Application of Low Coverage Genotyping by Sequencing in Selectively Bred Arctic Charr (Salvelinus alpinus)

Christos Palaiokostas,*,1 Shannon M. Clarke,† Henrik Jeuthe,*,‡ Rudiger Brauning,† Timothy P. Bilton,†,§ Ken G. Dodds,† John C. McEwan,† and Dirk-Jan De Koning*

*Department of Animal Breeding and Genetics, Swedish University of Agricultural Sciences, Box 7090, 750 07 Uppsala, Sweden, †Invermay Agricultural Centre, AgResearch, Private Bag 50034, Mosgiel 9053, New Zealand, ‡Aquaculture Center North, Ävägen 17, 844 61 Kälame, Sweden, and §Department of Mathematics and Statistics, University of Otago, Dunedin 9054, New Zealand

ORCID IDs: 0000-0002-4480-4612 (C.P.); 0000-0003-3434-5202 (H.J.); 0000-0002-9347-6379 (K.G.D.); 0000-0003-4801-6207 (J.C.M.); 0000-0001-6343-8155 (D.-J.D.K.)

ABSTRACT

Arctic charr (Salvelinus alpinus) is a species of high economic value for the aquaculture industry, and of high ecological value due to its Holarctic distribution in both marine and freshwater environments. Novel genome sequencing approaches enable the study of population and quantitative genetic parameters even on species with limited or no prior genomic resources. Low coverage genotyping by sequencing (GBS) was applied in a selected strain of Arctic charr in Sweden originating from a landlocked freshwater population. For the needs of the current study, animals from year classes 2013 (171 animals, parental population) and 2017 (759 animals; 13 full sib families) were used as a template for identifying genome wide single nucleotide polymorphisms (SNPs). GBS libraries were constructed using the PstI and MspI restriction enzymes. Approximately 14.5K SNPs passed quality control and were used for estimating a genomic relationship matrix. Thereafter a wide range of analyses were conducted in order to gain insights regarding genetic diversity and investigate the efficiency of the genomic information for parentage assignment and breeding value estimation. Heterozygosity estimates for both year classes suggested a slight excess of heterozygotes. Furthermore, FST estimates among the families of year class 2017 ranged between 0.009 – 0.066. Principal components analysis (PCA) and discriminant analysis of principal components (DAPC) were applied aiming to identify the existence of genetic clusters among the studied population. Results obtained were in accordance with pedigree records allowing the identification of individual families. Additionally, DNA parentage verification was performed, with results in accordance with the pedigree records with the exception of a putative dam where full sib genotypes suggested a potential recording error. Breeding value estimation for juvenile growth through the usage of the estimated genomic relationship matrix clearly outperformed the pedigree equivalent in terms of prediction accuracy (0.51 opposed to 0.31). Overall, low coverage GBS has proven to be a cost-effective genotyping platform that is expected to boost the selection efficiency of the Arctic charr breeding program.

Genomic information is being incorporated at an ever increasing rate in aquaculture breeding programs, guiding selection decisions (Yanez et al. 2015). Use of genome wide genetic markers has the potential to unravel the genetic factors that control traits of interest, increase the accuracy of estimated breeding values (Meuwissen et al. 2001) and optimize inbreeding management (D’Ambrosio et al. 2019). Current knowledge supported by both simulation (Sonesson and Meuwissen 2009) and real data (reviewed in Palaiokostas and Houston 2017; Lhorente et al. 2019) in a wide range of aquaculture species suggests that substantial improvements for key production traits can be obtained through use of genomic information. In particular, benefits are most pronounced for disease challenges, sex-limited-, late-in-life-, and post-slaughter traits.

Implementation of genomics in both animal and plant breeding programs is commonly performed through the usage of single nucleotide polymorphism (SNP) arrays (reviewed in Hickey et al.
germplasm has ever been included in the breeding nucleus. The Hornavan Arctic charr demonstrated superior growth capacity compared to other Arctic charr populations in Sweden and was thus selected for the establishment of a selective breeding program (Nilsson et al. 2010). Currently, selection candidates are chosen using best linear unbiased prediction (BLUP) methodology (Henderson 1975) for growth related traits. Typically, the breeding program has been based on 45 – 125 full sib families (Nilsson et al. 2010) reared in separate tanks until a size suitable (30 – 60 g) for identification using passive integrated transponders (PIT) tags. Thereafter the breeding candidates are reared communally in inland facilities.

Compared to the ample genomic information available in farmed salmonids like Atlantic salmon (Salmo salar) and rainbow trout (Oncorynchus mykiss), genomic resources for Arctic charr have only started to become available very recently (Nugent et al. 2017, 2019; Christensen et al. 2018). Nevertheless, no prior work has ever attempted to investigate the genetic diversity status and the potential for further improving the selectively bred Arctic charr strain in Sweden through the use of genome-wide genetic markers. The aim of the current study was to apply low coverage GBS in the aforementioned breeding strain using 930 animals from two-year classes (2013; 2017). Genome wide SNPs were identified and subsequently utilized for estimating genetic diversity metrics, verifying available parentage records and for estimation of breeding values through genomic BLUP (GBLUP).

MATERIALS AND METHODS

Sample background

Animals used in this study originated from two discrete year classes (2013 and 2017) of the Swedish Arctic charr breeding program. The breeding nucleus is located in the facilities of Aquaculture Center North (ACN; Kålarne, Jämtland, Sweden) where selection is operated using non-overlapping generations of approximately 4 years each. As such, the 2017 year class was formed through artificial crosses among selected animals from the 2013 year class. In the current study, 171 animals from year class 2013 (selected based on DNA availability) and 759 animals from year 2017 were genotyped using GBS. Genotyped animals from year class 2017 originated from crosses between 11 sires and 13 dams. Due to the aforementioned breeding scheme the genotyped animals of 2017 year class consisted of 13 full-sib families and two pairs of paternal half-sib families. Family size ranged between 10 – 90 offspring.

According to available pedigree data, five sires and four dams from the genotyped animals of year class 2013 were the parents of eight genotyped families from year class 2017. In particular, two families had both their putative parents genotyped, six had only one parent genotyped (two families had only their dam and four families had only their sire genotyped) and the remaining five families had neither of their parents genotyped (Table 1). Moreover, upon PIT-tagging fin-clips were collected from each animal for DNA extraction and stored in absolute ethanol at -20°C. The entire study was conducted in accordance to Swedish legislation for conducting animal research as described in the Animal Welfare Act 2018:1192 (ethics permit: 5.2.18 – 09859/2019).

GBS library preparation and sequencing

A tissue plug of approximately 3 mm diameter from each fin clip was used for DNA extraction. Prior to extraction, the tissue was air dried overnight to remove all traces of ethanol and then DNA was extracted following a salt-based extraction protocol (Clarke et al. 2014). A subset of the DNA extracted samples was visually assessed via 1.0% agarose gel in order to ensure the existence of high molecular weight
DNA. Quantification was performed using Picogreen (Quant-iT Picogreen dsDNA Reagent, Cat P11495, Life Technologies, Carlsbad, California, United States) fluorescence. Template DNA was digested with PstI (recognizing the CTGCA|G motif) and MspI (recognizing the C|CGG motif) restriction enzymes. Subsequent library preparation followed the method outlined in Elshire et al. (2011). Constructed libraries were size selected between 193 – 318 bp using a BluePippin (Sage Science). In total 10 GBS libraries were constructed containing 90 – 94 individuals each. Sequencing was performed in 5 lanes of an Illumina HiSeq 2500 using 100 cycles single end (SE) V4 chemistry.

QC filtering and SNP identification

Reads of low quality (average phred33 score below 30) were discarded using trimmomatic v0.39 (Bolger et al. 2014). SNP identification was performed using the UNEAK, Tassel v3.0.174 software (Lu et al. 2013) using the following settings: -UFastqToTagCountPlugin -e PstI-MspI; -UMergeTaxaTagCountPlugin -c 10; -UTagCountToTagPairPlugin -e 0.03; -UMapInfoToHapMapPlugin -mnMAF 0.05 -mxMAF 0.5 -mnC 0.1. SNPs with mean coverage below 0.5X were discarded. Finally, SNPs with Hardy-Weinberg disequilibrium (observed frequency of the reference allele homozygote minus its expected value) below -0.05 were removed using the KGD v0.8.7 software.

Genomic relationships

A genomic relationship matrix (GRM) was estimated using the KGD v0.8.7 software (https://github.com/AgResearch/KGD). The constructed GRM was the equivalent of G5, as described in Dodds et al. (2015). In short, an initial GRM using the SNPs passing the QC filtration and SNP identification step was followed using the xvalDapc function to select the optimal number of PC for the DAPC. Thereafter, a discriminant analysis step was conducted using clusters determined from the principal components. The Bayesian Information Criterion (BIC) was used for selecting the optimal number of clusters (K) based on the elbow method (Jombart et al. 2010).

Parentage assignment

SNPs passing QC filters were used for parentage assignment purposes. Assignment to most probable sire and dam was performed taking into account the sequence depth of the corresponding individuals as described in Dodds et al. (2019). Specifically, the excess mismatch rate (EMM) metric was considered for parentage verification using a maximum threshold of 0.025. Furthermore, successful assignment was accepted only when the relatedness (according to the GRM) among putative parents and offspring was above 0.30.

Estimation of heritability for growth traits – breeding value accuracy

Heritability estimates of juvenile weight and length were obtained using the constructed GRM. Variance components were estimated using AIREMLF90 (Miszal et al. 2014) with the following animal model:

\[
y = Xb + Zu + Tc + e
\]

where \(y\) is the vector of recorded phenotypes, \(X, Z\) and \(T\) are the incidence matrices relating phenotypes with fixed and random effects. \(b\) is the vector of the fixed effects (intercept and age), \(u\) the vector of random animal effects \(\sim N(0, G \sigma^2_u)\) with \(G\) corresponding to the genomic relationship matrix (Dodds et al. 2015), \(c\) the additive genetic variance, \(e\) the vector of random effect representing the full-sib common environmental effect due to rearing each family in separate tanks \(\sim N(0, I \sigma^2_e)\), where \(\sigma^2_e\) the corresponding variance, \(e\) the vector of residuals \(\sim N(0, I \sigma^2_e)\), \(I\) the identity matrix and \(\sigma^2\) the residual variance.

The common full-sib effect was estimated using the following formula:

\[
c^2 = \frac{\sigma^2}{\sigma^2_u + \sigma^2_e + \sigma^2_c}.
\]
Heritability for juvenile growth and length was estimated using the following formula:

\[
h^2 = \frac{\sigma_y^2}{\sigma_y^2 + \sigma_s^2 + \sigma_e^2},
\]

The prediction accuracy of genomic breeding values (GEBVs) was calculated and benchmarked against the accuracy of EBVs using traditional pedigree-based best linear unbiased prediction (BLUP) (Henderson 1975). Accuracy comparisons between BLUP and GBLUP were performed using a threefold cross validation scheme. For this, the PIT-tagged individuals were split into 3 groups (the number of animals within each group ranged between 241 – 263) with an equal family representation among groups. Each of these groups in turn was used as a validation set, while the other two groups were used in the training set. The cross validation procedure was repeated 10 times in order to reduce random sampling effects.

GEBVs were estimated with GBLUP (Meuwissen et al. 2001) using the BLUPF90 suite (Misztal et al. 2014) updated for genomic analyses (Aguilar et al. 2011). Pedigree-based BLUP was applied to calculate breeding values using the same software. The general form of the fitted models was as in equation (1).

The prediction accuracy was approximated as:

\[
r = \frac{\text{correlation(GEBV, y)}}{h},
\]

where y is the vector of recorded phenotypes, (G)EBV is the vector of (genomic) estimated breeding values and h is the square root of the previously estimated heritability. In order to have meaningful comparisons between BLUP and GBLUP we used in both cases the heritability estimated through the genomic relationship matrix.

DATA AVAILABILITY

Obtained sequences are available in the form of FASTQ files from NCBI repository under project ID PRJNA607181. Information regarding the utilized barcode and the sequencing lane of each library is available in supplementary file S1. Genotypic and pedigree information is available in the supplementary files S2 and S3. Phenotypic information for the genotyped animals of 2017 year class is available in the supplementary file S4. Supplemental material available at figshare: https://doi.org/10.25387/g3.12151725.

RESULTS

Dataset filtering

More than 1.35 billion SE reads were produced. Approximately 97% of the above reads (~1.32 billion) had an average phred33 score above 30 and were kept for downstream analysis. Reads without the expected enzymatic cut site and no recognizable barcode were discarded, resulting in ~964 million SE reads. Thereafter reads were merged in 662,978 unique tags that were used as a template for SNP identification. We removed 2 animals (year class 2013) that were probably incorrectly recorded. On the other hand, the putative sire of one parent (from year class 2013) was genotyped insufficiently (mean coverage < 0.1X). In total 17,652 SNPs were identified across 928 genotyped individuals. 70 SNPs with mean coverage below 0.5X were removed. The mean SNP coverage across animals was approximately 5X. Finally, 3,134 SNPs did not fulfill the chosen Hardy-Weinberg equilibrium (HWE) threshold and were removed. Overall, the final dataset was comprised of 14,518 SNPs (Figure 1) genotyped across 928 animals.

Genomic relationships

Among the genotyped full-sib pairs, six animals from four families had low genomic relationships with their full-sibs (below 0.10) and were removed from subsequent analysis. The mean genomic relationship among all full-sib pairs was 0.42 (Figure 2). Furthermore, since the estimated genomic relationship matrix was non positive definite, a bending approach was applied where a predefined threshold of 0.001 was used to replace eigenvalues below the aforementioned threshold (Schaeffer 2014) constituting the obtained matrix invertible. The ‘bent’ GRM was used for estimating GEBVs using GBLUP (Figure S1).

Genetic diversity – population structure

Heterozygosity metrics were estimated both in the raw scale and by taking into account the read depth of the corresponding SNP alleles. Higher estimates of heterozygosity were found in the 2013 year class. Observed heterozygosity (\(H_o\)) when the read depth was not taken into account was 0.21 and 0.19 in year class 2013 and 2017 respectively, while the corresponding estimates of expected heterozygosity (\(H_e\)) were 0.19 and 0.17. When the read depth was taken into account the \(H_o\) was found equal to 0.32 and 0.31 for year class 2013 and 2017 respectively. Moreover, the \(H_o\) for the above year classes was 0.29 and 0.28. \(F_{ST}\) based calculations were performed among the families of year class 2017. The median \(F_{ST}\) ranged between 0.009 – 0.066, with the lowest estimate being observed between families 7 and 8 and the highest between families 7 and 11 (Table 2). Principal component analysis was used as a dimensionality reduction technique both for visualization purposes and to infer potential clustering among genotyped animals. Regarding animals from the 2013 year class the first and second principal components accounted for 13% and 11% of the total variance respectively (Figure 3). Moreover, in the case of animals from the 2017 year class the first and second principal components accounted for 23% and 16% of the total variance (Figure 3). DAPC was applied in order to decipher the genetic structure of 2017 animals and identify potential clusters (Figure 4). Cross validation suggested that the optimal number of principal components for clustering was 20 (Figure S2). Moreover, according to the obtained BIC the optimal K was found to be 13 (Figure S3). Results from DAPC were generally in accordance with the preliminary structure suggested by PCA. According to DAPC families 9 and 10 were indistinguishable. The aforementioned families shared the same sire. A similar pattern was observed between families 5 and 7, who had the same grandfathers and granddams (Table 1). On the other hand, families 2 and 11 formed unique clusters (Figures 3, 4).

Parentage assignment

Parentage assignment was performed for the eight families where at least one parent (from year class 2013) was genotyped. The results suggested that the identity of the putative dam in family 2 was most probably incorrectly recorded. On the other hand, the putative sire of the same family was confirmed by 98.9% of the offspring. Regarding the other seven families, the genotypic data verified the available parentage pedigree records. Between 97.8–100% of offspring in each family were in accordance with the pedigree data. EMM was below 1% for all families apart from family 2 were EMM for the putative dam was approximately 5.4% (Table 3).

Estimation of heritability for growth traits – breeding value accuracy

The estimated heritability for juvenile weight and length was 0.21 (SE 0.07) and 0.19 (SE) respectively. The corresponding full sib effect for weight and length was 0.11 (SE 0.08) and 0.12 (SE 0.08). Genetic
correlation between weight and length was 0.94 (SE 0.13). For the subsequent breeding value accuracy estimation only the weight was used due to the high correlation between the two traits. The mean accuracy using GBLUP was 0.51, while the corresponding accuracy using pedigree BLUP was 0.31. In the case of GBLUP the obtained accuracy of the cross validation scheme used ranged between 0.42 – 0.56, while in the case of pedigree BLUP accuracy ranged between 0.28 – 0.35 (Table 4).

DISCUSSION
Genomic technologies can be utilized to estimate relationship values among the selection candidates at a higher resolution than a traditional pedigree-based analysis would allow. Relationships based on pedigree records will assign the same value to all full-sib members of individual families, making it impossible to utilize the within family variance for selection. Therefore, the information obtained through genomics can result in an increased accuracy of breeding values and a more efficient management of inbreeding.

A plethora of recent studies have already highlighted the advantages of including genomic information in aquaculture breeding programs (Robledo et al. 2018; Houston and Macqueen 2019; Yoshida et al. 2019; Boison et al. 2019; Saura et al. 2019; Vallejo et al. 2019; Barria et al. 2018; Tsairidou et al. 2019; Joshi et al. 2020). Nevertheless, genotyping related costs often limit the implementation of genomic technologies in practice. Cost effective genotyping approaches like GBS appear particularly suitable for small – medium sized breeding programs as is the case of Arctic charr breeding in Sweden.

Low coverage GBS was applied in our study aiming to further improve the selection efficiency of the Swedish Arctic charr strain. We inferred genomic relationships among the genotyped animals using a SNP derived genomic relationship matrix. The average genomic relationship among full sibs in our study was found to be 0.42 (Figure 1) which is lower than the expected theoretical value. In general, the average relationship among full sibs in the absence of inbreeding is expected to be 0.50. Prior application of low coverage GBS in selectively bred Atlantic salmon using varying SNP densities (~24K – 30K) estimated an average relationship ranging between 0.36 - 0.45 among full sibs (Dodds et al. 2015). In comparison, an average genomic relationship of 0.47 was found among full sibs in selected lines of poultry through the usage of a 60K SNP array (Lourenco et al. 2015). Therefore, our study has likely underestimated the realized genomic relationships. Moreover, it has to be pointed out that when the genomic relationship matrix is constructed using allele frequencies estimated from the genotyped animals (as is the case in the current study) and not from the founders, genomic relationships seem to be underestimated (Legarra 2018). Overall though, the fact that the obtained distribution of genomic relationships did not exhibit long tails (typical pattern in the presence of genotyping or pedigree errors) suggests that genotyping through low coverage GBS in Arctic charr is efficient in providing insights regarding the realized genomic relationships among selection candidates and allowing therefore the exploitation of within family variance for selection purposes.

Genetic diversity
As already mentioned, efficient management of inbreeding accumulation is of the utmost importance for the long-term sustainability of any breeding program. Therefore, genomic information can be particularly useful for guiding selection decisions that would minimize the loss of genetic diversity as has been already demonstrated in salmonids among other species (Barria et al. 2019). Even though inbreeding accumulation in the Arctic charr breeding program has been below 1% per generation according to available pedigree data, genomic tools can provide valuable insight regarding the existing genetic diversity and inform for optimal crosses among selection candidates.

The obtained heterozygosity metrics in our study showed that the \( H_s \) was lower than the \( H_e \), indicating an excess of heterozygotes which at first sight should correlate positively with the existence of ample
genetic variation. In general, a breeding program would be expected to be more resilient to the loss of genetic diversity in comparison to commercial hatcheries where no pedigree records are kept. Even though our dataset was comprised only of related individuals derived from a closed breeding nucleus (particularly in the case of year class 2017 where the studied dataset was comprised of a small number of full-sib families) the obtained heterozygosity values were higher in comparisons to previous studies where we used GBS type methodologies in studying the diversity of farmed tilapia populations from commercial hatcheries with no pedigree recordings (Kajungiro et al. 2019; Mbiru et al. 2019).

Nevertheless, it would seem premature to infer that due to the excess of heterozygotes the existing genetic diversity in the Arctic charr breeding program is sufficient. Interestingly, according to literature an excess of heterozygotes has been observed in small and relatively recently founded populations of both animals and plants with separate sexes (Allendorf and Luikart 2007). Naturally, the above phenomenon would be ephemeral, since in the long-term heterozygosity would eventually decline. The background history of the Arctic charr breeding program suggests that the founder population consisted of a small number of fish (Eriksson et al. 2010). It has to be stressed, that a potential pitfall of the current study lies on the fact that the methodology for obtaining heterozygosity metrics through low coverage GBS is still under development. However, available data from high coverage ddRAD from a different subset of 2017 year class Arctic charr, suggested observed and expected heterozygosity values in close accordance with the values we obtained after depth adjustment in the current study ($H_o = 0.33$, $H_e = 0.34$; unpublished data).

| Table 2 Median Fst values (14,518 SNPs) among families |
|------------------------------------------------------|
| F1 | F2 | F3 | F4 | F5 | F6 | F7 | F8 | F9 | F10 | F11 | F12 | F13 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| F1 | — | 0.063 | 0.031 | 0.041 | 0.039 | 0.046 | 0.037 | 0.043 | 0.045 | 0.032 | 0.056 | 0.053 | 0.036 |
| F2 | — | 0.049 | 0.035 | 0.056 | 0.060 | 0.057 | 0.034 | 0.052 | 0.027 | 0.067 | 0.056 | 0.033 |
| F3 | — | 0.049 | 0.028 | 0.041 | 0.035 | 0.038 | 0.032 | 0.038 | 0.046 | 0.046 | 0.046 |
| F4 | — | 0.043 | 0.017 | 0.042 | 0.044 | 0.029 | 0.050 | 0.039 | 0.027 | 0.052 |
| F5 | — | 0.055 | 0.012 | 0.038 | 0.040 | 0.034 | 0.063 | 0.056 | 0.039 |
| F6 | — | 0.059 | 0.051 | 0.047 | 0.029 | 0.063 | 0.045 | 0.029 |
| F7 | — | 0.009 | 0.040 | 0.032 | 0.066 | 0.058 | 0.043 |
| F8 | — | 0.048 | 0.029 | 0.059 | 0.063 | 0.043 |
| F9 | — | 0.010 | 0.048 | 0.046 | 0.027 |
| F10 | — | 0.032 | 0.040 | 0.049 |
| F11 | — | 0.060 | 0.029 |
| F12 | — | 0.040 |
| F13 | — | — |

Figure 2 Realized relationships among full-sib pairs.
Despite the fact that the Arctic charr breeding program has been operational for almost 40 years, no prior study attempted to investigate the genetic diversity of the selected population. The $F_{ST}$ metric is commonly applied in studying the genetic differentiation among populations (Cockerham and Weir 1984). Therefore, strictly on technical terms the current dataset is not expected to be highly

**Figure 3** A) Principal component analysis for the 2013 year class. B) Principal component analysis for the 2017 year class.

**Figure 4** Discriminant analysis of principal components in the 2017 year class.
informative for such analysis. Furthermore, no \( F_{ST} \) calculations were performed for the animals from year class 2013 since those animals constituted a representative sample of the breeding program for the aforementioned generation. However, \( F_{ST} \) comparisons can provide valuable insights regarding the differentiation of the genotyped families from year class 2017. In order to minimize the possibility of obtaining inflated \( F_{ST} \) values due to the reduced accuracy of genotype calling in low coverage GBS we estimated median \( F_{ST} \) values, since the latter are less sensitive to outliers (as a result of genotypic errors). The obtained comparisons among the genotyped families indicated low genetic differentiation among the tested families (0.009 – 0.066) which appears to be in line with the history of the breeding program.

Moreover, in the current study we attempted to study in higher depth the genetic differentiation among the tested animals using PCA and DAPC. Applying PCA to the animals from the 2013 year class did not indicate the existence of distinct clusters. On the other hand the corresponding application of PCA in animals from year class 2017 provided indications regarding the existence of distinct clusters. The underlying algorithm of PCA aims to summarize the total variation of the tested dataset in a reduced dimension. Furthermore, a major advantage of PCA is its computational efficiency. Nevertheless, the above approach is not optimal for distinguishing different clusters and PCA does not qualify strictly speaking as a clustering algorithm. DAPC on the other hand retains the computational advantages of PCA, but at the same time offers higher resolution for detecting genetic clusters (Jombart et al. 2010). In addition, being an unsupervised learning approach, DAPC has the potential of providing valuable genetic insight in samples of unknown origin. Over the years, genetic material from the Arctic char breeding program has been disseminated in various farms across the country (Nilsson et al. 2010). Potential crossbreeding with other Arctic char strains is therefore likely to have occurred in commercial farms. As such, DAPC could assist in distinguishing the selectively bred Arctic char from other farmed strains or potential crossbreds in Sweden. DAPC was successful in identifying clusters that were in agreement with pedigree records. In particular, families originating from the same grandsire and granddam were indistinguishable (e.g., families 5, 7, and 9, 10) and inseparable in comparison to other families that were more distantly related according to pedigree records. Overall, through GBS application informative decisions regarding selection crosses can be obtained in the future even in the absence of pedigree records.

### Parentage assignment

An additional advantage of genetic markers, most relevant for selective breeding, is their use for parentage assignment purposes.

### Table 3 Verification of suggested parentage from pedigree records

| Family Id | Mean Dam relationship | Mean Dam EMM % (SE) | Mean Sire relationship | Mean Sire EMM % (SE) | Dam verified offspring (%) | Sire verified offspring (%) |
|-----------|-----------------------|---------------------|------------------------|---------------------|---------------------------|---------------------------|
| 1         | N/A                   | 0.41                | N/A                    | 0.24 (0.08)         | N/A                       | 98.4                      |
| 2         | 0.01                  | 0.41                | 0.41                   | 0.23 (0.04)         | N/A                       | 98.9                      |
| 3         | N/A                   | 0.39                | N/A                    | 0.33 (0.13)         | N/A                       | 97.1                      |
| 7         | 0.35                  | 0.44 (0.03)         | N/A                    | N/A                 | 100                       | N/A                       |
| 8         | 0.37                  | 0.25 (0.04)         | N/A                    | N/A                 | 100                       | N/A                       |
| 9         | 0.38                  | 0.34 (0.08)         | N/A                    | 0.21 (0.04)         | 97.8                      | 97.8                      |
| 10        | N/A                   | 0.37                | N/A                    | 0.04 (0.08)         | N/A                       | 100                       |
| 13        | N/A                   | 0.50                | N/A                    | 0.21 (0.03)         | N/A                       | 100                       |

EMM refers to excess mismatch rate.

Unfortunately, in the current study it was not possible to obtain tissue samples from all the putative parents for the animals of the 2017 year class (only for 2 full sib families we genotyped both parents). The analysis conducted in the subset of families with at least one parent genotyped (8 families) demonstrated that the derived genomic information was sufficient for verifying putative parentage records. Apart from the obtained discrepancy between genotypes and pedigree for the putative dam of family 2, the obtained genotypic information verified the pedigree parentage records implying that low coverage GBS is an effective approach for parentage assignment as already demonstrated in red deer (Dodds et al. 2019). Despite the fact that genotype calling in low coverage GBS entails higher uncertainty compared with high coverage approaches (Dodds et al. 2015) all full-sibs of family 2 indicated the existence of a pedigree error for that particular dam. Furthermore, the relationship among the full sibs of family 2 was in the range we recorded for all tested full sib pairs (0.35 – 0.45). As such, the above results indicate that a pedigree recording error is the most likely explanation.

### Heritability for growth traits - accuracy of breeding value estimation

Moderate heritabilities (~0.20) for juvenile growth-related traits were obtained in the current study suggesting that ample genetic variance exists for further improving the Swedish Arctic char strain. A high common full-sib effect was found (0.11) which might be confounding with the additive genetic variance. As such the obtained heritability might be underestimated. Nevertheless, it has to be pointed out that a small number of families (13) was used in the current study. In addition, the number of animals genotyped per family varied widely (16 – 90). Therefore the obtained heritability metrics should be treated with caution.

Even though genomic selection through GBS related approaches has proven to consistently outperform pedigree BLUP in aquaculture species (Barria et al. 2018; Aslam et al. 2018; Kyriakis et al. 2019) no prior study attempted to investigate the efficiency of low coverage GBS on prediction accuracy in farmed fish. The results from the genomic prediction approach were encouraging for a future practical implementation of GBLUP in the selected strain. The inclusion of

### Table 4 Accuracy comparison between GBLUP and PBLUP using threefold cross validation (10 replicates)

| Validation Id | Group size | GBLUP accuracy (SE) | PBLUP accuracy (SE) |
|---------------|------------|---------------------|---------------------|
| 1             | 249        | 0.56 (0.03)         | 0.35 (0.03)         |
| 2             | 241        | 0.54 (0.03)         | 0.29 (0.03)         |
| 3             | 263        | 0.42 (0.04)         | 0.28 (0.03)         |
| Overall       | 753        | 0.51 (0.03)         | 0.31 (0.03)         |
genomic relationships resulted in an improved breeding value estimation accuracy clearly outperforming the corresponding pedigree BLUP based model. Additionally, our results appear to be in accordance with prior simulation derived data where low coverage GBS strategies were considered promising for delivering high prediction accuracies as opposed to traditional pedigree based approaches (Gorjanc et al. 2015). Nevertheless, we would have to acknowledge that the small dataset we used for genomic prediction (759 offspring from year class 2017) limits our ability of drawing definite conclusions.

CONCLUSIONS

Overall, low coverage GBS has been proven to be an efficient and cost-effective approach for obtaining a wide range of essential information for selective breeding purposes. Applying low coverage GBS in the selected Arctic charr strain allowed us to gain insightful information regarding the genetic diversity of the stock, verify (or reject) parentage records and estimate genomic breeding values. Further studies focusing on application of GBS in studying resistance of Arctic charr to common encountered diseases would be deemed particularly promising since it would be possible to exploit the within family variance during selection. Finally, studies aiming to combine low coverage GBS with single-step BLUP approaches (Aguilar et al. 2011; Legarra et al. 2014) where only a subset of the selection candidates is genotyped, could be particularly valuable regarding cost efficiency.

ACKNOWLEDGMENTS

The authors acknowledge support from FORMAS under grant agreement 2018-00809 (NextGenCharr) and the SLU Aquaculture platform. The Arctic charr breeding program is supported by funding from the Swedish government under grant agreement no. N2017/02366/SUN. The Ministry of Business, Innovation and Employment (New Zealand) via its funding of the “Genomics for Production & Security in a Biological Economy” programme (Contract ID C10X1306) contributed to the development of the software used in this study.

LITERATURE CITED

Aguilar, I., I. Misztal, A. Legarra, and S. Tsuruta, 2011 Efficient computation of the genomic relationship matrix and other matrices used in single-step evaluation. J. Anim. Breed. Genet. 128: 422–428. https://doi.org/10.1111/j.1439-0388.2010.00912.x

Allendorf, F. W., and G. H. Luikart, 2007 Conservation and the Genetics of Populations. Blackwell, Oxford.

Aslam, M. L., R. Carraro, A. Bestin, S. Cariou, A. K. Sonesson et al., 2018 Genetics of resistance to photobacteriosis in gilthead sea bream (Sparus aurata) using 2b-RAD sequencing. BMC Genet. 19: 43. https://doi.org/10.1186/s12863-018-0631-x

Baird, N., P. D. Etter, T. S. Atwood, M. C. Currey, A. C. Shiver et al., 2008 Rapid SNP discovery and genetic mapping using sequenced RAD markers. PLoS One 3: e3376. https://doi.org/10.1371/journal.pone.0003376

Barria, A., K. A. Christensen, G. M. Yoshida, K. Correa, A. Jellicki et al., 2018 Genomic Predictions and Genome-Wide Association Study of Resistance Against Piscirickettsia salmonis in Coho Salmon (Oncorhynchus kisutch) Using ddRAD Sequencing. G3 (Bethesda) 8: 1183–1194. https://doi.org/10.1534/g3.118.200053

Barria, A., K. A. Christensen, G. Yoshida, A. Jellicki, J. S. Leong et al., 2019 Whole Genome Linkage Disequilibrium and Effective Population Size in a Coho Salmon (Oncorhynchus kisutch) Breeding Population Using a High-Density SNP Array. Front. Genet. 10: 498. https://doi.org/10.3389/fgene.2019.00498

Bilton, T. P., A. J. Chappell, S. M. Clarke, R. Brauning, K. G. Dodds et al., 2019 Using genotyping-by-sequencing to predict gender in animals. Anim. Genet. 50: 307–310. https://doi.org/10.1111/age.12782

Bilton, T. P., J. C. McEwan, S. M. Clarke, R. Brauning, T. C. van Stijn et al., 2018a Linkage Disequilibrium Estimation in Low Coverage High-Throughput Sequencing Data. Genetics 209: 389–400. https://doi.org/10.1534/genetics.118.300831

Bilton, T. P., M. R. Schofield, M. A. Black, D. Chagné, P. L. Wilcox et al., 2018b Accounting for Errors in Low Coverage High-Throughput Sequencing Data When Constructing Genetic Maps Using Biparental Outcrossed Populations. Genetics 209: 65–76. https://doi.org/10.1534/genetics.117.300627

Boison, S., J. Ding, E. Leder, B. Gjerde, P. H. Bergtun et al., 2019 QTLs Associated with Resistance to Cardiomyopathy Syndrome in Atlantic Salmon. J Hered 110: 727–737. https://doi.org/10.1037/hered/ezo42

Bolger, A. M., M. Lohse, and B. Usadel, 2014 Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 30: 2114–2120. https://doi.org/10.1093/bioinformatics/btu170

Brown, J. R., J. B. Taggart, M. Bekaert, S. Palaisoktas et al., 2016 Mapping the sex determination locus in the hapaku (Polyprion oxygenius) using ddRAD sequencing. BMC Genomics 17: 448. https://doi.org/10.1186/s12864-016-2773-4

Chagné, D., J. Ryan, M. Saeed, T. Van Stijn, R. Brauning et al., 2018 A high density linkage map and quantitative trait loci for tree growth for New Zealand mānuka (Leptospermum scoparium). N. Z. J. Crop Hortic. Sci. 47: 261–272.

Christensen, K. A., E. B. Rondeau, D. R. Minckley, J. S. Leong, C. M. Nugent et al., 2018 The Arctic charr (Salvelinus alpinus) genome and transcriptome assembly. PLoS One 13: e0204076. https://doi.org/10.1371/journal.pone.0204076

Clarke, S. M., H. M. Henry, K. G. Dodds, T. W. D. Iottow, T. R. Manley et al., 2014 A High Throughput Single Nucleotide Polymorphism Multiplex Assay for Parentage Assignment in New Zealand Sheep. PLoS One 9: e93392. https://doi.org/10.1371/journal.pone.0093392

Cockerham, C. C., and B. S. Weir, 1984 Covariances of relatives stemming from a population undergoing mixed self and random mating. Biometrics 40: 157–164. https://doi.org/10.2307/2530754

D’Ambrosio, J., F. Phocas, P. Haffray, A. Bestin, S. Brard-Fudulea et al., 2019 Genome-wide estimates of genetic diversity, inbreeding and effective size of experimental and commercial rainbow trout lines undergoing selective breeding. Genet. Sel. Evol. 51: 26. https://doi.org/10.1186/s12711-019-0468-4

Davey, J. W., P. A. Hohenlohe, P. D. Etter, J. Q. Boone, J. M. Catchen et al., 2011 Genome-wide genetic marker discovery and genotyping using next-generation sequencing. Nat. Rev. Genet. 12: 499–510. https://doi.org/10.1038/nrg3012

Dodds, K. G., J. C. McEwan, R. Brauning, R. M. Anderson, T. C. van Stijn et al., 2015 Construction of relatedness matrices using genotyping-by-sequencing data. BMC Genomics 16: 1047. https://doi.org/10.1186/s12864-015-2252-3

Dodds, K. G., J. C. McEwan, R. Brauning, T. C. van Stijn, S. J. Rowe et al., 2019 Exclusion and Genomic Relatedness Methods for Assignment of Parentage Using Genotyping-by-Sequencing Data. G3 (Bethesda) 9: 3239–3247.

Elsheire, R. J., J. C. Glaubitz, Q. Sun, J. A. Poland, K. Kawamoto et al., 2011 A Robust, Simple Genotyping-by-sequencing (GBS) Approach for High Diversity Species. PLoS One 6: e19379. https://doi.org/10.1371/journal.pone.0019379

Eriksson, L.-O., A. Alanarä, J. Nilsson, and E. Brännås, 2010 The Arctic charr story: development of subarctic freshwater fish farming in Sweden. Hydrobiologia 650: 265–274. https://doi.org/10.1007/s10750-010-0248-1

Faville, M. J., S. Ganesh, M. Cao, M. Z. Z. Jahufer, T. P. Bilton et al., 2018 Predictive ability of genomic selection models in a multi-population perennial ryegrass training set using genotyping-by-sequencing. Theor. Appl. Genet. 131: 703–720. https://doi.org/10.1007/s00122-017-4303-1

Gorjanc, G., M. A. Cleveland, R. D. Houston, and J. M. Hickey, 2015 Potential of genotyping-by-sequencing for genomic selection in livestock populations. Genet. Sel. Evol. 47: 12. https://doi.org/10.1186/s12711-015-0102-z

Henderson, C. R., 1975 Best linear unbiased estimation and prediction under a selection model. Biometrics 31: 423–447. https://doi.org/10.2307/2529430
Hickey, J. M., T. Chiurugwi, I. Mackay, W. Powell and Implementing Genomic Selection in CGIAR Breeding Programs Workshop et al., 2017 Genomic prediction unifies animal and plant breeding programs to form platforms for biological discovery. Nat. Genet. 49: 1297–1303. https://doi.org/10.1038/ng.3920

Houston, R. D., and D. J. Macqueen, 2019 Atlantic salmon (Salmo salar L.) genetics in the 21st century: taking leaps forward in aquaculture and biological understanding. Anim. Genet. 50: 3–14. https://doi.org/10.1111/age.12748

Jombart, T., S. Devillard, and F. Balloux, 2010 Discriminant analysis of principal components: a new method for the analysis of genetically structured populations. BMC Genet. 11: 94. https://doi.org/10.1186/1471-2156-11-94

Joshi, R., T. H. E. Meuwissen, J. A. Wooldiams, and H. M. Gjeen, 2020 Genomic dissection of maternal, additive and non-additive genetic effects for growth and carcass traits in Nile tilapia. Genet. Sel. Evol. 52: 1. https://doi.org/10.1186/s12711-019-0522-2

Kajungiro, R. A., C. Palaiokostas, F. A. L. Pinto, A. J. Mmochi, M. Mtolera et al., 2019 Population Structure and Genetic Diversity of Nile Tilapia (Oreochromis niloticus) Strains Cultured in Tanzania. Front. Genet. 10: 1269. https://doi.org/10.3389/fgene.2019.01269

Kyriakis, D., A. Kanterakis, T. Manousaki, A. Tsakogiannis, M. Tsagris et al., 2017 Applications of genotyping by sequencing in aquaculture breeding and genetics. Rev. Aquacult. 0: 1–13.

Lourenco, D. A. L., B. O. Fragomeni, S. Tsuruta, I. Aguilar, B. Zumbach et al., 2014 Single Step, a covariance matrix-free method to compute genomic predictions. Front. Genet. 5: 58. https://doi.org/10.3389/fgene.2014.00539

Legarra, A., O. F. Christensen, I. Aguilar, and I. Misztal, 2014 Single Step, a general approach for genomic selection. Livest. Sci. 166: 54–65. https://doi.org/10.1016/j.livsci.2014.04.029

Llorente, J. P., M. Araneda, R. Neira, and J. M. Yáñez, 2019 Advances in genetic improvement for salmon and trout aquaculture: the Chilean situation and prospects. Rev. Aquacult. 11: 340–353. https://doi.org/10.1111/raq.12335

Lourenco, D. A. L., B. O. Fragomeni, S. Tsuruta, I. Aguilar, B. Zumbach et al., 2015 Accuracy of estimated breeding values with genomic information on males, females or both: an example on broiler chicken. Genet. Sel. Evol. 47: 56. https://doi.org/10.1186/s12711-015-0137-1

Lu, F., A. E. Lipka, J. Glauheit, R. Elshire, J. H. Cherney et al., 2013 Switchgrass Genomic Diversity, Ploidy, and Evolution: Novel Insights from a Network-Based SNP Discovery Protocol. PLoS Genetics 9: e1003215. https://doi.org/10.3717/journal.pgen.1003215

Mewissen, T. H. E., B. J. Hayes, and M. E. Goddard, 2001 Prediction of Total Genetic Value Using Genome-Wide Dense Marker Maps. Genetics 157: 1819–1829.

Misztal, I., S. Tsuruta, D. Lourenco, I. Aguilar, A. Legarra et al., 2014 Manual for BLUPF90 family of programs, University of Georgia, Athens.

Mburu, M., M. S. P. Motlera, L. J. Chauka, F. A. Lopes, D. J. de Koning et al., 2019 Characterizing the genetic structure of introduced Nile tilapia (Oreochromis niloticus) strains in Tanzania using double digest RAD sequencing. Aquacult. Int. https://doi.org/10.1007/s10750-019-00472-5

Nilsson, J., E. Brännäs, and L. O. Eriksson, 2010 The Swedish Arctic charr breeding programme. Hydrobiologia 650: 275–282. https://doi.org/10.1007/s10750-010-0232-9

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. https://doi.org/10.1534/g3.116.038036

Nugent, C. M., J. S. Leong, K. A. Christensen, E. B. Rondeau, M. K. Brachmann et al., 2019 Design and characterization of an 87k SNP genotyping array for Arctic charr (Salvelinus alpinus). PLoS One 14: e0215008. https://doi.org/10.1371/journal.pone.0215008

Olk, T. R., H. J. Jetha, H. Thorarinsen, J. Wollebek, and E. Lydersen, 2019 Brood-stock management and early hatchery rearing of Arctic charr (Salvelinus alpinus (Linnaeus)). Reviews in Aquaculture.12400.

Palaiokestas, C., and Houston, R. D. 2017 Genome-wide approaches to understanding and improving complex traits in aquaculture species. Perspect. Agric. Vet. Sci. Nutr. Nat. Resour. 12: 1–10.

Palaiokestas, C., M. Bekaert, M. Khan, J. B. Taggart, K. Gharbi et al., 2015 A novel sex-determining QTL in Nile tilapia (Oreochromis niloticus). BMC Genomics 16: 171. https://doi.org/10.1186/s12864-015-1383-x

Pomianowski, K., M. Jankun, and K. O’cawegicz, 2019 Cytogenetic identification of genetic elements of brook trout (Salvelinus fontinalis) in the karyotype of Arctic char (Salvelinus alpinus) from the aquaculture broodstock. Journal of Applied Ichthyology 35: 963–969.

Robledo, D., O. Matika, A. Hamilton, and R. D. Houston, 2018 Genome-Wide Association and Genomic Selection for Resistance to Amoebic Gill Disease in Atlantic Salmon. G3 (Bethesda) 8: 1195–1203. https://doi.org/10.1534/g3.118.200075

Robledo, D., C. Palaiokestas, L. Bargelloni, P. Martínez, and R. Houston, 2017 Applications of genotyping by sequencing in aquaculture breeding and genetics. Rev. Aquacult. 0: 1–13.

Rochette, N. C., A. G. Rivera-Colón, and J. M. Catchen, 2019 Stacks 2: Analytical methods for paired-end sequencing improve RAĐseq-based population genomics. Mol. Ecol. 28: 4737–4754. https://doi.org/10.1111/mec.15253

Saura, M., M. J. Carabaňo, A. Fernández, S. Cabaleiro, A. B. Doeschl-Wilson et al., 2019 Disentangling Genetic Variaton for Resistance and Endurance to Sctitococciatosis in Turbot Using Pedigree and Genomic Information. Front. Genet. 10: 539. https://doi.org/10.3389/fgene.2019.00539

SCB, 2017 Statistiska meddelanden-Vattenbruk 2017. https://www.scb.se/contentassets/5b627981d8bb40b29d0d1ee7c2a533f6/jo1201_2017a01_C24_lrs/ELARES/PDforce.pdf

Schaeffer, L., 2014 Making Covariance Matrices Positive Definite. http://animalbiosciences.uoguelph.ca/~lrs/ELARES/PDforce.pdf

Sonesson, A. K., and T. H. Meuwissen, 2009 Testing strategies for genomic selection in aquaculture breeding programs. Genet. Sel. Evol. 41: 37. https://doi.org/10.1002/bies.201300014

Yoshida, G. M., J. P. Lhorente, K. Correa, J. Soto, D. Salas et al., 2014 Scanning of Genetic Variants and Genetic Mapping of Phenotypic Traits in Gilthead Sea Bream Through ddRAD Sequencing. Front. Genet. 5: 56. https://doi.org/10.3389/fgene.2015.00539

VanRaden, P. M., 2008 Efficient methods to compute genomic predictions. J. Dairy Sci. 91: 4414–4423. https://doi.org/10.3168/jds.2007-0980

Yanez, J. S., Newman, R. D. Houston, 2015 Genomics in aquaculture to better understand species biology and accelerate genetic progress. Front. Genet. 6: 128.

Yoshida, G. M., J. P. Lhorente, K. Correa, J. Soto, D. Salas et al., 2019 Genome-Wide Association Study and Cost-Efficient Genomic Predictions for Growth and Fillet Yield in Nile Tilapi (Oreochromis niloticus). G3 (Bethesda) 9: 2597–2607.

Communicating editor: José M. Yáñez