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Overview of a SALSEA-SALMAN II Symposium and Workshop organized by the IASRB and sponsored by the TOTAL Foundation 20 February 2008, Paris, France

Report of the symposium on population structuring of Atlantic salmon: from within rivers to between continents

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Symposium objectives: *To review current understanding of the structuring of Atlantic salmon into genetic populations and of the latest molecular genetic marker based methodologies for assigning the origin of Atlantic salmon caught at sea to continent, region, river and population of origin.*

Symposium summary: *The information presented at the symposium confirmed that wild Atlantic salmon populations show genetic structuring between continents, among regions and rivers and even within rivers and that assigning salmon caught at sea, at least to region of origin, should be possible provided adequate genetic baseline data has been identified. Considerable progress is being made in this regard and these data will need to be available to the SALSEA programme through the development of an international database. Consideration of this was the focus of the workshop that followed the symposium.*

The symposium was opened by Dr Malcolm Windsor, Secretary of NASCO's International Atlantic Salmon Research Board (IASRB), who welcomed participants to Paris. He stressed that the symposium and subsequent workshop are vital to the success of the SALSEA programme since the objective is to use genetic stock identification (GSI) techniques to assign the origin of all salmon caught during marine surveys scheduled for 2008 and 2009 in both the North-East and North-West Atlantic to their region or river of origin. He noted that the intended output from the workshop will be a framework for integrating genetic data into an international database on which the SALSEA programme can draw. He thanked the TOTAL Foundation for sponsoring the meetings and for contributing essential funding for the SALSEA-Merge project. He also thanked Patrick Martin, Gina Sardelli-Sadiki and Eric Verspoor for the arrangements made. A list of participants at the symposium and workshop is contained in Annex 1 and the Programme for the meetings in Annex 2.

Mr Guy Sallavuard of the TOTAL Foundation welcomed participants and stressed that the TOTAL Foundation was pleased to sponsor the symposium and workshop in such a prestigious venue. The Foundation was established in 1992, shortly after the Earth Summit in Rio de Janiero, and its activities are focussed on research, education and rehabilitation in the field of biodiversity and the marine environment.

Dr Ken Whelan, Chairman of the IASRB, presented an overview of the SALSEA Programme and the SALSEA-Merge project further details of which are available at www.salmonatsea.com.

Dr Eric Verspoor provided the final introductory presentation. He referred to the first Salmon Microsatellite Analysis Network (SALMAN) workshop held in 2004. This had reviewed information on patterns of microsatellite variation in Atlantic salmon, considered its implications for use in GSI and assessed standardised screening methods with a view to developing an international database on microsatellite variation for use in GSI work. The outcome of that workshop had been an agreed panel of microsatellite loci for use in future population work (the so-called 'Virginia Panel'). He indicated that the objective of the symposium was to review current understanding of the structuring of Atlantic salmon into genetic populations and the latest molecular genetic marker-based methodologies for assigning the origin of Atlantic salmon caught at sea to continent, region, river and population of origin. Then, in the follow-on workshop, the objective would be to define a practical, optimal framework for integrating existing and future molecular population genetic data into an international database which could be used to assign the origin of salmon caught during the SALSEA programme marine surveys and agree a strategy for its implementation.

The first keynote speaker was Dr Tim King of the US Geological Survey. Dr King first presented the compelling range of heritable cytological, protein and DNA evidence showing the highly evolved genetic differentiation of European and North American salmon stocks, and of a lesser but still striking regional divergence among regions within these two areas. He argued that the differences between stocks in the Eastern and Western Atlantic that have been revealed by a host of researchers provide the basis for a strong case that the populations in these two parts of the species' range should be viewed as distinct subspecies.

Dr King then showed how he has exploited evolved genetic differences between North American and European salmon in their nuclear DNA (microsatellites) to assign salmon caught off West Greenland over the last decade to their continent of birth and determine the relative contribution of the two continents to the fishery; overall assignment accuracy to continent of origin of more than 99% was achieved. Over 7,000 fish were genotyped over this period and the analysis showed that overall ~74% of the catch was shown to be from the western Atlantic. However, the relative proportions showed consistent differences between different fishing areas as well as some annual variation within areas. For fish of western Atlantic origin, analysis has shown that the majority of the catch originates from Canada and within the US the majority of the catch is from the Penobscot River. Wild salmon in Maine are genetically distinct from Canadian salmon and genetic divergence exists among populations of wild salmon in rivers in Maine. The effect of the harvest on US stocks varies annually but even small harvests could have significant impacts on stocks experiencing low abundance.

Dr King concluded his talk by highlighting the renaissance that is occurring in systematic biology and the role it can play in the conservation of Atlantic salmon. He referred to the substantially increased utility of newly developed microsatellite markers and emerging molecular techniques such as the analysis of single nucleotide polymorphisms (SNPs).

The second keynote speaker was Professor Craig Primmer, University of Turku, Finland. He explored in more detail the evidence for population genetic structuring between rivers within regions, focusing on his group's work in the White and Barents Sea region. The differences in the relative importance of the different evolutionary forces (mutation, selection, migration, genetic drift) underlying genetic differentiation at the different geographic scales were discussed. In his study of nuclear DNA variation (microsatellites) in over 1,350 individuals from 30 rivers from this region, he concluded that salmon populations could be divided into four clearly defined sub-regions (Barents Sea/Atlantic, Kola Peninsula, White Sea and eastern Barents Sea), a view also supported by studies of mitochondrial DNA variation. Differentiation was sufficient to allow more than 90% of fish to be successfully assigned to sub-region level with the fourteen microsatellite makers used. Similar levels of assignment were also obtained to river of origin in the larger rivers that characterise the Eastern Barents Sea and White Sea sub-regions. He suggested that for conservation purposes these sub-groups should be considered as distinct management units. He concluded that establishing population structure to the 'sub-region' level was eminently realistic and individual assignment was relatively straightforward. However, he pointed out that establishing higher resolution population structure requires considerably more effort and stretches the limit of assignment methodologies or requires many times more loci. Professor Primmer concluded with a brief discussion of the factors contributing to the observed genetic structuring among the different parts of the Barents-White Sea region. He highlighted post-glacial influences, including the Komi Ice Lake, historical migration and current day demographic dynamics, and suggested that the evidence indicated that the relative importance of these factors appeared to vary in the different sub-regions studied.

Dr Melanie Dionne, Université Laval in Québec, Canada, reviewed the extent, nature and causes of within-river population structure and its relevance for Atlantic salmon conservation. She indicated that there is a large body of evidence for within-river population structure encompassing studies of allozymes, microsatellite DNA, and mitochondrial DNA. However, she noted that little attention has been given to the patterns and their implications for understanding the evolutionary basis of structure particularly with regard to its adaptive significance.

Dr Dionne went on to consider patterns observed by the Laval group in four Quebec and New Brunswick rivers. The work, based on microsatellite DNA analysis, showed dramatic variation for these markers among rivers in the extent of variation, from little detectable variation to substantive differences among tributaries. She then considered the two main evolutionary models proposed to account for variation. The first is the member-vagrant model under which population differentiation evolves as a consequence of natural selection maximising homing and juvenile survival to optimise the utilisation of a heterogeneous environment. This predicts temporally stable structuring with genetic differentiation positively correlated with geographic distance. The second is the meta-population model where, given unstable environmental conditions, the evolution of genetically distinct and locally adapted populations is restricted by recurrent local extinctions. This predicts weaker genetic structure, temporal instability of structure and no correlation of genetic and geographic distances. The group's work on the Ste-

Marguerite River in Quebec showed temporal variation to be greater than spatial variation but that temporal stability was variable among sites within the river, supporting the meta-population model. In contrast, work by Professor Primmer's group showed strong isolation by distance within the Varzuga River in Russia, more consistent with the member-vagrant model. Dr Dionne concluded that within-river population structure is common, sometimes as pronounced as between rivers; there is much variation in patterns of differentiation; the population structuring can be explained by the potential for homing to precise locations and variations in habitat stability.

Dr Dionne then went on to consider the more limited evidence bearing on the question of the extent to which structuring is adaptive. She cited one of the main lines of evidence from work on the allozyme MEP-2 by Dr. Verspoor that shows strong geographical correlations in both Europe and North America between variation at this locus and latitude, and more strongly with temperature. This occurs among as well as within river systems and provides a compelling case that some genetic structuring is adaptive. She went on to discuss work by the Laval group on the Ste-Marguerite River that showed higher levels of differentiation at MHC genes, known to be important in disease resistance, than at microsatellite loci, this also supporting the existence of adaptive genetic adjustment of populations. She then cited work on this river that suggested the evolution of adaptively distinct sub-populations in this river within six generations and that local adaptation is possible at the scale of tributaries; this included evidence of heritable differences in gene expression which were ~ 4 times greater than differences in microsatellite variant frequencies. She ended the discussion on adaptation by considering work on the association of traits such as body size on male reproductive tactics and for adaptive variation in growth, and by highlighting the conditions required for local adaptation to evolve. This seems most likely in tributaries and less likely in the main stems of rivers.

Taking all the evidence into account, Dr Dionne concluded that, in terms of conservation, it is important that: within-river diversity is taken into account; supportive breeding programmes should aim to maintain within-river genetic diversity; managers should consider that selective exploitation is likely if different sub-populations run at different times; and as there is no general rule, there is a need for genetic monitoring of individual rivers.

The topic of temporal stability of genetic differentiation and structuring was the focus of the next talk by Professor Danielle Ruzzante, of Dalhousie University in Nova Scotia. He started by outlining the basic biological issues related to this topic. He then considered the evidence related to this topic derived from recent work carried out by his group on Newfoundland rivers. He referred to the wealth of ecological and life-history data from rivers in Newfoundland. This work showed consistently that there was very substantially more spatial differentiation than temporal differentiation and indicated relative strong stability of population structure was not necessarily expected from considerations of the species biology.

The results of Professor Ruzzante's group mirrored those seen for many rivers elsewhere and he concluded that there is compelling evidence of widespread temporal stability in larger rivers, but smaller rivers may be less stable over time. Despite the observed stability of spatial differentiation in Newfoundland's rivers, the work of his group showed clear evidence of low level gene flow among populations, both contemporary and historical. This gene flow often appeared to be asymmetric, with greater flow into small

populations. He pointed out that estimates of migration are time scale dependent, that the extent of migration is a function of population size and population dynamics, including whether a population is part of a larger meta-population.

Professor Ruzzante then considered the use of temporal data for deriving estimates of migration rates and of the effective breeding size of populations. These two parameters, assuming no selection on the variation studied, will determine the extent of genetic differentiation among populations. He pointed out that the levels and patterns of genetic diversity among populations are differentially affected by these two aspects of the biology of the populations and that by considering these, estimates of the two parameters can be obtained; this was illustrated by the analysis of the genetic data from the Newfoundland rivers. He suggested that estimates of the breeding size of a population will be upwardly biased if one assumes no migration when in fact there is migration occurring; this appeared to be particularly true where migration is high or populations are less differentiated. He showed how the derivation of these estimates is complicated in the case of species such as the Atlantic salmon where populations are age structured and generations are overlapping, or where there is demographic instability e.g. in sex ratios. His analysis of Newfoundland data suggested that there have been no substantive changes in effective breeding sizes of populations associated with declines in numbers of adults in rivers in the 1980s. He considered that demographic mechanisms, such as decreased variance in family size, may act to maintain effective breeding sizes and genetic diversity in small populations.

Dr. Marja-Liisa Koljonen of the Finnish Game and Fisheries Research Institute presented her seminal work on the use of molecular genetic markers in the assessment of the contribution of different river stocks, both wild and ranched, to the Baltic Sea fisheries: hatchery production of ranched fish occurs as compensation for lost production due to hydroelectric dams. She presented the results of her work in relation to:

- simulation studies of the potential accuracy and precision of stock proportion estimates and individual assignments using genetic data;
- assessments of annual variation in stock proportions in different areas of the Baltic based on microsatellite DNA analyses;
- the use of stock proportion estimation for assessing the total catch of wild fish.

Dr. Koljonen described the 17 microsatellite loci used, the baseline information, and the statistical assignment method used. This took into account the structuring of rivers into three well defined regional stock groups and the ability to assign to these groups as compared to individual rivers. Simulations with the baseline data showed a very high accuracy of assignment averaging over 97%. This was true even with regard to wild and hatchery populations from the same river. Sampling requirements for obtaining stock proportion estimates within acceptable limits were considered as well as the nature and extent of estimation biases. The ability to assign individuals accurately to population of origin ranged from 100% down to ~50% depending on the population and the nature of the mixture being analysed; small proportions are more difficult than large proportions. In general acceptable results could be obtained using baseline information on 40 individuals per population.

Actual assignment work encompassed baseline information on 2,900 fish from 33 river populations. Catches were analysed from six locations throughout the Baltic. The analysis showed that considerable variation in stock proportions was seen between years.

In the Gulf of Finland, the majority of fish are from Finnish hatchery releases while over 60% of main basin fish are from wild rivers with the remainder equally from Finnish and Swedish hatcheries. In the Åland Sea, the analysis showed a dramatic increase in the proportion of wild fish from 2000 to 2002 of 20 to ~70% that was sustained until the present. However, this was not correlated with an increase in overall catches which have in general declined. Indeed, the analysis shows that absolute numbers of wild fish have declined but the decline in hatchery fish has been even more marked.

From her pioneering work on genetic stock assessment in the Baltic, Dr Koljonen concluded that the information gained has provided valuable management insights on the relative contributions of different Atlantic salmon stock groups and populations to the Baltic fisheries. She also concluded that, combined with other information, the insights could be used to provide insight into numbers caught in some situations and help to provide an index of abundance to monitor stock status.

Dr Paul Moran of the Northwest Fisheries Science Center in Seattle, Washington gave a presentation on his experiences associated with setting up a practical international collaborative genetic stock identification programme for Pacific salmon, where the use of GSI has been widespread for more than two decades. He gave an overview of the GAPS (Genetic Analysis of Pacific Salmon) programme in respect of the baseline database, and its mechanics and use for GSI, focusing on what they have learned and the continuing challenges they face.

Dr Moran first looked at the emergence of GSI programmes in the 1980s to address problems associated with stock harvest estimates derived from coded wire tagging. Key among these were the need for complete marking of hatchery stocks, the lack of information on wild stocks, and the unrealistic assumption that the performance of wild and hatchery stocks is the same. He indicated that the GAPS consortium, now comprising 32 different research labs in Western North America, was formed in 2002 to create an internationally shared and standardised microsatellite database for GSI for Pacific salmon. He illustrated GAPS using Chinook and Coho salmon GSI programmes as examples.

A major focus of GAPS was indicated to be the standardisation of microsatellites screened by different groups, facilitation of future expansion to include new genetic markers such as SNPs, and the development of a database application to support the dissemination and growth of the baseline data set. The first phase of the project covered two years with standardisation of marker sets and genetic nomenclature, and identification of baseline populations for sampling in the first year. Year 2 encompassed baseline development, quality control and analysis of the power of the database to achieve assignment. These issues were illustrated by examples from the GAPS programme and he noted a number of technical challenges encountered and how these were resolved.

Dr Moran then went on to describe the use of the databases developed in estimating proportions in mixed stocks and for individual assignment. He gave an example of the use of the databases developed in the management of the Chinook fishery in the Strait of Juan de Fuca, illustrating how the genetic methods were able to show major temporal shifts in the populations being fished. He further showed how they were used to regulate Chinook fisheries so as to increase escapement in the Klamath River where numbers of returning fish were below minimum requirements for stock conservation. In particular, he focused on how the genetic insights were integrated with other management tools to achieve overall management objectives.

Dr Moran then touched on the use of GSI to extend understanding of the marine ecology of Pacific salmon, analogous to the application proposed for the EU SALSEA-Merge project. This provided insight into factors such as the importance of disease in marine mortality and on the differences in the use of the marine environment by different stock groups and populations. He also suggested that genetic methods could be used to assess the benefits of management actions to specific stocks, and to provide basic biological insights. However, he pointed out that with fixed funds, development of these applications would compete with management initiatives.

Dr Moran then set out the main current challenges faced by GAPS. These included improved marker selection and statistical mixture algorithms, sustained funding for a permanent, safe, secure and convenient web based database. Additionally, a major challenge, given limited resources, would be to decide on how, when and where to implement genetic methods in management. He also pointed out that genetic methods were not a panacea but had strengths and weaknesses compared to tagging methods. The latter included no age information, probabilistic estimates, and poor estimates at low encounter rates. However, he also pointed out that perceived problems with DNA based methods may to a significant degree be related to the retention of management approaches conditioned by tagging paradigms e.g. how important is age structure information to effective management.

Dr Moran concluded with an overview of the points made. These included: the importance of international standardization of methodologies to multilateral fisheries management initiatives; striking a balance between integration and independence, focusing on the added value of collaboration; and exploiting the strengths of genetic methods within an integrated framework of management science.

A talk by Professor Willie Davidson on the Atlantic Salmon Genome Project followed, highlighting recent endeavours to advance molecular understanding of this species' genetic nature, current molecular work being based on the analysis of only a small randomly selected part of its genome. The Consortium for Genomics Research on All Salmon Project (cGRASP), involving more than a dozen partners, aims to coordinate all aspects of genomics research on Atlantic salmon, using it as a model organism for all salmonids. The project will extent understanding of the physical and genetic maps of the genome and integrate this to provide significant insights relevant to understanding the heritable basis of growth, development, disease susceptibility and performance variation, relevant to considerations associated with salmonid aquaculture, environmental risk assessment, and the conservation and management of wild stocks.

With regard to salmon conservation and management, Professor Davidson, pointed out that the increased understanding will:

- assist conservation and enhancement of wild populations;
- benefit commercial harvesting through the identification of specific stocks;
- enable fundamental scientific questions concerning the evolution of salmonid genomes to be answered;
- facilitate monitoring the expression of genes and proteins in a wide variety of natural and altered environments by regulatory agencies

He also indicated that understanding would be advanced with respect to:

- the number of genes in the genome;
- the metabolic and developmental expression of different genes;
- the biological traits influenced by different genes;
- the extent of heritable gene variation among individuals, populations and species.

Professor Davidson pointed out that genomic understanding of the Atlantic salmon was already significantly advanced and was already the 17th most characterised with regard to expressed genome sequences, much more than is the case for all other salmonids combined. He pointed out the role that cGRASP and its various partners had played in moving understanding forward, and how this understanding had been exploited in the development of technologies such as "Gene chips" and microarrays that allow routine screening of gene expression variation within and among individual fish. Studies carried out show that these gene chips can be used in studies of most salmonid fishes from grayling to Pacific salmon through to charrs.

Professor Davidson indicated that the expressed sequence regions identified by the Project and other researchers are a rich source of potentially useful genetic markers for studying population structuring and adaptation. In particular, he noted that they are proving a valuable resource for identifying single nucleotide polymorphisms.

The audience was told of ASalBase, a website for Atlantic salmon genomic resources that cGRASP has developed. This web based tool displays information such as the current physical genome map based on the bacterial artificial chromosome (BAC) libraries and linkage maps for genes based on family studies of the degree of co-inheritance of gene variation. It was pointed out that the BAC libraries developed contain 350,000 cloned DNA fragments, and how these connect and overlap to encompass the entire salmon genome. Additionally, it was noted that the database contains comparative genomic data on other species that helps to understand the nature of the salmon genome. Examples of the information were shown as were the efforts underway to integrate the different types of information into a holistic genomic understanding.

Professor Davidson concluded progress in the first three years has been significant and the project is on schedule. He then highlighted the considerable advances that would be realised by the availability of complete sequence information for the Atlantic salmon genome. He outlined the proposed future cGRASP programme, the extent of funding support so far obtained to achieve this end and the need for multi-national support if this objective is to be achieved. He invited new partners to join in the cGRASP programme and share in its aims and achievements.

Dr Sigbjørn Lien of the Centre for Integrative Genetics (CIGENE) gave the concluding talk of the day of the emerging genetic marker technology surrounding single nucleotide polymorphisms (SNPs). The Centre was established in 2003 and was funded by the Norwegian Research Council to serve as a national SNP genotyping facility. The Centre is predominantly involved in research related to food production with a specific focus on livestock and Atlantic salmon. Dr Lien followed this overview of CIGENE with a brief review of the Centre's high through-put molecular genotyping and data processing facilities.

Salmon related projects within CIGENE include those to:

- detect a large number of salmon SNPs and construct a dense genome wide SNP map;

- develop technology and resources to resolve duplicated genome regions;
- determine linkage disequilibrium and haplotype block structures in the genome;
- detect genes and gene pathways affecting economically important traits;
- compare levels of SNP variation in wild populations and domesticated strains.

His talk then went on to detail the approach CIGENE was taking to build up a large library of Atlantic salmon SNPs. This focused on the use of expressed sequence regions identified from RNA studies of different salmon tissues by various research groups and on the resequencing of salmon BAC fragment ends. So far they had identified ~16,000 potential SNPs from which they hoped to identify ~9,000 which could be used for genetic work. He outlined the process involved in doing so which involved a range of validation methods. A low resolution linkage map based on the detailed analysis of patterns of family inheritance for 300 SNPs was shown and showed the potential for advancing understanding of genome structure using SNPs.

Dr Lien concluded by highlighting the new sequencing technologies that are now available to researchers, touching on their relative merits as regards to cost per unit of information. He then indicated how one of these, known as 454 sequencing, will be married by CIGENE to the SNP discovery approaches they use to accelerate the rate of SNP identification. Finally, he indicated the process by which suites of SNPs would be able to be exploited by researchers by providing these to producers of DNA chips to develop custom chips for various applications, including population studies.

The symposium ended with a brief overview of the days talks by Dr. Verspoor and their relevance to the discussions in the workshop of the next two days. Additonally, for interested parties, Dr Paul Moran gave a demonstration of the web-based genetic database system developed for genetic stock identification of Pacific salmon. This provided a clear steer on the practical scope for developing and exploiting molecular markers in genetic stock identification in Atlantic salmon to advance understanding of population specific marine movements and the causes of increases in marine mortality over the last decades.

Report of the workshop on developing a scientific framework for trans-range stock identification of Atlantic salmon

Workshop objectives: *To define a practical, optimal framework for integrating existing and future molecular population genetic data into an international database which can be used for the identification of the continent, region and river of origin of Atlantic salmon caught at sea during their marine migrations, and agree a strategy for its implementation as part of the EU SALSEA-Merge project*

Workshop summary: *The specific aims for the work programme were to build on the review of existing biological information presented in the Symposium and consider the way forward for defining a practical framework and strategy for the development of marker panels and the integration of existing data sets and new regional markers into functioning GSI/MSA European, North American and trans-Atlantic databases. This was achieved by focused keynote presentations which examined 1) where we are compared to where we need to be, 2) the scope for developing new molecular markers for improved regional and river assignment, 3) the challenges in building a practical data base for genetic stock identification, and 4) how this would be achieved within the SALSEA framework. The main outcome of the workshop was affirmation of the shared conviction among researchers that the vision was both realistic as well as practically achievable, and of a consensus to move this forward for both the Eastern and Western Atlantic within the NASCO IASRB SALSEA framework. Additionally, the workshop reaffirmed the value and merit of continuing the SALMAN network and bringing it within the overall SALSEA programme. As such a major outcome was the renewing and strengthening of working relationships among Atlantic salmon geneticists and key elements in the broader research community, central to achieving the shared research objective. New important scientific links were established with researchers working on the Atlantic salmon genome project and with those working on similar genetic applications in relation to Pacific salmon. The workshop stimulated North American workers to initiate a programme of work to integrate North American data sets as will be achieved for Europe under the EU SALSEA-Merge project. Potential for linking North American and European efforts through trans-national co-operative links associated with EU 7th Framework programmes were explored and will be pursed. Finally, the workshop brought together the EU SALSEA-Merge researchers and updated everyone on the latest developments in the field and helped to build a consensus on the best way to move the genetic components of the project forward to ensure its ability to help advance understanding of the marine ecology of the Atlantic salmon to improve conservation and management programmes.*

The workshop was opened by Dr Eric Verspoor, Fisheries Research Services Scotland who welcomed the participants to the second part of the SALSEA-SALMAN II meeting. The relationship of the workshop objectives within the overall SALSEA and SALSEA-Merge programmes was restated and the specific aims for the work programme reviewed briefly. These were to build on the Symposium which considered the biological implications of existing information, and consider the way forward for defining a practical framework and strategy for the development of marker panels and the integration of existing data sets and new regional markers into functioning GSI/MSA European, North American and trans-Atlantic databases. The four topic areas to be covered were set out, with each topic area assigned a half day over the two days of the workshop and opened by a keynote paper related to the topic considered. A brief discussion was held amongst participants and it was agreed that it would be more efficient

and effective to hold each workshop discussion entirely in plenary rather than to have breakout groups following each keynote paper; this format was then followed.

Existing information: where we are compared to where we need to be

The keynote talk was given by Dr Carlos Garcia de Leaniz, Swansea University. He reviewed the state-of-the-art in 2004 at the time of the first SALMAN (Atlantic Salmon Microsatellite Analysis Network) meeting in Virginia, and gave a preliminary overview of progress since then based on initial meta-data summaries submitted by participants prior to the meeting. The paper touched on the considerations related to the use of different types of markers such as the use of microsatellite markers under selection and the assumption of neutrality in many genetic stock identification methodologies, and noted the lack of information on selection for most markers being used. Considerable progress had been achieved since 2004 aided by the formation of SALMAN. Despite its informal and voluntary nature, the meeting and informal network had promoted greater collaboration among researchers and considerable uptake in new research work of part, or all, of the microsatellite loci recommended for use at the meeting. No overall integrated database had as yet been developed but some bilateral linkages of data sets among research groups had occurred. Geographical coverage had increased as had the numbers of shared loci among groups.

In the ensuring discussion, it was noted that all the "Virginia" suite of markers are now on the Atlantic salmon genome linkage map with two shown to be linked to each other and a third showing linkage to a functional gene. The value of more detailed mapping work to examine linkage of loci to functional genes was agreed, as was the need for a standardized nomenclature for both population and genomic studies. The discussion also ranged to more general issues related to baseline development e.g. the need to take into account intra-river variation when sampling, focusing of within river sampling on spawning and associated juvenile rearing areas, the relative merits of juvenile versus adult samples, sample sizes, temporal variation, effects of farm escapes on baseline data sets, and changing the Virginia suite in light of experience since 2004. Different strategies for linking databases were noted.

Dr Tim King, USGS, Leetown, Virginia, led a focused discussion on recent work on North American stocks of Atlantic salmon with respect to the spatial coverage of current data sets and the overlap in microsatellites used. Studies of 12 American rivers, the oldest, are based on 11 microsatellites, and were carried out by a single US Laboratory. They show a 4 locus overlap with the loci used in more recent work on 90 Canadian rivers, carried out by 3 laboratories with a fourth starting, where 5-10 loci are used in common. Some standardization and inter-calibration among laboratories has already been carried out but a large data gap exists with respect to salmon rivers in Newfoundland and Labrador.

Dr Phil McGinnity, Marine Institute, Ireland, led a discussion on work carried out and currently underway in Europe. This showed a large part of the work carried out in Europe has occurred since 2004 and encompasses the SALMAN I recommended Virginia suite of microsatellites. Most of the European range of the species has been studied to some degree though coverage is highly variable with >90% of Irish rivers characterised. While many parts of Norway and Scotland have only a small proportion of their rivers characterised. In Norway, a wide range of 40 rivers have been screened for the full set of 15 Virginia markers by one group, with a further, smaller set of Norwegian rivers

screened by another group overlapping for 4 loci. A wide range of rivers in Northern Finland and Russia have been screened for 6 of the Virginia suite and representative rivers from Spain, France and Western Britain for 10 of these microsatellites. Many Icelandic rivers have been characterised for all 15 of the microsatellites and rivers in parts of Scotland for 14 of the Virginia set.

The general view was that existing work provided a good basis for development of a GSI baseline, with work focusing on determining the right balance in future work of extending geographical coverage and increasing the number of shared loci across all data sets. The broader value of including the Baltic data set in the European database was noted though it encompasses only a small proportion of the Virginia markers. The need to calibrate, standardise and test baseline data sets to achieve effective integration was emphasised and the best way forward was to do this at the same time as collecting new data and screening new loci. Options for extending coverage and keeping genotyping costs down were considered as were strategies to extend baseline information and the relative merits of extending sampling and increasing locus overlap. As regards, extending spatial coverage, prioritisation of rivers and dealing with within river variation were discussed, the relative contribution of a river to the overall regional stock being one of the key criteria. Based on Pacific salmon GSI experience, there is unlikely to be much gain in extending the baseline genetic marker set beyond a certain number of market alleles (350-400).

The session ended with a discussion of IPR issues when integrating data sets for GSI work. It was recognised that there was a need to balance individual needs, for first right to exploit their own data sets, particularly for academic participants and where PhD students are concerned, against the broader public need in support of management, both or applications such as GSI and for advancing science generally. The general consensus was that these potentially conflicting needs could be addressed by defining clear use and time constraints, both in respect of individuals within the SALSEA programmes and outsiders, and by developing mutually beneficial collaborations. The Pacific experience highlighted the importance of deciding these things in advance to avoid misunderstandings.

Developing new markers for improved regional and river assignment

The keynote address was delivered by Dr Patrick O'Reilly, Department of Fisheries and Oceans, Canada. He considered the relative merits of using a range of different types of molecular markers from restriction enzyme detected mitochondrial variants, to nuclear microsatellite markers, and single nucleotide polymorphisms (SNPs) in both nuclear and mitochondrial DNA (mtDNA) based on his studies of genetic variation within and among salmon rivers in eastern Canada and most particularly those in the Bay of Fundy region. He considered the levels of variation which could be resolved from within and between families to among populations within rivers, among rivers and among regions. The advantages of using both nuclear and mitochondrial markers in his studies were pointed out. His talk ended by highlighting recent technological advances which could be exploited for rapid, cost-effective screening of SNPs. Specific questions on the work presented were entertained before breaking for coffee.

Following coffee, Dr Vidar Wennevik, Institute of Marine Research, Norway, led a discussion on microsatellites. It was noted that the salmon genome contained tens of thousands of potentially useful microsatellite loci but that they varied in their levels of variability, the extent to which they were linked to functional loci, and the technical ease

with which they could be resolved. Existing microsatellite suites were generally chosen by what was available and by the technical ease of screening realised in a given lab using its particular screening platform. The Virginia panel represented the first attempt to develop an optimal set but its actual optimality needed to be reviewed as regards ease of use and levels of population differentiation. There was agreement that other, newer and better markers might be found, given evidence of considerable variation in regional and population differentiation among loci. The possible merit of replacing linked markers with additional unlinked markers in the Virginia panel was noted. However, overall it was concluded that microsatellites, and the Virginia panel in particular, remain the best current markers for the development of a trans-range GSI methodology.

Professor Tom Cross led a discussion on the potential for using nuclear SNPs. The general view was that, it was clear from work to date, particularly on other species, that this was likely to be the method of choice in the future, as it was likely to offer considerable cost and technical advantages. However, while considerable advances have been made, much development work is still needed. As such the benefits of basing GSI in Atlantic salmon on this class of genetic variation remain to be demonstrated and it would not be prudent at present to base development of an Atlantic salmon GSI programme entirely on nuclear SNPS. Needed development work was being carried out and, as part of SALSEA-Merge, was likely to advance rapidly over the next two years, making it likely that at least some use could be made of this class of markers in the SALSEA programme. Within the last 6 months, a prototype DNA chip with a range of SNPs has been produced which could be assessed for markers useful in population and regional discrimination to increase the GSI capacity of existing microsatellite markers, by a preliminary screening of index rivers. The value of the Matis/Prokaria plate of DNA samples from across the species range, currently being developed by the SALMAN network, for this purpose was highlighted. Developing the required baseline data set on which to base a purely SNP-based GSI would take time, even if a SNP-based method proves to be feasible, and would not be achievable in the time frame of SALSEA-Merge.

Before breaking for the day, Dr Eric Verspoor led a brief discussion on the use of mtDNA markers. The relative merits of using restriction enzymes, sequencing or other SNP detection methods were touched on. A significant potential for using mtDNA SNPs along with other markers was agreed as in principal mtDNA differentiation can be expected to be greater than at nuclear gene loci. However, their overall usefulness remained unclear. However, existing work makes clear that mtDNA variation may be of use in certain situations as illustrated by work in the Inner Bay of Fundy. The actual potential for identifying regional markers could be established quite easily by sequencing larger regions of the mtDNA in a set of river samples representative of the species range such as the Matis/Prokaria SALMAN sample set. Where regionally restricted variation can be found, it could be exploited along with microsatellites or nuclear SNPs to produce a more efficient and cost-effective methodology. Such work will be carried out under the SALSEA-Merge project.

Following the workshop session, the group attended a private viewing and buffet dinner at the superb Museum d'histoire naturelle de Paris "Abysses" exhibition in the Galerie de la Minéralogie, hosted by the TOTAL Foundation.

Specific challenges in building a practical Atlantic salmon GSI/MSA database

The session's keynote talk was given by Dr Jamie Coughlan, University College Cork, Ireland. He provided a general overview of the issues and challenges faced, many of which had been touched upon in the previous day's discussion. He then illustrated these in relation to the development of the Irish National Salmon Genetics Database, the most extensive national database developed to date and its linkage to the broader south-western European database developed as part of the EU funded ASAP programme. His talk finished with results obtained from the Irish programme which illustrate the potential power of microsatellite based GSI at both the stock and individual assignment levels. He concluded that, with detailed baseline information, it should be possible to cost-effectively achieve both regional and river specific determination of proportional stock contribution, as well as individual assignment, with a high degree of accuracy using as few as 12 microsatellite markers. Specific questions were entertained on the talk before breaking for coffee.

Following the break, discussions focused on four areas. These were defining sampling needs within and among rivers, led by Eric Verspoor, linking existing databases in North America, led by Tim King, linking existing databases in Europe, led by Vidar Wennevik, and the development of new regional markers, led by Phil McGinnity.

Sampling needs were agreed to depend on the GSI questions being addressed. With regard to regional assignment, this would be determined by how salmon are in fact regionally structured at the evolutionary level, something about which some insight has been gained from existing work, including allozyme studies. Existing insight into regional differentiation can help to establish the required geographical coverage for underpinning regional assignment. Within regions, it was agreed that partial sampling was likely to be adequate for regional assignment provided it encompassed the main populations. In contrast, to achieve river assignment, it was felt that detailed data on all rivers and populations within rivers would be needed. In the absence of prior knowledge of population structuring, sampling should target discrete spawning areas and associated habitat, or be guided by aspects of the physical structuring of the river such as branching, general habitat distribution and habitat discontinuities which appear to control population structuring. The relative merits of sampling juveniles as opposed to adults were discussed, and advantages and problems seen to be associated with both, though there was general agreement of the merit of sampling multiple age classes to help assess the stability of spatial differentiation. In general the view was that most sampling already carried out can contribute to the baseline data needed.

Tim King reported on deliberations among the North American researchers at the meeting which saw agreement to work towards linking data for salmon in the Western Atlantic in parallel with European work. It was noted that there was potential for using linkages with the European SALSEA-Merge programme to access monies for extending North American studies in parallel with European work. The overall advantage of using a common set of loci with Europe, for advancing general biological understanding, was recognised but this was not seen as essential to development of a working GSI system. North American groups agreed to consider during the meeting how to develop their own GSI framework to match that being developed for Europe as part of the EU SALSEA-Merge project, and how this might be progressed following the workshop. Ken Whelan offered the support of the IASRB as part of the SALSEA initiative.

Merging the already considerable body of work done on genetic variation in European salmon was seen as both technically feasible and practical, particularly given EU funding support under SALSEA-Merge. Different approaches which could be taken were discussed and their relative merits considered. In general the development of a common, integrated database for the whole of Europe was seen as the best approach but might only be able to achieve regional assignment in many areas if river sampling could not be achieved for the majority of rivers. Linkage and extension of existing data sets to achieve this was not seen as a major obstacle in principal, as illustrated by the relative ease of linking the Irish and ASAP databases for the common microsatellites. The main challenge was carrying out the calibration using allele ladders or reference samples, standardising allele nomenclature, and putting in place a working shared database, all issues to be addressed by SALSEA-Merge.

One of the main challenges in the development of new regional markers was seen as being understanding the hierarchy of regional differentiation which exists and the boundaries of regional evolutionary groupings of salmon populations, and targeting the appropriate level for marker development. It was suggested that existing ICES regional divisions, in combination with general insights gained from what limited work has already been carried out e.g. allozymes, be used as a starting point. Initial development work to identify diagnostic markers was agreed to be usefully focused on a test panel of ~50 populations with ~10 individuals per population which could be screened for microsatellite, nuclear SNP as well as mitochondrial SNP variation. At the same time, once existing data sets are linked, an analysis should be carried out to look for regional signals in those markers already used. Insights learned could then be fed back and help guide sampling programmes and further development work.

Building the database under SALSEA

The keynote paper for the session was given by Dr Jamie Stevens, Exeter University, England, who described the ASAP database which they developed to carry out GSI analyses in south-western Europe in conjunction with Oviedo University, with funding from the EU ARC programme. The work provided a clear overview of the challenges faced in developing a broader scale programme and highlighted the problems encountered when there was inadequate coverage of populations in the region considered. At the same time, the work clearly demonstrated the potential for using the methodology at the larger scale and provided significant insights into the nature and extent of larger scale regional structuring in this part of the species range. It was clear that the work carried out provided a solid basis on which to build a suitable baseline for the region as part of an overall European GSI database.

Following a brief discussion, Dr Carlos Garcia de Leaniz presented a more detailed preliminary meta-analysis of existing microsatellite data sets based on data summaries entered into a spreadsheet by participants during the meeting. This showed that very considerable advances had been made since 2004 both in the number of populations screened (now > 500 versus < 200 in 2004) and in the number loci screened in common among populations. This showed the majority of populations screened having been screened for at least 4 loci in common and a large proportion for at least 8 common loci. This suggested that the work required to extend the analysis of existing samples, on both sides of the Atlantic, to a set of 8-12 common loci was likely to be tractable.

In lieu of a further discussion on developing an integrated database, the meeting ended by taking up the offer of Dr Paul Moran, NOAA, USA, and had a real time demonstration of the web-based genetic database currently under development for Pacific salmon GSI. The demonstration proved highly informative and it was agreed that the database would serve as a very useful model for the one which would be required for Atlantic salmon GSI. To facilitate this, Dr Moran extended an invitation to the SALSEA consortium to send those setting up the Atlantic salmon database to visit his laboratory in Seattle to discuss the possibility of doing so with the database developers.

Following the demonstration, there was a brief discussion regarding the future of SALMAN. The consensus was that SALMAN should continue as it provided a valuable and productive vehicle for advancing studies on Atlantic salmon both at the academic and applied levels. It was also agreed that it would be of benefit to all if the Network operated within the framework of IASRB, with NASCO and that its name be modified to SALMMAN (Atlantic **SAL**mon **M**olecular **M**arker **A**nalysis **N**etwork) to encompass its broader genetic interest in SNPS as well as microsatellites. Having done so, the sponsors and the organisers of the workshop were thanked by the participants and the workshop called to a close.

Annex 1

List of Participants

Bernatchez, Louis	Laval University, Canada
Consuegra, Sonia	Swansea University, UK
Coughlan, Jamie	University College Cork, Ireland
Cross, Mary	University College Cork, Ireland
Cross, Tom	University College Cork, Ireland
Crozier, Walter	Agri-Food and Biosciences Institute, UK
Davidson, Willie	Simon Fraser University, Canada
Dillane, Eillen	University College Cork, Ireland
Dionne, Melanie	Laval University, Canada
Finnegan, Anna	University of Exeter, UK
Garcia de Leaniz, Carlos	Swansea University, UK
Gauthier, Marika	Laval University, Canada
Gilbey, John	Fisheries Research Services, UK
Griffiths, Andrew	University of Exeter, UK
Gross, Riho	Institute of Veterinary Medicine and Animal Science, Estonia
Gudjonsson, Sigurdur	Institute of Freshwater Fisheries, Iceland
Heffernan, Peter	Marine Institute, Ireland
Hindar, Kjetil	Norwegian Institute for Nature Research, Norway
Hutchinson, Peter	NASCO, UK
Hynes, Rosaleen	Queen's University Belfast, UK
Koljonen, Marja-Liisa	Finnish Institute of Game Research, Finland
King, Tim	US Geological Survey, USA
Lien, Sigbjorn	Centre for Integrative Genetics, Norway
Lubinski, Barb	US Geological Survey, USA
Martin, Patrick	Conservatoire Nationale du Saumon Sauvage, France
McGinnity, Phil	Marine Institute, Ireland
Meulien, Paul	GENOME British Columbia, Canada
Moran, Paul	National Marine Fisheries Service, USA
Olafsson, Kristin	MATIS, Icelandic Food Research, Iceland
O'Reilly, Pat	Department of Fisheries and Oceans, Canada
Palstra, Friso	Dalhousie University, Canada
Primmer, Craig	University of Turku, Finland
Prodohl, Paulo	Queen's University Belfast, UK
Ruzzante, Daniel	Dalhousie University, Canada
Sallavuard, Guy	TOTAL Foundation, France
Sanchez, Jose Antonio	University of Oviedo, Spain
Sardella-Sadiki, Gina	TOTAL Foundation, France
Sheehan, Tim	NOAA Fisheries Service, USA
Stevens, Jamie	University of Exeter, UK
Vähä, Juha-Pekka	University of Turku, UK
Vazquez, Eva Garcia	University of Oviedo, Spain
Verspoor, Eric	Fisheries Research Services, UK
Wennevik, Vidar	Institute of Marine Research, Norway

Whelan, Ken	Marine Institute, Ireland
Windsor, Malcolm	NASCO, UK

Symposium and Workshop Steering Committee

Eric Verspoor (Convenor) - Tim King
Carlos García de Leániz - Patrick Martin
Phil McGinnity - Vidar Wennevik
Malcolm Windsor - Ken Whelan

Annex 2

Programme

Population Structuring of Atlantic salmon: from within rivers to between continents

The Symposium will set the stage for the workshop, the main focus of the meeting. It will encompass a set of targeted keynote talks interspersed by poster sessions which it is hoped will stimulate discussions on the "current state of the art".

All participants are encouraged to present posters of their relevant work, to bring all participants up to date, including of primary studies and topical new syntheses of old or synoptic data sets, insightful considerations of new or existing concepts or theory relevant to the general subject of population structuring and genetic stock identification in salmonid fishes generally or Atlantic salmon specifically. Consideration is currently being given to inviting poster submissions in the second category to expand their posters into papers for publication in symposium proceedings, subject to peer-review, as a volume of Reviews in Fish and Fisheries. This will be discussed at the symposium.

0900 Opening Remarks: IASRB - Malcolm Windsor
TOTAL Foundation - Guy Sallavuard
0915 Introduction SALSEA and SALSEA-Merge – Ken Whelan
0930 Introduction to Symposium and Workshop – Eric Verspoor

0945 Keynote Talk: Continents and Broad regions:
1015 Keynote Talk: Within Regions, among rivers:
1045 Coffee and Posters

1130 Keynote Talk: Within Rivers, among tributaries:
1200 Keynote Talk: Temporal Stability of Structuring

1230 Buffet Lunch and Poster Session

1400 Keynote Talk: Atlantic Salmon GSI/MSA in the Baltic Sea
1430 Keynote Talk: GSI/MSA in Pacific Salmon:
1515 Coffee and Posters

1545 Keynote Talk: Atlantic Salmon Genome Project
1615 Keynote Talk: Atlantic Salmon Genome Variation
1645 Summation and Workshop forward look
1730 Close and Posters

Developing a Scientific Framework for Trans-range Stock Identification of Atlantic salmon

Workshop Day 1 (21 February)

Evaluating existing Atlantic salmon genetic databases and assessing development needs

Existing information: where we are compared to where we need to be

0830 Plenary Keymote: Overview of Existing Information– C. Garcia de Leaniz
0915 Discussion and formation of break-out groups
0930 Break-out Groups: Approaches and Strategies for integrating existing data
1. North America (Tim King: Leader)
2. Europe (Phil McGinnity: Leader)
3. IPR Issues (Eric Verspoor: Leader)

1130 Group Presentations and general discussion

1230 – 1400 Lunch

Developing new markers for improved regional and river assignment

1400 Keynote: Challenges and opportunities – Patrick O'Reilly (Canada)
1445 Discussion and formation of break-out groups
1500 Break-out Groups and Coffee
1. Microsatellites (Vidar Wennevik: leader)
2. nuclear SNPs (Tom Cross: leader)
3. mtDNA (Eric Verspoor: leader)

1700 Group Presentations and general discussion

1745 End of first day

1800 - 2030 Private visit to "Abysses" Exhibition and Buffet Dinner, Galerie de la Minéralogie

Workshop Day 2 (22 February)

Defining a practical framework and strategy for the development of marker panels and the integration of existing data sets and new regional markers into functioning GSI/MSA European, North American and trans-Atlantic databases

Specific challenges in building a practical Atlantic salmon GSI/MSA database

0900 Keynote Talk – Challenges and issues of integration – Tom Cross

0945 Discussion and formation of break-out groups

1000 Break-out Groups and Coffee

1. defining sampling needs within and among rivers (Eric Verspoor: leader)
2. linking existing databases in North America (Tim King: Leader)
3. linking existing databases in Europe (Vidar Wennevik: Leader)
4. Development of new regional markers, (Phil McGinnity: leader)

1130 Group Presentations and general discussion

1230 – 1400 Lunch

Building the database under SALSEA

1400 Keynote: Development of a practical database for GSI/MSA

1445 Discussion and formation of break-out groups

1500 Break-out Groups and Coffee

1. North America (Tim King: Leader)
2. Europe (Phil McGinnity: Leader)
3. Reference samples and Standardisation of nomenclature

1630 Group Presentations and general discussion

1745 Discussion on formalisation of SALMAN under SALSEA

1800 End of workshop

- strategies for development of new regional markers, including a transnational set of samples for development work (Phil McGinnity: leader)