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**CNL(14)66**

***Practical application of genetics in conserving the biological integrity (diversity) of populations of Atlantic salmon***

***Philip McGinnity, University College Cork***



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### ***Practical application of genetics in conserving the biological integrity (diversity) of populations of Atlantic salmon Philip McGinnity, University College Cork***

Below is provided a short outline of a talk prepared for the theme-base special session CNL(14)13 on the 'Management of single and mixed stock fisheries, with particular focus on fisheries on stocks below their conservation limit' and presented at the NASCO Annual General Meeting in St. Malo, France in June 2014. The aim of the paper is to provide some examples of the application of genetic methods for managers and is not meant to be a comprehensive review of the subject. Most of the examples presented in the paper pertain to work (much as yet unpublished) undertaken under the auspices of the Beaufort Marine Research Award in Fish Population Genetics Group held jointly by University College Cork and Queens University Belfast, the Marine Institute (Ireland) and Agri-Food and Biosciences Institute (Northern Ireland) and funded by the Irish Government under the Sea Change programme. Many of the projects were supported by and carried out in collaboration with Inland Fisheries Ireland. Studies at an early stage include a collaboration led by Dr Martin Llewellyn, University of Bangor, Wales and Université de Laval, Quebec, Canada, on Atlantic salmon micro-biomes. Other examples of the application of genetic methods presented are referenced from reports based on the work of a variety of International research groups.

#### **Background**

The objective of Atlantic salmon management is the protection of the species integrity ensuring long-term sustainable natural productivity and resilience. Biological integrity has been described as consisting of two elements (Angermeier and Karr, 2004). The first element is biological diversity, which is usually perceived as a hierarchical scheme of increasing ecological and genetic complexity, commencing with allelic variants of genes, the combination of genes giving rise to a genome or individual, individuals as an isolated breeding entity comprising a population of interbreeding individuals, inter-related populations combining to form population complexes or meta-populations and ultimately the aggregate of populations to make a species. Within the context of biological diversity, the level of most interest from management is usually the population or population complex. The population can be readily associated with an individual river or a major tributary within a river system. The population, or at least the fish within an individual river system, is basis upon which the conservation limits are established for the regulation of fisheries and which designations important for fisheries conservation, such as evolutionary significant units (ESUs), are defined.

The second component of biological integrity is the evolutionary and ecological processes, both natural and anthropogenic, associated with contemporary and historical environments that shape and have shaped the observed diversity in separate populations. Increasingly, within the broader definition of biological integrity, managers have an appreciation of the genetic and evolutionary impacts of man's activities on salmon productivity, population resilience and adaptability to changing environments and to the occurrence of and necessity to protect uniquely evolved and irreplaceable genetic solutions for life a range of environments. For example, changes in size at age and age at maturation in response to size

and run time selective fisheries; genetic changes in recipient wild populations subsequent to escapes from farm populations or deliberate introductions of cultured fish through stocking; environmental changes close to and beyond the biological tolerance of populations as a consequence of global warming; losses of distinct genetic diversity due to habitat loss from impoundments for hydroelectric installations. These all have are assumed to have a negative impact on population abundance and persistence on affected populations in dynamic environments. Results of recent studies (Hilborn et al. 2003; Schindler et al. 2010) demonstrate the critical importance of maintaining population diversity for stabilizing ecosystem services and securing the economies and livelihoods that depend on them and for enabling adaptation to changing environments.

### **Genetic Toolbox**

Population genetic techniques have provided a number of important additions to the fisheries management arsenal: resolution of genetic population structure; parentage assignment; mixed stock fisheries analysis; and pedigree reconstruction. These include the ability, classically, to determine population structure, to differentiate between individual populations and quantify inter population differences and to establish the status and genetic parameters pertaining to those populations such as levels of inbreeding, gene flow between individual populations, genetic drift, sex determination and consequently sex ratios, including, potentially, surrogate demographic attributes such as effective population size and number of breeders.

The capacity to assign individuals back to their parents, **parentage assignment**, has enabled salmon biologists to address a plethora of complex questions that were previously considered to be intractable. Within a common garden experimental framework in the wild, for example, .assessment of the relative fitness of the progeny of local wild and farm parents and their hybrids under natural conditions has been made (McGinnity et al. 2003); also the scale at which local adaptation occurs in salmon populations and its magnitude (McGinnity et al. 2004); and the role of variation in immune response genes as a basis for local adaptation (deEyto et al. 2011).

One of the most tangible benefits of genetic methods has been in the application of **genetic stock identification for mixed stock fisheries analysis**. With comprehensive baselines of potentially contributing populations, highly accurate assignments of individuals can be made to their river or region of origin. However, it very important to state here that while genetic markers can be considered to be analogous to physical markers, they are statistically derived assignments rather than absolute. As a consequence the quality of assignment will be a function of the quality of the baseline, which depends on coverage and molecular differentiation of the elements. The key advantage is that all samples in a mixed fishery provide biologically useful information, rather than just those from which a physical tag can be retrieved. In addition information is acquired from wild fish rather than for the most part data retrieved from fish that have be hatchery reared first in order to be large enough to successfully retain a tag.

**Pedigree based analysis**, facilitated by advances in statistical analysis and computing power, is a powerful method for separating genetic effects from environmental effects on the phenotype, as demonstrated by their common use in domestic animal breeding programmes. Pedigrees in the wild have rarely been constructed in any vertebrates because of the logistical and technical difficulties in identifying and following the performance of individual families. The molecular pedigree reconstruction approach, as with any in the field of quantitative genetics, requires knowledge of the relatedness of individuals in a population. Such

information, although challenging to come by in field populations, is increasingly available for studies of a range of taxa (Pemberton, 2008), fuelling a growing interest in the application of quantitative genetics to the multigenerational study of natural, rather than laboratory or domestic populations such as Atlantic salmon (Aykanat et al. 2014). In order to understand whether populations can adapt to human induced environmental change we need to understand the genetic basis of the phenotypic traits on which selection acts. To this end the skill sets associated with quantitative genetics are increasingly being employed in combination with those of population genetics. Questions being addressed by this approach include the impact of stocking and responses to climate variability, using fundamental quantitative genetics estimates of the heritability of important life history traits; selection; measuring evolutionary change e.g. human induced changes in critically important salmon population characteristics for ecological and fisheries management such as the age of reproduction and run time.

### **Some additional examples of the application of genetics for salmon management**

The combined genetic and ecological study developed within the SALSEA-Merge project has demonstrated how this approach can provide new insights into the population specific biology of salmon in the sea. Knowledge has been acquired of distribution and migration patterns of post-smolts from individual populations in addition to measures of their growth rates and feeding preferences (Jensen et al. submitted). A recent study of the West Greenland fishery, exploiting the baselines developed in SALSEA (P. Prodöhl, QUB, *pers. comm.*) indicated that those European salmon caught there originated primarily from southern European rivers, predominantly from Scottish rivers with significant contributions from English and Irish rivers. Also, significantly, there was considerable agreement between estimates of non-maturing one-sea-winter salmon or potential multi-sea-salmon for southern European stocks and the ICES predictions of pre-fisheries abundance, thus adding considerable confidence in the veracity of both estimates. What was noteworthy was not those fish from regions that were present, but those fish from regions that were not. Surprisingly, there were few Icelandic fish observed and very few fish from the rivers of northern Europe. It must be assumed that they are migrating to some other part of the North Atlantic. The ability to locate salmon from different regions and stock complexes offers the prospect of linking geographically delineated oceanographic phenomena with the past performance of regional stocks and potential of making predictions about future performance (Friedland et al. 2014).

One of the most controversial salmon fisheries until recently was the Irish off-shore drift net fishery. A decision was taken by the Irish government in 2007 to close the fishery. Subsequently, one of the first and largest studies until recently undertaken in Europe (P. McGinnity, UCC, *pers. comm.*) showed that the fishery was indeed catching fish from multiple Irish river systems and vindicated the Government's decision to close the fishery. However, contrary to expectations its impact on non-Irish fish was found to be minimal. Coincidental to the mixed stock analysis some insights into the return migration behaviour of Irish salmon were acquired. The propensity for capturing mixed stocks declined from north to south suggesting the homeward migration for the majority of Irish stocks was from a similar direction. As might have been expected the closer the fishery is prosecuted to its home river the more likely the fish are to be from that river, for example, Moy fish constituted 20% of the salmon caught of the Northwest coast of Ireland. Within Killala Bay, the inshore coastal district closest to the Moy river, Moy fish represented 60% of the catch. Significantly within the river itself at the transition from sea to river, Moy fish comprised 100% of the catch.

Modern fisheries management and stock assessment programmes require high accuracy census data to populate predictive models and to determine the success of management initiatives, relative to the achievement of conservation goals. It is difficult to count salmon in large river systems. In the Moy the potential of a different and innovative strategy whereby a counting facility is established in a small and manageable tributary, but critically one chosen because it has a genetically distinct salmon population compared to the rest of the system, was explored (P.McGinnity, UCC, *pers. comm.*). In collecting a representative mixed stock sample at the head of tide, for which the contributing stock components could be distinguished using genetic stock identification, it was possible to determine the total number of fish entering the river as the multiplication of the proportion that was accurately counted in the genetically distinct tributary relative to the genetically determined composition in the rest of the mixed sample.

On the basis of anecdotal evidence of distinct temporal patterns in run time among stocks using the estuary, an experimental fishery was operated in Castlemaine Harbour in Co. Kerry to enable samples to be collected for genetic stock identification to inform management in terms of operating a commercial fishery while safeguarding the spawning requirements of the salmon populations that were below conservation limit requirements (J. Coughlan, UCC, *pers. comm.*). Managers using a combination of genetic and biological data derived from this study found that there were indeed distinct and predominantly non-overlapping differences in run time among the various local populations indicating that the fishery could be prosecuted in a manner that only single stocks were targeted. The fishery was opened accordingly enabling a commercial salmon catch on sustainable populations that might otherwise have been foregone on the basis of designation as a mixed stock fishery.

While the extensive Irish genetic baseline for salmon has proven to be of great value for the analysis of mixed stock fisheries located around Ireland in terms of determining stock proportions in each fishery, the baseline has also been used to specifically identify the population of origin of fish that have undergone some level of processing (e.g. smoked salmon). In this respect, there is substantial interest in identification of farmed salmon, when processed, can be fraudulently sold (at considerable profit) as being of wild origin. Since 2005, all food businesses in Ireland are required to have traceability systems and must be able to authenticate the origin of all food products. In respect of salmon, these must be declared as being of wild or farmed origin. A test case taken in respect of smoked salmon by the Food safety Authority of Ireland (FSAI), falsely identified by the processors allegedly, using DNA profiling to confirm if salmon were of wild or farmed origin, showed that genetic data was admissible and acceptable as evidence in court ([http://www.fsai.ie/uploadedFiles/News\\_Centre/Newsletters/Newsletters\\_Listing/Final\\_mar\\_apr\\_09.pdf](http://www.fsai.ie/uploadedFiles/News_Centre/Newsletters/Newsletters_Listing/Final_mar_apr_09.pdf)). It was successfully argued in court that this was possible. The knowledge among food producers that the regulatory authorities can trace the origin of what was essentially a processed product should reduce the incidence of future mis-labeling.

In addition to tracing the origin of material in the food chain, genetic methods have come to the fore in identifying the origin of salmon that escape from aquaculture facilities into the wild. Trial studies in Norway by Glover et al. (2013a) show that this can be done very effectively. As stated by Glover et al. 2013 the point of identifying the origin of an escapee is that it also allows the identification of the cause of the escape, implementation measures to prevent its recurrence in order to reduce the extent of escapes, and learn from what has happened. At the same time, the authorities can decide whether there are circumstances associated with an escape that need to be further investigated, in case a fish farmer should be made responsible for an escape and its potential environmental and economic consequences.

Glover and his colleagues (2013b) have also deployed genetic methods to quantify cumulative introgression of farmed salmon in native Atlantic salmon populations and the genetic changes that occur as a consequence. They show levels of introgression varying between 2 and 47% among 20 Norwegian rivers assessed. The estimations demonstrate that the level of introgression has been population-specific, and that the level of introgression is not solely predicted by the frequency of escapees observed in the population. However, some populations have been strongly admixed with farmed salmon, and these data provide policy makers with unique information to address this situation. Whatever about the propensity of farm salmon established from wild Norwegian populations freely introgressing with salmon in Norwegian rivers, some early data from a recent, as yet unpublished study, by the Beaufort group, indicates the intriguing possibility of Norwegian origin farm salmon establishing a discrete sustaining population within an Irish river. However, for the most part recent genetic surveys in Irish rivers suggest very little genetic carryover from the farms in affected areas.

There have been a series of incredibly powerful measures of the relative fitness of captive bred fish facilitated by pedigree reconstructions of long-term sampling programmes, particularly for a number of Pacific salmon species. A recent review of these studies by Christie et al. 2014 indicate that for the most part (i) early-generation hatchery fish averaged only half the reproductive success of their wild-origin counterparts when spawning in the wild, (ii) the reduction in reproductive success was more severe for males than for females, and (iii) all species showed reduced fitness due to hatchery rearing. These studies have been very useful for managers in the continuing debate on the appropriateness of and the risks to wild populations of stocking hatchery fish into wild. Studies employing this type of analysis, particularly where efforts have been made to collect biological material such as scales will be common practice and become an integral part of the Europe's long-term monitoring efforts for Atlantic salmon.

It can be difficult in some instances to decide in mitigation programmes or stock rehabilitation programmes between persisting with genetic material that has been maintained within a hatchery, resampling from depressed wild populations or sourcing fish from other non-native populations. The capacity to examine the structure of historical salmon populations by genetic profiling of archive scale collections can provide important starting point for the design of salmon restoration programmes and an assessment of the material available. For example in a recent restoration ecology project involving the Shannon River system in Ireland, genetic analysis of archive scale material prior to the installation of the rivers hydro-electric facility in the 1920's showed that it was possible to identify the elements of biodiversity and genetically distinct populations with different life history profiles that have been lost in the intervening period (P. McGinnity, UCC, *pers. comm.*). Current discussions in respect of the rivers rehabilitation, informed by the genetic data, centre on options for ecological as well as genetic matching to provide best chances of success.

### **Future developments**

A full sequence of the salmon genome has been recently completed (<http://www.icisb.org/salmonsequencing>). Where up until recently a panel of 20 or so microsatellite type genetic markers were the norm, population geneticists now have ready access to panels of quarter of a million SNP markers. Probably one of the most exciting areas emerging in genetic and genomic methods is an increasing ability to assess patterns in the distribution of adaptive diversity and how it is adding to our understanding of the nature and extent of local adaptation (Hemmer-Hansen et al., 2014). It is now becoming increasingly feasible to link genes with life histories and to measure levels of expression and to gain new

fundamental insights into the ecology and evolution of Atlantic salmon. Also most population genetic studies previously concentrated and depended on estimates of neutral genetic variation. Now there is a shift to the usage of molecular markers influenced by selection; so called adaptive or gene associated markers (Neilsen et al. 2012). Markers under selection typically display elevated levels of differentiation, potentially enabling the discrimination of salmon populations exhibiting low genetic differentiation, something which has been a feature of some of the large Irish and Scottish rivers.

Further new applications of genetics and genomics in fisheries management are constantly being developed and deployed, for example, the study of Atlantic salmon micro-biomes and the detection of environmental DNA (eDNA) are good examples. Like the human gut micro-biome project, an understanding of the salmon micro-biome will provide insight into the role microbial species have in nutrient absorption and metabolism, and in immunity and disease response and consequently might provide an excellent window into our understanding of the health of salmon in marine and freshwater environments and their interaction with pathogens. Unlike in humans, where much of the micro-biome is transmitted to juveniles within families and social groups, salmon must selectively recruit all their commensal bacteria from the external environment (M. Llewellyn, Bangor University, *pers. comm.*; Llewellyn et al. 2014).

Environmental DNA (eDNA) is DNA that is released from an organism into the environment. Sources of eDNA include secreted feces, mucous, and gametes; shed skin and hair; and carcasses <http://pubs.usgs.gov/fs/2012/3146/>. In aquatic environments, eDNA is diluted and distributed by currents within a given water body. Depending on the environment eDNA lasts about 7–21 days. Protocols using eDNA will allow for rapid, cost-effective, and standardized collection of data about species distribution and relative abundance, but probably most powerfully deployed for early detection of aquatic invasive species. Aquatic invasive species, where they occur with Atlantic salmon will most certainly represent a potent evolutionary force on the species.

### **Summary**

Presented above are just a few examples of the application of genetic methods to the conservation of the biological integrity of the Atlantic salmon resource. Genetic markers provide an extraordinary powerful tool for identifying and delineating biologically significant management and conservation units in Atlantic salmon; the biology of species lends itself well to genetic population structuring with high, river specific, homing fidelity to discontinuously within river distributed spawning habitats. Importantly genetic methods play an important role essential to identifying the most vulnerable populations; according them with appropriate protections. The application of genetic methods has brought valuable new information on the extent of anthropogenic impacts (fishing; climate; habitat; aquaculture) on population productivity and resilience. The incorporation of eco-evolutionary concepts such as bio-complexity/portfolio effects are now central to the managers understanding of the factors that determine sustainable abundance and adaptability to dynamic environments.



## References

Angermeier, P.L. and Karr, J.R. (1994). Biological integrity versus biological diversity as policy directives: protecting biotic resources. *Bioscience*, Vol. 44, No 10, p690-697.

Aykanat, T., Johnston, S., Cotter, D., Cross, T., Poole, R., Prodohl, P.A., Reed, T., Rogan, G., McGinnity, P., Primmer, C. (2014) Molecular pedigree reconstruction and estimation of evolutionary parameters in a wild Atlantic salmon river system with incomplete sampling: a power analysis. *BMC Evolutionary Biology*, 14:68

Christie, M.R., Ford, M.J. & Blouin, M.S. (2014). On the reproductive success of early-generation hatchery fish in the wild. *Evolutionary Applications* doi:10.1111/eva.12183.

deEyto et al. 2011; de Eyto, E., McGinnity, P., Huisman, J., Coughlan, J., Consuegra, S., Farrell, K., Tufto, J., Megens, H.J., Jordan, W., Cross, T., Stet, R.J.M. (2011). Varying disease-mediated selection at different life history stages of Atlantic salmon in freshwater. *Evolutionary Applications*. 4: 6: 749-762.

Friedland, K.D., Shank, B.V., Todd, C.D., McGinnity & P., Nyee, J. (2014). Differential response of continental stock complexes of Atlantic salmon (*Salmo salar*) to the Atlantic Multi-decadal Oscillation. *Journal of Marine Systems* 133, 77-87.

Glover, K.A. & Skaala, Ø. (2013a). On the origin of escaped farm salmon. *ICES INSIGHT*, Issue No. 50. September 2013, 2-14.

Glover, K.A., Pertoldi, C., Besnier, F., Wennevik, V., Kent, M. & Skaala, Ø. (2013b); Atlantic salmon populations invaded by farmed escapees: quantifying genetic introgression with a Bayesian approach and SNP. *BMC Genetics* 2, 14:74.

Hilborn R, Quinn TP, Schindler DE, Rogers DE (2003) Biocomplexity and fisheries sustainability. *Proceedings of the National Academy of Sciences*, 100, 6564–6568.

Llewellyn, M., Boutin, S., Hoseinifar, S.H., Derome, N. (2014). Teleost microbiomes: progress towards their characterisation, manipulation, and applications in aquaculture and fisheries. *Frontiers in Microbiology*, 5, 207.

McGinnity et al. 2003; McGinnity P.; Prodöhl P.; Ferguson A.; Hynes R.; Maoiléidigh N., Baker N.; Cotter D.; O’Hea B.; Cooke D.; Rogan G.; Taggart J.; Cross T. (2003). Fitness reduction and potential extinction of wild populations of Atlantic salmon, *Salmo salar*, as a result of interactions with escaped farm salmon. *Proceedings of the Royal Society: Biological Sciences*, 270: 2443-2450.

McGinnity et al. 2004; McGinnity, P., Prodöhl, P., Ó Maoileidigh, N., Hynes, R., Cotter, D., Baker, N., O’Hea, B., Ferguson, A. (2004). Differential lifetime success and performance of native and non-native Atlantic salmon examined under communal natural conditions. *J. Fish Biol.* 65 (Suppl. A), 173-187.

Neilsen, E., Carini, A., MacAoidh and 22 others including the Fishpoptrace Consortium (2012). Genetic associated markers provide tools for tackling illegal fishing and false eco-certification. *Nature Communications*, 3: 851.

Pemberton, J.M. (2008). Wild pedigrees: the way forward. *PRSB*, 275, 613-621.

Schindler DE, Hilborn R, Chasco B *et al.* (2010) Population diversity and the portfolio effect in an exploited species. *Nature*, 465, 609–612.

