Genetic Status of Coastal Cutthroat Trout at the NAVBASE Kitsap Bangor, Manchester Fuel Department, NAS Whidbey Island Crescent Harbor

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Project Background

Puget Sound is home to four species of salmonids (Chinook Salmon, Hood Canal summer-run Chum Salmon, steelhead, and Bull Trout) that are afforded legal protection under the Endangered Species Act (ESA). In an effort to determine whether occurrence of these ESA-listed species has the potential to affect operations in the waters adjacent to the Naval Base (NAVBASE) Kitsap Bangor, the Manchester Fuel Department, and Naval Air Station (NAS) Whidbey Island Lake Hancock, the Naval Facilities Engineering Command Northwest (NAVFAC NW) and the Washington Department of Fish and Wildlife (WDFW) entered into a cooperative agreement whereby the WDFW agreed to survey these waters with a beach seine to evaluate both the seasonal and resident presence of ESA-listed fish. For further details regarding the ESA-listed fish surveys conducted with a beach seine at Naval installations in 2015 and 2016, see the final reports for each location (Frierson et al. 2017a, 2017b, 2017c). The ESA-listed fish species stock most relevant to this study is Puget Sound steelhead (*Oncorhynchus mykiss*). Hybridization between Coastal Cutthroat Trout (*O. clarkii*) and steelhead (or Rainbow Trout) has been documented in several streams within Puget Sound, including Big Beef Creek in Hood Canal (Campton and Utter 1985, Hawkins 1997, Young et al. 2001, Moore et al. 2010). In watersheds where Cutthroat Trout, steelhead, and Cutthroat-steelhead hybrids are known to co-occur, field identification can be challenging and inaccurate. Past studies have paired phenotypic characteristics with genetic analysis to aid in identification of trout and trout hybrids in freshwater systems (Weigel et al. 2002, Baumsteiger et al. 2005, Kennedy et al. 2009). They reported that phenotypic characteristics for steelhead were the most distinct from hybrids and Cutthroat Trout, and that hybrids were most often misidentified as pure Cutthroat Trout. Many Coastal Cutthroat Trout of various life stages were captured with a beach seine during surveys for ESA-listed salmonids and forage fish at the NAVBASE Kitsap Bangor, Manchester Fuel Department, and NAS Whidbey Island Crescent Harbor Naval properties during 2016 sampling (Figure 1). This project provided genetic samples to detect any hybridization in Coastal Cutthroat Trout captured in 2016. These data may also provide some evidence to aid visual identification of the hybrid or pure species status of Coastal Cutthroat Trout in the marine environment based on phenotypic traits.

Methods

A total of 100 tissue samples (2mm non-lethal pelvic fin clips) were collected from up to ten Coastal Cutthroat Trout of any length for each day of sampling with a beach seine, at each site, from February through August in 2016 (Table 1). Phenotypic traits specific to identification between Cutthroat and

steelhead were recorded for each sample: 'jaw slash intensity', 'maxillary extent past eye', 'hyoid teeth' present (see Kennedy et al. 2009). Individual fork lengths were also recorded with each sample number (Table 2).

Tissue samples from Coastal Cutthroat Trout were genotyped at seven microsatellite loci and 96 single nucleotide polymorphism loci (SNP) – the SNP loci included three species ID loci for distinguishing between Cutthroat and Rainbow trout (see Appendix A).

Coastal Cutthroat Trout genotypic data were examined for evidence of Rainbow Trout genetic ancestry. Genomic DNA was extracted from tissue samples using Clone-tech® extraction kits. Microsatellite alleles were PCR-amplified using fluorescently labeled primers. PCRs were conducted in 96 well plates in 10 µl volumes employing 1 µl template with final concentrations of 1.5 mM $MgCl_2$, 200µM of each dNTP, and 1X Promega PCR buffer. The following microsatellite loci were used at the following concentrations (concentration in µM after locus name): One-108 [0.075], Ots-103 [0.037], Omy-77 [0.075], Ots-1 [0.08], Ots-3M [0.05], Ogo-3 [0.07], and Omm-1138 [0.08]). After initial two minute denature at 94°, there were 3 cycles consisting of 94° denaturing for 30 seconds, 60° annealing for 30 seconds, at 72° extension for 60 seconds. These were followed by 30 cycles with the same parameters but the annealing temperature was dropped to 50° and then there was a final 10-minute extension at 72°. Samples were run on an ABI 3730 automated DNA Analyzer and alleles were sized (to base pairs) and binned using an internal lane size standard (GS500Liz from Applied Biosystems) and GeneMapper software (Applied Biosystems).

Samples were also genotyped at 96 SNPs (see Table 2 for list) through PCR and visualized on Fluidigm EP1 integrated fluidic circuits (chips). Nineteen of the SNP loci were developed to discriminate among trout species (three of these distinguish between Coastal Cutthroat and Rainbow Trout) and 77 of the SNP loci have been used to identify population structure and other genetic attributes of Coastal Cutthroat Trout. To enhance SNP locus DNA in preparation for PCR, specific target amplification (STA) reactions were conducted using 96-well plates in 5 ul volumes with 1.25 ul of DNA template and pooled TaqMan® assays concentrated at 1X. Samples were run for 15 minutes at 95.0°C, followed by 14 cycles of 15 second denaturing at 95.0°C and 4 minute annealing at 60.0°C. Protocols followed Fluidigm's recommendations for TaqMan SNP assays as follows: assay loading mixture contains 1X Assay Loading Reagent (Fluidigm), 2.5X ROX Reference Dye (Invitrogen) and 10X custom TaqMan Assay (Applied Biosystems); sample loading mixture contains 1X TaqMan Universal PCR Master Mix (Applied Biosystems), 0.05X AmpliTaq Gold DNA polymerase (Applied Biosystems), 1X GT sampling loading reagent (Fluidigm) and 6.5 µL STA. Four µL assay loading mix and 5 µL sample loading mix were pipetted onto the chip and loaded by the IFC loader (Fluidigm). PCR was conducted on a Fluidigm thermal cycler using a two-step profile. Initial mix thermal profile was 70°C for 30min, 25°C for 5 min, 52.3° for 10 sec, 50.1°C for 1 min 50sec, 98°C for 5 sec, 96°C for 9 min 55 sec, 96°C for 15 sec, 58.6°C for 8 sec, and 60.1°C for 43 sec. Amplification thermal profile was 40 cycles of 58.6°C for 10 sec, 96°C for 5 sec, 58.6°C for 8 sec and 60.1°C for 43 sec with a final hold at 20°C. The TaqMan assays were visualized on the Fluidigm EP1 machine using the BioMark data collection software and analyzed using Fluidigm SNP genotyping analysis software. All data were scored by two researchers.

Results

Genotyping was mostly successful for the two marker types (see Table 2). Samples with missing data were rerun to try and complete genotypes. One sample (16DS0062) was excluded from analyses due to missing 85% genotypic data. Most samples were genetically unique with the exception of four pairs of samples collected near Manchester that had identical genotypes: 16DS0003 and 16DS0080; 16DS0024 and 16DS0081, 16DS0052 and 16DS0086, 16DS0019 and 16DS0078. The four pairs of genetically matching samples indicate these four fish were first caught in the spring (except one) and recaptured in

summer. Each of these four recaptured fish expressed the same phenotypic characteristics as their first capture and grew an average of 0.5mm/day. Three of the Species ID SNPs have proven useful for identifying Cutthroat-Rainbow hybrids (ASpI002, ASpI014, AspI018, WDFW unpublished data) and genotypes at these loci indicated that all but three samples were pure Coastal Cutthroat Trout. The three samples with Rainbow Trout (RBT) alleles were 16DS0009 (1RBT allele), 16DS0045 (3RBT alleles), and 16DS0051 (1 RBT allele). The sample with 3 RBT alleles was likely a first generation hybrid (F1) and the samples with 1 RBT allele may be a second generation hybrid (F2).

The single F1 hybrid captured in May was detected out of 33 sampled Cutthroat Trout at Bangor in 2016 (87 trout captured in total). This fish expressed phenotypic traits of both Cutthroat Trout (light jaw slash, maxillary jaw extends post orbit) and steelhead (no teeth on hyoid) (Table 3). Each of the F2 hybrids were captured in different locations and months; one at Manchester in March, and one at Whidbey Island (Oak Harbor) in May. Both of their phenotypic traits were consistent with Cutthroat Trout sampled and confirmed in this study (light jaw slash, maxillary jaw extends past eye, hyoid teeth present). For Manchester, 59 Cutthroat Trout were sampled out of 173 total trout captured in 2016. For Whidbey Island, 8 Cutthroat Trout were sampled out of 9 total trout captured in 2016.

Conclusions

Overall, hybridization between Coastal Cutthroat Trout and steelhead was detected at very low frequencies (3%) for all three locations combined. The hybridization rate for smolts in Big Beef Creek has been documented to be somewhat stable at 20-25%, but the rate for returning adults is either undocumented or undetected in the marine environment (Moore et al. 2010, Losee et al. 2017). These data may provide a baseline for the occurrence of adult Cutthroat-steelhead hybrids in the nearshore marine environment in Puget Sound.

In regards to phenotypic traits expressed by Cutthroat-steelhead hybrids, there were not enough hybrids detected to make any definitive conclusions. The F2 hybrids sampled in this study were more likely to express traits consistent with Cutthroat Trout than steelhead, and the pure Cutthroat Trout expressed some variation in their key features (see Table 3). The single F1 hybrid indicates that what appears to be a Cutthroat Trout, but lacks hyoid teeth, may in fact be a hybrid.

Future work using genetics to assign Cutthroat Trout captured at Bangor to population of origin would provide important information regarding Cutthroat Trout migration and biology in Hood Canal, and allow managers to prioritize habitat work focused on streams disproportionally contributing to Hood Canal Cutthroat Trout captured in the marine environment. While the tools to address these data gaps have been developed for South Puget Sound (Losee et al. 2017), funding does not currently exist for Hood Canal Cutthroat Trout.

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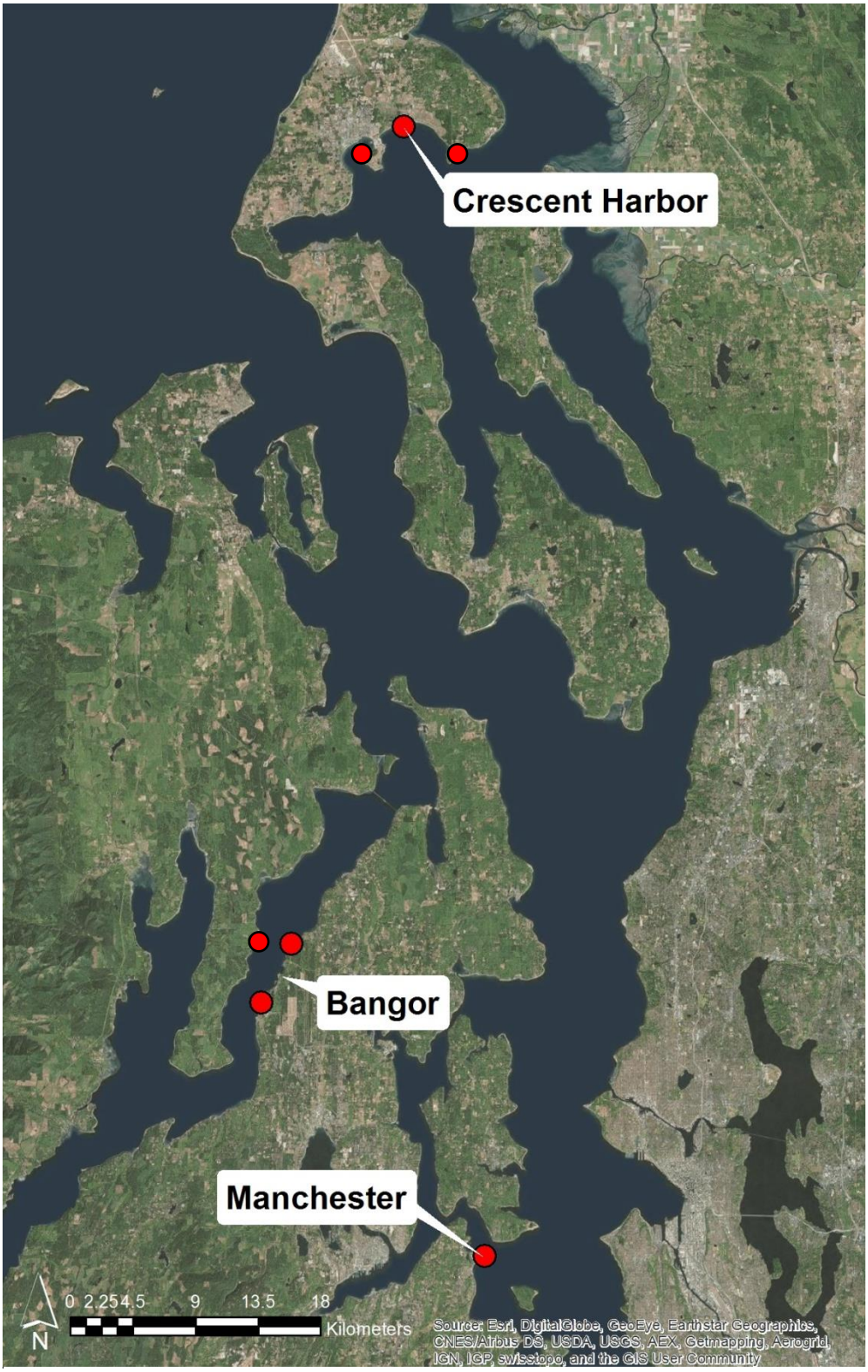

Figure 1. Map of Coastal Cutthroat Trout collection locations in Hood Canal (Bangor), Whidbey Island (Crescent Harbor), and central Puget Sound (Manchester).

Table 1. List of WDFW codes, collection locations, and Cutthroat Trout tissue samples.

WDFW code	Location	N
16_DS	Bangor	33
16_DS	Manchester	59
16_DS	Whidbey	8
	TOTAL	**100**

Table 2. List of Coastal Cutthroat Trout samples, biological data, and genetic status (Cutthroat or hybrid). Matching samples are indicated under "Genetic Match". Fish too small to detect hyoid teeth with a finger were recorded as 'n/a'.

Location	Date	Site ID	Fork Length (mm)	Jaw Slash Intensity	Maxillary Extent Past Eye	Hyoid Teeth	Code	Status	Genetic Match
Bangor	2/2/2016	Ba-South	252	Medium	NO	YES	16_DS01	Cutthroat	
Manchester	3/3/2016	Man-West	278	Light	YES	YES	16_DS02	Cutthroat	
Manchester	3/3/2016	Man-West	287	Light	YES	YES	16_DS03	Cutthroat	16_DS80
Manchester	3/3/2016	Man-West	285	Light	NO	YES	16_DS04	Cutthroat	
Manchester	3/3/2016	Clam Bay-East	335	Light	NO	YES	16_DS05	Cutthroat	
Manchester	3/3/2016	Clam Bay-East	320	Light	NO	YES	16_DS06	Cutthroat	
Manchester	3/3/2016	Clam Bay-East	320	dark	YES	YES	16_DS07	Cutthroat	
Manchester	3/3/2016	Clam Bay-East	320	Medium	YES	YES	16_DS08	Cutthroat	
Manchester	3/3/2016	Clam Bay-East	315	Medium	YES	YES	16_DS09	F2 hybrid	
Manchester	3/3/2016	Clam Bay-East	334	Light	YES	YES	16_DS10	Cutthroat	
Manchester	3/3/2016	Clam Bay-East	322	Light	YES	YES	16_DS11	Cutthroat	
Manchester	3/3/2016	Clam Bay-East	314	Light	YES	YES	16_DS12	Cutthroat	
Manchester	3/3/2016	Clam Bay-East	290	Light	YES	YES	16_DS13	Cutthroat	
Manchester	3/3/2016	Clam Bay-East	298	Light	YES	YES	16_DS14	Cutthroat	
Manchester	3/31/2016	Clam Bay-East	324	Light	YES	YES	16_DS15	Cutthroat	
Manchester	3/31/2016	Clam Bay-East	340	Light	YES	YES	16_DS16	Cutthroat	
Manchester	3/31/2016	Clam Bay-East	335	Light	YES	YES	16_DS17	Cutthroat	
Manchester	3/31/2016	Clam Bay-East	312	Light	YES	YES	16_DS18	Cutthroat	

Manchester	3/31/2016	Man-East	308	Light	YES	YES	16_DS19	Cutthroat	16_DS78
Manchester	3/31/2016	Man-East	302	Light	NO	YES	16_DS20	Cutthroat	
Manchester	3/31/2016	Man-East	298	Light	NO	YES	16_DS21	Cutthroat	
Manchester	3/31/2016	Man-East	320	Light	NO	YES	16_DS22	Cutthroat	
Manchester	3/31/2016	Man-West	316	Medium	YES	YES	16_DS23	Cutthroat	
Manchester	3/31/2016	Man-West	292	Light	NO	YES	16_DS24	Cutthroat	16_DS81
Whidbey	4/15/2016	Tom-East1	285	Light	YES	YES	16_DS25	Cutthroat	
Whidbey	4/15/2016	Tom-East1	385	Light	YES	YES	16_DS26	Cutthroat	
Whidbey	4/15/2016	Tom-East1	370	Light	NO	YES	16_DS27	Cutthroat	
Manchester	5/2/2016	Man-West	128	Light	NO	n/a	16_DS28	Cutthroat	
Manchester	5/2/2016	Man-West	151	dark	NO	YES	16_DS29	Cutthroat	
Manchester	5/2/2016	Man-East	169	Light	NO	YES	16_DS30	Cutthroat	
Manchester	5/2/2016	Man-East	165	Light	NO	YES	16_DS31	Cutthroat	
Manchester	5/2/2016	Man-East	181	Light	NO	YES	16_DS32	Cutthroat	
Manchester	5/2/2016	Man-East	147	Medium	NO	YES	16_DS33	Cutthroat	
Manchester	5/2/2016	Man-East	195	Dark	NO	YES	16_DS34	Cutthroat	
Manchester	5/2/2016	Man-East	159	Light	NO	YES	16_DS35	Cutthroat	
Manchester	5/2/2016	Clam Bay-West	343	Light	YES	YES	16_DS36	Cutthroat	
Manchester	5/2/2016	Clam Bay-West	315	Light	YES	YES	16_DS37	Cutthroat	
Bangor	5/13/2016	Ba-South	150	Light	YES	YES	16_DS38	Cutthroat	
Bangor	5/13/2016	Ba-South	160	Medium	YES	YES	16_DS39	Cutthroat	
Bangor	5/13/2016	Ba-South	116	Light	YES	n/a	16_DS40	Cutthroat	
Bangor	5/13/2016	Ba-South	169	Light	YES	YES	16_DS41	Cutthroat	
Bangor	5/13/2016	Ba-South	137	Dark	NO	YES	16_DS42	Cutthroat	
Bangor	5/13/2016	Ba-South	126	Light	NO	YES	16_DS43	Cutthroat	
Bangor	5/13/2016	Ba-South	187	Light	YES	YES	16_DS44	Cutthroat	
Bangor	5/13/2016	Ba-North	287	Light	NO	NO	16_DS45	F1 hybrid	
Bangor	5/13/2016	Ba-North	202	Light	NO	YES	16_DS46	Cutthroat	
Bangor	5/13/2016	Ba-North	233	Light	YES	YES	16_DS47	Cutthroat	
Bangor	5/13/2016	Ba-North	115	Medium	NO	n/a	16_DS48	Cutthroat	
Whidbey	5/17/2016	Lag-E	185	Light	NO	YES	16_DS49	Cutthroat	
Whidbey	5/17/2016	OH-West	192	Medium	NO	YES	16_DS50	Cutthroat	

Whidbey	5/17/2016	OH-East	304	Light	YES	YES	16_DS51	F2 hybrid	
Manchester	6/13/2016	Man-West	189	Light	NO	YES	16_DS52	Cutthroat	16_DS86
Manchester	6/13/2016	Man-West	183	Medium	YES	YES	16_DS53	Cutthroat	
Manchester	6/13/2016	Man-West	174	Light	YES	YES	16_DS54	Cutthroat	
Manchester	6/13/2016	Man-West	153	Light	YES	YES	16_DS55	Cutthroat	
Manchester	6/13/2016	Man-West	182	Light	YES	YES	16_DS56	Cutthroat	
Manchester	6/13/2016	Man-West	170	Light	YES	YES	16_DS57	Cutthroat	
Manchester	6/13/2016	Man-West	170	Light	YES	YES	16_DS58	Cutthroat	
Manchester	6/13/2016	Man-East	157	Light	YES	YES	16_DS59	Cutthroat	
Manchester	6/13/2016	Beaver Creek-West	212	Light	NO	YES	16_DS60	Cutthroat	
Manchester	6/13/2016	Clam Bay-East	329	Medium	YES	YES	16_DS61	Cutthroat	
Manchester	6/13/2016	Clam Bay-East	350	Medium	YES	YES	16_DS62	(missing data)	
Manchester	6/13/2016	Clam Bay-East	398	Light	YES	YES	16_DS63	Cutthroat	
Manchester	6/13/2016	Clam Bay-East	343	Medium	YES	YES	16_DS64	Cutthroat	
Manchester	6/13/2016	Clam Bay-East	378	Medium	YES	YES	16_DS65	Cutthroat	
Bangor	6/14/2016	Ba-South	208	Light	NO	YES	16_DS66	Cutthroat	
Bangor	6/14/2016	Ba-South	157	Light	NO	YES	16_DS67	Cutthroat	
Bangor	6/14/2016	Ba-South	149	Light	NO	n/a	16_DS68	Cutthroat	
Bangor	6/14/2016	Ba-South	145	Medium	YES	YES	16_DS69	Cutthroat	
Bangor	6/14/2016	Ba-South	147	Light	NO	YES	16_DS70	Cutthroat	
Bangor	6/14/2016	Ba-South	132	Light	NO	n/a	16_DS71	Cutthroat	
Bangor	6/14/2016	Ba-South	199	Light	NO	YES	16_DS72	Cutthroat	
Bangor	6/14/2016	Ba-West	393	Light	YES	YES	16_DS73	Cutthroat	
Bangor	6/14/2016	Ba-West	137	Light	NO	n/a	16_DS74	Cutthroat	
Bangor	6/14/2016	Ba-West	197	Light	YES	YES	16_DS75	Cutthroat	
Whidbey	6/15/2016	Tom-West	384	Light	YES	YES	16_DS76	Cutthroat	
Whidbey	6/15/2016	Tom-West	300	Light	YES	YES	16_DS77	Cutthroat	
Manchester	7/11/2016	Man-West	330	Light	YES	YES	16_DS78	Cutthroat	16_DS19
Manchester	7/11/2016	Man-West	173	Light	YES	YES	16_DS79	Cutthroat	
Manchester	7/11/2016	Man-West	350	Light	YES	YES	16_DS80	Cutthroat	16_DS03
Manchester	7/11/2016	Man-West	332	Light	NO	YES	16_DS81	Cutthroat	16_DS24
Manchester	7/11/2016	Man-West	352	Light	YES	YES	16_DS82	Cutthroat	

Manchester	7/11/2016	Man-West	216	Light	NO	YES	16_DS83	Cutthroat	
Manchester	7/11/2016	Man-West	222	Light	NO	YES	16_DS84	Cutthroat	
Manchester	7/11/2016	Man-West	170	Medium	NO	YES	16_DS85	Cutthroat	
Manchester	7/11/2016	Man-West	208	Light	NO	YES	16_DS86	Cutthroat	16_DS52
Manchester	7/11/2016	Man-West	228	Light	NO	YES	16_DS87	Cutthroat	
Bangor	7/12/2016	Ba-South	214	Light	NO	YES	16_DS88	Cutthroat	
Bangor	7/12/2016	Ba-South	230	Light	NO	YES	16_DS89	Cutthroat	
Bangor	7/12/2016	Ba-South	189	Light	NO	YES	16_DS90	Cutthroat	
Bangor	7/12/2016	Ba-South	210	Light	NO	YES	16_DS91	Cutthroat	
Bangor	7/12/2016	Ba-North	181	Light	YES	YES	16_DS92	Cutthroat	
Bangor	7/12/2016	Ba-North	216	Light	YES	YES	16_DS93	Cutthroat	
Bangor	7/12/2016	Ba-North	247	Light	YES	YES	16_DS94	Cutthroat	
Bangor	7/12/2016	Ba-North	145	Light	YES	YES	16_DS95	Cutthroat	
Bangor	7/12/2016	Ba-North	131	Medium	NO	YES	16_DS96	Cutthroat	
Bangor	7/12/2016	Ba-North	176	Light	NO	YES	16_DS97	Cutthroat	
Bangor	7/12/2016	Ba-North	141	Light	YES	YES	16_DS98	Cutthroat	
Manchester	8/8/2016	Clam Bay-West	358	Medium	YES	YES	16_DS99	Cutthroat	
Manchester	8/8/2016	Clam Bay-West	182	Medium	YES	YES	16_DS100	Cutthroat	

Table 3. Description and composition of categorical phenotypic characteristics for pure Cutthroat Trout, F1 hybrid, and F2 hybrid groups.

		Composition (%) within group		
Phenotype	**Description**	**Cutthroat Trout**	**F1 Hybrid**	**F2 Hybrid**
Jaw slash intensity	Light	79.2	100	50.0
	Medium	16.7	0	50.0
	Dark	4.2	0	0
Maxillary extent past eye	Yes	54.2	0	100
	No	45.8	100	0
Hyoid teeth present	Yes	100	0	100
	No	0	100	0

Appendix A: List of microsatellite (msat) and single nucleotide polymorphism (SNP) loci genotyped in the study. The SNP loci were assigned a WDFW nickname (WDFW name).

Type	WDFW name	Locus ID	Type	WDFW name	Locus ID
msat		Ogo-3	SNP	AOcl050	Ocl_120751c
msat		Omm-1138	SNP	AOcl051	Ocl_123048c
msat		Omy-77	SNP	AOcl052	Ocl_123205c
msat		One-108	SNP	AOcl053	Ocl_124454c
msat		Ots-1	SNP	AOcl055	Ocl_128302c
msat		Ots-103	SNP	AOcl056	Ocl_128757c
msat		Ots-3M	SNP	AOcl057	Ocl_128923c
SNP	AOcl001	Ocl_gdh-33	SNP	AOcl058	Ocl_128996c
SNP	AOcl003	Ocl_94903c	SNP	AOcl059	Ocl_129144c
SNP	AOcl004	Ocl_95769c	SNP	AOcl060	Ocl_129170c
SNP	AOcl005	Ocl_96127c	SNP	AOcl061	Ocl_130524c
SNP	AOcl006	Ocl_96500c	SNP	AOcl062	Ocl_131460c
SNP	AOcl007	Ocl_97077c	SNP	AOcl063	Ocl_131785c
SNP	AOcl008	Ocl_97865c	SNP	AOcl064	Ocl_131802c
SNP	AOcl009	Ocl_98188c	SNP	AOcl065	Ocl_impa1ya
SNP	AOcl010	Ocl_98409c	SNP	ASpI029	Ocl_impa1-189
SNP	AOcl011	Ocl_101704c	SNP	ASpI030	Ocl_ca050-39
SNP	AOcl012	Ocl_102420c	SNP	ASpI032	Ocl_gh1-633
SNP	AOcl013	Ocl_102510c	SNP	ASpI033	Ocl_MK3p-145
SNP	AOcl014	Ocl_103122c	SNP	ASpI040	Ocl_cin-90
SNP	AOcl015	Ocl_104216c	SNP	ASpI042	Ocl_hbad-264
SNP	AOcl016	Ocl_105385c	SNP	AOmy004	Omy_ALDOA_1
SNP	AOcl017	Ocl_105407c	SNP	AOmy048	Omy_113490-159
SNP	AOcl018	Ocl_105768c	SNP	AOmy049	Omy_114315-438
SNP	AOcl019	Ocl_105897c	SNP	AOmy063	Omy_97660-230
SNP	AOcl020	Ocl_106172c	SNP	AOmy064	Omy_97865-196
SNP	AOcl022	Ocl_106747c	SNP	AOmy210	OMS00153
SNP	AOcl023	Ocl_107074c	SNP	AOmy252	Omy_114976-223
SNP	AOcl024	Ocl_107607c	SNP	AOmy258	Omy_117540-259
SNP	AOcl025	Ocl_108007c	SNP	AOmy330	Omy_109894-185
SNP	AOcl026	Ocl_109243c	SNP	AOmy342	Omy_GH1-prom1-1
SNP	AOcl027	Ocl_109894c	SNP	AOcl002	Ocl_myo1b-16
SNP	AOcl028	Ocl_110064c	SNP	AOcl034	Ocl_113109c
SNP	AOcl029	Ocl_110495c	SNP	AOcl043	Ocl_117144c
SNP	AOcl030	Ocl_111084c	SNP	AOcl054	Ocl_125998c
SNP	AOcl031	Ocl_111312c	SNP	ASpI002	Ocl_Oku202
SNP	AOcl032	Ocl_111383c	SNP	ASpI014	Omy_F5_136
SNP	AOcl033	Ocl_112419c	SNP	ASpI018	Omy_Omyclmk436-96
SNP	AOcl035	Ocl_113128c	SNP	ASpI037	Ocl_fKbp2-62
SNP	AOcl036	Ocl_113600c	SNP	ASpI038	Ocl_mx1-129
SNP	AOcl037	Ocl_114315c	SNP	ASpI044	Ocl_gshpx-104
SNP	AOcl038	Ocl_114336c	SNP	ASpI046	Ocl_mk3pro-69
SNP	AOcl039	Ocl_114448c	SNP	ASpI048	Ocl_hsc71p-71
SNP	AOcl040	Ocl_115987c	SNP	ASpI053	Ocl_bcAKala-259
SNP	AOcl041	Ocl_116865c	SNP	ASpI055	Ocl_msra-168
SNP	AOcl042	Ocl_116938c	SNP	AOmy180	OMS00048
SNP	AOcl044	Ocl_117259c	SNP	AOmy279	OMS00015
SNP	AOcl045	Ocl_117370c	SNP	ASpI052	Ocl_aldB-79
SNP	AOcl046	Ocl_117432c	SNP	ASpI027	Ocl_arp-117
SNP	AOcl047	Ocl_117540c	SNP	ASpI056	Ocl_metB-106
SNP	AOcl048	Ocl_118654c	SNP	AOcl021	Ocl_106419c