Evolution of thermal physiology alters predicted species distributions under climate change

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Species distribution models (SDMs) are widely adopted to predict range shifts but can be unreliable under climate change scenarios\(^1\) because they do not account for evolution. The thermal physiology of a species is a key determinant of range\(^2,3\) but the impact of thermal trait evolution on SDMs has not been addressed. We identified a genetic basis for physiological traits that evolve in response to temperature change in threespine stickleback. Using these data, we created geographic range projections under two climate change scenarios where trait data was either static (‘no evolution’ model), allowed to evolve in agreement with published evolutionary rates for the trait (‘evolution’ model)\(^4\), or allowed to evolve with the rate of evolution scaled in association with the variance that is explained by QTL (‘PVE’ model). Here, we show that incorporating these traits and their evolution into SDMs substantially altered the predicted ranges for a widespread panmictic marine population, with increases in area of over 7-fold. Evolution-informed SDMs should therefore improve the precision of forecasting range dynamics under climate change, thereby aiding in their application to management and the protection of biodiversity\(^5-7\).

Temperature is a powerful driver of global biogeography and organism distributions frequently reflect temperature gradients in both aquatic and terrestrial habitats\(^8\). Many species adopt thermal strategies (such as thermoregulation or acclimation) that determine their thermal niche\(^9-11\) and thermal traits can provide a target for directional selection if the environment changes to include temperatures outside the range encompassed by the thermal niche. Adaptation may thus permit species to persist at temperatures that would have previously led to extirpation\(^12,13\). Under moderate climate change scenarios, mean global oceanic temperature is predicted to increase in excess of 2°C by the end of the century\(^14\), with more extreme changes predicted in localized regions\(^15\). Predicting species distribution patterns under climate change...
therefore requires data for temperature-associated adaptive trait evolution, which can vary by
species and population. While there have been recent steps to incorporate theoretical trait
evolution into SDMs\(^3\), to date, no model has used empirical estimates of evolutionary rate to
inform predictions about future species distributions.

Due to widespread phenotypic variation\(^16\), genomic resources\(^17\), the availability of
temperature-associated ecological and evolutionary trait data\(^4\), and the ability to artificially breed
multiple hybrid generations in a common garden lab environment, threespine stickleback fish
\((Gasterosteus aculeatus\), Fig. 1a) are a useful vertebrate species for understanding the impact of
adaptation on range dynamics under climate change. Here, we incorporate the specific capacity
of marine populations to adapt their physiology to rapidly changing climate conditions to
characterize how adaptive trait variation affects projections of species range distributions under
climate change\(^18\).

Results

We collected and reared wild marine and freshwater stickleback from two marine and
two freshwater locations (Fig. 1b). These stickleback exhibited a wide thermal tolerance range
bounded by a mean CTmin of 2.09 °C (+/- 1.13 °C SD) and a mean CTmax of 30.4 °C (+/- 2.64
°C SD)(Fig. 1c) and also tolerated a wide range of temperatures within which there was no
observable stress response (5.0 – 25.0 °C). To determine if these measured thermal traits have a
genetic basis and could therefore be subject to adaptive evolution, we raised hybrid marine-
freshwater F1 (N=2) and F2 (N=4) families under common garden conditions and used these fish
for genome-wide linkage map construction (Table S1) and quantitative trait loci (QTL) mapping.
Using 25,001 high-quality single nucleotide variants generated from restriction site-associated
DNA (RAD) sequencing, we identified one significant QTL for each thermal tolerance trait (Fig. 2b) that explained a high percentage of trait variance (PVE; $CT_{\text{min}} = 54\%$, $CT_{\text{max}} = 64\%$).

We used these genetically based traits to inform the boundaries of three distinct environmental regions in species distribution models (SDMs) for marine stickleback based on varying levels of physiological performance: i) a ‘normal behaviour’ envelope with environmental temperatures associated with an absence of an observable behavioural stress response (5.0 to 25.0 °C), ii) a ‘within physiological limits’ envelope with environmental temperatures that fall within the range of the measured physiological limits (0.85 to 31.9 °C), and iii) an ‘outside of physiological limits’ envelope with environmental temperatures that fall outside the measured physiological limits (below 0.85 and above 31.9 °C). Based on sea ice extent and bathymetry alone, our present-day correlative SDM suggests a marine range distribution for these stickleback from the southern Bering Sea to northern Washington state, and along the southeast Alaskan Panhandle (combined shaded area in Fig. 3a). When we include species-specific thermal trait data from the wild marine populations, nearly the entire range of suitable habitat was unaffected by thermal tolerance limits, with the exception of a slight restriction at the northern end of the range (Fig. 3a). However, when restricted to the Normal Behaviour area, the range becomes confined to the west of the northern tip of Kodiak Island (Fig. 3a), a limit coinciding with the northern-most known marine population in the Pacific Northwest genetic cluster\textsuperscript{19}.

We next generated SDMs based on predicted end-of-century environmental variables according to the Intergovernmental Panel on Climate Change (IPCC) representative concentration pathways (RCPs) 4.5 and 8.5 from the Fifth Assessment Report\textsuperscript{14,20}. End-of-century IPCC predictions resulted in a substantial increase in the overall suitable habitat area for
stickleback, with a 2.25-fold or 1,338,219 km$^2$ increase (combined shaded area in Fig. 3b-e) in association with a reduction in sea ice concentration at the northern end of the range. When temperature increases as predicted by RCP 4.5 in the ‘no evolution’ model, there is a 5.86-fold (1,011,949 km$^2$) increase in the Normal Behaviour area within this newly suitable habitat (Fig. 3b) when compared to the current day model. Under RCP 8.5 in the ‘no evolution’ model, the entirety of suitable habitat area remains within tolerable limits (Fig. 3c), with a smaller proportion of the range falling outside of the Normal Behaviour area compared to RCP 4.5 (10.7%) ‘no evolution’ model.

Incorporating the evolution of CT$\min$ into the SDMs (‘evolution’ model) results in a large increase in the proportion of suitable habitat that falls within the Normal Behaviour area. We allowed CT$\min$ to evolve at a rate of 0.63 haldanes, which is equal to the rate observed for CT$\min$ in marine stickleback$^4$ (there are currently no empirical estimates of evolutionary rate for CT$\max$). Under RCP 4.5, almost all (99.9%) of the suitable habitat range falls within the Normal Behaviour area (Fig. 3d), while under the RCP 8.5 ‘evolution’ model, the entire range of suitable habitat is within the Normal Behaviour area (Fig. 3e). These represent a 7.45-fold increase (1,336,123 km$^2$) for RCP 4.5 and 7.46-fold increase (1,338,219 km$^2$) for RCP 8.5 in the Normal Behaviour area when compared to the current day SDM.

We next considered the effect of limiting the evolutionary rate of CT$\min$ based on the observed genetic architecture of a single major effect locus (‘PVE’ model). Under the RCP 4.5 ‘PVE’ model, we observed a 1.08-fold reduction in the Normal Behaviour area (109,120 km$^2$, Fig. S3) when compared to the ‘evolution’ model (Fig. 3d). Under RCP 8.5 projections with the ‘PVE’ model, we observed a 3,905 km$^2$ decrease in the Normal Behaviour area (Fig. S3) when compared to the ‘evolution’ model (Fig. 3e). This relatively small reduction in Normal
Behaviour area with adjusted PVE for the thermal traits under RCP 8.5 still results in a
1,334,314 km² increase in area compared to the current day SDM.

Discussion

We assessed the critical thermal minimum (CTmin) and maximum (CTmax) for
threespine stickleback from wild marine and freshwater populations, as well as F1 and F2
families in order to determine the genetic basis underlying traits that will be important for
population persistence under climate change. We incorporated the empirical ecological and
evolutionary trait data from wild marine populations into mechanistic species distribution models
under two climate change scenarios. We estimated the species distribution in the Pacific
Northwest marine environment while these traits were held constant (‘no evolution’), allowed
CTmin to evolve in accordance with evolutionary rate estimates (‘evolution’), and allowed
CTmin to evolve at a constrained rate associated with the percent of trait variance explained by
the single QTL we detected (‘PVE’). The geographic ranges predicted for the end-of-century
species distributions increased by over 7-fold (RCP 4.5: 1,336,123 km²; RCP 8.5: 1,338,219
km²) when CTmin was allowed to evolve, a substantial increase over the ‘no evolution’ model.
Additionally, when CTmin evolution was constrained in the PVE model, there remained a ~6-
fold increase (RCP 4.5: 1,227,002 km²; RCP 8.5: 1,334,314 km²) in the geographic range
compared to current day. These differences in the predicted distributions underline the
significance of incorporating empirical evolutionary data into SDMs⁵,²¹,²², and in particular the
need to consider behaviour in addition to physiology when predicting range shifts²³.

While the results presented here showcase the importance of creating more robust and
informed SDMs, they also highlight a number of aspects that will benefit from additional
consideration when interpreting these models. The existing estimate of CTmin evolution in
sticklebacks considered the change in phenotypic variation across generations, rather than evolution at underlying loci. As such, it is likely that some proportion of the observed phenotypic change was due to plastic responses. In our PVE model, we take a conservative approach by restricting phenotypic evolution of CTmin to only occur through heritable change via the locus shown to be associated with the trait. The efficiency of translating the selection acting on a trait into evolutionary response across generations can depend on the genetic architecture of the trait. The large effect loci that we identified here are consistent with expectations from theory suggesting that prolonged bouts of adaptation with gene flow (as expected in this system) should favour architectures characterized by fewer, larger effect, more tightly linked alleles. However, it should be noted that the effects of the two QTL identified here are likely overestimated and other loci might have gone undetected (sensu the Beavis Effect). The joint action of plastic effects and evolution at undetected loci might therefore result in range distributions that are more similar to those predicted in our ‘evolution’ models.

Climate change is leading to an increase in the frequency of extreme temperature events, including both extreme heat and extreme cold, which could drive selection on both CTmin and CTmax. Our models reveal that the evolution of cold tolerance can have a significant impact on predicted range distributions despite most end-of-century climate change scenarios involving an overall warmer, not cooler world. This counterintuitive result occurs because climate change opens up newly available thermal niche space in waters north of the current day geographic range, and the evolution of CTmin extends this range expansion further still. Northward range expansion with climate change due to increasing habitat availability has also been documented in birds, plants, other fishes, and pest species (such as ticks and mountain pine beetle), as well as in large scale analyses of diverse taxa assessing the
‘fingerprints’ of climate change impacts\textsuperscript{51,52}. However, it is likely that evolution of CTmax will also play a role in responses to environmental change\textsuperscript{53–55}. Although we have no empirical estimates of CTmax evolution, it is interesting to explore how distributions would shift if we observed the same rate of evolution in this trait as in CTmin. Using the same rate of haldanes and incorporating our observed PVE for the locus associated with CTmax, we find that geographic ranges predicted for the end-of-century species distributions also increased by over 7-fold (RCP 4.5: 1,227,002 km\textsuperscript{2} increase; RCP 8.5: 1,334,314 km\textsuperscript{2} increase; Fig. S4). Further investigations to test the empirical rate of evolution of thermal behaviour, physiology and the molecular underpinnings of these key traits would be well served by assessing additional samples along the latitudinal gradient inhabited by stickleback to gain a more detailed understanding of these temperature-associated traits over a wider environmental range.

Collectively, the inclusion of thermal traits and their evolution alters the projected ranges of threespine stickleback, with a substantial increase in the predicted area that the species will occupy under climate change forecasts. Many traits are evolving in response to climate change\textsuperscript{56–59} and SDMs that do not take trait data (and trait evolution) into account could provide inaccurate predictions about future species distributions under climate change\textsuperscript{3} - an issue of particular concern for species at risk and pest species undergoing range expansion\textsuperscript{60–62}. Our results provide a framework for addressing this problem, which will have critical implications for the application of these models in policy and resource management, and the protection of biodiversity in a changing climate.
Materials and Methods

Sample collection and husbandry

We collected adult *Gasterosteus aculeatus* (Fig. 1a) from two marine populations (Bamfield, M1, 48°49'12.69"N 125° 8'57.90"W; Garden Bay Lagoon, M2, 49°37'52.84"N 124° 1'49.26"W) and two freshwater populations (Hotel Lake, FW1, 49°38'26.94"N 124° 3'0.69"W; Klein Lake, FW2, 49°43'32.47"N 123°58'7.83"W) in southwestern British Columbia (Fig. 1b). Individuals were maintained in a flow-through system and photoperiod that mimicked the source populations during collection periods before transport. We transported the fish to our aquatics facility in the Life and Environmental Sciences Animal Resources Centre at the University of Calgary, where we separated the fish into population-specific 113 L glass aquaria at a density of approximately 20 fish per aquarium. We acclimated marine individuals to freshwater salinity over one week and maintained fish in a common environment (salinity of 4-6 ppt, water temperature of 15 ± 2 °C, and a photoperiod of 16L:8D). Individuals were allowed to acclimate for at least 2 weeks before experiments (1 week for stress reduction post-transfer, 1 week for common garden environment acclimatation and salinity ramp). Each common garden aquarium was on a closed system with individual filters, air stones, and water supply. We fed all adult fish *ad libitum* once per day with thawed bloodworms (Hikari Bio-Pure Frozen Bloodworms). All collections and transfers were approved by the Department of Fisheries and Oceans (marine collections and transfers), the Ministry of Forests, Lands, and Natural Resource Operations (freshwater collections), and the Huu-ay-aht First Nations (marine collections).

Crossing design for marine and freshwater F1 families

We collected eggs from females and fertilized the eggs with extracted testes from euthanized males. We transferred the fertilized egg mass to a mesh-bottomed egg incubator...
suspended in a 37 L aquarium for hatching. Each hatching aquarium was maintained with a single air stone and a filter. Once hatched, we reared the larval fish in 37 L hatching aquaria until they reached a total length (TL) of approximately 1 cm, after which we split the families into family-specific 113 L aquaria to maintain suitable densities. We fed the larval fish ad libitum twice daily with live *Artemia spp.* nauplii, and then gradually transitioned the diet to chopped, thawed bloodworms (Hikari Bio-Pure Frozen Bloodworms) ad libitum once daily as they reached approximately 2 cm TL. The F1 families were maintained in a common garden environment identical to that of the F0 populations. We produced one F1 family for each population (M1_F1, M2_F2, FW1_F1, and FW2_F1).

**Crossing design for hybrid mapping families**

To generate genetically heterogeneous marine-freshwater F1 families from wild F0 parents, we collected eggs from marine females and fertilized the eggs with extracted testes from euthanized freshwater males. Egg masses were hatched, and juveniles were reared, as detailed above. Overall, we produced one F1 family of M1xFW1 hybrids (hereafter referred to as H1_F1) and three F1 families of M1xFW2 hybrids (hereafter referred to as H2_F1). The hybrid F1 families were maintained in a common garden environment identical to that of the F0 populations. To generate F2 families for linkage map construction, we crossed individuals from the same F1 family with the same methodology used to generate the F1 families. Overall, we produced one F2 family of H1xH1 hybrids (referred to as H1_F2) and three families of H2xH2 hybrids (referred to as H2_F2_1, H2_F2_2, and H2_F2_3). All F2 individuals were raised as described above in a common garden environment identical to that of the F0 and F1 individuals to ensure consistent history and use for QTL mapping.
Thermal tolerance experiments

To assess the lower and upper limits of physiological thermal tolerance, we conducted standard critical thermal minimum (CTmin) and maximum (CTmax) experiments on adult fish. At these sublethal limits, the fish experiences a loss of equilibrium (LOE) at which they lose the ability to escape conditions that would ultimately lead to their death in nature. Our experimental tank held 1000 mL glass beakers aerated individually to prevent thermal stratification. Before each experiment, individuals were fasted for 24 hours. After a 15-minute acclimation to the experimental apparatus in the individual beakers, we cooled or heated the water (for CTmin or CTmax, respectively) at a rate of approximately 0.33 °C min⁻¹. We assessed wild F0 individuals (nM1 = 32, nM2 = 14, nFW1 = 15, nFW2 = 16, N = 77; Fig. 1c) and lab raised F1 (nM1_F1 = 13, nM2_F1 = 15, nFW1_F1 = 15, nFW2_F1 = 15, N = 58; Fig. S1) and F2 individuals (nH1_F2 = 28, nH2_F2_1 = 36, nH2_F2_2 = 21, nH2_F2_3 = 17, N = 102; Fig. S2). All individuals were assessed for CTmin, allowed to recover for at least three days, then assessed for CTmax to keep thermal stress history consistent. The onset of erratic behaviours associated with a behavioural stress response occurred below 5.0 °C and above 25.0 °C during CTmin and CTmax experiments, respectively. Normal behaviour was observed between 5.0 °C and 25.0 °C, whereas outside of those temperatures, individuals gradually exhibited more extreme stress responses (e.g., increased gilling rate, erratic movement, muscle spasms, listing, as outlined by the Canadian Council of Animal Care guidelines) until reaching LOE and the inability of an individual to right itself (the experimental endpoint). At the time of data collection for thermal trait experiments, all individuals were adults.
Isolation and characterization of single nucleotide polymorphisms (SNPs)

Genomic DNA was extracted from caudal fin tissue using a phenol-chloroform-based protocol. We digested tissues overnight in digestion buffer and proteinase K at 55 °C, then performed multiple phenol-chloroform and ethanol washes to isolate the DNA. We assessed the quantity of the extracted DNA using the Quant-iT PicoGreen dsDNA assay kit (ThermoFisher Scientific, Waltham, MA, USA) and Synergy HT plate reader with the Gen5 associated software (BioTek, Winooski, VT, USA). We prepared restriction site-associated DNA (RAD) libraries (Peterson et al. 2012) using MluCl and NlaIII restriction (New England Biolabs, Ipswich, MA, USA), ligation of individual barcodes, and pooling of 48 individuals per library at equimolar concentrations. We performed a final PCR to amplify DNA and add library-specific indices to allow for pooling of multiple libraries. We sequenced three libraries at McGill University and Génome Québec Innovation Center on one lane of Illumina HiSeq 4000 (Illumina Inc., San Diego, CA, USA).

Assembly of genetic linkage map

After barcode demultiplexing and filtering out low quality reads in STACKS\textsuperscript{66}, we removed PCR duplicates from the raw sequences and aligned to the \textit{G. aculeatus} reference genome\textsuperscript{17} using the Burrows-Wheeler transform\textsuperscript{67}. Individual libraries were concatenated and filtered\textsuperscript{68} using \textit{vcftools} v3.0\textsuperscript{69} and then split into chromosome-specific VCF files to assemble the linkage maps chromosome by chromosome. We assigned markers to a linkage group with an initial LOD score of 3 after filtering out markers that showed high levels of segregation distortion and missing observations (> 20% missing data) in Lep-MAP3\textsuperscript{70}. Unassigned markers were subsequently added to the existing linkage group at a LOD score of 3 and a size limit of 5 markers per linkage group. We ordered the markers using a minimum posterior value of 0.001
and collapsed multiple markers when the probability difference between markers was < 0.01.

The final linkage map was subset for use in R with a custom Python script to visualize the linkage map and to generate a list of informative SNPs to use in subsequent analyses with the qtl v1.44 and qtlTools v1.2.0 packages. Linkage maps were visualized using LinkageMapView in R. The final linkage maps were similar across families in number of markers, length, and spacing between markers, though the H1_F2 map did have a higher density of markers (Table S1).

Quantitative trait loci (QTL) mapping

We analysed families separately with the same methodology to assess the presence of QTL associated with the thermal traits. We calculated conditional genotype probabilities using hidden Markov model technology and simulated genotypes based on the observed marker data (allowing for possible genotyping errors at a level of 0.0001 using a Kosambi mapping function with a fixed step width) prior to running genome scans with a single QTL model. We determined the logarithm of the odds (LOD) score significance thresholds for each trait through permutation tests for each family (100,000 permutations) (Fig. 2a). We pulled significant QTL above the genome-wide significance threshold ($\alpha = 0.05$), calculated confidence intervals of QTL location based on nearby markers, and estimated the percent variance explained by each QTL peak marker. We identified two QTL on linkage group 4 (which corresponds to chromosome 4 of the BROAD assembly) associated with CTmin and CTmax (Fig. 2b).

Environmental variables and species distribution models (SDMs)

We compiled environmental data widely used in the construction of SDMs to estimate suitable habitat in both present day and end-of-century forecasts, including bathymetry, sea ice extent and concentration, salinity, and sea surface temperature. We used 2014 data as our
baseline year to match the forecasting baseline of the Fifth Assessment Report\textsuperscript{14}. We assumed a suitable habitat range for this species in the Pacific Northwest to consist of coastal areas (where the water depth is less than 200 m) where sea ice is never present (\textit{i.e.}, no sea ice at the maximum extent). The salinity tolerance for \textit{G. aculeatus} is very wide\textsuperscript{79,80} and salinity was not limiting in any of the habitat\textsuperscript{81}, therefore salinity was not included in the final present day or forecasted models. We obtained bathymetry data from the General Bathymetric Chart of the Oceans (GEBCO) of the British Oceanographic Data Centre\textsuperscript{82}, and maximum sea ice extent data from the Multisensory Analyzed Sea Ice Extent – Northern Hemisphere (MASIE-NH) product\textsuperscript{83}. We obtained maximum and minimum daily mean sea surface temperature (SST)\textsuperscript{84}. Sea surface temperature was used as a proxy for water temperature. Stickleback thermal trait data were used to set the limits of the distribution within the possible area delineated by sea ice free water of a suitable depth (Table S2). The thermal trait measurements were all based on our experimental findings reported here.

In the end-of-century forecast for suitable habitat, we assumed bathymetry to be consistent with the modern scenario. However, the Arctic Ocean is predicted to be predominantly free of sea ice in the summer by the end of the century\textsuperscript{85}, with significant end-of-century reductions in winter/spring sea ice concentration (reduced to a concentration of 0.1 at the Seward Peninsula\textsuperscript{85}), so we conservatively set the maximum northern extent of the suitable habitat to be 65°35’ N, which corresponds to the western tip of the Seward Peninsula (near Wales, AK). The extent of sea ice was kept consistent between scenarios to control for area in calculations of range expansion. The water temperatures were increased based on projections for large marine ecosystems of Northern Oceans from global climate models\textsuperscript{14,20}. Maps were created in R\textsuperscript{71} using the packages \textit{raster} v. 3.3-13\textsuperscript{86} and \textit{rgeos} v. 0.5-3\textsuperscript{87}. 
We incorporated the experimental data from the critical thermal minimum and maximum trials on the wild marine populations (Fig. 1c) to understand how trait inclusion may affect range projections under climate change. These trait-defined envelopes were overlain on the suitable habitat background to delineate projected presence based on thermal traits in both current day and IPCC predicted RCPs 4.5 and 8.5. The trait values were kept constant (i.e., not changed) in the ‘no evolution’ projections, but in the ‘evolution’ projections, we allowed CTmin to evolve an improvement of 2.5 °C (i.e., 2.5 °C lower than CTmin boundary in the ‘no evolution’ projection) by the end of the century based on a rate of 0.63 haldanes from a selection experiment previously conducted on populations from this same genetic cluster\(^4\). The ‘evolution’ model assumes whole-organism tolerance evolution with selection acting on 100% of the loci affecting CTmin evolution. Therefore, to account for the observed genetic architecture of a single, large effect locus associated with CTmin, we next considered a ‘PVE’ model, where CTmin was allowed to evolve to only 54% of the total estimated trait value from the ‘evolution’ model (i.e., 2.5 °C * 0.54). However, a notable restriction in the evolution of CTmin for both models (‘evolution’ and ‘PVE’) was a hard boundary drawn at 0 °C under the assumption that population persistence in a sub-zero environment would require many additional adaptations alongside CTmin improvement (e.g., extreme adaptations observed in Antarctic notothenionid fishes\(^88-90\)).

To quantify the differences in estimated suitable habitat under current day and end-of-century conditions, we compared areas for each warming scenario to the equivalent scenario under current conditions. Similarly, to compare the differences in evolutionary scenarios, the area of each end-of-century evolutionary trajectory was compared to either the contrasting RCP projection or adjusted PVE projection. For these comparisons, we used North Pole Lambert...
azimuthal equal area projection for all maps, and georeferenced to known landmarks in ArcGIS v10.8\textsuperscript{91} to calculate area from the maps generate in R\textsuperscript{71} (conversion ratio of 7873.42).

The datasets generated and analysed during the current study are available from the corresponding author upon reasonable request. The annotated code, including all parameter thresholds, for the above QTL analyses and SDM construction is publicly available on Github (github.com/sjswuitchik/gasAcu_qtl_sdm).

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Author Contributions

This study was designed by SJSW, RDHB, and SMR; fish husbandry and breeding by SJSW and TNB; experimental data collection by SJSW; DNA sequencing and initial processing by AP; bioinformatic and QTL analyses by SJSW; species distribution modelling by SJSW and SM; the manuscript was written by SJSW, RDHB, and SMR, with input from authors; the study was funded by HAJ, RDHB, and SMR.

Competing Interests: The authors declare no competing interests.
**Figures and Tables**

![Figure 1](image-url)

Figure 1. a) Adult threespine stickleback (*Gasterosteus aculeatus*) from a single genetic cluster were sampled from b) two marine and two freshwater populations in the Canadian Pacific Northwest. These populations were assayed for c) critical thermal minima and maxima. Thermal trait values for marine populations (M1 and M2) were incorporated into the species distribution models, while marine and freshwater populations (M1, FW1, and FW2) were used to generate F1 and F2 generations for linkage map construction and quantitative trait loci (QTL) analyses.
Figure 2. a) Quantitative trait loci (QTL) scan of linkage group 4 with trait-specific significance thresholds for LOD scores, showing a significant LOD peak for CTmin (dark purple) and CTmax (light blue) with b) an inset of linkage group 4 highlighting the position of the significant QTL for upper and lower thermal tolerances (CTmax and CTmin, respectively).
Figure 3. Changes in the distribution of marine threespine stickleback (*Gasterosteus aculeatus*) as a result of incorporating thermal traits in a) current day environmental conditions and under IPCC end-of-century projections RCP 4.5 and 8.5 without trait evolution (‘no evolution’ model, b & c, orange box) and with trait evolution (‘evolution’ model, d & e, red box).
Supplementary Figures and Tables

**Figure S1.** Critical thermal minimum (CTmin) and maximum (CTmax) measurements for adult threespine stickleback (*Gasterosteus aculeatus*) from pure F1 marine (M*F1) and freshwater (FW*F1) families raised in a common garden under a constant thermal environment.
Figure S2. Critical thermal minimum (CTmin) and maximum (CTmax) measurements for adult threespine stickleback (Gasterosteus aculeatus) from hybrid marine-freshwater F2 families raised in a common garden under a constant thermal environment.
Figure S3. Changes in the distribution of marine threespine stickleback (*Gasterosteus aculeatus*) as a result of incorporating thermal traits under IPCC end-of-century projections RCP 4.5 and 8.5 with trait evolution constrained by the underlying genetic architecture of critical thermal minimum (CTmin) as determined from hybrid F2 mapping families.
Figure S4. Changes in the distribution of marine threespine stickleback (Gasterosteus aculeatus) as a result of incorporating thermal traits under IPCC end-of-century projections RCP 4.5 and 8.5 with trait evolution constrained by the underlying genetic architecture of critical thermal minimum (CTmin) and critical thermal maximum (CTmax) as determined from hybrid F2 mapping families.
Table S1. Summaries of family-specific linkage maps constructed for quantitative trait loci (QTL) analyses.

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Table S2. Thermal trait data from *Gasterosteus aculeatus* used to inform the species distribution envelopes (rows) in the varied evolutionary scenarios (columns) projected for end-of-century conditions.

<table>
<thead>
<tr>
<th></th>
<th>Current day</th>
<th>No evolution</th>
<th>Adjusted PVE</th>
<th>Evolution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Behaviour</td>
<td>(5 - 25)</td>
<td>(5 - 25)</td>
<td>(3.6 - 25)</td>
<td>(2.5 - 25)</td>
</tr>
<tr>
<td>Within Physiological Limits</td>
<td>(0.9 - 31.9)</td>
<td>(0.9 - 31.9)</td>
<td>(0 - 31.9)</td>
<td>(0 - 31.9)</td>
</tr>
<tr>
<td>Outside Physiological Limits</td>
<td>31.9 &gt; x &lt; 0.9</td>
<td>31.9 &gt; x &lt; 0.9</td>
<td>31.9 &gt; x &lt; 0</td>
<td>31.9 &gt; x &lt; 0</td>
</tr>
</tbody>
</table>