S2 Discrimination between codfish (cod and saithe) using DNA

A major problem in the analysis of gastro-intestinal contents in seals is that fish otoliths are often very eroded making the species identification difficult, particularly between fish species in the same family, in which the otoliths have similar structure.

Saithe probe

The development of a species-specific TaqMan assay for saithe was pursued by extracting DNA from ethanol preserved gill tissue using the commercial kit Omega E-Z 96 Tissue DNA Kit (Omega Bio-Tek). The ATPase gene was amplified in a 10 µl PCR comprising 2 µl DNA, 250 µM dNTPs, 1 X PCR buffer, 0.5 µM of each of the primers 8.2_L8331 and CO3.2_H9236 (Taylor et al. 2002) and 1.5 U of Taq polymerase. The PCR profile was adopted from (Nash et al. 2012). The PCR products were subsequently purified using Illustra (Merck KGaA, Darmstadt; Germany) according to the manufacturer. The following Sanger sequencing was performed on an ABI 3130 XL sequencer (Applied Biosystems), using the BigDye™ Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems). Approximately 6 µl of Illustra treated PCR product was used as template for sequencing both directions of the ATPase gene. The 10 µl sequencing reaction comprised 0.5µl BigDye™ Terminator 3.1 Ready Reaction Mix, 3.2 pmol primer (GAD-F or GAD-RII), 2 µl BigDye™ Terminator v1.1 & v3.1 5X Sequencing Buffer. The sequencing reaction was run on a GeneAmp 9700 PCR system using 28 cycles with the temperature profile recommended by the manufacturer.

The TaqMan-MGB probe for identification of saithe (*Pollachius virens*) was designed using the Primer Express Software (Applied Biosystems) and a BLAST search was performed to ensure the specificity of the probe.

Real-Time PCR assays on otolith DNA

DNA was isolated from otoliths collected in grey seal (*Halichoerus grypus*) stomachs and intestines by using QIAamp DNA micro kit (Qiagen), where we extended the incubation step to 24 hours. The volume of the lysate was adjusted to ensure that the otolith was completely soaked. The final elution volume was set to 20 µl. The PCR was performed using a nested strategy. As the gDNA content in our samples was low (1-5 ng/µl) we performed our analysis in two reactions. In the first PCR, to increase the template DNA, we used the sequencing primers developed by (Taylor et al. 2002) (Table 1) to amplify the approximately 900 bp ATPase gene for the species Atlantic cod (*Gadus morhua*), haddock (*Melanogrammus aeglefinus*), whiting (*Merlangius merlangus*) and saithe. This was done in a 5 µl reaction volume comprising 1X Qiagen multiplex PCR mix, 0.5 µM of each primer and 1 µl of gDNA. For the second PCR (species specific assay) we used the product from the first PCR as a template and added the degenerate primers GAD-F and GAD-RII (Table 1), to amplify a shorter fragment within the ATPase gene, together with the species-specific probes (Table 1) and 1X Quanta PerfeCta QPCR master mix (Quanta BioSciences). The Real-Time PCR assay was analyzed using Roche 480 LigthCycler II (Roche Diagnostics). Positive and negative controls were included in both PCR reactions.

Results

A total of 52 (43.3%) out of 120 otoliths (56 in Nordland and 64 in Finnmark) could reliably be scored in the species-specific assay. Thus, 68 otoliths were classified as “unidentified”, as the assay failed to detect any signal from either of the species included. The classification to species is summarized in Table 2.

Nash, R. D. M., P. J. Wright, I. Matejusova, S. P. Dimitrov, M. O'Sullivan, J. Augley, and H. Hoffle. 2012. Spawning location of Norway pout (*Trisopterus esmarkii* Nilsson) in the North Sea. Ices Journal of Marine Science **69**:1338-1346.

Taylor, M. I., C. Fox, I. Rico, and C. Rico. 2002. Species-specific TaqMan probes for simultaneous identification of (*Gadus morhua* L.), haddock (*Melanogrammus aeglefinus* L.) and whiting (*Merlangius merlangus* L.). Molecular Ecology Notes **2**:599-601.

Table 1. Overview of the sequencing primers and TaqMan probes used in this study.

Primer/probe	**Primer/probe sequence 5' -3'**	**Reporter**	**Quencher**	**Reference**
Sequencing primer				
Forward (8.2_L8331)	AAA GCR TYR GCC TTT TAA GC	N.A	N.A	Taylor et al. 2002
Reverse (CO3.2_H9236)	GTT AGT GGT CAK GGG CTT GGR TC	N.A	N.A	Taylor et al. 2002
TaqMan primer				
GAD-F	GCA ATC GAG TYG TAT CYC TWC AAG GAT	N.A	N.A	Taylor et al. 2002
GAD-R II	GCA AGW AGY GGH GCR CAT TTG TG	N.A	N.A	Nash et al. 2012
Cod	CTT TTT ACC TCT AAA TGT GGG AGG	6-FAM	Non-fluorescent	Taylor et al. 2002
Saithe	CTT TCT CCC CTT AAA TGT AGG	VIC	Non-fluorescent	This study
Haddock	CTT TCT TCC TTT AAA CGT TGG AGG	TET	Non-fluorescent	Taylor et al. 2002
Whiting	GTT TAT YCC TCT AAA CGT AGG AGG	VIC	Non-fluorescent	Taylor et al. 2002

R = AG; Y = CT; K = GT; W =AT; H = AGT

Table 2. The frequency of occurrence for the different species within each region and total estimated from the genetic analysis.

	Finnmark		Nordland		Total	
	n	Frequency	n	Frequency	n	Frequency
Atlantic cod	9	0,36	8	0,30	17	0,33
Saithe	16	0,64	19	0,70	35	0,67
Haddock	0	0	0	0	0	0
Total	25	n.a	27	n.a	52	n.a