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Research article

Preliminary study of dietary interactions between invading Ponto-Caspian gobies and some native fish species in the River Danube near Bratislava (Slovakia)

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Abstract

Dietary breadth and diet overlap were examined in two native fish species (Eurasian perch *Perca fluviatilis*, Balon's ruffe *Gymnocephalus baloni*) and two invading species (round goby *Neogobius melanostomus*, bighead goby *Neogobius kessleri*) in the Middle Danube (Slovakia) at dawn and dusk over two consecutive 24-h periods in late summer 2004. Dietary breadth was narrow in all species/age classes except perch of ages 4 and 5. Dietary overlap between native fishes and the invading gobies was very high, except for age 3 perch. This high overlap was due to a preference, high in some cases, for gammarids. Our preliminary results suggest the potential for competition between the invading and native fishes, which requires further study.

Key words: dietary overlap, round goby *Neogobius melanostomus*, bighead goby *Neogobius kessleri*, Eurasian perch *Perca fluviatilis*, Balon's ruffe *Gymnocephalus baloni*

Introduction

Four species of Ponto-Caspian gobies have recently been expanding upstream into the middle and upper courses of the River Danube (reviewed in Copp et al. 2005a): bighead *Neogobius kessleri* (Günther, 1861), racer *N. gymnotrachelus* (Kessler, 1857), monkey *N. fluviatilis* (Pallas, 1814) and round *N. melanostomus* (Pallas, 1814). For bighead goby and round goby, this range expansion represents an invasion (Erős et al. 2005), probably facilitated by the dispersal of the fish as 'hitch hikers', or hull fouling, of river container ships (Wiesner 2005). Bighead goby, as the first Ponto-Caspian gobiid invader of the middle Danube, having been recorded in 1994 in Austria (Zweimüller et al. 1996) and then in 1996 in Slovakia (Stráňai 1998), and previously the most abundant and widely distributed of the invading gobiids, has been recently outnumbered in both abundance and distribution dynamics by a subsequent invader, the round goby (recorded in 2003 in Slovakia).

The high frequency and abundance of the round goby and bighead goby (Copp et al. 2005a; Jurajda et al. 2005; personal observations in August 2007) have raised concerns that these invading species may be having an impact on native fish species. Indeed, the rise in numbers of these invading gobies has coincided with a progressive decline in some native benthic fishes (Jurajda et al. 2005): bullhead *Cottus gobio* Linnaeus, stone loach *Barbatula barbatula* (Linnaeus), and the white-finned gudgeon *Gobio albipinnatus* (Lukasch). The aim of this preliminary investigation was to evaluate the potential dietary interactions between the alien and native fish species, with the specific objective of determining the dietary preferences of the non-native gobies, which are poorly described for the upper and middle Danube, and the extent of dietary overlap between the invading gobies and native species.

Study area, Material and Methods

Because many fish species in the Danube feed mainly at dusk and dawn (Copp et al. 2005c), a pattern observed elsewhere (Copp et al. 2005b), fish sampling was undertaken at 06:00 and 21:00 over two consecutive 24-h periods between 30 August and 1 September 2004 along three stretches of bank in the old main channel of the Danube: 1) just downstream of the weir at Čunovo (river km 1851), 2) at 50 m downstream of stretch 1, and 3) further downstream along a wing dam (river km 1845). The hydrology and geomorphology of the Slovak/Hungarian flood plain have been described elsewhere (e.g. Copp et al. 1994; Černý et al. 2004). Stretch 1 was characterised mainly by boulders (rip-rap) mixed with sand, with water depth ranging from 0.5 to 2.5 m, slow-to-moderate water velocities and moderate turbidity (level 4 on a scale of increasing turbidity: 1–6). Stretch 2 had a similar substratum, but with moderate-to-high water velocities, turbidity levels 4 to 5, steep banks and rapidly increasing depths to >1.5 m. Stretch 3 was characterised by boulders (rip-rap) and stones with muddy patches. Water was mainly shallow, ranging from 0.5 to 1.2 m depth, the current was moderate or even slow and turbidity reached the level 3.

Fish were collected by continuous electro-fishing (1 hour) on six occasions (four in the evening, two in the morning). The fish were killed with an overdose of anaesthetic and preserved in 4% formaldehyde for analysis in the laboratory. The numbers of aquatic invertebrates in the benthos (i.e. available in the environment) were determined by quantitative Surber sampling. The substratum was disturbed and the macrozoobenthos individuals washed into the rear part of the Surber sampler by the water current and/or by hand. The contents of the Surber sampler was washed into a 500 µm sieve and preserved with formalin in the field. In the laboratory, the specimens were measured using vernier calliper to the nearest 0.01 mm –standard length (SL), total length (TL) and fork length (FL) were taken – and weighted to the nearest 0.1 g both before (body weight) and after dissection (eviscerated body weight). The gut of each specimen was opened and its content analysed. To determine the age of the individuals examined, three to six intact, well-developed scales were removed from the left side of each specimen. The scales were cleaned and compressed between two glass slides. The age of specimens was determined by counting the number of completely developed annual rings. Although five is the recommended number of specimens (of fish that contain prey) to obtain a reliable impression of a fish species diet in at any given time (e.g. Copp and Mann 1993), one species (round goby) was included, based on four specimens due the lack of information on the diet of this non-native species in the middle Danube.

Data analysis

The fish data were categorized according to species and age class, with data analysis limited to the most abundant species for which the age classes were combined in all cases except Eurasian perch: bighead goby of ages 1 to 3 combined (NkA), round goby of ages 3 and 4 combined (Nm3), Balon's ruffe *Gymnocephalus baloni* Holcik et Hensel 1974 of ages 3 and 4 combined (Gb3), and Eurasian perch *Perca fluviatilis* Linnaeus of ages 1 and 2 combined (Pf2), perch age 3 (Pf3), and perch age 4 (Pf4).

To assess the contribution of individual prey selection (i.e. variations in resource use by each individual within a species) to diet composition and overlap, covariance matrix principle components analysis (de Crespin de Billy et al. 2000) was applied to the data matrices consisting of the numbers of each prey type found in the gut of each individual converted to a proportion of the total number of items found in that gut. All prey types were included. Because the use of proportions removes the unequal weight among individuals, semi-quantitative investigations are more appropriate for analyses at the individual level (de Crespin de Billy et al. 2000). In the analysis, each prey type is linked to the 'sample population' centroid by an arrow, whose length is proportional to the relative abundance of that taxon. Additionally, the length of the arrow also depends on the variation of use of the corresponding prey type among individuals. Thus, prey taxa dominant in the diet are ordinated along the principal components (resource gradients) whereas rarely taken prey types are concentrated near the origin of the biplot (de Crespin de Billy et al. 2000). For each species, 90% contour ellipses (Green 1971) were generated to aid the interpretation of dietary overlap. Note that narrow ellipses (i.e. dietary breadth) may appear to be straight lines. The analysis and graphics were undertaken using software by Thioulouse and Chessel (1990-2003).

To complement the dietary overlap suggested in the PCA biplots, the Zaret and Rand (1971) overlap index was calculated for each of the species classes using the formula:

$$C\lambda = 2 \cdot \sum_{i=1}^{s} X_i \cdot Y_i / \left(\sum_{i=1}^{s} X_i^2 + \sum_{i=1}^{s} Y_i^2\right)$$

where s is the total number of all food categories distinguished, X_i , and Y_i are the proportions of food item i in the guts of species X and Y respectively. Zaret and Rand (1971) assumed that $C\lambda$ values ≥ 0.6 were significant, but gave no test statistics.

Dietary electivities of each fish species class were calculated using Jacob's (1974) modified version of Ivlev's electivity index ($D = r\text{-}p \div [r+p - 2rp]$) in which r is the proportion of a given prey type used by each species class and p is the proportion of that prey type available in the environment. Prey types of terrestrial origin were did not included in the analysis. The electivity values for fish prey and invertebrate prey were calculated separately, using the combined totals for all sampling periods for the two prey groups, respectively. The numbers of fish prey types (Cyprinidae, Gobiidae) available to a potential predator during a given sampling excursion were estimated from the numbers of fish captured during that excursion, with 'available' fish prey being the number of potential prey specimens that were ≤60% of the size of the potential predator. Graphical illustrations of the D values for each fish species were generated using software by Thioulouse (1990), whereby values approaching or equal to '1' correspond to prey types taken proportionately more than their availability in the environment (i.e. preference), whereas values approaching or equal to '-1' correspond to prey types taken proportionately less than their availability in the environment (i.e. avoidance; Jacob's 1974).

Results

In total, 246 fish were captured during the study period, representing 14 species (Annex 1). The numerically most abundant species were, in decreasing order, Eurasian perch, bleak *Alburnus alburnus*, bighead goby, roach, round goby, and Balon's ruffe. The other eight species occurred in low numbers (<5 individuals). The age and size distributions of these fish varied. Ages 0 to 4 were observed in bleak and bighead goby only (Annex 1), with ages 1–4 observed in round goby and perch. All other species were observed in one or two age classes only, except pikeperch *Sander lucioperca* for which specimens of ages 1, 2 and 4 were captured.

Prey availability differed between fish and invertebrates, with the former being variable and the latter relatively consistent, among the six sampling excursions (Annex 2). Most notable in the available fish prey was the disappearance of small gobies and the increase in small cyprinds during the study period. There was considerable overlap in the diet of the six species/age classes examined for resource use (Annex 3, Figure 1A), with Gammaridae, Corixidae and Oligochaeta being the principal taxa (Figure 1B) in the biplot of principle components (PC) one and two, which accounted for 77% of the variation (Figure 1C). The ordinations reflect which prey types were taken by the fish species/age classes proportionately less (avoidance) or more (preference) than that prey's availability in the environment (Figure 2). Many of the species/size classes avoided, or were indifferent to, most prey types and demonstrate a strong preference for a few prey types only (e.g. Balon's ruffe for Corixidae, bighead goby and round goby for Gammaridae only, age 2 perch for Oligochaeta only). Only perch of ages 3 and 4 demonstrated more than one strong preference (Figure 2).

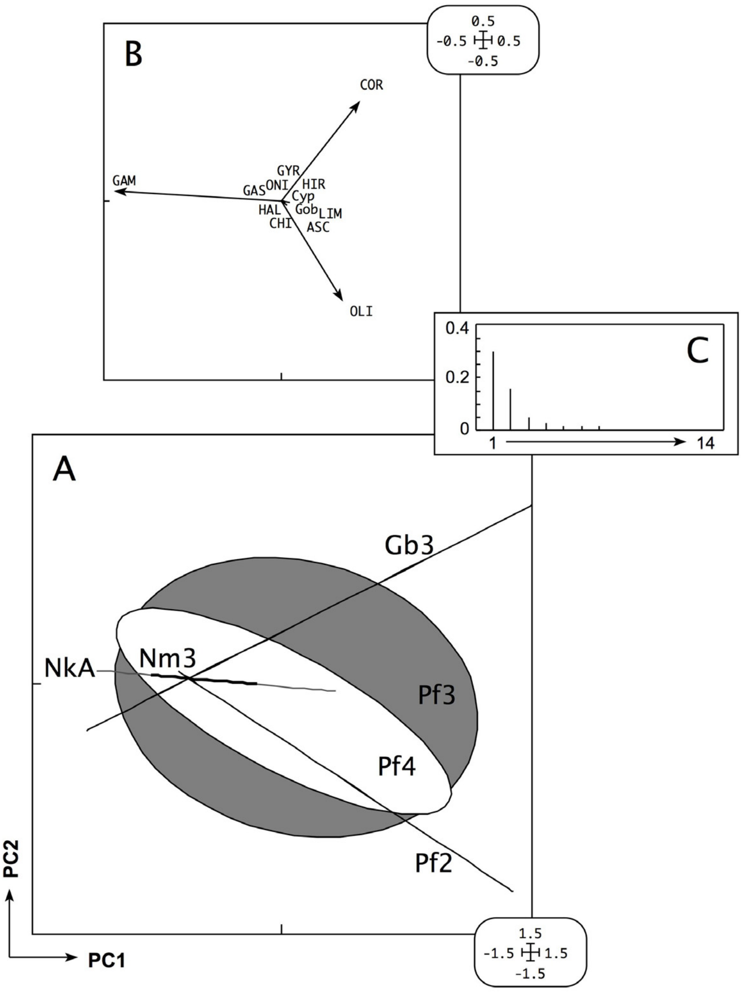

Figure 1. Principal components (PC) analysis biplot of diet variation (de Crespin de Billy et al. 2000) in individual specimens of fish captured in the River Danube in August 2004: A) PC1 and PC2 biplot, with 90% contour ellipses (Green 1971) given for common age classes (note that a narrow ellipse, i.e. narrow dietary breadth, may appear to be a straight line); B) correlation vectors of the dietary items whereby arrow length reflects the relative contribution of that prey item to the ordination; C) eigen values of which PC1 and PC2 account for 77% of the variation. Fish and prey species codes given in Annex 2

These two species/age classes were the only ones to demonstrate a preference for fish prey (i.e. Cyprinidae), but their preferences for invertebrate prey types differed, with age 4 perch showing a moderate preference for Gammaridae and Oligochaeta only, whereas age 3 perch was the only species/age class to express preferences for Asellidae, Gyrinidae, Haliplidae, and Hirudinae (Figure 2). All native fish size classes except age 2 perch demonstrated significant dietary overlap with the invading gobies, with overlap also noted between the invading and between the size classes of native species (Annex 3).

Discussion

Owing to its invasion of numerous hydrosystems in Europe and North America, the diet of the round goby has attracted much study in the recent decades. Molluscs, and especially bivalves, have been observed to be a major food category of round goby invading both European (Simonović et al. 1998; Simonović et al. 2001; Skora and Rzeznik 2001) and North American water (French and Jude 2001). Indeed, in the Bay of Gdansk round goby > 13 cm TL fed almost exclusively on molluscs (Skora and Rzeznik

2001), and an increasing proportion of molluscs in the diet with increasing fish size has been observed in the Great Lakes of North America (French and Jude 2001). However, molluscs were in low abundance in the studied stretches of the Middle Danube (Annex 2), and this is reflected in their rarity (Figure 1) and in the prey preferences of round goby (Figure 2).

Other prey items taken by round goby include crustaceans, worms and insect larvae, with fish being observed in low numbers in the present study (Figure 2) and in many (French and Jude 2001; Skora and Rzeznik 2001) but not all (Simonović et al. 1998, 2001) studies elsewhere. Indeed, the diet of round goby is known to vary both spatially and by season (French and Jude 2001; Skora and Rzeznik 2001). According the present study, round goby strongly preferred gammarids (Figure 2) but took gastropods proportional to their availability (Annex 2, Figure 2). All other potential prey items were taken proportionately less than their availability, resulting in a relatively narrow diet breadth during our preliminary investigation.

Bighead goby diet resembled that of round goby (Figure 1), with gammarids as preferred prey, but the species used chironomid larvae as second food resource, resulting in a narrow food breadth (Figure 2) and a high dietary overlap between the goby species (Annex 3). This high overlap in diet of invading gobies has also been observed in the Serbian sector of the Danube (Simonović et al. 2001).

Of the native fishes, the diet breadth of Balon's ruffe and young perch were similar to the gobies in that it was very narrow and only one prey preference was observed (Figure 2). As predicted from an early study of native species diet in side channels of the Slovak Danube, where Rotifers were a major dietary component for young perch (Copp et al. 2005c), perch were abundant along the rip-rap shores of our study sites (Annex 1). With increase age (size), perch diet increased in breadth (Figure 1) and number of preferred prey types, including fish prey types (Figure 2). We did not sample available zooplankton prey, but the diet was dominated by benthic invertebrates plus Corixidae (Figure 1).

Dietary overlap between native fishes and the invading gobies was very high (Annex 3), suggesting the potential for competition between the invading and native fishes. This high overlap is due to a preference, high in some cases, for gammarids. Indeed, five of the six fish species/age classes examined demonstrated weak-to-strong preferences for this benthic taxon. Round goby shared one prey type with small perch (Pf2), gastropods, a prey type avoided by all other fish species/age classes except round goby (Figure 2). Although our investigation was restricted to a few 24-h periods during summer, our results are consistent with longer-term studies of invasive round goby in North America (French and Jude 2001), where diet overlap with native North American benthic

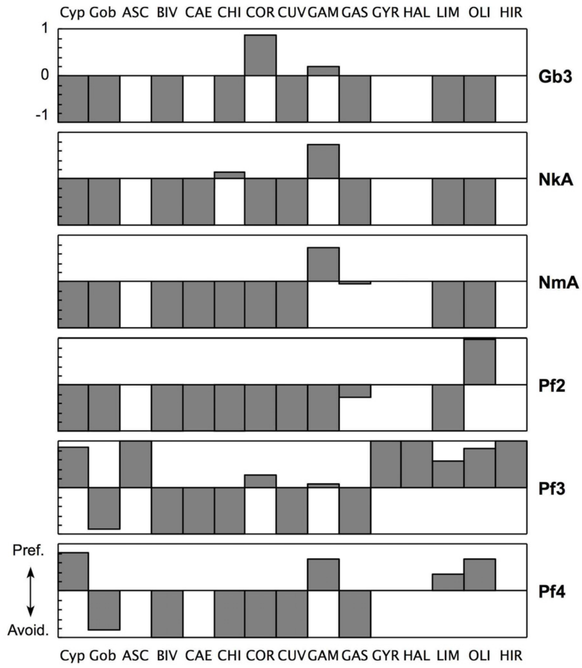

Figure 2. Dietary electivities (Jacob's 1974 version of Ivlev's '*D*') of each fish species/age class for prey types in the River Danube (Slovakia) in August 2004. Values approaching 1.0 indicate preference (i.e. taken proportionately more), those approaching -1.0 indicate avoidance (i.e. taken proportionally less). Fish species codes given in Annex 1

fishes was high on several occasions. However, smaller round gobies tended to have higher overlap values than larger specimens (French and Jude 2001). Molluscs were in low abundance; they were observed in the first Surber sample only (Annex 2); and hence they were not a preferred prey (Figure 2). For round goby, this contrasts the introduced populations in the Great Lakes, where zebra mussels are a common food of larger round gobies but an infrequent prey of native fishes (French and Jude 2001). An important attribute that has facilitated the invasions by Ponto-Caspian gobies is their plasticity in life-history (Balážová-L'avrinčíková and Kováč 2007) and diet (e.g. Grabowska and Grabowski 2005; Kakareko et al. 2005), and our preliminary results suggest narrow dietary breadth in the invading gobies and the native Balon's ruffe, but with high overlap between the invading and native species. Whether this overlap constitutes competition or repartition of the available resources requires further study.

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References

Balážová-L'avrinčíková M, Kováč V (2007) Epigenetic context in the life history traits of the round goby, *Neogobius melanostomus*. In Gherardi F (ed) Freshwater Bioinvaders: Profiles, Distribution, and Threats. Springer, Berlin, pp 275-287

Černý J, Copp GH, Kováč V, Gozlan RE, Vilizzi L (2003) Initial impact of the Gabčíkovo hydroelectric scheme on 0+ fish assemblages in the Slovak flood plain, River Danube. River Research and Applications 19: 749-766

Copp GH, Mann RHK (1993) Comparative growth and diet of tench *Tinca tinca* (L.) larvae and juveniles in river floodplain biotopes in France and England. Ecology of Freshwater Fish 2: 58-66

Copp GH, Bianco PG, Bogutskaya N, Erős T, Falka I, Ferreira MT, Fox MG, Freyhof J, Gozlan RE, Grabowska J, Kováč V, Moreno-Amich R, Naseka AM, Peňáz M, Povž M, Przybylski M, Robillard M, Russell IC, Stakėnas S, Šumer S, Vila-Gispert A, Wiesner C (2005a) To be, or not to be, a non-native freshwater fish? Journal of Applied Ichthyology 21: 242-262

Copp GH, Guti G, Rovný B, Černý J (1994) Hierarchical analysis of habitat use by 0+ juvenile fish in the Hungarian/Slovak flood plain of the River Danube. Environmental Biology of Fishes 40: 329-348

Copp GH, Spathari S, Turmel M (2005b) Consistency of diel behaviour and interactions of stream fishes and invertebrates during summer. River Research and Applications 21: 75-90

Copp GH, Vranovský M, Černý J, Kováč V (2005c) Diel dynamics of young fishes and zooplankton in a lentic side-channel of the River Danube. Biología (Bratislava) 60: 179-188

de Crespin de Billy V, Dolédec S, Chessel D (2000) Biplot presentation of diet composition data: an alternative for fish stomach content analysis. Journal of Fish Biology 56: 961-973

Erős T, Sevcsik A, Tóth B (2005) Abundance and night-time habitat use patterns of Ponto-Caspian gobiids species (Pisces, Gobiidae) in the littoral zone of the River Danube, Hungary. Journal of Applied Ichthyology 21: 350-357

Green RH (1971) A multivariate statistical approach to the Hutchinsonian niche: bivalve molluscs of central Canada. Ecology 52: 543-556

Jacobs J (1974) Quantitative measurement of food selection: a modification of the forage ratio and Ivlev's electivity index. Oecologia 14: 413-417

Grabowska J, Grabowski M (2005) Indications on the diel feeding activity in early summer of racer goby *Neogobius gymnotrachelus* (Gobiidae) - a new invader in the Baltic basin. Journal of Applied Ichthyology 21: 282-286

Kakareko T, Żbikowski J, Żytkowicz J (2005) Preliminary results on diet partitioning in summer of two syntopic neogobiids from two different habitats of the lower Vistula River, Poland. Journal of Applied Ichthyology 21: 292-295

Kováč V (1994) Updated red list of Slovak fish and cyclostomes. Zborník Ochrana biodiverzity na Slovensku. Bratislava, pp 153-158

Simonović P, Valković B, Paunović M (1998) Round goby *Neogobius melanostomus*, a new Ponto-Caspian element for Yugoslavia. Folia Zoologica 47: 305-312

Simonović P, Paunović M, Popović S (2001) Morphology, feeding, and reproduction of the round goby, *Neogobius melanostomus* (Pallas), in the Danube River Basin, Yugoslavia. Journal of Great Lakes Research 27: 281-289

Skora KE, Rzeznik J (2001) Observations on diet composition of *Neogobius melanostomus* Pallas 1811 (Gobiidae, Pisces) in the Gulf of Gdansk (Baltic Sea). Journal of Great Lakes Research 27: 290-299

French JRP, Jude DJ (2001) Diets and diet overlap of nonindigenous gobies and small benthic native fishes co-inhabiting the St. Clair River, Michigan. Journal of Great Lakes Research 27: 300-311

Stráňai I (1998) Morphometry study on *Neogobia kessleri* (Günther 1861) from the main stream of the Danube river. Czech Journal of Animal Science 43: 289-292

Thioulouse J (1990) MacMul and GraphMu: two Macintosh programmes for the display and analysis of multivariate data. Computers and Geoscience 8: 1235-1240

Thioulouse J, Chessel D (1990–2003) ADE Programme library for the analysis of environmental data on Macintosh System 10. Université Lyon I, Villeurbanne. http://biomserv.univ-lyon1.fr/ADE-4.html

Wiesner C (2005) New records of non-indigenous gobies (*Neogobius* spp.) in the Austrian Danube. Journal of Applied Ichthyology 21: 324-327

Zaret TM, Rand AS (1971) Competition in tropical stream fishes: support for the competitive exclusion principle. Ecology 52: 336-342

Zweimüller I, Moidl S, Nimmervoll H (1996) A new species for the Austrian Danube – *Neogobius kessleri*. Acta Universitatis Carolinae, Biologica 40(1–2): 213-218

Annex 1
Total number of specimens (N), numbers by age class (n), and the minimum (Min.) and maximum (Max.) values of standard length (SL) for the 246 fish captured in the River Danube near Bratislava (Slovakia) over two consecutive 24-h periods in August 2004

N	Species	Age 0			Age 1			Age 2			Age 3			Age 4		
		n	Min	Max	n	Min	Max	n	Min	Max	n	Min	Max	n	Min	Max
45	*Alburnus alburnus*	6	28.3	31.9	14	34.3	42.2	6	70.4	88.5	14	82.2	107.1	5	96.8	104.9
2	*Aspius aspius*							2	98.1	105.0						
2	*Blicca bjoerkna*							1	78.8	78.8				1	50.8	50.8
2	*Esox lucius*							1	227.4	227.4	1	199.2	199.2			
18	*Gymnocephalus baloni*							5	82.4	98.1				13	84.3	105.5
1	*Lepomis gibbosus*													1	77.4	77.4
2	*Leuciscus idus*										2	106.9	111.2			
4	*Leuciscus leuciscus*													4	114.7	178.9
43	*Neogobius kessleri*	6	30.8	40.0	20	43.1	56.7	6	59.4	89.8	7	87.7	107.9	4	108.5	146.8
33	*Neogobius melanostomus*				7	28.9	36.9	3	36.3	57.7	15	63.4	88.1	8	85.8	102.0
56	*Perca fluviatilis*				2	56.8	63.7	8	89.2	109.0	28	78.5	138.3	18	104.1	178.1
34	*Rutilus rutilus*							4	90.5	95.0	16	100.5	128.7	14	100.6	159.0
3	*Rutilus rutilus*				1	148.7	148.7	1	162.3	162.3				1	241.0	241.0
1	*Vimba vimba*							1	116.3	116.3						

Annex 2

Numbers of prey items available in the environment to fish predators in the River Danube near Bratislava (Slovakia) in August 2004

	Excursion number						Totals
	1	2	3	4	5	6	
Cyprinid fishes available as prey to:							
Gb3 (*G. baloni* ages 3 & 4)	1	1		1	0	17	20
NkA (*N. kessleri* ages 1-3)	0	0	0	0	0	6	6
NmA (*N. melanostomus* ages 3 & 4)	0	0	0	0	0	6	6
Pf2 (*P. fluviatilis* ages 1 & 2)	1	1	0	1	0	17	20
Pf3 (*P. fluviatilis* age 3	1	1	0	1	0	17	20
Pf4 (*P. fluviatilis* age 4)	2	1	0	7	2	22	34
Totals:	5	4	0	10	2	85	106
Gobiid fishes available as prey to:							
Gb3	4	26	1	2	0	0	33
NkA	4	26	1	2	0	0	33
NmA	1	4	1	0	0	0	6
Pf2	4	26	1	2	0	0	33
Pf3	7	55	1	6	0	0	69
Pf4	13	47	5	11	0	0	76
Totals:	33	184	10	23	0	0	250
All fish prey totals:	38	188	10	33	2	85	356
Invertebrates available:							
Asellidae (ASE)	0	0	0	0	0	0	0
Bivalvia (BIV)	3	0	0	0	0	0	3
Caenidae (CAE)	0	0	0	0	1	0	1
Chironomidae (CHI)	16	0	23	8	0	0	47
Corixidae (COR)	0	2	0	0	9	8	19
Corophium curvispinum (CUV)	0	57	0	0	0	0	57
Gammaridae (GAM)	0	0	29	0	115	20	164
Gastropoda (GAS)	67	0	0	0	0	0	67
Gastropoda (GAS)	0	0	0	0	0	0	0
Haliplidae (HAL)	0	0	0	0	0	0	0
Limnomysis benedeni (LIM)	0	0	3	0	1	2	6
Oligochaeta (OLI)	0	0	0	1	0	13	14
Hirudinae (HIR)	0	0	0	0	0	0	0
Totals:	86	59	55	9	126	43	378

Annex 3

Number of fish captured (nT) and examined (nD), whereby the difference is the number of fish with empty digestive tracts, as well as the dietary overlap (as per Zaret and Rand 1971) of fish species/age classes from the River Danube (Slovakia) whereby dietary overlap value ≥ 0.6 are considered statistically significant (see Zaret and Rand 1971). Fish codes given in Annex 2. Values associated with <5 specimens are given in italics to highlight their low reliability

	nT	nD	Dietary overlap				
			NkA	NmA	Pf2	Pf3	Pf4
Gb3	18	14	**0.74**	**0.74**	0.09	**0.69**	**0.69**
NkA	43	11	-	**0.96**	0.12	**0.73**	**0.87**
NmA	33	4		-	*0.12*	***0.72***	***0.86***
Pf2	10	5		-		0.55	0.28
Pf3	28	19			-		**0.88**
Pf4	18	8				-	