

Trophic dynamics within a hybrid zone – interactions between an abundant cyprinid hybrid and sympatric parental species

BRIAN HAYDEN^{*,†}, ALEXIA MASSA-GALLUCCI^{*}, JOE CAFFREY[‡], CHRIS HARROD[‡], STEFANO MARIANI^{*}, MARTIN O'GRADY[‡] AND MARY KELLY-QUINN^{*}

^{*}School of Biology and Environmental Science, University College Dublin, Dublin, Ireland

[†]School of Biological Sciences, Queens University Belfast, Northern Ireland, U.K.

[‡]Central Fisheries Board, Swords Business Campus, Swords, Co., Dublin, Ireland

SUMMARY

1. Recent proliferation of hybridisation in response to anthropogenic ecosystem change, coupled with increasing evidence of the importance of ancient hybridisation events in the formation of many species, has moved hybridisation to the forefront of evolutionary theory.
2. In spite of this, the mechanisms (e.g. differences in trophic ecology) by which hybrids co-exist with parental taxa are poorly understood. A unique hybrid zone exists in Irish freshwater systems, whereby hybrid offspring of two non-native cyprinid fishes often outnumber both parental species.
3. Using stable isotope and gut content analyses, we determined the trophic interactions between sympatric populations of roach (*Rutilus rutilus*), bream (*Abramis brama*) and their hybrid in lacustrine habitats.
4. The diet of all three groups displayed little variation across the study systems, and dietary overlap was observed between both parental species and hybrids. Hybrids displayed diet, niche breadth and trophic position that were intermediate between the two parental species while also exhibiting greater flexibility in diet across systems.

Keywords: cyprinid, ecology, hybridisation, stable isotopes, trophic niche

Introduction

Although hybrid offspring are typically considered less fit than their parental taxa, numerous studies in the last decade have highlighted the adaptive potential of hybrids, whilst others have demonstrated ancient hybridisation events in the development of many species (Arnold & Hodges, 1995; Bell & Travis, 2005). Consequently, hybridisation has moved to the forefront of evolutionary ecology, and its role in

speciation is receiving growing attention (Seehausen, 2004; Arnold & Martin, 2010; Nolte & Tautz, 2010).

However, the majority of studies to date concern the genetic mechanisms that facilitate hybridisation and molecular evidence of same (Schwenk, Brede & Streit, 2008). While this is of key importance to understanding the role of hybridisation in evolution, the ecological interactions between hybrid offspring and parental species often remain overlooked, particularly in vertebrate taxa. For instance, for a hybrid genotype to gain a selective advantage, the corresponding phenotype must be able to forage effectively in habitats containing both parental species (Grant & Grant, 1996). However, evidence to date of trophic interactions between hybrids and parental taxa

Correspondence: Brian Hayden, School of Biological Sciences, Medical Biology Centre, Queens University, Belfast BT7 1LN, Ireland. E-mail: b.hayden@qub.ac.uk

remains sparse (Costedoat *et al.*, 2007). Such studies are hindered by the relative infrequency of vertebrate hybrid populations in nature (Scribner, Page & Barton, 2000), while the small number of offspring in the majority of hybrid zones exacerbates difficulties in obtaining meaningful sample sizes (Wyatt, Pitts & Butlin, 2006). In spite of this, ecological investigations are vital to provide an insight into the trophic interactions between hybrid offspring and their parental taxa, unravelling the mechanisms of ecological hybrid vigour (Rosenfield *et al.*, 2004).

Hybridisation is more frequent within the teleost family Cyprinidae than any other family of vertebrates (Scribner *et al.*, 2000). Various studies have documented the occurrence of hybridisation among several cyprinid species and genera across Europe (Crespin, Berrebi & Lebreton, 2002; Costedoat *et al.*, 2005; Nolte *et al.*, 2005). However, fish communities found in Irish freshwater ecosystems contain an abundance of cyprinid hybrids rarely seen elsewhere. Following the colonisation of Irish freshwater systems by invasive populations of roach (*Rutilus rutilus* L.), hybrids were observed in all waterbodies containing non-native populations of bream (*Abramis brama* L.) (Kennedy & Fitzmaurice, 1973). Hybridisation has been recorded throughout the native ranges of both species (Regan, 1911; Wheeler, 1969; Adams & Maitland, 1991), but rarely in significant numbers (Jeppesen *et al.*, 2000; Frear, 2002; Olin *et al.*, 2002). The hybrid zone in Irish waters is unique as the abundance of roach \times bream hybrids often exceeds those of both parent species. Kennedy & Fitzmaurice (1973) first observed roach \times bream hybrids in Ireland and estimated that hybrids accounted for 50% of the fish community of one lake. Similar proportions (c. 40%) were recorded by Fahy, Martin & Mulrooney (1988) in Leixlip Reservoir, while more recent studies indicate that such abundances occur throughout Irish lakes (Kelly, Connor & Champ, 2008). Irish lacustrine systems thus provide an ideal opportunity to quantify the trophic interactions between roach, bream and roach \times bream hybrids.

Relative to other hybrid groups, the roach \times bream hybrid is comparatively well studied. Hybrids are fertile and readily backcross with parental taxa, although no evidence exists of F2 individuals in natural populations (Yakovlev *et al.*, 2000; Wyatt *et al.*, 2006). Recent studies of Irish hybrid populations have indicated that the overwhelming majority of fish are

first generation crosses between female bream and male roach, and while no evidence of second generation hybrids has been produced, significant rates of back-crossing have been detected in certain populations (For details see Hayden *et al.*, 2010).

Previous authors have reported similarities in diet between hybrids and both roach (Fahy *et al.*, 1988) and bream (Cowx, 1983), although a recent account indicated that hybrids may exhibit a broader trophic niche than either roach or bream in eutrophic conditions (Toscano *et al.*, 2010). In spite of this, evidence of trophic interactions within the hybrid zone remains sparse, and this study is the first to incorporate a multisystem sample design to investigate trophic ecology. Furthermore, to determine the possible effect of lake productivity on the ecological interrelationships between parental species and hybrids, populations were sampled in mesotrophic and eutrophic systems.

As true species are, in general, ecologically superior to hybrids, it has been hypothesised that one of the underlying facilitators of hybrid success is an ability to utilise a trophic niche unoccupied by either parental species, thereby avoiding competition with ecologically adept true species (Seehausen, 2004). This is particularly plausible in locations outside the native range of the parental species, where they may not be well adapted to all available food sources. Hence, the principal hypothesis in this investigation was that roach \times bream hybrids, the progeny to two introduced fishes, would utilise a separate prey resource than either parental species. In addition to this ontogenetic, temporal and spatial variations in diet were investigated to identify the potential for niche segregation between the three fishes.

Methods

Study sites

Sampling was carried out in four cyprinid-dominated lakes in Ireland, two of which, Leixlip Reservoir (4 km², 53°21'N, 6°32'W) and Lough Ramor (7.5 km², 53°49'N, 7°03'W), have undergone cultural eutrophication (Toner *et al.*, 2005) and are dominated by roach and roach \times bream hybrids (Fahy *et al.*, 1988; Toscano *et al.*, 2010). Fish stock assessments conducted in Lough Ramor in 2005 using gill nets estimated population sizes (number of fish per metre of net) of

0.5 for roach, 0.1 for roach × bream hybrids and 0.001 for bream (Inland Fisheries Ireland, unpublished data). The remaining two lakes, Lough Corrib (53°27'N, 9°17'W) and Ross Lake (53°20'N, 9°04'W), are mesotrophic (Toner *et al.*, 2005). Lough Corrib (182 km²) consists of two geological basins, a lime-rich lower basin and a silica-dominated upper basin which contain different macrophyte and invertebrate communities (Krause & King, 1994). Ross Lake is considerably smaller (1.5 km²) and is situated on the same limestone bedrock as the lower basin of Lough Corrib. Fish stock assessments conducted in Lough Corrib in 2008 estimated density (number of fish per metre of net) of roach as 0.24, roach × bream hybrids as 0.01 and bream as 0.003 (Kelly *et al.*, 2009). Similar assessments in Ross lake in 2007 estimated roach density at 0.4, hybrids at 0.1 and bream at 0.06 (Kelly *et al.*, 2008).

Sampling

Fish and invertebrates were sampled from Lough Corrib, Lough Ramor and Leixlip Reservoir in both April and August 2006. Ross Lake was sampled during April and August of 2007. Fish were sampled using benthic gill nets set in the littoral zone in each lake. A combination of Nordic multimesh monofilament gillnets, 30 m in length, 1.5 m in height, comprising of 12 equidistant, 2.5 m panels with stretched mesh sizes ranging from 5 to 55 mm (Appelberg, 2000; Holmgren & Appelberg, 2000), together with larger multifilament mesh nets consisting of eight, 20 m panels with mesh sizes of 50, 63, 76, 89, 102, 114, 127 and 165 mm (O'Grady, 1981; Roche, 1999) were used. Two sets of each net were set at midday and retrieved the following morning. Where possible, 40 individual roach, beam and hybrids, encompassing the entire size range of each population were sampled, although 0+ fish (under 5 cm in length) are generally under-represented by these methods. Fish were visually identified (roach, bream and hybrids are clearly distinguishable), fork length was measured (± 1 mm), and subsamples of fish were retained for dietary and stable isotope analysis.

Invertebrate sampling was conducted either immediately prior to, or at the same time as fish sampling to ensure comparable datasets. Prey items encompassing three principal lotic food webs were collected: filter feeding bivalves were selected to represent the pelagic food web, gastropods, isopods and amphipods rep-

resented the littoral food web and chironomid larvae represented the profundal food web (Post, 2002; Harrod, Mallela & Kahilainen, 2010). Littoral invertebrates and bivalves were collected using kick sampling techniques, and an Eckman grab was used to collect chironomid larvae.

Gut content analysis (GCA)

The digestive tract of fish was removed and preserved in 70% ethanol for later dissection. Because of the macerating effects of pharyngeal teeth, prey types were divided into broad trophic groups, namely molluscs, detritus, diptera larvae, trichopterans, plant material, crustaceans, zooplankton, unidentified insects and oligochaetes. The points method (Hynes, 1950) was used to estimate the relative abundance of each group in the diet. Contents of the digestive tract were spread over a Petri dish and the relative proportions of each prey type were recorded and scored as 16, 8, 4, 2 or 1, where 16 is most abundant and 1 is present. The score for each food group was divided by the total score for the individual to estimate the proportion of each prey group in the gut (Hyslop, 1980).

Stable isotope analysis (SIA)

Fish muscle tissue was excised from the dorsal flank of each fish and frozen on site. For all invertebrate groups included in the analysis, 3–7 individuals were collected from stony, weeded and silted littoral habitats in each lake and were ground together to give a mean amalgamated value for that group in each lake. Obtaining individuals from different microhabitats reduced the possibility of confounding results because of the presence of a non-sampled resource. Owing to the effects of water chemistry on isotope values, separate invertebrate samples were obtained from the two basins in Lough Corrib. Invertebrate samples were stored in 70% ethanol, which has little effect on $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values (Syväranta *et al.*, 2008). Molluscs were dissected from their shells and shells discarded. Fish and invertebrate samples were oven-dried (60 °C for 24 h) and ground using a pestle and mortar. Sub-samples (1 ± 0.1 mg) were removed from the ground tissue and stored in pre-weighed tin capsules. Stable isotope analysis was performed using a Europa Scientific 20–20 Isotope Ratio Mass

Spectrometer with a Europa Scientific Roboprep-CN preparation module at Iso-Analytical Ltd (Sandbach, U.K.).

As lipid stores are ^{13}C depleted (De Niro & Epstein, 1977), $\delta^{13}\text{C}$ values of fish muscle tissue were lipid corrected to remove the influence of variable lipid concentrations prior to analysis (Kiljunen *et al.*, 2006).

Data analysis

Spearman rank correlations of the proportion of zooplankton observed in the gut contents against fork length for each fish taxon were performed to test for an ontogenetic diet shift. The same test was carried out between both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values and fork length, to identify ontogenetic changes in trophic position. Correlations were calculated using SPSS 10.1 (SPSS 2003).

A Bray–Curtis similarity matrix (Bray & Curtis, 1957) was created based on non-transformed proportion GCA data. Subsequently, a four-factor PERMANOVA (PRIMER 6; Clarke & Gorley, 2006) was performed on the data to test the effect of ‘lake trophic condition’ (2 levels, fixed), ‘site’ (4 levels, random), ‘sample time’ (2 levels, random) and ‘group’ (3 levels, fixed), with ‘site’ nested within ‘trophic condition’, on variation within the dataset. PERMANOVA is a nonparametric probability-based analogue of analysis of variance between two or more groups based on a distance measure, in this case the Bray–Curtis similarity matrix (Anderson, 2001; Mcardle & Anderson, 2001).

Levels of inter-group dietary overlap within lakes were visualised using a multidimensional scaling ordination (MDS) and dietary similarity was calculated using analysis of similarities (ANOSIM), performed using the PAST statistical package (Hammer, Harper & Ryan, 2001). Dietary niche breadth was calculated using the standardised Levins index (β), and values were compared using ANOVA. To ensure comparable values, niche width comparisons were standardised to the smallest sample size; where a larger number of samples was available, a subsample was obtained using random number generation.

ANOSIM was preferred to PERMANOVA for within-lake analysis as it allows a direct comparison between the results of gut content and stable isotope analyses. As isotopic baseline values vary according to underlying geology and water chemistry, trophic interac-

tions between groups must be measured independently in each system, and hence a PERMANOVA-based approach was not best suited to this analysis. A Euclidean distance matrix was created from non-transformed $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values for fish analysed from all lakes, and ANOSIM was subsequently used to identify potential differences in isotopic values between taxa.

The relative contribution of prey derived from the littoral, pelagic and profundal zones to the diet of each consumer group was estimated using the stable isotope analysis in R (SIAR) Bayesian mixing model (Parnell *et al.*, 2010). Finally, a measure of isotopic niche breadth was constructed based on the Euclidean space occupied by a convex hull encompassing the full distribution of isotopic values of each species, standardised randomly to the smallest available sample size (Layman *et al.*, 2007). Isotopic niche breadth values were subsequently compared using ANOVA.

Results

Gut content analysis

The most prevalent food items in the diet of all three taxa were molluscs (note that because of the actions of pharyngeal teeth, gastropod and bivalve shells were often indistinguishable in gut contents) and dipteran larvae (Table 1). However, in some cases Spearman rank correlations revealed a significant decrease in the levels of zooplankton consumed with increasing fish size (Table 2). In Lough Ramor, such ontogenetic variation was difficult to assess as bream under 20 cm were absent from the sample. However, a shift from zooplankton to an invertebrate-based diet was observed in bream and hybrids in both Lough Corrib and Leixlip Reservoir (Table 2). Roach, while displaying a homogenous diet throughout their size range in both these lakes, exhibited a plankton-to-macroinvertebrate shift in Ross Lake and Lough Ramor.

PERMANOVA of the gut content dataset failed to identify significant variation between eutrophic and mesotrophic systems or among all the lakes studied (Table 3). Interactions tested within the PERMANOVA reveal that although significant temporal variation was observed in the diet, dietary overlap between groups remained constant at each sampling time

Table 1 Results of trophic analyses of roach, bream and roach × bream hybrids

Lake	Taxon	n	Fork length (mm)				$\delta^{13}\text{C}$		$\delta^{15}\text{N}$		Gut contents										Isotopic niche	Dietary niche
			Mean	Min	Max	Mean (\pm SD)	Mean (\pm SD)	Mean (\pm SD)	Moll	Det	Dipt	Tric	Plant	Crust	Zoo	Insect	Oligo					
Ramor	Bream	29	315	178	475	-28.6 (0.2)	13.8 (0.4)	0.21	0.07	0.56	0.01	0.03	0.05	0.06	0.0	0.0	0.8	0.2				
	Hybrid	48	323	95	444	-28.1 (0.6)	14.5 (0.6)	0.23	0.13	0.23	0.04	0.09	0.26	0.01	0.01	0.0	4.1	0.5				
	Roach	50	184	58	302	-28.4 (0.6)	14.3 (0.6)	0.25	0.12	0.15	0.01	0.10	0.15	0.21	0.01	0.0	4.3	0.6				
Leixlip	Bream	50	223	135	312	-29.8 (0.8)	13.5 (0.5)	0.36	0.16	0.29	0.00	0.06	0.04	0.08	0.0	0.0	3.2	0.4				
	Hybrid	60	228	129	423	-29.1 (0.8)	14.3 (0.9)	0.46	0.20	0.17	0.01	0.03	0.07	0.05	0.01	0.01	4.6	0.3				
	Roach	61	202	75	305	-28.6 (0.9)	15.3 (0.7)	0.29	0.21	0.09	0.01	0.03	0.16	0.08	0.09	0.04	4.3	0.6				
Ross	Bream	83	322	144	432	-28.2 (0.8)	13.2 (1.4)	0.28	0.32	0.06	0.05	0.15	0.02	0.10	0.02	0.0	7.3	0.5				
	Hybrid	54	317	160	460	-28.4 (0.8)	13.1 (1.6)	0.26	0.30	0.04	0.10	0.23	0.01	0.04	0.01	0.0	9.3	0.4				
	Roach	72	201	58	350	-27.9 (1.3)	12.5 (1)	0.25	0.20	0.05	0.07	0.28	0.01	0.12	0.02	0.0	15.8	0.5				
Corrib	Bream	55	270	105	434	-32.1 (1.4)	13 (0.5)	0.39	0.12	0.19	0.06	0.09	0.05	0.09	0.0	0.0	6.8	0.4				
	Hybrid	45	236	117	375	-31.8 (1.8)	13.1 (0.8)	0.35	0.11	0.19	0.12	0.05	0.11	0.05	0.01	0.0	22.8	0.5				
	Roach	39	158	70	310	-31.8 (1.5)	12.4 (0.8)	0.34	0.00	0.23	0.17	0.06	0.09	0.02	0.08	0.0	20.8	0.5				

Stable isotope values are given as means with standard deviations in parenthesis. Gut content values represent mean proportions of each prey group: molluscs (Moll), detritus (Det), tricoptera (Tric), diptera larvae (Dipt) plant material (Plant), crustacean (Crust), zoo plankton (Zoo), insects (Insect) and oligochaetes (Oligo). Values of isotopic and dietary niche breadth are also provided.

(Table 3). Levels of within-lake dietary overlap between species were high: MDS ordinations showed little evidence of dietary segregation (Fig. 1), while ANOSIM analysis revealed significant differences between the gut contents of the three groups in only four cases, each of which was countered by low R values, indicating minimal dissimilarity (Table 4).

Dietary niche width measurements (Table 1) displayed no variation relative to the trophic status of the study site ($F_{1,10} = 0.86$, $P = 0.37$). Niche breadth of hybrids did not vary significantly from that of either bream ($F_{1,6} = 0.4$, $P = 0.5$) or roach ($F_{1,6} = 5$, $P = 0.07$), although roach did display a significantly broader niche than bream ($F_{1,6} = 6.4$, $P = 0.04$).

SIA – invertebrate baseline

In all lakes, invertebrate taxa could be distinguished by their $\delta^{13}\text{C}$ values (Table 5). Invertebrate $\delta^{13}\text{C}$ variation within a lake typically spanned 5–6‰, although chironomid larvae in Leixlip Reservoir were considerably depleted in $\delta^{13}\text{C}$ relative to all other invertebrates. In Lough Corrib, the apparent influence of geology was evident in the $\delta^{13}\text{C}$ values of gastropods: although little variation was observed between the mean values of specimens from either basin, a 10‰ difference was recorded between the minimum and maximum $\delta^{13}\text{C}$ values evident in both basins. Similarly, the $\delta^{13}\text{C}$ values recorded for bivalves of both basins spanned 6.5‰.

SIA – consumers

Ontogenetic variation in $\delta^{15}\text{N}$, whereby values increased with fork length, was observed in the stable isotope values of all bream and the majority of hybrid populations studied (Table 2). Ontogenetic variation in $\delta^{13}\text{C}$ was less consistent across the sample: in Lough Corrib, no significant variation was observed in any group, while in Ross Lake both roach and roach × bream hybrids displayed increasing $\delta^{13}\text{C}$ with increasing fork length. A similar trend was observed in the eutrophic lakes: roach × bream hybrids in Lough Ramor and bream in Leixlip Reservoir exhibited similar ontogenetic enrichment in $\delta^{13}\text{C}$, while the opposite trend was uniquely observed in roach populations from Leixlip Reservoir. No variation was observed between spring and summer isotope values of consumers (ANOSIM, $R = 0.014$, $P = 0.1$).

Table 2 Spearman rank correlations comparing fish fork length (ontogeny) with proportion of zooplankton (Zoo) in the diet, and $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$

	Eutrophic						Mesotrophic					
	Lough Ramor			Leixlip Reservoir			Lough Corrib			Ross Lake		
	Zoo	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	Zoo	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	Zoo	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	Zoo	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$
Bream	-0.1	-0.2	0.6**	-0.4**	0.5**	0.6**	-0.6**	-0.03	0.7**	-0.4**	0.2	0.4**
Hybrid	-0.1	0.5**	0.4**	-0.3**	0.2	0.5**	-0.5**	-0.05	0.7**	-0.03	0.6**	-0.1
Roach	-0.4**	0.1	0.2	-0.2	-0.3*	0.2	-0.05	-0.06	0.02	-0.5**	0.6**	0.4**

Significant values are presented in bold.

*Significant difference of $P < 0.05$.

**Significant difference of $P < 0.01$.

Table 3 PERMANOVA examining the effects of trophic condition (Trophic), study site (Site), time and group on variation in gut content

Source	df	MS	Pseudo- <i>F</i>	<i>P</i> (perm)
Group	2	15305	0.84	0.66
Time	1	99291	3.79	0.03
Trophic	1	55195	0.96	0.54
Site	2	61283	2.35	0.10
Group \times Time	2	14720	3.26	0.01
Group \times Trophic	2	11049	1.21	0.30
Time \times Trophic	1	19234	0.76	0.65
Group \times Site	4	6792	1.57	0.16
Time \times Site	2	22697	10.52	<0.01
Group \times Time \times Trophic	2	5031	1.19	0.38
Group \times Time \times Site	4	3883	1.80	0.02
Residual	622	2158		

With the exception of bream and roach in Leixlip Reservoir and bream and hybrids in Lough Ramor, both of which were clearly distinguishable based on their isotope values, trophic overlap was observed between both parental species and hybrids (Fig. 2). In Ross Lake, bream and hybrids could not be distinguished statistically, while in the remaining lakes significant levels of variation were countered by low *R* values (Table 3).

Results of the SIAR mixing model revealed that although proportions of littoral, pelagic and profundal prey varied between lakes, in each lake minimal differences were observed between the relative inputs from each source to each consumer (Fig. 3).

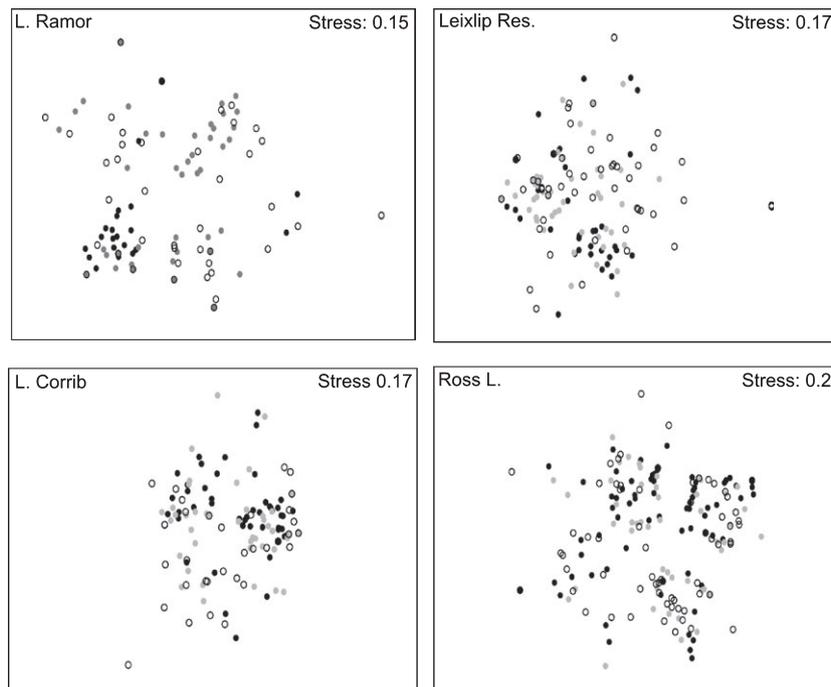
**Fig. 1** Multidimensional scaling ordination (MDS) of Bray–Curtis similarity matrix derived from gut content values of bream (black circle), roach (white circle) and roach \times bream hybrids (grey circle) in Lough Ramor, Leixlip Reservoir, Lough Corrib and Ross Lake.

Table 4 Measure of trophic overlap between each taxon in eutrophic and mesotrophic conditions

	Eutrophic				Mesotrophic			
	L. Ramor		Leixlip Res		L. Corrib		Ross L.	
	GCA	SIA	GCA	SIA	GCA	SIA	GCA	SIA
Bream–Roach	0.08	0.18**	0.06**	0.56**	0.13**	0.07**	-0.01*	0.08**
Bream–Hybrid	0.06	0.33**	0.04	0.15**	0.02	0.14**	0	0.03
Roach–Hybrid	0.04	0.04*	0.05**	0.17**	0.04	0.12**	0	0.12**

R values detail results of ANOSIM analysis of gut content (GCA) and stable isotope (SIA) values, respectively. *R* = 1 indicates complete dissimilarity.

Significant values are presented in bold.

*Significant difference of *P* < 0.05.

**Significant difference of *P* < 0.01.

Table 5 Stable isotope values for macroinvertebrates sampled in each lake

	Bivalve		Gastropod		<i>Gammarus</i>		<i>Asellus</i>		Chironomid	
	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$								
Leixlip	-32.6 (0.5)	10.1 (1.2)	-27.2 (3.3)	11 (0.8)	-26.6 (3.2)	11.2 (3.2)	-28.9	10.8	-44.7 (4.2)	1.9 (3.7)
Ramor	-32.1 (0.1)	9.9 (0.05)	-27.5 (0.06)	10.3 (0.05)	-26 (0.9)	9.7 (0.5)	-	-	-34.5 (6.6)	7.6 (2.4)
Corrib (Upr)	-30.8 (1.5)	8.1 (0.3)	29.1 (2.4)	8.7 (0.2)	-24.6 (1.2)	9.3 (0.2)	-27.7	9.9	-	-
Corrib (Lwr)	-27.7 (1.3)	8 (0.1)	-26.7 (1.9)	8.6 (0.3)	-25 (1.4)	8.1	-27.8	7.7	-25.6	8.2
Ross	-33.9 (0.84)	7.58 (1.18)	-29.1 (2.4)	8.4 (0.2)	-28 (2.4)	7.66 (0.5)	-29.1 (1.64)	8.67 (1.1)	-32.4 (0.9)	9.8 (0.9)

Data presented are mean values of combined sampling events and microhabitats with standard deviation in parentheses.

The isotopic niche of each taxon was smaller in the eutrophic lakes than the mesotrophic systems (ANOVA, $F_{1,10} = 12.3$, $P < 0.01$). No significant variation in niche width was evident between roach and hybrids (ANOVA, $F_{1,6} = 0.03$, $P = 0.9$) but, in all lakes, both roach and hybrids exhibited a larger isotopic niche than bream (Table 1).

Discussion

Trophic interrelationships

In all four lakes, regardless of trophic status, the diet of roach \times bream hybrids bore a remarkable similarity to that of both parental taxa. There was no evidence to support the principal hypothesis that hybrids exploited a food resource that was not utilised by their parental taxa. However, subtle differences in ontogenetic diet shifts and niche dynamics of the three groups illustrate potential trophic interactions sustaining large hybrid populations.

Both stable isotope and gut content analyses revealed large dietary overlap between bream and roach in three of the four lakes studied. This is in

contrast to numerous other studies that have detailed dietary segregation between bream, which specialise on zooplankton and chironomid larvae (Van Den Berg *et al.*, 1992, 1994), and roach, which are generalists often utilising a large trophic niche (Persson, 1983; Svanback *et al.*, 2008). Furthermore, dietary overlap appears to be consistent across sampling periods. Although temporal variation in diet was observed in gut contents, considerable dietary overlap was evident between the three groups during both sampling periods. Similarly, the absence of such variation in the stable isotope values, which provide a longer-term indication of dietary overlap than gut contents, indicates that seasonal variation in diet is unlikely to be a defining characteristic of the studied populations. Thus, the proliferation of hybrids in the absence of niche segregation suggests a number of hypotheses for the ecological success of hybrids in these systems.

Firstly, an abundance of prey, readily obtainable by all three groups, would make niche segregation unnecessary (Matthews, 1998). Although no measurement of prey availability was carried out, a scenario

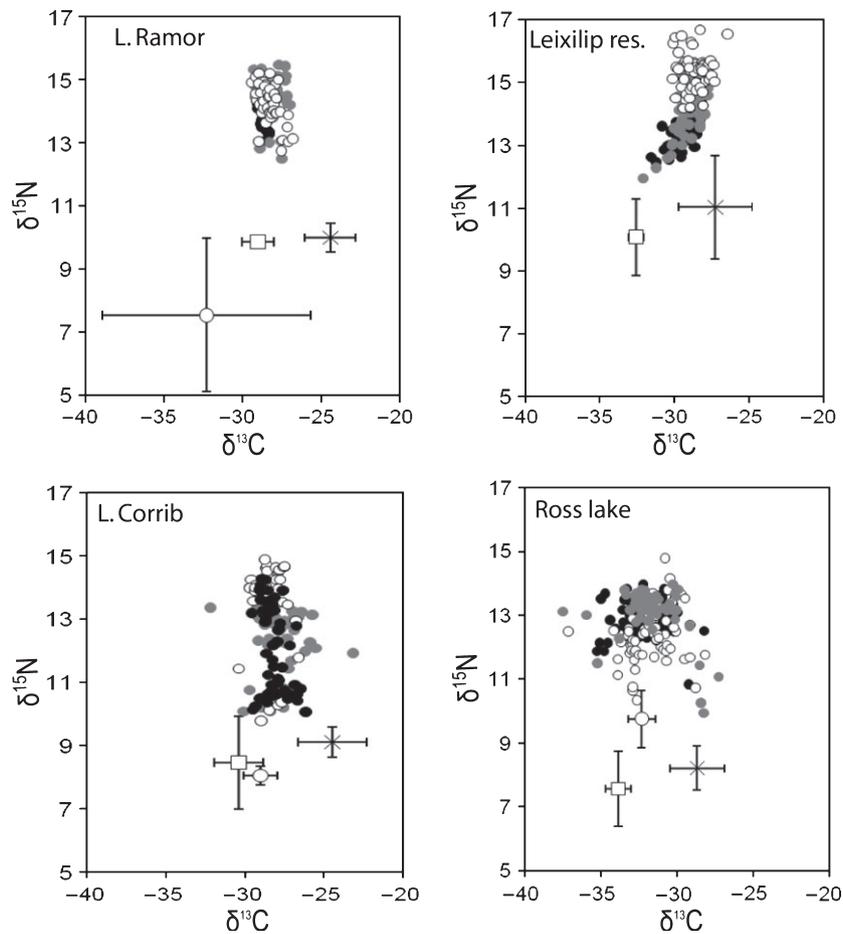


Fig. 2 Isotope bi-plots detailing the trophic position of bream (black circle), roach (white circle) and roach × bream hybrids (grey circle) in Lough Ramor, Leixlip Reservoir, Lough Corrib and Ross Lake. Isotopic baseline data are presented as mean \pm SD of pelagic (open square), littoral (cross) and profundal (open circle) prey types.

whereby roach, bream and their hybrids are feeding on the same prey items suggests that prey is not a limiting resource. As such, the intermediate morphology of a hybrid is unlikely to confer an ecological disadvantage. A similar situation is observed in Galapagos finches (e.g. *Geospiza* spp.) where hybrid progeny survive during wet summers when food is plentiful but are largely absent in drier, leaner years (Grant & Grant, 1996). Secondly, given that Irish fish communities are depauperate relative to those within the native range of both species (Wheeler, 1969; Fitzsimons & Igoe, 2004), it is plausible that hybrids in Irish waters may exploit a niche that would be occupied by other taxa elsewhere, thereby avoiding competition with their parental species should prey becomes limiting. As juvenile fish were not sampled in this investigation, the potential remains for niche segregation between the three groups during the sensitive first summer of life when mortality rates are high and access to food for gape-limited larvae and

fry may be limited (Persson *et al.*, 2000, 2004). The limited number of roach × bream hybrids in other European waters, including areas of similar environmental conditions to Ireland but with a greater diversity of cyprinid species, lends weight to this hypothesis.

A third hypothesis concerns hybrid vigour and combines these data and the observation of an increased propensity of hybrid zones in areas novel to both parental species. In such conditions, non-native taxa may not be well adapted to the new habitat; hence, the mixing of parental genomes in the intermediate hybrid may provide the basis for a more locally adapted phenotype. Similar instances of hybrid vigour have been recorded many times in other hybrid zones (Barton, 2001), and though this may explain the apparent success of hybrids in Ireland, it would fail to account for the paucity of roach × bream hybrids within the native ranges of both species.

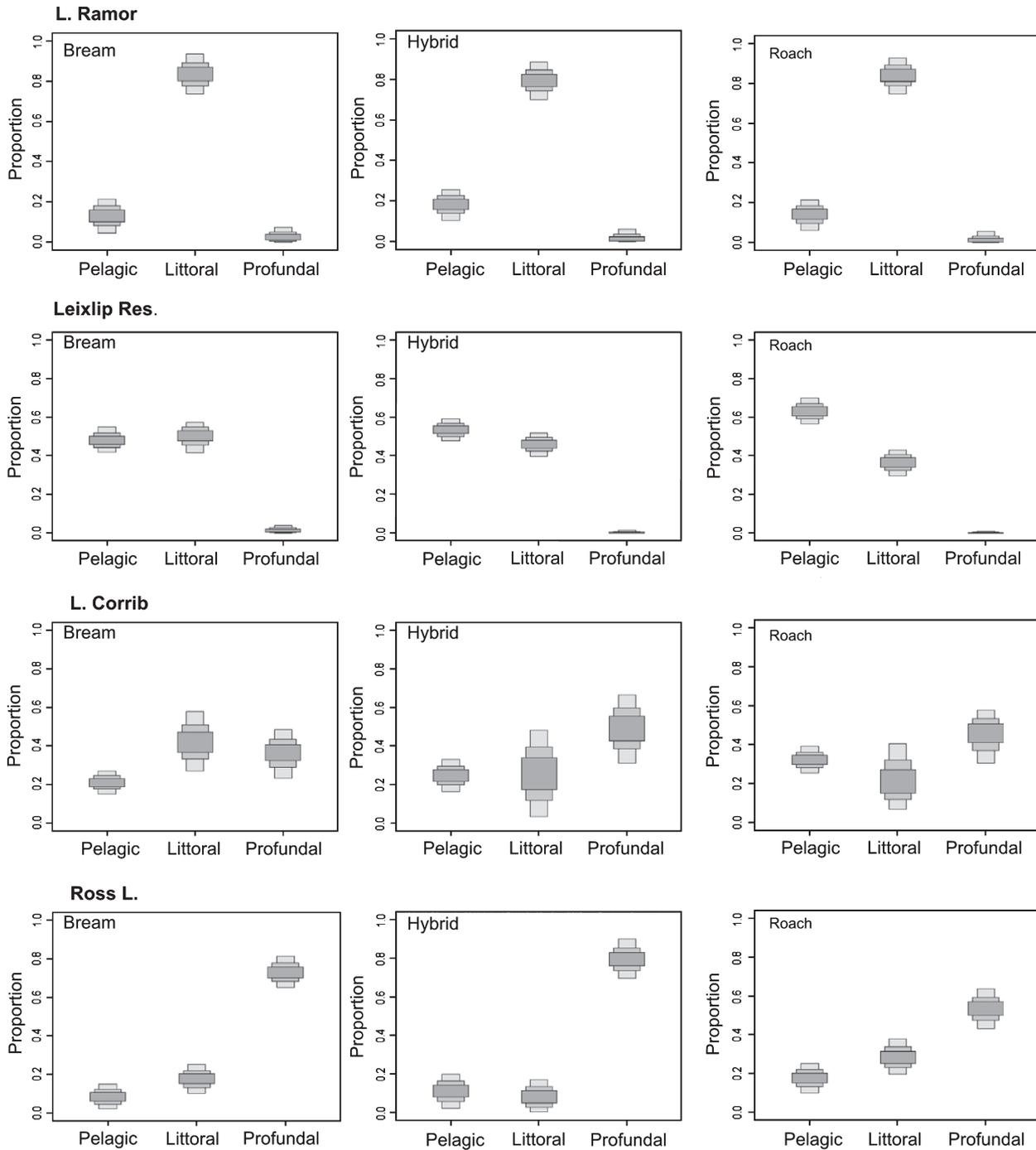


Fig. 3 Proportional input from littoral, pelagic and profundal prey sources based on the stable isotope analysis in R (SIAR) mixing model. Boxes represent 95, 75 and 25% credibility intervals.

While these hypotheses may account for the success of hybrids in the systems studied, differences in diet observed between the groups, most notably regarding niche breadth and ontogenetic diet shifts, may also shed light on the role played by trophic ecology in the

maintenance of a large hybrid population. Although our analyses excluded juvenile fish, it is apparent from both gut content and stable isotope data that both bream and hybrids exhibit an ontogenetic shift from zooplankton to macro-invertebrates during their life

history. Conversely, ontogenetic variation was not a feature of roach populations. With the exception of Ross Lake, no significant variation was recorded in either the diet or isotope values of roach, although the absence of individuals smaller than 5 cm fork length (i.e. 0+ individuals) may be a factor (Persson, 1990; Nunn, Harvey & Cowx, 2007). These results emphasise the generalist ecology of roach in contrast to the more specialised strategy of bream. Considering this, ontogenetic variation displayed by hybrids may be a key consideration in its ecological success. Such an ability to specialise on prey items, allied with the large trophic niche also observed in hybrids, conveys a flexibility in diet absent from either parental species. As such, hybrids may be best suited to obtain maximum benefit from a situation of high prey availability.

Hybrid trophic flexibility was particularly evident in the eutrophic systems. In Lough Ramor, bream exhibited an extremely small isotopic niche and a diet dominated by chironomid larvae, while roach fed on a wide variety of prey types. Hybrids in Lough Ramor exhibited a generalist ecology and wide trophic niche similar to roach, while the diet of both included the same prey resources as bream (see also Toscano *et al.*, 2010). In contrast, in Leixlip Reservoir, where significant isotopic segregation was observed between bream and roach, the diet of hybrids bore greater similarity to that of bream.

Furthermore, in Leixlip Reservoir, a discrepancy between the dietary and isotopic overlap between bream and roach was observed, which may reflect the potential influence of microbially derived carbon in the stable isotope values of consumers and provides a useful indication of the dietary potential of the hybrids. Chironomid larvae sampled in Leixlip Reservoir were markedly depleted in both $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$, indicative of organisms utilising methane-derived carbon (Grey *et al.*, 2004; Harrod & Grey, 2006). Isotope values of some bream, and to a lesser extent hybrids, were similarly depleted relative to roach, hence the isotopic segregation between the species. However, this contradicts the results of the gut content analysis, which showed that all three groups were feeding on similar amounts of chironomids. Only certain species of chironomid feed on microbially produced carbon, and it can be surmised that bream and hybrids are feeding on these species while roach are not. However, fish feeding solely on such chironomids display isotope values further depleted

than that evident in Leixlip Reservoir (Ravinet *et al.*, 2009), and it is likely that these species only comprise a relatively minor part of the diet of bream and hybrids, although crucially they are not consumed by roach.

Niche breadth

The role of stable isotopes in providing a measure of trophic niche has been an area of great recent interest (Bearhop *et al.*, 2004; Newsome *et al.*, 2007). As they provide a long-term representation of the diet, a metric of niche width based on stable isotope ratios has a significant advantage over one derived from gut content alone (Dalerum & Angerbjorn, 2005). The joint analyses performed here provide a valuable insight into the relative benefits of both methodologies and the pitfalls associated with studies based uniquely on one method.

Isotopic niche size of each taxon was consistently larger in the mesotrophic lakes than the eutrophic waters, a trend not mirrored in the gut content analysis. In all lakes, the variation in $\delta^{15}\text{N}$ was quite consistent, typically spanning one trophic level, and therefore differences in niche size are because of greater variation in $\delta^{13}\text{C}$ values. In Lough Corrib, the increased $\delta^{13}\text{C}$ variation is probably due to the presence of fish feeding in both the lime-rich and silica-rich basins of the lake. Bivalve and gastropod populations in both basins contained individuals with markedly different $\delta^{13}\text{C}$ values, therefore a sample of fish drawing individuals from both basins or indeed individuals that feed in both basins would be likely to be biased towards greater variation in $\delta^{13}\text{C}$.

Fish in Ross Lake displayed a range of $\delta^{13}\text{C}$ values far exceeding those recorded in the eutrophic lakes. This was principally because of the presence of fish considerably depleted in $\delta^{13}\text{C}$. As the minimum $\delta^{13}\text{C}$ values recorded for each taxon fell outside the range of invertebrates sampled, these fish must be drawing carbon from another, unsampled food source. This may be accounted for by fish drawing on a microbial source of carbon as was demonstrated in Leixlip Reservoir. However, the absence of this signal in bream populations, typically most likely to forage on such prey, relative to roach and hybrids appears to undermine such a hypothesis (Harrod & Grey, 2006). A second possibility derives from the gut content analysis which revealed that bream, roach and

roach × bream hybrids in Ross Lake consumed considerably more plant material than was found in any of the other populations. Although the particular plant species in the gut were not identifiable, many aquatic plants exhibit $\delta^{13}\text{C}$ values low enough to account for the $\delta^{13}\text{C}$ values observed in fish sampled in Ross Lake (Keeley & Sandquist, 1992). Thus, it is possible that a diet containing such materials would lead to the observed depleted $\delta^{13}\text{C}$ values. However, in such instances fish $\delta^{15}\text{N}$ values would probably be similarly depleted, which was not the case for the fish sampled in Ross Lake.

The situation outlined here does not resolve the question of how an abundant population of roach × bream hybrids can co-exist in sympatry with parental species. Future investigations will need to focus on assessing the trophic dynamics of juvenile fish in these systems. Understanding the relationship between the three fish groups during the first year of life will be key to accurately determining the reasons for the success of hybrids in Irish waters. In conjunction with this, a series of tank experiments to determine feeding behaviour of the three groups in a variety of prey availability conditions would be of fundamental benefit in assessing any potential ecological advantages of the hybrid phenotype.

The trophic dynamics of the roach × bream hybrid zone does, however, highlight the conditions in which an intermediate hybrid can succeed in sympatry with its parental species. Numerous, previously cited, authors have outlined the methods by which a hybrid may be able to avoid competition by utilising a niche unoccupied by its parental species. Our findings highlight a novel hypothesis, supported across a variety of lacustrine systems; in the absence of niche segregation between the parental species, an intermediate morphology will not convey any significant disadvantage to the hybrid.

Acknowledgments

The authors are indebted to the staff of Inland Fisheries Ireland for assistance in obtaining samples. BH and AMG were funded by Inland Fisheries Ireland. Our sincerest gratitude is also extended to David Balata, John Coyne and Fran Igoe for assistance rendered and also to two anonymous reviewers and Colin Townsend for their insightful comments on the manuscript.

References

- Adams C.E. & Maitland P.S. (1991) Evidence of further invasions of Lough Lomond by nonnative fish species with the discovery of a roach × bream, *Rutilus rutilus* (L.) × *Abramis brama* (L.) Hybrid. *Journal of Fish Biology*, **38**, 961–963.
- Anderson M.J. (2001) Permutation tests for univariate or multivariate analysis of variance and regression. *Canadian Journal of Fisheries and Aquatic Sciences*, **58**, 626–639.
- Appelberg M. (2000) Swedish standard methods for sampling freshwater fish with multi-mesh gillnets. *Fiskeriverket Information*, **1**, 1–32.
- Arnold M.L. & Hodges S.A. (1995) Are natural hybrids fit or unfit relative to their parents. *Trends in Ecology & Evolution*, **10**, 67–71.
- Arnold M.L. & Martin N.H. (2010) Hybrid fitness across time and habitats. *Trends in Ecology & Evolution*, **25**, 530–536.
- Barton N.H. (2001) The role of hybridization in evolution. *Molecular Ecology*, **10**, 551–568.
- Bearhop S., Adams C.E., Waldron S., Fuller R.A. & Macleod H. (2004) Determining trophic niche width: a novel approach using stable isotope analysis. *Journal of Animal Ecology*, **73**, 1007–1012.
- Bell M.A. & Travis M.P. (2005) Hybridization, transgressive segregation, genetic covariation, and adaptive radiation. *Trends in Ecology & Evolution*, **20**, 358–361.
- Bray J.R. & Curtis J.T. (1957) An Ordination of the Upland Forest Communities of Southern Wisconsin. *Ecological Monographs*, **27**, 326–349.
- Clarke K.R. & Gorley R.N. (2006) *PRIMER v6: User Manual*. PRIMER-E, Plymouth.
- Costedoat C., Pech N., Salducci M.D., Chappaz R. & Gilles A. (2005) Evolution of mosaic hybrid zone between invasive and endemic species of Cyprinidae through space and time. *Biological Journal of the Linnean Society*, **85**, 135–155.
- Costedoat C., Pech N., Chappaz R. & Gilles A. (2007) Novelty in Hybrid Zones: crossroads between Population Genomic and Ecological Approaches. *PLoS ONE*, **2**, e357.
- Cowx I.G. (1983) The Biology of bream, *Abramis-brama* (L), and its natural hybrid with roach, *Rutilus-rutilus* (L), in the River Exe. *Journal of Fish Biology*, **22**, 631–646.
- Crespin L., Berrebi P. & Lebreton J.D. (2002) Spatially varying natural selection in a fish hybrid zone. *Journal of Fish Biology*, **61**, 696–711.
- Dalerum F. & Angerbjorn A. (2005) Resolving temporal variation in vertebrate diets using naturally occurring stable isotopes. *Oecologia*, **144**, 647–658.

- De Niro M.J. & Epstein S. (1977) Mechanism of carbon isotope fractionation associated with lipid synthesis. *Science*, **197**, 261–263.
- Fahy E., Martin S. & Mulrooney M. (1988) Interactions of Roach and Bream in an Irish Reservoir. *Archiv Fur Hydrobiologie*, **114**, 291–309.
- Fitzsimons M. & Igoe F. (2004) Freshwater fish conservation in the Irish Republic: a review of pressures and legislation impacting on conservation efforts. *Biology and Environment*, **104B**, 17–32.
- Frear P.A. (2002) Hydroacoustic target strength validation using angling creel census data. *Fisheries Management and Ecology*, **9**, 343–350.
- Grant B.R. & Grant P.R. (1996) High survival of Darwin's finch hybrids: effects of beak morphology and diets. *Ecology*, **77**, 500–509.
- Grey J., Kelly A., Ward S., Sommerwerk N. & Jones R.I. (2004) Seasonal changes in the stable isotope values of lake-dwelling chironomid larvae in relation to feeding and life cycle variability. *Freshwater Biology*, **49**, 681–689.
- Hammer O., Harper D.A.T. & Ryan P.D. (2001) PAST: paleontological statistics software package for education and data analysis. *Palaentologia Electronica*, **4**, 9.
- Harrod C. & Grey J. (2006) Isotopic variation complicates analysis of trophic relations within the fish community of Plußsee: a small, deep, stratifying lake. *Archiv Fur Hydrobiologie*, **167**, 281–299.
- Harrod C., Mallela J. & Kahilainen K.K. (2010) Phenotype-environment correlations in a putative whitefish adaptive radiation. *Journal of Animal Ecology*, **79**, 1057–1068.
- Hayden B., Pulcini D., Kelly-Quinn M., O'Grady M., Caffrey J., Mcgrath A. *et al.* (2010) Hybridisation between two cyprinid fishes in a novel habitat: genetics, morphology and life-history traits. *BMC Evolutionary Biology*, **10**, 169.
- Holmgren K. & Appelberg M. (2000) Size structure of benthic freshwater fish communities in relation to environmental gradients. *Journal of Fish Biology*, **57**, 1312–1330.
- Hynes H.B.N. (1950) The food of freshwater sticklebacks (*Gasterosteus aculeatus* and *Pygosteus pungitius*), with a review of methods used in studies of the food of fishes. *Journal of Animal Ecology*, **19**, 36–58.
- Hyslop E.J. (1980) Stomach contents analysis – A review of methods and their application. *Journal of Fish Biology*, **17**, 411–429.
- Jeppesen E., Jensen J.P., Sondergaard M., Lauridsen T. & Landkildehus F. (2000) Trophic structure, species richness and biodiversity in Danish lakes: changes along a phosphorus gradient. *Freshwater Biology*, **45**, 201–218.
- Keeley J.E. & Sandquist D.R. (1992) Carbon – fresh water plants. *Plant Cell and Environment*, **15**, 1021–1035.
- Kelly F., Connor L. & Champ T. (2008) *WFD Surveillance Monitoring – Fish in Lakes 2007*. Central Fisheries Board, Dublin.
- Kelly F., Connor L., Wightman G., Matson R., Morrissey E., O'callaghan R. *et al.* (2009) *Sampling Fish For the Water Framework Directive – Summary Report 2008*. Inland Fisheries Ireland, Dublin.
- Kennedy M. & Fitzmaurice P. (1973) Occurrence of cyprinid hybrids in Ireland. *Irish Naturalists Journal*, **17**, 349–351.
- Kiljunen M., Grey J., Sinisalo T., Harrod C., Immonen H. & Jones R.I. (2006) A revised model for lipid-normalizing delta C-13 values from aquatic organisms, with implications for isotope mixing models. *Journal of Applied Ecology*, **43**, 1213–1222.
- Krause W. & King J.J. (1994) The ecological status of Lough Corrib, Ireland, as indicated by physiographic factors, water chemistry and macrophytic flora. *Vegetatio*, **110**, 149–161.
- Layman C.A., Arrington D.A., Montana C.G. & Post D.M. (2007) Can stable isotope ratios provide for community-wide measures of trophic structure? *Ecology*, **88**, 42–48.
- Matthews W.J. (1998) *Patterns in Reshwater Fish Ecology*. Chapman & Hall, New York.
- Mcardle B.H. & Anderson M.J. (2001) Fitting multivariate models to community data: a comment on distance-based redundancy analysis. *Ecology*, **82**, 290–297.
- Newsome S.D., Del Rio C.M., Bearhop S. & Phillips D.L. (2007) A niche for isotopic ecology. *Frontiers in Ecology and the Environment*, **5**, 429–436.
- Nolte A.W. & Tautz D. (2010) Understanding the onset of hybrid speciation. *Trends in Genetics: TIG*, **26**, 54–58.
- Nolte A.W., Freyhof J., Stenshorn K.C. & Tautz D. (2005) An invasive lineage of sculpins, *Cottus* sp (*Pisces, Teleostei*) in the Rhine with new habitat adaptations has originated from hybridization between old phylogeographic groups. *Proceedings of the Royal Society B-Biological Sciences*, **272**, 2379–2387.
- Nunn A.D., Harvey J.P. & Cowx I.G. (2007) Benefits to 0 + fishes of connecting man-made waterbodies to the lower River Trent, England. *River Research and Applications*, **23**, 361–376.
- O'Grady M.F. (1981) Some direct gill net selectivity tests for the brown trout populations. *Irish Fisheries Investigations Series A*, **22**, 1–9.
- Olin M., Rask M., Ruuhijarvi J., Kurkilahti M., Ala-Opas P. & Ylonen O. (2002) Fish community structure in mesotrophic and eutrophic lakes of southern Finland: the relative abundances of percids and cyprinids along a trophic gradient. *Journal of Fish Biology*, **60**, 593–612.

- Parnell A.C., Inger R., Bearhop S. & Jackson A.L. (2010) Source partitioning using stable isotopes: coping with too much variation. *PLoS ONE*, **5**, e9672.
- Persson L. (1983) Effects of intraspecific and interspecific competition on dynamics and size structure of a perch *Perca fluviatilis* and a roach *Rutilus rutilus* population. *Oikos*, **41**, 126–132.
- Persson L. (1990) A Field Experiment on the Effects of Interspecific Competition from Roach, *Rutilus rutilus* (L), on Age at Maturity and Gonad Size in Perch, *Perca fluviatilis* (L.). *Journal of Fish Biology*, **37**, 899–906.
- Persson L., Bystrom P., Wahlstrom E., Nijlunsing A. & Rosema S. (2000) Resource limitation during early ontogeny: constraints induced by growth capacity in larval and juvenile fish. *Oecologia*, **122**, 459–469.
- Persson L., Bystrom P., Wahlstrom E. & Westman E. (2004) Trophic dynamics in a whole lake experiment: size-structured interactions and recruitment variation. *Oikos*, **106**, 263–274.
- Post D.M. (2002) Using stable isotopes to estimate trophic position: models, methods, and assumptions. *Ecology*, **83**, 703–718.
- Ravinet M., Syvaranta J., Jones R.I. & Grey J. (2009) Atrophic pathway from biogenic methane supports fish biomass in a temperate lake ecosystem. *Oikos*, **119**, 409–416.
- Regan T.C. (1911) *The Freshwater Fish of the British Isles*. Methuen & Co., Ltd., London.
- Roche W. (1999) *Fish Studies in Pollaphuca Reservoir, Co. Wicklow*. PhD Thesis, University College Dublin.
- Rosenfield J.A., Nolasco S., Lindauer S., Sandoval C. & Kodric-Brown A. (2004) The role of hybrid vigor in the replacement of Pecos pupfish by its hybrids with sheepshead minnow. *Conservation Biology*, **18**, 1589–1598.
- Schwenk K., Brede N. & Streit B. (2008) Introduction. Extent, processes and evolutionary impact of interspecific hybridization in animals. *Philosophical Transactions of the Royal Society B-Biological Sciences*, **363**, 2805–2811.
- Scribner K.T., Page K.S. & Barton M.L. (2000) Hybridization in freshwater fishes: a review of case studies and cytonuclear methods of biological inference. *Reviews in Fish Biology and Fisheries*, **10**, 293–323.
- Seehausen O. (2004) Hybridization and adaptive radiation. *Trends in Ecology & Evolution*, **19**, 198–207.
- Svanback R., Eklov P., Fransson R. & Holmgren K. (2008) Intraspecific competition drives multiple species resource polymorphism in fish communities. *Oikos*, **117**, 114–124.
- Syväranta J., Vesala S., Rask M. & Ruuhijärvi J. (2008) Evaluating the utility of stable isotope analysis of archived freshwater sample materials. *Hydrobiologia*, **600**, 121–130.
- Toner P., Bowman J., Clabby K., Lucey J., McGarrigle M., Concannon C. et al. (2005) *Water Quality in Ireland 2001–2003*. Environmental Protection Agency, Ireland.
- Toscano B.J., Pulcini D., Hayden B., Russo T., Kelly-Quinn M. & Mariani S. (2010) An ecomorphological framework for the coexistence of two cyprinid fish and their hybrids in a novel environment. *Biological Journal of the Linnean Society*, **99**, 768–783.
- Van Den Berg C., Sibbing F.A., Osse J.W.M. & Hoo-genboezem W. (1992) Structure, development and function of the branchial sieve of the common bream, *Abramis brama*, white bream, *Blicca bjoerkna*, and roach, *Rutilus rutilus*. *Environmental Biology of Fishes*, **33**, 105–124.
- Van Den Berg C., Van Den Boogaart J.G.M., Sibbing F.A. & Osse J.W.M. (1994) Zooplankton feeding in common bream (*Abramis brama*), white bream (*Blicca bjoerkna*) and roach (*Rutilus rutilus*) experiments, models and energy intake. *Netherlands Journal of Zoology*, **44**, 15–42.
- Wheeler A. (1969) *The Fishes of the British Isles and Northwest Europe*. MacMillan & Co., London.
- Wyatt P.M.W., Pitts C.S. & Butlin R.K. (2006) A molecular approach to detect hybridization between bream *Abramis brama*, roach *Rutilus rutilus* and rudd *Scardinius erythrophthalmus*. *Journal of Fish Biology*, **69**, 52–71.
- Yakovlev V.N., Slyn'ko Y.V., Grechanov I.G. & Krysanov E.Y. (2000) Distant Hybridization in Fish. *Journal of Ichthyology*, **40**, 298–311.

(Manuscript accepted 7 March 2011)