Mapping of quantitative trait loci associated with size, shape, and parr mark traits using first and second generation backcrosses between European and North American Atlantic salmon (*Salmo salar*).

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Running title: Mapping of QTL in TransAtlantic Salmon Crosses

Word count: 6552 without abstract or references

Abstract: Little is known about the genetic architecture of traits important for salmonid restoration ecology. We mapped quantitative trait loci (QTL) using single nucleotide polymorphisms (SNPs) for juvenile body length, weight, shape, and vertical skin pigmentation patterns (parr marks) within three hybrid backcross families between European and North American subspecies of Atlantic salmon. Amounts of variation in skin colour and pattern quantified in the two second-generation transAtlantic families exceeded the ranges seen in purebred populations. GridQTL analyses using low-density female linkage maps detected QTL showing experiment-wide significance on *Ssa*02, *Ssa*03, *Ssa*09, *Ssa*11, *Ssa*19, and *Ssa*26/28 for both length and weight, on *Ssa*04 and *Ssa*23 for parr mark number, on *Ssa*09, *Ssa*13 for parr mark contrast, and on *Ssa05, Ssa*07, *Ssa*10, *Ssa*11, *Ssa*18, *Ssa*23, and *Ssa*26/28 for geometric morphometric shape co-ordinates. Pleiotrophic QTL on *Ssa*11 affected length, weight, and shape. No QTL was found that explained more than 10% of the phenotypic variance in pigmentation or shape traits. Each QTL was approximately positioned on the physical map of the Atlantic salmon genome. Some QTL locations confirmed previous studies but many were new. Studies like ours may increase the success of salmon restoration projects by enabling better phenotypic and genetic matching between introduced and extirpated strains.

Key words: conservation genetics, geometric morphometrics, parr marks, quantitative trait mapping, single nucleotide polymorphisms

Introduction

Atlantic salmon (*Salmo salar*) is an economically important species, generating large revenues in the aquaculture industry, and in the recreational angling industry. There has been an overall decline of wild anadromous Atlantic salmon stocks, with the result that much of the world's salmon biomass is a result of aquaculture (Asche and Bjorndal 2011). Studies of the genetic architecture and molecular basis of appearance traits are not only important to the Atlantic salmon aquaculture industry (Colihueque and Araneda 2014) but are also of interest to restoration ecologists trying to re-establish extirpated salmon populations (Donnelly and Whoriskey 1993).

The success of efforts to reintroduce juvenile salmon into the wild may be affected by population-specific genetic variation in their body shape and skin pigmentation. Recent geometric morphometrics studies have shown parr and smolt shape of salmonids differs among streams at different distances to the ocean (Billman et al. 2014) and that it is partially genetically determined (Boulding et al. 2008; Laporte et al. 2015). Parr marks, which are dark vertical oval markings along the sides of juveniles called parr, provide camouflage in streams with pebble and gravel bottoms and reduce visual detection by predators (Donnelly and Whoriskey 1993). Across individuals and populations, there is variation in the number of parr marks and in their colour intensity relative to the background skin (Culling et al. 2013). The number of parr marks per individual has a large genetic component (Kudo et al. 2002; Boulding et al. 2008) but environmental factors and acclimation also play a role in matching juveniles to their background (Mezzera et al. 1997). Donnelly and Whoriskey (1993) showed that parr marks of Atlantic salmon are an anti-predator adaptation to stream environments, and that the salmon's ability to change their colouration to match their surroundings is limited. Kawamura et al. (2012) suggested that predators preyed more heavily upon nonindigenous stocked hatchery salmon than on purebred native amago salmon because of differences in their skin colour traits that caused the former to be more conspicuous. Parr mark number has shown to be heritable in amago salmon (Kudo et al. 2002). If loci controlling a large proportion of the variation in parr skin colour phenotype can be detected and mapped, then future restoration efforts can identify populations that are suitably camouflaged for a specific river environment.

The use of single nucleotide polymorphisms (SNPs) for detecting quantitative trait loci (QTL) is becoming increasingly more popular due to the development of high-throughput SNP

genotyping assays that enables large numbers of individuals to be machine-scored for few hundred SNPs (Yáñez et al. 2015). In Atlantic salmon, the use of a 6K SNP chip and next generation sequencing has allowed estimation of moderate density linkage maps in both the North American (Brenna-Hansen et al. 2012) and European subspecies (Lien et al. 2011; Gonen et al. 2014) which possess 29 chromosomal pairs (NF=74) and 27 chromosomal pairs (NF=72) respectively (Brenna-Hansen et al. 2012). High density Atlantic salmon SNP chips (Houston et al. 2014; Yáñez et al. 2016) are now used to make high density maps (Tsai et al. 2016).

Our objective was to detect QTL associated with parr mark number and contrast, size (length and weight) and shape (geometric morphometric landmarks) in second generation backcrosses between the two subspecies of Atlantic salmon. We used both sib-pair and half-sib regression methods that are optimized for mapping QTL showing divergence between populations or subspecies (Haley et al. 1992; Haley et al. 1994; Haley et al. 2004). We expected that divergent QTL found in second generation crosses would be on the same chromosome arms but more accurately located (Hayes et al. 2006) those that found using first generation backcrosses because more recombination between the parental chromosomes would have taken place (Boulding et al. 2008; Pedersen et al. 2013). We also tried to increase accuracy by mapping QTL by focussing on the female map. Female Atlantic salmon typically show recombination along the entire length of the chromosome arms with exception of the telomeres, whereas male salmon typically show recombination only at the telomeres (Lien et al. 2011).

Materials and methods

Creation of transAtlantic hybrid backcrosses

The three families used in this study were the product of first and second generation crosses between European Mowi and Canadian Saint John River Atlantic salmon. The original goal was to produce F2 crosses between full siblings from a previously studied transAtlantic F1 cross that was used for backcrosses (Boulding et al. 2008). In January 2011 four females in the European “hybrid” tank were observed to be in spawning condition. Shortly thereafter, each female was crossed to a different male from the same tank. All fish were spawned using the dry method, with eggs being stripped from the female, and sperm being manually added and the gametes gently mixed. The offspring hatched in March 2011. The fish from Cross 2 did not survive likely because the gametes of one parent were overripe. All fish from Crosses 1, 3 and 4

were kept and maintained at the Saint Andrews Biological Stations (SABS) (Department of Fisheries and Oceans Canada) in Saint Andrews, New Brunswick, Canada in dechlorinated freshwater. Parr were reared from eggs indoors (Boulding et al. 2008) and then moved to outdoor 3 metre diameter tanks (Pedersen 2013).

The initial QTL mapping of the crosses assumed that they were F2 crosses between full-siblings (Pedersen 2013) but this assumption was questioned because the parr from one of the crosses were observed to have very different skin pigmentation than those from the other two surviving crosses (Table 1). Fortunately, the genetic origin of all six parents had been estimated using their 4K SNP genotypes and the program STRUCTURE (Pritchard et al. 2000) in a previous study (Liu et al. 2017). The STRUCTURE results suggested that the parents of Cross 1 were both the result of different F1 hybrids backcrossed to different purebred Mowi (the dam, Dam 1, was estimated to be 0.247 North American, 0.753 European, while the sire, Sire 5, was estimated to be 0.174 North American and 0.826 European). The parents of Cross 3 were estimated to be a pure Mowi dam (Dam 4) and a sire (Sire 7) that was an F1 hybrid (STRUCTURE results: 0.472 European, 0.528 North American). Finally, the parents of Cross 4 were estimated to be a pure Mowi dam (Dam 3), and a sire that was the result of an F1 hybrid backcrossed to Mowi (Sire 8, STRUCTURE results: 0.215 North American, 0.785 European).

Subsequent parentage analysis with the Colony software (Jones and Wang 2010) using 1000 SNPs showed that only one of the six parents was from the original F1 cross (Boulding et al. 2008). This confirmed that the new crosses were not F2 crosses. The parentage analysis also showed that each parent of Cross 4 was a full sibling to one parent from another cross. Dam 3 of Cross 4 was a full sibling of Dam 4 of Cross 3 consistent with them being from a purebred European family (family F studied by Boulding et al. 2008). In addition, Sire 8 of Cross 4 was a full sibling with Dam 1 of Cross 1 which combined with the STRUCTURE results is consistent with them being from a family created by backcrossing an F1 hybrid male to European salmon female (family C or D studied by Boulding et al. 2008). The STRUCTURE and the Colony analyses thus confirmed the recollection by BG that the European “hybrid” tank had contained all surviving parents and offspring with European ancestry from crosses A through F from Boulding et al. 2008.

SNP assay development

Two separate assays of four Agena MassARRAY™ SNP multiplexes were used in this study. The first custom assay had been used to genotype the transAtlantic backcross families B-D (Boulding et al. 2008) so was included to compare QTL locations between the two studies. This first set of multiplexes was designed at the Centre for Integrative Genetics (CIGENE) in Ås, Norway using Spectro-DESIGNER v3.0 from Agena and consisted of 129 SNPs chosen from a subset of 700 candidate SNPs that had been discovered by aligning EST contigs (Hayes et al. 2007).

To identify candidate markers for our second custom MassARRAY™ SNP assay, the parents of each cross were first genotyped for 4000 SNP markers using a 6K SNP chip. Candidate SNP assay markers that were heterozygous in all six parents were chosen over markers that were polymorphic in fewer parents. Markers were chosen at 10cM intervals by EG Boulding after visually scanning female and male maps for Canadian Atlantic salmon (Brenna-Hansen et al. 2012). A higher density of candidate markers was added near the telomeres where most of the recombination in males takes place (Lien et al. 2011). A higher density of markers was also added near the centromere, which when combined with the higher density at the telomeres, increased the possibility of detecting recombination in females. Any markers showing non-Mendelian segregation (multisequence variants or paralogous sequence variants (Gidskenhaug et al. 2011) were omitted from the list of candidate markers. Sets of four PCR multiplexes were designed at CIGENE by Arne Roseth using Spectro-DESIGNER using the pool of 329 candidate SNPs. The maximum number of SNPs that could be amplified *in silico* in any set of four PCR multiplexes was 121. A set containing some highly-desired SNPs (e.g., *PACA*) was selected for genotyping the three transAtlantic crosses.

All offspring from the three crosses were genotyped using Agena MassARRAY™ iPLEX Gold chemistry at the Centre for Clinical Genomics in Toronto. SNPs were analysed and any that failed or genotyped poorly were deleted. Where necessary, the genotype cluster-definition ellipses were edited manually by S. Pedersen using Agena Spectro-TYPER. A total of 188 SNP markers were successfully genotyped for all individual offspring and most were also genotyped for the parents.

Morphometric and skin pigmentation traits

Offspring from the three crosses reached an average weight of 8-9 grams in November 15th-25th, 2011. Fish were then non-lethally anesthetised (using tricaine methanesulfonate (TMS) at 0.07g/L), photographed for "parr" length and morphometric data, weighed (wet weight to the nearest 0.1g), pit-tagged, and fin clipped. Fin clips were kept in 1.5 mL tubes with "O"-ring screw caps, filled with 0.5mL of 95% EtOH at 4°C. A total of 1047 fish were weighed and photographed during the first measurement period. Tank space at SABS was limited therefore 447 fish were euthanized with a lethal dose of TMS (0.7g/L). To keep the families as large as possible, approximately 300 individuals were kept at random from Crosses 1 and 4 but all of family 3 were euthanized. During November 28th-30th, 2012, when fish were an average of 100g and beginning to display signs of undergoing the parr-smolt transformation, they were weighed and photographed for a second time. Of the 600 fish that were retained, 499 survived to the second measurement period. Fish were anesthetised in the same manner as the first measurement, weighed (wet weight to the nearest 0.5g), and photographed for morphometric analysis.

Geometric morphometric landmark software (Rohlf 2015) were used to measure body shape traits. The photographs of individual fish were digitized using tpsDig2 2.16 (Rohlf 2010) and 12 landmarks were recorded (Figure 1). All photographs were individually scaled, aligned and rotated using tpsRegr 1.37 (Rohlf 2009), to the same centroid size, maintaining the geometry of the landmark positions. Centroid size was used as a covariate to try and separate variation in landmark position that was based on shape from that based on size. Both x and y coordinates of each landmark were analysed as to specify the directional variation (vertical vs. horizontal) for the specific anatomical feature. After all photographs had been digitized, outliers were determined using aligned coordinates in tpsRegr 1.37 (Rohlf 2009) removing any outliers, and re-digitizing all fish with any mistakes in landmark position. For both the "parr" and "near smolt" measurement periods, landmark 8 (AnPCF: the most anterior point of the pectoral fin) was removed due to the extremely high degree of variation in landmark positioning when landmarks from multiple individuals were simultaneously plotted with tpsDig2 2.16.

The contrast between skin and parr marks was quantified from the measurement time 1 photographs that were also used for morphometrics (Supplementary Figure S1). Using Adobe Photoshop 7.0, the eyedropper tool was used to measure the brightness (L/A/B) of a 5x5 pixel area. The brightness was measured from the centre of the parr mark at the lateral line (or the

symmetrical centre of the parr mark if not along the lateral line) and on the skin in between parr marks along the lateral line. Individual values were recorded for the brightness of each parr mark as well as the adjacent skin. Relative contrast was calculated as the ratio of each parr mark to skin brightness, and then averaged over all parr marks for each individual fish. The contrast data were not transformed and natural outliers were retained. All statistical analyses on raw data were performed using StatPlus:mac© in Microsoft Excel or R, and were interpreted using graphs created in R.

Linkage maps

SNP linkage maps were created for the three hybrid backcrosses, as we observed that recombination frequencies differed significantly from both the pure North American (Brenna-Hansen et al. 2012) and European populations (Lien et al. 2011). Maps were created using JoinMap® 4.0 with https://www.kyazma.nl/index.php/JoinMap/ the CP option and the program defaults for other parameters. Marker grouping was done at a minimum LOD of 4.0 except for the individual genot.freq option where the LOD groupings threshold was lowered from 2.0 to 3.0. Linkage maps were first created separately for each family and sex, and subsequently combined into two sex-specific maps based on all crosses. The cross-specific male and female maps enabled separate QTL analyses for each cross whereas the composite male and female maps enabled simultaneous QTL analyses for all three crosses. To enable QTL analysis with GridQTL, very short linkage groups were joined with one other linkage group at a distance of 50cM, which is the expected recombination rate for unlinked markers (Lynch and Walsh 1998). Finally, a "Genomic-corrected linkage map" for females was made by comparing order of the SNPs on each of the chromosome of the composite female linkage map to their order on the physical map of the European Atlantic salmon (ICSASG_v2). This involved rearranging closely-spaced SNPs on some linkage groups so that their order matched the physical map. It also involved completely reversing some linkage groups so that the p arm end was at 0cM. The Genomic-corrected linkage map was then used to compare QTL positions found in separate analyses of individual families. This was necessary because the lengths of linkage maps made by Join Map for the same chromosome varied for different parents because of differences in which SNPs were informative in a particular family (Lynch and Walsh 1998).

In the linkage maps for individual parents up to three groups of linkage groups: 1) *Ssa*01p and *Ssa*23, 2) *Ssa*08 and *Ssa*29, and 3) *Ssa*26 and *Ssa*28 were joined together by Join Map showing that a hybrid parent was polymorphic for one or more of the three chromosome fusions typical of North American Atlantic salmon (Brenna Hansen et al. 2012) rather than the 29 linkage groups that are expected for European Atlantic salmon (Lien et al. 2011).

QTL detection

GridQTL software was used to detect QTL for the traits of interest. http://gridqt1.cap.ed.ac.uk/gridqtl_project.htm . GridQTL is an online web application (Hernández-Sánchez et al. 2009; Allen et al. 2014) used for mapping QTL within families for crosses between divergent outbred populations or subspecies (Andersson et al. 1994; Knott et al. 1996; Haley et al. 2004). Its Sib-pair (SP) module ("portlet") and its Half-sib (HS) module were used to analyze all traits in steps of 1.0cM. In addition, for Cross 3 only it was also possible to use the Backcross-F2 (BC-F2) module where we assumed that each offspring inherited one of two alternative alleles at the putative QTL from its hybrid male parent. GridQTL performs regression analyses using flanking molecular markers to detect QTL using similar algorithms to an earlier version, QTL Express (Seaton et al. 2006). A separate analysis with the 250 iterations of bootstrapping was used to determine 95% confidence limits for all QTL detected that explained a high proportion of the phenotypic variance (PEV). PEV was calculated for the BC-F2 analysis and but could only be approximately calculated for the HS analyses and could not be calculated at all for the SP analyses (S. Knott, pers. comm.). Permutation tests using the "experiment-wide option" with 250 iterations to estimate the minimum F value for significance at α=0.05 and for α=0.01 were performed separately for each trait, at both the experiment-wide (≈genome-wide) and the chromosome-wide levels for all traits in all analyses. To reduce our use of shared computer resources, permutation for the morphometric traits all 22 morphometric trait analyses were initially analyzed in a single run using composite size as a covariate without permutations. The analysis was then repeated with permutations of 250 iterations only for traits that with the largest LOD values in the first analysis. Separate QTL analyses were performed for each trait as follows: size data (length and weight) at both measurement periods; morphometric aligned co-ordinates at both measurement periods (using composite size as a covariate); and for parr mark number and contrast. Analyses were performed using composite female maps

(incorporating "combined" data from all three crosses), and for each of the three individual family crosses. A total of 18 different QTL analyses using both the Sib-pair module and dam-based analyses with the Half-sib module were undertaken. We also did 18 sire-based analyses using the Half-sib module that we are only briefly describe in the main text as our objective was to focus on female linkage map analyses. To enable comparison of our results with those of Boulding et al. (2008) we did three additional analyses for Cross 3 with the BC-F2 module using the same male linkage map that they used for their combined family analysis.

Physical map and candidate genes

Each significant QTL was positioned on the Atlantic salmon genome using the nearest SNP (or SNPs) to its estimated position on the linkage map. This was done by first entering the rs number for the nearest SNP (Moen et al. 2008; Lien et al. 2011; Brenna Hansen et al. 2012) into dbSNP https://www.ncbi.nlm.nih.gov/snp to find the largest continuous DNA sequence containing the SNP. Often the physical map position on the Atlantic salmon genome (ICSASG_v2) was available through a link on dbSNP. Otherwise the blast algorithm (https://blast.ncbi.nlm.nih.gov/Blast.cgi) was used to search for the contig's physical position. Finally, the Atlantic salmon genome browser SalmoBase (Samy et al. 2017) coupled with information on gene function (https://www.ncbi.nlm.nih.gov/gene/) was used to identify putative candidate genes upstream or downstream from the SNP's location (Supplementary Appendix 1). Although the candidate gene approach has limitations (Mäki-Tanila 2010), SNP markers associated with traits or genes (e.g. Christensen et al. 2017) can now be directly incorporated into genomic selection indices (L.R. Schaeffer, unpubl. data).

Results

Phenotype statistics

Length and weight were weakly correlated in young parr (r=0.41, $P<0.001$, N=1047) with length only explaining 16% of the phenotypic variance in weight. It was therefore decided to analyse length and weight as separate traits for the first measurement period. Length and weight were more highly correlated in fish near smelting (hereafter "near-smolts"; r=0.90, $P<0.001$, N=499); nevertheless, they were also analyzed as separate traits at the second measurement period for consistency.

During the first measurement period, lengths across all families ranged between 5.39-12.17cm (mean±se, 9.05cm ±0.04), and weights varied between 1.6-21.4 grams (mean±se, 8.74g±0.12) (Table 1) and were normally distributed (length: skewness=0.02, kurtosis=2.20; weight: skewness=0.55, kurtosis=2.58). Significant differences were found among the three crosses for both traits (one-way ANOVA, $P<0.001$ df=2), with individuals of Cross 1 being significantly shorter and lighter than the other two families (Tukey post-hoc, $P<0.001$).

The two families maintained through the second measurement period, had total lengths ranging from 10.61-28.55cm (mean±se, 19.18±0.14), and weight ranging from 15.0-236.5 grams (mean±se, 95.10±1.73) (Table 1) and were normally distributed (length skewness=0.18, kurtosis=2.42; weight skewness=0.69, kurtosis=3.17). Cross 1 was significantly lighter (Welsh's t-test, $P<0.001$ df=495) and significantly shorter (Welsh's t-test, $P<0.001$, df=495) than was Cross 4 (Table 1).

The number of parr marks per individual at the first measurement period ranged from 6 to 12, (mean±se, 9±0.03, Table 1) and were normally distributed (skewness=0.19, kurtosis=2.52). The relative contrast of skin colouration to parr mark pigmentation ranged from 1.08-6.29 (mean±se, 1.88±0.01), (Table 1). The distribution of skin/parr mark colouration was positively skewed, with few individuals showing high contrast, and a greater number with lower contrast values (skewness=2.56, kurtosis=15.90, Figure S2). Significant differences between families for both parr mark number and contrast was determined (one-way ANOVA, $P<0.001$, df=2). Cross 4 had significantly more parr marks and a significantly higher contrast between the dark parr mark colouration and the light skin than the other two families (Tukey post-hoc, $P<0.001$; Table 1). The variation in skin pigmentation was even more dramatic at the second measurement period (Figure S3).

Linkage map:

The female-specific composite linkage map contained a total of 29 linkage groups (Supplementary Appendix S1). *Ssa*01p and *Ssa*01q were kept as separate linkage groups because of differences in the karyotypes of chromosome in the hybrid parents of the three crosses. All QTL were successfully assigned to an approximate position on the physical map of the Atlantic genome (Appendix S1) and genes containing the SNP markers were identified for many of the traits (Appendix 1). All candidate genes were regarded as provisional because the QTL in our

study were only approximately located. Most QTL had confidence intervals that were smaller than 100% but larger than 25% of the entire mapped length of the linkage group (Tables S2 & S3).

QTL length

Several QTL for length using the female linkage map were found to be highly significant (α=0.01) at the experiment-wide level in both the sib-pair (Tables 2-5) and the dam-based half-sib (Table S2) analyses with GridQTL. Large QTL for parr length at measurement time 1 were detected on *Ssa*26/28 for cross 1 using the half-sib module, and on *Ss*a11 in Cross 1 in combined family dataset using both modules. Smaller QTL (α <0.05) significant at the experiment-wide level for length at measurement time 1 were found on *Ssa*02 in Cross 4 and in the combined family dataset and on *Ssa*18 in Cross 1 using the in the sib-pair module. In the dam-based half-sib analyses, significant QTL were found to be located on *Ssa*09 in Cross 4 and composite females (Table S2).

Highly significant QTL detected at measurement time 2 for near smolt length using sib-pair analyses were mapped to *Ssa*11 in Cross 1 and to *Ssa*19 in Cross 4 and the combined family dataset (Table 4 & 5). Half-sib analyses resulted in the detection of highly significant QTL on *Ssa*03 in Cross 4 and *Ssa*11 in Cross 1. Smaller QTL (α <0.05) that were significant at the experiment-wide level for length at measurement time 2 using Sib-pair module were found on *Ssa*02 and *Ssa*03 in Cross 4, on *Ssa*15 in Cross 1, and on *Ssa*02 in the combined family dataset. Three small QTL were found on *Ssa*03, *Ssa*04, and *Ssa*11 for combined family dataset using the Half-sib module. The most interesting candidate gene for length was *Androgen receptor beta 2* on *Ssa*04 (Table S6). Figure 2 shows that many of the experiment-wide significant QTL for length were similar for the two measurement periods.

QTL Weight

Several QTL for parr weight at the first measurement period were highly significant at the experiment-wide level using the sib-pair (Tables 2-5) and the dam-based half-sib (Figure 2; Table S2) analyses. Sib-pair analyses detected large QTL on *Ssa*02 in Cross 4 and in the combined dataset for all three families, *Ssa*11 in Crosses 1, 3, 4 and the combined dataset, *Ssa*16

in Cross 4 and the combined dataset, *Ssa*18 in Cross 3, and *Ssa*19 in the combined dataset. Smaller QTL (α <0.05) were detected on *Ssa*03 in Cross 4, *Ssa*07 and *Ssa*09 in Cross 3 as well as on *Ssa*03 and *Ssa*26/28 in the combined dataset. Half-sib analyses detected large QTL for parr weight on *Ssa*03 in Cross 4 and in combined family dataset, and *Ssa*11 in Cross 1, Cross 3, and the combined dataset. Smaller QTL were detected on *Ssa*07 in Cross 3, *Ssa*09 in Cross 3 and the combined dataset, as well as on *Ssa*26/28 in Cross 1, Cross 4, and the combined dataset. Generally, QTL that accounted for higher amounts of the phenotypic variance were more likely to be highly significant at the experiment-wide level. For example, in the backcross analysis of Cross 3, highly significant QTL accounted for 4.1% to 5.9% of the phenotypic variance (PEV) whereas significant QTL only accounted for 3.0% to 3.7% PEV (Table S1) and the same trend was seen in the half-sib analyses (Table S2).

QTL for weight in near-smolts at the second measurement period that were highly significant at the experiment-wide level using sib-pair analyses were detected on *Ssa*02, *Ssa*11 and *Ssa*18 in Cross 1, on *Ssa*19 in Cross 4, and on *Ssa*11 and *Ssa*19 in the combined analysis. Smaller QTL (α<0.05) for near smolt weight that were significant at the experiment-wide level were found on *Ssa*02, *Ssa*03, and *Ssa*26/28 in Cross 4 and on *Ssa*02 in the combined dataset. With dam-based analyses, large QTL were found on *Ssa*03 in Cross 4 and *Ssa*11 in Cross 1 and the combined dataset (Table S2). Most but not all experiment-wide significant QTL for weight were also QTL for length (Figure 2). The most interesting candidate gene for weight was *Steroid receptor RNA activator 1 (sra10)* on *Ssa*11 (Table S6).

QTL parr mark number and contrast

In the sib-pair analysis, QTL on *Ssa*23 were found to have experiment-wide significance for parr mark number in Cross 4 (Table 4). In addition, three suggestive QTL for parr mark number were found on *Ssa*07, *Ssa*13, and *Ssa*17 using the composite dataset (Table 5). Sire-based half-sib analyses also found significant QTL for number on *Ssa*04 in Cross 3 and on *Ssa*7 and *Ssa*17/24 (α <0.01) in the composite dataset (Figure 2, Table S3).

With sib-pair analyses, significant QTL for the contrast between the parr marks and the skin were found on *Ssa*23 in Cross 4. Three suggestive QTL for contrast were detected on *Ssa*18 in Cross 1 (Table 2), on *Ssa*09 and *Ssa*11 in Cross 3, and on *Ssa*01/23 in the combined analysis.

Using dam-based half-sib analyses, three significant QTL ($\alpha<0.05$) were found on *Ssa*09 in Cross 3 and in the combined family analyses, as well as on *Ssa*13 in Cross 1 (Table S2). Sire-based half-sib analyses also found highly-significant QTL for contrast on *Ssa*01 and *Ssa*21/23 in Cross 4, on *Ssa*13 in Cross 1, and on *Ssa* 01/23 in the combined analysis (Table S3).

The backcross analysis of Cross 3 using the current study's female-linkage map found a QTL for parr mark number on *Ssa*04 (explaining 4.1 PEV) and three suggestive QTL for parr mark contrast on *Ssa*09, *Ssa*11, and *Ssa*18 (explaining 1.6, 2.4 and 2.1% PEV, respectively; Table S1).The backcross analysis of Cross 3 using the slightly-modified male linkage map of Boulding et al. (2008) found a QTL for parr mark number on *Ssa*03 that was highly significant at the experiment-wide level and a QTL for parr mark contrast on *Ssa*11 that was significant.

QTL morphometric landmarks

Several QTL for 22 morphometric co-ordinate traits (Figure 1a) across all chromosomes were significant at the experiment-wide levels in GridQTL analyses that used centroid size as a covariate (Table 6). Overall it was found that chromosomes *Ssa*05 at both measurement periods, *Ssa*11 in parr and *Ssa*26/28 in near-smolts had the most significant QTL in the sib-pair analyses. In parr, *Ssa*11 contained QTL for four morphometric traits that were significant at the experiment-wide level, suggesting large effect of this region of the genome on juvenile shape. Dam-based half-sib analysis showed parr shape to be influenced by *Ssa*18, while *Ssa*07 had a significant ($\alpha<0.05$) effect on shape of near-smolts in Cross 1 (Table S4). In Cross 4, *Ssa*23 was found to have significant influence on shape at both measurement periods (Table S4). The most interesting candidate gene for shape on *Ssa*23 was *thyroid hormone receptor alpha* (Table S7). The backcross analysis of Cross 3 using the slightly-modified male linkage map of Boulding et al. (2008) found a QTL for PLFx (x7) on *Ssa*24 that was significant at the experiment-wide level.

Discussion

Skin pigmentation related traits

A major contribution of this study was to locate novel QTL for skin pigmentation traits - parr mark number and contrast, using a female linkage map, and to verify that both traits are partially controlled by multiple loci. Boulding et al. (2008) found QTL for parr mark number on

412 *Ssa*08 (as LG19), *Ssa*17 (as LG22), and *Ssa*23 (as LG18) using a male-linkage map and first
413 generation backcrossed transAtlantic families. Our new analysis of Cross 3 using a slight
414 modification of their male linkage map found a highly significant novel QTL for parr mark
415 number on *Ssa*03. This suggests that some of the differences seen between their study and the
416 current study are a product of different QTL segregating in different parents (the hybrid sire of
417 Cross 3 is a full sibling brother to the four sires used by Boulding et al. 2008) rather than
418 differences between studies in the markers that are genotyped, the marker density or whether a
419 male or female linkage map is used. Using the female linkage map of the three newly-created
420 transAtlantic hybrid crosses we found more precisely located two QTL (*Ssa*23 and *Ssa*17) found
421 in the previous study. Additionally, we discovered two novel QTL for parr mark number on
422 *Ssa*07 (a homeolog of *Ssa*17) and on *Ssa*13.
423 In addition, we discovered novel QTL for parr mark contrast. Although Boulding et al.
424 (2008) detected two significant QTL for parr mark contrast on *Ssa*02 (LG1) and *Ssa*07 (LG24)
425 neither of these were found in the current study even when a very similar linkage map was used
426 for Cross 3. Instead QTL on *Ssa*01/23 (at 56cM) in the composite female dataset, on *Ssa*09 in
427 Cross 3, on *Ssa*18 in Cross 1, and on *Ssa*23 in Cross 4 were detected in the new transAtlantic
428 families using the female linkage maps. Further one novel QTL on *Ssa*17 was detected in Cross
429 3 using the male linkage map.
430 Our use of three backcross families with varying proportions of European and North
431 American lineages allowed for the detection of a greater number of QTL for two skin
432 pigmentation traits than has been found previously. Cross 4 had a significantly higher number of
433 parr marks and a higher contrast between the parr marks and the surround skin than did Crosses
434 1 and 3 even though all crosses were randomly split in two tanks in the same environment. The
435 higher number of parr marks may be a result of Cross 4 being a second-generation backcross to a
436 European parent and therefore having a smaller amount of North American ancestry than the
437 other two crosses. The pure Saint John River North American strain is known to have
438 significantly fewer parr marks (mean 5.9 (s.d. 2.4)) than the pure Mowi European strain (mean
439 8.3 (s.d.: 1.1)) parr marks and less parr mark contrast even when reared in the same environment
440 (Boulding et al. 2008).
441 Previous studies regarding pigmentation in fish have frequently involved supplementing
442 the study species' diet with carotenoids. It has often been found that carotenoids and even

carotenoid equivalents can have significant effects on skin pigmentation (Kalinowski et al. 2004. These results, along with the equality of feed amounts and types in the tanks of all three transAtlantic families does not infer that nutritional content is the reason for the variation of colour phenotypes seen. Similarly, while parr mark contrast does show some phenotypic plasticity in response to the colour of the background environmental (Donnelly and Whoriskey 1993), our experimental design averaged tank effects and therefore we do not believe that environmental conditions significantly affected parr colouration in the transAtlantic families.

Greenwood et al. (2011) found a major pigment candidate gene, *Gja5*, that collocated with the presence of vertical bars in F2 hybrids between freshwater (barred) and marine (unbarred) threespine stickleback populations. They located significant QTLs for two pigmentation traits - degree of melanization of melanophores and spatial variation in melanophore number - on two different linkage groups that together explained 26.6% of the variance in barring. Synteny analysis of the stickleback cross found colocation between the barring QTL and a candidate gene *Gja5*. On the current version of the Atlantic salmon genome *Gja5* is on *Ssa*17 between 52.2 and 52.8 Mb. We did not have any SNP markers near this locus but we did find a QTL for number of parr marks on *Ssa*17 that was located 21 Mb upstream (Table S6).

Studies on zebrafish support the hypothesis that the dark parr marks on salmon juveniles are likely created by vertical bands of melanophores. In zebrafish, embryonic and early metamorphic melanophore progenitors only develop normally where there is signalling by Mast/stem cell growth factor receptor "Kit" (Fig. 2F in Singh and Nüsslein-Volhard 2015). However, in Atlantic salmon the *kita* locus on *Ssa*23 20.14 and 20.18 is 9 Mb downstream from the QTL for number of parr marks on *Ssa*23 in Cross 4 (Table S6). Similarly, mutations in *Tyrosinase-related protein 1a* (*Trp1a*) are known to affect melanophore survival (Singh and Nüsslein-Volhard 2015) and this gene was 1 Mb downstream from a QTL that we found for parr mark contrast on *Ssa*18 (Table S6).

Length and weight

Several studies have mapped QTL for growth related traits in Atlantic salmon due to their economic importance in cultured fish (Reid et al., 2005; Boulding et al. 2008; Houston et al. 2009; Baranski et al., 2010; Gutierrez et al. 2012; Pedersen et al. 2013; Besnier et al. 2015; Tsai

et al. 2015). A recent analysis of growth QTL mapping studies shows that QTL for length, weight, and condition factor often mapped to the same chromosome in different studies (Fig. 3c in Liu et al. 2017). In our study *Ssa*02, *Ssa*11, *Ssa*15, *Ssa*18, and *Ssa*19 were found to contain growth QTLs showing experiment-wide significance so it is not surprising that they have been detected in other studies. *Ssa*02 has been previously reported to contain growth QTL (Reid et al. 2005; Boulding et al. 2008; Houston et al. 2009; Gutierrez et al. 2012; Petersen et al. 2013). *Ssa*11 was also found to contain growth QTL in the trans-Atlantic backcross families of Boulding et al. (2008), and Pedersen et al. (2013), and in previous studies using European salmon (Houston et al. 2009; Gutierrez et al. 2012; Besnier et al. 2015). *Ssa*15 (Reid et al. 2005; Gutierrez et al. 2012), *Ssa*18 (Gutierrez et al. 2012; Tsai et al. 2015) and *Ssa*19 (Gutierrez et al. 2012) have also previously been reported to contain growth QTLs.

It has been found in numerous studies that QTL for similar traits often map to the same chromosome. QTL for two traits being on the same chromosome could be due to the linkage of separate QTL for each trait, or due to one QTL with pleiotropic effects accounting for both traits. In our transAtlantic families, it was found that length and weight at both measurement periods mapped together on *Ssa*11 in Cross 1, and length and weight at the second measurement period mapped to *Ssa*03 and *Ssa*04 in Cross 4 and composite females, respectively. We also found that QTL for the same trait mapped to homeologous chromosomes. Many of the duplicated chromosome arms present in Atlantic salmon have been described, making it possible to ascertain the potential duplicated QTL positions (Danzmann et al. 2008; Lien et al. 2011; Lien et al. 2016). For example, it was found that the homeologous chromosomes *Ssa*11/*Ssa*26, both contained mapped QTL for early length in Cross 1, early weight using the composite map, and weight in Cross 4 (though significant at different measurement periods). Significant QTL for parr mark number were found on homeologs *Ssa*7/*Ssa*17, possibly indicating duplicated QTL.

A previously suggested candidate gene for length and weight QTL is insulin growth factor 2 (*igf2bp1*; Reinecke et al. 2005; Pedersen et al. 2013; Tsai et al. 2014) which is located on Ssa03 (57.61 to 57.67 Mb). However, *igf2bp1* is an equivocal candidate gene in the current study because it was 9.2 Mb upstream from QTL segregating in Cross 4 for parr weight and for near-smolt length, and for weight1, weight 2 and length 1 on *Ssa*03 (Table S6).

Morphometric shape traits

The results of QTL analyses of the morphometric shape traits revealed several loci showing experiment-wide significance. The three linkage groups containing the most significant of the morphometric landmark QTL across the three transAtlantic families were *Ssa*05, *Ssa*11, and *Ssa*26/28. Boulding et al. (2008) also found that shape QTL on *Ssa*02, *Ssa0*3, *Ssa*07, *Ssa*11, and *Ssa*23. Both studies found that *Ssa*11 contains the most significant morphometric QTL and that there are multiple QTL for each trait, indicating that even specific morphological features are complex and perhaps controlled by polygenic genes.

Along with being associated with numerous landmarks in transAtlantic backcrosses and hybrids, *Ssa*11 was similarly linked to length in near-smolts (Boulding et al. 2008; Pedersen et al. 2013). We had three closely-spaced SNP markers (34.64cM, 35.49 cM, 36.26 cM) exactly located in the region of the pleiotrophic QTL at 35cM on *Ssa*11 but the positions of the markers ranged covered 10 Mb (Table S6) and the QTL itself has wide confidence limits (Tables S2, S4). Physiological or development mechanisms can be helpful in identifying candidate genes for shape. For example, thyroid hormones have recently been shown to affect shape differently in flatfishes and in zebrafish (Xu et al. 2016). This suggests that the *thyroid hormone receptor alpha* is a plausible candidate gene for the shape QTL we found on *Ssa*23 (Table S7).

Although environmental factors can strongly influence shape in Atlantic salmon (Von Cramon-Taubade et al. 2005), previous morphometric studies in other fish species have also shown that shape has a large genetic component. The genetic basis of shape traits in cichlids can involve major QTL (Franchini et al. 2014; Fruciano et al. 2016). Zhang et al. (2013) found that the genetic architecture of shape in the common carp had a large genetic component. Erickson et al. (2016) quantified over 100 QTL associated with skeletal morphology in the threespine stickleback suggesting strong polygenetic control. Laine et al. (2013) identified numerous significant and age-specific QTL associated with size and shape in the nine-spined stickleback.

Conclusions and future work

The use of second-generation backcrosses, a physical map of the SNP markers, and use of a female linkage map allowed more accurate mapping of QTL associated with length, weight, body shape, parr mark number, and contrast than was possible in two previous transAtlantic salmon studies (Boulding et al. 2008; Pedersen et al. 2012). Most notably, this study validates

previous QTL mapping studies in showing multiple regions of the genome partially determine pigmentation traits as well as body shape traits. Our results may encourage restoration ecologists to use salmon stocks with a body shape that matches the stream flowrates and parr mark colouration that matches the stream bottom substrate. All QTL were located relative to a physical map of the Atlantic salmon genome allowing putative candidate genes to be identified and our QTL to be compared with those found in future studies. Finer QTL mapping and GWAS (Gutierrez et al. 2015; Tsai et al. 2015) will be necessary to see if the candidate genes proposed to underlie these traits continue to be associated with them in future studies.

Acknowledgments

The authors would like to thank R. R. Danzmann for comments on the thesis version of the manuscript, S. Knott for her suggestions about the GridQTL analysis, and C. Rochus for helping SP with DNA extractions, and use R for statistical analysis. We also thank technicians at the Toronto Clinical Genomics Centre for running the MassARRAY™ assay. Also, the Director and technicians at the Department of Fisheries and Oceans Canada's St. Andrews Biological Station for assistance with animal care. University of Guelph work-study students S. Denome-Brown, J. Brimmer and A. Chan assisted with digitizing, E.N. Hay and I. Smith helped redraft figures, and F.N. Shizari helped EGB with finding SNP locations on the physical map and checking references. Funding was from a Natural Sciences and Engineering Research Council of Canada Strategic Grant STPGP 381643 – 2009 to E.G. Boulding and colleagues and from an NSERC Discovery grant to E.G. Boulding.

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Table 1. Summary of means for 6 phenotypic traits measured in each full-sibling back-cross family of transAtlantic salmon progeny between North American (NA) and European (EU) subspecies of Atlantic salmon (*Salmo salar*).

Cross	Dam	Sire	N_{t1}	N_{t2} [c]	Length1 (cm)[a]	Weight1 (g)[a]	Length2 (cm)[b]	Weight2 (g)[b]	No. Parr Marks[a]	Skin Contrast[a]
1	$BC1_{EU}$[d]	$BC1_{EU}$[d]	300	237	8.48±0.06	7.14±0.18	18.35±0.19	82.78±2.00	8.67±0.06	1.79±0.02
3[c]	EU	F1[e]	347	N/A	9.38±0.08	9.83±0.22	N/A	N/A	8.79±0.05	1.78±0.03
4	EU	$BC1_{EU}$[d]	400	262	9.18±0.07	9.01±0.21	19.89±0.19	105.9±2.57	9.41±0.05	2.03±0.02

[a] Measurement 1 "parr" (N_{t1}) took place November 15th-25th, 2011 and included a colour photograph used for morphometrics and parr mark traits. Phenotypic distributions for each trait are available in Figures 5 to 10 of Pedersen (2013).
[b] Measurement 2 "near smolt" (N_{t2}) took place November 28th-30th, 2012 and included a colour photograph used for morphometrics.
[c] Cross 3 were euthanized at the end of the first measurement period because of tank shortage. (Note that cross 3 and cross 4 were reversed in Petersen 2013).
[d] Parent most likely from backcross family with F1 hybrid male parent and EU female parent used by Boulding et al. (2008).
[e] Sire was an F1 hybrid between NA and EU subspecies from the same full sibling family used by Boulding et al. (2008).

Table 2. Linkage groups associated with significant QTL in Cross 1 using map based on female meiosis (FC1) with the Sib-Pair module of GridQTL. The suffixes on each trait refer to the measurement period (Table 1).

Chr[a]	LG[b]	Trait	Genome[c]	Linkage[d]	F[e]	LOD	df
2	1	Weight2	16cM	16cM	31.18**	26795	18.7994
11	9	Length 1	36cM	35cM	568.04**	123.35	44549
11	9	Weight 1	35cM	35cM	482.03**	104.67	44549
11	9	Length 2	35cM	33cM	99.56**	21.62	26794
11	9	Weight 2	35cM	33cM	107.18**	23.27	26794
15	8	Length2	65cM	24cM	10.29*	26795	2097.3
18	16	Length1	22cM	8cM	13.06*	44550	2.442
18	16	Contrast	22cM	0cM	34.84^	7.57	44549

[a] Atlantic salmon *Ssa* chromosome number Phillips et al. 2009 as used for North American chromosomes by Brenna-Hansen et al. 2012

[b] Linkage group using numbering system in Phillips et al. 2009

[c] Position on Genome-corrected Composite Linkage Map for all three females.

[d] Position on Linkage Map for dam of family 1 respectively.

[e] F-test significant at: * experiment-wide level at P<0.05, ** experiment-wide level at P<0.01 using permutation options in GridQTL. For parr mark traits only: ^ chromosome-wide level at P<0.05, # chromosome-wide level at P<0.01.

Table 3. Linkage groups associated with significant QTL in Cross 3 using map based on female meiosis (FC3) with the Sib-pair module of GridQTL. The suffixes on each trait refer to the measurement period (Table 1).

Chr[a]	LG[b]	Trait	Genome[c]	Linkage[d]	F[d]	LOD	df
9	10	Contrast	46cM	0cM[f]	31.73#	6.89	59683
11	9	Weight 1	35cM	9cM[f]	91.24*	19.81	60029

[a] Atlantic salmon *Ssa* chromosome number Phillips et al. 2009 as used for North American chromosomes by Brenna-Hansen et al. 2012

[b] Linkage group using numbering system in Phillips et al. 2009

[c] Position on Genome-corrected Composite Linkage Map for all three females

[d] Position on Linkage Map for dam of family 3

[e] F-test significant at: * experiment-wide level at P<0.05, ** experiment-wide level at P<0.01) using permutation options in GridQTL. For pigmentation traits only: ^ chromosome-wide level at P<0.05, # chromosome-wide level at P<0.01

[f] This QTL was also found by the BC-F2 module analysis although not necessary at the exact same position (See Table S1).

Table 4. Linkage groups associated with significant QTL in Cross 4 using map based on female meiosis (FC4) with the Sib-Pair module of GridQTL. The suffixes on each trait refer to the measurement period (Table 1).

Chr[a]	LG[b]	Trait	Genome[c]	Linkage[d]	F[e]	LOD	df
2	1	Weight1	6cM	24cM	31.4**	6.81	79799
2	1	Length2	13cM	16cM	32.55*	7.07	34190
2	1	Weight2	16cM	17cM	26.25*	5.67	34190
3	11	Weight 1	29cM	144cM	82.86*	17.99	79798
3	11	Length 2	30cM	144cM	63.91*	13.88	34189
3	11	Weight 2	30cM	144cM	38.33*	8.32	34189
11	9	Weight 1	35cM	0cM	127.2**	27.62	79798
19/16	26/23	Weight1	0cM	20cM	74.91**	16.27	79799
19/16	26/23	Length2	0cM	0cM	75.68**	16.43	34190
19/16	26/23	Weight2	0cM	0cM	135.12**	29.34	34190
23	18	Number	4cM	89cM	47.66**	10.35	79798
23	18	Contrast	1cM	0cM	77.67*	16.87	79798
26/28	21/33	Weight 2	0cM	0cM	61.28*	13.31	79798

[a]Atlantic salmon *Ssa* chromosome number Phillips et al. 2009 as used for North American chromosomes by Brenna-Hansen et al. 2012

[b] Linkage group using numbering system in Phillips et al. 2009

[c] Position on Genome-corrected Linkage Map for all three females

[d] Position on raw Linkage map for dam of family 4.

[e] F-test significant at: * experiment-wide level at P<0.05, ** experiment-wide level at P<0.01 using permutation options in GridQTL. For parr mark traits only: ^ chromosome-wide level at P<0.05, # chromosome-wide level at P<0.01.

Table 5. Linkage groups associated with significant QTL for all three families combined using composite map based on female meiosis (CompFem) with the Sib-Pair module of GridQTL. The suffixes on each trait refer to the measurement period (Table 1).

Chr[a]	LG[b]	Trait	Genome[c]	Linkage[d]	F[e]	LOD	df
1/23	17/18	Contrast	1q @0cM	56cM	49.28#	10.70	183689
2	1	Weight1	6cM	6cM	42.14**	9.151	184035
2	1	Length2	13cM	13cM	42.19*	9.162	60986
2	1	Weight2	16cM	16cM	56.02*	12.16	60986
7	24	Number	0cM	61cM	50.36^	10.94	183689
11	9	Weight 1	35cM	35cM	233.08**	50.61	184034
11	9	Weight 2	35cM	21cM	74.16**	16.10	60985
13	5	Number	32cM	0cM	57.93^	12.58	183689
17	22	Number	17@≈0cM	0cM	44.39#	9.64	183689
19/16	13/23	Weight1	0cM	0cM	48.55**	10.54	184035
19/16	13/23	Length2	0cM	0cM	94.06**	20.43	60986
19/16	13/23	Weight2	0cM	0cM	130.05**	28.24	60986

[a] Atlantic salmon *Ssa* chromosome number Phillips et al. 2009 as used for North American chromosomes by Brenna-Hansen et al. 2012

[b] Linkage group using numbering system in Phillips et al. 2009

[c] Position on chromosome on Genome-corrected composite female Linkage Map

[d] Position on chromosome on raw composite Linkage Map for all three females

[e] F-test significant at: * experiment-wide level at P<0.05, ** experiment-wide level at P<0.01 using permutation options in GridQTL. For parr mark traits only: ^ chromosome-wide level at P<0.05, # chromosome-wide level at P<0.01.

Table 6 Linkage groups associated with significant morphometric QTL at the first and second measurement periods (Table 1) based on female meiosis when centroid size was used as a covariate with the Sib-Pair module of GridQTL.

Period	Cross	Chr[a]	LG[b]	Trait	Genome[c]	Linkage[d]	F[e]	LOD	df
1	FC1	5	12	dPCFx	0cM	0cM	32.7*	7.102	44251
1	FC1	11	9	vPCFy	36cM	35cM	29.05*	6.308	44251
1	FC1	11	9	dPCFx	16cM	35cM	34.28*	7.443	44251
1	FC3	11	9	CFx	16cM	0cM	127.02*	27.583	59338
1	FC4	11	9	CPx	16cM	42cM	45.56*	9.893	79399
1	FC4	23	18	AFx	4cM	89cM	34.79*	7.554	79399
2	FC4	23	18	FKx	4cM	116cM	28.35*	6.155	18719

[a] Atlantic salmon *Ssa* chromosome number Phillips et al. 2009 as used for North American chromosomes by Brenna-Hansen et al. 2012

[b] Linkage group using numbering system in Phillips et al. 2009

[c] Position on chromosome on Genome-corrected Linkage Map for all three females

[d] Position on chromosome on vs raw Linkage Map for the dam of family 1, 3 or 4 respectively.

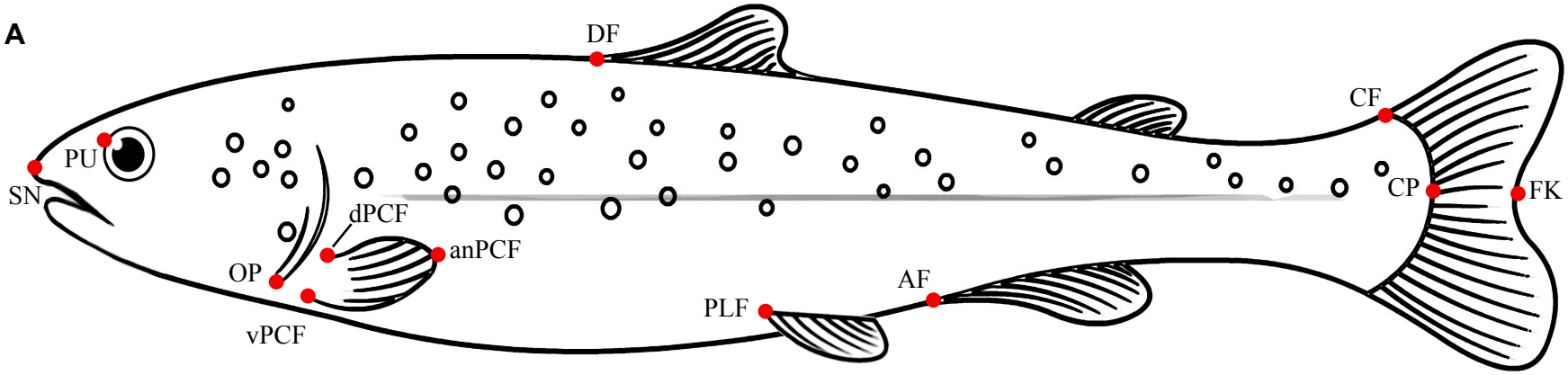
[e] F-test significant at: * experiment-wide level at P<0.05, ** experiment-wide level at P<0.01 using permutation options in GridQTL.

Figure Legends

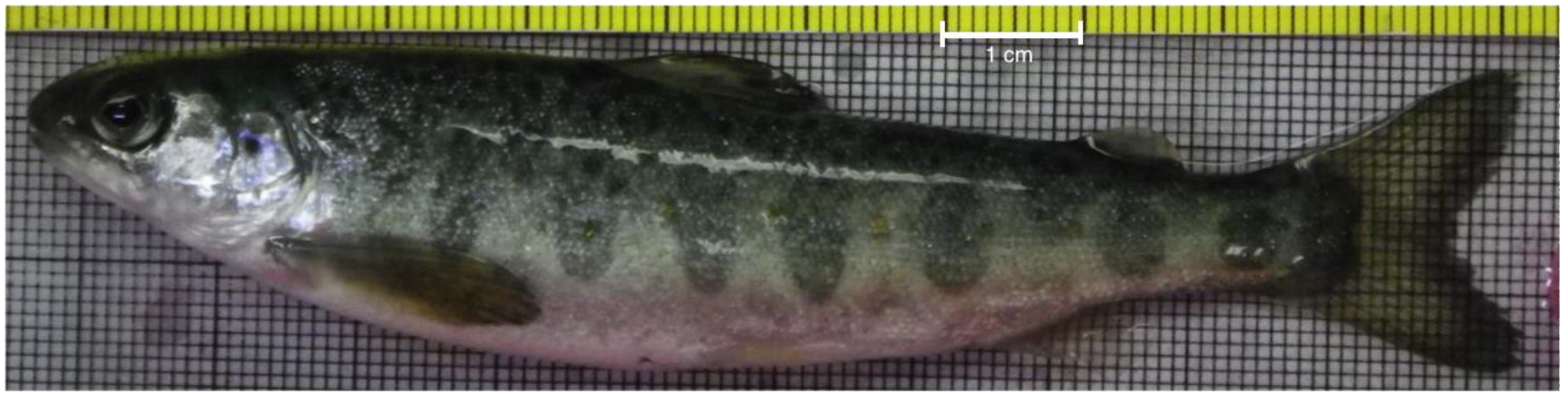
Figure 1. A. Landmarks for geometric morphometrics traits: 1. Tip of snout (SN), 2. Anterior insertion of dorsal fin (DF), 3. Dorsal insertion of caudal fin (CF), 4. End of caudal peduncle at the lateral line (CP), 5. Fork of caudal fin (FK), 6. Anterior insertion of anal fin (AF), 7. Anterior insertion of pelvic fin (PLF), 8. Most anterior point of pectoral fin (AnPCF), 9. Most ventral and posterior insertion of pectoral fin (vPCF), 10. Most anterior and dorsal insertion of pectoral fin (dPCF), 11. Point where the operculum meets the ventral body line (OP), 12. Most anterior point of pupil (PU). **B**. Actual photograph of fish that was digitized at first measurement period that also shows the vertical bars of darker skin-pigmentation called parr marks.

Figure 2. Summary of QTL found for weight, length, parr mark number and contrast, and 22 geometric morphometric shape traits.

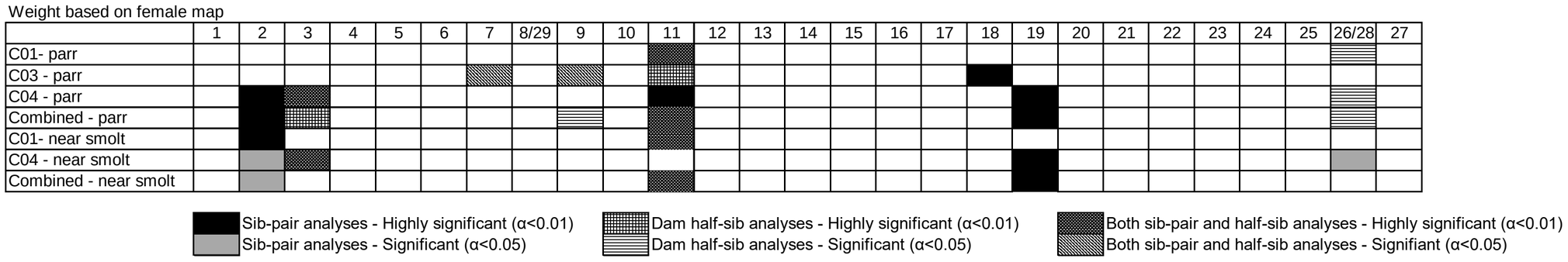
A



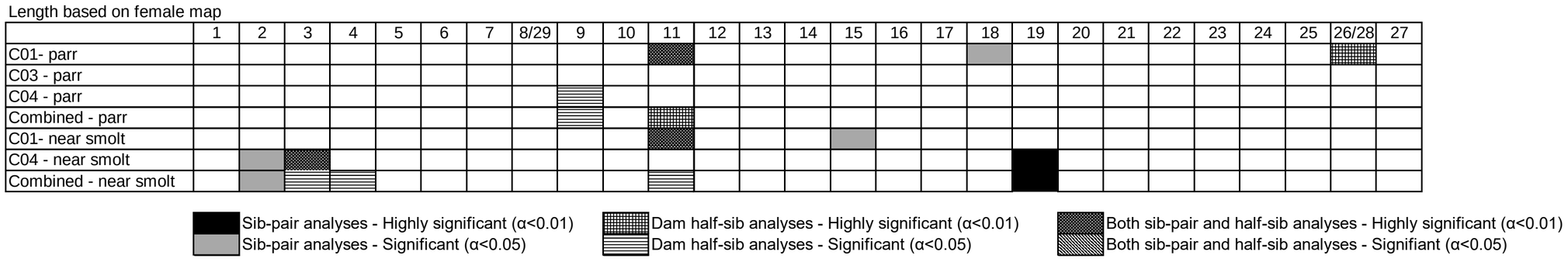
B



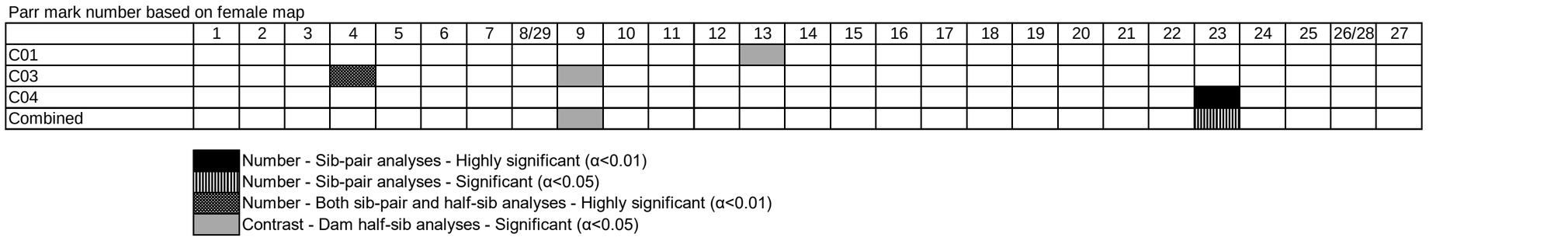
Weight based on female map



Length based on female map



Parr mark number based on female map



Experiment-wide significant QTL for morphometric landmarks based on female map