Journal of FISH BIOLOGY
Published for the Fisheries Society of the British Isles

Journal of Fish Biology (2013) **82**, 1103–1118
doi:10.1111/jfb.12035, available online at wileyonlinelibrary.com

Morphological variability of black bullhead *Ameiurus melas* in four non-native European populations

A. Novomeská*†, S. Katina‡§, G. H. Copp§, G. Pedicillo‖, M. Lorenzoni‖, L. Pompei‖, J. Cucherousset¶ and V. Kováč*

**Department of Ecology, Faculty of Natural Sciences, Comenius University, Mlynská dolina, 842 15, Bratislava, Slovakia, ‡Department of Probability and Mathematical Statistics, Faculty of Mathematics and Physics and Informatics, Comenius University, Mlynská dolina, 842 48, Bratislava, Slovakia, §Centre for Environment Fisheries and Aquaculture Science, Pakefield Road, Lowestoft, NR33 0HT, Suffolk, U.K., ‖Dipartimento di Biologia Cellulare e Ambientale, Università degli Studi di Perugia, Via Elce di Sotto, 06123, Perugia, Italy and ¶Laboratoire Évolution et Diversité Biologique, UMR 5174 – University Paul Sabatier/CNRS, 118 Route de Narbonne, F-31062, Toulouse, France*

(Received 11 October 2011, Accepted 22 November 2012)

External morphology in black bullhead *Ameiurus melas*, a fish species considered to have high invasive potential, was studied in its four non-native European populations (British, French, Italian and Slovak). The aim of this study was to examine this species' variability in external morphology, including ontogenetic context, and to evaluate its invasive potential. Specimens from all non-native populations reached smaller body size compared to individuals from native populations. Juvenile *A. melas* were found to have a relatively uniform body shape regardless of the population's origin, whereas adults developed different phenotypes depending upon location. Specimens from the U.K., Slovak and French populations appeared to be rather similar to each other, whereas the Italian population showed the most distant phenotype. This probably results from the different thermal regime in the Italian habitat. *Ameiurus melas* from non-native European populations examined in this study showed some potential to alter the body shape both within and between populations. The phenotypic plasticity of *A. melas*, however, was not found to be as significant as in other invasive fish species. The results suggest that morphological variability itself is not necessarily essential for invasive success. The invasiveness of *A. melas* is therefore probably favoured by variations in its life-history traits and reproduction variables, together with some behavioural traits (*e.g.* voracious feeding and parental care) rather than by phenotypic plasticity expressed in external morphology.

© 2013 The Authors
Journal of Fish Biology © 2013 The Fisheries Society of the British Isles

Key words: external morphology; geometric morphometrics; growth changes; invasive species; ontogeny.

INTRODUCTION

Variability in growth, development and maturation creates a variety of body shapes within a species (Cadrin, 2000) that, along with ecological interactions of organisms, are directly or indirectly influenced by environmental conditions (Norton *et al.,* 1995). Some ontogenetic features are obscured or displaced by subsequent development, but

†Author to whom correspondence should be addressed. Tel.: +421 2602 96 324; email: kovac@fns.uniba.sk
§Present address: *School of Mathematics and Statistics, University of Glasgow, G12 8QQ, Glasgow, U.K.*

© 2013 The Authors
Journal of Fish Biology © 2013 The Fisheries Society of the British Isles

others persist as a record of an individual's life history. Moreover, interactive effects of environment, selection and genetics on individual ontogenies produce variation in the morphology of a species (Cadrin, 2000). Particularly in the case of introduced species, the ability to adapt to a novel environment plays an important role in determining their invasion success (Sakai *et al.,* 2001; McMahon, 2002). Phenotypic plasticity allows the same genotype to produce a variety of phenotypes in response to different local conditions (Komers, 1997; Schlichting & Pigliucci, 1998). In other words, phenotypic plasticity allows introduced species to respond effectively to environmental changes by modification of morphology, reproduction or survival that mitigate the effects of environmental variation (Stearns, 1983; Meyer, 1987). The detailed examination of external morphology of rapidly dispersing species usually shows a high degree of phenotypic plasticity (Balážová-Ľavrinčíková & Kováč, 2007; Tomeček *et al.,* 2007; Záhorská *et al.,* 2009), and morphometry is one of the most easily accessible means of assessing a species' environmental plasticity (Kováč *et al.,* 1999).

The black bullhead *Ameiurus melas* (Rafinesque 1820) was introduced to Europe in the late 19th and early 20th centuries from North America and is now established in many European countries (Wheeler, 1978; Copp *et al.,* 2005). *Ameiurus melas* possesses several characteristics associated with successful invaders (Ribeiro *et al.,* 2008), such as high reproductive potential, parental care, omnivory, aggressive behaviour and considerable tolerance to water pollution, turbidity, low oxygen concentration, elevated temperatures and a range of pH values (Scott & Crossman, 1973; Karp & Tyus, 1990; Brown *et al.,* 1999; Vila-Gispert *et al.,* 2005; Leunda *et al.,* 2008; Ribeiro *et al.,* 2008; Novomeská & Kováč, 2009). The species, however, has received little attention regarding its external morphology and phenotypic plasticity, attributes that may be important for successful invasion (Záhorská *et al.,* 2009). As geographic variation in morphometry has been used to discriminate local forms of fishes (Cadrin, 2000), the aim of this study was to examine variability in the external morphology of *A. melas* from non-native populations in four European countries (France, Italy, Slovakia and U.K.). Evaluated within an ontogenetic context, morphological variability is discussed in terms of this species' invasive potential.

MATERIALS AND METHODS

Specimens of *A. melas* were collected from four European sites (Fig. 1): Tom's Pond (51° 42′ 26″ N; 0° 10′ 58″ E), Essex, south-east U.K. ($n = 163$); Brière Marsh (47° 22′ N; 2° 11′ W), River Loire drainage, north-west France ($n = 93$); Lake Trasimeno (43° 05′ 21″ N; 12° 09″ 18″ E), Umbria region, central Italy ($n = 342$) and from an oxbow of the River Váh (47° 48′ 30″ N; 18° 07′ 30″ E) in Komárno, south-western Slovakia [$n = 231$; evaluation of the last is based on reanalysed data already published by Novomeská *et al.* (2010)]. The fish were killed with an overdose of anaesthetic and immediately chilled to freezing for subsequent analysis in the laboratory. Once defrosted, all specimens were photographed from the lateral and dorsal perspectives using a Nikon D40 digital camera (www.nikon.com), with 32 morphometric characters (Fig. 2), including standard length (L_S) and total length (L_T), subsequently measured using Impor 2.31E software (Kvant s.r.o.; www.kvant.sk). To examine the patterns of relative growth (ontogenetic aspect), raw values from 30 morphometric characters were plotted against L_S (Kováč *et al.,* 1999). Inter-population comparisons of the distance-based measurements (Table I) were based on values of these 30 morphometric characters expressed in % L_S (Snedecor, 1946). All specimens (females,

© 2013 The Authors

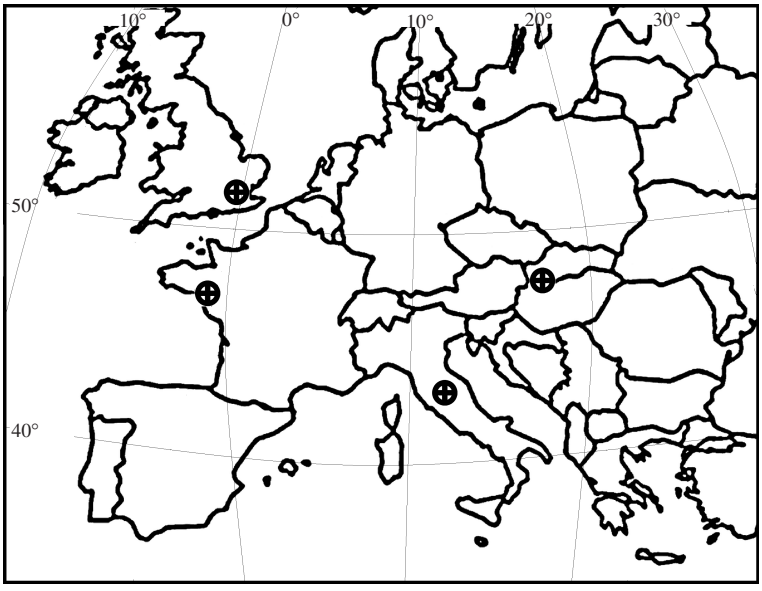

FIG. 1. Sampling sites of four non-native European *Ameiurus melas* populations (U.K., French, Italian and Slovak).

males and juveniles) were analysed together as no sexual dimorphism has previously been found in *A. melas* (Novomeská *et al.,* 2010).

Using triple-regression analysis (TRA), the development of body proportions was assumed not to deviate from isometry (linear regression; the null hypothesis H_0), with gradual allometry (quadratic regression; H_{a_1}) and isometry with an abrupt change (split linear regression; H_{a_2}) being the alternative hypotheses. In isometric development, the morphometric characters develop in proportion to L_S, while in allometric development the growth rate is always different from that of L_S. In isometric with abrupt change, a character develops isometrically up to a specific L_S, then changes to develop allometrically within a short L_S interval and subsequently shifts to another isometric relationship. Shifts, which separated two intervals of isometric growth, were indicated by a breakpoint between two linear regressions (Nickerson *et al.,* 1989). Finally, the model that provided the best fit was assessed using the F-test (Sokal & Rohlf, 1981; see Tables II and III).

The results of TRA (predominant type of development in each population) were used in correspondence analysis (CA) to find out multivariate mutual relationship between populations. The findings were visualized as CA scores and loadings.

As morphological variability can be explained by transformations of homologous features in co-ordinate space (Thompson, 1917), geometrical shape analysis of landmarks [co-ordinates-based measurements in a two-dimensional space (Katina, 2003)] was used. A wide range of body shapes can be described as regular compressions or elongations in a rectangular co-ordinate system (Thompson, 1917), and thus coordinates of 18 landmarks (Fig. 2) in each specimen were marked in the ImporPro 3.2 software (Kvant s.r.o., Bratislava, Slovakia) and evaluated in R software (R Development Core Team; www.r-project.org) with programme routines as established by Katina (2007). Subsequently, the landmark-point co-ordinates were transformed to Procrustes shape co-ordinates using generalized Procrustes analysis (Dryden & Mardia, 1999). First, the centroid of each form was found, and its root-mean-square distance to the landmarks (*i.e.* centroid size, C_S) was identified. Second, the forms were rescaled to $C_S = 1$, the centroids superimposed and the forms rotated around one another about the common centroid until the sum-of-squared distances between corresponding landmarks achieved a minimum over all such rotations. This resulted in Procrustes shape co-ordinates.

© 2013 The Authors

TABLE I. Mean, minimum (Min), maximum (Max) and C.V. of morphometric characters of four non-native European populations of *Ameiurus melas* expressed in % L_S

	U.K.				FRA				ITA				SVK			
Character	Mean	Min	Max	C.V.	Mean	Min	Max	C.V.	Mean	Min	Max	C.V.	Mean	Min	Max	C.V.
Total length (mm)	113·7	72·7	228·0	25·0	152·5	57·7	231·0	30·0	182·1	34·5	261·3	24·1	120·2	20·8	219·7	33·9
L_S (mm)	94·5	60·3	193·8	26·0	130·5	49·1	200·8	30·6	158·3	29·5	229·4	24·3	101·8	17·6	184·9	34·0
% of L_S																
1. Head length	29·0	23·5	35·8	8·5	25·6	23·4	33·2	7·6	29·8	25·8	35·7	5·5	27·2	20·1	34·4	8·2
2. Preorbital distance	7·2	4·8	10·3	13·9	7·4	5·5	9·9	10·9	9·3	6·0	12·6	11·7	7·1	4·8	9·0	12·6
3. Eye diameter	3·8	2·4	5·6	14·9	3·0	2·3	4·3	15·1	2·5	1·7	5·1	18·1	4·0	2·8	6·9	18·7
4. Postorbital distance	18·3	12·2	22·4	11·6	17·3	13·8	20·8	9·4	18·1	14·7	22·3	7·7	16·8	13·7	20·9	7·2
5. Head depth	22·0	15·8	25·9	7·5	21·9	17·5	26·7	8·8	20·7	16·5	26·4	10·1	21·5	18·3	25·9	7·0
6. Predorsal distance	41·6	33·0	48·6	4·4	41·8	38·6	45·9	4·0	40·6	36·4	45·5	3·7	41·0	35·5	45·9	4·5
7. Prepelvic distance	50·1	33·2	55·3	5·1	50·2	43·9	55·0	4·2	51·0	46·4	58·2	3·8	49·9	45·0	57·4	3·9
8. Preanal distance	63·5	52·4	75·8	5·2	64·1	55·5	74·1	4·0	63·7	56·7	71·5	3·0	62·1	58·8	71·2	2·8
9. Postdorsal distance	56·2	44·6	62·9	4·9	56·5	52·1	63·6	4·0	55·5	50·0	60·3	3·1	56·6	50·8	62·7	3·3
10. V–A distance	13·8	8·7	22·3	17·0	14·4	10·0	20·6	13·9	13·3	7·8	18·7	11·6	12·7	9·6	15·5	9·5
11. D–A distance	37·4	30·5	42·7	5·5	38·4	33·4	44·1	5·5	36·0	32·2	42·9	4·6	36·5	32·0	40·4	3·8
12. D–adip distance	35·7	26·2	44·4	8·0	37·2	30·2	46·3	7·4	37·3	32·0	44·5	5·7	36·3	30·3	43·1	5·4
13. Adip–A distance	26·3	20·0	30·6	7·2	27·7	22·9	31·1	6·2	25·3	20·3	32·2	6·9	26·9	22·6	30·7	5·6
14. Adip–posterior of A distance	22·9	16·8	27·9	7·9	22·5	18·1	27·9	8·5	20·8	13·2	26·7	7·4	23·0	18·6	27·3	6·8
15. Posterior of adip–C base	19·2	14·9	23·0	7·5	18·7	15·4	21·6	6·5	17·6	14·2	23·5	7·1	19·8	16·4	23·6	5·7
16. Caudal-peduncle length	17·0	13·8	19·6	6·5	16·4	14·2	19·7	7·4	16·1	11·8	20·6	7·7	16·4	13·7	19·5	6·1
17. Caudal-peduncle depth	14·7	11·3	17·7	6·6	14·5	12·7	16·6	6·3	13·8	10·6	17·8	8·6	14·6	11·8	17·2	5·9
18. Minimum body depth	14·2	10·7	16·2	5·6	13·7	12·3	15·8	5·4	12·5	10·1	16·2	7·1	14·1	11·2	16·4	5·9
19. Body depth	29·7	18·9	40·6	10·4	29·9	24·4	39·1	11·3	26·5	18·7	37·3	12·6	29·8	24·3	36·2	8·6
20. D height	19·9	13·2	25·8	9·2	17·3	12·1	21·0	11·1	16·0	10·7	26·4	13·1	19·3	11·7	23·3	7·8
21. V height	13·4	8·4	17·3	14·2	13·0	8·4	17·5	13·6	13·2	9·0	16·7	9·2	13·8	9·4	18·3	9·6
22. A height	11·5	7·7	15·8	14·2	11·2	6·0	15·8	16·9	11·2	7·1	14·9	13·3	13·3	9·1	17·4	11·2
23. C height	28·5	20·6	38·3	12·7	24·8	18·8	31·1	10·3	21·3	15·0	30·0	14·1	25·2	16·3	31·5	11·2
24. D-base length	9·3	6·8	13·7	11·0	9·0	6·3	11·2	10·3	9·2	5·8	13·5	11·2	9·3	7·0	15·6	13·2
25. Adip-base length	11·0	6·8	15·4	16·1	10·3	7·0	15·0	14·9	10·8	6·7	15·0	13·9	11·7	8·1	22·6	13·7
26. A-base length	22·2	14·3	26·7	10·6	23·0	18·5	27·3	7·8	22·9	17·4	26·6	6·0	23·7	16·2	27·6	6·0
27. C length	20·9	15·9	23·3	6·8	17·3	15·0	21·7	8·4	15·2	11·3	20·9	9·2	18·4	14·0	21·3	6·5
28. P length	15·5	12·1	18·6	7·3	14·1	10·7	18·1	9·9	13·0	9·0	19·5	12·4	15·7	10·8	21·6	8·9
29. Interorbital distance	18·2	13·6	21·7	6·9	19·2	16·6	24·0	7·9	18·4	14·7	23·6	9·3	19·5	15·7	23·3	6·7
30. Head width	26·2	21·5	29·3	4·1	26·1	23·0	29·9	4·9	24·8	20·2	30·3	8·3	27·2	24·2	33·4	5·0

U.K., United Kingdom; FRA, French; ITA, Italian; SVK, Slovak; L_T, total length; D, dorsal fin; P, pectoral fin; V, pelvic fin; A, anal fin; C, caudal fin; adip, adipose fin.

© 2013 The Authors

TABLE II. Triple-regression analysis for morphometric characters (see Table I) of *Ameiurus melas* from U.K. and France

	U.K.									France								
Character	*F*(Q/L)	*P*	*F*(S/Q)	*P*	*F*(S/L)	*P*	Fit	BP	S.E.	*F*(Q/L)	*P*	*F*(S/Q)	*P*	*F*(S/L)	*P*	Fit	BP	S.E.
1. Head length	2·77	NS	−2·23	NS	0·24	NS	L			4·08	<0·05	−0·87	NS	1·56	NS	Q		
2. Preorbital distance	2·59	NS	−6·17	NS	−1·84	NS	L			0·56	NS	0·38	NS	0·47	NS	L		
3. Eye diameter	1·22	NS	−12·86	NS	−5·87	NS	L			3·75	NS	−15·28	NS	−6·11	NS	L		
4. Postorbital distance	0·68	NS	1·17	NS	0·93	NS	L			2·14	NS	−1·38	NS	0·35	NS	L		
5. Head depth	0·69	NS	−0·20	NS	0·24	NS	L			0·98	NS	2·14	NS	1·56	NS	L		
6. Predorsal distance	8·33	<0·01	−29·16	NS	−11·20	NS	Q			9·48	<0·01	6·37	<0·05	8·21	<0·01	S	157·97	6·29
7. Prepelvic distance	4·18	<0·05	−2·44	NS	0·82	NS	Q			0·00	NS	0·04	NS	0·02	NS	L		
8. Preanal distance	1·81	NS	3·11	NS	2·47	NS	L			0·62	NS	1·00	NS	0·81	NS	L		
9. Postdorsal distance	2·71	NS	5·38	<0·05	4·08	<0·05	S	190·70	1·63	0·00	NS	1·39	NS	0·69	NS	L		
10. V–A distance	8·82	<0·01	−31·54	NS	−12·26	NS	Q			2·68	NS	−28·31	NS	−13·25	NS	L		
11. D–A distance	0·69	NS	10·46	<0·01	5·59	<0·05	S	182·85	3·45	0·30	NS	6·74	<0·05	3·53	NS	L		
12. D–adip distance	0·00	NS	0·75	NS	0·37	NS	L			0·15	NS	1·47	NS	0·81	NS	L		
13. Adip–A distance	3·70	NS	−1·80	NS	0·91	NS	L			0·22	NS	1·34	NS	0·78	NS	L		
14. Adip–posterior of A dist.	0·47	NS	7·01	<0·01	3·75	NS	L			0·12	NS	3·07	NS	1·60	NS	L		
15. Posterior of adip–C base	0·77	NS	13·93	<0·01	7·38	<0·01	S	185·81	3·07	3·58	NS	1·87	NS	2·75	NS	L		
16. Caudal-peduncle length	0·43	NS	7·81	<0·01	4·13	<0·05	S	177·21	6·55	1·02	NS	2·19	NS	1·61	NS	L		
17. Caudal-peduncle depth	0·99	NS	18·36	<0·01	9·73	<0·01	S	185·79	2·69	0·20	NS	6·22	<0·05	3·22	NS	L		
18. Minimum body depth	0·00	NS	0·30	NS	0·15	NS	L			0·54	NS	5·42	<0·05	3·00	NS	L		
19. Body depth	6·75	<0·05	−23·58	NS	−8·93	NS	Q			0·10	NS	1·71	NS	0·91	NS	L		
20. D height	4·81	<0·05	2·77	NS	3·82	NS	Q			5·82	<0·05	−29·92	NS	−13·05	NS	Q		
21. V height	2·82	NS	1·01	NS	1·92	NS	L			0·06	NS	0·36	NS	0·21	NS	L		
22. A height	2·08	NS	−9·33	NS	−3·69	NS	L			0·19	NS	−18·62	NS	−9·23	NS	L		
23. C height	3·71	NS	0·61	NS	2·15	NS	L			10·21	<0·01	1·74	NS	6·02	<0·05	Q		
24. D-base length	6·12	<0·05	−12·82	NS	−3·61	NS	Q			8·51	<0·01	−29·02	NS	−11·68	NS	Q		
25. Adip-base length	0·04	NS	−21·59	NS	−10·77	NS	L			0·28	NS	−18·27	NS	−9·02	NS	L		
26. A-base length	1·11	NS	6·19	<0·05	3·67	NS	L			0·65	NS	−32·56	NS	−16·07	NS	L		
27. C length	28·56	<0·01	−3·37	NS	12·19	<0·01	Q			14·93	<0·01	−2·76	NS	5·77	<0·05	Q		
28. P length	2·41	NS	4·28	<0·05	3·37	NS	L			1·96	NS	1·62	NS	1·80	NS	L		
29. Interorbital distance	0·29	NS	11·41	<0·01	5·86	<0·05	S	186·15	3·01	9·20	<0·01	0·96	NS	5·08	<0·05	Q		
30. Head width	0	NS	1·85	NS	0·92	NS	L			6·35	<0·05	2·02	NS	4·22	<0·05	Q		

L, linear; Q, quadratic; S, split-linear development; Fit, best fitting model; BP, breakpoint; D, dorsal fin; P, pectoral fin; V, pelvic fin; A, anal fin; C, caudal fin; adip, adipose fin; NS, non-significant ($P > 0·05$).

© 2013 The Authors
Journal of Fish Biology © 2013 The Fisheries Society of the British Isles, *Journal of Fish Biology* 2013, **82**, 1103–1118

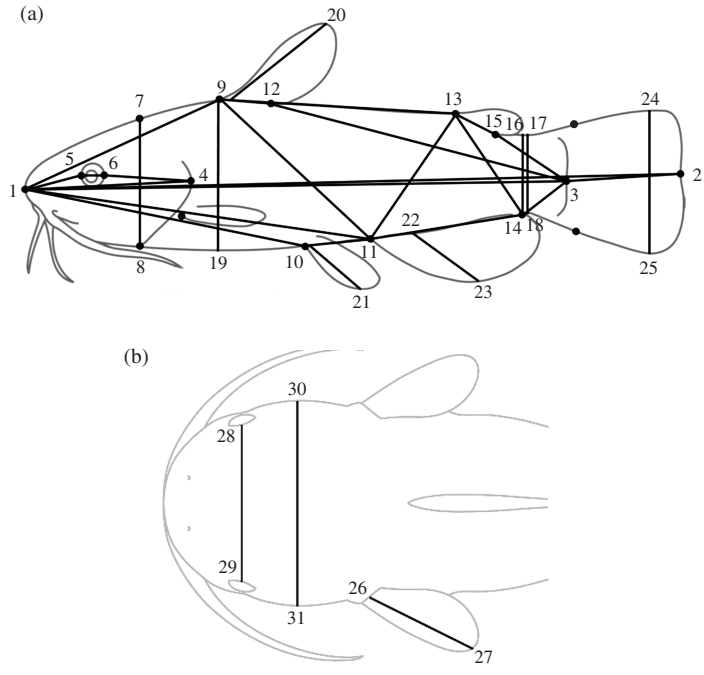

FIG. 2. Schematic illustration of the distance-based morphometric characters (——) and the 18 landmarks (●) used in geometrical shape analysis of *Ameiurus melas*. (a) Lateral and (b) dorsal view: total length (1–2), standard length (1–3), head length (1–4), preorbital distance (1–5), eye diameter (5–6), postorbital distance (6–4), head depth (7–8), predorsal distance (1–9), prepelvic distance (1–10), pre-anal distance (1–11), post-dorsal distance (12–3), V–A distance (10–11), D–A distance (9–11), D–adip distance (9–13), adip–A distance (13–11), adip–posterior of A distance (13–14), posterior of adip–C base (15–3), caudal-peduncle length (14–3), caudal-peduncle depth (14–16), minimum body depth (17–18), body depth (9–19), D height (9–20), V height (10–21), A height (22–23), C height (24–25), D-base length (9–12), adip-base length (13–15), A-base length (11–14), C length (2–3), P length (26–27), interorbital distance (28–29), head width (30–31) [dorsal (D), pectoral (P), pelvic (V), anal (A) and caudal (C) fins and adipose fin (adip)].

To explore the nature of size and shape (form) variability, form space was decomposed into: (1) size-related and (2) size-adjusted sub-spaces. In the size-related sub-space, the allometric pattern (shape changes during growth) was analysed *via* form-space principal component analysis (PCA; relative warp analysis, RWA). In the form-space PCA, the approximate tangent co-ordinates (centred Procrustes shape co-ordinates) were augmented by $\ln C_S$, and variability was decomposed into orthogonal components of size-and-shape variation, which were examined. The first principal component (PC1) usually reflects changes in external morphology associated with growth changes (allometry), which is measured by Pearson product moment correlation coefficient of PC1 scores and $\log_{10} C_S$. In the size-adjusted sub-space, the non-allometric pattern was analysed *via* size-adjusted PCA. In this form-space decomposition, size-adjusted variability is orthogonal (perpendicular) to the growth direction, geometrically speaking, and both sub-spaces are independent; thus, the allometric and non-allometric biological signals are decomposed and analysed separately. In both PCA methods, the first two PCs accounted for a sufficient amount of variability (97·53%).

To explore the nature of differences between the populations, a linear discriminant analysis (LDA) was used. As in PCA, the variability was decomposed into two components: (1) size-related, *i.e.* analysed with shape-space LDA and (2) size-adjusted, *i.e.* analysed with size-adjusted shape-space LDA. In both LDA methods, the first two linear discriminants

© 2013 The Authors

TABLE III. Triple-regression analysis for morphometric characters of *Ameiurus melas* from Italy and Slovakia (see Table II)

	Italy									Slovakia								
Character	*F* (Q/L)	*P*	*F* (S/Q)	*P*	*F* (S/L)	*P*	Fit	BP	S.E.	*F* (Q/L)	*P*	*F* (S/Q)	*P*	*F* (S/L)	*P*	Fit	BP	S.E.
1. Head length	6·22	<0·05	−4·85	NS	0·62	NS	Q			0·34	NS	11·02	<0·01	5·68	<0·05	S	151·12	4·42
2. Preorbital distance	0·62	NS	−17·67	NS	−8·54	NS	L			4·30	<0·05	−21·00	NS	−8·55	NS	Q		
3. Eye diameter	0·85	NS	−15·62	NS	−7·40	NS	L			11·48	<0·01	1·18	NS	6·34	<0·05	Q		
4. Postorbital distance	10·10	<0·01	7·16	<0·01	8·72	<0·01	S	172·27	7·65	0·00	NS	0·10	NS	0·05	NS	L		
5. Head depth	22·97	<0·01	−8·52	NS	6·90	<0·01	Q			5·76	<0·05	−2·26	NS	1·71	NS	Q		
6. Predorsal distance	15·79	<0·01	1·13	NS	8·46	<0·01	Q			1·68	NS	12·35	<0·01	7·05	<0·01	S	150·09	4·09
7. Prepelvic distance	23·51	<0·01	−10·24	NS	6·24	<0·05	Q			6·96	<0·01	0·72	NS	3·83	NS	Q		
8. Preanal distance	32·70	<0·01	−18·97	NS	5·90	<0·05	Q			11·94	<0·01	7·07	<0·01	9·67	<0·01	S	88·52	7·83
9. Postdorsal distance	10·62	<0·01	2·02	NS	6·34	<0·05	Q			5·82	<0·05	−2·21	NS	1·76	NS	Q		
10. V–A distance	2·20	NS	−0·65	NS	0·78	NS	L			3·68	NS	−11·39	NS	−3·95	NS	L		
11. D–A distance	9·38	<0·01	−1·49	NS	3·91	<0·05	Q			5·63	<0·05	5·37	<0·05	5·56	<0·05	S	132·74	7·91
12. D–adip distance	0·60	NS	−0·14	NS	0·23	NS	L			11·00	<0·01	4·64	<0·05	7·91	<0·01	S	155·35	3·62
13. Adip–A distance	4·43	<0·05	5·29	<0·05	4·89	<0·05	S	166·89	10·56	16·09	<0·01	7·31	<0·01	11·92	<0·01	S	86·83	6·90
14. Adip–posterior of A dist.	9·64	<0·01	−0·24	NS	4·68	<0·05	Q			3·41	NS	2·76	NS	3·10	NS	L		
15. Posterior of adip–C base	3·27	NS	−2·83	NS	0·20	NS	L			18·29	<0·01	2·97	NS	10·71	<0·01	Q		
16. Caudal peduncle length	5·83	<0·05	2·99	NS	4·43	<0·05	Q			0·00	NS	8·48	<0·01	4·24	<0·05	S	153·53	4·55
17. Caudal peduncle depth	2·65	NS	−0·23	NS	1·20	NS	L			23·71	<0·01	8·04	<0·01	16·24	<0·01	S	106·85	5·97
18. Minimum body depth	3·86	NS	1·98	NS	2·93	NS	L			34·41	<0·01	10·99	<0·01	23·46	<0·01	S	98·27	4·99
19. Body depth	2·34	NS	4·66	<0·05	3·51	NS	L			0·00	NS	7·47	<0·01	3·73	NS	L		
20. D height	1·74	NS	−329·94	NS	−164·95	NS	L			2·83	NS	−0·56	NS	1·12	NS	L		
21. V-height	0·89	NS	−0·57	NS	0·15	NS	L			0·35	NS	1·61	NS	0·98	NS	L		
22. A height	0·15	NS	−25·78	NS	−12·82	NS	L			14·06	<0·01	11·35	<0·01	13·03	<0·01	S	120·47	5·68
23. C height	0·25	NS	9·14	<0·01	4·70	<0·05	S	226·40	1·13	32·59	<0·01	2·79	NS	17·81	<0·01	Q		
24. D-base length	12·64	<0·01	0·51	NS	6·56	<0·05	Q			13·85	<0·01	5·32	<0·05	9·72	<0·01	S	77·76	6·30
25. Adip-base length	8·79	<0·01	−27·35	NS	−9·64	NS	Q			0·79	NS	−2·14	NS	−0·67	NS	L		
26. A-base length	13·35	<0·01	1·48	NS	7·42	<0·01	Q			0·74	NS	3·31	NS	2·03	NS	L		
27. C length	5·47	<0·05	−17·34	NS	−6·08	NS	Q			2·65	NS	1·85	NS	2·26	NS	L		
28. P length	2·17	NS	0·58	NS	1·37	NS	L			16·96	<0·01	6·33	<0·05	11·84	<0·01	S	98·20	7·06
29. Interorbital distance	20·33	<0·01	−14·60	NS	2·39	NS	Q			2·81	NS	4·67	<0·05	3·76	NS	L		
30. Head width	14·41	<0·01	−7·55	NS	3·24	NS	Q			1·50	NS	10·11	<0·01	5·83	<0·05	S	159·95	4·84

L, linear; Q, quadratic; S, split-linear development; Fit, best fitting model; BP, breakpoint. D, dorsal fin; P, pectoral fin; V, pelvic fin; A, anal fin; C, caudal fin; adip, adipose fin; NS, not significant ($P > 0.05$).

© 2013 The Authors
Journal of Fish Biology © 2013 The Fisheries Society of the British Isles, *Journal of Fish Biology* 2013, **82**, 1103–1118

(LD) covered sufficient amount of variability (90·44 %). Interpretation of the PCs and LDs followed Mitteroecker *et al.* (2004) and Katina (2007), where the effects of each PC and LD were visualized (*i.e.* back-projected into the configuration space) *via* thin-plate spline (TPS) deformation grids, for both directions of negative (left) and positive (right) change, corresponding to the particular PC and LD direction. To ease the visualization, the differences were magnified two (PCA) or three (LDA) times.

The coefficient of difference (C_{dif}) was used to compare L_S as well as the other morphometric characters among the populations examined: $C_{dif} = (x_2 - x_1)(s_1 + s_2)^{-1}$,where x_1 and x_2 are the arithmetic averages, and s_1 and s_2 are the S.D. Real differences between 90% of the populations or morphometric characters exist when $C_{dif} > 1{\cdot}28$ (Mayr *et al.,* 1953). For ontogenetic evaluations, as well as for the inter-population comparisons of distance-based morphometric characters, two sub-samples of 30 specimens with similar range of L_S (*i.e.* $C_{dif} < 1{\cdot}28$) were selected from each population. These two sub-samples consisted of smallest and largest, or juvenile and adult specimens, respectively, depending on the type of analysis. Similarly, a sub-sample of 30 specimens with a comparable range of L_S (specimens with 103–148 mm L_S) was chosen from each population.

To test the assumption that the morphological differences result from different temperatures, latitude was used as a surrogate of temperature regimes, as proposed for one of the most successful of the introduced North American nest-guarding species, pumpkinseed *Lepomis gibbosus* (L. 1758) (Copp *et al.,* 2002; Copp & Fox, 2007). Thus, to examine the potential latitudinal differences among the four populations, morphometric characters (expressed as the mean values of % L_S) were sorted following the co-ordinates of each site from north to south. Pearson correlation coefficients (r) were computed and linear regression analysis (least-square method, $P < 0{\cdot}05$) was applied.

RESULTS

BASIC MORPHOMETRY WITHIN ONTOGENETIC CONTEXT

Specimens of *A. melas* collected from the U.K. ranged from 60·3 to 193·8 mm L_S (mean = 94·5 mm), specimens from the French population ranged from 49·1 to 200·8 mm L_S (mean = 130·5 mm), Italian *A. melas* ranged from 29·5 to 229·4 mm L_S (mean = 158·3 mm) and *A. melas* from Slovakia ranged from 17·6 to 184·9 mm L_S (mean = 101·8 mm (Table I).

In the TRAs, *A. melas* from the U.K. demonstrated isometric development in 17 morphometric characters, allometric development in seven characters and isometric development with an abrupt change in six characters (Table II) that occurred between 177·2 and 190·7 mm L_S [Fig. 3(a)], *i.e.* very late in ontogeny. In *A. melas* from France, the majority (22) of morphometric characters developed isometrically [Fig. 3(b) and Table II], whereas seven characters demonstrated allometric development and only one morphometric character developed isometrically with abrupt change (breakpoint occurring at 158 mm L_S). In the Italian *A. melas,* 12 morphometric characters developed isometrically, 15 allometrically and three isometrically with abrupt changes that occurred between 166·9 and 226·4 mm L_S [Fig. 3(c) and Table III], *i.e.* only in adult specimens. In *A. melas* from Slovakia, 10 characters developed isometrically, seven allometrically and 13 isometrically with abrupt changes that occurred between 77·7 and 159·9 mm L_S [Fig. 3(d) and Table III).

According to the geometric-shape analysis (allometric form-space PCA), adults in all four populations appeared to have a significantly different body shape than juveniles (Fig. 4). During ontogeny, the head became relatively shorter, the eyes migrated anteriorly, the abdominal area became relatively larger and the caudal fin deepened. The development of body proportions in all four populations, however,

© 2013 The Authors

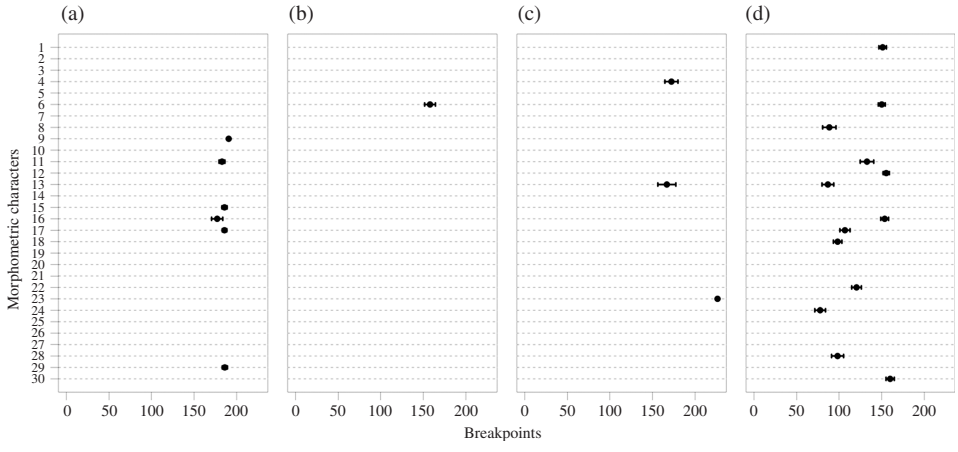

FIG. 3. Estimates of breakpoints and their S.E. (see Tables II and III) in four non-native European populations of *Ameiurus melas*. Breakpoints in the morphometric characters (listed in Table I) of four non-native European *Ameiurus melas* populations demonstrate significant abrupt changes in slope when plotted against standard length (L_S) (Kováč *et al.,* 1999): (a) U.K., (b) France), (c) Italy and (d) Slovakia.

appeared equal and parallel (Fig. 4). Differences between full-model (each of the four populations taken into account separately) and any particular sub-model (all four populations pooled together) revealed that all populations had the same initial shape position ($F_{2,805} = 1{\cdot}6933$, $P > 0{\cdot}05$) and identical developmental slopes, *i.e.* the same developmental directions ($F_{2,805} = 3{\cdot}3862$, $P > 0{\cdot}05$). In all four populations examined, the influence of PC1 highly predominates (96·67%). The effect of developmental changes associated with growth dominated, whereas the inter-population non-allometric variability was almost negligible (only 3·33%; Fig. 4). Even if the direction of the developmental changes was identical in all populations (changes related to the longitudinal axis of body), their intensity in the developmental trajectories was not. As a result, developmental changes associated with growth led to different overall phenotypes among populations.

No significant difference (C_{dif}) was found in body proportions between sub-samples of the 30 smallest specimens from each population. In sub-samples of the 30 largest specimens, however, some statistically significant differences were observed among populations: preorbital distance was smaller in French than in Italian specimens; C-fin length and eye diameter were larger in U.K. than in Italian specimens; eye diameter and C-fin length were significantly larger and preorbital distance significantly shorter in Slovak than in Italian specimens (all differences also visible in Figs 5 and 6). To sum up, juvenile *A. melas* were found to have a relatively uniform body shape regardless of the population's origin, whereas adults developed different phenotypes depending upon location.

INTER-POPULATION COMPARISONS

The highest similarity in the development of individual distance-based morphometric characters was found between U.K. and French populations, with 17 (predominantly isometric) characters showing the same developmental pattern

© 2013 The Authors

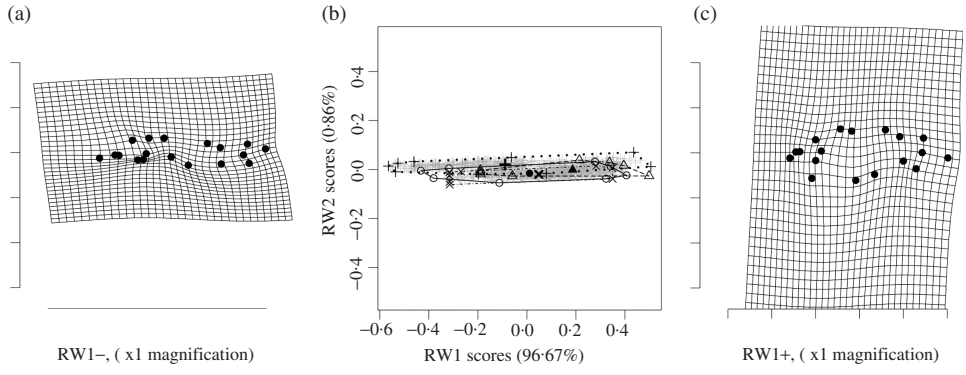

FIG. 4. (b) Growth changes during ontogeny in the four populations of *Ameiurus melas* (—●—, U.K.; –▲–, France; ··+··; Italy, ·-×-·, Slovakia), illustrated on thin-plate spline (TPS) grids of form-space principal component analysis (PCA)-relative warp analysis (RWA) (96·67%). Form-space PCA (RWA) explained variability is 97·53%. The body shape of (a) immature and (c) adult specimens (both in direction of RW1).

(Fig. 7 and Table II). A different pattern was found in the Italian population, with most characters developing allometrically (Fig. 7 and Table III); whereas in the Slovak population, the majority of characters developed isometrically with abrupt changes [Figs 3(d) and 7 and Table III]. In all examined populations, the breakpoints appeared late in ontogeny, *i.e.* in adults (Fig. 3 and Tables II and III).

Size-adjusted shape-space PCA of the geometric analysis (Fig. 5), size-adjusted shape space LDA (Fig. 6) and the C_{dif} results (see above) indicated that Italian *A. melas,* relative to the U.K. and Slovak populations had proportionally smaller and more posteriorly situated eyes, a larger head, anteriorly placed dorsal fin, posteriorly placed adipose fin, shallower caudal peduncle and shorter and smaller caudal fin. The French *A. melas* represented an intermediate form between the U.K. and Slovak

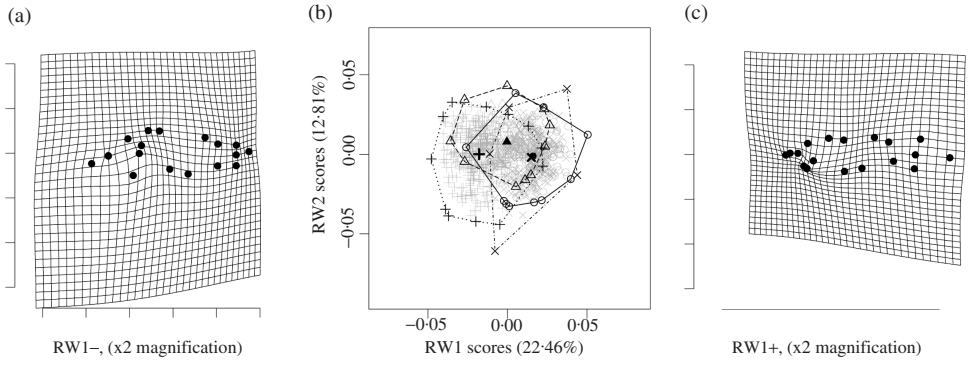

FIG. 5. (b) Inter-population differences among the four populations of *Ameiurus melas* (—●—, U.K.; –▲–, France; ··+··; Italy, ·-×-·, Slovakia), illustrated on thin-plate spline (TPS) grids of size-adjusted shape-space principal component analysis (PCA)-relative warps analysis (RWA). The shape–space PCA (RWA) size–adjusted shape space explained variability is 35·27%. The most discriminative body shape is shown in the (a) Italian and (c) U.K. and Slovak populations of *A. melas* (both in the direction of RW1).

© 2013 The Authors

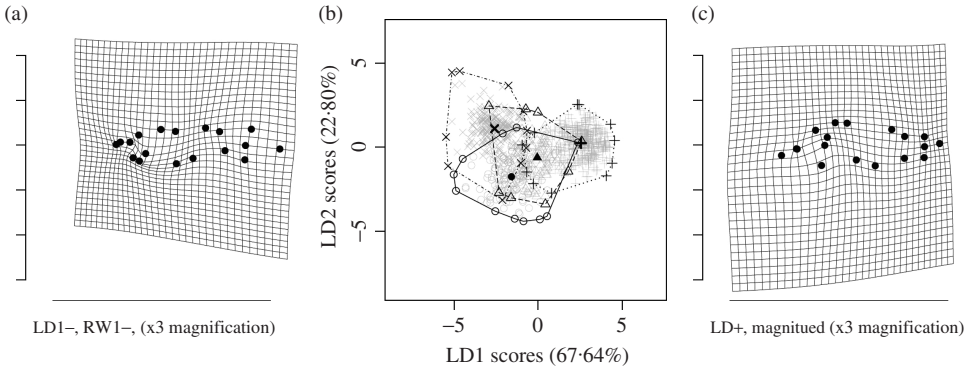

FIG. 6. (b) Inter-population differences among the four populations of *Ameiurus melas* (-●-, U.K.; -▲-, France; ··+··; Italy, ··×··, Slovakia), illustrated on thin-plate spline (TPS) grids of size-adjusted shape-space linear discriminant analysis (LDA). Linear discriminant analysis size–adjusted shape space explained variability = 90·44%. The most discriminative body shape is shown in the (a) U.K. and Slovak and (c) Italian populations of *A. melas* (both in the direction of LD1).

and Italian phenotypes (Figs 5 and 6). In the test of latitudinal effect, a statistically significant difference ($P < 0{\cdot}05$) was found in two morphometric characters: caudal-fin depth and caudal-fin length.

DISCUSSION

One of the typical attributes of successful invasive fish species appears to be their high morphological variability and phenotypic plasticity (Tomeček *et al.,* 2005; Záhorská *et al.,* 2009), as body shape is directly or indirectly influenced by the ambient environmental conditions (Norton *et al.,* 1995). This has been observed in

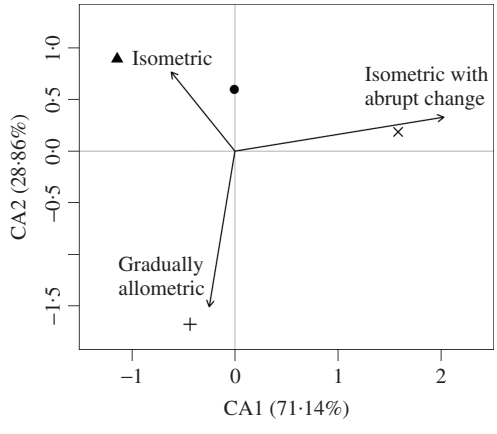

FIG. 7. Correspondence analysis of the four populations of *Ameiurus melas* (●, U.K.; ▲, France; +, Italy; ×, Slovakia). Graph shows the predominant type of development of particular morphometric characters in all populations examined in this study and their reciprocal relativeness.

© 2013 The Authors

several widespread species of fishes, such as sticklebacks *Gasterosteus* spp. (Kováč *et al.,* 2002), *L. gibbosus* (Tomeček *et al.,* 2005) or topmouth gudgeon *Pseudorasbora parva* (Temminck & Schlegel 1846) (Záhorská *et al.,* 2009).

Freshwater invasive fishes in newly occupied areas usually tend to attain smaller body size compared to individuals from native populations (Tomeček *et al.,* 2005; Záhorská *et al.,* 2009). This is often caused by modification of their life histories, especially by a shift in allocation of their resources to reproduction and maintenance of offspring rather than to somatic growth (Novomeská *et al.,* 2010). Indeed, individuals from all four European populations of *A. melas* examined in this study are considerably smaller relative to those from native North American populations, where *A. melas* grows up to 467 mm L_T or even 610 mm L_T (Carlander, 1969; Scott & Crossman, 1973). Individuals from populations with the longest period of introduction, *i.e.* from the Italian population established in the Lake Trasimeno for at least 40 years (Gianotti *et al.,* 1975) and from the French population established in Brière Marsh for at least 70 years (Cucherousset *et al.,* 2006), show larger body size (both maximum and mean values) compared to more recently established U.K. and Slovak populations (see Table I). Therefore, it might seem that a longer time of establishment gradually leads back to the body size values typical for native populations. Nevertheless, body size in all four populations examined still remains smaller compared to native *A. melas.* The same phenomenon has been recorded in North America for non-native round goby *Neogobius melanostomus* (Pallas 1814), which attains a smaller body size than specimens from native Ponto-Caspian populations (MacInnis & Corkum, 2000), or in *L. gibbosus* from some non-native European sites that were found to be smaller than *L. gibbosus* from native North American populations even after 100 years of establishment (Copp *et al.,* 2004; Copp & Fox, 2007). In this case, the differences are likely to be related to differences in temperature regime, which influences both the amount of food available and timing of somatic growth.

In all populations examined, breakpoints occur only in adult specimens and in most cases quite late in ontogeny. This suggests that *A. melas* attains its definitive phenotype early in ontogeny and no substantial body shape changes appear later. It may be associated with a relatively uniform life history of *A. melas* throughout its ontogeny from early juvenile to adult periods. In many species, ontogenetic changes in body shape go hand in hand with adaptation to different habitat use (*e.g.* lentic *v.* lotic and pelagic *v.* benthic) (Tomeček *et al.,* 2005), diet type (insects *v.* larger prey) (Watson & Balon, 1984; Kováč *et al.,* 1999) or other ecological requirements. In such cases, the abrupt changes in morphometric characters can be considered a potential tool to identify thresholds in the fish's life history (Copp & Kováč, 1996; Kováč *et al.,* 1999). On the other hand, breakpoints in morphometry can be considered to be developmental thresholds only if they coincide with each other and with other morphological, physiological, ecological or behavioural changes during ontogeny (Kováč *et al.,* 1999). This is not the case in *A. melas,* however, and the breakpoints found late in its ontogeny do not seem to have any substantial functional meaning, especially if the importance of changes in external morphology decreases with size and age of fish (Kováč *et al.,* 1999).

A previous study of the Slovak population concluded that ontogenetic changes in external morphology of this non-native population occur continuously, and that both juveniles and adults appeared to show little morphological variation between individuals (Novomeská *et al.,* 2010). Such uniformity within the population may

© 2013 The Authors

reflect a possible founder effect and low phenotypic plasticity in this population. Similarly, no statistically significant differences (see C_{dif} results) were found among juveniles when all four non-native populations were compared. Continuous developmental changes in these populations, however, result in increased variation between adult individuals of the same population, as well as between populations. In other words, low morphological variability appears to be restricted to the Slovak population, whereas a pattern of continuous ontogenetic change in external morphology (*i.e.* without apparent thresholds) appears to be typical for all non-native *A. melas* populations. Such a pattern was also observed in other introduced species in Europe, *e.g. P. parva* (Záhorská *et al.,* 2009) or *L. gibbosus* (Tomeček *et al.,* 2005; Copp & Fox, 2007), where different adult morphotypes are formed, whereas the morphology of juveniles appear rather uniform across the geographical range of the species (*i.e.* both in its native and non-native populations). In *P. parva,* for example, the phenotypic plasticity appears to be much higher than that in *A. melas*, as it is expressed not only in the formation of different definite phenotypes but also in the manner by which the phenotypes are achieved (Záhorská *et al.,* 2009). Indeed, in *A. melas* the ontogenetic trajectories were found to be parallel, and the overall morphological variability is affected by interindividual noise rather than by differences between populations. Moreover, high variability in *P. parva* can result from the very short lifespan of this species, which allows very fast alteration of generations and thus a potential to generate novel phenotypes faster.

The Italian phenotype was found to be the most distinct among the four populations of *A. melas* (Figs 5–7). In general, variations in external morphology can result from different genetic attributes of the populations, from different environmental conditions, or from the combination of both. Populations of *A. melas* were imported to Europe (mainly to Italy, Germany, the Netherlands and Belgium) for experimental purposes, aquaculture and stocking ponds in the late 19th and early 20th centuries (Wheeler, 1978; Copp *et al.,* 2005). The fish probably originate from various sites in North America. Different sources of introduction could thus introduce genetic diversity in these non-native European populations of *A. melas,* with potential effects on external morphology. Unfortunately, no relevant information on genetic differences of *A. melas* in Europe have been available until now; only a preliminary genetic analysis of specimens from Belgium, France and the Netherlands has been undertaken (Verreycken *et al.,* 2010). On the other hand, in *L. gibbosus* it has been found that only 14% of morphological variability is caused genetically, while as much as 53% is induced by environmental conditions (Robinson *et al.,* 2000). Indeed, body shape in fishes can be influenced by various factors such as temperature (Martin, 1949; Beacham, 1990; Šumer *et al.,* 2005), food ratio (Currens *et al.,* 1989) and type of food or feeding mode (Day *et al.,* 1994; Robinson & Wilson, 1996). Moreover, phenotypic plasticity is regarded to be more beneficial in variable environments than in constant environments, where a single optimal phenotype may be favoured instead of maintaining plasticity in traits (Schlichting & Pigliucci, 1998). The thermal conditions associated with lower latitudes could be responsible for the distinct patterns in the morphology of the Italian population, as the water temperature in Lake Trasimeno often exceeds 30° C in summer (Lorenzoni *et al.,* 2010) and thus differs considerably from the other sites where *A. melas* for this study were sampled.

It has been demonstrated that *A. melas* from non-native European populations have some potential to alter their body shape both within and between populations.

© 2013 The Authors

The phenotypic plasticity of *A. melas,* however, was not found to be as significant as in other invasive fish species, *e.g. P. parva* or *L. gibbosus.* The findings of this study suggest that the morphological variability itself is not necessarily essential for invasive success. The invasiveness of *A. melas* is therefore probably favoured by variations in its life-history traits and reproduction variables, together with some behavioural traits, *e.g.* voracious feeding, parental care (Scott & Crossman, 1973; Ribeiro *et al.,* 2008) rather than by phenotypic plasticity expressed in external morphology.

The authors thank several fishermen for their assistance in the field, M. J. Godard, G. Zięba and E. Franchi for their help in the laboratory, and D. Gruła for his help with graphical processing. This study was supported by VEGA Projects 1/0226/08 and 1/0641/11, Comenius University grant UK/218/2011 and the National Scholarship Programme of the Slovak Republic.

References

Balážová-L'avrinčíková, M. & Kováč, V. (2007). Epigenetical context in the life-history of round goby *Neogobius melanostomus* from Slovak stretch of the Danube. In *Freshwater Bioinvaders: Profiles, Distribution, and Threats* (Gherardi, F., ed.), pp. 275–287. Dordrecht: Springer Verlag.

Beacham, T. D. (1990). A genetic analysis of meristic and morphometric variation in chum salmon (*Oncorhynchus keta*) at three different temperatures. *Canadian Journal of Zoology* **68,** 225–229. doi: 10.1139/z90-033

Brown, M. L., Willis, D. W. & Blackwell, B. G. (1999). Physicochemical and biological influences on black bullhead populations in Eastern Dakota glacial lakes. *Journal of Freshwater Ecology* **14,** 47–60.

Cadrin, S. X. (2000). Advances in morphometric identification of fishery stocks. *Reviews in Fish Biology and Fisheries* **10,** 91–112. doi: 10.1023/A:1008939104413

Carlander, K. D. (1969). *Handbook of Freshwater Fishery Biology.* Ames, IA: Iowa State University Press.

Copp, G. H. & Fox, M. G. (2007). Growth and life history traits of introduced pumpkinseed (*Lepomis gibbosus*) in Europe, and its relevance to its potential invasiveness. In *Biological Invaders in Inland Waters: Profiles, Distribution, and Threats* (Gherardi, F., ed.), pp. 289–306. Dordrecht: Springer Verlag.

Copp, G. H. & Kováč, V. (1996). When do fish with indirect development become juveniles? *Canadian Journal of Fisheries and Aquatic Sciences* **53,** 746–752. doi: 10.1139/f95-252

Copp, G. H., Fox, M. G. & Kováč, V. (2002). Growth, morphology and life history traits of a coolwater European population of pumpkinseed *Lepomis gibbosus. Archiv für Hydrobiologie* **155,** 585–614.

Copp, G. H., Fox, M. G., Przybylski, M., Godinho, F. & Vila-Gispert, A. (2004). Life-time growth patterns of pumpkinseed *Lepomis gibbosus* introduced to Europe relative to native North American populations. *Folia Zoologica* **53,** 237–254.

Copp, G. H., Bianco, P. G., Bogutskaya, N. G., Erős, T., Falka, I., Ferreira, M. T., Fox, M. G., Freyhof, J., Gozlan, R. E., Grabowska, J., Kováč, V., Moreno-Amich, R., Naseka, A. M., Peňáz, M., Povž, M., Przybylski, M., Robillard, M., Russell, I. C., Stakėnas, S., Šumer, S., Vila-Gispert, A. & Wiesner, C. (2005). To be, or not to be, a non-native freshwater fish? *Journal of Applied Ichthyology* **21,** 242–262. doi: 10.1111/j.1439-0426.2005.00690.x

Cucherousset, J., Paillisson, J.-M., Carpentier, A., Eybert, M.-C. & Olden, J. D. (2006). Use of an artificial wetland by the invasive catfish *Ameirus melas. Ecology of Freshwater Fish* **15,** 589–596. doi: 10.1111/j.1600-0633.2006.00199.x

Currens, K. P., Sharpe, C. S., Hjort, R., Schreck, C. B. & Li, H. W. (1989). Effects of different feeding regimes on the morphometrics of Chinook salmon (*Oncorhynchus tshawytscha*) and rainbow trout (*O. mykiss*). *Copeia* **1989,** 689–695.

© 2013 The Authors

Day, T., Pritchard, J. & Schluter, D. (1994). A comparison of two sticklebacks. *Evolution* **48,** 1723–1734.

Dryden, I. L. & Mardia, K. V. (1999). *Statistical Shape Analysis.* New York, NY: John Wiley & Sons.

Gianotti, F. S., Giovinazzo, G. & Gori, L. (1975). Il quadriennio di pesca 1968–1971 nel lago Trasimeno. *Rivista di Idrobiologia* **14,** 209–262.

Karp, C. A. & Tyus, H. M. (1990). Behavioral interactions between young Colorado squawfish and six fish species. *Copeia* **1990,** 25–34.

Katina, S. (2003). Application of allometric shape analysis to fish growth with geometrical notes. *Tatra Mountains Mathematical Publications* **26**, 323–336. Available at http://www.defm.fmph.uniba.sk/∼katina/KatinaTMMP2003.pdf.

Katina, S. (2007). Shape analysis in the light of simplical depth estimators. In *Systems Biology and Statistical Bioinformatics* (Barber, S., Baxter, P. D. & Mardia, K. V., eds), pp. 51–54. Leeds: Leeds University Press.

Komers, P. E. (1997). Behavioural plasticity in variable environments. *Canadian Journal of Zoology* **75,** 161–169. doi: 10.1139/z97-023

Kováč, V., Copp, G. H. & Francis, M. P. (1999). Morphometry of the stone loach, *Barbatula barbatula*: do mensural characters reflect the species' life history thresholds? *Environmental Biology of Fishes* **56,** 105–115. doi: 10.1023/A:1007570716690

Kováč, V., Copp, G. H., Dimart, Y. & Užíková, M. (2002). Comparative morphology of threespine *Gasterosteus aculeatus* and ninespine *Pungitius pungitius* sticklebacks in lowland streams of southeastern England. *Folia Zoologica* **51,** 319–336.

Leunda, P. M., Oscoz, J., Elvira, B., Agorreta, A., Perea, S. & Miranda, R. (2008). Feeding habits of the exotic black bullhead *Ameiurus melas* (Rafinesque) in the Iberian Peninsula: first evidence of direct predation on native fish species. *Journal of Fish Biology* **73,** 96–114. doi: 10.1111/j.1095-8649.2008.01908.x

Lorenzoni, M., Dolciami, R., Ghetti, L., Pedicillo, G. & Carosi, A. (2010). Fishery biology of the goldfish *Carassius auratus* (Linnaeus, 1758) in Lake Trasimeno (Umbria, Italy). *Knowledge and Management of Aquatic Ecosystems* **396,** 1. doi: 10.1051/kmae/20010001

MacInnis, A. J. & Corkum, L. D. (2000). Fecundity and reproductive seasons of the round goby *Neogobius melanostomus* in the upper Detroit River. *Transactions of the American Fisheries Society* **129,** 136–144.

Martin, W. R. (1949). *The Mechanics of Environmental Control of Body Form in Fishes.* Toronto, ON: University of Toronto Press.

Mayr, E., Linsley, E. G. & Usinger, R. L. (1953). *Methods and Principles of Systematic Zoology*. New York, NY: McGraw-Hill.

McMahon, R. F. (2002). Evolutionary and physiological adaptations of aquatic invasive animals: r selection versus resistance. *Canadian Journal of Fisheries and Aquatic Sciences* **59,** 1235–1244. doi: 10.1139/f02-105

Meyer, A. (1987). Phenotypic plasticity and heterochrony in *Cichlasoma managuense* (Pisces, Cichlidae) and their implications for speciation in Cichlid fishes. *Evolution* **41,** 1357–1369.

Mitteroecker, P., Gunz, P., Bernhard, M., Schaefer, K. & Bookstein, F. L. (2004). Comparison of cranial ontogenetic trajectories among great apes and humans. *Journal of Human Evolution* **46,** 679–697.

Nickerson, D. M., Facey, D. E. & Grossman, G. D. (1989). Estimating physiological thresholds with continuous two-phase regression. *Physiological Zoology* **62,** 866–887.

Norton, S. F., Luczkovich, J. J. & Motta, P. J. (1995). The role of ecomorphological studies in the comparative biology of fishes. *Environmental Biology of Fishes* **44,** 287–304. doi: 10.1007/BF00005921

Novomeská, A. & Kováč, V. (2009). Life-history traits of non-native black bullhead *Ameiurus melas* with comments on its invasive potential. *Journal of Applied Ichthyology* **25,** 79–84. doi: 10.1111/j.1439-0426.2008.01166.x

Novomeská, A., Kováč, V. & Katina, S. (2010). Morphometry of non-native black bullhead *Ameiurus melas* from Slovakia. *Central European Journal of Biology* **5,** 888–893. doi: 10.2478/s11535-010-0069-2

© 2013 The Authors

Ribeiro, F., Elvira, B., Collares-Pereira, M. J. & Moyle, P. B. (2008). Life-history traits of non-native fishes in Iberian watersheds across several invasion stages: a first approach. *Biological Invasions* **10,** 89–102. doi: 10.1007/s10530-007-9112-2

Robinson, B. W. & Wilson, D. S. (1996). Genetic variation and phenotypic plasticity in a trophically polymorphic population of pumpkinseed sunfish (*Lepomis gibbosus*). *Evolutionary Ecology* **10,** 631–652. doi: 10.1007/BF01237711

Robinson, B. W., Wilson, D. S. & Margosian, A. S. (2000). A pluralistic analysis of character release in pumkinseed sunfish (*Lepomis gibbosus*). *Ecology* **81,** 2799–2812. doi: 10.1890/0012-9658

Sakai, A. K., Allendorf, F. W., Holt, J. S., Lodge, D. M., Molofsky, J., With, K. A., Baughman, S., Cabin, R. J., Cohen, J. E., Ellstrand, N. C., McCauley, D. E., O'Neil, P., Parker, I. M., Thomson, J. N. & Weller, S. G. (2001). The population biology of invasive species. *Annual Review of Ecology and Systematics* **32,** 305–332.

Schlichting, C. D. & Pigliucci, M. (1998). *Phenotypic Evolution: A Reaction Norm Perspective*. Sunderland, MA: Sinauer Associates, Inc.

Scott, W. B. & Crossman, E. J. (1973). Freshwater fishes of Canada. *Bulletin of the Fisheries Research Board of Canada* **184**.

Snedecor, G. W. (1946). *Statistical Methods.* Ames, IA: Iowa State College Press.

Sokal, R. R. & Rohlf, F. J. (1981). *The Principles and Practice of Statistics in Biological Research*. San Francisco, CA: W. H. Freeman.

Stearns, S. C. (1983). A natural experiment in life-history evolution: field data on the introduction of mosquitofish (*Gambusia affinis*) to Hawaii. *Evolution* **37,** 601–617.

Šumer, S., Kováč, V., Povž, M. & Slatner, M. (2005). External morphology of a Slovenian population of pumpkinseed *Lepomis gibbosus* (L.) from a habitat with extreme thermal conditions. *Journal of Applied Ichthyology* **21,** 306–311. doi: 10.1111/j.1439-0426.2005.00691.x

Thompson, D. W. (1917). *On Growth and Form*. Cambridge: Cambridge University Press.

Tomeček, J., Kováč, V. & Katina, S. (2005). Ontogenetic variability in external morphology of native (Canadian) and non-native (Slovak) populations of pumpkinseed *Lepomis gibbosus* (Linnaeus 1758). *Journal of Applied Ichthyology* **21,** 335–344. doi: 10.1111/j.1439-0426.2005.00678.x

Tomeček, J., Kováč, V. & Katina, S. (2007). Biological flexibility of pumpkinseed, a successful coloniser throughout Europe. In *Freshwater Bioinvaders: Profiles, Distribution, and Threats* (Gherardi, F., ed.), pp. 307–336. Dordrecht: Springer Verlag.

Vila-Gispert, A., Alcaraz, C. & García-Berthou, E. (2005). Life-history traits of invasive fish in small Mediterranean streams. *Biological Invasions* **7,** 107–116. doi: 10.1007/s10530-004-9640-y

Watson, D. J. & Balon, E. K. (1984). Ecomorphological analysis of fish taxocenes in rainforest streams of northern Borneo. *Journal of Fish Biology* **25,** 371–384. doi: 10.1111/j.1095-8649.1984.tb04885.x

Wheeler, A. (1978). *Ictalurus melas* (Rafinesque, 1820) and *I. nebulosus* (LeSueur, 1819): the North American catfishes in Europe. *Journal of Fish Biology* **12,** 435–439. doi: 10.1111/j.1095-8649.1978.tb04186.x

Záhorská, E., Kováč, V., Falka, I., Beyer, K., Katina, S., Copp, G. H. & Gozlan, R. E. (2009). Morphological variability of the Asiatic cyprinid, topmouth gudgeon *Pseudorasbora parva,* in its introduced European range. *Journal of Fish Biology* **74,** 167–185. doi: 10.1111/j.1095-8649.2008.02121.x

Electronic Reference

Verreycken, H., Belpaire, C. & De Gelas, K. (2010). Status of two North American *Ameiurus* species (Ictaluridae) in Flanders (Belgium) with preliminary genetic screening of specimens from Belgium, France and the Netherlands. *Proceedings of the 17th International Conference on Aquatic Invasive Species,* p. 174. Available at http://www.icais.org/pdf/abstracts_2010.pdf/

© 2013 The Authors