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Telomere Length Differences Indicate Climate Change-Induced Stress and Population Decline in a Migratory Bird

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ABSTRACT

Genomic projections of (mal)adaptation under future climate change, known as genomic offset, faces limited application due to challenges in validating model predictions. Individuals inhabiting regions with high genomic offset are expected to experience increased levels of physiological stress as a result of climate change, but documenting such stress can be challenging in systems where experimental manipulations are not possible. One increasingly common method for documenting physiological costs associated with stress in individuals is to measure the relative length of telomeres—the repetitive regions on the caps of chromosomes that are known to shorten at faster rates in more adverse conditions. Here we combine models of genomic offsets with measures of telomere shortening in a migratory bird, the yellow warbler (*Setophaga petechia*), and find a strong correlation between genomic offset, telomere length and population decline. While further research is needed to fully understand these links, our results support the idea that birds in regions where climate change is happening faster are experiencing more stress and that such negative effects may help explain the observed population declines.

1 | Introduction

The ability of populations to persist when threatened by climate change depends on their capacity for range shift (Tingley et al. 2009) or adaptation to climate in place (Beever et al. 2016; Nicotra et al. 2015; Nogués-Bravo et al. 2018). In the past, assessments of vulnerability to climate change mostly focused on forecasting distributional changes using ecological niche modelling approaches (e.g., Cianfrani et al. 2018; Saunders et al. 2020; Thuiller, Lavorel, and Araújo 2005), but such approaches do not consider the capacity for organisms to adapt (Beever et al. 2016; Nicotra et al. 2015; Rellstab 2021). In contrast, landscape genomic approaches like genomic offset use the difference between current gene–environment relationships and those modelled under future climate change scenarios to identify which populations will have the most difficulty adapting to changing climate conditions (Bay et al. 2018; Capblancq et al. 2020; Ruegg et al. 2018). These approaches use associations between genomic variation and climate variables measured where individuals are sampled to determine the genomic variation needed to adapt to current climate conditions. These associations are then projected across future-predicted climate scenarios to determine the genetic shift that would be needed by populations to adapt to future conditions. The amount of genetic shift needed

is the genomic offset measure, where regions with the highest genomic offset are predicted to experience the most climate-induced stress in the future and, in some cases, may have already experienced population decline (Bay et al. 2018). While genomic offset methods are becoming increasingly popular for predicting climate change responses, their broadscale utility is often limited by the inability to validate model predictions.

One of the biggest challenges with genomic offset approaches is the difficulty with validating the predicted costs associated with maladaptation. In plant systems, past genomic offset studies have validated fitness effects by using phenotypic data in common garden or reciprocal transplant studies (e.g., Borrell et al. 2020; Fitzpatrick et al. 2021). For example, Borrell et al. (2020) measured fitness-related phenotypic traits in a common garden and found a significant negative relationship between genomic offset and reproductive output in *Betula nana*. Similarly, Fitzpatrick et al. (2021) showed a negative association between genomic offset and performance in two common garden experiments. They also found that genomic offset was ultimately a better predictor of performance than differences in climate alone. Despite their value for validating genomic offset predictions, common garden studies are infeasible for many wild non-model organisms. Thus, a method for validating the predicted negative effects of genomic offset in non-model organisms would help increase its broadscale utility.

An increasingly common method for assessing the relative influence of stress on individuals across taxa that has not yet been applied to test predictions from genomic offset models is telomere length. Telomeres are nuclear protein structures made of non-coding tandem repeats of base pairs at the end of linear chromosomes (Blackburn 1991, 2005). Telomeres shorten with each DNA replication cycle (Blackburn 2005) and telomere shortening can be accelerated due to stress experienced by the individual, also known as allostatic load (McEwen and Wingfield 2003). Many environmental factors can be labelled as stressors in that they cause a physiological and behavioural change that prioritises survival at the expense of a physiological cost (Romero 2004). Acute and chronic stress is associated with increased metabolic rate, which in turn is associated with increased mitotic and mitochondrial activity (Silverin 1986; McEwen and Wingfield 2003; Boonstra 2004; Haase, Long, and Gillooly 2016). This increased mitochondrial activity is responsible for increased oxidative stress (Cadenas and Davies 2000), which has been labelled as one of the main drivers of telomere shortening (von Zglinicki 2000, 2002; Sozou and Kirkwood 2001; Houben et al. 2008). For example, factors such as intra and interspecies competition, pollutant exposure and severe weather events are a few examples of adverse environmental factors that trigger an environmentally induced stress response (Casagrande and Hau 2019; Chatelain, Drobniak, and Szulkin 2020; Reichert and Stier 2017; Von Zglinicki 2002).

Telomere length measurements are increasingly being used as a biomarker for important life-history traits such as lifespan (Bichet et al. 2020; Heidinger et al. 2012), lifetime reproductive success (Eastwood et al. 2019) and individual quality (Angelier et al. 2019; Cheron et al. 2021; Rollings et al. 2017) across a variety of taxa from humans, to birds, to small mammals. Telomeres seem to be particularly sensitive to environmental variation at early life stages, including prior to birth/hatching (Casagrande et al. 2020; Haussmann et al. 2012; Noguera, da Silva, and Velando 2022) via maternal effects and parental life histories (Haussmann et al. 2012; McLennan et al. 2018). Consequently, in wild populations, environmental conditions experienced during early life can generate long-lasting cohort effects on telomere length and may even have a larger impact on telomere length than current conditions (Debes et al. 2016). Thus, telomere length may represent both generational and contemporary effects of the stress induced by maladaptation to the changing climate and can serve as a method for validating predictions from genomic offset models.

The objective of this study is to expand the application of genomic offset as a tool for predicting the impacts of climate change by developing methods that can be used to test key model predictions. To achieve this, we will investigate the relationship between genomic offset, telomere length and population decline in the yellow warbler (*Setophaga petechia*). The yellow warbler is a migratory songbird that breeds in various habitats throughout North America and is an excellent system for this study because earlier work by Bay et al. (2018) identified patterns of genomic offset across the species range. The researchers then used the correlation between genomic offset and past population declines (1970s to present) to suggest that climate change is negatively affecting populations. However, as noted by Fitzpatrick, Keller, and Lotterhos (2018), a key assumption of this work is that past population declines resulted from past climate change-induced stress. The authors, however, failed to provide a metric for documenting such stress and did not establish the necessary correlation between past and future climate change.

In this study, we aim to test the hypothesis that genomic offset can help identify regions where climate change is negatively impacting wild populations using telomere length as a biomarker for the impacts of climate induced stress. To test this hypothesis, we measure telomere length and approximate past population trends in yellow warbler populations across regions of high and low genomic offset. If telomere length, genomic offset and population declines are strongly correlated even after accounting for other important variables that can influence telomere shortening (such as age, sex, body size and population-level effects), then we can conclude that genomic offset results in increases in adverse conditions. Additionally, we will investigate the specific climate factors contributing to shorter telomeres and population declines in the yellow warbler system and test the relationship between past and future climate change. We focus on precipitation because previous research indicates that precipitation was highly weighted in genomic offset predictions (Bay et al. 2018). If precipitation is a main driver climate-induced stress, both population trends and telomere length should also be correlated with past precipitation. Overall, this study will help test assumptions at the core of many genomic offset predictions, thereby increasing their utility for predicting climate change impacts in wild populations.

2 | Methods

2.1 | Study Design and Sampling

We used genotypes derived from restriction-site associated DNA sequencing (RAD-Seq) data from Bay et al. (2018) on 229 individuals from 21 locations across the yellow warbler breeding range. To estimate genomic offset, we then ran gradient forest

(Fitzpatrick et al. 2021), a machine-learning regression tree-based approach implemented in the R package gradientforest (Ellis, Smith, and Pitcher 2012) on a subset of 1,694 unlinked candidate single-nucleotide polymorphisms (SNPs) that were significantly associated with climatic variables based on LFMM analysis from Bay et al. (2018). We built a gradient forest model with average monthly precipitation, temperature maximum, temperature minimum, latitude and longitude values for the months of May through July (breeding months for the yellow warbler) as environmental response variables and the candidate SNPs as predictors. Precipitation data was obtained from the CRU-TS 4.06 dataset (Harris et al. 2020) downscaled with WorldClim 2.1 (Fick and Hijmans 2017). We then used the predict function within gradient forest to weight the environmental response variables for both current (2021–2040) and future (2041–2060) predicted climates at 10,000 random locations across the yellow warbler breeding range. We then interpolated across the entire breeding range to form a continuous map of genomic offset (Figure 1).

We selected sample sites for telomere measurements by using the continuous map of genomic offset and choosing areas across a gradient of genomic offset in addition to precipitation and elevation. Estimates of genomic offset were then calculated for each specific sample site. We collected blood samples for telomere measurements from each sample location spanning the breeding range of the yellow warbler once over the course of 2020 and 2021 breeding seasons. Birds were captured via mist-nets, and once in hand, individuals were banded, morphological measurements were taken, and age and sex were recorded. Age was determined using plumage characteristics outlined in Pyle (1997). Due to rapid telomere shortening and variation in telomere length during early life, hatch year birds were not included in the study. Between 10 and 30 μL of blood was collected using brachial venipuncture and stored in Queen's lysis buffer on ice until reaching the lab where they were stored in −20°C until extraction, which occurred within 6 months of collection (Criscuolo et al. 2009). Birds that could not be sexed in the field were sexed using polymerase chain reaction (PCR) with the primer set CHD1F and CHD1R (Çakmak, Akın Pekşen, and Bilgin 2017). Our sampling resulted in blood samples from 416 yellow warblers spanning 39 sample sites across the breeding range of the species (Figure 1).

2.2 | Telomere Measurement

DNA was extracted from blood using DNeasy Blood and Tissue kit (Qiagen, Valencia, California) following the manufacturer's protocol. Telomere analysis was conducted within three months following DNA extraction. We used a NanoDrop 8000 spectrophotometer (Thermo Scientific) to measure DNA concentration and quality on the same day as the telomere analysis took place. The average ratio of absorbance at 260 nm over 280 nm was used to check for protein contamination and the average ratio of absorbance at 260 nm over 230 nm was used to check for salt contamination. If either ratio of absorbance was <1.8, the extract was excluded from further analysis (Morinha et al. 2020). DNA integrity was visually assessed on an agarose gel as recommended by Seeker et al. (2016). Following the protocol of Criscuolo et al. (2009), we quantified telomere length by quantitative real-time PCR (qPCR). Telomere length is calculated as the ratio (T/S) of telomere repeat copy number (T) to a control single gene copy number (S), which is standardised to a reference sample and expressed as relative telomere length.

We used the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as the single control gene. The primers we used to amplify the telomere region were as follows: Tel1b (5′-CGGTTTGTTTGGGTTTGGGTTTGGGTTTGGGTT-3′) and Tel2b (5′-GGCTTGCCTTACCCTTACCCTTACCCTTACCCTTACCCT-3′), and for the amplification of the GAPDH we used specific GADPH-F (5′-GGTAGATGGGAGTTCAGTTGTG-3′) and GAPDH-R (5′-AGAAACAAAGCACTGTCAGGG-3′). We used a multiplexed qPCR using 3 μL of sample DNA at 3 ng/μL, Tel1b/Tel2b primers at a concentration of 900 nM, and

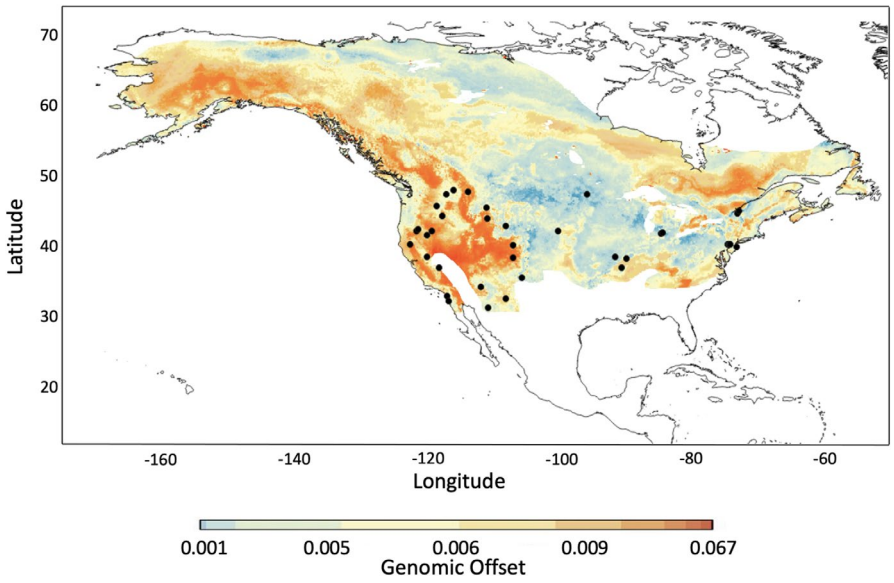

FIGURE 1 | Map of the yellow warbler breeding distribution showing estimates of genomic offset based on 2050 SSP 885 projections, where cool colours indicate low genomic offset and warm colours indicate high genomic offset. Black dots are yellow warbler sampling locations.

GAPDH-F/GAPDH-R primers at a concentration of 400 nM in a final volume of 25 μL containing 10 μL of GoTaq qPCR Master Mix (Promega, Madison, Wisconsin, USA). qPCR was ran on a CFX96 touch real-time PCR detection system machine (Bio-Rad) and the conditions under which we ran the telomere qPCR were 10 min at 95°C followed by 30 cycles of 15 s at 95°C, 30 s at 54.5°C and 30 s at 72°C. For the GAPDH amplification, we used 10 min at 95°C followed by 40 cycles of 15 s at 95°C, 30 s at 60.5°C and 30 s at 72°C. DNA samples were run in triplicate and a reference sample was run on every plate to compare measurements between plates. qPCR plates included serial dilutions (0.2, 0.4, 2, 10, 30 and 50 ng) of DNA from the same reference bird to create a reference curve to control for amplifying efficiency of the qPCR. All plates had standard curves within acceptable ranges. We used a Yellow Warbler sample from our study site (not included in our study) as the reference sample. Overall, we included 416 samples across 22 qPCR plates. Samples were randomly distributed across plates, though sample triplicates were all run on the same plate. Coefficient of determination was > 0.99 and efficiencies within 100 ± 10% (Telomere standard curve: mean = 1.99, standard deviation = 0.02; GAPDH standard curve: mean = 1.99, standard deviation = 0.02). Within sample triplicates, if the coefficient of variation was > 0.14 for a sample, one triplicate was dropped. Samples were excluded if the remaining sample duplicate coefficient of variation was still > 0.14 (Nettle et al. 2019). Average repeatability (standard deviation) of T/S values was 0.067 across all samples and was 0.045 for reference samples across plates. Interplate repeatability based on samples measured across plates was 1.02 (95% CI [1.0026, 1.0334]).

Following the methods used in Kärkkäinen et al. (2022), we validated the use of our qPCR approach at the between-population level by evaluating whether populations varied in control single gene Cq, in addition to qPCR efficiencies for both control single gene and telomere assays (Table S1). Control gene Cq values differed across populations ($F_{38,337} = 8.137$, $p < 0.001$), as did both control gene and telomere assay efficiencies (Control gene: $F_{38,377} = 9.95$, $p < 0.001$; Telomere: $F_{38,377} = 7.527$, $p < 0.001$). Thus, we added qPCR plate as a random effect in all downstream telomere analyses. In addition, relative telomere length (T/S) was calculated based on plate-specific efficiencies (Table S2) using the mathematical model presented in Pfaffl (2001).

2.3 | Statistical Analysis

Statistical analyses were carried out using R 4.1.2 (R Core Team, 2021). The findings in the Bay et al.'s (2018) study imply a connection between genomic offset and the decline in yellow warbler populations. To validate this assertion, it is crucial to investigate the correlation between past and future climate changes. To test this assumption, we analyse the correlation between historic and future changes in climate for the top three uncorrelated climate variables found to be associated with yellow warbler genomic variation according to Bay et al. (2018). These climate variables were bio18 (mean monthly precipitation amount of the warmest quarter), bio15 (precipitation seasonality) and bio13 (precipitation amount of the wettest month).

The distribution of relative telomere length was right-skewed, so log relative telomere length was used in subsequent analyses. Log relative telomere length was then standardised with z-transformation using the scale()-function in R to facilitate comparison to future studies (Verhulst 2020). We first tested for variation in telomere length across sample populations by fitting a linear mixed model with telomere length as the dependent variable, sample population as the independent variable and qPCR plate as a random effect. We then examined whether potential population differences in telomere length could be explained by genomic offset, by using an information-theoretic framework in R. We used estimates of genomic offset calculated for each sample location. For this analysis, we constructed a global model consisting of variables that could be influencing telomere length within and across populations of yellow warblers on their breeding range. These predictor variables included genomic offset, age class, sex, elevation, latitude, tarsus length and date. Our candidate model set consisted of linear mixed effects models (lmer in lme4; Bates et al. 2015) where we included study site and qPCR plate as random effects in each model.

As all our predictor variables have potential for additive effects, all combinations of the predictor variables included in the global model were compared and ranked based on AICc using the package MUMIN (Bartoń 2009). Two-way interactions were only included in the global model if such relationships were considered plausible a priori. A null model was included in the candidate model set. Akaike weights were used to assess the support for each model. All continuous predictors were centered and scaled but were back-transformed for plotting. Prior to model comparison, to determine independence of predictor variables, correlations between all predictor variables were checked and variance inflation factors (VIF) of the global model were checked to assess multicollinearity: all VIFs were less than 3 (Fox and Weisberg 2011). Model residuals of the global models were assessed to confirm compliance with model assumptions. The top model was estimated using maximum likelihood and the Kenward–Roger method was used to calculate degrees of freedom of fixed factors and to assess parameter estimates and their standard errors.

To test the assumption that historical changes in precipitation are associated with population trends and telomere lengths we again used an information–theoretic framework. We constructed a global model consisting of variables that could be influencing yellow warbler abundance trends and telomere lengths across populations on their breeding range. These variables included the top three uncorrelated BIOCLIM variables found to be important to yellow warbler local adaptation in Bay et al. (2018). BIOCLIM measures are a collection of monthly climate variables made to capture environmentally important climate variation (Fick and Hijmans 2017). The predictor variables were historic changes of precipitation amount of the wettest month (bio13), precipitation seasonality (bio15) and mean monthly precipitation amount of the warmest quarter (bio18), in addition to elevation and latitude variables. In analysing telomere length, our candidate model set consisted of linear mixed effects models (lmer in lme4; Bates et al. 2015) where we included sample site as a random effect in each model. In analysing abundance trends, our candidate model set consisted of linear models. Each candidate model set included a null model. Global model construction and model selection were conducted using the same methods described earlier. Yellow warbler abundance trend

values for each sample location were extracted from Breeding Bird Survey data (Ziolkowski et al. 2023).

Finally, we test the association between telomere length and abundance trends across yellow warbler populations to find if populations in decline also have shorter average telomere length. To do this, we analysed the correlation between abundance trends and average telomere length across sample sites.

3 | Results

Because genomic offset is based on future climate, and Bay et al. (2018) used future climate in their model, but current stress and past population decline are a result of past climate, we tested the assumption that past and future climates are correlated. To test this assumption, we compared changes in each of the top climate variables in Bay et al. (2018) and found significant correlations among two of the three primary bioclimatic variables (bio13, R^2 0.76; bio15, R^2 0.76) as well as a positive trend in the third bioclimatic variable (bio18, R^2 0.08; Figure 2).

3.1 | Telomere Length, Genomic Offset and Population Trends

We found significant variation in telomere length across sampled populations of breeding yellow warblers ($F_{38,331} = 0.996$, $p < 0.001$, Figure S1). We then found that significant population differences in telomere length could be explained by genomic offset. Using an information theoretic approach, we found that the top ranked model, which also carried a majority of the model weight, included interactive effects of genomic offset and elevation along with an additive effect of tarsus length (Akaike; $w_i = 0.46$; Table S3). Conducting a standard linear regression on telomere length versus genomic offset revealed evidence of a significant decline in telomere length with increasing genomic offset estimates ($p \leq 0.018$, Figure 3). The interaction between genomic offset and elevation ($p = 0.002$) provides evidence that in high elevation areas with high genomic offset, telomere length is shorter than in low elevation areas (Figure S2). The additive effect of tarsus length is likely related to body size differences, supporting the idea that larger birds with longer tarsi have shorter telomeres relative to smaller birds with shorter tarsi (Figure S3). Despite the influence of body size and elevation on telomere length, results support the idea that genomic offset is significantly linked to telomere length. Overall, while factors such as population, elevation and body size influence telomere length, there remains a strong correlation between telomere length and genomic offset in the direction predicted, indicating that birds in locations with high genomic offset may be experiencing more climate-induced stress.

3.2 | Association Between Precipitation, Population Trends and Telomere Length

To test the assumption that changes in climate are associated with abundance trends we conducted AIC model selection. The top ranked model was an interactive model between bio15 (precipitation seasonality) and latitude (Akaike; $w_i = 0.33$; Table S4), suggesting a significant decline in abundance trends with increasing precipitation seasonality (between the years 1960–2021) and latitude ($R^2 = 0.38$, p-value = 0.044; Figure 4a). We also performed AIC model selection to find the most important climate variables associated with telomere length and found that, similar to the analysis on abundance, the top-ranking model was an interactive model between bio15 (precipitation seasonality) and latitude (Akaike; $w_i = 0.14$; Table S5), suggesting a significant decline in telomere length with increasing precipitation seasonality (years 1960–2021) and latitude ($R^2 = 0.41$, p-value = 0.03; Figure 4b). Finally, we test the association between

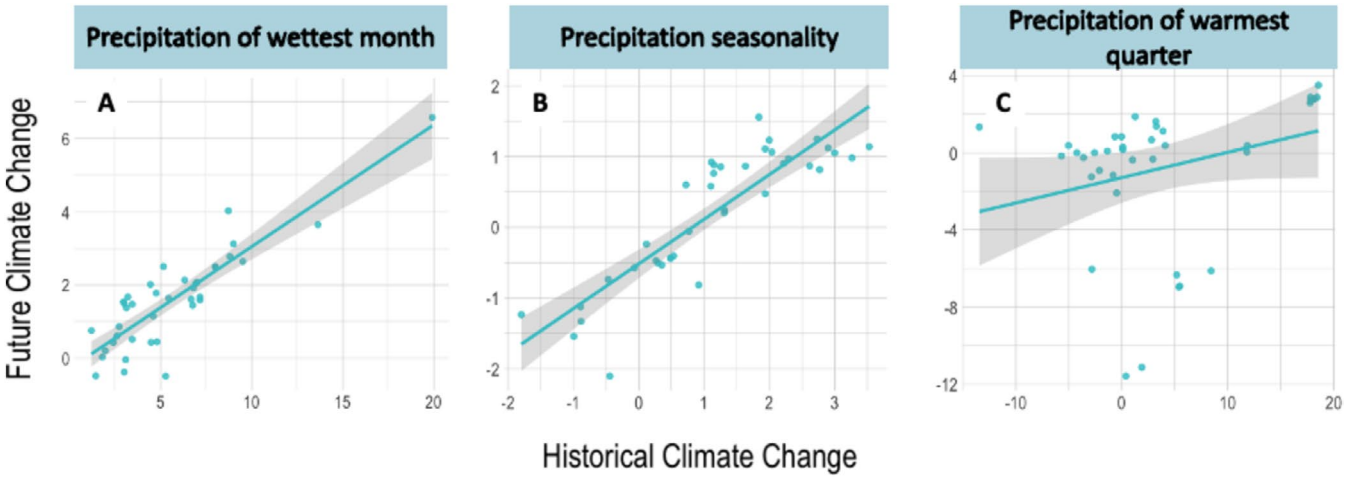

FIGURE 2 | Correlation between historic and future changes in climate at 39 yellow warbler sample sites for bioclimatic variables associated with yellow warbler genomic variation of the breeding grounds. Historical climate change were calculated by subtracting historic bioclimatic variables (1970–2000) from those bioclimatic variables calculated for 2021–2040. Similarly, future changes were calculated by subtracting bioclimatic variables from 2021 to 2040 from future bioclimatic variables (2041–2060). We show that (A) changes in historical precipitation amounts of the wettest month are associated with future projected changes ($R^2 = 0.76$, p-values ≤ 0.001), (B) changes in historical precipitation seasonality are associated with future projected changes ($R^2 = 0.76$, p-value ≤ 0.001), and (C) changes in precipitation of the warmest quarter have an insignificant but positive trending association with future projected changes ($R^2 = 0.08$, p-value = 0.078).

FIGURE 3 | Yellow warblers ($n = 416$) sampled in areas with high genomic vulnerability had lower standardised relative telomere length compared to those in areas of low genomic vulnerability ($R^2 = 0.35$).

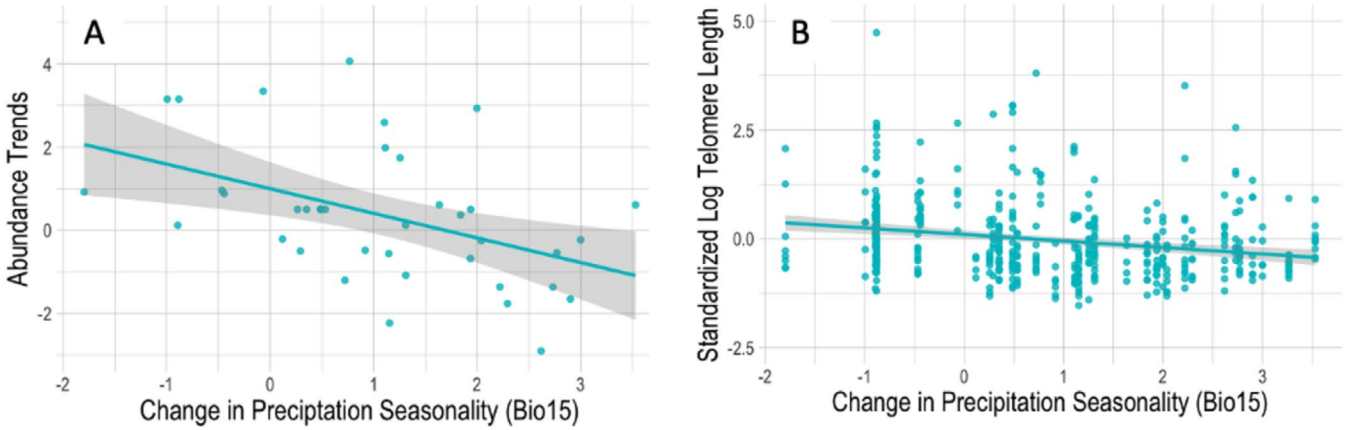

FIGURE 4 | Yellow warbler abundance trends and telomere lengths in response to historical climate change using bioclimatic variables associated with genomic variation on the breeding grounds. (A) a significant negative association between abundance trends and historical changes in precipitation seasonality across 39 sample sites ($R^2 = 0.38$, p-value = 0.044). (B) a significant negative association between historic changes in precipitation seasonality and telomere length in 451 yellow warbler samples across 39 sample sites ($R^2 = 0.41$, p-value = 0.03).

telomere length and abundance trends across yellow warbler populations and find a significant positive correlation, with abundance trends increasing with increasing telomere length ($p = 0.005$; Figure S4).

4 | Discussion

The integration of adaptation into models of vulnerability to climate change, known as genomic offset (e.g., Hoffmann, Weeks, and Sgrò 2021; Layton et al. 2021; Ruegg et al. 2018), faces limitations in its widespread application due to challenges associated with validating model predictions. Specifically, organisms inhabiting regions predicted to experience high genomic offset are also projected to encounter increased climate-induced stress and decreased fitness due to climate change, but estimating such responses is challenging in most systems. Our findings suggest that the yellow warblers in regions predicted to undergo significant climate change are situated in areas that have historically encountered substantial climate change. Furthermore, birds in these regions have experienced the most significant declines in abundance and have the shortest telomeres, which may be a sign of increased stress. Overall, this work provides a framework for validating assumptions at the core of genomic offset models in cases where reciprocal transplants are not possible. The work supports the idea that while genomic offset models predict how organisms will fare under future climate change, they can also be used to identify regions where populations may already be experiencing climate change-induced stress in cases where past and future climate changes are correlated.

Previous studies that have used telomere shortening to assess the impacts of climate change often concentrated on single populations or lacked essential information about environmental variables crucial for local adaptation. Our approach is an advance because it deliberately considers specific variables recognised as important for climate adaptation. For instance, studies by Zhang et al. (2023) and Eastwood et al. (2022) identified associations between temperature and telomere length within specific populations, but did not establish whether temperature

is a significant factor in local adaptation, nor did they investigate how local adaptation across the landscape is predicted to change in the future. In contrast, our method links telomere length to precipitation which we know from our analyses is the environmental variable that is most strongly associated with genomic variation across space in yellow warbler. As such, we have a strong reason to expect that variation in precipitation across the landscape helps shape patterns of local adaptation in the yellow warbler and, correspondingly, changes in precipitation over the last 30 years may result in stress if populations are unable to evolve fast enough to keep pace with such changes. Further, unlike past research, we show that telomere length is not only associated with changes in precipitation—the environmental variable most likely important to local adaptation in yellow warblers—but also with past population declines. While further work is needed, the result that populations which have been declining also have shorter telomeres supports the idea that climate-induced stress may have long-term fitness effects in the yellow warbler. Overall, we demonstrate that measuring telomere length alongside genomic offset provides a robust framework for evaluating the impacts of climate change across diverse populations.

In birds, changes in precipitation have frequently been linked to population declines (e.g., Cruz-McDonnell and Wolf 2016; Iknayan and Beissinger 2018; Senapathi et al. 2011), but reasons for these association remain unclear. Precipitation plays a vital role in determining the availability of food resources for birds (e.g., Ferger et al. 2014; Wagner 2020; Zhu et al. 2014) and when precipitation patterns deviate from optimal conditions, populations may experience reduced reproductive success and subsequent population declines (Anctil, Franke, and Bêty 2014; Coe et al. 2015; Conrey et al. 2016). Building upon findings in other bird species of the relationship between precipitation, resource availability and fitness, one hypothesis to explain our results is that climate change induced increases in aridity across the yellow warbler breeding grounds have led to shifts in the insect and plant communities upon which yellow warblers depend for survival. While further work is necessary, such shifts in food availability may be placing increased selective pressure on bill morphology which previous work suggests is correlated with precipitation across the breeding range in this species (Bay et al. 2021). To further investigate these linkages, future research will focus on identifying recent changes in the food resources crucial for yellow warblers during the breeding season and determining whether such changes may be placing selective pressure on bill size.

In addition to illustrating a negative association between telomere length and genomic offset, our results provide evidence for an effect of elevation, where high elevation populations in high genomic offset regions have the shortest telomeres. While the negative relationship between telomere length and elevation has been found in prior research (Stier et al. 2016), the interaction between genomic offset and elevation could be explained by mountainous areas being considered as climate 'hotspots', where effects of climate change can be amplified or accelerated (Pepin et al. 2022). Therefore, yellow warbler populations that are unable to adapt to climate change may be facing even higher fitness consequences at high elevation where changes in climate are occurring more rapidly. Further, we demonstrate that genomic offset may be used to identify current regions of climate-induced stress, in cases where past and future climate change are correlated, as is the case with the yellow warbler. In addition to validating the genomic offset concept, these results support the use of genomic offset as an important tool for understanding impacts of climate change on current and future populations and informing conservation efforts.

Climate change has been observed to impact the fitness of various wild species (e.g., Benito Garzón et al. 2018; Huang and Pike 2011; Lane et al. 2012), but identifying specific ecological drivers of fitness loss can be challenging. Overall, our work supports the idea that combining telomere length measurements with data on past population trends and changes in environmental variables important to local adaptation provides a framework for assessing the impacts of climate change on wild populations. Future work will aim to clarify the relationships between genotype, phenotype and natural selection by identifying the genes underlying bill morphology and assessing whether genetic variation within these genes has shifted due to recent climate change.

Author Contributions

M.D.R. and K.C.R. conceived of the idea. M.D.R. collected the data. M.D.R. did the laboratory work. M.D.R. and R.A.B. conducted the analysis with guidance from K.C.R. M.D.R. wrote the paper and K.C.R. and R.A.B. reviewed the manuscript.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are openly available via Dryad at https://datadryad.org/stash/share/koV-2biWSCDV5UjFjK7xdO9Z6HS16uw6QZf3gChR22c.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.