

# Morphological change and phenotypic plasticity in native and non-native pumpkinseed sunfish in response to competition

Stan Yavno · Anna C. Rooke · Michael G. Fox

Received: 30 December 2013 / Revised: 9 April 2014 / Accepted: 12 April 2014 / Published online: 26 April 2014  
© Springer-Verlag Berlin Heidelberg 2014

**Abstract** Non-indigenous species are oftentimes exposed to ecosystems with unfamiliar species, and organisms that exhibit a high degree of phenotypic plasticity may be better able to contend with the novel competitors that they may encounter during range expansion. In this study, differences in morphological plasticity were investigated using young-of-year pumpkinseed sunfish (*Lepomis gibbosus*) from native North American and non-native European populations. Two Canadian populations, isolated from bluegill sunfish (*L. macrochirus*) since the last glaciation, and two Spanish populations, isolated from bluegill since their introduction in Europe, were reared in a common environment using artificial enclosures. Fish were subjected to allopatric (without bluegill) or sympatric (with bluegill) conditions, and differences in plasticity were tested through a MANOVA of discriminant function scores. All pumpkinseed populations exhibited dietary shifts towards more benthivorous prey when held with bluegill. Differences between North American and European populations were observed in body dimensions, gill raker length and pelvic fin position. Sympatric treatments induced an increase in body width and a decrease in caudal peduncle length in native fish; non-native fish exhibited longer caudal peduncle lengths when held in sympatry with bluegill. Overall, phenotypic plasticity influenced morphological divergence less than genetic factors, regardless of population. Contrary to predictions, pumpkinseeds from Europe exhibited

lower levels of phenotypic plasticity than Canadian populations, suggesting that European pumpkinseeds are more canalized than their North American counterparts.

**Keywords** Centrarchidae · Common-garden experiments · Functional morphology · Interspecific competition · Reaction norm · Resource partitioning

## Introduction

During a biological invasion, organisms are oftentimes transplanted across large spatial scales, exposing populations to biotic and abiotic characteristics that differ from those in their native range. Adaptive phenotypes can help an organism to maintain its fitness under novel environmental conditions, but the genetic process to acquire them can take time (Fraser et al. 2011), and in some cases, is even inhibited (e.g., Kolbe et al. 2004). Instead, mechanisms such as phenotypic plasticity can more rapidly provide individuals with adaptive traits (e.g., Robinson and Wilson 1996), facilitating survival until local selective pressures allow the new phenotypes to become genetically acquired by the population (Crispo 2007). Several authors have hypothesized that the invasion success of non-indigenous species (NIS) is partially due to their ability to exhibit a strong degree of phenotypic plasticity (e.g., Caño et al. 2008; Chun 2011), and recent studies have indeed identified that non-indigenous flora exhibit higher plasticity than their native counterparts (Matesanz et al. 2010). Some comparisons have been conducted on higher order taxa (e.g., Chown et al. 2007), but few experimental studies addressing this question have included vertebrates.

Competitive pressures can induce phenotypic changes in many taxa, but the interspecific interactions between pumpkinseed (*Lepomis gibbosus*) and bluegill (*L. macrochirus*), two congeneric sunfish, are particularly well-documented

---

Communicated by: Sven Thatje

---

S. Yavno (✉) · A. C. Rooke  
Environmental and Life Sciences Graduate Program,  
Trent University, 1600 West Bank Drive, Peterborough, ON,  
Canada K9J 7B8  
e-mail: stanyavno@trentu.ca

M. G. Fox  
Environmental and Resources Studies Program and Department of  
Biology, Trent University, Peterborough, Ontario, Canada K9J 7B8

(Werner and Hall 1976, 1977, 1979; Arendt and Wilson 1999; Robinson et al. 2000). Both species are endemic to eastern North America, where their range distributions overlap (Scott and Crossman 1973). In sympatry, the two species occupy vegetated areas during early ontogeny, decreasing the risk of predation while simultaneously increasing competition for soft-bodied invertebrates (Keast 1980; Osenberg et al. 1988). At larger sizes, each species exhibits a diet shift: pumpkinseed prey on more hard-bodied molluscs in the littoral zone, whereas bluegill move into open water and predominantly prey on zooplankton (Mittelbach 1984). Pumpkinseeds will occupy pelagic habitats and feed on prey typically consumed by bluegill, but only in allopatric systems (Robinson et al. 2000). Overall, the two congeners exhibit a strong level of phenotypic plasticity, and both species exhibit plasticity and morphological change in response to competitive pressures (e.g., Arendt and Wilson 1999).

In the early 1890s, the pumpkinseed was introduced to Europe and it is now present in over 28 countries (García-Berthou and Moreno-Amich 2000; Copp and Fox 2007). The species has continued to spread rapidly through the Iberian Peninsula (Elvira and Almodóvar 2001), where it was recently detected in approximately half of all sampled reservoirs (Clavero et al. 2013). Native fish communities in this region are nearly devoid of piscivorous species; aquatic systems throughout Spain and Portugal contain several endemic taxonomic groups (e.g., Cyprinidae) that feed heavily on invertebrates and zooplankton (Clavero et al. 2004). Juvenile pumpkinseeds also consume invertebrates and zooplankton, but the species has a strong capacity to shift to different trophic levels and diets in response to competition (Werner and Hall 1976; Arendt and Wilson 1999; Robinson et al. 2000). Robinson and Wilson (1996) used trophic levels to test for morphological plasticity in native pumpkinseed, and found that over half of the observed morphological divergence was the result of phenotypic plasticity. Yavno et al. (2013) found inherited morphological differences between native Canadian and non-native Iberian pumpkinseed populations, and proposed that they were the result of adaptive phenotypic plasticity. The invasion success of pumpkinseeds throughout the Iberian Peninsula could be explained by the ability of these populations to exhibit strong, adaptive morphological plasticity, which helps to release them from the effects of competition.

Given the consistent differences in plasticity observed in other native/non-native conspecific pairs, and that non-native populations are nearly always more plastic than native counterparts (reviewed by Davidson et al. 2011), we tested for differences in morphological plasticity of native and non-native pumpkinseed. Using a common garden approach to examine pumpkinseed responses to interspecific competition from bluegill, we hypothesized that native and non-native pumpkinseeds would differ in their ability to exhibit plasticity, and predicted that (1) all populations would exhibit overall

diet shifts in response to competition; (2) there would be morphological diversification in all populations, consistent with any observed diet shifts; and (3) there would be more pronounced morphological shifts in non-native populations, reflecting an overall higher degree of phenotypic plasticity. In assessing 12 external morphological traits that are functionally significant for locomotion, Yavno et al. (2013) identified geographic differences between native and non-native pumpkinseed populations, which were hypothesized to be the result of adaptive plasticity. Those 12 traits are also functionally significant for foraging, given that the acquisition of prey necessitates navigation, maneuverability and speed (e.g., Higham 2007). This study tests the hypothesis proposed by Yavno et al. (2013) from the perspective of competition, and incorporates six additional morphological traits that are known to vary in pumpkinseed, and other fishes, as a result of different foraging strategies (Day et al. 1994; Bhagat et al. 2006; Svanbäck and Schluter 2012).

## Materials and methods

### Experimental animals and study sites

In August 2012, native pumpkinseeds (*L. gibbosus*) were collected from two lakes in Ontario, Canada, that are devoid of bluegill (*L. macrochirus*), but located within a geographic region containing pumpkinseed populations used in previous morphological comparisons (Jastrebski 2001; Gillespie and Fox 2003; Parsons and Robinson 2006). The populations from these systems have likely been isolated from bluegill for approximately 13,000 years, which is when the region was covered by temporary glacial waterbodies (Mandrak and Crossman 1992). Ashby Lake (44°09' N, 77°66' W; 260 ha surface area) has a relatively small littoral zone with a low abundance of aquatic macrophytes, and a well-developed pelagic zone with islands and rocky shoals. Anstruther Lake (44°74' N, 78°20' W; 530 ha surface area) has a pelagic zone that is free of islands or shoals, and intermittent littoral areas that contain dense patches of aquatic macrophytes. Pumpkinseeds were collected in littoral areas of each lake using a 9-m bag seine (mesh size=3 mm). Juvenile pumpkinseeds were transported to an indoor facility for a 14-day isolation period in 57–76 l aquaria at 22 °C with a carbon filter system and aeration, and fed ad libitum for 10 min daily with dry fish flakes.

The bluegill is not found in Europe, and given that the pumpkinseed has been present in the Catalonia region of Spain for approximately 100 years and has a mean age at maturity of less than 2 years (Fox et al. 2007), populations in that area have likely been isolated from bluegill for more than 50 generations. During the spring of 2007, adult pumpkinseeds ( $N \geq 20$ ) were collected from two Catalanian

waterbodies (Ter River, 42°01' N, 3°12' E; Susqueda Reservoir, 42°58' N, 2°30' E; 466 ha surface area), flown to Canada, and held in an indoor facility over the winter. In early June 2008, pumpkinseeds from each population were released into their own artificial outdoor pond (9 m [length] × 3 m [width] × 3 m [depth]) containing aquatic macrophytes and two common species of cyprinid (*Phoxinus eos*; *Pimephales promelas*). Juvenile progeny from these ponds have been used in previous morphological comparisons (Yavno et al. 2013; Yavno and Fox 2013). For this study, juvenile non-native pumpkinseeds were collected from the aforementioned ponds in August 2012 and transported to an indoor holding facility for the same 14-day isolation period as the native juveniles. Standard length (SL) measurements varied from 29 to 41 mm, with a mean of 32.0 mm across all populations; these sizes are less than or equal to the initial length of pumpkinseeds used in previous morphological plasticity experiments (e.g., Arendt and Wilson 1999; Mittelbach et al. 1999; Parsons and Robinson 2007).

In their juvenile stage, both pumpkinseed and bluegill inhabit the littoral zone, feed on the same prey types, and are considered strong congeneric competitors (Mittelbach 1984; Osenberg et al. 1988). Bluegill were collected from littoral habitats in the Otonabee River (Ontario, Canada; 44°36' N, 78°29' W), where they co-occur with pumpkinseeds, using a 9-m bag seine. Collected bluegill were transported to the holding facility, and held under conditions identical to those of the pumpkinseeds. Bluegills and pumpkinseeds had no visual or olfactory contact during the isolation period.

#### Rearing experiment

Rearing was conducted in a fishless outdoor pond (150 × 30 m), with mud/silt substrate, and an established invertebrate community comparable to that of nearby natural systems. Juveniles were reared in 14 artificial enclosures (1 m [length] × 1 m [width] × 0.5 m [height], lined with 3.2-mm hardware cloth) that were partially depressed into the substrate to permit access to benthic prey, while the enclosure mesh size did not restrict access to zooplankton. Enclosures were assigned to one of the four populations (native: Ashby Lake, Anstruther Lake; non-native: Ter River, Susqueda Reservoir) and one of two competition treatments (allopatry: bluegill absent; sympatry: bluegill present). All treatment pairs were replicated twice, with the exception of Ashby Lake, which had only one replicate due to sample size limitations.

Our experimental design is analogous to replacement series experiments that are commonly employed in competition studies with fauna, wherein the total density of individuals remains constant in both allopatric and sympatric treatments, but the species composition changes (Snaydon 1991). This

design has been criticized because it confounds the effects of intra- and interspecific competition (e.g., Firbank and Watkinson 1985). On the other hand, an additive design holds the effects of intraspecific competition steady across all treatments, which helps to better test the effects of interspecific competition (Reynolds 1999). In a previous competition study between two centrarchid species, the presence of bluegill, but not their relative density, was sufficient to induce phenotypic divergence (Marchetti 1999). Altering densities across allopatry and sympatry treatments would have enabled us to better disentangle the effects of intra- and interspecific competition, but given the constraints in number of fish available and number of suitable sites available for enclosure treatments, and because our study aimed to determine whether non-native pumpkinseeds are, at the very minimum, responding specifically to a novel competitor and not competition due to increased fish density, we elected to standardize density (i.e., the replacement design). To standardize density, 30 sunfishes were distributed into each enclosure: allopatric treatments contained 30 pumpkinseeds, and sympatric treatments contained 15 pumpkinseeds and 15 bluegills. In each sympatric enclosure, bluegills were visually size-matched to pumpkinseeds to match stocking densities (Hanson and Leggett 1986), and better replicate the competitive conditions that juvenile sunfish typically experience in the wild (e.g., Osenberg et al. 1988). Juveniles were reared in the enclosures for 75 days to ensure that a sufficient period of time had transpired for morphological divergence to occur (see Robinson and Wilson 1996; Yavno and Fox 2013).

A total of 32 pumpkinseeds were lost across all populations over the course of the experiment (~10 % mortality). Final external morphological changes in pumpkinseed were assessed at the end of the rearing period by euthanizing all remaining fish ( $n=283$ ) with MS-222, and individually photographing their left sides and dorsal aspects. The ventral pharyngeal jaw and the first branchial arch were excised, stained with alizarin red (Springer et al. 2000), and photographed using a Leica S8AP0 dissection scope equipped with a Lumenera Infinity1-1C camera.

#### Stomach content analysis

After digital photographs were taken, stomach contents were examined in all individuals to help confirm that any observed morphological trait differentiation was consistent with differences in prey consumption. Prey were classified into major taxonomic groups (amphipods, cladocerans, copepods and ephemeropterans; all other prey types were grouped together under 'other' as they did not make up a large portion of the diet) and the percent volume of each group was estimated using a gridded Petri dish. To identify differences in niche

overlap, Levins' index (Levins 1968; Keast 1978) was calculated as follows

$$\alpha_{ji} = \frac{\sum_{h=1}^s p_{ih} p_{jh}}{\sum_{h=1}^s p_{ih}^2},$$

where  $\alpha_{ji}$  represents the niche overlap of species  $j$  on species  $i$  relative to the niche breadth of species  $i$  for all  $h$  samples of resource  $s$ ;  $p_{ih}$  and  $p_{jh}$  are the proportion of a particular item  $h$  in the diets of species  $i$  and  $j$  (respectively), while  $p_i$  is the breadth of prey use by species  $i$ . A lower overlap value indicates that populations have different diets. General diet differences among pumpkinseed populations were assessed using the overlap between pairs of populations held only in allopatry, and differences among pumpkinseed and bluegill were assessed using the overlap of the two species when held in sympatry. Evidence of a diet shift in response to bluegill competition was assessed by comparing the dietary overlap of pumpkinseed in allopatric and sympatric treatments, calculated separately for each population.

### Morphometric analyses

A distance-based approach was used for identifying morphological differences between groups. All internal and external photographs included a scientific ruler, which permitted a standardization of scale among morphometric measurements across all digital photographs (see Yavno and Fox 2013). Morphology was analysed using a modified box truss design, which comprises homologous points that form inter-landmark distances (Strauss and Bookstein 1982). A centroid (CS), used as a measure of body size, was calculated to take the effect of allometry into account during the statistical analysis (Robinson et al. 2000). A residual analysis performed on a regression of SL against CS identified 15 individuals as outliers ( $>2\sigma$ ); these fish were subsequently removed from the dataset prior to statistical analysis. A generalized linear mixed model was performed on the original dataset, and revealed that the fish that were not included in the final dataset (i.e., mortalities and outliers) belonged to allopatry treatments for Anstruther, Ashby and Susqueda. Sixteen morphological characters (12 external, four internal), identified as adaptive for known feeding strategies exhibited in the presence or absence of this congener, and previously examined in pumpkinseed because of their functional significance (Bhagat et al. 2011; Yavno et al. 2013), were compared among the four populations.

Morphometric measurements taken on day 75 were first  $\log_{10}$ -transformed to meet the assumption of normality, and then regressed against  $\log_{10}$ -transformed CS in an analysis of covariance (ANCOVA). Internal and external measurements were tested separately; because of the multiple paired comparisons, probabilities were adjusted using Bonferroni correction (external traits,  $\alpha \leq 0.0042$ ; internal traits,  $\alpha \leq 0.0125$ ). If there was no significant

interaction between CS and a main effect, the particular interaction term was removed from the ANCOVA and the test rerun. Student Neuman–Keuls analyses and Tukey's HSD multiple comparisons were used on traits that had significant main effects.

Multivariate analyses were performed using measurements that were statistically adjusted to minimize the effects of body size (e.g., Bhagat et al. 2006). Adjusted values were first analysed in multivariate analysis of variance (MANOVA), and then analysed in multivariate discriminant function analysis (DFA) to test for morphological differences between groups. Differences among populations were evaluated using Wilk's  $\lambda$  and the associated  $P$  statistic.

### Analysis of phenotypic plasticity

Canonical scores obtained from the DFA were used in an independent analysis to provide a representation of the relative contribution of genetic (population origin) and environmental (competition) factors towards morphological differentiation. Population origin must be controlled in order to properly assess differences in phenotypic plasticity, so the size-adjusted morphological measurements were separated by geographic origin, and two new sets of canonical scores were generated by performing DFAs for native and non-native populations; the analysis described below was performed independently on each of the new sets of canonical scores for native and non-native populations. Following Robinson and Wilson (1996), the mean canonical scores for each factor were first run in ANOVA to provide an estimate of the variance components for each factor. This variance was then multiplied by the canonical correlation (also obtained from each DFA) of each factor in the multivariate discriminant function. The resulting values were then summed and expressed as a percentage of the total variation to assess the relative importance of genetic and environmental factors.

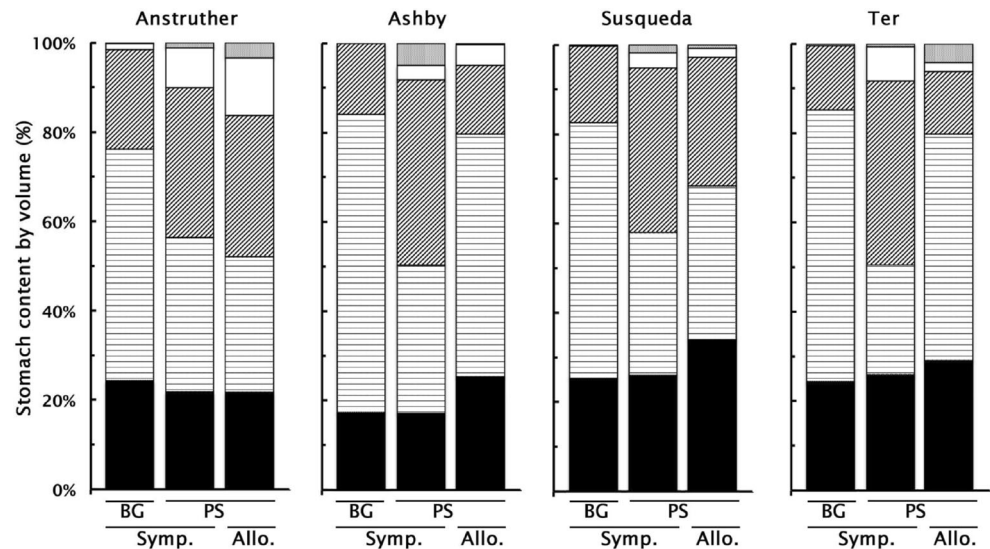
## Results

### Differences in prey consumption

In sympatry, non-native pumpkinseeds (*L. gibbosus*) consumed more ephemeropterans and amphipods, while decreasing their consumption of copepods and cladocerans (Fig. 1). In contrast, native populations under sympatric conditions reduced their consumption of amphipods. In all treatments, bluegill (*L. macrochirus*) consumed mostly copepods and few amphipods. Levins' index confirmed that diet shifts occurred following the introduction of bluegill (Table 1a). Niche overlap between pumpkinseed and bluegill pairs (Table 1b) was lower than that seen among pumpkinseed populations under allopatry (Table 1a), which reflects the higher consumption of copepods and overall narrower diet breadth of bluegill (Fig. 1).



**Fig. 1** Mean percent volume of prey (black box, cladocerans; box with horizontal lines, copepods; box with diagonal lines, ephemeropterans; white box, amphipods; gray box, other) consumed by bluegill (*L. macrochirus*) and pumpkinseed (*L. gibbosus*), when held in either allopatry or sympatry



Contrary to our predictions, a geographic native/non-native pattern was not evident. Pumpkinseeds from Ashby and Ter were the most influenced by the interspecific competition. Both populations have the least similar diets to bluegill, and they each exhibit the least diet overlap with their allopatric counterpart.

#### Morphological trait differentiation

Five of 12 external morphological traits showed significant among-population differences: two body and caudal peduncle traits and three fin traits differentiated native from non-native pumpkinseeds (Fig. 2). Tukey's HSD test revealed that, regardless of treatment, Iberian pumpkinseed had significantly longer anal fin bases and more anteriorly placed pelvic fins than North

American pumpkinseed (Table 2). Iberian populations exhibited longer dorsal fin bases, shorter caudal peduncles and narrower body depths, but these differences were not statistically significant. When held in sympatry with bluegill, native populations developed deeper and wider bodies and shorter caudal peduncles, while Iberian populations developed longer caudal peduncles and more anteriorly positioned pelvic fins. Moreover, the presence of bluegill induced shorter pectoral fins in all four populations, and with the exception of the Ashby population, more anteriorly positioned pectoral fins (Fig. 2).

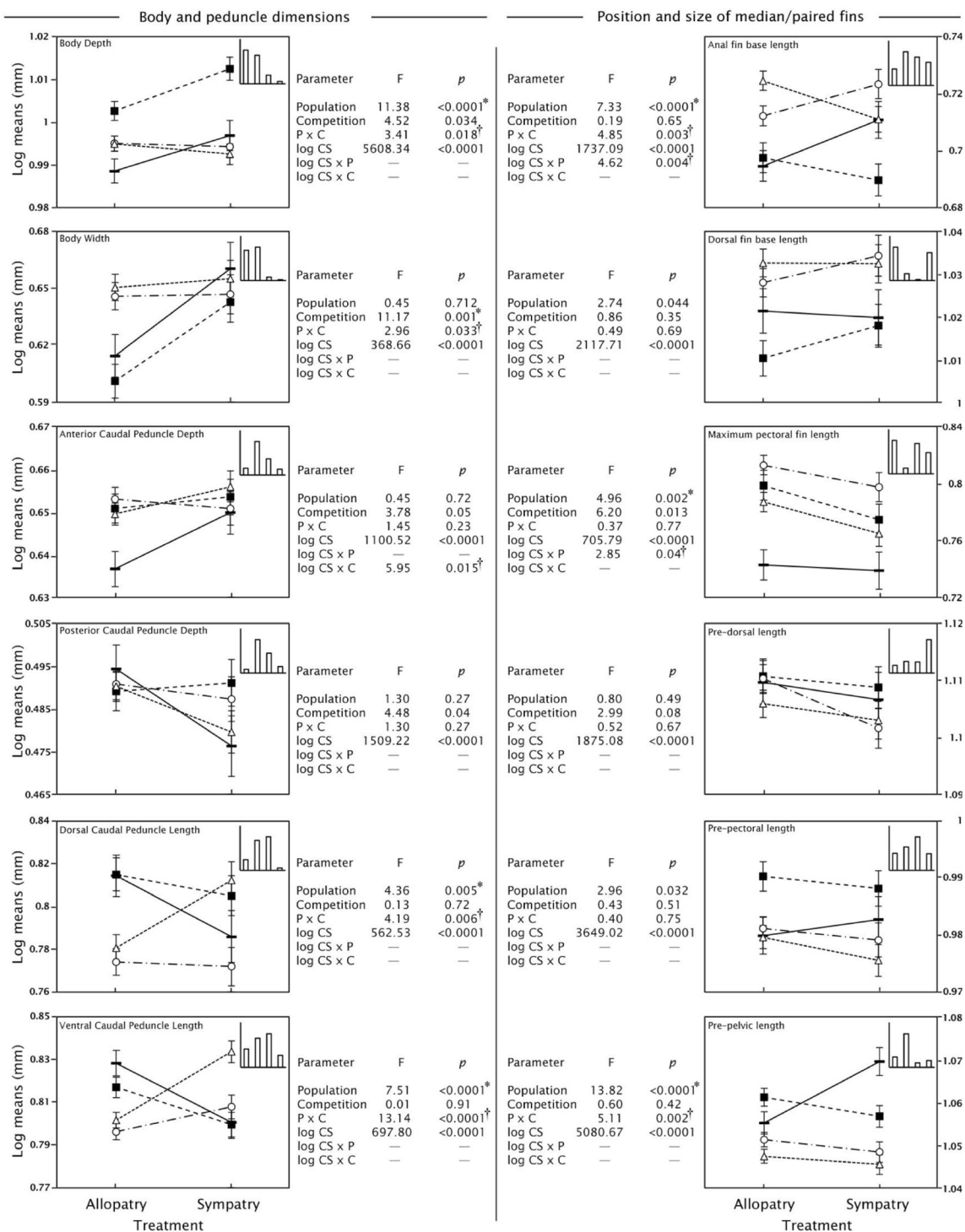
All populations developed longer gill rakers when held with bluegill, while non-native pumpkinseeds exhibited shorter second gill raker lengths, regardless of whether bluegills were present or not (Fig. 3). The distance

**Table 1** (a) Dietary overlap (Levins' index) among native and non-native pumpkinseed (*L. gibbosus*) populations held under only allopatry (i.e., without bluegill, *L. macrochirus*), versus those held under either allopatry or sympatry (i.e., with bluegill); the latter is representative of the

observed diet shift following the introduction of a novel competitor. The first and second values represent  $\alpha_{ij}$  and  $\alpha_{ji}$ , respectively (see Keast 1978). (b) Dietary overlap between pumpkinseed populations held in sympatry with bluegill, and the bluegill within the same treatment cage

		(j) Pumpkinseed (allopatry)				(j) Pumpkinseed (sympatry)			
		AS	AN	TR	SQ	AS	AN	TR	SQ
(a)									
(i) Pumpkinseed (allopatry)	AS	1.0	1.07/0.71	1.02/0.96	1.00/0.82	0.74/0.92			
	AN		1.0	0.72/1.03	0.85/1.06		1.05/0.93		
	TR			1.0	0.99/0.86			0.71/0.85	
	SQ				1.0				0.96/0.99
(b)									
		(j) Bluegill							
(i) Pumpkinseed (sympatry)	AS	0.65/0.98							
	AN	0.82/1.07							
	TR	0.60/0.90							
	SQ	0.74/1.02							

AN Anstruther Lake, AS Ashby Lake, SQ Susqueda Reservoir, TR Ter River



**Fig. 2** Reaction norm plots to changes in competition for 12 external morphological traits identified as having a functional significance for foraging. Log<sub>10</sub>-transformed measurements were regressed against the log<sub>10</sub>-transformed centroid (CS) in an ANCOVA. Interactions between CS and a main effect (*P* population, *C* competition) that were not significant (i.e., homogeneous slopes) were removed from the analysis, and probabilities were Bonferroni adjusted (\**P* < 0.0042; †*P* < 0.05).

Native pumpkinseed (*L. gibbosus*) populations were Anstruther Lake (solid square and broken line) and Ashby Lake (solid horizontal bar and solid line); non-native populations were Susqueda Reservoir (open triangle and dotted line) and Ter River (open circle and dash-dotted line). Insets provide a graphical representation of the magnitude of the slope (i.e., absolute value of the difference between the allopatric and sympatric treatment) for each population (*L* to *R*: Anstruther, Ashby, Susqueda, Ter)

between gill rakers and the length of the lower jaw were also affected by competition from bluegill, as these traits

appeared to converge towards a common size in all pumpkinseed populations.

**Table 2** Results of post hoc analyses of morphological traits (a, body and peduncle dimensions; b, position and size of median/paired fins; c, jaw bone and gill rakers) that exhibited significant differentiation under Bonferroni-corrected ANCOVA (a and b,  $P < 0.0042$ ; c,  $P < 0.0125$ )

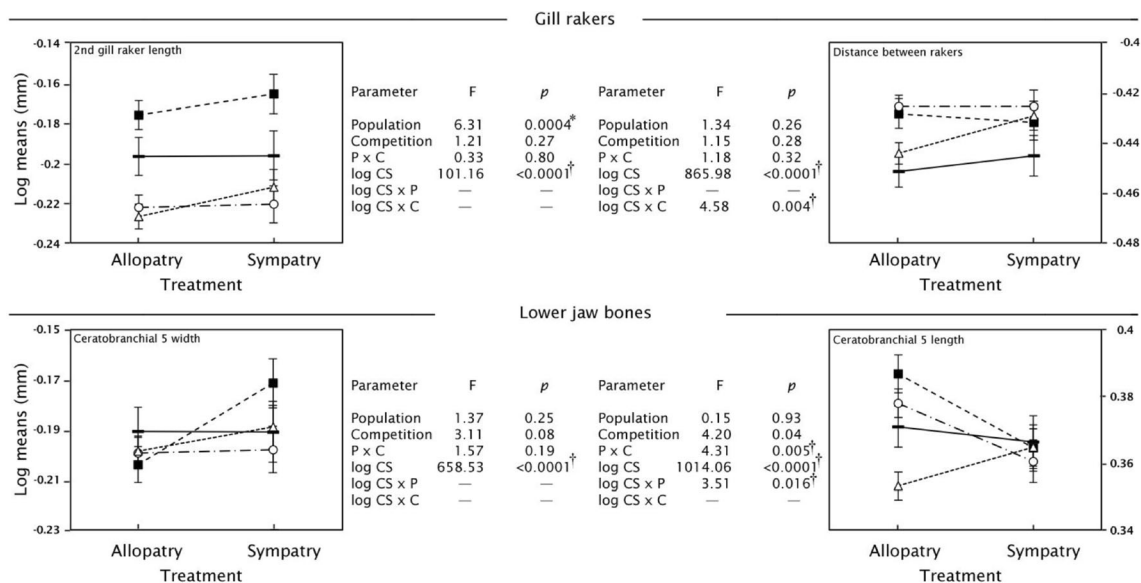
	Population				Competition	
	AN	AS	SQ	TR	Allopatry	Sympatry
(a) Body depth	A	B	B	B	A	A
Body width	A	A	A	A	B	A
Anterior caudal peduncle depth	A	A	A	A	A	A
Posterior caudal peduncle depth	A	A	A	A	A	A
Dorsal caudal peduncle length	A	AB	A	B	A	A
Ventral caudal peduncle length	B	B	A	B	A	A
(b) Anal fin base length	B	AB	A	A	A	A
Dorsal fin base length	A	A	A	A	A	A
Maximum pectoral fin length	AB	B	AB	A	A	A
Pre-dorsal length	A	A	A	A	A	A
Pre-pectoral length	A	A	A	A	A	A
Pre-pelvic length	B	A	C	BC	A	A
(c) Second gill raker length	A	AB	B	B	A	A
Distance between gill rakers	A	A	A	A	A	A
Ceratobranchial 5 width	A	A	A	A	A	A
Ceratobranchial 5 length	A	A	A	A	A	A

Differences due to population (AN Anstruther Lake, AS Ashby Lake, SQ Susqueda Reservoir, TR Ter River) were examined with Tukey's HSD multiple comparisons tests, while differences due to competition (without bluegill, *L. macrochirus*, allopatry; with bluegill, sympatry) were identified through separate Student Neuman–Keuls analyses. Letters earlier in the alphabet represent larger means and identical uppercase letters indicate no significant difference ( $P > 0.05$ )

### Multivariate analysis

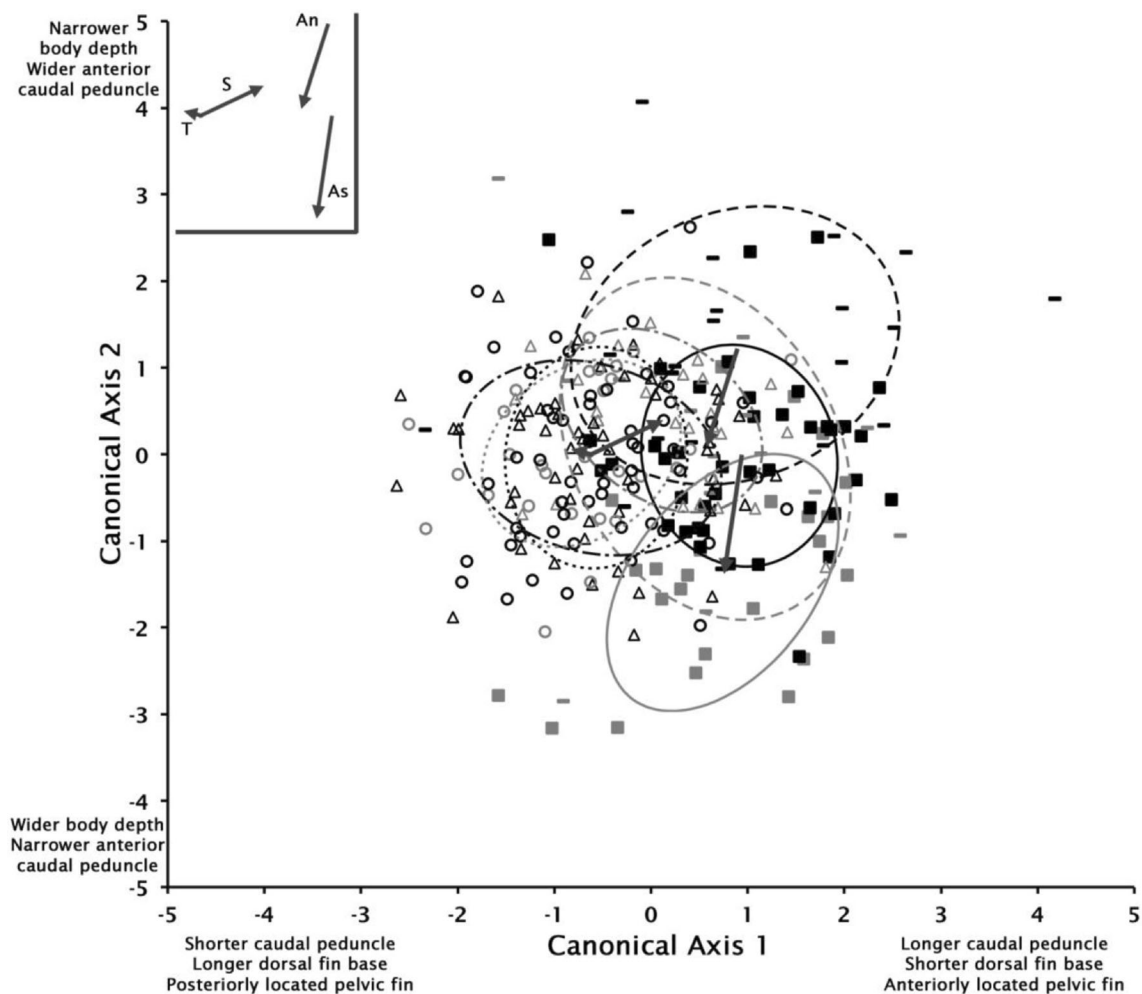
Significant morphological differentiation of external traits was first confirmed by the two-factor MANOVA of size-adjusted measurements (Population, Wilk's  $\lambda = 0.504$ ,  $F = 5.33$ ,  $P < 0.0001$ ; Competition, Wilk's  $\lambda = 0.862$ ,  $F = 3.30$ ,

$P < 0.001$ ; Population–Competition interaction, Wilk's  $\lambda = 0.683$ ,  $F = 2.81$ ,  $P < 0.0001$ ). The subsequent DFA revealed significant morphological differentiation of external traits among the four populations and two treatments (Wilk's  $\lambda = 0.30$ ,  $F = 3.92$ ,  $P < 0.0001$ ). The first canonical axis separated native and non-native populations (Fig. 4). The second axis



**Fig. 3** Reaction norm plots to changes in competition for four internal morphological traits identified as having functional significance for foraging. Measurements were  $\log_{10}$ -transformed; probabilities were

Bonferroni-corrected (\* $P < 0.0125$ ; † $P < 0.05$ ). Symbol and line designations correspond to those in Fig. 2



**Fig. 4** Scatterplot of pumpkinseed (*L. gibbosus*) canonical scores from the multivariate discriminant function analysis, performed on 12 size-adjusted external morphological traits, with 50 % ellipsoids about the centroid of each group for allopatric (black) and sympatric (grey)

competition treatments. Arrows denote the shift of the centroid between allopatric and sympatric treatments for each population, and are recreated in the inset for clarity. Symbol and line designations correspond to those in Fig. 2

did not provide a clear separation of groups by either geographic origin or competition treatment. Mahalanobis distances indicate that allopatry/sympatry treatments for Ter did not show significant separation ( $P > 0.05$ ), whereas significant separation was evident in the three remaining populations ( $P < 0.05$ ). The first two canonical axes explained 60.5 % of the total variation between the eight treatment groups (Table 3). The length of the caudal peduncle, and the base lengths of the anal and dorsal fins significantly correlated with the first canonical axis, whereas body depth and the anterior depth of the caudal peduncle were significantly correlated with the second axis. The multivariate and univariate analyses were both consistent in demonstrating that Iberian populations exhibited shorter caudal peduncles and longer anal/dorsal fin bases.

For internal traits, morphological differentiation was again first confirmed by the two-factor MANOVA of size-adjusted measurements (Population, Wilk's  $\lambda = 0.812$ ,  $F = 4.52$ ,  $P < 0.0001$ ; Competition, Wilk's  $\lambda = 0.95$ ,  $F = 3.46$ ,  $P < 0.01$ ;

Population–Competition interaction, Wilk's  $\lambda = 0.894$ ,  $F = 2.49$ ,  $P < 0.01$ ). A subsequent DFA also revealed significant morphological differentiation (Wilk's  $\lambda = 0.67$ ,  $F = 4.05$ ,  $P < 0.0001$ ), but the scatterplot (not shown) did not provide a clear separation of populations by either geographic origin or competition treatment. Moreover, Mahalanobis distances indicated no significant differentiation between allopatric and sympatric treatments for Ashby, Ter or Susqueda ( $P > 0.05$ ). The first two canonical axes explained 80.4 % of the total variation between the eight treatment groups (Table 3). The distance between gill rakers and the length of the lower jaw significantly correlated with Axis 1, while the length of the second gill raker correlated with Axis 2.

#### Phenotypic plasticity

The MANOVA and multivariate discriminant analyses revealed that the greatest separation between competition



**Table 3** Canonical loadings (i.e., correlations) between 12 functionally significant morphological traits and each canonical axis in the overall multivariate discriminant function analysis (DFA)

Discriminant function analysis					
External traits			Internal traits		
Morphological trait	Axis 1	Axis 2	Morphological trait	Axis 1	Axis 2
Body depth	0.198	<b>−0.730</b>	Second gill raker length	0.241	<b>−0.926</b>
Body width	−0.223	−0.105	Distance between rakers	<b>0.580</b>	−0.187
Anterior caudal peduncle depth	−0.156	<b>−0.412</b>	Ceratobranchial 5 width	−0.229	−0.369
Posterior caudal peduncle depth	−0.056	−0.047	Ceratobranchial 5 length	<b>0.575</b>	0.052
Dorsal caudal peduncle length	<b>0.440</b>	0.122			
Ventral caudal peduncle length	0.309	<b>0.435</b>			
Anal fin base length	<b>−0.551</b>	0.064			
Dorsal fin base length	<b>−0.405</b>	0.022			
Maximum pectoral length	−0.347	−0.189			
Pre-dorsal length	0.056	−0.077			
Pre-pectoral length	0.190	−0.256			
Pre-pelvic length	0.374	−0.113			
% of total variance explained	37.1	60.5		50.2	80.4

Traits with correlations/loadings that are greater than or equal to an absolute value of 0.4 are considered to have biologically significant relationships (Yavno and Fox 2013), and are shown in boldface

treatments occurred for external, rather than internal traits. Thus, the canonical scores from the external DFA were used in the plasticity analyses. ANOVA analyses of the canonical scores for each factor, separated by geographic origin, indicated

that genetic and competitive effects significantly influenced the first canonical axis for non-native pumpkinseeds and both canonical axes for native pumpkinseeds (Table 4). Based on the variance components of the DFA, the percent variation of

**Table 4** Results of Analyses of Variance (ANOVA) on the canonical scores for DFA axes, after controlling for population origin (i.e., performing separate DFAs on native and non-native populations, and analyzing those canonical scores independently)

Analysis of variance <sup>a</sup>						
Model parameter	Axis 1			Axis 2		
	<i>F</i>	<i>P</i>	Components of variance	<i>F</i>	<i>P</i>	Components of variance
Native populations						
Population	42.96	<0.001	0.766 (32.4)	14.54	<0.001	0.302 (17.3)
Competition	21.48	<0.001	0.526 (22.0)	21.57	<0.001	0.441 (25.3)
<i>P</i> × <i>C</i>	3.21	0.07	0.1	0.46	0.50	0
Error			1.000			1.000
Total			2.392 (100)			1.743 (100)
Non-native populations						
Population	41.46	<0.001	0.398 (22.1)	0.00	0.98	0
Competition	29.11	<0.001	0.336 (18.6)	0.16	0.69	0
<i>P</i> × <i>C</i>	3.51	0.06	0.068	16.73	<0.001	0.429 (30.0)
Error			1.000			1.000
Total			1.802 (100)			1.429 (100)

The contribution towards morphological differentiation in native and non-native pumpkinseeds (*L. gibbosus*) is based on the discriminatory power (i.e., the percent of total variance explained) of the DFA, following an adjustment to reflect the variance components on each axis. The third canonical axis was excluded from the analyses because it added little to the discriminatory power of the DFA

<sup>a</sup> Variance components were obtained by analysing the canonical scores for each DFA axis in separate two-way ANOVAs. Values in parentheses represent the percent of total variance for each component

morphology explained by genetic factors and phenotypic plasticity were 22.2 % and 19.0 %, respectively, for native populations. Contrary to our predictions, non-native populations exhibited 28 % less plasticity (13.7 % explained by phenotypic plasticity due to competition) than native populations; non-native populations also exhibited 27 % less genetic differentiation (16.3 % explained by genetic factors).

## Discussion

Before they can become established and spread through a new environment, NIS must be capable of adjusting to novel biotic and abiotic conditions (Kolar and Lodge 2001). The pumpkinseed (*L. gibbosus*) feeds predominantly on benthic invertebrates (Osenberg et al. 1988), and yet the species continues to spread through rivers and reservoirs in the Iberian Peninsula, which are dominated by other species that consume similar prey (Magalhães 1993; Clavero et al. 2013). While previous studies have suggested that competitive interactions exist between pumpkinseeds and other fishes in Spain and Portugal (Godinho et al. 1997), no study has yet been able to confirm whether resource competition within Iberian waterbodies have any detectable effects on the species. The present findings suggest that, like their native counterparts (e.g., Arendt and Wilson 1999), Iberian pumpkinseeds are capable of exhibiting morphological plasticity in response to interspecific competition, which may help to explain their invasion success.

In their native range, the pumpkinseeds exhibit a high level of plasticity, and are capable of exhibiting phenotypic divergence in response to the surrounding environment (e.g., Robinson et al. 1993; Gillespie and Fox 2003). As a direct result of competitive interactions between congeneric bluegill (*L. macrochirus*) and green sunfish (*L. cyanellus*), pumpkinseeds shift their foraging strategy and habitat preference, with juveniles transitioning from a diet consisting of vegetation prey, to one that is more benthivorous (Werner and Hall 1979). In a later study, Robinson et al. (1993) showed that in lakes where pumpkinseed co-occurred with bluegill, the former remained in the littoral zone where they fed on insects and larvae, whereas in allopatry, some adult pumpkinseeds also occupied the pelagic zone and fed primarily on planktonic prey.

Our study confirmed that the presence of a native competitor is capable of inducing ecological divergence in both native and non-native pumpkinseed populations. Shifts in prey preference of North American and Iberian populations were observed when individuals were held in sympatry with bluegill, resulting in the consumption of fewer pelagic cladocerans and an increase in the consumption of benthic amphipods and ephemeropterans. Diet shifts have been well documented in many species (Brabrand 1985; Persson and Brönmark 2002; Liso et al. 2011), and they can be attributed to either ontogeny

or changes in resource availability (Persson and Hansson 1999). Bluegills enclosed with pumpkinseeds consumed a large quantity of copepods, few ephemeropterans, and no amphipods. Thus pumpkinseeds held in sympatry with bluegills would likely have experienced a decrease in the availability of copepods, which resulted in a shift in preference towards benthic prey that were not heavily consumed by bluegill.

Robinson et al. (2000) examined adaptive divergence in pumpkinseeds from lakes where they occur in allopatry and sympatry with bluegill, and found that sympatric pumpkinseeds consume a more littoral diet. However, the authors also found that allopatric and sympatric pumpkinseeds exhibited divergent morphological traits, corresponding to habitat-specific foraging strategies. Allopatric pumpkinseeds exhibited a body shape that was more slender, while sympatric populations were more gibbose. Streamlined body forms reduce hydrodynamic drag (Sagnes et al. 2000) and are common when fish inhabit open-water environments (e.g., Robinson and Parsons 2002; Svanbäck et al. 2008). Searching for pelagic prey can necessitate long periods of sustained swimming, which is less energetically demanding in fish with more streamlined body forms (sensu Crossin et al. 2004). A deep body facilitates maneuvering through complex habitats, or searching for submerged prey (Ehlinger and Wilson 1988; Svanbäck and Eklöv 2002). In our study, native pumpkinseeds developed deeper bodies when reared with bluegill, which is consistent with the observed dietary shift to more benthivorous prey. Iberian pumpkinseeds reared with bluegill also exhibited a diet shift towards more benthic macroinvertebrates; however, body depth did not significantly differ across treatments. Body depth is functionally significant for foraging, and, among species that exhibit trophic polymorphism, the size of this trait tends to diverge towards a state that is considered adaptive for foraging within a specific habitat (e.g., Schluter 1993; Arbour et al. 2010). The lack of phenotypic divergence in response to changing environmental conditions suggests that a trait has become canalized, and likely represents a morphological form that has been assimilated by the genotype because it is adaptive (Crispo 2007).

Phenotypic plasticity produces changes to traits that, in perturbed environments, are more beneficial in terms of fitness, and over time, a genotype can assimilate a divergent adaptive trait in a manner that no longer requires the original environmental perturbation to produce the phenotypic change (Crispo 2007). Afterwards, the genotype may resist changes to the environment and exhibit canalization, which helps to prevent the formation of maladaptations to traits that are adaptive (Ghalambor et al. 2007). In the presence of a competitor, native pumpkinseeds exhibited marked increases in body width, while Iberian pumpkinseeds do not express any significant change in body width. Moreover, the increase exhibited by native fish clearly converges towards the form already exhibited by Iberian fish, and one that has been

observed in other species that feed in littoral habitats (e.g., Svanbäck and Eklöv 2002). This is reflective of genetic assimilation, and suggests that Iberian pumpkinseeds have assimilated an external morphological form that is more optimal for foraging in littoral habitats. Yavno and Fox (2013) observed similar canalization while testing for morphological plasticity in response to water velocity; however, they identified canalization in caudal peduncle and median fin traits, rather than those pertaining to body shape. In the present study, caudal peduncle length and median fin position responded to the presence of a congeneric competitor, which suggests that these traits are not necessarily canalized.

In our multivariate DFA, genetic and environmental factors influence the variance of each canonical axis; as the total variance is additive, the analysis can summarize how each factor influences phenotypic divergence. This approach is, for instance, similar to the additive components of variance that are obtained from a heritability analysis, and used to determine the genetic contribution towards phenotypic change (e.g., Messiaen et al. 2012). While the use of variance from a DFA is less robust than using variance from a heritability analysis, the analysis nevertheless provides a good summation of how each individual factor contributes towards divergence. In the present study, the results revealed that morphological divergence due to environmental factors was 28 % weaker in Iberian populations than in native fish, supporting the univariate reaction norms in that non-native pumpkinseeds possess traits that are less plastic, which could indicate an increase in canalization. Because this value is relative, one could also interpret this finding as an increase in the plasticity of native fish, rather than canalization in non-native fish. In the absence of bluegill, North American pumpkinseeds occupy broader niches than their sympatric counterparts (e.g., Robinson et al. 2000), which may necessitate a need to exhibit a high degree of phenotypic plasticity. However, Arendt and Wilson (1997), in their analysis of growth, found no evidence that allopatric pumpkinseeds exhibited higher plasticity than fish collected from waterbodies where bluegill were present. Yavno and Fox (2013) also tested for differences in plasticity between native and non-native pumpkinseed. In their study, native populations originated from waterbodies that contain bluegill, and yet their findings were also congruent with those of the present study in that Iberian pumpkinseeds possess traits that are less plastic than those of native fish.

Small populations tend to possess less genetic variation (Frankham 1996), which many authors have argued can lead to a decreased potential for adaptive evolutionary responses to environmental change (reviewed in Willi et al. 2006). By using 20 founders to establish the artificial ponds, breeding would have produced no more than ten families, and so the standing genetic variation of pumpkinseeds in the ponds may have been less than that of pumpkinseeds from the native lakes. Decreases in genetic variation can lead to the decreased

potential for exhibiting adaptive phenotypic plasticity (Auld and Relyea 2010). With no information pertaining to relatedness among progeny, the decrease in phenotypic plasticity of juveniles from Susqueda and Ter could be attributed to a decrease in the standing genetic variation of pumpkinseeds within each artificial pond. However, the native F1 progeny used by Yavno and Fox (2013) were collected from the same artificial pond colonies, initially established with only wild 20 adults. In their study (all populations born in artificial ponds), the amount of morphological divergence due to plasticity was 32 % smaller in Iberian juveniles, relative to that of native fish. This is consistent with the present study, in which pond progeny from the Iberian Peninsula were estimated to be 27 % less plastic relative to North American fish born in the wild. Given the similarities in the relative plasticity values between the two studies, we could reasonably surmise that the F1 Iberian progeny produced in the artificial ponds possess a level of genetic variation that would have been comparable to that of progeny had they been collected directly from the source waterbodies. Nevertheless, we cannot rule out the possibility that genetic drift reduced the plasticity of Iberian populations. Few records describe the origin of pumpkinseeds introduced into Europe, and so populations established in the Iberian Peninsula during the 1890s may have been comprised largely of fish that originated from North American populations that are less plastic relative to those of the wild populations used in the present study. We suggest that future plasticity studies that use individuals from artificial sources incorporate genetic analyses, and attempt to determine the level of genetic variation among their experimental treatments.

Our study is unable to discount that the differences in phenotypic plasticity we observed may be linked to the pre-experimental conditions of our populations: native pumpkinseeds were collected in the wild, whereas the non-native pumpkinseeds are pond-reared, and represent progeny from wild-caught Iberian adults. While our findings revealed that the Iberian fish exhibited less phenotypic plasticity than the native pumpkinseeds, recent experimental comparisons have demonstrated that populations held under stable, homogeneous environments (e.g., laboratory conditions) exhibit a loss of plasticity in their phenotypic traits (Schleicherová et al. 2013). In terms of biotic and abiotic characteristics, Anstruther and Ashby lakes are noticeably more heterogeneous than the artificial ponds, which have may favoured the progeny from those populations to express higher levels of adaptive phenotypic plasticity in response to novel competitive pressures.

The present study is the first to attempt to experimentally induce changes to internal traits in multiple pumpkinseed populations, and while we did find that Iberian pumpkinseeds exhibit shorter second gill raker lengths than either of the North American populations, our results failed to provide any conclusive evidence for differences in the morphological

plasticity of internal feeding structures. The experimental time frame of the present study was similar to that of previous comparisons (e.g., Robinson et al. 1996; Yavno and Fox 2013), and while we induced morphological change to external traits, changes to internal traits may require a longer experimental period for the development of detectable differences (e.g., 240 days in Hegrenes 2001). Internal morphological traits are important for foraging, and divergent functional forms have been documented in several species. For instance, the size and number of gill rakers in *Coregonus* and *Gasterosteus* spp. are directly related to the acquisition of prey type (Day et al. 1994; Matthews et al. 2010; Kahilainen et al. 2011; Svanbäck and Schluter 2012). Pumpkinseeds also exhibit variation in feeding morphology with respect to prey and habitat preference. Gillespie and Fox (2003) observed that pelagic pumpkinseeds inhabiting several North American lakes exhibited longer, more tightly spaced gill rakers, facilitating the capture of small prey items. Bluegills naturally possess more gill rakers than pumpkinseed, but Robinson et al. (1993) determined that, in the absence of bluegill, pelagic pumpkinseeds became more morphologically similar to the congener. The pumpkinseed developed thicker gill rakers, rather than more gill rakers, which the authors speculate can achieve the same biological filtering function as the latter by decreasing the space between individual rakers.

Pumpkinseed morphology has a clear, well-documented tendency to react to changes in both biotic and abiotic characteristics (e.g., Robinson et al. 2000; Yavno and Fox 2013), and during range expansion, non-indigenous fishes are likely to experience novel selective pressures with respect to water flow, prey diversity, predation and competition. While simultaneous comparisons between native and non-native populations can help to reveal whether introduced organisms are better able to respond to novel environmental perturbations (Caño et al. 2008), few studies have done so using vertebrates. Thus far, non-native pumpkinseeds appear to be more canalized than some native populations with respect to interspecific competition (present study), and water velocity (Yavno and Fox 2013). However, fish morphology can interact with multiple biotic and abiotic factors, so Iberian populations may, for example, be more plastic than native fish in response to changes in predator communities or thermal regimes. Experimental comparisons can disentangle interactions between genetic and environmental factors, and may be key to help us understand the invasion success of NIS.

**Acknowledgments** We thank C. Corkery and his family for generously providing access to their pond, and D. Algera, J. Gobin, A. Istead, and L. Masson for their field assistance. We also thank three anonymous reviewers for providing constructive comments on an earlier draft of this paper. Funding was provided by the Natural Sciences and Engineering Research Council of Canada to MGF and by the Ontario Ministry of Training, Colleges and Universities in the form of Ontario Graduate Scholarships to SY and ACR.

## References

- Arbour JH, Hardie DC, Hutchings JA (2010) Morphometric and genetic analyses of two sympatric morphs of Arctic char (*Salvelinus alpinus*) in the Canadian High Arctic. *Can J Zool* 89:19–30
- Arendt JD, Wilson DS (1997) Optimistic growth: competition and an ontogenetic niche-shift select for rapid growth in pumpkinseed sunfish (*Lepomis gibbosus*). *Evolution* 51:1946–1954
- Arendt JD, Wilson DS (1999) Countergradient selection for rapid growth in pumpkinseed sunfish: disentangling ecological and evolutionary effects. *Ecology* 80:2793–2798
- Auld JR, Relyea RA (2010) Inbreeding depression in adaptive plasticity under predation risk in a freshwater snail. *Biol Lett* 6:222–224
- Bhagat Y, Fox MG, Ferreira MT (2006) Morphological differentiation in introduced pumpkinseed *Lepomis gibbosus* (L.) occupying different habitat zones in Portuguese reservoirs. *J Fish Biol* 69: 79–94
- Bhagat Y, Fox MG, Ferreira MT (2011) Trophic polymorphism in introduced pumpkinseed (*Lepomis gibbosus*) inhabiting Iberian reservoirs. *Environ Biol Fish* 91:203–217
- Brabrand Å (1985) Food of roach (*Rutilus rutilus*) and ide (*Leusiscus idus*): significance of diet shift for interspecific competition in omnivorous fishes. *Oecologia* 66:461–467
- Caño L, Escararé J, Fleck I, Blanco-Moreno JM, Sans FX (2008) Increased fitness and plasticity of an invasive species in its introduced range: a study using *Senecio pterophorus*. *J Ecol* 96:468–476
- Chown SL, Slabber S, McGeoch MA, Janion C, Leinaas HP (2007) Phenotypic plasticity mediates climate change responses among invasive and indigenous arthropods. *Proc R Soc B Biol Sci* 274: 2531–2537
- Chun YJ (2011) Phenotypic plasticity of introduced versus native purple loosestrife: univariate and multivariate reaction norm approaches. *Biol Invasions* 13:819–829
- Clavero M, Blanco-Garrido F, Prenda J (2004) Fish fauna in Iberian Mediterranean river basins: biodiversity, introduced species and damming impacts. *Aquat Conserv* 14:575–585
- Clavero M, Hermoso V, Aparicio E, Godinho FN (2013) Biodiversity in heavily modified waterbodies: native and introduced fish in Iberian reservoirs. *Freshw Biol* 58:1190–1201
- Copp GH, Fox MG (2007) Growth and life history traits of introduced pumpkinseed (*Lepomis gibbosus*) in Europe, and the relevance to its potential invasiveness. In: Gherardi F (ed) *Biological invaders in inland waters: profiles, distribution, and threats*. Springer, Dordrecht, pp 289–306
- Crispo E (2007) The Baldwin effect and genetic assimilation: revisiting two mechanisms of evolutionary change mediated by phenotypic plasticity. *Evolution* 61:2469–2479
- Crossin GT, Hinch SG, Farrell AP, Higgs DA, Lotto AG, Oakes JD, Healey MC (2004) Energetics and morphology of sockeye salmon: effects of upriver migratory distance and elevation. *J Fish Biol* 65: 788–810
- Davidson AM, Jennions M, Nicotra AB (2011) Do invasive species show higher phenotypic plasticity than native species and, if so, is it adaptive? A meta-analysis. *Ecol Lett* 14:419–431
- Day T, Pritchard J, Schluter D (1994) A comparison of two sticklebacks. *Evolution* 48:1723–1734
- Ehlinger TJ, Wilson DS (1988) Complex foraging polymorphism in bluegill sunfish. *Proc Natl Acad Sci U S A* 85:1878–1882
- Elvira B, Almodóvar A (2001) Freshwater fish introductions in Spain: facts and figures at the beginning of the 21st century. *J Fish Biol* 59: 323–331
- Firbank LG, Watkinson AR (1985) On the analysis of competition within two-species mixtures of plants. *J Appl Ecol* 22:503–517



- Fox MG, Vila-Gispert A, Copp GH (2007) Life-history traits of introduced Iberian pumpkinseed *Lepomis gibbosus* relative to native populations. Can differences explain colonization success? J Fish Biol 71:56–69
- Frankham R (1996) Relationship of genetic variation to population size in wildlife. Conserv Biol 10:1500–1508
- Fraser DJ, Weir LK, Bernatchez L, Hansen MM, Taylor EB (2011) Extent and scale of local adaptation in salmonid fishes: review and meta-analysis. Heredity 106:404–420
- García-Berthou E, Moreno-Amich R (2000) Introduction of exotic fish into a Mediterranean lake over a 90-year period. Arch Hydrobiol 149:271–284
- Ghalambor CK, McKay JK, Carroll SP, Reznick DN (2007) Adaptive versus non-adaptive phenotypic plasticity and the potential for contemporary adaptation in new environments. Funct Ecol 21:394–407
- Gillespie GJ, Fox MG (2003) Morphological and life history differentiation between littoral and pelagic forms of pumpkinseed. J Fish Biol 62:1099–1115
- Godinho F, Ferreira MT, Cortes RV (1997) The environmental basis of diet variation in pumpkinseed sunfish, *Lepomis gibbosus*, and largemouth bass, *Micropterus salmoides*, along an Iberian river basin. Environ Biol Fish 50:105–115
- Hanson JM, Leggett WC (1986) Effect of competition between two freshwater fishes on prey consumption and abundance. Can J Fish Aquat Sci 43:1363–1372
- Hegrenes S (2001) Diet-induced phenotypic plasticity of feeding morphology in the orangespotted sunfish, *Lepomis humilis*. Ecol Freshw Fish 10:35–42
- Higham TE (2007) Feeding, fins and braking maneuvers: locomotion during prey capture in centrarchid fishes. J Exp Biol 210:107–117
- Jastrebski CJ (2001) Divergence and selection in trophically polymorphic pumpkinseed sunfish (*Lepomis gibbosus*). PhD thesis. University of Guelph, Guelph
- Kahilainen KK, Siwertsson A, Gjelland KØ, Knudsen R, Bohn T, Amundsen PA (2011) The role of gill raker number variability in adaptive radiation of coregonid fish. Evol Ecol 25:573–588
- Keast A (1978) Feeding interrelations between age-groups of pumpkinseed (*Lepomis gibbosus*) and comparisons with bluegill (*L. macrochirus*). J Fish Res Board Can 35:12–27
- Keast A (1980) Food and feeding relationships of young fish in the first weeks after the beginning of exogenous feeding in Lake Opinicon, Ontario. Environ Biol Fish 5:305–314
- Kolar CS, Lodge DM (2001) Progress in invasion biology: predicting invaders. Trends Ecol Evol 16:199–204
- Kolbe JJ, Glor RE, Schettino LR, Lara AC, Larson A, Losos JB (2004) Genetic variation increases during biological invasion by a Cuban lizard. Nature 431:177–181
- Levins R (1968) Evolution in changing environments. Princeton Univ Press, Princeton
- Liso S, Gjelland KØ, Reshetnikov YS, Amundsen PA (2011) A planktivorous specialist turns rapacious: piscivory in invading vendace *Coregonus albula*. J Fish Biol 78:332–337
- Magalhães MF (1993) Feeding of an Iberian stream cyprinid assemblage: seasonality of resource use in a highly variable environment. Oecologia 96:253–260
- Mandrak NE, Crossman EJ (1992) Postglacial dispersal of freshwater fishes into Ontario. Can J Zool 70:2247–2259
- Marchetti MP (1999) An experimental study of competition between the native Sacramento perch (*Archoplites interruptus*) and introduced bluegill (*Lepomis macrochirus*). Biol Invasions 1:55–65
- Matesanz S, Gianoli E, Valladares F (2010) Global change and the evolution of phenotypic plasticity in plants. Ann N Y Acad Sci 1206:35–55
- Matthews B, Marchinko KB, Bolnick DI, Mazumder A (2010) Specialization of trophic position and habitat use by sticklebacks in an adaptive radiation. Ecology 91:1025–1034
- Messiaen M, Janssen CR, Thas O, De Schampheleere KAC (2012) The potential for adaptation in a natural *Daphnia magna* population: broad and narrow-sense heritability of net reproductive rate under Cd stress at two temperatures. Ecotoxicology 21:1899–1910
- Mittelbach GG (1984) Predation and resource partitioning in two sunfishes (Centrarchidae). Ecology 65:499–513
- Mittelbach GG, Osenberg CW, Wainwright PC (1999) Variation in feeding morphology between pumpkinseed populations: phenotypic plasticity or evolution? Evol Ecol Res 1:111–128
- Osenberg CW, Werner EE, Mittelbach GG, Hall DJ (1988) Growth patterns in bluegill (*Lepomis macrochirus*) and pumpkinseed (*L. gibbosus*) sunfish: environmental variation and the importance of ontogenetic niche shifts. Can J Fish Aquat Sci 45:17–26
- Parsons KJ, Robinson BW (2006) Replicated evolution of integrated plastic responses during early adaptive divergence. Evolution 60:801–813
- Parsons KJ, Robinson BW (2007) Foraging performance of diet-induced morphotypes in pumpkinseed sunfish (*Lepomis gibbosus*) favours resource polymorphism. J Evol Biol 20:673–684
- Persson A, Brönmark C (2002) Foraging capacity and resource synchronization in an ontogenetic diet switcher, pikeperch (*Stizostedion lucioperca*). Ecology 83:3014–3022
- Persson A, Hansson LA (1999) Diet shift in fish following competitive release. Can J Fish Aquat Sci 56:70–78
- Reynolds HL (1999) Plant interactions: competition. In: Pugnaire F, Valladares F (eds) Handbook of functional plant ecology. Dekker, New York, pp 649–676
- Robinson BW, Parsons KJ (2002) Changing times, spaces, and faces: tests and implications of adaptive morphological plasticity in the fishes of northern postglacial lakes. Can J Fish Aquat Sci 59:1819–1833
- Robinson BW, Wilson DS (1996) Genetic variation and phenotypic plasticity in a trophically polymorphic population of pumpkinseed sunfish (*Lepomis gibbosus*). Evol Ecol 10:631–652
- Robinson BW, Wilson DS, Margosian AS, Lotito PT (1993) Ecological and morphological differentiation of pumpkinseed sunfish in lakes without bluegill sunfish. Evol Ecol 7:451–464
- Robinson BW, Wilson DS, Shea GO (1996) Trade-offs of ecological specialization: an intraspecific comparison of pumpkinseed sunfish phenotypes. Ecology 77:170–178
- Robinson BW, Wilson DS, Margosian AS (2000) A pluralistic analysis of character release in pumpkinseed sunfish (*Lepomis gibbosus*). Ecology 81:2799–2812
- Sagnes P, Champagne JY, Morel R (2000) Shifts in drag and swimming potential during grayling ontogenesis: relations with habitat use. J Fish Biol 57:52–68
- Schleicherová D, Sella G, Lorenzi MC (2013) Do stable environments select against phenotypic plasticity in hermaphroditic sex allocation? Ital J Zool 80:358–363
- Schluter D (1993) Adaptive radiation in sticklebacks: size, shape, and habitat use efficiency. Ecology 74:699–709
- Scott WB, Crossman EJ (1973) Freshwater fishes of Canada. Fisheries Research Board of Canada. Ottawa, Ontario
- Snaydon RW (1991) Replacement or additive designs for competition studies? J Appl Ecol 28:930–946
- Springer VG, Johnson GD, Schaefer SA (2000) Use and advantages of ethanol solution of alizarin red S dye for staining bone in fishes. Copeia 2000:300–301
- Strauss RE, Bookstein FL (1982) The truss: body form reconstructions in morphometrics. Syst Zool 31:113–135
- Svanbäck R, Eklöv P (2002) Effects of habitat and food resources on morphology and ontogenetic growth trajectories in perch. Oecologia 131:61–70
- Svanbäck R, Schluter D (2012) Niche specialization influences adaptive phenotypic plasticity in threespine stickleback. Am Nat 180:50–59

- Svanbäck R, Eklöv P, Fransson R, Holmgren K (2008) Intraspecific competition drives multiple species resource polymorphism in fish communities. *Oikos* 117:114–124
- Werner EE, Hall DJ (1976) Niche shifts in sunfishes: experimental evidence and significance. *Science* 191:404–406
- Werner EE, Hall DJ (1977) Competition and habitat shift in two sunfishes (Centrarchidae). *Ecology* 58:869–876
- Werner EE, Hall DJ (1979) Foraging efficiency and habitat switching in competing sunfishes. *Ecology* 60:256–264
- Willi Y, Buskirk JV, Hoffmann AA (2006) Limits to the adaptive potential of small populations. *Annu Rev Ecol Evol Syst* 37:433–458
- Yavno S, Fox MG (2013) Morphological change and phenotypic plasticity in native and non-native pumpkinseed sunfish in response to sustained water velocities. *J Evol Biol* 26:2383–2395
- Yavno S, Fox MG, Vila-Gispert A, Bhagat Y (2013) Morphological differences between native and non-native pumpkinseed in traits associated with locomotion. *Environ Biol Fish* 96:507–518