

Appendix 1. Microsatellite loci used in the three different PCR multiplexes.

Locus	Forward primer	Reverse primer	Multiplex	Number of alleles
Ssa85 ¹	AGGTGGGTCCTCCAAGCTAC	gtttACCCGCTCCTCACTTAATC	1, 3	13
mOne102a.b ²	GGGATTATTCTTACTTTGGCTGTT	gtttCCTGGTTGGGAATCACTGC	1	18
Str2QUB ³	CTGGGGTCCACAGCCTATAA	gtttGAGCTACAACCTGATCCACCA	1	46
Ssa416UoS ⁴	TGACCAACAACAAACGCACAT	gtttCCCACCCATTAACACAACATAT	1	4
mOne101 ²	TGCTAAATGACTGAAATGTTGAGA	gtttGAGAATGAATGGCTGAATGGA	1	7
Cocl-lav-4 ⁵	TGGTGTAAATGGCTTTTCCTG	gtttGGGAGCAACATTGGACTCTC	1	11
Oneμ ⁹⁶	CTCTCTTTGGCTCGGGGAATGTT	gtttGCATGTTCTGACAGCCTACAGCT	1	14
CA048828 ⁷	GAGGGCTTCCCATAACAACAA	gtttGTTTAAGCGGTGAGTTGACGAGAG	1	29
BG935488 ⁷	gtttTGACCCACCAAGTTTTTCT	AAACACAGTAAGCCCATCTATTG	2	unreliable scoring
SsaD71 ⁸	AACGTGAAACATAAATCGATGG	gtTTAAGAATGGGTTGCCTATGAG	2	16
Sasa-TAP2A ⁹	gtttGTCCTGATGTTGGCTCCCAGG	GCGGGACACCGTCAGGGCAGT	2	10
MHC-I ⁹	AGGAAGGTGCTGAAGAGGAAC	gtttCAATTACCACAAGCCCGCTC	2	19
Ssa410UoS ⁴	gtttGGAAAATAATCAATGCTGCTGGTT	CTACAATCTGGACTATCTTCTTCA	2	40
Str3QUB ³	CTGACCGCTGCACACTAA	gtttGGCTCTAATCGACTGGCAGA	2	12
CA060177 ⁷	CGCTTCCTGGACAAAAATTA	gtttGAGCACACCCATTCTCA	2	17
Ssa197 ¹	GGGTTGAGTAGGGAGGCTTG	gttTGGCAGGGATTTGACATAAC	2, 3	18
SsOsl417 ¹⁰	TTGTTCAAGTGTATATGTGTCCCAT	GTTTGATCTTCACTGCCACCTTATGACC	3	14
Ssa87NVH ¹¹	CTGTAAACATCACAGGCG	GTTTCTCCACTAATAGTCTGAAGG	3	16
Ssa24NVH ¹¹	TGAGTCACACCTGTCACG	GTTTGTGATGATGATTAAAGCCAG	3	6
BS-131 ¹²	CACATCATGTTACTGCTCC	GTTTCAGCCTAATTCTGAATGAG	3	13
Ssa408 ⁴	AATGGATTACGGGTACGTTAGACA	GTTTCTCTTGTGCAGGTTCTTCATCTGT	3	28

Exon2 ¹³	TGATGGATGGGATCCCCGTCATCTCTCTCCCAAAG	TAGAGCTTAAAACCACTCCACCCTCCATGAGGGA	3	-
Exon4 ¹³	AGTTGGAACGCTTCAGCAGAGCAGATGG	AGATTGGTGCCTGAGTGATGAGTCTTGTCC	3	-

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Appendix 2: Polymerase Chain Reaction conditions. **A:** PCR mixes used for the 3 microsatellite loci multiplexes. Exon 2 and Exon 4 stand for the gender marker primers. Reaction mixes were made in order to get 10µL per well. **B:** PCR programs used for the 3 multiplexes.

A	Multiplex 1			Multiplex 2			Multiplex 3		
	Concentration			Concentration			Concentration		
	DNA	ca 33	ng	DNA	ca 33	ng	DNA	ca 33	ng
	Buffer	1	x	Buffer	1	x	Buffer	1	x
	MgCl ₂	2	mM	MgCl ₂	2	mM	MgCl ₂	3	mM
	dNTP	0.2	mM	dNTP	0.2	mM	dNTP	0.2	mM
	GoTaq pol	0.5	U	GoTaq pol	0.5	1U	GoTaq pol	0.5	1U
	Ssa85	0.2	mM	BG935488	0.2	mM	Ssa85	0.125	mM
	mOne102a_b	0.4	mM	SsaD71	0.5	mM	SsOsl417	0.2	mM
	Str2QUB	0.4	mM	Sasa-TAP2A	0.6	mM	Ssa87NVH	0.15	mM
	Ssa416UoS	0.4	mM	MHC-I	0.3	mM	Ssa24NVH	0.3	mM
	mOne101	0.4	mM	Ssa410UoS	0.7	mM	BS-131	0.3	mM
	Cocl-Lav-4	0.4	mM	Str3QUB	0.15	mM	Ssa408	0.3	mM
	One_u9	0.4	mM	CA060177	0.8	mM	Ssa197	0.125	mM
	CA048828	0.4	mM	Ssa197	0.2	mM	Exon2-Vic	0.05	mM
							Exon4-Vic	0.05	mM

B	Multiplexes 1 & 2	Multiplex 3
	95°C 15min	94°C 5min
	95°C 45sec 55°C 1min30sec 72°C 1min } x5	94°C 45sec 55°C 1min30sec 72°C 1min } x3
	95°C 45sec 57°C 1min30sec 72°C 1min } x22	94°C 1min 57°C 1min30sec 72°C 1min } x25
	60°C 30min	72°C 10min
	4°C ∞	4°C ∞

B

[illegible]

Appendix 4: Proportion of anadromous trout ascending Guddal River according to the week number between 2006 and 2017. Individuals from 2017 were not genotyped, so the data might contain some Atlantic salmon as well as individuals that ascended multiple times. Week 20 is mid-May, week 45 beginning of November.

