Evaluation of otolith shape variability in hatchery-reared brook trout (*Salvelinus fontinalis*), Black Sea trout (*Salmo trutta labrax*) and their hybrid

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Abstract

In aquaculture, the process of hybridization is used to produce animals that have better growth rates, meat quality, and higher resistance and tolerance to environmental variations than their parental species. The identification of a hybrid fish may require a DNA-based technology that is an expensive process. In this study, the sagittal otolith shape of hatchery-reared brook trout (*Salvelinus fontinalis*), Black Sea trout (*Salmo trutta labrax*) and their hybrid (*S. fontinalis ♂ × S. t. labrax ♀*) were studied and compared to elucidate the variation between their morphometric values. The otoliths were measured by image analysis and used to calculate shape descriptors: form-factor, roundness, and aspect ratio. Based on the morphometric measurements, the hybrid fish were not statistically intermediate between the parents and share most of the similarities with the female parent. The relationships between fish size and otolith size were best described by the exponential function ($r^2=0.90$).

Keywords: Morphology, Otolith, Salmonidae, Shape descriptors

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Introduction
Otoliths are used to establish the age of fish on both daily and yearly scales (Campana and Thorrold 2001). Also, estimating fish size from the size of its otolith has been established for several fish species (Echeverria 1987; Martin-Smith, 1996; Granadeiro and Silva, 2000; Waessle et al., 2003; Bilge, 2013; Jawad et al., 2017). In a majority of fish species, the relationship between fish size and otolith size are described by either linear (Mugiya and Tanaka, 1992; Harvey et al., 2000; Waessle et al., 2003) or by curvilinear regression (West and Larkin, 1987; Lombarte and Lleonart, 1993; Sadighzadeh et al., 2014) depending upon the growth pattern of the fish species.

Otolith structure has been a useful tool to distinguish between wild and hatchery-reared populations of the same fish species (Hindar and L’abée-Lund, 1992; Hendricks et al., 1994; Zhang et al., 1995; Barnett-Johnson et al., 2007). Their shape is mainly controlled genetically, however, environmental factors such as feeding, water quality, and photoperiod have been assumed to influence their shape as well (Neilson and Geen, 1982; Campana and Neilson, 1985; Cardinale and Arrhenius, 2004; Fey and Hare, 2012; Rebaya et al., 2017). Hence, the chemical composition of otoliths has been used to reconstruct the life history of fishes (Kalish 1989).

In this study, the morphometry of otoliths (in this paper, otolith refers to the sagittal otolith) and the relationships between otolith size and fish size of hatchery-reared brook trout (Salvelinus fontinalis), Black Sea trout (Salmo trutta labrax) and their hybrid were studied. They were compared in order to determine the similarities in otolith shape between the hybrid and parents.

Materials and methods
Fishes were acquired from the KTÜ Sürmene Faculty of Marine Sciences, Department of Fisheries Technology Engineering during 2013. Prior to extracting the otolith, the individual fish were weighed (to the nearest 0.01 g) and their total lengths (TL, to the nearest mm) were measured. The extracted otoliths (right) were then washed with fresh water to remove any soft tissue and were stored in plastic tubes (labelled with fish information). The digitized otolith images were produced using a camera coupled to a MZ75 LEICA binocular microscope (Fig. 1). A digitized image of the otolith was then analysed by image analysis software (LAS V3.3) to outline the otolith morphological parameters: otolith length (OL, mm), otolith height (OH, mm), area (mm$^2$), perimeter (mm), and otolith weight (mg). These values were then used to calculate three-dimensional shape descriptors according to Tuset et al. (2003):

The irregularity of surface area was compensated by means Form-factor (FF) as:

$$FF = \frac{4\pi \times \text{Area}}{\text{Perimeter}^2}$$

(1)

The Roundness (RD) was estimated to give detailed information on the similarity of various features with regard to a perfect circle:
The proportional relationship between the otolith length-height:

\[
(AR)\text{ Aspect Ratio } = \frac{\text{Length}}{\text{Height}}
\]

(3)

Figure 1: The measurements of otolith morphometric values

The relationship between fish and otolith sizes were described by simple power and exponential functions. The best fit model was determined using the coefficient of determination \((r^2)\) and the one that provided a higher \(r^2\) value was selected as the best fit model to the data.

The ANOSIM and SIMPER tests (based on Bray–Curtis method) were used to determine the similarity between the otolith morphologies of all three fishes. The StatSoft 5.5 and Past 3.14 (Hammer et al., 2001.) were used for statistical analysis.

Results

A total of 144 otoliths were selected to elucidate variations in the otolith morphometry of *S. fontinalis*, *Salmo trutta labrax* and their hybrid. The length, weight and height of *S. fontinalis* otoliths were relatively smaller than *S. t. labrax* and their hybrid (Table 1). The simple power model adequately described the correlation between otolith length and its weight, which showed that the increment in otolith size occurred with a negative allometric pattern (Table 1), while the relationship between fish and otolith sizes were better described by the exponential model than a simple power model (Table 2). Based on the results of the exponential model, hybrid fish had higher values of slope indicating a relatively faster increment in otolith size than their parental species. The mean (±S.E.) values of shape descriptors are given in Table 3. Most of the shape descriptor values were slightly, but not significantly, higher for hybrid fish and *S. t. labrax*.

The results of ANOSIM and SIMPER tests revealed a high percentage of similarity (>94%) between *S. t. labrax* and the hybrid fish. *Salvelinus fontinalis* otolith differed from them with >17% dissimilarity (Table 4). Moreover, SIMPER analyses showed that otolith weight contributed the most to the differences among the morphometric measurements. The results showed that the hybrid fish were not statistically intermediate between the *S. fontinalis* and *S. t. labrax*. The hybrid fish share most of their similarities with the mother (*S. t. labrax*).
Table 1: Fish and otolith sizes along with simple power relationship \((y = ax^b)\) between otolith length \((OL)\) and weight \((OW)\) of hatchery-reared brook trout \((Salvelinus fontinalis)\), Black Sea trout \((Salmo trutta labrax)\) and their hybrid \((S. fontinalis × S. t. labrax)\). Mean and standard deviation are given in parentheses.

<table>
<thead>
<tr>
<th>Fishes</th>
<th>Weight ((g))</th>
<th>Length ((mm))</th>
<th>Weight ((mg))</th>
<th>Height ((mm))</th>
<th>Relationship (b/w)</th>
<th>Regression parameters</th>
<th>Correlation coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(L_T)</td>
<td>(W_T)</td>
<td>(OL)</td>
<td>(L_T)</td>
<td></td>
<td>(a) (\pm) (SD)</td>
<td>(r^2)</td>
</tr>
<tr>
<td>Brook trout</td>
<td>20.1–28.2</td>
<td>82.1–265.3</td>
<td>2.4–4.3</td>
<td>1.8–5.3</td>
<td>1.8–2.9</td>
<td>(OL \times OW)</td>
<td>0.912 (\pm) 0.032</td>
</tr>
<tr>
<td></td>
<td>(24.2 (\pm) 0.1)</td>
<td>(174.6 (\pm) 0.3)</td>
<td>(3.5 (\pm) 0.1)</td>
<td>(2.5 (\pm) 0.1)</td>
<td>(2.4 (\pm) 0.1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Black Sea trout</td>
<td>21.9–29.6</td>
<td>128.3–250.8</td>
<td>3.6–4.5</td>
<td>3.9–7.0</td>
<td>2.3–2.9</td>
<td>(OL \times OW)</td>
<td>0.248 (\pm) 0.028</td>
</tr>
<tr>
<td></td>
<td>(25.9 (\pm) 0.1)</td>
<td>(194.6 (\pm) 0.2)</td>
<td>(4.0 (\pm) 0.1)</td>
<td>(5.2 (\pm) 0.1)</td>
<td>(2.5 (\pm) 0.1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hybrid</td>
<td>19.4–32.8</td>
<td>88.9–432.8</td>
<td>3.6–4.9</td>
<td>3.8–7.4</td>
<td>2.1–2.9</td>
<td>(OL \times OW)</td>
<td>0.693 (\pm) 0.055</td>
</tr>
<tr>
<td></td>
<td>(27.9 (\pm) 0.1)</td>
<td>(244.4 (\pm) 0.3)</td>
<td>(4.0 (\pm) 0.1)</td>
<td>(5.4 (\pm) 0.2)</td>
<td>(2.5 (\pm) 0.1)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(L_T\), total length of fish; \(W_T\), minimum and maximum values observed; \(n\), sample size; \(b/w\), between.

Table 2: The relationship between the morphometric measurements of hatchery-reared brook trout \((Salvelinus fontinalis)\), Black Sea trout \((Salmo trutta labrax)\) and their hybrid \((S. fontinalis \times S. t. labrax)\). Simple power \((y = ax^b)\) and exponential functions were used to define the relationship \((\text{estimate} \pm \text{SD})\).

<table>
<thead>
<tr>
<th>Fishes</th>
<th>Relationship between</th>
<th>Simple power function</th>
<th>Exponential function</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(OL \times W_T)</td>
<td>(a \pm b \pm \text{SE})</td>
<td>(L_T) (= ) (\text{estimate} \pm \text{SD})</td>
</tr>
<tr>
<td>Brook trout</td>
<td>(OH \times L_T)</td>
<td>10.929 (\pm) 0.381</td>
<td>0.886 (\pm) 0.038</td>
</tr>
<tr>
<td>Black Sea trout</td>
<td>(OL \times W_T)</td>
<td>6.518 (\pm) 1.593</td>
<td>2.441 (\pm) 0.174</td>
</tr>
<tr>
<td></td>
<td>(OH \times L_T)</td>
<td>12.112 (\pm) 0.328</td>
<td>0.464 (\pm) 0.016</td>
</tr>
<tr>
<td>Hybrid</td>
<td>(OL \times W_T)</td>
<td>3.927 (\pm) 0.920</td>
<td>2.948 (\pm) 0.163</td>
</tr>
<tr>
<td></td>
<td>(OH \times L_T)</td>
<td>10.795 (\pm) 0.485</td>
<td>0.563 (\pm) 0.026</td>
</tr>
</tbody>
</table>

\(L_T\), fish total length; \(W_T\), total fish weight; \(OH\), otolith height.

Table 3: Mean \((\pm \text{SE})\) and minimum–maximum values of the shape descriptors for hatchery-reared brook trout \((Salvelinus fontinalis)\), Black Sea trout \((Salmo trutta labrax)\) and their hybrid \((S. fontinalis \times S. t. labrax)\).

<table>
<thead>
<tr>
<th>Shape descriptors</th>
<th>Brook trout</th>
<th>Black Sea trout</th>
<th>Hybrid</th>
</tr>
</thead>
<tbody>
<tr>
<td>(A)</td>
<td>2.64–7.30</td>
<td>4.92 (\pm) 0.91</td>
<td>4.34–9.81</td>
</tr>
<tr>
<td>(P)</td>
<td>7.01–13.25</td>
<td>9.86 (\pm) 1.31</td>
<td>8.93–14.38</td>
</tr>
<tr>
<td>(FF)</td>
<td>0.38–0.76</td>
<td>0.64 (\pm) 0.07</td>
<td>0.39–0.73</td>
</tr>
<tr>
<td>(RD)</td>
<td>1.24–2.44</td>
<td>1.49 (\pm) 0.19</td>
<td>1.29–2.44</td>
</tr>
<tr>
<td>(AR)</td>
<td>1.26–1.74</td>
<td>1.51 (\pm) 0.11</td>
<td>1.26–2.05</td>
</tr>
</tbody>
</table>

\(A\), area; \(P\), perimeter; \(FF\), form factor; \(RD\), roundness; \(AR\), aspect ratio.
Table 4: The average dissimilarities between the otolith morphology of hatchery-reared brook trout (Salvelinus fontinalis), Black Sea trout (Salmo trutta labrax) and their hybrid (S. fontinalis ♂ × S. t. labrax ♀). The ANOSIM and SIMPER tests are based on Bray–Curtis method.

<table>
<thead>
<tr>
<th></th>
<th>F(1, 1)</th>
<th>p value</th>
<th>Average Dissimilarity</th>
<th>Discriminating structure</th>
<th>Contribution (%)</th>
<th>Discriminating structure</th>
<th>Contribution (%)</th>
<th>Discriminating structure</th>
<th>Contribution (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brook trout – Black Sea trout</td>
<td>0.92</td>
<td>0.0001</td>
<td>17.46</td>
<td>OW</td>
<td>76.4</td>
<td>OL</td>
<td>16.4</td>
<td>OH</td>
<td>6.8</td>
</tr>
<tr>
<td>Brook trout – Hybrid</td>
<td>0.90</td>
<td>0.0001</td>
<td>18.64</td>
<td>OW</td>
<td>76.7</td>
<td>OL</td>
<td>16.5</td>
<td>OH</td>
<td>6.8</td>
</tr>
<tr>
<td>Black trout – Hybrid</td>
<td>0.00</td>
<td>0.470</td>
<td>06.00</td>
<td>OW</td>
<td>62.2</td>
<td>OL</td>
<td>23.6</td>
<td>OH</td>
<td>14.2</td>
</tr>
</tbody>
</table>

OW, otolith weight; OL, otolith length; OH, otolith height.

Discussion

The relationship between fish and otolith size (OL×W and OH×L) was described in accordance to most of the previously performed studies such as Battaglia et al. (2010, 2015) Harvey et al. (2000), Waessle et al. (2003), Giménez et al. (2016) and Aneesh Kumar et al. (2017).

In all three fishes, the shape descriptors did not show any significant differences which may be due to the fact that all the fishes were stocked under similar conditions and fed with the same commercial pellets for two years. Parmentier et al. (2001) found that different fish species occupying the same ecological niche show resemblances in otolith shape. For S. fontinalis, Morat et al. (2008) and Lombarte et al. (2006) reported smaller values of FF and RD than the present study. Their studies included S. fontinalis caught from the wild whereas the present study had hatchery-reared S. fontinalis fed on formulated diets. It can further be confirmed that Barnett-Johnson et al. (2007) carried out an otolith morphometric study on hatchery-reared and wild Chinook salmon Oncorhynchus tshawytscha and reported smaller morphometric values for wild Chinook salmon than hatchery-reared. The differences in the otolith of farmed and wild fish are mainly due to the environments they experience (Reimer et al., 2016). According to Glover et al. (2017) farmed Atlantic salmon (Salmo salar L.) have displayed a range of genetic differences to wild conspecifics. This is because farmed fish grow at a relatively faster rate than wild population causing abnormal vaterite formation in the farmed fish otoliths (Reimer et al., 2017).

In conclusion, this study was the first approach to elaborate the otolith shape of hatchery-reared S. fontinalis and S. t. labrax including their hybrid offspring. The data obtained from otolith shapes of S. fontinalis and S. t. labrax should provide a useful tool in predicting fish size using their otoliths. Furthermore, the results of this study will assess the identification of hatchery-reared S. fontinalis and S. t. labrax.

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