Age and growth of European eel, *Anguilla Anguilla* (Linnaeus, 1758), in Estonian lakes

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Summary
To investigate the difference in growth rates of eels in the studied water bodies (Võrtsjärv, Ülemiste, Saadjärv, Vagula, Kaiavere and Kuremaa lakes, all in Estonia), 828 individual eel otoliths from the years 1999–2004 and 2008–2012 were examined for age determination. *Anguilla anguilla* captured in Lake Võrtsjärv mostly by professional fyke nets (mesh size >36 mm in the cod end) showed the best growth rate ($g_R = 6.9$ cm/year) and had a high Fulton condition factor ($K = 0.19$), indicating suitable growth conditions in the lake. However, samples caught using the same type of gear from the smaller lakes situated near Võrtsjärv, showed much slower $g_R$ ($3$ cm/year) and lower $K$ (0.17) growth rates. The slowest $g_R$ (2.6 cm/year) was observed for specimens caught in Lake Kuremaa. Stocking densities are much higher in the small lakes of the region and have a direct effect on the growth rates.

1 | INTRODUCTION

Aging of fish is principally determined by counting and verifying the growth zones (annual marks, *annuli*) in the hard parts of the fishes (Bagenal & Tesch, 1978). Different structures can be used, such as the scales, bones or otoliths. To determine the age of European eel, *Anguilla anguilla*, researchers use sagittal otoliths (Vøllestad, Lecomte-Finiger, & Steinmetz, 1988). Age determination for *anguillids* is usually based on observing the number of annual rings present on the otolith (Beentjes & Jellyman, 2015). Otoliths can characterize the entire life-cycle of the studied fish. Depending upon the environment (ICES, 2009) the formation of annual rings on the otolith may also differ, making it possible to draw parallels between different biotopes inhabited by the eel.

Age determination based on examination of otoliths is relatively new in Estonian fisheries science. Apart from Rohtha et al. (2014), Rohtha, Taal, Swirgsden, and Veteema (2015) with research on burbot (*Lota lota*) and ide (*Leuciscus idus*), respectively, there are no other recent scientific publications. A paper was published by Kangur (1998) regarding the growth rates of eels in Lake Võrtsjärv as a part of a larger study concerning this species. Unfortunately, the methodology used to measure the growth rates was not mentioned.

Eel landings have decreased rapidly throughout Europe since the 1960s (ICES, 2014). The same applies for the coastal waters of Estonia, where eel landings have declined by over 90% compared to the beginning of the 2000s (Bernotas et al., 2015). However, eel landings in freshwaters have remained steady for the past 5 years (averaging 15 tonnes/year; ICES, 2015), as these populations rely completely on stocking (ICES, 2014). Apart from Lake Ülemiste, all studied waterbodies (Figure 1) have annual stocking programmes (Table 1). According to the Estonian Eel Management Plan (EMP; Järvalt, 2008), Estonia is divided into two eel management units – West-Estonian River Basin District (RBD) and Narva RBD (Järvalt, 2008). Eels have been stocked in Lake Võrtsjärv since the 1950s and in other Narva RBD waterbodies since the beginning of 2000s (ICES, 2014). Both glass eels and evers have been stocked in these lakes. As the purpose of stocking eels in the Narva RBD water bodies is to secure the reproduction of the species (Järvalt, 2008), an understanding of age and growth of the eels in freshwater habitats is necessary. In this paper we present an analysis of the different growth parameters of *A. anguilla* in Estonian lakes and discuss the environmental and anthropogenic factors influencing these parameters.

2 | MATERIAL AND METHODS

2.1 | Study area

Our study area consisted of six lakes, of which five are situated in Narva RBD and one in the West-Estonian RBD (Figure 1; Table 2).
The eels inhabiting Lake Ülemiste were stocked in 1986 as glass eels and therefore of known ages – 17 and 18 years. These glass eels originating from France were originally destined for stocking in Lake Võrtsjärv, but due to logistical complications were released into the closest waterbody to the airport, in this case the Ülemiste. The exact amount of eels stocked in the Ülemiste is not known.

2.2 | Data

The 828 otoliths used in this study originate from the eel otolith collection of the Centre for Limnology of Estonian University of Life Sciences dating back to 1999 (Table 3). Based on the life stage at stocking (glass eel or elver), the otoliths collected from the Võrtsjärv eels were divided into two groups. The glass eel group (Võrtsjärv G) contains otoliths collected during the period 1999–2003. The elver group (Võrtsjärv E) includes otoliths from 2010 to 2012. All individuals were caught with professional fyke nets (mesh size >36 mm in the cod end). The eels were measured for total length (TL, cm) and total weight (TW, g), and the date of capture recorded. Fulton’s condition factor \( K \) (Fulton, 1904) was calculated for each analysed specimen. The annual gain in length was calculated according to the formula:

\[
g_R = \frac{(L - X)}{t}
\]

where \( g_R \) is the mean annual length increment in cm per year. \( L \) is the total length of the eel, \( X \) is the total length of the eel at stocking and \( t \) is the established otolith age (Svedäng, Neuman, & Wickström, 1996). \( X = 7 \) cm for glass eels and \( X = 15 \) cm for elvers.

Analysis of variance (ANOVA) and the Tukey HSD test were used to assess differences between observed variables (mean length and age of eels, Fulton condition factor and annual growth, coefficient of variation differences) in the different lakes; Pearson correlation coefficient was used to test the relationship between annual growth

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**TABLE 1** Average annual number of stocked eels (*Anguilla anguilla*) per hectar (period 1999–2012) in five studied Estonian lakes (excluding Ülemiste; ICES, 2015)

<table>
<thead>
<tr>
<th>Lake</th>
<th>Period</th>
<th>sp/ha/year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vortsjarv</td>
<td>1999–2012</td>
<td>12</td>
</tr>
<tr>
<td>Saadjarv</td>
<td>2000–2012</td>
<td>33</td>
</tr>
<tr>
<td>Kaiavere</td>
<td>2010–2012</td>
<td>58</td>
</tr>
<tr>
<td>Kuremaa</td>
<td>2001–2012</td>
<td>21</td>
</tr>
</tbody>
</table>

**TABLE 2** Characteristics of studied eutrophic water bodies in Estonia (Järvet, 2004; Mäemets, 1977; Tamre, 2006)

<table>
<thead>
<tr>
<th>Name</th>
<th>Catchment area (km²)</th>
<th>Surface area (km²)</th>
<th>Mean depth (m)</th>
<th>Max depth (m)</th>
<th>Stratified</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vortsjarv</td>
<td>3.104</td>
<td>270</td>
<td>2.8</td>
<td>6</td>
<td>No</td>
</tr>
<tr>
<td>Ülemiste</td>
<td>99.2</td>
<td>9.6</td>
<td>2.5</td>
<td>6</td>
<td>No</td>
</tr>
<tr>
<td>Saadjarv</td>
<td>31.9</td>
<td>7.2</td>
<td>8</td>
<td>25</td>
<td>Yes</td>
</tr>
<tr>
<td>Vagula</td>
<td>495</td>
<td>5.1</td>
<td>5.3</td>
<td>11.5</td>
<td>Yes</td>
</tr>
<tr>
<td>Kaiavere</td>
<td>92.2</td>
<td>2.5</td>
<td>2.5</td>
<td>4.5</td>
<td>No</td>
</tr>
<tr>
<td>Kuremaa</td>
<td>25.4</td>
<td>4.0</td>
<td>5.9</td>
<td>13.8</td>
<td>Yes</td>
</tr>
</tbody>
</table>

**TABLE 3** Number of *Anguilla anguilla* otoliths used for age determination in six studied Estonian lakes, including years when sampled

<table>
<thead>
<tr>
<th></th>
<th></th>
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<th></th>
<th></th>
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<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Vortsjarv</td>
<td>43</td>
<td>90</td>
<td>72</td>
<td>4</td>
<td>6</td>
<td>3</td>
<td>148</td>
<td>42</td>
<td>408</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ülemiste</td>
<td>11</td>
<td>12</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>23</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saadjarv</td>
<td>14</td>
<td>1</td>
<td>6</td>
<td>19</td>
<td>10</td>
<td>22</td>
<td>45</td>
<td>117</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vagula</td>
<td>7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>46</td>
<td>19</td>
<td>17</td>
<td>89</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kaiavere</td>
<td>61</td>
<td>18</td>
<td>6</td>
<td>12</td>
<td>15</td>
<td>25</td>
<td>106</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kuremaa</td>
<td>10</td>
<td>27</td>
<td>17</td>
<td>12</td>
<td>13</td>
<td>33</td>
<td>132</td>
<td>222</td>
<td>135</td>
<td>828</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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Statistical analyses were conducted using R 2.14.1 (R Core Team, 2014) and Statsoft STATISTICA 12. (http://www.statsoft.com/Products/STATISTICA/Product-Index).

3.2.3 Extraction and preparation of otoliths. Age determination

Sagittae otoliths were extracted, cleaned and stored in microtubes filled with glycerine. Slight improvements were made to the ‘burning and cracking’ method (Hu & Todd, 1981), whereby the otolith was fractured through the core. If the core was not revealed, additional polishing of the otolith was needed. Burning was done in the flame of a spirit lamp on the blade of a scalpel. After cooling, the otolith was fixed to a base filled with modelling paste.

and age. A t test was used for testing differences between different Coefficient of variation (CV) groups.

The Motic SMZ-143-FBGG stereomicroscope with a Moticam 2000 and Moticam 5 camera was used for viewing and photographing the otoliths. Motic Images Plus 2.0ML (http://www.motic.com/As_microscope_software_r/product_230.html) software was used for image post-processing.

Three to four different persons participated in the age reading process. A mean value was calculated using all age readings associated with the otolith. To measure the aging precision, the methodology described by Campana (2001) was used. Coefficient of variation (CV) was calculated using the formula:

\[
CV_b = \frac{100\% \times \sqrt{\frac{\sum_{a=1}^{R} (X_{ab} - X_{b})^2}{R - 1}}}{X_{b}}
\]

where CV<sub>b</sub> is the age precision estimate for the bth fish, X<sub>ab</sub> is the age determination of the bth fish and X<sub>b</sub> is the mean age estimate of the bth fish, and R is the number of times the age of each fish was determined. Campana (2001) also recommended using CV of 5% as an indicator of good aging precision.

3. RESULTS

The length distribution of eels (N = 828) was compared among the studied lakes (ANOVA F<sub>5,828</sub> = 100.6, p < .001). Specimens caught in Lake Ülemiste had a significantly higher mean TL (TL = 87.8 cm; N = 23) than eels in the other lakes (Tukey HSD, Q = 18; p < .01; Figure 2a). The shortest eels were found in the samples from lakes Kuremaa (TL = 61.6 cm; N = 106) and Võrtsjärv (TL = 63.1 cm; N = 408).

Comparing the calculated Fulton condition factors among the studied specimens revealed highest mean values for eels caught in lakes Võrtsjärv and Ülemiste (K = 0.19, N = 401; Figure 2b).

The mean determined age (t) of an eel (Figure 2c) did not differ significantly for specimens caught in lakes Saadjärv, Kuremaa, Kaiavere and Vagula (t = 21.2 years, N = 399, ANOVA F<sub>3,395</sub> = 0.9, p = .5). In Võrtsjärv G the mean age of captured individuals was t = 8.44 (N = 215).
years, but was 0.94 years younger ($t = 7.5, N = 193$) in the Võrtsjärv E group, with the difference statistically significant ($p = .03$). In Lake Ülemiste the known stocking-based age of the eels (17–18 years) was overestimated by 2–3 years (average $t = 20.2$).

The relationship between annual growth and age in our entire database ($N = 828$) showed a strong negative correlation ($r^2 = - .92, p < .001$, Figure 3). When annual growth in the studied lakes was compared, we did not find any statistical differences in Kaiavere, Saadjärv and Vagula (ANOVA $F_{2,294} = 2.2$, $p = .1$), Saadjärv ($N_{\text{reader}} = 3$; ANOVA $F_{2,294} = 1.4, p = .2$), and Vagula ($N_{\text{reader}} = 3$; ANOVA $F_{2,294} = 0.6, p = .5$). Age determination did not vary significantly among age estimators for Ülemiste ($N_{\text{reader}} = 3$; ANOVA $F_{2,66} = 2.2, p = .1$), Saadjärv ($N_{\text{reader}} = 3$; ANOVA $F_{2,294} = 1.4, p = .2$), and Vagula ($N_{\text{reader}} = 3$; ANOVA $F_{2,294} = 0.6, p = .5$).

### 4 | DISCUSSION

In Lake Võrtsjärv our estimations of the growth rate for *A. anguilla* that were stocked as glass eels (group Võrtsjärv G, period 1999–2003, mean $gR = 6.9$ cm/year) were higher than those observed by Kangur (1998) (5.9 cm/year). According to Sinha and Jones (1967) and Tesch (2003), population density can have a considerable effect on the eel growth rate: in more crowded populations the growth rate tends to be lower. In Võrtsjärv, the population density decreased from 35 sp/ha in 1965–2001 to 12 sp/ha in 2002–2010 (ICES, 2010). The legal size limit for commercial fishery of eel was lowered in 2002 from TL = 60 cm to TL = 55 cm. Allowing stronger fishing pressure and younger individuals to be caught by the fishermen could have caused the rise in the growth rate after this change. When compared to eels caught in small German lakes (Simon, 2007), our estimated growth rates (excluding Võrtsjärv) were exceptionally lower. Age of the studied individuals could play a crucial role here, since the growth rate decreases with age (Figure 3). The difference was most probably caused by the age difference of the studied specimens in the different lakes, as growth of the older individuals is assumed to be slower (Simon, 2007). The maximum age class of eels examined by Simon (2007) was 14 years. The mean estimated age for the individuals studied in the small lakes of Narva RBD exceeded 20 years, while the mean determined age for individuals caught in Lake Võrtsjärv was 8 years. Thus, it is not surprising that the estimated mean growth rate of *A. anguilla* was highest in Lake Võrtsjärv and lowest in the small lakes of Narva RBD.

### TABLE 4 Number of age readers and samples of *Anguilla anguilla* analysed in six studied Estonian lakes

<table>
<thead>
<tr>
<th>Water body</th>
<th>$N_{\text{reader}}$</th>
<th>$N_{\text{samples}}$</th>
<th>CV mean (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ulemiste</td>
<td>3</td>
<td>23</td>
<td>3</td>
</tr>
<tr>
<td>Vortsjärv</td>
<td>4</td>
<td>408</td>
<td>4</td>
</tr>
<tr>
<td>Kuremaa</td>
<td>3</td>
<td>106</td>
<td>4</td>
</tr>
<tr>
<td>Saadjärv</td>
<td>3</td>
<td>117</td>
<td>7</td>
</tr>
<tr>
<td>Kaiavere</td>
<td>2</td>
<td>85</td>
<td>7</td>
</tr>
<tr>
<td>Vagula</td>
<td>3</td>
<td>89</td>
<td>8.5</td>
</tr>
</tbody>
</table>

Mean coefficient of variation (CV) of the average percent error describes the precision of age determination among the age readers.
The estimated growth rate of eels in Lake Võrtsjärv was also higher than the mean values documented in Norway (6.2 cm/year; Vellestad & Jonsson, 1986), Poland (4.1 cm/year; Nagiec & Bahnsway, 1990), Denmark (3.6 cm/year; Bisaard & Pedersen, 1991), France (5.3 cm/year; Panfilii, Ximenes, & Crivelli, 1994) Ireland (1.5–6.2 cm/year; Arai, Kotake, & McCarthy, 2006), and Germany (4.5 cm/year; Simon, 2007).

Previous studies by Simon and Dörner (2013), and Simon, Dörner, Scott, Schreckenbach, and Knösche (2013) showed that A. anguilla stocked into lakes as elvers (TW = 6.6 g at stocking) had some difficulties adapting to natural prey in the first years, reflected by lower growth rates when compared to the group stocked as glass eels. However, after the third or fourth year post-stocking, the growth rates between the two different groups equalized. Our results showed that the condition factors of the specimen in the Võrtsjärv and Kaiavere lakes as well as in Lake Ülemiste were very close (0.19 ± 0.002).

From May to September, the water temperature usually stays above 10°C in Lake Võrtsjärv (Järvet, 2004). Eels feed actively during this period (Sinha & Jones, 1967). Due to the shallowness and large surface area, the waters in lakes Võrtsjärv and Ülemiste are well mixed and aerated in the ice-free period. Nevertheless, wind-free and hot periods in the summer can cause hypoxia in the bottom layer of both lakes (Järvet, 2004; Ott, 2012). Hypoxic conditions can relate to increasing levels of stress in fish. Tzeng, Wu, and Wickström (1994) found that false annuli formation on eel otoliths is related to short-term stress. This condition could also explain the false annuli observed on eel otoliths in the Võrtsjärv and Kaiavere lakes as well as in Lake Ülemiste (Figure 5). In the Ülemiste, cyanobacterial blooms have been common in past summer decades (Pedusaar, 2010). Harmful effects of cyanobacterial blooms on fishes are widely acknowledged (Havens, 2008). Eels have a pH optimum of 7–8 (Tesch, 2003), but during cyanobacterial blooms the water pH can rise above 10 (Gao, Cornwell, Stoecker, & Owens, 2012) and remain at that level for weeks, which could add to the stress caused by the low oxygen content and form false annuli on an otolith.

In Lake Kaiavere, in addition to a fluctuating oxygen content and high water pH (mean value = 8.6 in 2011; Ott, 2011) in the summer months, an unidentified bacterial infection has been observed on eels. This disease has also been found on specimens collected from lakes Kuremaa, Saadjärv and Vagula. It can be assumed that the infection occurs when the immune system of the eel is weakened. Changes in water temperature, population density and stress all influence the immune system parameters (Magnadottir, 2006; Uribe, Folch, Enriquez, & Moran, 2011). The rise in the level of stress potentially contributes to the formation of false annuli on otoliths, thus causing an overestimation of the eel age.

The age determination variation (CV) values were above the recommended 5% for most of the studied small lakes. The combination of false annuli and high age of the fish could lead to some aging errors. However, analysing the age reading data showed that the age determination CV values of samples from Ülemiste, Saadjärv and Vagula did not differ significantly among the age readers. No variation between the age readers in samples considering Lake Ülemiste was expected since the sample size was low (N = 23) and the known eel age was 17–18 years. In the studied small lakes a different method of age verification is needed to confirm the relatively high ages (t = 21 years) determined. As such, mass marking was already carried out in both Võrtsjärv and the small lakes of Narva RBD in 2014–2015 (Silm, Järvalt, Mäe, & Bernotas, 2015) and 2016 (M. Siml, unpublished data), and the growth rates of the eel can be corrected as soon as the first marked eels begin to appear in official catches, anticipated to be in the year 2019.

The difference in the condition factor (K) values shows that between the studied lakes, eel growth conditions are most suitable in lakes Võrtsjärv and Ülemiste. These lakes share similar characteristics when it comes to feeding objects and water quality (Järvet, 2004; Ott, 2012; Pedusaar, 2010). However, the length variation was greater among the specimens from Ülemiste (Figure 2a). As the age of eels in Ülemiste is known from the stocking history (17–18 years) with little variation, this shows that the growth rate can vary significantly among specimens stocked in the same year.

5 | CONCLUSION

Võrtsjärv has very good growth conditions for eels, considering the observed mean K-value (0.19) and mean growth rate of glass eels (6.9 cm/year) and elvers (6.5 cm/year). Our study showed that the eels in the small lakes of Narva RBD grew much more slowly (mean growth rate 2.9 cm/year), but in these lakes age overestimation due to false annuli on the otoliths might have occurred. A possible solution for the problem would be mass marking of all individuals before stocking into these lakes. Such mass marking was already carried out in both Võrtsjärv and the small lakes of Narva RBD in 2014–2016, and the eel growth rates can be corrected as soon as the first marked eels begin to appear in official catches, anticipated in the year 2019.

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