Research Article

Ontogenetic phenomena, temporal aspect, and ecological factors in the successful invasion of round goby *Neogobius melanostomus* in the River Danube

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Abstract

Plasticity in life-history traits is thought to be one of the key factors that contribute to adaptation of invasive species to the novel environment at the beginning of an invasion. It has been found that round gobies *Neogobius melanostomus* from freshly invaded areas (invasion front) exhibit different life-history traits than individuals from core or central areas with well-established populations. The theory of alternative ontogeny and invasive potential predicts such differences. Nevertheless, it is difficult to distinguish between the effects of intrinsic ontogenetic phenomena and the effects of particular ecological factors on life-history traits of populations at various stages of invasion. Thus, the main aim of this study was to test the prediction that two sub-populations of round gobies from two habitats exposed to a different intensity of anthropogenic pressures in the River Danube, but at the same stage of invasion, will have significant differences in their reproductive parameters. Females from the relatively undisturbed habitat were predicted to have 1) larger oocytes, 2) lower absolute number of oocytes, and 3) lower real absolute fecundity. For two parameters (1 and 3), differences were statistically significant, as predicted. For parameter 2, the difference was as predicted, though not significant. Several studies suggest that changes in life-history traits observed over the progressing invasions of fishes are typical rather than accidental. They appear to be related to the stage of the invasion and closely associated with ontogenetic phenomena (the capability to generate alternative ontogenies). However, ecological factors also play an important role in this process. If the habitat is exposed to permanent disturbances, the established invasive population will probably keep the same life-history traits as at the beginning of the invasion.

Key words: round goby, stage of invasion, alternative ontogenies, phenotypic plasticity

Introduction

Changes in life-history traits (LHT) of invasive species resulting from biological flexibility and/or phenotypic plasticity appear to play an important role in biological invasions during the colonization of new areas. Variable traits allow invasive species to better adapt to new local conditions which are often different from those in their native regions (Agrawal 2001; Fox et al. 2007; Kováč et al. 2009). Therefore plasticity in LHT is thought to be one of the key factors which contribute to adaptation of invasive species to the novel environment at the beginning of an invasion (Ľavrinčíková and Kováč 2007; Feiner et al. 2012; Záhorská et al. 2014). In terms of classic ecological theory this refers to r- and K-selection (Charlesworth 1971; Roughgarden 1971), and in terms of life history theory (Winemiller 1989; Winemiller and Rose 1992) this means shifts along the continuum between the opportunistic and equilibrium strategies. Both the ecological r/K-selection concept and the life history theory have a parallel in developmental biology – the concept of alternative ontogenies (Balon 2004). Phenotypic plasticity of organisms is a function of epigenesis, the process that shapes developing individuals and has the potential to generate alternative ontogenetic trajectories and life-histories. This is usually expressed through the formation of both generalized and specialized forms within and/or among populations. Generalized forms are characterised by production of a larger number of smaller oocytes with less dense yolk,

i.e. larger number of offspring with reduced fitness, compared to specialized forms that typically produce fewer number of more capable offspring, i.e. a smaller number of larger oocytes with dense yolk (Balon 1989; Flegler-Balon 1989; see also Hôrková and Kováč 2014)".

One of the most successful invasive species, spreading rapidly from its native Ponto-Caspian region and invading diverse habitats in Europe and in North America, is the round goby *Neogobius melanostomus* (Pallas, 1814). Over the last two decades, the round goby has colonized both marine (Baltic Sea coastal areas) and fresh-water ecosystems (European rivers such as the Danube and the Rhine; Borcherding et al. 2011; Kornis et al. 2012) and its rapid expansion across Europe still continues (Kalchhauser et al. 2013; Verreycken 2013). The invasive character of its spread has stimulated a number of studies aimed at LHT variability. It has been found that round gobies can exhibit variability in LHT between native and invasive populations (Hôrková and Kováč 2014), but also within a single population through time (e.g., Kováč et. al 2009; Gutowsky and Fox 2012; Brandner et al. 2013). The latter two papers showed that round goby individuals from freshly invaded areas (invasion front) exhibited different LHT compared to individuals that inhabited established areas for a long period of time (core or central areas). This can be explained using the theory of alternative ontogeny and invasive potential (Kováč 2010) which predicts that successful invaders, producing more specialized forms in their native areas (i.e., under stable organism-to-environment interactions that have resulted from evolution), will shift toward more generalized forms and life histories in unknown and potentially less favourable environments in areas they have invaded. However, once they have established, they will shift back to their original native-like forms (Kováč 2011).

This theoretical approach postulates that regardless of concrete ecological factors, the invasive population has to cope with an unknown environment never experienced in the species' evolutionary history, and the success of such a population depends on its capability to generate phenotypes with more generalized LHT (bet-hedging for more numerous offspring), often substantially different from those of the native population. This is because novelty of the environment itself (regardless of environment characteristics) acts as a stressor on the invading population, resulting in an epigenetically induced LHT shift to a more generalized form. Over

time, the environment will no longer appear novel, hence 'stress' is reduced and the population will return to a more specialized LHT. Early stages of invasion should thus be viewed as an acclimatization period, which is accompanied by considerable changes in gene expression (see e.g. Somero 2010). Once the acclimatization has completed, the phenotypic reaction of the following generations of invading organisms will differ from that of the very first generations. Several hypotheses derived from this theory have been tested successfully in our previous studies (Záhorská and Kováč 2013; Záhorská et al. 2013; Hôrková and Kováč 2014; Záhorská et al. 2014).

Although changes in LHT, including repro-ductive parameters, have been found to be related to stage of invasion, density-dependent processes, as well as other biotic and abiotic factors are also likely to interact with the population and influence its observed LHT (Gutowsky and Fox 2012). Therefore, it is difficult to distinguish between the effect of the intrinsic ontogenetic phenomena (as described in the theory of alternative ontogenies, Balon 2004; Vilizzi and Kováč 2014) and the effect of particular ecological factors on changes in LHT in populations at various stages of an invasion. One way to tackle this problem is to compare LHT of populations or sub-populations at the same stage of invasion but from habitats with different attributes. In such a case, the impact of the stage of invasion (i.e., the factor of time) on changes in LHT can be eliminated. However, the theory of alternative ontogeny and invasive potential (Kováč 2010) predicts that if one of the habitats is exposed to permanent disturbances, the established invasive population is challenged and stressed by the less favourable environment even after a period of time since the beginning of the invasion, and as such should still keep its generalized LHT instead of shifting toward the more specialised form.

Thus, the main aim of this study was to test the hypothesis that sub-populations of round gobies from two different habitats in the Middle Danube, but at the same stage of invasion, will differ in their reproductive parameters. This hypo-thesis predicts that females from the relatively undisturbed habitat (the Karlova Ves side-arm) will have significantly larger oocytes, lower absolute number of oocytes, and lower real absolute fecundity (specialized forms) than females from the disturbed habitat exposed to several anthropo-genic pressures (generalized forms). If not rejected, the hypothesis supports the idea that the observed temporal aspect of the changes in LHT
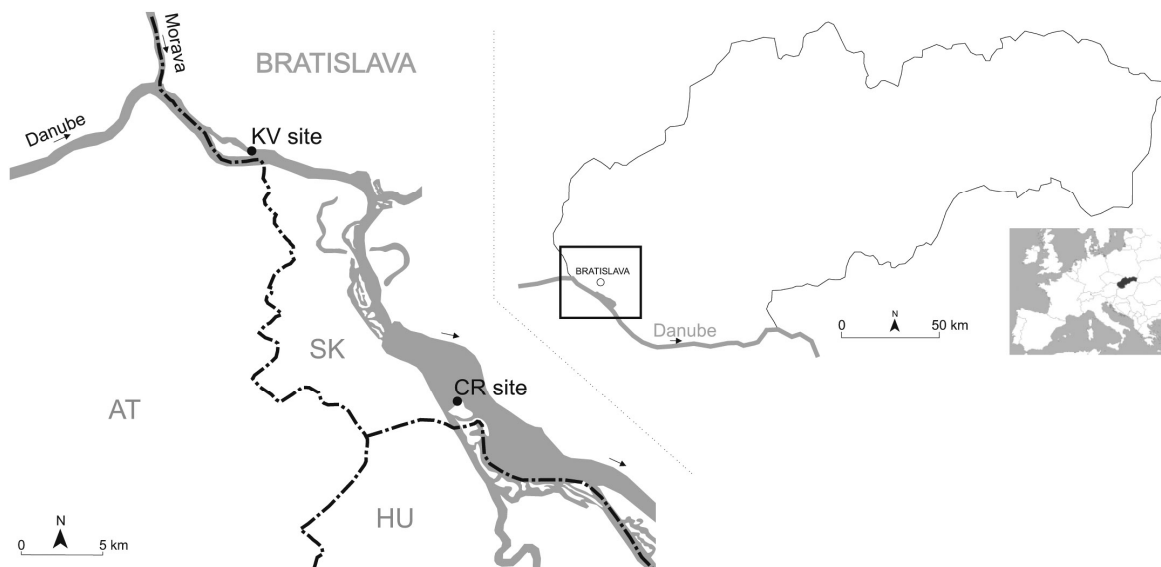
Figure 1. Sampling sites (KV - Karlova Ves side-arm and CR - Čunovo Reservoir, Bratislava, Slovakia) of the two invasive sub-populations of round goby. AT - Austria, HU - Hungary, SK - Slovakia.

(Gutowsky and Fox 2012; Brandner et al. 2013) can be explained with the theory of alternative ontogenies and invasive potential. Nevertheless, because the two habitats examined differed in several ecological factors, and were exposed to contrasting disturbance regimes (natural and anthropogenic), their possible effects on the reproductive parameters examined are also discussed.

Methods

Sampling sites and the evaluation of habitats

Samples of round gobies were collected at two sites located in the middle section of the River Danube (Slovakia): the Karlova Ves side-arm (KV) and the Čunovo Reservoir (CR; Figure 1), both invaded in 2003. Intensity of disturbances at the two habitats was evaluated following the approach of pressure assessment developed for the process of intercalibration for implementing the European Union Water Framework Directive, where a pressure is defined as physical expression of human activities that change the status of the environment (van de Bund 2008). Habitats of the two sub-populations examined in this study were thus characterised using the following nine criteria of pressures: impoundment, riparian vegetation, local habitat alteration, dykes (flood protection), national water quality index (segment scale),

water quality alteration (local scale), navigation, recreational use with high intensity, and heavy predation (cormorants and other predatory birds supported by human-induced habitat alterations). Each pressure was assessed based on a four-scale modality (no, low, medium, high) expressed numerically as 1, 2, 3, 4, respectively. The final pressure index, that is considered to reflect the intensity of disturbances, was then calculated as a sum of all nine criteria values, which provides the possible range from 9 (minimum disturbance) to 36 (maximum disturbance).

Habitats at the two study sites differed from each other in the overall type of water body, substrate, width, depth and current velocity (Table 1). Furthermore, biotic factors, such as composition of the fish community and trophic conditions (Table 1; χ^2-test, $P<0.01$), i.e. diet diversity (Shannon-Wiener index; t-test, $P<0.01$) and relative abundance of prey items (Mann-Whitney U-test, $P<0.01$ for each: Chironomidae larvae, *Corophium* spp., and Cladocera; B. Števove, unpublished data), were also significantly different.

In June 2010, a sudden extreme increase (plus 4 m) in water level of the River Danube at KV occurred, accompanied by highly increased current velocities and a sudden temperature decline. Because the CR water body was controlled by an impoundment, the water level there did not fluctuate (Figure 2).

Table 1. Abiotic and biotic factors at the sampling sites and the differences between the relatively undisturbed (the Karlova Ves side-arm) and disturbed (the Čunovo Reservoir) habitats evaluated by Pressure Index, European Fish Index Plus (EFI+), Fish Index Austria (FIA) and Fish Index of Slovakia (FIS).

Parameter	Karlova ves side-arm (KV)		Čunovo reservoir (CR)	
	Abiotic Parameters			
Coordinates	48°08'43" N; 17°04'10" E		48°02'01" N; 17°13'50" E	
River km	1872		1852	
Description of habitat	parapotamal type side-arm		reservoir	
Bottom	gravel and/or silt		gravel	
Banks	natural banks strengthened with rip-rap stones		artificial banks covered by rip-rap stones	
Width at sampling sites	56 m		2,600 m	
Depth at sampling site	approximately 3.5 m		from 1 to 4 m	
Current velocity	from 0 m.s⁻¹ (dry periods) to 1 m.s⁻¹ (higher water levels)		from 0 to 0.2 m.s⁻¹ (depending on water level and weather conditions)	
	Fish Community			
2007[1]	23 species		19 species	
	Alburnus alburnus (Linnaeus, 1758)	51.1 %	*Neogobius melanostomus*	54.3 %
	Neogobius melanostomus	27.2 %	*Alburnus alburnus*	21.8 %
	Abramis brama (Linnaeus, 1758)	3.5 %	*Carassius gibelio* (Bloch, 1782)	8.3%
	Neogobius kessleri (Günther, 1861)	3.1 %	*Neogobius kessleri*	6.4 %
	Barbus barbus (Linnaeus, 1758)	2.7 %	*Rutilus rutilus* (Linnaeus, 1758)	3.4 %
	Gymnocephalus schraetser (Linnaeus, 1758)	2.6 %	other	< 1 %
	other	< 2 %		
2013[2]	32 species		27 species	
	Blicca bjoerkna (Linnaeus, 1758)	28.8 %	*Alburnus alburnus*	63.9 %
	Neogobius melanostomus	23.4 %	*Neogobius melanostomus*	20.1 %
	Alburnus alburnus	21.8 %	*Blicca bjoerkna*	4 %
	Leuciscus cephalus (Linnaeus, 1758)	5.6 %	*Neogobius kessleri*	2.4 %
	Lota lota (Linnaeus, 1758)	3.4 %	*Abramis brama*	2 %
	Abramis brama	2.2 %	other	< 2 %
	other	< 2 %		
	Round Goby Diet			
Diet composition[3]	a total of 49 food types		a total of 43 food types	
Relative abundance[3]	most important food types:Chironomid larvae, *Corophium* sp. Latreille, 1806		most important food types:Cladocera, chironomid larvae	
Biomass[3]	Bryozoans, *Corophium* sp.		Bryozoans, chironomid larvae	
	Ecological Status			
Pressure index	14/36 (relatively undisturbed)		29/36 (disturbed)	
Fish comunities assessment[1, 2]	EFI+ 2007 = 0.40 (class 3, moderate)		EFI+ 2007 = 0.20 (class 4, poor)	
	EFI+ 2013 = 0.39 (class 3, moderate)		EFI+ 2013 = 0.24 (class 4, poor)	
	FIA 2007 = 3.04 (class 3, moderate)		FIA 2007 = 5.0 (class 5, bad)	
	FIA 2013 = 2.37 (class 2, good)		FIA 2013 = 2.72 (class 3, moderate)	
	FIS 2013 = 0.51 (class 3, moderate)		FIS 2013 = 0.16 (class 5, bad)	

[1]Wiesner et al. 2007;

[2]Joint Danube Survey 3, unpublished (igor.liska@unvienna.org);

[3]B. Števove, unpublished (stevove@fns.uniba.sk).

Processing of samples and statistical tests

Fish samples were collected by electrofishing and fishing rod from March to October 2010 (Table 2). Round goby females were killed with an overdose of 2-phenoxyethanol and subsequently preserved in 4% formaldehyde solution for further analyses. Both total length (TL) and standard length (SL) were measured to the nearest 1 mm using a vernier calliper. Body weight, eviscerated body weight, and gonad weight were measured to the nearest 1 mg, using a KERN ABJ balance. The gonado-somatic index (GSI) was defined as gonad weight/eviscerated body-weight percentage. Diameters of 50 randomly chosen oocytes per individual were measured using an ocular micrometer to the nearest 0.0025 mm, and/or from photographs using the AxioVision 4.8.2 software, Carl Zeiss

Table 2. Sampling dates, numbers of round goby females (n) collected from the Karlova Ves side-arm (KV) and from the Čunovo Reservoir (CR), and methods of sampling – fishing rod (FR) and electrofishing (EF).

KV sub-population			CR sub-population		
Sampling date	n	Equipment	Sampling date	n	Equipment
March 26	16	FR	March 26	9	FR
April 08	27	FR	April 09	18	FR
April 24	23	EF	April 23	26	EF
April 30	28	EF	May 07	21	EF
May 06	23	FR	June 19	26	FR
July 01	14	FR	July 02	29	FR
July 15	17	FR	July 16	14	FR
September 20	29	FR	August 11	21	FR
October 11	2	FR	September 23	30	FR
October 15	5	FR	October 14	3	FR
Total	184			197	
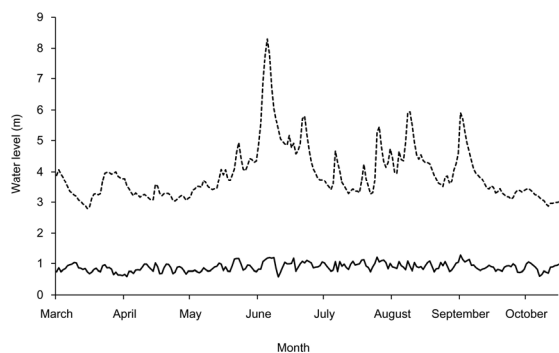
Figure 2. Water levels in the Karlova Ves side-arm (dashed line) and the Čunovo Reservoir (solid line) in 2010. Note the extreme increase of water level in June. Data from the Bratislava Hydrometeorological Station provided by the Slovak Hydrometeorological Institute, Bratislava, 2011.

MicroImaging GmbH. Oocyte size groups were determined based on the oocyte-diameter frequency distribution.

Based on seasonal variation of GSI (Figure 3), the pre-spawning, spawning and post-spawning periods for the samples were determined. The absolute number of oocytes (ANO) was calculated in specimens from pre-spawning and post-spawning periods. Real absolute fecundity (RAF; i.e., the real number of eggs released within a season) was also calculated. During the post-spawning period, ovaries of both sub-populations contained almost no oocytes >0.59 mm diameter. Therefore, RAF was determined based on the number of oocytes >0.59 mm diameter present in ovaries of females during the pre-spawning and spawning periods. By analysing the size distribution of oocytes, two or three spawning batches per season were identified for round goby females. Thus, the RAF was calculated using the formula:

$$RAF = n_o \times n_b$$

where n_o is the number of oocytes >0.59 mm and n_b is the number of spawning batches per season. Absolute number of oocytes and RAF were determined gravimetrically (Holčík and Hensel 1972).

Based on results of the F-test (homogeneity of variance) and Kolmogorov-Smirnov test, either a Mann-Whitney U-test or t-test was used to evaluate differences in fecundity parameters between KV and CR sub-populations. Oocytes in the last stage of maturation were tested because only this category of oocytes corresponds to the size of spawned eggs and thus provides the biological signal about the character of LHT and subsequent ontogeny. All statistical analyses were performed using Past (version 2.15, Hammer and Harper, 2012) and Statistica (version 7.0, StatSoft, 2004).

Results

No significant differences between KV and CR females in the somatic parameters were found (Mann-Whitney U-test, P>0.05; Table 3), which allows testing for differences in their reproductive parameters without any bias related to body size and/or sampling method.

Ovary weight ranged from 0.012 to 1.907 g (mean 0.34 g) in KV females and from 0.004 to 3.194 g (mean 0.37 g) in CR females (Mann-

Table 3. Somatic and reproductive parameters of round goby females from the Karlova Ves side-arm (KV) and the Čunovo reservoir (CR): total length (TL), standard length (SL), body weight (BW), eviscerated body weight (EBW), diameter of oocytes (DO), absolute number of oocytes (ANO), real absolute fecundity (RAF), (n) number of females and/or oocytes, and (SD) standard deviation.

Parameter	KV sub-population					CR sub-population				
	Min	Max	Mean	n	SD	Min	Max	Mean	n	SD
	Somatic parameters									
TL (mm)	60	130	80	184	12.04	54	136	80	197	15.28
SL (mm)	48	107	65	184	9.85	44		65	197	12.61
BW (g)	2.91	37.11	8.44	184	4.58	2.43	41.98	8.89	197	6.10
EBW (g)	2.52	32.47	7.17	184	3.95	2.10	33.68	7.54	197	5.04
	Reproductive parameters									
DO – two size-groups (mm)										
Size-group I	0.06	1.18	0.34	3 060	0.20	0.05	1.17	0.32	2 319	0.22
Size-group II	0.54	2.58	1.54	1 240	0.44	0.53	2.62	1.66	831	0.45
DO – three size-groups (mm)										
Size-group I	0.08	0.54	0.22	118	0.09	0.06	0.45	0.18	187	0.07
Size-group II	0.45	1.09	0.71	69	0.14	0.49	1.16	0.70	75	0.19
Size-group III	1.67	2.73	2.18	63	0.25	1.57	2.38	1.98	88	0.19
DO – whole reproductive cycle (mm)	0.04	2.73	0.53	9 200	0.51	0.04	2.62	0.42	9 850	0.47
DO – last phase of maturation (mm)	1.53	2.73	2.05	411	0.22	1.50	2.62	2.01	505	0.21
ANO	1 613	10 605	4 279	50	1 506	1 578	8 812	4 436	43	2 083
RAF (two batches)	665	7 572	1 952	116	1 071	679	6 561	2 394	86	1 124
RAF (three batches)	998	11 359	2 928	116	1 607	1 018	9 842	3 591	86	1 687

Figure 3. Variation of gonadosomatic index of round goby females from the two invasive sub-populations from the Karlova Ves side-arm (KV, dashed line) and the Čunovo reservoir (CR, solid line) during 2010. Dots indicate mean values, vertical lines indicate the range. Both the KV and CU samples from 3/26 were assigned to pre-spawning period, KV samples 4/8-7/15 and CU samples 4/9-7/16 to spawning period, and the rest of samples to post-spawning period. See Table 2 for sample size.
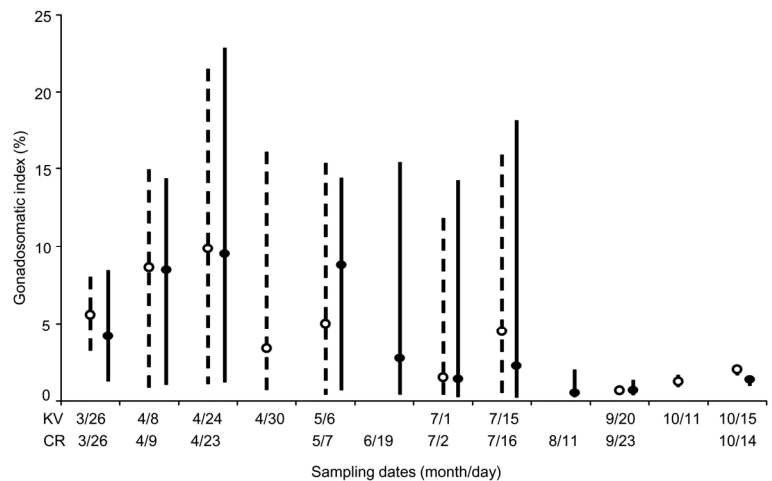
Whitney U-test, $P<0.01$). During the entire reproductive cycle, values of GSI varied from 0.31 to 21.51 % in KV females, and from 0.15 to 22.84 % in CR females. The seasonal variation in GSI indicates that KV females spawned from early April to mid July, whereas in the CR sub-population the greatest portion of females spawned from early April to mid June and only a few females were able to spawn after this period (Figure 3).

Two or three groups of oocytes were determined in both sub-populations during pre-spawning and spawning periods (Table 3). In KV, two size-groups of oocytes were clearly distinguished in 57 % of the females, whereas three size groups of oocytes were found in 3.3 % of the females. In the rest of females (39.7 %), the largest group of oocytes was absent (either because these females were juveniles or they were collected just after they were spent) and/or the next batch was just

under development, i.e., not distinguishable from size-group I. In CR, two size-groups of oocytes occurred in 49.4 % of the females, and three size-groups of oocytes in 5.1 % of the females. In the rest of the females (45.5 %), the largest group of oocytes was absent (for the same reason as in KV sub-population). Egg diameters in the last phase of maturation (mature oocytes that are to be spawned soon) were significantly larger in females from KV than in those from CR (t-test, P<0.01; Table 3).

The values of ANO were found to be lower in KV females than in CR females, though not significantly (t-test, P>0.05; Table 3). The RAF calculated for both two and three batches per season was significantly lower in KV females than in CR females (t-test, P<0.01; Table 3).

The smallest mature female measured 49 mm SL in KV, and 46 mm SL in CR; all females from both sites >59 mm SL were found to be mature.

After the sudden water level increase in KV, a new extra spawning batch was formed in 42 % of the KV females but only in 16 % of the CR females. Values of GSI varied from 0.31 to 15.94 % (mean 3.18 %, SD = 4.39, n = 31) in KV females, and from 0.15 to 18.12 % (mean 1.63 %, SD = 3.57, n = 43) in CR females (Mann-Whitney U-test, P<0.01).

Discussion

Following the hypothesis derived from the theory of alternative ontogenies and invasive potential, females from the relatively undisturbed habitat (KV) were predicted to have 1) larger oocytes, 2) lower absolute number of oocytes, and 3) lower real absolute fecundity than females from the disturbed habitat (CR). This is based on the assumption that after a period of time since the beginning of the invasion, individuals from undisturbed habitats should develop more specialized forms than individuals from disturbed habitats (Balon 2004; Kováč 2010; Hôrková and Kováč 2014; Vilizzi and Kováč 2014), such as habitats exposed to anthropogenic pressures (Záhorská et al. 2013). Specialized forms are characterised by production of a smaller number of larger oocytes with dense yolk i.e., fewer number of more capable offspring (Balon 1989; Flegler-Balon 1989).

For two parameters (1 and 3), differences were statistically significant, as predicted. For parameter 2, the difference was as predicted, though not significant. Summing up, all three reproductive parameters were found not to be at odds with the hypothesis tested. Apart from that, as expected, the smallest mature female from KV was larger than the smallest mature female from CR, though the data did not allow testing this difference. Assuming that both sub-populations had identical LHT at the beginning of the invasion (2003), and assuming that these LHT were the same as LHT found in the CR sub-population in 2010, these findings would demonstrate that in the case of the KV sub-population the LHT started to change as new generations appeared over time. This is the temporal aspect of the invasions demonstrated by the changes in LHT, and it has also been observed elsewhere (Feiner et al. 2012; Gutowsky and Fox 2012; Brandner et al. 2013; Záhorská et al. 2013). Thus, it appears that the time that has elapsed since the beginning of the invasion plays a substantial role in shaping phenotypic plasticity of the invasive species, and this can be explained by ontogenetic phenomena, i.e., the capability of species to generate alternative ontogenies (Balon 2004). In contrast to KV, the CR sub-population was continually exposed to permanent disturbances (expressed by the Pressure Index), and therefore, females have kept such LHT as they probably had at the beginning of the invasion. In other words, the LHT of the two sub-populations, presumably identical at the beginning of invasion, diverged over time, as several generations of round gobies turned in both habitats. Of course, this scenario describing how the two sub-populations achieved different LHTs is only one of a number of alternatives, and further research (especially experimental studies) is necessary to verify the theory of alternative ontogenies and invasive potential.

Nevertheless, even if the results were found to be as predicted, it does not necessarily mean that the ontogenetic phenomenon described by the theory of alternative ontogenies (Balon 2004) always prevails over the effects of particular ecological factors (see e.g., Brownscombe and Fox 2012; Gutowsky and Fox 2012). The sudden extreme increase in water level in June 2010, accompanied with highly increased current velocities and a sudden temperature decline, provided an extra opportunity to test whether this unexpected natural perturbation that occurred at KV, but not at CR, would result in a different response between the two sub-populations. A substantial proportion of females from the KV sub-population responded by increasing their reproductive effort (higher mean GSI, extra batch of eggs), which probably helped to compensate

for the loss which occurred from the interrupted spawning season. Such behaviour was observed in a much lower proportion of females from CR, which were not impacted by the increased water level changes.

Although both sustained habitat degradation (CR) and natural flood disturbance (KV) can induce changes in LHT, they differ in the intensity and duration of effects on a population. Thus, the difference in perturbation duration and timing is the key to explaining the ontogenetic response of the gobies rather than the nature of the perturbation. The rapid response of the KV gobies in the form of a post-flood batch of eggs in fact reflects a shift towards more generalized LHT (increased fecundity) stimulated by a sudden change in environmental cues (short-term perturbation). In other words, the KV females followed the same principle of alternative ontogenies as those from CR. However, because the water level fluctuation affected the KV sub-population for a limited time only, the effect in LHT was not as strong as the effect of permanent disturbance on CR sub-population.

This also demonstrated that the scenario of the temporal aspect described above can be blurred by unexpected environmental perturbations. Nevertheless, the rapid response of the KV round goby females also highlighted the phenotypic plasticity of this species. Without the sudden extreme increase in water level of the River Danube at KV followed by the extra batch of eggs, the contrast in the values of RAF between the KV and CR sub-populations would be even higher. Thus, unexpected environmental perturbations can basically slow down the otherwise inevitable transition from the generalized phenotypes (smaller oocytes, higher fecundity, earlier maturation) at the beginning of the invasion towards the specialized phenotypes (larger oocytes, lower fecundity, later maturation), which normally occurs over a period of time, as the population establishes in the novel environment of the invaded areas.

Of course, under field conditions, it is never possible to have a perfect control over all ecological factors. In fact, apart from the human-induced differences in environmental variables of the two habitats examined as expressed by the Pressure Index, some important differences in natural ecological factors were also obvious. Therefore, we are aware that all these ecological factors also could be responsible for the differences found in the two sub-populations examined in our study, which makes the simple idea proposed

by the theory of alternative ontogenies and invasive potential more complicated.

In conclusion, results of this study together with results of several other studies that were carried out independently (Feiner et al. 2012; Gutowsky and Fox 2012; Brandner et al. 2013; Záhorská et al. 2013) suggest that changes in LHT observed over the progressing invasions of fishes are typical rather than accidental. They appear to be related to the stage of the invasion and closely associated with ontogenetic phenomena (the capability to generate alternative ontogenies). However, local ecological factors also play an important role in this process. For example, if the habitat is exposed to permanent disturbances, the established invasive population will probably keep the same LHT as it did at the beginning of the invasion.

Acknowledgements

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