

## **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 Leonart, 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<sup>2</sup>), 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:

$$FF = 4\pi * Area \div Perimeter^2$$

(1)

The Roundness (*RD*) was estimated to give detailed information on the similarity of various features with regard to a perfect circle:

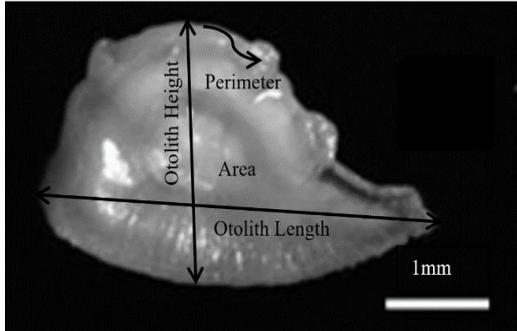
$$RD = 4 * Area \div \pi Max R^2$$

(2)

The proportional relationship between the otolith length-height:

$$(AR) Aspect Ratio = Length \div Height$$

(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 ( $\pm$ 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.**

Fishes	n	Fish size		Otolith size			Relationship b/w	Regression parameters (estimate ± SD)		Correlation coefficient $r^2$
		$L_T$ (cm)	Weight (g)	Length (mm)	Weight (mg)	Height (mm)		a	b	
		Min–Max	Min–Max	Min–Max	Min–Max	Min–Max				
Brook trout	49	20.1–28.2 (24.2 ± 0.1)	82.1–265.3 (174.6 ± 0.3)	2.4–4.3 (3.5 ± 0.1)	1.8–5.3 (2.5 ± 0.1)	1.8–2.9 (2.4 ± 0.1)	OL × OW	0.912 ± 0.032	0.670 ± 0.028	0.928
Black Sea trout	46	21.9–29.6 (25.9 ± 0.1)	128.5–250.8 (194.6 ± 0.2)	3.6–4.5 (4.0 ± 0.1)	3.9–7.0 (5.2 ± 0.1)	2.3–2.9 (2.5 ± 0.1)	OL × OW	0.248 ± 0.028	2.182 ± 0.080	0.945
Hybrid	49	19.4–32.8 (27.9 ± 0.1)	88.9–432.8 (244.4 ± 0.3)	3.0–4.9 (4.0 ± 0.1)	3.8–7.4 (5.4 ± 0.2)	2.1–2.9 (2.5 ± 0.1)	OL × OW	0.493 ± 0.055	1.712 ± 0.079	0.912

$L_T$ , total length of fish; Min–Max, 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* ♂ × *S. t. labrax* ♀). Simple power ( $y = ax^b$ ) and exponential functions were used to define the relationship (estimate ± SD).**

Fishes	Relationship between	Simple power function			Exponential function	$r^2$
		a	b	$r^2$		
Brook trout	OL × $W_T$	6.769 ± 0.613	2.578 ± 0.069	0.972	$W_T = 13.209e^{0.727OL}$	0.959
	OH × $L_T$	10.929 ± 0.381	0.886 ± 0.038	0.924	$L_T = 10.177e^{0.355 \times OH}$	0.929
Black Sea trout	OL × $W_T$	6.518 ± 1.593	2.441 ± 0.174	0.824	$W_T = 13.660e^{0.659OL}$	0.914
	OH × $L_T$	12.112 ± 0.328	0.464 ± 0.016	0.948	$L_T = 9.748e^{0.388 \times OH}$	0.953
Hybrid	OL × $W_T$	3.927 ± 0.920	2.948 ± 0.163	0.884	$W_T = 6.900e^{0.872OL}$	0.902
	OH × $L_T$	10.795 ± 0.485	0.563 ± 0.026	0.909	$L_T = 7.832e^{0.502 \times OH}$	0.918

$L_T$ , fish total length;  $W_T$ , total fish weight; OH, otolith height.

**Table 3: Mean (± 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* ♂ × *S. t. labrax* ♀).**

Shape descriptors	Brook trout		Black Sea trout		Hybrid		One-Way ANOVA	
	Min–Max	Mean ± SE	Min–Max	Mean ± SE	Min–Max	Mean ± SE	$F_{2,147}$	P
A	2.64–7.30	4.92 ± 0.91	4.34–9.81	6.23 ± 0.84	4.60–8.03	6.50 ± 0.841	0.955	0.387
P	7.01–13.25	9.86 ± 1.31	8.93–14.38	11.27 ± 1.21	9.03–14.12	11.31 ± 1.000	0.490	0.614
FF	0.38–0.76	0.64 ± 0.07	0.39–0.73	0.62 ± 0.07	0.50–0.72	0.64 ± 0.047	0.033	0.967
RD	1.24–2.44	1.49 ± 0.19	1.29–2.44	1.53 ± 0.22	1.30–1.87	1.48 ± 0.116	0.022	0.979
AR	1.26–1.74	1.51 ± 0.11	1.26–2.05	1.67 ± 0.15	1.52–2.03	1.69 ± 0.096	0.699	0.499

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.**

Fishes	One-way ANOSIM			SIMPER					
	R value	p value	Average Dissimilarity %	Discriminating structure I	Contribution (%)	Discriminating structure II	Contribution (%)	Discriminating structure III	Contribution (%)
Brook trout – Black Sea trout	0.92	0.0001	17.46	OW	76.4	OL	16.4	OH	6.8
Brook trout – Hybrid	0.90	0.0001	18.64	OW	76.7	OL	16.5	OH	6.8
Black trout – Hybrid	0.00	0.470	06.00	OW	62.2	OL	23.6	OH	14.2

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

## Discussion

The relationship between fish and otolith size ( $OL \times W_T$  and  $OH \times L_T$ ) 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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