THEME SESSION J: IS THERE MORE TO EELS THAN SLIME?

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Stock discrimination of the European eel A. anguilla in Lithuania waterbodies by otolith Sr:Ca ratios and other elemental signatures

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Abstract

Glass eels purchased in the UK and France have been directly released into Lithuanian inland waters and mixed with naturally recruited eels for several decades. The study aimed to discriminate between both stocked and naturally recruited eels, to evaluate the contribution and interaction of the two possible eel origins to each population of studied sites. To distinguish the restocked eels from naturally recruited ones and to understand the migratory environmental history of the eel, Sr:Ca ratio in otolith were examined by wavelength dispersive X-ray spectrometry with an electron probe microanalyzer; laser-ablation ICPMS was used for Na:Ca, Mg:Ca, Mn:Ca and Ba:Ca analyses, to distinguish between eels from the freshwater lagoon and lake. The eels were sampled from the brackish Baltic Sea, a freshwater Curonian lagoon and inland lake in the eastern part of the country. Stocked eels were identified by the freshwater signature (Sr:Ca <2.24×10⁻³) on the otolith after the glass eel stage. Naturally recruited eels were characterized by an extended sea/brackish water signature (Sr:Ca >3.23×10⁻³) after the glass eel stage. Stocked eels accounted for 20% of the eels from the Curonian Lagoon and 2% of eels sampled in Baltic coastal waters, while all eels from the inland lake were of stocked origin. One-way ANOVA tests indicated that both Ba:Ca and Na:Ca ratios in eel otoliths were significantly higher for the eels in the lagoon than those of the lake (p<0.05) and enables correctly assign 83% of the eels to their freshwater sampling locations, however, otolith Mg:Ca and Mn:Ca ratios were similar for eels from the two environments.

Keywords: Anguilla anguilla; European eel; Migratory history; Stocking; Otolith microchemistry; Elemental composition, Laser-ablation ICP-MS

Introduction

Catadromous eels are commercially valuable species and support worldwide eel aquaculture and eel fisheries. The abundance of several species (i.e., Anguilla anguilla Linnaeus, 1758, A. rostrata Lesueur, 1817, and A. japonica Temminck & Schlegel, 1847) have declined throughout their distribution ranges due to over-fishing, anthropogenic activities and changes of oceanic currents induced by global weather anomalies (Castonguay et al., 1994; Dekker, 2003; Tatsukawa, 2003). In addition, the eel’s mysterious life cycle further compounds its fate and makes conservation and recovery more difficult.

Declines in eel recruitment in Scandinavia have been noted since the 1940s (Moriarty, 1996), but the greatest decreases in the recruitment of eels throughout Europe have occurred since the early 1980s (Dekker,
2000). Recent recruitments of *A. anguilla* glass eel were estimated to be only 1% of the level before the 1980s (Dekker, 2004). Poor natural recruitment from the oceanic migration phase, exacerbated by habitat degradation, pollution, artificial physical barriers to migration and high fishing pressure on glass eels (Moriarty & Dekker, 1997) has led to the need for stocking to maintain, enhance, restore or establish stocks. Intensive stocking programmes have been undertaken in the Baltic Sea region over the past 50 years. The most intense stocking programmes have been implemented in the Baltic Sea drainage using eels originating from western Europe.

The metabolically inert otolith records biological as well as environmental information throughout the fish’s life. Fish can absorb Sr in the ambient water and substitute for Ca in the process of \( \text{CaCO}_3 \) deposition in the otolith. The positive relationship between salinity and otolith Sr:Ca ratios has been validated for different species including eels (e.g., Tzeng 1996; Secor *et al.*, 1998; Kraus & Secor, 2003). Accordingly, seawater-resident fish uptake and deposit more Sr in the otolith than do freshwater fish. Otolith Sr:Ca ratios in combination with age data have been used to elucidate the migratory environmental history of diadromous fishes, including anguillid eels (Tsukamoto & Arai 2001; Jessop *et al.*, 2002; Shiao *et al.*, 2003). Some other elemental ratios, such as Ba:Ca can be used to assign fish from different freshwater habitats, whose otoliths contain similar Sr:Ca ratios (Elsdon & Gillanders, 2005b).

Elvers purchased in the United Kingdom and France are directly released into Lithuanian freshwater lakes and the Curonian Lagoon. These stocked eels do not experience the long migratory journey through the North and Baltic Sea and thus they should show a freshwater signature of low otolith Sr:Ca ratios immediately after the elver stage. In contrast, eels naturally recruited to Lithuania must pass the North and Baltic Seas and should show an extended sea/brackish water signature of high (North Sea) and intermediate (Baltic Sea) otolith Sr:Ca ratios after the elver stage. Thus, a life history scan of otolith Sr:Ca ratios should be able to discriminate between both stocked and naturally recruited eels. Clarification of the eel’s migratory history will also help to evaluate the contribution and interaction of the two possible eel origins (stocked or naturally recruited) to each population along the Baltic coasts and in the Curonian Lagoon and inland lakes.

**Materials and Methods**

**Fish collection and sampling sites**

Silver and yellow-stage European eels *A. anguilla* were collected by fyke nets and long lining from Baltic coastal waters, the Curonian Lagoon in western Lithuania and the freshwater Lake Baluošai in eastern Lithuania in 2003-2005 (Fig. 1). The lake is about 300 km from the Curonian Lagoon and about 350 km from the Baltic Sea, to which it is connected via a system of small streams, lakes, the river Nemunas and the Curonian Lagoon. Natural recruitment to these lakes has never been reported and may not occur; however, the possibility cannot be excluded. Elvers have been regularly stocked since 1960 into the system of lakes in the Baluošai Lake region.

The shallow Curonian Lagoon (mean depth - 3.7 m) is separated by a narrow sand spit (0.5 – 4.0 km wide) from the Baltic Sea and is connected to the Baltic Sea through the narrow (0.5 km wide) Klaipėda Strait. The salinity of the Baltic Sea adjacent to Lithuania varies from 4.9 to 6.8 (Dubra & Dubra, 1998). The Lagoon is 1584 km² in area and is a fresh water basin. Rivers supply the lagoon with about 3.6 times more freshwater than the water volume in the lagoon itself and the mean water level in the lagoon is 15 cm higher than sea level. Therefore, brackish water penetration into the Lagoon is rare. The salinity fluctuates from 0.03 in the southern part of the Lagoon up to 2.7 in the Klaipėda Strait. During stormy inflows of brackish water the salinity may episodically rise to 5-6 in the northern areas (Olenin, 1996).

The total length (\( L_T \)) and weight (W) of each eel was measured to the nearest 1.0 mm and 1.0 g. Sexes were determined macroscopically from the gross morphology of the gonads, where eels with thin, regularly lobed organs (Syrski’s organ) were considered males, while individuals with more broad and folded curtain-like gonads were females (Tesch, 2003). The eels were classified as yellow and silver eels, by their external colour, fin shape and eye size.

Water Sr and Ca concentrations around the eel sampling locations were determined by atomic absorption spectrophotometer (Hitachi Z-5000). Standard solutions from Merck (Darmstadt, Germany) were used to make the standard curve. Sr and Ca concentrations of the water collected from the Baltic coast (salinity 5.8) were approximately \( 1.67 \times 10^{-5} \) and \( 2.60 \times 10^{-3} \) M, respectively (\( 6.44 \times 10^{-3} \) for the Sr:Ca ratio). Water collected in the Curonian Lagoon (salinity 0) contained approximately \( 1.36 \times 10^{-6} \) M of Sr and \( 1.51 \times 10^{-3} \) M of Ca (\( 0.90 \times 10^{-1} \) for the Sr:Ca ratio). Water Sr and Ca concentrations in the Baltic coast and Curonian Lagoon were in the range of normal brackish and fresh water.
Fig. 1 A. Northern Europe, showing the North and Baltic Seas, and B. Sampling locations (dots) in the Baltic Sea, Curonian Lagoon and Lake Baluošai.
**Otolith preparation and Sr:Ca analysis**

The largest pair of eel otoliths (sagittae) was removed, dried in air, embedded in Epofix resin, ground and polished until the core was exposed. For electron probe microanalysis, the polished otoliths were coated with carbon under a high-vacuum evaporator. Sr and Ca concentrations in the otolith were measured from the otolith core to the edge at 10 µm intervals. Quantitative analyses were conducted with an electron probe microanalyzer (JEOL JXA-8900R), using beam conditions of 15 kV for the acceleration voltage, 3 nA for the current, and a 5 x 4 µm rectangular scanning beam. The quantitative data were corrected by the PRZ (phi-rho-z) method to calculate oxide compositions (e.g., Goldstein et al., 1984; Reed, 1993). The peak concentration of Sr Lα was counted for 80 s with background measurements for 20 s on each side. The peak concentration of Ca Kα was counted for 20 s and each background for 10 s. A synthesized aragonite (CaCO₃) and strontianite ([Sr₀.95Ca₀.05]CO₃; NMNH R10065) were used as calibration standards. Since aragonite-structure carbonates are similar to otoliths, the standards have smaller matrix corrections than other types of standards such as oxide or silicate (Jarosewich & White, 1987). The standards were mounted in epoxy resin and polished. The carbon coating for the standards and otoliths had the same thickness (25-35 nm). After microchemical analysis, the otolith was polished to remove the carbon layer, then etched with 5% EDTA for 1 to 2 min to reveal the annual rings for age determination. The duration of the eel in freshwaters and sea/brackish waters was estimated by relating the otolith Sr:Ca ratio profile to the otolith annuli. The criteria for the discrimination between freshwater- and sea/brackish water-residents are defined in the results.

**Na:Ca, Mg:Ca, Mn:Ca and Ba:Ca elemental ratio analyses**

Some other elemental ratios, including Na:Ca, Mg:Ca, Mn:Ca and Ba:Ca, were analyzed in 22 eel otoliths from different habitats (Table IV), by laser-ablation ICPMS (inductively coupled plasma mass spectrometry). The eel otoliths were re-polished with a micro cloth and 0.05 µm alumina paste to remove the carbon coating then triple rinsed with Milli Q water and dried in clean room before the consequence chemical analyses. The LA-ICPMS system consisted of a Merchantek LUV 266 Nd:YAG UV laser microprobe (New Wave Research, Inc.) connected to a Finnigan MAT ELEMENT 2 high resolution ICPMS. Otoliths were ablated inside a sealed chamber. The sampled gas was extracted from the chamber in an argon and helium gas stream, and transported to the ICP-MS for analyzing isotopes of ²³Na, ²⁴Mg, ⁴⁴Ca, ⁵⁵Mn, ⁸⁸Sr, and ¹³⁸Ba. Calcium was used as an internal standard, and the concentrations of other elements were estimated against the Ca concentration. The laser was pulsed at 20 Hz across the transverse section of the otolith, moving from the primordium to the otolith edge with a speed of 15 µm per second. Each data point takes about 2.46 sec and represents the chemical composition over 37 µm. The average of the last three point from the otolith edge represented the recent deposition on the otolith before the fish was collected.

**Data analysis**

Data are expressed as means ± SD (n = number of fish). Statistical differences among groups (locations) were evaluated by one-way analysis of variance (ANOVA) or Mann-Whitney Rank Sum Test. Differences among groups were identified by Tukey’s pairwise multiple comparison test. Significance was set at p < 0.05. Canonical discriminant analysis was used to distinguish eels from different habitats by otolith elemental compositions (Na:Ca, Mg:Ca, Mn:Ca and Ba:Ca ratios).
Fig. 2 *Anguilla anguilla*. Otoliths of European eels showing the metamorphosis check (MC), elver check (EC) and annuli (remaining dots). Panel a: restocked eel no. 3 (yellow eel, 63 cm TL) collected from Curonian Lagoon, showing rapid growth as inferred from wide otolith annuli. Panel b: restocked eel no. 105 (silver eel, 74 cm TL) collected from Lake Baluošai showing slow growth inferred from narrow annuli. A conspicuous check (indicated by the arrow) fused with the second annuli is regarded as a false annulus in eel no. 3. Scale bar = 1mm.
Results

**Total length, body weight, ages and sexes of the eels among locations**

All 48 eels collected in the coastal waters of the Baltic Sea were at the yellow eel stage as were the 49 eels collected at the Curonian Lagoon, with the exception of 1 silver eel (Table I). All 10 eels collected in the Lake Baluošai were migrating silver eels. All eels collected in the 3 sites were all females except one male in Lake Baluošai. There were no significant differences in mean length (F = 1.56, df = 2, p = 0.21) and weight (F = 1.54, df = 2, p = 0.22) among sampling locations. However, the mean ages of the eels from Lake Baluošai were significantly greater than those from the Curonian Lagoon and the Baltic coast (F = 84.8, df = 2, p < 0.001). This implies that the eel grows faster in the Baltic coasts and Curonian Lagoon than in Lake Baluošai.

Table 1. Biological characteristics (means ± SD) of the European eels collected from Lithuanian sites.

<table>
<thead>
<tr>
<th>Sampling Location</th>
<th>Sampling period</th>
<th>Developmental stage</th>
<th>Sample size*</th>
<th>Total length (cm)</th>
<th>Body weight (g)</th>
<th>Age (year)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baltic coast</td>
<td>June-September</td>
<td>Yellow eel</td>
<td>48</td>
<td>63.0 ± 7.3 (47.5 - 81.0)</td>
<td>582.4 ± 274.6 (180.0 - 1400.0)</td>
<td>11.0 ± 1.8 (8 - 16)</td>
</tr>
<tr>
<td>Curonian Lagoon</td>
<td>June-August</td>
<td>Yellow eel (except one silver eel)</td>
<td>50</td>
<td>66.3 ± 10.4 (49.0 - 92.0)</td>
<td>691.4 ± 441.7 (201.0 - 2126.0)</td>
<td>10.8 ± 1.7 (6 - 15)</td>
</tr>
<tr>
<td>Lake Baluošai</td>
<td>April</td>
<td>Silver eel</td>
<td>10</td>
<td>64.7 ± 11.0 (43.3 - 80.0)</td>
<td>519.9 ± 266.2 (127.0 - 930.0)</td>
<td>19.0 ± 3.0 (15 - 24)</td>
</tr>
</tbody>
</table>

* All female except one male in Lake Baluošai

**Life history scan of otolith Sr:Ca ratios**

Sr:Ca ratios in eel otoliths increased from approximately 8 - 10 × 10⁻³ in the core to a peak of approximately 18 - 24 × 10⁻³ about 60 - 100 μm from the core. Otolith Sr:Ca ratios then sharply decreased (Figs. 3 - 6), which corresponds to the metamorphosis from leptocephalus to glass eel (Arai et al., 1997). Otolith Sr:Ca ratios before the elver stage were similar among individuals since the eels have similar migratory histories at the leptocephalus and glass eel stages. The patterns of otolith Sr:Ca ratios beyond the elver stage were variable, indicating diverse migratory histories during the yellow eel to silver eel stages. The migratory patterns of the eels were classified as follows:

**Freshwater pattern:** There were 16 eels (10 from Lake Baluošai and 6 from the Curonian Lagoon) which showed consistently low otolith Sr:Ca ratios from the elver to silver eel stage (Fig. 3). No eels collected in the Baltic Sea had a pattern of consistently low otolith Sr:Ca ratios. The mean otolith Sr:Ca ratio of these 16 eels after the elver stage was 0.72 ± 0.76 × 10⁻³, which is consistent with previous studies on European and American eels (Tzeng et al., 1997; Cairns et al., 2004). This pattern suggested that these eels resided in freshwater from the elver to the yellow or silver eel stage.

Table 2. Migratory patterns of European eels as inferred from otolith Sr:Ca ratios. Freshwater residents, Sr:Ca ratios consistently <2.24 × 10⁻³; Seawater residents, Sr:Ca ratios consistent >3.23 × 10⁻³; Inter habitat shifters, Sr:Ca ratios covering the ranges of freshwater and seawater values.

<table>
<thead>
<tr>
<th>Habitat</th>
<th>Sample size</th>
<th>Migratory patterns of the eels (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Freshwater residents</td>
</tr>
<tr>
<td>Baltic coast</td>
<td>48</td>
<td>-</td>
</tr>
<tr>
<td>Curonian Lagoon</td>
<td>50</td>
<td>12</td>
</tr>
<tr>
<td>Lake Baluošai</td>
<td>10</td>
<td>100</td>
</tr>
<tr>
<td>Total</td>
<td>108</td>
<td>14.8</td>
</tr>
</tbody>
</table>
Sea/brackish water pattern: Twenty-three eels (48%) collected in the Baltic coastal waters had otolith Sr:Ca ratios consistently above $3 \times 10^{-3}$ between the elver check and the otolith edge (Fig. 4). The consistently high otolith Sr:Ca ratios suggested that these eels resided in brackish waters or seawaters without entering freshwater from the elver stage through the yellow eel stage. Eels with this kind of otolith Sr:Ca ratio pattern were considered to be sea/brackish water eels (Tsukamoto et al., 1998; Tzeng et al., 2000). The mean otolith Sr:Ca ratio from the elver check to the otolith edge of the sea/brackish water-resident eels was $4.84 \pm 1.61 \times 10^{-3}$, which was significantly higher than that of the freshwater-resident eels ($0.72 \pm 0.76 \times 10^{-3}$) (Fig. 3). Therefore, the mean otolith Sr:Ca ratios of the eels collected in the freshwater Lake Baluošai and in the Baltic Sea were used as criteria to classify different migratory environmental histories of the eel. Eels with otolith Sr:Ca ratios lower than $2.24 \times 10^{-3}$ (mean otolith Sr:Ca ratios of 16 freshwater eels + 2 SD) were considered as freshwater residents while eels with ratios larger than $3.23 \times 10^{-3}$ (mean otolith Sr:Ca ratios of 23 sea/brackish water eels - 1 SD) were considered as sea/brackish water residents. Intermediate values were regarded as transition between freshwater and sea waters. No eels collected in the Curonian Lagoon demonstrated the sea/brackish water pattern; all showed freshwater residency (< $2.24 \times 10^{-3}$) for all or part of their life’s span.

Some sea/brackish water-resident eels showed relatively high otolith Sr:Ca ratios between the elver and yellow eel stages and gradually decreased to lower otolith Sr:Ca ratios in the later part of the yellow stage (Fig. 4). For example, eel no. 34 had higher Sr:Ca ratios ($5 - 12 \times 10^{-3}$) before 600 μm (age 5) and lower ratios ($3 - 5 \times 10^{-3}$) between 700 and 1000 μm (age 6 - 10) (Fig. 4a). Eel no. 44 showed otolith Sr:Ca ratios that decreased from $6 - 10 \times 10^{-3}$ at 350 μm (age 2) to $3 - 4 \times 10^{-3}$ around 800 μm (age 8) (Fig. 4d). Decreasing trends of otolith Sr:Ca ratios were found in 20 sea/brackish water eels that indicated a habitat shift from high to low salinity by these eels (Figs. 4, 5). The pooled profile of these 20 sea/brackish water-resident eels showed a relatively large mean otolith Sr:Ca ratio ($5.51 \pm 1.57 \times 10^{-3}$, range $4 - 8 \times 10^{-3}$, n = 1240) between 160 - 770 μm from the core and a small otolith Sr:Ca ratio ($3.64 \pm 1.10 \times 10^{-3}$, range $3 - 5 \times 10^{-3}$, n = 1119) between 780 - 1500 μm (Fig. 7). The gradient of mean otolith Sr:Ca ratios in Fig. 7 may reflect the migratory history of the eel from the full-strength sea water in North Sea to the brackish waters in the southeastern Baltic coasts.

Table 3. Relative contribution of restocked and naturally recruited European eels in different habitats.

<table>
<thead>
<tr>
<th>Habitat</th>
<th>Sample size</th>
<th>Origin of the eel (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Restocked</td>
</tr>
<tr>
<td>Baltic coasts</td>
<td>48</td>
<td>2</td>
</tr>
<tr>
<td>Curonian Lagoon</td>
<td>50</td>
<td>20</td>
</tr>
<tr>
<td>Lake Baluošai</td>
<td>10</td>
<td>100</td>
</tr>
<tr>
<td>Total</td>
<td>108</td>
<td>19</td>
</tr>
</tbody>
</table>
Fig. 4. Sr:Ca ratio transects from eel otolith cores to the edge illustrating consistent seawater residence after the glass eel stage. The arrows and numerals indicate the annuli corresponding to the peaks of Sr:Ca ratios. Eels were collected from the Baltic Sea.

Patterns of interhabitat shifters: Most eels caught in the Curonian Lagoon (88%) and half from the Baltic Sea (52%) migrated between habitats (Table II) i.e., their otolith Sr:Ca ratios fluctuated between freshwater and seawater levels. Most interhabitat shifters (eels migrating between fresh water and sea/brackish water) showed high otolith Sr:Ca ratios (>3.23 × 10^{-3}) for several years after the elver stage that were then followed by relatively low (<2.24 × 10^{-3}) or fluctuating ratios between the freshwater and seawater levels (Fig. 5). Eel no. 1 resided in sea/brackish water for the first five years (otolith Sr:Ca ratios range: 4 - 12 × 10^{-3}) and invaded freshwater at age 5 (Fig. 5d). This eel showed seasonal peaks in otolith Sr:Ca ratios at each annulus, suggesting that it wintered in brackish water but spent the remainder of each year in fresh water (Tzeng et al., 1997; 1999). The coincidence of otolith Sr:Ca ratio peak and annulus was also found in other eels e.g., no. 4, 34 and 41 (Figs. 4, 5). In light of the high plasticity of eel phenotypes, an explicit description or classification of the diversified migratory behaviors is not feasible and seems not necessary. Briefly, these varying patterns suggested that the eels recruited to freshwater and resided there until being caught or moved seasonally/irregularly between fresh water and sea water.
Fig. 5. Sr:Ca ratio transects from eel otolith cores to the edge illustrating eel movement into freshwaters after a period of seawater residence. Eel no. 4 demonstrate a gradual decline of Sr:Ca ratio profile corresponding to the movement from high salinity through low salinity to freshwater. Eel no. 1 shows seasonal migration between high salinity and low salinity or freshwaters. The arrows and numerals indicate the annuli corresponding to peaks in the Sr:Ca ratios. The X-ray intensity mapping display high Sr content from age 1-5 and high Sr rings at annuli 6 to 10. Eels were collected from the Curonian lagoon.
Fig. 6. Sr:Ca ratio transects from eel otolith cores to the edge illustrating rapid entry into fresh water at the elver stage then a return to seawater (eels no. 45 and 100) or movement between freshwater and seawater (eels no. 5 and 7). Eels no. 5 and 7 were collected from the Curonian Lagoon. Eels no. 45 and 100 were collected from the Baltic Sea.

Fig. 7. The pooled otolith Sr:Ca ratio profile of 20 European eels that showed a gradual decline in Sr:Ca ratios during movement from the North Sea to the Baltic Sea. The 20 eels were collected in the coastal waters of the Baltic Sea.
Migratory patterns and origin of the eels among locations

The eels collected along the Baltic coast were either sea/brackish water-resident eels (48%) or interhabitat shifters (52%); none were freshwater eels (Table II). The eels collected from the Curonian Lagoon were primarily interhabitat shifters (88%) while freshwater-resident eels accounted for about 12% (Table II). Eels from the freshwater Lake Baluošai were all freshwater eels (Table II).

The presence or absence of an extended sea/brackish water signature (otolith Sr:Ca ratios > $3.23 \times 10^{-3}$) after the glass eel stage was used to distinguish stocked eels from naturally recruited eels. The 6 eels from the Curonian Lagoon and 10 eels from Lake Baluošai showing a consistent freshwater signature throughout their life after the glass eel stage must be the stocked eels. Another 85 eels with extended large otolith Sr:Ca ratios after glass eel stage should be the naturally recruited eels if the stocked elvers have not migrated immediately to seawater.

In a few interhabitat shifters ($N = 7$), a period of low otolith Sr:Ca ratios ($< 2.24 \times 10^{-3}$) appeared before the extended high otolith Sr:Ca ratio ($>3.23 \times 10^{-3}$), making it difficult to tell whether these 7 eels were stocked eels or naturally recruited eels (Fig. 6). This may indicate that the eels had invaded freshwater at the elver stage for a period of time, then returned to sea/brackish water. Eels no. 5, 6 and 7 resided in fresh water for approximately 2 years, returned to sea/brackish waters for 1 - 4 years, and then moved back to freshwater (Fig. 6a, b). Eels no. 14, 45 and 100 resided in freshwater for approximately 1 - 4 years and subsequently returned to sea/brackish waters for their remaining life (Fig. 6c, d). Differences in their migratory histories were evident. Eels no. 45 and 100, collected on the Baltic coast, (Figs. 6c, d) showed relatively high otolith Sr:Ca ratios (400 - 700μm for eel no. 45 and 350 - 800μm for eel no. 100) at the early stage and a decreasing trend in otolith Sr:Ca ratio in the later stage, which is very similar to the pattern of naturally recruited eels. Therefore, eels no. 45 and 100 might be naturally recruited eels that have entered freshwater somewhere prior to entering Lithuanian waters. In contrast, eels no. 5, 6, 7, 14 collected in the Curonian Lagoon (Figs. 6a, b) and eel no. 60 from Baltic coast might be stocked eels that stayed in a freshwater for a few years then returned to the Baltic coast or migrated between both sites. This interpretation was based on otolith Sr:Ca ratios for the sea/brackish water signature that were smaller, intermittent or shorter than that for naturally recruited eels and that showed no decline in otolith Sr:Ca ratios. Overall, 87 individuals were naturally recruited eels while 21 eels were stocked eels among the samples collected in the Curonian Lagoon ($N = 10$), Baltic coasts ($N = 1$) and Lake Baluošai ($N = 10$) (Table III). Stacked eels accounted for about 20% of the eels in the Curonian Lagoon and 2% on the Baltic coast; however, the eels in the Lake Baluošai were 100% of stocked origin (Table III).

Geographic differences in otolith elemental composition among eels from different locations

Otoliths of 22 eels (Table IV) were analyzed by laser-ablation ICPMS. Otolith Sr:Ca ratios successfully discriminated restocked individuals from naturally-recruited ones. Laser-ablation ICPMS method was applied for otolith Na:Ca, Mg:Ca, Mn:Ca and Ba:Ca ratios analysis, to distinguish between eels from different habitats and primarily from the freshwater lagoon and lake, whose otoliths contain similar low Sr:Ca ratios. Overall 91.3 % of the individuals could be correctly assigned by otoliths edges elemental compositions using canonical discriminant analysis (Figs. 8, 9). A total of 83% of the eels could be correctly assigned to their freshwater sampling locations. One-way ANOVA tests indicated that both Ba:Ca and Na:Ca ratios in eel otoliths were significantly higher for the eels in the lagoon than those of the lake ($p<0.05$), but otolith Mg:Ca and Mn:Ca ratios were similar for eels from the two environments ($p>0.05$).

Table 4. Origin of eels used for laser-ablation ICPMS.

<table>
<thead>
<tr>
<th>Type</th>
<th>N</th>
<th>Sampling site</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (Restocked, freshwater eels)</td>
<td>5</td>
<td>Curonian Lagoon</td>
</tr>
<tr>
<td>II (Restocked, freshwater eels)</td>
<td>6</td>
<td>Lake Baluošai</td>
</tr>
<tr>
<td>III (Naturally-recruited, sea/brackish water residents)</td>
<td>6</td>
<td>Baltic Sea</td>
</tr>
<tr>
<td>IV (Naturally-recruited, freshwater residents)</td>
<td>5</td>
<td>Curonian Lagoon</td>
</tr>
</tbody>
</table>
Fig. 8. Elemental ratios (Na:Ca, Ba:Ca) on the otolith edges from different migratory type European eels

Fig. 9. Canonical discriminant analysis of European eel otolith edges elemental compositions

**The ages on arrival in Lithuanian waters**

Sixty-three naturally recruited eels initially entered freshwater at ages of 1 to 10 yrs with a mean age of 5.2 ± 2.1 yrs (Fig. 10). This implies that after reaching the Baltic, the eels spent a number of years in marine/brackish waters before entering freshwater. Twenty-two of these 63 eels showed consistent low otolith Sr:Ca ratios after extended high Sr:Ca ratios, indicating that they continuously resided in fresh waters after freshwater entry (Figs. 5a, b). Mean age of these 22 eels at freshwater entry was 4.6 ± 2.2 yrs (Fig. 10b), not significantly different from the mean ages of the other 41 eels (5.6 ± 1.9) that entered fresh water but showed subsequent movements between freshwater and sea/brackish waters (p = 0.16, Mann-Whitney Rank Sum Test). This indicated these eels spent several years in the Baltic Sea then entered fresh water at the area of capture. After the initial freshwater entry, the eels spent 5 – 6 years (range: 1 - 10 years) in the Curonian Lagoon or Baltic coast before capture (Fig. 10).
Fig. 10. Frequency distribution of ages of naturally recruited eels at initial entry into freshwater entry (panel a), Panel b, only naturally recruited eels continuously reside in Lithuanian freshwaters after initial entry.

**Discussion**

**Interpretation of eel migration by otolith Sr:Ca ratios**

Bath *et al.* (2000) and Kraus & Secor (2004) pointed out that it was Sr:Ca ratios in the water rather than salinity that primarily determined the incorporation of Sr into the otolith. This finding implied that otolith Sr:Ca ratios, as the proxy of salinity, should be interpreted based on knowledge of the ambient water chemistry. This is because fish living in Sr-rich fresh water may incorporate Sr at the same level or even higher than the fish living in normal sea/brackish water. However, Kraus & Secor (2004) also admitted that natural Sr-rich fresh water, if it can be found, is rarely seen. Generally, Sr is about 100-fold greater in seawater \((8.7 \times 10^{-5} \text{ M})\) than in fresh water \((9 \times 10^{-7} \text{ M})\) (Campana, 1999). We measured more than 10-fold higher water Sr concentration or 7-fold higher water Sr:Ca ratios in the Baltic coast than in the Curonian Lagoon. Elsdon & Gillanders (2005) found significantly increased otolith Sr:Ca ratios by enhancing ambient water Sr:Ca ratios by 2 to 4 fold. Their experimental results justified the use of otolith Sr:Ca ratios in interpreting migratory history of the eels across different salinity environments.

Salinity decreases gradually from about 35 in the North Sea to about 15 in the southwestern Baltic Sea and to around 6 in the coastal waters of the southeastern Baltic Sea. The migration from a high salinity (North Sea) to low salinity environment (southeastern Baltic) was clearly imprinted in 20 naturally recruited eels with decreasing otolith Sr:Ca ratios (Fig. 7). Owing to the asymptotic relationship between otolith Sr:Ca ratios and salinity (Tzeng, 1996; Limburg *et al.*, 2003), there is limited ability to distinguish full-strength and half-strength sea water salinities by otolith Sr:Ca ratios. However, freshwater eels showed very low otolith Sr:Ca ratios, distinct from those in sea/brackish water eels. This result is consistent with the Zimmerman (2005) finding that
salmonid otolith Sr:Ca ratios were linearly related to salinity but the sensitivity of otolith Sr:Ca ratios was only enough to discriminate between fresh water, brackish water and seawater.

**Contribution of stocked and naturally recruited eels to the population**

The composition of stocked and naturally recruited eels differed among locations. Based on the patterns of otolith Sr:Ca ratios, eels caught in Lake Baluošai are all of stocked origin, suggesting that few, if any naturally recruited eels reach this area and that eel fisheries in the inland lakes are all based on the stocked eels. Further studies with larger sample sizes are required to determine if there is any natural recruitment to the inland waters of eastern Lithuania. The proportions of stocked eels decreased to 20% and 2% in the Curonian Lagoon and Baltic coast, respectively (Table III). There was no evidence to suggest that stocked eels from inland lakes of eastern Lithuania will emigrate downriver and contribute to the eel stock in the Curonian Lagoon or the Baltic coast. It seems unlikely that stocked eels undergo the long migration, approximately 300 km, from Lake Baluošai or other lakes from the same water basin to the Curonian Lagoon during the yellow eel growth-phase. The eels caught in Lake Baluošai showed narrow otolith annuli (Fig. 2b), which is distinct from that of residents in the Curonian Lagoon and Baltic coast (Fig. 2a). The narrow annuli indicate a slow growth rate due to the limited prey available in Lake Baluošai, determined by higher eel population density based on catch-per-unit-effort in the stocked lakes of eastern Lithuania (L. Ložys, unpubl. data). The growth differences might also be determined by lower lake productivity and by feeding differences at the sampling sites. The Curonian Lagoon is eutrophic (Jašinskaitė, 1998) while Lake Baluošai is meso/oligothrophic (K. Arbačiauskas, pers. comm.). Eel dietary studies demonstrated that Lagoon and Baltic Sea eels eat a high proportion of fish while Lake Baluošai eels eat largely invertebrates (E. Bacevičius, pers. comm.). None of the 11 stocked eels found in the Curonian Lagoon and Baltic coast showed narrow otolith annuli, suggesting that few, if any, stocked eels migrate downriver to the Curonian Lagoon or Baltic coast waters until the spawning migration. Moreover, statistical tests indicated that both Ba:Ca and Na:Ca ratios in eel otoliths were significantly higher for the eels in the lagoon than those of the lake, supporting the hypothesis that the eel populations in inland lakes of the eastern Lithuania and the Curonian Lagoon are independent during the growth phase. Energy costs, density-dependent migration and variable habitat quality could influence the geographic distribution as well as the migration of eels within the river (Feunteun et al., 2003).

Some glass or small yellow eels have been released in Curonian Lagoon, but the stocking rate has been low: (only 1.7 eels ha⁻¹ in 1995 - 2003). Only one stocked eel (no. 60) was found to have emigrated from the Curonian Lagoon to the nearby Baltic coast while 4 stocked eels (no. 5, 6, 7, 14) eventually returned to the Curonian Lagoon after short movement to the Baltic coast. This suggests that stocked eels prefer to settle in the location where they are released. Accordingly, 91% (n = 10) of the stocked eels (n = 11) remained in the Curonian Lagoon where they were released, assuming that no or very few eels had descended from lakes via the Nemunas River. In addition, about 9% (n = 1) of the stocked eels in the Curonian Lagoon emigrated to the Baltic coast and constituted about 2% of the local eel population.

Eels are important commercial and recreational species in central and eastern Europe and make important contributions to local and regional economies. Hence, the original aim of stocking programmes in the Lithuania and other Baltic countries was enhancement of inland fisheries. Intensive exploitation of the stocked eels presumably led to high fishing mortality and a low rate of escapement by silver eels. However, Limburg et al., (2003) found that 26.7% of the migrating silver eels at the Baltic Sea exit were of stocked origin, comparable to our finding that stocked eels account for 20% of the population in the Curonian Lagoon. Without related information on natural recruitment as well as the survival rate of the stocked eels, it is difficult for this first evaluation of the contribution of stocked eels to the naturally recruited population in the southeastern Baltic Sea to determine stocking effectiveness. However, it is possible that the stocked glass eels in Lithuania (e.g., stocked to the Curonian Lagoon) or other Baltic countries migrate to the Baltic Sea too soon after release to allow the freshwater signature to be recorded in the otolith. If so, the proportion of stocked eels in the Baltic Sea will be underestimated. Alternatively, most stocking programmes in the Baltic countries are focused on fisheries enhancement in inland lakes and quick migration over long distances to the Baltic Sea without creating a freshwater signature in the otoliths seems unlikely.

Sea/brackish water-resident eels accounted for only 23% of eels examined from Baltic coastal waters and the lagoon, while interhabitat shifters comprised approximately 70% of the eels. The proportion of naturally-recruited eels that have experienced fresh water is more than twice as high as found in the migrating silvers at the exit of the Baltic Sea (Limburg et al., 2003). If only the eels collected in the Lithuanian Baltic coast were considered, the proportion of the eels experiencing freshwater is still as high as 50%. The differences between these 2 independent studies may be due to the different geographical and habitat constraints or different behaviors of the eels. Long-distance migratory eels may be more active in exploring different habitats than their counterparts that settle down earlier. After entry into Baltic Sea, eels trapped in this closed system may explore optimal habitats at minimal energy cost to benefit maximal growth. Diversified habitats usually provide more
foods and shelters than does a less diversified habitat, which presumably encourages euryhaline fish to explore different habitats. This may explain the flexible and complex migratory behavior of the eels, which reflects their environmental and evolitional adaptation.

**Ages of eel on arrival in SE Baltic Sea**

To our knowledge, this is the first study that estimates the ages of the eels arriving in the southeastern Baltic area. Most naturally-recruited eels showed an initial freshwater entry at age 1 - 10 years (5.2 ± 2.1 years). High variability in the age at initial freshwater entry indicates that some eels might migrate quickly through the Baltic Sea and into freshwater within one or a few years, while some eels showed very slow migration eastward. The broad ranges of age at initial freshwater entry also suggest a random distribution of the eels in the Baltic Sea rather than size or age dependent distribution. The eel density in the North Sea and Skagerrak/Kattegat Sounds may influence ages at arrival in the southeastern Baltic. Low eel density in the Baltic Sea due to low recruitment of young eels (Westin, 2003) may discourage eastward migration due to low intraspecific competition, so the eels arrive at older ages. It is known that populations in the lower reaches of rivers achieve high densities but as eels grow, relative biomass and hence competition for food and space increase. Agonistic encounters may then act as a stimulus for further upstream migration (Knights, 1987). Interestingly, on the Baltic coast of Denmark, at the monitoring site of eel recruitment at the Harte hydropower station, 50% of trapped eels were glass eels in the 1960s, while glass eels are rarely seen today. Thus, the mean size of recruiting eels has probably increased over the years due to the delayed recruiting process. In Vester Vedsted brook on the Danish North Sea coast, glass eels, elvers and yellow eels are found, however pigmented glass eels are most common at the lower part of the brook and are considered as new recruits (Pedersen, 2002). More to the south of the European coast, in the Netherlands, new recruits are partly but never fully pigmented glass eels (Dekker, 2002), while in coastal Germany at recruitment monitoring sites both true glass eel and fully pigmented elvers are found (Kuhlmann *et al.*, 2002). In southwest Norway in the river Imsa, all sampled eels are fully pigmented elvers or young yellow eels that have stayed for one winter or more in marine or estuarine waters (Vøllestad, 2002). In Sweden, the catch in the River Viskan discharging to the North Sea consists mainly of elvers; however, at other rivers of the same coast age ranged from 0 to about 8 (Wickström, 2002). Overall, eels from rivers along the eastern coast of Sweden (Baltic Sea) are older than in rivers closer to the coast of Skagerrak/Kattegat sound (Wickström, 2002) i.e., the sound between the Baltic and North Seas. Hence, presumably eels to the southeastern Baltic should not be glass eels on arrival. Our observed arrival ages clearly support the hypothesis of eel recruitment to the southeastern Baltic at the yellow eel stage and explains why regional ichthyologists and managers were so uncertain about the contribution of natural recruitment, i.e., the absence of truly glass eels in the coastal waters led to hypotheses of natural recruitment weakness or even overall absence in the region.

Stocking programmes are a common and usually effective strategy to mitigate population decline, restore fisheries or to create new fisheries. The majority of stocked lakes in Latvia, Lithuania and Poland were almost devoid of eels before intensive stocking programmes began in the 1950s. The stocking programmes did create new eel fisheries in these inland lakes and also partially support the eel population in the Curonian Lagoon. However, many stocking programmes, including eel stocking, are carried out without evaluation of their effectiveness or actual success (Cowx, 1999). The use of otolith Sr:Ca ratios enabled this study to discriminate stocked eels from naturally recruited eels and to evaluate their contribution to the population. The understanding of population structure is fundamental for successful management and conservation.

**Stock discrimination by otolith Na:Ca, Mg:Ca, Mn:Ca and Ba:Ca ratio signatures**

Otolith elemental ratios, including Na:Ca, Mg:Ca, Mn:Ca and Ba:Ca were used aiming to distinguish between eels of the freshwater lagoon and lake, whose otoliths contain similar low Sr:Ca ratios. A total of 83% of the eels could be correctly assigned to their sampling locations. One-way ANOVA tests indicated that both Ba:Ca and Na:Ca ratios in eel otoliths were significantly higher for the eels in the lagoon than those of the lake, but otolith Mg:Ca and Mn:Ca ratios were similar for eels from the two environments. These findings indicate that chemical signatures in eel otoliths can distinguish not only between freshwater and seawater habitat use but also between different freshwater habitats.

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