MOLECULAR ECOLOGY

Molecular Ecology (2013) doi: 10.1111/mec.12240

Thermal adaptation and acclimation of ectotherms from differing aquatic climates

SHAWN R. NARUM,*† NATHAN R. CAMPBELL,* KEVIN A. MEYER,‡ MICHAEL R. MILLER§ and RONALD W. HARDY†

*Columbia River Inter-Tribal Fish Commission, 3059F National Fish Hatchery Road, Hagerman, ID, 83332, USA, †University of Idaho, 3059F National Fish Hatchery Road, Hagerman, ID, 83332, USA, ‡Idaho Department of Fish & Game, 1414 East Locust Lane, Nampa, ID 83686, USA, §Institute of Molecular Biology, University of Oregon, Eugene, OR 97403, USA

Abstract

To elucidate the mechanisms of thermal adaptation and acclimation in ectothermic aquatic organisms from differing climates, we used a common-garden experiment for thermal stress to investigate the heat shock response of redband trout (*Oncorhynchus mykiss gairdneri*) from desert and montane populations. Evidence for adaptation was observed as expression of heat shock genes in fish from the desert population was more similar to control (unstressed) fish and significantly different ($P \leq 0.05$) from those from the montane population, while F1 crosses were intermediate. High induction of heat shock proteins (Hsps) in the montane strain appeared to improve short-term survival during first exposure to high water temperatures, but high physiological costs of Hsp production may have led to lower long-term survival. In contrast, the desert strain had significantly lower heat shock response than the montane fish and F1 crosses, suggesting that these desert fish have evolved alternative mechanisms to deal with thermal stress that provide better balance of physiological costs. Genomewide tests of greater than 10 000 SNPs found multiple SNPs that were significantly associated with survival under thermal stress, including Hsp47 which consistently appeared as a strong candidate gene for adaption to desert climates. Candidate SNPs identified in this study are prime targets to screen more broadly across this species' range to predict the potential for adaptation under scenarios of climate change. These results demonstrate that aquatic species can evolve adaptive responses to thermal stress and provide insight for understanding how climate change may impact ectotherms.

Keywords: adaptation, climate change, ectotherms, fish, gene expression, RAD-seq

Received 20 August 2012; revision received 2 November 2012; accepted 16 November 2012

Introduction

Environmental temperatures have extensive biological implications for all organisms, but ectotherms in aquatic systems are particularly affected by thermal profiles and climate regimes. This is because aquatic ectotherms primarily exchange heat with their environment through conduction and convection, and their body temperature closely follows the temperature of surrounding water. Thus, environmental temperatures greatly influence the distribution, physiology and behaviour of aquatic organisms, and local adaptation to thermal systems is the norm (e.g. Schluter 2000). Further, many aquatic organisms have developed capacities for thermal acclimation that provide greater tolerance to chronic exposure to stressful temperatures (Tomanek & Somero 1999; Hoffmann *et al*. 2003; Sinclair & Roberts 2005).

The processes of thermal acclimation and adaptation are often interdependent and distinct strategies enable species to occur over broad geographic ranges with highly variable climate regimes. Empirical studies have shown that populations at thermal extremes consist of specialists, and populations elsewhere contain generalists that function over a wider range of temperatures (reviewed in Pörtner 2010). However, temperatures

Correspondence: Shawn R. Narum, Fax: 208 837 6047;
E-mail: nars@critfc.org

may affect organisms differently, with shifts in reaction norms that cause changes in niche width, mean performance or optimal temperature (Knies *et al.* 2006). In the case of thermal adaptation within a species that occurs over a wide geographical range with variable climate, reaction norms for performance (i.e. fitness) could include multiple changes such as horizontal shifts related to environmental temperature along with reduction of niche width as shown in the theoretical example in Fig. 1. Further, initial exposure to extreme temperatures often causes greater tolerance of subsequent thermal extremes ('acclimatization', 'preconditioning' or 'hardening'; Beitinger & Bennett 2000; Hoffmann *et al.* 2003; Sinclair *et al.* 2003; Sinclair & Roberts 2005; Pörtner 2010). For this study, we define thermal adaptation as *evolution* of a population to an altered reaction norm for temperature, whereas thermal acclimation is any *phenotypic response* of individuals to environmental temperature that alters performance and plausibly changes fitness (following Angilleta 2009). Therefore, acclimation responses of individuals can produce fitness advantages that result in evolutionary change in a population over generations (e.g. Pörtner 2010).

Anthropogenic climate change (IPCC 2007) has caused concerns that some fishes may be extirpated or need to relocate due to limitations related to thermal tolerance (e.g. Perry *et al.* 2005; Pörtner & Knust 2007). However, thermal acclimation and adaptation of fishes from variable environments have not been well studied, and therefore, the ability to predict the adaptive potential of natural populations under scenarios of climate change is limited. Current molecular and genomic tools provide the opportunity to investigate the heat shock response of fishes from varying thermal regimes and link that information with adaptive regions of the genome that is under selection. Specifically, heat shock proteins (Hsps) have been demonstrated to be induced and act as molecular chaperones in all organisms to a variety of stressors including heat stress (Feder & Hofmann 1999; Basu *et al.* 2002; Sørensen *et al.* 2003), and thus, quantitative gene expression can provide a basis for the physiological response of individuals under experimental thermal conditions. Next-generation sequencing technologies allow for dense genome-wide association mapping of quantitative and binary phenotypic traits (i.e. heat shock response and survival/mortality). These approaches offer the power of genome-wide association in combination with the physiological response of heat stress to identify patterns of acclimation and adaptation.

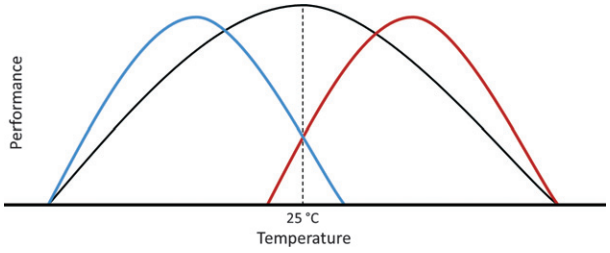

Fig. 1 Reaction norms for performance may shift (width, mean or peak) to become locally adapted to environmental conditions such as temperature. In the example shown here, the mean reaction norm for the species has shifted from the standard condition (black line) to either cool (blue line) or warm (red line) environments, and niche width has narrowed.

In this study, we investigated patterns of thermal acclimation and adaptation in populations of redband trout (*Oncorhynchus mykiss gairdneri*) from differing aquatic climates. Redband trout are native and common in the interior region of the Pacific Northwest of the United States and occupy streams in both desert and montane climates with significantly different habitat characteristics (Meyer *et al.* 2010). Optimal temperature for this species has been found to be approximately 13.6 °C (Bear *et al.* 2007), but redband trout have been observed in desert streams with water temperatures exceeding 30 °C (Zoellick 1999; Rodnick *et al.* 2004). Previous studies of this species have demonstrated local adaptation of populations to desert and montane climates (Gamperl *et al.* 2002; Narum *et al.* 2010), and the current study investigates specific populations that appear to be adapted to different thermal regimes. A common-garden experiment for thermal stress was used to investigate the heat shock response of redband trout from desert and montane populations, and their F1 crosses. We tested hypotheses related to the heat shock response and identified genes associated with survival under thermal stress that was designed to represent natural diel conditions during summer months in a desert stream environment. Results demonstrate three primary findings: (i) acclimation of the heat shock response over time; (ii) adaptive heat shock response in redband trout from the desert population relative to those from a cooler environment and (iii) adaptive signatures of selection at genes relevant to thermal stress.

Methods and materials

To investigate thermal acclimation and adaptation of redband trout (*O. mykiss gairdneri*) from desert and montane populations, fry of approximately 4 months of age from each environment and their F1 crosses were exposed to diel temperature cycles (peaking at 28 °C) over a 6-week period in a controlled setting. Liver and gill tissues were collected from euthanized individuals on day 1, 3, 7 and 28 to quantify mRNA expression of six heat shock genes. Mortality of each strain was recorded daily, and fin tissue was sampled from all

mortalities and survivors over the course of the study for genome-wide association tests.

Fish populations

Gametes and fry were collected from two locations (desert climate—Little Jacks Cr., Idaho, USA, and montane climate—Keithly Cr., Idaho, USA), cross-mated and reared in the laboratory to represent populations adapted to different environments. These two sites were chosen for further study based on previous tests of six desert and six montane streams (Narum *et al.* 2010). Gametes were fertilized to produce half-sibling progeny of pure desert strain, pure montane strain and F1 crosses. Fry were reared in constant 15 °C spring water until they reached an average weight of 2 g, and then, each strain was divided into treatment and control groups. Three replicate tanks were used to estimate survival for all treatment and control groups for each strain (3 tanks × 3 strains × 2 treatments equals a total of 18 tanks), with an average of 43 fish per tank. Fish were fed a diet of Soft Moist pellets (Rangen Inc.) to satiation twice per day, and photoperiod was fixed at 14 h light and 10 h dark. Fish in recirculating treatment tanks experienced diel temperature cycles over 6 weeks that reached a maximum of 28.5 °C in the afternoon and a minimum of 17.0 °C at night (mean temperature gradient of ~1.5 °C per hour; Fig. S1, Supporting information), while fish in control tanks were held at a constant temperature of 15 °C. All experimental protocols were approved in advance by the University of Idaho's Institutional Animal Care and Use Committee.

Gene expression

To investigate heat shock response over the course of the experiment, the expression of six heat shock genes was measured at four time periods over the course of the experiment for each of the three strains. Gill and liver tissues were sampled from fish euthanized with 250 mg/L of tricaine methanesulfonate (MS-222) at the time of peak temperatures from both treatment and control tanks on four separate days during the 6-week thermal stress experiment (day 1, 3, 7, 28). Three fish were netted from each replicate tank for a total of nine samples representing each strain for each control and treatment group on each of the four sampling days. Tissues were stored in RNA-later and frozen at −80 °C. Total RNA was isolated with RNeasy kits (Qiagen), and cDNA was prepared with kits from Ambion. Quantitative real-time polymerase chain (qRT-PCR) reactions with Syber Green assays (LifeTechnologies) were designed for six heat shock genes (Table S3, Supporting information) with primer sequences from previous work (Almany *et al.* 2009). All qRT-PCRs were completed with a standard curve and included both elongation factor and beta-actin as a reference gene, but beta-actin was found to provide the most consistent data and so beta-actin was the final reference gene chosen for this study. Expression values for each individual were normalized to beta-actin, and mean expression was calculated for each strain for control and treatment groups on each of the four sampling days. The mean expression of unstressed control fish was subtracted from mean expression of treatment fish of each strain to correct for constitutive expression and any potentially confounding signals of expression (e.g. handling stress). Significance between strains and days was tested with analysis of variance (ANOVA). Heat shock protein levels have been demonstrated to be elevated in redband trout in response to heat stress in previous studies (Cassinelli & Moffitt 2010).

Genome-wide association

To test for genome-wide association with survival to heat stress, fin tissue was sampled from survivors and mortalities through the experiment and genotyped at thousands SNP markers. Fin tissue was collected from all fish mortalities by date during the course of the 6-week thermal stress experiment and from all surviving fish at the end of the experiment. Tissues were preserved by dry storage on Whatman chromatography paper until DNA extractions were completed with DNeasy kits (Qiagen). Template DNA from all individuals was quantified with a spectra-fluorometer (Victor3V, Perkin Elmer) and normalized across samples.

To genotype tissues at thousands of SNPs, samples were prepared for library construction with restriction-site-associated DNA (RAD) protocols (Baird *et al.* 2008; Miller *et al.* 2012). Briefly, DNA was digested with Sbf1 and subsequently ligated with both a barcode adapter and an Illumina sequencing adapter. Barcoded adapters allowed an average of 29 individuals to be pooled in single libraries for sequencing on an Illumina HiSeq 2000 instrument with single-end 100 reads. Pooling strategy was determined based upon the number of expected reads from reagents available from Illumina for sequencing on the HiSeq 2000 instrument. Samples were sequenced to reach a minimum target of 1.5 million reads per individual, and data were analysed following the pipeline by Miller *et al.* (2012) as described in detail by Hecht *et al.* 2012; (this issue). Briefly, sequence reads of 100 bp were sorted by barcode, trimmed to 80 bp and filtered to remove low-quality reads.

Reads from 10 individuals were used for initial SNP discovery, and reads from all other individuals were subsequently aligned against the discovered SNPs. Sequence data are available through accession number SRA057008

in the NCBI Short Read Archive database. Scoring criteria were developed to filter out false SNPs caused by sequencing error but also to adequately score heterozygotes in Hardy–Weinberg proportions. Genotypes for each SNP were scored using criteria that required a minimum of five reads to call homozygotes, and an allelic ratio of less than 10:1 to call a heterozygote. Read counts that did not meet these criteria were scored as missing genotypes. All SNPs were tested for Hardy–Weinberg equilibrium within each population in PLINK, and those that deviated significantly were removed from further analyses. Another 188 SNPs from expressed sequence tags (ESTs) of interest were genotyped with Taqman assays run on 96.96 integrated fluidic chips from Fluidigm with standard protocols as described in Narum *et al.* (2010). In total, 10,685 SNPs were genotyped across samples, and tests for quality control and genomewide association were completed in PLINK (Purcell *et al.* 2007). Tests for deviation from Hardy–Weinberg equilibrium were used to identify anomalous loci and potential paralogous sequence variants, and these SNPs were ignored for association tests.

Two separate strategies were used for genome-wide association tests with mortality: first testing for association over all three populations taking stratification into account and second testing for association within each of the three strains individually (Little Jacks Cr., Keithly Cr., and F1 crosses). To reduce false positives, BY-FDR corrections for multiple tests (False Discovery Rate of Benjamini & Yekutieli 2001) were used to identify significant associations. A BLASTn search (NCBI) was completed for significantly associated SNPs, and only hits with high sequence similarity (coverage >90%, identity >88%, and *e*-value < 1.00 E-10) were annotated according to this method.

Results

A strong signal for acclimation of the heat shock response was observed in all strains of redband trout as measured by qRT-PCR in six heat shock genes. In five of the six heat shock genes (Fig. 2a–f), mRNA expression was highly induced at day 1 but was significantly ($P \leq 0.05$) reduced in the remainder of the 6-week thermal stress experiment (peak temperatures reaching ~28 °C daily). By day 3, levels of expression for Hsp70, Hsp90, Hsp47 and Hsp27 (Fig. 2a–d) were significantly lower than day 1 ($P \leq 0.05$) and more similar to control fish that were held at a constant temperature of 15 °C. In contrast to the strongly induced upregulation observed in the four Hsp genes, the two transcription factors Hsf1 and Hsf2 (Fig. 2e–f) were downregulated, and the signal of acclimation was either weak (Hsf2) or absent (Hsf1). In the case of Hsf2, expression was not significantly different from day 1 until day 7 or day 28, depending on the strain (Fig. 2f).

In addition to acclimation, patterns of adaptation were also observed in the heat shock response between strains of redband trout from desert and montane streams as well as their F1 crosses. In most cases, the relative expression of heat shock genes in fish from the desert population of Little Jacks Cr. was more similar to control (unstressed) fish and significantly different ($P \leq 0.05$) from those from the montane population of Keithly Cr. (Fig. 2a–f). The F1 crosses typically had levels of expression that were intermediate to the pure strains and were significantly different ($P \leq 0.05$) from at least one of the pure strains at day 1 in four of the genes (Hsp70, Hsp47, Hsf1 and Hsf2). These patterns of adaptation were most prevalent at first exposure to high temperatures on day 1, but Hsp47 (Fig. 2c) and Hsf2 (Fig. 2f) showed consistently significant differences among strains over the course of the experiment even after acclimation. This was not the case for Hsp70 (Fig. 2a) and Hsf1 (Fig. 2e) as relative expression between strains was only significant on day 1, but not at day 3, day 7 or day 28. Inconsistent differences in gene expression among strains were observed in Hsp90 (Fig. 2b) and Hsp27, and no clear patterns of significance were evident.

Tissue samples were also collected from individual fish to test for genome-wide association with mortality over the course of the 6-week thermal stress experiment. In general, mortality was highest during the first 24 h of exposure to high temperatures. Interestingly, redband trout from the desert population of Little Jacks Cr. had higher mortality ($P = 0.05$) in the first 24 h than those from the montane population of Keithly Cr., but montane fish had highest mortality over the remainder of the 6-week experiment (no significant differences among strains; Fig. 3). Mortality of F1 crosses was not significantly different from either of the pure strains despite higher rates in the first 24 h and lower rates in the remainder of the experiment than Keithly Cr. fish (Fig. 3). Total mean mortality for each strain was 76.7% for Little Jacks Cr., 72.2% for F1 crosses and 56.5% for Keithly Cr, but these total mortality levels accumulated at different rates.

Tests over all strains for genome-wide association with mortality revealed regions of the genome that were under selection for thermal stress. More than 10 000 SNPs were discovered and genotyped in individuals with restriction-site-associated DNA (RAD) sequencing, and tests for stratified allelic association across all strains identified 18 SNPs that were highly significant after corrections for multiple tests to reduce false positives (Fig. 4; Table S1, Supporting information). The majority of these associated SNPs were from unknown or unannotated loci (Table S1, Supporting information), but the most significant SNP was from the 3′ UTR region of a known EST for Hsp47 (involved in heat

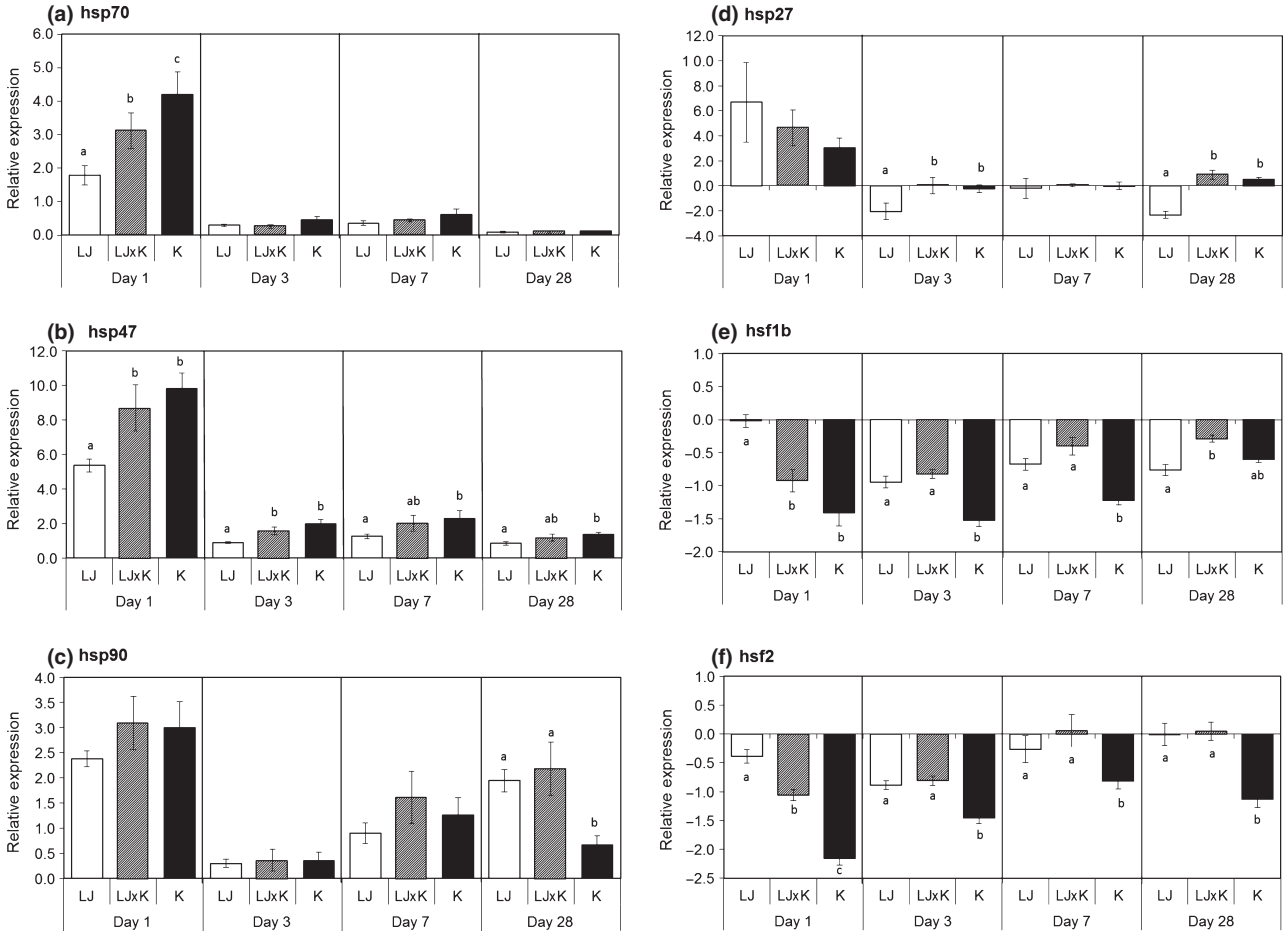

Fig. 2 Mean relative gene expression of six heat shock genes in liver tissue for three redband trout strains reared under chronic diel thermal stress, (a) heat shock protein 70, (b) heat shock protein 47, (c) heat shock protein 90, (d) heat shock protein 27, (e) heat shock factor 1b and (f) heat shock factor 2. Mean values ± SE are shown for each of four collection dates for fish from Little Jacks Cr. (LJ, desert), Keithly Cr. (K, montane), and their F1 cross (LJ × K). Values shown are the difference between fish reared at treatment and control temperatures such that control values are equal to zero, and treatment values that are closer to zero are more similar to controls. Significant differences between strains at each time period are shown by letters above the bars.

shock response). Several additional SNPs appeared to be from immune response genes related to MHC I and II regions. These are strong candidate regions for further studies to pinpoint polymorphisms responsible for adaptation to thermal stress.

Association of SNPs within each strain followed an expected pattern of adaptation among strains where there was much greater standing variation for selection to act upon in montane fish from Keithly Cr. and F1 crosses than in the desert strain from Little Jacks Cr. There were 9 SNPs significantly associated with mortality in the Keithly Cr. strain and 14 in the F1 crosses as opposed to none in the desert strain from Little Jacks Cr. (Table S2, Supporting information). Of the nine significantly associated SNPs from Keithly Cr. and the F1 crosses, many were from unknown genes, but Hsp47 was identified as one of the most significantly associated genes (P = 9.86 e-09), and several others were consistent with immune response MCH genes. Two other heat shock genes were associated at lower significance levels (Hsf2 and Hsc71) along with other candidates such as Na–K-ATPase-α3.

Discussion

When organisms in natural populations are exposed to thermal stress, there are limited possibilities for response including that the population may adapt over generations to tolerate the stressor, individuals may attempt to avoid the stressor (through movement, behaviour or physiological changes) or the population may become extirpated. These responses are often summarized as 'adapt, move, or die'. The current study in redband trout demonstrates that a portion of individual fish may acclimate to thermal stress and survive, but also that populations have evolved an adaptive heat shock response in desert environments. Overall, results suggest that aquatic populations with

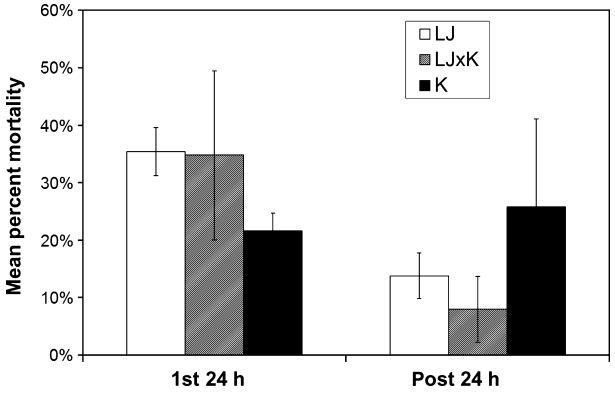

Fig. 3 Mean percentage mortality (±SE) for three strains of redband trout reared under chronic temperature stress. Results are shown for initial mortality after first 24 h of exposure to diel thermal stress and for the remaining 6 weeks of the experiment (post 24 h). Strains are from Little Jacks Cr. (LJ, desert), Keithly Cr. (K, montane) and their F1 cross (LJ × K). No significant differences (alpha = 0.05) were observed among strains.

adequate standing genetic variation can be expected to adapt to climate change over generations.

Acclimation of the heat shock response occurred rapidly in all strains of redband trout under repeated thermal stress over a period of 6 weeks. The acclimation response occurred within day 3 of exposure to elevated temperatures and continued through the remainder of the study. However, studies of the heat shock response in model species such as *Drosophila* (Sørensen *et al.* 1999) and other aquatic ectotherms (Tomanek & Somero 1999; Podrabsky & Somero 2004; Pörtner 2010) have shown that acclimation can occur more quickly, and it is possible that finer-scale temporal sampling would identify more precise information about the initiation of heat shock response in redband trout. Acclimation and reduction of the heat shock response have been shown to occur because the benefit of increased stress resistance becomes hampered by physiological costs of producing Hsps such as high energy demands, impaired growth and reduced fitness (Sørensen *et al.* 2003).

In addition to acclimation, results from our study demonstrate that an adaptive heat shock response has evolved in redband trout in desert environments to reduce energetic demands of Hsp production and potential costs to development and fitness. High induction of Hsps in the montane strain improved short-term survival during the first exposure to high temperatures, but demands of Hsp production may have led to high mortality with chronic exposure to heat stress. In contrast, the desert strain had significantly lower heat shock response than the montane fish and F1 crosses, suggesting that these desert fish may not have mounted sufficient response at first exposure to heat and have evolved alternative mechanisms to deal with thermal stress with less physiological costs. While low Hsp response in the desert strain was probably responsible for relatively high mortality at first exposure to elevated temperatures, stochastic temperature profiles from nature could not be exactly replicated in a laboratory environment, and an abrupt shift to experimental temperatures may have exceeded upper thermal tolerance limits of all strains. Fish in the laboratory were reared at a constant temperature near their optimum (15 °C) for several weeks before being moved directly into diel temperature regimes that increased approximately 1.5 °C per hour to a peak of nearly 28 °C (Fig. S1, Supporting information). While this was an effective study design to induce Hsps in controlled environments to demonstrate differences among strains, more gradual seasonal changes are encountered in nature even in desert environments and may produce different patterns of Hsp expression. In general, the results suggest that more complex adaptive mechanisms are involved than simply induction of heat shock genes.

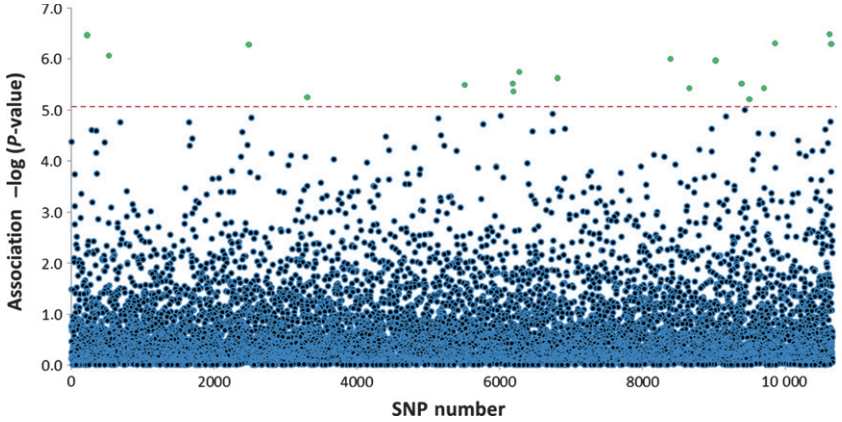

Fig. 4 Plot of SNPs associated with mortality overall three strains of redband trout reared under diel thermal stress. The dashed line indicates the critical value for significant association (with BY-FDR correction). Dots that are coloured green highlight SNPs with strong association.

Similar adaptive heat shock response has been observed in both laboratory experiments (Bettencourt *et al.* 1999; Sørensen *et al.* 1999; Lansing *et al.* 2000) and studies of natural populations (Sørensen *et al.* 2001; Fangue *et al.* 2006) of thermal stress, with populations from warmer environments expressing lower levels of Hsps than those from cooler environments due to evolution of alternative mechanisms to deal with stress response and costs related to Hsp expression (Sørensen *et al.* 1999; Sørensen & Loeschcke 2002). The phenomenon of adaptive heat shock response observed in redband trout and other organisms indicates that physiological costs of expressing Hsps are common in nature, and organisms evolve additional mechanisms to lower these costs. It is yet unknown whether parallel solutions among organisms are made to reduce these costs, but oxygen transport (Pörtner 2010) and cardiac/aerobic scope (Eliason *et al.* 2011) are expected to be critical in aquatic organisms.

Results from genomewide association tests validated the adaptive heat shock response and showed significant association at a SNP in the 3′UTR of the Hsp47 gene, but also identified multiple other genes that were associated with survival under thermal stress. These genes are strong candidates for alternative mechanisms regulating the response to thermal stress. Previous studies in marine fishes have shown that oxygen delivery is limiting in climate-related stressors (Pörtner & Knust 2007), and thus, genes involved in oxygen transport are expected to play a significant role. Additionally, we expect that metabolic and immune pathways could be involved given the energy demands and potential for disease under thermal stress (e.g. Sørensen & Loeschcke 2007), and results from our study are consistent with this expectation.

Climate-induced thermal stress is expected to increase in future generations and may have significant impacts on marine (Perry *et al.* 2005) and freshwater fishes (Ficke *et al.* 2007). However, freshwater fishes typically have less potential to alter their distribution due to natural and anthropogenic barriers (e.g. waterfalls and dams, respectively) and may be forced to adapt or become extirpated. Therefore, studies that investigate the adaptive responses of thermal stress are critical for understanding the potential for adaptation in species such as redband trout that currently occupy broad geographic regions with different climates. Our results complement recent evidence in sockeye salmon (*O. nerka*) that demonstrate that salmonid populations may segregate by temperature tolerance (Eliason *et al.* 2011). We also identify genes under selection for thermal stress, and these may be utilized to screen broadly across the species' range to predict the potential for adaptation under scenarios of climate change. Our study indicates that populations in desert environments have evolved an adaptive heat shock response, and populations from cool climates may retain suitable genetic diversity to adapt to changing climate. The largest conservation concern is expected to be for populations that no longer retain adequate genetic variation to adapt to shifting climates and have limited potential for dispersal. Ultimately, genetically diverse ectotherms in aquatic environments appear capable of adaptation and acclimation to thermal stress, but climate change is expected to cause local extirpation in genetically depauperate populations, reduce range distributions and result in unusually high rates of extirpation.

Acknowledgements

We thank Steve Elle, Liz Mamer, Chris Sullivan, Carson Watkins, Lindsay Maier, Mike Casten, Amanda Matala, Vanessa Morman, Stephanie Harmon and Jeff Stephenson for assistance with this study. Ben Hecht assisted with nomenclature of RAD tags and documentation of the analysis pipeline. Funding was provided by the Bonneville Power Administration through grant 200900500 to S.R.N.

References

Almany GR, DeArruda MP, Arthofer W (2009) Identification and characterization of heat shock response related single nucleotide polymorphisms in *O. mykiss* and *O. tschawytscha*. *Molecular Ecology Resources*, **9**, 1460–1466.

Angilleta MJ (2009) *Thermal Adaptation: A Theoretical and Empirical Synthesis*. Oxford University Press, Oxford.

Baird NA, Etter PD, Atwood TS *et al.* (2008) Rapid SNP discovery and genetic mapping using sequenced RAD markers. *PLoS ONE*, **3**, e3376.

Basu N, Todgham AE, Ackerman PA *et al.* (2002) Heat shock protein genes and their functional significance in fish. *Gene*, **295**, 173–183.

Bear EA, McMahon TE, Zale AV (2007) Comparative thermal requirements of westslope cutthroat trout and rainbow trout: implications to species interactions and development of thermal protection standards. *Transactions of the American Fisheries Society*, **136**, 1113–1121.

Beitinger TL, Bennett WA (2000) Quantification of the role of acclimation temperature in temperature tolerance of fishes. *Environmental Biology of Fishes*, **58**, 277–288.

Benjamini Y, Yekutieli D (2001) The control of false discovery rate under dependency. *Annals of Statistics*, **29**, 1165–1188.

Bettencourt BR, Feder FE, Cavicchi S (1999) Experimental evolution of Hsp70 expression and thermotolerance in *Drosophila melanogaster*. *Evolution*, **53**, 484–492.

Cassinelli JD, Moffitt CM (2010) Comparison of growth and stress in resident redband trout held in laboratory simulations of montane and desert summer temperature cycles. *Transactions of the American Fisheries Society*, **139**, 339–352.

Eliason EJ *et al.* (2011) Differences in thermal tolerance among sockeye salmon populations. *Science*, **332**, 109–112.

Fangue NA, Nann AF, Hofmeister M, Schulte1 PM, (2006) Intraspecific variation in thermal tolerance and heat shock protein gene expression in common killifish. *Fundulus heteroclitus*. *Journal of Experimental Biology*, **209**, 2859–2872.

Feder ME, Hofmann GE (1999) Heat-shock proteins, molecular chaperones, and the stress response: evolutionary and ecological physiology. *Annual Reviews of Physiology*, **61**, 243–282.

Ficke AD, Myrick CA, Hansen LJ (2007) Potential impacts of global climate change of freshwater fisheries. *Reviews in Fish Biology and Fisheries*, **17**, 581–613.

Gamperl AK, Rodnick KJ, Faust HA *et al.* (2002) Metabolism, swimming performance, and tissue biochemistry of high desert redband trout (*Oncorhynchus mykiss* ssp.): evidence for phenotypic differences in physiological function. *Physiological and Biochemical Zoology*, **75**, 413–431.

Hecht BC, Campbell NC, Holecek DE, Narum SR (2012) Genome-wide association reveals genetic basis for the propensity to migrate in wild populations of rainbow and steelhead trout. *Molecular Ecology*, doi: 10.1111/mec.12082.

Hoffmann AA, Sørensen JG, Loeschcke V (2003) Adaptation of *Drosophila* to temperature extremes: bringing together quantitative and molecular approaches. *Journal of Thermal Biology*, **28**, 175–216.

Intergovernmental Panel on Climate Change (2007) Climate Change 2007: Synthesis Report, Forth Assessment Report of the Intergovernmental Panel on Climate Change.

Knies JL, Izem R, Supler KL, Kingsolver JG, Burch CL (2006) The genetic basis of thermal reaction norm evolution in the lab and natural phage populations. *PLoS Biology*, **4**, 1–8.

Lansing E, Justesen J, Loeschcke V (2000) Variation in the expression of Hsp70, the major heat-shock protein, and thermotolerance in larval and adult selection lines of *Drosophila melanogaster*. *Journal of Thermal Biology*, **25**, 443–450.

Meyer KA, Lamansky J, Schill DJ (2010) Biotic and abiotic factors related to redband trout occurrence and abundance in desert and montane streams. *Western North American Naturalist*, **70**, 67–76.

Miller MR, Brunelli JP, Wheeler PA *et al.* (2012) A conserved haplotype controls parallel adaptation in geographically distant salmonid populations. *Molecular Ecology*, **21**, 237–249.

Narum SR, Campbell NR, Kozfkay CC, Meyer KA (2010) Adaptation of redband trout in desert and montane environments. *Molecular Ecology*, **19**, 4622–4637.

Perry AL, Low PJ, Ellis JR, Reynolds JD (2005) Climate change and distribution shifts in marine fishes. *Science*, **308**, 1912–1915.

Podrabsky JE, Somero GN (2004) Changes in gene expression associated with acclimation to constant temperatures and fluctuating daily temperatures in an annual killifish *Austrofundulus limnaeus*. *Journal of Experimental Biology*, **207**, 2237–2254.

Pörtner HO (2010) Oxygen- and capacity-limitation of thermal tolerance: a matrix for integrating climate-related stressor effects in marine ecosystems. *Journal of Experimental Biology*, **213**, 881–893.

Pörtner HO, Knust R (2007) Climate change affects marine fishes through the oxygen limitation of thermal tolerance. *Science*, **315**, 95–97.

Purcell S, Neale B, Tod-Brown K *et al.* (2007) PLINK: a tool-set for whole-genome association and population-based linkage analysis. *American Journal of Human Genetics*, **81**, 559–575.

Rodnick KJ, Gamperl AK, Lizars KR, Bennett MT, Rausch RN, Keeley ER (2004) Thermal tolerance and metabolic physiology among redband trout populations in south-eastern Oregon. *Journal of Fish Biology*, **64**, 310–335.

Schluter D (2000) *The Ecology of Adaptive Radiation*. Oxford University Press, Oxford.

Sinclair BJ, Roberts SP (2005) Acclimation, shock and hardening in the cold. *Journal of Thermal Biology*, **30**, 557–562.

Sinclair BJ, Vernon P, Klok CJ, Chown SL (2003) Insects at low temperatures: an ecological perspective. *Trends in Ecology and Evolution*, **18**, 257–262.

Sørensen JG, Loeschcke V (2002) Natural adaptation to environmental stress via physiological clock-regulation of stress resistance in *Drosophila*. *Ecology Letters*, **5**, 16–19.

Sørensen JG, Loeschcke V (2007) Studying stress responses in the post-genomic era: its ecological and evolutionary role. *Journal of Bioscience*, **32**, 447–456.

Sørensen JG, Michalak P, Justesen J, Loeschcke V (1999) Expression of the heat-shock protein HSP70 in *Drosophila buzzatii* lines selected for thermal resistance. *Hereditas*, **131**, 155–164.

Sørensen JG, Dahlgaard J, Loeschcke V (2001) Genetic variation in thermal tolerance among natural populations of *Drosophila buzzatii*: down regulation of Hsp70 expression and variation in heat stress resistance traits. *Functional Ecology*, **15**, 289–296.

Sørensen JG, Kristensen TN, Loeschcke V (2003) The evolutionary and ecological role of heat shock proteins. *Ecology Letters*, **6**, 1025–1037.

Tomanek L, Somero GN (1999) Evolutionary and acclimation-induced variation in the heat-shock responses of congeneric marine snails (genus *Tegula*) from different thermal habitats: implications for limits of thermotolerance and biogeography. *Journal of Experimental Biology*, **202**, 2925–2936.

Zoellick BW (1999) Stream temperatures and the elevational distribution of redband trout in southwestern Idaho. *Great Basin Naturalist*, **59**, 136–143.

S.N. leads a research group interested in population and ecological genomics of fishes. N.C. is a genomics researcher involved with developing genomics resources for use in various fish species. K.M. is a fisheries biologist evaluating stream habitat and occurrence of native inland fishes of the Pacific Northwest USA. M.M. is a scientist involved in animal genomics, bioinformatics development, and quantitative genetics. R.H. is renowned for his research in fish nutrition and aquaculture.

Data accessibility

SNP genotypes and sample information in PLINK format, gene expression and sample information for heat shock genes and SNP number with RAD tag ID and allele sequences: DRYAD entry doi:10.5061/dryad.kh7f2.

Illumina Single Read 100 sequence reads available through accession number SRA057008 in the NCBI Short Read Archive database.

Supporting information

Additional supporting information may be found in the online version of this article.

Table S1. Syber green qRT-PCR assay information.

Table S2. List of SNPs associated overall strains.

Table S3. List of SNPs associated within each strain.

Fig. S1. Diel temperatures during 6-week thermal stress experiment.

Figure S1. Diel water temperatures of recirculating treatment tanks for a six week thermal stress experiment of redband trout. Control tanks were maintained at a constant 15°C throughout the experiment (not shown). Asterisks indicate the four dates when samples (liver and gill) were collected from fish for gene expression analyses. Two arrows show dates when recirculating water was exchanged with fresh water.

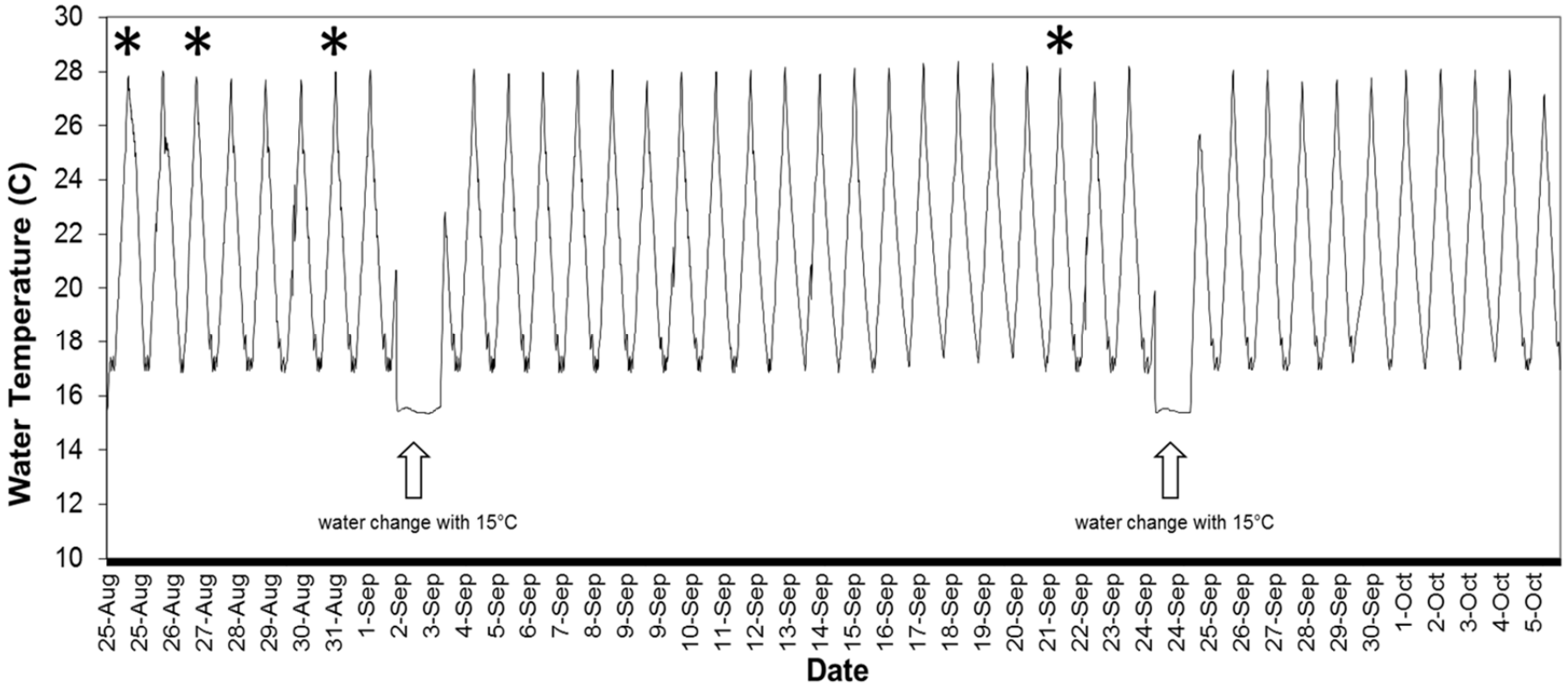

Table S1. List of SNPs overall redband trout strains that were associated with survival/mortality under thermal stress.

SNP	Association p-value	BLAST annotation	Species	% coverage	% identity	e-value
10619	3.25E-07	heat stock protein 47	O. mykiss	100	100	na
219	3.39E-07	PHF1 near MHCII-alpha	O. mykiss	100	100	3.00E-32
9854	4.91E-07	zinc transporter near MHCI antigen	O. mykiss	98	89	1.00E-18
10644	4.98E-07	P9 genomic near sex chromosome	O. mykiss	100	100	na
2484	5.16E-07	unknown	na	na	na	na
524	8.47E-07	MHCI antigen	S. salar	91	90	1.00E-17
8390	9.82E-07	unknown	na	na	na	na
9024	1.07E-06	unknown	na	na	na	na
6275	1.78E-06	unknown	na	na	na	na
6806	2.34E-06	MHC IA core region	O. mykiss	97	97	2.00E-28
9387	2.99E-06	unknown	na	na	na	na
6182	3.02E-06	unknown	na	na	na	na
5508	3.19E-06	unknown	na	na	na	na
9702	3.70E-06	unknown	na	na	na	na
8658	3.72E-06	unknown	na	na	na	na
6192	4.30E-06	unknown	na	na	na	na
3301	5.58E-06	transmembrane domain	S. salar	100	94	3.00E-13
9498	6.03E-06	unknown	na	na	na	na

Table S2. List of SNPs within each strain of redband trout that were associated with survival/mortality under thermal stress.

Population	SNP	Association p-value	BLAST annotation	species	% coverage	% identity	e-value
Little Jacks Cr.	none	na	na	na	na	na	na
Keithley Cr.	10589	1.81E-09	MHCI-a region	O. mykiss	86	86	1.00E-67
	10645	7.56E-09	unknown	na	na	na	na
	10619	1.53E-08	heat stock protein 47	O. mykiss	100	100	
	10532	1.72E-08	unknown	na	na	na	na
	10505	1.03E-07	unknown	na	na	na	na
	10644	8.13E-07	P9 genomic near sex chromosome	O. mykiss	100	100	
	10530	2.19E-06	unknown	na	na	na	na
	1645	1.57E-06	Raftlin near MHCII-alpha	O. mykiss	97	100	2.00E-24
	3367	3.96E-06	unknown				
F1 LittleJacks X Keithley	219	2.34E-10	PHF1 near MHCII-alpha	O. mykiss	100	100	3.00E-32
	2918	1.20E-08	unknown	na	na	na	na
	5956	8.13E-08	SYPG1 near MCHII-alpha; MHC 1b	O. mykiss	100	92	5.00E-27
	9024	1.82E-07	unknown	na	na	na	na
	2978	2.31E-07	Raftlin near MHCII-alpha	O. mykiss	100	94	2.00E-13
	10038	2.59E-07	Raftlin near MHCII-alpha; Hsp70	O. mykiss	75	82	4.00E-09
	524	5.00E-07	MHCI antigen	S. salar	91	90	1.00E-17
	9326	9.63E-07	unknown	na	na	na	na
	8133	1.78E-06	unknown	na	na	na	na
	6388	2.56E-06	unknown	na	na	na	na
	3265	2.56E-06	MHCI antigen	O. mykiss	98	87	2.00E-20
	7569	2.61E-06	Raftlin near MHCII-alpha	O. mykiss	100	100	4.00E-21
	1650	4.07E-06	PBX2 near MHCII-alpha	O. mykiss	95	95	6.00E-13
	3427	4.12E-06	unknown	na	na	na	na

Table S3. Primer and probe sequences for quantitative real-time PCR assays.

Gene Target	Forward primer	Reverse Primer	Taqman Probe or SYBR-green
Heat Shock Protein 70 (targets both isoforms)*	AGGGAGATCGCTGAGGCTTAC	AAGTAGGCAGGGACTGTGATGAC	6FAM-CAGAAGGTGTCCAATGC-MGB
Heat Shock Protein 90B (targets both isoforms)	ACCTCTGCAAGCTCATGAAGGAGA	ATGCAGCAGGGCGACGACAC	SYBR-green
Heat Shock Cognate 71	GCAGTCGGCATCGATCTCGGG	TCCTGTTGCCTTGGTCGTTGGC	SYBR-green
Heat Shock Factor 1b	CCGCCCACTCCTTGCCCAAG	TAGCAGGCGAGCCCTGCAGA	SYBR-green
Heat Shock Factor 2	TGCCCTCTGCTGGCCTTCCT	TGCAGCAGGCTGTCTCCCAGT	SYBR-green
Heat Shock Protein 47	GGGCGGCCAAGTCGACAGAC	CGGGTCACCAGGAAGCCACG	SYBR-green
Heat Shock Protein 27	TGCCCCCTATGGCTGACGCT	GCAGCCTTGATGGCCTGCCT	SYBR-green
Elongation Factor (EF1a)	GAGAACCATTGAAAAGTTCGAGAAG	GCACCCAGGCATACTTGAAAG	SYBR-green
Beta Actin	AGCTGAGGGTGGCTCCAGAGGA	AACACGGCCTGGATGGCCAC	SYBR-green