International Atlantic Salmon Research Board

SAG(15)3

Joint Application for Funding to the IASRB by the Atlantic Salmon Trust and the School of Biology and Environmental Science, University College Dublin

To investigate the application of eDNA technology in the assessment of pelagic by-catch of Atlantic salmon

APPLICATION FOR FUNDING TO THE INTERNATIONAL ATLANTIC SALMON RESEARCH BOARD

JOINT APPLICATION: ATLANTIC SALMON TRUST AND THE SCHOOL OF BIOLOGY AND ENVIRONMENTAL SCIENCE, UNIVERSITY COLLEGE DUBLIN

To investigate the application of eDNA technology in the assessment of pelagic by-catch of Atlantic salmon

Background

Research has shown that pelagic vessels may inadvertently take a significant by-catch of juvenile Atlantic salmon. In terms of direct protection of migrating post-smolts and adult salmon perhaps the most practical action we can take is to quantify and manage the level of by-catch from the large pelagic fisheries taking place in the North East Atlantic. These fisheries are carried out by fleets from the EU, Iceland, the Faroe Islands, Russia and Norway. They account for the harvest of millions of tonnes of fish a year, mainly comprising mackerel, herring and blue whiting. The fisheries use a range of gear including surface trawls, pair trawls and large purse seines. The quantities of fish taken in each sweep of these very large nets are immense. Locating individual post-smolts, between 12cm and 20cm long, often squashed and descaled, amongst such quantities of fish has proven exceptionally difficult. What has become clear is that over the past two decades these increasingly efficient pelagic fisheries may, at times, have a direct impact on the overall survival of Atlantic salmon at sea.

The SALSEA Programme and the AST's Ocean Silver Conference clearly identified what actions need to be taken to quantify and minimise the impacts of pelagic trawling on salmon smolts. The following key actions have been recommended:

Communication:

- Communicate with the pelagic industry to outline the existence of key migration corridors for Atlantic salmon post-smolts; the need to designate such areas as marine protected areas; to emphasise the need for a new approach and the development of new techniques to identify, and where possible quantify, the presence of salmon in the catch of these pelagic trawlers.

Management of Catch Records:

- The need for improved by-catch records

Research:

- Carefully designed and controlled experiments using commercial fishing vessels to quantify the potential impacts
- Gear studies and post-smolt behaviour studies to minimise the level of by catch by commercial pelagic fleets
- More detailed, temporal information on the relative diets of salmon, mackerel and herring and where overlap occurs

It is clear that manually searching for the bodies of post-smolts in the catches of pelagic vessels is likely to be a very long-term and costly strategy. New and innovative approaches are urgently required. As part of a broader strategy to engage with the EU pelagic fisheries community and to keep them appraised of the latest research on survival at sea and the migration and distribution patterns of salmon at sea, AST has been closely associated with ground breaking research in University College Dublin to develop environmental-DNA or eDNA probes (Gustavson et al. under revision) . The initial pilot research project was funded by Inland Fisheries Ireland and used eDNA probes to identify the presence or absence of spawning sea lamprey (*Petromyzon marinus*) in an Irish river. Water samples were taken from stretches of the river over the spring and summer period and the probes were used to identify the presence of sea lamprey in the area where the water sample was taken. This work has proven highly successful and it is our belief that the techniques developed could be used to test for the presence of Atlantic salmon DNA in the holds of ships or on the pelagic trawls themselves. Work is ongoing at the present time not alone to identify the presence or absence of a range of marine target species but to quantify the mass of animals present in a given quantitative sample. This approach is intended as a surrogate for estimating the overall numerical abundance of the target species present in the samples.

The proposal

AST has developed a three part project proposal to assess the efficacy of eDNA in assessing the level of by-catch in the chosen pelagic fisheries (http://www.atlanticsalmontrust.org/latest-news/pelagic-bycatch-project.html). Given how sophisticated, fast and cost effective genetic screening technology has become, any DNA salmon fragments collected during the study can also be used to broaden our knowledge in relation to the distribution patterns of Atlantic salmon populations across the North Atlantic.

There are three parts to the Project

Proof of Concept

1. **Phase 1:** Pelagic trawler operational processes will be observed and recorded on board a selected pelagic trawler, in an area of the ocean where salmon may be present. An Atlantic salmon specific eDNA probe will be used to test samples taken from a range of locations on board (hold, nets, deck etc) and also, as a control, from inshore boats fishing commercially for salmon.

2. **Phase 2***:* The laboratory preparatory phase will include testing of the Atlantic salmon eDNA probe and analysis of eDNA samples from Phase 1. It will also include: the overall development of techniques for sampling, analysing and recording Atlantic salmon eDNA; preparation of a report on Phases 1 and 2, including a SWOT analysis of the use of the technique and its potential efficacy in monitoring salmon by-catch and distribution at sea of wild salmon populations.

Experimental Programme

3. **Phase 3:** Assuming that the Proof of Concept stage is successful and using protocols established at that stage, design and implement a broadly based programme to sample a range of pelagic trawlers and other pelagic vessels in the North Atlantic. Assess the incidence of by-catch by these vessels using the eDNA protocols developed and tested in the earlier phases of the project. Record a range of data on location, sea conditions, time of year, ocean temperature etc. Identify key locations and time periods where pelagic by-catch is occurring.

As currently envisaged an important outcome of the project will be subsequent discussions with the pelagic fisheries sector on management / avoidance protocols, to eliminate or reduce by- catch of salmon in the North Atlantic. This research will build on earlier work carried out under SALSEA Programme, where migration corridors for post-smolts were identified in the North East Atlantic. Recent experience off the Icelandic coast has shown that pelagic fisheries may also come across the larger, feeding salmon at sea. We believe this technique has the potential to monitor by-catch across wide areas of the ocean and to identify where fleets may be accidentally encountering salmon at all of their marine life stages: post-smolts, feeding salmon and returning adults.

Time Frame

It is envisaged that the Project will take nine months to complete. Assuming a commencement date of 1.7.15, the completion date would be 1.4.16

Timetable

The timetable will be dependent on negotiations and agreement with the Pelagic skippers and the commercial salmon fishermen, who will host the field work. In general, we would hope to have the field work completed by late autumn 2015 and the laboratory analysis complete by the end of February 2016, leaving us the month of March to compile a report for the IASRB.

The Research Team and Funding Required

The research team will comprise Dr Jens Carlsson and his team from the School of Biology and Environmental Science, University College Dublin (http://www.ucd.ie/research/people/biologyenvscience/drjenscarlsson/) and Jeanette Carlsson MSc, in her role as Research Scientist to the project. The team will also be supported by Professor Ken Whelan, Research Director of the Atlantic Salmon Trust and an Adjunct Professor in SBES, UCD (http://www.ucd.ie/bioenvsci/ourstaff/academic/whelanken/).

It is estimated that the initial Phase of the project (elements 1 and 2 above) will require £12,000 in funding. To date AST has raised £6,000 towards these costs from the core AST budget and funding

from supporters. We are seeking a further matching £6,000 from the International Atlantic Salmon Research Board (see indicative budget attached).

While the current application is focused on the use of eDNA probes to address the issue of pelagic by-catch, the technique has the potential to address a far broader range of research and management issues, both in the freshwater and the marine environments and may well become a forensic tool of choice in the years to come.

Scientific Background – eDNA

{Extracts taken from – **Gargan, L. (2104) - Literature Review: Environmental DNA in ecology and evolution – potential and pitfalls. UCD, School of Biology and Environmental Science}**

Effective conservation management depends on knowledge of the distribution of species (Dejean et al. 2012). There is currently a global decline in biodiversity due to many factors including climate change, habitat loss, pollution, over-exploitation of natural resources and invasive species (Hui, 2013). Consequently, there is a need for fast, effective monitoring of threatened species and data to drive conservation actions (Thomsen et al 2012a).

Environmental DNA (eDNA) is the collective term for DNA molecules that are released from living or dead organisms into the environment, which can come from sources as diverse as blood, skin, mucous, sperm, eggs and faeces. Subsequently eDNA can be extracted from an environmental sample such as water, air or soil (Taberlet et al. 2012). Techniques employing eDNA are non-invasive (i.e. do not require the direct observation or sampling of an organism), instead relying on DNA found in the environment as a source of information. As a result, eDNA is rapidly emerging as a valuable tool for biodiversity monitoring, especially where traditional surveying methods (e.g. transect counting, trapping, netting, electrofishing, visual observation, etc.) may not be feasible. For instance, these methods can injure the target species or damage the surrounding environment, and this often conflicts with the reasons for surveying in the first place. In addition, such techniques can be costly, require considerable effort and may be insensitive for cryptic or rare species, as well as requiring specialist knowledge to identify species once they have been seen or sampled (Darling and Mahon 2011, Thomsen et al 2012a). For the reasons outlined above, the non-invasive option of sampling eDNA is becoming an increasingly attractive choice for ecologists.

eDNA Properties and Detection

Where eDNA is to be relied on as a proxy for directly observing or sampling of an organism, an understanding of its properties is important. The DNA found in an environmental sample is often degraded into small fragments (Taberlet et al. 2012). The degradation of eDNA is affected by factors such as temperature, UV radiation, pH, salinity, and endo /exo-nucleases (Pilliod et al. 2013). Variable degradation rates of eDNA have been reported, depending on the taxa and environment being studied. eDNA detection studies have shown that, both under natural and controlled conditions, there is a relationship between animal density and the concentration of DNA molecules, which can be quantified over time (Thomsen et al. 2012a). eDNA is becoming increasingly used by ecologists who wish to capture the DNA of macro-organisms from the environment. For over two decades, this has had a variety of applications in a wide range of fields such as invasion biology (Jerde et al. 2011), for monitoring rare and endangered species (Zhu et al. 2011), for the detection of cryptic species (Piaggio et al. 2014), in the study of diet (Deagle et al. 2013) and paleoecology (Willerslev et al. 2004, Jorgensen et al. 2012).

As methods and technologies give rise to cheaper and more efficient ways to generate and store genetic data from environmental samples, it is likely that more applications will be found.

Detecting species in low numbers

A successful use of eDNA has been for the detection of rare or invasive species, in both terrestrial and aquatic environments. This has been particularly applied to the latter (e.g. Ficetola et al. 2008, Goldberg et al. 2011, Jerde et al. 2011, Dejean et al. 2012) where detection of populations with low numbers is extremely difficult under water and where traditional monitoring techniques may not be sensitive enough to detect low numbers, typical of an incipient invading population or a rare species. eDNA as a tool for the detection of aquatic species was identified as one of 15 topics in a 2013 annual horizon scan focusing on global biodiversity and conservation (Sutherland et al. 2013).

Early detection of potentially harmful organisms

The detection of potentially harmful organisms in ships ballast water is another important application of this technology. Necessary for shipping activity, ballast water is one of the primary vectors for marine alien invasive species into ports. The environmental and economic consequences can be extensive, so whether it is for detection for single toxic dinoflaggelate (Patil et al. 2004), or for identifying the entire species profile contained in a sample from ballast water (Harvey et al. 2009), the use of eDNA can be an inexpensive and efficient method to mitigate the ecological, health and economic impacts of potentially harmful species from ballast water through transoceanic shipping.

Describing Biodiversity

An important application of eDNA is the ability to identify the species composition from an environmental sample, and this has been demonstrated in a number of studies. Thomsen et al. (2012a) both detected and quantified a diverse range of endangered species in freshwater environments using water samples taken from ponds, lakes and streams. eDNA has also been used to detect a diverse array of marine fish fauna from seawater samples (Thomsen et al. 2012b). This was the first study to use eDNA in the marine environment and this not only has implications for biodiversity monitoring and conservation, but also fisheries management. Thomsen et al. (2012b) also investigated the efficiency of the eDNA approach compared to nine different traditional survey methods, and found that eDNA was better or equal to these methods at uncovering the diversity of fish at their study site.

Next Generation Sequencing

High throughput, *Next Generation Sequencing* (NGS) platforms, have made it possible to analyse environmental samples from a variety of habitats including freshwater, marine, and soil. PCR technology combined with NGS, which can produce thousands to millions of sequences for analyses of biodiversity from eDNA, means that highly degraded fragments (eDNA can often be less than 150bp) can be identified from an environmental sample (Herder et al. 2014). New generation platforms are being developed that have the ability to sequence directly individual DNA molecules, without the need for prior PCR amplification and the potential bias inherent to that process (Glenn 2011). These advances could have significant positive impacts in eDNA studies, which rely on PCR technology.

Conclusion

Environmental DNA (eDNA) has the potential to become a valuable tool for biodiversity monitoring, particularly where traditional sampling methods are not feasible. (Pilliod et al. 2013). This is because the eDNA approach does not involve invasively sampling or observing species, but utilising DNA found in the environment as a source of information. However, its widespread application has been hindered to date as

there are many open questions remaining and technical challenges to overcome, including how the detection of eDNA is affected by environmental conditions, as well as field methods and laboratory procedures (Pilliod et al. 2013, Bohmann et al. 2014) and whether it can provide reliable abundance estimates for target organisms in the natural environment. While some researchers have investigated the detection thresholds of eDNA under control and natural conditions, there is a lack of clarity and standardisation for eDNA studies that prevents comparison of results. The extent to which eDNA acts as a proxy for traditional sampling and direct observation is an open research question that will depend on the detectability of DNA molecules and there are many biotic and abiotic factors to consider. However, it is clear that eDNA provides a sensitive and quick method to detect species where little taxonomic knowledge is required, and it can be applied across a broad spectrum of habitat types. The widespread adoption of barcoding and initiatives such as Barcoding Life will facilitate the ability to identify species composition with minimum effort, without the need to invasively sample or observe species thereby reducing the need for trained taxonomists, man-hours in the field, the risk of injury to the organism or damage to the environment. These advantages need to be considered in light of the inherent problems associated with the techniques involved in sampling, processing and analysing eDNA. As other authors have noted (Darling and Mahon 2011, Pilliod et al. 2013, Bohmann et al. 2014) there is a need to set standards for the use of eDNA in order to reduce error being introduced along the pipeline and to facilitate comparison of data among researchers. As Bohmann et al. (2014) point out, it is certainly exciting to think that with future developments a real-time eDNA monitoring system could be realised. The studies highlighted in this review have demonstrated how eDNA can be used to effectively detect biodiversity in an environmental sample. Should eDNA research address the current gaps that have been identified (i.e. reliability of abundance estimates, eDNA detection limits and degradation rates, standardisation of protocols), then this method is likely to become an integral ecological tool and provide data to inform conservation management decisions.

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AST and the Pelagic Fisheries Community

Following AST's Ocean Silver Conference in December 2011, the Trust has concentrated on raising awareness of the potential risk from the expanding pelagic fisheries.

- ***European pelagic trawlers and other commercial vessels***. In the autumns of 2012 to 2014 AST attended the Annual Meeting of the Pelagic Advisory Council (http://www.pelagic-ac.org/) and gave detailed presentations on various aspects of the SALSEA Programme results and on the by-catch issue. In 2014 AST was accompanied by Dr Phil McGinnity who provided the PelAC with an overview of genetic stock identification and how it is used to identify populations of salmon at sea. The Trust also provided to the PelAC recommendations on practical steps which might be taken by the commercial fleets to overcome some of the potential by-catch problems.
- ***EU meeting in Brussels***. An AST delegation also met with DG Mara and DG Environment in 2012 to brief them on the results of the SALSEA Programme and to make them aware of the problems which may be associated with the expansion of pelagic fisheries across the North Atlantic.
- ***AST is a full member of the Pelagic Advisory Council and of its Executive Committee.*** AST attends meetings of both the PelAC Executive Committee and the relevant Working Groups.

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Phases 1 and 2 - Budget Proposal eDNA Atlantic Salmon

LAB COSTS		
Consumables		£
Taqman kit x 2		
Qiagen Dneasy kit (50 rxn)		
Qia chredder		
Micro amp PCR plates		
Primers		
Probes		
Sequencing		
Taq		
General comsumables, plasticware, tips, gloves, tubes etc.		
Filters		
	Sum:	1800.00
	VAT (23%)	414.00
	Sum including VAT:	2214.00
Lab Work		
Staff Time - Research Scientist and Supervision (6 weeks)		5000.00
	TOTAL:	7214.00

FIELD COSTS (over two weeks)	
Field Work	£
Travel	500.00
Accommodation	200.00
Subsistence	100.00
Research Assistant and Supervision	1000.00
TOTAL:	1800.00

Total Lab and Field Costs	**9,014.00**
University Overheads	**2704.00**

Total Project Cost (£): **11,718.00**