GENETIC STOCK IDENTIFICATION
OF THE CROMANE SALMON FISHERY 2010

CLIENT CONFIDENTIAL REPORT

TO INLAND FISHERIES IRELAND

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Background and aims

The Minister of State at the Department of Communications, Energy & Natural Resources requested advice on how a commercial salmon fishery could be operated on stocks in the Castlemaine Harbour Special Area of Conservation in a sustainable manner, maximizing the opportunities for commercial fishing whilst ensuring that stocks are not overexploited. In this context, a pilot fishery was operated, under section 18 of the Fisheries Act 1980, in a particular area of Castlemaine Harbour in order to determine exploitation rates of the various stocks in the fishery. The results of this analysis are intended to inform management in terms of operating a commercial fishery while safeguarding the spawning requirements of the different rivers in Castlemaine Harbour.

The specific aims of the present research were to identify the proportion of salmon from the rivers entering the Castlemaine Harbour/Dingle Bay area (Behy, Caragh, Laune, Maine, Emalgh and Owenascaul) and, if present, rivers from outside this area, in catches from the Cromane fishing area. The results were to be presented on a temporal (to investigate whether stock proportions vary over the fishing season) and spatial (to examine whether stock proportions vary between different areas of the fishery) basis.

Contributors from the Beaufort Fish Genetics Research Group to this report were as follows: Post Doctoral Fellow: Dr E Dillane (Laboratory analysis), Research assistant: Ms. Mary Cross (Laboratory analysis), Senior Researcher: Dr J. Coughlan (Statistical Analysis and initial report drafting), Principal Investigator: Dr P McGinnity (Report drafting and editing), Scientific co-ordinator: Professor TF Cross (Report drafting and editing).

Executive summary

A total of 783 Atlantic salmon individuals caught in the Cromane fishery between the 11th June and 27th August 2010 were genetically typed for variation at fifteen microsatellite DNA loci. These were tested against a baseline of approximately 8300 individuals from 160 Irish Atlantic salmon populations sampled from 104 rivers located throughout Ireland.

Taken at face value, the results from analysis of the fishery as a whole indicate that approximately 93.5% of the fishery is made up of stocks from Castlemaine Harbour rivers with the Laune (64.4%), Maine (18.2%) and Caragh (5.6%) being to most substantial contributors. Small contributions from the Behy, Emlagh and Owenascaul were observed in the fishery as a whole. It is noted, however, that analysis of temporal stability in the samples taken from the Behy and Emlagh indicates that these rivers may have low genetic integrity over generations (see Temporal Stability report to the IFI 2011). We recommend assessment of the Owenascaul river for temporal stability.

Approximately 2% of the fishery appeared to be originating from rivers close to but outside Castlemaine Harbour (Inny, Ferta and Tralee Lee in particular), whereas 3.8% appeared to originate from further distant locations (3.4% to Waterford/Lismore rivers and 0.4% to Donegal). However, analysis of simulated fishery samples suggests that these apparent contributions from rivers outside Castlemaine Harbour may result from experimental error associated with mis-assignment from correct population of origin.

Analysis of the fishery samples on a temporal basis appears to indicate substantial variations in proportions of Caragh and Maine fish in particular, throughout the fishing season. The proportion of Caragh origin fish was highest in the samples taken in June (17.7%) and lowest in the August samples (1.9%) suggesting that this river is composed of predominantly early-run fish. However, the proportion of Maine fish was lowest in June (5.5%) and highest in August (22.4%) suggesting that Maine fish run later into the fishery. There appeared to be less variation in stock proportions between the different fishing areas although there was slightly higher proportions of Caragh fish in areas A and

B (6.9%) compared to C, D and E (5%) and conversely slightly less Maine fish in areas A and B (17.3%) compared to C, D and E (18.5%). This suggests fishing time may be more important than fishing area when considering management of these stocks.

The results of all analyses (using mixed stock fishery proportions and individual assignment approaches) are presented without making any judgement with respect to management concerns. Additional supporting information (catch details and assignment proportions to baseline rivers for each individual fish) is also supplied on the attached spreadsheet so that more detailed analysis can be undertaken if desired. We are available for consultation or clarification with respect to any of the work presented in this report.

Methods and analysis

Sampling and genotyping

A total of 785 tissue samples (gills and/or muscle stored in alcohol) from individual fish were delivered to University College Cork on 2nd September 2010 by Anthony Holmes. Details for each sample (capture date, area of capture, length, weight and sex (where known) were also provided on a spreadsheet. The entire fishery sample included fish which were caught between the 11th June and 27th August 2010 in the different fishing areas presented in Figure 1.

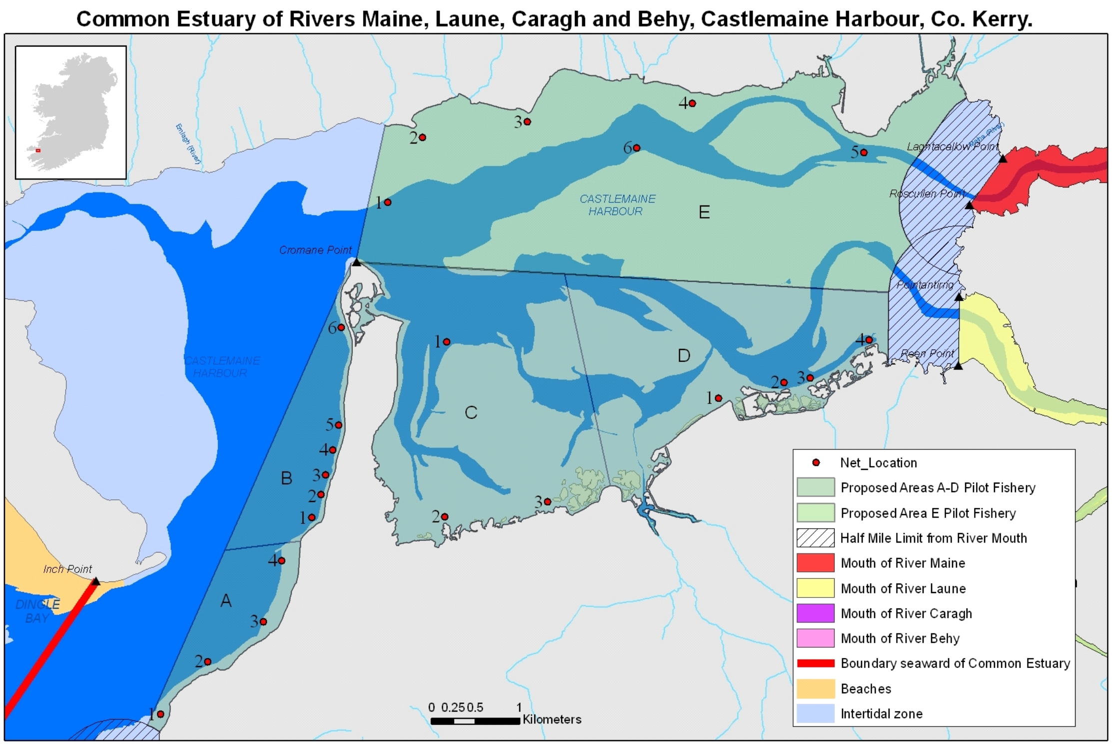

Figure 1 Locations of 2010 Castlemaine Harbour pilot fisheries.

DNA from each sample was extracted and quantified using the methods described in Appendix 1. The DNA from each sample was used to amplify 15 microsatellite DNA loci which were genotyped on a Licor DNA autosequencer (screening methods also described in the appendix). Genotypes at each locus for each individual were scored by eye and double-checked to ensure accuracy. A subset of samples (approximately 12%) were re-screened to ensure that scoring was consistent and accurate (no scoring errors were detected). Among the samples, one trout (Tag number E056, area E, 6th July) and one salmon/trout hybrid (Tag number B076, area B, 24th June) were identified based on DNA profiles. These were excluded from further analysis. Among the remaining salmon samples, data quality was very high in that all samples were scored for at least 13 of the 15 DNA loci (in fact 758 (97%) were scored for all loci).

Mixed stock fishery analysis (MSA) and individual assignment (IA)

The software package cBAYES was used here to estimate the proportions of stocks contributing to the Cromane fishery (MSA) as revealed by the samples provided above. The program was also used to assign each individual fish to river of origin (IA). The samples were tested against the National genetic stock identification (NGSI) baseline which currently consists of over 8300 individuals from 160 populations (or sampling locations/temporal replicates) from 104 rivers located across the Irish Republic. Each population sample was typically composed of a minimum of 48 individuals. For rivers most likely to contribute (being geographically adjacent) to the Cromane fishery, the baseline includes samples from the Behy (two temporal samples), the Caragh (two population samples), the Laune (eight population samples), the Maine (two population samples), the Emlagh (two temporal samples) and the Owenascaul (one population sample).

To validate and assess the potential accuracy of MSA and IA for the Cromane fishery, a simulated fishery sample composed of individuals in varying proportions from rivers in the Castlemaine Harbour area was constructed. Details of the results of MSA and IA analysis of this sample are presented in Appendix 2. The simulated sample revealed that the baseline used here could correctly estimate stock proportions of Castlemaine Harbour rivers in a real fishery with a high degree of accuracy. The results of MSA of the 783 individuals sampled from Cromane salmon fishery 2010 (total fishery sample) are presented in Table 1.

Table 1 MSA results of the Cromane salmon fishery 2010 sample (783 fish) showing estimated proportions of each river in the NGSI baseline as revealed by cBAYES (SD is the standard deviation for each estimate) Bold values are significant contributors (proportions capable of indicating the presence of one whole fish).

		MSA	SD			MSA	SD
1	Castletown	-	0.0002	43	Kilcogan	-	0.0002
2	Fane	-	0.0002	44	Corrib	0.0002	0.0005
3	Glyde	-	0.0003	45	Owenboliska	-	0.0002
4	Dee	0.0001	0.0004	46	Cashla	-	0.0002
5	Boyne	0.0002	0.0007	47	Screebe	-	0.0002
6	Liffey	0.0002	0.0006	48	Owenmore	0.0001	0.0003
7	Dargle	0.0001	0.0007	49	Dawros	-	0.0002
8	Vartry	-	0.0002	50	Culfin	-	0.0002
9	Avoca	-	0.0003	51	Erriff	0.0002	0.0008
10	Slaney	0.0001	0.0004	52	Bundorragh	0.0001	0.0006
11	SEPC	**0.0339**	**0.0130**	53	Bunowen	0.0008	0.0026
12	Lee	0.0005	0.0018	54	Newport	-	0.0002
13	Bandon	0.0004	0.0022	55	Srahmore	-	0.0002
14	Ilen	-	0.0002	56	Owenduff	0.0001	0.0003
15	Owvane	-	0.0002	57	Owenmore	0.0001	0.0006
16	Glengariff	-	0.0002	58	Cloonaghmore	-	0.0002
17	Adrigole	-	0.0002	59	Moy	0.0010	0.0023
18	Croanshagh	-	0.0002	60	Brusna	-	0.0003
19	Owenshagh	-	0.0002	61	Easkey	-	0.0003
20	Cloonee	-	0.0003	62	Ballysadare	0.0001	0.0003
21	Sheen	-	0.0002	63	Garvogue	-	0.0002
22	Roughty	0.0001	0.0003	64	Duff	0.0004	0.0010
23	Finnihy	-	0.0002	65	Drowes	-	0.0002
24	KerryBlackwater	-	0.0002	66	Erne	-	0.0002
25	Sneem	-	0.0002	67	Abbey	0.0001	0.0004
26	Owreagh	-	0.0002	68	Laghy	-	0.0002
27	Currane	0.0001	0.0004	69	Eske	-	0.0002
28	Inny	**0.0021**	**0.0027**	70	Eany	0.0001	0.0003
29	Ferta	**0.0066**	**0.0044**	71	Glen	-	0.0002
30	Behy	**0.0318**	**0.0134**	72	Owenwee	-	0.0002
31	Caragh	**0.0557**	**0.0099**	73	Owenea	**0.0044**	**0.0039**
32	Laune	**0.6435**	**0.0371**	74	Gweebarra	-	0.0002
33	Maine	**0.1820**	**0.0293**	75	Gweedore	-	0.0002
34	Emlagh	**0.0051**	**0.0063**	76	Clady	-	0.0002
35	Annascaul	**0.0170**	**0.0062**	77	Lackagh	-	0.0003
36	Milltown	0.0001	0.0003	78	Leannan	0.0001	0.0003
37	Feoghanagh	-	0.0003	79	Swilly	-	0.0003
38	TraleeLee	**0.0110**	**0.0130**	80	Crana	-	0.0002
39	Feale	0.0001	0.0003	81	NorthernIreland	0.0002	0.0009
40	Galey	-	0.0002	82	Aquagen	-	0.0002
41	Maigue	0.0001	0.0003	83	Fanad	-	0.0002
42	Mulcair	0.0001	0.0005				

As expected from previous genetic studies of the Cromane fishery (2006 and 2008), the 2010 fishery sample was dominated by contributions from the Laune (in particular), Maine and Caragh. The total contribution of Castlemaine Harbour rivers (Behy, Caragh, Laune, Maine, Emlagh and Owenascaul) to the fishery was just over 93.5% and included small contributions from the Behy, Emlagh and Owenascaul. Minor contributions were also revealed for rivers nearby but outside Castlemaine Harbour, the Inny, Ferta and Tralee Lee. There were also significant proportions of fish originating from the SEPC area (Waterford and Lismore) and from the Owenea in Donegal. The overall proportions of these were however, quite low and within the tolerance range of mis-assignment as determined from analysis of the simulated sample (i.e. these proportions may be over-estimated due to incorrect assignment).

The MSA output proportions were then divided based on different criteria (time, space and time-space). In this respect, samples were classified by date of capture (June, July or August), area of capture (A, B, C, D or E, see Figure 1) and by date of capture for each area (e..g. June-area A etc.). MSA proportions of fishery samples collected in June, July and August, and in each area, respectively are presented in Table 2. Stock estimates in terms of capture date in each area are presented in Table 3. Examination of these proportions reveals substantial temporal variations in the contributions of Caragh and Maine stocks, in particular, throughout the fishing season. Caragh stocks appear to have highest proportions in the fishery during June (17.7%) which then decrease to 7.9% in July and 1.9% in August. In contrast, Maine stocks appear to have lowest proportions in June (5.5%) but have increased representation in the July (15.1%) and August (22.4%). These results seem to indicate that Caragh fish are more likely to be encountered in the fishery early in the season whereas Maine fish appear to run later. The Behy seems to show a similar distribution of proportions in the fishery as the Maine although the overall contribution of the Behy to the fishery is much lower. There appears to be substantially less variation of stock proportions in each separate fishing area although there is some indication that proportional contribution of stocks is influenced by the closeness of contributing rivers to a particular fishing area (e.g. A and B, versus C, D and E).

Table 2 MSA results of the Cromane salmon fishery 2010 indicating estimated proportions for each river in the Castlemaine Harbour area and for rivers outside this area demarcated by month and area capture.

		Outside Castlemaine Harbour	Behy	Caragh	Laune	Maine	Emlagh	Owenascaul
	n							
June	**78**	0.019	0.000	0.177	0.743	0.055	0.005	0.002
July	**271**	0.067	0.019	0.079	0.654	0.151	0.002	0.028
August	**434**	0.062	0.045	0.019	0.630	0.224	0.007	0.013
A	**127**	0.082	0.054	0.087	0.583	0.185	0.005	0.005
B	**133**	0.060	0.025	0.053	0.671	0.168	0.006	0.017
C	**187**	0.048	0.018	0.063	0.682	0.165	0.003	0.020
D	**162**	0.050	0.042	0.021	0.641	0.225	0.007	0.014
E	**174**	0.062	0.029	0.058	0.651	0.170	0.005	0.026

Table 3 MSA results of the Cromane salmon fishery 2010 indicating estimated proportions for each river in the Castlemaine Harbour area and for rivers outside this area demarcated by month of capture in each area.

		Outside Castlemaine Harbour	Behy	Caragh	Laune	Maine	Emlagh	Owenascaul
	n							
June-A	**15**	0.046	0.000	0.366	0.506	0.074	0.000	0.008
July-A	**39**	0.116	0.022	0.049	0.613	0.191	0.002	0.008
August-A	**73**	0.072	0.075	0.049	0.590	0.205	0.007	0.002
June-B	**29**	0.010	0.001	0.171	0.781	0.024	0.013	0.000
July-B	**31**	0.055	0.005	0.064	0.656	0.209	0.001	0.009
August-B	**73**	0.082	0.044	0.002	0.633	0.207	0.006	0.027
June-C	**12**	0.012	0.000	0.138	0.841	0.009	0.000	0.000
July-C	**78**	0.062	0.015	0.099	0.639	0.142	0.001	0.032
August-C	**97**	0.042	0.015	0.025	0.697	0.204	0.005	0.013
June-D	**16**	0.013	0.000	0.043	0.856	0.086	0.000	0.001
July-D	**25**	0.047	0.036	0.040	0.717	0.092	0.001	0.066
August-D	**121**	0.056	0.049	0.015	0.597	0.270	0.009	0.004
June-E	**6**	0.025	0.000	0.167	0.647	0.162	0.000	0.000
July-E	**98**	0.061	0.015	0.088	0.665	0.139	0.004	0.028
August-E	**70**	0.066	0.051	0.006	0.632	0.213	0.007	0.024

Where detailed stock of origin information is required for individual fish in a mixed stock fishery, it is possible to investigate individual assignment scores. For example, in a hypothetical fishery sample consisting of 100 fish with stock contributions from rivers A (90%), B (5%) and C (5%), interrogating IA output scores can indicate which individual fish came from populations A, B or C and also the degree of probability associated with each assignment. As indicated from the analysis of the simulated sample in Appendix 2, higher IA scores are associated with increased accuracy whereas as scores become lower, identifying river of origin correctly becomes less certain. IA scores for each fish in the Cromane salmon fishery 2010 are provided in the attached spreadsheet and a summary of the number of individual samples (including a proportion of the total fishery) are presented in Table 4.

Table 4 Numbers of individuals with assignment scores in specific ranges for the Cromane salmon fishery 2010. (The number of individuals in each assignment score range is also given).

IA score	n	Propn. of total
100	168	0.215
95-100	386	0.493
90-100	472	0.603
80-100	567	0.724
70-100	645	0.824
66-100	665	0.849
50-100	742	0.948
0-49	41	0.052

The majority of IA assignment scores for the Cromane salmon fishery 2010 are greater than 50% and analysis of the simulated sample indicates that these assignments are likely to be correct in 96% of cases. Therefore, results of individual assignment of samples based on the whole fishery, by month of capture, by area of capture and by month of capture in each area, are presented in Tables 5 and 6. Due to the high proportion of high assignment scores in the fishery (as presented in Table 4), it was neither expected or observed that fishery proportions based on IA would differ substantially from the MSA proportions *per se* as given in Table 1.

Table 5 Individual assignment of Cromane salmon fishery 2010 samples to river of origin for the whole fishery and demarked by month and area of capture. Numbers assigning to each stock, expressed as proportions, are given in

	n	**SEPC**	**Inny**	**Ferta**	**Behy**	**Caragh**	**Laune**	**Maine**	**Emlagh**	**Owenascaul**	**Tralee Lee**	**Owenea**
All	**783**	17 (0.022)	2 (0.003)	4 (0.005)	25 (0.032)	30 (0.038)	468 (0.598)	138 (0.176)	4 (0.005)	11 (0.014)	3 (0.004)	3 (0.004)
June	**78**	1 (0.013)	- (0.000)	- (0.000	- (0.000)	14 (0.179)	58 (0.744)	4 (0.051)	1 (0.013)	- (0.000)	- (0.000)	- (0.000)
July	**271**	9 (0.033)	1 (0.004)	1 (0.004)	5 (0.018)	21 (0.077)	185 (0.683)	40 (0.148)	- (0.000)	6 (0.022)	1 (0.004)	2 (0.007)
August	**434**	8 (0.018)	(0.002)	3 (0.007)	20 (0.046)	9 (0.021)	283 (0.652)	98 (0.226)	4 (0.009)	5 (0.012)	2 (0.005)	1 (0.002)
A	**127**	6 (0.047)	- (0.000)	- (0.000)	7 (0.055)	11 (0.087)	75 (0.591)	25 (0.197)	1 (0.008)	- (0.000)	2 (0.016)	- (0.000)
B	**133**	2 (0.015)	2 (0.015)	1 (0.008)	3 (0.023)	7 (0.053)	94 (0.707)	20 (0.150)	2 (0.015)	2 (0.015)	- (0.000)	- (0.000)
C	**187**	3 (0.016)	- (0.000)	2 (0.011)	3 (0.016)	12 (0.064)	134 (0.717)	28 (0.150)	- (0.000)	3 (0.016)	1 (0.005)	1 (0.005)
D	**162**	3 (0.019)	- (0.000)	1 (0.006)	6 (0.037)	4 (0.025)	106 (0.654)	39 (0.241)	1 (0.006)	2 (0.012)	- (0.000)	- (0.000)
E	**174**	4 (0.023)	- (0.000)	- (0.000)	6 (0.034)	10 (0.057)	117 (0.672)	30 (0.172)	1 (0.006)	4 (0.023)	- (0.000)	2 (0.011)

Table 6 Individual assignment of Cromane salmon fishery 2010 samples to river of origin demarked by capture month in each area of capture.

	n	SEPC	Inny	Ferta	Behy	Caragh	Laune	Maine	Emlagh	Owenascaul	Tralee Lee	Owenea
June-A	**15**	1				6	7	1				
July-A	**39**	3			1	1	24	9			1	
August-A	**73**	2			6	4	44	15	1		1	
June-B	**29**					5	23		1			
July-B	**31**		1			2	22	6				
August-B	**73**	2	1	1	3		49	14	1	2		
June-C	**12**					2	10					
July-C	**78**	2		1	2	8	53	9		2		1
August-C	**97**	1		1	1	2	71	19		1	1	
June-D	**16**						14	2				
July-D	**25**	1				1	18	3		2		
August-D	**121**	2		1	6	3	74	34	1			
June-E	**6**					1	4	1				
July-E	**98**	3			2	9	68	13		2		1
August-E	**70**	1			4		45	16	1	2		1

However, stock proportions revealed by using the IA assignment method compared to MSA differ in one important respect. The total proportion of fish now assigning to the SEPC rivers is lowered from 3.4% to 2.2%, (possibly still higher than expected-see Appendix 2). The proportion of stocks from other rivers outside Castlemaine Harbour also tends to be reduced.

It is possible therefore, that using the IA method gives a slightly more realistic indication of fishery proportions than the MSA approach. In addition, with further more detailed simulations (including fish from populations across Ireland), it should be possible to define a probability of assignment cut-off level, so individuals that fail to be assigned above that level can be excluded. If such a probability cut-off level does not result in significantly different proportions in the simulated samples, then this could be used to further define not only presence of stocks in the fishery with a very high degree of confidence but also improved estimates of stock proportions.

Notes for interpretation of MSA and IA data to aid management

Previous studies of the Cromane fishery

Our previous analysis of Cromane fishery samples in 2006 and 2008 largely agree with the findings presented here. The 2006 fishery (n = 224 caught in June and July) as presented in the NGSI report suggested that approximately 94% of the catch was composed of contributions from Castlemaine Harbour rivers and was dominated by the Laune (78.4%) and the Caragh (15.4%). There was also a small apparent contribution from the SEPC (Waterford and Lismore) rivers detected at that time. The proportion of Maine fish at that time was very low (0.2%). The 2008 fishery (n = 369 mostly caught in July and fishing areas included the Back Beach and Caragh River only) was also dominated by contributions from the Laune and the Caragh and, although there was a small number of Maine and Owenascaul fish, these were only detected among samples collected towards the end of July.

Apparent assignment of fish to rivers outside Castlemaine Harbour

Our current understanding of regional stock structure of Atlantic salmon in Irish rivers suggests that some rivers from Castlemaine Harbour (the Laune in particular) have a shared ancestry with rivers from the SEPC area. This is unusual since other rivers in southwest Ireland generally cluster with other south-western or western Ireland stocks. However, this may also result in mis-assignment of a small number of fish between these areas as indicated from the analysis of the simulated fishery sample. Therefore, it is speculated that at least some of the fish identified in the 2010 Cromane fishery as being of SEPC river origin may be considered as mis-assigned and the real proportion of SEPC fish in the Cromane fishery is likely to be lower than reported here.

There is less certainty associated with the samples that assign to the Tralee Lee, Inny, Ferta and, in particular, the Donegal Owenea. It is conceivable that fish from the Kerry rivers would be present in the Cromane area, although the presence of Donegal fish is unlikely. It is speculated that the Owenea fish have been mis-assigned due to their having rare alleles or genotypes not encountered in the Castlemaine Harbour portion of the baseline samples as included here. In addition, it may be that not all populations present in Castlemaine Harbour rivers have been sampled. The samples used here demonstrate that the Laune in particular has substantial within river population structure and it may be that not all these populations have been identified or described. If there is undetected structure or unsampled populations within Castlemaine Harbour rivers, fish originating from these other populations may not assign to other local populations. Increasing the baseline sizes of the Laune, in particular but also the Caragh and Maine might reveal additional population structure and would also increase the likelihood of detecting rare alleles which may help to resolve this issue and will improve assignment precision and accuracy within the Castlemaine Harbour area.

Temporal stability and population integrity

The two population samples from both the Behy and Emlagh rivers included in the baseline of this study samples show significant evidence of a high degree of temporal instability suggesting that these rivers have low population size and inter-annual integrity. Therefore, these rivers may constitute genetically ephemeral populations related to varying and typically small numbers of spawners in each generation (number of effective breeders was estimated to be 14 and 25 for the two Emlagh temporal replicates, respectively, and 8 and 123 for two Behy replicates). This is an important consideration for management of these rivers in a mixed stock fishery and although temporal samples from other rivers in the Castlemaine Harbour area (e.g. Owenascaul), have not been screened, it is recommended that analysis of these be undertaken.

Stock estimates in MSA and IA

The results of national and international Mixed Stock Fishery Analysis of both simulated and real samples in Irish Atlantic salmon and other species, indicates that while it is most accurate to accept point estimates of stock proportions, there is increasing uncertainty associated with the estimates of small contributors. Our tolerance analysis based on the simulated samples indicates that proportion estimates can vary by between 1 and 3% by using 95% confidence intervals, which is an important consideration for the smallest contributors. In this respect, we maybe slightly over or underestimating the proportional contributions of the Behy, Emlagh and Owenascaul, and of rivers located outside Dingle

Bay. Examination of individual assignments and scores given in the attached spreadsheet may help to resolve the presence of absence of these stocks in the fishery.

Appendix1

Genotyping

Microsatellite locus amplification and screening

DNA was extracted from all samples as follows: individual alcohol preserved tissue samples were blotted on tissue paper to remove alcohol and placed in a 1.5ml Eppendorf tube. 300µl of Cell Lysis Solution and 4µl of Proteinase K (20mg/ml) were added and the tube placed in incubator at 37ºC overnight. The tube was cooled to room temperature and 100µl of Protein Precipitation Solution was added. It was then vortexed for 20 seconds and placed on ice for 10 minutes before centrifuging at 13,000rpm for 5 minutes. A new 1.5ml Eppendorf tube was prepared for each sample containing 300µl of 100% isoproponal (2-proponal). The supernatant of the spun sample (the pellet is protein to be discarded) was removed and added to the isopropanol containing Eppendorf. The DNA pellet was precipitated by inverting the tube. The isopropanol was then decanted off and the pellet was washed in 70% ethanol for 2 hours. The 70% ethanol was then poured off and the pellet was allowed to air dry for 30 minutes. 50-100µl of 0.1X TE buffer was added to resuspend the DNA. DNA was quantified using a nanodrop spectrophotometer and diluted to a concentration of 100ng/ul for PCR.

Fifteen microsatellite loci (the same as those used in the recent Irish National Genetic Stock Identification Programme – see Appendix 1 Table 1) were used to screen each DNA sample. Amplifications for each locus were carried out in a total volume of 10uL and contained 1uL of DNA, 1X reaction buffer with 0.5U Taq polymerase (Promega), 250uM dNTPs and 1uM forward and reverse primers (one of which was 5'- end-labelled with IRD800 or IRD700 to enable detection of DNA fragments on a Licor DNA sequencer). Total $MgCl_2$ concentration for each locus was 2.0mM. All loci were amplified separately using a PCR profile of 95C for 3m followed by 30 cycles of 95C for 30s, 56C for 30s and 72C for 30s. Amplified fragments were stored at 4C until use.

All loci were screened separately or mixed in multiplexes on 18 or 25cm 6% polyacrylamide gels on Licor automated DNA sequencers. Allele sizes and genotypes for each individual at each locus were determined by reference to DNA size markers and allele-standards which were loaded with the samples on each gel. All genotypes were scored by eye and double checked for correctness.

Appendix 1 **Table 1** Primer sequences for microsatellites used to screen samples. Label refers to the type of infra-red label attached to primers to facilitate screening on the double-dye Licor system.

Locus	**Reference**	**Primer sequence**	**Label**
MHC I	Grimholt *et al*. (2002)	For AGGAAGGTGCTGAAGAGGAAC Rev CAATTACCACAAGCCCGCTC	800
MHC II	Stet *et al*. (2002)	For GATGGCAAAGAGGAAAGTGAG Rev TTGTTATGCTCTACCTCTGAA	700
*Ssa*197	O'Reilly *et al*. (1996)	For TGGCAGGGATTTGACATAAC Rev GGGTTGAGTAGGGAGGCTTG	800
*Ssa*171	O'Reilly *et al*. (1996)	For TTATTATCCAAAGGGGTCAAAA Rev GAGGTCGCTGGGGTTTACTAT	700
*Ssa*202	O'Reilly *et al*. (1996)	For CTTGGAATATCTAGAATATGGC Rev GTTCATGTGTTAATGTTGCGTG	800
*Ssa*D170	EMBL accession no: AF525205	For GGAGGCAGTTAAGAGAACAAAAG Rev TCACCTACCCTTCTCATTCAAG	700
*Ssa*D157	King *et al*. (2005)	For ATCGAAATGGAACTTTTGAATG Rev GCTTAGGGCTGAGAGAGGAATAC	800
*Ssa*D71	King *et al*. (2005)	For AACGTGAAACATAAATCGATGG Rev TTAAGAATGGGTTGCCTATGAG	800
*Ssa*D48	King *et al*. (2005)	For GAGCCTGTTCAGAGAAATGAG Rev CAGAGGTGTTGAGTCAGAGAAG	700
*Sssp*2210	Paterson *et al*. (2004)	For AAGTATTCATGCACACACATTCACTGC Rev CAAGACCCTTTTTCCAATGGGATTC	800
*Sssp*2201	Paterson *et al*. (2004)	For TTAGATGGTGGGATACTGGGAGGC Rev CGGGAGCCCCATAACCCTACTAATAAC	800
*Sssp*2216	Paterson *et al*. (2004)	For GGCCCAGACAGATAAACAAACACGC Rev GCCAACAGCAGCATCTACACCCAG	700
*Sssp*1605	Paterson *et al*. (2004)	For CGTAATGGAAGTCAGTGGACTGG Rev CTGATTTAGCTTTTTAGTGCCCAATGC	800
*Sssp*3016	Paterson *et al*. (2004)	For GACAGGGCTAAGTCAGGTCA Rev GATTCTTATATACTCTTATCCCCAT	800
*SSOSL*85	Slettan *et al*. (1995)	For TGTGGATTTTTGTATTATGTTA Rev ATACATTTCCTCCTCATTCAGT	700

Appendix 2

Validation of MSA and IA using simulated fishery samples

To validate and assess the potential accuracy of MSA and IA for the Cromane fishery, a simulated mixture sample was constructed using genetic data from the rivers most likely to contribute to this fishery (based on previous studies of Cromane fisheries and geographic location of rivers). Simulated genotypes were created by drawing alleles (with replacement) based on allele frequencies in specified population samples. The final simulated mixture was composed of 1700 fish and included simulated genotypes from the Behy (200), Caragh (200), Laune (800), Maine (200), Emlagh (200) and Owenascaul (100) rivers.

MSA of simulated fishery sample

This simulated data set was then tested against the entire NGSI baseline using the cBAYES software. Actual and estimated proportions of contributing populations to this simulated fishery sample are presented in Appendix 2 Table 1. The proportions of contributing rivers as estimated by cBAYES are very close to the actual proportions in the mixture (within 0.5%) which suggests, with a high degree of confidence, that this software will correctly estimate stock proportions in the real fishery. However, the software also indicated that there were small contributions from other rivers outside the Castlemaine Harbour area, in particular from rivers in the so called south eastern population complex (Barrow, Nore, Suir, Blackwater and Bride).

Appendix 2 Table 1 MSA of a simulated fishery sample showing actual proportions of each river and the proportions estimated by cBAYES (SD is the standard deviation for each estimate). Proportions in bold are considered to be significant

		Actual	MSA	SD			Actual	MSA.	SD
1	Castletown	-	-	-	43	Kilcogan	-	-	-
2	Fane	-	-	-	44	Corrib	-	0.0001	-
3	Glyde	-	-	-	45	Owenboliska	-	-	-
4	Dee	-	-	-	46	Cashla	-	-	-
5	Boyne	-	0.0001	-	47	Screebe	-	-	-
6	Liffey	-	-	-	48	Owenmore	-	0.0001	-
7	Dargle	-	0.0001	-	49	Dawros	-	-	-
8	Vartry	-	-	-	50	Culfin	-	-	-
9	Avoca	-	-	-	51	Erriff	-	0.0001	-
10	Slaney	-	0.0001	-	52	Bundorragh	-	-	-
11	SEPC	-	**0.0024**	**0.002**	53	Bunowen	-	-	-
12	Lee	-	0.0001	-	54	Newport	-	-	-
13	Bandon	-	0.0001	-	55	Srahmore	-	-	-
14	Ilen	-	-	-	56	Owenduff	-	0.0001	-
15	Owvane	-	-	-	57	Owenmore	-	0.0001	-
16	Glengariff	-	-	-	58	Cloonaghmore	-	-	-
17	Adrigole	-	-	-	59	Moy	-	0.0002	-
18	Croanshagh	-	-	-	60	Brusna	-	-	-
19	Owenshagh	-	-	-	61	Easkey	-	-	-
20	Cloonee	-	-	-	62	Ballysadare	-	0.0001	-
21	Sheen	-	-	-	63	Garvogue	-	-	-
22	Roughty	-	0.0001	-	64	Duff	-	0.0001	-
23	Finnihy	-	-	-	65	Drowes	-	-	-
24	KerryBlackwater	-	-	-	66	Erne	-	-	-
25	Sneem	-	-	-	67	Abbey	-	-	-
26	Owreagh	-	-	-	68	Laghy	-	-	-
27	Currane	-	0.0001	-	69	Eske	-	-	-
28	Inny	-	-	-	70	Eany	-	-	-
29	Ferta	-	-	-	71	Glen	-	-	-
30	Behy	0.1176	**0.1151**	**0.008**	72	Owenwee	-	-	-
31	Caragh	0.1176	**0.1237**	**0.009**	73	Owenea	-	-	-
32	Laune	0.4706	**0.4691**	**0.013**	74	Gweebarra	-	-	-
33	Maine	0.1176	**0.1129**	**0.008**	75	Gweedore	-	-	-
34	Emlagh	0.1176	**0.1183**	**0.008**	76	Clady	-	-	-
35	Annascaul	0.0558	**0.0558**	**0.006**	77	Lackagh	-	-	-
36	Milltown	-	-	-	78	Leannan	-	0.0001	-
37	Feoghanagh	-	-	-	79	Swilly	-	-	-
38	TraleeLee	-	-	-	80	Crana	-	-	-
39	Feale	-	0.0001	-	81	NorthernIreland	-	0.0001	-
40	Galey	-	-	-	82	Aquagen	-	-	-
41	Maigue	-	-	-	83	Fanad	-	-	-
42	Mulcair	-	0.0001	-					

The results of this analysis indicate likelihood that fish from the real Cromane fishery may in a small number of cases, wrongly assign to, or reveal non-contributing stock proportions from, rivers outside the Castlemaine Harbour area. However, using 95% confidence intervals of the proportion estimates in the simulated fishery sample, we can estimate the proportions of contributing stocks that are likely to be detected in error. Appendix 2 Table 2 gives stock estimates for the simulated sample as revealed by cBAYES and associated 95% confidence intervals.

Appendix 2 Table 2 MSA proportions of contributing stocks for the simulated fishery with 95% confidence intervals (proportions summed for rivers not included in the simulation)

	MSA proportion	95% CI
Outside Castlemaine Harbour area	0.005	0.000 – 0.052
Behy	0.115	0.100 – 0.131
Caragh	0.124	0.108 – 0.141
Laune	0.469	0.445 – 0.495
Maine	0.113	0.097 – 0.130
Emlagh	0.118	0.103 – 0.135
Owenascaul	0.056	0.045 – 0.068

Using the confidence intervals, we can assess the likelihood of over- or under-estimating the proportions of stocks from inside and outside Castlemaine Harbour (see Appendix 2 Table 3). Although point estimates of stock proportions are the most reliable, this tolerance analysis of the MSA estimates suggests that there is a 95% probability that fish from outside Dingle Bay could be wrongly detected up to a proportion of 5.2%. This means that a minimum proportion of 5.3% of fish from outside Castlemaine Harbour need to be detected in the fishery to be 95% sure that these are present.

Appendix 2 Table 3 Precision estimates of stock proportions based on analysis of 95% confidence intervals

	Potential for over-estimation	Potential for under-estimation
Outside Castlemaine Harbour area	0.052	-
Behy	0.013	0.018
Caragh	0.023	0.010
Laune	0.024	0.026
Maine	0.012	0.021
Emlagh	0.017	0.015
Owenascaul	0.009	0.014

In addition, this analysis suggests a likelihood of both under- and over-estimating the proportions of Castlemaine Harbour rivers-originating fish in the Cromane Fishery. Proportions estimates for these rivers may be 0.009 to 0.024 lower than point estimates or 0.01 to 0.026 higher. For each of the rivers in Castlemaine Harbour, detected proportions in the Cromane fishery need to be greater than the potential for over-estimation (see Table A2.3) to be 95% confident that these rivers are indeed contributing to the fishery. However, it is worth noting that the potentials for under-estimation reveal that we may also fail to detect the presence of very small contributors.

IA of simulated fishery sample

The cBAYES software also provides individual assignment scores for each fish to each population in the baseline. The can be summed where there are multiple populations or samples within a river to give an overall assignment score for each river. The results of IA on the simulated sample are presented in Appendix 2 Table 4. The overall correctly assigned proportion is 95.8% and for each river in Castlemaine Harbour, a minimum of 90% (Maine) of the simulated fish are correctly assigning to their origin.

Appendix 2 Table 4 Individual assignment of the fish in the simulated sample (origin of fish is given in the first column, where the assign to is given in the other columns). (% refers to the proportion of fish that are correctly assigned; non-CH refers to rivers other than those specified in the simulated mixture).

	n	Behy	Caragh	Laune	Maine	Emlagh	Owenascaul	non-CH	%
Behy	**200**	**191**	2	5	2				0.955
Caragh	**200**		**192**	3	3	1	1		0.960
Laune	**800**	5	13	**773**	5	1		3	0.966
Maine	**200**	2	2	13	**180**	2		1	0.900
Emlagh	**200**				1	**199**			0.995
Owenascaul	**100**	1	1	3	1	1	**93**		0.930
								Total	**0.958**

The majority of mis-assignments among the Castlemaine Harbour rivers appear to be wrongly classified as Laune fish but each river sample has at least some mis-assignment (lowest for the Emalgh). The worst case appears to be the Caragh where 6.5% of samples are wrongly classified as being of Laune origin.

For the simulated sample a very small number of individuals assign to rivers outside the Castlemaine Harbour area. These are typically assigned to rivers in the SEPC area indicating that some mis-assignment of fish to these rivers is likely to be encountered in the analysis of the real fishery.

Assignment testing attempts to estimate the probability of finding the genotype of a particular individual from a fishery mixture in any of the baseline populations. The individual is then assigned a range of assignment scores which are based on the probability of that individual belonging to each baseline stock. These scores range from 0 to 100% with the lowest values being associated with low probability of belonging to a particular stock and increasing scores associated with increasing probabilities of belonging to a specific stock. Typically, the highest score (first choice) for a particular stock or baseline population is taken as the population of origin for each individual fish. However, it must be stressed that as 'first choice' assignment scores decrease, the likelihood of being correct decreases.

For the simulated sample, the number is individuals that fall into different ranges of assignment scores is presented in Appendix 2 Table 5. It can be seen that individuals with the highest assignment scores are most likely to be correct and scores of 100% are always correct.

Appendix 2 Table 5 Assignment scores for simulated individuals. (IA score is the individual assignment score range for each individual, n is the number of individuals that have scores within that range, n correct is the number of individuals that were correct for that assignment score range, Propn. Correct in the number correct expressed as a ratio).

IA score	n	Propn. of total	n correct	Propn. correct
100	1198	0.705	1198	1.000
95-100	1469	0.864	1464	0.997
90-100	1526	0.898	1515	0.993
80-100	1591	0.936	1564	0.983
70-100	1631	0.959	1590	0.975
66-100	1638	0.964	1594	0.973
50-100	1692	0.995	1625	0.960
<50	8	0.005	3	0.375

For the simulated sample, there are a high proportion of fish (>70%) with 100% assignment scores. However, these results also indicate the low accuracy associated with assignments scores of less than 50%. In the simulated sample, this number is very low but as these individuals were specifically drawn from specified baseline populations, it is likely that a higher proportion of individuals in the lower assignment scores ranges will be encountered. This is because fish in a real fishery situation may have alleles that are at low frequency in the baseline samples of contributing stocks and may not have been sampled when the baseline was constructed. Improving and updating the baseline will help to resolve this situation.