Projects of Interest to the Board and its Work

Purpose

The purpose of this paper is to provide an update on projects of interest to the Board and its work, namely the Likely Suspects Framework, the ROAM Programme, the SMOLTrack projects and the PINKTrack project.

Decisions

- no decisions are required.

Background

At the 2020 Annual Meeting of the International Atlantic Salmon Research Board (the Board), ICR(20)16, it was agreed that an Agenda item would be retained in future years that focused on projects of interest to the Board and its work. This would include information on projects where NASCO has some ownership (such as EU-funded projects and the Likely Suspects Framework) and other relevant projects; those researchers could be invited to contribute information. The Board asked the Secretary to provide updates on projects where NASCO has some ownership and, through the Board and SAG members, to seek information on projects that would be of interest to the Board and its work.

This paper contains the information provided in response to the Secretariat's request for information in relation to projects of interest to the Board.

LIKELY SUSPECTS FRAMEWORK PROGRAMME

Background

At its 16th Annual Meeting (2017) the Board agreed to part-fund a workshop on The Likely Suspects Framework (LSF) concept. An Atlantic Salmon Trust (AST) workshop in 2017 developed the concept and the proceedings were published in the AST Blue Book series.

The 35th Annual Meeting of NASCO (2018) endorsed the LSF concept and agreed to request the Secretary to explore with ICES how best to integrate Atlantic salmon marine survival and population data with relevant ICES marine databases, and to suggest that a number of joint workshops might be convened. The first of these workshops (WKSalmon) was held in June 2019 with the second completed in 2022.

The LSF project has been developed by the UK's Missing Salmon Alliance[1] since 2019, and since 2021 has an agreed five-year implementation plan to:

- provide and mobilise new knowledge on the drivers of salmon mortality from across the full life-cycle in sea and freshwater; and
- generate new stock forecasting and scenario-testing capacities to support salmon managers' appraisal of options and guide their activities.

[1] The Missing Salmon Alliance brings together leading salmon conservation organisations across the UK -the Atlantic Salmon Trust, Game & Wildlife Conservation Trust, Fisheries Management Scotland, The Rivers Trust and the Angling Trust. It aims to reverse the devastating collapse in wild salmon populations around the UK. By combining expertise, coordinating activities and advocating effective management solutions MSA is focused on ensuring that wild Atlantic salmon populations survive and thrive in rivers, lochs and seas for the next generation.

Over the last four years, reports were delivered to the Board outlining project progress. The current report provides an update on LSF project progress over the past 12 months.

Progress in 2024

Supporting co-operative research and the ecosystems-based approach for Atlantic salmon was assisted in 2024 by publication of primary scientific articles from the IYS Symposium on salmon risk assessment frameworks in the North Pacific Anadromous Fish Commission Bulletin 7 (Bull and Luedke 2024), and challenges for data mobilisation in salmon management (Diack *et al.* 2024) . In 2024, presentations by members of the LSF team were made at the World Fisheries Congress meeting (Seattle, USA), The Fisheries Society of the British Isles Annual Conference, Sustaining Arctic Observing Networks, (Anchorage, USA), Marine Alliance for Science and Technology for Scotland Annual Science Meeting and Advances in Marine Ecosystem Modelling Research meeting.

Researching the drivers of salmon mortality at sea has advanced with the discovery of new links between salmon marine return rates and changing zooplankton energy (Tyldesley *et al.* (2024) and the development of individual-based models for the initial months of post-smolt migration from a range of UK and Irish populations routes taken by post smolts (Borland *et al.* 2024). Both have been published in the ICES Journal of Marine Science and enable more focused understanding and evaluation of regionally appropriate conditions and pressures facing migrating salmon at sea. A new analysis of the possible impact of changing water temperatures during smolt transition from freshwater to marine has been submitted to Journal of Fish Biology (Tyldesley *et al.* submitted). The international database for sharing salmonid PIT (Passive Integrated Transponder) tagging records has now recorded four PIT-tagged salmon smolts from French and Norwegian rivers in landings from commercial fishing vessels, providing new marine migration route knowledge.

During 2024 progress was made by the LSF team in collaboration with others to work up the commercial pelagic fish landings data from the 2022 NASCO-ICES WKSalmon2 workshop. Funding received from The Fishmongers Company enabled this to be finalised with a report provided to ICES WGNAS annual meeting (ICES 2025 in prep) and a paper outlining the findings to be submitted for publication (Borland *et al.* in prep).

Development of an underlying salmon mortality framework and provision of a Salmon Management Decision Support Tool has advanced in 2024 by finalising a stage and state responsive population model for the River Bush (NI) salmon population (Sivel *et al.* submitted to Fisheries Management and Ecology). The base model is now being developed as a priority workstream in 2025 to utilise regional signals from both the freshwater and marine phases and reflect the range of salmon life histories and influences on growth and survival. The decision support tool has advanced in line with the development of the underlying model in 2024 to provide new support for salmon managers and scenario-testing capacity. The tool shall continue to be developed and made available to the public in the coming year.

ROAM APPROACH TO MARINE TRACKING

RAFOS Ocean Acoustic Monitoring (ROAM) is an acoustic tracking system where low frequency long ranging sound wave ‘pongs’ are emitted from ocean moored sound sources and received by a tag equipped with a hydrophone attached to the study animal. A primary advantage of the ROAM approach is the long range of the ‘pongs’, which could result in more accurate geolocation over a wider spatial and temporal range compared to traditional light-based methods. The development of the ROAM approach to marine tracking is being led by researchers at the Woods Hole Oceanographic Institute (WHOI) and the University of Rhode Island, USA.

ROAM was first brought to the attention of the Board in 2017 (CNL(17)9) and annual updates have been provided since (ICR(18)06; CNL(19)09; ICR(20)16; CNL(21)12; CNL(22)10; CNL(23)10; CNL(24)08). In addition, a workshop involving researchers from the North Atlantic and Pacific oceans was held in 2018, which provided a detailed overview of the ROAM approach (https://repository.library.noaa.gov/view/noaa/22044). ROAM is a re-design and re-purposing of a common oceanographic monitoring technique. As such, efforts have been focused on evolving the monitoring approach to be suitable for tracking marine animals in the ocean and on conducting field trials to verify that the equipment performs as expected. Unfortunately, the project has experienced numerous delays associated and caused by the COVID-19 pandemic, equipment / supply shortages, equipment failure and a lack of dedicated funding.

Field trials were conducted in 2021, 2022 and 2024. The trials were largely unsuccessful due to a variety of issues unrelated to the functionality of the ROAM approach. However, the preliminary data that were obtained from those efforts were encouraging. In spite of these setbacks, all collaborators remained keen on the project and progress continues. As such, interest remained high and researchers continue to pursue the field-testing of the approach so that informed decisions can be made on the next step.

In 2023, a project proposal to Canada First Research Excellence Fund (CFREF), which included a ROAM subcomponent, was selected for funding. The project proposal contains two phases, with Phase II's funding being contingent on the successful completion of Phase I. Phase I activities are focused on further chip development and field testing. The ROAM chip will be redesigned to bring it in line with contemporary chip manufacturing protocols while also incorporating the ability for multi-frequency monitoring and detection. This will allow for the chip to be commercially produced while also being able to detect 'pongs' of different frequencies. Generally speaking, low frequency 'pongs' provide increased accuracy whereas high frequency 'pongs' helps to increase detection range.

Field trials are scheduled to occur in 2027 in the Pacific Ocean. Field trials will be combined with a fully funded oceanographic monitor survey, which will be utilising ROAM sound sources in support of carbon sensing and monitoring project. Although the testing will be 'piggybacked' onto this survey, the fact that the survey is fully funded and is utilising ROAM sound sources provides a unique and cost-effective opportunity for a 'dedicated' field trial for the ROAM tags. The field testing will likely focus on dedicated glider missions, although detailed plans are still being developed.

Despite the various setbacks noted above, interest still remains high in further testing and developing the ROAM approach to aquatic animal tracking. If ROAM approach performs as expected, it could be used to monitor the movements of numerous aquatic species. However the primary focus remains on developing a tracking system suitable for smolt and adult Atlantic salmon.

SMOLTRACK PROJECTS

The EU has provided funding to the Board to support the following SMOLTrack projects:

- Understanding and comparing early mortality of European salmon populations at sea (SMOLTrack I – completed);
- Comparing mortality of European salmon populations at sea using multiple-method telemetry studies (SMOLTrack II – completed);
- Quantifying smolt survival from source to sea: informing management to optimise returns (SMOLTrack III – completed);

- Quantifying salmon survival from river exit to return as adult: Collecting thermal and behavioural data to refine smolt to adult survival indices (SMOLTrack IV – completed); and
- Studies of behaviour and survival of salmon during their migration – development of tagging programmes to follow the marine migration of salmon to oceanic feeding areas and back (SMOLTrack V – ongoing. Update below).

The website for the projects is: SMOLTRACK. There is also information on the Board website.

SMOLTRACK V: Studies of behaviour and survival of salmon during their migration – development of tagging programmes to follow the marine migration of salmon to oceanic feeding areas and back.

Recent marine survival estimates for Atlantic salmon are amongst the lowest recorded in decades and indicate that as little as 3% of wild smolts now survive to return as adults. In recent decades, survival at sea has widely been accepted as the key determinant of river stock abundance. However, recent research conducted in Denmark, England, Ireland, Portugal, Spain, Sweden and Northern Ireland through the EU-funded SMOLTrack and related initiatives, have indicated that smolt mortality during the early outward migratory phase from 'source to sea' is much higher than previously assumed. Thus, early smolt mortality may be more important than marine mortality. To support this hypothesis, various studies have demonstrated that a reduction in negative pressures (e.g. predation, aquaculture) on smolts in the relevant zone of influence can ultimately boost associated numbers of adult returns.

Therefore, it has become apparent that significant knowledge gaps remain to understand, quantify and partition the principal cumulative factors responsible for Atlantic salmon smolt survival during this critical life stage. In addition to the high mortality for smolts and post-smolts, there is also a significant mortality in the oceanic phase of Atlantic salmon.

It is important to investigate if any part of this mortality may be managed. The SMOLTrack V project is building on the work of the previous SMOLTrack projects, enabling studies of behaviour and survival rates of salmon during their migration through the lower parts of rivers, estuaries and coastal areas. Thus, the project will provide data on smolt run timing and migration behaviour, as well as generate ocean migration data through tagging of smolt with nano-DSTs (Data Storage Tags). SMOLTrack V will further expand on the development of a 'fit for purpose tagging programme' to be able to follow the return migration of salmon from feeding areas in the Arctic Sea (East Greenland).

NASCO staff are involved in the administration and co-ordination of each work package (WP) in SMOLTrack V. A 12-month extension to the timeline of the project has been secured by NASCO, moving the end date to April 2026, to enable a further expedition to East Greenland to take place in 2025.

WP1: Tagging in East Greenland

Objective 1

WP1 is testing a 'fit for purpose tagging program' developed in SMOLTrack-IV, to be able to follow the return migration of salmon from the feeding areas in the North Atlantic and Arctic Sea.

Background

The WP targets salmon in East Greenland, catching live salmon and establishing holding facilities to enable genetic assignment before subsequent tagging and release. This requires a quick genetic assignment and the project aims to establish ways to accomplish this. Such a setup could be of great value for many salmon studies.

Key findings

A third ‘expedition’ to East Greenland took place in the summer of 2024 and a further expedition is planned for 2025. The 2024 expedition was successful in capturing, sampling and tagging Atlantic salmon in eastern Greenland. Nets and trolling were found to be effective capture methods, while longlines were not found to be effective. The use of the trolling technique was found to make it possible to hold, tag and release adults. Researchers were able to hold Atlantic salmon in live wells for extended periods of time. This creates future opportunities to genetically assign salmon and tag them according to the facilities available in their home rivers.

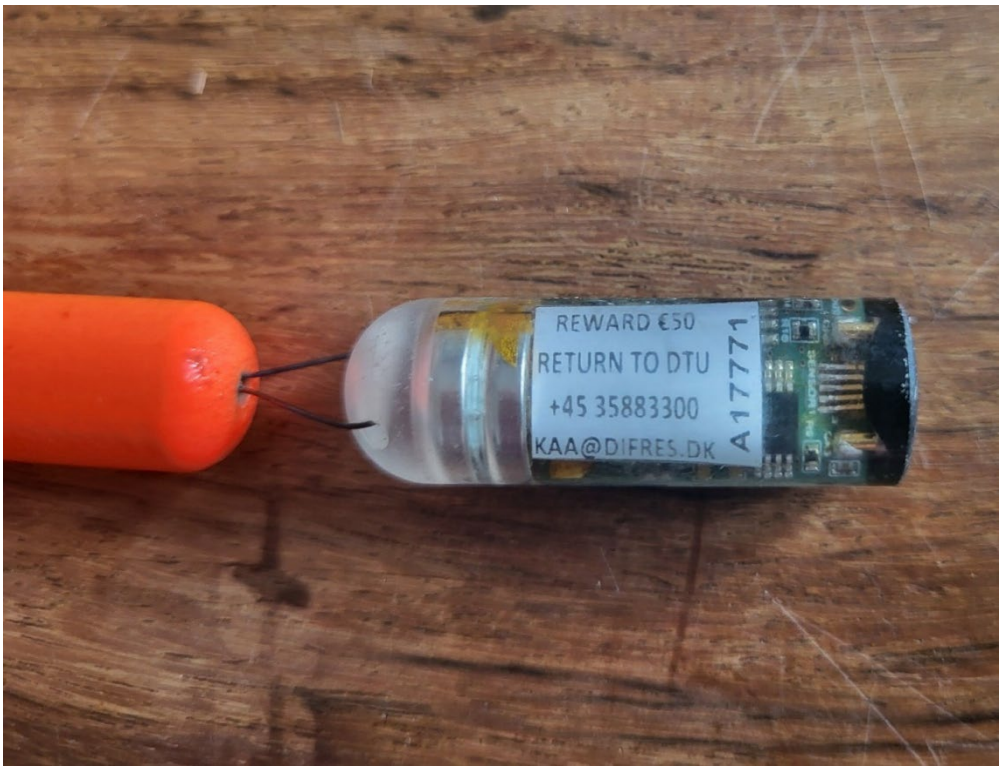

Figure 1. *Returned tag, Scotland July 2024.*

WP2: Full marine migration temperature of salmon smolts, gender dependant behaviour, smolt-runs in River Minho and test of impacts of handling, tagging

Objective 2.i

The first objective of WP2 is to continue the effort to obtain several full marine migration temperature datasets from salmon. Few data are available due to the small size of salmon smolts which, to date, could not accommodate internal implantation of temperature logger tags which typically exceed the tag burden smolts can tolerate. Recent advances in logger technology have resulted in increased miniaturisation and the opportunity to collect key thermal data for migrating salmon smolt and returning adults.

Background

The project aimed to tag 200 outgoing smolts in the River Bush in Northern Ireland and River Erriff in Ireland with miniature Data Storage Tags (DST) that record temperature.

Figure 2. *Location of River Bush in Northern Ireland and River Erriff in Ireland.*

The goals of the study were to get as many adult returns as possible (rendering full-datasets upon retrieval) and record the range of temperatures that the migrating salmon, from smolt stage to adult river return, experienced over their full oceanic migration.

Outgoing smolts implanted with DST were a minimum > 12.5 cm fork length. The two river sites have full trapping facilities for both out-migrating smolts and returning adults. This allowed for removal and processing of returned tagged fish. Smolts were also implanted with a PIT tag to trigger DST tagged fish removal at each trap site.

Figure 3. *An Atlantic salmon smolt.*

Figure 4. *A miniature Data Storage Tag, a Star-Oddi NanoT DST.*

Smolts were captured with a Wolf trap in the River Bush and with a screw trap in the River Erriff. Adult returns were captured by upstream trap, commercial fishery and angler catch.

Key findings

Tagging was conducted from 22 April – 13 May 2024 for the Bush & Erriff Rivers, following on from previous tagging conducted in April – May in the years 2021 – 2023.

Table 1. *DST Tagging Biometrics*

	River Erriff		River Bush	
Year	**n**	**Mean Fork Length (±S.D.) (cm)**	**n**	**Mean Fork Length (±S.D.) (cm)**
2021	100	13.6 (±0.7)	99	16.2 (±1.2)
2022	100	13.1 (±0.6)	100	16.5 (±0.9)
2023	84	13.0 (±0.6)	116	16.1 (±1.1)
2024	70	13.7 (±0.7)	130	16.2 (±0.7)
TOTAL	**354**		**445**	

So far, one adult salmon has been recaptured, providing the first dataset of a full marine cycle. The poor adult returns to date show the challenge of current poor marine survival.

The collection of high-quality data has shown that the study design worked and can be further refined. The tag is effective and it is recommended that the tag burden needs to be approximately 4.5%. The minimum size of V7-4L tags should be 40 g, and the minimum size of nano tags should be 30 g. Tagging activity should target the early-peak migration period.

In the future the project aims to maintain 'index' smolt survival time-series for the Rivers Bush and Erriff. The existing facilities at tagging and return sites can continue to be used.

The studies have also provided important insight on how to optimize the return of tagged adult salmon. Plans are underway for a future tagging-retention study utilizing dummy DST-tags to test different insertion sites for optimal retention.

Kennedy *et al*. (2023) showed that by activating and deactivating acoustics tags, it is possible to detect both seaward smolt migration and adult salmon returning from the sea. This method may increase our understanding of adult return and survival without any additional cost.

Objective 2.ii

The second objective of WP2 is to increase knowledge of smolt run size and behaviour data for the most Southern salmon populations in the River Mouro (Portuguese Minho River Basin). This will be accomplished with smolt-trapping and PIT tagging.

Background

The aim of this part of WP2 was to record the range of temperatures that migrating salmon, from smolt stage to adult river return, experience over their full oceanic migration. Few data are available due to the small size of salmon smolts which, to date, could not accommodate internal implantation of temperature logger tags which typically exceed the tag burden smolts can tolerate. Recent advances in logger technology have resulted in increased miniaturisation and the opportunity to collect key thermal data for migrating salmon smolt and returning adults.

Key findings

A rotary screw trap was visited daily between 1 March to 31 May in 2024. Biometric measures were taken (tail length and width). Tissue samples were collected for genetic analyses, and scales. Tagging with PIT tags was conducted for potential recapture.

Figure 5. *Rotary screw trap on the River Mouro.*

A total of 29 smolts were captured in 29, with a migration peak in April. Results were highly affected by permanent floods and significant damage to the rotary screw trap, which is being repaired for 2025 monitoring.

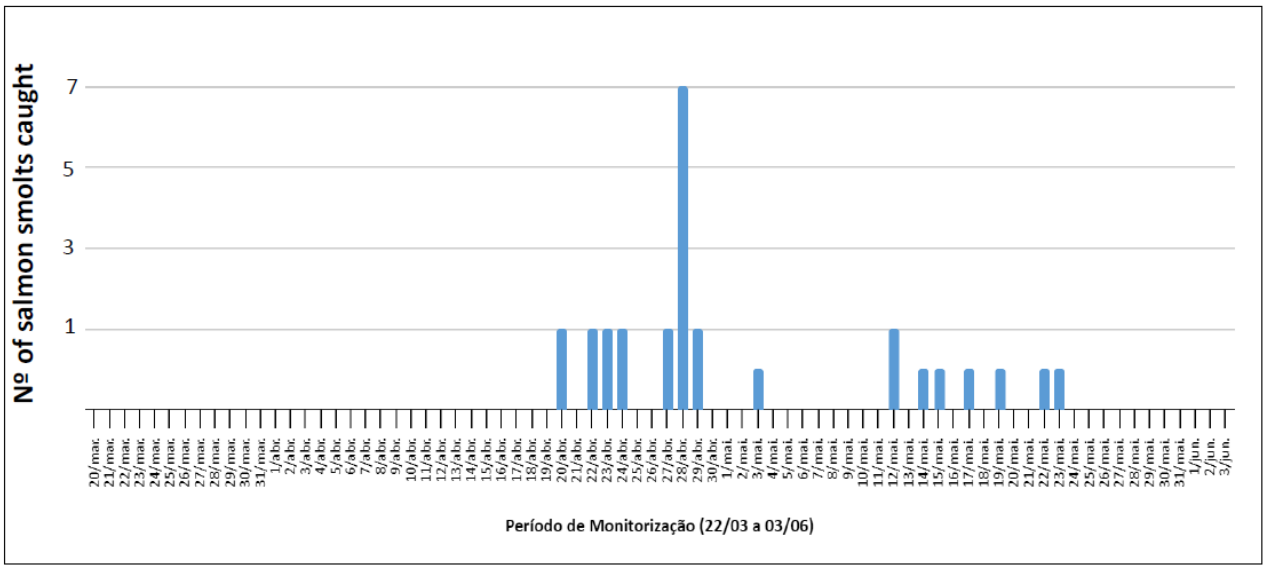

Figure 6. *Salmon smolts caught in 2024 in the River Mouro rotary screw trap.*

Several next steps are recommended to continue increasing the knowledge about Atlantic salmon populations in the most southern part of their range. The recommended next steps are:

- continue monitoring of salmon smolt migration to understand the patterns of smolt production in the River Mouro;
- continue to evaluate the relationship between environmental factors with smolt migration (e.g. flow, temperature, rainfall and photoperiod);
- analyse temperature data loggers operating in Minho and Lima river basins; and
- proceed to study salmon parr and smolt age, through scale reading.

Objective 2.iii

The third objective of WP2 is to investigate if there is sex-based differences in smolt outmigration survival and / or in return rates of 1SW and MSW.

Background

The aim of this part of WP2 was to record the genders of migrating salmon, from smolt stage to adult river return. Length and sex are both factors that affect survival. Few data are available due to the small size of salmon smolts which, to date, could not accommodate internal implantation of temperature logger tags which typically exceed the tag burden smolts can tolerate. Recent advances in logger technology have resulted in increased miniaturisation and the opportunity to collect key data for migrating salmon smolt and returning adults.

Out-migrating smolts will be captured and tagged. Their sex will be determined by genetic analyses of a tissue sample. The project aim is to have 100 smolts gender-determined and tagged in River Bush or River Erriff and a (much) higher number sex assigned and PIT-tagged to evaluate return rates.

Key findings

The study is concluded. The results of this work are not available at the date of submission.

Objective 2.iv

The fourth objective of WP2 is to ensure that overall results are not significantly impacted by the effects of capture, handling and tagging.

Background

This work consists of a series of parallel laboratory / field studies on the performance of smolts, with different treatment groups in terms of capture, handling and holding. The hypotheses used were:

1. Slower initial progression in trapped smolts, then similar to comparator;
2. Similar nocturnal migration; and
3. Lower survival in trapped smolts.

Two rivers were used in the study, the River Ballycastle in Northern Ireland and the River Skjern in Denmark.

Key findings

For the comparator group, 152 smolt were electrofished and tagged about two weeks before migration, giving time to recover. For the trapped smolts a total of 366 individuals were trapped during migration (Table 2).

Table 2. *Information on numbers and biometrics of smolts tagged in River Skjern (2020 and 2022) and River Ballycastle (2022).*

River (year)	Treatment	Release date	Re-captured	*N*	Mean length mm	Mean weight g	Mean condition	Mean tag burden %
Skjern (2020)	Comparator	01.04	0	29	173.5 (10.6)	47.6 (8.1)	0.91 (0.06)	4.0 (0.7)
Skjern (2020)	Trapped	16.04 and 20.04	0	51	168.7 (12.4)	37.1 (8.6)	0.76 (0.06)	5.1 (1.1)
Skjern (2022)	Comparator	14.03 and 21.03	13	97	157.9 (9.7)	36.0 (6.8)	0.91 (0.05)	4.6 (0.7)
Skjern (2022)	Trapped	06.04-03.05	9	279	155.7 (11.2)	31.4 (7.3)	0.82 (0.04)	5.1 (1.0)
Ballycastle (2022)	Comparator	24.03	16	26	146.4 (8.5)	35.9 (6.7)	1.13 (0.14)	5.2 (0.8)
Ballycastle (2022)	Trapped	16-26.04	4	36	161.1 (9.8)	44.3 (7.0)	1.06 (0.14)	4.2 (0.7)

Note: Recaptured indicates the number of smolts that were captured twice, and N represents the final sample size (excluding recaptures) used for data analysis. Numbers represent mean average with standard deviation in brackets. Length represents the total length (TL) of smolt. All comparator smolts were captured by electrofishing, while trapped smolts were captured by rotary screw traps.

The study found no significant difference in descent trajectories between the 'comparator' group of smolts with pre-migration tagging and the 'trapped' smolts, in both Denmark and Northern Ireland, once migration was initiated (Figure 7). No significant difference was found between day and night.

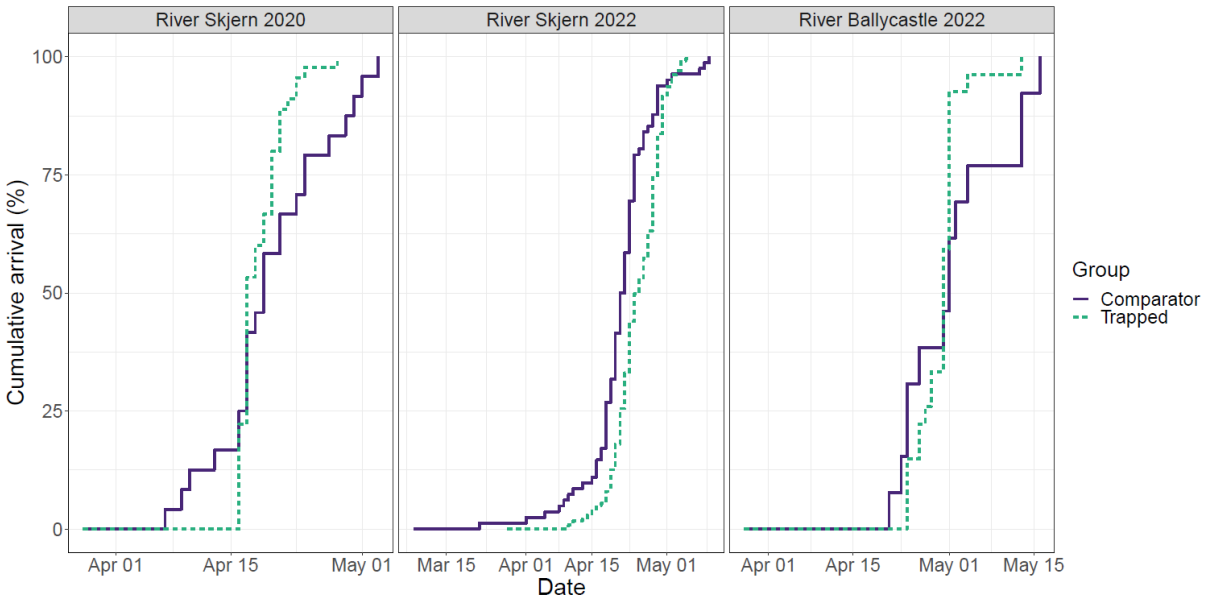

Figure 7. *Descent trajectories of the 'comparator' group of smolts with pre-migration tagging and the 'trapped' smolts, in the River Skjern, Denmark and River Ballycastle, Northern Ireland, once migration was initiated.*

The study concluded that the timing of capture was important, with the comparator group of smolts observed to migrate earlier in the River Skjern in 2022. However, once migration commenced there was no difference in descent trajectories, diel patterns, progression rates or survival between trapped and pre-migration non-delayed (comparator) fish.

WP3: Project Workshop

Objective 3

The objective of WP3 is the organisation of a physical 2-3 day project workshop for partners to meet and discuss the execution of the plans, enhancing methodologies and the dissemination of results. NASCO staff participated to discuss how NASCO can implement novel scientific information discovered in the SMOLTrack research.

The existing SMOLTrack partnership, which has been operating since 2017, together with its new partners, has extensive experience in migratory fish research and particularly telemetry studies, often using their existing telemetry arrays. The distribution of experienced project partners, across the wide latitudinal distribution of salmon in Europe, allows the project to investigate salmon under varying climatic ranges. It ensures a fast and wide international uptake of the results produced by the studies. SMOLTrack I, II, III and IV have already produced important research outputs (peer reviewed papers) and SMOLTrack V aims to further advance understanding.

The workshop was held over two days in October 2024 at NASCO's headquarters in Edinburgh. Project partners attended in person and virtually to present SMOLTrack findings. External experts from Scottish Government, CEFAS and the Atlantic Salmon Trust also attended.

Key discussion points included:

- post-spawning kelt survival;
    - protecting kelts after spawning can be vital for boosting salmon populations;
    - previous attempts to recondition kelts through feeding trials have been costly and ineffective, underscoring the need for better approaches; and
- southern salmon population monitoring;

    - there is ongoing monitoring of the most southern salmon population in Portugal;
    - this population is critically endangered, with very few salmon remaining;
    - the population exhibit unique genetic variability, highlighting the importance of targeted conservation efforts; and
- handling and tagging protocols;
    - a recent study by SMOLTrack partners found that trapping did not adversely impact the behaviour or survival of migrating smolts, suggesting trapping remains a low-impact capture method (Sortland *et al.* 2024); and
- innovative tagging technology;
    - Kennedy *et al.* (2023)[2] showed that by activating and deactivating acoustics tags, it is possible to detect both seaward smolt migration and adult salmon returning from the sea. This method may increase our understanding of adult return and survival without any additional cost;
    - SMOLTrack partners in Ireland and Northern Ireland have tagged salmon smolt with miniature data storage tags (DST) that record temperature. So far, one adult salmon has been recaptured, providing the first dataset of a full marine cycle;
    - the studies have also provided important insight on how to optimize the return og tagged adult salmon;
    - plans are underway for a future tagging-retention study utilizing dummy DST-tags to test different insertion sites for optimal retention; and
- Greenland expedition;
    - the expedition was successful in capturing and sampling Atlantic salmon in eastern Greenland;
    - researchers were able to hold Atlantic salmon in live wells for extended periods;
    - this creates future opportunities to genetically assign salmon and tag them according to the facilities available in their home-rivers. The possible use is limited by insufficient resolution of the current genetic assignment methods.

PINKTRACK: CO-ORDINATED eDNA SURVEILLANCE FOR PINK SALMON IN THE EU.

The EU has provided funding to the Board to support the PINKTrack project.

At the 2022 and 2023 meetings of the North Atlantic Salmon Conservation Organisation (NASCO) serious concerns were raised about the ongoing and future threat of non-native pink salmon to native wild Atlantic salmon stocks in the Convention areas. There has been an unprecedented occurrence of pink salmon over a wide geographical scale in all Convention areas since 2017, predominately in odd-years. Of particular concern is that self-sustaining pink salmon populations have become established in northern Norwegian rivers in recent years and in some rivers there they have been reported to out-number co-existing Atlantic salmon stocks. Since 2019, an explosive population growth in pink salmon stocks has been observed both in northern Norway and in the adjacent White Sea basin of northwestern Russia, where this non-native species was originally introduced. This has raised additional concern, that as a consequence, pink salmon may be encountered more commonly and have the potential to establish populations in adjacent and more southerly EU member states. This project intends to address these concerns under the beneficiary of NASCO, through a consortium comprised of state agencies and research institutes based in EU jurisdictions which is supported by

technical expertise from Norway. The project is undertaking work to better understand the extent of occurrence of pink salmon in EU waters through the use of environmental DNA (eDNA), which will enable it to elucidate temporal and geographic patterns of spread and provide an 'early warning system' of their presence to inform appropriate management responses.

A funding application for a further stage of the PINKTrack project is in preparation to continue the current work beyond its end date of 31 October 2026.

WP1: eDNATrack – Development of standardised protocols for eDNA

Objective 1

The first objective of the PINKTrack project is to develop standardised protocols for eDNA sampling and standardised approaches for the analyses of eDNA samples for the detection of pink salmon in EU member states, with the intention that such methods can continue to be utilised in routine national monitoring programmes after the project concludes. This WP includes preparatory work to evaluate different approaches to sampling and analyses and their effect on the results for detection. In addition it includes the establishment of a repository of eDNA samples collected during the project and in subsequent years to provide valuable material for future assessments as analytical technologies develop.

Key findings

The efficacy of a range of eDNA filter types (Table 3) were tested in project partner jurisdictions (Figure 8) in 2024 using Atlantic salmon and brown trout as proxy species for pink salmon.

Table 3. *Details of the DNA water filters included in WP1 – eDNATrack in 2024.*

Filter	Pore size (µM)	Membrane material
Sterivex GP 0.45 µm	0.45	PES
Merck millipore glassfiber filter 2.0 µm	2.0	Glass fiber
Sylphium Dual Filter 0.8 µm	0.8	PES
Sylphium Dual Filter 5 µm	5.0	PES
Waterra Filter 1.2 µm	1.2	PES
Waterra Filter 5 µm	5.0	PES
Smith-Root self preserving 1.2 µm	1.2	PES
Smith-Root glassfiber 5 µm	1.5	Glass fiber

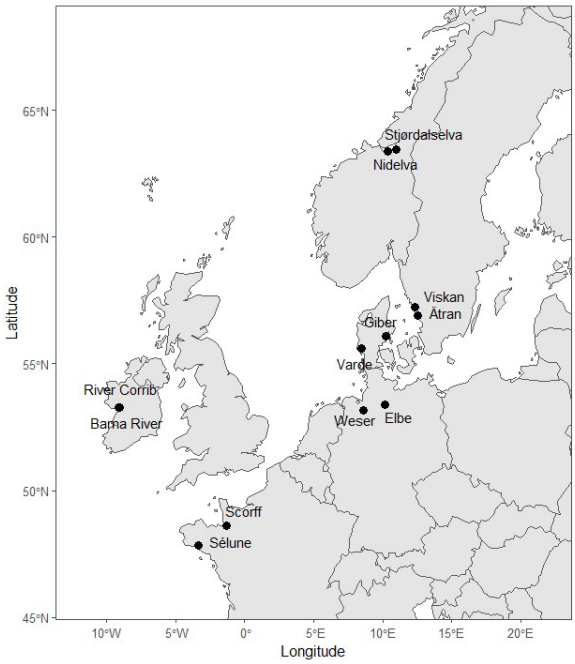

Figure 8. *Map showing the 12 rivers sampled in the WP1 – eDNA project in 2024.*

Three project partner laboratories (based in Germany, Ireland and Norway) were used to analyse the samples taken. Samples from Norway and Sweden were analysed by the Centre for biodiversity genetics (NINAGEN) at the Norwegian Institute for Nature Research (NINA), samples from Denmark and Germany were analysed by the Thünen Institute of Fisheries Ecology in Germany, and samples from France and Ireland were analysed by the University College Dublin (UCD) in Ireland.

Identical methods were used across all three labs to standardise the genetic analyses across the three different laboratories. DNA was extracted using the Nucleospin Plant II Midi kit columns (Macherey-Nagel GmbH, Düren, Germany) in combination with lysis and washing buffers from the Qiagen Blood & Tissue kit. DNA concentrations (ng/uL) and Optical Density (OD) were measured for all extracts using Nanodrop. A qPCR assay was used to detect and quantify trout (*Salmo trutta*) and Atlantic salmon (*S. salar*) DNA in the DNA extracts.

The variation in environmental conditions across rivers influenced both sampling logistics and analytical outcomes. Filter-specific handling requirements, such as differences in ease of applying preservation buffers, affected workflow efficiency. For instance, the Waterra filters allowed filtration of larger water volumes, but required correspondingly large buffer volumes for DNA preservation. These findings underscore the importance of balancing filter capacity, robustness, and ease of handling when selecting methods for large-scale surveillance efforts.

During fieldwork, differences in water characteristics had a pronounced effect on filtration performance. While in clearer, more oligotrophic rivers, larger volumes could be filtered, filters clogged rapidly in turbid, eutrophic environments with high loads of suspended organic material and fine sediments. The volume filtered thus varied considerably between sites, depending on the combination of local water characteristics and filter type, including membrane material, pore size, and housing design. High turbidity and suspended sediment loads, particularly in estuarine and lowland river systems like the Elbe and Weser, posed challenges for filter performance and affected DNA yield and PCR success rates, potentially due to co-extracted inhibitors. Conversely, in clearer and nutrient-poorer rivers in Norway and Ireland, such issues were less prevalent. In some rivers, lower expected densities of target species such as Atlantic salmon and brown trout may have limited the amount of eDNA recovered.

In addition to environmental variability, practical challenges with some filter types emerged. Breakage and leakage issues, particularly with the Sylphium filters under pressure from the Vampire pump system, led to difficulties during fieldwork and subsequent laboratory processing due to reduced handling efficiency and occasional sample loss during lab processing. The use of preservation buffers also presented some challenges. While smaller filters were fixated with minimal buffer volumes, larger filter types such as the Waterra capsules required substantially more buffer solution to ensure effective DNA preservation.

Differences in fish abundance between rivers affected detection probabilities, highlighting the need to test methods under a wide range of eDNA concentrations. For the pink salmon surveillance planned in 2025, this is especially relevant, as the expected abundance of pink salmon will likely differ substantially between countries − with higher returns anticipated in Norwegian and Swedish rivers and lower densities expected in Denmark, Germany or France.

The results suggest that there are important differences related to user-friendliness and manufacturing quality in addition to differences in the quantity of DNA obtained from each filter. Larger differences in DNA-output were observed among filters from different manufacturers than differences related to pore size. However, the results also suggest that several filters provide satisfactory results and several filters can be recommend based on the cross-country pilot study. The Sterivex filter performance was quite low for water volume filtered and seems to be problematic for turbid rivers. The Smith-Root self preserving filter performance was quite low for DNA-quantity (OD) and qPCR analysis for salmon and, therefore, seems less good for detecting rare species. The Sylphium filters appeared to have low plastic quality, and leakage problems were experienced with many filters. There is however a new version of these filters for 2025, and the plastic quality is expected to be improved.

WP2: SurveillTrack – Establishment and undertaking of an eDNA sampling programme for detection of pink salmon in EU member states

Objective 2

The second objective of the PINKTrack project is to establish and undertake an eDNA sampling programme for detection of pink salmon in EU member states, in order to elucidate temporal and geographic patterns of spread and provide an 'early warning system' of their presence to inform appropriate management responses. The intention for this programme of work is to provide a basis for continued routine national programmes after the project concludes. The work also includes the analyses of any samples taken by the project partners in 2023.

Background

Five EU jurisdictions as well as Norway and Greenland will participate in the pink salmon eDNA surveillance programme in 2025. It is envisaged that the eDNA sampling will be undertaken from mid-summer to mid-autumn and will coincide with the anticipated occurrence of pink salmon in each jurisdiction. A maximum of ten rivers will be monitored in each jurisdiction.

The eDNA samples will typically be taken in the lowermost section of an index river (duplicates with a control) but additional sites may be sampled within index rives or in other rivers as required. The frequency of sampling will be monthly. A project meeting will be held in Q2 of 2025 in advance of the commencement of the surveillance programme to finalise all relevant details including the procurement of the sampling consumables. Once the sampling is complete, all samples will be sent for eDNA analyses. It is intended that samples will be analysed in a maximum of five project partner laboratories (in Denmark, Germany, France, Ireland and Norway), the results of which will later be compiled, circulated and published for relevant stakeholders.

WP3: ProjectTrack: Organisation and hosting of a two-day virtual project workshop after project commencement

Objective 3

A formal project planning and development workshop for PINKTrack was held in January 2024. This workshop aimed to identify the methodologies required for pink salmon:

- to evaluate approaches to eDNA sampling and analyses
- to establish standardised approaches and protocols for eDNA sampling; and
- to inform methods for an eDNA sampling programme for detection of pink salmon in EU Member States.

A series of follow-up meetings were held in this regard in Q2 of 2024. As a result, it was decided to test the efficacy of a range of eDNA filter types in summer 2024 in rivers in project partner jurisdictions. As adult pink salmon were not expected to be present in significant abundance in 2024 in the North-East Atlantic outside its non-native established range (owing to the dominance of odd-year cohorts), Atlantic salmon and brown trout were used as proxy species for the study. Three project partner laboratories (based in Germany, Ireland and Norway) were used to analyse the samples taken. The output of this work is intended to inform the operation of an EU Member States surveillance programme for pink salmon in summer 2025.

A project wrap-up virtual workshop will also be held as the project concludes in 2026 to review outputs and inform their further adoption by EU member states.

Secretariat
Edinburgh
9 May 2025