

NATIONAL RIVERS AUTHORITY
AWDURDOD AFONYDD CENEDLAETHOL

WELSH REGION
RHANBARTH CYMRU



WELSH REGION LIBRARY COPY



NRA

Guardians of the Water Environment
Diogelwyr Amgylchedd Dŵr

ASiantaeth yr Amgylchedd Cymru
ENVIRONMENT AGENCY WALESGWASANAETH LLYFRGELL A
GWYBODAETH CENEDLAETHOLNATIONAL LIBRARY &
INFORMATION SERVICE

PRIF SWYDDFA/MAIN OFFICE

Plas-yr-Afon/Rivers House
Parc Busnes Llaneirwg/
St Mellons Business Park
Heol Fortran/Fortran Road
Llaneirwg/St Mellons
Caerdydd/Cardiff CF3 0LTNRA WALES
FISHERIES REPORTSSALMONID GENETICS IN WATERREPORT OF INTERIM REVIEW MEETINGST. MELLONS, 17/4/91CIRCULATION

Prof. J.A. Beardmore	U.C. Swansea
Dr. D. Skibinski	U.C. Swansea
M. O'Connell	U.C. Swansea
L. Hauser	U.C. Swansea
H. Hall	U.C. Swansea (based at U.C. London)
D. Benbo	U.C. Cardiff
Dr. D. Thompson	MAFF
Dr. D. King	DANI
S. Hovey	NRA (Wessex)
J. Ravenscroft	University of Birmingham
Dr. K. O'Grady	NRA (London)
Dr. D. Jordan	NRA (London)
A. Barber	NRA (Wessex)
W.J. Ayton	NRA (Wales)
Dr. C. Pattinson	NRA (Wales)
Dr. D. Clarke	NRA (Wales)
Dr. R. Cresswell	NRA (Wales)
A. Winstone	NRA (Wales)

Prepared by
N. Milner
6/5/91

Ref: EAN/91/04

ENVIRONMENT AGENCY
WELSH REGION CATALOGUE
ACCESSION CODE AEWW
CLASS No _____

CONTENTS

SUMMARY

1. INTRODUCTION
2. AIMS OF GENETICS PROGRAMME
3. RESEARCH OBJECTIVES
4. SUMMARY OF PREVIOUS OR CURRENT GENETICS WORK IN WALES
5. MAIN POINTS
6. CONCLUSIONS

TABLE 1 DISTRIBUTION OF WELSH SAMPLING SITES

APPENDIX A MEETING DETAILS

APPENDIX B PRESENTATION SUMMARIES

SUMMARY

1. This report describes proceedings of a meeting held to review progress of NRA funded research into genetics of Welsh salmon and sea trout stocks. This work is being achieved by PhD studentships at Swansea University; but a number of other studies, completed or in progress, were also reported.
2. The primary objective of the studies is to describe the nature of genetic variation in wild stocks. A number of secondary objectives, dependent on the outcome of the first, are also identified. The aim is to support genetic aspects of fisheries policy development and management in the NRA.
3. Main points are discussed under the headings: spatial genetic variation, effects of stocking, genetic markers, seasonal run differences and methodology. More data are available for trout than for salmon. In both, evidence for between catchment differences is accumulating. Although some mtDNA data indicate lack of variation, the reasons for this are being explored.
4. Good evidence of hybridisation between stocked "sea" trout and resident "brown" trout on the Conwy demonstrates the need for cautious management of stocking or opening access to migratory trout.
4. The NRA-funded component is on course to achieve its primary objective, and the results of other studies have been of great value in building a region-wide picture of genetic variation. Full value of these studies will come from their integration with other programmes at national and international level. A means to achieve this needs to be established.

SALMONID GENETICS IN WALES

REPORT OF INTERIM REVIEW MEETING, ST MELLONS, 17/4/91

1. INTRODUCTION

A significant amount of genetics work has recently taken place in Wales, meeting a need to support or develop regional NRA (formerly Welsh Water) fisheries policy and improve fisheries management practice. The initiative to pursue this originally came from the Chairman's Working Party Report on Salmon and Sea Trout (Welsh Water, 1985), and was developed into specific recommendations by the Review of Fisheries Genetics Research Requirements in Welsh Water (WW, 1987). Shortly thereafter the CFRD working group on fish genetics was established, allowing Welsh needs to be set in a national context, and leading to the association with University College of Wales, Swansea, through which most present work is being directed.

In addition, a number of other projects have arisen in which Welsh salmonid stocks have been investigated as part of both large scale surveys and postgraduate research projects.

The purpose of this meeting (Appendix A) was to review progress with the Swansea work, fully or part funded through the NRA R&D programme (see (3) and (9), section 4 below), to ensure it is proceeding satisfactorily and continues to be appropriate to NRA aims and objectives. Because most current genetic projects are linked through methodology or objectives, it was considered useful to invite other workers to contribute to the meeting. Their cooperation in this is greatly appreciated.

2. AIMS OF GENETICS PROGRAMME (Swansea studies)

- 1) To enable development of effective policy on stock transfers (e.g. stocking, fish farm escapes), hatchery use and conservation of natural genetic resources.
- 2) To explore the application of genetic methods in monitoring stocking programmes and describing the makeup of mixed stock fisheries.

3. RESEARCH OBJECTIVES

The principle objective of current studies is to describe prevailing patterns of genetic variation in Welsh salmon and sea trout stocks. Depending on the outcome of this, the nature and stability of variation, a number of secondary objectives may be feasible.

- ...to identify genetic tags suitable for following progress of stocked fish and makeup of mixed stock fisheries.
- ...to describe the effects of stocking on genetics of recipient stocks
- ...to describe genetic variation between seasonal runs.

4. SUMMARY OF PREVIOUS OR CURRENT GENETICS WORK IN WALES

Studies referring specifically to Welsh stocks are listed below, the numbers () are references used in the text, * indicates presentations given at the meeting and summarised in Appendix B:

4.1 SALMON

- (1) **DAFS**(1987-88). Studies as part of UK-wide survey, Usk and Conwy sites included; allozyme and mtDNA.
- (2)***MAFF/University of Buckingham** (D.Thompson,D.King, start 1988). Studies including Conwy with English rivers; allozyme and mtDNA.
- (3)***NRA/University College of Wales,Swansea** (UCS). PhD studentship (M.O'Connell, start Oct 1989). Genetics of Atlantic Salmon in Wales; allozyme and mtDNA.

4.2 TROUT

- (4) **WW/University College of North Wales,Bangor** (UCNW). MSc (J.Anderson,1987). Genetics of trout in three Snowdonia lakes. Examined genetic variations in stocks from isolated lakes in relation to acidity; allozyme.
- (5) **NRA/UCNW** MSc (G.Marshall, 1989) Genetics of trout in Conwy tributaries. Preliminary survey of differences in migratory and non-migratory trout in the Conwy catchment, in relation to consequences of opening up Conwy Falls; allozyme.
- (6) **University of Buckingham** PhD (J.Ravenscroft, start Aug.1989). Genetics of brown trout,inc. Conwy sites; allozyme.
- (7)***NRA/University of Wales, Cardiff** (UCC). PhD (D.Bembo,1989). Part of wider study of natural recruitment of brown trout on the Usk, identification of localised breeding populations; mtDNA.
- (8)***NRA/UCNW** MSc (L.Hauser,1990) Effects of sea trout stocking on resident non-migrant trout in the Conwy Catchment. examined evidence for gene introgression; allozyme.
- (9)***NRA/UCS** PhD (H.Hall, start Oct 1989). Genetics of trout in Wales; primarily aimed at sea trout, this work also necessarily includes studies on non-migrant trout; allozyme and mtDNA.

5. MAIN POINTS

NB in most cases the conclusions so far available are provisional, and may be revised in the light of continuing studies. Summaries of the presentations included in this report should not be quoted without reference to the authors concerned.

5.1 Spatial Genetic Variation

5.1.1 For salmon, mtDNA data indicate distinct substocks within the Wye and Teifi, but Usk stocks appear less variable (3). Allozyme frequency data for three polymorphisms demonstrate similarities between the Conwy and rivers in NW England, but these two are different from rivers in Southern England (2).

5.1.2 In the case of "trout", significant between-tributary variation has been identified for the Conwy (5,8), Dee (9) and Usk (7) catchments. Significant differences have also been seen in some lake

populations (4). Compared with migrant "sea" trout, heterozygosity is more variable between "brown" trout populations but less variable within populations (8).

5.1.3 mtDNA results for trout in a region-wide study (9) indicate little variability between stocks so far examined. This is in contrast to allozyme data and is surprising in view of the general perception of high variability within this species; it may be a feature of the particular part of the genome examined (see section 5.5). These results will be compared with allozyme electrophoretic analysis to be carried out on the same set of samples.

5.1.4 The extent of surveys to date is shown in Table 1, along with catchments remaining to be sampled.

5.2 Effects of Stock Mixing

5.2.1 No information yet available for salmon

5.2.2 Allozyme studies on the Conwy (8) clearly show hybridisation between introduced sea trout and resident brown trout above Conwy Falls. This is detectable within 3 yrs of stocking (seen in Ogp fish) and producing genetic features intermediate between resident and stocked fish. The biological implications of shifts in gene frequency are still unknown, but the result is clear evidence that genetic changes can rapidly occur.

5.3 Genetic Markers

5.3.1 Genetic markers have application for monitoring the progress of stocked fish and measuring the composition of mixed stock fisheries. It is worth noting that their practical application may require significant resources to screen exploited/stocked populations and their donor stocks.

Rare alleles, identified by allozyme analysis, have been identified in lake populations of brown trout (4). Unique mtDNA variants have been found in Wye and Teifi salmon samples (3), and may prove suitable as markers

5.4 Seasonal Run Differences

No data are yet available on this. There are sampling difficulties which have not yet been resolved.

5.5 Methodology

A feature of contemporary work is the shift away from allozyme analysis to various DNA techniques. In the case of salmon this has been necessary because of the comparatively low number of known enzyme polymorphisms. In most studies restriction fragment length polymorphisms have been employed, but polymerase chain reaction (PCR) and direct sequencing have been used in the regional trout study (9), which has the advantage that it can be used on very small amounts of tissue taken by non-lethal sampling. In the case of PCR, lack of variability may be just a feature of the DNA section selected for study and further work is planned to look at the cytochrome b gene(9).

mtDNA analysis is still a comparatively new method, and its use in this programme has inevitably resulted in some exploratory development work. However, there is appropriate communication between the workers to avoid unnecessary duplication and "reinvention of wheels". Single locus probes may be used in genetic "fingerprinting" of salmon(3) and sea trout(9).

6. CONCLUSIONS

6.1 The NRA funded programme is on course to achieve its principle objective. Some early difficulties with methodology have been resolved, and there is intended overlap, where appropriate, between the more traditional allozyme analysis and DNA analysis.

6.2 It is still too early to know how the secondary objectives will develop, or if they can be achieved within this programme.

6.3 The evidence of genetic consequences of stocking (5,8) should prompt a rapid review of regional policy towards opening up previously inaccessible areas to migrant trout, and conservation of brown trout genetic resources.

6.4 The concurrence of several different projects, involving different institutions and approaches, has been valuable in tackling the overall problem in Wales. Although this programme is specifically directed at Welsh stocks, the results will be of value to other NRA regions having salmonid fisheries. However, the full benefits of the salmon and sea trout work will emerge when these studies are set in the context of UK and international programmes. A means for achieving this needs to be established.

TABLE 1 - DISTRIBUTION OF WELSH SAMPLING SITES

Project Reference (see text) and No. of Sampling Sites

<u>Div.</u>	<u>River/Tributary</u>	<u>SALMON</u>			<u>TROUT</u>				
		(1)	(2)	(3)	(4)	(5)/(8)	(6)	(7)	(9)
N	Dee			6*					6
N	Conwy	1	3			8	3		
N	Braint (Anglesey)								2
N	Crigyll (Anglesey)								3
N	Soch			3*					
N	Erch			3*					
N	Mawddach								3
N	Wnion								3
N	Dyfi			5*					
SW	Teifi			1					4
	Tywi			5*					1
SE	Usk	1		8				6	
	Wye			5					
Snowdonia Lakes					3				

* Intended Sampling (1991)

APPENDIX A

SALMONID GENETICS IN WALES
REVIEW MEETING

Venue: NRA Offices, St Mellons, Cardiff
Date: 17/4/91

AGENDA

1000 COFFEE

1015 Introduction Nigel Milner (NRA)

"TROUT"

1030 Conwy Stocking Lorenz Hauser (UCS, exUCNW)

1050 Afon Usk Dave Bembo (UCC)

1110 Welsh Region Heather Hall (UCS)

1130 Discussion

1230-1330 LUNCH

SALMON

1330 MAFF/Buckingham Univ. Studies written report only

1350 Welsh Region Mike O'Connell (UCS)

1410 Discussion

1530 End

Attendance:

Prof. J.A.Beardmore (UC Swansea)
Dr D.Skibinski "
Mrs H.Hall "
Mr M.O'Connell "
Mr L.Hauser "
Mr D.Benbo (UC Cardiff)
Dr N.Milner (NRA, Welsh)
Mr A.Winstone "
Dr D.Clarke "

Apologies from Dr D.Thompson(MAFF), Dr D.King,Mr S.Hovey (written report enclosed); Ms J.Ravenscroft.

EFFECTS OF STOCKING ON THE POPULATION GENETICS OF BROWN TROUT (*SALMO TRUTTA* L.) IN THE CONWY RIVER SYSTEM, NORTH WALES

L. Hauser

The objective of this study was to assess the effects of the introduction of fry of anadromous sea trout on the genetics of landlocked brown trout populations. Own data and data collected by Marshall (1989) were used. Samples were taken from six brown trout populations of streams above impassable waterfalls in the Conwy river system (North Wales). Three of these streams (Nant-y-Foel, Cadnant, Machno) had no known stocking history and three (Merddwr, Nug, Iwrch) had been stocked with sea trout fry from the lower Conwy system over the last few years. Representatives of these sea trout were collected from two streams in the lower Conwy system (Nant-y-Goron, Roe) and from a hatchery (Mawddach Hatchery). Allele frequencies at 13 loci, six of which were polymorphic, were determined by starch gel electrophoresis.

There were marked differences in allele frequencies between sea trout and unstocked brown trout populations, with the stocked populations showing intermediate allele frequencies. However, as shown by both allele frequencies and Nei's genetic identities, the degree, to which the stocked populations were affected, varied. A principal component analysis (PCA) suggested significant numbers of introduced fish in the Merddwr, but not in the Nug, indicating a failure of the recent stocking programme in the latter. Hybrids between introduced and native fish were shown by the PCA in all of the stocked streams. In the Merddwr they were only found in the 0+ age class, suggesting a recent onset of hybridization. In the other stocked streams the hybrids were probably due to earlier stocking.

The results show that the success of the stocking programme varied considerably between sites. They also show that some of the introduced sea trout did not migrate down the falls to the sea, but stayed in freshwater and hybridized with the local brown trout population.

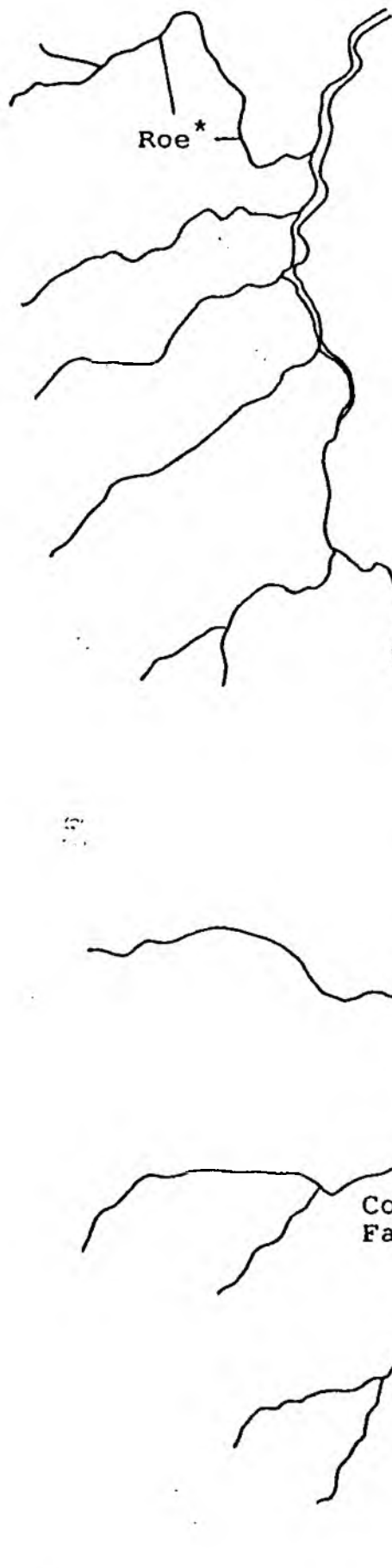


Table of sampling sites:

Stream	Grid ref	N	Age	Date
Nant-y-Foel	SH869525	50	>0+	21.6.'90
Cadnant	SH864525	15	0+	7.8.'90
		41	>0+	
Machno	SH788501	50	>0+	7.-8.'89
Nug	SH896514	47	0+	28.8.'90
		15	>0+	
Merddwr	SH875513	31	0+	10.7.'90
		30	>0+	
Iwrch	SH828528	50	>0+	7.-8.'89
Roe	SH755716	25	>0+	7.-8.'89
	SH768698	25	>0+	7.-8.'89
Nant-y-Goran	SH809609	50	>0+	7.-8.'89
Hatchery	-----	57	0+	15.8.'90

▲ Impassable waterfall



1 km

Simplified Map of the Conwy river system showing the sampling sites and relevant impassable waterfalls.
 *: Sites of Marshall (1989)

Genetics of River Usk Brown trout (*Salmo trutta* L.)

This research forms part of a 3yr NERC/NRA funded PhD project on the biology of brown trout in the River Usk catchment.

The objective of this study was to investigate the degree of heterogeneity within the stock, and also whether spawning isolation leads to genetically distinct 'sub-stocks' within the catchment.

Methods

Samples of 6 fish (10-15cm) were taken in March/April 1990 from 6 sites on the Usk and also from one site on the River Wye.

Usk sampling sites were:-

1. River Bran	SN933383
2. River Senni	SN255928
3. River Menascin, above a large weir	S0064243
4. River Menascin, below the weir	S0088253
5. River Grwyne Fawr	S0267204
6. River Gavenny	S0311163

(See accompanying map)

Wye sampling site :- River Hiernant S0002259

Mitochondrial DNA (mtDNA) was extracted from the liver and heart tissue of trout, using differential centrifugation and a cesium chloride gradient (45,000rpm, 40hrs). The mtDNA was cut with restriction endonucleases recognising 4,5 or 6 base sites, ³²P endlabelled, and run on agarose or polyacrylamide gels, depending on fragment size. Fragments were sized by comparison with fragments of known molecular weight. Enzymes used were *AvaII*, *HaeIII*, *HindIII*, *HinfI*, *Sau3AI*, and *XbaI*.

Results

Only two of the six enzymes tested, *AvaII* and *HinfI*, revealed polymorphisms. The 42 fish sampled were split into 5 genotypes :-

<u>GENOTYPE</u>	<u>HinfI morph</u>	<u>AvaII morph</u>
I	A	A
II	B	A
III	C	A
IV	D	A
V	B	B

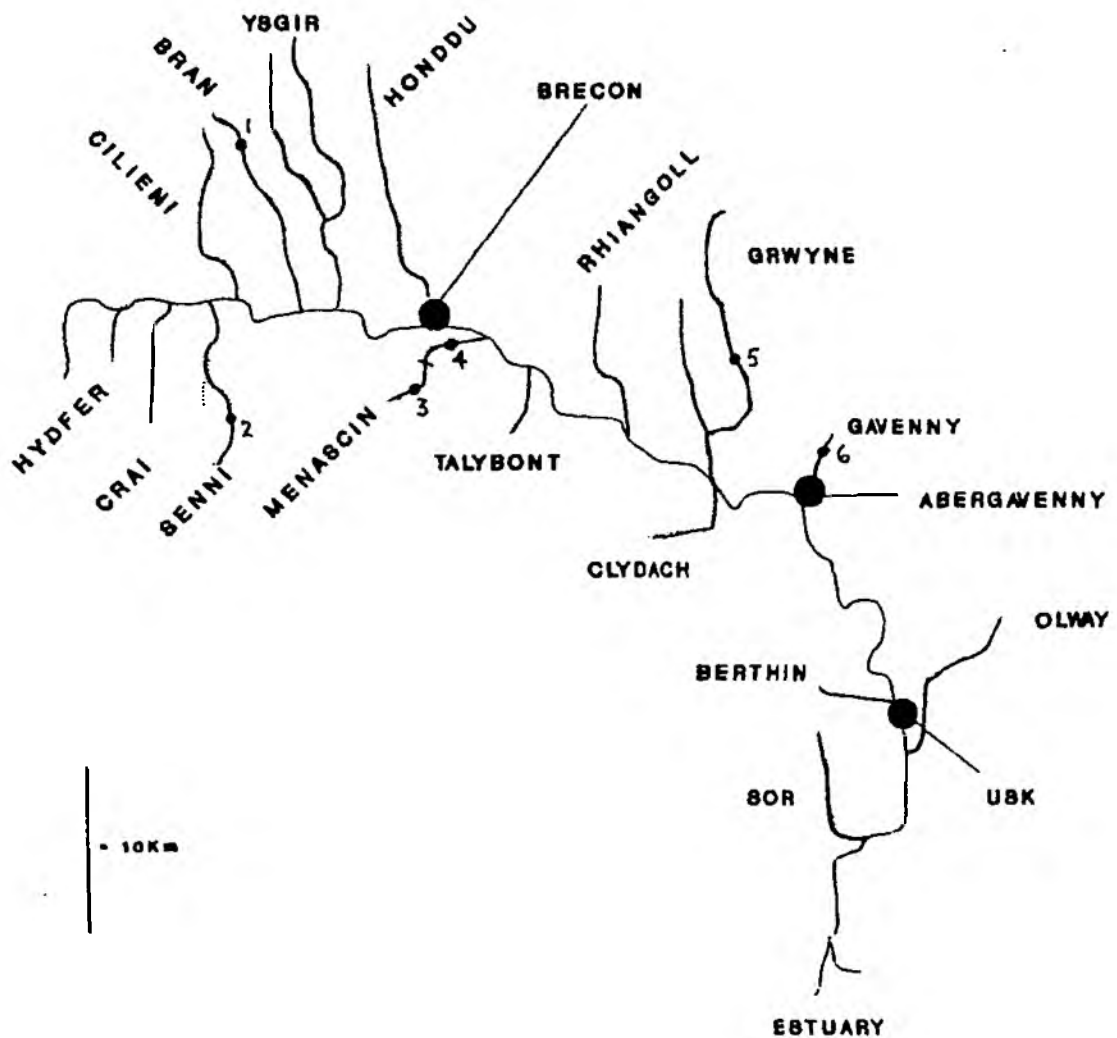
Genotype I was found at least once in every sample, and made up 100% of the Grwyne, Gavenny, Bran and Menascin (above weir) samples. Genotypes III and IV each occurred in a single fish in the Senni and Menascin (below weir) samples respectively. Genotype II occurred in 1 Wye fish and in 4 out of 6 Senni fish, but nowhere else on the Usk. Genotype V occurred only in the Wye sample.

Conclusions and work in progress

The work has so far shown an encouraging degree of heterogeneity within this lightly stocked catchment. The results from the River Senni (along with supporting data on the fecundity and maturation characteristics of the stock) indicate that this may be a sub-stock with its own genetic characteristics.

Further samples for mtDNA restriction work have been taken from the Senni, Wye (Hiernant) and also from some previously unstudied Usk tributaries. Tissue samples from these fish are being stored for DNA fingerprinting work using synthetic oligonucleotide probes.

MAP OF RIVER USK



A study of genetic variation in trout (*Salmo trutta* L.) in Welsh rivers.

Heather J. Hall

*University College London, Department of Biology,
Medawar Building, Gower St., London WC1E 6BT*

Introduction

The aim of this study is to carry out a genetic survey of trout, *Salmo trutta* L., in Welsh rivers, in order to establish the degree of variation among and within populations from different river systems.

Trout were sampled from seven rivers which are considered among the most important for trout in Wales, and which also cover a wide geographic range. Trout were collected by electrofishing a 50-100m stretch of river, and killed with an overdose of benzocaine. In most cases the fish were kept on ice until reaching the laboratory, where the liver and heart were removed and stored at -70°C. A summary of the sampling programme is outlined in Table I.

Table I. A summary of the rivers and sites sampled in this study.

RIVER	TRIBUTARY	GRIDREF.	NO.FISH
DEE	Hirnant	SH 958 334	10
	Hirnant	SH 949 366	10
	Meloch	SH 962 394	45
	Meioch	SH 956 378	10
	Mynach	SH 911 418	48
	Ceidiog	SJ 27 343	10
	BRAINT	Mynach	SH 668 454
CRIGYLL	Dic	SH 680 481	10
	Dic	SH 682 483	10
	Cymunod	SH 775 341	10
WNION	Cae Hywel	SH 771 338	10
	Treban	SH 772 364	10
	Clywedog	SH 188 753	10
MAWDDACH	Newydd	SH 199 759	10
	Hengwt	SH 222 796	10
	Ceirw	SH 284 766	10
TEIFI	Gain	SH 310 733	10
	Eden	SH 303 910	10
	Brenig	SN 674 590	10
	Groes	SN 702 606	9
	Brefi	SN 681 546	9
	Creuddyn	SN 571 482	10

contd.

	Grannell	SN 516 509	10
	Cledlyn	SN 502 455	10
	Banc	SN 355 418	10
	Bargoed	SN 358 380	10
	Cych	SN 270 376	10
	Piliiau	SN 181 441	10
TYWI	Nant y Ffin	NS 787 473	10

Allozyme Work

This work has been limited to the River Dee. Of fourteen enzymes tested in trout muscle and liver tissues, seven were successfully resolved. Two of these were monomorphic (PGM, ME) and the other five were polymorphic (GPI, G3PDH, MDH, AAT, LDH). Significant differences were found between two sites on the Dee for the enzymes GPI and MDH.

Mitochondrial DNA Analysis

The use of restriction fragment polymorphisms (RFLP) to detect genetic variation in the mitochondrial genome has proved to be technically difficult. Rapid techniques for the isolation and purification of mtDNA resulted in very low yields which were still contaminated. The use of caesium chloride gradients can produce pure DNA, but this technique is so time consuming that it considerably limits a large study of this kind. This approach was therefore abandoned.

The polymerase chain reaction (PCR) can be used to amplify a specific DNA sequence from the mitochondrial (or nuclear) genome. For this research, the control region was chosen as an area of the mtDNA genome that has been widely reported as highly variable and suitable for intrapopulation studies. This region was amplified using conserved primers from a number of different trout from all the rivers that have been sampled. The control region was then sequenced using double stranded sequencing techniques, and the sequences recorded and analysed using a sequence analysis computer program.

At present, the mtDNA control regions from fourteen trout have been amplified and sequenced. The evidence so far suggests that the variation between different trout populations in the different rivers is negligible. Controls run at all stages of the experiments have shown that this is not due to contamination. A possible explanation is that there has been a lack of time for significant evolutionary diversification since trout recolonised Welsh rivers after the last ice age approximately 10 000 years ago.

The use of PCR and direct sequencing provides a relatively easy means of obtaining sequence data from a large number of trout. These procedures have significant advantages over other genetic techniques: as PCR can amplify mtDNA from very small amounts of tissue, sequence data can be obtained by non-lethal sampling techniques, such as adipose fin clipping.

Future Work

The sequence data will be completed and extended to include samples of salmon, and trout from other areas of the U.K. to obtain a measure of the levels of variation. In addition, the cytochrome b gene of the mtDNA genome will be amplified and sequenced to provide additional data.

Professor of Biochemistry

E. G. Brown, Ph.D., D.Sc., C.Chem., F.R.S.C. (295374)

Professors of Genetics

J. A. Beardmore, Ph.D., C.Biol., F.I.Biol. (295382)

J. M. Parry, Ph.D., D.Sc. (295385)

Professor of Marine Biology

J. S. Ryland, Ph.D., D.Sc., F.I.Biol. (295440)

Professors of Zoology

P. F. Brain, Ph.D., F.I.Biol. (295444)

N. A. Ratcliffe, Ph.D., D.Sc., F.I.Biol. (295454)



UNIVERSITY COLLEGE OF SWANSEA

Singleton Park
Swansea SA2 8PP
Wales U.K.

Telephone: 0792 205678
Telex: 48149 UICS G
Fax: (0792) 295447 (School)
Fax: (0792) 295618 (Campus)

SCHOOL OF BIOLOGICAL SCIENCES

Head of School

Professor J. A. Beardmore, Ph.D., C.Biol., F.I.Biol. (Tel: 0792 295382)

GENETICS OF WELSH SALMON - M. O'CONNELL

Introduction

This Ph.D. represents part of a three year research program funded by the NRA. The main aim is to investigate the presence of salmon stocks between and within the major salmon rivers in Wales. To date the Wye, Usk, and Teifi have been sampled representing over twenty individual sites.

Materials and Methods

Included in this report are a total of fifteen sites from the three aforementioned rivers. The Wye sampling sites are;

Llynfi (Talgarth)	SO 146 336
Wye (Glasbury)	SO 180 394
Garth Dulas (Dolderwen)	SN 946 514
Cledan (Glanchedan)	SN 867 448
Edw (Hundred Hse.)	SO 114 544

The Usk sampling sites are;

Usk	SN 858 278
Senni (Abersenni)	SN 931 206
Cilieni	SN 913 313
Menascin	SN 077 257
Grywne	SO 241 223
Ysgir (Fawr)	SN 995 372
Ysgir (Fechan)	SN 995 334
Tarrell (Abergwdi)	SO 027 277
Cynrigg (Hatchery)	SO 066 265

Pontrhydfendiag represents the single site sampled within the Teifi. Fish of approximately 10cms were taken back to the lab and fresh liver and heart were used as a source of mtDNA. The mtDNA was extracted using conventional CsCl gradients and then digested with two four base cutters, MboI and HinfI, end-labelled using ³²P and run on a five percent acrylimide gel. Comparisons between gels could be made by reference to marker fragments of a known size.

Results

Using HinfI a total of five different genotypes have been noted with two being restricted to the Wye and one to the Teifi. With MboI three different patterns have so far been observed. Due to the relatively low sample sizes (Av. sample size is 5.4) a Nass's χ^2 was carried out for both enzymes. Initially tests for differences in genotype frequency were performed over all test sites for the two enzymes separately. Tests proved to be highly significant for both enzymes.

HinfI

MboI

$\chi^2=130.242^{***}$ dF 56

$\chi^2=54.636^{***}$ dF 28

Nass's $\chi^2=55.346^{***}$ dF 24.1 Nass's $\chi^2=72.836^{***}$ dF 37.874

Test for significance between Usk, Wye, and Teifi;

HinfI

MboI

$\chi^2=24.314^{**}$ dF 8

$\chi^2=26.018^{***}$ dF 4

Nass's $\chi^2=18.116^{**}$ dF 6.026 Nass's $\chi^2=28.121^{***}$ dF 4.385

Test for significance between tributaries within the Usk;

HinfI

MboI

$\chi^2=5.669$ dF 8 N.S.

$\chi^2=17.708$ dF 16 N.S.

Nass's $\chi^2=2.869$ dF 4.123 N.S. Nass's $\chi^2=17.558$ N.S.

Test for significance between tributaries within the Wye;

HinfI

MboI

$\chi^2=35.889^{***}$ dF 12

$\chi^2=8.881^*$ dF 8

Nass's $\chi^2=41.86^{***}$ dF 14.5 Nass's $\chi^2=13.664$ dF 13.128 N.S.

Discussion

Both enzymes tested show a surprising amount of variation. Within the Usk, although both enzymes were variable neither suggested the presence of sub-stocks within the catchment as a whole.

With the analysis of the Wye, significance was noted between HinfI genotypes within the drainage. One observed genotype, designated E, has only been observed within the Edw, suggesting the possible presence of a distinct sub-stock in this tributary. Another variant, genotype C has only been found to date within the Wye catchment and thus may prove to be a useful marker. Finally, although only one site has been looked at on the Teifi, this population appeared to have a unique HinfI genotype designated D. Although this has to be repeated for validation it may also prove to be a useful river-specific marker.

We have also conducted a preliminary series of experiments with a new technique, Polymerase Chain Reaction (PCR) finger-printing and are beginning to get encouraging results. We have also tested DNA extracted from Usk samples and have obtained interpretable DNA fingerprints. Thus it is planned that a limited set of experiments will be carried out, using single locus probes, to assay variation in certain populations.

MAFF Atlantic Salmon Genetics Programme

D. Thompson, D. King, S. Hovey

Objectives:

When this programme commenced in 1988 the initial problem we addressed was to what extent do the salmon stocks of different rivers in England and Wales represent genetically distinct and isolated populations.

We decided that two further problems, firstly, what would the consequences of mixing different populations, either in a stock enhancement programme or as a consequence of escapes of hatchery reared fish from fish farms, and secondly, to what extent would genetic variation within a population be lost by poor husbandry practices in hatchery-based stock enhancements could only be answered when information was available on the degree of genetic differentiation between populations and the levels of genetic variation within populations.

Progress:

Two techniques, each at a different stage of development and application, were adopted. These were the established techniques of gel electrophoresis and allozyme frequency variations and the more pioneering restriction fragment length polymorphism analysis of mitochondrial DNA.

Allozyme frequency data have been obtained for 15 different rivers in England and Wales. Where possible more than one site and/or tributary have been sampled for each river system and for some rivers more than one year class have been sampled. (Table 1)

Allozyme frequency data for three polymorphic loci for the 15 rivers are reported. Lower levels of polymorphism have been observed at two other loci; nine loci have been found to be monomorphic. Data have been pooled where no significant differences occurred between samples collected from different sites on the same day or between different year classes in any of the samples.

Differences between rivers occur at all three loci although the major differences are at the AAT-4 locus. The chalk streams of the South of England differ from all other sampled rivers.

There is considerable variation in allozyme frequencies between the rivers in the South-West region of England. The rivers of North Wales and the North of England have similar allozyme frequencies which are distinct from those of the previously mentioned areas. Differences also occur, in some instances, between sites on the same river.

At present there are gaps in the data which can be filled by more sampling in 1991. This is particularly true for the South West of England.

We are interested in sampling from other areas provided there are no clashes of interest between NRA funded projects and ourselves.

With respect to the mt.DNA study, differences in the frequency of occurrence of particular material lines have been found both between two rivers and between spawning sites on one river. We are continuing with this study at Lowestoft and hope to extend our research to include behavioural studies on the Rivers Test and Itchen.

TABLE 1

SALMON POPULATIONS SAMPLED DURING GENETICAL STUDIES 1987-1990

RIVER	SAMPLE NO.	DATE	N	AGE	COMMENTS
ITCHEN	1	Sept 1988	77	"0"parr	Site 1
	2	Sept 1988	95	"0"parr	Site 2
	3	April 1989	52	"1"&"2"smolts	Sites 2,3,4&5
	4	Sept 1989	50	"0"&"1"parr	
	5	March 1990	40	"1"smolts	Sites 4&5
TEST	1	Oct 1989	55	"0"&"1"parr	Sites 1,2,3&4
	2	Oct 1990	48	"0"&"1"parr	Sites 1,2,3,4&5
FROME		Oct 1989	43	"0"parr	
TEIGN	1	June 1990	25	"1"parr	Site 1
	2	June 1990	25	"1"parr	Site 2
LYD (TAMAR)	1	Oct 1990	25	"0"parr	Site 1
	2	Oct 1990	25	"0"parr	Site 2
LYNHER	1	Oct 1990	25	"0"parr	Site 1
	2	Oct 1990	25	"0"parr	Site 2
CAMEL	1	June 1990	24	"1"parr	Site 1
	2	June 1990	26	"1"parr	Site 2
OKEMENT (TORRIDGE)		Oct 1990	53	"0"parr	
TAW		Oct 1990	53	"0"&"1"parr	
RHIW (SEVERN)		Sept 1990	50	"0"parr	
TANAT (SEVERN)	1	May 1990	60	"0"&"1"parr	Site 1
	2	Sept 1990	50	"0"parr	Site 1
CONWY	1	Nov 1987	25	"0"parr	Site 1
	2	Aug 1989	60	"0"parr	Site 1
	3	Aug 1989	50	"0"parr	Site 2
HODDER (RIBBLE)	1	Nov 1988	50	"0"parr	Site 1
	2	Sept 1989	50	"0"parr	Site 1
RIBBLE		Sept 1989	50	"0"parr	
LUNE	1	Nov 1988	56	"0"parr	Site 1
	2	Sept 1989	49	"0"parr	Site 1
CRAKE	1	Nov 1988	59	"0"parr	
	2	Nov 1989	44	"0"parr	
	3	Sept 1990	49	"0"parr	
CALDEW (EDEN)		Sept 1989	50	"0"parr	