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

Bascinar N.S.[1*]

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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

1-Central Fisheries Research Institute, Vali Adil Yazar Ave. No:14 Kasustu, Yomra, 61250, Trabzon, Turkey
*Corresponding author's Email: seldabascinar@gmail.com

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:

$$FF = 4\pi * Area \div Perimeter^2 \qquad (1)$$

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

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

The proportional relationship between the otolith length-height:

$$(AR) Aspect\ Ratio = Length \div Height \qquad (3)$$

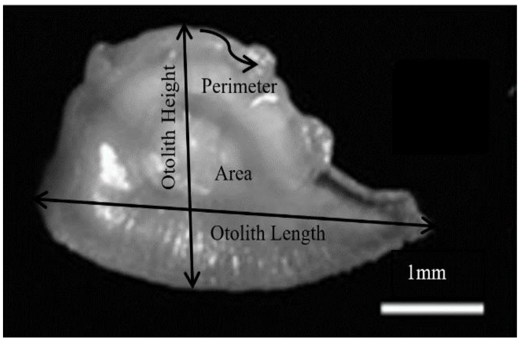


Figure 1: The measurements of otolith morphometric values

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.

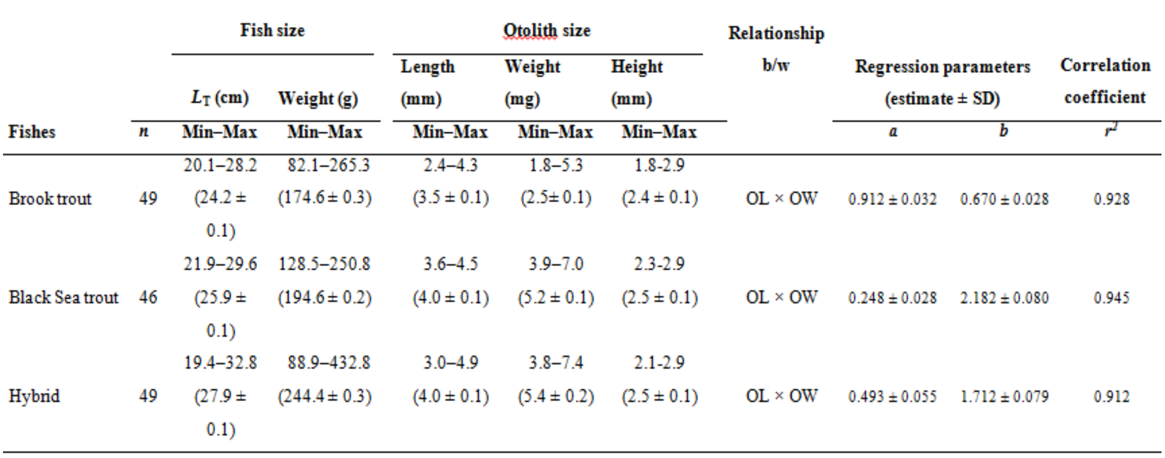
The ANOSIM and SIMPER tests (based on Bray–Curtis method) were used to determine the similarity between the otolith morphologies of all three fishes. The StatSoft 5.5 and Past 3.14 (Hammer *et al.*, 2001.) were used for statistical analysis.

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.



		Fish size		Otolith size			Relationship			
		L_T (cm)	Weight (g)	Length (mm)	Weight (mg)	Height (mm)	b/w	Regression parameters (estimate ± SD)		Correlation coefficient
Fishes	*n*	Min–Max	Min–Max	Min–Max	Min–Max	Min–Max		*a*	*b*	r^2
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	
		a	*b*	r^2		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.

	One-way ANOSIM		SIMPER						
Fishes	*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×W_T and OH×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*.

References

Aneesh Kumar K., Deepa K., Hashim M., Vasu C. and Sudhakar M., 2017. Relationships between fish size and otolith size of four bathydemersal fish species from the south eastern Arabian Sea, India.

Journal of Applied Ichthyology, 33(**1**), 102-107. DOI: 10.1111/jai.13250

Barnett-Johnson, R., Grimes, C.B., Royer, C.F. and Donohoe, C.J., 2007. Identifying the contribution of wild and hatchery Chinook salmon (*Oncorhynchus tshawytscha*) to the ocean fishery using otolith microstructure as natural tags. *Canadian Journal of Fisheries and Aquatic Sciences*, 64(**12**), 1683-1692. DOI: 10.1139/F07-129

Battaglia, P., Malara, D., Romeo, T. and Andaloro, F., 2010. Relationships between otolith size and fish size in some mesopelagic and bathypelagic species from the Mediterranean Sea (Strait of Messina, Italy). *Scientia Marina*, 74(**3**), 605-612. DOI: 10.3989/scimar.2010.74n3605

Battaglia, P., Malara, D., Ammendolia, G., Romeo, T. and Andaloro, F., 2015. Relationships between otolith size and fish length in some mesopelagic teleosts (Myctophidae, Paralepididae, Phosichthyidae and Stomiidae). *Journal of Fish Biology*, 87(**3**), 774-782. DOI: 10.1111/jfb.12744

Bilge, G., 2013. Otolith size-fish size relations in the jewel lanternfish *Lampanyctus crocodilus* (Actinopterygii: Myctophiformes: Myctophidae), from deep water environment of the Southern Aegean Sea. *Acta Ichthyologica et Piscatoria*, 43(**4**), 293-296. DOI: 10.3750/AIP2013.43.4.05

Campana, S.E. and Neilson, J.D., 1985. Microstructure of fish otoliths. *Canadian Journal of Fisheries and Aquatic Sciences*, 42(**5**), 1014-1032.

Campana, S.E. and Thorrold, S.R., 2001. Otoliths, increments, and elements: keys to a comprehensive understanding of fish populations? *Canadian Journal of Fisheries and Aquatic Sciences*, 58(**1**), 30-38. DOI: 10.1139/f85-127

Cardinale, M. and Arrhenius, F., 2004. Using otolith weight to estimate the age of haddock (*Melanogrammus aeglefinus*): a tree model application. *Journal of Applied Ichthyology*, 20(**6**), 470-475. DOI: 10.1111/j.1439-0426.2004.00576.x

Echeverria, W., 1987. Relationship of otolith length to total length in rockfishes from northern and central California. *Fishery Bulletin*, 85(**2**), 383-387.

Fey, D.P. and Hare, J.A., 2012. Temperature and somatic growth effects on otolith growth of larval Atlantic Menhaden, *Brevoortia tyrannus* (Actinopterygii: Clupeiformes: Clupeidae). *Acta Ichthyologica et Piscatoria*, 42(**3**), 215-222. DOI: 10.3750/AIP2011.42.3.05

Giménez, J., Manjabacas, A., Tuset, V.M. and Lombarte, A., 2016. Relationships between otolith and fish size from Mediterranean and north-eastern Atlantic species to be used in predator–prey studies. *Journal of Fish Biology*, 89(**4**), 2195-2202. DOI: 10.1111/jfb.13115

Glover, K.A., Solberg, M.F., McGinnity, P., Hindar, K., Verspoor, E., Coulson, M.W.,

Hansen, M.M., Araki, H., Skaala, Ø. and Svåsand, T., 2017. Half a century of genetic interaction between farmed and wild Atlantic salmon: Status of knowledge and unanswered questions. *Fish and Fisheries*, 18(**5**), 890-927.

Granadeiro, J.P. and Silva, M.A., 2000. The use of otoliths and vertebrae in the identification and size-estimation of fish in predator-prey studies. *Cybium*, 24 (**4**), 383-393.

Hammer Ø., Harper D. and Ryan P. 2001. Paleontological statistics software: Package for education and data analysis. *Palaeontologia Electronica*, 4, 9.

Harvey, J.T., Loughlin, T.R., Perez, M.A. and Oxman, D.S., 2000. Relationship between fish size and otolith length for 63 species of fishes from the eastern North Pacific Ocean. NOAA Technical Report NMFS. 150, 35.

Hendricks, M.L., Torsello, D.L. and Backman, T.W., 1994. Use of otolith microstructure to distinguish wild from hatchery-reared American shad in the Susquehanna River. *North American Journal of Fisheries Management*, 14(**1**), 151-161. DOI: 10.1577/1548-8675(1994)014<0151:UOOMTD>2.3.CO;2

Hindar, K. and L'ABÉE-LUND, J., 1992. Identification of hatchery-reared and naturally produced Atlantic salmon, *Salmo salar* L., juveniles based on examination of otoliths. *Aquaculture Research*, 23(**2**), 235-241. DOI: 10.1111/j.1365-2109.1992.tb00614.x

Jawad, L.A., Park, J.M., Kwak, S.N. and Ligas, A., 2017. Study of the relationship between fish size and otolith size in four demersal species from the south-eastern Yellow Sea. *Cahiers De Biologie Marine*, 58(**1**), 9-15. DOI: 10.21411/CBM.A.645C2013

Kalish, J.M., 1989. Otolith microchemistry: validation of the effects of physiology, age and environment on otolith composition. *Journal of Experimental Marine Biology and Ecology*, 132(**3**), 151-178. DOI: 10.1016/0022-0981(89)90126-3

Lombarte, A. and Lleonart, J., 1993. Otolith size changes related with body growth, habitat depth and temperature. *Environmental Biology of Fishes*, 37, 297–306. DOI: 10.1007/BF00004637

Lombarte, A., Chic, Ò., Parisi-Baradad, V., Olivella, R., Piera, J. and García-Ladona, E., 2006. A web-based environment for shape analysis of fish otoliths. The AFORO database. *Scientia Marina*, 70(**1**), 147-152.

Martin-Smith, K., 1996. Length/weight relationships of fishes in a diverse tropical freshwater community, Sabah, Malaysia. *Journal of Fish Biology*, 49(**4**), 731-734. DOI: 10.1006/jfbi.1996.0201

Morat, F., Betoulle, S., Robert, M., Thailly, A., Biagianti-Risbourg, S. and Lecomte-Finiger, R., 2008. What can otolith examination tell us about the level of perturbations of

Salmonid fish from the Kerguelen Islands? *Ecology of Freshwater Fish*, 17(**4**), 617-627. DOI: 10.1111/j.1600-0633.2008.00313.x

Mugiya, Y. and Tanaka, S., 1992. Otolith development, increment formation, and an uncoupling of otolith to somatic growth rates in larval and juvenile goldfish. *Nippon Suisan Gakkai*, 58(**5**), 845-851.

Neilson, J.D. and Geen, G.H., 1982. Otoliths of Chinook salmon (*Oncorhynchus tshawytscha*): daily growth increments and factors influencing their production. *Canadian Journal of Fisheries and Aquatic Sciences*, 39(**10**), 1340-1347. DOI: 10.1139/f82-180

Parmentier, E., Vandewalle, P. and Lagardère, F., 2001. Morpho-anatomy of the otic region in carapid fishes: Eco-morphological study of their otoliths. *Journal of Fish Biology*, 58(**4**), 1046-1061. DOI: 10.1111/j.1095-8649.2001.tb00554.x

Rebaya, M., Ben Faleh, A.R., Allaya, H., Khedher, M., Trojette, M., Marsaoui, B., Fatnassi, M., Chalh, A., Quignard, J.P. and Trabelsi, M., 2017. Otolith shape discrimination of *Liza ramada* (Actinopterygii: Mugiliformes: Mugilidae) from marine and estuarine populations in Tunisia. *Acta Ichthyologica et Piscatoria*, 47(**1**), 13–21.
DOI: 10.3750/AIEP/02006

Reimer, T., Dempster, T., Warren-Myers, F., Jensen, A. and Swearer, S., 2016. High prevalence of vaterite in sagittal otoliths causes hearing impairment in farmed fish. *Scientific Reports*, 6, 25249.

Reimer, T., Dempster, T., Wargelius, A., Fjelldal, P.G., Hansen, T., Glover, K.A. and Swearer, S.E., 2017. Rapid growth causes abnormal vaterite formation in farmed fish otoliths. *Journal of Experimental Biology*, 220(**16**), 2965-2969.

Sadighzadeh, Z., Valinassab, T., Vosugi, G., Motallebi, A.A., Fatemi, M.R., Lombarte, A. and Tuset, V.M., 2014. Use of otolith shape for stock identification of John's snapper, *Lutjanus johnii* (Pisces: Lutjanidae), from the Persian Gulf and the Oman Sea. *Fisheries Research*, 155, 59–63. DOI: 10.1016/j.fishres.2014.02.024

Tuset, V., Lozano, I., Gonzalez, J., Pertusa, J. and García-Díaz, M., 2003. Shape indices to identify regional differences in otolith morphology of comber, *Serranus cabrilla* (L., 1758). *Journal of Applied Ichthyology*, 19(**2**), 88-93. DOI: 10.1046/j.1439-0426.2003.00344.x

Waessle, J.A., Lasta, C.A. and Favero, M., 2003. Otolith morphology and body size relationships for juvenile Sciaenidae in the Río de la Plata Estuary (35-36 S). *Scientia Marina*, 67(**2**), 233-240.

West, C.J. and Larkin, P., 1987. Evidence for size-selective mortality of juvenile sockeye salmon (*Oncorhynchus nerka*) in Babine Lake, British Columbia. *Canadian Journal of Fisheries and Aquatic Sciences*, 44(**4**), 712-721.
DOI: 10.1139/f87-086

Zhang, Z., Beamish, R.J. and Riddell, B., 1995. Differences in otolith microstructure between hatchery-reared and wild Chinook salmon (*Oncorhynchus tshawytscha*). *Canadian Journal of Fisheries and Aquatic Sciences*, 52(**2**), 344-352. DOI: 10.1139/f95-035