

## **Pilot study on the morphological identity of wild brown trout (*Salmo trutta*) stocks in the streams of the Danube river basin (Serbia)**

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**A b s t r a c t.** This study aimed to define the morphological characters useful to discriminate wild from stocked brown trout *Salmo trutta* in Serbia. Rainbow trout *Oncorhynchus mykiss* and Ohrid Lake's belvica *Salmothymus ohridanus* were also examined as outgroup taxa in order to understand better the overall variability and to reduce bias in the methodology applied. Certain continuous external morphological characters were found useful to distinguish clearly the wild and stocked brown trout in particular streams. Molecular analysis is needed to validate this result, which could permit rapid field assessment and subsequent conservation of wild or stocked origin of as yet uninvestigated brown trout stocks in Serbia.

**Key words:** aboriginality, field assessment, morphometrics, conservation

### **Introduction**

Morphological variability in salmonid fishes has been extensively discussed both in Europe and worldwide. However, these studies used analytic approaches so out-of-date that they are no longer worthy of citation. Whereas, progress in biochemical and molecular methods has enabled the clear definition of distinct taxonomic and phyletic lineages of the brown trout *Salmo trutta* L., 1758, including the reconstruction of their evolutionary history. Within the recognized lineages, however, particular isolates remain insufficiently explained in a taxonomic sense as regards the discrepancy between morphological and molecular data. Kottelat (1997) has denied the relevance initially given to taxonomic results based exclusively on biochemical and molecular data (i.e. ignoring information on morphology, zoogeography and dispersal). The genetic character and purity of brown trout stocks in Europe have received much attention in the last 30 years, with contradictory conclusions about the impact of stocking on the native brown trout populations (Morán et al. 1995, Krieg & Guyonard 1985). The main aim of the present study was to compare the morphology of wild and stocked brown trout in Serbia to determine whether there are any morphological characters in wild brown trout that can be used as a quick field criterion for determining the status (wild or stocked) of captured fish.

### **Materials and Methods**

Specimens of brown trout *Salmo trutta*, rainbow trout *Oncorhynchus mykiss* and Lake Ohrid belvica *Salmothymus ohridanus* were collected in the 1998–2000 from the streams located in Eastern and Western Serbia and the former Yugoslav Republic of Macedonia. The fish were captured by electrofishing, angling and gill netting between May 1996 and October

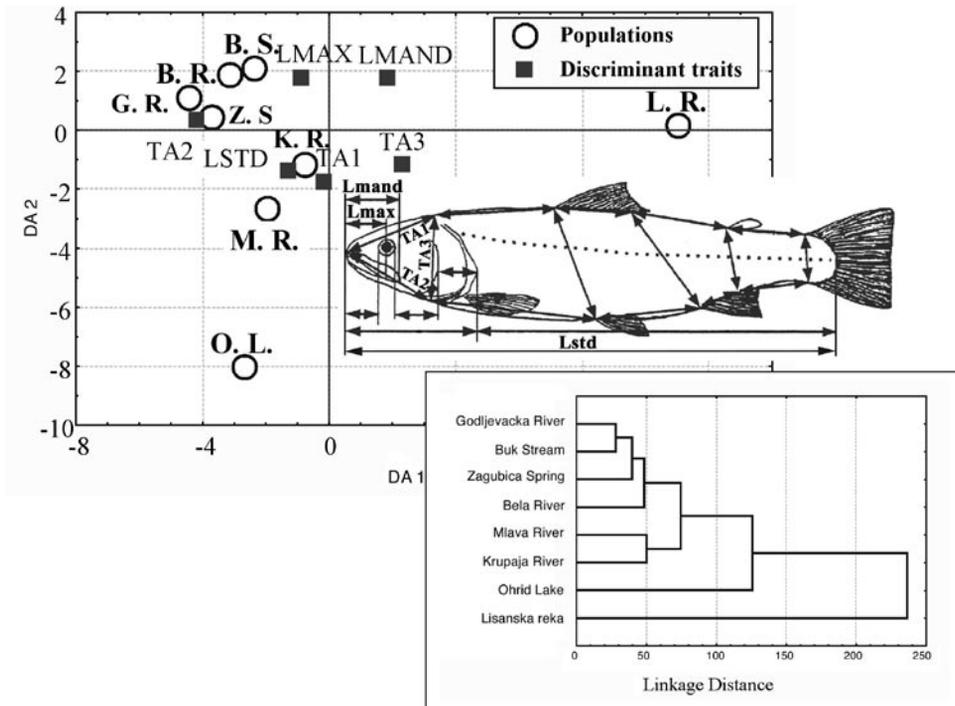
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1999 from Buk Stream and Krupaja River (tributaries of the Mlava River, which drains into the Danube River), Lišanska and Bela Rivers (tributaries of the Ibar River, Western Morava drainage), and Lake Ohrid (Adriatic Sea basin).

Analysis of external morphology (34 mensural and meristic characters) was undertaken on 88 individuals in total, defrosted from deep frozen state. Most of these specimens were sexually immature, hence the sex was not taken into account as a source of variability. Twenty-three mensural characters were measured using digital calipers, accuracy to 0.01 mm. Eleven meristic characters were counted. Both kinds of characters were chosen following Holčík (1989) and Bookstein et al. (1985, slightly modified). All measures and counts for bilateral characters were taken from the right side of the fish except when that was impossible due to the loss, or damage of the character concerned.

The morphometric data were tested for normality of distribution by Kolmogorov-Smirnov normality test (Lilliefors 1967) and then analyzed using univariate (ANOVA, with the Scheffé *post-hoc* test) and multivariate statistical methods: Discriminant Canonical Analysis, MANOVA, and UPGMA Cluster Analysis (Sokal & Rohlf 1995). Discrete traits were analyzed by Correspondent Analysis (Zar 1984). The analyses were performed using STATISTICA v. 5.1 for Windows 95.

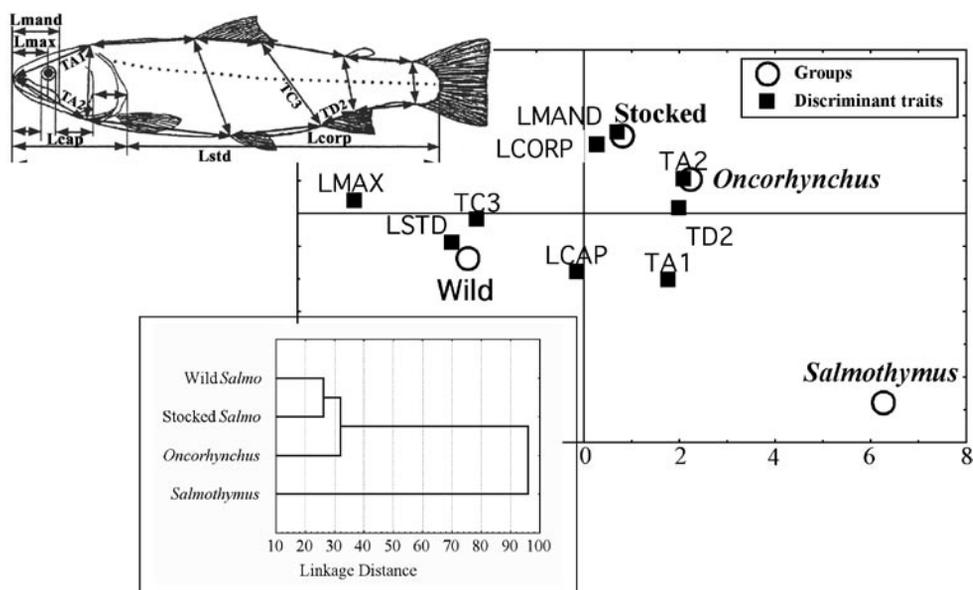


**Fig. 1.** Relative position of sample centroids and continuous morphological traits (in salmonids) in the projection of the first and second Discriminant Axes (sampling localities: G.R., Godljevačka River *Salmo trutta*, n = 13; L.R., Lišanska River *Salmo trutta*, n = 18; B.R., Bela River *Salmo trutta*, n = 6; M.R., Mlava River *Salmo trutta*, n = 9; B.S., Buk Stream *Salmo trutta*, n = 17; K.R., Krupaja River *Salmo trutta*, n = 5; *Oncorhynchus*, Zagubica Spring *Oncorhynchus mykiss*, n = 14; O.L., Lake Ohrid *Salmothymus ohridanus*, n = 6), with the set of mensural characters (discriminant ones denoted) and relationship between samples derived using UPGMA Cluster Analysis on Squared Mahalanobis Distances between samples.

## Results

Kolmogorov-Smirnov test for normality revealed normal distribution of data in all characters (values of range 0.052–0.102, Lilliefors  $P > 0.1$ ). Discriminant Analysis of locality-related samples has brought out nine characters of strong discriminating power (Fig. 1) from the set of 23 external morphological characters, whereas the same analytic method applied on taxa emphasized six discriminating characters (Fig. 2). The remarkable reduction obtained by multivariate approach simplified the further interpreting.

The significant differences for morphometric characters were shown on accomplishing the MANOVA test, both on nominal taxa (Wilks  $\lambda=0.03$ , Rao  $R=12.01$ ,  $P < 0.01$ , Pillai – Bartlett  $\text{Trac}=1.62$ ,  $V=11.63$ ,  $P < 0.01$ ) and on groups of samples (Wilks  $\lambda=0.11$ , Rao  $R=14.35$ ,  $P < 0.01$ , Pillai – Bartlett  $\text{Trac}=0.89$ ,  $V=14.35$ ,  $P < 0.01$ ). The Mahalanobis  $D^2$  distances as a measure of difference between OTUs (both taxa and stocks) of brown trout and related salmonid fishes revealed that indigenous brown trout samples (from the Godljevačka and Bela Rivers and Buk Stream) are the most similar (Fig. 1). That supports the *a priori* adopted statement on their aboriginality. The samples from the populations that contain the stocked material of brown trout (Lišanska, Mlava and Krupaja Rivers) are clustered separately. The phenetic differentiation between particular genera and species of salmonids (*Salmo trutta*, *Oncorhynchus mykiss* and *Salmothymus ohridanus*) is also good. The analysis of external morphology based on the aboriginality criterion revealed that differences between indigenous and non-indigenous brown trout stocks are less than those between species and genera (Fig. 2). The statistical significance of both differences between taxa and stocks in mensural characters indicated the prominent differentiation of samples, since on the ANOVA test results the between-group variability in 9 of 10 characters of strong



**Fig. 2.** Relative position of group centroids (groups: wild *Salmo*, stocked *Salmo*, *Oncorhynchus mykiss* and *Salmothymus ohridanus*) and continuous morphological traits in the projection of the first and second Discriminant Axes with the set of mensural characters (discriminant ones denoted) and relationship between samples derived using UPGMA Cluster Analysis on Squared Mahalanobis Distances between samples.

discriminating power was significant (Table 1). The *Post hoc* Sheffe test on these characters (Appendix I) clarified which of them can be used for distinguishing between indigenous and non-indigenous stocks, e.g., **tB1**, together with **tA1**, **tC3** and **tD1**.

On the external meristic characters, according to both taxonomic and aboriginality criteria, brown trout and related salmonine taxa were distinguishable on the complex of characters with the specific states, but without the prominent expression of any of them particularly.

**Table 1.** ANOVA tests between analysed taxa (morphometric characters) of salmonids.

Characters	F(df1,2)	P
LSTD	6.52	***
LCORP	5.81	***
LMAX	4.57	**
LMAND	4.50	**
TA1	6.92	***
TA2	1.71	ns
TA3	4.02	**
TB1	6.85	***
TC3	9.91	***
TD1	17.92	***

\*\*\*  $P < 0.001$ ; \*\*  $P < 0.01$ ; ns – no significant

## Discussion

The external morphology of salmonids was scarcely studied in last 30 years (Wilkins et al. 1994). However, biochemical (Crozier & Ferguson 1986) and molecular approaches (Osinov & Lebedev 2000) to the systematics and reconstruction of phylogenetic relationships in this group has frequently been applied. For example, a general feature in brown trout populations is an apparent lack of correspondence between geographic distance and genetic identity (Ryman 1983, Crozier & Ferguson 1986, Karakousis & Triantaphyllidis 1990). The between-population gene flow estimated in neighboring freshwater and anadromous brown trout populations from Austrias, Northern Spain, revealed that a high mean level of gene flow occurred in populations from the same drainage, but also that gene flow found between populations from different drainages was negatively related to the distance between river mouths (Morán et al. 1995). Sometimes, populations living in rivers a few km apart (or even in the same river system) show more differences than others 500 km apart (Ferguson 1989), even when migration does not appear to be prevented by the apparent physical isolation of tributaries (Morán et al. 1995) that results from recent obstacles (e.g. dams built 20 year ago represent isolation for five or six generations), or incomplete barriers, which are surpassed occasionally by floods, allowing upstream migration of adults in each of generations.

The difference observed between populations of brown trout not subjected to stocking and those that are supports the presumption that breeding between aboriginal and stocked lineages might impact, i.e. alter, the phenotype (e.g. see the relationship between samples from the Buk Stream and Mlava and Krupaja Rivers, Fig. 1). In spite of uncertainty on the

**Appendix I.** *Post hoc* (Scheffe) tests between groups for mensural characters (wild *Salmo*, n = 30; stocked *Salmo*, n = 38; *Oncorhynchus*, n = 14; *Salmothymus*, n = 6; m, means and se, standard errors in percent of total length, except for Lmax, Lmand, TA1, TA2 and TA3, which are in percent of head length; \* denotes significant difference at P < 0.05).

<b>Lstd</b>	m ± se	wild <i>Salmo</i>	stocked <i>Salmo</i>	<i>Salmothymus</i>	<i>Oncorhynchus</i>
wild <i>Salmo</i>	89.09 ± 0.521		0.003*	0.191	0.993
stocked <i>Salmo</i>	85.21 ± 0.714			0.999	0.014*
<i>Salmothymus</i>	85.08 ± 1.422				0.189
<i>Oncorhynchus</i>	89.45 ± 1.419				
<b>Lcorp</b>	m ± se	wild <i>Salmo</i>	stocked <i>Salmo</i>	<i>Salmothymus</i>	<i>Oncorhynchus</i>
wild <i>Salmo</i>	73.91 ± 1.188		0.002*	0.389	0.067
stocked <i>Salmo</i>	67.11 ± 1.364			0.979	0.993
<i>Salmothymus</i>	68.45 ± 0.241				0.998
<i>Oncorhynchus</i>	67.75 ± 1.179				
<b>Lmax</b>	m ± se	wild <i>Salmo</i>	stocked <i>Salmo</i>	<i>Salmothymus</i>	<i>Oncorhynchus</i>
wild <i>Salmo</i>	48.07 ± 0.747		0.879	0.000*	0.993
stocked <i>Salmo</i>	47.33 ± 0.593			0.000*	0.990
<i>Salmothymus</i>	37.82 ± 1.171				0.000*
<i>Oncorhynchus</i>	47.72 ± 0.886				
<b>Lmand</b>	m ± se	wild <i>Salmo</i>	stocked <i>Salmo</i>	<i>Salmothymus</i>	<i>Oncorhynchus</i>
wild <i>Salmo</i>	53.31 ± 0.795		0.014*	0.000*	0.469
stocked <i>Salmo</i>	48.72 ± 1.021			0.000*	0.819
<i>Salmothymus</i>	36.58 ± 1.442				0.000*
<i>Oncorhynchus</i>	50.41 ± 1.772				
<b>TA1</b>	m ± se	wild <i>Salmo</i>	stocked <i>Salmo</i>	<i>Salmothymus</i>	<i>Oncorhynchus</i>
wild <i>Salmo</i>	63.61 ± 1.046		0.258	0.000*	0.001*
stocked <i>Salmo</i>	66.22 ± 0.798			0.002*	0.041*
<i>Salmothymus</i>	75.53 ± 1.791				0.403
<i>Oncorhynchus</i>	71.09 ± 1.497				
<b>TA2</b>	m ± se	wild <i>Salmo</i>	stocked <i>Salmo</i>	<i>Salmothymus</i>	<i>Oncorhynchus</i>
wild <i>Salmo</i>	64.69 ± 1.259		0.000*	0.917	0.831
stocked <i>Salmo</i>	46.74 ± 2.671			0.067	0.000*
<i>Salmothymus</i>	60.92 ± 1.218				0.656
<i>Oncorhynchus</i>	68.28 ± 1.409				
<b>TA3</b>	m ± se	wild <i>Salmo</i>	stocked <i>Salmo</i>	<i>Salmothymus</i>	<i>Oncorhynchus</i>
wild <i>Salmo</i>	62.80 ± 0.783		0.692	0.000*	0.000*
stocked <i>Salmo</i>	61.56 ± 0.703			0.000*	0.000*
<i>Salmothymus</i>	71.64 ± 0.722				0.649
<i>Oncorhynchus</i>	74.26 ± 1.121				
<b>TB1</b>	m ± se	wild <i>Salmo</i>	stocked <i>Salmo</i>	<i>Salmothymus</i>	<i>Oncorhynchus</i>
wild <i>Salmo</i>	26.03 ± 0.279		0.416	0.848	0.000*
stocked <i>Salmo</i>	26.76 ± 0.228			0.334	0.000*
<i>Salmothymus</i>	25.33 ± 0.869				0.000*
<i>Oncorhynchus</i>	30.62 ± 0.725				
<b>TC3</b>	m ± se	wild <i>Salmo</i>	stocked <i>Salmo</i>	<i>Salmothymus</i>	<i>Oncorhynchus</i>
wild <i>Salmo</i>	20.88 ± 0.212		0.989	0.397	0.868
stocked <i>Salmo</i>	20.98 ± 0.198			0.485	0.946
<i>Salmothymus</i>	21.80 ± 0.383				0.789
<i>Oncorhynchus</i>	21.20 ± 0.339				
<b>TD1</b>	m ± se	wild <i>Salmo</i>	stocked <i>Salmo</i>	<i>Salmothymus</i>	<i>Oncorhynchus</i>
wild <i>Salmo</i>	20.26 ± 0.403		0.816	0.258	0.000*
stocked <i>Salmo</i>	20.71 ± 0.285			0.512	0.000*
<i>Salmothymus</i>	21.96 ± 0.674				0.000*
<i>Oncorhynchus</i>	16.39 ± 0.375				

impact of general pattern, e.g. difference between brown trout samples from adjacent Buk Stream and Mlava River (Fig. 1), the differences found between wild and stocked brown trout (Appendix) emphasizes the need for caution in future stocking policies.

Our study provides the fisheries manager with a useful tool for the assessment of stock status using a small set of characters that are easy-to-operate with and that are easily accessible in field work. This approach could open the possibility for different grading in the evaluation of stocks, and consequently, for the mitigation of impacts on aboriginal and non-indigenous stocks, or for the restriction of angling on pure, i.e., aboriginal brown trout stocks.

#### A c k n o w l e d g m e n t s

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