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A molecular approach to identifying the relationship between resource use and availability in Eurasian otters (*Lutra lutra*)

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Abstract

In South Korea, the Eurasian otter (*Lutra lutra* Linnaeus, 1758), a semi-aquatic carnivore, is found mainly in lower-order streams that tend to have a low abundance of preferred prey fish species. To investigate the relationship between resource use and availability, we used DNA barcoding to identify otter diet items in 24 otter spraints (faeces) from 16 sites along the Nakdong River basin during June 4 to 6, 2014. At these sites fish availability was assessed using scoop-nets and casting nets. Fish formed the bulk of otter diet, which included also frogs, mammals and reptiles. By DNA barcoding (success rate: 72.38%), we identified 79 prey items from 105 bone remains. The diet comprised mostly fish, but frogs, mammals, and reptiles were also identified. The fish fauna and otter diet composition differed significantly. Across the study sites, members of the Cyprinidae dominated in netted samples, but occurred less frequently in otter diet. Because most Cyprinidae are fast swimmers, otters also fed on benthic fishes and frogs, suggesting limited foraging flexibility in otters and specialisation on more slowly moving prey.

Key words: Eurasian otter; *Lutra lutra*; diet; food availability; home range size; DNA barcoding

Introduction

Resource availability and foraging ability are a major factors affecting resource use, home range characteristics of carnivores, fecundity, and inter- and intra-species competition (MacArthur and Pianka 1966; Doncaster and Woodroffe 1993; Eide et al. 2004; Ruiz-Olmo et al. 2011; Crowley et al. 2013). Several endangered carnivore populations have recovered in response to improved environmental conditions and strengthened protection laws, however, resource availability remains a key driver for recovery (Carss 1995; Sjoasen 1997; Almeida et al. 2012).

The Eurasian otter (*Lutra lutra* Linnaeus, 1758) displays either generalist or specialist feeding behaviour, depending on resource availability (Clavero et al. 2003). Most otter species prefer easily catchable fish, which typically are relatively slow-swimming large fish (Cote et al. 2008 for river otter [*Lontra Canadensis* Shrebbler, 1776]; Rheingantz et al. 2012 for Neotropical otter [*Lontra longicaudis* Olfers, 1818] Erlinge 1968; Sulkava 1996; Ayres and Garcia 2011 for Eurasian otter). For example, when Salmonidae were released to restock wild populations, otters specialized on the stocked fish which were less adapted to natural streams (Jacobsen 2005; Sittenthaler et al. 2015). However, otters show considerable flexibility in adjusting their diet to the local fish fauna composition and abundance (Clavero et al. 2003). When fish are scarce, other taxa, such as frogs, crayfish, and other are eaten more frequently (Bouros and Murariu 2017) and home ranges are typically larger (Eide et al. 2004; Crowley et al. 2013). Otter diet is also known to vary with season, altitude a.s.l., and stream order (main river vs. tributaries), mirroring variation in food availability (Je, drzejewska et al. 2001; Brzeziński et al. 2006; Georgiev 2006; Crowley et al. 2013).

In the last two decades, populations of the endangered, semi-aquatic Eurasian otter have recovered throughout its wide range in parts of Europe and in South Korea, facilitated by their ability to disperse up to 40 km to establish new territories (Sjoasen 1997). In South

Korea, the Eurasian otter (termed otter, henceforth) has substantially recovered, assisted by habitat restoration and forest development, and improved protection laws (Hong 2018). However, resource abundance and availability may still affect the long-term recovery of otters and need to be furtherly investigated (Ruiz-Olmo et al. 2011).

DNA barcoding has been used widely to identify consumed prey to species level (Folmer et al. 1994; Jo et al. 2014) and can be used to analyse the relationship between resource availability and use (Sheppard et al. 2004; Kruger et al. 2014). Although numerous studies using visual identification of prey items in otter faeces indicated a certain degree of uncertainty (Carss 1995), DNA barcoding has not previously been applied to analyse otter diet. A total of 213 freshwater fish species occur on the Korean peninsula, including 67 endemic species, a diversity much higher than that recorded in Europe or central Asia (Kim et al. 2005; Yoon et al. 2018). High diversity of prey species is likely to complicate visual identification of food items in faecal samples. Therefore, in this study, we aimed (i) to apply DNA barcoding to identify otter prey items, and (ii) to assess the relationship between resource availability and use in Korean otter populations.

Materials and methods

Study area and sample collection

The Nakdong River flows for approximately 520 km and has a catchment of about 23,800 km² (a quarter of South Korea's land area). In the upper reaches of the river, there are four dams, low human population density, and mostly undisturbed streams. Previously, we investigated otter distribution at 250 sites along the Nakdong river catchment, counting spraints (otter faeces) along 600 m transects to calculate spraint densities (Fig. 1). We then selected 16 sites located in upper stream regions and tributaries connected to the East Sea to analyse otter feeding habits. The selected sites tended to have higher densities of spraints and

higher fish and benthic macro-invertebrate assessment indices than other sites (Table 1). Nine of the selected sites were in the upper-middle reaches of the Nakdong River, and seven sites were located in small streams near the East Sea (Fig. 1a). Stream orders of study sites varied from 2nd to 5th order (Fig. 1b). At the selected sites, we collected spraints from June 4 to 6, 2014. The animal protocol used in this study was reviewed by the Pusan National University–Institutional Animal Care and Use Committee (PNU-IACUC) and approved (Approval Number PNU-2018-2112).

Molecular analysis of otter diet from faecal samples

For the molecular analyses, we collected 105 bones (mostly jaw bones) from 24 spraints. Counts of jaw bones allowed us to estimate the number of prey individuals (Remonti et al. 2009). We distinguished between individuals by comparing the size and colouration of bones. After visual quantification of the bones, ethanol was volatilized from the samples before the DNA extraction process. The bones were then frozen in liquid nitrogen and manually ground to a fine powder using mortar and pestle. DNA extraction and amplification procedures were conducted as described by Jo et al. (2016).

Specifically, we used a mitochondrial 12S rRNA fragment for universal vertebrate identification (Fuller et al. 1998). The PCR thermal regime consisted of 10 min at 94 °C, 35 cycles of 1 min at 94 °C, 1.5 min at 50 °C, 1 min at 72 °C, and a final step of 5 min at 72 °C, using a Mastercycler (Eppendorf, Hamburg, Germany). PCR products were separated using 1.5 % agarose gels. When no sufficient PCR amplification was achieved, re-amplification was performed using 1 µL of the first PCR product and the same thermocycling protocol. DNA sequencing was performed at Macrogen, Inc. (Seoul, Republic of Korea). Sequence alignments were produced using Clustal W 2.0 (Larkin et al. 2007). A BLASTn search was performed to identify the sequences with the best hits.

After the species in spraints were identified, we reviewed published information on the identified species' geographical distribution. When the identified species was not known to occur in South Korea, we assigned it to the highest possible taxonomical grade. For example, sequences identified as *Bombina variegata* Linnaeus, 1758 which is not known to occur in South Korea were assigned at genus level, but not considered to be *Bombina orientalis* Boulenger, 1890, the only species of this genus occurring in South Korea (Table 3).

Use-availability relationship

To analyse the relationship between stream order and spraint density, we compared the average and variance spraint density of each stream order using an analysis of variance (ANOVA) and compared spraint density in upper (stream order 1-4) and lower (stream order > 4) streams by the Mann-Whitney test using SPSS 18 software (IBM Inc., Chicago, IL, USA).

We then identified the representative fish species according to stream orders. We used clustering techniques (non-metric multidimensional scaling, NMDS) to analyse fish communities (MOE/NIER 2014) at each of the 16 selected sites. We used the vegan package of the software R v. 3.2.3 (Oksanen et al. 2018). We excluded sites where less than ten fish were caught ($n = 17$). We used Bray-Curtis coefficients to ordinate fish fauna association in two dimensions using 20 random starts. We used the function 'stressplot' of the vegan package to draw a Shepard plot in which ordination distances were plotted against community dissimilarities, and the fit was shown as a monotone step line for goodness of fit ($R^2 = 1 - S^2$). To determine the association of fish communities according to stream orders and predict the expected fish species at the sites, we used information on the stream order to represent the community using the ggplot2 package (Wickham 2016). The most representative species in relation to stream order were those identified as highly significant (p

< 0.001) and with a square sum of values of two axes > 0.1 as analysed by NMDS (Fig. 2). We also classified fish as species living in upper (stream order 1-4) or lower streams (stream order > 4) (Table S1). Identified fish items were assigned to upper stream order (U), lower stream order (L), or not caught in the survey (NC) with reference to the entire fish community using NMDS.

Because otter feeding success is influenced by prey swimming traits, we also differentiated between swimming fishes (agile swimmers) and benthic fishes (slow swimmers) (Erlinge 1968). Swimming fishes included the families Cyprinidae, Osmeridae, Centrarchidae, and Mugilidae. Benthic fishes comprised the Cobitidae, Gobiidae, Centropomidae, Odontobutidae, and Amblycipitidae families that mostly live on the riverbed or under stones (Kim and Park 2002). We compared the composition of diet and fish diversity using chi-square tests in SPSS 18 (IBM Inc., Chicago, IL, USA; Zar 1999). In this analysis, the Mugilidae were classified as ocean fish, according to their primary habitat.

Results

Fish communities according to stream orders

Overall, we defined fish communities over the stream orders using 20,791 fish caught at 233 sites (Fig. 2). With stress values below 0.2 (0.19), the axis of NMDS1 clearly differentiated fish communities into two groups, consisting of (i) first, second, third, and fourth orders streams, and (ii) fifth, sixth, and seventh order streams. In addition, we were able to discriminate representative fish species according to stream order (Fig. 2). Chinese minnow (*Rhynchocypris oxycephalus* Sauvage & Dabry de Thiersant, 1874) were representative of first and second order streams, Korean chub (*Zacco koreanus* Kim, Oh & Hosoya, 2005) of third order streams, *Pungtungia herzi* Herzenstein 1892, *Coreoperca herzi* Herzenstein 1896, and *Coreoleuciscus splendidus* Mori 1935 of fourth order streams;

Pseudogobio esocinus Temminck & Schlegel, 1846 of fifth order streams, and *Hemibarbus labeo* Pallas, 1776 and *Opsariichthys uncirostris* Temminck & Schlegel, 1846 of sixth order streams. Furthermore, two invasive species (largemouth bass, *Micropterus salmoides* Lacepède, 1802 and bluegill, *Lepomis macrochirus* Rafinesque, 1819) represented seventh order streams. Representative species of lower-order streams were mostly predators of benthic invertebrates and diatoms (except for *C. herzi* which is piscivorous and normally hides among rocks), while larger and more slowly swimming piscivores predominated in higher-order streams (Kim et al. 2005).

Fish communities at selected sites included 28 species. Most belonged to the families Cyprinidae (18; 64.29%) and Cobitidae (3; 10.71%). Only one species of each of the families Centropomidae, Gobiidae, Odontobutidae, Centrarchidae, Mugilidae, Osmeridae, and Amblycipitidae was collected (Table 2). Ten species (35.71%) were endemic, one was an invasive species (3.57%), and two species (7.14%) were migratory species in brackish streams. One species (*Microphysogobio koreensis* Mori, 1935) was a designated critically endangered species (Ministry of Environment of Korea 2018). The Korean chub (*Z. koreanus*) was the dominant species at all but three sites. The next most dominant species were the Korean dark sleeper (*Odontobutis platycephala* Iwata & Jeon, 1985) and the pale chub (*Zacco platypus* Temminck & Schlegel, 1846; 10/28, 32.14 %).

Otter diet as assessed by DNA barcoding

Spraint densities differed significantly among stream orders ($F = 2.91$, $p < 0.01$, Fig. 1b) and were the highest at second-order streams (40.58 ± 9.06 spraints per km). Spraint densities of upper streams (28.44 ± 3.10) were significantly higher than those of lower streams (11.17 ± 2.29 ; $z = -3.10$, $p < 0.01$).

From the 105 bone samples, we identified 76 prey items (success rate: 72.38 %),

excluding two contaminated samples. We identified animals of 4 classes, 7 orders, 13 families, 28 genera, and 34 species (Table S2). Most identified animals were fish (52, 68.42 %), followed by frogs (20, 26.32 %), mammals (2, 2.63 %), and reptiles (2, 2.63 %). Using the Korean fish species inventory, we identified ten fish, four anurans and one mammal to species-level. Eleven fish were identified to genus level, five to subfamily, two to family, and one to suborder level. Most fish not identified to species level did not live in South Korea (15, 78.95 %) or at the study sites (3, 15.79 %). Most sites provided high rates of identification (more than 60 %) but some sites (N13 (25 %), N16 (44.44 %), and N89 (12.5 %)) did not (Table 2). Of the 29 identified fish prey items, 18 items inhabited upper streams (62.07%, Table 2).

The proportions of the main fish families in the communities of selected sites and otter diet differed significantly ($X^2 = 35.50$, $df = 4$, $p < 0.01$). Across all study sites, swimming fish were dominant in the fauna surveys (1,069; 88.4%) but not significantly less so in the diet (30/52; 57.69%; $X^2 = 2$, $df = 1$, $p > 0.05$). Comparing fish fauna and otter diet, none of the fish species recorded in the faunal survey were identified as diet items at more than half of the sampling sites (62.5 %).

Discussion

Diet identification using faecal analyses has been criticised in the past (Carss and Parkison 1996; Lanszki et al. 2014), particularly due to the lack of geographic correlation of sampling and occurrence of prey species (Clavero et al. 2006; Taastrom and Jacobsen 2006; Sales-Luis et al. 2007). With our study, we addressed this criticism and achieved a relatively high success rate (72.38 %) of species identification from faecal DNA by amplifying mitochondrial 12S rRNA fragments. Because of the difficulty of visually discriminating fish remains on species level, DNA barcoding is the preferable method to identify otter prey

species (Jo et al. 2014). However, both methods are important, as physical examination of prey remnants in faeces can provide additional information such as estimates of fish size inferred from measurements of the jaw bones (Radke et al. 2000; Hajkova et al. 2003).

Prey identification depends on sample quality (Table 2; Sheppard et al. 2004). Amplification of a different fragment may have improved the identification; however, identification resolution remains an issue (Clare et al. 2009). In this study, by combining the results of DNA barcoding with available information on the geographic distribution of fish species, we improved the identification-level (Clare et al. 2009).

Otter have been reported to spraint more frequently at sites where prey items occur at a higher abundance (Kruuk et al. 1992; Remonti et al. 2011). In the Nakdong River basin, however, spraint densities were higher at upper streams (which are inhabited by smaller and fast swimming fishes) than at lower streams. This finding suggests two possibilities; (i) slow or large fish also occur in upper streams but at lower densities than in lower streams. Otters actively select slow fish from upper streams, and mark in the pools where slow fish live (Remonti et al. 2011). (ii) Otters rest on upper, relatively undisturbed stretches, but move nightly downstream to forage in more profitable habitats (Beja 1996). In the second ordered streams where spraint densities were highest, the percentage occurrence of slow or large fishes, which mostly live in the lower streams, was 12.54 % (upper streams: 21.7 %). The percentages were positively correlated with stream orders, but slow or large fishes were found in all stream orders, as reflected by otter foraging. Meanwhile, because otters showed a flexible feeding strategy related to human disturbance, the possibility of flexible habitat use, and otter spatial use in general needs further study using radio telemetry (Clavero et al. 2006; Weinberger et al. 2016).

The foraging flexibility in this study was relatively low, compared with the results of previous studies. The otter is often characterised as an opportunistic forager because its dietary

spectrum widens when preferred fish are not abundant (Clavero et al. 2003; Brzeziński et al. 2006); however, dietary flexibility is limited (Erlinge 1968; Crait et al. 2015). In our study, the small-sized and strong swimming Cyprinidae were the dominant fish family in most of the sampled streams, however, slow and benthic fishes and frogs contributed more to otter diet (Fig. 3). This suggests that otters are partially limited to eating slower swimming fish. Thus, while Cyprinidae were as dominant a food type in our study sites as in Mediterranean habitats, the reduced contribution of Cyprinidae to diet comparing with availability suggests lower-order stretches might not be profitable foraging sites for that prey (Clavero et al. 2003; Remonti et al. 2009).

Additionally, our finding of ocean fish in otter diet at a site (N214) far from the sea (24.2 km linear distance) supports the assumption that the home range of otters expands when suitable prey fish are scarce (Crowley et al. 2013; Lanszki et al. 2014). Although a false-positive identification of ocean fish cannot be ruled out, similar findings have been reported previously for otters (Mulville 2015) and other small and medium-sized carnivores (Doncaster and Woodroffe 1993; Eide et al. 2004). The fish species characteristics used for NMDS classified 37.93 % of the eaten items as living in higher-order streams, although 93.75% of sampling sites were located on lower-order streams. This suggests the large home ranges of otters reflect the need to forage widely or selective foraging for slow or larger fish living in lower-order streams.

More detailed diet information on otter diet using DNA barcoding and extensive prey sampling will help understand the relationships between food availability and otter distribution. The comparison of otter diet, as assessed by DNA barcoding and food availability could therefore be a useful approach for defining the suitability of river catchments for otters which, in turn, would be a useful aid to the identification of sites for preservation for natural recolonization or reintroduction.

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386 **Figure Legends**

387 Fig. 1. (a) Location of the 16 spraint collection sites (thicker black outline circles) out of the
388 250 surveyed sites in the Nakdong River basin. Circle sizes indicate the sizes of otter spraint
389 densities (index of population density) indicated on a land cover map (from black (urban) to
390 white (water) gradient). (b) Spraint densities (Average $\pm$ S.E.) of stream orders. Thicker
391 black outlined bars represent stream orders including 16 selected study sites.

392 Fig. 2. Fish communities according to stream orders (1st to 7th) using 233 sites in the
393 Nakdong River basin. Representative fish species of each order are indicated by black
394 arrows. The 233 sites clustered by stream orders with 95% confidence. The clusters are
395 represented by each number and circle with different colours (from 1st (black) to 7th (white)
396 gradient).

397 Fig. 3 Comparison of fauna of fish families recorded in river surveys and fish families eaten
398 by otters. (a) Percentages of prey groups eaten identified by DNA barcoding, (b) Total fish
399 families consumed, and (c) Fish fauna identified to family level.

400

401 Table 1. Comparison of aquatic health and environmental variables (Mean $\pm$ S.E) between
 402 sites for diet analysis ($n=16$) and all sites surveyed along Nakdong River ($n=250$).

Environmental variables	Selected sites	All sites
Spraint densities (per km)	26.57 $\pm$ 7.13	24.50 $\pm$ 2.49
No. Fish individuals	73 $\pm$ 7	81 $\pm$ 5
Human densities (no. human individuals per km ²)	3000 $\pm$ 1556	11819 $\pm$ 1870
Distance to forest (m)	111.91 $\pm$ 20.81	181.00 $\pm$ 9.99
Altitude (m)	204.5 $\pm$ 36.85	124.58 $\pm$ 8.41
BMI, Benthic Macro-Invertebrate Index	74.08 $\pm$ 4.38	68.91 $\pm$ 1.34
FAI, Fish Assessment Index	60.58 $\pm$ 4.18	55.15 $\pm$ 1.37

404 **Table 2.** Fish fauna at the study sites. Bold font indicates fish at sites found in both fish fauna and fish diet.

Sites	Upper streams connected to main Nakdong Rivers									Short tributaries near East Sea						
	91	94	12	14	15	90	13	16	89	222	218	221	215	217	213	214
Cephalaspidomorphi																
Cypriniformes																
Cobitidae																
<i>Cobitis hankugensis</i>	10				6			6	9				3			
<i>Misgurnus anguillicaudatus</i>	11											2			2	
<i>Niwaella multifasciata</i>				1												
Cyprinidae																
<i>Squalidus gracilis majimae</i>					7	10			21							
<i>Opsarichthys uncistrostris</i>		2														
<i>amurensis</i>																
<i>Pungtungia herzi</i>	2				12		7	7	3	6				6		
<i>Microphysogobio yaluensis</i>				7	9			9	10							
<i>Pseudogobio esocinus</i>				4												
<i>Microphysogobio koreensis</i>				3												

<i>Rhynchocypris oxycephalus</i>			17			7			1					43	17
<i>Carassius auratus</i>	1	15			2			3	2		2				
<i>Coreoleuciscus splendidus</i>				1							9				
<i>Cyprinus carpio</i>		1													
<i>Squalidus multimaculatus</i>											16	9		27	
<i>Zacco koreanus</i>	35		36	38	28	22	38	34	37				78	8	34 39 15
<i>Hemibarbus longirostris</i>				9											
<i>Squalidus chankaensis tsuchigae</i>		1						2							
<i>Pseudorasbora parva</i>									9					35	
<i>Hemiculter eigenmanni</i>														42	
<i>Acheilognathus koreensis</i>				9	1										
<i>Zacco platypus</i>	27	39		22	6			12	5	77			12	3	
Perciformes															
Centropomidae															
<i>Coreoperca herzi</i>				3										7	
Periophthalmidae															
<i>Chaenogobius urotaenia</i>												4			

Odontobutidae										
<i>Odontobutis platycephala</i>	11	1	2	6	3	3	3	12	4	
Centrarchidae										
<i>Micropterus salmoides</i>	1									
Mugilidae										
<i>Mugil cephalus</i>								17		
Osmeridae										
<i>Plecoglossus altivelis altivelis</i>							21	8		
Amblycipitidae										
<i>Liobagrus mediadiposalis</i>									2	

405

406 **Table 3.** Identified items using DNA barcoding. When identification level to species, the identified species were categorised as from upper
 407 streams (U), lower streams (L), or un-caught (NC) by reference to the entire fish community association in the Nakdong River using NMDS
 408 (Table S1).

Sites	Sprints	Samples	Gene	AC	Score	Match	Pct (%)	Identification level (U, L, NC)
N12	1	1	<i>Misgurnus anguillicaudatus</i>	KC881110.1	643	384	96	Species (U)
		2	<i>Koreocobitis nakdongensis</i>	HM535625.1	723	400	99	Species (NC)
		3	-	-	-	-	-	-
		4	<i>Misgurnus anguillicaudatus</i>	KC881110.1	691	399	97	Species (U)
		5	-	-	-	-	-	-
N13	1	1	-	-	-	-	-	-
		2	-	-	-	-	-	-
		3	<i>Rana rugosa</i>	AB430346.1	649	379	97	Species
		4	-	-	-	-	-	-
N14	1	1	<i>Odontobutis platycephala</i>	DQ010651.1	682	391	98	Species (U)
		2	<i>Odontobutis platycephala</i>	DQ010651.1	682	391	98	Species (U)
		3	<i>Odontobutis platycephala</i>	DQ010651.1	682	391	98	Species (U)
N15	1	1	<i>Homo sapiens</i>	KF148592.1	710	394	99	-
		2	<i>Rana chosonica</i>	JF730436.1	654	373	98	Genus (Pelophylax)
		3	<i>Carassius auratus</i>	KF147851.1	730	398	99	Species (L)
		4	<i>Leucaspis delineatus</i>	AP009307.1	673	384	98	Subfamily (Leuciscinae)
		5	<i>Misgurnus bipartitus</i>	KF562047.1	174	168	84	Genus (Misgurnus)
	2	1	-	-	-	-	-	-
		2	<i>Zacco platypus</i>	KF683339.1	701	396	98	Species (L)
		3	-	-	-	-	-	-
		4	<i>Bombina orientalis</i>	DQ925753.1	680	390	98	Species
		5	<i>Phoxinus oxycephalus</i>	AB626852.1	669	382	98	Species (U)

N16	1	1	<i>Pelecus cultratus</i>	AB239597.1	675	383	98	Subfamily (Leuciscinae)
		2	<i>Bombina orientalis</i>	DQ925753.1	712	395	99	Species
		3	-	-	-	-	-	-
		4	<i>Abramis brama orientalis</i>	KC894466.1	135	155	80	Subfamily (Leuciscinae)
		5	<i>Belligobio nummifer</i>	KJ413052.1	219	149	91	Subfamily (Gobioninae)
		6	-	-	-	-	-	-
		7	-	-	-	-	-	-
		8	-	-	-	-	-	-
		9	-	-	-	-	-	-
N89	1	1	-	-	-	-	-	-
		2	<i>Rana rugosa</i>	AB430346.1	597	367	95	Species
	2	1	-	-	-	-	-	-
		2	-	-	-	-	-	-
		3	-	-	-	-	-	-
		4	-	-	-	-	-	-
N90	1	1	<i>Cyprinus carpio color</i>	JX188253.1	712	390	99	Species (L)
		2	<i>Cyprinus carpio color</i>	JX188253.1	719	403	99	Species (L)
		3	-	-	-	-	-	-
		4	<i>Odontobutis platycephala</i>	DQ010651.1	652	380	97	Species (U)
N91	1	1	<i>Belligobio nummifer</i>	KJ413052.1	300	179	96	Subfamily (Gobioninae)
		2	<i>Paramisgurnus dabryanus</i>	KJ027397.1	176	167	84	Species
		3	<i>Pseudopungtungia nigra</i>	EU332752.1	582	335	97	Subfamily (Gonioninae)
	2	1	<i>Pelodiscus sinensis</i>	JQ688041.1	739	405	99	Species
		2	<i>Hyla japonica</i>	AB303949.1	274	173	94	Species
		3	<i>Homo sapiens</i>	JX303794.1	141	121	85	-
	3	1	<i>Misgurnus anguillicaudatus</i>	KC881110.1	719	396	99	Species (U)

N94	1	2	<i>Misgurnus anguillicaudatus</i>	KC881110.1	723	398	99	Species (U)
		1	<i>Phoxinus oxycephalus</i>	AB626852.1	704	398	98	Species (U)
		2	<i>Phoxinus perenurus</i>	AP009061.1	303	176	97	Genus (Rhynchocypris)
	2	1	<i>Squalidus argentatus</i>	KF926824.1	686	386	98	Genus (Squalidus)
		2	<i>Pelodiscus sinensis</i>	JQ688041.1	749	410	99	Species
		1	<i>Odontobutis platycephala</i>	DQ010651.1	226	176	88	Species (U)
N213	1	2	<i>Rana chosonica</i>	JF730436.1	664	378	98	Genus (Pelophylax)
		3	<i>Odontobutis obscura</i>	AB095530.2	169	146	85	Genus (Odontobutis)
		4	<i>Odontobutis platycephala</i>	DQ010651.1	315	191	96	Species (U)
	1	5	<i>Eliomys quercinus</i>	Y16896.1	167	106	93	Suborder (Sciuromorpha)
		1	-	-	-	-	-	-
		2	<i>Phoxinus oxycephalus</i>	AB626852.1	582	365	94	Species (U)
	1	3	<i>Leucaspis delineatus</i>	AP009307.1	671	386	97	Subfamily (Leuciscinae)
		4	<i>Zacco platypus</i>	KF683339.1	673	386	97	Species (L)
		5	<i>Phoxinus oxycephalus</i>	AB626852.1	460	281	95	Species (U)
	2	6	<i>Carassius auratus</i>	KF147851.1	673	381	98	Species (L)
		7	<i>Carassius auratus</i>	FJ710918.1	411	242	96	Species (L)
		8	<i>Carassius auratus</i>	KF147851.1	678	392	97	Species (L)
	2	9	<i>Myocastor coypus</i>	AF520669.1	575	341	96	Species
		10	<i>Misgurnus anguillicaudatus</i>	KC884745.1	307	179	97	Species (U)
		1	<i>Odontobutis platycephala</i>	DQ010651.1	675	388	97	Species (U)
	2	2	-	-	-	-	-	-
		3	<i>Rana chosonica</i>	JF730436.1	658	370	98	Genus (Pelophylax)
		4	<i>Zacco platypus</i>	KF683339.1	682	389	98	Species (L)
	2	5	<i>Rana pyrenaica</i>	EU746401.1	150	154	82	Genus (Rana)
		6	-	-	-	-	-	-
		7	<i>Rana chensinensis</i>	KF898356.1	612	375	95	Genus (Rana)
	2	8	<i>Hyla japonica</i>	AB303949.1	664	374	98	Species

		9	<i>Rana nigromaculata</i>	AY322305.1	174	165	83	Species
		10	-	-	-	-	-	-
		11	-	-	-	-	-	-
	3	1	<i>Pristipomoides typus</i>	AY484974.1	734	409	99	Family (Lutjanidae)
		2	<i>Phoxinus oxycephalus</i>	AB626852.1	706	396	99	Species (U)
		3	<i>Misgurnus anguillicaudatus</i>	KC881110.1	712	392	99	Species (U)
		4	<i>Caesio erythrogaster</i>	AY484973.1	723	396	99	Suborder (Percoidei)
		5	<i>Phoxinus perenurus</i>	AP009061.1	313	184	97	Genus (Rhynchocypris)
N215	1	1	<i>Rana rugosa</i>	AB430346.1	649	370	98	Species
		2	<i>Pristipomoides typus</i>	AY484974.1	684	393	97	Family (Lutjanidae)
		3	<i>Rana chensinensis</i>	KF898356.1	614	369	95	Genus (Rana)
		4	<i>Bombina orientalis</i>	DQ925753.1	333	197	97	Species
N217	1	1	<i>Leucaspius delineatus</i>	AP009307.1	688	395	97	Subfamily (Leuciscinae)
N218	1	1	<i>Caesio erythrogaster</i>	AY484973.1	710	403	98	Suborder (Percoidei)
		2	<i>Bombina variegata</i>	JX893180.1	189	132	91	Genus (Bombina)
		3	-	-	-	-	-	-
		4	<i>Bombina variegata</i>	JX893180.1	163	93	98	Genus (Bombina)
		5	-	-	-	-	-	-
	2	1	<i>Platichthys stellatus</i>	EF424428.1	326	181	99	Species
		2	<i>Rana rugosa</i>	AB430346.1	610	374	95	Species
N221	1	1	<i>Silurus soldatovi</i>	AB860299.1	226	169	89	Genus (Silurus)
		2	<i>Belligobio nummifer</i>	KJ413052.1	287	180	94	Subfamily (Gobioninae)
		3	<i>Silurus soldatovi</i>	AB860299.1	303	183	96	Genus (Silurus)
		4	<i>Cobitis striata</i>	AP010782.1	169	157	84	Genus (Cobitis)
N222	1	1	<i>Rana nigromaculata</i>	AF205548.1	387	277	89	Species
		2	<i>Rana nigromaculata</i>	DQ283137.1	651	381	97	Species
		3	<i>Tridentiger obscurus</i>	AB022901.1	106	63	95	Species (L)

Fig. 1. (a) Location of the 16 spraint collection sites (thicker black outline circles) out of the 250 surveyed sites in the Nakdong River basin. Circle sizes indicate the sizes of otter spraint densities (index of population density) indicated on a land cover map (from black (urban) to white (water) gradient). (b) Spraint densities (Average $\pm$ S.E.) of stream orders. Thicker black outlined bars represent stream orders including 16 selected study sites.

416x369mm (300 x 300 DPI)

Fish communities according to stream orders (1st to 7th) using 233 sites in the Nakdong River basin. Representative fish species of each order are indicated by black arrows. The 233 sites clustered by stream orders with 95% confidence. The clusters are represented by each number and circle with different colours (from 1st (black) to 7th (white) gradient).

Comparison of fauna of fish families recorded in river surveys and fish families eaten by otters. (a) Percentages of prey groups eaten identified by DNA barcoding, (b) Total fish families consumed, and (c) Fish fauna identified to family level.

615x145mm (300 x 300 DPI)