

Size-selection drives genomic shifts in a harvested population

Daniel Sadler¹, Tiina Sävilammi², Stephan van Dijk², Phillip Watts³, and Silva Uusi-Heikkilä²

¹University of Jyväskylä Department of Biological and Environmental Science

²University of Jyväskylä

³University of Oulu

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Abstract

Overfishing not only drastically reduces the number of fish in an exploited population but is often selective for body size removing the largest individuals from a population. Here, we study experimentally the evolutionary effects of size-selective harvesting using whole genome sequencing on a model organism; the zebrafish, which also allows us to quantify gene ontology terms due to the high-resolution reference genome. We demonstrate genomic shifts in the populations exposed to size-selective harvesting for five generations and show reduced genetic diversity in all harvested lines, including control line (non-size-selected). We also determine differences in gene ontology, with enrichment in nervous system related genes. Our results illuminate the biological processes underlying fisheries-induced genetic changes, hence contribute towards the understanding of the changes potentially associated with the vulnerability of an exploited population to future stressors.

Introduction

Harvesting of animals frequently exceeds natural mortality rates and can cause drastic demographic changes in a population (Jørgensen et al., 2007; Festa-Bianchet et al., 2011). Overfishing is a particularly severe example of harvesting, as adult fish are removed from populations at an unprecedented rate, often exceeding fishing mortality rates of 75% (Lewin et al., 2006). Alongside population decline, selective pressures, which are operating on growth, reproduction, and other fitness-related traits are being exerted (Allendorf & Hard 2009; Alós et al., 2012; Uusi-Heikkilä et al., 2015). Fisheries selection often acts on size and size associated traits, as fisheries target the largest individuals in a population. The largest individuals usually have the highest reproductive fitness and economic value (Kuparinen & Merilä 2007; Law 2007). Though demographic and phenotypic changes are often clearly visible over time in an exploited population, size-selective harvesting can also cause genetic changes underlying the phenotypic ones (van Wijk et al., 2013; Uusi-Heikkilä 2015; 2017; Therikildsen 2019). Identifying genetic changes caused by fishing is important as they are slow to reverse, if indeed can be reversed at all (Lacy 1987; Conover et al., 2009). Now that we are in the age of genomics, we can look further into the mechanisms and associated functions induced by size-selective fisheries using next generation sequencing technology.

Size-selective fishing can result in decreased genetic diversity through demographic loss (Pinsky & Palumbi 2014; Marty et al., 2015). A reduction in genetic diversity can be problematic in fast changing environments because it can lead to a loss of adaptive potential (Fisher 1958; Allendorf et al., 2008). Evidence for erosion of genetic diversity in exploited fish stocks is accumulating, showing increases in inbreeding coefficient or reductions in effective population size (N_e) (Hauser et al., 2002; Hoarau et al., 2005; Hare et al., 2011; see Pinsky & Palumbi 2014 for meta-analysis). Despite this some studies show no declines in genetic diversity caused by fishing (Ruzzante et al., 2001; Hutchinson et al., 2003; Poulsen et al., 2006; Therikildsen 2010)

potentially because many fish populations are so large, that even collapsed populations are resistant to the loss of genetic diversity (Beverton 1990; Anderson & Brander 2009).

Alongside demographic reduction, size-selective fisheries also exert directional selection, which could magnify loss of genetic diversity and cause shifts in genomic structure. Experimental studies have demonstrated significant genetic changes in artificially harvested fish populations after only three (van Wijk et al. 2013), four (Therkildsen et al. 2019) or five generations (Uusi-Heikkilä et al. 2015; 2017) of size-selective harvesting. The cause of these changes can be challenging to manifest in natural populations because fishing occurs in a constantly changing environment where biotic and abiotic conditions can create simultaneous selection pressures to fish populations. In an experimental system, confounding factors such as these environmental changes can be removed, to isolate the effects of size-selection alone, however, even in experimental studies these changes can be difficult to replicate. Therkildsen et al. (2019) demonstrated genomic changes in different size-selected experimental populations of Atlantic silverside (*Menidia menidia*). Each experimental population had two replicates, which showed both parallel and idiosyncratic effects on the genome, but not phenotypic, responses to the exact same selection pressure. These results highlight how different evolutionary trajectories from different islands of genomic architecture can lead to the same phenotypes and potentially complicate the predictions of the outcomes of fisheries-induced evolution due to the added stochasticity of genomic changes. It is therefore fundamental that we assess whether this stochasticity is repeatable across other systems, if so then it will make genomic shifts more difficult to comprehend for fisheries management.

Gene ontology describes functions of genes and gene products (Ashburner et al, 2000), thus can help to interpret fisheries-induced genetic changes and link them to potential phenotypic changes including growth, metabolism, and behaviour. However, this is only possible when using a model organism with high-quality, high-resolution reference genome, such as zebrafish (*Danio rerio*) (Howe et al., 2013). As such, whilst an earlier size-selective harvesting experiment has described large-scale genomic changes (Therkildsen et al. 2019), no study has utilised this framework within a model system. By assessing gene ontology, we are able to determine the underlying functionality of hitchhiker genes that could be associated with selection of body size, that are unclear when assessing phenotypic changes alone. Thus, we are able to expand on previous studies and identify key differences in gene functions and molecular processes, further allowing us to predict the pathways associated with enriched genes.

In the present study we use populations of wild zebrafish that were exposed to five generations of size-selective harvesting pressures in the laboratory: small-selected (75% of the largest fish were removed/harvested; simulating typical fisheries selection), large-selected (75% of the smallest fish were removed/harvested), and random-selected (75% of fish were randomly harvested; acting as a control line). Here, we assess the large-scale genomic changes caused by intensive size-selective harvesting at the whole genome level. We predict that (i) size-selective harvesting will cause a shift in genomic architecture in a contemporary time scale, (ii) directional selection (large- and small-selected) will have a greater loss of genetic diversity after five generations of harvesting than demographic loss alone (random-selected) and, (iii) associated gene ontology terms will relate to growth associated functions, and be more enriched in size-selected populations.

Materials and methods

Experimental design

We used wild-caught zebrafish originating from West Bengal, India as the F_0 -generation (founder population), in this size-selective harvesting experiment (Uusi-Heikkilä et al., 2015). To examine the genomic changes caused by size-selective harvesting, we used three selection lines; small-selected (75% of the smallest fish were randomly harvested; mimicking fisheries selection), large-selected (75% of the largest fish harvested; giving a full range of responses), and random-selected (75% randomly chosen fish were harvested as the control line) with two replicate populations for each selection line. Selection-line replicates are from now on called as SS1, SS2, LS1, LS2, RS1 and RS2. Harvesting continued for five generations. Full details of the experimental design can be found in Uusi-Heikkilä et al., (2015).

DNA extraction and sequencing

We extracted DNA altogether from 267 individuals (Table S1). We used a modified salt extraction protocol to extract the genomic DNA (Aljanabi & Martinez, 1997). Library construction and sequencing was conducted at the Novogene Biotech Co. (Beijing, China). The random fragmentation of the genomic DNA was skipped because the DNA was fragmented during the storage period. The DNA fragments were end polished, A-tailed, and ligated with the full-length Illumina adapters, followed by further PCR amplification with P5 and indexed P7 oligos. The PCR products were purified with AMPure XP system. The size distribution of the finished libraries was inspected by Agilent 2100 Bioanalyzer (Agilent Technologies, CA, USA), and quantified by real-time PCR. The quality-controlled libraries were pooled and sequenced using the Illumina NovaSeq 6000 platform to sequence 150-base paired end reads producing the average of 7696 megabase pairs of data per sample.

Data assembly

We filtered the raw sequence for adapters and poor-quality bases using fastp v. 0.20.0 (parameters `-g -q 5 -u 50 -n 15 -l 30 -overlap_diff_limit 1 -overlap_diff_percent_limit 10`). During trimming, we removed bases from read ends when they represented adapter sequence, or when they were of low quality. All assembly tools utilised htslib 1.10. We mapped the trimmed sequences against reference genome with bwa mem v. 1.10. We used the primary assembly sequences of zebrafish GRCz11, obtained from Ensembl (Howe et al., 2021) as a reference. We excluded the potential PCR duplicates using samtools markdup 1.10. SNP calling was done using bcftools mpileup v. 1.10 (parameter `-a "FORMAT/DP"`) to extract the per-sample sequencing depths and bcftools call v. 1.10 (parameters `-m -f GQ`). The SNPs were filtered using bcftools filter v. 1.10 (`-i Qual>20 && AVG(FMT/DP)>3 && AVG(FMT/DP)<10`). Finally, we required that each SNP was genotyped in > 70% of the samples and had minor allele frequency (MAF) > 0.05. We further excluded loci with more than two alleles, or alleles other than 0 or 1. The sequencing resulted in the median of 7,498 and 7,338 megabases of raw and filtered sequence data per sample, respectively. We excluded 14 individuals from further analysis due to low mapping back rate and low coverage. After filtering, 3.5 million SNPs were called.

SNP annotation

The functional associations between zebrafish genes and each SNP were predicted using snpEff software v. 5.0 (default parameters; Cingolani et al., 2012) and the GRCz11 genome annotation of the zebrafish. The gene annotation with most severe effect was selected for each SNP. If the SNP did not associate with any gene, it was annotated as intergenic.

Assessing genomic shifts

To visualize the genomic differences among the selection lines and the founder population, we used 1M randomly sampled SNPs in a principal component analysis. To this end, we imputed the missing values with the median allele for each SNP. We required that the subsets had MAF>0.05. Based on Cattell's graphical rule and the broken stick method (Figure S1), we determined the principal components that contained the relevant relatedness information and population structure for further analyses utilizing principal components with PCAdapt.

Parallel effects

We used diffstat statistic to quantify the differentiation between small- and large-selected lines against the random-selected lines (Turner et al., 2011). We extracted the minimum absolute difference between the allele frequencies of each contrasting comparison to explore if the differences were consistent (i.e., the direction of allele frequency change was the same among the selection-line replicates) among all comparisons and we set the diffstat of the loci with non-consistent allele frequency changes to zero. We then binned the observed founder population allele frequencies and estimated empirical one-tailed P -values for the observed diffstat based on comparing to the null distributions in the respective bins. Confidence intervals were estimated parametrically using t-test function.

Differentiation-based outlier detection

To identify diverged loci among all selection-line replicates and the founder population, we used two different outlier approaches. First, to detect variation that was causing divergence between the individuals regardless of the selection line assignment or relatedness between individuals, we used PCAdapt v. 4.3.3 (Luu et al., 2017) with K based on Cattell's rule (Figure S1; K indicates principal component level). We repeated the analysis for all samples and subsets of samples in the small- and random-selected and large- and random-selected comparisons. We used the component-wise analysis, which uses principal component loadings as a test statistic as the components clearly separate the selection lines and time (F_0 - F_6). The resulting P -values were corrected for multiple comparisons using the Benjamini-Hochberg approach (Benjamini & Hochberg 1995). Second, to screen the genomes for signatures of response to harvesting while simultaneously controlling for random effects due to factors such as variability in population structure, relatedness of the individuals and selection-line replicates, we used latent factor mixed model (LFMM), implemented in the `lfmm` R package v. 1.0 (Frichot et al., 2013). The model includes SNP matrix as a response, selection line as a predictor, as well as latent factors, which are inferred from the data by the software and used to correct the model for confounding effects of unobserved variables. Based on principal component significance, which may be used to detect the number of latent factors to consider, we used $K=3$, which corresponds to the number of latent factors evaluated (i.e., selection lines), and is the number of expected genetic clusters. We repeated the LFMM analysis for all samples and subsets of samples in the small- and random-selected and large- and random-selected comparisons. We required that the final set of outliers were present in both outlier analyses (PCAdapt and LFMM).

Gene ontology analysis

We sought gene ontology enrichments among the genes associated with outlier SNPs related to the differentiation between large- and random-selected, and between small- and random-selected lines as well as between replicates. For this, we compared the lists of all genes associated with the final sets of outlier SNPs (large vs random and small vs random). We used a list of all genes associated with SNP(s) as a background and used the standard hypergeometric statistics, as implemented in the Gene Ontology Enrichment analysis and Visualization tool (GOrilla; Eden et al., 2009).

Genetic diversity

N_e was calculated for each population using the linkage disequilibrium method (Waples & Do 2010) with NeEstimator v. 2.1 using random mating (Do et al., 2014). VCFtools (v0.1.16) was used to calculate nucleotide diversity (π) and heterozygosity across all SNPs (Table S2). Polymorphism (%) was also calculated using a subset of 100,000 SNPs (Table S2).

Results

SNP annotation

85.8% of all SNPs were associated with genes. Most of the SNPs were found in the introns and intergenic regions followed by up- or downstream regions of genes (Figure S2). SNPs outside the coding region had lower divergence than SNPs associated with genes (Figure S2). Interestingly, synonymous SNPs and 5' UTR variants were highly diverged (Figure S2). This may suggest that directional selection has targeted functionally relevant SNPs, regulating gene expression levels.

Genetic changes caused by size-selective harvesting.

Both size-selected (small and large) and the random-selected lines formed distinct clusters in the principal component analysis indicating a genomic difference among them. PC1 separated the small-selected line from the other two lines (large- and random-selected) and PC2 the random-selected and founder population (F_0 -generation) from the size-selected lines (Figure 1). Furthermore, size-selected lines diverged more from the founder population than random-selected line as evidenced by the *diffstat* values (Figure S3). Small-selected and large-selected lines were more diverged from each other than either were diverged from random-selected line.

Outlier detection

PCAdapt approach resulted in 93,397 and 15,635 outlier SNPs for the two first principal components, respectively ($FDR < 0.01$; Figure 2). Of those, 2,990 were common to both PC1 and PC2 outlier sets. When comparing small- and random-selected lines, 13,022 outlier SNPs (associated with 5,092 genes) were significant in both the LFMM and PCAdapt analysis. When comparing large- and random-selected lines, 7,108 outliers were significant (associated with 2,908 genes).

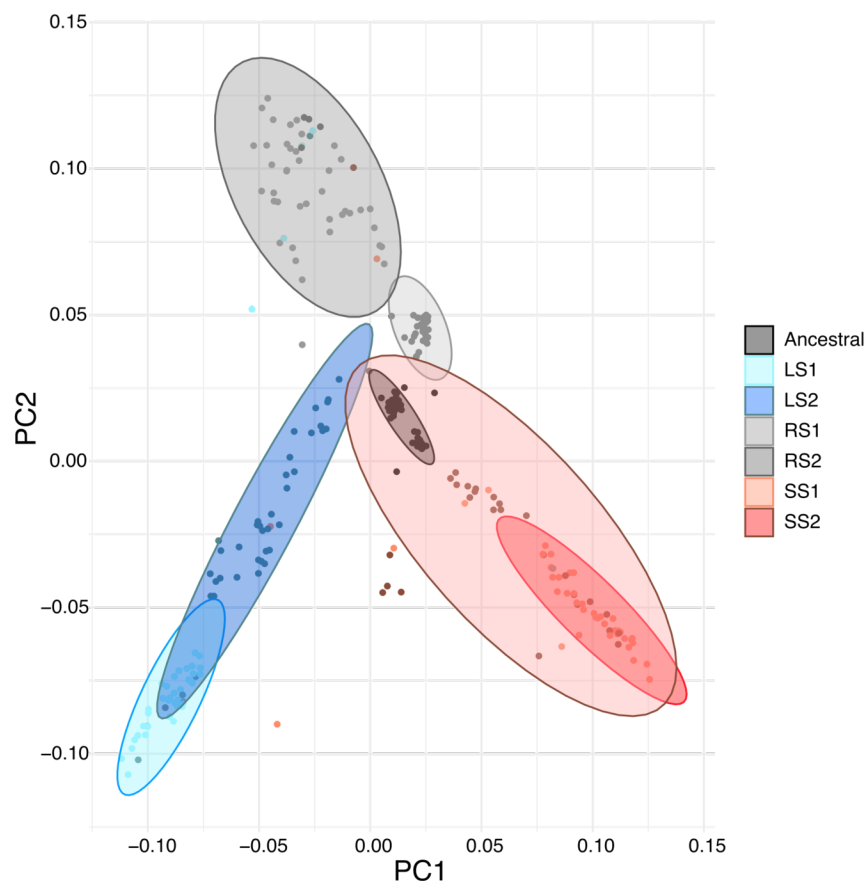


Figure 1 . Principal component analysis based on random subset of SNPs amongst ancestral, small-selected replicates (SS1, SS2), large-selected replicates (LS1, LS2), random-selected replicates (RS1, RS2), and ancestral population. PC1 and PC2 explain 3.5 and 2.5% of the variation, respectively. Points indicate individuals. Ellipses are 95% confidence intervals around the mean and highlight selection-line replicates and the founder population.

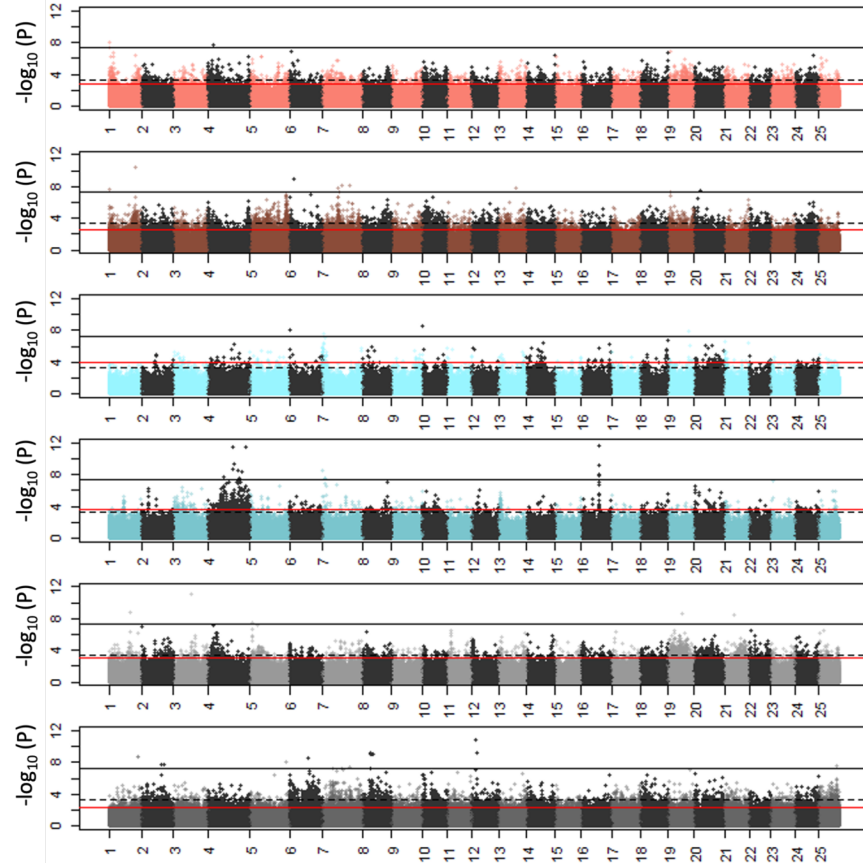


Figure 2: Genome wide distribution of p -values, FDR-corrected and $-\log_{10}$ -transformed for LFMM associated SNPs for the selection line replicates against the founder population across the 25 chromosomes. (a) Small-selected (SS1); (b) Small-selected, (SS2); (c) Large-selected (LS1); (d) Large-selected (LS2); (e) Random-selected (RS1); (f) Random-selected (RS2).

Genetic diversity

Genetic diversity, measured as % polymorphism, expected heterozygosity, effective population size (N_e), and inbreeding coefficient was higher in founder population compared to small-, large and random-selected lines (Table S2; Figure S4). However, this pattern was not evident in nucleotide diversity (Table S2). Despite high harvest rate (75%) that was applied for five generations, diversity measures did not differ vary among the selection lines.

Gene ontology

We found that 212, 76 and 65 terms were significantly enriched in the small-selected, large-selected and random-selected line respectively (Figure S5). After filtering for genes with the highest enrichment (over one), 25, 14, and 11 genes were enriched respectively in small-selected, large-selected and random selected (Figure 3). Large-selected fish had several enriched terms related to phosphorylation and nervous system development, whilst small-selected fish showed enrichment in structural morphogenesis and locomotion (Figure 3). Despite having a fewer enriched terms, the random-selected line had the highest individual enrichments associated with anion transport, morphogenesis, and molecular regulation (Figure 3).

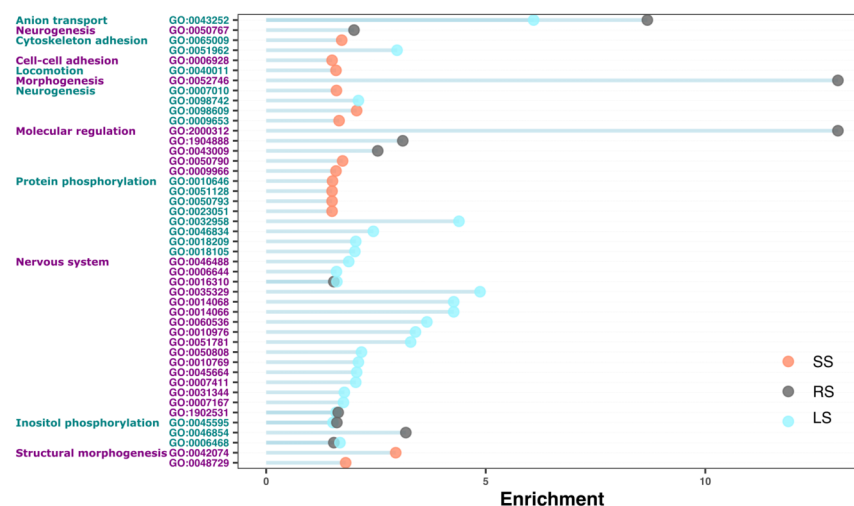


Figure 3: Outlier Gene Ontology (GO) terms with significant enrichment ($FDR < 0.05$) from the size-selected lines: large-selected (LS), small-selected (SS), and random-selected (RS) clustered by descriptor terms. The clusters of GO terms are indicated with colour changes in the term list based on term category.

Discussion

Size-selective harvesting can lead to substantial shifts in genomic architecture due to directional selection (Uusi-Heikkilä et al., 2017; Therkildsen et al., 2019). Furthermore, size-selective harvesting has been shown to lead to reduced genetic diversity (Pinsky & Palumbi 2014). Here we show that experimental size-selective harvesting led to large-scale genomic shifts, which were responsible for different gene enrichments among selection lines. Genetic diversity decreased during the experiment (i.e., all selection lines had lower genetic diversity than the founder population), however, in contrast to our predictions there were no clear differences in genetic diversity between the size-selected (small- and large-selected) and random-selected lines (no directional selection). Moreover, we find a large region of enriched gene ontology terms associated with nervous system in large-selected fish.

Our results follow a suite of previous studies that demonstrate a reduction in genetic diversity after over-harvesting (see Pinsky and Palumbi 2014 for meta-analysis). Though it seems evident that genetic diversity should decrease following demographic loss and selective sweeps, some studies show no loss of genetic diversity following overexploitation (e.g., Pinsky et al. 2021). Indeed, we show clear loss in overall genetic diversity but no loss of nucleotide diversity. Interestingly, here we show genetic diversity did not differ between populations exposed to directional selection (small- and large-selected) and populations experiencing only demographic loss (random-selected) despite substantial harvest rate (75%). This is contrary to Therkildsen et al., (2019) who showed that size-selection amplified genetic loss compared to demographic loss only after a harvesting rate of 90%. Though genetic loss can be indicative of reduced adaptive potential (Allendorf et al., 2008), it is important to delve into what variation of alleles are being selected against and how genomic structure changed dependent on harvesting protocol (i.e., size-selective or not size-selective).

While the phenotypic effects of size-selective harvesting (reduced body size, earlier maturation, and reduced reproductive output) have been demonstrated earlier (Conover & Munch 2002; van Wijk et al., 2013; Uusi-Heikkilä 2015), it has remained less clear what is the magnitude of genetic changes caused by harvesting. Previous studies have demonstrated this by utilizing relatively small number of genetic markers (van Wijk et al. 2013; Uusi-Heikkilä et al., 2015) whereas whole-genome approaches have been less common (but see Therkildsen et al., 2019; Pinsky et al., 2021). Here, we show that size-selection shifts genomic structure (Figure 1), with size-selection (large- and small-selected) having different evolutionary trajectories from demographic

loss alone (random-selected). Directional selection can cause over time genetic changes through genetic hitchhiking and selective sweeps. Genetic hitchhiking is a process where allele frequency is changed because the locus is linked to another gene that is under selection. This can reduce the amount of genetic variation in a population, especially near the selected site. A selective sweep, on the other hand, is a process where the frequency of beneficial alleles is increased and in the most extreme case the allele become fixed (Stephan 2019). Here, selective sweeps could be driving differences in genomic structure of the large-selected, small-selected and random-selected lines, though due to the polygenic nature of body size, it is difficult to pin down specific alleles. Small-selected lines were most different from other selection lines as shown by PC1 (Figure 1), this follows the pattern of phenotypic differences previously shown among the selection lines (Uusi-Heikkilä et al., 2015). We also show that directional selection (small- and large-selected line) drove genomic shifts in a different direction to genetic loss alone (random-selected line). Furthermore, the change in genomic structure and genetic diversity means intensive harvesting could lead to severe loss in adaptive potential (Hollins et al., 2018) which may act in tandem with phenotypic changes to erode population resilience to environmental change and further fishing events. Despite experiencing the same selective pressure, genomic changes differed between population replicates (see also Therkildsen et al., 2019) suggesting different evolutionary trajectories towards different genomic architecture. Though differences in genomic structure occur despite experiencing the same selective pressure, the genetic variation is likely high enough to maintain genetic redundancy, with different genes producing the same phenotype, with different pathways to the same function (Barghi et al., 2019).

Due to the extensive zebrafish reference genome annotations, we were able to produce high quality, accurate gene ontology associations to the outlier SNPs (Howe et al., 2013). This allowed us to predict the functions being selected for as a result of size selection. We showed large clusters of enriched genes associated with nervous system function in large-selected lines, which could be indicative of for example changes in behaviour or cognition. It was demonstrated earlier that the small-selected fish were less explorative and bold than large-selected individuals (Uusi-Heikkilä et al., 2015) and they have also been shown to differ in their personalities and cognitive functions even after harvesting had been halted for generations (Sbragaglia et al., 2019; Roy et al., 2023). Lowered activity and increased cautiousness of small-selected individuals has been evident in other studies (Walsh et al., 2006) and this can lead to increased vulnerability to fishing gear (Alós et al., 2012; Härkönen et al., 2014; Diaz Pauli et al., 2015). Here, we may provide evidence of supporting genetic changes to behaviour via nervous system developmental changes, though we can only show inference with GO terms without gene expression data. Furthermore, we see more gene enrichment in lines exposed to directional selection (small- and large-selected) than demographic loss *per se* (random-selected), potentially showing an increase in selective pressure and differing shifts in genomic function. Indeed, differentiation of gene ontology could allow us to predict which genes are being hitchhiked along with size related genes during size selection. Moreover, we show that the different line replicates differ in their gene ontogeny despite undergoing the same selective pressure (Figure S5), further supporting theory that size-selection can drive different evolutionary trajectories resulting in differing islands of genomic architecture.

We show that harvesting, whether being size-selective or not, decreases genetic diversity and causes divergence in genomic structure, with directional selection favouring small fish causing greater shifts than demographic loss *per se* (random-selected). While this may imply that size-selective harvesting removing the largest individuals from the population causes greater genomic changes than balanced harvesting (i.e., no size-selection), it is noteworthy that it did not cause greater losses in genetic diversity. However, type of selection should be considered in fisheries management, as changes in genomic architecture can potentially lead to shifts in population vulnerability through changes of functions and loss of adaptive alleles. Here, we show detailed gene ontology from genomic data to understand associated genes that have been selected for alongside size related phenotypes. However, future studies should consider other approaches such as metabolomics to get an in-depth picture of differentiation of pathways leading to phenotypic change. Furthermore, as we provide further evidence of divergence of genomic structure following the same size-selection regime (Therkildsen et al., 2019), it would be plausible to see if this is a consistent effect over multiple selection lines and whether the effects are truly stochastic. Overall, reductions in genetic diversity and changes in genomic structure can

lead to populations losing adaptive alleles making them more prone to future environmental stressors and fisheries events.

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Data accessibility statement

Raw data reads of whole genome data are archived in the SRA (BioProject ID: PRJNA1013179)

Benefits sharing statement

Benefits from this research result from the sharing of our genetic data and results on appropriate public databases. All collaborators are listed as co-authors.

Author contributions

SUH, PCW, TS and DES conceived the ideas and designed methodology; DES and SvD collected the data; DES and TS analysed the data; DES led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

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