

## BIOMETRY ANALYSIS OF EUROPEAN GRAYLING (*THYMALLUS THYMALLUS* L.) FRY, BREEDING IN RECIRCULATING AQUACULTURE SYSTEM (RAS)\*

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### Abstract

The research objective has been to achieve biometric characterisation of the European grayling (*Thymallus thymallus* L.), aged 0+, cultured in the recirculating aquaculture system (RAS). The obtained results were referred to the biometric data relating to wild graylings published in literature. The body shape variation of grayling fry breeding under RAS conditions, coincides with body shape variation of the analysed wild populations in Poland.

The closest similarity to graylings from the Odra River basin was demonstrated in: the lateral length of the head, preanal length, length of pectoral fins, predorsal length, and from the Vistula River drainage basin in: preanal length, body height, distance between the pectoral and ventral fins, length of ventral fins. The biggest differences between the plastic traits of the analysed fish versus graylings from the natural environment concerned: the height of the dorsal and anal fins, length of the base of the dorsal fin, length of the base of the tail, lengths of folds of the tail fin. Analysis of the measurements revealed internal variation in the biometric characteristics of European grayling. The highest variation was observed for the parameters: anal fin height, head width, maximum body height and the smallest for: caudal fin length, eye diameter and lateral head length. The analysis results, show that the breeding of European grayling fry in RAS does not affect the variability of meristic traits.

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Principal component analysis (PCA) for the linear measurements of distances revealed that the area between the anal fin base and the head has high component loadings. As a result, it was possible to quantify the co-variation in morphological measurements in fish cultured in RAS. The PCA demonstrated three principal components, which together explained 77% of the body shape variation. The first component explains 44% of the variation, and it is composed of the postdorsal lengths, head width and maximum body height, while the second component explains 19% of the variation, and comprises the size of the eye and length of the postorbital space. The third principal component explains 14% of the variation and includes only one trait – the head lateral length. It seems that based values of the traits these three components PCA, can be carried out selection work while breeding grayling in RAS.

## Introduction

European grayling (*Thymallus thymallus* L.) is a typical representative of rheophilic riverine fish (MALLETT et al. 2000). In general, this species occurs in all Euroasia and North America, in cool, clean and well-oxygenated mountainous and sub-mountainous rivers (KOTTELAT and FREYHOF 2007). In Poland, it dwells in some rivers and larger streams in the Pomeranian Lake District, the southern belt of uplands, foothills and mountains, and in tributaries of the Pregoła River. Moreover, it has been introduced to some rivers outside its natural occurrence area, such as the Wel, Pasłęka, Tanew and San (WITKOWSKI et al. 1984).

For a few decades now, the number of European grayling in natural habitats have been diminishing. With the high environmental requirements of this species, the changes that take place in its habitats have an adverse influence on the stability of the species' populations. Worth mentioning are anthropogenic modifications implemented in channels and valleys of rivers (PENCZAK and KRUK 2000, OVIDIO and PHILIPPART 2002, WIŚNIEWOLSKI et al. 2004) or pollution (HONKANEN et al. 2005). Furthermore, European grayling is exposed to intense angling and poaching pressure (HOLČIK 2004, AUGUSTYN and NOWAK 2014).

Seen in this light, it appears that adequately designed and consistent fish stocking efforts should be taken in parallel to the amelioration of the aquatic environment so as to sustain the presence of European grayling in our waters. Programmes designed for the active protection of this species should have a well-defined aim and include an assessment of potential and actual outcomes (COWX 1994). For each population of endangered or overfished species, or sometimes even part of such a population, a customised approach should be implemented. To reach the presumed goals and achieve maximum environmental benefits, fish populations should be managed separately (GRIMES et al. 1987). Hence, the necessity to monitor and constantly check the efficiency of applied measures, i.e. the purposefulness of stocking efforts (TUREK et al. 2010, HORKÁ et al. 2015)

Fish stocking practice ought to strive towards the creation of stable, self-reproducing populations (FRASER 2009), and should not interfere with its genetic separateness (OCALEWICZ et al. 2013, WEISS et al. 2013). Introduction to open waters the fish stocking material that does not demonstrate the characteristics of indigenous populations leaves a permanent genetic trace (DUFTNER et al. 2005), and may have a significant influence on their current status and condition. This is particularly important because populations inhabiting various river catchments can be observed to present distinct differences with respect to some features (WITKOWSKI et al. 1984). Similar observations can be made among populations from rivers with different flow rates.

Considering the above, a species breeding system can have an effect on subsequent differences in the growth and survivability of fish, which – after stocking – may translate into their reproductive success and angling appeal. This applies to both stocking material production of endangered fish species, and in this case, grayling in open (SZMYT et al. 2013) and recirculation water systems. Intensive development of aquaculture, including water management systems, leads to an increasing popularity of recirculating aquaculture systems (RAS) in fish rearing. It is therefore justifiable to examine the fish stocking material obtained in such systems, both in terms of their growth and health parameters as well as measurable attributes. This is particularly important at present, when identification of intraspecies individuals or flocks of species with unique morphological traits has become more efficient and enables both better management of fish stocks – including analysis of the restocking programs effectiveness – and more effective fish protection (TUREK et al. 2010, 2018, LEPIČ et al. 2019).

Biometric traits are an essential component of fish taxonomic research. For every species, there are patterns of scales and symbols of fins, with descriptions given as numerical values of measurable traits. They are not usually constant and are assigned a certain range of variability within a species.

It has been proven experimentally that temperature is one of the main factors that affect values of the calculable features (ORSKA 1956). It has also been demonstrated that such characteristics are affected by water parameters. Changes in the numerical values of meristic traits are mostly associated with environmental conditions and emerge in early developmental stages of fish (BEACHAM and MURRAY 1986). Studies on shapes of fish bodies prove that this feature depends on numerous factors, including the technique of swimming, availability of food, or quality of the environment. Morphometric features play an important role in identification of intraspecies variation, i.e. they can be applied as a tool to distinguish

populations adapted to various environmental or fish rearing conditions (WIŚNIEWSKA 2008, PULCINI et al. 2013). Therefore, biometric research is necessary, especially in the context of stocking material production different fish species. For example KUPREN et al. (2015) carried out allometric tests on the chub juveniles. The authors analysed the morphological development and allometric growth patterns in the aspect of the potential possibility for determining the quality of chub in restocking programs. In the case of European grayling there is little research results on biometry of this species in the wild, but there is lack information about the biometry in controlled conditions, especially in RAS.

The objective of this research has been to provide characterisation of biometric traits of European grayling (*Thymallus thymallus* L.) reared up in a recirculating aquaculture system (RAS). The research hypothesis assumed no differences in the values of meristic traits between grayling fry from RAS controlled conditions in relation to fish from the wild.

## Material and Methods

The material for fry rearing was obtained from the Fish Breeding Centre of the Polish Angling Association in Paliwoda. It originated from a broodstock, spawning for the first time under controlled conditions. The broodstock in Paliwoda was created on a base reproduction material obtained from wild graylings from Biała Głucholaska river. The experimental rearing up of the European grayling fry was conducted at the Aquaculture and Ecological Engineering Centre, the Faculty of Environmental Sciences, the University of Warmia and Mazury in Olsztyn, Poland.

Fish aged 0+, of an average initial weight of  $1.5 \text{ g/individ.} \pm 0.56 \text{ SD}$  and initial length (l.t.)  $5.9 \text{ cm} \pm 0.73 \text{ SD}$ , were kept in plastic tanks, each having the capacity of  $0.32 \text{ m}^3$ , in a recirculating aquaculture system set up on a semi-technical scale. The average input stocking density of fish in a tank was  $0.94 \text{ kg m}^{-3}$  (200 individuals). During the fish rearing, the water temperature was maintained at  $16\text{--}17^\circ\text{C}$ , oxygen content was within  $9.4\text{--}9.9 \text{ mg O}_2 \text{ dm}^{-3}$ , and oxygen saturation was  $90\text{--}95\%$ . Fish rearing period was 156 days. Graylings were fed by Aller Futura EX and Aller Futura commercial feed in size  $1.3\text{--}2.0 \text{ mm}$ .

All fish after being caught were, immediately anaesthetized with MS-222 ( $300 \text{ mg dm}^{-3}$ ) and killed by brain destruction with sharp scissors, measured and weighted. This procedure is in accordance with the guidelines in annex IV of the directive European Union number 201/63/UE.

The methodology chosen for our analysis of biometric traits was the one referred to as canonical morphometry (MARCUS 1990), as it enabled us

to make references to the literature data. Literature data cited in this work concern wild grayling of similar age and size and living in rivers with similar hydrological and environmental conditions.

The research material was analysed in terms of the variation of biometric features. Individual measurements of fish ( $n = 100$ ) were taken according to the procedure illustrated in Figure 1. All measurements were performed by the same person, using a digital calliper set at 0.1 mm accuracy, except for TL, Fl and Sl where the accuracy range was 1 mm.

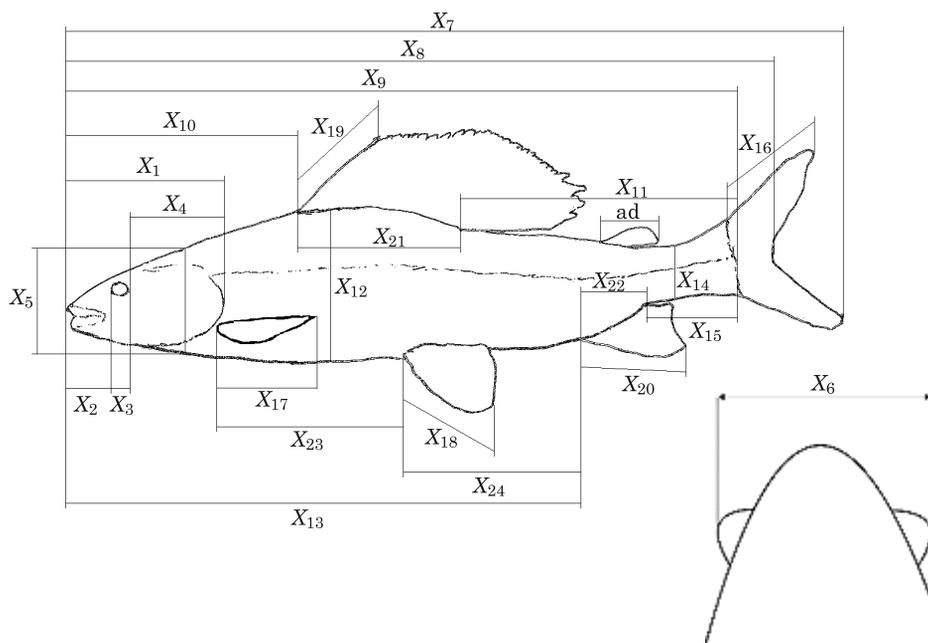


Fig. 1 Schematic design of morphometric measurements performed in the research.

Description of the symbols: • shape of the head [mm]:  $X_1$  – lc, lateral head length (*longitudo capitis lateralis*);  $X_2$  – prO, preorbital space (*spatium praeorbitale*);  $X_3$  – O, eye diameter (*diaemeter oculi*);  $X_4$  – poO, postorbital space (*spatium postorbitale*);  $X_5$  – hc, head height (*altitudo capitis*);  $X_6$  – lac, head width (*latitudo capitis*); • body shape [mm]:  $X_7$  – TL, total length (*longitudo totalis*);  $X_8$  – Fl, caudal length (*longitudo caudalis*);  $X_9$  – Sl, body length (*longitudo corporis*);  $X_{10}$  – pD, predorsal length (*longitudo praedorsale*);  $X_{11}$  – poD, postdorsal length (*longitudo postdorsale*);  $X_{12}$  – H, maximum body height (*altitudo corporis maxima*);  $X_{13}$  – pA, preanal length (*longitudo praeanal*);  $X_{14}$  – h, minimum body height (*altitudo corporis minima*);  $X_{15}$  – lpc, length of the caudal peduncle (*longitudo pedunculi caudae*); • shape and position of fins [mm]:  $X_{16}$  – IC, caudal fin length (*longitudo pinnae C*);  $X_{17}$  – IP, pectoral fin length (*longitudo pinnae P*);  $X_{18}$  – IV, ventral fin length (*longitudo pinnae V*);  $X_{19}$  – hD, dorsal fin height (*altitudo D*);  $X_{20}$  – hA, anal fin height (*altitudo A*);  $X_{21}$  – lDb, length of dorsal fin base (*longitudo basis D*);  $X_{22}$  – lAb, length of anal fin base (*longitudo basis A*);  $X_{23}$  – P-V, distance between the pectoral and ventral fins (*distancia P - V*);  $X_{24}$  – V-A, distance between the ventral and anal fins (*distancia V - A*); ad – adipose fin length (*longitudo pinnae adiposa*)

For all measured characteristics, descriptive statistics (mean, min., max., standard deviation) were calculated and presented, alongside the variability coefficient  $V_L$  of the analysed fish, which was derived from:

$$V_L = 100 \text{ SD } L^{-1}$$

where:

$V_L$  – variability coefficient [%]

SD – standard deviation

$L$  – average length (l.t.) of an individual fish [mm].

For a length-measured dimension ( $L$ ), a growth-related proportional change pattern is given by the relationship between base dimension [e.g., fork length (Fl) or head length (lc)] and the  $L$  proportion ( $L/Fl$  or  $L/lc$ ). The relationship between Fl (or lc) and  $L$  is allometric growth. Variation coefficient for ratio allometric was calculated following to the same formula as the total length variation coefficient. The linear measurements of distances were submitted to principal component analysis. As a result, it was possible to quantify the co-variation in morphological measurements (WIŚNIEWSKA 2008). Transformation of data was performed with the use of an allometric method postulated by ELLIOTT et al. (1995), whose purpose is to assign observed variability to differences in the body shape, not correlated to the relative size of fish. This method adjusts for residual size effects. In this paper, the method served to correct the size-dependent variability of morphometric traits, using the formula:

$$\text{Madj} = M (L_s/L_0)^b$$

where:

$M$  – the original measurement

Madj – the size-adjusted value

$L_s$  – the average value of the same trait for all fish

$L_0$  – is the value of a given trait

$b$  – estimated for each trait from the observed data as the slope of the regression of  $\log M$  on  $\log L_0$  using all fish in the given group.

All analyses were supported by Statistica 12.0 software.

## Results

Our analysis of the measurements revealed internal variation in the biometric characteristics of European grayling (Table 1).

Table 1

Values of descriptive statistics of the analysed characteristics [mm]

MM	$M$	Min.	Max.	$\sigma$	$V_L$ [%]
$X_1$	27.6	17.50	42.0	4.92	17.85
$X_2$	7.6	4.40	14.1	1.77	23.44
$X_3$	7.7	5.80	10.1	1.20	15.53
$X_4$	12.2	7.10	18.8	2.35	19.24
$X_5$	19.5	10.20	32.7	4.46	22.88
$X_6$	13.5	7.60	20.3	3.65	27.16
$X_7$	139.7	81.00	213.0	27.58	19.74
$X_8$	129.6	75.00	204.0	26.82	20.69
$X_9$	120.1	69.00	192.0	25.84	21.50
$X_{10}$	44.7	6.90	65.8	12.06	26.99
$X_{11}$	49.1	27.40	86.2	11.39	23.20
$X_{12}$	26.3	13.00	43.5	7.11	27.07
$X_{13}$	89.5	51.50	137.1	19.25	21.51
$X_{14}$	8.2	4.10	12.8	1.77	21.60
$X_{15}$	18.9	11.50	34.8	4.64	24.57
$X_{16}^a$	18.7	11.90	24.5	2.93	15.65
$X_{16}^b$	19.2	12.10	25.0	2.87	14.90
$X_{17}$	19.6	10.40	30.4	4.05	20.65
$X_{18}$	18.1	10.40	27.8	4.17	23.07
$X_{19}$	19.7	11.10	31.1	4.61	23.39
$X_{20}$	20.9	10.10	46.8	10.03	48.03
$X_{21}$	20.5	9.60	30.5	4.80	23.46
$X_{22}$	11.3	5.70	17.5	2.40	21.27
$X_{23}$	35.1	19.90	53.7	8.45	24.10
$X_{24}$	31.4	15.40	52.9	7.68	24.43

Explanations: MM – morphometric measurements,  $M$  – mean value of the trait,  $\sigma$  – standard deviation  $V_L$ % – variability coefficient

The lowest variation was found for the body length SI ( $X_9$ ) ( $V\% = 1.51$ ) and preanal length pA ( $X_{13}$ ) ( $V\% = 2.20$ ). The highest variation was determined for the anal fin height hA ( $X_{20}$ ) ( $V\% = 29.27$ ) and length of the dorsal fin base lDBs ( $X_{21}$ ) ( $V\% = 28.81$ ). Relative to the caudal length Fl ( $X_8$ ), the highest values were determined for the body length SI ( $X_9$ ) and preanal length pA ( $X_{13}$ ). The smallest values of the proportion of a given trait to the caudal length Fl ( $X_8$ ) were identified for the minimum body height  $h$  ( $X_{14}$ ) and length of the anal fin base lABs ( $X_{22}$ ).

However, the literature on the subject there is no data regarding the above. Therefore, for reference the results to the literature data, in the subsequent step of our analysis, the data were transformed relative to the caudal length  $Fl$  ( $X_9$ ) (Table 2) and lateral head length  $lc$  ( $X_1$ ) (Table 3).

Table 2  
Values of the plastic traits of European grayling (*Thymallus thymallus* L.) ( $n = 100$ ) in relation to the caudal length  $Fl$  ( $X_9$ )

MM	Morphometric measurements as a % of caudal length $Fl$ ( $X_9$ )				
	min.	max.	$M$	$\sigma$	$V_L$ [%]
$X_9$	89.58	98.31	92.6	1.4	1.51
$X_{13}$	65.07	73.37	69.07	1.52	2.20
$X_{10}$	31.63	41.81	36.57	1.62	4.44
$X_1$	19.49	24.55	21.63	1.09	5.04
$X_{23}$	23.52	32.24	26.88	1.61	5.98
$X_{18}$	11.72	16.17	13.84	0.84	6.07
$X_{24}$	20.53	29.32	24.15	1.49	6.18
$X_{17}$	13.48	17.55	15.22	0.98	6.41
$X_{14}$	4.13	7.42	6.28	0.45	7.20
$X_{22}$	6.31	11.3	8.53	0.80	9.36
$X_{19}$	12.4	19.33	15.13	1.53	10.13
$X_{15}$	10.56	23.98	14.48	1.52	10.53
$X_{12}$	10.96	25.19	19.87	2.61	13.14
$X_{21}$	8.93	24.03	16.55	4.77	28.81
$X_{20}$	9.25	22.16	14.98	4.38	29.27

Explanations: MM – morphometric measurements;  $M$  – mean value of the trait;  $\sigma$  – standard deviation;  $V_L$ % – variability coefficient

Table 3  
Values of the plastic traits of European grayling (*Thymallus thymallus* L.) ( $n = 100$ ) in relation to the lateral head length  $lc$  ( $X_1$ )

MM	Morphometric measurements as a % of lateral head length $lc$ ( $X_1$ )				
	min	max.	$M$	$\sigma$	$V$ [%]
$X_4$	34.36	51.99	43.87	2.69	6.13
$X_3$	21.67	34.39	28.68	2.31	8.06
$X_2$	20.49	33.59	27.34	2.31	8.43
$X_5$	33.6	86.6	69.49	7.42	10.68

Explanations: MM – morphometric measurements;  $M$  – mean value of the trait;  $\sigma$  – standard deviation;  $V_L$ % – variability coefficient

The length of the body Sl ( $X_9$ ) among the analysed European grayling specimens equalled 92.6% of the caudal length Fl ( $X_8$ ). The minimum  $h$  ( $X_{14}$ ) and the maximum  $H$  ( $X_{12}$ ) body height of the analysed graylings was 6.28% and 19,87% of the caudal length Fl ( $X_8$ ) respectively. The analysed specimens were characterised by the lateral head length lc ( $X_1$ ) corresponding to 21.63% of the caudal length Fl ( $X_8$ ). Predorsal length pD ( $X_{10}$ ) and preanal length pA ( $X_{13}$ ) of the analysed fishes equalled 36.57% and 69.07% of the caudal length Fl ( $X_8$ ) respectively.

The highest value relative to the lateral head length lc ( $X_1$ ) was achieved by the characteristic describing of the height head hc ( $X_5$ ). The lowest approximately the same values were scored by the preorbital space prO ( $X_2$ ) and eye diameter O ( $X_3$ ). The highest variability coefficient values were obtained for the trait denoted as the head height hc ( $X_5$ ), while the lowest one was achieved for the postorbital space poO ( $X_4$ ) – Table 3.

The data transformed as explained above were submitted to a factorial analysis, in the course of which three principal components were distinguished that explained 77% of the variability of the body shape. The results of Principal Component Analysis are presented in Table 4.

Table 4

Values of the principal components and explained variation

	Component value	% of the total variance	Cumulative own value	Cumulated [%]
PC1	10.23	42.62	10.23	42.62
PC2	4.60	19.15	14.82	61.77
PC3	3.57	14.89	18.40	76.65

Contributions of principal components to explaining the variation are collated in Table 4. Loads of principal components are set in Table 5.

Table 5

Loads of principal components

Morphometric characteristics	PC2	PC2	PC3
$X_1t$	–	–	-0.780341
$X_3t$	–	0.841196	–
$X_4t$	–	0.802514	–
$X_5t$	0.71287	–	–
$X_6t$	0.91957	–	–
$X_8t$	0.87417	–	–

$X_9^t$	0.91217	–	–
$X_{10}^t$	–	0.741789	–
$X_{11}^t$	0.86080	–	–
$X_{12}^t$	0.90026	–	–
$X_{13}^t$	0.93279	–	–
$X_{14}^t$	0.77588	–	–
$X_{15}^t$	0.72859	–	–
$X_{16}^{at}$	–	0.763493	–
$X_{16}^{bt}$	–	0.768419	–
$X_{20}^t$	0.85632	–	–
$X_{23}^t$	0.93550	–	–
$X_{24}^t$	0.81332	–	–
<b>Variance</b>	<b>10.22789</b>	<b>4.595951</b>	<b>3.573120</b>
<b>Percent</b>	<b>43</b>	<b>19</b>	<b>14</b>

The first component explains 44%, while the second one explains 19% of the variation. The highest contribution to PC1 is made by the values of the following traits: head width lac ( $X_6$ ), body length Sl ( $X_9$ ), maximum body height  $H$  ( $X_{12}$ ), preanal length pA ( $X_{13}$ ), distance between the pectoral and ventral fins P-V ( $X_{23}$ ). The highest contribution to the second component was assigned to the traits: eye diameter O ( $X_3$ ), and postorbital space poO ( $X_4$ ). This outcome proves that 44% of the variation in the body shape of domesticated European grayling arises from the postdorsal lengths poD ( $X_{11}$ ), head width lac ( $X_6$ ) and maximum body height  $H$  ( $X_{12}$ ), while 19% depends on the eye diameter O ( $X_3$ ) and postorbital space poO ( $X_4$ ). It is interesting to note that the third component has only one contributor – lateral head length lc ( $X_1$ ), yet it explains 14% of the variation. This proves the high influence of the said trait on the body shape. No contribution to the principal components is made by values of these traits: preorbital space prO ( $X_2$ ), length of the dorsal fin IP ( $X_{17}$ ), length of the ventral fin IV ( $X_{18}$ ), height of the dorsal fin hD ( $X_{19}$ ), which seems to indicate the lack of any influence of these traits on the differentiation of the body shape.

## Discussion

Studies on morphological, morphometric and meristic traits, carried out to define and characterise populations, have long been performed in ichthyology. Salmonids exhibit large scale plasticity in overall body shape (CURRENS et al. 1989, BEACHAM 1990, VON CRAMON-TAUBADEL et al. 2005).

Fish populations present in areas where the water flow is rapid are typically slimmer and sleeker than those living in waters with less intensive water flow (FRANSSEN 2011, GASTON and LAUER 2015). In hatcheries, especially under RAS conditions, any phenotypic shifts will be mostly due to plasticity of traits (STRINGWELL et al. 2014). In natural conditions, in addition to the plasticity of traits the differences are probably the result of the quality of the aquatic environment. Even within a single population it is possible to identify the conditions and parameters which affect the body shape of fish (Table 5). According to PULCINI et al. (2013), compared to fish living in the wild, phenotypes of domesticated salmonid fish are characterised by a larger head, longer dorsal and anal fins and a generally less sleek body. A similar tendency in the changing body shape was detected in the course of our study on European grayling reared under controlled conditions (Table 5).

Studies conducted on several populations have shown certain differentiation of morphological traits of this species across Poland (WITKOWSKI et al. 1984). Discriminant multivariate analysis of meristic and morphometric characteristics clearly distinguished three neighbouring French populations of *T. thymallus* from the Rhone drainage basin (SURRE et al. 1986, BAJIĆ et al. 2018). In this study, multivariate analysis was used to identify the traits responsible for body shape variation, of which three main components were distinguished, which explained 77% of the observed variability (Table 4).

The length of the body SI ( $X_9$ ) among the analysed European grayling specimens equalled 92.6% of the caudal length FI ( $X_8$ ). This is less than determined in fish dwelling in two rivers from the Odra River catchment, such as the Nysa Kłodzka and the Kaczawa (93.34% and 93.37%, respectively), the Dunajec River (the Vistula River basin) (93.45%), and the Mesna River (95.38%). Approximately the same values of this proportion were found in the graylings from the Vltava River (the River Elbe tributary) and in the Danube River tributaries (91.70% and 91.44%, respectively) (WITKOWSKI and KOWALEWSKI 1979).

The lateral head length lc ( $X_1$ ) of the European grayling equals 18–21% of the caudal length FI ( $X_8$ ) (WITKOWSKI et al. 1984). The analysed specimens were characterised by the lateral head length lc ( $X_1$ ) corresponding to 21.63% of the caudal length FI ( $X_8$ ). This is more than the head lateral length lc ( $X_1$ ) of European graylings from rivers in the Odra River basin, i.e. the Nysa Kłodzka (21.32%) and the Kaczawa (21.34%), rivers in the Danube River basin (20.83%), from the Vltava River (20.59%), the Dunajec (the Vistula River's tributary; 20.05%) or the Mesna (18.53%) (WITKOWSKI and KOWALEWSKI 1979). The result obtained in this study lies in the range

established for the Mongolian grayling (*Thymallus brevirostris* K.), in which the lateral head length  $lc$  ( $X_1$ ) equals 19–24% of the caudal length  $Fl$  ( $X_8$ ) (WITKOWSKI et al. 1984).

The measured predorsal length  $pD$  ( $X_{10}$ ) of the analysed fishes equalled 36.57% of the caudal length  $Fl$  ( $X_8$ ) – Table 2. This is higher than in European grayling caught in the Dunajec, Nysa Kłodzka, Elbe, Danube or Mesna rivers (35.43%, 35.39%, 33.94%, 35.10%, 32.51%). The European grayling caught in Kaczawa River scored a comparable value of this trait, namely 36.61% (WITKOWSKI and KOWALEWSKI 1979).

In representatives of the Mongolian grayling, this trait scores higher, at 37.5% on average, but it is lower in specimens of the Kosogol grayling (*Thymallus nigrescens* D.), between 32–34% (WITKOWSKI et al. 1984).

The preanal length  $pA$  ( $X_{13}$ ) of the fish analysed was 69.07% of the caudal length  $Fl$  ( $X_8$ ) – Table 2. This is less than in European grayling fish from the Mesna River (71.35%), rivers in the Danube basin (69.5%), the Dunajec River (69.55%), the Nysa Kłodzka (69.41%), or the Kaczawa (69.28%). In the fish living in the Vltava River, this trait scored lower (68.87%) (WITKOWSKI and KOWALEWSKI 1979).

The maximum body height  $H$  ( $X_{12}$ ) is a trait that can reflect nutritional conditions in a water body from which given individuals originate. The maximum body height  $H$  ( $X_{12}$ ) of the European grayling varied highly, from 14% to 25% of the caudal length  $Fl$  ( $X_8$ ) (WITKOWSKI et al. 1984). In other representatives of the genus *Thymallus*, the value of this characteristic equals: 19–20% in the Kosogol grayling, and 15.5–24.5% in the Baikal grayling (*Thymallus baicalensis* D.) (WITKOWSKI et al. 1984). In the fish analysed in our study, the maximum body height  $H$  ( $X_{12}$ ) corresponded to 19.87% of the caudal length  $Fl$  ( $X_8$ ) – Table 2. Similar results were found in fish caught from the rivers: Mesna (19.68%), Dunajec (19.87%) and in the Danube River basin (20.09%). The European grayling populating the Nysa Kłodzka, Kaczawa and Vltava is distinguished by higher values of this trait (21.04%, 21.21% and 20.51%, respectively), (WITKOWSKI and KOWALEWSKI 1979). The highest values of this parameter present WITKOWSKI (1975) for graylings from Nysa Kłodzka and Kaczawa rivers – 22.56% and 22.7% respectively. Variation coefficient ( $V_L$ ) for this parameter was 7.48% and 6.88% for graylings from Nysa Kłodzka and Kaczawa rivers respectively (WITKOWSKI 1975). These values are about twice lower than those obtained for graylings from RAS – 13.14% (Table 2).

The minimum body height  $h$  ( $X_{14}$ ) of the analysed graylings was 6.28% of the caudal length  $Fl$  ( $X_8$ ) – Table 2. This is less than found in other rivers, like the Mesna (6.65%), Veltava (7.09%), Dunajec (6.83%), Nysa Kłodzka (6.94%), Kaczawa (6.87%) or in the Danube River basin (7.12%)

(WITKOWSKI and KOWALEWSKI 1979). Similar to the previous parameter the highest values of minimum body height  $h$  ( $X_{14}$ ) was reported by WITKOWSKI (1975) for graylings from Kaczawa river – 7.38% and Nysa Kłodzka river – 7.44% in the Odra River basin. The same author indicates the value of variation coefficient ( $V_L$ ) for this parameter at the level 7.05% and 8.30% for graylings from Nysa Kłodzka and Kaczawa rivers respectively. This results there are similar to calculations related to our research – 7.20% (Table 2).

The distance between the pectoral and ventral fins P-V ( $X_{23}$ ) in the analysed fish equalled 26.88% of the caudal length Fl ( $X_8$ ) (Table 2) and is comparable to the value of this trait in graylings from the Vltava River (27.17%). The value of this trait determined in this study is lower than the ones obtained for fish caught in the rivers Mesna (27.98%), Dunajec (28.05%), Nysa Kłodzka (28.10%), Kaczawa (28.03%) or the rivers in the Danube River drainage basin (28.8%) (WITKOWSKI and KOWALEWSKI 1979). Variation coefficient ( $V_L$ ) for  $X_{23}$  in our investigations (5.98%) was similar to WITKOWSKI (1975) publication data. The author obtained 6.13% and 5.58% of this parameter for graylings from Nysa Kłodzka and Kaczawa rivers respectively.

The space between the ventral fins and the anal V-A ( $X_{24}$ ) fin in the analysed fish was 24.15% of the caudal length Fl ( $X_8$ ) – Table 2. This is comparable to the values obtained for the fish living in the Dunajec (24.16%). Graylings in the Nysa Kłodzka, Kaczawa, Vltava and Mesna are characterised by higher values of this parameter (25.48%, 25.11%, 25.25% and 26.49%. respectively). The fish caught from rivers in the Danube River basin presented a slightly lower value of this trait (23.56%) (WITKOWSKI and KOWALEWSKI 1979).

The length of dorsal fins IDbs ( $X_{21}$ ) analysed fish equalled 15.22% of the caudal length Fl ( $X_8$ ) – Table 2. The value of this trait was similar in the fish originating from the Vltava River (15.32%), but lower in the grayling populations from the rivers Mesna (14.53%), Dunajec (14.58%), Nysa Kłodzka (15.01%) and Kaczawa (15.04%). The graylings living in the Danube River basin presented this trait at a higher value of 16.16% (WITKOWSKI and KOWALEWSKI 1979).

The length of ventral fins IV ( $X_{18}$ ) in the analysed fish was 13.84% of the caudal length Fl ( $X_8$ ) – Table 2. The closest result can be found in the fish caught from the Mesna River (14.01%), Dunajec (14.01%) and Nysa Kłodzka (14.04%). The highest value of this parameter was determined in graylings from the Kaczawa (14.54%), Vltava (15.0%) and the Danube's tributaries (15.16%) (WITKOWSKI and KOWALEWSKI 1979).

The height of the dorsal fin hD ( $X_{19}$ ) in the European grayling equals 13–14% of the caudal length Fl ( $X_8$ ) (WITKOWSKI et al. 1984). In the fish

measured in our study, it reached 15.13%. This is higher than in graylings from the rivers: Mesna (13.98%), Dunajec (13.05%), Nysa Kłodzka (13.95%), Kaczawa (13.93%), Vltava (13.57%), or in the catchment of the Danube (13.6%) (WITKOWSKI and KOWALEWSKI 1979).

In the fish we examined, the height of the anal fin  $hA$  ( $X_{20}$ ) was 14.98% of the caudal length  $Fl$  ( $X_8$ ) – Table 2. The fish from the Kaczawa River (14.47%) and the Danube River basin (14.11%) scored the closest values of this trait. Graylings from the other rivers presented lower values of this parameter, namely 11.2% in the Mesna River, 12.59% in the Dunajec, 13.3% in the Nysa Kłodzka, 13.26% in the Vltava 13.26%, (WITKOWSKI and KOWALEWSKI 1979). The length of the dorsal fin base  $lDbs$  ( $X_{21}$ ) in the European grayling is 20–23% of the caudal length  $Fl$  ( $X_8$ ) (WITKOWSKI et al., 1984). In the analysed grayling individuals, it equalled 16.55%. This was less than determined for graylings dwelling in the rivers Mesna (22.15%), Dunajec (20.6%), Nysa Kłodzka (22.2%), Kaczawa (21.64%), Vltava (22.25%), or in the tributaries of the Danube River (22.08%) (WITKOWSKI and KOWALEWSKI 1979).

The length of the anal fin base  $lAbs$  ( $X_{22}$ ) in the analysed graylings equalled 8.53% of the caudal length  $Fl$  ( $X_8$ ) – Table 2. This was less than in European grayling individuals from the rivers Mesna (9.05%), Dunajec (9.37%), Nysa Kłodzka (9.51%), Kaczawa (12.17%), Vltava (9.44%) or in rivers from the Danube River basin (9.47%) (WITKOWSKI and KOWALEWSKI 1979).

The length of the tail base  $lpc$  ( $X_{15}$ ) in the analysed fish was 14.48% of the caudal length  $Fl$  ( $X_8$ ) – Table 2. A similar value of this trait was found in graylings from the Vltava River (14.24%). Fish of the same species living in the Danube River basin have a slightly lower value of this parameter (14.03%). In graylings from the rivers Mesna, Dunajec, Nysa Kłodzka or Kaczawa, this trait scored higher values, i.e. 16.22%, 16.34%, 16.27%, respectively (WITKOWSKI and KOWALEWSKI 1979). The Mongolian grayling shows an even higher value of this trait, between 17–19% (WITKOWSKI et al. 1984).

The length of the upper fold of the tail fin  $lC$  ( $X_{16}$ ), was around 14.63% and that of the lower fold stood at 14.96% of the caudal length  $Fl$  ( $X_8$ ) – Table 2. These values are smaller than found in graylings from the rivers Mesna (16.31% the upper fold and 15.97% the lower fold), Dunajec (15.52% and 17.15%), Nysa Kłodzka (16.82% and 16.63%), Kaczawa (16.93% and 17.83%), Vltava (19.18% and 18.86%) and the Danube River basin (18.06% and 18.73%) (WITKOWSKI and KOWALEWSKI 1979).

The preorbital space  $prO$  ( $X_2$ ) in the analysed graylings was around 27.34% of the lateral head length  $lc$  ( $X_1$ ) – Table 3. This was less than in

graylings from the rivers Dunajec (34.48%), Nysa Kłodzka (31.47%) and Kaczawa (31.19%) according to data who was presented Witkowski et al. (1984). In other studies, similar data is given WITKOWSKI (1975) for graylings from Kaczawa river (31.07%) and Nysa Kłodzka (31.52%).

The eye diameter  $O$  ( $X_3$ ) in the analysed graylings equalled 28.68% of the lateral head length  $lc$  ( $X_1$ ) – Table 3. This was a higher value than determined for graylings from the Dunajec (23.78%) and Nysa Kłodzka (23.41%). Graylings inhabiting the Kaczawa River presented a similar value of the eye diameter proportion (27.79%) (WITKOWSKI et al. 1984). The postorbital space  $poO$  ( $X_4$ ) in the analysed graylings was around 43.87% of the lateral head length  $lc$  ( $X_1$ ). This was less than in graylings from the rivers Dunajec (47.78%), Nysa Kłodzka (48.21%) and Kaczawa (48.33%) (WITKOWSKI et al. 1984).

The head height  $hc$  ( $X_5$ ) in the analysed graylings was around 69.49% of the lateral head length  $lc$  ( $X_1$ ) – Table 3. The value of this trait in graylings from the rivers Dunajec (69.42%), Nysa Kłodzka (68.1%) and Kaczawa (68.91%) was similar (WITKOWSKI et al. 1984).

Breeding of European grayling fry under RAS conditions, is not only possible, but simply indicated and our results, show, that morphometric analyses can serve as a useful comparative tool in the implementation of species protection programmes. The body shape variation of grayling fry breeding under RAS conditions, coincides with body shape variation of the analysed wild populations in Poland. The closest similarity to graylings from the Odra River basin was demonstrated in: the lateral length of the head  $lc$  ( $X_1$ ), preanal length  $pA$  ( $X_{13}$ ), length of pectoral fins  $IP$  ( $X_{17}$ ), predorsal length  $pD$  ( $X_{10}$ ), and from the Vistula River drainage basin in: preanal length  $pA$  ( $X_{13}$ ), body height  $H$  ( $X_{12}$ ), distance between the pectoral and ventral fins  $P-V$  ( $X_{23}$ ), length of ventral fins  $IV$  ( $X_{18}$ ). The biggest differences between the plastic traits of the analysed fish versus graylings from the natural environment concerned: the height of the dorsal  $hD$  ( $X_{19}$ ) and anal  $hA$  ( $X_{20}$ ) fins, length of the base of the dorsal fin  $lDbs$  ( $X_{21}$ ), length of the base of the tail  $lpc$  ( $X_{15}$ ), lengths of folds of the tail fin  $lC$  ( $X_{16}$ ).

Analysis of covariance matrix for the transformation linear measurements of distances indicated that the first three PCA explained about 77% of variance of the morphometric characters (Table 4). Based on the PCA analysis, it can be concluded that:

– 44% of breeding European grayling body shape variation arises from the postdorsal lengths  $poD$  ( $X_{11}$ ), head width  $lac$  ( $X_6$ ) and maximum body height  $H$  ( $X_{12}$ ),

– 19% of the observed variation depends on the eye size  $O$  ( $X_3$ ) and postorbital space length  $poO$  ( $X_4$ ),

– lateral head length  $lc (X_1)$  has a big influence on the variability of the body shape, maximum body height  $H (X_{12})$  explains 14% of the variation the body shape.

It seems that based values of the traits these three components PCA, can be carried selection work out while breeding grayling in RAS.

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## References

- AUGUSTYN L., NOWAK M. 2014. *Assessment of populations of European grayling Thymallus thymallus (L.), in The Dunajec river catchment based on recreational catch records.* Kom. Ryb., 141(4): 9–15.
- BAJIĆ A., JOJIĆ V., SNOJ A., MILJANOVIĆ B., ASKEYEV O., ASKEYEV I., MARIĆ S. 2018. *Comparative body shape variation of the European grayling Thymallus thymallus (Actinopterygii, Salmonidae) from wild populations and hatcheries.* Zool. Anz., 272: 73–80.
- BEACHAM T.D. 1990. *A genetic analysis of meristic and morphometric variation in chum salmon (Oncorhynchus keta) at three different temperatures.* Can. J. Zool., 68(2): 225–229.
- BEACHAM T.D., MURRAY C.B. 1986. *The effect of spawning time and incubation temperature on meristic variation in chum salmon (Oncorhynchus keta).* Can. J. Zool., 64(1): 45–48.
- COWX I.G. 1994. *Stocking strategies.* Fisheries Manag. Ecol., 1: 15–30.
- CRAMON-TAUBADEL N. VON, LING E.N., COTTER D., WILKINS N.P. 2005. *Determination of body shape variation in Irish hatchery-reared and wild Atlantic salmon.* J. Fish Biol., 66(5): 1471–1482.
- CURRENS K.P., SHARPE C.S., HJORT R., SCHRECK C.B., LI H.W. 1989. *Effect of different feeding regimes on the morphometrics of chinook salmon (Oncorhynchus tshawytscha) and rainbow trout (O. mykiss).* Copeia, 3(8): 689–695.
- DUFTNER N., KOBLMÜLLER S., WEISS S., MEDGYESY N., STURMBAUER CH. 2005. *The impact of stocking on the genetic structure of European grayling (Thymallus thymallus, Salmonidae) in two alpine rivers.* Hydrobiologia, 542: 121–129.
- ELLIOTT N.G., HASKARD K., KOSLOW J.A. 1995. *Morphometric analysis of orange roughy (Hoplostethus atlanticus) off the continental slope of southern Australia.* J. Fish Biol., 46(2): 202–220.
- FRANSSSEN N. 2011. *Anthropogenic habitat alteration induces rapid morphological divergence in a native stream fish.* Evol. Appl., 4: 791–804.
- FRASER D.J. 2008. *How well can captive breeding programs conserve biodiversity? A review of salmonids.* Evol. Appl., 1: 535–586.
- GASTON K., LAUER T. 2015. *Morphometric variation in bluegill Lepomis macrochirus and green sunfish Lepomis cyanellus in lentic and lotic systems.* J. Fish Biol., 86 (1): 317–332.
- GRIMES C.B., JOHNSON, A.G., FABLE W.A. JR. 1987. *Delineation of king mackerel (Scomberomorus cavalla) stocks along the US east coast and in the Gulf of Mexico.* In: *Proceedings of the stock identification workshop.* Eds. H.E. Kumpf, R.N. Vaught, C.B. Grimes, A.G. Johnson, E.L. Nakamura. NOAA technical memorandum NMFS-SEFC, pp. 186–187.
- HOLČIK J. 2004. *Fishes of the Poprad River. Current status and utilization.* Arch. Pol. Fish., 12 (suppl. 2): 91–102.
- HONKANEN J.O., KOSTAMO A., KUKKONEN J.V.K. 2005. *Toxicity of a phytosterol mixture to grayling (Thymallus thymallus) during early developmental stages.* Arch. Environ. Con. Tox., 48: 391–396.
- HORKÁ P., HORKÝ P., RANDÁK T., TUREK J., RYLKOVÁ K., SLAVÍK O. 2015. *Radio-telemetry shows differences in the behaviour of wild and hatchery-reared European grayling Thymallus thymallus in response to environmental variables.* J. Fish Biol., 86 (2): 544–557.
- KOTTELAT M., FREYHOF J. 2007. *Handbook of European Freshwater Fishes.* Publishing Kottelat. Cornol, Switzerland. Freyhof. Berlin, Germany.
- KUPREN K., NOWOSAD J., ŽARSKI D., TARGOŃSKA K., HAKUĆ-BŁAŻOWSKA A., KUCHARCZYK D. 2015. *Early development and allometric growth in Laboratory-Reared European Chub Leuciscus cephalus (Linnaeus, 1758).* Turk. J. Fish. Aquat. Sc., 15(3): 391–398.

- LEPIĆ P., BLECHA M., KOZÁK P. 2019. *Intensive winter culture of Chondrostoma nasus (Linnaeus, 1758) and Vimba vimba (Linnaeus, 1758) for spring restocking*. Turk. J. Fish. Aquat. Sc., 20(2): 97–102.
- MALLET J.P., LAMOUROUX N., SAGNES P., PERSAT H. 2000. *Habitat preferences of European grayling in a medium size stream, the Ain river, France*. J. Fish Biol., 56(6): 1312–1326.
- MARCUS L.F. 1990. *Traditional morphometrics*. In: *Proceedings of the Michigan morphometrics workshop*. Eds. F.J. Rohlf, F.L. Bookstein. Special Publication Number 2. University of Michigan Museum of Zoology. Ann Arbor., pp. 77–122.
- OCALEWICZ K., FURGALA-SELEZNIOW G., SZMYT M., LISBOA R., KUCINSKI M., LEJK A.M., JANKUN M. 2013. *Pericentromeric location of the telomeric DNA sequences on the European grayling chromosomes*. Genetica, 141(10–12): 409–416.
- ORSKA J. 1956. *The influence on temperature on the development of the skeleton in teleost*. Zool. Pol., 7: 261–326.
- OVIDIO M., PHILIPPART, J.C. 2002. *The impact of small physical obstacles on upstream movements of six species of fish*. Hydrobiologia, 483(1–3): 55–69.
- PENCZAK T., KRUK A. 2000. *Threatened obligatory riverine fishes in human – modified Polish rivers*. Ecol. Freshw. Fish, 9(1–2): 109–117.
- PULCINI D., WHEELER P.A., CATAUDELLA S., RUSSO T., THORGAARD G.H. 2013. *Domestication shapes morphology in rainbow trout Oncorhynchus mykiss*. J. Fish Biol., 82(2): 390–407.
- STRINGWELL R., LOCK A., STUTCHBURY C.J., BAGGETT E., TAYLOR J., GOUGH P.J., GARCIA DE LEANIZ C. 2014. *Maladaptation and phenotypic mismatch in hatchery-reared Atlantic salmon Salmo salar released in the wild*. J. Fish Biol., 85(6): 1927–1945.
- SURRE C., PERSAT H., GAILLARD J.M. 1986. *A biometric study of three populations of the European grayling, Thymallus thymallus (L.), from the French Jura Mountains*. Can. J. Zool., 64(11): 2430–2438.
- SZMYT M., GORYCZKO K., GRUDNIEWSKA J., LEJK A.M., WIŚNIEWSKA A.M., WOŹNIAK M. 2013. *Preliminary results of European grayling (Thymallus thymallus L.) fry rearing to the autumn fingerlings stage*. Pol. J. Natur. Sc., 28(4): 471–483.
- TUREK J., HORKY P., VELISEK J., SLAVIK O., HANAK R., RANDAK T. 2010. *Recapture rate and growth of hatchery-reared brown trout (Salmo trutta v. fario, L.) in Blanice River and the effect of stocking on wild brown trout and grayling (Thymallus thymallus, L.)*. J. Appl. Ichthyol., 26(6): 881–885.
- TUREK J., ŽILÁBEK V., VELÍSEK J., LEPIĆ P., ČERVENY D., RANDAK T. 2018. *Influence of geographic origin on post-stocking survival and condition of European grayling (Thymallus thymallus) in a small river*. Aquat. Living Resour., 31, 29.
- VLADYKOV V.D. 1934. *Environmental and taxonomic characters of fishes*. Biological Board of Canada Royal Canadian Institute, 43: 99–140.
- WEISS S. J., KOPUN T., SUSNIK BAJEC S. 2013. *Assessing natural and disturbed population structure in European grayling Thymallus thymallus: melding phylogeographic, population genetic and jurisdictional perspectives for conservation planning*. J. Fish Biol., 82(2): 505–521.
- WIŚNIEWSKI W., AUGUSTYN L., BARTEL R., DEPOWSKI R., DĘBOWSKI P., KLICH M., KOLMAN R., WITKOWSKI A. 2004. *Restitution of migratory fish and the patency of Polish rivers*. Warszawa, Poland: WWF.
- WIŚNIEWSKA A.M. 2008. *Application of multidimensional exploration techniques in morphological studies of fish on the example of carp (Cyprinus carpio L.)*. In: *Application of statistical methods in scientific research*. Eds. J. Jakubowski, J. Wątroba. StatSoft, Kraków, Poland, pp. 379–393.
- WITKOWSKI A. 1975. *The grayling (Thymallus thymallus L.) from the rivers of Lower Silesia*. Acta Hydrobiol., 17: 355–370.
- WITKOWSKI A., KOWALEWSKI M. 1979. *Biometrics of the grayling Thymallus thymallus (L.) (Os-teichthyes: Thymallidae) from the River Dunajec basin*. Acta Hydrobiol., 21: 301–312.
- WITKOWSKI A., KOWALEWSKI M., KOKUREWICZ B. 1984. *Lipień*. Państwowe Wydawnictwo Rolnicze i Leśne, Warszawa.

