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Age and growth of non-native monkey goby *Neogobius fluviatilis* (Teleostei, Gobiidae) in the River Ipeľ, Slovakia

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Abstract. Amongst the non-native Ponto-Caspian gobies that have invaded the Middle Danube is the monkey goby *Neogobius fluviatilis* (Pallas, 1814). Despite a strong specialization towards sandy substratum, revealed in a previous study, monkey goby is considered an invasive species and therefore should demonstrate great plasticity in its biological traits. The main aim of the present study was to evaluate the age and growth of a non-native population of monkey goby from the River Ipeľ in Slovakia. Six age groups (0 – V) were observed in the sample from the River Ipeľ ($n = 165$), with males demonstrating faster growth than females, which was reflected both in the smoothing cubic B-spline model and in the annual increment. The cubic B-spline model indicated a reverse Lee's phenomenon, especially in males, which is likely to have arisen from the size selective mortality. Growth of invading monkey goby from the River Ipeľ was slower than that reported for the species' native range. Together with other growth patterns, this may suggest (among other possible explanations) a greater allocation of resources to reproduction than to somatic growth, which is a life-history pattern typical for newly established populations of successful invaders.

Key words: Ponto-Caspian, invasive, length-weight relationship, reverse Lee's phenomenon

Introduction

The monkey goby *Neogobius fluviatilis* (Pallas, 1814) is one of four Ponto-Caspian gobies of the genus *Neogobius* (Il'jin, 1927) that have invaded upstream sections of the Rivers Volga and Danube (Copp et al. 2005). Expansions of gobies have been facilitated by plasticity of phenotype (Kováč & Siryová 2005, Balážová-Lavrinčíková & Kováč 2007), of reproductive characteristics (e.g. extended spawning period, protracted spawning, nest construction and parental care; Kostrzewa & Grabowski 2002, Kostrzewa et al. 2004, Grabowska 2005), as well as low parasite loads relative to

native fish species (Ondračková et al. 2005, Koščo et al. 2006). In Slovakia, the monkey goby was first observed in 2001 in the Danube and its tributaries, including the River Hron (Straňai & Andreji 2002). The morphological differences among three goby species (monkey, bighead and round) demonstrate that monkey goby is not expected to spread to new areas as fast as the round and bighead gobies, and their distribution is likely to be limited to sandy and/or sandy-gravel substrata (Čáповá et al. 2008). Indeed, strong specialization to sandy substratum has been reported from several other areas (Berg 1949, Kazanchev 1963, Svetovidov 1964, Erős et al. 2005).

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Nevertheless, the monkey goby is still considered to be an invasive species (Copp et al. 2009), and one means of determining whether a species is attempting to establish numerous populations is to evaluate the age and growth of recently-established populations (see Copp & Fox 2007). Indeed, growth provides an integrated assessment of the environmental and endogenous conditions (Wootton 1998).

Thus, as a part of a more comprehensive project (see Čápková et al. 2008), the main objective of this study was to 1) describe the basic growth patterns in a non-native monkey goby population including the comparison between males and females; 2) compare these with growth patterns of monkey goby from native areas; and 3) discuss the results within the context of the species' invasiveness.

Material and Methods

Specimens of monkey goby ($n = 182$) were collected by electrofishing on 9 October 2007 from the River Ipel' near the Village of Chľaba, Slovakia ($47^{\circ}49'53.44''$ N, $18^{\circ}50'05.71''$ E). In the laboratory, the specimens were preserved in 4% formaldehyde, and standard length (SL) was measured using digital photographs and the Impor 2.31E software to the nearest 0.01 mm. Body weight was measured to the nearest 1 mg, using a RADWAG balance WPS 360/C/2. Five to eight intact, well developed scales were removed from the left side of each specimen, below the pectoral fin. Sex of the specimens was determined by gonad examination during dissection. The scales were cleaned and compressed between two glass slides. Subsequently, photographs of one scale from each specimen were made by the Canon S 80 camera attached to a Leica MZ 95 stereomicroscope. The age of specimens was determined by counting the number of completely developed annual rings. To check the accuracy of ageing, another reader did a blind analysis of age based on the same approach. Both the scale size and annual increments were measured using the software Motic 2000 to the nearest 0.001 mm.

As the linear model ($R = 0.012SL + 0.159$) of the relationship between scale size (R) and fish length (SL) provided a negative size at which the scales would start to form (-13.25 mm SL), this model was rejected, and therefore, as an alternative, the structural allometric model ($R = 0.0219SL^{0.8981}$) was applied (see e.g. Ricker 1975). Finally, the data were log-transformed into a linear form as $\ln(R) = \ln(a) + \beta \ln(SL) + \varepsilon$.

SL at which the scales start to form and the back-calculated standard lengths were determined using the equation $SL_n = a + (SL - a)R_n / R$, where SL_n is back-

calculated standard length of the fish at the time when the annulus of age n was formed; SL is the standard length of the fish when the scale sample was obtained; R_n is radius of annulus n ; R is total scale radius; a is the SL at which the scales starts to form (estimated as $\ln(R) = \ln(a) + \beta \ln(SL) + \varepsilon$; Holčík & Hensel 1972).

Two growth models were applied to the back-calculated SL data: the smoothing cubic B-spline model (Eilers & Marx 1996, Wood 2006) and the von Bertalanffy growth model (Ricker 1975). The smoothing cubic B-spline (CS) is a method of smoothing (fitting a smooth curve to a set of noisy observations) using a CS function that appears to be an optimal estimator for smooth functions (Wood 2006). The von Bertalanffy growth model (VBGM), i.e. a nonlinear ordinary least square regression model, was calculated according to the equation $SL_t = \max(SL_n)(1 - e^{-k(t-t_0)}) + \varepsilon$, where SL_t is the theoretical SL (in mm; estimated SL in Fig. 1) at age t ; $\max(SL_n)$ is the maximum back-calculated length; k is the growth rate and t_0 is the theoretical age (in years) at length zero. Because of controversial results provided by VBGM when asymptotic length was used (problem of its overestimation, see e.g. Živkov et al. 1999, Copp et al. 2004, Boroń et al. 2008, Zanella et al. 2008), maximum back-calculated length was applied in the present study.

Since the smoothing CS model fits the real data better than VBGM by definition (see above), the CS fit was used as a reference model to test the validity of VBGM for monkey goby. In this test, if significant differences between CS and VBGM fits (i.e. a break of the VBGM curve behind the 95%-confidence interval of the CS) are found, the use of VBGM to fit the data or to predict future growth of monkey goby population examined is considered unreliable.

Because of statistically significant deviation from normality, median length at age plots for male and female of monkey goby were compared with Mann-Whitney U-tests. To characterise the growth, the annual increment ($AI = SL_n - SL_{n-1}$) was calculated as a difference between mean back-calculated SL.

In order to examine Lee's phenomenon (indicated by the CS model), back-calculated standard lengths at the specific ages were compared from one age group to another in a subsequent order (Pedicillo et al. 2008), using t-test.

To examine, whether the relationship between SL and weight (w), which is usually expressed by the equation $w = aSL^{\beta} + \varepsilon$ is isometric ($\beta_1 = 3$) or allometric ($\beta_1 \neq 3$; Wootton 1998), the t-test was applied using the log-log regression model $\log(w) = \log \beta_0 + \beta \log(SL) + \varepsilon$ (Zar 1984). This analysis was performed both on the whole

sample (all specimens caught) and on the sample stratified by sex.

Results

From 182 specimens of monkey goby collected, only 165 were used for age and growth analyses due to unreadable scales of the other 17 specimens. SL of females ($n = 87$) were in interval from 35.88 to 88.71 mm and the SL of males ($n = 78$) were in interval from 33.44 to 123.16 mm (Tables 1 and 2).

Based on the linearized structural allometric regression model $\ln(R) = -3.58 + 0.90\ln(SL)$ ($r^2 = 0.85$), the SL at which the scales start to form was estimated to be 5.41 mm.

Six age groups were observed in the monkey goby population, from age group 0 to age group V (females from 0 to IV, males from 0 to V; Tables 1 and 2). Females were found to dominate in the length classes from 51 to 65 mm SL (Fig. 2), which corresponds mostly to the age groups II and III (Table 1). On the other hand, most of the males attained length class from 46 to 50 mm SL (Fig. 2), i.e. the age group I and II (Table 2), followed by a considerable decline in the

subsequent length frequencies.

The VBGM parameters were $k = 0.30$, $\max(SL_n) = 80.37$, $t_0 = -0.95$, $r^2 = 0.80$ for females and $k = 0.23$, $\max(SL_n) = 111.29$, $t_0 = -0.77$, $r^2 = 0.76$ for males. As expected, the CS model fitted the growth data better both in females ($r^2 = 0.82$) and males ($r^2 = 0.83$). The VBGM curve was found to break the 95%-confidence interval of CS in the second and fifth years in males, and in the fourth year in females (Fig. 1), and therefore, the von Bertalanffy model is not considered a reliable growth model for the monkey goby population examined.

In general, males grew faster than females, especially in the higher ages, which were reflected both by smoothing cubic B spline model (Fig. 1) and annual increment (Tables 1 and 2). However, mean back-calculated standard lengths differed significantly between males of different ages, when subsequent age groups were compared (Table 2), though in females such a significant difference was found only in the third year between specimens of the age groups III and IV (Table 1).

Back calculated lengths showed that median SL of

Table 1. Back-calculated standard lengths (SL in mm) of monkey goby *N. fluviatilis* females from the River Ipeľ in certain years of their life obtained from scales measurements (top); minimum, maximum, mean and standard error of SL in females of age groups 0 – IV at capture, minimum, maximum, mean and standard error for back-calculated SL of the same females at each age, and the number of individuals examined (middle); mean values of SL at each age obtained by the two growth models (bottom). A significant difference in mean back-calculated SL between specimens of subsequent age groups was observed in the third year of life between age groups III and IV ($t = -3.66$, $P = 0.0003$). This difference reflects reverse Lee's phenomenon.

age group	number of annuli	n	SL at capture	mean back-calculated SL at age in years			
			mean	1	2	3	4
0	0+	5	39.45	-	-	-	-
I	1+	9	42.02	36.09	-	-	-
II	2+	20	53.91	36.31	46.88	-	-
III	3+	42	60.85	35.58	45.43	*53.98	-
IV	4+	11	75.39	37.24	48.31	*59.53	68.54
minimum	-	-	35.88	29.54	39.47	46.33	58.87
maximum	-	-	88.71	44.07	55.78	67.3	80.37
mean	-	-	57.92	36.04	46.27	55.13	68.53
standard error	-	-	1.17	0.36	0.49	0.68	2.15
n	-	-	87	82	73	53	11
annual increment	-	-	-	36.04	10.23	8.86	13.40
cubic B-spline	-	-	-	36.04	46.27	55.14	68.54
von Bertalanffy	-	-	-	35.56	47.17	55.77	62.14

Table 2. Back-calculated standard lengths (SL in mm) of monkey goby *N. fluviatilis* males from the River Ipeľ in certain years of their life obtained from scales measurements (top); minimum, maximum, mean and standard error of SL in males of age groups 0 – V at capture, minimum, maximum, mean and standard error for back-calculated SL of the same males at each age, and the number of individuals examined (middle); mean values of SL at each age obtained by the two growth models (bottom). Significant differences in mean back-calculated SL between specimens of subsequent age groups were observed in the first and the second year (between age groups II and III, $t_1 = -2.20$, $P_1 = 0.017$, $t_2 = -1.85$, $P_2 = 0.036$; between age groups IV and V, $t_1 = -4.14$, $P_1 = 0.000$, $t_2 = -3.71$, $P_2 = 0.001$) in the third year (between age groups III and IV, $t_3 = -2.42$, $P_3 = 0.011$, between age groups IV and V $t_3 = -4.50$, $P_3 = 0.000$) and in the fourth year (between age groups IV and V, $t_4 = -4.60$, $P_4 = 0.000$); lower indices denote the particular year of life). These differences reflect reverse Lee's phenomenon.

age group	number of annuli	n	SL at	mean back-calculated SL at age in years				
			capture	1	2	3	4	5
0	0+	5	37.40	-	-	-	-	-
I	1+	12	44.34	37.62	-	-	-	-
II	2+	22	51.61	36.48	45.16	-	-	-
III	3+	16	65.40	38.81	48.12	57.52	-	-
IV	4+	19	82.08	39.89	50.95	63.03	73.39	-
V	5+	4	114.19	48.73	61.31	78.30	91.44	104.40
minimum	-	-	33.44	31.28	37.82	48.25	61.03	98.33
maximum	-	-	123.16	52.58	63.64	82.74	93.92	106.65
mean	-	-	63.04	38.74	48.79	62.34	76.53	104.40
standard error	-	-	2.27	0.50	0.81	1.40	2.06	2.87
n	-	-	78	73	61	39	23	4
annual increment	-	-	-	38.74	10.05	13.55	14.19	27.87
cubic B-spline	-	-	-	38.66	48.96	61.88	77.82	100.45
von Bertalanffy	-	-	-	36.92	52.06	64.11	73.72	81.36

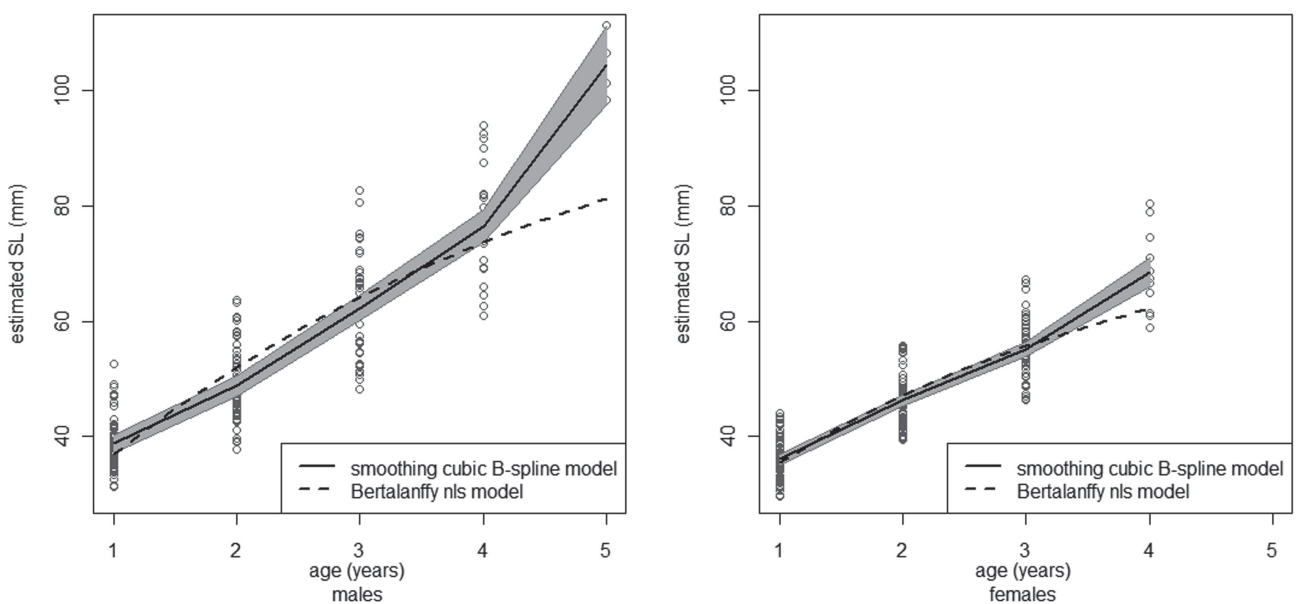


Fig. 1. Growth models of monkey goby from the River Ipeľ, Slovakia: smoothing cubic B-spline model with 95% confidence interval and von Bertalanffy model (nls – nonlinear least squares).

males were significantly larger than median SL of females in all years ($Z_1 = 4122$, $n_1 = 155$, $p_1 < 0.01$; $Z_2 = 2714$, $n_2 = 134$, $p_2 < 0.05$; $Z_3 = 1541$, $n_3 = 92$, $p_3 < 0.01$; $Z_4 = 188$, $n_4 = 34$, $p_4 < 0.05$; Z_i , $i = 1, 2, 3, 4$, where i means particular year). Based on the annual increment, females grew the slowest in their third year of life (8.86 mm), and males in their second year (10.05 mm; Tables 1 and 2).

The relationship between body weight and SL was found to be isometric in females [$\log(w) = -4.79 + 3\log(SL)$, $r^2 = 0.99$, $t = -0.0146$, $n = 87$, $p = 0.506$] but allometric in males [$\log(w) = -5.05 + 3.14\log(SL)$, $r^2 = 0.99$, $t = 7.029$, $n = 78$, $p < 0.001$], as well as in all specimens together [$\log(w) = -4.97 + 3.10\log(SL)$, $r^2 = 0.99$, $t = 5.352$, $n = 165$, $p < 0.001$].

Discussion

The monkey goby is one of the species in which males take care about the eggs in the nests (Dmitrieva 1966, Biľko 1968) and usually reach their sexual

after the age group II (Table 2, Fig. 2), may be the result of increased mortality resulting from their high investment into the parental care (Begon et al. 1990). Males at similar SL achieved greater weight than females (slope β in the length and log weight equation is 3.14 for males and 3 for females), that suggests better condition of males, which may favour their longer live span compared to females, though their decline after the age group II is higher (Tables 1 and 2).

The oldest specimens collected from the River Ipel' were males in the age group V (number of annuly 5+, the sixth year of their life, $n = 4$). However, in their native area, 5+ monkey goby have been reported only from the Molochny Estuary (the River Tashchenak, Tarnavsky 1960), whereas in other areas, specimens not older than 3+ (Biľko 1965) or 4+ (Pavlov 1960 cited from Smirnov 1986) were found. Thus, it appears that in the River Ipel', non-native monkey goby have a relatively long life-span, which increases their chance to reproduce more times and may improve their

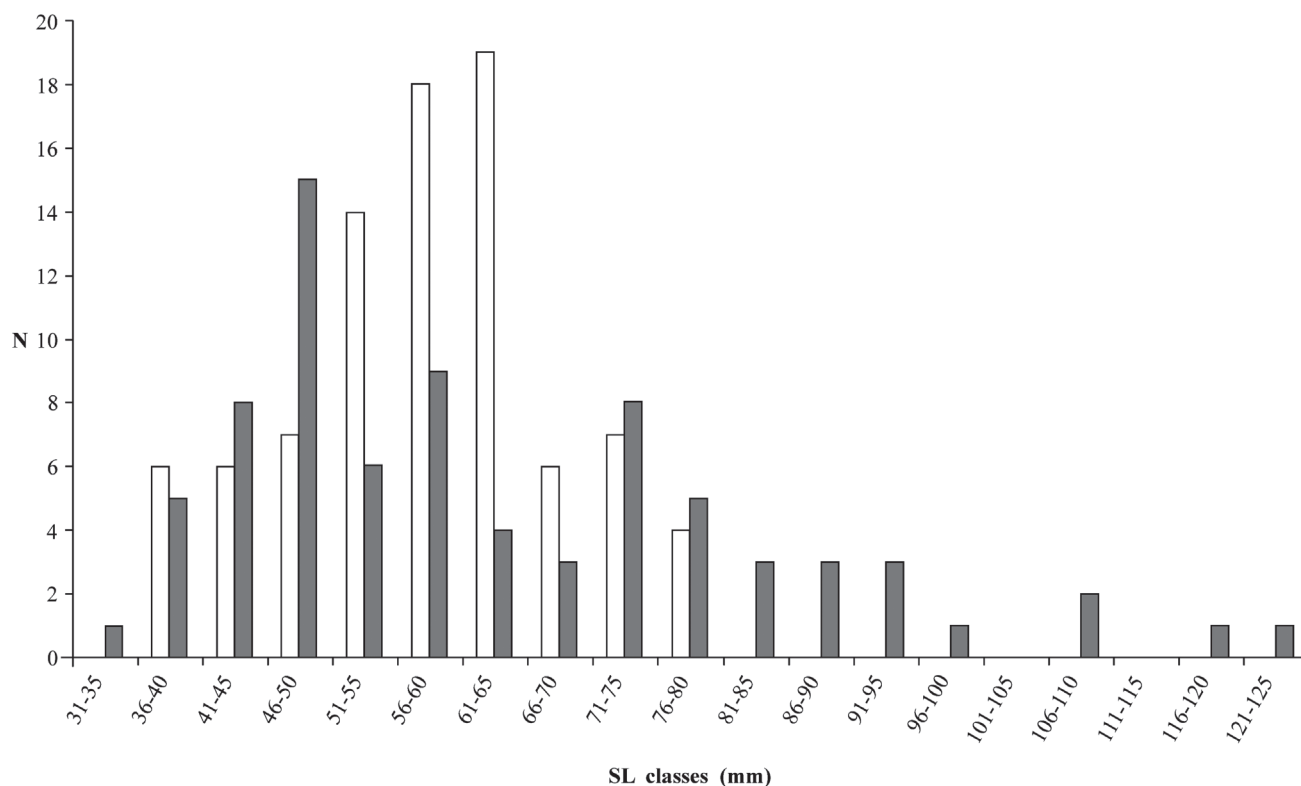


Fig. 2. Length-frequencies (observed standard length) of females (white columns) and males (grey columns) of monkey goby from the River Ipel', Slovakia.

maturity in their second year of life (Smirnov 1986). Furthermore, these males have been reported to stop eating for a long time during the spawning season (Berg 1949, Dmitrieva 1966). Therefore, the shift observed in the age structure, i.e. the decline of males

invasive potential.

Nevertheless, monkey goby belongs to species with a relatively short reproduction cycle, which, together with asynchronous maturation of oocytes and protracted spawning, makes any growth analysis extremely difficult. Indeed, the growth pattern of

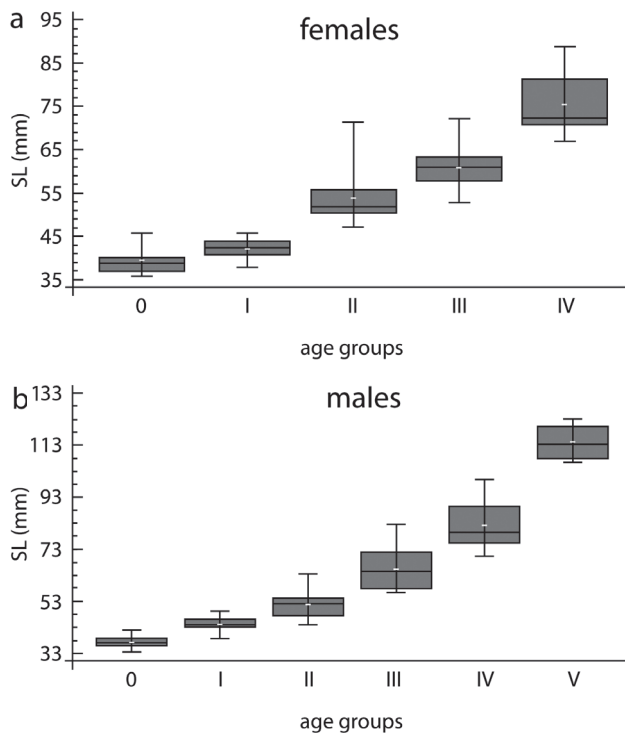


Fig. 3. Observed standard length (SL in mm) distribution of females (a) and males (b) in each age group. Dark boxes enclose middle 50% of observed data, black horizontal lines inside each box represent median, white marks denote mean, and the whiskers represent the range of observed SL.

monkey goby from the River Ipeľ, especially in males (Table 2), differs from a usually expected pattern, as it is characterised with continued, even accelerated, growth in older fish (see also the cubic B-spline model, Fig. 1). This is generally known as the reverse Lee's phenomenon (Tesch 1968). It appears that selective mortality of smaller males at most age groups (0, I, III,

IV, V) caused that mostly larger individuals survived (Fig. 3b). Higher mortality of smaller males from these age groups can hardly result from a continuous predation (or any other) pressure, because in such a case, not only smaller individuals of an age group but also larger individuals of a preceding age group would be a subject of the same mortality rate. Therefore, the selective mortality of smaller individuals from most of the age groups appears more likely to be associated with reproduction, namely with nest guarding. Indeed, larger males of gobies have higher capacity to protect eggs in larger nests (Magnhagen 1995, Lindström 1998). Smaller males guarding their nests can be an easier prey for predators, as well as an object of attack by larger males of their own species (intra-specific competition, e.g. Wootton 1998).

On the other hand, the reverse Lee's phenomenon in females (in their third year of life, Fig. 3a) may be caused by better survival of bigger individuals (Tesch 1968) that allocate more sources to growth than to reproduction. Whereas, the overall slower growth of females than males may be associated with their earlier maturation (Biľko 1971).

Non-native monkey gobies from the River Ipeľ were also found to grow slower than those from native areas (Table 3). In principle, this may have two possible explanations: 1) there are less energy sources available in the River Ipeľ compared to native areas; or 2) the energy sources available are more or less equal but non-native monkey gobies allocate more of their energy resources to reproduction than monkey gobies in their native areas. If the latter explanation was true, then the life-history traits of newly established non-native monkey goby would be similar to the pattern found in other invaders, as

Table 3. Mean observed standard lengths of monkey goby *N. fluviatilis* in specific age groups from native (N) and non-native (NO) areas of distribution.

authors	areas	sex	mean standard length (mm) in specific age groups					
			0	I	II	III	IV	V
Plachá et al. (2010)	River Ipeľ (NO)	female	39	42	54	61	76	-
		male	37	44	52	65	82	104
Biľko (1965)	Dnieper (N)	female	-	61	84	113	-	-
		male	-	62	105	116	-	-
Biľko (1965)	Bug (N)	female	-	73	106	122	-	-
		male	-	76	115	121	-	-
Trifonov (1955)	Azov see (N)	female	-	88	113	-	-	-
		male	-	108	135	-	-	-
Pavlov (1960) cited from Smirnov (1986)	Utlyusky estuary (N)	-	-	97	109	116	-	-

it has been found that allocation of more sources into reproduction than into somatic growth appears to be one of the characteristics of successful invasive organisms (L'avrinciková & Kováč 2007, Kováč et al. 2009, Novomeská & Kováč 2009, Záhorská & Kováč 2009). Indeed, although monkey goby do not spread as quickly as the other invasive goby species [round goby, *Neogobius melanostomus* (Pallas, 1814) and bighead goby *Neogobius kessleri* (Günther, 1861); see Biró 1972, Stráňai & Andreji 2002, Copp et al. 2005, Čápvová et al. 2008 for details], they continue to extend their non-native area of distribution (for example the recent record of monkey goby from the Netherlands; Kessel et al. 2009).

In conclusion, a decline in the ratio of males after the age group II was observed, which may reflect their increased mortality resulting from high investment into the parental care. The von Bertalanffy growth model is not considered a reliable growth model for back-calculated data of the monkey goby population

examined, because the curve has a predefined shape and does not reveal the reverse Lee's phenomenon. Non-native monkey goby from the River Ipeľ grew slower than those from native areas, and this may theoretically be a result of a shift in allocation of sources towards reproduction rather than somatic growth, that appears to be one of the characteristics of the successful invasive fishes. Although monkey goby do not spread as quickly as the other invasive goby species, they continue on extending their non-native area of distribution.

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