A quantitative PCR based environmental DNA assay for detecting Atlantic salmon (*Salmo salar* L.)

Siobhán Atkinson[1,2], Jeannette E.L. Carlsson[2], Bernard Ball[2], Damian Egan[1], Ken Whelan[1,3], Mary Kelly-Quinn[1], Jens Carlsson[2]

1. *School of Biology & Environmental Science, University College Dublin, Dublin, Ireland*
2. *Area52 Research Group, School of Biology & Environmental Science/Earth Institute, University College Dublin, Dublin, Ireland*
3. *Atlantic Salmon Trust, 11 Rutland Square, Edinburgh, Scotland, UK*

Corresponding author: siobhan.atkinson@ucdconnect.ie

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 (Taberlet et al. 2012).

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

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.*

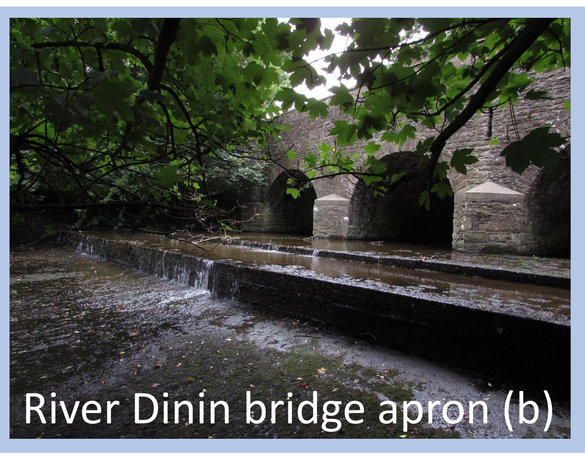
Methods

Primer Express 3.0 (Applied Biosystems-Roche, Branchburg, NJ) was used to design the **species-specific** primers and 5' NED labelled 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.

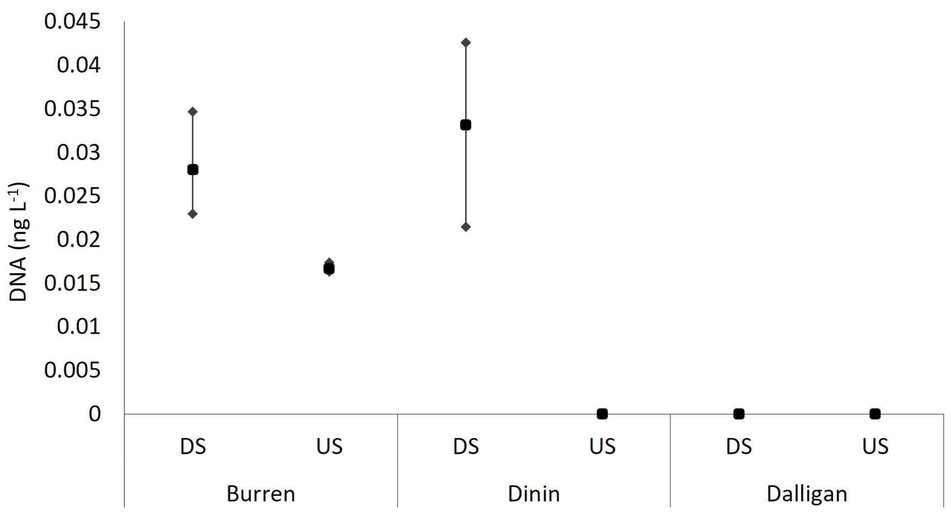


Figure 2. Graph showing the mean and range (maximum and minimum) of *S. salar* eDNA concentrations (ng L^{-1}) at each location (downstream (DS) or upstream (US) of the river obstacle) within each river sampled.

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

River	Obstacle Type	*S. salar* detection Upstream		*S. salar* detection Downstream	
		Electrofishing	eDNA	Electrofishing	eDNA
Burren	Weir	✓	✓	✓	✓
Dalligan	Weir	✗	✗	✗	✗
Dinin	Bridge Apron	✗	✗	✓	✓

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.

References

Fisher, A. C., Volpe, J. P., & Fisher, J. T. (2014). Occupancy dynamics of escaped farmed Atlantic salmon in Canadian Pacific coastal salmon streams: implications for sustained invasions. *Biological Invasions*, *16*(10), 2137–2146.

Friedland, K. D., MacLean, J. C., Hansen, L. P., Peyronnet, A. J., Karlsson, L., Reddin, D. G., … McCarthy, J. L. (2009). The recruitment of Atlantic salmon in Europe. *ICES Journal of Marine Science*, *66*(2), 289–304.

Piccolo, J. J., & Orlikowska, E. H. (2012). A biological risk assessment for an Atlantic salmon (*Salmo salar*) invasion in Alaskan waters. *Aquatic Invasions*, *7*(2), 259–270.

Taberlet, P., Coissac, E., Hajibabaei, M., & Rieseberg, L. H. (2012). Environmental DNA. *Molecular Ecology*, *21*(8), 1789–1793.

Acknowledgements: The authors would like to thank the Atlantic Salmon Trust and the Irish EPA for funding this project. We would also like to thank Inland Fisheries Ireland for carrying out all the electrofishing that supported this research.