A European whitefish linkage map and its implications for understanding genome-wide synteny between salmonids following whole genome duplication

Rishi De-Kayne *,†, Philine G.D. Feulner *,†

*Department of Fish Ecology and Evolution, Centre of Ecology, Evolution and Biogeochemistry, EAWAG Swiss Federal Institute of Aquatic Science and Technology, Switzerland
†Division of Aquatic Ecology and Evolution, Institute of Ecology and Evolution, University of Bern, Switzerland
Abstract:
Genomic datasets continue to increase in size and ease of production for a wider selection of species including non-model organisms. For many of these species highly contiguous and well-annotated genomes are unavailable due to their prohibitive complexity and cost. As a result, a common starting point for genomic work in non-model species is the production of a linkage map, which involves the grouping and relative ordering of genetic markers along the genome. Dense linkage maps facilitate the analysis of genomic data in a variety of ways, from broad scale observations regarding genome structure e.g. chromosome number and type or sex-related structural differences, to fine scale patterns e.g. recombination rate variation and co-localisation of differentiated regions. Here we present both a sex-averaged and sex-specific linkage maps for Coregonus sp. “Albock” containing 5395 single nucleotide polymorphism (SNP) loci across 40 linkage groups to facilitate future investigation into the genomic basis of whitefish adaptation and speciation. The map was produced using restriction-site associated digestion (RAD) sequencing data from two wild-caught parents and 156 F1 offspring in Lep-MAP3. We discuss the differences between our sex-averaged and sex-specific maps and identify synteny between C. sp. “Albock” linkage groups and the Atlantic salmon (Salmo salar) genome. Our synteny analysis confirms that many patterns of homology observed between Atlantic salmon and Oncorhynchus and Salvelinus species are also shared by members of the Coregoninae subfamily.

Introduction:
Although advances in sequencing technology continue to increase the yield and lower the cost of genomic data acquisition, the curation of this data into a usable format can still be challenging (Ellegren 2014). Understanding the relative positions of genetic markers is often essential for the
detailed analysis of genomic datasets, and is carried out in many model organisms by mapping
reads to a reference genome (Sarropoulou 2011; Wolf and Ellegren 2017). However, marker
ordering in the absence of a reference genome can also be carried out using a linkage map, which
provides a measure of recombination distance rather than a physical difference, and as a result
their production has become a common early step in the analysis of large genomic datasets
(Lander and Green 1987; Lander and Schork 1994; Gross et al. 2008). Linkage maps are
produced by observing recombination events which have occurred in parents by sequencing many
offspring of that parental cross. Recombination events, which break up parental combinations of
alleles, are used to assign markers to, and then order within, linkage groups, elucidating the
relative location of thousands of markers along the genome (Sturtevant 1913; Rastas et al. 2013).
The resulting maps hold information on the broad genome structure e.g. number and length of
linkage groups (i.e. chromosomes) and can be used to evaluate synteny with related taxa to
investigate genome evolution (Sarropoulou 2011; Hale et al. 2017; Leitwein et al. 2017).
Linkage maps can be used to associate phenotypes and genotypes through quantitative trait locus
(QTL) mapping (Doerge 2002). Linkage maps also hold the information to investigate the
colocalization of regions under selection e.g. $F_{ST}$ outliers identified from genome scans and the
recombination landscape itself (Sakamoto et al. 2000; Johnston et al. 2017). Empirical evidence
has shown recombination to vary between species, populations, sexes and even individuals,
highlighting the importance of its investigation in existing and new study organisms (Smukowski
and Noor 2011; Kawakami et al. 2014; Stapley et al. 2017).

Linkage maps have become an essential tool in investigating evolution in non-model systems
where the use of existing reference genomes is limited and the assembly of new de novo
genomes is neither logistically nor financially feasible (Ellegren 2013; da Fonseca et al. 2016; Sutherland et al. 2016; Kubota et al. 2017; Sun et al. 2017; Zhigunov et al. 2017; Matz 2018).

Many non-model organisms have specific ecological and evolutionary characteristics which make them particularly interesting for asking targeted evolutionary questions (Matz 2018). These features can include high speciation rate, remarkable numbers of species living in sympatry, high phenotypic and genomic diversity within or between populations, and unique ecological characteristics (Garvin et al. 2010; Ekblom and Galindo 2011; Hornett and Wheat 2012; Matz 2018). Carrying out studies to understanding the genomic basis of these phenomena relies upon the development of new primary genomic resources in these non-model systems (Matz 2018). Linkage maps are therefore an ideal starting point to study evolution in new systems and open the door for the future production of more complex genomic resources including de novo genomes. Scaffolds produced during de novo genome assembly can be anchored to a linkage map, improving the contiguity and accuracy of the assembly (Fierst 2015; Lien et al. 2016; Feulner et al. 2018).

Salmonids are a particularly interesting family of teleost fishes in terms of their ecology and evolution, having colonised and adapted to a huge range of freshwater habitats (Nelson et al. 2006). They also have an interesting evolutionary history, influenced by a whole genome duplication which occurred ~80 Mya in the shared ancestor of all salmonids (Macqueen and Johnston 2014). The family Salmonidae comprises of two main clades, which diverged ~52 Mya (Macqueen and Johnston 2014). One clade is made up of the subfamily Salmoninae which includes salmon, trout and char species and the other contains the two subfamilies Thymallinae, containing grayling, and Coregoninae, containing whitefish and ciscos (Near et al. 2012;
Macqueen and Johnston 2014). Whitefish exhibit remarkable phenotypic diversity and high speciation rate, with multiple sympatric species having evolved post-glaciation in the last 15000 years (Lu and Bernatchez 1999; Kottelat and Freyhof 2007; Hudson et al. 2011). Two main whitefish species complexes exist, one in North America and the other in Europe. The North American whitefish complex comprises of *C. clupeaformis* species including sympatric ‘dwarf’ and ‘normal’ morphs which have arisen since the last glacial maximum (Bernatchez and Dodson 1990). The European species complex was previously described under the umbrella term ‘*C. laveratus* species complex’, however ongoing work to formally describe the many species which are found across Europe is being undertaken by taxonomists (Douglas et al. 1999; Østbye et al. 2005; Kottelat and Freyhof 2007; Hudson et al. 2011). In Europe, whitefish are naturally found as far north as Finland and as far south as the Alps, with a particularly speciose monophyletic clade known as the ‘pre-alpine’ whitefish which are distributed throughout Switzerland and its surrounding countries (Østbye et al. 2005; Hudson et al. 2011). Over 30 whitefish species have been described in Switzerland alone (Steinmann 1950) and some lakes continue to harbour up to six sympatric whitefish species despite the reduction of genetic and phenotypic differences between many species and the extinction of others following lake eutrophication in the 1980s (Vonlanthen et al. 2012). Sympatric whitefish species are monophyletic in many Swiss lakes and occupy a variety of ecological niches and exhibit a range of morphological differences (including body size, gill raker number and spawning season and depth; Douglas et al. 1999; Hudson et al. 2011; Vonlanthen et al. 2012; Hudson et al. 2017). It is the repeated ecological differentiation in sympatry that makes Swiss whitefish a particularly interesting radiation in which to study the genomic basis of adaptation. Although multiple studies have investigated the genetic basis of
adaptation in other salmonids those carried out on the Coregoninae subfamily are comparatively scarce.

The complex evolutionary history of salmonids, specifically the effect of the salmonid specific whole genome duplication (Ss4R), makes the genetic basis of adaptation difficult to study in this family (Lien et al. 2016). Dense linkage maps have been produced to address these difficulties for a variety of Salmoninae, including Arctic char, Brook trout, Brown trout and Chinook salmon (McKinney et al. 2016; Sutherland et al. 2016; Leitwein et al. 2017; Nugent et al. 2017). These studies typically pair the use of dense linkage maps with the Atlantic salmon (Salmo salar) reference genome to improve the genomic resolution of their analyses. However, due to the ~50 million year divergence time between Salmoninae and Coregoninae, and the limited number and density of whitefish linkage maps, the current analysis of genomic whitefish datasets is complex and limited (Rogers et al. 2001; Rogers and Bernatchez 2004; Rogers and Bernatchez 2007; Gagnaire et al. 2013). Only one whitefish linkage map produced using a restriction-site associated digestion (RAD) sequencing approach is available and was produced using data from North American whitefish (C. clupeaformis; Gagnaire et al. 2013). It includes 3438 single nucleotide polymorphism (SNP) markers resolved into 40 linkage groups (matching the karyotype of C. clupeaformis; Phillips and Ráb 2007) and was successfully used to investigate expression QTLs in C. clupeaformis (Gagnaire et al. 2013). However, studies which later described synteny patterns between salmonid genomes struggled to confidently resolve the relationships between lake whitefish linkage groups and other salmonid chromosomes using this map (Sutherland et al. 2016). The use of this map for investigating the remarkable European adaptive radiation of whitefish is also limited, due to the specificity of RAD markers and lack of
knowledge about genetic differentiation between *C. clupeaformis* and European whitefish (*C. laveratus* spp. complex) (Østbye *et al.* 2005; Hudson *et al.* 2011). The production of a European whitefish linkage map is therefore essential to study evolution within these extraordinary radiations.

In this study we produce a detailed linkage map for pre-alpine whitefish using a RAD sequencing approach. We produced both sex-specific and sex-averaged linkage maps for *Coregonus sp. “Albock”*, one member of the pre-alpine whitefish clade, from one F1 lab-bred cross. Here, we describe the sex-averaged and sex-specific linkage maps of *C. sp “Albock”* and use our sex-averaged linkage map to identify synteny between *C. sp. “Albock”* and Atlantic salmon (*Salmo salar*). We identify rearrangements present between the two species which reflect the occurrence of fission and fusion events following the Ss4R whole genome duplication, some of which were confidently identified to be shared only between members of the Salmoninae subfamily in past studies. This *Coregonus* linkage map will facilitate future research regarding the genomic basis of adaptation in the adaptive radiation of Swiss whitefish as well as assisting in the *de novo* assembly of the whitefish genome.

**Materials and methods:**

**Experimental cross**

One F1 family comprising of two parents and 156 offspring was used for linkage map construction. Both parent whitefish were sexually ripe, adult, *Coregonus sp. “Albock”*, a formally undescribed species, which likely originates from an introduction of whitefish from Lake Constance and for which taxonomic description is in progress. The parental whitefish
collected from Lake Thun in December 2016 and were crossed in vitro by mixing sperm and
eggs (obtained from the cantonal hatchery) together before adding cold water to harden
successfully fertilized eggs. Fertilized eggs were then placed in a flow-through system which ran
5°C lake water over the eggs for 11 weeks until they began to hatch. Before larvae had fully
utilized their yolk sac they were sedated and euthanized with MS222 (50 mg/l for sedation; 200
mg/l for euthanization; buffered with sodium bicarbonate 500 mg/l) and preserved in 100%
ethanol (February 2017; Animal Permit number LU03/15).

DNA extraction, library preparation and sequencing

DNA for both parental whitefish was extracted from muscle tissue. Progeny DNA was extracted
following the digestion of 176 whole larvae. Both parent and progeny DNA was extracted using
QIAGEN DNeasy Blood and Tissue extraction kit. The DNA concentration of each extract was
measured using the Qubit 1.0 Fluorometer. In total five RAD libraries were constructed, one
comprising only of the two parental individuals and the other four comprising of 44 offspring
each, following the protocol of Baird et al. (2008) with slight modifications. Equal amounts of
DNA from each offspring (1 µg) were pooled prior to restriction enzyme digestion. Since the
parental library contained only two individuals, to achieve higher sequencing depth, 18 µg DNA
from each parent was used for the digestion. The restriction enzyme digestion was carried out
using the Sbf-1 enzyme, which has been shown to digest salmonid DNA effectively (Gonen et al.
2014; Gagnaire et al. 2013; Sutherland et al. 2016), before the digested genomic DNA was
ligated to the first Illumina adaptor and individual-specific barcodes. Size selection then took
place using a SageELF to retain only DNA fragments between 300 and 700 base pairs. These
fragments were then amplified in a PCR after the ligation of the second Illumina adaptor. Each
library was spiked with PhiX DNA (~10% of reads) before being single-strand sequenced, each on a single lane of Illumina HiSeq 2500 with 100 cycles at the Lausanne Genomic Technologies Facility (Switzerland).

**Sequence processing and genotyping**

The first step of processing the sequenced reads was to remove all PhiX reads using a Bowtie2 mapping approach (using default parameters except for the number of allowed mismatches which we set to 1; Langmead and Salzberg 2012). Next, all reads from the parental library were filtered for quality using TRIMMOMATIC v.0.35 (Bolger et al. 2014). Bases were trimmed from the beginning and end of reads if they were below quality 3, a sliding-window approach was used with a 4 base wide window to trim bases below a quality score of 15. Reads were only retained if they had an average quality of 30 and if they were still less than 50 base pairs (bp) in length. Reads from the parental library and four offspring libraries were then demultiplexed and offspring reads were trimmed to 90 bp using the `process_radtags` module in Stacks (Catchen et al. 2013). Next, 20 offspring with < 1 million reads were discarded to leave both parents and 156 F1 offspring for analysis. A de novo reference assembly was produced by combining only reads from both parents, running the `ustacks` module in Stacks (Catchen et al. 2013) to identify putative SNP loci present in the parents of the cross (with a minimum coverage depth of 20) and the concatenation of these consensus stacks (Catchen et al. 2013). An index of this reference was then produced with Bowtie2 (Langmead and Salzberg 2012). Both parental and all offspring fasta files were aligned to the parental de novo reference assembly using Bowtie2 (using default parameters except for the number of allowed mismatches which we set to 1) resulting in individual alignment files. The GATK Haplotype Caller (Poplin et al. 2017) was used to call
genotypes, producing a VCF file retaining only SNPs genotyped with a minimum base quality score of 20 and a minimum confidence threshold of 20, i.e. p-error 0.01. This genotype file was further filtered with VCFtools (Danecek et al. 2011) to leave 20635 biallelic SNPs with a minimum phred quality score of 30 with indels removed. Since only one generation of offspring are included in an F1 linkage map, the most informative loci are those that are heterozygous in one parent and homozygous in the other (e.g. maternal Aa, paternal aa or maternal aa, paternal Aa). Offspring can therefore be heterozygous or homozygous (e.g. Aa or aa in an expected ratio of 1:1) and the phasing/origin of each allele is known. In addition to these highly informative loci, loci for which both parents are heterozygous can also provide information in the offspring in certain linkage mapping programs (e.g. maternal Aa, paternal Aa). In these cases, three offspring genotypes may be observed e.g. AA, Aa, aa in an expected ratio of 1:2:1 with only homozygous offspring being informative since we know that one copy of each allele is from each parent (e.g. AA offspring or aa offspring have received one A from each parent or one a from each parent, respectively). Heterozygous offspring genotypes are uninformative since the origin of each allele is unknown (e.g. Aa offspring may have received A or a from either parent). Loci were then filtered in R leaving only informative loci segregating in these two ways as well as removing any loci with missing data in either parent. All SNPs from RAD loci with more than three SNPs were removed and one SNP was chosen at random from those RAD loci with two SNPs. Remaining loci with over 20% missing data were also removed, leaving 9757 loci for linkage mapping (R Core Team 2017).

228

Linkage mapping
Linkage Map construction was carried out using Lep-MAP3 (Rastas 2017). First custom R and python scripts were used to convert the VCF file containing informative loci to Lep-MAP3 format before it was converted to a posterior probability table using the script linkage2post.awk and the Transpose module (Lep-MAP2; Rastas et al. 2016). Next Lep-MAP3 modules were used starting with the ParentCall2 module identifying 7800 informative markers. The Filtering2 module was then used to remove markers with significant segregation distortion (dataTolerance=0.001). Linkage groups were then identified using SeparateChromosomes2 with a logarithm of odds (LOD) score of 16 (lodLimit = 16) and the minimum number of markers per linkage group set to 25, resolving 40 linkage groups (corresponding to the 40 whitefish chromosomes identified by karyotyping; Phillips and Ráb 2007) containing 5395 loci before within-group ordering of markers was carried out (Rastas 2017). Due to the slight stochastic variation in marker distances between runs, the OrderMarkers2 module was used, specifying a sex-specific map (sexAveraged=0), three times on each linkage group to produce a male and a female linkage map. This procedure was then repeated specifying a sex-averaged map (sexAveraged=1). The marker orders with the highest likelihoods for each linkage group for each type of map were combined to produce the final most likely male and female sex-specific maps and one final sex-averaged map, each positioning the same 5395 SNP markers. A custom R script was used to calculate differences in the marker densities and lengths between maps and the sex-averaged map was plotted using MapChart (Voorrips 2002; R Core Team 2017).

Synteny analysis

To identify synteny between the 29 salmon chromosomes and the 40 whitefish linkage groups, the RAD loci sequenced from the two parents of the cross, which were used for de novo
reference production, were mapped to the *Salmo salar* genome using Stampy v. 1.0.22 (Lunter and Goodson 2010) to produce an alignment file for all reference reads. Since whitefish and Atlantic salmon are ~60 million years divergent and transcript analysis has shown them be 93% similar, a divergence percentage of 7% (substitution rate=0.07) was specified during read mapping (Koop et al. 2008). A custom R script was then used to match the 5395 SNP loci within the complete sex-averaged map to the corresponding loci in the reference whitefish - Atlantic salmon alignment file, extracting the salmon chromosome, base pair position and mapping quality. Mapped loci were then stringently filtered by their mapping quality score (MAPQ > 30) and the salmon chromosome with the most hits was noted. Linkage groups were then ordered to reflect their synteny with salmon chromosomes (Table 1) and renamed with the prefix ‘W’ to match salmon chromosome ordering. Synteny was visualized out using the *circlize* package (Gu 2014) in R plotting all links from reads with MAPQ > 30 to the corresponding salmon chromosome and position within this chromosome (Figure 2).

**Data availability**

Fastq files for all 156 offspring and both parents are deposited in the NCBI short read archive (SRA accession xxxx available upon publication). The genotype file (VCF) and the Lep-MAP input file are available at Dryad (doixxx, available upon publication). All R, Python and bash scripts used can be accessed at https://github.com/RishiDeKayne/.

**Results and Discussion**

**Linkage mapping**
Our F1 cross comprising of two *C. sp. “Albock”* adults and 156 offspring was successfully genotyped using a RAD-seq approach. The assignment of SNPs to linkage groups and the subsequent ordering of SNPs within linkage groups was carried out using Lep-Map3 (Rastas 2017). In total 9757 SNPs were retained following stringent quality control and loci filtering steps, with 7800 identified as informative in Lep-MAP3. Finally, 5395 SNPs were assigned to, and arranged within, linkage groups in both sex-averaged and sex-specific maps (Table 1; Figure 1). With the LOD score of 16, 40 linkage groups, corresponding to the 40 chromosomes observed in karyotype studies of European whitefish (*C. laveratus*; Phillips and Ráb 2007), were formed with an average of 135 markers per linkage group (Table 1). Map lengths varied from 2293.86 cM in the sex-averaged map to 2460.10 cM and 2263.05 cM in the female and male maps, respectively. All three maps produced in this study were considerably shorter than a previously published *C. clupeaformis* linkage map containing 3438 RAD markers, which had a total map length of 3061 cM (Gagnaire et al. 2013). Our sex-averaged *C. sp. “Albock”* map had an average linkage group length of 57.35 cM with the female and male sex-specific maps showing average linkage group lengths of 61.50 cM and 56.58 cM, respectively.

The number of SNPs per linkage group varied from 31 to 253 and the lengths of linkage groups varied from 15.20 cM to 83.57 cM in the sex-averaged map. Two linkage groups, Calb38 and Calb39, were comprised only of male-informative loci and therefore had lengths of 0 cM in the female map, with the longest linkage group in the female map being Calb02 at 101.33 cM. In the male map linkage groups vary in length from 7.41 cM to 88.06 cM for linkage groups Calb40 and Calb07.
Our sex-averaged map has high resolution, with a low average cM per marker of 0.46 cM,
varying from 0.27 cM in Calb04 to 0.77 cM in Calb34. The linkage map of the close relative C.
clupeaformis, a representative of the North American whitefish lineage, had a marker resolution
across the map of 0.89 cM, around half the density of our C. sp “Albock” map. In the female
map the average cM per marker was 0.48 cM varying in linkage groups (only considering
linkage groups > 0 cM) from 0.31 cM in Calb04 to 0.99 cM in Calb35. The average cM per
marker in the male map was 0.46 cM with the smallest and largest ratios found in Calb12 and
Calb39 respectively with 0.18 cM and 1.05 cM.

Sex differences can be observed by comparing our sex-specific linkage maps for C. sp.
“Albock”. Comparing total map lengths for the female and male maps gives a sex-ratio
(female: male) of 1.09 however, this does not account for the two whitefish linkage groups which
have length 0 cM in our female map (Calb38 and Calb39). Calculating this length sex-ratio for
each linkage group separately, including only those > 0 cM in both maps, results in a sex-ratio of
1.25. Salmonid species have been shown to have sexual dimorphisms in recombination rate with
female: male map length ratios for these species varying from 1.38 in Atlantic salmon (Lien et al.
2011) to 2.63 in Brown trout (Gharbi et al. 2006) and therefore sexual dimorphism in whitefish
appears to be low in comparison to other salmonids. However, since each sex-specific linkage
map represents the recombination landscape in one individual, in our case each parent of the F1
cross, more than one linkage map is required to disentangle individual variation in recombination
rate and consistent sex specific recombination rate variation (Sakamoto et al. 2000; Moen et al.
2004; Lien et al. 2011). Although our sex-ratio does not conclusively show variable
recombination rates between females and males it still reveals a striking difference in map length
considering the inclusion of the same set of markers for each. Studies on other teleost species, including stickleback, have reported detailed empirical evidence of sexually dimorphic recombination rates, calculating sex-ratios of linkage map lengths to be 1.64 (Sardell et al. 2018). Future work should aim to compare and contrast the recombination landscape of whitefish to the detailed sexually dimorphic recombination patterns observed in drosophila, mice, deer and various fish species (Dunn 1920; Sakamoto et al. 2000; Lenormand and Dutheil 2005; Johnston et al. 2017; Kubota et al. 2017; Sardell et al. 2018).

Synteny analysis

Synteny analysis was carried out to investigate broad scale genome structural variation, such as fission and fusions of chromosomes or chromosome arms, within the Salmonidae family. Stringent filtering of mapped RAD-seq reads to the salmon genome was applied to identify synteny whilst excluding uncertain mappings. From 5395 loci included in our linkage map we retained 839 mappings of high quality, which were spread across all 40 whitefish linkage groups (Figure 2). Homology between salmon chromosomes and whitefish linkage groups was determined by identifying the most common salmon chromosome the markers on each whitefish linkage group mapped to. Only a small number of markers on each whitefish linkage group mapped to a different salmon chromosome than the identified homologous chromosome (shown by the low abundance of non-parallel links from each linkage group; Figure 2). The largest of these deviations is a series of links (16) from W02 (which was identified as homologous to Ssa01 with 18 links) to Ssa19. Due to the similar abundance of links to two different salmon chromosomes these are unlikely to be erroneous or uncertain mappings and might rather reflect a whitefish specific fusion of two Atlantic salmon chromosomes Ssa01 and Ssa19, although future
research is required to confirm this. Three salmon chromosomes, Ssa02, Ssa08 and Ssa26 were not identified as homologs to any of our whitefish linkage groups, with Ssa08 having no significant mappings at all. This might be due to the fact that this salmon chromosome is small (26.43 Mb) and may also be small in whitefish, decreasing the number of RAD loci sequenced within it by chance (Lien et al. 2016). However, the C. clupeaformis linkage map also failed to identify synteny to Ssa08, raising the possibility this chromosome has been lost in whitefish (Gagnaire et al. 2013; Sutherland et al. 2016). We identified two salmon chromosomes which were each homologous to three different whitefish linkage groups; Ssa01 to W01, W02 and W03 and Ssa09 to W11, W12 and W13 (Figure 2). These Atlantic salmon chromosomes have been identified to map to three linkage groups in other salmonids including Brook Charr, Arctic Charr, Coho salmon and various Oncorhynchus species, however, synteny with C. clupeaformis, the only member of Coregoninae included in these comparisons, was less clear (Kodama et al. 2014; Sutherland et al. 2016; Hale et al. 2017; Nugent et al. 2017). This syntenic pattern has been attributed to fusion events which were unique to the Atlantic salmon lineage only. Here we add to the evidence provided by the C. clupeaformis linkage map that this synteny is also consistent with Coregoninae despite their significant divergence from members of the Salmoninae. Synteny analysis between members of Salmonidae also identified a number of Atlantic salmon chromosomes which each show homology to two linkage groups (Sutherland et al. 2016; Hale et al. 2017). We find a similar pattern of synteny between Salmo salar and Coregonus for many of these salmon chromosomes including Ssa03 (to W04 and W05), Ssa10 (to W14 and W15), Ssa13 (to W19 and W20), Ssa15 (to W22 and W23), Ssa16 (to W24 and W25), Ssa18 (to W27 and W28) and Ssa20 (to W30 and W31) (Figure 2). Our synteny analysis also identified Ssa04 as homologous to W06 and W07 and Ssa11 as homologous to W16 and W17, although W07 and
W17 have very few links (1 and 2, respectively). We also find that the multiple one to one relationships between salmon chromosomes and salmonid linkage groups identified by Sutherland et al. (2016) are also consistent with our map including those to Ssa12 (W18), Ssa22 (W33), Ssa23 (W34), Ssa24 (W35), Ssa25 (W36), Ssa27 (W37) and Ssa29 (W40; Table 1). We identify one possible whitefish-specific fission event with markers from both W38 and W39 mapping to Ssa28, which is homologous to only one linkage group in each salmonid species compared by Sutherland et al. (2016) including C. clupeaformis. It is therefore possible that a fission event has occurred in the European whitefish lineage, however, due to relatively low number and density of markers on W38 and W39 future investigation should aim to clarify this pattern. Our synteny analysis also highlights possible fusion events which have occurred only in European whitefish and Atlantic salmon. Whilst the C. clupeaformis, Rainbow trout, and multiple salmonid linkage maps, identified synteny of Ssa05, Ssa06, Ssa14, Ssa17 and Ssa19 to two linkage groups each, we only identify synteny to W08, W09, W21, W26 and W29, respectively (Table 1; Gagnaire et al. 2013; Sutherland et al. 2016). Two salmon chromosomes, Ssa07 and Ssa21 were shown by Sutherland et al. (2016) to be homologous to two linkage groups in C. clupeaformis but only one linkage group in all other salmonids. Our C. sp. “Albock” map identifies Ssa07 as homologous to W10 and Ssa21 to W32 suggesting the pattern of synteny may not be conserved between Coregonus species. Further work must therefore be carried out to determine genome structure similarity between C. sp. “Albock” and C. clupeaformis.

Both broad and small scale structural variations, including inversions, duplications and deletions, have been observed between closely related species and the mis-segregation which can occur
during meiosis as a result of these variations is thought to be able to play a role in the speciation process (Feulner and De-Kayne 2017). It is therefore possible that European and North American whitefish lineages (and even species within these lineages) have unique structural variations which may underpin reproductive isolation in sympathy. Without information on genome wide synteny and the occurrence of structural variation between these two lineages it is difficult to determine whether the observed variation in synteny patterns to the Atlantic salmon (e.g. with regards to Ssa05, Ssa06, Ssa14, Ssa17 and Ssa19) represents true variation between these species or variation in linkage mapping resolution and accuracy. A comparison of synteny between our *C. sp. “Albock”* map and the Atlantic salmon (using our synteny mapping approach) and the *C. clupeaformis* map to the Atlantic salmon (compared by Sutherland *et al.* 2016) can be found in Table S1.

The development of genomic resources for European whitefish

A wealth of genomic resources used to study adaptation and speciation are now available for a variety of systems. Multiple species from popular model radiations including Galapagos finches and Lake Victoria cichlids now have highly contiguous, well curated and annotated, reference genomes (Brawand *et al.* 2014; Lamichhaney *et al.* 2015). These resources provide the opportunity to ask specific questions about intra and inter-species genomic differences with many studies focusing on understanding the genomic basis of adaptation and reproductive isolation. Studies can now utilize high throughput whole-genome sequencing to achieve high depth of coverage and are able to map these reads to a reference genome to understand the distribution of genomic variation along the genome.
However, many interesting organisms including the many ecologically diverse salmonids have limited reference genomes available. Although both the Atlantic salmon (*Salmo salar*) and Rainbow trout (*Oncorhynchus mykiss*) genomes have been assembled (Berthelot *et al.* 2014; Lien *et al.* 2016), they are distantly related to many species within the salmon family, specifically the diverse clade of whitefish. Whitefish show extreme phenotypic diversity, although the degree of differentiation in sympatry varies. In North America a variety of lakes harbour sympatric pairs of ‘normal’ and ‘dwarf’ whitefish (Bernatchez and Dodson 1990). In Europe the diversity of whitefish species is much greater, and in Switzerland specifically up to six species of pre-alpine whitefish exist in sympatry in some post-glacial lakes (Hudson *et al.* 2011; Hudson *et al.* 2017). These species differ in many phenotypic traits including gill-raker number, body size and body shape. Reproductive isolation between species is thought to be largely maintained by different spawning seasons and depths between sympatric species (Hudson *et al.* 2017). Because of the rapid post-glaciation diversification of whitefish, the genomic basis of adaptation and speciation in pre-alpine whitefish is of particular interest. However, the best assembled, most closely related reference genome to this whitefish radiation is that of the Atlantic salmon. Since these two clades are so distantly related, new whitefish-specific resources are needed. Despite the production of multiple *Coregonus clupeaformis* linkage maps (Rogers *et al.* 2001; Rogers and Bernatchez 2004; Rogers and Bernatchez 2007), only one map to-date (Gagnaire *et al.* 2013) has utilized a high throughput sequencing technique. Although this map contains many SNP loci its use is largely restricted to the North American system, since the genome-wide synteny between North American and European whitefish is unknown and RAD markers are anonymous.
Our linkage map fills a gap in the resources available to analyse European whitefish genetic data allowing investigation into this species rich, ecologically diverse, lineage. The patterns of synteny between European whitefish and Atlantic salmon reported here should be further investigated once whitefish genomes become available to identify synteny at a finer scale, identifying chromosome fission and fusion events and possible inversions also within the *Coregonus* genus. Our linkage map can also be paired with future resources to investigate the outcome of whole genome duplication including estimations of the rediploidized proportion of the genome, already calculated in Atlantic salmon and Rainbow trout. Future work should further aim to identify regions of the genome which may underpin reproductive isolation in whitefish to better understand the speciation mechanism in this adaptive radiation.

In conclusion, we have produced the densest *Coregonus* linkage map to date, with a total sex-averaged map length of 2293.86 cM containing 5395 SNP loci. We have found evidence of sex-specific recombination rate variation within *C. sp. “Albock”* by calculating the sex-ratio in female and male linkage map lengths. The level of heterochiasmy inferred by this sex-ratio is reflected in other species with known sex-specific recombination variation, including other salmonids (Gharbi *et al.* 2006; Lien *et al.* 2011). We also show that *C. sp. “Albock”* linkage groups exhibit synteny with Atlantic salmon chromosomes, in some cases following a pattern of synteny shared with other salmonid species. This linkage map will facilitate a host of future studies into the genomic basis of adaptation in pre-alpine whitefish including those on the identification of QTLs for traits of interest, the interpretation of genome-wide divergence data and the colocalization of regions under selection e.g. *F_{ST}* outliers identified from genome scans. It also has the potential to assist in future scaffolding of pre-alpine whitefish reference genomes.
Acknowledgements

Thanks to Benjamin Gugger and team from Lake Thun whitefish hatchery for providing us with the breeding pair of *C. sp. “Albock”*. Also thanks to Anna Feller, David Frei, Andreas Taverna and Erwin Schäffer for their help breeding and maintaining the whitefish larvae and Oliver Selz for his taxonomic expertise. This project is funded by the Swiss National Science Foundation (SNSF project 31003A_163446/1 awarded to PGDF).

References

Baird, N. A., P. D. Etter, T. S. Atwood, M. C. Currey, A. L. Shiver *et al.*, 2008 Rapid SNP Discovery and Genetic Mapping Using Sequenced RAD Markers. PLoS One 3: e3376.

Bernatchez, L., and J. J. Dodson, 1990 Allopatric origin of sympatric populations of lake whitefish (*Coregonus clupeaformis*) as revealed by mitochondrial-DNA restriction analysis. Evolution (N. Y). 44: 1263–1271.

Berthelot, C., F. Brunet, D. Chalopin, A. Juanchich, M. Bernard *et al.*, 2014 The rainbow trout genome provides novel insights into evolution after whole-genome duplication in vertebrates. Nat. Commun. 5: 3657.

Bolger, A. M., M. Lohse, and B. Usadel, 2014 Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 30: 2114–2120.

Brawand, D., C. E. Wagner, Y. I. Li, M. Malinsky, I. Keller *et al.*, 2014 The genomic substrate for adaptive radiation in African cichlid fish. Nature 513: 375–381.

Catchen, J., P. A. Hohenlohe, S. Bassham, A. Amores, and W. A. Cresko, 2013 Stacks: an analysis tool set for population genomics. Mol. Ecol. 22: 3124–3140.

Danecek, P., A. Auton, G. Abecasis, C. A. Albers, E. Banks *et al.*, 2011 The variant call format and VCFtools. Bioinformatics 27: 2156–2158.
Doerge, R. W., 2002 Mapping and analysis of quantitative trait loci in experimental populations. Nat. Rev. Genet. 3: 43-52.

Douglas, M. R., P. C. Brunner, and L. Bernatchez, 1999 Do assemblages of Coregonus (Teleostei: Salmoniformes) in the Central Alpine region of Europe represent species flocks? Mol. Ecol. 8: 589–603.

Dunn, L. C., 1920 Linkage in mice and rats. Genetics 5: 325-343.

Ekblom, R., and J. Galindo, 2011 Applications of next generation sequencing in molecular ecology of non-model organisms. Heredity (Edinb). 107: 1–15.

Ellegren, H., 2014 Genome sequencing and population genomics in non-model organisms. Trends Ecol. Evol. 29: 51–63.

Ellegren, H., 2013 The Evolutionary Genomics of Birds. Annu. Rev. Ecol. Evol. Syst 44: 239–259.

Feulner, P. G. D., and R. De-Kayne, 2017 Genome evolution, structural rearrangements and speciation. J. Evol. Biol. 30: 1488–1490.

Feulner, P. G. D., J. Schwarzer, M. P. Haesler, J. I. Meier, and O. Seehausen, 2018 A dense linkage map of Lake Victoria cichlids improved the Pundamilia genome assembly and revealed a major QTL for sex-determination. bioRxiv 275388.

Fierst, J. L., 2015 Using linkage maps to correct and scaffold de novo genome assemblies: methods, challenges, and computational tools. Front. Genet. 6: 220.

da Fonseca, R. R., A. Albrechtsen, G. E. Themudo, J. Ramos-Madrigal, J. A. Sibbesen et al., 2016 Next-generation biology: Sequencing and data analysis approaches for non-model organisms. Mar. Genomics 30: 3–13.

Gagnaire, P.-A., S. A. Pavey, E. Normandeau, and L. Bernatchez, 2013 The genetic architecture of reproductive isolation during speciation-with-gene-flow in lake whitefish species pairs assessed by RAD sequencing. Evolution (N. Y). 67: 2483–2497.

Garvin, M. R., K. Saitoh, and A. J. Gharrett, 2010 Application of single nucleotide polymorphisms to non-model species: a technical review. Mol. Ecol. Resour. 10: 915–934.
Gharbi, K., A. Gautier, R. G. Danzmann, S. Gharbi, T. Sakamoto et al., 2006 A Linkage Map for Brown Trout (Salmo trutta): Chromosome Homeologies and Comparative Genome Organization With Other Salmonid Fish. Genetics 172: 2405-2419.

Gonen, S., N. R. Lowe, T. Cezard, K. Gharbi, S. C. Bishop et al., 2014 Linkage maps of the Atlantic salmon (Salmo salar) genome derived from RAD sequencing. BMC Genomics 15: 166.

Gross, J. B., M. Protas, M. Conrad, P. E. Scheid, O. Vidal et al., 2008 Synteny and candidate gene prediction using an anchored linkage map of Astyanax mexicanus. Proc. Natl. Acad. Sci. U. S. A. 105: 20106–20111.

Gu, Z., L. Gu, R. Eils, M. Schlesner and B. Brors, 2014. circlize implements and enhances circular visualization in R. Bioinformatics 30: 2811-2812.

Hale, M. C., G. J. McKinney, C. L. Bell, and K. M. Nichols, 2017 Using Linkage Maps as a Tool To Determine Patterns of Chromosome Synteny in the Genus Salvelinus. G3 (Bethesda). 7: 3821–3830.

Hornett, E. A., and C. W. Wheat, 2012 Quantitative RNA-Seq analysis in non-model species: assessing transcriptome assemblies as a scaffold and the utility of evolutionary divergent genomic reference species. BMC Genomics 13: 361.

Hudson, A. G., B. Lundsgaard-Hansen, K. Lucek, P. Vonlanthen, and O. Seehausen, 2017 Managing cryptic biodiversity: Fine-scale intralacustrine speciation along a benthic gradient in Alpine whitefish (Coregonus spp.). Evol. Appl. 10: 251–266.

Hudson, A. G., P. Vonlanthen, and O. Seehausen, 2011 Rapid parallel adaptive radiations from a single hybridogenic ancestral population. Proceedings. Biol. Sci. 278: 58–66.

Johnston, S. E., J. Huisman, P. A. Ellis, and J. M. Pemberton, 2017 A High-Density Linkage Map Reveals Sexual Dimorphism in Recombination Landscapes in Red Deer (Cervus elaphus). G3 (Bethesda). 7: 2859–2870.

Kawakami, T., L. Smeds, N. Backström, A. Husby, A. Qvarnström et al., 2014 A high-density linkage map enables a second-generation collared flycatcher genome assembly and reveals the patterns of avian recombination rate variation and chromosomal evolution. Mol. Ecol. 23: 4035–4058.

Koop, B. F., K. R. von Schalburg, J. Leong, N. Walker, R. Lieph et al., 2008 A salmonid EST genomic study: genes, duplications, phylogeny and microarrays. BMC Genomics 9: 545.
Kottelat, M., and J. Freyhof, 2007 *Handbook of European freshwater fishes*. Publications Kottelat. Switzerland

Kubota, S., A. Longloy, A. Singhabun, W. Khammee, K. Kessuwan *et al.*, 2017 Quantitative trait locus mapping of growth-related traits in inter-specific F₁ hybrid grouper (*Epinephelus fuscoguttatus* × *E. lanceolatus*) in a tropical climate. Aquac. Res. 48: 5913–5927.

Lamichhaney, S., J. Berglund, M. S. Almén, K. Maqbool, M. Grabherr *et al.*, 2015 Evolution of Darwin’s finches and their beaks revealed by genome sequencing. Nature 518: 371–375.

Lander, E. S., and P. Green, 1987 Construction of multilocus genetic linkage maps in humans. Proc. Natl. Acad. Sci. 84: 2363-2367.

Lander, E. S., and N. J. Schork, 1994 Genetic Dissection of Complex Traits. Science 265: 2037-2048.

Langmead, B., and S. L. Salzberg, 2012 Fast gapped-read alignment with Bowtie 2. Nat. Methods 9: 357–359.

Leitwein, M., B. Guinand, J. Pouzadoux, E. Desmarais, P. Berrebi *et al.*, 2017 A Dense Brown Trout (*Salmo trutta*) Linkage Map Reveals Recent Chromosomal Rearrangements in the *Salmo* Genus and the Impact of Selection on Linked Neutral Diversity. G3 (Bethesda). 7: 1365–1376.

Lenormand, T., and J. Dutheil, 2005 Recombination Difference between Sexes: A Role for Haploid Selection. PLoS Biol. 3: e63.

Lien, S., L. Gidskehaug, T. Moen, B. J. Hayes, P. R. Berg *et al.*, 2011 A dense SNP-based linkage map for Atlantic salmon (*Salmo salar*) reveals extended chromosome homeologies and striking differences in sex-specific recombination patterns. BMC Genomics 12: 615.

Lien, S., B. F. Koop, S. R. Sandve, J. R. Miller, M. P. Kent *et al.*, 2016 The Atlantic salmon genome provides insights into rediploidization. Nature 533: 200–205.

Lu, G., and L. Bernatchez, 1999 Correlated trophic specialization and genetic divergence in sympatric lake whitefish ecotypes (*Coregonus clupeaformis*): support for the ecological speciation hypothesis. Evolution (N. Y) 53: 1491-1505.
Lunter, G., and M. Goodson, 2011 Stampy: a statistical algorithm for sensitive and fast mapping of Illumina sequence reads. Genome Res. 21: 936–9.

Macqueen, D. J., and I. A. Johnston, 2014 A well-constrained estimate for the timing of the salmonid whole genome duplication reveals major decoupling from species diversification. Proceedings. Biol. Sci. 281: 20132881.

Matz, M. V., 2018 Fantastic Beasts and How To Sequence Them: Ecological Genomics for Obscure Model Organisms. Trends Genet. 34: 121–132.

McKinney, G. J., L. W. Seeb, W. A. Larson, D. Gomez-Uchida, M. T. Limborg et al., 2016 An integrated linkage map reveals candidate genes underlying adaptive variation in Chinook salmon (Oncorhynchus tshawytscha). Mol. Ecol. Resour. 16: 769–783.

Moen, T., B. Hoyheim, H. Munck, and L. Gomez-Raya, 2004 A linkage map of Atlantic salmon (Salmo salar) reveals an uncommonly large difference in recombination rate between the sexes. Anim. Genet. 35: 81–92.

Near, T. J., R. I. Eytan, A. Dornburg, K. L. Kuhn, J. A. Moore et al., 2012 Resolution of ray-finned fish phylogeny and timing of diversification. Proc. Natl. Acad. Sci. U. S. A. 109: 13698–13703.

Nelson, J. S., T. Grande, and M. V. H. Wilson, 2006 Fishes of the world. John Wiley and Sons, Inc. Hoboken, New Jersey.

Nugent, C. M., A. A. Easton, J. D. Norman, M. M. Ferguson, and R. G. Danzmann, 2017 A SNP Based Linkage Map of the Arctic Charr (Salvelinus alpinus) Genome Provides Insights into the Diploidization Process After Whole Genome Duplication. G3 (Bethesda). 7: 543–556.

Østbye, K., L. Bernatchez, T. F. Naesje, K.-J. M. Himberg, and K. Hindar, 2005 Evolutionary history of the European whitefish Coregonus lavaretus (L.) species complex as inferred from mtDNA phylogeography and gill-raker numbers. Mol. Ecol. 14: 4371–4387.

Phillips, R., and P. Rab, 2007 Chromosome evolution in the Salmonidae (Pisces): an update. Biol. Rev. 76: 1–25.

Poplin, R., V. Ruano-Rubio, M. A. DePristo, T. J. Fennell, M. O. Carneiro et al., 2017 Scaling accurate genetic variant discovery to tens of thousands of samples. bioRxiv 201178.
R Core Team, 2014 R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/.

Rastas, P., 2017 Lep-MAP3: robust linkage mapping even for low-coverage whole genome sequencing data. Bioinformatics 33: 3726–3732.

Rastas, P., F. C. F. Calboli, B. Guo, T. Shikano, and J. Merilä, 2016 Construction of Ultradense Linkage Maps with Lep-MAP2: Stickleback F2 Recombinant Crosses as an Example. Genome Biol. Evol. 8: 78–93.

Rastas, P., L. Paulin, I. Hanski, R. Lehtonen, and P. Auvinen, 2013 Lep-MAP: fast and accurate linkage map construction for large SNP datasets. Bioinformatics 29: 3128–3134.

Rogers, S. M., and L. Bernatchez, 2007 The genetic architecture of ecological speciation and the association with signatures of selection in natural lake whitefish (Coregonus sp. Salmonidae) species pairs. Mol. Biol. Evol. 24: 1423–1438.

Rogers, S. M., and L. Bernatchez, 2004 FAST-TRACK: Integrating QTL mapping and genome scans towards the characterization of candidate loci under parallel selection in the lake whitefish (Coregonus clupeaformis). Mol. Ecol. 14: 351–361.

Rogers, S. M., D. Campbell, S. J. E. Baird, R. G. Danzmann, and L. Bernatchez, 2001 Combining the analyses of introgressive hybridisation and linkage mapping to investigate the genetic architecture of population divergence in the lake whitefish (Coregonus clupeaformis, Mitchill). Genetica 111: 25–41.

Sakamoto, T., R. G. Danzmann, K. Gharbi, P. Howard, A. Ozaki et al., 2000 A Microsatellite Linkage Map of Rainbow Trout (Oncorhynchus mykiss) characterized by large sex-specific differences in recombination rates. Genetics 155: 1331-1345

Sardell, J. M., C. Cheng, A. J. Dagilis, A. Ishikawa, J. Kitano et al., 2018 Sex Differences in Recombination in Sticklebacks. G3 (Bethesda). g3.200166.2018.

Sarropoulou, E., 2011 Comparative genomics in teleost species: Knowledge transfer by linking the genomes of model and non-model fish species. Comp. Biochem. Physiol. Part D Genomics Proteomics 6: 92–102.

Smukowski, C., and M. Noor, 2011 Recombination rate variation in closely related species. Heredity (Edinb). 10744: 496–508.
Stapley, J., P. G. D. Feulner, S. E. Johnston, A. W. Santure, and C. M. Smadja, 2017 Variation in recombination frequency and distribution across eukaryotes: patterns and processes. Philos. Trans. R. Soc. Lond. B. Biol. Sci. 372: 20160455.

Steinmann, P., 1950 Monographie der schweizerischen Koregonen. Schweizerische Zeitschrift für Hydrol. 12: 340–491.

Sturtevant, A. H., 1913 The linear arrangement of six sex-linked factors in Drosophila, as shown by their mode of association. J. Exp. Zool. 14: 43–59.

Sun, C., Y. Niu, X. Ye, J. Dong, W. Hu et al., 2017 Construction of a high-density linkage map and mapping of sex determination and growth-related loci in the mandarin fish (Siniperca chuatsi). BMC Genomics 18: 446.

Sutherland, B. J. G., T. Gosselin, E. Normandeau, M. Lamothe, N. Isabel et al., 2016 Salmonid chromosome evolution as revealed by a novel method for comparing RADseq linkage maps. Genome Biol. Evol. 8: evw262.

Vonlanthen, P., D. Bittner, A. G. Hudson, K. A. Young, R. Müller et al., 2012 Eutrophication causes speciation reversal in whitefish adaptive radiations. Nature 482: 357–362.

Voorrips, R. E., 2002 MapChart: Software for the graphical presentation of linkage maps and QTLs. J. Hered. 93: 77–78.

Wolf, J. B. W., and H. Ellegren, 2017 Making sense of genomic islands of differentiation in light of speciation. Nat. Rev. Genet. 18: 87–100.

Zhigunov, A. V., P. S. Ulianich, M. V. Lebedeva, P. L. Chang, S. V. Nuzhdin et al., 2017 Development of F1 hybrid population and the high-density linkage map for European aspen (Populus tremula L.) using RADseq technology. BMC Plant Biol. 17: 180.
Table 1: Table comparing statistics for the sex-averaged, female and male C. sp. “Albock” linkage maps. The results of synteny analysis are included, showing the homologous salmon chromosome for each whitefish linkage group and the re-ordered whitefish linkage group name.

Figure 1: European whitefish linkage map showing the grouping and position of 5395 SNPs within a sex-averaged linkage map. The length of each of the 40 linkage groups is indicated by the scale in cM with linkage groups ordered by marker number from highest to lowest.

Figure 2: Synteny plot identifying homologous whitefish linkage groups and Atlantic salmon (Salmo salar) chromosomes denoted by the colouring of linkage groups and chromosomes. Links represent the location of 839 markers within the whitefish linkage map which were successfully mapped to the Atlantic salmon genome. Black salmon chromosomes Ssa02 and Ssa26 represent chromosomes with no homologous whitefish linkage groups.

Table S1: Table comparing the synteny identified between North American lake whitefish (Coregonus clupeaformis) and Atlantic salmon (Salmo salar) by Sutherland et al. (2016) using MapComp and our identified synteny between pre-alpine whitefish (C. sp. "Albock") and Atlantic salmon using a mapping approach.
| Linkage Group | Number of SNPs | LG length (cM) | Female SNPs/cM | Male LG length (cM) | Male SNPs/cM | Homologous Salmon Chromosome | Synteny LG | LG Length Sex Ratio (F:M) |
|---------------|----------------|---------------|----------------|---------------------|--------------|----------------------------|-----------|---------------------------|
| Calb01        | 253            | 75.96         | 0.30           | 91.07               | 0.36         | 63.67                      | W02       | 1.43                      |
| Calb02        | 228            | 83.57         | 0.37           | 101.33              | 0.44         | 69.58                      | W03       | 1.46                      |
| Calb03        | 220            | 78.51         | 0.36           | 84.40               | 0.38         | 87.95                      | W32       | 0.96                      |
| Calb04        | 214            | 58.45         | 0.27           | 66.69               | 0.31         | 50.05                      | W15       | 1.33                      |
| Calb05        | 190            | 66.93         | 0.35           | 63.63               | 0.33         | 71.66                      | W18       | 0.89                      |
| Calb06        | 187            | 53.16         | 0.28           | 70.69               | 0.38         | 37.88                      | W20       | 1.87                      |
| Calb07        | 181            | 71.53         | 0.40           | 68.13               | 0.38         | 88.06                      | W06       | 0.77                      |
| Calb08        | 173            | 52.28         | 0.30           | 56.37               | 0.33         | 45.30                      | W14       | 1.24                      |
| Calb09        | 170            | 79.41         | 0.47           | 73.03               | 0.43         | 91.75                      | W10       | 0.80                      |
| Calb10        | 165            | 62.43         | 0.38           | 60.45               | 0.37         | 65.05                      | W01       | 0.93                      |
| Calb11        | 164            | 65.01         | 0.40           | 64.04               | 0.39         | 66.05                      | W16       | 0.97                      |
| Calb12        | 164            | 51.09         | 0.31           | 70.15               | 0.43         | 30.22                      | W33       | 2.32                      |
| Calb13        | 162            | 69.34         | 0.43           | 71.26               | 0.44         | 63.49                      | W40       | 1.12                      |
| Calb14        | 157            | 65.11         | 0.41           | 61.78               | 0.39         | 72.14                      | W19       | 0.86                      |
| Calb15        | 156            | 64.90         | 0.42           | 63.19               | 0.41         | 71.73                      | W24       | 0.88                      |
| Calb16        | 154            | 56.17         | 0.36           | 55.30               | 0.36         | 65.75                      | W31       | 0.84                      |
| Calb17        | 151            | 65.53         | 0.43           | 69.40               | 0.46         | 61.63                      | W34       | 1.13                      |
| Calb18        | 149            | 61.50         | 0.41           | 65.22               | 0.44         | 62.38                      | W11       | 1.05                      |
| Calb19        | 147            | 62.15         | 0.42           | 68.25               | 0.46         | 55.50                      | W21       | 1.23                      |
| Calb20        | 144            | 66.36         | 0.46           | 79.08               | 0.55         | 56.52                      | W37       | 1.40                      |
| Calb21        | 143            | 71.78         | 0.50           | 69.37               | 0.49         | 83.01                      | W36       | 0.84                      |
| Calb22        | 137            | 71.12         | 0.52           | 74.56               | 0.54         | 67.96                      | W04       | 1.10                      |
| Calb23        | 127            | 64.80         | 0.51           | 68.96               | 0.54         | 69.78                      | W09       | 0.99                      |
| Calb24        | 127            | 52.57         | 0.41           | 58.54               | 0.46         | 54.23                      | W22       | 1.08                      |
| Calb25        | 124            | 57.74         | 0.47           | 61.62               | 0.50         | 60.81                      | W35       | 1.01                      |
| Calb26        | 123            | 64.59         | 0.53           | 70.67               | 0.57         | 62.12                      | W29       | 1.14                      |
| Calb27        | 118            | 46.03         | 0.39           | 61.06               | 0.52         | 30.24                      | W27       | 2.02                      |
| Calb28        | 115            | 59.05         | 0.51           | 63.68               | 0.55         | 59.73                      | W23       | 1.07                      |
| Calb29        | 114            | 62.40         | 0.55           | 61.31               | 0.54         | 70.58                      | W12       | 0.87                      |
| Calb30        | 112            | 62.75         | 0.56           | 68.12               | 0.61         | 63.96                      | W08       | 1.07                      |
| Calb31        | 111            | 53.35         | 0.48           | 63.62               | 0.57         | 42.48                      | W30       | 1.50                      |
| Calb32        | 104            | 56.67         | 0.54           | 63.47               | 0.61         | 53.94                      | W28       | 1.18                      |
| Calb33        | 97             | 67.73         | 0.70           | 70.46               | 0.73         | 66.40                      | W13       | 1.06                      |
| Calb34        | 79             | 61.12         | 0.77           | 71.34               | 0.90         | 62.97                      | W05       | 1.13                      |
| Calb35        | 56             | 36.88         | 0.66           | 55.57               | 0.99         | 21.14                      | W38       | 2.63                      |
| Calb36        | 45             | 24.18         | 0.54           | 15.92               | 0.35         | 30.75                      | W26       | 0.52                      |
| Calb37        | 37             | 27.48         | 0.74           | 34.82               | 0.94         | 21.51                      | W17       | 1.62                      |
| Calb38        | 34             | 11.86         | 0.35           | 0.00                | 0.00         | 24.01                      | W25       | 0.00                      |
| Calb39        | 32             | 17.17         | 0.54           | 0.00                | 0.00         | 33.66                      | W07       | 0.00                      |
| Calb40        | 31             | 15.20         | 0.49           | 23.55               | 0.76         | 7.41                       | W39       | 3.18                      |
| Total         | 5395           | 2293.86       | 2460.10        | 2263.05             | 1.09         |                            |           |                           |
| Average       | 134.88         | 57.35         | 0.46           | 61.50               | 0.48         | 56.58                      |           | 0.46                      |
Atlantic salmon – *Salmo salar*

Whitefish – *C. sp. "Albock"*
| Lake Whitefish (Gagnaire et al. 2013) | Atlantic Salmon | C. sp. Albock “Calb” | C. sp. Albock “W” |
|--------------------------------------|-----------------|----------------------|-------------------|
| Cclu05b?                             | Ssa01a          | Calb10               | W01               |
| Cclu04b                              | Ssa01c          | Calb02               | W03               |
| Cclu01a?                             | Ssa02a          | NA                   | NA                |
| Cclu06b                              | Ssa02b          | NA                   | NA                |
| Cclu26                               | Ssa03a          | Calb22               | W04               |
| missing                              | Ssa03b          | Calb34               | W05               |
| missing                              | Ssa04a          | Calb07               | W06               |
| Cclu08                               | Ssa04b          | Calb39               | W07               |
| Cclu29                               | Ssa05a          | Calb30               | W08               |
| Cclu01a?                             | Ssa05b          | NA                   | NA                |
| Cclu18                               | Ssa06a          | Calb23               | W09               |
| Cclu17                               | Ssa06b          | NA                   | NA                |
| Cclu24b                              | Ssa07a          | Calb09               | W10               |
| Cclu02a?                             | Ssa07b          | NA                   | NA                |
| missing                              | Ssa08           | missing              | missing           |
| Cclu31                               | Ssa09a          | Calb18               | W11               |
| Cclu16                               | Ssa09b          | Calb29               | W12               |
| Cclu35                               | Ssa09c          | Calb33               | W13               |
| Cclu06a                              | Ssa10a          | Calb08               | W14               |
| Cclu30                               | Ssa10b          | Calb04               | W15               |
| Cclu04a?                             | Ssa11a          | Calb11               | W16               |
| Cclu33                               | Ssa11b          | Calb37               | W17               |
| Cclu38b                              | Ssa12a          | Calb05               | W18               |
| Cclu38a                              | Ssa12b          | NA                   | NA                |
| Cclu27                               | Ssa13a          | Calb14               | W19               |
| Cclu13                               | Ssa13b          | Calb06               | W20               |
| Cclu25                               | Ssa14a          | Calb19               | W21               |
| Cclu24a                              | Ssa14b          | NA                   | NA                |
| Cclu40                               | Ssa15a          | Calb24               | W22               |
| Cclu14                               | Ssa15b          | Calb28               | W23               |
| Cclu11                               | Ssa16a          | Calb15               | W24               |
| Cclu19?                              | Ssa16b          | Calb38               | W25               |
| Cclu19?                              | Ssa17a          | Calb36               | W26               |
| Cclu01b?                             | Ssa17b          | NA                   | NA                |
| Cclu05b?                             | Ssa18a          | Calb27               | W27               |
| Cclu23                               | Ssa18b          | Calb32               | W28               |
| Cclu39                               | Ssa19a          | Calb26               | W29               |
| Cclu05a                              | Ssa19b          | NA                   | NA                |
| Cclu37                               | Ssa20a          | Calb31               | W30               |
| Cclu28                               | Ssa20b          | Calb16               | W31               |
| Cclu02 + 03                          | Ssa21           | Calb03               | W32               |
| Cclu21                               | Ssa22           | Calb12               | W33               |
| Cclu36                               | Ssa23           | Calb17               | W34               |
| Cclu34                               | Ssa24           | Calb25               | W35               |
| Cclu32                               | Ssa25           | Calb21               | W36               |
| Cclu04a?                             | Ssa26           | NA                   | NA                |
| Cclu10                               | Ssa27           | Calb20               | W37               |
| Cclu15                               | Ssa28           | Calb35, Calb4 | W38, W39 |
| Cclu12                               | Ssa29           | Calb13               | W40               |