



## Age and Growth of the Bulgarian Minnow, *Phoxinus strandjae* (Drensky, 1926) (Actinopterygii: Cypriniformes: Cyprinidae) Living in Melen River Basin (Düzce, Turkey)

İlkay Altınışık<sup>1</sup> , Deniz Yağlıoğlu<sup>2\*</sup> 

<sup>1</sup>Graduate School of Natural and Applied Sciences, Department of Biology, Duzce University, Duzce, Turkey

<sup>2</sup>Department of Biology, Faculty of Arts and Science, Duzce University, Duzce, Turkey

### Abstract

This study aims to present the growth parameters of Endangered (EN) (IUCN Redlist). *Phoxinus strandjae* which until very recently has been believed to be existing only in a very limited area around Turkey's Thrace region and in Bulgaria (in Europa continent). At the same time, this study is the first to be conducted on an Asian continent population. The study was performed on 159 individuals of *P. strandjae* species obtained during fauna follow-up project conducted in October, 2015 in Kucuk Melen creek in Melen River basin. The examined samples include 55 (34.59%) female and 104 (65.41%) male specimens. Female to male ratio was found as 1:1.89 among 159 specimens. As a result of examining otolith and scale readings of individuals, the age distributions were determined between 0 to 3 for females and 0 to 4 for males. The lengths of females examined within the study was determined as 78.16 mm max., while of the males was determined 90.00 mm in male; the maximum weights for females and males were 5.54 g and 7.78 g, respectively. The length-weight relationship for female and male individuals of *P. strandjae* were  $W=0.00786 \times L^{3.1689}$  and  $W=0.0083 \times L^{3.1339}$ , respectively. The longitudinal growing parameters calculated according to von Bertalanffy formulation for female and male individuals, and asymptote value for female individuals ( $L_{\infty}$ ) was calculated as 17.509 cm, Brody's growth coefficient (K) as  $0.1191 \text{ year}^{-1}$ , hypotheticage of fish before hatching ( $t_0$ ) as  $-1.61512$ ; while the same values for male were calculated as  $L_{\infty}=18.356 \text{ cm}$ ,  $K=0.1102 \text{ year}^{-1}$ ,  $t_0=-1.63994 \text{ year}$  and for all populations as  $L_{\infty}=17.642 \text{ cm}$ ,  $K=0.1179 \text{ year}^{-1}$ ,  $t_0=-1.5942 \text{ year}$ . This is the first study performed about age and growth parameters of *P. strandjae* on Anatolia population. At the end of the study, it was found that Bulgarian minnow (*P. strandjae*) in Melen River basin demonstrating positive allometric growth.

### Keywords:

*Phoxinus strandjae*, Bulgarian minnow, Age, Growth parameters, Melen River basin.

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\*Corresponding Author: Deniz YAĞLIOĞLU, E-mail: denizyaglioglu@duzce.edu.tr

## Introduction

In relation to very few numbers of scientists studying freshwater fishes, there is an apparent lack of studies on biological features in Anatolia, besides there still are uncertainties about many species' living areas' borders and on species' systematic distinction. Recently, new species are being recorded in Turkey's inland waters and seas, existing species systematic statuses are being revised and new species are being added to world fish literature. Today, "using the nature sustainably" that overemphasized by scientists, environmental organizations and fishery industry managers, is not just being exploiting the fishery sources but plays an important role for benefitting from nature totally in an economic manner. Also, supporting scientific studies on species being important bio-monitors those constituting vital parts of the ecosystem, not having an economical significance but important in for understanding the nature, plays an important role in understanding the nature and determining the degraded ecosystem via various human activities.

Although, *Phoxinus strandjae* (Drensky, 1926), which has been thought to exist only in a very limited area including the Thrace region of Turkey and Bulgaria until very recently, exists in different basins throughout the Anatolia (Sac and Ozulug, 2015). *P. strandjae*, also was catalogued in red list by International Union for Conservation of Nature and Natural Resources (IUCN) and evaluated as an endangered (threatened) species. The facts that the species being determined in 9 different streams in a recent bio-diversity inventory study performed for the species in Duzce (Anonymous, 2014) and being determined the only species in some streams in follow-up studies (Anonymous, 2015), make the scheduling of this study inevitable and it is thought that the results may provide important data in terms of following this species and the regional ecosystem in future.

This study was performed to determine the growth parameters of Duzce Melen river basin population of *P. strandjae* existing in Anatolia (in Asia continent).

## Materials and Method

In the present study, the *P. strandjae* individuals, those were obtained by T.R. Ministry of Forestry and Water Affairs, 9th Regional Directorate, Duzce Branch Office within the scope of "Duzce Ecosystem, Fauna and Flora Monitoring Project" in October 2015. Sampling was done through Kucuk Melen Creek in Melen River Basin and totally 159 pieces of *P. strandjae* individuals were obtained. Study was performed in Duzce University, Faculty of Arts and Science, Biology Department, Marine Biology Research Laboratory. Sampling data of the species was given in Table 1 and sampling location on map was shown in Figure 1.

Table 1. Sampling data of *Phoxinus strandjae*. Standard deviations of mean standart length (TL) of samples are given brackets.

Sampling area	Locations	N (Male/Female)	Mean TL (mm) (±SD)
Küçük Melen (Saklıkent linn)	40° 56' 30.4" N 31° 29' 18.5" E	159 (104/55)	46.84 (17.06)

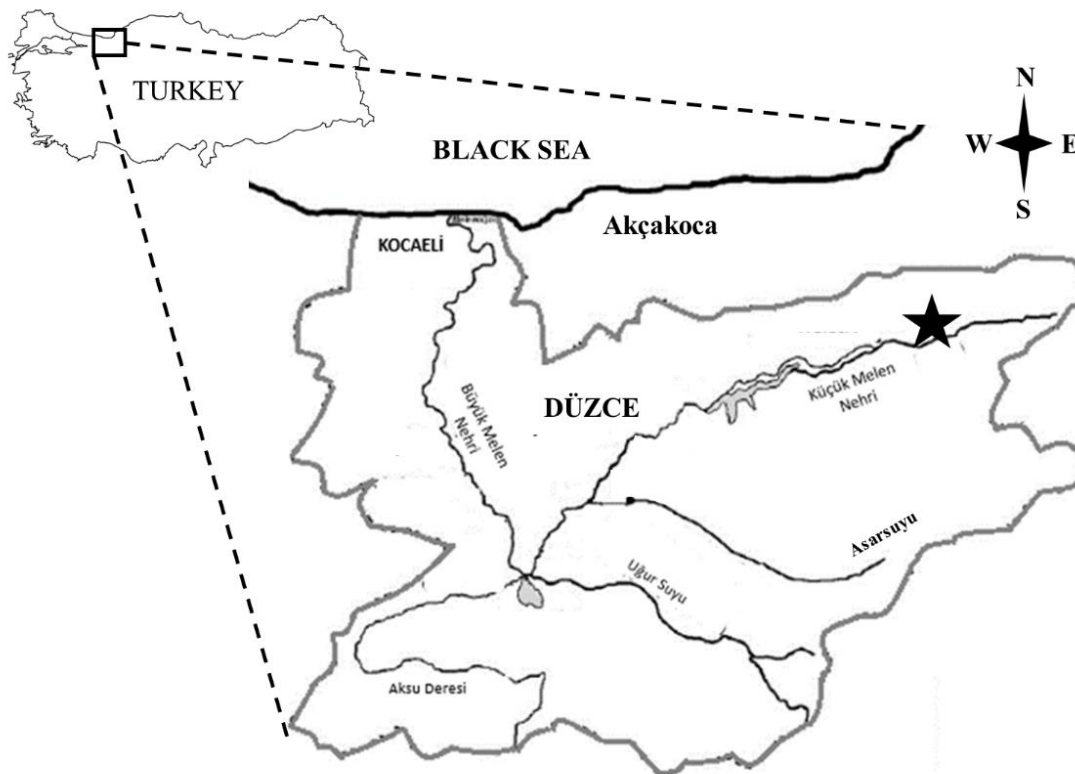


Figure 1. Sampling area maps of *P. strandjae* on the Melen River Basin

*P. strandjae*, from Cyprinidae family, has colors ranging from light green to dark brown and a characteristic line including dark dots on a lateral line from the eye to the beginning of tail. It has a single dorsal fin. The number of scales on lateral line ranges between 75-84, and vertebra count was determined as 34.

Upon catching, *P. strandjae* individuals were transferred to laboratory in cold chain and their total lengths (TL) and total weights (TW) were recorded by using a caliper and a scale which were having sensitivity of 0.01 mm and 0.01 g, respectively. Gender of 159 individuals were determined via gonads. Ages were read by using otoliths and scales. Otoliths and scales were removed and put into envelopes for age reading afterwards. In age determination, otoliths, after being washed and cleaned, were examined by independent readers under stereo microscope (with reflected light), and 3 different readings were compared with age data originally determined (Williams and Bedford, 1974). Length-weight relationship model was determined according to Ricker (1975):  $W = aL^b$  where  $W$  is weight in g,  $L$  is TL in cm,  $b$  represents the length-weight factor and  $a$  is a constant. The relationship between total length and weight was calculated for all specimens as well as for males and females separately. The Student's  $t$ -test was applied to determine the significance of differences between the isometric growth ( $b = 3$ ) and the estimated  $b$  value of the equation (Sokal and Rohlf, 1981). The von Bertalanffy function  $L_t = L_\infty (1 - e^{-K(t-t_0)})$  was used to describe growth in size and weight, where  $L$  is total length ( $L_t$ ) at time  $t$ ;  $K$  is a growth constant;  $L_\infty$  is the asymptotic length; and  $t_0$  the theoretical age at length 0.

**Results**

Among the 159 *P. strandjae* individuals examined in study, 55 (34.59%) were females, 104 (65.41%) were males, and Female:Male (F:M) ratio was 1:1.89 (Table 2). Also, in gender distribution according to age groups, it is observed that male individuals are dominant in all age groups. According to X<sup>2</sup> test; apart from the “0” age group, Female:Male ratios in all age groups were found significant. The maximum age was 4 in both sexes. No female individual in 4 years age group in study was observed. The age-length key is given in Table 2. Total length of individuals ranged from 23.32 to 90.00 mm; weight ranged from 0.12 to 7.92 g (Table 3). The maximum lengths were 78.16 mm in females and 90.00 mm in males. In Melen basin *P. strandjae* population examined in study, the oldest female was 3 years old and oldest male was 4 years old. 29 (52.73%) of female individuals, 32 (30.77%) of male individuals, and total 61 (38.36%) of all individuals were in 0 age group. The “4 years” age group having 5 individuals was determined the smallest group (Table 2).

Table 2. Age-length key of *P. strandjae* caught in Melen River Basin. (\* X<sup>2</sup> significant status).

Length intervals (mm)	Age groups					Total
	0	1	2	3	4	
20.01-25.00	3					3
25.01-30.00	20					20
30.01-35.00	34					34
35.01-40.00	4	12				16
40.01-45.00		15				15
45.01-50.00		12				12
50.01-55.00		8				8
55.01-60.00		2	1			3
60.01-65.00			15			15
65.01-70.00			7	13		20
70.01-75.00				5		5
75.01-80.00				2		2
80.01-85.00				1	2	3
85.01-90.00					3	3
<b>Total</b>	61	49	23	21	5	159
<b>%</b>	38.36	30.82	14.47	13.21	3.14	100.00
<b>Mean TL±SD</b>	30.61±3.04	44.49±5.74	63.96±2.60	71.47±4.10	85.53±3.53	46.84±17.05
<b>Mean TW±SD</b>	0.28±0.09	0.99±0.60	2.86±2.03	4.00±0.95	6.66±1.67	1.56±1.71
<b>Males (%n)</b>	32(20.13)	33(20.75)	19(11.95)	15(9.43)	5(3.14)	104
<b>Females (%n)</b>	29(18.24)	16(10.06)	4(2.52)	6(3.77)	0(0)	55
<b>F:M</b>	01:1.10	01:2.06	01:4.75	01:2.50	0	01:1.89
<b>X<sup>2</sup> (a=0.05)</b>	0.14	5.88*	9.88*	3.84*	5*	15.1*

Length-weight relationship regressions were calculated for all individuals (N = 159) as males, females and combined. Length-weight relationships for *P. strandjae* ( $W = aL^b$ ) were calculated for females, males and combined as:  $W = 0.00786xL^{3.1689}$  ( $R^2=0.9897$ );  $W=0.0083xL^{3.1339}$  ( $R^2=0.9741$ );  $W=0.0081xL^{3.1502}$  ( $R^2=0.9802$ ) respectively. In this study, the b value was calculated as 3.1689 for female individuals, 3.1339 for male individuals, 3.1502 for combined sexes and positive allometric growth was detected in all of them. All regression values were found to be highly significant ( $P<0.05$ ). In addition, the length-weight relationships of all *P. strandjae* individuals are presented in Figure 2.

Table 3. Descriptive statistics and length–weight relationships for *P. strandjae*. (*n* is the sample size; *a* and *b* are the parameters of the length-weight relationship; *SE* (*b*) is the standard error of the slope *b*; *CI* is the confidence interval; and *r* is the coefficient of determination).

Sex	n	TL(mm) Lmin- max	TW(g) Wmin- max	Parameters of the L-W relationship					
				a	b	SE (b)	95% CI of b	r	W = a L <sup>b</sup>
Female	104	23.19- 78.16	0.12- 5.54	0.0078	3.1689	0.044	3.080- 3.258	0.9948	$W=0.0078xL^{3.1689}$
Male	55	27.65- 90.00	0.17- 7.92	0.0083	3.1339	0.051	3.034- 3.234	0.9870	$W=0.0083xL^{3.1339}$
Combined	159	23.19- 90.00	0.12- 7.92	0.0081	3.1502	0.036	3.080- 3.221	0.9901	$W=0.0081xL^{3.1502}$

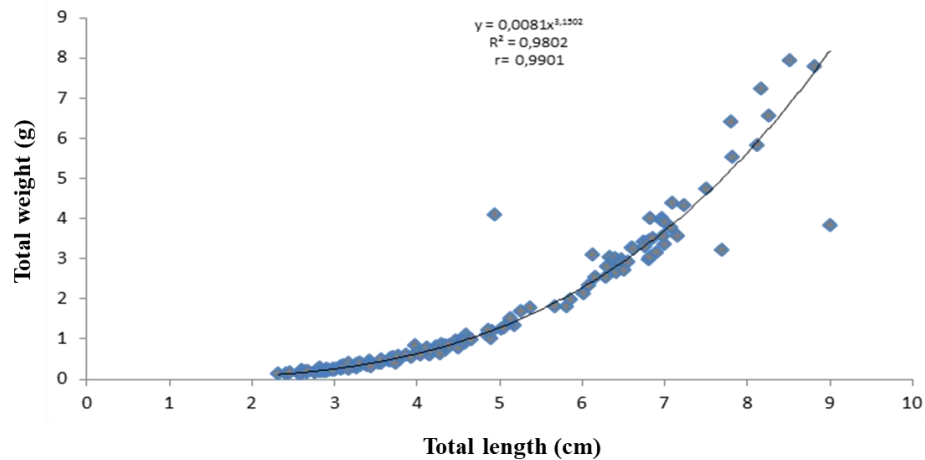


Figure 2. Length-weight relationships of all *P. strandjae* individuals.

In study, von Bertalanffy (1938) longitudinal growing equations calculated separately for females, males and all individuals by using average total length per age data is given in Table 4, growth / length graphic for females and males is given in Figure 3.

Table 4. The von Bertalanffy (1938) growth equations and parameters of *P. strandjae*.

Sex	Growth parameters				Growth Equations
	$L_{\infty}$	K	$t_0$	$\phi$	$L_t = L_{\infty}(1 - e^{-K(t-t_0)})$
Female	17.509	0.119121	-1.61512	1.562511	$L_t=17.509[1-e^{-0.119121(t+1.61512)}]$
Male	18.356	0.110261	-1.63994	1.569978	$L_t=18.356[1-e^{-0.110261(t+1.63994)}]$
Combined	17.642	0.117996	-1.5942	1.564963	$L_t=17.642[1-e^{-0.117996(t+1.5942)}]$

In study, for *P. strandjae* female individuals,  $L_{\infty}=17.509$  cm,  $K=0.119121$  year<sup>-1</sup> and  $t_0=-1.61512$  year,  $\phi=1.562511$  cm values were calculated according to von Bertalanffy (1938) method. For male individuals,  $L_{\infty} = 18.356$  cm,  $K=0.110261$  year<sup>-1</sup> and  $t_0= -1.63994$  year,  $\phi= 1.569978$  cm values were calculated. And for all individuals,  $L_{\infty} = 17.642$  cm,  $K=0.117996$  year<sup>-1</sup> and  $t_0 = -1.5942$  year,  $\phi=1.564963$  cm values were calculated.

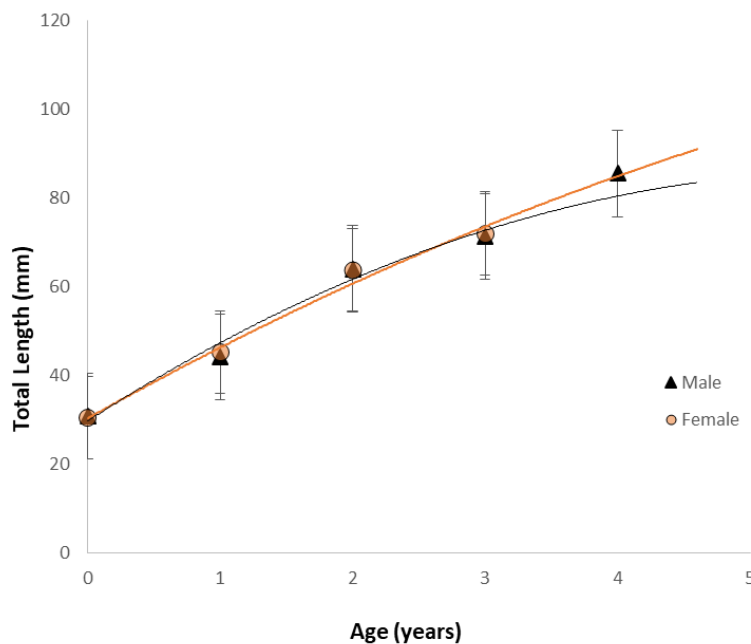


Figure 3. Growth-length graphic for female and male individuals of *P. strandjae*.

Total length values of females, males and all individuals were evaluated for each age group separately, and according to age readings of *P. strandjae* individuals obtained from the researched region, the average length values were 3.06 cm in 0 years age group, 4.45 cm 1 year age group, 6.40 cm in 2 years age group, 7.15 cm in 3 years age group, 8.55 cm years age group. No 4 years old female individual were observed in the population. In other age groups, average length values were found similar for both genders.

Average weight values for each age group of Duzce Melen basin *P. strandjae* population are 0.28 g in 0 years age group, 0.99 g in 1 year age group, 3.86 g in 2 years age group, 4.00 g in 3 years age group, 6.66 g in 0 years age group for female+male individuals. In study, average weights are similar in 0-1 years age group and female individuals' average weights are more than of the

male individuals in 2-3 years age group. Growth/weight graphic obtained for female and male individuals is given in Figure 4.

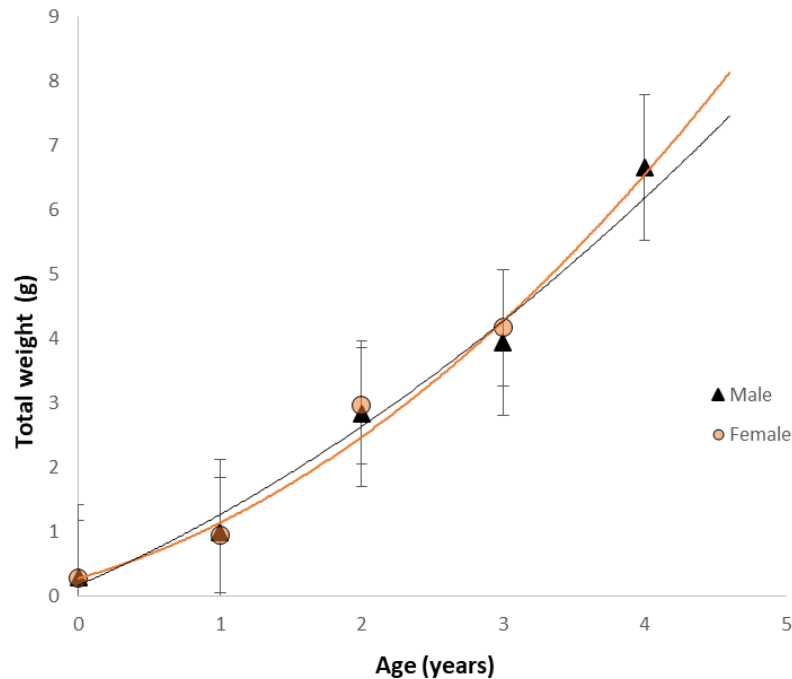


Figure 4. Growth-weight graphic for female and male individuals of *P. strandjae*.

## Discussion

Until very recent *Phoxinus strandjae* was thought to exist only in Bulgaria and Thrace region of Turkey. In recent years, the studies of Sac and Ozulug (2015) and Yigit and Yaglioglu (2017) revealed that the species also exists in different streams in Anatolia. This gap in literature until very recent have resulted in only limited number of studies on this species. In their recent study, Sac and Ozulug (2020) have examined *P. strandjae* Istranca creek population (Thrace, Europa) and they have obtained important data for the growth parameters. Due to that is the only study on the species' age and growth parameters, the data obtained in their study could be discussed in this study. This study is also important as it providing the possibility to discuss the data for Asian and European populations of the species geographical isolated.

In this study, *P. strandjae*'s maximum age is 4, maximum total length is 90 mm and maximum weight is 7.92 g. On the other hand, Sac and Ozulug (2020) have reported maximum age as 6, maximum standard length as 72 mm and maximum weight as 10.596 g in Istranca creek. The reason of the maximum weight difference is that the Istranca population includes 6 years old individuals.

While Sac and Ozulug (2020) have observed 0-1 and 2 years old juvenile individuals in Istranca creek, reproduction of 0 years old females were observed in Melen river basin population. The reasons of the reproduction biology difference between Melen river population and Thrace population were thought that there being no competitive species in Kucuk Melen creek where the

sampling conducted, partly being in a distance from contaminants, and a differently developed evolution progress because of habitat and ecologic niche due to being on the other side of a hydrogeographic barrier such as Bosphorus. The individuals of Istranca creek population were reported mostly 2 (29%) and 3 (33%) years old (Sac and Ozulug, 2020), on the other hand the individuals of Melen river population are mostly 0 (38.36%) and 1 (30.82%) year old.

In length-weight relation, a “b” parameter being above 3 demonstrates an allometric growth. According to the data obtained from this study, a positive allometric growth is shown for *P. strandjae*'s females, males and all individuals in Melen river basin. In Istranca population, it was reported that male individuals having isometric growth, and females and all individuals having, as in our study, positive allometric growth. That difference observed in male individuals may be referenced into age, sexual maturity and sampling areas from different habitats of different continents, so that, in this study female/male ratio was reported as 1:1.15 and in performed analysis it is explained that this ratio is not significantly different from 1:1 ratio ( $X^2 = 1.47$ ;  $p > 0.05$ ) (Sac and Ozulug, 2020). In our study, female:male ratio in all individuals is 1:1.89 ( $X^2 = 15.1$ ;  $p > 0.05$ ). Except the 0 years age group, the difference between female:male ratios in all ages are significant ( $X^2 p > 0.05$ ).

In this study, determined von Bertalanffy growth parameters  $L_t = 17.642(1 - e^{-0.117996(t+1.5942)})$  are different from the other study;  $L_t = 11.78(1 - e^{-0.106(t+2.103)})$  (Sac and Ozulug, 2020). Also, in our study (like in the other study), it is determined that male individuals having a longer asymptotic length ( $L_\infty$ ) and “K” value in females is greater than of males, even by a narrow margin. This difference may be explained with time and geographic difference. Fish populations of the same species from different geographical regions may exhibit highly variable, individual growth rates (Wotton, 1990; Erguden et al., 2010).

As a result, it is thought that this study includes important and beneficial information about a species whose existence in Asia continent (Anatolia) was determined, recently. However, more comprehensive and detailed studies on this population of the species are important in understanding species' evolutionary isolation in different geographic regions.

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### Author Contributions

D.Y.: Designed the study, Validation, Investigation, Formal analysis, Writing - original draft, Writing, I.K.: Data curation, Validation, Supervision, Investigation, original draft, Writing - review & editing.

### Conflict of Interest

The authors declare that they have no competing interests.



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