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Daniel Sadler

So Long and Thanks for All the Fish

Fisheries Erode Adaptive Potential

UNIVERSITY OF JYVÄSKYLÄ
FACULTY OF MATHEMATICS
AND SCIENCE

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ABSTRACT

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Fisheries not only deplete fish populations but are size-selective, removing the largest individuals in the population. The effects of directional selection are little known compared to bottlenecks and loss of diversity associated with overharvesting. This directional selection for body size can lead to loss of genomic and phenotypic diversity, leading to a loss of adaptive potential. To alleviate population loss, fisheries may halt harvesting to allow for population recovery. It is unknown whether a period of recovery prevents further genomic divergence and loss of adaptive potential. To address these questions, I used a model zebrafish system that had been exposed to five generations of size-selective harvesting, followed by ten generations of recovery. Two lines had experienced directional selection for either large or small body size, whilst one line was subject to random removal of individuals. I used a combination of molecular approaches and a long-term experimental study system to (1) determine the genomic change after an overharvesting event, and whether directional selection exacerbates such change, (2) whether a period of recovery prevents further genomic divergence, and (3) how directional selection interacts with exposure to thermal stress to influence physiology, life history, behaviour, genomic markers, and skin microbiota. I found that the change in genomic architecture depended on the direction of selection after harvesting and was stochastic between line replicates. Furthermore, I found that despite a recovery period, genomic architecture continues to change and genomic diversity decrease. Moreover, I found that directional selection increases susceptibility to thermal stress, decreasing fitness based on phenotypic measurements and genomic markers. I also find that a legacy of directional selection does not influence skin microbiota. Taken together, a legacy of directional selection can alter genomic architecture, and degrade adaptive potential of a population, reducing fitness of individuals. Crucially, I find that direction of selection does not matter as much as the act of selection itself, and that a balanced harvesting approach may be the optimum strategy to manage fisheries.

Keywords: Directional selection; fitness components; fisheries; multiple stressors; population genomics; size-selection; thermal stress

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TIIVISTELMÄ

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Kokoon kohdistuva kalastus heikentää kalojen adaptiivista potentiaalia
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Kalastus poistaa suuria määriä ja useimmiten isoja kaloja populaatiosta, koska kalastajat suosivat suuria yksilöitä. Tätä kutsutaan suuntaavaksi kalastusvalinnaksi ja sen vaikutuksista kalapopulaatioihin tiedetään vasta vähän. Kokoon kohdistuva suuntaava valinta saattaa vähentää perinnöllistä ja ilmiasuvaihtelua populaatiossa, mikä puolestaan saattaa vähentää populaation kykyä sopeutua muutoksiin. Kun kalakannan koko pienenee, kalastus voidaan kieltää, jotta populaatio toipuisi. Ei kuitenkaan tiedetä, tapahtuuko toipumista ja miten kalastuksen lopettaminen vaikuttaa populaation kykyyn sopeutua muutoksiin. Käytin kokeellisia seeprakalapopulaatioita (*Danio rerio*) ymmärtääkseni kokoon kohdistuvan kalastuksen ja kalastuksen lopettamisen vaikutuksia kalapopulaatioihin. Populaatiot oli viiden sukupolven ajan altistettu kokoon kohdistuvalle kalastukselle, jonka jälkeen niiden oli annettu toipua kymmenen sukupolven ajan. Kokoon kohdistuva valinta suosi joko suurta tai pientä kokoa tai populaatiosta poistettiin satunnaisen kokoisia kaloja. Tutkin (1) kalastuksen aiheuttamia muutoksia perimässä ja etenkin vertailin muutoksia suuntaavan valinnan ja satunnaisesti valittujen linjojen välillä, (2) muutoksia perimässä, kun kalastus lopetettiin ja (3) millaisia vaikutuksia suuntaavalla valinnalla yhdessä lämpötilastressin kanssa oli kalojen elinkierto-ominaisuuksiin, fysiologiaan, käyttäytymiseen, perimään ja ihon mikrobistoon. Muutokset perimässä riippuivat valinnan suunnasta ja niissä oli satunnaisuutta. Kalastuksen lopettamisen jälkeen perimä muuttui edelleen ja perinnöllinen vaihtelu väheni. Populaatiot, jotka altistuivat suuntaavalle valinnalle, olivat herkempiä lämpötilastressille. Suuntaava valinta ei vaikuttanut kalojen ihon mikrobistoon. Väitöskirjani osoittaa, että suuntaava valinta voi vaikuttaa populaatioiden perimään ja vähentää populaation kykyä sopeutua muutoksiin sekä yksilöiden kelpoisuutta. Kokoon kohdistuvan valinnan suunnalla ei niinkään ole vaikutusta, vaan itse valinnalla. Täten kokoon kohdistumaton valinta saattaa olla ihanteellisempi strategia kalastuksen säätelyssä, kuin kokoon kohdistuva.

Avainsanat: Kalastus; kelpoisuuden mittaaminen, kokoon kohdistuva valinta; lämpötilastressi; moninaiset stressitekijät; populaatiogenomiikka; suuntaava valinta.

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LIST OF ORIGINAL PUBLICATIONS

The thesis is based on the following original papers, which will be referred to in the text by their Roman numerals I–V.

I Sadler DE, Sävilammi T, van Dijk S, Watts PC, Uusi-Heikkilä S. Size-selective harvesting drives genomic shifts in a harvested population. Submitted manuscript.

II Sadler DE, Sävilammi T, van Dijk S, Watts PC, Uusi-Heikkilä S. Population genomics of an overharvested population after a period of recovery. Manuscript.

III Sadler DE, van Dijk S, Karjalainen J, Watts PC, Uusi-Heikkilä S. 2024. Does size-selective harvesting erode adaptive potential to thermal stress? *Ecology and Evolution* 14, e11007.

IV Sadler DE, Watts PC, Uusi-Heikkilä S. Directional selection, not the direction of selection, affects telomere length and copy number at ribosomal RNA loci. Submitted manuscript.

V Sadler DE, Watts PC, van Dijk S, Uusi-Heikkilä S. Skin microbiota remains resilient under thermal stress in a teleost. Manuscript.

Author contributions to the original papers within the thesis.

Study	**I**	**II**	**III**	**IV**	**V**
Study design	DS, SvD, TS, PW, SUH	DS, TS, SvD, PW, SUH	DS, SvD, JK, PW, SUH	DS, PW, SUH	DS, PW, SUH
Data collection	DS, SvD, SUH	DS, SvD	DS, SvD	DS	DS, SvD
Laboratory work	DS, SvD	DS, SvD	DS, SvD	DS	DS
Data analyses	DS, TS	DS, TS	DS	DS	DS, PW
Manuscript writing	DS, SvD, TS, PW, SUH	DS, TS, PW, SUH	DS, SvD, JK, PW, SUH	DS, PW, SUH	DS, PW, SvD, SUH

Daniel E. Sadler (DS), Stephan van Dijk (SvD), Tiina Sävilammi (TS), Phillip C. Watts (PW), Silva Uusi-Heikkilä (SUH), Juha Karjalainen (JK)

The planet has survived everything, in its time.
It will certainly survive us.
-Dr Ian Malcom

1 INTRODUCTION

1.1 Overharvesting and its evolutionary consequences

On the rich coasts of South Africa, we find the meeting point of two oceans; Atlantic and Indian. It is also the collision point of the Benguela and Agulhas currents which drive nutrient productivity through upwelling, driving huge productivity for the marine ecosystem. This region of the world is also crucially important for us, as it is the birthplace of us all; the cradle of humankind (Dirks and Berger, 2013). Theory suggests that access to the rich fatty acids of coastal fish kick-started the evolution of human intelligence, but at the very least it ignited the human desire to exploit our oceans. From the development of simple bone hooks used by early hominids, to woven nets of the ancient Egyptians, to today's industrial use of long lines, trawlers and factory ships, humans have always exploited resources from the seas to stock their larders. However, with the advent of the industrial revolution, exploitation of these stocks has increased exponentially, becoming unsustainable through overharvesting. Indeed, capture fishing provides 90 million tonnes of food per annum, representing some 17 % of global animal protein consumed by humans (FAO, 2022). However, the percentage of fish stocks that are sustainably caught has reduced from 90 % in 1990 to 65 % in 2019 (FAO, 2022), leading to overharvesting of world fish stocks. Overharvesting can lead to drastic population declines, with some worst-case predictions suggesting that fish stocks may become exhausted by 2050 (Worm et al., 2006; Worm 2016). Indeed, in many coastal areas around the world, populations of large vertebrates including sharks, rays and cod are functionally extinct due to severe overharvesting events (Jackson et al., 2001).

Rapid population declines as a result of overharvesting can cause a loss of genetic (Marty et al., 2015; Pinsky and Palumbi, 2014; Sadler et al., 2023; Therkildsen et al., 2019) and phenotypic variation (Olsen et al., 2009; Therkildsen et al., 2019). Alongside such population loss, fisheries are often size-selective, removing the largest, and therefore most economically valuable fish in the population (Jørgensen et al., 2007; Law, 2007; Lewin et al., 2006). The effect of

overharvesting is well studied, especially on commercially important fish such as cod (Hutchinson et al., 2003; Therkildsen et al., 2010; Pinsky et al., 2021) and herring (Trochta et al., 2020). However, these studies often focus on the loss of genetic and phenotypic diversity caused by the bottleneck effect of overharvesting, ignoring the more subtle and understudied effects of directional selection.

1.1.1 What selection should we select?

Selection on a population can be (1) stabilising, whereby a population stabilises around a non-extreme mean phenotype, (2) disruptive, where extreme phenotypes are favoured over mean, often driving distinct populations (e.g., a population with very large and very small body size) and (3) directional (i.e., size-selective fishing) where the act of selecting for an extreme phenotype (e.g., large body size), causes allele frequency to shift, favouring the new phenotype. Directional selection may magnify any effects of phenotypic and genomic changes than expected under a simple reduction in population size alone (random selection). Directional selection can drive a faster loss of genetic diversity compared with population loss alone (Frankham, 2012), as it favours a specific phenotype and causes a directional shift in allele frequency (Quinn et al., 2007; but see Pinsky et al., 2021). Such directional selection on body size can drive evolutionary change towards specific phenotypic traits that correlate with size (Conover and Munch, 2002; Uusi-Heikkilä et al., 2015), such as faster juvenile growth rate, earlier age at maturation, and altered behaviour (Olsen et al., 2004; Mollet et al., 2007; van Wijk et al., 2013; Uusi-Heikkilä et al., 2015; Therkildsen et al., 2019). It is therefore crucial to understand not only the phenotypic and genomic effects of population loss (overharvesting), but also how directional selection (size-selective harvesting) could magnify the effects of overharvesting and what are its consequences on fitness.

An alternative fishing strategy to size-selective harvesting is balanced harvesting in which a quota of the targeted species is removed but without regard to a particular trait, such as body size (Zhou et al., 2010). Balanced harvesting is predicted to be more sustainable than harvesting involving directional selection on exploited populations, as balanced harvesting may maintain phenotypic diversity (Garcia et al., 2012). However, the efficacy of balanced harvesting to mediate effects of directional selection is currently unknown (Zhou et al., 2019) and needs to be carefully studied.

I examine the overlooked influence of directional selection and how that compares to bottlenecks and loss of diversity alone. I used selection lines of zebrafish (*Danio rerio*) which have been exposed to directional selection for body-size (small- or large-selection) or random-selection which is analogous to balanced harvesting. Using this model system, I aimed to determine whether directional selection and direction of such selection (small- or large-) exacerbated loss of fitness compared to population loss alone (random-selection).

1.2 Does halting harvesting allow for phenotypic and genomic recovery?

Fisheries management may close a fishery when a population becomes severely depleted to allow for population recovery. A moratorium is then put in place, whereby fish are not permitted to be harvested during this period. Cessation of harvesting may facilitate restoration of genetic and phenotypic diversity and halt the effects of directional selection which may have magnified loss of phenotypic and genomic diversity during overharvesting (Frankham, 2012). Similarly, marine protected areas (MPA) can be implicated to halt fishing in certain areas (analogous to a moratorium). Evidence suggests phenotypic responses to protection by MPA's (i.e., no harvesting) differed from non-protected areas (harvested) with cod (*Gadus morhua*) having greater phenotypic fitness in MPA's (Moland et al., 2013). However, overharvesting may have reached beyond a tipping point, eroding diversity to such an extent that individuals cannot return to the pre-harvested phenotypic and genomic architecture (Walsh et al., 2006; Pinsky and Palumbi, 2014; Dakos et al., 2019). Legacy effects of population loss and directional selection may persist in the population, suggesting populations may never fully recover. It is difficult to empirically study the phenotypic and genomic recovery of a population as many fished populations have such a long generation time (e.g., tuna), and many other biological and environmental variables can influence the severity of the effect of fisheries (Sadler et al., 2023). Furthermore, we seldom have phenotypic or genomic data of natural populations before harvesting.

It has been predicted that triple the generations of harvesting is needed for phenotypic recovery (e.g., four generations of harvesting would need 12 generations of recovery as per Conover et al., 2009). However, directional selection can drive more severe phenotypic differences associated with growth rate compared to population loss alone, even after a period of recovery (Conover et al., 2009). As such, phenotypic fitness may be affected by legacy effects of directional selection, meaning even if a population experienced a cessation of harvesting, it may not be enough to allow phenotypic recovery.

Despite some knowledge of phenotypic recovery in experimental populations, little is known about the possibility of genomic recovery of a population. Evidence suggests that genomic recovery is much slower than phenotypic recovery in populations that have experienced bottlenecks (Adams and Edmands, 2023). Even less work has been done on genomic recovery of populations exposed to directional selection except some work on bighorn sheep (Miller et al., 2012). However, to the best of my knowledge no research has been done on genomic recovery in the context of fisheries.

The recovery potential of a population's genomic architecture is currently unknown and could potentially follow one of three patterns (Fig. 1). (1) genomic stability: after recovery, genomic architecture stabilises, remaining the same as pre-recovery, due to lack of directional selection, (2) genomic divergence: after

recovery, genomic architecture continues to diverge (from the structure prior to overharvesting) due to assortative mating, and genetic drift, (e.g., in bacteria Papadopoulos et al., 1999), and (3) genomic convergence: after recovery genomic architecture begins to converge back to its original structure prior to overharvest due to recombination and/or selection for low frequency alleles. How genomic architecture changes after a period of recovery likely relies on the interplay of inbreeding, assortative mating and other selective pressures in the absence of any immigration. Moreover, the length of a moratorium and the intensity of initial selection regime will influence a population's ability to fully recover. A moratorium may therefore not be enough to stimulate a full phenotypic recovery and prevent further genomic divergence.

Using the aforementioned selection lines of zebrafish (small-, large- and random-selected), I can quantify the changes in genomic architecture that occur after a period of recovery (i.e., cessation of harvesting).

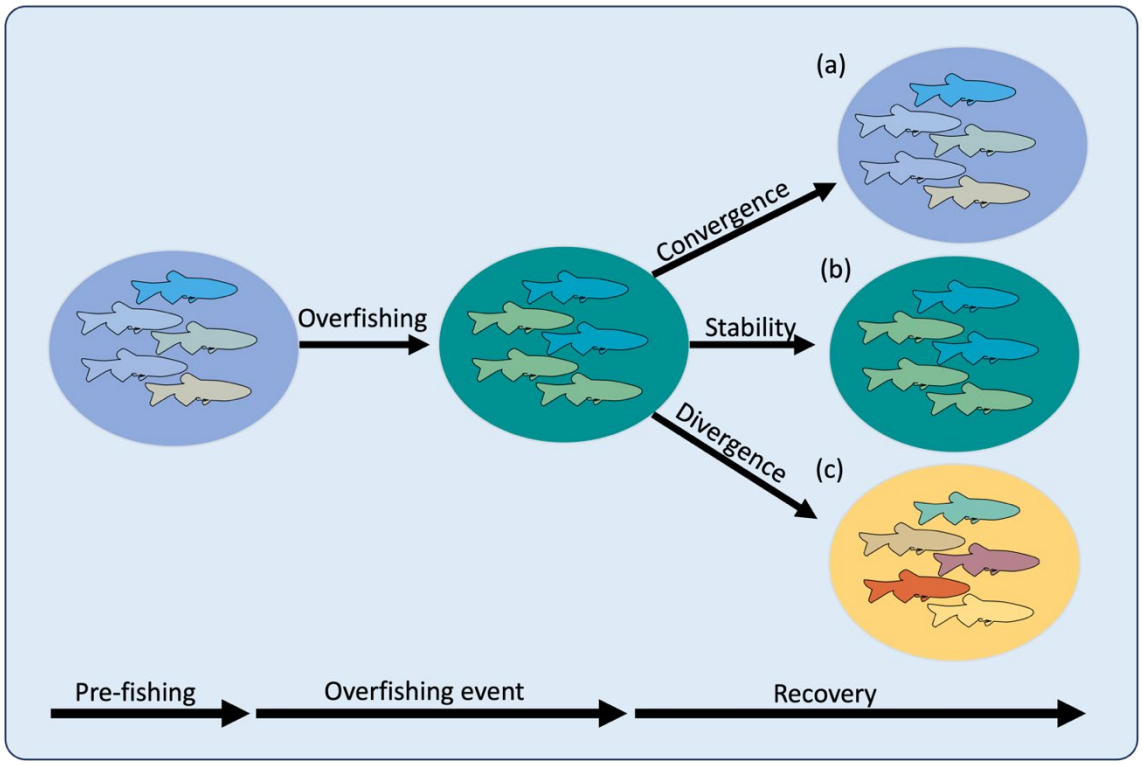

FIGURE 1 Overview of the three possible types of change in genomic architecture that occur in fish stocks that experience a period of overharvesting and then recovery (cessation of harvesting representative of a moratorium): (a) genomic convergence, (b) genomic stability, and (c) genomic divergence. Coloured fish represent different combinations of genotypes. (**II**).

1.3 Loss of genomic diversity and adaptive potential

Vulnerability of populations after a period of size-selective harvesting is poorly known. We do not know whether legacy effects of harvesting persist on genomic

and phenotypic traits even after many generations of recovery. Population loss alone will likely increase susceptibility to future environmental stressors through random loss of adaptive alleles (Thompson et al., 2019; Petrou et al., 2021), as well as a reduction in phenotypic (Anderson et al., 2008; Morrongiello et al., 2019) and genomic (Pinsky and Palumbi, 2014) diversity. Directional selection may lead to further reductions in genomic and phenotypic diversity (Marty et al., 2015; Groth et al., 2018; Therkildsen et al., 2019). As high genomic and phenotypic diversity allows for an adaptive response to a change in environment (Pörtner and Farrell, 2008), a loss of phenotypic and genomic diversity from directional size-selective fishing is expected to associate with increased vulnerability to environmental stressors (Anderson et al., 2008; Morrongiello et al., 2019).

Population recovery through a moratorium may mitigate loss of adaptive potential caused by overharvesting. However, genomic and phenotypic recovery may be extremely slow due to long generation times of fish species (Heino, 1998; Allendorf et al., 2008). Additionally, mutations cannot be relied on as a source of new genetic variation as generation times are too long and fish populations too small, so humans will likely overharvest populations before mutation becomes relevant. Directional size-selection can leave a legacy effect on populations for many generations without harvesting, leading to increased susceptibility to environmental stressors (Conover and Munch 2002; Morrongiello et al., 2019). It is therefore crucial to understand how a period of recovery (i.e., cessation of harvesting) influences genomic architecture and whether it influences susceptibility to other coinciding environmental stressors.

I was able to examine how directional selection on body size influences genomic architecture and diversity between founder population (pre-fishing) and harvested populations to determine how genomic architecture may change depending on selection regime (**I**). Additionally, I was able to determine change in genomic architecture following a recovery period (**II**). Moreover, I examined how adaptive potential is affected by exposing the recovered population to a novel stressor (**III–V**).

1.4 Multiple stressors in a changing world

We live in an era of unprecedented environmental change, driving the sixth mass extinction (Ceballos et al, 2015; Cowie et al., 2022). Aquatic systems are under increasing stress from a plethora of environmental stressors including habitat destruction (Walker and Kendrick, 1998), climate change induced warming (Thomas et al., 2004; Craig, 2012; Poloczanska et al., 2016), ocean acidification (Caldeira and Wickett, 2003), pollution (Young et al., 2016) and overharvesting (Scheffer et al., 2005). A high level of environmental stress can influence a species' physiology, life history, behaviour, and overall population dynamics. Environmental stressors do not act in isolation, rather, the natural environment is multifaceted, and it is therefore important to consider the interaction of these stressors. Multiple stressors can act synergistically or antagonistically on a

population to drive changes in population structure and ecology. For example, thermal stress from climate change can cause oxygen depletion, increased pH, and ocean acidification, resulting in a multifaceted complex stressor. Organisms can respond to environmental stressors through acclimation and adaption (DeMarche et al., 2019). However, adaption and acclimation to multiple stressors is difficult to quantify, as selective pressures on a trait can be opposing. For example, one stressor may favour large body size, whilst a second stressor favours small body size when acting independently of each other, so it is difficult to predict the outcome. High genetic variation is important for a population's persistence as it can increase adaptive potential to environmental stress (Lande and Shannon, 1996; Thompson et al., 2019; Petrou et al., 2021). A benefit of high genetic diversity is genetic redundancy, in which different genes produce the same phenotype, resulting in different pathways to the same functions (Barghi et al., 2019). As such, population loss and directional selection may reduce genetic redundancy through the loss of diversity.

As overharvested populations may also experience human-induced changes in their environment, an important question arises: how does an overharvested population exposed to directional selection interact with an environmental stressor and how does this affect fitness? Arguably the most important of these environmental stressors in the aquatic realm is thermal stress driven by climate change (Planque et al., 2010; Wootton et al., 2021).

I was able to tackle the interaction between directional size selective harvesting and thermal stress, to better understand how these two major stressors in the aquatic realm influence fitness. Studying the interaction between size-selective fisheries and environmental stressors is challenging in natural environments, therefore it is crucial to utilise laboratory studies such as the one presented here to shed light on the interaction of multiple stressors.

1.4.1 Thermal stress as an environmental stressor

Climate change is leading to long-term warming of the environment (Parmesan and Yohe, 2003; Mittelbach et al., 2007) and temperatures are predicted to continue rising (Pörtner et al., 2019). Climate change is also causing more extreme weather scenarios (e.g., heatwaves), leading to sea surface temperature increases of 2–4°C and sometimes > +5°C (Oliver et al., 2018; Sen Gupta et al., 2020). Additionally, climate change may cause an increase in cold winters (Williams et al., 2015) which can drive fish deaths worldwide (Gunter, 1951; Pörtner and Peck 2010). Aquatic species such as fish can cope with thermal stress by (1) short-term acclimation, (2) long-term adaptation, or (3) migration towards more optimal temperatures (Dahms and Killen, 2023).

Thermal stress from long-term warming and extreme weather events will likely exceed thermal limits of fish physiology (Perry et al., 2010; Hollowed et al., 2013), negatively affecting growth (Boltaña et al., 2017), reproduction (Pankhurst and Munday, 2011), metabolic function (Clarke and Fraser, 2004; Seebacher et al., 2014), and behaviour (Neubauer and Andersen, 2019). One interesting prediction is that elevated temperature will select for fast growth and small adult body size

following the temperature size rule (TSR; Atkinson, 1994). Following the TSR, directional selection for small body size (as in fisheries) may act in synergy with thermal stress also selecting for small body size (Audzijonyte et al., 2016). It is therefore crucial to understand how the interaction of directional selection induced by fisheries and thermal stress affects fitness.

1.5 Fitness components

There are many different definitions of biological fitness, but the most useful is fitness being an organism's survivability and reproductive success, as well as the subsequent reproductive success of their offspring (Orr, 2009). It is rarely possible to track fitness across many generations, particularly in vertebrates, so here I use fitness components (Hutchings, 2021). In fish, relevant fitness components include: (1) life history traits such as growth rate (Ahti et al., 2020), reproduction (Jonsson and Jonsson, 2019; Alix et al., 2020), metabolic rate (Killen et al., 2016) and, behaviour (Biro et al., 2010; Neubauer and Andersen, 2019), (2) genomic markers including telomeres, rDNA and mtDNA (Monaghan and Haussmann, 2006; Näslund et al., 2015; Wilbourn et al., 2018; Filograna et al., 2021) and (3) microbiota composition (Koskella et al., 2017).

1.5.1 Life history, physiology, and behaviour

Arguably the most important markers of fitness relate to an organism's physiology and life history that directly influence survivability and reproductive success. Life history theory indicates that reduced survivability will lead to earlier maturation and increased fecundity (Gadgil and Bossert 1970; Law, 1979) demonstrated in both experimental (Uusi-Heikkilä et al., 2015) and field (Reznick et al., 1990) systems. Such life-history traits including growth, maturation age, and fecundity are also severely affected by both directional size-selection (Conover and Munch, 2002; van Wijk et al., 2013; Uusi-Heikkilä et al., 2015) and thermal stress (Atkinson 1994; Pörtner and Farrell, 2008). However, the possible interaction of directional selection and thermal stress is understudied. Directional selection and thermal stress (following the TSR) can both select for small body size in fish. Therefore, these stressors may act in synergy to magnify such selection towards smaller body size and altered growth rate, along with other associated phenotypic traits. The combined effects of directional selection and thermal stress are unpredictable, but may negatively affect fitness (Planque et al., 2010; Rouyer et al., 2012; Wootton et al., 2021).

Metabolic rate is also a critical component of fitness (Killen et al., 2016) and is positively correlated with temperature in ectotherms (Clarke and Fraser, 2004; Seebacher et al., 2014; Morgan et al., 2022). However, beyond a critical temperature outside the thermal niche of an organism, metabolic rate starts to decline (Schulte et al., 2011; Schulte, 2015). Moreover, metabolic rate is strongly associated with body size (Urbina and Glover, 2013; Kraskura et al., 2023), as a

result, directional selection for body size may drive differences in metabolic rate. As such it may be expected that thermal stress and directional size-selection interact to drive alterations in metabolic rate and other physiological functions.

Behaviour is an important aspect of an organism's biology and interlinked with physiology and life history. Crucially, organisms can alter their behaviour almost immediately following an interaction with a stressor, and behaviour is highly plastic compared with morphological and physiological traits (Mousseau and Roff, 1987; Duckworth, 2009). Thermal stress and fisheries stress can independently alter exploration, boldness, activity, and feeding behaviour (Walsh et al., 2006; Uusi-Heikkilä et al., 2015; Neubauer and Andersen, 2019; Pilakouta et al., 2023). The interplay of these stressors is complex to predict and could cause decreased fitness at least in some cases. For example, an increase in boldness and exploration could increase catchability, magnifying the effect of overharvesting on fitness.

Using the selection lines of zebrafish, I was able to expose these fish to thermal stress of ±6°C compared to an ambient control treatment. This allowed me to examine how the legacy of directional selection and thermal stress interacted and potentially affected phenotypic traits (**III**).

1.5.2 Telomere and copy number variation

Directional selection (i.e., size-selection) impacts diversity at single-copy regions such as microsatellite loci (van Wijk et al., 2013) or SNPs within and among protein-coding regions (Therkildsen et al., 2019; Sadler et al., 2023). However, the impact of size-selective harvesting on other types of genomic changes, such as variation in copy number, is not known. What is known however, is that some genomic regions exhibit variation in their copy number when exposed to environmental stress. These regions include: telomeric DNA (Kotrschal et al., 2007; Simide et al., 2016), ribosomal RNA cassette (rDNA; Kobayashi, 2011; Salim and Gerton, 2019), and mitochondrial DNA (mtDNA; Bateson, 2016).

In vertebrates, telomeres are tandem repeats of TTAGGG capping the end of linear chromosomes (Zakian, 2012). Environmental stress affect telomeres by causing short telomeres and/or an accelerated rate of telomere attrition (von Zglinicki, 2002; Kotrschal et al., 2007; Monaghan, 2010; Reichert and Stier, 2017; Barnes et al., 2019) unless repaired, for example by telomerase (Aubert and Lansdorp, 2008; Webb et al., 2013). As telomere shortening is associated with cell senescence, telomere length can reduce fitness in some animals (Monaghan and Haussmann, 2006; Horn et al., 2010; Näslund et al., 2015; Wilbourn et al., 2018). The effect of environmental stress on telomere length is well studied (Chatelain et al., 2020), but it is unknown how directional selection for body size may affect telomere length. As telomeres shorten with growth (cell division; Allsopp et al., 1995), directional selection on body size (and indirectly on growth rate) could be expected to accelerate telomere attrition, and thermal stress may magnify such effects.

Ribosomal DNA is comprised of tandem arrays of the rRNA cassette (18S, 5.8S, and 28S rRNA loci). Transcription of rDNA is necessary for ribogenesis and

protein synthesis. Like telomere length, rDNA is sensitive to environmental stress, causing variation in copy number (Kobayashi, 2011; Paredes et al., 2011; Salim et al., 2017; Jernfors et al., 2021). Though not as well studied as telomeres, rDNA could be an emerging fitness marker, representing an environmental sensor that may regulate response to environmental cues (Kwan et al., 2013; Jack et al., 2015; Salim and Gerton, 2019; Symonová, 2019). How thermal stress influences rDNA copy number is little studied, and to my knowledge no other study has examined the effect of an environmental stressor on rDNA copy number in teleosts. As rDNA copy number, is sensitive to cell division (Kobayashi, 2014), directional selection on body size could affect rDNA copy number variation. Alongside directional selection, thermal stress may have an additive effect on rDNA copy number variation, which may lead to reductions in fitness.

Mitochondria contain their own genome; mitochondrial DNA (mtDNA) which have essential metabolic roles, notably, mitochondria are described as the powerhouse of the cell, supplying most of a cells energy requirements (Filograna et al., 2021). Content of mtDNA, like telomeres and rDNA can vary with age (Hartmann et al., 2011), growth rate (Quéméneur et al., 2022), and environmental stress (Chung and Schulte, 2020; Kesäniemi et al., 2020). Reduction in mtDNA content can result in increased cellular malfunction and disease, hence, could drive a reduction in fitness (Clay Montier et al., 2009; Reznik et al., 2016). Mitochondrial DNA is another understudied genomic marker, with only one other study examining the effect of temperature on mtDNA content in teleosts, finding an increase in mtDNA content in stickleback (*Gasterosteus aculeatus*) eggs at warmer temperatures (Kim et al., 2023). As mtDNA content is associated with growth rate, directional selection for body size is expected to affect mtDNA content, which may be amplified by thermal stress.

These genomic regions are correlated, affecting each other, for example, telomeres have been linked with mitochondrial content (Metcalfe and Olsson, 2022), so it is important to understand interrelatedness of these markers. As each of these genomic regions are associated with growth and are sensitive to environmental stress, I was able to examine the interaction of these regions within the context of size-selective fisheries and thermal stress (**IV**), an area previously unexplored.

1.5.3 Beyond the host: the microbiota

Beyond the phenotype and genotype of an organism, environmental stressors can also alter associated microbial communities, which can be crucial for host fitness (Koskella et al., 2017). The microbiota represents all microorganisms including bacteria, archaea, and fungi at a particular site on an organism (e.g., gut microbiota) or habitat (e.g., soil microbiota). The microbiota is associated with the health of a host species by interacting with host immune system (Guardiola et al., 2014; Yu et al., 2021; Wang et al., 2023), also commensal bacteria in the microbiota may help protect the host against pathogens (Balcázar et al., 2007). Alterations in the microbiota can increase incidence of disease (Ghosh et

al., 2022) and mortality (Mohammed and Arias 2015; Gomez and Primm 2021). Hence, an intact microbiota is crucial for health and fitness of the host.

In fish, the skin microbiota is particularly important as it is associated with immune defence, protecting fish against pathogens through competition and acting alongside the innate immune system within the mucosal layer (Balcázar et al., 2007; Sanford and Gallo, 2013; Guardiola et al., 2014). Comparatively little is known about how the skin microbiota of fish can shift in response to environmental stress compared to, for example, gut microbiota (Gomez and Primm, 2021). Some studies suggest environmental stress can disrupt abundance and diversity of skin microbiota communities (Krotman et al., 2020). For example, salinity (Schmidt et al., 2015; Lokesh and Kiron, 2016), hypoxia (Wang et al., 2021), and temperature (Huyben et al., 2018; Ghosh et al., 2022) have been shown to influence fish skin microbiota diversity and abundance. Dysbiosis of the skin microbiota can reduce fitness and increase the prevalence of key fish pathogens such as *Vibrio* spp. (Neuman et al., 2016). Host genotype strongly influences recruitment of the microbiota (Boutin et al., 2014), raising the potential that directional selection may influence recruitment through, for example, selective sweeps. Thus, directional size-selection and thermal stress may therefore interact driving a reduction in fitness through disruption of the microbiota and the promotion of pathogen causing microbes.

I was able to use the thermal stress experiment to additionally explore how a legacy of directional selection and contemporary thermal stress influenced skin microbiota (**V**). Exploring the microbiota in this context allowed me to examine fitness beyond the phenotype to quantify change in a highly dynamic system.

1.6 Zebrafish as a model of size-selective fisheries

The model organism in the experiments is the zebrafish (*Danio rerio*). Zebrafish are a freshwater cyprinid fish from India, found in a range of habitats from flooded rice fields to narrow streams and large rivers such as the Ganges (Neff et al., 2020).

Zebrafish were selected as a model organism as early as the 1960s, used as a research subject in toxicology, biomedical science, and evolutionary biology (Grunwald and Eisen, 2002). The zebrafish is an ideal model species due to its short generation time, ability to breed all year round, high number of offspring produced and ease of maintenance (Grunwald and Eisen, 2002). Additionally, zebrafish has a well-annotated, well studied genome (Howe et al., 2013), and it has been utilised as a genetic model for decades.

Although used in a variety of research fields, zebrafish as a fisheries model has only been developed in recent years. Indeed, the current lines used in the present study are from Uusi-Heikkilä et al., (2015) original study demonstrating the effect of size-selective harvesting on genetic and phenotypic traits of zebrafish. Although other studies have been run on other teleosts including Atlantic silversides (Conover and Munch 2002), guppies (van Wijk et al., 2013),

and pike (Edeline et al., 2007), these zebrafish lines have the advantage of being long running (almost 20 years) and having a well-annotated genome meaning their populations genetics can be analysed at a high resolution.

Zebrafish used in these experiments were of wild origin from the West Bengal region of India (Uusi-Heikkilä et al., 2010). After the founder population (F_0) was collected and acclimated in the laboratory, they were exposed to three selection regimes: (1) small-selected (experiencing directional selection for small body size, typical of size-selective fisheries targeting the largest individuals), (2) large-selected (experiencing directional selection for large body size) and (3) random-selected (experiencing no directional selection, the control line). This study design allowed me to compare directional selection (large- and small-selected) against a reduction in population size alone (random-selected). Fish were harvested for five generations (F_1–F_6) after which fish were shown to phenotypically differ (Uusi-Heikkilä et al., 2015). I took DNA samples from these fish for whole genome sequencing to assess the genomic differences among the selection lines (**I**). After five generations of harvesting, fish populations were left to recover (no harvesting) for ten generations (twice the length of harvesting), at which point I again took DNA samples for whole genome sequencing to determine genomic differences among the selection lines after recovery (**II**). I then conducted a thermal stress experiment to assess the interaction of a legacy of directional size-selection and thermal stress exposing fish to three separate temperature treatments: 28°C (ambient, the standard temperature they are kept in the lab), 34°C (elevated temperature) and 22°C (low temperature). During the thermal experiment, I monitored fish physiological, behavioural and life history traits (**III**). From these same fish in the thermal experiment, I also collected DNA to study the effect of directional selection and thermal stress on telomere length, mtDNA content, and rDNA copy number variation (**IV**), and took skin samples to study differences in their skin microbiota (**V**).

1.7 Objectives

My aim was to better understand how (1) size-selective harvesting (i.e., directional selection) influences the genomic architecture of exploited populations, (2) whether cessation of harvesting allows for genomic recovery, and (3) whether directional selection interacts with a novel environmental stressor (thermal stress) to influence fitness. The fitness components I used include phenotypic traits (life-history, physiological, and behavioural traits), genomic markers, and host skin microbiota.
Specifically, I will address the following research questions (Fig. 2):

I. Does size-selective harvesting influence the genomic architecture of a population?

II. Does cessation of harvesting after an overharvesting event prevent further genomic divergence?

III. Does directional selection (i.e., size-selective harvesting) magnify the effects of thermal stress on phenotypic traits?

IV. How does the interaction of directional selection and thermal stress influence genomic regions associated with stress?

V. Does directional selection and thermal stress influence the skin microbiota; a marker of host fitness

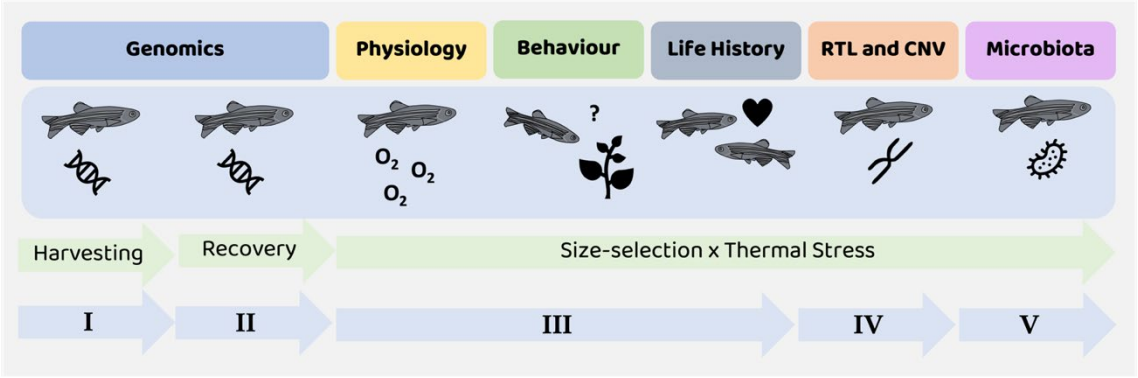

FIGURE 2 Overview of the research objectives of the thesis (**I–V** indicate manuscripts).

2 METHODS

2.1 Harvesting and subsequent recovery

The zebrafish founder population was exposed to three size-selection regimes over five generations: small-selected (75 % of the largest fish were removed, leaving the smallest fish in the spawning stocks; simulating typical size-selective fisheries), large-selected (75 % of the smallest fish were removed, leaving the largest fish in spawning stocks; the opposite directional selection regime), and random-selected (75 % randomly chosen fish were kept in spawning stocks; Fig. 3). Each line had two replicates with a population size of 450 individuals each. Populations were exposed to harvesting stress for five generations and DNA samples kept for later processing (**I**). The selection lines were allowed to recover for 10 generations (twice the harvesting length) where no harvesting took place (Fig. 3), after which DNA samples were taken (**II**), and a thermal stress experiment was conducted (**III–V**).

2.2 DNA processing

All fish used for the experiments were euthanised from the selection experiment then frozen for DNA extraction (–80°C). Genomic DNA was extracted from dissected muscle tissue from founder (**I**), harvested (**I**) and recovered (**II**) fish using a modified salt extraction method to extract genomic DNA (Aljanabi and Martinez, 1997). Genomic DNA was extracted for qPCR of biomarkers (**IV**) using Qiagen DNeasy Blood and Tissue kit according to manufacturers instructions. DNA was collected from skin swab samples and three 150 ml water samples per tank which was filtered through 0.22 µm membranes. Subsequent DNA from the swabs and water samples was extracted for amplicon sequencing (**V**) using a Qiagen DNeasy PowerSoil Pro Kit. Sequencing of the genomic DNA was conducted by Novogene Illumina Novoseq 6000 using whole genome sequencing of 150 base paired end reads (**I, II**) and 250 paired end reads for the

amplification of the V3–V4 region of the 16S ribosomal RNA (rRNA) in bacteria (**V**).

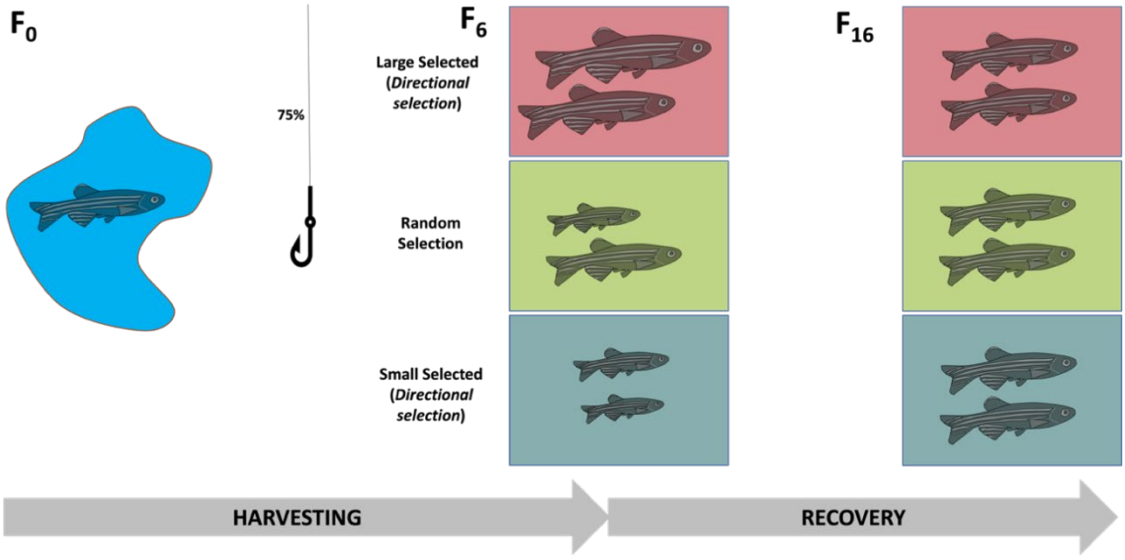

FIGURE 3 Overview of harvesting regime. F0 represents founder population from West Bengal which after generation of acclimation in the lab were harvested (F_6; 75 % removed, 25 % left in the spawning stock) according to three harvest regimes (large-selected, random-selected, and small-selected) with two replicates per line. Selection lines were then allowed to recover (no harvesting) for ten generations (F_{16}).

2.3 Whole genome sequencing processing

Raw reads were filtered for adapters and quality using fastp v. 0.2 (Chen et al., 2018). Sequences were mapped against the reference genome (Zebrafish GRCz11; Howe et al., 2021) using bwa mem v. 1.10 (Li and Durbin, 2010). Data was processed through a custom pipeline explained fully in manuscripts. Briefly, SNP were called and filtered using bcftools (Li, 2011). Number of SNPs called varied between the studies, full details in the manuscripts. Finally, SNPs were annotated with snpEff (Cingolani et al., 2012). Genomic diversity was calculated as effective population size (N_e), nucleotide diversity and nucleotide polymorphism (%).

2.4 Thermal experiment

Fish from each selection line replicate were exposed to three different thermal stress treatments: low (22°C), ambient (28°C), and elevated (34°C) for a total of 250 days (Fig. 4). Ambient temperature (28°C) had been the standard rearing temperature in the laboratory, acting as the control temperature. Elevated temperature (+6°C from the ambient temperature) is representative of temperature increase from extreme weather events (Oliver et al., 2018; Sen Gupta et al., 2020). Whilst –6°C from ambient temperature was representative of potential cold snaps and allowed an observation of the full range of responses. The range of temperatures used are representative of thermal stress in zebrafish (Åsheim et al., 2020; Morgan et al., 2022). To be able to collect individual data for the fish in the experiment, prior to the thermal experiment, all fish were tagged with visible implant tags (VIE; Northwest Marine Technologies, 162 Shaw Island, WA, USA). 360 individuals were chosen at random from the selection line replicates for the experiment (n=20 per selection line replicate, per temperature treatment). The same fish were consistently used across phenotypic and behavioural assays as well for the genomic markers and microbiota. Fish were acclimated at 28°C for two weeks, after which the temperature was altered by ±1°C per day for six days according to temperature treatment. Full details of the thermal experiment and measured phenotypic traits are described in **III.**

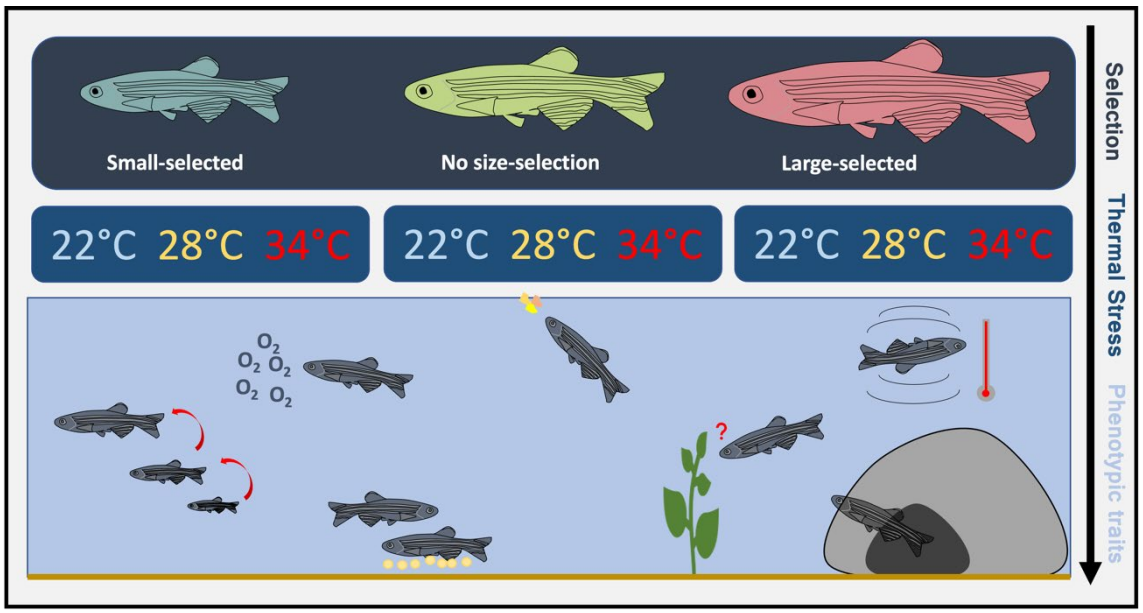

FIGURE 4 Overview of the thermal stress experiment lasting 250 days and covering a variety of phenotypic traits including (A) growth, (B) metabolic rate, (C) reproduction, (D) feeding behaviour, (E) exploration, (F) boldness, and (G) CT_{max}.

2.4.1 Growth rate

Fish were put under anesthesia (2-phenoloxyethanol, 1.5 % concentration), and standard length (SL) and wet mass (WM) of each fish were recorded weekly. Fish were individually identified by their VIE, and then photographed (against millimeter paper for scale) using a Canon EOS 90D DSLR Camera affixed with a Sigma 105 mm DG Macro HSM lens. ImageJ was used to measure the subsequent images to obtain SL (Schneider et al., 2012). An analytical balance was used to weigh fish to obtain WM (Mettler AE240). Weekly growth rate and specific growth rate were calculated. Specific growth rate was calculated as follows:

$$(\ln \text{final length (or weight)} - \ln \text{initial length (or weight)} / \text{days} \times 100)$$

2.4.2 Reproductive success

Fish were subsetted (n = 12 per selection line replicate per temperature treatment) and paired in 1 l breeding boxes attached to 3.5 l tanks (one male and one female). Spawning occurred for one week (seven days). At the end of the spawning period, eggs were collected and quantified fertilised, unfertilised, and dead eggs. Mean number of eggs per breeding fish pair were used to calculate fecundity. Using a microscope (Olympus SZ61 with a SC50 camera mount) SL of egg, egg yolk, and larvae was measured. Eggs were incubated at the same thermal stress treatment their parents experienced until larvae hatched.

2.4.3 Metabolic rate

A subset of fish (n = 10 per selection line replicate per temperature treatment) were taken to calculate metabolic rate. WM and SL were taken to adjust metabolic rate for mass. Fish were placed in acrylic cylindrical chambers for the intermittent-flow respirometer (Loligo® Systems, SY21020, Viborg, Denmark). The respirometer chambers were submerged in water and kept at the same temperature as the corresponding experimental temperature. Oxygen consumption was measured using the OXY-4 mini oxygen meter system and AutoResp-software (Loligo Systems, Viborg, Denmark). Mass-specific respiration rates ($mgO_2\,h^{-1}$) were obtained by dividing the individual respiration rates by individual mass (g WM). Standard metabolic rate (SMR) and maximum metabolic rate (MMR) were calculated based on the oxygen consumption. MMR was defined as the handling stress induced maximum metabolic rate which has been previously shown to be equivalent of true maximum metabolic rate of fish (Karjalainen et al., 1995). Absolute aerobic scope (AAS) was also calculated (MMR–SMR).

2.4.4 Behaviour

Exploration, boldness and feeding behaviour were measured in a subset of fish (n = 10 per selection line replicate per temperature treatment). Behavioural trials

were conducted in 30 l glass tank, which had been split into two distinct sections. One section was covered and acted as a refuge; in contrast, the other section contained stones, plastic plants and bright coloured tiles. Fish were placed in the refuge compartment for 10 minutes. The divider was then lifted which allowed the fish to explore the other section (a novel environment) for 20 minutes. Exploration was quantified as time spent exploring a new environment (Le Roy et al., 2021). Boldness was quantified as time taken to emerge (Krause et al., 1998). Feeding behaviour was recorded by adding flake food. Feeding behaviour was quantified based on the frequency at which individuals consumed food from surface of the water as well as the latency time for the fish to begin feeding. These behaviour proxies were filmed at two angles (above and in front of the tank) using a GoPro 7 Silver and a Canon EOS 90D. The behavioural data generated included: exploration time (s), number of emergences, time of first emergence (s), feeding frequency, probability to feed, and time of first feed (s).

2.4.5 Critical thermal maximum (CT_{max})

A subsample of fish (n = 6 per selection line replicate per temperature treatment) were placed in a 20 l glass tank attached to a Lauda E100 1.6Kw heater. Water temperature at the beginning of the experiment was the same as the corresponding rearing temperature. Water temperature was increased by 0.3°C min^{-1} (Åsheim et al., 2020). Individuals were removed after they had experienced loss of equilibrium for three seconds (Becker and Genoway, 1979). This temperature was recorded as CT_{max}. Thermal scope was also calculated (CT_{max} –rearing temperature).

2.4.6 Genomic markers of stress

At the end of the thermal experiment, all fish were euthanized with 2-phenoloxyethanol and stored at –20°C. Extracted DNA (described previously; Section 2.2) was then used for qPCR on a CFX96 thermal cycler (BioRad). Each reaction contained 20 ng DNA, 0.3 µM of each primer and 10 µl of iQ SYBR green supermix (BioRad). A negative control (the same standard DNA) and a serial dilution (1:2 from 80 ng/µl) to calculate qPCR efficiency was also included. Relative telomere length (RTL) was assessed by using standard vertebrate telomere primers (tel1, tel2; Cawthon et al., 2002) and the single copy gene (SCG; Moore and Whitmore, 2014) *c-fos*. rDNA copy number was measured using 18S rDNA primer (Tao et al., 2020) and the SCG. mtDNA copy number was calculated by comparing mtDNA against a nuclear target (Hunter et al., 2010). Full details of the qPCR primers (for each locus and for the SCG) are in **IV**. Relative copy number (RCN) or RTL were calculated per sample using:

$$\text{RCN or RTL} = E(\text{target})^{(Ct\ GS\ -\ Ct\ SAMPLE)} / E(\text{control})^{(Ct\ GS\ -\ Ct\ SAMPLE)}$$

Where E(target) and E(control) are the qPCR efficiencies of the target (i.e., telomere, rDNA, and mtDNA) and the single copy gene respectively. Ct^{GS} and

Ct^{SAMPLE} are the critical cycle thresholds for the golden standard and sample DNAs, respectively (Cawthon, 2002; Pfaffl, 2001).

2.4.7 Microbiota sampling

DNA extraction protocol and sequencing are described in section 2.2. Sequence data was processed using QIIME2 (Bolyen et al., 2019). Chimeras were removed using UCHIME (Edgar et al., 2011). Taxonomy was assigned using amplicon sequence variants (ASVs) on the SILVA v.132 database (Yilmaz et al., 2014). Low abundance and unassigned ASVs were removed as well as ASVs classified as mitochondria, chloroplasts, or Archaea. Low frequency ASVs (<10 reads) were removed. The resulting output was then loaded into R for further analysis (Section 2.5.3).

2.5 Statistical analysis

All statistics were performed using R 4.1.2 (R Core Team, 2022) within the CSC computing cluster.

2.5.1 Shifts in genomic architecture (I, II)

To visualise shifts in genomic architecture a principal component analysis was conducted, basing principal components on Cattell's graphical rule (Cattell, 1966) and broken stick method (Jackson, 1993). To quantify the differences in genomic architecture between small- and large-selected lines against the random-selected line the diffstat statistic was used (Turner et al., 2011). Outliers were detected using PCAdapt (Luu et al., 2017) and latent factor mixed model (LFMM; Frichot et al., 2013). The final set of outliers was required to be present in both outlier analyses for downstream analyses. Gene ontology enrichments were generated with the final set of outlier SNPs using Gene Ontology Enrichment analysis and Visualization tool (GOrilla; Eden et al., 2009) (**I**), and TopGO (Alexa and Rahnenfuhrer, 2023) (**II**).

2.5.2 Thermal stress experiment: life history, physiology, behaviour, and genomic markers of stress (III, IV)

Linear mixed models (LMM) and generalized linear mixed models (GLMM) were used to analyse the effect of thermal treatment and selection line on life history (growth and reproduction), physiological (SMR, MMR, AAS, CT_{max}), behavioural traits (boldness, exploration, and feeding behaviour) and genomic markers of stress (telomeres, rDNA, and mtDNA). Temperature treatment, selection line and their interaction were used as fixed effects. Selection line replicate and rearing tank were used as random effects in the model. For growth (weight and length) A log-log in the model was used to consider the non-linearity

of growth and measuring time as a fixed effect and the individual as a random effect. Analyses used the *lmer, glmer* and functions within the lme4 package (Bates et al., 2015) and *lmertest* within the lmerTest package (Kuznetsova et al., 2017). Post hoc pairwise comparisons of significant interactions were made using Tukey contrasts with *emmeans* function within the emmeans package (Lenth et al., 2018). Pearson's correlation was assessed between pairs of relative telomere length, rDNA copy number and mtDNA content within each treatment using *cor.test* within GGally (Schloerke et al., 2024).

Permutational multivariate analysis of variance (PERMANOVA) was performed to test for individual variation in multivariate phenotypic responses to treatments (temperature treatment and selection line). Pairwise Gower distances were calculated using *vegdist* within the vegan package (Oksanen et al., 2013) to take into account the differences in scale between variables. The matrices produced were used in PERMANOVAs run for 9999 permutations using *adonis2* within the vegan package Principal component analysis (PCA) was used to visualise the multivariate phenotypes.

2.5.3 Thermal stress experiment: microbiota (V)

Negative controls were used to remove potential decontaminants within the sample data using decontam (Davis et al., 2018) and read lengths below 200 were removed before import into phyloseq (McMurdie and Holmes, 2013). Alpha diversity was calculated using observed richness and Shannons index. Significant differences between alpha diversity were calculated using Kruskal-Wallis and Wilcoxons test. Beta diversity was estimated using BrayCurtis, Jaccards and Unifrac (weighted and unweighted). Differences amongst individuals were visualised using PCoA and significant differences calculated using permutation multivariate analysis of variance (PERMANOVA) in *adonis2* within the vegan package (Oksanen et al., 2013). Temperature and selection line were set as fixed factors and tank as a random factor in the PERMANOVA model. Beta dispersion and permutation test was used to determine significant differences in dispersion. To calculate differential expression amongst taxa ANCOM-BC2 (Lin and Peddada, 2024) and DESeq2 (Love et al., 2014) were used. Random forest analysis was used to assess predictive outcome of taxa structure and determine abundance of discriminative taxa. Finally, FEAST (Shenhav et al., 2019) was used to assess uptake of microbial community from the water column.

3 RESULTS AND DISCUSSION

3.1 Key findings

Genomic architecture shifted and genomic diversity decreased after overharvesting, diverging from the population prior to overharvesting, i.e. founder population (**I**). Surprisingly, although the genomic architecture differed between selection lines, the extent genomic diversity decreased did not. Through a period of recovery (cessation of harvesting), genomic architecture continued to diverge, and genomic diversity continued to decrease, suggesting no genomic stability or genomic recovery despite ten generations of no harvesting (**II**). When assessing whether adaptive potential eroded with size-selection directional selection (i.e., small- and large-selected) magnified the effect of an environmental stressor (thermal stress) compared to population loss alone (random-selection) on life history and physiological traits, but not behaviour (**III**). Moreover, a similar pattern was observed in genomic markers that are indicators of stress, as directional selection reduced rDNA copy number and relative telomere length. Whilst thermal stress increased mtDNA content regardless of selection pressure, acting as a stress marker for high temperature (**IV**). Finally, thermal stress caused a shift in microbial communities on the fish skin, but surprisingly, only had a mild effect. A mild effect may suggest that fish skin microbiota is relatively resilient, although thermal stress potentially promoted the colonisation of pathogenic bacteria (**V**).

Taken together, these five manuscripts investigate how a legacy of size-selective harvesting affects genomic architecture to influence susceptibility to thermal stress, altering a plethora of fitness components.

3.2 Overharvesting causes a shift in genomic architecture and loss of genetic diversity (I)

Size-selective harvesting led to substantial shifts in genomic architecture following directional selection (Fig. 5).

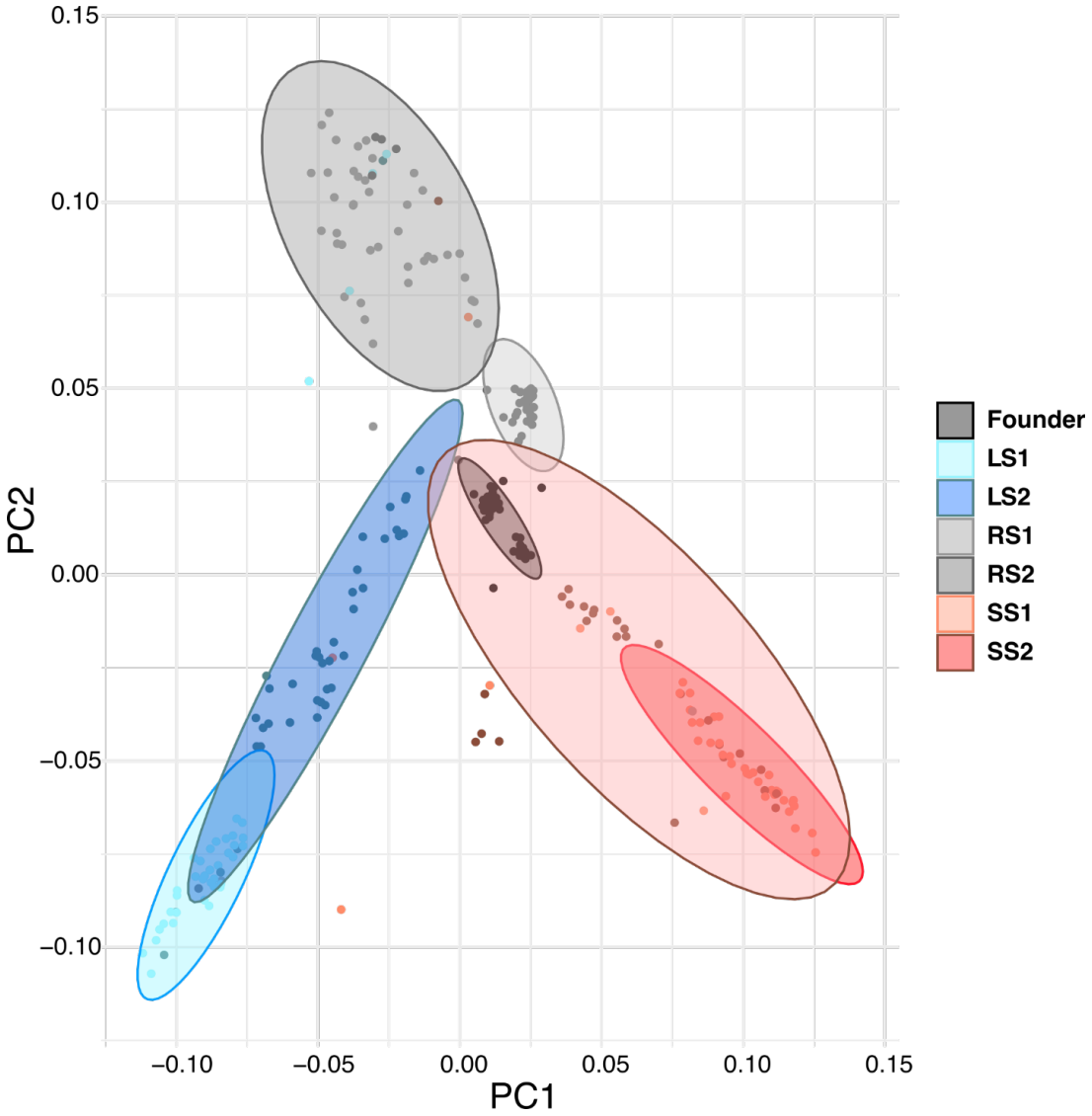

FIGURE 5 Principal component analysis based on random subset of one million SNPs amongst zebrafish models of size-selection. Selection lines include: (1) founder population, (2) large-selected replicates (LS1, LS2), (3) random-selected replicates (RS1, RS2), and (4) small-selected replicates (SS1, SS2). PC1 and PC2 explained 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. (I).

Moreover, genomic diversity decreased compared to the founder population, as expected following a severe bottleneck event (in this case 75 % harvesting rate) and evidenced in previous studies on overharvesting (see Pinsky

and Palumbi, 2014 for meta-analysis). Specifically, a reduction of nucleotide polymorphism (%) and effective population size was observed. Surprisingly, although genomic diversity declined in all selection lines compared to the founder population, it did not differ between selection lines. As zebrafish are a model organism with a high-resolution reference genome (Howe et al., 2013), high quality gene ontology enrichments were obtained. 212, 76 and 65 significantly enriched terms in small-, large-, and random-selected lines, respectively were observed. Within these, a large suite of gene ontology terms associated with the nervous system in large-selected fish, potentially corresponding with differences in behavioural traits across the lines previously shown (Uusi-Heikkilä et al., 2015; Sbragaglia et al., 2019). Overharvesting (population loss) and directional selection (small- and large-selected) drive a change in genomic architecture and subsequent loss of genomic diversity, leading to different gene ontogenies between the line replicates. Furthermore, there is stochasticity between the line replicates despite being exposed to the same selection pressure, making the effects of size-selective harvesting on genomic architecture unpredictable. Whilst genomic change and loss of genomic diversity is perhaps unsurprising after a 75 % population decline, it is important to highlight that divergence of fish experiencing directional selection (as in size-selective fisheries) from fish experiencing random selection is worrying as it suggests that genomic changes might occur in contemporary time scales also in exploited natural populations.

3.3 Cessation of harvesting does not prevent further population divergence (II)

Although the impacts of cessation of harvesting have been assessed at the phenotypic level (e.g., Conover et al., 2009), they have not been assessed at the genomic level. Genomic shifts after 10 generations of recovery were dependent on prior size-selective pressure. Small-selected fish showed signs of genomic divergence and reduced variation (Fig. 6a,b). Random-selected fish showed the greatest genomic differentiation from post harvesting to post recovery and evidence of genomic divergence (Fig. 6c,d). In contrast, in one replicate the large-selected fish remained stable in their genomic architecture (LS1: Fig. 6e) whilst the other replicate converged back towards the pre-harvest state in the other replicate (LS2; Fig. 6f). It therefore seems that change in genomic architecture after a period of recovery can be unpredictable. Moreover, genomic diversity continued to decline during recovery period in all selection line replicates except LS1, which showed stability in genomic architecture.

As with (**I**) the highly annotated zebrafish genome was used to obtain high quality gene ontologies. After a period of recovery, gene ontologies were shown to be associated with bone morphogenesis and cartilage development in the fish exposed to directional selection, but not in those exposed to random selection.

Interestingly, this could correspond with the differences in growth rate and adult body size between fish experiencing directional selection and random-selection (**III**).

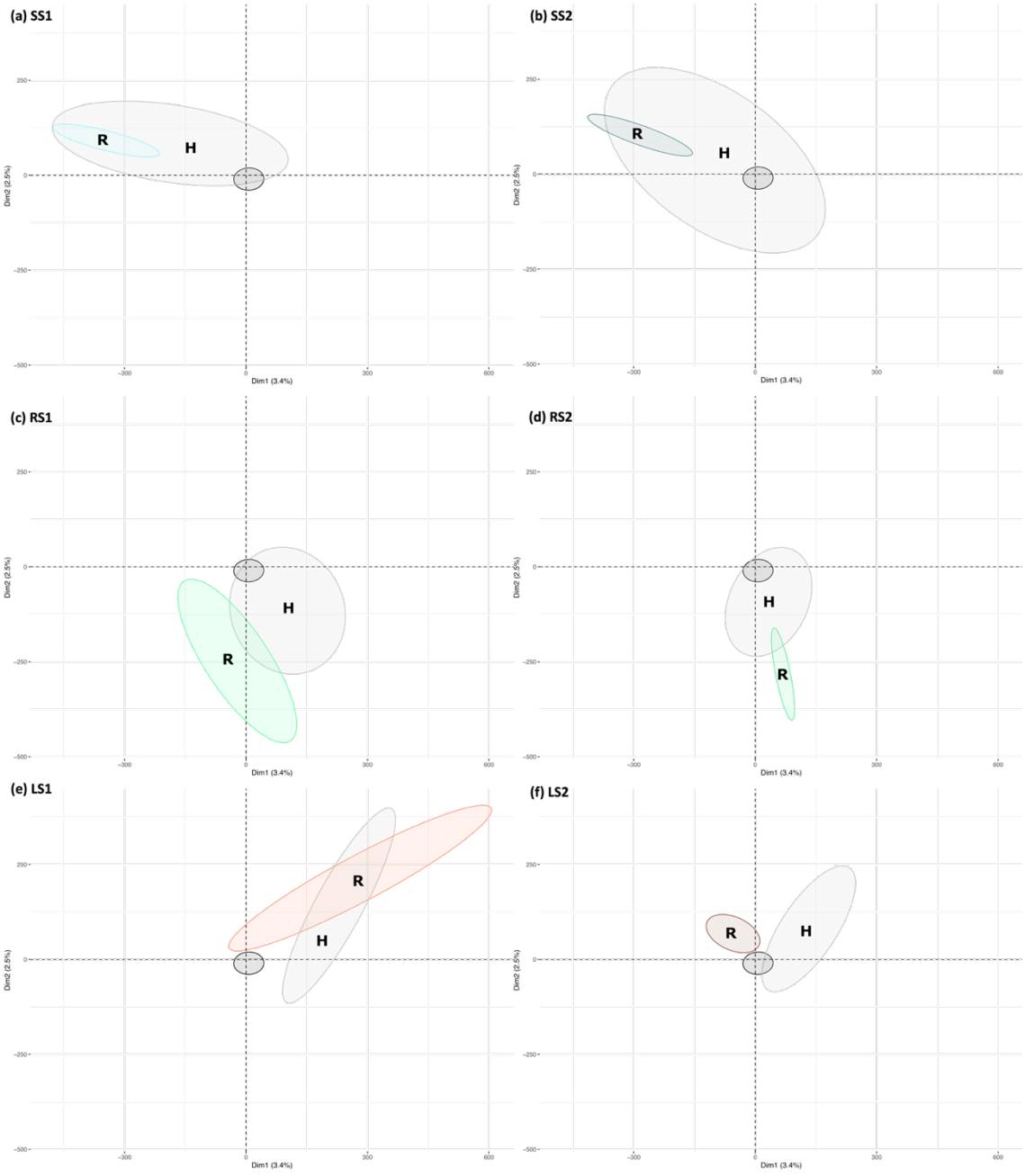

FIGURE 6 Principal component analysis of five generations of (size-selectively) harvested (H) zebrafish compared to recovered (R) individuals (after 10 generations of no harvesting) in small-selected (a, b), random-selected (c,d), and large-selected (e,f) replicates. PC1 and PC2 explained 3.4 and 2.5 % of the variation, respectively. Ellipses are 95 % confidence intervals around the mean and highlight selection-line replicates. (**II**).

That the selection-line replicates can differ, demonstrates the possibility of different evolutionary trajectories despite experiencing the same selective

pressure. It also demonstrates the importance of genetic redundancy as a mechanism to buffer against anthropogenic pressures (Barghi et al., 2019).

3.4 Thermal stress acts in tandem with size-selection (III)

Directional selection (i.e., large- and small-selected) exacerbated a population's vulnerability to extreme and rapid thermal stress. Random-selected fish exhibited a differential shift in multivariate phenotype (Pigliucci and Preston, 2004) compared to fish experiencing directional selection (Fig. 7). Moreover, growth rate is a crucial component of fitness (Ahti et al., 2021), and is indirectly selected for during overharvesting (Uusi-Heikkilä et al., 2015). Here, growth rate was higher in random-selected lines at low and ambient temperature, but not at high temperatures where all lines had an equally low growth rate. Contrary to expectations, fish did not follow the temperature size rule (Atkinson, 1994) as both large- and small-selected fish had similar phenotypic responses to suboptimal temperatures.

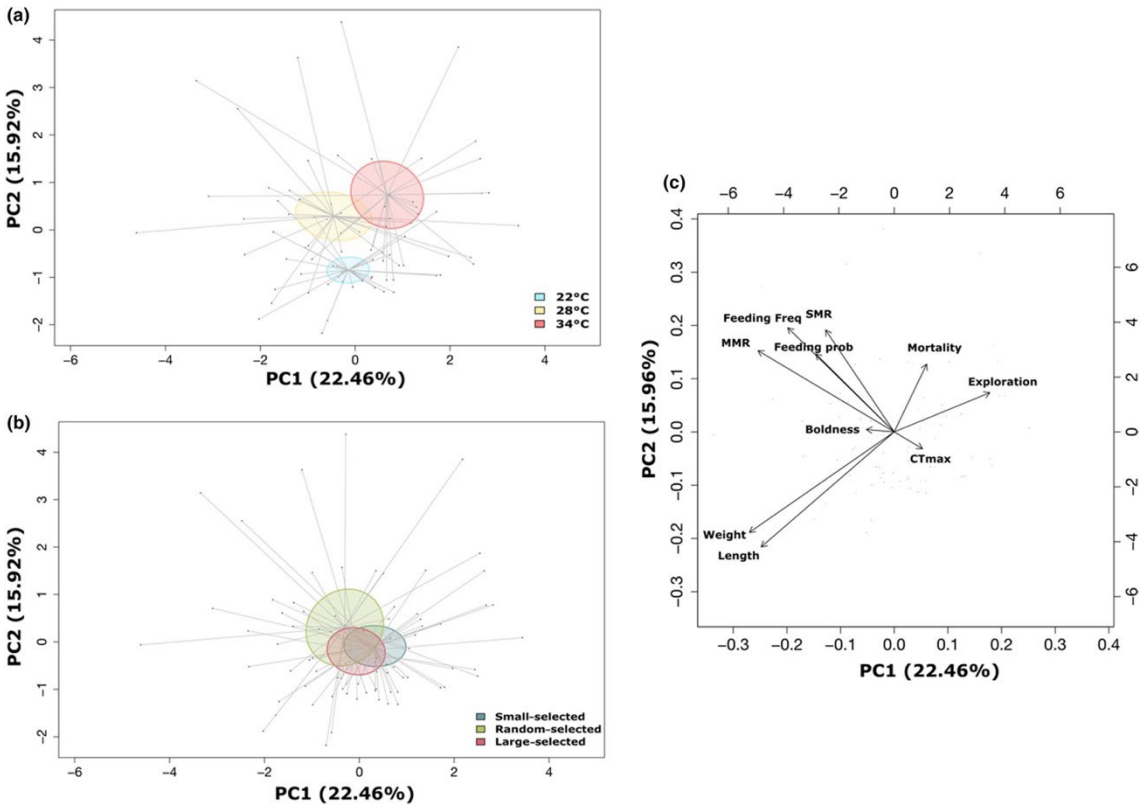

FIGURE 7 Principal Component Analysis of multiple measured traits of the zebrafish model of size-selection with 95 % confidence intervals across (a) selection lines and, (b) temperature treatments. Contributions to principal component space shown in biplot (c). Lengths of lines indicate distance of each individual from respective group centroid. (**III**).

Reproduction is another key component of fitness, although there was little difference between the selection lines in reproductive performance. Elevated temperature caused the cessation of any reproduction likely due to the extreme thermal stress on energetic requirements. Such energetic requirements can be quantified using metabolic rate. Here, there was a significant difference in metabolic rate, whereby metabolic rate was highest at ambient temperature and for random-selected lines. However, noticeably, metabolic rate was lower at elevated temperatures and the effect sizes were small, potentially evidence of metabolic acclimation occurring to the rearing temperature during the long-term experimental period (Sandblom et al., 2014; Pilakouta et al., 2020).

Behavioural response to thermal stress depended on the type of directional selection, small-selected fish were less bold, consistent with previous work on the same fish lines (Sbragaglia et al., 2019; Uusi-Heikkilä et al., 2015). Current results suggest that behavioural responses to altered temperatures act differently than other phenotypic responses and are more dependent on the direction of selection, potentially due to the high plasticity of behavioural traits (Mousseau and Roff, 1987; Duckworth, 2009).

Overall, random-selected fish (i.e., no directional selection for body size) had the highest phenotypic variability in response to thermal stress (Fig. 7). As such, directional selection may magnify loss of phenotypic diversity through selective sweeps and hitchhiking on covarying traits. Crucially, the direction of size-selection (small- or large-selection) appears less important than the act of directional selection alone.

3.5 Directional selection and thermal stress influence genomic markers of stress (IV)

Directional selection (both small- and large-selected lines) caused a reduction in relative telomere length and rDNA copy number, but not mtDNA content, compared with random-selection (absence of size-selection; Fig. 8). In contrast, mtDNA content was increased at elevated temperature, whilst thermal stress did not influence rDNA copy number or relative telomere length (Fig. 8).

Loss of genetic diversity (Therkildsen et al., 2010; Pinsky and Palumbi, 2014; Sadler et al., 2023) and potentially inbreeding (Hoarau et al., 2005; O'Leary et al., 2013) is expected in many overharvested fish stocks (see also **I**). Such directional selection may be driving a faster loss of genetic diversity compared to random selection (Frankham, 2012). Inbreeding is thought to affect telomere length (Bebbington et al., 2016; Pepke and Eisenberg, 2022; but see Olsson et al., 2022), as such, it may be a driving mechanism of short telomeres. However, differences in inbreeding coefficient in (**I**) or (**II**), meaning other factors may be more important such as differences in growth rate. Short relative telomere length was associated with directional selection on body size, which corresponds with directionally selected fish having lower growth rates and reaching a smaller adult body size than random-selected fish (**III**). It could therefore be speculated that fish under directional selection are less capable of telomere maintenance.

Ribosomal DNA copy number was also reduced in fish experiencing directional selection (Fig. 8). As with the telomeres, inbreeding/reductions in population size could drive differences in rDNA copy number (Veiko et al., 2007). Indeed, rDNA copy number and relative telomere length were positively correlated, suggesting these regions could be sensitive to similar stressors (Valeeva et al., 2023). rDNA is sensitive to environmental variation (Kobayashi, 2011; Paredes et al., 2011; Salim et al., 2017; Jernfors et al., 2021), it was therefore surprising to see no influence of thermal stress on rDNA copy number.

The lack of association between mtDNA content and selection may be expected in some cases, as mitochondria have a separate genome and mitochondrial mass is dynamic (e.g. independent of cell division; Ding et al., 2021). Mitochondrial DNA content was strongly associated with high temperature, potentially corresponding with an increase in mitochondrial content (Lee and Wei, 2000) and metabolic rate (Clarke and Fraser, 2004; Johansen and Jones, 2011). mtDNA content is also important as it associates with reactive oxygen species (Abele et al., 2002; Olsson et al., 2018; Metcalfe and Olsson, 2022) that can damage telomeres (von Zglinicki et al., 2002; Reichert and Stier 2017; Barnes et al., 2019) and rDNA (Kobayashi and Sasaki, 2017).

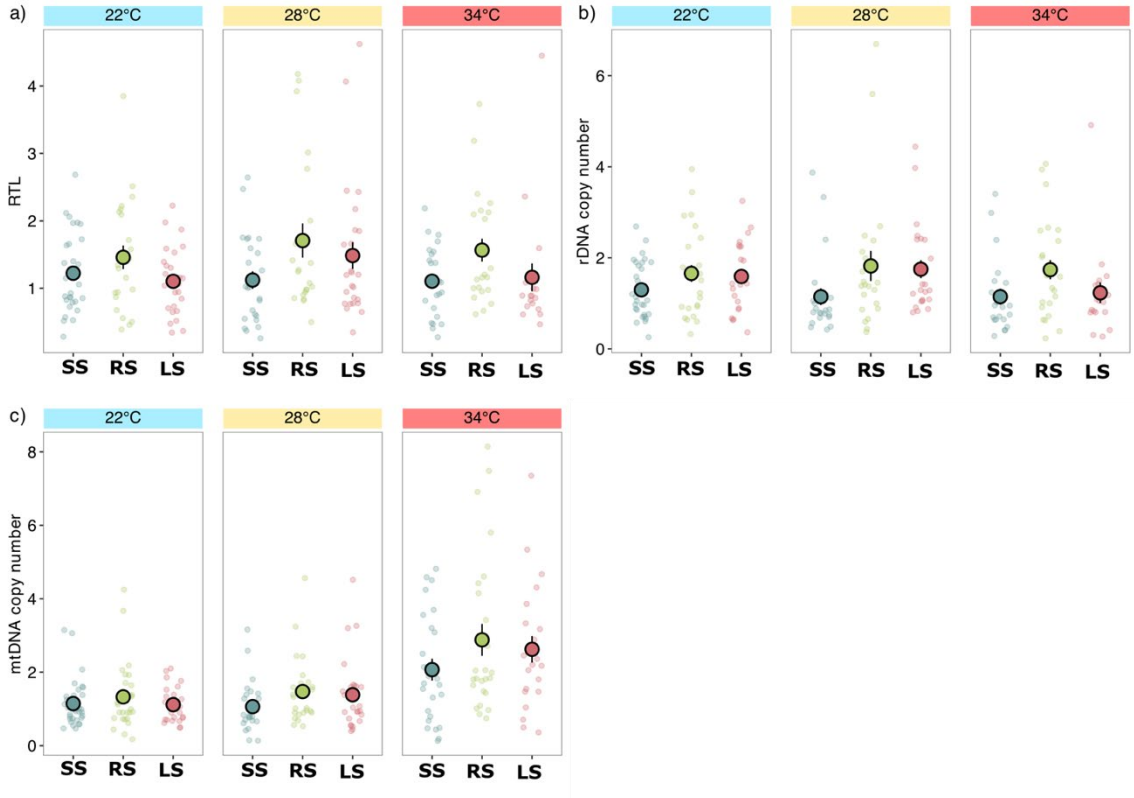

FIGURE 8 Variation in genomic markers of stress amongst size-selection lines: small-selected (SS), random-selected (RS), and large-selected (LS) zebrafish lines between the three temperature treatments (22°C, 28°C, and 34°C). (a) 18S rDNA copy number, (b) relative telomere length (RTL), and (c) mtDNA content. Data are shown as individual observations per fish (dots) and the mean with standard errors within each treatment combination. (**IV**).

Overall, it seems that directional selection for body size has a greater effect on rDNA and telomeres than population loss alone (random-selection). Whilst mtDNA content is driven by the increase of thermal stress. Crucially, rDNA copy number and telomere length follow a similar pattern to **III**, as regardless of direction (small- or large-selection), directional selection erodes fitness to a similar extent compared to random selection.

3.6 Thermal stress but not directional selection influence fish skin microbiota (V)

Thermal stress can disrupt the microbiota of aquatic species (Huyben et al., 2018; Ghosh et al., 2022), affecting composition and diversity of the host microbiota. Additionally, thermal stress may increase pathogenic taxa, potentially leading to a reduction in host fitness (Grice and Segre, 2011; Gomez and Primm, 2021). Here, an increase in thermal stress (cold and warm) caused a mild shift in beta diversity (Fig. 9), but not beta dispersion following other studies (Li et al., 2023). Indeed, the fact there was only a small effect size, and no change in alpha diversity or dispersion may be indicative of resilience to thermal stress, demonstrating the flexibility of the fish skin microbiota. However, thermal stress (cold and warm) increased the prevalence of pathogenic bacteria such as *Vibrio* and *Carnobacterium* which may lead to decreased fitness under future climatic scenarios.

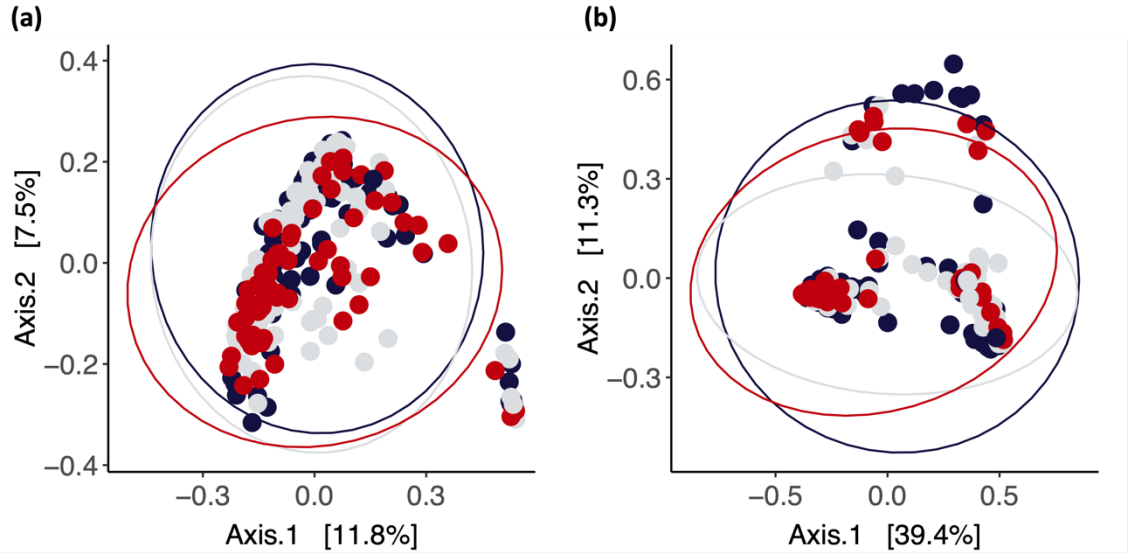

FIGURE 9 Variation in beta diversity of zebrafish between thermal treatments (22°C, 28°C and 34°C). (a) beta diversity using Jaccards distance and, (b) beta diversity using Bray-Curtis distance. Points represent individuals within each treatment, and ellipses represent 95 % confidence intervals. (**V**).

Interestingly, in contrast to Boutin et al., (2014) who found a strong effect of genotype on microbiota recruitment, here, there was no effect of selection line on

vulnerability to microbiota dysbiosis under thermal stress. As the skin microbiota is in constant contact with the surrounding environment, any environmental change likely has a much more overarching effect on microbiota composition than genomic differences among the selection lines (Woodhams et al., 2020). Though there was no differentiation in microbiota composition and diversity between lines, the lines are still different in their genomic architecture (**I, II**). Differences in genomic architecture may mean that immune response was different between the selection lines (although not measured), leading to increased/decreased susceptibility to disease from the uptake of opportunistic pathogens under thermal stress.

4 LIMITATIONS AND FUTURE DIRECTIONS

Although my data provide an insight into directional selection for body size and its interaction with thermal stress, there are still limitations to the extent I am able to draw broader conclusions, to better inform fisheries management and guide future research.

A key component that I do not include is a completely unfished line (i.e., no population decline at all) which would allow a baseline alongside population loss alone (random-selection) to compare the effects of size-selection. Previous evidence had showed that growth rate was decreased in a harvested vs unharvested line (Silliman, 1975), but most studies exclude such a baseline. It would therefore be prudent to assess how an unfished line compares with those that have experienced population loss and directional selection to understand the magnitude of change. Additionally, an unfished population would allow us to disentangle any effects of domestication caused by long term laboratory experiments. Such domestication effects could be caused by differences in laboratory conditions (i.e., a change in institute), though comparisons between the selection lines experiencing directional selection and the random selected (i.e., the control line) circumvents this issue to some extent.

As with Conover and Munch (2002), I was also only able to maintain two replicates per selection line, which gave some idea of stochasticity between lines experiencing the same treatment. However, it would be insightful to have more line replicates to see if this stochasticity is maintained, for example, if I could maintain ten lines per selection regime, would all line replicates show different evolutionary trajectories? Moreover, additional lines may disentangle any potential maladaptation effects caused by the laboratory set up and spawning regime. Such parallelism would be interesting to study, but likely only feasible in organisms such as *Drosophila*, *Daphnia* and yeasts that require much less laboratory space.

Here I used low-coverage whole genome sequencing as a tool to examine the effects of overharvesting and size-selective fishing (Therkildsen et al., 2019; Lou et al., 2021). However, low-coverage calling does have its limitations, for example, ability to call heterozygotes and to quantify inbreeding is reduced, and

there is a lack of ability to study structural rearrangement. That I did not find differences in genomic diversity between selection lines may be explained by the low-coverage in this instance. This could potentially be solved using a mix of long and short read genomic sequencing (e.g., Mérot et al., 2022) to get a higher resolution look into genomic architecture. Whole genome analyses remain underutilised in a fisheries context, with a large research gap, particularly in wild populations to assess the current and past state of genomic architecture of fish populations.

For the thermal stress experiment, I took DNA samples for telomeres, rDNA, mtDNA, and skin microbiota at the end of the experiment. However, if I had more time and resources, it would be interesting to examine the longitudinal change of these fitness components. Due to the difficulty using non-destructive sampling such as blood (e.g., Olsson et al., 2018), I was not able to determine starting telomere lengths and could not quantify rate of erosion to see whether all fish had similar length telomeres at hatching or whether telomere maintenance/repair differed longitudinally. Nevertheless, short telomeres are a known biomarker for stress, and likely associated with a reduction in fitness (Näslund et al., 2015; Bateson 2016; Wilbourn et al., 2018).

No other study has assessed the change in genomic architecture after a period of recovery following an overharvesting event. Though my results are insightful, they pave way for the next step, which would be to assess genomic recovery in wild populations, for example, a comparison between fishing grounds and a marine protected area. Additionally, I observe genomic changes in an isolated population with no influx of genetic material, whilst in a natural environment genomic recovery may be hastened through immigration and genetic rescue (Chevin et al., 2013; Whiteley et al., 2015). However, new genotypes from immigration would depend on the composition and reproductive success of the new individuals, meaning genomic recovery may still not occur even with high gene flow. Though a critical question, integrating immigration into a laboratory study of vertebrates remains a challenge.

My data provide new knowledge of the effects of directional selection on adaptive potential across many fitness components. Yet it is a mere starting point to understand how humankind are shaping the aquatic system and how future stressors will shape the aquatic world.

5 CONCLUSIONS

Overharvesting has the capacity to be destructive, physically decimating landscapes, and causing unprecedented population declines. Size-selection (i.e., directional selection) exacerbates phenotypic and genomic changes resulting from fisheries (Therkildsen et al., 2019; Uusi-Heikkilä et al., 2017, 2015). A strategy for coping with a fisheries crash is to halt harvesting and impose a moratorium. Here, I show that despite cessation of harvesting for 10 generations and subsequent phenotypic recovery (van Dijk et al., unpublished), a moratorium does not allow genomic recovery in the absence of new gene flow (i.e., immigration from refugia), which no other study has examined (**II**). As such, it is likely that differences in genomic architecture persist or indeed continue to differentiate after intensive harvesting events, which can lead to reductions in adaptive potential and subsequent vulnerability to other environmental stressors. Indeed, here after exposing the selection lines to a novel stressor (thermal stress), lines exposed to directional selection had lower fitness (in terms of body size and growth) (**III**) and altered genomic fitness markers (**IV**). Crucially, previous studies have focused on how overharvesting causes loss of diversity through bottlenecks and population loss, whilst here I show evidence that directional selection has a key impact. Additionally, direction of selection appears to be less important than the act of directional selection itself. Evidently the process of size-selection is detrimental, and I provide evidence that balanced harvesting (random-selected) may be a better strategy of fishing than the typical size-based fishing. Though these data show some worrying patterns, there is perhaps some hope in the microbiota, as it remains resilient to prior selective pressure, as well as thermal stress (**V**), and as microbiota can be directly related to fish fitness, this could be a beneficial buffer mechanism under future climatic scenarios.

Although I answer crucial questions related to state of our natural world, I generate even more questions and open new research avenues for future studies to pursue. Even though the aquatic world looks bleak in the face of the ravenous hunger of humankind, perhaps, in the light of evolution, and a little human intervention, some hope can be maintained for the state of future fish populations.

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ORIGINAL PAPERS

I

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by

Daniel Sadler, Tiina Sävilammi, Stephan van Dijk, Phillip C. Watts &
Silva Uusi-Heikkilä

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II

POPULATION GENOMICS OF AN OVERHARVESTED POPULATION AFTER A PERIOD OF RECOVERY

by

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III

DOES SIZE-SELECTIVE HARVESTING ERODE ADAPTIVE POTENTIAL TO THERMAL STRESS?

by

Daniel Sadler, Stephan van Dijk, Juha Karjalainen, Phillip C. Watts &
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RESEARCH ARTICLE

Does size-selective harvesting erode adaptive potential to thermal stress?

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Abstract

Overharvesting is a serious threat to many fish populations. High mortality and directional selection on body size can cause evolutionary change in exploited populations via selection for a specific phenotype and a potential reduction in phenotypic diversity. Whether the loss of phenotypic diversity that accompanies directional selection impairs response to environmental stress is not known. To address this question, we exposed three zebrafish selection lines to thermal stress. Two lines had experienced directional selection for (1) large and (2) small body size, and one was (3) subject to random removal of individuals with respect to body size (i.e. line with no directional selection). Selection lines were exposed to three temperatures (elevated, 34°C; ambient, 28°C; low, 22°C) to determine the response to an environmental stressor (thermal stress). We assessed differences among selection lines in their life history (growth and reproduction), physiological traits (metabolic rate and critical thermal max) and behaviour (activity and feeding behaviour) when reared at different temperatures. Lines experiencing directional selection (i.e. size selected) showed reduced growth rate and a shift in average phenotype in response to lower or elevated thermal stress compared with fish from the random-selected line. Our data indicate that populations exposed to directional selection can have a more limited capacity to respond to thermal stress compared with fish that experience a comparable reduction in population size (but without directional selection). Future studies should aim to understand the impacts of environmental stressors on natural fish stocks.

KEYWORDS
adaptive potential, fisheries, phenotypic diversity, size selection, thermal stress

TAXONOMY CLASSIFICATION
Evolutionary ecology

1 | INTRODUCTION

Overharvesting can lead to rapid population declines (McCauley et al., 2015) causing a loss of genetic (Marty et al., 2015; Pinsky & Palumbi, 2014; Sadler et al., 2023; Therkildsen et al., 2019) and phenotypic variation (Olsen et al., 2009; Palumbi et al., 2019). A key aspect in many fisheries is the size selective removal of the largest individuals from the population (Jørgensen et al., 2007; Law, 2007; Lewin et al., 2006). Such directional selection on body size can drive evolutionary change towards specific phenotypic traits (Conover & Munch, 2002; Uusi-Heikkilä et al., 2015), such as faster growth rate, earlier age at maturation and altered behaviour (Mollet et al., 2007; Olsen et al., 2004; Reid et al., 2023; Therkildsen et al., 2019; Uusi-Heikkilä et al., 2015, 2017; van Wijk et al., 2013). A key question is whether altered phenotypic variation caused by directional selection and/or population decline decreases the resilience of species to changing environmental conditions (Morrongiello et al., 2021; Pörtner & Peck, 2010).

Population decline alone is likely to increase susceptibility to future stressors through random loss of adaptive alleles (Petrou et al., 2021; Thompson et al., 2019) and a reduction in phenotypic diversity (Anderson et al., 2008; Morrongiello et al., 2019). Many fisheries not only reduce population sizes substantially but also expose the targeted populations to size selection (i.e. directional selection), which can magnify the loss of genetic variation (Frankham, 2012), as favouring a specific phenotype can cause a concomitant directional shift in allele frequency (Quinn et al., 2007; but see Pinsky et al., 2021). While the effects of fisheries-induced directional selection on phenotypic variation remain relatively understudied (but see Olsen et al., 2009; Palumbi et al., 2019), it is crucial to understand what consequences the additive effect of population decline and directional selection has on the average phenotype in a changing environment. Thus, not only do fish stocks have to cope with a loss of phenotypic diversity caused by reductions in population size but they may also be further limited by the reductions in phenotypic diversity resulting from directional selection (Groth et al., 2018; Marty et al., 2015; Therkildsen et al., 2019). It is unknown whether this loss of phenotypic diversity from directional selection is important, and how this could influence resilience to environmental stressors.

Exploited populations experience diverse stressors alongside fishery impacts (Planque et al., 2010; Wootton et al., 2021). An important stress experienced in many natural populations is temperature change (Mittelbach et al., 2007; Parmesan et al., 2003). Environmental temperature will likely rise beyond the tolerable limits of many fish species due to climate change and associated extreme weather events (Hollowed et al., 2013; Perry et al., 2010). Indeed, extreme weather events can lead to sea surface temperature increases of 2–4°C, and sometimes >5°C (Sen Gupta et al., 2020). Thermal stress can negatively affect fish populations through, for example, changes in life-history traits including growth and reproduction (Pörtner & Farrell, 2008).

The temperature size rule (TSR) describes the growth response of ectotherms to temperature, with higher temperature predicted to select for fast growth and small adult body size (Atkinson, 1994; Cheung et al., 2013). Therefore, size-selective harvesting and increased water temperature may interact and favour small body size (Audzijonyte et al., 2016). This possible combined effect of directional selection for body size and thermal stress may further negatively affect population growth and recruitment, and associated phenotypes (Planque et al., 2010; Rouyer et al., 2012; Wootton et al., 2021). It is important to understand how populations exposed to size-selective fisheries cope with thermal stress and whether fisheries-induced selection interacts with thermal stress to accelerate the effects of directional selection.

We studied experimentally how zebrafish (*Danio rerio*) that had experienced three size selection regimes responded to thermal stress. Size-selection lines consisted of (1) small-selected fish (a treatment that mimics the selection pattern typical to fisheries), (2) large-selected fish (to understand the full range of responses caused by size selection) and (3) random-selected fish (no size selection). We exposed fish from each selection line for 250 days to three temperatures—low (22°C), ambient (28°C) and elevated (34°C)—to determine if (1) directional selection impacted the response to thermal stress and whether (2) selection for a distinct (i.e. small or large) body size interacted with the response to a change in temperature. To provide a multivariate assessment of response to thermal stress, we monitored the growth, reproductive success, metabolic rate, behaviour and critical thermal maximum (CT_{max}) of zebrafish. We hypothesised that (1) size-selected fish (small- and large-selected) would perform worse than random-selected fish under thermal stress due to, for example, the potentially stronger loss of (genetic and phenotypic) diversity that occurs when there is directional selection compared with a reduction in population size, or, alternatively, that (2) size selection may interact with temperature, favouring small body size. The latter would lead us to hypothesise that small-selected fish would perform better in high temperatures than large- and/or random-selected fish following the TSR. We found that directional selection on body size (whether for large or small body size) magnified the negative consequences of thermal stress in terms of growth compared with a comparable population reduction but no directional selection (random-selected fish). However, we found no evidence of an interaction between size selection and TSR.

2 | MATERIALS AND METHODS

2.1 | Study design

Selection lines were created using wild-caught zebrafish (*Danio rerio*), from the West Bengal region of India (Uusi-Heikkilä et al., 2010). In this experiment, we used fish from the three previously established selection lines (with two replicates each) that had been subject to 75% fishing mortality rate (Uusi-Heikkilä et al., 2015): (1) small-selected (where 25% of the smallest fish were kept in spawning stocks), (2) large-selected (where 25% of the largest fish were kept in spawning stocks) and (3) random-selected

(where 25% randomly chosen fish were kept in spawning stocks). In each generation, all fish in a spawning stock (i.e. 25% of the population) were allowed to spawn (using multiple spawning boxes that each contained two females and four males). Harvesting continued for five generations, after which the selection lines phenotypically and genetically differed (Uusi-Heikkilä et al., 2015, 2017). Next, the selection lines were allowed to recover (i.e. no harvesting) for 10 generations. We used these lines in the current experiment, as they exhibited persistent genetic differences (Uusi-Heikkilä et al., 2017) and behavioural differences (Roy & Arlinghaus, 2022; Sbragaglia et al., 2019).

Prior to the thermal stress experiment, adult zebrafish were kept at 28°C with a 14:10 light cycle and fed ad libitum with a mixture of dry food (TetraMin XL) and live *Artemia salina*. During the experiment, fish were fed dry food ad libitum twice daily. Fish from each selection line replicate were exposed to three different temperature regimes at age 50 days post fertilisation (dpf): low (22°C), ambient (28°C) and elevated (34°C) for 250 days. To avoid high mortality at early (<50 dpf) life stages, we focused on later life stage effects. Ambient temperature is the control temperature, as it has been the standard rearing temperature in the laboratory for 15 generations as well as representing the natural environment of zebrafish (Sundin et al., 2019). Elevated and low temperatures were chosen as ±6°C from the ambient temperature, representing thermal stress on zebrafish physiology (Åsheim et al., 2020; Morgan et al., 2022) with +6°C also representing a potential temperature rise in extreme weather events (Sen Gupta et al., 2020).

Fish were tagged with visible implant tags (VIE; Northwest Marine Technologies, 162 Shaw Island, WA, USA) so that we could measure individual-level data for all fish. We picked 360 individuals randomly from the selection line replicates for the experiment ($n = 20$ per selection line replicate, per temperature treatment). For each temperature treatment, we had three replicate 30 L glass tanks. In each tank, there were eight cylindrical wire mesh cages and, in each cage, we placed five zebrafish randomly distributed accorded to line (i.e. all individuals from the same line were not in the same tank, preventing confounding effects). Individuals were used as the unit of replication. Experimental fish were acclimated for 2 weeks at 28°C. Temperature was altered by ±1°C day^{-1} until it reached either 22°C or 34°C in the low and elevated temperature treatments, respectively.

2.2 | Growth rate

Standard length (SL) and wet mass (WM) of 360 fish ($n = 20$ per selection line replicate per temperature treatment) were recorded weekly by placing individuals under anaesthesia (2-phenoloxyethanol, 1.5% concentration). Fish were identified using a UV light on the VIE, and photographed (against millimetre paper for scale) using a Canon EOS 90D DSLR Camera affixed with a Sigma 105 mm DG Macro HSM lens. Images were measured using ImageJ (Schneider et al., 2012). WM was measured using an analytical balance (Mettler AE240). After approximately 100 days, the growth rate began to plateau and length/weight measurements were taken biweekly. Alongside weekly growth rate, specific growth rate was calculated for mass and length (ln final length (or weight) − ln initial length (or weight)/days × 100).

2.3 | Reproductive success

Fish ($n = 12$ per selection line replicate per temperature treatment) were placed in pairs (one female, one male) in 1 L breeding boxes attached to 3.5 L tanks on a Techniplast housing rack and allowed to spawn for 7 days. Eggs were collected and counted for the number of fertilised, unfertilised and dead eggs. Fecundity was determined as an average number of fertilised eggs per breeding couple across the 7-day spawning trial. We measured egg and egg yolk diameter of the fertilised eggs using a microscope (Olympus sz61 with a sc50 attachment) and then placed the eggs in a 24-well plate incubated at the corresponding treatment temperature using the same tank water. Eggs were then checked once per day to monitor mortality rate and age at hatching (d). When larvae hatched, we measured the larval SL using the same microscope as with measuring the eggs.

2.4 | Metabolic rate

We kept individuals ($n = 10$ per selection line replicate per temperature treatment) without food for 24 h before taking body mass and SL to allow calculation of mass-specific metabolic rates. Oxygen consumption of four fish was measured per time interval individually in four replicate acrylic cylinders (volume 108 cm^3) of the intermittent-flow respirometer (Loligo® Systems, SY21020, Viborg, Denmark). The system was submerged in a heated water bath regulated with an E100 1.6 Kw heater, set according to treatment temperature, and placed in a climate regulated room in complete darkness (with fish unable to see each other during the experiment). Oxygen was measured using a fibre-optic sensor on the OXY-4 mini oxygen meter system and AutoResp software (Loligo Systems, Viborg, Denmark). Measurements were taken using an intermittent flow system every 25 min (including 220 s flush, 230 s delay and 1050 s closed measuring periods). Duration of measurement period for each set of four fish was 8 h. After each treatment, the system was cleaned with bleach and water replenished. Background bacterial oxygen consumption was measured before and after the experimental period by measuring the empty chamber without fish and subtracted from the oxygen consumption values of the fish. AutoResp calculated the decrease in oxygen consumption during closed periods, converted them automatically to respiration rates (mgO_2h^{-1}) per fish during each closed period and mass-specific rates were obtained by dividing the respiration rates by fish mass (g WM). Standard

metabolic rate (mass-specific $mgO_2g^{-1}h^{-1}$; SMR) was calculated using the average of the three lowest respiration rates while maximum metabolic rate (mass-specific $mgO_2^{-1}gh^{-1}$; MMR) used the average of the three highest respiration rates. MMR in the intermittent respirometer was the handling stress induced maximum metabolic rate which has been observed to be near the maximum metabolic rate of fish in the swimming respirometer (Karjalainen et al., 1995). Absolute aerobic scope (mass-specific $mgO_2g^{-1}h^{-1}$; AAS) was calculated as the difference between SMR and MMR to estimate the fish's ability to increase metabolic rate above maintenance level.

2.5 | Behaviour

Exploration, boldness and feeding behaviour were measured ($n = 10$ per selection line replicate per temperature treatment). Fish were kept without food for 24 h before the trials. Behavioural trials occurred in a glass tank (30 L) divided into two sections by an opaque plastic sheet. One compartment was darkened and acted as a refuge, while the other compartment contained stones and a novel object (coloured tiles) and thus acted as an area for exploration (Figure S1). Fish were acclimated in the refuge area for 10 min before the divider was removed and then fish were allowed to explore the novel environment for 20 min. Exploration and boldness were measured as time exploring a novel environment (Le Roy et al., 2021) and the latency of emergence from a refuge (Krause et al., 1998), respectively. Feeding behaviour was recorded for 5 min by adding approximately 6 mg of flake food (TetraMin XL) and measuring the frequency fish took food from the water surface and the time taken to start feeding. After each trial, excess food was removed from the tank. Behaviour was filmed throughout the duration with an overhead view and a side view using a GoPro 7 Silver and a Canon EOS 90D. All trials were performed within an isolated, temperature-controlled room to prevent disturbance. Behaviour videos were viewed at 1.5x speed to measure exploration time (s), number of emergences, latency of emergence (s), feeding frequency (number of feeding events), probability to feed and latency to feed (s).

2.6 | Critical thermal maximum (CT_{max})

Six fish per selection line replicate per temperature treatment were simultaneously placed in a 20 L thermal tank with a Lauda E100 1.6Kw heater separated with a mesh divider. The starting temperature corresponded to the experimental rearing temperature, and the water was heated at 0.3°C min^{-1} (Åsheim et al., 2020). Fish were observed until they experienced loss of equilibrium for 3 s (Becker & Genoway, 1979). Temperature of fainting (CT_{max}) was then recorded, and the fish were immediately euthanized using an overdose of 2-phenoloxyethanol. Thermal scope was calculated by subtracting the rearing temperature from the CT_{max}.

2.7 | Statistical analysis

2.7.1 | Univariate

All statistics were performed using R v.4.1.2 (R Core Team, 2022) within RStudio (Posit team, 2022). We used linear mixed models (LMMs) and generalised linear mixed models (GLMM) to analyse the effect of temperature treatment and selection line on life history (growth and reproduction), physiological (SMR, MMR, AAS and CT_{max}) and behavioural traits (boldness, exploration and feeding behaviour) using temperature, selection line and their interaction as fixed effects. Individual fish were used as unit of replication in the growth rate analysis. Selection line replicate and rearing cage (and spawning tank for reproductive measures) were set as random effects (Trait ~ Temperature * Selection line + (1|cage) + (1|selection line replicate)). For growth (weight and length), we utilised log–log in the model to consider the non-linearity of growth and used time as a fixed effect and the individual as a random effect (log(Size) ~ log(Week) * Temperature * Selection line + (Week|Cage) + (1|selection line replicate) + (1|individual)). If a model was in singularity and/or did not converge, we removed the corresponding random factor from the model preventing overfitting of the model after confirming no significant effect on the result. Analyses used *lmer* and *glmer* within the lme4 package (Bates et al., 2015) and the *lmertest* function within the lmerTest package (Kuznetsova et al., 2017). For data that did not fit the assumptions of the LMM, we used GLMM (details of the models in Tables S1 and S2). Post hoc pairwise comparisons of significant interactions were made using Tukey contrasts with *emmeans* function within the emmeans package (Length, 2023). Coefficients of variance (CVs) were calculated to assess differences in trait variability. CVs were bootstrapped (10,000 times) and compared using modified signed-likelihood ratio tests within the cvequality package (Marwick & Krishnamoorthy, 2019).

2.7.2 | Multivariate

Permutational multivariate analysis of variance (PERMANOVA) was used to test for individual variation in multivariate phenotypic responses to treatments (temperature and selection line). Pairwise Gower distances were calculated using *vegdist* within the vegan package (Oksanen et al., 2013) to account for differences in scale between variables (Gower, 1971). The matrices produced were used in PERMANOVAs run (9999 permutations) using *adonis2*. Principal component analysis (PCA) was used to visualise multivariate phenotypes.

3 | RESULTS

3.1 | Growth rate

Weekly growth rate varied significantly among selection lines and temperatures in both SL ($F_{2,265} = 4.80$, $p < .01$, Table S1, Figure 1)

and WM ($F_{2,263}=11.38$, $p<.001$, Table S1, Figure S2). Specific growth rate and weekly growth rate were greater at 22°C and 28°C compared to the elevated 34°C temperature treatment (Figure 1, Figure S3, Table S1). Random-selected fish had a faster growth rate than fish experiencing directional selection (small- and large-selected fish) at 22°C and 28°C but not at 34°C, when all lines had comparable growth rates (Figure 1, Figure S3, Table S1). Both lines experiencing directional selection had similar growth rates across all temperatures (Figure 1, Figure S3, Table S1).

3.2 | Reproductive success

Fish housed at 34°C did not spawn regardless of the selection line. At 22°C and 28°C, fish did not significantly differ in fecundity among temperature treatments or selection lines (Figure 2a, Table S1).

Eggs produced by females exposed to the low temperature treatment (22°C) were significantly larger ($F_{1,666}=271.48$, $p<.001$, Table S1, Figure 2b) than eggs produced at ambient temperature (28°C). Selection line also influenced egg size within both temperature treatments, with the random-selected line having the smallest egg size at 22°C but the largest egg size at 28°C ($F_{2,666}=54.19$, $p<.001$, Table S1, Figure 2b).

Larvae took a significantly longer time ($t=0.01$, $p<.001$, Figure 2c, Table S2), by an average of 4 days, to hatch at 22°C than at ambient temperature (28°C). At 28°C, there were no differences in larval age at hatch among selection lines, but at 22°C, larvae from small-selected lines hatched significantly earlier than larvae from random- and large-selected lines ($t=0.015$, $p<.001$, Figure 2c, Table S2).

Larvae hatched at 22°C were significantly larger than larvae hatched at 28°C ($F_{1,442}=19.57$, $p<.001$, Figure 2d, Table S1). However, larval size at hatch did not significantly differ among selection lines (Table S1, Figure 2d).

3.3 | Metabolic rate

There was no significant interaction between temperature and selection line in SMR, MMR or AAS. SMR was the highest at the ambient (28°C) temperature ($F_{2,166}=4.41$, $p<.05$; Table S1, Figure 3a). Random-selected lines showed a higher SMR than the lines experiencing directional selection: large-selected (emmean contrast: −0.056, $p<.05$) and small-selected (emmean contrast: 0.060, $p<.01$), corresponding with higher growth rate at low and ambient temperature ($F_{2,166}=5.36$, $p<.01$; Table S1, Figure 3a). MMR differed between lines ($F_{2,166}=2.76$, $p<.05$; Table S1, Figure 3b), with random-selected having a significantly higher MMR than small-selected (emmean contrast: −0.095, $p<.05$), but unlike SMR, it did not significantly differ across the three temperatures (Table S2, Figure 3b). AAS did not differ between lines or temperature (Table S2, Figure 3c).

3.4 | Behaviour

Probability to feed differed among temperatures and selection lines, with feeding most likely to occur at ambient (28°C) and least likely at the low (22°C) temperature ($z=-1.10$, $p<.05$, Figure 4a, Table S2). Feeding latency differed significantly with temperature but not among selection lines ($F_{2,160}=1.56$, $p<.05$, Figure 4b, Table S1), with

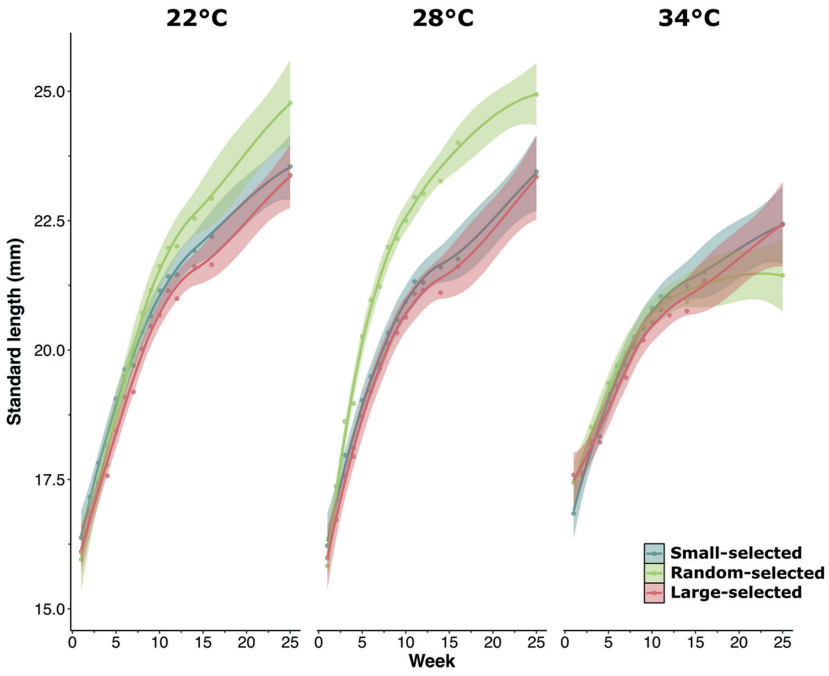

FIGURE 1 Differences in growth (standard length; mm) among zebrafish selection lines (large-selected, random-selected and small-selected; selection-line replicates combined) in the three temperature treatments: 22°C, 28°C and 34°C. Mean points and error around the lines represent standard error across housing cages within each treatment combination.

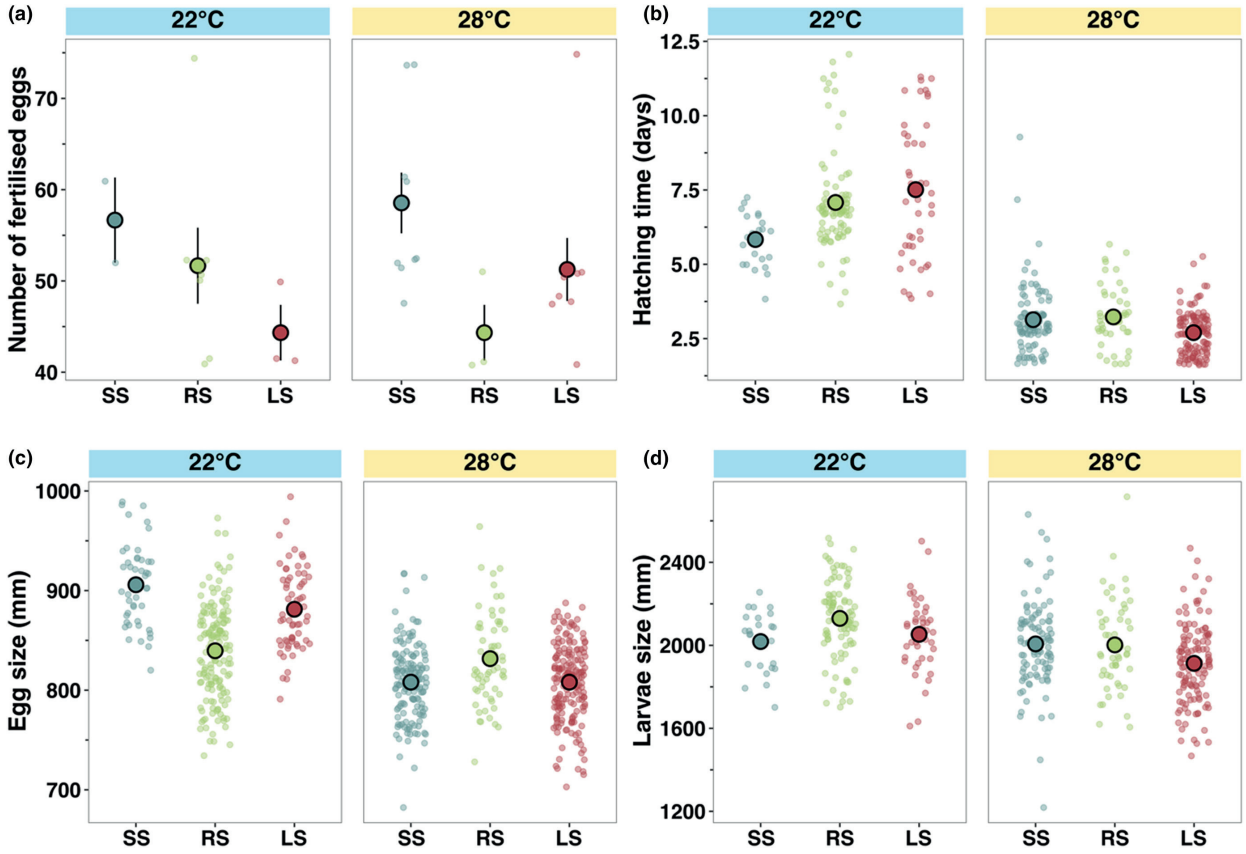

FIGURE 2 Differences in reproductive success among zebrafish selection lines (large-selected (LS), random-selected (RS) and small-selected (SS); selection-line replicates combined) in three temperature treatments. (a) The average number of fertilised eggs per female per spawning day, (b) larval age at hatch (days), (c) egg size (mm) and (d) larval size at hatch (mm). Data are shown as individual observations per fish (dots) and the mean with standard errors across housing cages within each treatment combination.

fish at the lowest temperature taking longer to feed than at 28°C and 34°C (emmean contrast: 74.7, $p < .05$). Number of feeding events was significantly different between temperatures ($F_{2,160} = 6.11$, $p < .05$, Figure 4c, Table S1), with the highest being at ambient temperature and the lowest at 22°C regardless of the selection line (emmean contrast: −5.34, $p < .001$), since, at 22°C, many individuals did not feed (Figure 4c, Table S1).

The number of times fish emerged from the shelter was most frequent at 34°C ($z = 2.01$, $p < .05$, Figure 5a, Table S2). Selection line had a significant effect as random-selected fish emerged most frequently at 22°C ($z = 2.46$, $p < .05$, Figure 5a, Table S2). The latency time to emerge from the refuge (a proxy for boldness) was the highest at the low temperature ($z = 2.39$, $p < .05$, Figure 5b, Table S2), and the boldest individuals were from random and large-selected lines, with the small-selected lines taking the longest to emerge ($z = 2.39$, $p < .05$, Figure 5b, Table S2). Fish from the random-selected and large-selected lines were less explorative than fish from the small-selected line at 28°C (RS; $z = 4.80$, $p < .001$, LS; $z = 2.303$, $p < .05$, Figure 5c, Table S2), but selection lines did not differ in explorative behaviour at the elevated or lower temperature (Figure 5c, Table S1).

3.5 | Thermal tolerance

CT_{max} followed a typical temperature-dependent relationship, whereby CT_{max} increased with the rearing temperature ($F_{2,99} = 359.40$, $p < .001$; Figure 6a, Table S1). However, selection line had no significant effect on CT_{max} (Figure 6a, Table S1). Thermal scope (rearing temperature − CT_{max}) decreased as temperature increased, suggesting fish at 34°C were close to their thermal limit ($F_{2,99} = 534.60$, $p < .001$; Figure 6b, Table S1). In contrast to CT_{max}, there was a significant interaction between temperature treatment and selection line ($F_{2,99} = 2.84$, $p < .05$; Figure 6b, Table S1), whereby small-selected fish had the lowest thermal scope at 28°C (emmean contrast: −5.41, $p < .001$) while the random-selected line had the lowest thermal scope at 34°C (emmean contrast: −2.52, $p < .001$).

3.6 | Mortality of experimental fish

Mortality increased as temperature increased, with the lowest number of mortalities at 22°C and the highest at 34°C ($z = -2.723$, $p < .01$, Table S2).

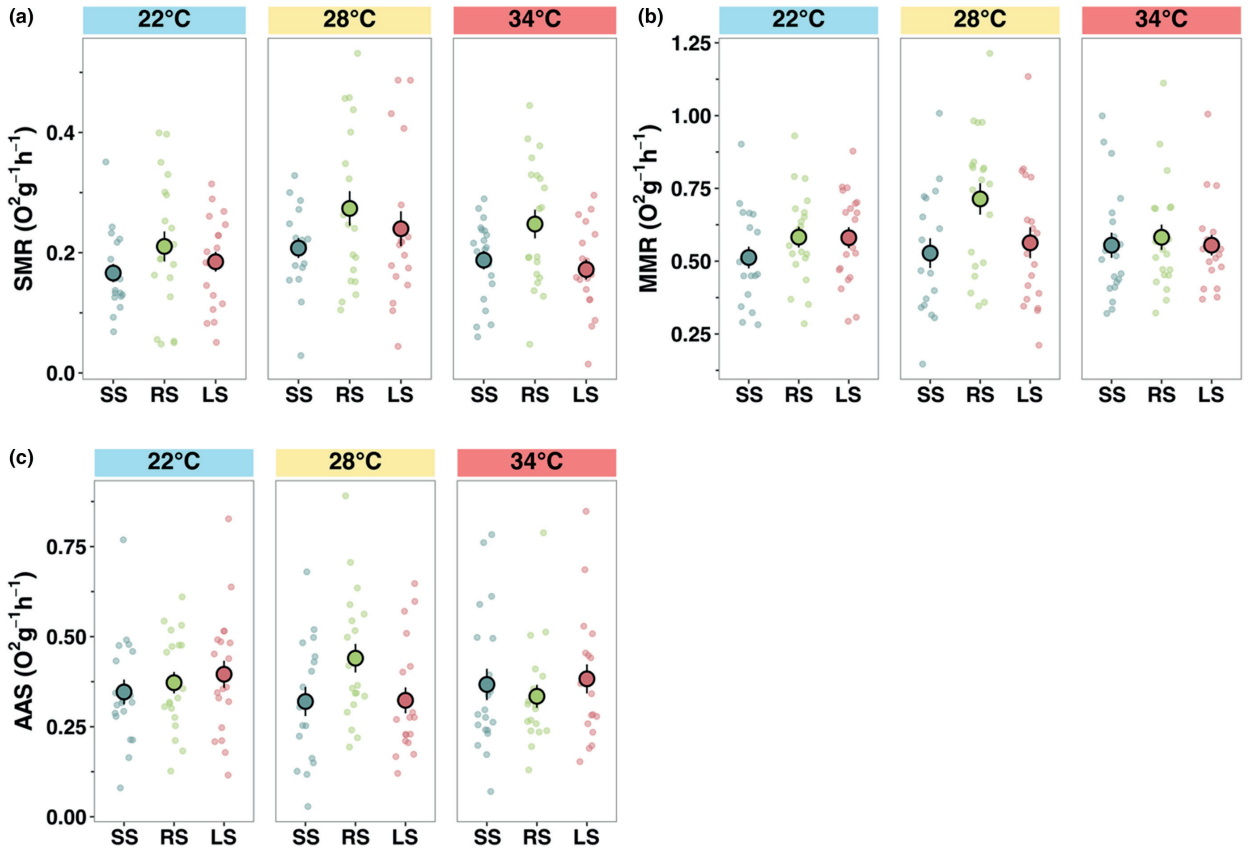

FIGURE 3 Differences in metabolic function among zebrafish selection lines (large-selected (LS), random-selected, (RS) and small-selected (SS); selection-line replicates combined) in three temperature treatments. (a) Mass-specific standard metabolic rate (SMR), (b) maximum metabolic rate (MMR) and (c) absolute aerobic scope (AAS). Data are shown as individual observations per fish (dots) and the mean with standard errors across housing cages within each treatment combination.

3.7 | Multivariate phenotype

Assessing coefficient of variation across univariate traits showed a small difference in variation across lines (Table S5); however, when all life-history, physiological and behavioural traits were analysed together, temperature had a significant effect on the multivariate phenotype (PERMANOVA, $F_{1,77}=6.56$, $p<.001$, Figure 7a, Table S3) but selection line did not (PERMANOVA, $F_{2,77}=1.79$, $p=.09$, Figure 7b, Table S3). Despite no significant difference among the selection lines, there was greater variation in phenotypic responses to thermal stress in the random-selected line as indicated by the larger ellipses (Figure 7b).

PC1 accounted for 22% of the phenotypic variation and mainly separated the mean phenotype at 34°C from the lower temperatures, while PC2 accounted for 16% of the variation, separating the mean phenotype at 22°C from the higher temperatures (Figure 7a). Individuals with higher PC1 scores were exposed to 34°C with higher mortality, greater exploration tendency and higher CT_{max} (Table S4). Individuals exposed to 28°C and 22°C exhibited faster growth (weight and length), were bolder, had higher metabolic rates (MMR and SMR), and greater feeding probability and frequency (Table S4). Individuals with high PC2 scores exposed to the 28°C and 34°C temperature treatments had higher mortality, metabolic rate, feeding probability and feeding frequency, boldness and exploration (Table S4, Figure 7c). While individuals at 22°C had a greater CT_{max} and growth rate (Table S4, Figure 7c). Moreover, when we subset PCAs by temperature, we show feeding rate is associated with growth rate at 28°C as expected under ambient (28°C) environmental conditions; however, this association weakens at the stressful temperatures (22°C and 34°C). Indeed, at 34°C, feeding rate becomes associated with metabolic rate instead (Figure S4).

4 | DISCUSSION

Directional selection on a phenotypic trait can change the mean value and reduce diversity of the target trait and any correlated phenotypic traits, with the loss of diversity potentially affecting the ability of a population to respond to future stressors. Here, we show that directional selection on body size can exacerbate a population's susceptibility to thermal stress beyond that which would occur in a population that had experienced a random loss of diversity. In

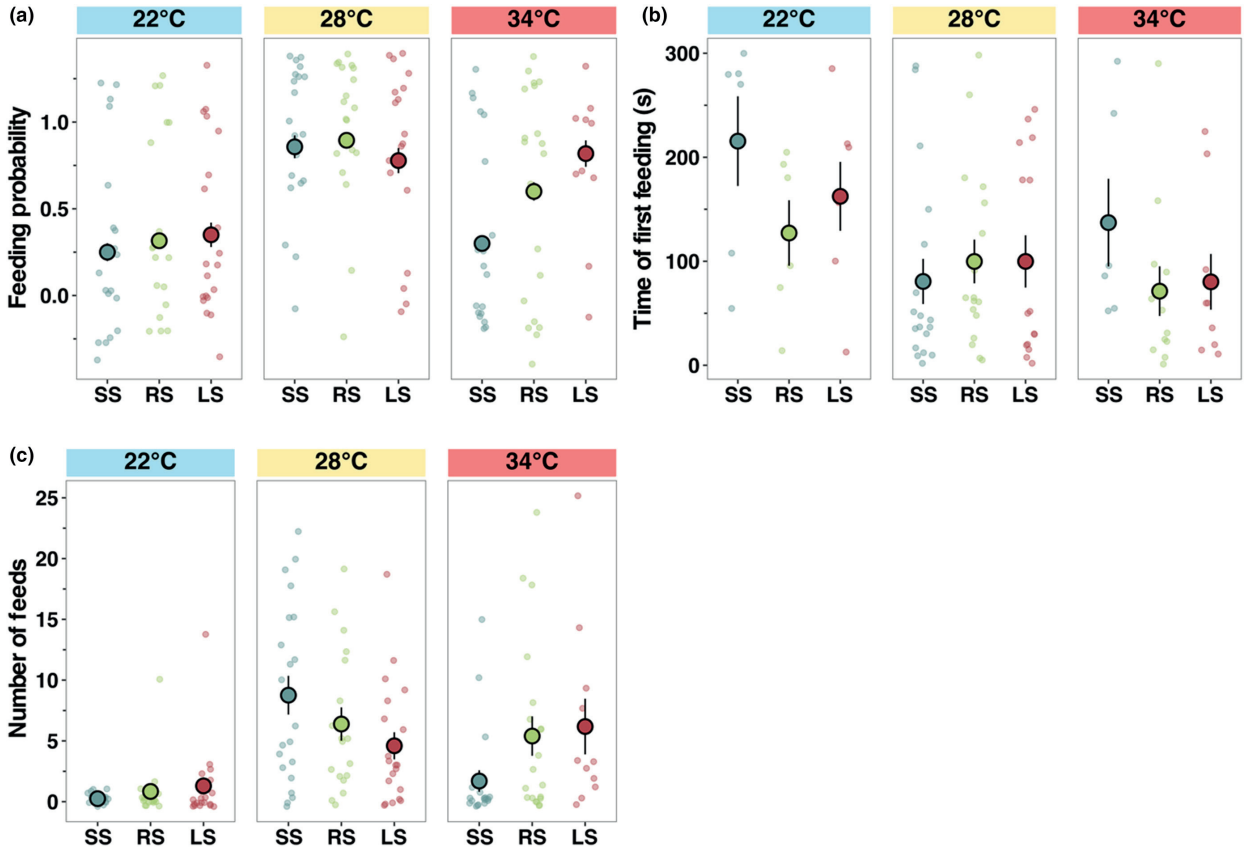

FIGURE 4 Differences in feeding behaviour among zebrafish selection lines (large-selected (LS), random-selected (RS) and small-selected (SS); selection-line replicates combined) in three temperature treatments. (a) Feeding probability, (b) time taken to reach first feeding attempt (s) and (c) number of feeding events within 5-min feeding trial. Data are shown as individual observations per fish (dots) and the mean with standard errors across housing cages within each treatment combination.

line with our first hypothesis, the random-selected line exhibited a differential shift in their multivariate phenotype (Figure 7b; Pigliucci & Preston, 2004) and had greater fitness in terms of growth rate. Counter to our second hypothesis, we did not find evidence that fish selected for large or small body size would perform better at low or elevated temperature.

Variation in temperature drives large differences in phenotypes in many vertebrates, including fitness-related traits such as growth (Atkinson, 1994; Killen et al., 2010), reproduction (Alix et al., 2020; Jonsson & Jonsson, 2019) and behaviour (Biro et al., 2010; Neubauer & Andersen, 2019). Metabolic rate is also a critical component of fitness (Killen et al., 2016) and is positively correlated with temperature in ectotherms (Clarke & Fraser, 2004; Morgan et al., 2022; Seebacher et al., 2014). However, beyond a critical temperature outside the thermal niche of an organism, metabolic rate starts to decline (Schulte, 2015; Schulte et al., 2011), as demonstrated in the present study. At the low temperature, metabolic rate was lower and the fish were less active than in the ambient temperature, potentially due to slowdown of biological processes (Volkoff & Rønnestad, 2020). Indeed, this slower pace of life is supported by longer developmental time (age at hatch), and larger larvae emergence at 22°C. Despite significant differences in metabolic rate (SMR and MMR), it is notable that our effect size was small, which could be evidence of metabolic acclimation occurring to the temperature during the long-term experimental period (Sandblom et al., 2014). Indeed, acclimation in a stable environment allows some resilience to extreme temperature differences (Seebacher et al., 2014), and future studies should explore different acclimation temperatures in more detail. That exposure to an elevated temperature (34°C) prevented zebrafish from reproducing potentially reflects more energy being allocated to metabolic maintenance at this temperature (Donelson et al., 2010) and is consistent with studies that found teleost spawning being sensitive to increases of 2–3°C (Alix et al., 2020; Hotta et al., 2001). We show that long-term exposure to elevated temperature had a significant negative effect on fish performance (growth, fecundity and survival) suggesting that their fitness may decrease under future extreme weather events.

Phenotypic traits covary genetically (Law, 1991) and phenotypically (Plaistow & Collin, 2014) and it is therefore important to assess multiple traits and understand their interconnectivity as a multivariate phenotype (Pigliucci & Preston, 2004), especially when a population undergoes selection, for example, fisheries selection.

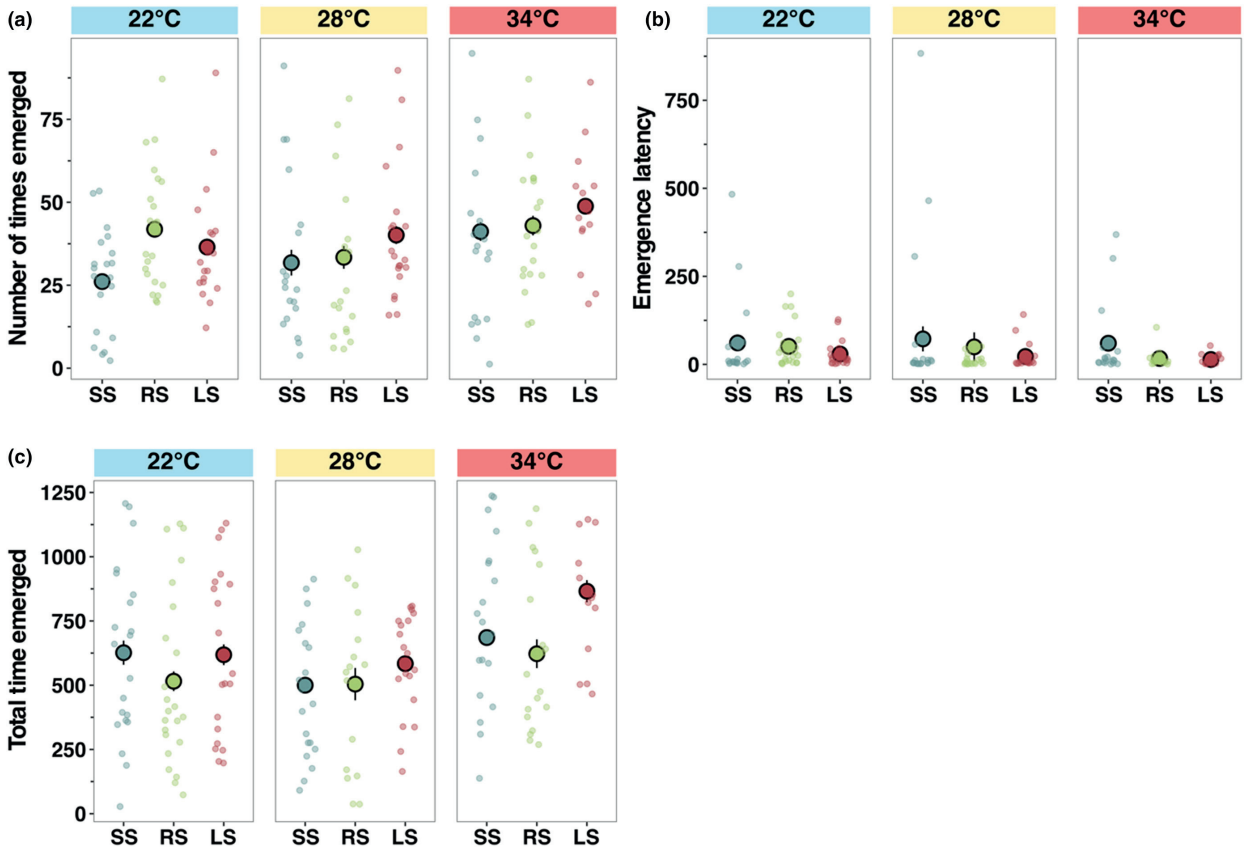

FIGURE 5 Differences in boldness and exploration among zebrafish selection lines (large-selected (LS), random-selected (RS) and small-selected (SS); selection-line replicates combined) in three temperature treatments. (a) Number of times emerged from the shelter within the 20-min time period, (b) proxies of boldness measured as time taken to emerge from the shelter and (c) total time spent emerged from the shelter (an indicator of exploration). Data are shown as individual observations per fish (dots) and the mean with standard errors across housing cages within each treatment combination.

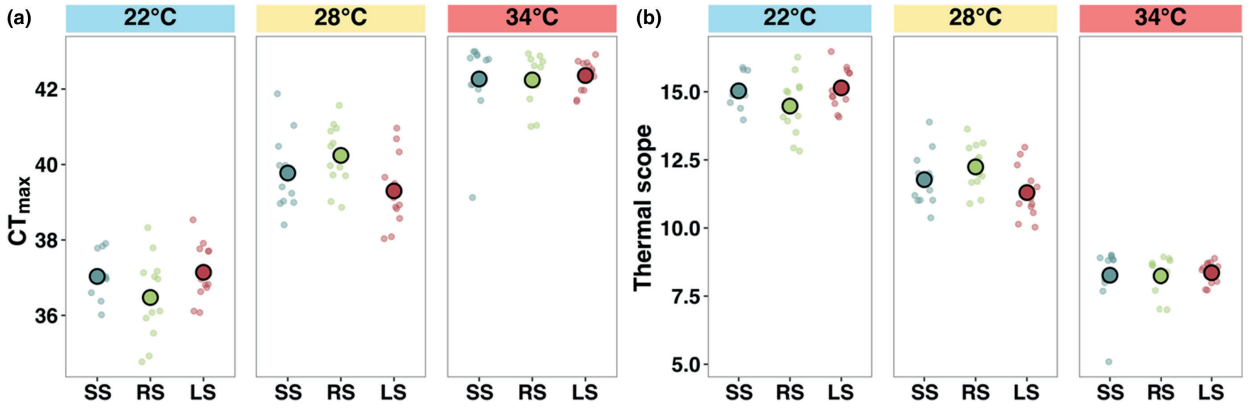

FIGURE 6 Differences in thermal limitations among selection lines of zebrafish (large-selected (LS), random-selected (RS) and small-selected (SS); selection-line replicates combined) in the three temperature treatments. (a) Critical temperature (CT_{max}) where the fish loses equilibrium and (b) thermal scope (CT_{max} – acclimation temperature). Data are shown as individual observations per fish (dots) and the mean with standard errors across housing cages within each treatment combination.

As such, although we show differing responses across the univariate phenotypic traits, studies on univariate traits may miss subtle but relevant phenotypic shifts (Plaistow & Collin, 2014). We show that covariation in phenotypic traits breakdown under stress, for example, growth rate was no longer correlated with food uptake at either lower or elevated thermal stress. The apparent breakdown

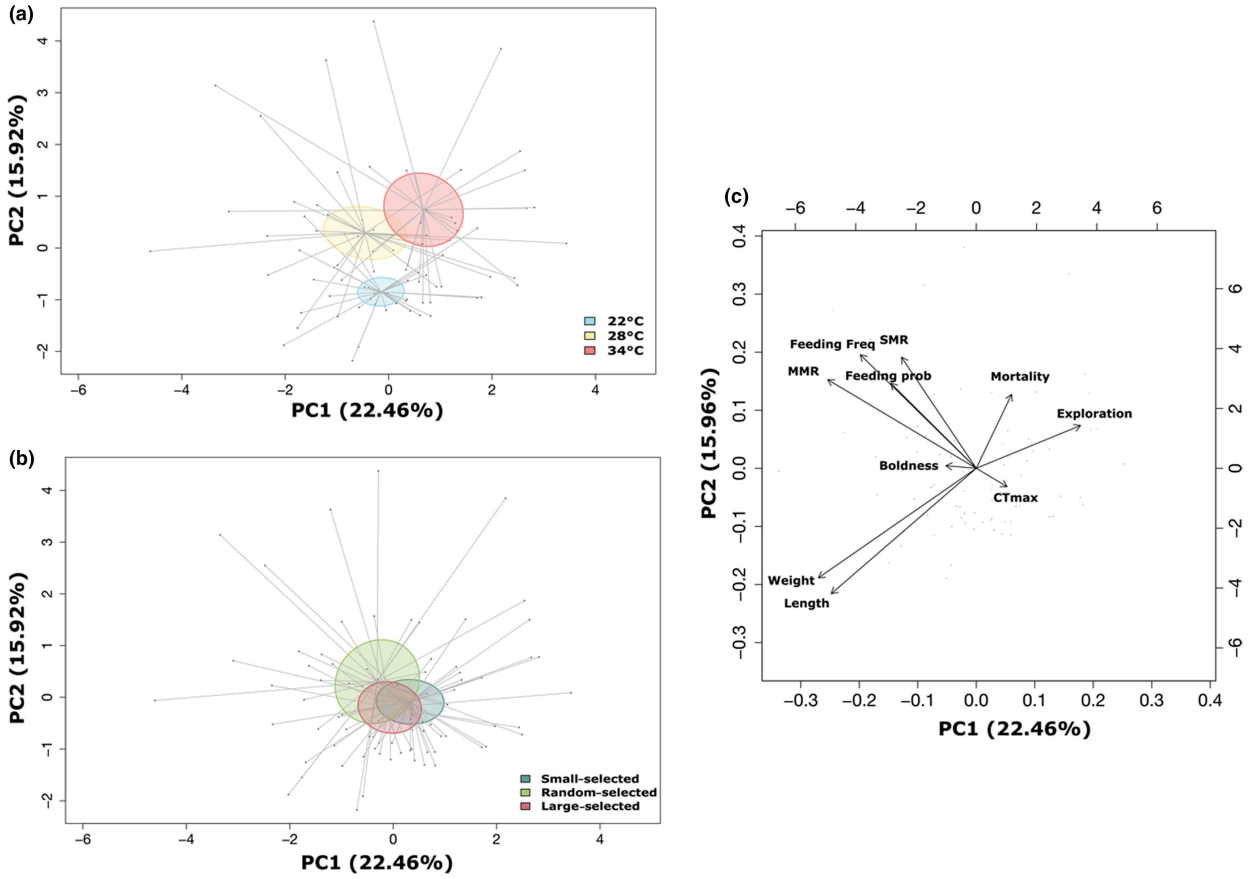

FIGURE 7 Principal component analysis of multiple measured traits with 95% confidence intervals across (a) zebrafish selection lines and (b) temperature treatments. Contributions to principal component space shown in biplot (c). Lengths of lines indicate distance of each individual from the respective group centroid.

in correlated traits in animals exposed to a stressful environment is comparable to a loss of correlation in copy number in genomes of mammals exposed to pollution (Jernfors et al., 2021). Here, we show that directional selection drives a shift in average phenotype (i.e. multivariate phenotype), leading to increased susceptibility to thermal stress, which in turn could alter phenotypic diversity (O'Dea et al., 2019).

Directional selection induces marked phenotypic changes in fish populations, such as reduced adult body size and earlier maturation at a smaller size (Conover & Munch, 2002; Uusi-Heikkilä et al., 2015; van Wijk et al., 2013). Directional selection has a greater impact on phenotypic traits than a population reduction alone because it causes selective sweeps and can magnify loss of diversity via genetic hitchhiking (Frankham, 2012; Stephan, 2019; Therkildsen et al., 2019). It is speculated that such selection for specific phenotypes (e.g. body size) is associated with further loss in diversity (Frankham, 2012). Notably, the direction of directional selection (i.e. for small or large size) in our study had little effect as the small- and large-selected lines had similar phenotypic responses to thermal stress in a number of traits. That both phenotypic outcomes of directional selection performed poorly suggests that some general mechanism might have reduced resilience to thermal stress, potentially related to inbreeding and further loss of diversity compared to random population reduction alone (Frankham, 2012). Our results therefore suggest the direction of selection per se may not matter and that size-selective fisheries may erode phenotypic diversity further than non-size-selective fisheries.

The effect of size-selective harvesting on fish populations is well studied (Pinsky & Palumbi, 2014; Therkildsen et al., 2019; van Wijk et al., 2013), but the interaction between reduced body size and warming is a relatively unexplored area in the fisheries context. At 34°C, random-selected fish showed similar growth reductions to the size-selected lines, suggesting this higher temperature had a severe effect upon all lines, regardless of prior selective pressures. Contrary to expectations following the TSR (Atkinson, 1994), both large- and small-selected fish had similar phenotypic responses to suboptimal temperatures and performed equally in terms of growth and reproduction. While small-selected fish were not more vulnerable to low or high temperatures compared with large-selected fish in our study, in more stochastic, natural environments size truncation may decrease demographic

buffering and population stability (Hočevar & Kuparinen, 2021; Kuparinen et al., 2016). Moreover, it is important to note that we used a large range in temperature (12°C) across the thermal scope of zebrafish (Morgan et al., 2022) that would likely be experienced by animals in nature for shorter infrequent timescales following extreme weather events (Sen Gupta et al., 2020). It would therefore be prudent for future studies to assess not only stable temperatures but also the impact of stochastic and variable temperature change that could disrupt possible acclimation.

Directional selection on body size can select for heritable behavioural traits that correlate with size, such as feeding behaviour, activity, exploration, boldness and aggression (Uusi-Heikkilä et al., 2015; Walsh et al., 2006). Differences in feeding behaviour may be important, as food intake will directly impact growth rate, therefore fish that show reduced feeding probability could have lower growth rate. Here our patterns do not always match (e.g. fish reared at low temperatures fed less, but had the similar growth rate to those reared at 28°C) potentially due to laboratory conditions where fish are fed ad libitum and do not need to spend energy on avoiding predators and/or parasites. Behavioural response to thermal stress depended on the type of directional selection, that small-selected fish were shyer than other lines is consistent with previous work (Monk et al., 2021; Sbragaglia et al., 2019; Uusi-Heikkilä et al., 2015). Furthermore, fish were less bold in elevated temperatures. This might suggest that at least with certain fishing gear (e.g. angling), selection favouring small body size could lead to increased vulnerability to fishing and predation, and reduced foraging success (Alós et al., 2012, 2015; Diaz Pauli et al., 2015; Härkönen et al., 2014; Klefoth et al., 2012; Stamps, 2007). Overall, we did not detect as clear patterns in behavioural traits than in, for example, growth and physiology, which could be caused by the relatively high plasticity of behavioural traits compared to morphological and physiological traits (Duckworth, 2009; Mousseau & Roff, 1987). Our results suggest that behavioural responses to altered temperatures act differently than life history and physiological responses and are more dependent on the direction of selection.

In balanced harvesting, fishing mortality is not applied to selected functional groups, species or size of individuals and balanced harvesting has been suggested to reduce the negative effects of fisheries on ecosystems and on targeted populations by mitigating the effects of directional selection (Garcia et al., 2012). Balanced harvesting can increase stock productivity (Zhou et al., 2015), aid the recovery of populations' natural size structures (Beamish et al., 2006) and improve the resilience of the populations to natural disturbances (Hixon et al., 2014). Balanced harvesting, especially when it comes to body size, should maintain more phenotypic (and potentially genetic) variation in an exploited stock compared to size-selective harvesting. In our experiment, the random-selected line corresponds with balanced harvesting; however, this line did not maintain more phenotypic variation (with traits considered as a multivariate phenotype) than the size-selected lines. However, random-selected fish had greater fitness than small- and large-selected fish, particularly when using adult body size and growth as a proxy for fitness (Barneche et al., 2018; White et al., 2013). Even if high fishing mortality without directional selection reduces phenotypic diversity through the reduction in population size, it should reduce less diversity than when combined with directional selection.

We show that random-selected fish (no directional selection for body size) had the highest phenotypic variability in response to thermal stress. Size-selective harvesting has a greater impact than the effects of a population reduction alone, with directional selection potentially magnifying loss of phenotypic diversity through selective sweeps and hitchhiking on covarying traits. Importantly, the direction of phenotypic change (either small- or large-selected phenotype) appears less important than the action of directional selection per se, as size-selective harvesting generally decreased the performance of fish exposed to thermal stress. A crucial next step would be to determine whether natural populations of exploited fish experience similar phenotypic changes under changing water temperature, and what are the underlying genetic mechanisms driving such changes. Our data suggest that selection regimes during harvesting should be reconsidered by utilising alternative harvesting strategies, aiming to reduce the magnifying effects of directional selection on fitness.

AUTHOR CONTRIBUTIONS

Daniel E. Sadler: Conceptualization (equal); data curation (lead); formal analysis (lead); investigation (lead); methodology (equal); visualization (lead); writing – original draft (lead); writing – review and editing (equal). **Stephan van Dijk:** Conceptualization (equal); data curation (supporting); methodology (equal); writing – review and editing (equal). **Juha Karjalainen:** Methodology (equal); resources (equal); writing – review and editing (equal). **Phillip C. Watts:** Conceptualization (equal); investigation (equal); project administration (equal); resources (equal); supervision (equal); writing – review and editing (equal). **Silva Uusi-Heikkilä:** Conceptualization (equal); funding acquisition (lead); investigation (equal); methodology (equal); project administration (lead); resources (equal); supervision (equal); writing – review and editing (equal).

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CONFLICT OF INTEREST STATEMENT

No conflict of interest.

DATA AVAILABILITY STATEMENT

All data are available in Dryad https://doi.org/10.5061/dryad.cvdncjt9v.

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IV

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