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BRIEF COMMUNICATION

Application of DNA metabarcoding on faeces to identify European catfish *Silurus glanis* diet

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In this study, the results of conventional stomach-content analysis are compared with the recent DNA metabarcoding approach on faeces to identify fish species consumed by non-native European catfish *Silurus glanis* in the Garonne River (south-western France), with a special emphasis on anadromous prey. Fourteen prey species were identified in the stomach contents or faeces, including four anadromous fish species. Despite higher intestine than stomach emptiness, more species were identified through faecal analysis (11 of 14) than through stomach-content analysis (five of 14) suggesting that DNA metabarcoding on faeces is an efficient, non-intrusive technique to study the diet of predatory fishes.

Key words: diet; DNA metabarcoding; excrement; predatory fish diet; stomach content analysis.

Understanding the feeding habits of predatory species provides the basis for understanding trophic interactions, managing stocks or conserving sensitive species (Vander Zanden *et al.*, 2000; Constable, 2001). In fisheries science, analysis of stomach contents has long been standard practice for the accurate description of predatory fish diet (Hynes, 1950; Hyslop, 1980). The efficiency of this approach, however, may be limited if predators have empty stomachs (Renones *et al.*, 2002; Vinson & Angradi, 2011). Even when the stomach contains material, identification of prey to the species level may be challenging and biased, because different prey items will be digested at different rates (Legler *et al.*, 2010). Alternative approaches have been developed to improve the accuracy of diet analysis. For instance, fatty-acid and stable-isotope analyses provide a broad picture of energy flow through the food web, but do not give information about species composition (Guest *et al.*, 2009). DNA-based techniques have been used

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to identify prey to the species level for predatory mammals (Gillet *et al.*, 2015) and birds (Wong *et al.*, 2015). For fishes, DNA-based techniques have been used to study diet using stomach or gut contents (Smith *et al.*, 2005; Jo *et al.*, 2013, 2016; Moran *et al.*, 2016) and faeces (Corse *et al.*, 2010; Taguchi *et al.*, 2014) and often provide better resolution than conventional stomach-content analysis.

The European catfish *Silurus glanis* L. 1758 is a large predatory fish known to have a wide dietary spectrum, consuming prey ranging from aquatic invertebrates to terrestrial birds (Copp *et al.*, 2009; Cucherousset *et al.*, 2012). *Silurus glanis* was introduced and has now established self-sustaining populations in many catchments of France (Poulet *et al.*, 2011; Guillerault *et al.*, 2015). Syväranta *et al.* (2009) demonstrated that in the Garonne River (south-western France), the diet of some *S. glanis* individuals mainly comprises anadromous fishes, without detailing the species concerned, which may be relevant for management of highly depleted stocks of anadromous species. Because of autecological factors (*e.g.* nocturnal piscivory), environmental conditions (*e.g.* temperature) or sampling artefacts (*e.g.* regurgitation on capture) (Arrington *et al.*, 2002; Copp *et al.*, 2009; Vinson & Angradi, 2011), *S. glanis* can show high rates of stomach emptiness (Wysujack & Mehner, 2005), which can limit the usefulness of stomach-content analysis. The present study aims to compare the outputs of the conventionally used stomach-content analysis and the recent DNA metabarcoding approach on faeces, in order to test a non-lethal and non-intrusive method to study fish diet, and to identify species consumed by *S. glanis* in the Garonne River, with a special emphasis on anadromous species.

Thirty one *S. glanis* ranging from 125 to 237 cm (mean ± S.D. total length, $L_T = 178 \pm 30$ cm) were caught and handled for faeces and stomach-content collection. They were collected by recreational anglers and commercial inland fishermen using baits, lures or trap nets in a 40 km stretch of the Garonne River located *c.* 210 km from the sea [44° 18′ 50·7″ N; 0° 19′ 56·6″ E; for more details on river characteristics see Syväranta *et al.* (2009)] from 16 March 2013 to 8 July 2013. Stomach contents were collected by manually and gently clearing out the fish stomach towards the mouth with moistened gloved hand. After sample collection angled fish were released back into the river. Stomach contents were identified in the field when prey were easily identifiable, or in the laboratory, through identification of bony structure. Faeces were collected by manually pressing the peritoneal cavity towards the rectum. Faeces were frozen (−20° C) for further examination.

DNA was extracted from 20 mg of faeces using the DNeasy Blood and Tissue Kit (Qiagen; www.qiagen.com) following the manufacturer's protocol in a room dedicated to processing degraded DNA samples. Two negative extraction controls were added and identical molecular analyses were performed upon these negative controls to monitor for possible contamination. DNA amplifications were performed in a final volume of 25 µl, using 3 µl of DNA extract as a template. The amplification mixture contained 1 U of AmpliTaq Gold DNA Polymerase (Applied Biosystems; www.appliedbiosystems.com), 10 mM of Tris-HCl, 50 mM of KCl, 2·5 mM of $MgCl_2$, 0·2 mM of each deoxynucleotide triphosphate (dNTP), 0·2 μM of teleo primers (Valentini *et al.*, 2016) for fish species or 18S_allshorts (Guardiola *et al.*, 2015) for eukaryotes and 0·2 µg µl^{-1} of bovine serum albumin (BSA; Roche Diagnostic; www.roche.com). In the mix containing teleo primers, 4 μM of human blocking primer for teleo primers (Valentini *et al.*, 2016) and 4 μM of *S. glanis* blocking primer (Blk_Sglanis 5′-CTCTCCCCTCCAATAAAAAATTTTATAC-SPC3I-3′) were added.

The primers were 5′ labelled with a unique seven nucleotide tag (with at least three differences between tags) allowing the assignment of sequences to the respective sample during the DNA sequence analysis. The PCR programme was: 95° C for 10 min, followed by 50 cycles of 30 s at 95° C, 30 s at 55° C for teleo primers or 45° C for 18S_allshort and 1 min at 72° C, followed by a final elongation at 72° C for 7 min. Two negative PCR controls (one per primer couple) were analysed in parallel with the samples to monitor for possible contamination during the PCR step. After amplification, the samples were purified, sequenced and the bioinformatics analysis were performed following the protocol described in Valentini *et al.* (2016). The purified PCR products were pooled in equal volumes, to achieve an expected sequencing depth of 10 000 reads per sample. Library preparation and sequencing were performed at Fasteris (www.fasteris.com). Libraries were prepared using the TruSeq Nano DNA genomic kit (Illumina; www.illumina.com) and a paired-end sequencing (2×100 bp) was carried out using an Illumina MiSeq sequencer (Illumina) using the Paired-end MiSeq Reagent Kit V2 (Illumina) following the manufacturer's instructions.

Of the 31 *S. glanis* individuals sampled, 19% did not deliver faeces, 35% did not deliver stomach contents, 48% delivered both materials and 3% delivered no material. Fourteen fish species were identified as prey of *S. glanis* in the Garonne River (Table I). Eleven of these species (including three anadromous species) were identified through faecal DNA metabarcoding. Fifty-one per cent of faecal samples did not allow detection of prey (mostly clear and in low-volume samples). Five species (including one anadromous species) were identified through stomach-content analysis (Table I). Stomach-content analysis outcomes were often restricted to crayfish (*Procambarus clarkii*) and therefore of limited efficiency in identifying prey in comparison with faecal analysis. This result is presumably due to slower digestibility of the crayfish exoskeleton in comparison with fish bones, leading to longer residence of crayfish remains in *S. glanis* stomachs and hence their detection.

Even in this limited sample, the *S. glanis* diet included almost all anadromous species migrating in the Garonne River: Twait shad *Alosa fallax* (Lacépède 1803), thinlip grey mullet *Liza ramada* (Risso 1810), sea lamprey *Petromyzon marinus* L. 1758 and Atlantic salmon *Salmo salar* L. 1758. A possible consumption of sea trout *Salmo trutta* L. 1758 by *S. glanis* cannot be excluded since an undetermined salmonid was detected in faeces. In addition, with the direct identification of prey, DNA metabarcoding provides indirect information about fish diet. For instance, the presence of the eel-specific parasite *Anguillicola crassus* in *S. glanis* faeces suggests the consumption of European eel *Anguilla anguilla* (L. 1758) [the occurrence of *A. anguilla* in *S. glanis* diet (Table I), was inferred by direct identification of *A. anguilla*]. DNA metabarcoding results, however, depend on the exhaustiveness of the barcode reference library of potential prey items.

This study confirms that DNA metabarcoding on faeces is an effective tool for studying fish diet (Carreon-Martinez *et al.*, 2011; Joly *et al.*, 2014; Taguchi *et al.*, 2014), as it greatly increases the number of species detected relative to stomach-content analysis only. Yet, DNA metabarcoding on faeces and stomach content analysis appear complementary, as only two species were detected by both techniques. Based on sampling experience, it is recommended that only thick faeces should be used, thus reducing cost of analysis without decreasing the number of prey detected. Analysis of faeces also has the advantage of being non-intrusive as gently pressing the fish's peritoneal cavity is sufficient to collect fish faeces. Predation by *S. glanis* affects most anadromous

TABLE I. Number of *Silurus glanis* individuals in which prey were found in stomach contents or faeces from March to August 2013 in the Garonne River, France

Prey		Prey identified by	
Common name	Scientific name	Faeces	Stomach contents
Anadromous species			
Twait shad	*Alosa fallax*	1	–
Thinlip mullet	*Liza ramada*	1	–
Sea lamprey	*Petromyzon marinus*	–	1
Atlantic salmon	*Salmo salar*	1	–
Other species			
Bleak	*Alburnus alburnus*	2	1
Common bream	*Abramis brama*	–	1
	Carassius sp.	1	–
Carp	*Cyprinus carpio*	1	–
Ruff	*Gymnocephalus cernuus*	1	–
Topmouth gudgeon	*Pseudorasbora parva*	2	–
Bitterling	*Rhodeus sericeus*	1	–
Roach	*Rutilus rutilus*	2	–
Chub	*Squalius cephalus*	–	2
European eel	*Anguilla anguilla*	2	1
Undetermined			
Crayfish		3	5
Salmonid		1	–
Cyprinid		–	5

species of the Garonne River, which concerns managers seeking to rebuild anadromous species' stocks. Further studies could refine DNA methodologies for faecal material, notably to quantify prey proportions and increasing sample sizes could allow quantification of the effect of predation by *S. glanis* on species of conservation interest.

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