threshold between the freshwater and marine environments is valid, because during spawning migration at least one specimen was caught that had remained long enough in the Hjortvard Å river to achieve a ^{88}Sr:^{44}Ca ratio of 0.17 at the edge of the scale (Fig. 5a).

The same migration pattern seems to be valid for Lake IJsselmeer houting of Type B. The mean ^{88}Sr:^{44}Ca ratio at the scale nucleus was significantly above the threshold that indicates a juvenile freshwater period long enough to achieve a ratio below 0.27. Consequently, the Type B specimens from Lake IJsselmeer must have reached the brackish/marine environment before they had grown to a size of about 4–5 cm. Is this conceivable for the River Rhine? Houting were regularly stocked at a size of about 2–3 cm in the lower Rhine and the River Lippe (Borcherding et al., 2006). These stocking locations are about 150–200 km upstream of the North Sea, considering a passage through the Waal/Nederijn/Lek branches of the Rhine delta that ends up in the Nieuwe Waterweg, i.e. the only freely accessible migration route (Breukelaar et al., 1998). Drifting with the water current would take at least about 5 days, depending on the water level and the related current velocity of the Rhine (cf. Borcherding and De Ruyter van Steveninck, 1992). A passage through the branch of the River Rhine known as the IJssel and Lake IJsselmeer is not conceivable in this case, as the juveniles would have had to swim a distance of about 70 km through Lake IJsselmeer from the outlet of the IJssel to the sluices of the Afsluitdijk dam to reach the sea. Taking into account the known growth rates of houting (Borcherding et al., 2006) and the swimming ability of such small fish, specimens should be much larger than 4–5 cm before reaching the sea and thus have ^{88}Sr:^{44}Ca ratios below 0.27 at the scale nucleus. Assuming a passage through the Nieuwe Waterweg implies, however, that the houting of Type B migrated at least about 150 km through the North Sea to the western part of the Wadden Sea before they entered Lake IJsselmeer through the sluices of the Afsluitdijk dam, where they were caught. Another possible explanation for the ^{88}Sr:^{44}Ca ratios of Type B may be that these fish originated from natural reproduction in the River Rhine.

In contrast to Type B specimens, houting of Type C that were caught in Lake IJsselmeer must have spent a certain period in freshwater as juveniles before they migrated to the marine environment. The ^{88}Sr:^{44}Ca ratios at the scale nucleus of these specimens were significantly lower than those of Type B and the Danish houting, and they were always below 0.27. One of the three Type C specimens apparently migrated to the marine environment at an estimated size of less than 25 cm TL. In the other specimens, higher ^{88}Sr:^{44}Ca ratios were found only near the edge of the scales. Comparing the distance from the nucleus to this point would mean a migration to the sea at a size of approximately 30–35 cm TL.

The majority of Lake IJsselmeer houting had ^{88}Sr:^{44}Ca scale ratios that were never above the threshold of 0.27 (Type A); so these specimens were probably never at sea or a possible period at sea was too short to achieve a ^{88}Sr:^{44}Ca ratio on the scales that would indicate the marine environment. The majority of these specimens were less than 30 cm TL (18 out of 28 specimens), which could mean for this size group that some of them might have shifted to Type C houting at larger sizes if they had not been caught by the fisherman. Ten specimens of Type A were larger than 30 cm TL, and three mature individuals larger than 40 cm TL. This evidence suggests that houting do not need to spent a certain period in brackish or marine environments to become mature, as also found for other anadromous species (e.g., Kalish, 1990; Rieman et al., 1994).

It is difficult to determine whether these specimens of Type A from Lake IJsselmeer can be considered a 'landlocked' part of the population that had no opportunity to migrate to the sea, or whether these specimens voluntarily stayed in freshwater. Resident and anadromous life styles are known to occur simultaneously in other diadromous species (Kalish, 1990; Rieman et al., 1994; Katayama et al., 2000; Howland et al., 2001). However, most of these observations were made on populations in which the fish were able to move without any barriers on their migratory routes. Thus, the different observations were used as arguments in the discussion of how far the coexistence or divergence between alternative life history styles has an evolutionary or even genetic basis and what the mechanisms could be. This is clearly not possible for the houting from Lake IJsselmeer as the large dams in the Rhine delta represents migratory barriers that can have a strong effect on diadromous fish species (Breukelaar et al., 1998; De Leeuw et al., 2005). Nevertheless, we conclude that the scale analysis using LA-ICP-MS is an appropriate and nonlethal method that revealed different migration patterns for the houting in Lake IJsselmeer. It provides evidence that this species (1) is sometimes able to pass the migratory barriers from and to the Wadden Sea, and (2) does not need to migrate to the sea to reach maturity.

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