

RESEARCH ARTICLE

Temperature-Related Effects on Disease Susceptibility and Immune Response in Redband Trout (*Oncorhynchus mykiss gairdneri*) Following Challenge With *Flavobacterium columnare*

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ABSTRACT

Heat stress can increase disease risk in fishes by reducing immune function. Interactions between redband trout (*Oncorhynchus mykiss gairdneri*) and *Flavobacterium columnare*, a causative agent of columnaris disease, provide an opportunity to investigate the effects of temperature on immune function and disease resistance during periods of thermal stress. We conducted three trials to characterise differences in immune function and mortality between redband trout held at 18°C and 21°C following challenge with *F. columnare*. In trial 1, cumulative per cent mortality (CPM) was low and not statistically different between 18°C and 21°C. In trials 2 and 2, we administered higher challenge doses and observed increased CPM overall and significantly greater CPM at 21°C than 18°C. Redband trout upregulated *il-8*, *tnf-α*, *igm* and *igt* following infection by *F. columnare*, suggesting that all of these genes may be involved in immune responses to *F. columnare* infection. We found no differences in the strength of the immune responses between fish held at 21°C versus 18°C. This indicated that 21°C did not elicit sufficient thermal stress to impair immune function and that increased CPM at 21°C versus 18°C was due to enhanced *F. columnare* virulence.

1 | Introduction

An emerging threat to wild fish populations resulting from anthropogenic increases in water temperatures is enhanced disease risk (i.e., sublethal fitness reductions and mortality caused by diseases; Chu, Mandrak, and Minns 2005; Karvonen et al. 2010; Isaak et al. 2012, 2018; Vezzulli, Colwell, and Pruzzo 2013). Pathogen outbreaks in captive populations of fishes often coincide with warm water temperatures and increasingly, pathogen outbreaks in wild populations of fishes are being linked to thermal stress (Glibert

et al. 2002; Karvonen et al. 2010; Martin et al. 2010; Reverter et al. 2020). The impacts of temperature on disease risk vary among pathogen–host pairs and temperature-driven changes in disease risk can be mediated by a variety of mechanisms (Karvonen et al. 2010; Martin et al. 2010; Tort 2011; Vezzulli, Colwell, and Pruzzo 2013; Whitney et al. 2016). Efforts to predict the impacts of environmental change on fish populations will benefit from characterising the mechanisms underpinning temperature-driven changes in disease risk (Karvonen et al. 2010; Whitney et al. 2016; Petitjean et al. 2020; Scharsack and Franke 2022).

Warm temperatures can increase disease risk by reducing the ability of fishes to prevent pathogen invasion and fight subsequent infections (Avtalion et al. 1973; Weyts et al. 1999; Martin et al. 2010; Tort 2011; Whitney et al. 2016; Rebl et al. 2020). For example, chronic heat stress can shrink aerobic power budgets, which, in turn, can diminish immune function. Aerobic power budgets are the accessible aerobic energy above basic maintenance costs and they decrease in size as ectotherms approach upper thermal tolerances due to the limited capacity of fish circulatory and ventilatory systems to meet oxygen demands (Pörtner, Mark, and Bock 2004; Pörtner and Knust 2007; Pörtner, Bock, and Mark 2017). Reductions in the sizes of aerobic power budgets can reduce immune function (Lochmiller and Deerenberg 2000; Kortet et al. 2003; Schwenke, Lazzaro, and Wolfner 2016; Wernicke von Siebenthal et al. 2018). Host fish behaviour can also modulate disease risk. For example, warm temperatures may encourage fishes to seek thermal refugia and the resulting increases in fish densities may enhance pathogen transmission potential (Marcogliese 2008; Karvonen et al. 2010; Marcos-López et al. 2010).

Warm temperatures can also increase disease risk by allowing pathogens to expand their geographic ranges and by altering pathogen physiology (Decostere et al. 1999; Zheng et al. 2004; Karvonen et al. 2010). For example, the pathogenic potential of *Flavobacterium columnare*, a causative agent of columnaris disease, is often increased at warm water temperatures (~15°C–30°C) that allow for optimal bacterial growth and adherence to gill epithelia (Wood 1974; Decostere et al. 1999; Austin and Austin 2007). Warming temperatures have facilitated expansion of the geographic ranges of a variety of aquatic pathogens that can be harmful to fishes, such as *Vibrio* spp. and *Flavobacterium* spp. (Karvonen et al. 2010; Vezzulli, Colwell, and Pruzzo 2013). Together, increased pathogen ranges, growth rates, pathogenicity and virulence can increase the likelihood of fishes coming into contact with pathogens, elevate disease transmission rates between hosts and cause fitness reductions in hosts (Vezzulli, Colwell, and Pruzzo 2013; Karvonen et al. 2010).

Host–pathogen interactions are governed by the physiology and immunity of the host, the virulence and pathogenicity of the pathogen and may also be impacted by environmental temperatures (Avtalion et al. 1973; Clayton and Price 1992; Decostere et al. 1999; Karvonen et al. 2010; LaFrentz et al. 2012; Vezzulli, Colwell, and Pruzzo 2013; Chen et al. 2018). Consequently, the relative importance of different mechanisms to increased disease risk at warm temperatures is predicted to vary widely among different pathogen/host species pairs (Whitney et al. 2016). Determining the mechanisms governing disease risk at warm water temperatures can inform conservation and natural resource management. For example, this information can be useful when selecting source populations for conservation aquaculture or assisted migration and when modelling the impacts of environmental change on fish populations (Escobar, Escobar-Dodero, and Phelps 2018; Chen et al. 2022; Bartholomew et al. 2023).

Interactions between redband trout (*Oncorhynchus mykiss gairdneri*) and *F. columnare*, a causative agent of columnaris disease, provide an opportunity for investigating the effects of temperature on immune function during periods of thermal stress. Redband trout are a subspecies of rainbow trout that occur in western North America in an array of habitat types and are

locally adapted to different thermal regimes across their range (Muhlfeld et al. 2015; Chen et al. 2018; Chen and Narum 2021; Andrews et al. 2023). *F. columnare* is widespread in freshwater ecosystems. Columnaris disease outbreaks resulting in high mortality in salmonid species, including in redband trout, have been linked to warm water temperatures in wild and cultured populations (Perkins, Kann, and Scoppettone 2000; Belchik, Hillemeier, and Pierce 2004; Suomalainen, Tirola, and Valtonen 2005; Karvonen et al. 2010; Kunttu et al. 2012; Declercq et al. 2013; Loch and Faisal 2015; Faisal et al. 2017). Redband trout can tolerate temperatures as high as 29°C for extended periods of time (Rodnick et al. 2004). However, aerobic power budgets of redband trout from Idaho, USA, begin to decline at ~20°C, indicating that at temperatures greater than 20°C redband trout will begin to experience thermal stress (Chen et al. 2018). Temperatures >20°C are increasingly encountered by redband trout in several populations during summer months and correspond to temperatures at which *F. columnare* grows rapidly and is highly virulent (Holt et al. 1975; Decostere et al. 1999; LaFrentz et al. 2012; Karvonen et al. 2010; Chen et al. 2018). This suggests that at temperatures >20°C, redband trout may have increased columnaris disease risk due to both pathogen and host effects. Declines in immune indicators at warm temperatures, such as the expression of immune genes, which typically (but not always) reflects changes in protein production (Frenette et al. 2023), would suggest increased disease risk due to impairment of host immune systems, potentially due to inadequate power budgets. Constant immune indicators across temperatures along with increased host mortality at warm temperatures would implicate increased disease risk via pathogen effects, potentially due to enhanced transmission and proliferation rates.

The present study investigated relationships between temperature and immune function and mortality in redband trout following exposure to *F. columnare*. Although redband trout mortality following *F. columnare* infection has been documented, the susceptibility of redband trout to columnaris disease and immune responses to infection are poorly understood (Perkins, Kann, and Scoppettone 2000). Therefore, our first objective was to quantify the mortality of redband trout from different source populations following *F. columnare* exposure at two temperatures (18°C and 21°C). Our second objective was to characterise the regulation of innate and adaptive immune system genes following *F. columnare* exposure. Our third objective was to test the hypothesis that immune responses will be stronger in redband trout infected at cooler temperatures relative to redband trout infected at warmer temperatures. We accomplished these objectives by conducting three experimental trials in which we experimentally infected redband trout with *F. columnare*, and then documented subsequent redband trout mortality and immune gene expression.

2 | Materials and Methods

2.1 | Experimental Redband Trout

To obtain juvenile redband trout, we manually spawned wild-caught broodstock collected in 2018 and held at the University of Idaho Aquaculture Research Institute. Previous genetic analyses confirmed that the broodstock used for this study

was redband trout (Chen et al. 2018; Chen and Narum 2021). Using all available mature fish, we made three crosses, one involving broodstock from populations inhabiting montane streams (cross 1 = one female from Keithley Creek, one female from Fawn Creek, one male from Mann Creek and one male from Keithley Creek) and two involving broodstock from populations inhabiting desert streams (cross 2 = one female from Pole Bridge, one male from Pole Bridge and two males from Williams Creek and cross 3 = one female from Williams Creek and one male from Duncan Creek; Figure 1; Table S1). For each cross, we anaesthetised fish with 50 mg/L buffered MS-222 (Syndel Ferndale, WA), then stripped gametes into a single metal bowl for fertilisation following the 'dry' spawning method (Piper 1982). Embryos were reared in Heath Trays and treated with formalin (~1500 μ L/L for 15 min 1 time per day on days 1–15) to prevent fungal growth. Embryos hatched ~20 days postfertilisation and the following day, we moved them to troughs for subsequent rearing. Fish were held at a constant temperature of 15°C, which is approximately the thermal optimum for *O. mykiss*, prior to acclimation to experimental temperatures (McMahon, Bear, and Zale 2008; Cocherell et al. 2014; Chen et al. 2015). Prior to experiments, half of the fish from each cross were acclimated from 15°C to 18°C and the other half, from 15°C to 21°C, at a rate of 0.5°C per day. We selected 18°C as the cooler experimental temperature because it does not impose thermal stress on most redband populations, but is in the temperature range at which *F. columnare* can be highly virulent (Holt et al. 1975; Decostere et al. 1999; LaFrentz et al. 2012; Karvonen et al. 2010). We selected 21°C for the warm experimental temperature because temperatures of $\geq 21^\circ\text{C}$ are currently only encountered by broodstock source populations during short periods in the summer, but are becoming more common, and are above temperatures at which redband trout populations begin to experience decreases in aerobic power budgets and thermal stress (Chen et al. 2018). Additionally, at 21°C *F. columnare* can be highly virulent (Decostere et al. 1999; LaFrentz et al. 2012; Karvonen et al. 2010). We did not select

warmer experimental temperatures for our study because we did not want acute mortality to be so high that it prevented us from measuring pathogen effects on gene expression.

2.2 | *F. columnare* Challenges

After temperature acclimation, and immediately prior to challenge with *F. columnare*, we evenly divided fish into replicate groups (four replicate groups challenged plus one mock-challenged group per temperature in trial 1 and 3 replicate groups challenged plus one mock-challenged group per temperature in trials 2 and 3; Table 1) and moved each group into a 2-L tank in a biosecure wet laboratory in the College on Natural Resources, at the University of Idaho, Moscow, USA. We conducted three trials in which we challenged redband trout with *F. columnare* and then held them at experimental temperatures for 28 days. Cross 1 fish were used in trial 1, cross 2 fish were used in trial 2 and cross 3 fish were used in trial 3. Due to logistical constraints associated with the availability of wild-caught broodstock and the timing of broodstock maturation, we were unable to include all three crosses in each trial or conduct additional trials.

For each trial, we immersed naïve redband trout with a virulent strain of *F. columnare* (051-10-S5) using a standardised challenge model for rainbow trout (LaFrentz et al. 2012). *Flavobacterium* isolates have recently undergone taxonomic revision and the classification of isolate 051-10-S5 remains *F. columnare* (LaFrentz et al. 2022). Briefly, we inoculated 100 mL of sterile TYES in 500-mL culture flasks with cryopreserved *F. columnare* isolates. We incubated the flasks on a shaker for 24 h at 21°C and then adjusted optical densities to $OD_{540} = 1.00$. We made spread plate dilutions to confirm culture purity by observing bacterial colony morphology and determine inoculum concentration: trial 1 = 1.79×10^8 CFU/mL and trials 2 and 3 = 1.35×10^8 CFU/mL. We then added bacterial inoculate to challenge tanks to achieve final doses of 8.95×10^4 CFU/mL in trial 1 (added 1 mL

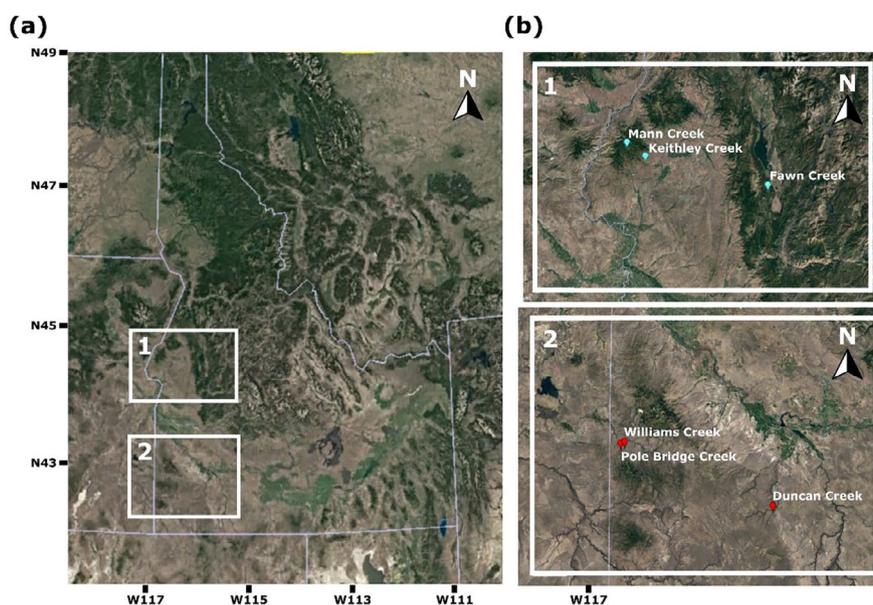


FIGURE 1 | Maps of study sites with terrain map from Google Earth Pro 7.3.6.9345 (2022). (a) Map centred on Idaho, USA showing the two regions containing broodstock source populations. (b) Higher resolution images of each broodstock sampling region: (1) montane sampling sites (blue markers) and (2) desert sampling sites (red markers).

TABLE 1 | Sampling in each redband trout *Flavobacterium columnare* challenge: Challenge = number of each challenge trial as described in the 'Experimental challenge with *F. columnare*' methods section.

Challenge	Stream type	Temp	0 wt.	28 wt.	<i>n</i> chal	<i>n</i> mock
One	Montane	18°C	1.40	1.25	63	14
		21°C	1.35	1.16	65	14
Two	Desert	18°C	1.16	NA	74	25
		21°C	1.35	NA	74	25
Three	Desert	18°C	1.30	NA	47	13
		21°C	1.23	NA	49	9

Note: Stream type = type of stream (desert or montane) where experimental trout were collected, Temp = temperatures used for experiments, 0 wt. = average fish weight in grams at the start of experiments on day 0, 28 wt. = average fish weight in grams on day 28, *n* chal = number of fish divided into replicate groups and challenged with *F. columnare*, and *n* mock = number of fish mock-challenged with sterile TYES broth (i.e., control fish). Average day 28 fish weights are not presented for trials 2 and 3 because mortality reached 100% before this sampling point.

of inoculate to 2-L water) and 4.5×10^5 CFU/mL in trials 2 and 3 (added 5-mL inoculate to 1.5-L water), then statically challenged fish for 1 h. We increased the challenge dose in trials 2 and 3 because the dose used in trial 1 did not elicit substantial mortality. In all trials, we included a control group of fish that was mock-challenged using sterile TYES broth in place of the bacterial culture. We measured the mean weights of fish on the day immediately prior to challenges (day 0) and on day 28 at the conclusion of each trial (Table 1).

Following static challenges, we transferred replicate groups of fish to identical 16-L tanks in flow-through aquaculture systems for the remainder of each trial (Table 1; Numbers of fish included in replicate groups are shown in Tables S3–S5). Half of the replicate groups were held at a constant temperature of $18^\circ\text{C} \pm 0.5^\circ\text{C}$ for the duration of the trial and the other half were held at a constant temperature of $21^\circ\text{C} \pm 0.5^\circ\text{C}$. All tanks experienced a 12-h photoperiod and received aerated, dechlorinated municipal water at a rate of approximately 2 L/min. We offered fish a commercial feed (1.5 mm Skretting, Toole, UT, USA) at ~1% of body weight per day. We removed and recorded mortalities twice daily. We streaked kidney, gill and spleen tissue from > 30% of daily mortalities on sterile TYES agar plates that we incubated at 21°C for 96 h to confirm *F. columnare* infection based on bacterial colony morphology.

2.3 | Tissue Sampling, RNA Isolation and Quantifying Gene Expression

For trial 1, we euthanised fish from both temperatures with 100 mg/L buffered MS-222 on day 0, prior to the challenge, and on days 1, 2, 7 and 28, postchallenge. For trials 2 and 3, we euthanised fish on days 0, 2, 7 and 28. We euthanised three fish from each temperature at each sampling point when possible, but there were some cases when fewer fish were sampled because there were less than three fish surviving (Table S2). We did not sample fish on day 1 for trials 2 and 3 in an attempt to ensure availability of fish for sampling at later time points. For all trials, we dissected head kidney, gill and spleen tissues from sampled fish and stored them in RNA later at -80°C .

To estimate the strength of immune responses to *F. columnare* infection, we quantified the mRNA expression of two innate

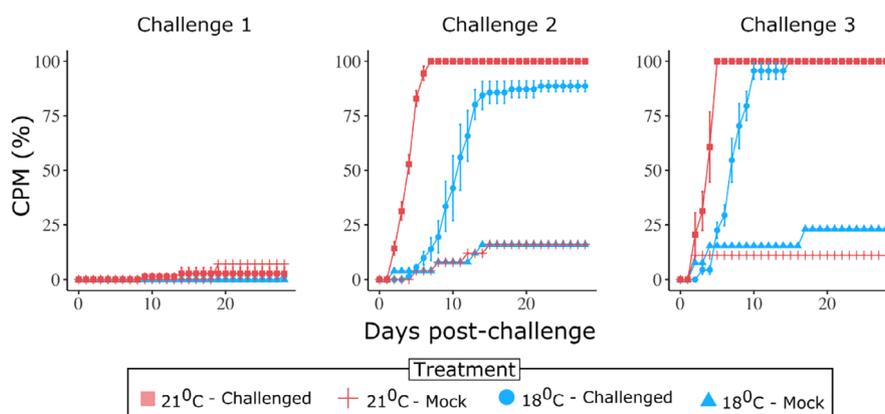
immune genes involved in pro-inflammatory response (*il-8* and *tnf- α*) and two immunoglobulin genes involved in humoral adaptive immunity (*igm* and *igt*; Table 2; Mokhtar et al. 2023). Additionally, we quantified the expression of β -actin as a housekeeping gene. The utility of β -actin as a housekeeping gene in fishes, including *O. mykiss*, has been demonstrated by numerous studies (e.g., Pérez-Cordón et al. 2014; Wei et al. 2018; Tong Sri et al. 2020). Mobile B lymphocytes and macrophages express *igm* and *igt* and *il-8* and *tnf- α* respectively (Lämmermann et al. 2008; Barros-Becker et al. 2017). Thus, our study design allowed us to quantify changes in gene expression within particular tissues, but was unable to distinguish the extent to which this reflected migration of immune cells into or out of tissues versus transcriptional regulation within resident immune cells. We extracted total RNA from 226 samples (Table S2) using a PureLink RNA Mini Kit (ThermoFisher Scientific). Then, we performed cDNA synthesis with a High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA) following the manufacturer's protocol. We measured the expression of target genes via 10- μL real-time quantitative polymerase chain reactions (qPCR) containing 1 μL of template cDNA, 0.3 μL each of forward and reverse primers, 5 μL of Power SYBR Green PCR Master Mix (ThermoFisher Scientific) and 3.4 μL of water. We ran all reactions in triplicate on an Applied Biosystems StepOnePlus Real-Time PCR System with the following steps: denaturation at 95°C for 2 min, 40 cycles of 95°C for 20 s and 60°C for 60 s and dissociation stage from 72°C to 95°C . We characterised relative gene expression levels using two approaches and β -actin as the internal control gene and unchallenged day 0 fish for normalisation: (1) $\Delta\Delta C_T$ values and (2) relative quantification (RQ) values using the $2^{-\Delta\Delta C_T}$ method (Livak and Schmittgen 2001; Schmittgen and Livak 2008). $\Delta\Delta C_T$ values characterise differences in the C_T values of genes of interest in treated fish (immersion-challenged fish) relative to untreated control fish (i.e., fish on day 0 not subjected to immersion challenges). Trial-specific day 0 values were used for calculations. RQ values describe the fold change in the expression of genes of interest in treated fish relative to untreated control fish.

2.4 | Statistical Analyses

We conducted all statistical analyses in program R v3.5.2 (R Core Development Team) and used a *p* value of 0.05 as the

TABLE 2 | Information associated with the primers used for qPCR.

Gene	Primer	Oligonucleotides [5'-3']	GenBank ID	References
β -Actin	Forward	GCCGGCCGCGACCTCACAGACTAC	AJ438158	Makesh, Sudheesh, and Cain (2015), Sanchez et al. (2018)
	Reverse	CGGCCGTGGTGGTGAAGCTGTAAC		
Immunoglobulin M	Forward	AAGAAAGCCTACAAGAGGGAGA	S63348	Makesh, Sudheesh, and Cain (2015), Sanchez et al. (2018)
	Reverse	CGTCAACAAGCCAAGCCACTA		
Immunoglobulin T	Forward	CAGACAACAGCACCTCACCTA	AY870264	Makesh, Sudheesh, and Cain (2015)
	Reverse	GAGTCAATAAGAAGACACAACGA		
Interleukin 8	Forward	CTCGCAACTGGACTGACAAA	AJ279069	Martin et al. (2012)
	Reverse	TGGCTGACATTCTGATGCTC		
Tumour necrosis factor α	Forward	GGGGACAAACTGTGGACTGA	AJ277604	Musharrafieh et al. (2014)
	Reverse	GAAGTTCTTGCCCTGCTCTG		

**FIGURE 2** | Line plots with standard error bars showing cumulative per cent mortality for challenged and mock-challenged fish at 21°C and 18°C in each trial. *Flavobacterium columnare* challenges were conducted on day 0 and mortality was monitored until trials ended on day 28.

threshold for statistical significance in all comparisons. For each trial, we tested for significant differences in mortality (expressed as cumulative per cent mortality (CPM)) between temperatures on days 2, 7, 14, 21 and 28 with Student's *t*-tests as these data conformed to the assumptions of homogeneous variances and normal distributions. We excluded fish euthanised to characterise gene expression from mortality calculations. We used $\Delta\Delta C_T$ values to test for differences in gene expression between time points within each trial following the recommendations of Yuan et al. (2006). Normal probability plots indicated that $\Delta\Delta C_T$ data were not normally distributed. We attempted to normalise data using a log transformation, but normal probability plots indicated that transformed $\Delta\Delta C_T$ data were still not normally distributed. Therefore, we used nonparametric one-way Kruskal–Wallis tests followed by post hoc Dunn's tests to test for temporal differences in gene expression. We used the Holm (1979) method to adjust *p* values for multiple comparisons. We used nonparametric Mann–Whitney *U* tests to test for differences in gene expression between temperature treatments at each time point.

3 | Results

3.1 | Cumulative Percent Mortality

In all trials, fish challenged with *F. columnare* exhibited clinical signs consistent with columnaris disease, including gill necrosis, fin erosion and yellow epithelial coloration on dorsal and lateral surfaces of the body (Austin and Austin 2007; Bruce et al. 2020). Additionally, we reisolated *F. columnare* from mortalities in tanks containing challenged fish. In trial 1 (montane fish/cross 1), there was little mortality in challenged fish and no statistically significant differences in CPM between temperatures on days 2 ($p=1.000$), 7 ($p=1.000$), 14 ($p=0.391$), 21 ($p=0.391$) or 28 ($p=0.720$; Figure 2; Table S3). We observed greater mortality in the desert fish challenged in trials 2 (cross 2) and 3 (cross 3), with CPM increasing more rapidly at 21°C than 18°C and most tanks experiencing 100% CPM by day 28 at both temperatures (Figure 2; Tables S4 and S5). In trial 2, there was significantly greater CPM in challenged fish at 21°C than 18°C on day 7 ($p=0.005$), but not on days 2 ($p=0.057$), 14 ($p=0.167$),

TABLE 3 | Trial 1 p values resulting from Kruskal–Wallis (K–W) tests and Dunn's post hoc tests (Dunn) for $\Delta\Delta C_p$ differences between time points for each gene (*igm*, *igt*, *tnf- α* , *il-8*) by temperature (Temp) and tissue type (Tissue).

Temp	Tissue	<i>igm</i>						<i>igt</i>						<i>tnf-α</i>						<i>il-8</i>					
		K–W		Dunn		Days	K–W	Dunn		Days	K–W	Dunn		Days	K–W	Dunn		Days	K–W	Dunn		Days	K–W	Dunn	
		Unadj.	Adj.	Unadj.	Adj.			Unadj.	Adj.			Unadj.	Adj.			Unadj.	Adj.			Unadj.	Adj.			Unadj.	Adj.
18°C	Gill	1–7	0.0315	0.018	0.176	0.0197	1–7	0.028	0.228	0.0285	1–28	0.001	0.010	0–2	0.0319	0.010	0.022	0–2	0.022	0.202	0–28	0.018	0.036	0.176	
		1–28	0.036	0.036	0.286	0.002	0.019	1–28	0.002	0.019	—	—	—	—	0–28	—	—	0.176	—	—	0–28	—	—	0.176	
	7–2	0.018	0.018	0.159	0.022	0.202	2–28	0.022	0.202	—	—	—	—	1–2	—	—	0.250	1–2	0.036	0.250	1–28	0.028	0.028	0.228	
	28–2	0.036	0.036	0.250	—	—	—	—	—	—	—	—	—	—	—	—	—	0.228	—	—	1–28	0.028	0.028	0.228	
21°C	Kidney	0–28	0.0498	0.014	0.137	0.0489	1–28	0.002	0.025	0.1344	—	—	—	0–2	0.0367	—	0.019	0–2	0.002	0.019	0–2	0.036	0.036	0.322	
		1–28	0.018	0.018	0.159	—	—	—	—	—	—	—	—	—	1–2	—	—	0.322	1–2	0.036	0.322	—	—	—	
	2–28	0.036	0.036	0.286	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
	Spleen	0.0769	—	—	—	0.1963	—	—	—	0.1015	—	—	—	—	0.1062	—	—	—	—	—	—	—	—	—	
Gill	0–28	0.0458	0.022	0.225	0.0729	—	—	—	—	0.0184	1–2	0.022	0.180	0–2	0.0181	0.180	0.003	0–2	0.003	0.035	0–2	0.003	0.003	0.035	
	1–28	0.022	0.022	0.202	—	—	—	—	—	—	1–7	0.008	0.081	2–28	—	—	0.006	2–28	0.006	0.056	—	—	—		
	2–28	0.028	0.028	0.228	—	—	—	—	—	—	2–28	0.028	0.199	—	—	—	—	—	—	—	—	—	—		
	—	—	—	—	—	—	—	—	—	—	7–28	0.011	0.095	—	—	—	—	—	—	—	—	—	—		
Kidney	2–7	0.0258	0.036	0.286	0.1177	—	—	—	—	0.1967	—	—	—	—	0.0672	—	—	—	—	—	—	—	—		
	0–28	0.045	0.045	0.312	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—		
	1–28	0.018	0.018	0.159	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—		
	2–28	0.006	0.006	0.062	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—		
Spleen	0–7	0.0154	0.019	0.173	0.5414	—	—	—	—	0.8589	—	—	—	—	0.1511	—	—	—	—	—	—	—	—		
	0–28	0.001	0.001	0.013	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—		
	2–28	0.025	0.025	0.198	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—		

Note: See Figure 3 and Table S2 for detailed overviews of sampling. Statistically significant p values are bold. We only show p values from Dunn's post hoc tests for statistically significant comparisons. For Dunn's tests, we include p values unadjusted for multiple testing (unadj) and p values adjusted for multiple testing (adj). See Table S7 for all p values, including nonsignificant values.

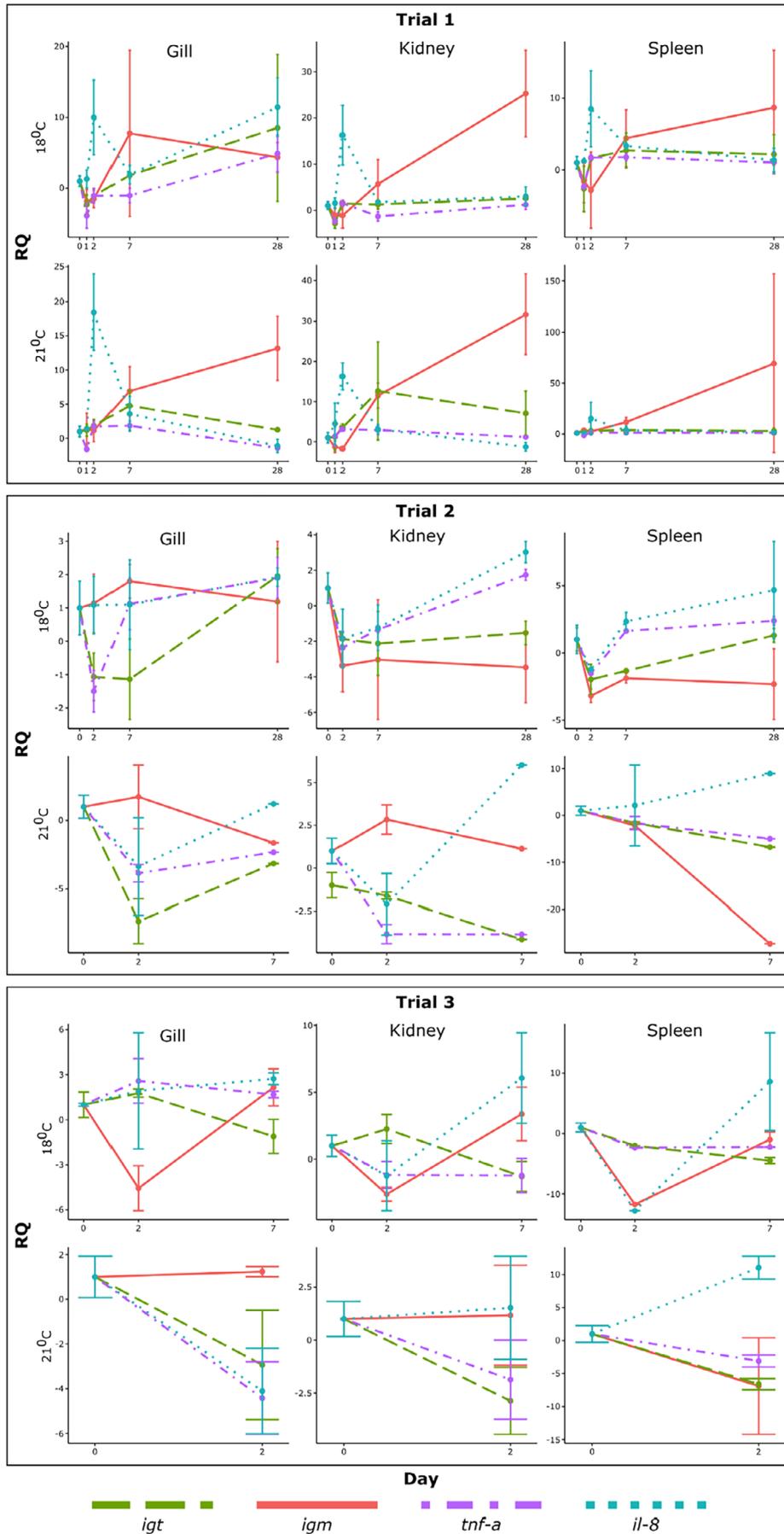


FIGURE 3 | Legend on next page.

FIGURE 3 | Line graphs showing temporal changes in gene expression for *igt*, *igm*, *tnf- α* and *il-8* measured as RQ values. RQ values describe the fold change in gene expression in challenged fish relative to mock-challenged control fish. For each gene, day 0 RQ values (gene expression prior to *F. columnare* challenges) were normalised to 1.0 to compare fold-changes in gene expression. Note that y- and x-axis ranges vary between individual plots.

21 ($p=0.104$) or 28 ($p=0.065$). In trial 3, there were no significant differences in CPM between challenged fish at 21°C and 18°C (day 2 $p=0.219$, day 7 $p=0.060$, day 14 $p=0.423$ and days 21 and 28 $p=1.000$). Mock-challenged fish exhibited low mortality in all trials and we did not reisolate *F. columnare* from any mortalities amongst mock-challenged fish (Figure 2; Tables S3–S5). Challenged fish were observed feeding, but lost weight in trial 1 at 18°C and 21°C (Table 1). High mortality prevented us from comparing average weight at the ends of trials 2 and 3.

3.2 | Temporal Changes in Gene Expression Following Challenge With *F. columnare*

In trial 1 (montane fish) at 18°C, there were statistically significant increases in the expression of *igm*, *igt* and *il-8* in gills and kidneys, but only significant increases in the expression of *tnf- α* in gills (Table 3). There were no statistically significant temporal changes in gene expression in spleens. The expression of *igm* peaked on day 7 in gills and on day 28 in kidneys (Figure 3). The expression of *igt* peaked on day 28 in gills and kidneys. The expression of *tnf- α* was greatest in gills on day 28 (Figure 3). In gills and kidneys, *il-8* expression peaked on day 28 (Figure 3). At 21°C, there were statistically significant increases in the expression of *igm* in gills, kidneys and spleens and *tnf- α* and *il-8* in gills (Table 3). At 21°C, there were no statistically significant temporal changes in the expression of *igt*. The expression of *igm* peaked on day 28 in gills, kidneys and spleens. In gills, *tnf- α* expression peaked on day 7 and *il-8* expression peaked on day 2 (Figure 3).

In trial 2 (desert fish) at 18°C, there were statistically significant decreases in the expression of *igt* in spleens and significant increases in the expression *tnf- α* in gills, kidneys and spleens and *il-8* in kidneys and spleens (Table 4). There were no statistically significant temporal changes in the expression of *igm* (Table 4). The expression of *igt*, *tnf- α* and *il-8* peaked on day 28 in gills, kidneys and spleens (Figure 3). At 21°C, there were no statistically significant temporal changes in gene expression (Table 4).

In trial 3 (desert fish) at 18°C, there were statistically significant increases in the expression of *igm* in gills and kidneys, but no significant changes in the expression of *igt*, *tnf- α* or *il-8* (Table 5). The expression of *igm* peaked in gills and kidneys on day 7 (Figure 3). At 21°C, there was a statistically significant decrease in the expression of *igt* in kidneys, but no significant changes in the expression of *igm*, *tnf- α* or *il-8* (Table 5). In kidneys, *igt* expression was greatest on day 0 (Figure 3).

3.3 | Impacts of Temperature on Gene Expression

Nonparametric Mann–Whitney *U* tests did not identify statistically significant differences in gene expression between temperatures at the same time points in any trial (Figure 4; Table 6).

In trial 1 (montane fish), RQ values were generally larger at 21°C compared to 18°C at the same time point (Table S6). In trials 2 and 3 (desert fish), there were slightly more cases in which RQ values were larger at 18°C compared to 21°C at the same time point (Table S6).

4 | Discussion

4.1 | Mortality and *F. columnare* Virulence

In trial 1 (montane fish), there was little mortality and no statistically significant differences in mortality between temperatures. In trials 2 and 3, cumulative mortality was greater for fish at 21°C than 18°C at most time points, although differences were only significant in trial 2 on day 7, and mortality was nearly 100% by day 28 in both trials at 21°C and 18°C. Our finding of increased *F. columnare* virulence at warmer temperatures is in agreement with previous research and suggests that, even if warming temperatures do not suppress redband trout immune systems, they could still increase the threat that columnaris disease poses to wild populations (Karvonen et al. 2010; Kunttu et al. 2012; Declercq et al. 2013; Loch and Faisal 2015). Although we did not observe high CPM in mock-challenged tanks, it was slightly higher than expected in trials 2 (18°C and 21°C = 16%) and 3 (18°C = 23% and 21°C = 11%) given what has been documented in previous studies using the same *F. columnare* challenge model (LaFrentz et al. 2012). This may have been because our work used undomesticated redband trout, which we have found to exhibit substantially higher mortality in captive-rearing conditions relative to domesticated rainbow trout.

Previous *F. columnare* challenges with the 051-10-S5 strain used domesticated rainbow trout (1.1 g–2.25 g), higher challenge doses (1.6×10^7 – 6.4×10^8 CFU/mL), lower temperatures (16°C–17°C), and in some cases made final CPM measurements earlier (days 24–28) than our study (LaFrentz et al. 2012; Bruce et al. 2020). It is important to consider these methodological differences as we compare resistance to the 051-10-S5 *F. columnare* strain between domesticated rainbow trout and redband trout below, particularly because *F. columnare* virulence is positively correlated with temperature (Holt et al. 1975; Decostere et al. 1999; Suomalainen, Tirola, and Valtonen 2005). LaFrentz et al. (2012) and Bruce et al. (2020) reported CPM ranging from 36% to 57%. We documented lower CPM (measured on day 28) than these studies in trial 1 and higher CPM in trials 2 and 3. It is possible that CPM was low in trial 1 compared to previous studies because the undomesticated redband trout from the montane population used in this trial are more resistant to columnaris disease than the domesticated rainbow trout used in previous studies. Columnaris disease resistance varies widely among *O. mykiss* populations (Evenhuis et al. 2015) and some strains of domesticated rainbow trout have been shown to be less resistant to columnaris disease than wild fish (Karvonen et al. 2016). Additionally, because a small number of parents were used in the crosses that generated

TABLE 4 | Trial 2 *p* values resulting from Kruskal–Wallis (K–W) tests and Dunn’s post hoc tests (Dunn) for $\Delta\Delta C_{T1}$ differences between time points for each gene (*igm*, *igt*, *tnf- α* , *il-8*) by temperature (Temp) and tissue type (Tissue).

Temp	Tissue	<i>igm</i>				<i>igt</i>				<i>tnf-α</i>				<i>il-8</i>			
		K–W		Dunn		K–W		Dunn		K–W		Dunn		K–W		Dunn	
		Days	Adj.	Days	Unadj.	Days	Adj.	Days	Unadj.	Days	Adj.	Days	Unadj.	Days	Unadj.	Days	Unadj.
18°C	Gill	0.3730	0.2214	—	—	—	—	0.0117	0.001	0.007	0.1383	—	—	—	—	—	—
	Kidney	0.3561	0.3340	—	—	—	—	0.0221	0.003	0.021	0.0441	2–28	0.013	0.077	2–28	0.031	0.154
	Spleen	0.5229	0.0347	2–28	0.008	0.051	0.216	0.0186	0.002	0.014	0.0318	2–28	0.006	0.035	2–28	0.006	0.035
21°C	Gill	0.0608	0.1173	—	—	7–28	0.043	—	—	—	—	—	—	—	—	—	—
	Kidney	0.3041	0.0764	—	—	—	—	0.1173	—	—	0.1717	—	—	—	—	—	—
	Spleen	0.1353	0.1353	—	—	—	—	0.1017	—	—	0.2765	—	—	—	—	—	—

Note: See Figure 3 and Table S2 for detailed overviews of sampling. Statistically significant *p* values are bold. We only show *p* values from Dunn’s post hoc tests for statistically significant comparisons. For Dunn’s tests, we include *p* values unadjusted for multiple testing (unadj.) and *p* values adjusted for multiple testing (adj.). See Table S8 for all *p* values, including nonsignificant values.

the experimental fish for this study, it is likely that family effects (i.e., disease resistance of the families we used for experimental crosses, which may not be the same as mean population-level disease resistance) contributed to the differences we observed in CPM among trials 1, 2 and 3 and between our study and previous studies (Sahoo et al. 2008). Although Bruce et al. (2020) did not find an association between *F. columnare* challenge dose and CPM, some studies using different strains of *F. columnare* have found positive correlations between challenge doses and CPM in rainbow trout (Evenhuis et al. 2015; Kinnula et al. 2015). This, in combination with the higher temperatures used in our study, may explain why we saw higher CPM in trials 2 and 3 than those reported by Bruce et al. (2020). Initial densities in experimental trial tanks varied slightly among trials (mean tank densities in trial 1 = 1.02 fish/L, trial 2 = 0.65 fish/L and trial 3 = 1.00 fish/L), which could have contributed to inter-trial variation in CPM because *F. columnare* can be transmitted via propagules shed into the water by infected fish (Mitiku 2018). However, we did not observe any overt relationships between CPM and fish densities in tanks. Additionally, it is possible that the desert fish used in trials 2 and 3 possessed lower columnaris disease resistance than the montane fish used in trial 1 due to either family or population effects, which may have also contributed to the higher CPM in trials 2 and 3.

4.2 | Temporal Changes in Gene Expression in Gills, Kidneys and Spleens

We found evidence that all four genes investigated in this study were significantly upregulated following exposure to *F. columnare*, and are likely part of a large suite of genes involved in immune responses to *F. columnare* and other bacterial pathogens. In some cases, changes in gene expression were large and likely resulted in changes in protein production, but in other cases, changes in gene expression were relatively small and may have not caused appreciable changes in protein levels (Frenette et al. 2023). It is also important to note that changes in gene expression within a particular tissue may, at least in part, reflect the migration of immune cells into or out of that tissue, rather than exclusively representing transcriptional regulation within resident immune cells (i.e., immune cell trafficking in addition to localised gene activation; Lämmermann et al. 2008; Barros-Becker et al. 2017). Previously, *igm* and *igt* have been identified as key components of humoral immune response to *F. columnare* in several fish species (Tian et al. 2009; Wei et al. 2018; Tongri et al. 2020). In fishes, *igt* and *igm* are primarily involved in mucosal and systemic immunity, respectively, and both immunoglobulins may be present in mucosal secretions of the gills, gut and skin, particularly *igt* (Gomez, Sunyer, and Salinas 2013; Xu et al. 2019). The kidney and spleen are key lymphoid tissues in teleost fishes and generally exhibit high *igm* and moderate *igt* expression levels (Press and Evensen 1999; Tian et al. 2009; Piazzon et al. 2016).

We identified multiple instances of *igm* upregulation and downregulation in gills, kidneys and spleens following *F. columnare* infection. In our study, *igm* was most consistently upregulated in gills, while the largest fold increases occurred in kidneys, followed by gills, then spleens. Tian et al. (2009) also documented larger fold increases in *igm* expression in kidneys than in gills

TABLE 5 | Trial 3 *p* values resulting from Kruskal–Wallis (K–W) tests and Dunn's post hoc tests (Dunn) for $\Delta\Delta C_T$ differences between time points for each gene (*igm*, *igt*, *tnf- α* , *il-8*) by temperature (Temp) and tissue type (Tissue).

Temp	Tissue	<i>igm</i>		Dunn		<i>igt</i>		<i>tnf-α</i>	<i>Il-8</i>
		K–W	Days	Unadj.	Adj.	K–W	Days	K–W	K–W
18°C	Gill	0.0273	2–7	0.007	0.022	0.1931	—	0.1133	0.2521
	Kidney	0.0439	2–7	0.014	0.042	0.1133	—	0.8371	0.0665
	Spleen	0.3425	—	—	—	0.1173	—	0.1173	0.1173
21°C	Gill	0.5637	—	—	—	0.0833	—	0.0833	0.0833
	Kidney	0.5127	—	—	—	0.0495	0–2	0.5127	0.8273
	Spleen	0.1213	—	—	—	0.1213	—	0.1213	0.1213

Note: See Figure 3 and Table S2 for detailed overviews of sampling. Statistically significant *p* values are bold. We only show *p* values from Dunn's post hoc tests for statistically significant comparisons. For Dunn's tests, we include *p* values unadjusted for multiple testing (unadj.) and *p* values adjusted for multiple testing (adj.). See Table S9 for all *p* values, including nonsignificant values. Note that we only had sampling from kidneys for time points 0 and 2 for the 21°C treatment. Therefore, there was only a single comparison of and no need to run a Dunn's post hoc test in this instance.

and spleens in the mandarin fish (*Siniperca chuatsi*) after exposure to *F. columnare*. We only identified upregulation of *igt* in gills and kidneys in trial 1 and observed the largest fold increase in *igt* expression in kidneys. In trials 2 and 3, *igt* was not upregulated in gills and was downregulated in spleens and kidneys. It is not surprising that *igt* was not upregulated in spleens given that previous work reports variation among host–pathogen combinations in the expression of *igt* in spleens following bacterial infection (Piazzon et al. 2016; Han et al. 2022). We expected *igt* to be upregulated in kidneys, and gills in particular, following *F. columnare* infection because research has found that *igt* is often upregulated in kidneys and gill lamellae following bacterial infection (Makesh, Sudheesh, and Cain 2015; Tongsri et al. 2020; Han et al. 2022). Previous work has shown particularly strong upregulation of *igt* in the gills of rainbow trout infected with *F. columnare* (Tongsri et al. 2020). Therefore, it was surprising that *igt* was not upregulated in gills in trials 2 and 3. This may have been because mortality occurred rapidly in these trials, possibly before *igt* could be upregulated. Like previous work, we found that when *igm* and *igt* were upregulated, expression generally increased within a few days of infection and, and typically peaked a few weeks postinfection when fish survived that long (Tian et al. 2009; Wei et al. 2018; Tongsri et al. 2020). Overall, our results indicate that *igt* and *igm* play central roles in adaptive immune responses to *F. columnare* and, in agreement with previous research, the upregulation of these genes in gills suggests that the mucosal immune system is key in responses to *F. columnare* infection (Tian et al. 2009; Tongsri et al. 2020).

The pro-inflammatory cytokines *il-8* and *tnf- α* are often rapidly upregulated following bacterial infection in teleost fishes (Pérez-Cordón et al. 2014; Wei et al. 2018). The expression of *il-8* and *tnf- α* following infection with *F. columnare* has not been investigated in *O. mykiss*, but these genes are upregulated in other species of fishes within a few days of *F. columnare* infection in gills, kidneys and spleens (Wei et al. 2018; Chen et al. 2020; Jia et al. 2021). In *O. mykiss*, *il-8* and *tnf- α* are also important components of innate immune responses to other bacterial pathogens (Fajardo et al. 2022). We found that *il-8* and *tnf- α* were upregulated in gills and kidneys in multiple trials at 18°C and

21°C, but were only upregulated in spleens in trial 2 at 18°C. This indicates that *il-8* and *tnf- α* are both important in *O. mykiss* immune responses to *F. columnare*. We observed that *il-8* and *tnf- α* generally showed increased expression by day 7 and sometimes as early as day 2, but we did not observe upregulation occurring as rapidly as reported by Wei et al. (2018), who reported statistically significant upregulation 1 day post infection by grass carp (*Ctenopharyngodon idellus*).

4.3 | Impacts of Temperature on Gene Expression

There is evidence that thermal stress can suppress immune responses in ectotherms (Kortet et al. 2003; Varsamos et al. 2006; Tort 2011; Schwenke, Lazzaro, and Wolfner 2016; Wernicke von Siebenthal et al. 2018). For example, Magnadóttir et al. (1999) found that cod (*Gadus morhua*) held at experimental temperatures of 1°C, 7°C and 14°C for 12 months exhibited the greatest mortality at 14°C (48.5% vs. 12.5% and 6% at 1°C and 7°C respectively) and serum protein concentrations of IgM were negatively correlated with temperature. Therefore, we hypothesised that redband trout held at cool temperatures close to the thermal optimum for *O. mykiss* would exhibit stronger immune responses to *F. columnare* infection than redband trout held at warmer temperatures. Our study found little evidence to support this hypothesis, despite implementing a warm temperature treatment of 21°C, which has been shown to cause thermal stress in redband trout (Chen et al. 2018). We did not identify any statistically significant differences in gene expression between cool and warm temperature treatments in any trial. In trials 2 and 3 (desert fish), there were more cases in which immune genes were upregulated to a greater degree at 18°C than 21°C at the same time point, providing a small amount of support for our hypothesis. However, we observed the opposite pattern in trial 1 (montane fish), in which immune genes were more frequently upregulated to a greater degree at 21°C than 18°C. One potential explanation for this discrepancy could be that the lower dose of *F. columnare* used in trial 1 resulted in less upregulation of immune genes. If trial 1 fish indeed exhibited weaker, less energetically expensive immune responses than

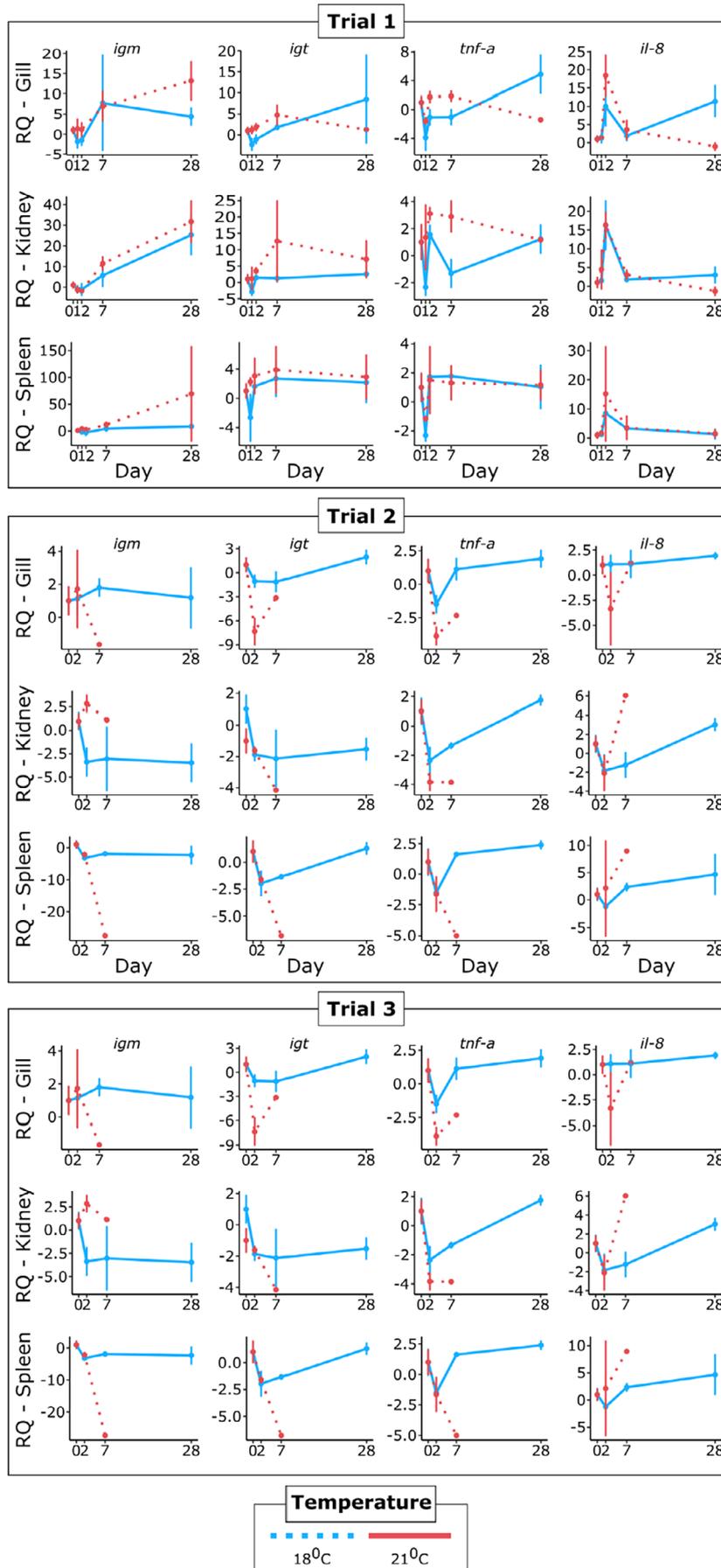


FIGURE 4 | Legend on next page.

FIGURE 4 | Line graphs showing differences between temperatures in gene expression over time for each tissue (gills, kidneys and spleens) and gene (*igt*, *igm*, *tnf- α* and *il-8*). Gene expression data are displayed as RQ values, which describe the fold change in gene expression in challenged fish relative to mock-challenged control fish. For each gene, day 0 RQ values (gene expression prior to *F. columnare* challenges) were normalised to 1.0 to compare fold-changes in gene expression. Note that y-axis ranges vary between individual plots.

TABLE 6 | *p* values from nonparametric Mann–Whitney *U* tests for differences between temperatures at same time points. Mann–Whitney *U* tests were only run if there was a significant change in gene expression detected by Kruskal–Wallis tests.

Challenge	Tissue	Days	<i>igm</i>	<i>igt</i>	<i>tnf-α</i>	<i>il-8</i>
1	Gill	1	0.70	0.10	0.20	0.70
		2	0.40	0.40	0.40	0.40
		7	1.00	0.10	0.40	0.40
		28	0.20	0.10	0.10	0.10
	Kidney	1	1.00	0.40	—	0.70
		2	0.70	0.20	—	0.70
		7	0.70	0.10	—	0.40
		28	0.70	0.20	—	0.10
	Spleen	1	0.20	—	—	—
		2	0.10	—	—	—
		7	0.70	—	—	—
		28	0.40	—	—	—
2	Gill	2	—	—	0.10	—
		7	—	—	0.50	—
	Kidney	2	—	—	0.40	1.00
		7	—	—	0.50	0.50
	Spleen	2	—	1.00	0.70	0.70
		7	—	0.67	0.67	0.67
3	Gill	2	0.20	—	—	—
	Kidney	2	0.40	0.10	—	—

trial 2 and 3 fish, they may have circumvented immunosuppression imposed by exhausted aerobic power budgets at 21°C. However, our data did not support this idea. We found that gene expression was typically greater at comparable time points in trial 1 than in trials 2 and 3 at both 18°C and 21°C. This indicates that trial 1 fish mounted stronger immune responses than trial 2 and 3 fish. This could have been the result of the higher *F. columnare* challenge doses in trials 2 and 3, which led to the rapid onset of columnaris disease signs and mortality at both trial temperatures, potentially compromising immune function. Additionally, interfamily and interpopulation differences in columnaris disease resistance could have contributed to the different relationships between temperature and gene expression in trial 1 versus trials 2 and 3. Additional research using an experimental design appropriate for characterising family and population differences in redband trout columnaris disease resistance and temperature effects is warranted. Previous work has also found positive correlations between temperature and the strength of immune responses in fishes (Avtalion et al. 1973;

Pérez-Casanova et al. 2008; Tort 2011; Zanuzzo et al. 2020; Beemelmans et al. 2021). For example, Zanuzzo et al. (2020) found that Atlantic salmon (*Salmo salar*) acclimated to a warm temperature (20°C) exhibited greater upregulation of immune genes following vaccine-induced immunostimulation, relative to fish held at a control temperature of 12°C. However, it is not known if positive correlations between temperature and immune function would have been maintained if these studies used warmer experimental temperatures that induced greater thermal stress, or if declines in immune function would have eventually been observed if temperatures were increased sufficiently. The ‘scheme of temperature effects on teleost immunity’ presented by Scharsack and Franke (2022) predicts weaker immune responses at low and high temperatures with the strength of immune responses peaking at intermediate temperatures. It is possible that studies finding positive correlations between immune function and temperature did not expose fishes to temperatures outside the ‘intermediate’ range that would be expected to suppress fish immune systems.

There are several possible reasons why redband trout did not mount stronger immune responses at cooler temperatures than warmer ones. It may have been because immune responses occurred before aerobic power budgets could become limiting. It is also possible that our experimental temperatures were not warm enough to suppress immune systems via reductions in aerobic power budgets or the release of cortisol. At 21°C, most of the source populations used in our study are thought to only experience moderate heat stress (Chen et al. 2018; Andrews et al. 2023). However, our mortality data suggest that for many redband trout populations, increased *F. columnaris* virulence may result in 100% mortality before the possibility of immunosuppression becomes relevant.

4.4 | Implications for the Conservation of Redband Trout

Columnaris disease has been linked to die-offs in wild populations of freshwater fishes, including redband trout, during periods of abnormally high temperatures that are stressful for fish and enhance the growth rates and virulence of *F. columnare* (Perkins, Kann, and Scoppettone 2000; Karvonen et al. 2010; Kunttu et al. 2012; Declercq et al. 2013; Loch et al. 2013; Loch and Faisal 2015; Faisal et al. 2017). *F. columnare* is common in freshwater aquatic environments worldwide (Austin and Austin 2007). Where *F. columnare* co-occurs with wild redband trout, columnaris disease may emerge as a significant source of mortality if environmental changes increase water temperatures. At temperatures experienced during the summer by wild redband trout populations, we found that *F. columnare* caused high mortality, but identified little evidence of temperature-mediated immunosuppression (Chen et al. 2018; Chen and Narum 2021). Therefore, modelling efforts focused on redband trout and other fishes may benefit most from focusing on the impacts of temperature on *F. columnare* transmission dynamics and virulence and intraspecific variation in the disease resistance of fishes, while considering the impacts of temperature on redband trout immune function may be less important. Future research could characterise gene expression and mortality following challenge with *F. columnare* at a wider range of temperatures and could use challenge doses that induce lower mortality than trials 2 and 3 in our study, allowing for chronic symptomology that may result in immunosuppression. Additionally, future studies could also improve our understanding about relationships between temperature and immune responses to *F. columnare* infection by using study designs that involve more family groups in each cross, include fish from all crosses in each replicate trial and conduct several replicate trials for each *F. columnare* challenge dose. Finally, our results also indicate that *F. columnare* transmission dynamics in wild populations of coldwater fishes warrant further investigation given predicted exposure to warming temperatures (e.g., Andrews et al. 2023).

Author Contributions

Joshua P. Egan: conceptualization, formal analysis, visualization, writing – original draft, writing – review and editing, investigation, project administration. **Jie Ma:** writing – review and editing, investigation, conceptualization. **Veronica L. Myrsell:** writing – review and editing, investigation. **Zhongqi Chen:** investigation. **Jonathan Masingale:**

investigation. **Christopher C. Caudill:** supervision, writing – review and editing, project administration, funding acquisition, resources. **Timothy Boyle:** investigation. **Julianna Browning:** investigation. **Shawn R. Narum:** writing – review and editing. **Kenneth D. Cain:** conceptualization, supervision, writing – review and editing, funding acquisition, resources, project administration. **Paul A. Hohenlohe:** conceptualization, supervision, project administration, writing – review and editing, funding acquisition, resources.

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Ethics Statement

All experimental procedures involving live fish were carried out with prior approvals from the Institutional Animal Care and Use Committees at the University of Idaho (protocol IACUC-2022-19). Laboratory pathogens were covered under the University of Idaho Institutional Biosafety Committee approval IBC-22-023.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available in the Tables S1–S9.

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Supporting Information

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