



# An ecomorphological framework for the coexistence of two cyprinid fish and their hybrids in a novel environment

BENJAMIN J. TOSCANO<sup>1,2†‡</sup>, DOMITILLA PULCINI<sup>3†</sup>, BRIAN HAYDEN<sup>2</sup>, TOMMASO RUSSO<sup>3</sup>, MARY KELLY-QUINN<sup>2</sup> and STEFANO MARIANI<sup>2\*</sup>

<sup>1</sup>Department of Ecology and Evolutionary Biology, University of Connecticut, 75 North Eagleville Road, Storrs, Connecticut 06269-3043, USA

<sup>2</sup>UCD School of Biology and Environmental Science, University College Dublin, Belfield, Dublin 4, Ireland

<sup>3</sup>Laboratory of Experimental Ecology and Aquaculture, Department of Biology, University of Rome 'Tor Vergata', via della Ricerca Scientifica s.n.c, 00133 Rome, Italy

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Niche variation between hybrid taxa and their parental species has been deemed imperative to the persistence of hybrid populations in nature. However, the ecological factors promoting hybrid establishment remain poorly understood. Through the application of a multidisciplinary approach integrating genetics, morphometry, life-history, and trophic ecology, we studied the hybrids of roach (*Rutilus rutilus* L.) and bream (*Abramis brama* L.), and their parental species inhabiting an Irish lake. The roach × bream hybrid exhibited a body shape intermediate of that of the parental species. Diet analyses depicted the hybrid as a generalist, feeding on all prey items consumed by either parental species. Stable isotope data confirm the trophic niche breadth of hybrids. A significant correlation between body shape and diet was detected, suggesting that the intermediate phenotype of hybrids might play a role in their feeding abilities, resulting in the utilization of a broader trophic spectrum than the parental species. Growth and age class structure analyses also yielded a scenario that is consistent with the ecological success of hybrids. Genetic analyses suggest that the majority of hybrids result from first-generation crosses between the parental species; however, a potentially significant proportion of back-crosses with bream were also detected. The recent introduction of roach and bream into Irish waters, as well as the climatic and ecological features of the colonized habitats, can explain the remarkable success of the roach × bream hybrid in Ireland. The adaptive significance of hybridization and its demographic consequences for the parental species are discussed. © 2010 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2010, 99, 768–783.

ADDITIONAL KEYWORDS: *Abramis brama* – adaptation – freshwater – hybridization – *Rutilus rutilus*.

## INTRODUCTION

Many studies have stressed that interspecific hybrids should exhibit significant reductions in viability, fertility, and fitness (Templeton, 1981; Barton & Hewitt, 1985) from the disruption of coadapted parental gene

complexes (Dobzhansky, 1951; Mayr, 1963; Waser & Price, 1991) or through the creation of morphologically intermediate offspring, adapted to neither parental habitat and outcompeted by nonhybrid individuals (Arnold & Hodges, 1995). Yet, hybridization is increasingly recognized as an evolutionary force that can lead to adaptation through the creation of novel genotypes and morphologies (Rieseberg, Van Fossen & Desrochers, 1995; Arnold, 1997). This phenomenon is particularly common when populations invade new environments, and potentially elevates the rate of

\*Corresponding author. E-mail: stefano.mariani@ucd.ie

†These authors contributed equally to this work.

‡Present address: Department of Biological Sciences, University of South Carolina, Columbia, SC 29208, USA.

response to selection by exposing colonizing populations to rapid adaptive diversification under disruptive or divergent selection (Seehausen, 2004; Hänfling, 2007). Empirical studies show that hybrid taxa may differ ecologically from both parents by occupying different spatial, temporal or diet niches (Demarais *et al.*, 1992; Rieseberg, 1997; Giessler, Mader & Schwenk, 1999; Lexer *et al.*, 2003).

The hybrid between roach (*Rutilus rutilus* L.) and bream (*Abramis brama* L.) is one of the most common cyprinid hybrids occurring in British (Pitts *et al.*, 1997) and especially Irish waters (Kennedy & Fitzmaurice, 1973; Wood & Jordan, 1987; Fahy, Martin & Mulrooney, 1988). Hybridization is facilitated by the temporal overlap of spawning activities and the fishes' preference for similar aquatic vegetation as a spawning habitat (Wheeler, 1969; Diamond, 1985; Pitts *et al.*, 1997). This tendency can probably be further enhanced by anthropogenic factors, such as habitat modification, which increases competition for spawning habitat (*sensu* Bianco, 1982). Genetic compatibility exists between the gametes of roach and bream (Wood & Jordan, 1987), and putative roach × bream hybrids were identified in several studies using meristic and genetic characters (Kennedy & Fitzmaurice, 1968; Brassington & Ferguson, 1976; Child & Solomon, 1977; Cross, 1978; Cowx, 1983; Mulrooney & Fahy, 1985).

In Ireland, roach was accidentally introduced as a baitfish in the late 19th Century to the Munster Blackwater (Went, 1950). Subsequently, they colonized all major river systems in the country during the 1960s and 1970s (Fitzmaurice, 1981). Bream are also non-native to Ireland (Went, 1957; Wheeler, 1969), although their exact origin is unknown and, prior to an extensive stocking programme beginning in 1955, they had a limited distribution in the country (Kennedy & Fitzmaurice, 1968). Both species are now well established in every major watercourse in the country. Roach × bream hybrids are present in large numbers in water bodies containing both parental species, often outnumbering them (J. Caffrey, unpubl. data). This situation is unique to Ireland. Although both parental species are found in large numbers throughout Central and Eastern Europe, roach × bream hybrids are rarely found in greater than incidental quantities (Cowx, 2001).

The rapid success of these species bears testament to the breadth of niche space available in Ireland to taxa well adapted to eutrophic conditions. Irish freshwaters have considerably fewer taxa than either the UK or mainland Europe: the only native species are cold-water diadromous fishes, namely salmonids, coregonids, eels, and shads, which colonized the country after the last glaciation (Fitzsimons & Igoe, 2004). The widespread eutrophication which occurred

in Ireland during the latter part of the 20th Century (Wilson, 1998) created many freshwater environments that were less suitable to the native fish species but to which cyprinids, particularly roach and bream, could inhabit successfully (Olin *et al.*, 2002; Mehner *et al.*, 2007). For both roach and bream, Irish lakes and reservoirs therefore represent a relatively novel environment (compared to the continental mainland) to which they have adapted for long evolutionary times. By examining the links between external morphology and resource use in roach, bream and their hybrids, we explored whether the morphological characters of the latter could allow them to exploit the available resources more successfully than the parental species, and hence play a role in their adaptation to Irish freshwaters.

## MATERIAL AND METHODS

### STUDY SITE AND SAMPLING

Lough Ramor is a hyper-eutrophic Irish lake (Toner *et al.*, 2005) situated on the Kells Blackwater River in the Irish midlands. It has a mean depth of 2.25 m with a maximum depth of approximately 5 m at the north end of the lake. The fish community is dominated by non-native species, particularly roach, bream, roach × bream hybrids, perch (*Perca fluviatilis* L.), pike (*Esox lucius* L.), and gudgeon (*Gobio gobio* L.).

Sampling was conducted during July of 2007. To achieve an unbiased coverage of all size classes present in the population, specimens were captured using Nordic multimesh gill nets (Appelberg, 2000; Holmgren & Appelberg, 2000). Each net measured 30 m in length, was 1.5 m high, and consisted of 12 equidistant 2.5-m panels with mesh sizes in the range 5–55 mm. Two nets were cast in random locations throughout the lake during the day and retrieved the following morning for four consecutive days. A total of 209 individuals (55 bream, 88 roach, and 66 hybrids) were collected. Captured fish were weighed to the nearest gram and measured to the nearest millimetre. Five to ten scales were removed from the dorsal surface of each fish for ageing. The first third of the digestive tract was removed and preserved in 70% ethanol for later dissection.

### AGEING

A sub-sample of bream ( $N = 43$ ) and roach ( $N = 61$ ) and all 66 hybrids were aged by scale reading. In the laboratory, scales were cleaned with a saline solution and viewed under a Bell & Howell MT633 microfiche reader. Annual checks were recorded at the end of areas where circuli became closely spaced followed by areas of far spaced circuli. An annual check was only

confirmed when associated structures could be viewed around the circumference of the check (Cragg-Hine & Jones, 1969). At least three scales were read for each fish to confirm the age and 20% of scales were cross-checked by a second scale analyst to avoid bias. Age data were used to identify year class structure for each taxon (Goldspink, 1978, 1981), whereas the growth rate of each fish was estimated by back calculating length-at-age (Bagenal & Telsch, 1978). To avoid the bias and possible error associated with back calculation (Francis, 1990), measurements were made using the body proportional hypothesis, whereby the body length of the fish was regressed based on the scale radius (Horppila, 2000). This method has been shown to provide the most accurate assessment of length at age via back calculation for roach (Horppila & Nyberg, 1999).

#### FEEDING ANALYSIS

The gut contents from a subset ( $N = 30$  for each taxon and hybrids) of the total sample were analysed. Gut contents were emptied into a Petri dish and observed under a dissecting microscope. Contents were classified to the lowest taxonomic level possible and recorded as present or absent.

To overcome the difficulties associated with observational dietary analysis (Bearhop *et al.*, 2004), stable isotope analysis (SIA) was performed. Over the last decade, SIA has emerged as an effective tool in the study of trophic niche ecology (Bolnick *et al.*, 2002; Layman *et al.*, 2007; Newsome *et al.*, 2007). As a predator assimilates a food item, the lighter isotopes of each element present in the prey are positively selected during metabolic reactions by a process of fractionation (Martinez del Rio & Wolf, 2005). Consequently, the muscle tissue of the predator is enriched in the isotope relative to its prey. Because fractionation occurs at known levels in nitrogen (approximately 3.4‰), it is possible to ascertain at what trophic level a consumer feeds (Post, 2002). Carbon isotopes, on the other hand, increase minimally during fractionation (approximately 1‰) but vary considerably between food types (Phillips & Gregg, 2001), allowing determination of the food source from which a consumer draws most of its carbon (Post, 2002; Harrod & Grey, 2006). An advantage of SIA over gut content analysis is the slow turnover of isotopes in the muscle tissue of predators, typically over 3–6 months (Dalerum & Angerbjorn, 2005; Newsome *et al.*, 2007). Therefore, isotopic values represent the seasonal trophic position of consumers, thus avoiding the temporal and subjective biases implicit in traditional gut content analysis.

Carbon and nitrogen SIA was performed on fish and invertebrates sampled during March and August of 2006. SIA was performed on 134 fish (33 bream, 52

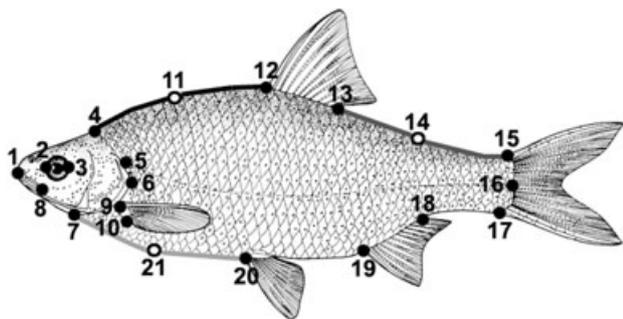
roach, and 49 hybrids). In addition to the fishes, molluscs, chironomid larvae, *Asellus*, and *Gammarus* were sampled to provide isotopic values for the major prey types available in Lough Ramor. Invertebrate samples were stored in 70% ethanol and washed thoroughly with double distilled H<sub>2</sub>O before processing. Both fish and invertebrate samples were oven dried (60 °C for 24 h), and dried samples were ground using a pestle and mortar. Sub-samples (1 mg ± 0.1 mg) were removed from the ground tissue and stored in pre-weighed tin capsules. For all invertebrate groups included in the analysis, three to seven individuals from each lake were ground together to give an amalgamated value for that group. Each group was sampled during both sampling events (March and August) and results are presented as the mean ± SD. Because lipid stores are <sup>13</sup>C depleted (DeNiro & Epstein, 1977), <sup>δ</sup><sup>13</sup>C values of fish were 'lipid fixed' using the lipid normalizing model outlined by Kiljunen *et al.* (2006).

SIA was performed using a Europa Scientific 20–20 IRMS with a Europa Scientific Roboprep-CN preparation module at Iso-Analytical Ltd. Reference samples run as quality control checks during the analysis were: powdered bovine liver (<sup>δ</sup><sup>13</sup>C<sub>V-PDB</sub> = -21.60‰, <sup>δ</sup><sup>15</sup>N<sub>Air</sub> = 7.65‰), sucrose (<sup>δ</sup><sup>13</sup>C<sub>V-PDB</sub> = -10.43‰), beet sugar (<sup>δ</sup><sup>13</sup>C<sub>V-PDB</sub> = -26.03‰), and two ammonium sulphate samples (<sup>δ</sup><sup>15</sup>N<sub>Air</sub> = -4.71‰, <sup>δ</sup><sup>15</sup>N<sub>Air</sub> = 20.30‰). In all cases, levels of experimental error, measured as the SD of replicates of reference samples, were less than 0.15.

#### MORPHOMETRICS

To perform shape analysis, each fish was photographed in a lateral view and digital images were processed: on each specimen, landmarks were digitized using the software TPSDIG (Rohlf, 2006). To guarantee an overall coverage of shape (Zelditch *et al.*, 2004; Perez, Bernal & Gonzalez, 2006), three outline curves (Fig. 1), encompassing two reference structures, were recorded on each specimen, and then sliding semi-landmarks were automatically chosen along each curve using TPSDIG. A 'sliding semi-landmark' is defined as a landmark that depends upon other points, so that the degree of freedom is reduced. The final configuration was represented by 18 conventional landmarks and three sliding semi-landmarks for each specimen (Fig. 1).

Landmarks and semi-landmarks were converted to shape coordinates by Procrustes superimposition (Rohlf & Slice, 1990), standardizing each specimen to unit centroid size, or an estimate of overall body size (Bookstein, 1991). Residuals from the registration were analysed with the thin-plate spline interpolating function (Bookstein, 1991).



**Figure 1.** Configuration of landmarks (black circles) and semi-landmarks (white circles) collected on roach (in the image), bream and hybrid. (1) Snout tip; (2) right and (3) left extremes of the eye circle; (4) boundary between the head and the dorsal curve; (5) anterior end of the lateral line; (6) point of maximum extension of the operculum on the lateral profile; (7) insertion of the operculum on the lateral profile; (8) posterior extremity of the premaxillar; (9) superior and (10) inferior insertions of the pectoral fin; (11) semi-landmark collected along the curve (in black) delimited by landmarks (4) and (12); (12) anterior and (13) posterior insertions of the dorsal fin; (14) semi-landmark collected along the curve (in dark gray) delimited by landmarks (13) and (15); (15) superior and (17) inferior insertions of the caudal fin; (16) posterior body extremity; (18) anterior and (19) posterior insertion of the anal fin; (20) insertion of the pelvic fin; (21) semi-landmark collected along the curve (in light gray) delimited by landmarks (20) and (7).

#### GENETIC IDENTIFICATION

The ITS1 nuclear ribosomal DNA region was used to genetically identify hybrids in accordance with the method described by Wyatt, Pitts & Butlin (2006). Screened specimens included 20 bream, 20 roach, and 40 hybrids, which were randomly chosen among the individuals analysed morphometrically. A multiplex polymerase chain reaction containing a universal forward primer and two species-specific reverse primers, which amplify fragments of approximately 386 bp and approximately 150 bp for roach and bream, respectively, was performed on all specimens. All individuals displaying both ITS1 bands on agarose gel were regarded as hybrids. Hardy–Weinberg assumptions for a biallelic locus ( $p^2 + pq + q^2$ ) were used as a basic framework to either identify hybrid morphotypes bearing only one of the parental fragments as post- $F_1$  hybrids, or detect parental morphotypes bearing both bands as back-crosses. In a neutral framework, back-crosses bearing only the parental fragment would be present at the same frequency as those exhibiting both bands.

#### STATISTICAL ANALYSIS

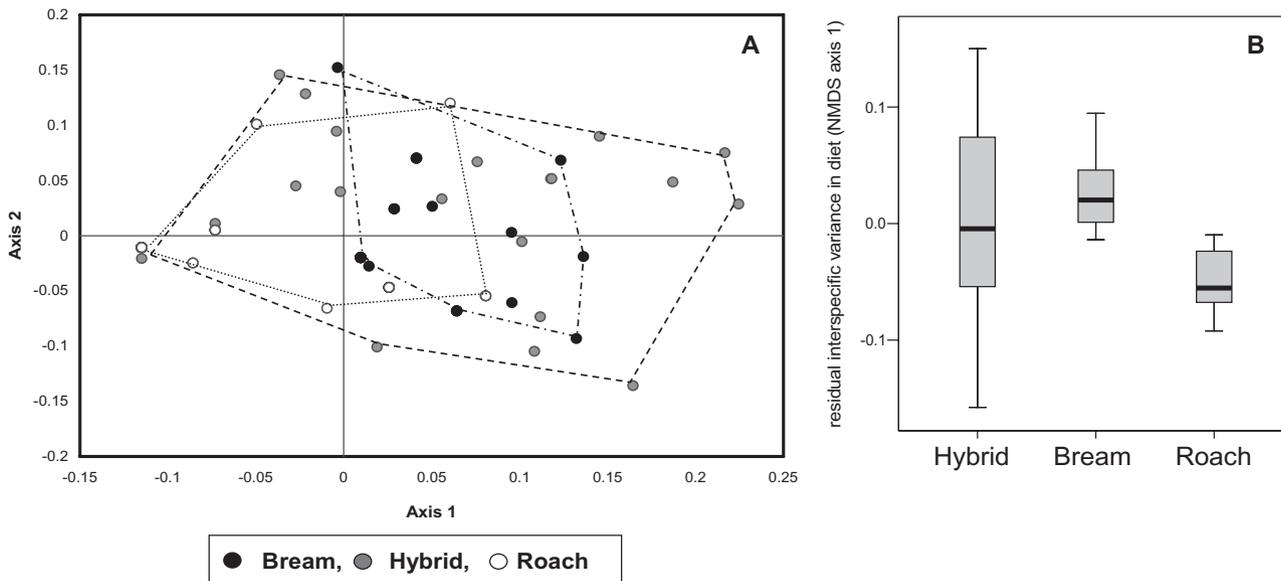
Variation in prey composition was analysed by performing a nonmetric multidimensional scaling

(NMDS) (Kruskal, 1964a, b; Schiffman, Reynolds & Young, 1981) on the Manhattan distance matrix of the stomach contents data from the fish taxa. The NMDS ordination provided a two-dimensional map of the distances between samples in a Euclidean space. Because the size of roach was significantly smaller than both bream and hybrids [analysis of variance (ANOVA),  $F = 33.1$ ;  $P < 0.001$ ], we used the Spearman rank coefficient of determination between length and the scores along the NMDS first axis to estimate the amount of variance in the diet explained by size. We then tested whether the residual variation was still accounted for by dietary differences between bream, roach, and hybrids.

To identify the taxa primarily responsible for observed differences in diet, similarity percentage analysis (SIMPER) (Clarke, 1993) was performed. This analysis implicitly uses Bray–Curtis similarity measure and employs the analysis of similarity test to assess the overall significance of the difference between groups. NMDS, Spearman correlation analysis, and SIMPER were performed using PAST (Hammer, Harper & Ryan, 2001).

Relative warp analysis was performed using TPSRELW (Rohlf, 2001) to synthesize and visualize the morphological variation between the three taxa. The pattern of covariation between shape and size was analysed using partial least square (PLS) analysis (Bookstein *et al.*, 2003; Zelditch *et al.*, 2004). This analysis is a multivariate correlation technique that helps to find correlated pairs of linear combinations (singular vectors) between two blocks of variables (in this case shape and size). The singular vectors computed by PLS are constructed in the form of new, paired 'latent' variables accounting for as much of the covariation as possible between the two original sets of variables (for details and computational aspects, see Bookstein, 1991; Rohlf & Corti, 2000; Fadda & Corti, 2001; Bookstein *et al.*, 2003). The amount of covariance explained by the paired singular vectors (i.e. the correlation ' $R$ ' of the scores of specimens along the singular axes of the two blocks; Rohlf, 1990) allows a statistical quantification of the correlations.

PLS was also employed to explore the pattern of covariation between shape and diet within the three taxa (Russo *et al.*, 2008). For this analysis, 90 individuals (30 for each taxon) were available (all those for which stomach contents were examined). Correlation coefficients were estimated and their significance tested by means of permutation as implemented in TPSPLS software. The outputs were represented by two-dimensional bubble plots, in which the size of the bubble was related to the size of the individuals, aiming to relate the pattern of shape–diet covariation with growth. Histograms of scores at the extreme of the latent vector of diet and splines showing the



**Figure 2.** A, nonmetric multidimensional scaling (NMDS) of the Manhattan distance computed on trophic data. The dashed, dash-dotted and dotted connecting lines enclose the areas occupied by points of hybrids, bream and roach, respectively. B, box-whisker plot of residual variance of the NMDS axis 1 among the three groups, after removing the effect of size. Boxes represent SDs; whiskers the 95% percentile distributions.

relevant deformation at the extreme of the latent vector of shape were used to visually explain the detected patterns.

A Euclidean distance matrix was created from non-transformed  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of fish analysed from all lakes. The levels of isotopic overlap between seasonal samples of each taxon as well as overlap between the three taxa were assessed using the one-way nonparametric (NP) multivariate analysis of variance (MANOVA) test in PAST (Hammer *et al.*, 2001). NPMANOVA is a nonparametric measure of variation between two or more groups based on a distance measure, in this case the Euclidean distance similarity matrix. The test assigns significance by calculating permutations (10 000) of group membership, and the  $F$ -value is analogous to the ANOVA  $F$ -ratio. PAST calculates pairwise  $P$ -values and Bonferroni corrected  $P$ -values as a post-hoc analysis. The conservative Bonferroni corrected  $P$ -values were used when assessing levels of variation between groups.

Finally, a measure of isotopic niche breadth (total area; TA) was constructed based on the Euclidean space occupied by a convex hull encompassing the full distribution of isotopic values of each species (Layman *et al.*, 2007).

## RESULTS

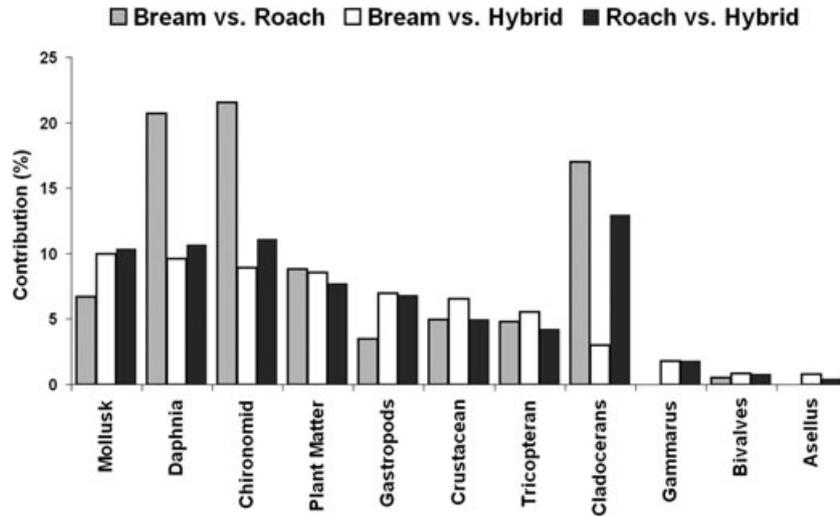
### FEEDING ECOLOGY

Hybrids were found to feed on all 11 prey items found in the gut contents, whereas bream and roach had

narrower food spectra, characterized by nine and eight food items, respectively. Bream did not consume cladocerans, whereas roach did not prey on bivalves and the amphipod *Gammarus*. Remarkably, the isopod *Asellus* was only found in the gut contents of hybrids.

The narrower food spectra of the parental species are clearly visible in the NMDS plot (Fig. 2A), where the roach specimens are scattered to the left of the ordination and are only partially overlapped with the bream individuals (to the right). The hybrids show a broader distribution, encompassing all the individual-points of the other two taxa. This apparent segregation along the first axis was positively correlated with the length of individual fish, with approximately 17% of the variance in diet explained by size. After removing the effect of size, the residual dietary variance showed even more pronounced differences between roach and bream (ANOVA,  $F = 4.5$ ;  $P < 0.01$ ) and confirmed that hybrids occupy the full breadth of the trophic space (Fig. 2B).

The overall average dissimilarity is higher for the bream versus roach comparison (88.63%) than in the comparisons between the hybrid and either one of the parental species (62.58% and 71.88% for bream/roach versus hybrid, respectively). SIMPER analysis pinpoints the contribution of single prey items in discriminating diet profiles (Fig. 3): it is revealed that chironomids, as eaten almost exclusively by common bream, and *Daphnia* and other cladocerans, as eaten mostly by roach, represent the food items most



**Figure 3.** Histogram showing the contribution of each food item to the differentiation of the diet of bream, roach, and their hybrid computed by SIMPER. Greater values refer to greater dissimilarity, between species pairs, in the consumption of a given food item.

**Table 1.** Results of carbon and nitrogen isotope analysis of fish and invertebrate prey groups sampled in Lough Ramor

	N	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	Niche width
		Mean $\pm$ SD	Mean $\pm$ SD	
<b>Fish</b>				
Bream	33	$-28.6 \pm 0.2$	$13.8 \pm 0.4$	0.8
Roach	52	$-28.4 \pm 0.6$	$14.3 \pm 0.6$	4.3
Roach $\times$ bream hybrid	49	$-28.1 \pm 0.57$	$14.5 \pm 0.62$	4.1
<b>Invertebrates</b>				
Bivalve		$-32.1 \pm 0.1$	$9.9 \pm 0.05$	
Gastropod		$-27.5 \pm 0.06$	$10.3 \pm 0.05$	
Gammarus		$-26 \pm 0.9$	$9.7 \pm 0.5$	
Chironomid		$-34.5 \pm 6.6$	$7.6 \pm 2.4$	

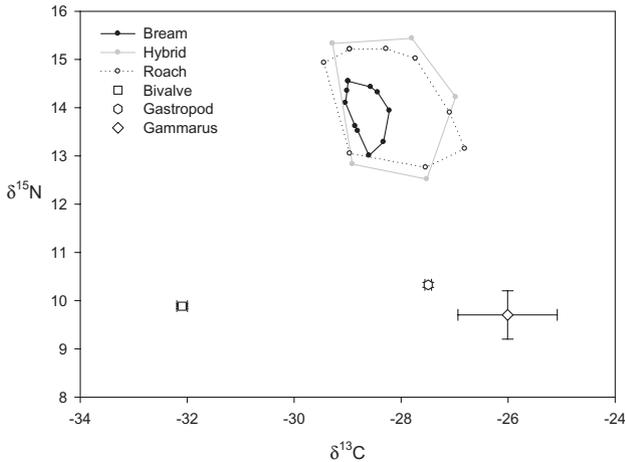
responsible for diet dissimilarity between the two species. In the bream versus hybrid comparison, the greatest difference in diet composition was a result of the presence of unidentified mollusks, consumed by hybrids, whereas, in the roach versus hybrid comparison, the absence of cladocerans other than *Daphnia* in the hybrids' diet differentiated the two taxa.

No temporal variation was observed between repeat SIA samples from March and August ( $P = 0.1$ ). Consequently, data from both samples were pooled and the presented results refer to the overall annual isotopic signatures for each taxon (Table 1). The isotopic distribution of bream varied significantly with that of both roach ( $P < 0.01$ ) and roach  $\times$  bream hybrids ( $P < 0.01$ ); however, no variation was observed between the isotopic distribution of roach and roach  $\times$  bream hybrids ( $P = 0.14$ ). Consistently, roach and hybrids also displayed fully overlapping isotopic

niches of similar size (TA = 4.3 and 4.6, respectively; Fig. 4) both of which largely exceeded that of bream (TA = 0.8).

#### SHAPE

The first two relative warps accounted for 78.4% of the total variance: the largest proportion of variance was accounted for by the first axis (RW1, 70%), which expressed morphological variation among the three taxa. The second axis (RW2, 8.4%) described within-species variability (Fig. 5). All bream specimens (in black) are located in the negative end of the first axis and roach specimens (in white) are in the positive one. The hybrid specimens (in grey) are positioned between the parental species, with no overlap between the three groups. RW1 describes a gradient in body shape from deeper (left) to slimmer (right)



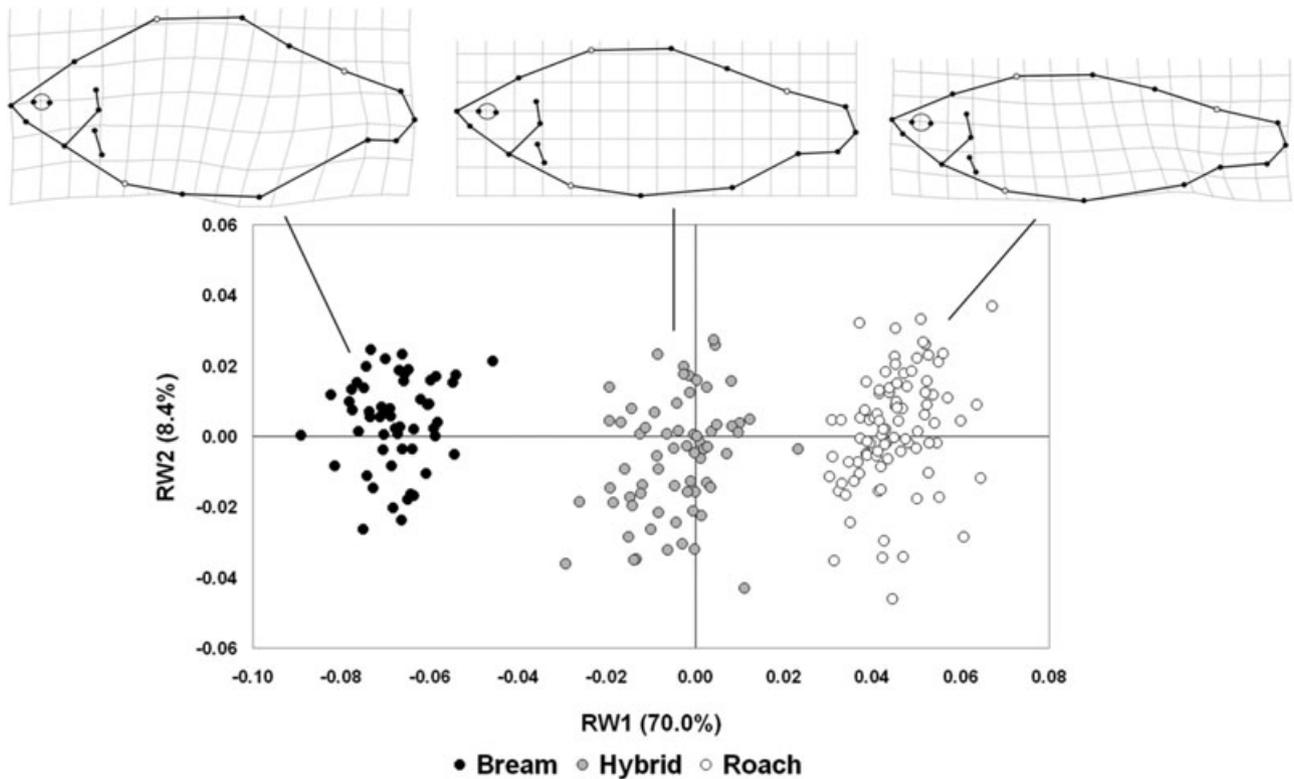
**Figure 4.** Isotope biplot of the distribution of isotope values of bream, roach and hybrids along with the mean  $\pm$  SD of major invertebrate groups. The isotopic niche of each taxon is represented by a convex hull encompassing the full range of isotope values for those fish. Because of their extremely depleted  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values, chironomids values were omitted from the plot.

proportions. No major morphological modifications occur in the regions of the head and tail, except for a slight shortening of the premaxillar length in roach. Almost all the variation is concentrated in the trunk area.

PLS revealed a significant covariance between shape and size ( $R = 0.64\%$ ;  $P < 0.001$ ). Also in this case, the hybrid's ontogenetic trajectory is positioned between the trajectories of the parental species, with no overlap (Fig. 6). Interestingly, this is paralleled in the comparison of growth rates (see below).

#### GENETICS

The vast majority of hybrids analysed were believed to be  $F_1$ . No homozygote fish with a hybrid phenotype were recorded (Table 2), and although using our methodology any heterozygous  $F_2$  progeny would be undistinguishable from the  $F_1$ , the absence of homozygous fish with a hybrid phenotype indicates that any post- $F_1$  hybridization taking place is negligible. Three fish with a bream phenotype and one fish with a roach phenotype amplified both bands; these three



**Figure 5.** Scatterplot representing the results of the relative warp analysis performed on shape data collected on bream (in black), roach (in white), and their hybrid (in grey). The splines are reported on the right and left of the plot, relative to the extreme configurations of the first axis (RW1: 70%). The consensus is represented at the top. Black circles on the splines represent the position of the traditional landmarks; white circles represent the positions of the sliding semi-landmarks.

specimens were considered back-crosses (based on their parental phenotype). In accordance with Hardy–Weinberg expectations, we estimated that four homozygote parental phenotypes (three bream-like and one roach-like) would also potentially be genetically undetectable back-crossings. This corresponds to a rate of back-crossing of 10% and 30% of the roach and bream populations, respectively.

#### CORRELATION BETWEEN SHAPE AND DIET

A highly significant covariance between ‘shape’ and ‘diet’ was detected with PLS for bream ( $R = 0.7$ ;  $P < 0.01$ ), roach ( $R = 0.9$ ;  $P < 0.01$ ), and hybrids ( $R = 0.6$ ;  $P < 0.01$ ). The model yielded by the first two latent vectors represents the coupled changes in diet and shape. The extremes of the bream linear pattern (Fig. 7A) appear very similar, suggesting that this species does not undergo remarkable changes in shape in the part of its life history examined in the present study. The most important shape change involves the head region, with larger individuals having proportionally larger heads and eyes. Also, the dorsal fin becomes shorter in larger individuals. Associated changes in the diet include an increase in

mollusk consumption and a decrease in crustaceans. The latent vector of shape for hybrids (Fig. 7B) revealed a decrease of head and eye size in larger individuals and a significant deepening of the trunk. As clearly visualized by the bubbles, shape co-varies with size (Spearman  $R = 0.77$ ,  $P < 0.001$ ), and a corresponding complete diet shift across the entire food spectrum is observed. To a lesser extent, a deepening of the trunk is also observed in roach (Fig. 7C), which is also associated with a complete shift in food preferences. Consequently, a PLS conducted with pooled data was also significant ( $R = 0.71$ ,  $P < 0.01$ ), confirming hybrids as the most trophically and morphologically variable group.

#### AGE AND GROWTH

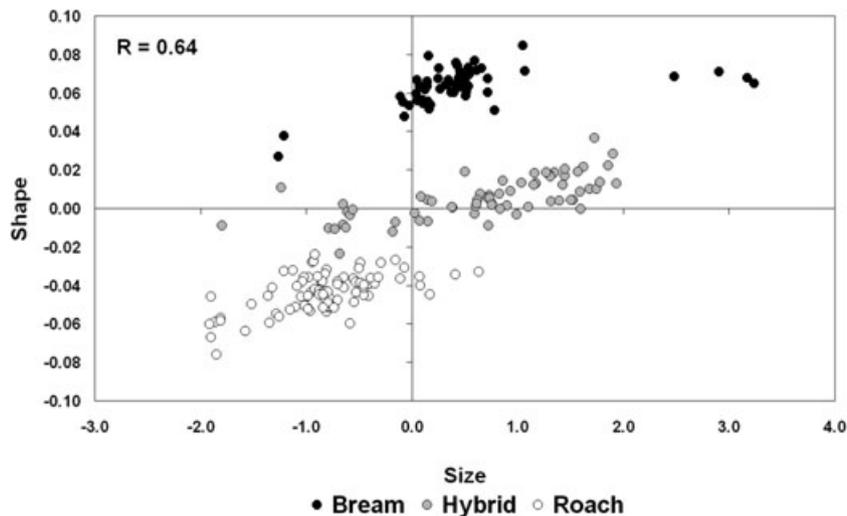
Roach  $\times$  bream hybrids exhibited a growth rate intermediate between the parental species (Fig. 8A) and higher than previously recorded for other populations in both Irish and British waters (Fig. 8B). The age frequency distributions reveal remarkable temporal variation in year class strength among taxa (Fig. 9). In certain years, such as 1995–2001, hybrids dominate recruitment, whereas strikingly in 2003, a very strong recruitment of bream was reflected in a very poor year for hybrids. The roach population consisted predominantly of fish less than 4 years of age, although little recruitment is evident for the 2005 year class.

**Table 2.** Results of banding patterns recovered after polymerase chain reaction amplification of the ITS1 marker in 80 fish phenotypically identified as hybrids ( $N = 40$ ), bream ( $N = 20$ ), and roach ( $N = 20$ )

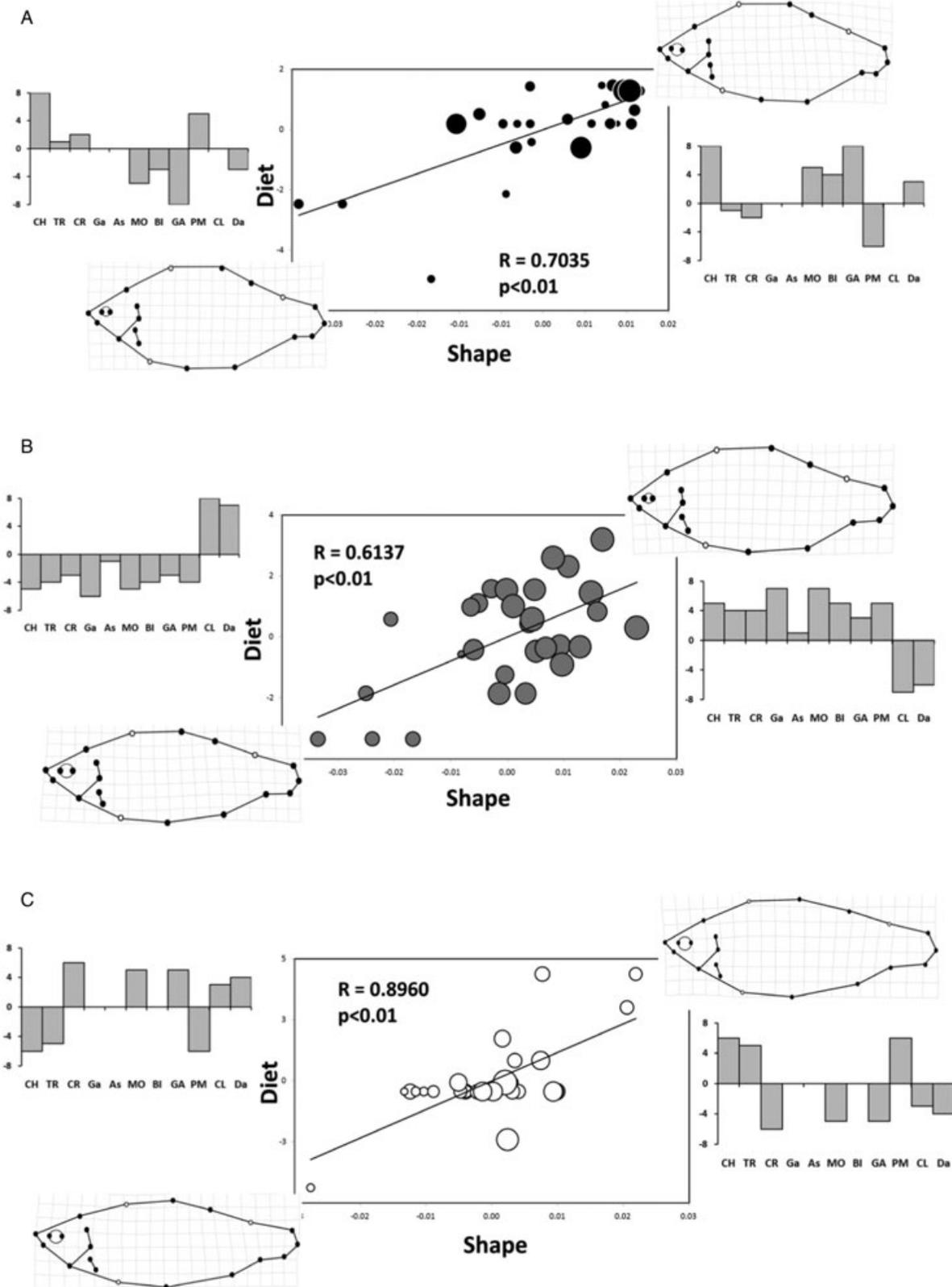
	Homozygote	Heterozygote
Bream	17	3
Roach	19	1
Hybrids	0	40

#### DISCUSSION

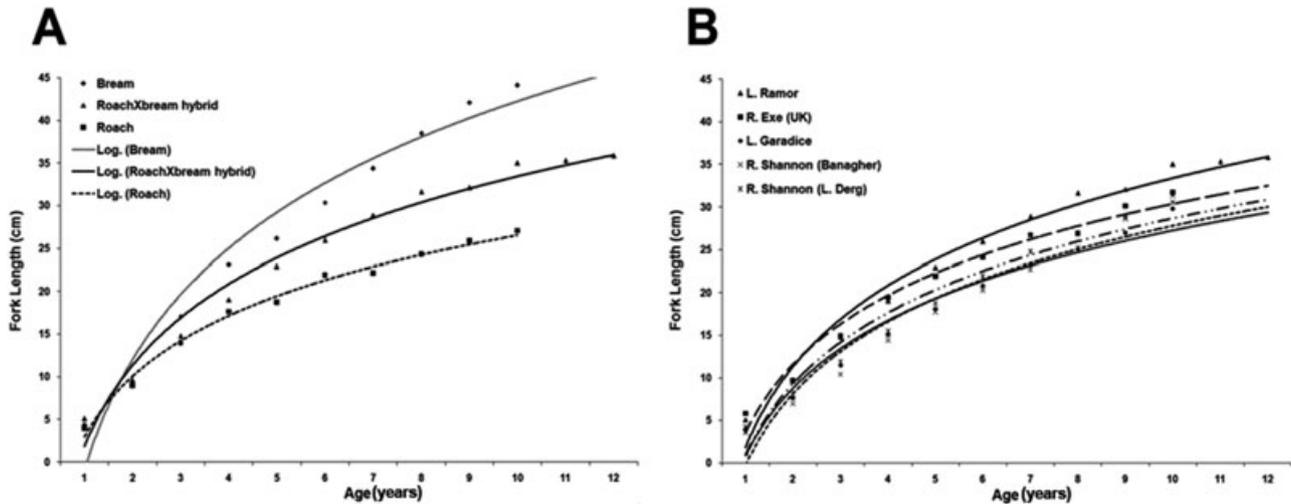
The topic of hybrid fitness has received much attention in the long-standing debate over the role of natural hybridization in animal evolution (Arnold &



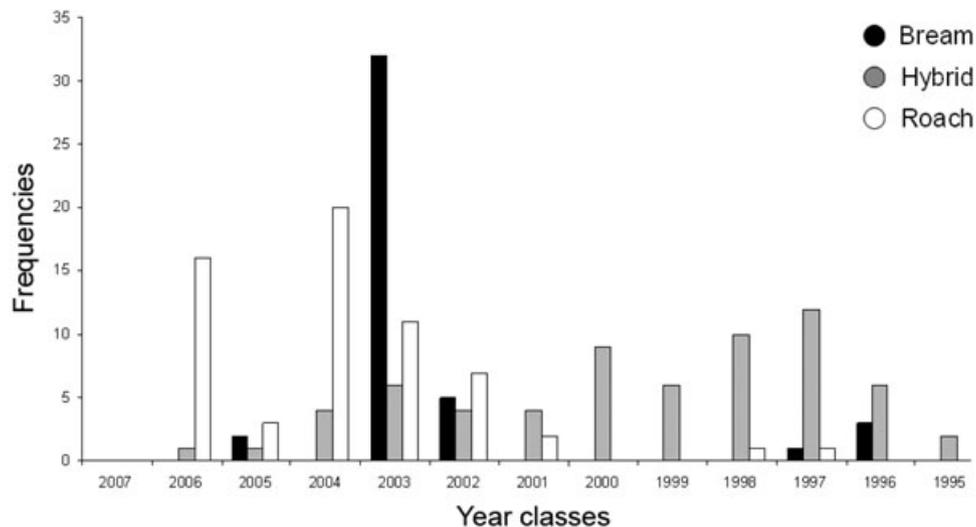
**Figure 6.** Scatterplot of the partial least square analysis computed on ‘shape’ and ‘size’ variables.



**Figure 7.** Two-dimensional bubble-plots of coupled partial least square latent vectors of shape and diet. The plot shows the relationship between whole shape and diet. The splines depict shape variation along the latent vector of shape, and histograms represent the correlation between the consumption of each single food item to these splines. A, bream; B, hybrid; C, roach; CH, chironomids; TR, tricopterans; CR, unidentified crustaceans; Ga, *Gammarus*; As, *Asellus*; MO, unidentified molluscs; BI, bivalves; GA, gastropods; PM, plant matter; CL, other cladocerans; Da, *Daphnia*.



**Figure 8.** Growth rates based on scale reading of bream, roach and roach  $\times$  bream hybrids from Lough Ramor (A) and from other roach  $\times$  bream hybrid comparable populations (B). River Exe (UK) values are taken from Cowx (1983), Lough Garadice and River Shannon values are reported with the permission of the Central Fisheries Board. Data points represent mean length at age and are fitted with logarithmic curves.



**Figure 9.** Age frequency distributions for bream, roach and roach  $\times$  bream hybrids in Lough Ramor in 2007. The number of 0+ fish were recorded; fish with one annual check were recorded as 2006 year class.

Hodges, 1995; Burke & Arnold, 2001). The historical view assumes the majority of hybrids to be inherently unfit, primarily as a result of genetic incompatibilities between divergent genotypes of the parental species, which can result in reduced viability and/or fertility, commonly referred to as hybrid inferiority (Mayr,

1963; Dobzhansky, 1970). By contrast, recent empirical studies have provided evidence for elevated fitness levels of hybrids relative to their parental species in certain circumstances (Arnold & Hodges, 1995; Emms & Arnold, 1997; Burke, Carney & Arnold, 1998; Pfennig, 2007; Reyer, 2008), which makes the under-

standing of the mechanisms underlying such variations in hybrid fitness a very crucial task in evolutionary biology (Burke & Arnold, 2001).

The present study focused on an ecomorphological process likely to have a key role in the proliferation of the roach × bream hybrid in a recently colonized habitat. It was shown that  $F_1$  hybrids dominate the hybrid population, indicating that there is little or no evidence of a self-sustaining hybrid lineage. Roach × bream hybrids in Lough Ramor displayed consistent strong growth throughout their life history, reaching a maximum size intermediate between the parental taxa, as perhaps would be expected based on morphological and ontogenetic data (Figs 5, 6). Moreover, their growth rate relative to hybrid populations in other waters reveals that roach × bream hybrids are thriving in Lough Ramor (Fig. 8B). Combined with the large population sizes recorded by Caffrey (2005), this indicates that the  $F_1$  roach × bream hybrid is vigorous and successful in coexisting with its parental species.

Gut content data show that hybrids may be able to exploit a wider spectrum of prey items than either bream or roach, and this might be aided by their intermediate shape. SIA analysis, which provides a more reliable medium- to long-term picture of feeding ecology, confirms the broad trophic niche of hybrids, although without detecting differences with roach (Fig. 4). Under strong selective conditions, an intermediate feeding strategy would often be selected against because the hybrid is not a specialist in any particular niche (Arnold & Hodges, 1995). However, Ireland has few cyprinid species relative to the native ranges of both parental taxa, and the other species present in Lough Ramor, such as perch and trout, are not particularly abundant in the hypereutrophic conditions found in the lake. As a result, there may be enough available niche space in which the intermediate hybrid can coexist successfully. This hypothesis is strengthened by comparisons with roach × bream hybrid populations in other systems both in England (Frear, 2002; Smith, 2002) and mainland Europe (Goldspink, 1979; Simons *et al.*, 2001) where hybrids are very rare whenever both parental species are found in sympatry with a number of other cyprinid taxa.

#### REPRODUCTIVE CONSIDERATIONS

The varying year class success rates raise interesting questions on the factors underlying hybridization in Lough Ramor. In each year prior to 2003, hybridization occurred at a consistently high level, whereas bream recruitment was negligible. This situation mirrors previous work performed by Hänfling *et al.* (2005), showing in other cyprinids that the extent of  $F_1$  hybridization can dramatically reduce the effective

population sizes of the parental species. On the other hand, the successful recruitment of bream in 2003 was associated with the poorest recruitment of hybrids recorded. Such variations in recruitment levels have previously been recorded in bream (Goldspink, 1981) and the proliferation of hybridization in unsuccessful years may explain why this occurs in Lough Ramor. All  $F_1$  roach × bream hybrids recorded contained bream mitochondrial DNA (data not shown), indicating they are the progeny of female bream and male roach. One plausible explanation is that unsuccessful bream recruitment is a result of male bream failing to become reproductively active during the spawning season, which is significantly shorter in Ireland than on the continent. Female bream release their eggs and, in the absence of bream sperm, they are fertilized by sympatric roach, giving rise to large numbers of  $F_1$  roach × bream hybrids. Mean summer air temperatures recorded in Mullingar, close to Lough Ramor, reveal that 2003 was the warmest summer for two decades (M. Eireann, unpubl. data). Favourable conditions may have allowed male bream to mature and reproduce successfully, resulting in excellent recruitment of bream and very little hybridization. Another factor that might play a role in bream being maternal during hybridization is the documented aggressive and territorial behaviour of male bream (Nzau Matondo *et al.*, 2009), which may result in these fighting each other at the same time as tolerating the presence of large numbers of male roach, which are not seen as a threat. Further studies are certainly needed to clarify these aspects, although it appears reasonable to expect that an interplay between climatic and behavioural factors may play a role in influencing the reproductive dynamics in these taxa, especially in the highly variable conditions of Irish freshwaters.

#### FEEDING ECOMORPHOLOGY

Clear variation in diet composition between species was apparent from the gut content data. The roach versus bream comparison showed the least amount of diet overlap between taxa, primarily as a result of the dominance of *Daphnia* and other cladocerans in roach stomachs, whereas chironomid larvae dominated the diet of bream. *Daphnia* and the other cladocerans are planktonic organisms, whereas chironomid larvae live buried in the substrate: their presence in roach and bream stomachs provides direct evidence that these fishes are foraging in the pelagic and benthic zones, respectively. This trophic distinction is not particularly surprising because it has been determined that roach are most efficient at particulate feeding in clear water (Lammens, Geursen & McGillivray, 1985), whereas bream are widely recognized as bottom

feeders, preferring crustaceans and insect larvae as prey (Lammens & Hoogenboezem, 1991). The generalist ecology of the roach is further demonstrated by the comparison of the stable isotope signatures of both parental taxa, which show the distribution of bream values falling within a small portion of the isotopic niches of roach and hybrids.

Collective evidence from gut contents and SIA indicates that hybrids have a generalized trophic niche, exhibiting a diet that spans the entire trophic resource continuum, suggesting a remarkable trophic plasticity. Prey item identification underestimates the niche breadth of roach detected through isotopic analysis, suggesting that both roach and hybrids are feeding as generalists in Lough Ramor with a diet consisting of zooplankton and various macroinvertebrates, whereas bream appear to employ a specialized strategy confined to chironomids larvae and a selection of benthic macroinvertebrates. The oral morphology of bream, specifically their highly protrusible jaw, enables them to feed successfully in deep sediment, far exceeding the efficiency of roach in such an environment (Lammens & Hoogenboezem, 1991). Moreover, a higher body profile, together with the steep angle of insertion of the pectoral fin (Lauder & Drucker, 2004), appears to fit the paired-fins model of swimming activity (Webb, 1984), enabling bream to move through restricted spaces and perform fine maneuvers rather than fast swimming. The larger eye and the streamlined body of roach is consistent with their trophic ecology: these features are commonly associated with transient propulsion swimming (Webb, 1984), where sudden changes of direction and acceleration enable them to forage for widely-dispersed pelagic prey (Ehlinger, 1990; Winemiller, 1991). The hybrid phenotype was precisely intermediate of the parental extremes in all characters, and the one exhibiting the broader spectrum of morphological and trophic diversity. Because pelagic and benthic feeding requires different searching strategies (Ehlinger, 1990), and the ability to implement these strategies is constrained by fish morphology, the intermediate phenotype of the hybrid appears to underlie its ability to utilize both pelagic and benthic food sources.

Fahy *et al.* (1988) found that roach and roach  $\times$  bream hybrids fed on nearly identical prey types, primarily aufwuchs and detritus, in the Leixlip reservoir (bream diet was not analysed). In the English River Exe, roach  $\times$  bream hybrids were found to have an omnivorous benthophagic habit which overlapped largely with the diets of roach and bream (Cowx, 1983). The present study strengthens previous findings on the trophic plasticity of hybrids and offers additional life-history and morphological evidence to help understand their flourishing in Irish waters.

#### EVOLUTIONARY CONSIDERATIONS

Hatfield & Schluter (1999) showed that morphologically and ecologically intermediate hybrid sticklebacks (*Gasterosteus aculeatus* complex) had inferior growth rates in the wild (assumed to be a result of reduced foraging efficiency) compared to the parental taxa adapted to limnetic and benthic habitats, respectively. By contrast, the results obtained in the present study yield a scenario whereby, rather than compromising their ability to utilize either foraging microhabitat, the roach  $\times$  bream hybrids in Lough Ramor are able to efficiently cover the full breadth of both food spectra of the parental species, indicating the ecological success of the intermediate phenotype. This process is likely to be reinforced by the overall low diversity of the fish community in Lough Ramor, which arguably provides hybrids with greater niche availability than other European habitats.

The environment-dependent aspect of hybrid success should not be overlooked in the present scenario. In addition to the forces of endogenous selection acting upon reduced viability, fertility, and other genetic defects (Burke & Arnold, 2001; Schluter, 2001), hybrids must contend with exogenous ecological selection pressures (Hatfield & Schluter, 1999). To avoid out-competition by parental species, particularly in parental habitats (Barton & Hewitt, 1985; Burke & Arnold, 2001), the hybrid must enter a previously unoccupied ecological niche (McCarthy, Asmussen & Anderson, 1995; Buerkle *et al.*, 2000), or be better able to utilize the niches of its parental species. On the basis of this premise, hybrids may be favoured in new or perturbed environments in which the parental species are not yet adapted (Dowling & Secor, 1997; Barton, 2001). This explains the commonly observed increased fitness of hybrids in laboratory settings, and a reduction in fitness when transferred to parental habitats (Hatfield & Schluter, 1999). Similarly, biological introductions, as with the present scenario, can expose species to previously inexperienced environmental conditions.

A significant level of back-crossing with bream was recorded. Wood & Jordan (1987) and Wyatt *et al.* (2006), based on laboratory experiments, concluded that there is considerable circumstantial evidence that hybrids can cross in the wild; yet, in the present study, we found that, even in an environmental scenario ecologically favourable to hybrids, post- $F_1$  hybridization is probably still a negligible phenomenon at the population level. This is likely to depend on the reduced fertility of  $F_1$  hybrids (Wood & Jordan, 1987; Pitts *et al.*, 1997), which therefore counters their apparent ecological success. Yet, the possibility of genetic swamping of the bream genome cannot be discounted in the long run, especially considering the

fluctuating and vulnerable population size of bream and the potential spatial and temporal reproductive overlap between roach and bream. In the present scenario, hybrids could inject new genetic material into their sympatric parental taxa through introgression because  $F_1$  roach  $\times$  bream hybrids are genetically compatible with both parental species (Pitts, 1994). The present study shows that hybrids are at least capable of back-crossing with both parental species in the wild, although the longer-term consequences of such introgression of genes from either parental species into the other are difficult to predict.

#### CONCLUSIONS

We have identified an ecomorphological mechanism whereby roach  $\times$  bream hybrids can thrive in sympatry with their parental species. It is likely that novel environmental conditions are playing a pivotal role in the success of the roach  $\times$  bream hybrid in Irish waters, although more Irish freshwater systems should be studied to strengthen this view. Judging by the relatively short time of inhabitation in Ireland of roach and the largely  $F_1$  nature of the hybridization (lack of 'hybrid swarming'), the observed hybrid generalist strategy is likely to be driven by niche selection based on phenotype, rather than an adaptive response to the environment (Lauder, 1981). However, life-history data and ecological considerations indicate that, under certain environmental conditions, large numbers of roach  $\times$  bream hybrids can be established and maintained in Irish freshwaters, with genetic data suggesting that the roach genome can introgress bream populations through back-crossing.

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