**What is eDNA?**

Environmental DNA is the collective term for DNA present freely in the environment which has been shed by organisms (in the form of mucus, faeces, gametes or blood, for example), and can be extracted (Taborlet et al. 2012).

**Atlantic Salmon Salmo salar L.**

The Atlantic salmon (Salmo salar L.) has worldwide ecological, cultural and economic importance. As a result, this species has been the subject of intense exploitation. Although S. salar is protected under Annex II and Annex V of the EU Habitats Directive, and efforts to reduce fishing pressure and restore freshwater habitats have been implemented, this once abundant species has continued to decline (Friedland et al., 2009).

This species is also used for intensive aquaculture outside its native range. Large escapes of S. salar happen with regularity in these areas, causing concerns about the species’ invasive potential (Fisher et al., 2014; Piccolo & Orlikowska, 2012). To adequately address these issues, and to achieve the conservation objectives of the species, it is vital to have knowledge on its distribution.

The aim of this study was to develop an MGB based qPCR assay to detect the presence of S. salar.

**Methods**

Primer Express 3.0 (Applied Biosystems-Roche, Branchburg, NJ) was used to design the species-specific primers and 5’ NED labeled TaqMan® minor groove binding (MGB) probe for S. salar, which targeted the mtDNA COI region.

To ensure species-specificity of the assay, the primers and probe were tested in silico, and in vitro with other fish species.

Three rivers located in the south of Ireland were selected for field validation of the eDNA assay: the Dalligan, Dinin and Burren rivers. Each of these rivers contains an obstacle or barrier, which has the potential to prevent or delay the migration of S. salar (Table 1). Environmental DNA samples were collected upstream and downstream of each obstacle. Following filtration (Fig. 1), the eDNA was extracted, and qPCR was carried out on the samples with the S. salar assay. As brown trout (S. trutta) were present in all rivers, both upstream and downstream of the obstacles, this species was used as a positive field control, to check for the presence of amplifiable DNA in the field samples.

Electrofishing was carried out by Inland Fisheries Ireland upstream and downstream of each obstacle to verify the presence or absence of S. salar at each site.

**Results**

The present assay was successful in detecting S. salar DNA in silico, in vitro and in situ. The assay did not amplify the DNA of closely related species (S. trutta) or any other species included in the specificity test.

The results of the eDNA analysis mirrored what was observed in the electrofishing surveys. At each site where the presence of S. salar was confirmed by electrofishing, its presence was confirmed by eDNA analysis also, and vice versa (Table 1). Detectable eDNA was confirmed at all sites including the sites where no S. salar DNA was detected, as amplification occurred when the same samples were run in qPCR with the S. trutta assay.

**Discussion**

The assay presented here is an efficient and effective method of detecting S. salar in rivers. This assay could be used to identify new conservation areas for the species, and additionally, provide evidence to support remediation action, for example removing river obstacles that may be preventing the migration of the species.

**Table 1. The different combinations of S. salar detection downstream and upstream of the river obstacles listed.**

<table>
<thead>
<tr>
<th>River</th>
<th>Obstacle Type</th>
<th>S. salar detection upstream</th>
<th>S. salar detection downstream</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Electrofishing</td>
<td>eDNA</td>
</tr>
<tr>
<td></td>
<td>Dinin bridge apron</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td></td>
<td>Dalligan bridge apron</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td></td>
<td>Dinin</td>
<td>✓</td>
<td>✔</td>
</tr>
<tr>
<td></td>
<td>Burren</td>
<td>✓</td>
<td>✔</td>
</tr>
</tbody>
</table>

**Figure 1. eDNA filtration being carried out in the field.**

**Figure 2. Graph showing the mean and range (maximum and minimum) of S. salar eDNA concentrations (ng L⁻¹) at each location (downstream (DS) or upstream (US) of the river obstacle) within each river sampled.**

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**References**


