THERMAL ADAPTATION OF WESTSLOPE CUTTHROAT TROUT

ONCORHYNCHUS CLARKII LEWISI

by

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ABSTRACT

Understanding local adaptations is a fundamental goal of evolutionary biology and would provide managers information necessary to better protect and conserve species. Salmonids are a particularly useful system for studying local adaptations as they often persist in disparate and isolated environments. In addition, their sensitivity to temperature provides a likely candidate for natural selection to act. I studied thermal adaptation in four wild populations and one hatchery stock of westslope cutthroat trout. Native stream mean summer temperatures ranged from 6.7° to 11.2°C. Embryos were collected from the wild and differences in embryonic development, embryonic survival, and juvenile growth were measured. I found a significant relationship between median embryonic survival and native stream temperatures at warm incubation temperatures (Rank test; $P = 0.04$). The change in embryonic survival across incubation temperatures was consistent for populations from warm streams, but changed drastically for populations from cool streams. This difference suggests that populations from warmer streams may be thermal generalists, and populations from cooler streams may be thermal specialists. Results have both short- and long-term implications. In the short-term, managers should use these data to support the consideration of local adaptations when performing translocation projects. In the long-term, these data suggest that global climate change may be detrimental for westslope cutthroat trout.

INTRODUCTION

Habitat heterogeneity across the range of a species may lead to varying importance of traits among populations. If these traits are heritable, populations will probably become locally adapted through natural selection. Understanding this process is a fundamental goal of evolutionary biology.

Salmonids are particularly useful for studying local adaptations as they often persist in disparate and isolated environments. In addition, their strong homing behavior and temporal differences in reproduction reinforce the isolation of populations (Quinn 1993; Quinn *et al.* 2000). Many salmonids are currently threatened or endangered and a better understanding of their local adaptations could be important to their long-term persistence. The westslope cutthroat trout *Oncorhynchus clarkii lewisi* is one such species.

The westslope cutthroat trout is an extant member of Salmonidae endemic to the northwestern United States and southwestern Canada. Historically, its range was the largest of any cutthroat trout and included the Missouri, Columbia, and Saskatchewan river basins (Behnke 1992). Populations are declining range-wide (Liknes & Graham 1988; Shepard *et al.* 1997; Shepard *et al.* 2005) and are estimated to inhabit only 60% of their historic range (Shepard *et al.* 2005). Habitat alterations, competition with nonnative fishes, overexploitation, and genetic introgression are all factors that have been attributed for the loss of westslope cutthroat trout (Allendorf & Leary 1988; Liknes & Graham 1988;

Rieman & Apperson 1989; Van Eimeren 1996). Of these potential causes, genetic introgression mainly from rainbow trout (*O. mykiss*), Yellowstone cutthroat trout (*O. c. bouvieri*), and golden trout (*O. m. aguabonita*) is believed to have had the most detrimental effect (Allendorf & Leary 1988; Liknes & Graham 1988). Only 35% of the total stream length currently occupied by westslope cutthroat trout is believed to be inhabited by genetically pure populations (Shepard *et al.* 2005). This equates to about 21% of the historic range.

Many of the remaining genetically pure populations are small and isolated above either natural or constructed barriers. These barriers prevent fish migrations and make the transfer of genetic material between populations impossible. Small, isolated populations such as these are at a greater risk of inbreeding, which has a detrimental effect in a number of taxa (Darwin 1892; Ralls *et al.* 1979; Gjerde *et al.* 1983; Berger 1990; Frankham 1995; Dunham 1997; Saccheri *et al.* 1998; Frankham *et al.* 2006). To counter the effects of inbreeding, artificial immigration projects have been considered by managers. Modeling simulations suggest that artificial immigration projects can be successful (Hilderbrand 2002) and they have been shown to have positive effects on reproductive fitness and population growth in various taxa (Westemeier *et al.* 1998; Frankham *et al.* 2006; Johnson *et al.* 2010). However, no studies of local adaptations have been performed on westslope cutthroat trout. Before any translocation projects are initiated, a better understanding of potential local adaptations is imperative. The introduction of individuals from a poorly adapted

population could result in further population depression through the introduction of poorly adapted genotypes.

Because fish are poikilothermic, temperature affects nearly every aspect of their lives (Brett 1970). Survival, development, growth, migration, and reproduction are all influenced by temperature (Embody 1934; Pankhurst *et al.* 1996; Swanberg 1997; Bear *et al.* 2007). As such, it likely has a strong selective force and could be an environmental factor to which populations are locally adapted. In addition, the study of thermal adaptations allows for additional inquiry into how climate change may affect populations. The Intergovernmental Panel on Climate Change estimates that by 2100 global air temperature will increase by 1.4 to 5.8°C (IPCC 2003). Warming trends have already been seen (Stewart *et al.* 2005) and extinctions are predicted to increase (Thomas *et al.* 2004). How future changes will affect already imperiled ecosystems and species is of great concern.

My objective was to investigate potential thermal adaptations of westslope cutthroat trout so as to better understand how natural selection influences divergence, to provide managers with additional information for conservation, and to explore potential effects of global climate change. The work explored both embryonic and juvenile responses to temperature by investigating embryonic survival and development rates and juvenile growth and composition.

MATERIALS AND METHODS

Study Area

Five populations of westslope cutthroat trout, four wild and one hatchery, were investigated. The wild populations came from Chamberlain, Cottonwood, O'Brien, and Ray creeks, all in the Missouri River drainage. Cottonwood Creek is the warmest of the four with an average summer temperature of 11.2℃ in 2009, followed by O'Brien (8.2℃), Chamberlain (7.3℃), and Ray creeks (6.7℃).

The hatchery population comes from the Montana Department of Fish, Wildlife and Parks' Washoe Park Trout Hatchery, Anaconda, Montana. Fish housed in this facility are of the MO12 strain. The MO12 strain was developed in 1983 and 1984 from populations in the Flathead and Clark Fork river drainages. The brood stock was infused with wild fish from the Flathead drainage from 2003 to 2005. Embryos at the Washoe Park Trout Hatchery are generally incubated and reared at 13.3℃. See Appendix A for more information on the five populations included in the study.

Embryonic Adaptation

Embryonic survival and development were investigated using offspring of the five study populations. Broodstock from all wild populations were collected starting in June 2009 and continuing throughout the spawning period using a

backpack electrofisher. Captured westslope cutthroat trout were retained and housed in 110-liter live-cars in their respective creeks. Female broodstock were checked every two days for signs of ripeness. A female was designated as ripe when gentle ventral pressure released eggs. Eggs from each ripe female were manually stripped and separated into two lots. Each lot was fertilized by one male and each pairing was considered one family (half-sib pairings). If a female had fewer than 50 eggs (estimated by observation), she was spawned with only one male. Eggs were exposed to milt for 60 seconds, after which time, excess milt was rinsed from each lot. Embryos were allowed to water harden for 30 minutes before being placed in coolers and transported to the U.S. Fish and Wildlife Service Bozeman Fish Technology Center (BFTC), Bozeman, Montana. After spawning, brood fish were returned to their respective streams. Embryos from each family were divided and incubated at one of three target temperatures: 8° (mean 8.08°C; 95% confidence interval 7.63 to 8.59°C), 10° (mean 10.11°C; 9.67 to 10.37°C), or 14°C (mean 14.53°C; 14.39 to 14.67°C) at the BFTC. Two Heath tray batteries (8-Tray Vertical Incubator, MariSource, Milton, WA) were maintained at each temperature. Each battery contained six trays. Each tray was divided into 48 individual compartments. Upon arrival at the BFTC, embryos were sterilized for 10 minutes in idophore Betadine (Piper *et al.* 1982). Next, embryos were acclimated to their treatment temperature at a rate of 1°C per 15 minutes. After acclimation, embryos were deposited into their assigned incubator locations. Assignments were made pseudo-randomly. Families were manually

assigned to 2 trays to prevent the same family being present on the same tray. Families were randomly assigned to a well location within each tray. Each family was present in two wells in each Heath stack.

Embryos were examined every other day, and a record was kept of total embryos, mortalities, and hatching. Embryos that died or hatched were removed from the system. A formalin treatment of 1666 ppm was applied for fifteen minutes every other day to prevent the occurrence of fungus (Piper *et al.* 1982). Water temperatures were manually monitored daily and hourly with a temperature logger (UA-002-64 and UA-002-08, Onset HOBO, Pocasset, MA).

Juvenile Adaptation

Differences in growth (weight, length, and condition) and composition (percent dry protein and percent dry fat) were investigated using the surviving individuals from the embryonic adaptation experiment. At the BFTC, 63 75-L aluminum tanks of 120 x 35 x 25 cm dimensions were used. Twenty-one tanks were assigned to each of three treatment temperatures: 8 (mean 8.34°C; 95% confidence interval of 8.16 to 8.52°C), 10 (mean 10.19°C; 10.05 to 10.32°C), and 14°C (mean 14.30°C; 14.21 to 14.38°C). Within each temperature group, three tanks were randomly assigned to each of the five populations (Chamberlain Creek, Cottonwood Creek, O'Brien Creek, Ray Creek, and MO12). Embryos were incubated at about the same temperature as the treatment groups, and upon hatching, were randomly assigned to a tank for their population. When fry

in a tank began actively seeking food, a 12-hour automatic belt-feeder was used to provide BioVita Starter pellet (Bio-Oregon, Longview, WA). Fish were fed to excess, defined as the presence of food remaining in the tank 24 hours after feeding. If no food remained, rations were increased daily until excess was reached. Tanks were cleaned daily. Fish were taken off feed 89 days after peak hatch date for each tank. Individuals were sacrificed the following day using an overdose of MS-222 (Argent Laboratories, Redmond, WA). Lengths and weights of all fish were measured immediately after euthanization. Fish were stored at -80℃ until they were processed using proximate analysis. Proximate analysis was performed to determine the mean percents dry protein and dry fat of fish in each tank.

All individuals from a tank were homogenized with a known amount of deionized water. Samples were immediately placed into a freezer. After freezing, samples were freeze dried using a Labconco FreeZone 12 (Labconco Corporation, Kansas City, MO). Samples remained in the freeze dryer until no weight change occurred over a 24-hour period. Protein analysis was performed using a LECO TruSpec CN (LECO Corporation, St. Joseph, Michigan). Samples were combusted at 950℃. The resulting product was measured for traces of nitrogen, which were converted to protein content by multiplication by a protein factor of 6.25, which was based on the nitrogen content of protein (16%). Protein was measured in duplicate for fish in each tank using samples of about 0.15 g. Fat content was analyzed using the Ankom^{XT10} Extractor (ANKOM Technology,

Macedon, NY). The extraction system is a modified soxhlet extractor that uses petroleum ether as the solvent. Duplicate samples of about 0.1 g from each tank were weighed and placed into the extractor. After extraction, samples were dried for 30 minutes at 135°C. Next, they were cooled in a vented desiccator for 20 minutes. Weights were measured again and percent fat was calculated by multiplying the estimated percent fat in the sub-sample by percent dry matter.

Statistical Methods

Westslope cutthroat trout require about 280 to 310 degree days to hatch. At higher temperatures, the range of days between the start and finish of hatching is expected to decrease. Also, my protocol of checking embryos every other day cause the date of hatch to be grouped by two-day intervals. Thus, the variance of these data was not constant across temperatures, requiring a non-parametric analysis. An average number of days to hatch was calculated for each family at each temperature. The averages were analyzed using Kruskal-Wallis non-parametric analysis of variance tests for each incubation temperature. When significant differences were detected, pairwise comparisons were made using a Mann-Whitney-Wilcoxon test with a Bonferroni adjustment for multiple comparisons. All calculations were performed using R version 2.11.1.

Embryonic survival was assessed in two ways. First, Kruskal-Wallis non-parametric analysis of variance tests were performed at each incubation temperature. These tests explored possible differences in embryonic survival

among the populations. When differences were detected, pairwise Mann-Whitney-Wilcoxon tests with a Bonferroni adjustment were used for multiple comparisons. Second, because the distribution of the data did not allow for direct comparisons of possible population x temperature interactions, I performed an additional analysis comparing multiplicative changes in embryonic survival across incubation temperatures based on native stream temperatures. That is, I calculated the proportional change in median survival of each population between 8 and 10°C and 10 and 14°C. Changes from 8 to 14°C were not assessed as they masked survival at 10°C. Family survival was pooled at each temperature. Pooled values were used for all embryonic survival analyses.

Growth metrics (weight, length, and Fulton condition factor (K)) were analyzed using both nested ANOVAs in which each tank was a random effect and ANOVAs using averages from each tank. Both analyses resulted in the same conclusions and ANOVAs using tank averages are reported here because of their simplicity. At each treatment temperature, one-way ANOVAs with population as the explanatory parameter were used to examine differences in growth. Across temperatures, a series of two-way ANOVAs was used to assess possible population x temperature interactions. Differences in K across treatment temperatures did not exist. Therefore, values were pooled before the final analysis was completed.

Both percent dry protein and percent dry fat were investigated only with fish raised at 8 and 10°C. Fish raised at 14°C were used for a different study and

were unavailable. Duplicate samples were run for both and an average percent protein and percent fat was calculated for each tank. Percent protein was analyzed using one-way ANOVAs with population as the explanatory parameter at each treatment temperature. Differences in percent dry protein across treatment temperatures did not exist. Therefore, values were pooled before the final analysis was completed. Outliers existed in percent dry fat data. Therefore, a Kruskal-Wallis non-parametric analysis of variance test was used at each treatment temperature. Percent dry fat did not differ across temperatures; the results were therefore pooled before the final analysis. Pairwise Mann-Whitney-Wilcoxon tests with a Bonferroni adjustment were used for multiple comparisons.

RESULTS

Embryonic Survival

I found a significant correlation between native stream temperature and embryonic survival at warm temperatures (Appendix B: Figure 3). Decline in median embryonic survival from 10 to 14°C was significantly greater for populations from colder native streams (Rank test, $P = 0.04$). A 1°C lower native stream temperature resulted in a multiplicative reduction in median survival of 0.21 ($F_{1,2} = 71.43$, $P = 0.01$; 95% confidence interval 0.12 to 0.32). The same pattern did not exist at colder incubation temperatures. No correlation in proportional change in median survival between 8 and 10°C and native stream temperature was detected ($F_{1,2} = 0.75$, $P = 0.48$).

Median embryonic survival was highest for all populations at colder temperatures (Appendix B: Figure 4). Median survival of Ray, Cottonwood, and hatchery embryos was best at 8°C. Median survival of Chamberlain and O'Brien creek embryos was slightly higher at 10°C than 8°C. Survival of all populations was lowest at 14°C. Survival of MO12 embryos was lower than that of all other populations at all temperatures. See Appendix C for more information on growth results.

Rate of Embryonic Development

All populations had a significant reduction in days to hatch with an increase in incubation temperature (KW_2 = 199.105, P < 0.001). Populations did not differ in days to hatch at 8 (KW_4 = 6.262, P = 0.18) or 10°C (KW_4 = 3.038, P = 0.55). At 14°C, MO12 embryos hatched in significantly fewer days than those from the warmest population, Cottonwood Creek (W = 31, P = 0.003). Embryos from Cottonwood Creek were estimated to take 0.8 more days to hatch than MO12 embryos with a bootstrapped 95% confidence interval of 0.2 to 1.6. No other significant differences in days to hatch were detected at 14°C. See Appendix C for more information on embryonic development results.

Fish Growth

Weight ($F_{1,45}$ = 146.93, P < 0.001) and length ($F_{1,45}$ = 180.12, P < 0.001) of all populations increased significantly with an increase in temperature. Fulton Condition Factors (K) did not differ among treatment temperatures ($F_{1,37}$ = 3.22, P = 0.08). Significant differences in weight were detected among populations at both 8 ($F_{4,12}$ = 3.82, P = 0.03) and 10°C ($F_{4,15}$ = 17.10, P < 0.001). At 8°C, the mean weight of Cottonwood Creek fish was 0.21 g more than that of O'Brien Creek fish (t_6 = 6.75, P < 0.001, 95% confidence interval 0.13 to 0.29) and 0.18 g more than that of Ray Creek fish (t_3 = 8.98, P = 0.002, 95% confidence interval 0.12 to 0.25). No other differences in weight were detected at 8°C. At 10°C, the

mean weight of Cottonwood Creek fish was estimated to be 0.60 g more than that of Chamberlain Creek fish (t_5 = 7.20, P = 0.001, 95% confidence interval 0.38 to 0.86), 0.58 g more than that of O'Brien Creek fish (t_7 = 14.12, P < 0.001, 95% confidence interval 0.48 to 0.68), and 0.43 g more than that of Ray Creek fish (t_3 = 9.33, P = 0.002, 95% confidence interval 0.28 to 0.57). No other differences in weight were detected at 10°C. Length differed among populations only at 10°C ($F_{4,15}$ = 8.61, P < 0.001). O'Brien Creek fish had a mean length 3.9 mm shorter than that of MO12 fish (t_7 = 4.82, P = 0.002, 95% confidence interval 2.0 to 5.8) and 6.3 mm. shorter than that of Cottonwood Creek fish (t_6 = 8.35, P < 0.001, 95% confidence interval 4.5 to 8.1). No other differences in length were detected at 10°C. Populations significantly differed in K ($F_{4,42}$ = 12.93, P < 0.001). Condition of Cottonwood Creek fish was estimated to be 0.07 (t_8 = 15.36, P < 0.001) greater than those of all other populations (95% confidence interval of 0.05 to 0.09). No differences in K were detected among the other populations.

Two-way ANOVAs showed no evidence of any population x temperature interactions for weight ($F_{4,37}$ = 1.7436, P = 0.16), length ($F_{4,37}$ = 1.337, P = 0.27), or condition ($F_{4,37}$ = 1.7436, P = 0.16). See Appendix D for more information on growth results.

Fish Composition

Percents dry protein ($F_{4,32}$ = 2.35, P = 0.14) and fat (KW_1 = 0.7442, P = 0.38) were not significantly different among temperatures. Differences existed in

percent dry protein among the populations after pooling population data across temperatures ($F_{4,29}$ = 6.98, P < 0.001). O'Brien Creek fish had 2.0% more protein than the Ray Creek population (t_{15} = 5.62, P < 0.001, 95% confidence interval 1.2 to 2.8%). No other differences in percent dry protein were detected. Percent dry fat also differed among the populations (KW_4 = 19.3648, P < 0.001). Cottonwood Creek fish had 3.0% more fat than O'Brien Creek fish (W = 66, P < 0.001, 95% confidence interval 2.1 to 3.5%). Cottonwood Creek fish had 2.5% more fat than Chamberlain Creek fish (W = 36, P = 0.002, 95% confidence interval 1.8 to 3.4%). Ray Creek fish had 1.6% more fat than O'Brien Creek fish (W = 5, P = 0.003, 95% confidence interval 0.5 to 2.4%). No other differences in percent fat were detected among the populations. See Appendix E for more information on growth results.

DISCUSSION

A thermal cline existed in embryonic survival at warm (14°C) incubation temperatures. Survival responses were different depending on the population, with fish from the coolest creek (Ray Creek) having a proportional reduction in median survival that was over three times that of fish from the warmest creek (Cottonwood Creek). In addition, warm-water populations survived more consistently at all incubation temperatures, whereas cold water populations survived substantially better at colder incubation temperatures. The proportional change in median survival of Cottonwood Creek embryos was nearly constant when changing from 8 to 10°C or 10 to 14°C (0.11 and 0.10), whereas a considerable difference existed for Ray Creek embryos (0.08 and 0.34). Embryos from the intermediate Chamberlain and O'Brien creeks had intermediate changes (-0.01 and 0.26 and -0.02 and 0.21). These results suggest that populations may have adapted into thermal generalists and specialists. Most Rocky Mountain streams experience near-freezing temperatures in winter, but their summer high temperatures are dependent on a wide range of factors (Poole & Berman 2001) and temperatures of neighboring streams can be quite different. As such, populations in streams with a high peak temperature experience a wider range of temperatures annually than populations in cooler streams. Consequently, natural selection would favor cool-water specialists for colder streams and thermal generalists for warm-water streams.

During the westslope cutthroat trout incubation period in spring and summer, temperatures in Cottonwood Creek reached a maximum of 19.9℃ whereas those in Ray Creek only reached 9.5℃. It is of note that the hatchery strain MO12 was created using broodstock from 20 different populations and is generalist by design. The proportional change in median survival from 10 to 14℃ for MO12 embryos was 0.13, which is nearly identical to that of Cottonwood Creek embryos, the warmest stream in the study.

Existence of the temperature effect only during embryogenesis is not surprising. Individuals are most sensitive to their environment during embryonic development (McKim 1977) and selection is believed to have a strong effect during this life stage. Throughout embryogenesis, cells are forming the precursors of the adult body (Kunz 2004) and abnormalities in gastrulation or neurulation could result in death or severe deformity. Individuals with genotypes poorly adapted to an incubation temperature would likely be removed. This would leave only individuals with adapted genotypes at later life stages. This could explain why no patterns of thermal adaptation were present in post-embryonic metrics.

The fact that the rate of embryonic development was the same for all wild populations is also unsurprising. Thus far, the only known intra-specific differences in development rate detected in salmonids were detected in conspecific salmon from different spawning seasons (Tallman 1986, Konecki *et al.* 1995). Westslope cutthroat trout all spawn at the same time of the year.

An anomaly in this study was the general poor embryonic survival of the hatchery population. Survival of MO12 embryos was the poorest of all populations at all incubation temperatures. Most hatchery broodstock used in this study were age two, which is their first spawning season. Gamete quality for the first spawning season is often poor, resulting in reduced survival (Bromage & Cumaranatunga 1988). In addition, gamete quality of hatchery populations is often poor compared to wild populations (Srivastava & Brown 1991). These factors probably contributed to the effect seen here.

Embryonic survival of all populations was best at cold or intermediate temperatures. This may highlight both the requirement of salmonids for cool temperatures and our current lack of knowledge of actual incubation temperatures within redds. However, the phenomenon detected in this study is highly unlikely if stream temperatures are not correlated with incubation temperatures.

It was unsurprising that juveniles of all populations increased in size with an increase in temperature between 8 and 14°C. Hatchery westslope cutthroat trout grow optimally between 13 and 15°C (Bear *et al.* 2007).

One caution I would be remiss not to mention is the small sample size of the study. As with most species of conservation concern, collecting large samples is often difficult and perilous for the species. This study was no different. The number of families created in each population ranged from six to twenty-five. Likely, this is not enough to provide a good estimate of underlying

population distributions, but more than enough to estimate medians, the statistic on which the significant results are based. In addition, only four wild populations were used to estimate the slope. Additional populations would provide more clarity to the estimated decline.

Implications

My findings have both short- and long-term management implications. In the short-term, managers are wrestling with how to properly manage isolated populations of westslope cutthroat trout. This study provides evidence that local adaptations should be considered before translocation projects are initiated. Embryonic survival at warm temperatures was greater for populations from warm native streams, and the greatest chance of translocation success would likely result from matching donor and recipient habitats. That being said, additional work is needed before any translocation projects are undertaken. Outbreeding depression, or the reduction in fitness from breeding distantly related individuals, has never been studied in westslope cutthroat trout. If outbreeding depression occurs in westslope cutthroat trout, careful consideration must be taken by managers to properly balance local adaptations with the need for additional genetic diversity. In addition, no studies have investigated inbreeding in westslope cutthroat trout. Although the majority of literature on the subject has shown that inbreeding depresses populations, evidence for inbreeding depression in westslope cutthroat trout is needed.

In the long-term, the persistence of westslope cutthroat trout, given the threat of global warming, must be considered. Global models predict an air temperature increase of 1.4 to 5.8℃ by 2100 (IPCC 2003). It is unclear exactly how this increase in air temperature will affect streams, but it will likely cause a wide range of effects (e.g. Crozier *et al.* 2008). Increases in air temperature have been correlated with increases in water temperature throughout the Northern Rocky Mountains (Isaak *et al.* 2010). Such increases will probably cause a shift in salmonid habitat use to more upstream locations. However, because many populations of westslope cutthroat trout are already isolated in headwater reaches, little upstream habitat is available. Instead, populations may be forced to live in warming waters.

My findings suggest that the colder the native stream, the greater the mortality at warm temperatures. Because many populations are isolated in cold headwater streams, an increase in water temperature caused by global warming could be detrimental to their long-term persistence. In areas where populations are still connected and immigration is above a certain threshold, re-colonization could be possible. However, the number of interconnected populations, especially in the Missouri River basin, is low.

Factors likely to be important to the long-term survival of westslope cutthroat trout in the face of global warming will be the degree of thermal increase and the evolutionary potential of the populations. If water temperature increases are small, temperatures for most populations will likely stay within an

acceptable range. Most changes in survival would be minimal, similar to the change in embryonic survival from 8 to 10°C seen in this study. In addition, a slight increase in temperature would likely result in increased fish growth and could potentially be beneficial, especially for populations now at high elevations (Cooney *et al.* 2005; Bear *et al.* 2007). If temperature increases are large, survival could decrease and the evolutionary potential of each population would be important. If the evolutionary potential of cold water populations is high, selection would probably cause a change in the genetic structure of the population, but the demographics could potentially rebound (e.g. Stockwell *et al.* 2003). Unfortunately, this information is unknown. A low amount of neutral genetic diversity exists within populations, particularly in the Missouri River drainage (Drinan *et al.* In review). It is unknown whether neutral diversity is correlated with adaptive diversity.

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APPENDICES

APPENDIX A

POPULATIONS STUDIED

Chamberlain Creek is located in the Lewis and Clark National Forest in Montana. The stream length is about 8.7 km. Westslope cutthroat trout in the stream were genetically tested in 2008 and determined to be 98.2% genetically pure (Montana Department of Fish, Wildlife and Parks 2009). A man-made barrier is located near the mouth of the stream, above which only westslope cutthroat trout and mottled sculpin *Cottus bairdii* are believed to exist. Brook trout, mottled sculpin, and westslope cutthroat trout are present below the barrier. Cottonwood Creek is in the Castle Mountains southeast of White Sulphur Springs, Montana. It has a stream length of about 9.8 km. The population had no introgression in 2000 (Montana Department of Fish, Wildlife and Parks 2009). Westslope cutthroat trout are the only known species in Cottonwood Creek. O'Brien Creek is located in the Lewis and Clark National Forest north of White Sulphur Springs, Montana. The stream length is about 8.8 km. Westslope cutthroat trout in O'Brien Creek were genetically pure in 2007 (Montana Department of Fish, Wildlife and Parks 2009). There are two barriers in O'Brien Creek. Above the second barrier, only westslope cutthroat trout are believed to persist. Brook trout, mottled sculpin, rainbow trout, and westslope cutthroat trout are present below the barrier. Ray Creek is located in Helena National Forest northeast of Townsend, Montana. The stream is about 19.1 km long. Westslope cutthroat trout are the only species present. Westslope cutthroat trout were genetically pure in 2008 (Montana Department of Fish, Wildlife and Parks 2009).

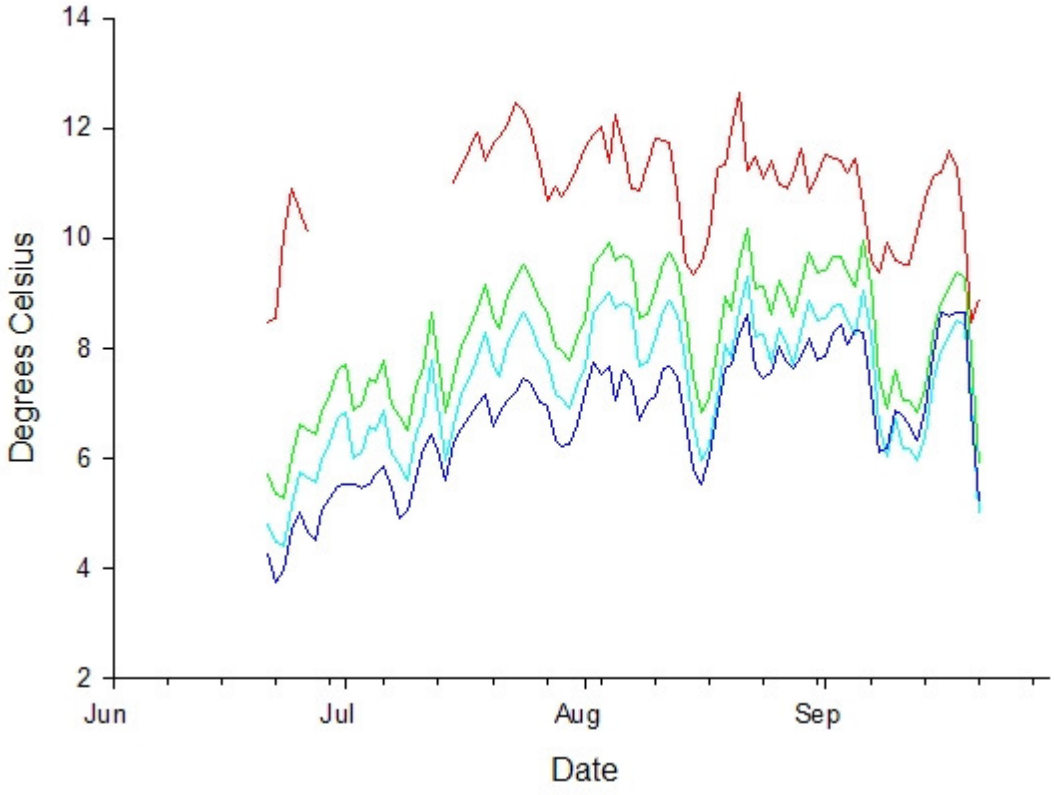

Figure 1: Daily average summer stream temperatures for Cottonwood (red), O'Brien (green), Chamberlain (light blue), and Ray (purple) creeks.

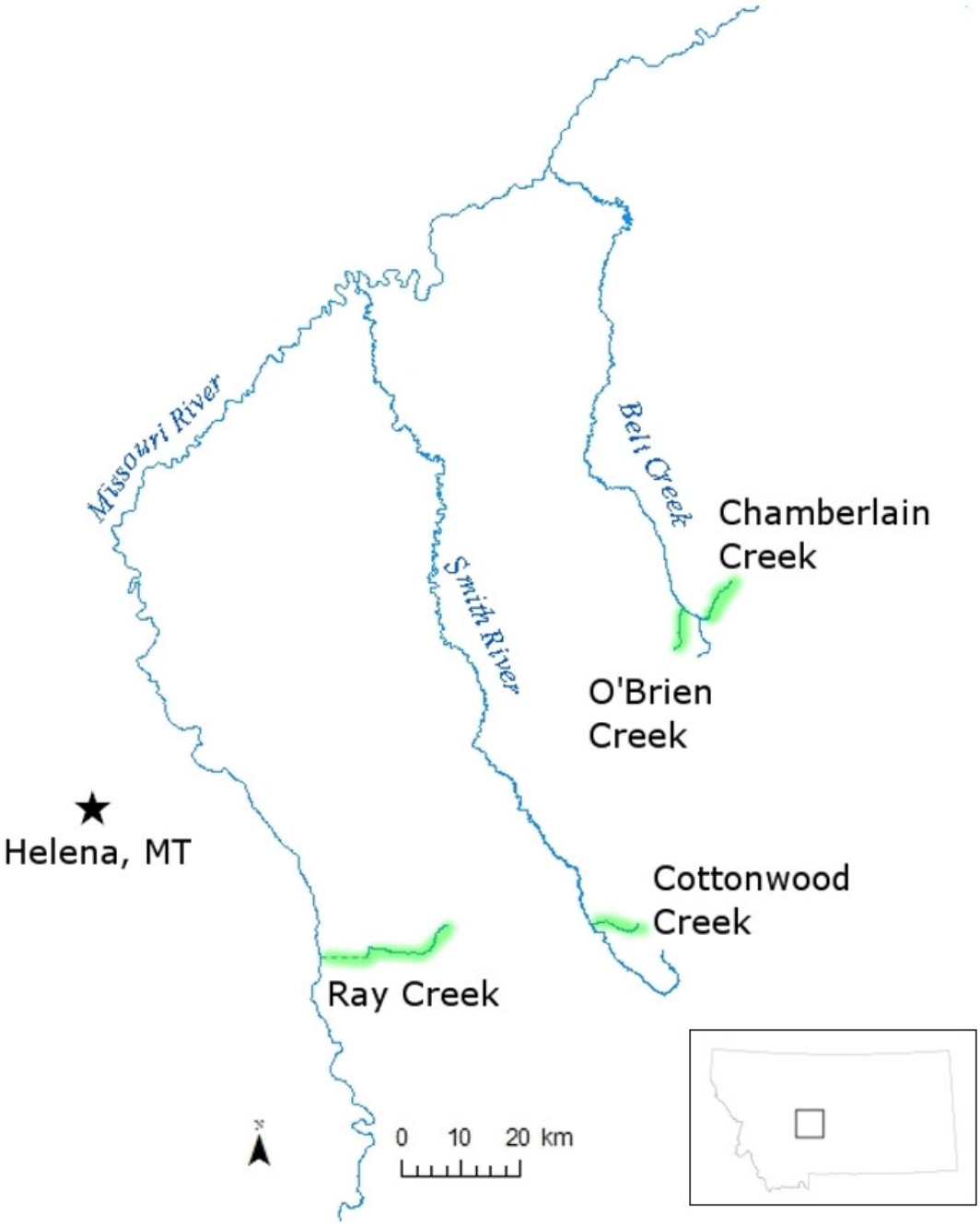

Figure 2: Sampled populations of *O. clarkii lewisi*, 1) Ray Creek, 2) Cottonwood Creek, 3) O'Brien Creek, and 4) Chamberlain Creek.

Table 1: Source populations and contributions for MO12 broodstock.

Source		Year and Collection				
Drainage	***Stream***	***1983***	***1984***	***2003***	***2004***	***2005***
Flathead	Ball Cr.	105				
	Branch Cr.	175	130			
	Connor Cr.	350	470			
	Battery Cr.	35	25			
	Quintonkon Cr.	150	365	40		
	Felix Cr.	200	100			
	Hungry Horse Cr.	230	150			
	Lost Mare Cr.	230	100			
	Tiger Cr.	345	200			
	Margaret Cr.	395	200			
	Emery Cr.	800	400			
Clark Fork	Tin Cr.		240			
	Marten Cr.		600			
	Vermillion River		450			
Flathead	Deep Cr.			37		
	Quintonkon Cr.			40		
	Paint Cr.				20	
	Addition Cr.					16
	South Cr.					8
	Goldie Cr.					8
	Twin Cr.					9

APPENDIX B

EMBYRONIC SURVIVAL

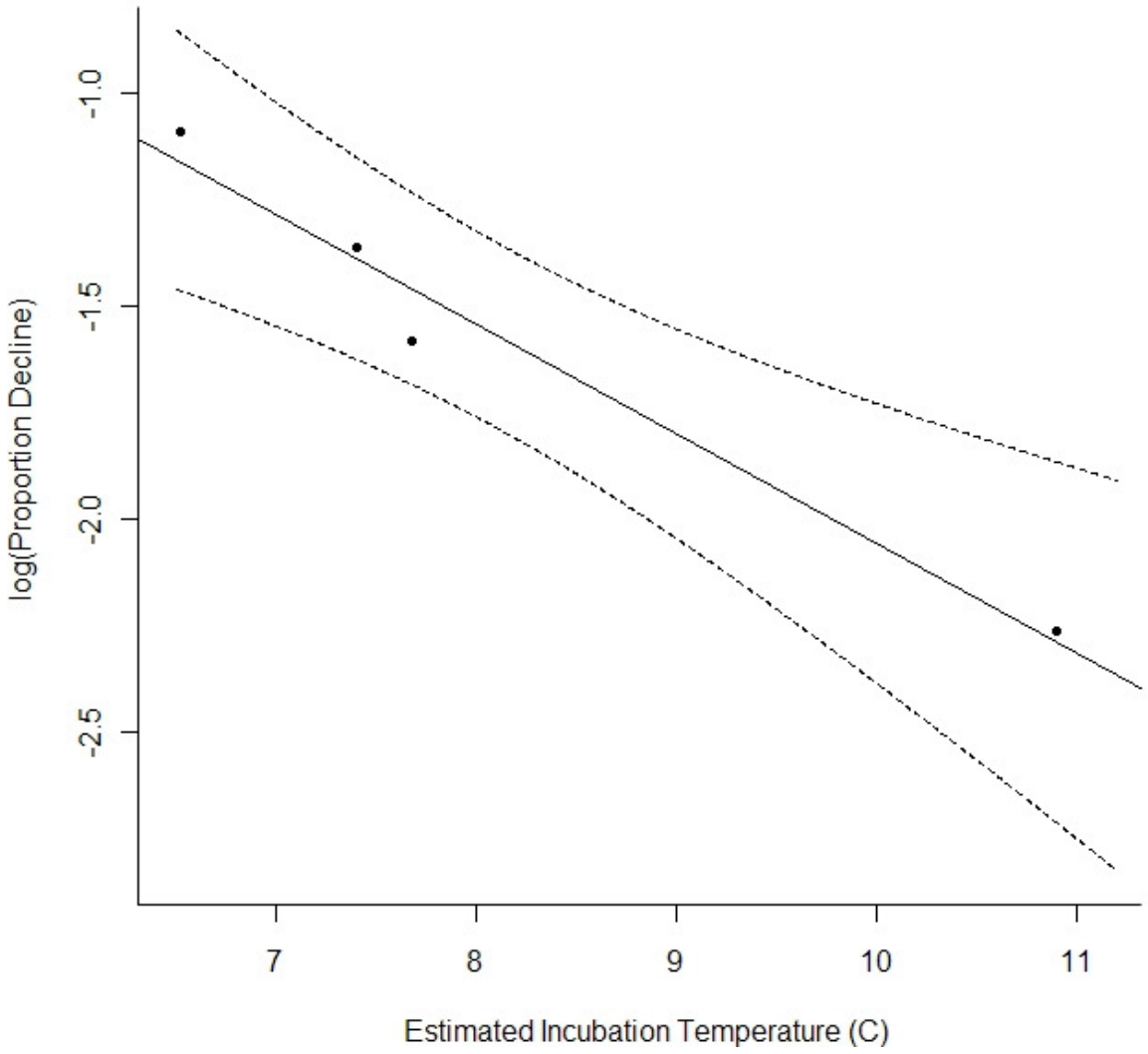

Figure 3: Log-transformed proportion decline from 10 to 14℃ with 95% confidence bands for the estimated regression. Each dot represents the log-transformed decline in median survival for one population. Estimated incubation temperatures were calculated by averaging native stream temperatures from the start of spawning to the estimated end of incubation. The end of incubation was estimated by calculating 305 accumulated thermal units (average accumulated thermal units to hatch for fish in this study) after the last spawning.

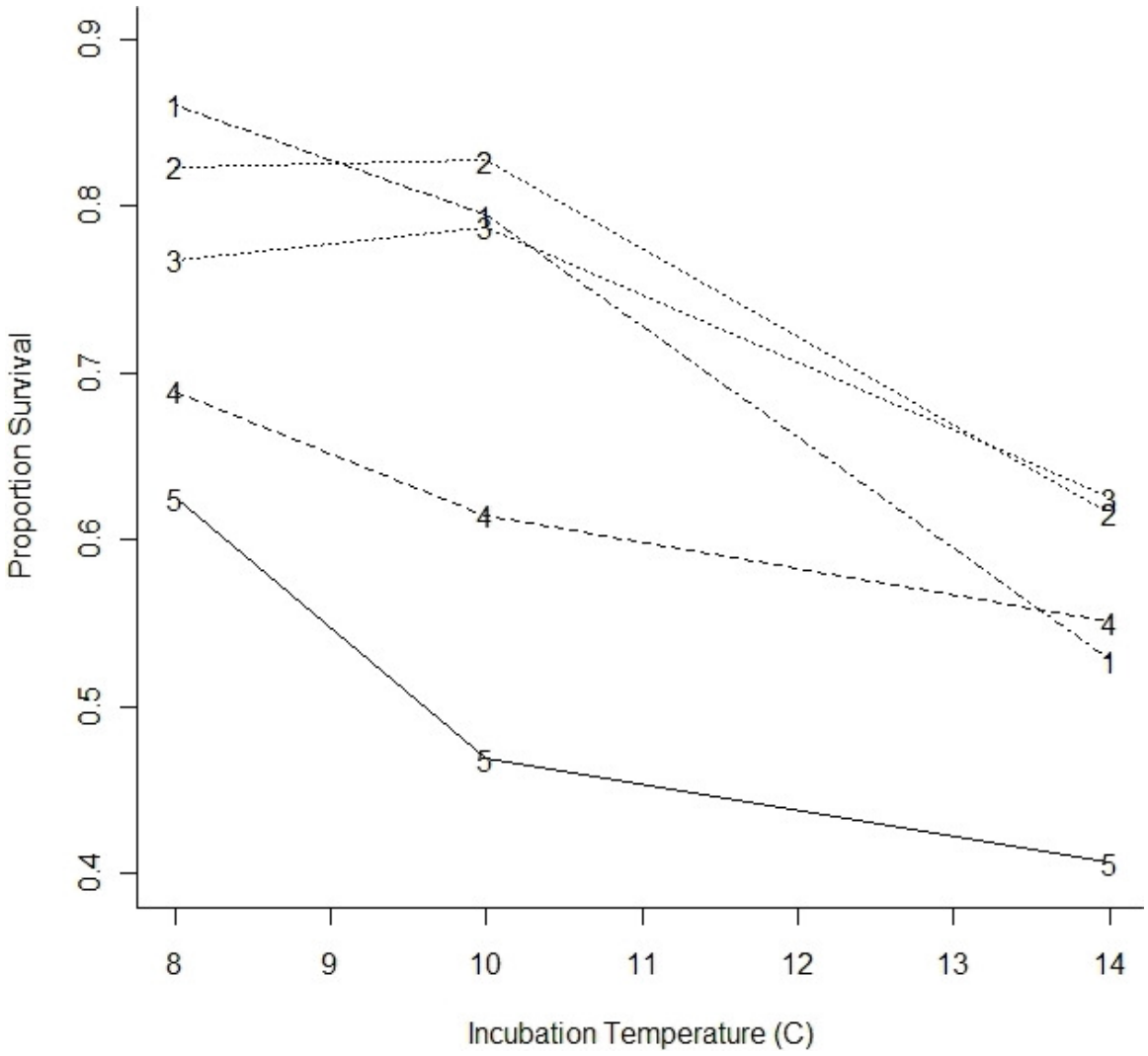

Figure 4: Median survival rates of each population at each incubation temperature, 1) Ray, 2) Chamberlain, 3) O'Brien, 4) Cottonwood creek, and 5) MO12. Average 2009 summer temperatures were 6.7° (Ray Creek), 7.3° (Chamberlain Creek), 8.2° (O'Brien Creek), and 11.2°C (Cottonwood Creek).

APPENDIX C

EMBRYONIC DEVELOPMENT

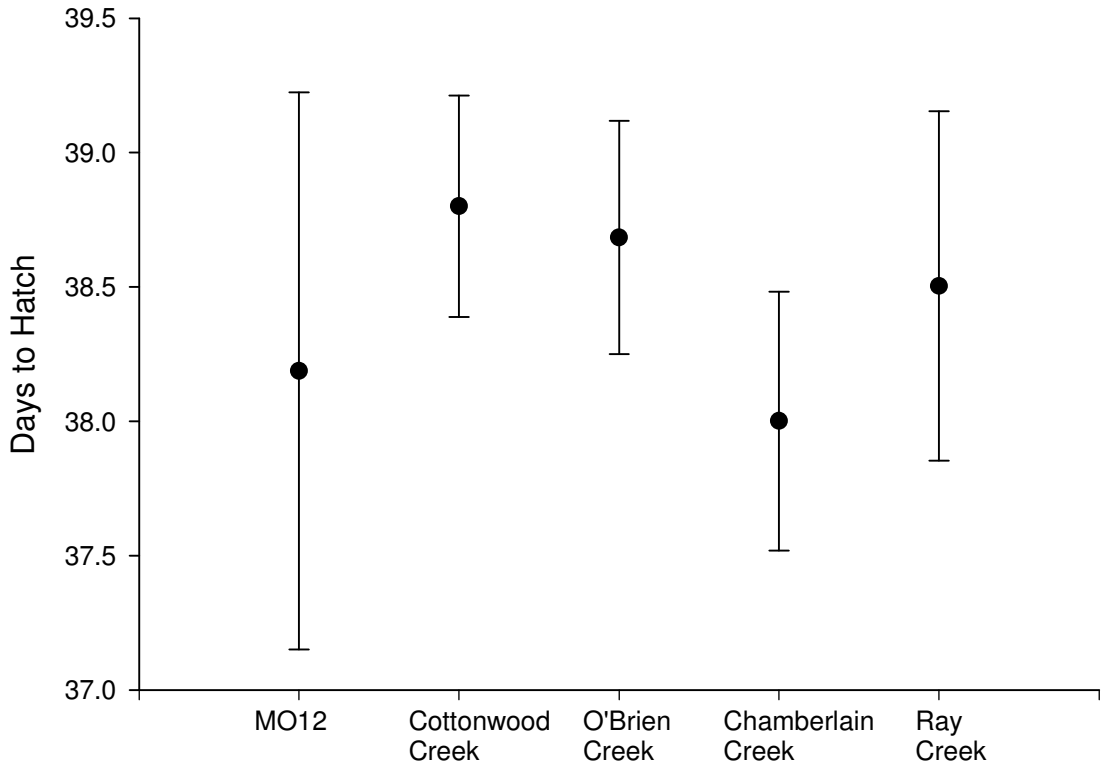

Figure 5: Mean days to hatch with 95% confidence intervals when incubated at 8℃. Average 2009 summer temperatures were 6.7° (Ray Creek), 7.3° (Chamberlain Creek), 8.2° (O'Brien Creek), and 11.2℃ (Cottonwood Creek).

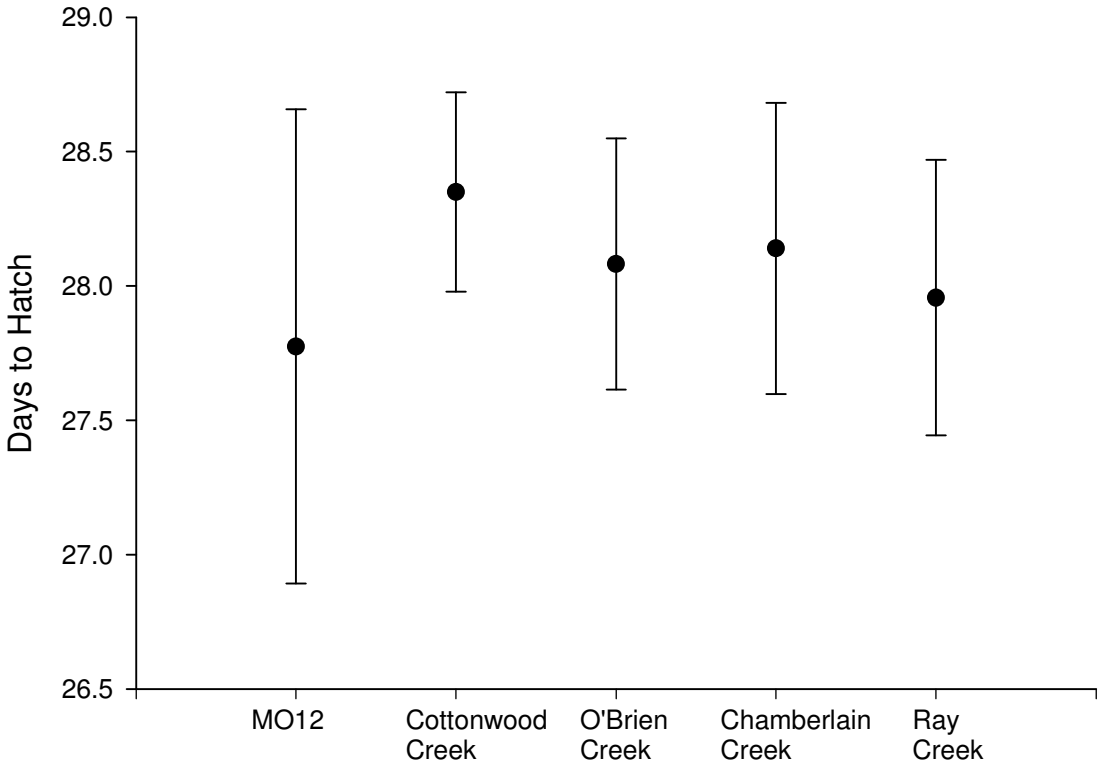

Figure 6: Mean days to hatch with 95% confidence intervals when incubated at 10℃. Average 2009 summer temperatures were 6.7° (Ray Creek), 7.3° (Chamberlain Creek), 8.2° (O'Brien Creek), and 11.2℃ (Cottonwood Creek).

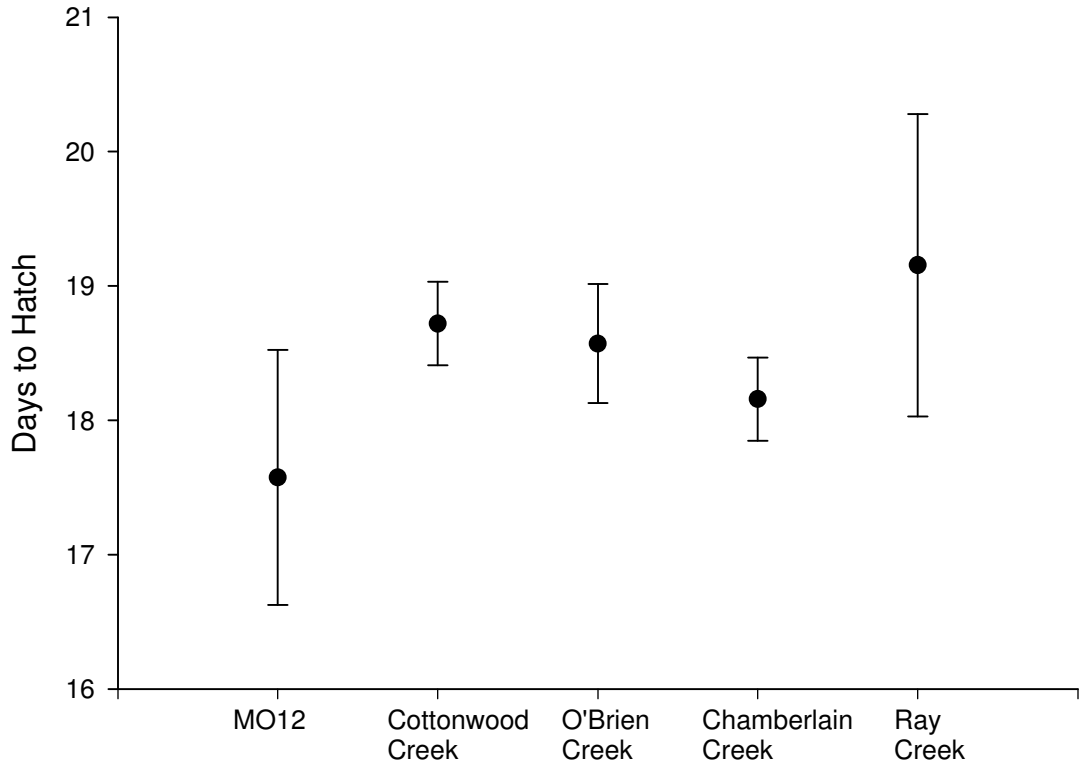

Figure 7: Mean days to hatch with 95% confidence intervals when incubated at 14℃. Average 2009 summer temperatures were 6.7° (Ray Creek), 7.3° (Chamberlain Creek), 8.2° (O'Brien Creek), and 11.2℃ (Cottonwood Creek).

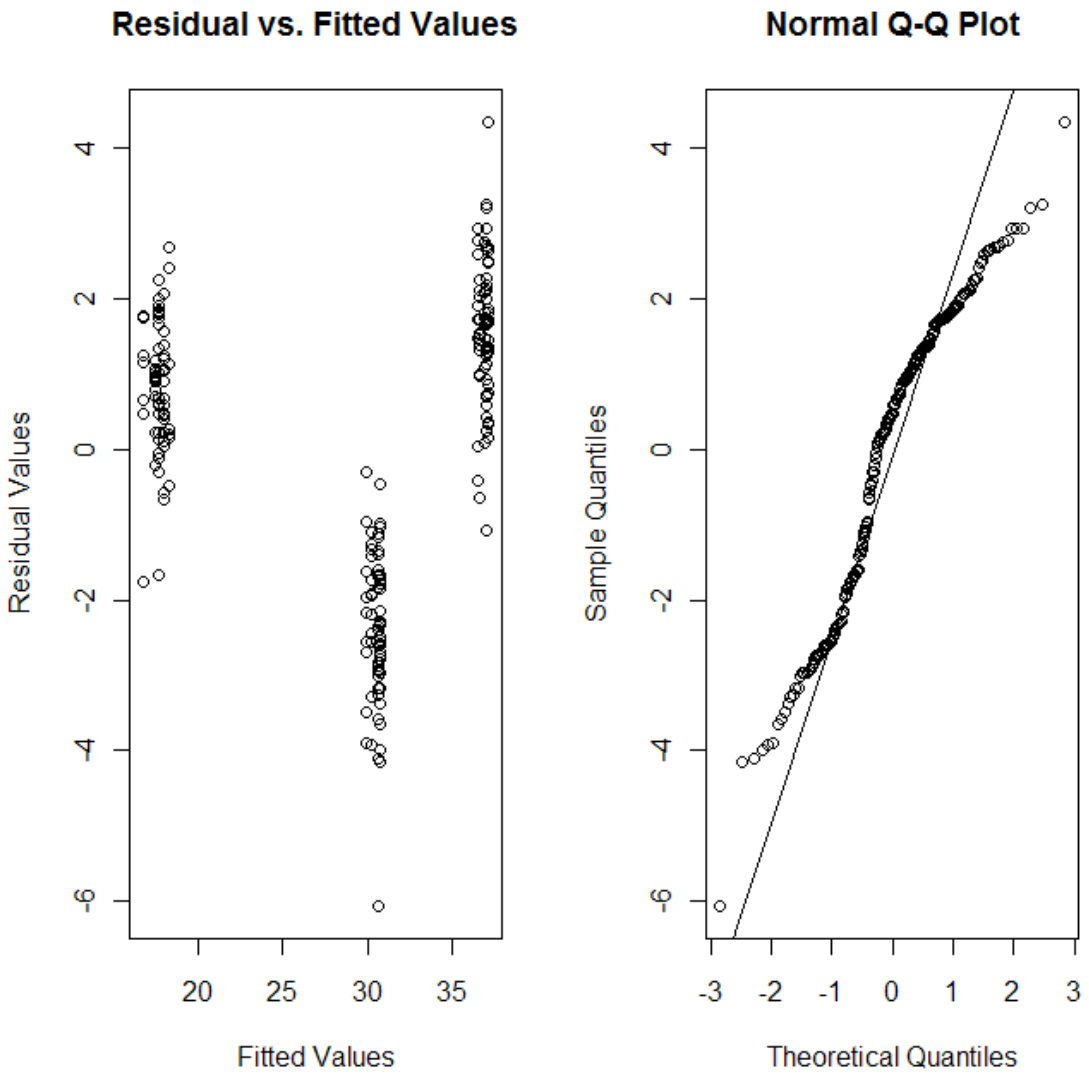

Figure 8: Residuals versus fitted values and QQ Plot for the model $\mu\{\text{Incubation Days} \mid \text{Population, Temperature}\} = \beta_0 + \beta_1\text{Population} + \beta_2\text{Incubation Temperature} + \beta_3\text{Population} * \text{Incubation Temperature}$. Residual versus fitted plot shows skew in the variance, thus making the use of a two-way ANOVA or linear regression inappropriate. QQ Plot shows near normality.

APPENDIX D

GROWTH

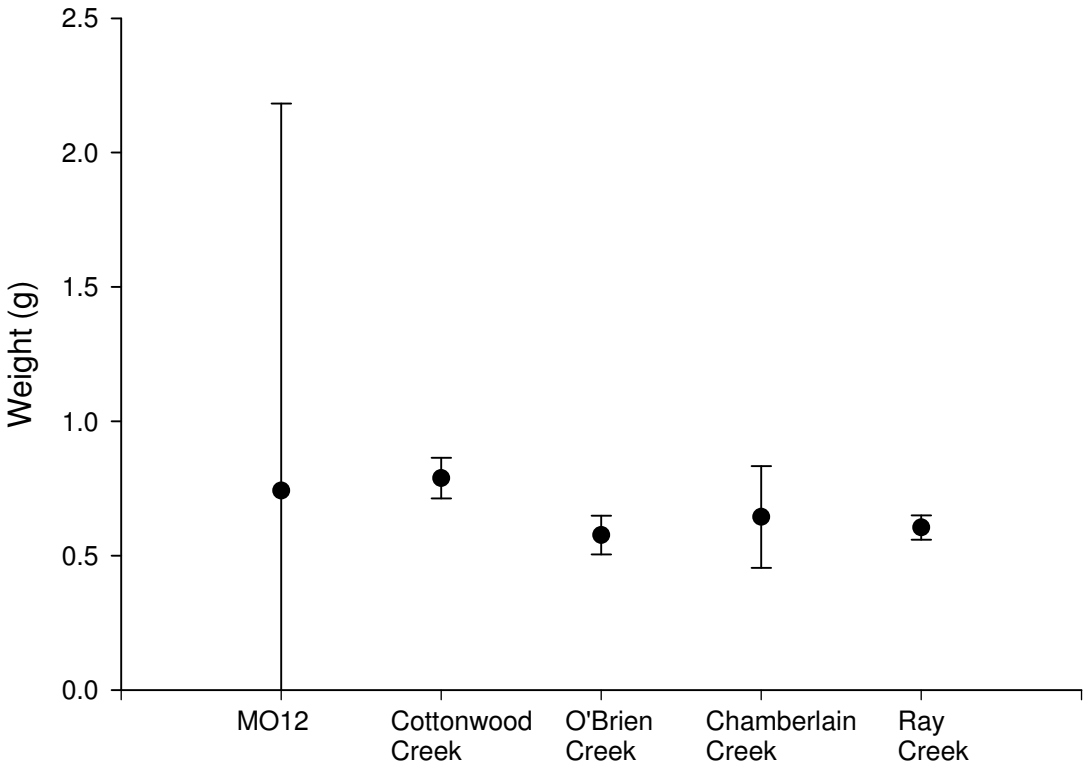

Figure 9: Mean fish weights from each tank with 95% confidence intervals when raised at 8℃. Average 2009 summer temperatures were 6.7° (Ray Creek), 7.3° (Chamberlain Creek), 8.2° (O'Brien Creek), and 11.2℃ (Cottonwood Creek).

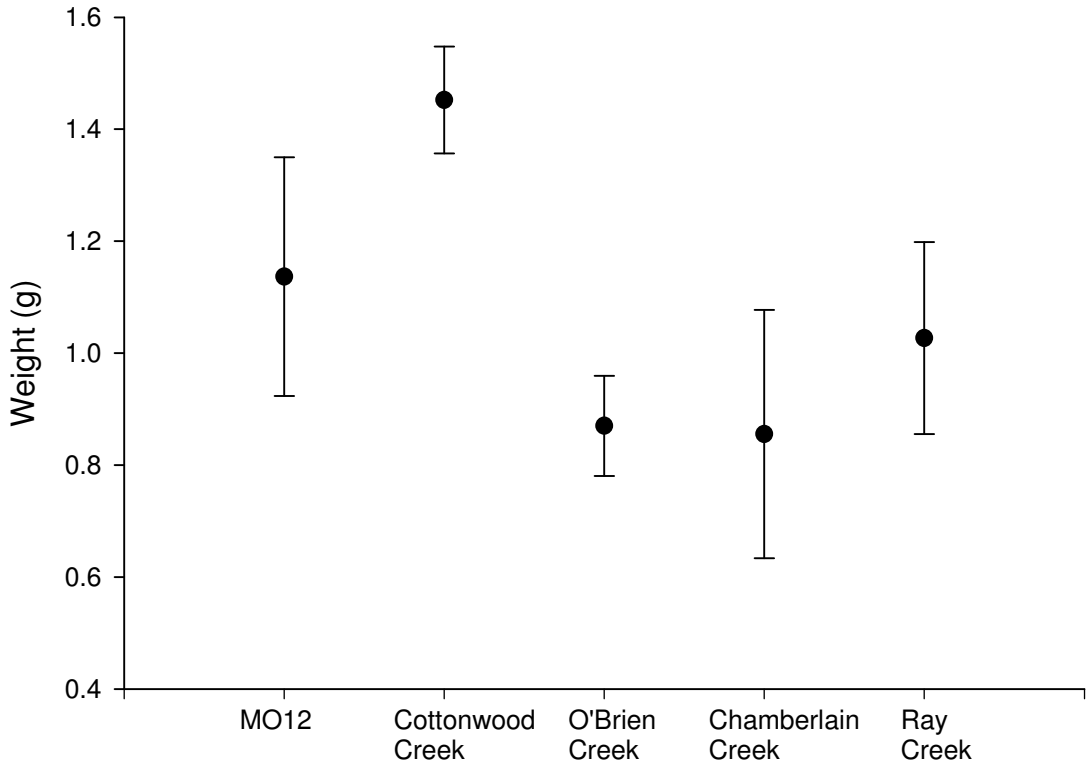

Figure 10: Mean fish weights from each tank with 95% confidence intervals when raised at 10℃. Average 2009 summer temperatures were 6.7° (Ray Creek), 7.3° (Chamberlain Creek), 8.2° (O'Brien Creek), and 11.2℃ (Cottonwood Creek).

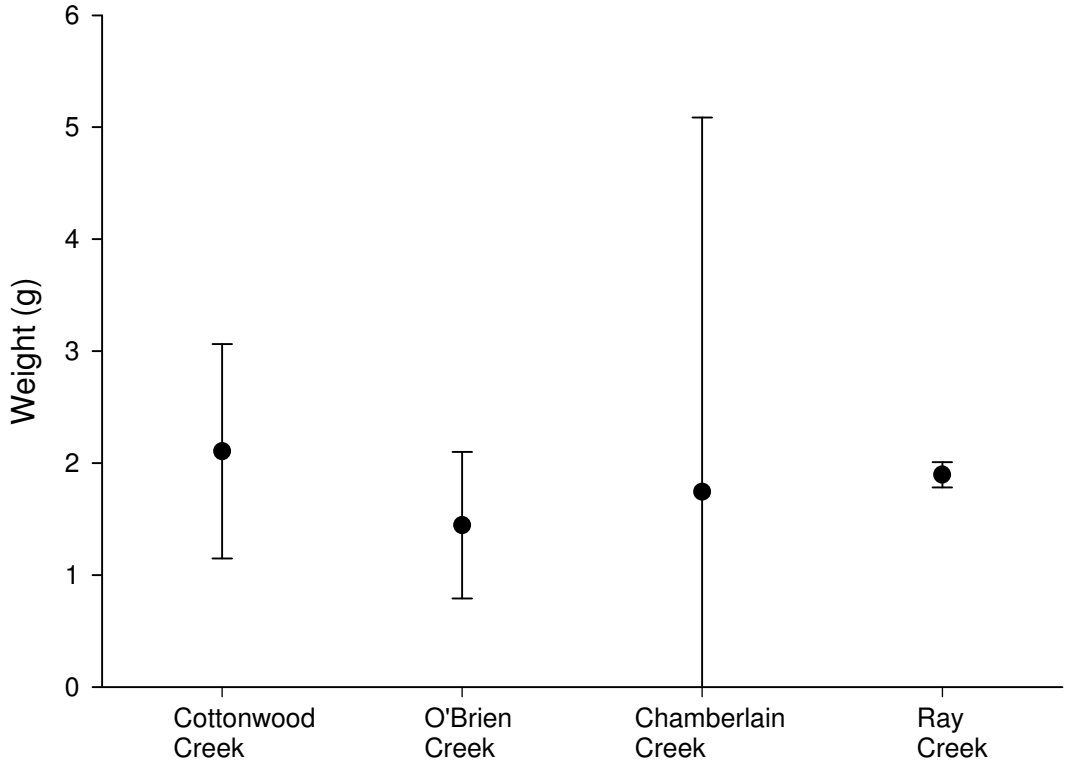

Figure 11: Mean fish weights from each tank with 95% confidence intervals when raised at 14℃. Average 2009 summer temperatures were 6.7° (Ray Creek), 7.3° (Chamberlain Creek), 8.2° (O'Brien Creek), and 11.2℃ (Cottonwood Creek).

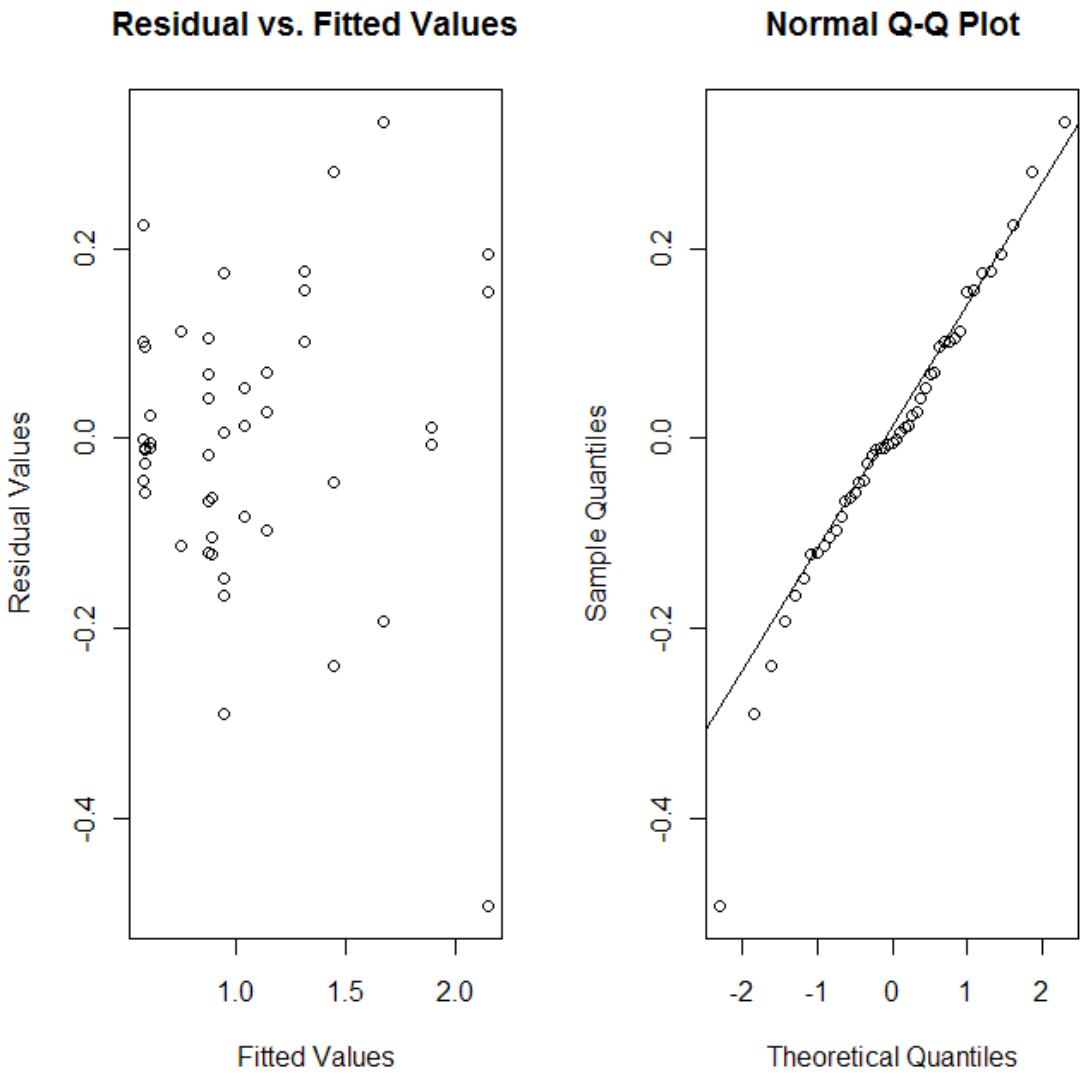

Figure 12: Residuals versus fitted values and QQ Plot for the model $\mu\{\text{Weight} \mid \text{Population, Temperature}\} = \beta_0 + \beta_1\text{Population} + \beta_2\text{Incubation Temperature} + \beta_3\text{Population} * \text{Incubation Temperature}$. The fitted versus residuals plot shows an equal variance and the QQ plot shows near normality. Therefore, the use of a two-way ANOVA is appropriate.

Table 2: Analysis of variance table for the model μ{Weight | Population, Temperature} = β_0 + β_1Population + β_2Incubation Temperature + β_3Population * Incubation Temperature. No evidence of population x temperature interactions was detected.

Analysis of Variance					
Response: Weight					
	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Population	4	1.97	0.49	17.97	2.82E-08
Temperature	1	7.55	7.55	276.20	< 2.2e-16
Pop. * Temp.	4	0.19	0.05	1.74	0.1612
Residuals	37	1.01	0.03		

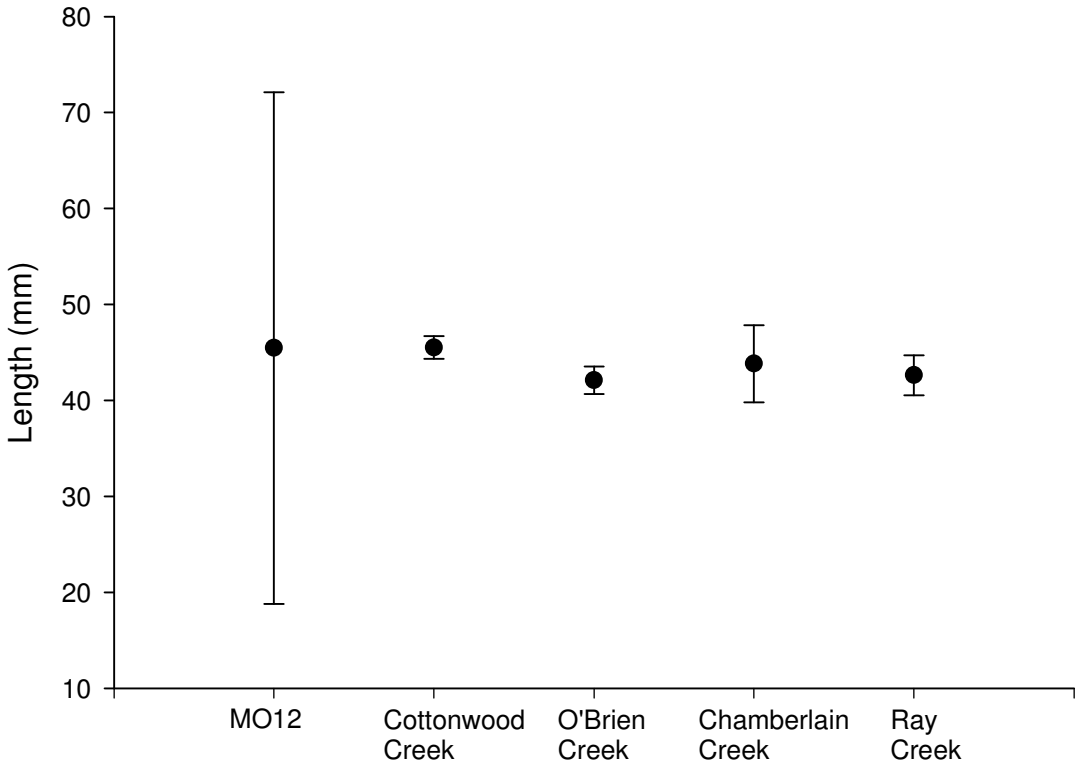

Figure 13: Mean fish lengths from each tank with 95% confidence intervals when raised at 8°C. Average 2009 summer temperatures were 6.7° (Ray Creek), 7.3° (Chamberlain Creek), 8.2° (O'Brien Creek), and 11.2°C (Cottonwood Creek).

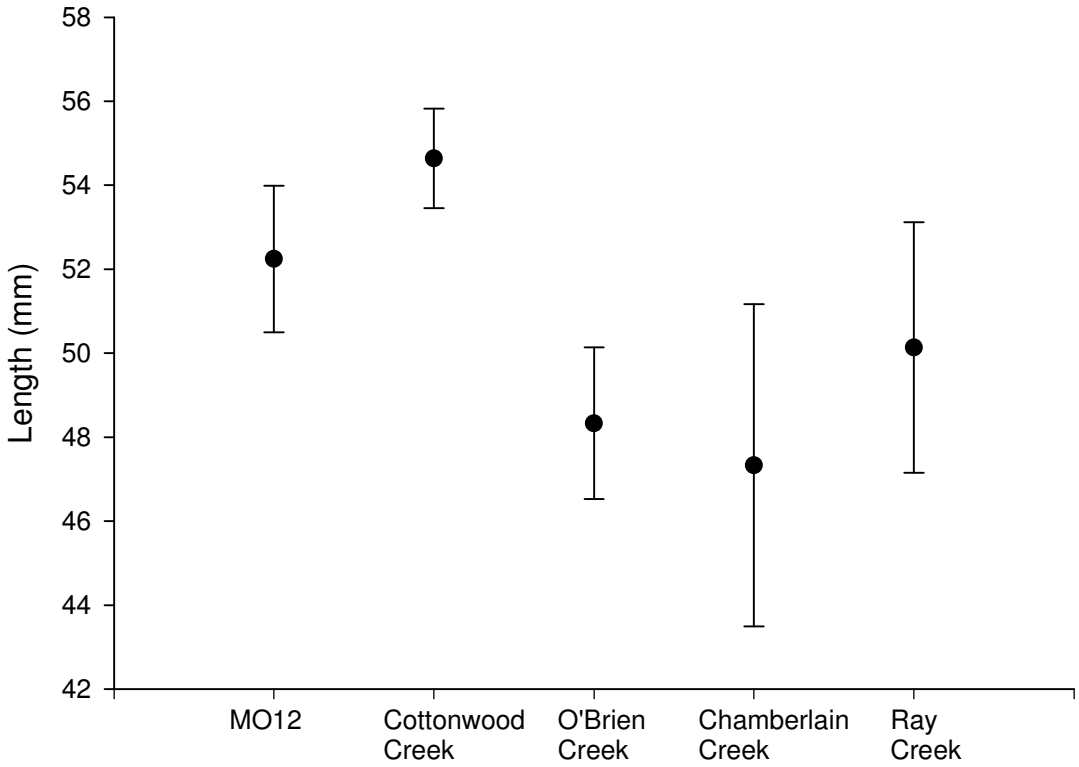

Figure 14: Mean fish lengths from each tank with 95% confidence intervals when raised at 10℃. Average 2009 summer temperatures were 6.7° (Ray Creek), 7.3° (Chamberlain Creek), 8.2° (O'Brien Creek), and 11.2℃ (Cottonwood Creek).

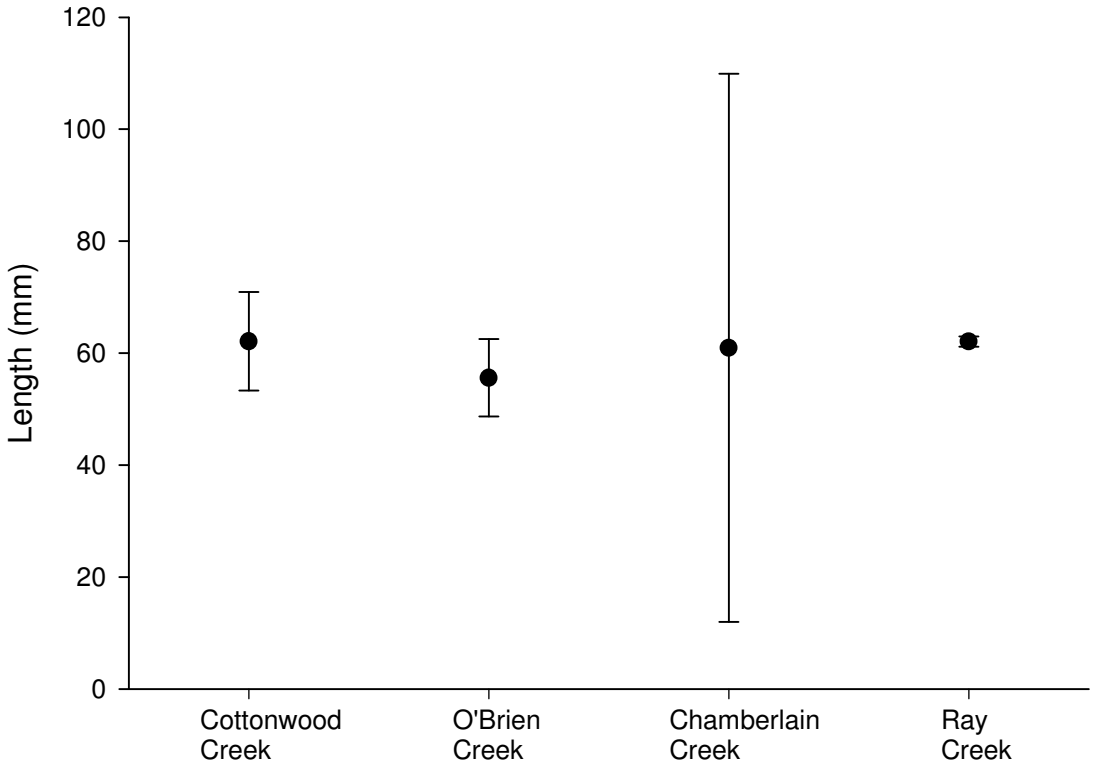

Figure 15: Mean fish lengths from each tank with 95% confidence intervals when raised at 14℃. Average 2009 summer temperatures were 6.7° (Ray Creek), 7.3° (Chamberlain Creek), 8.2° (O'Brien Creek), and 11.2℃ (Cottonwood Creek).

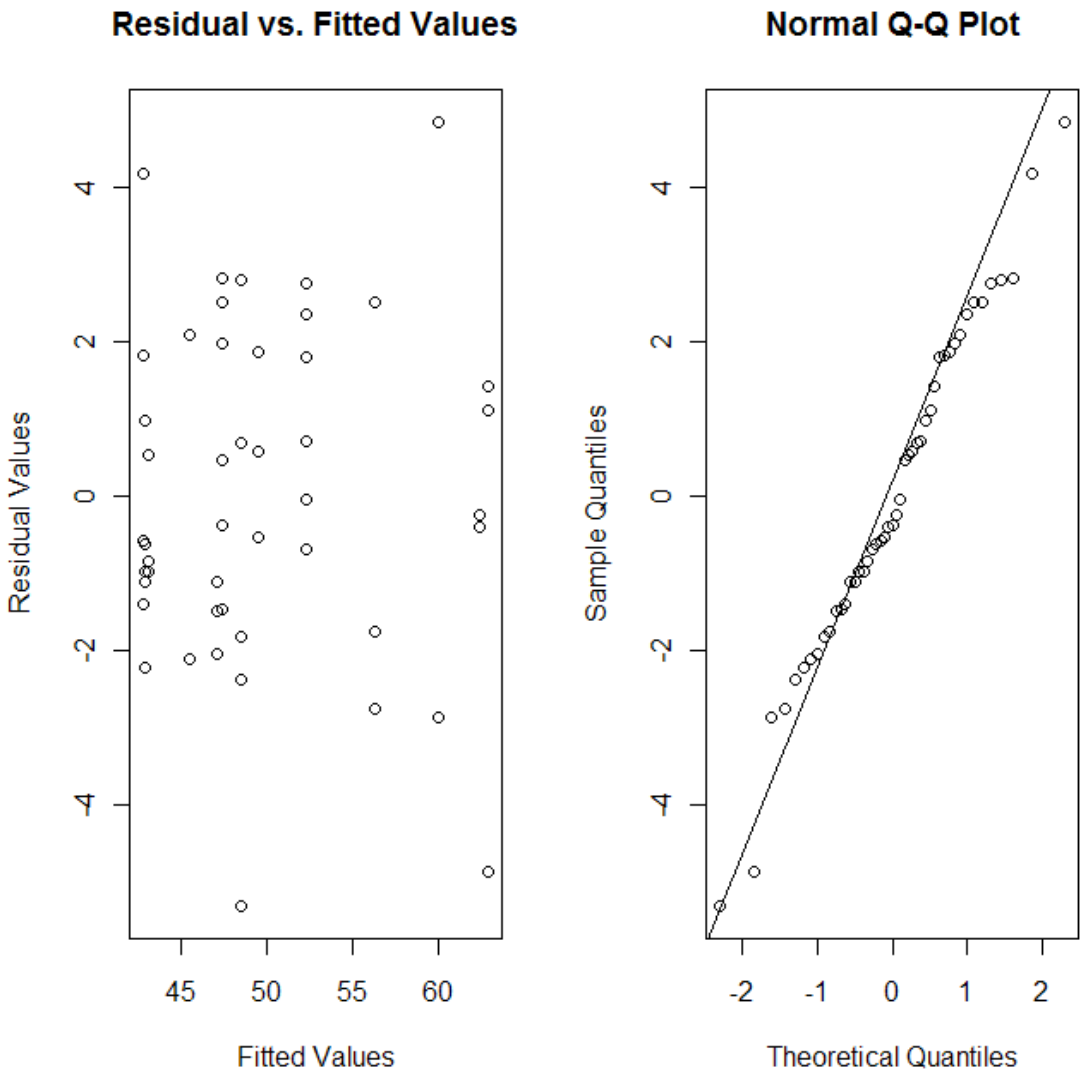

Figure 16: Residuals versus fitted values and QQ Plot for the model $\mu\{\text{Length} \mid \text{Population, Temperature}\} = \beta_0 + \beta_1\text{Population} + \beta_2\text{Incubation Temperature} + \beta_3\text{Population} * \text{Incubation Temperature}$. The fitted versus residuals plot shows an equal variance and the QQ plot shows near normality. Therefore, the use of a two-way ANOVA is appropriate.

Table 3: Analysis of variance table for the model μ{Length | Population, Temperature}= β0 + β1Population + β2Incubation Temperature + β3Population * Incubation Temperature. No evidence of population x temperature interactions was detected.

Analysis of Variance					
Response: Length					
	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Population	4	249.41	62.35	10.83	6.49E-06
Temperature	1	1592.75	1592.75	276.70	< 2.2e-16
Pop. * Temp.	4	30.8	7.7		
Residuals	37	212.98	5.76		

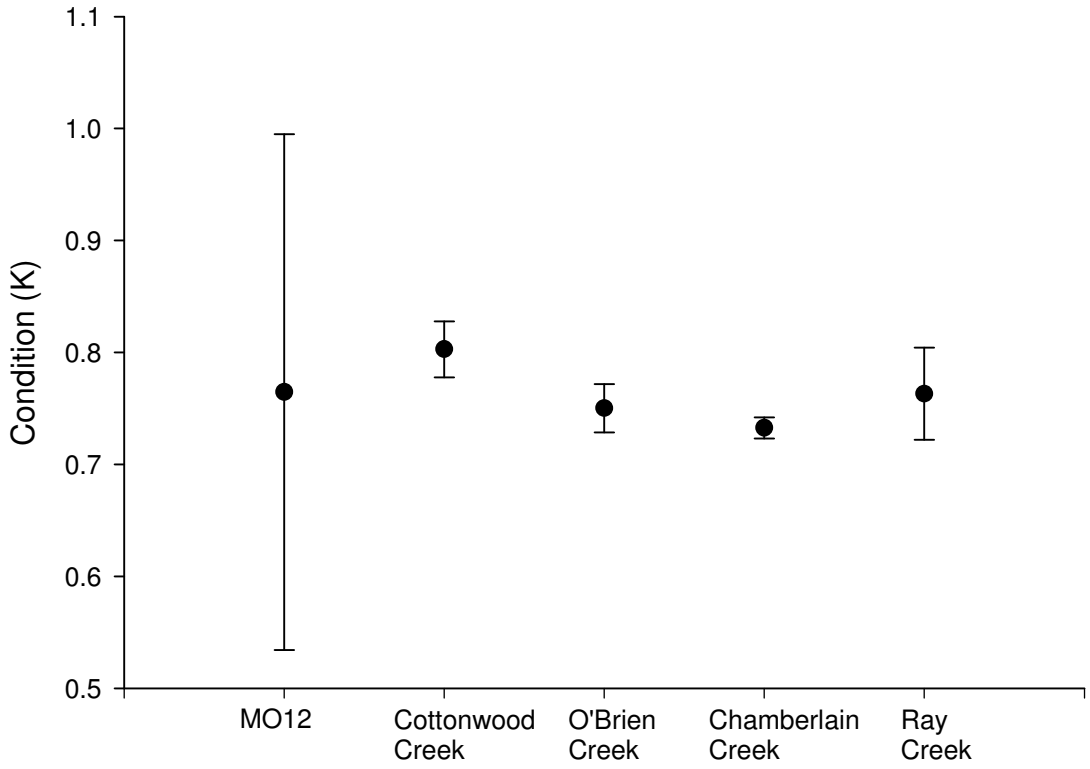

Figure 17: Mean fish conditions from each tank with 95% confidence intervals when raised at 8℃. Average 2009 summer temperatures were 6.7° (Ray Creek), 7.3° (Chamberlain Creek), 8.2° (O'Brien Creek), and 11.2℃ (Cottonwood Creek).

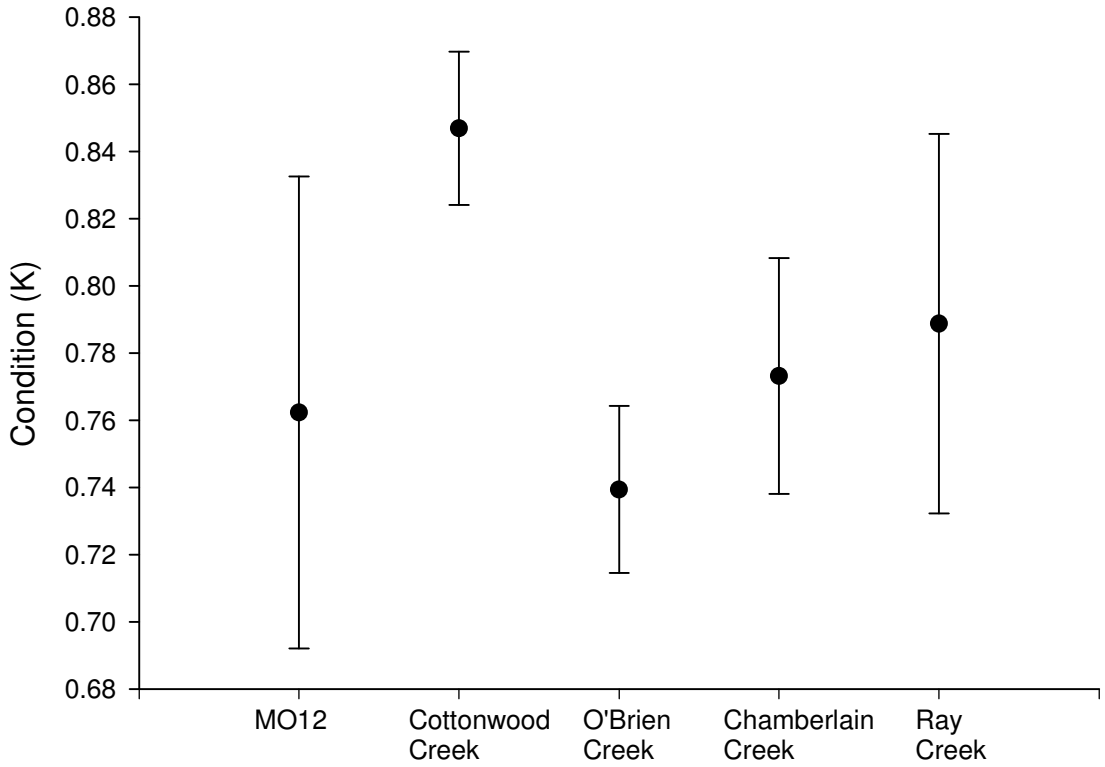

Figure 18: Mean fish conditions from each tank with 95% confidence intervals when raised at 10℃. Average 2009 summer temperatures were 6.7° (Ray Creek), 7.3° (Chamberlain Creek), 8.2° (O'Brien Creek), and 11.2℃ (Cottonwood Creek).

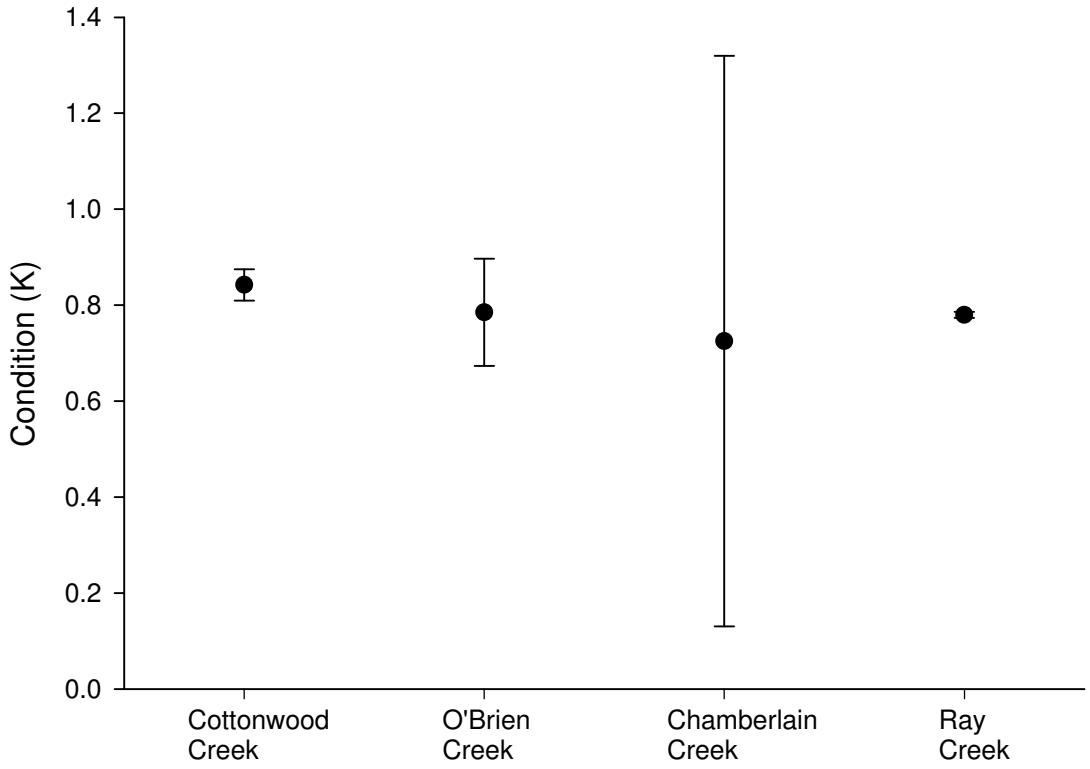

Figure 19: Mean fish conditions from each tank with 95% confidence intervals when raised at 14℃. Average 2009 summer temperatures were 6.7° (Ray Creek), 7.3° (Chamberlain Creek), 8.2° (O'Brien Creek), and 11.2℃ (Cottonwood Creek).

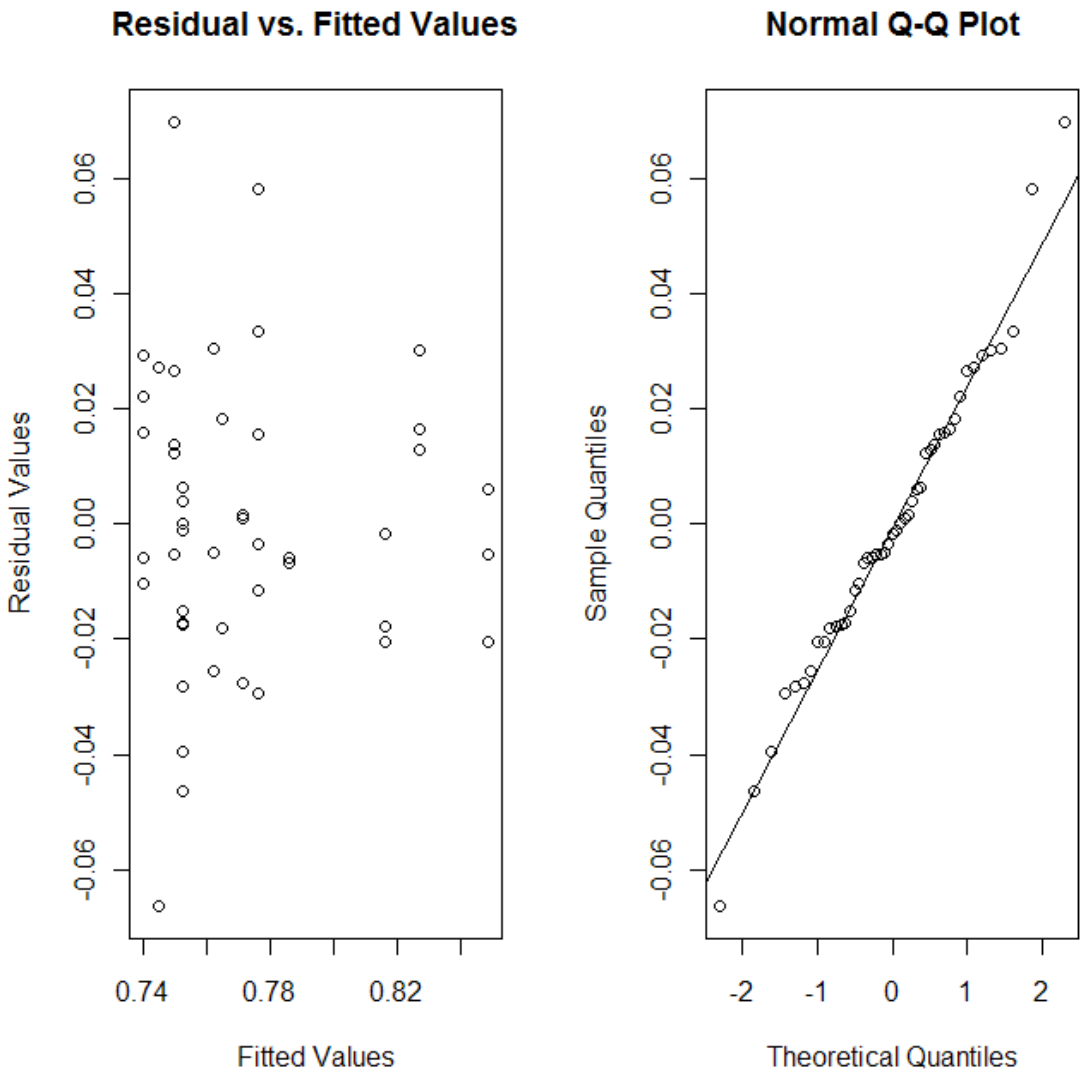

Figure 20: Residuals versus fitted values and QQ Plot for the model $\mu\{K \mid \text{Population, Temperature}\} = \beta_0 + \beta_1\text{Population} + \beta_2\text{Incubation Temperature} + \beta_3\text{Population} * \text{Incubation Temperature}$. The fitted versus residuals plot shows an equal variance and the QQ plot shows near normality. Therefore, the use of a two-way ANOVA is appropriate.

Table 4: Analysis of variance table for the model μ{Condition | Population, Temperature}= β0 + β1Population + β2Incubation Temperature + β3Population * Incubation Temperature. No evidence of population x temperature interactions was detected.

Analysis of Variance					
Response: Condition					
	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Population	4	0.04	0.01	13.12	9.50E-07
Temperature	1	0.00	0.00	3.22	0.08
Pop. * Temp.	4	0.00	0.00	0.60	0.67
Residuals	37	0.03	0.00		

APPENDIX E

COMPOSITION

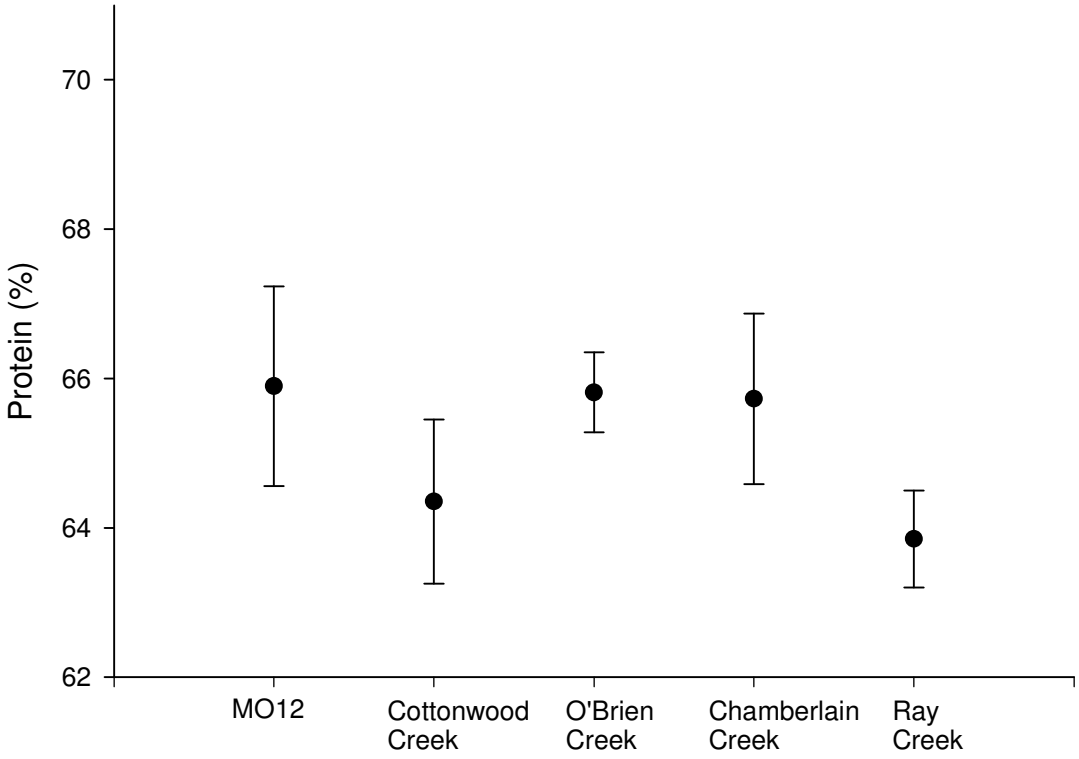

Figure 21: Mean fish percents protein from each tank with 95% confidence intervals across all temperatures. Average 2009 summer temperatures were 6.7° (Ray Creek), 7.3° (Chamberlain Creek), 8.2° (O'Brien Creek), and 11.2°C (Cottonwood Creek).

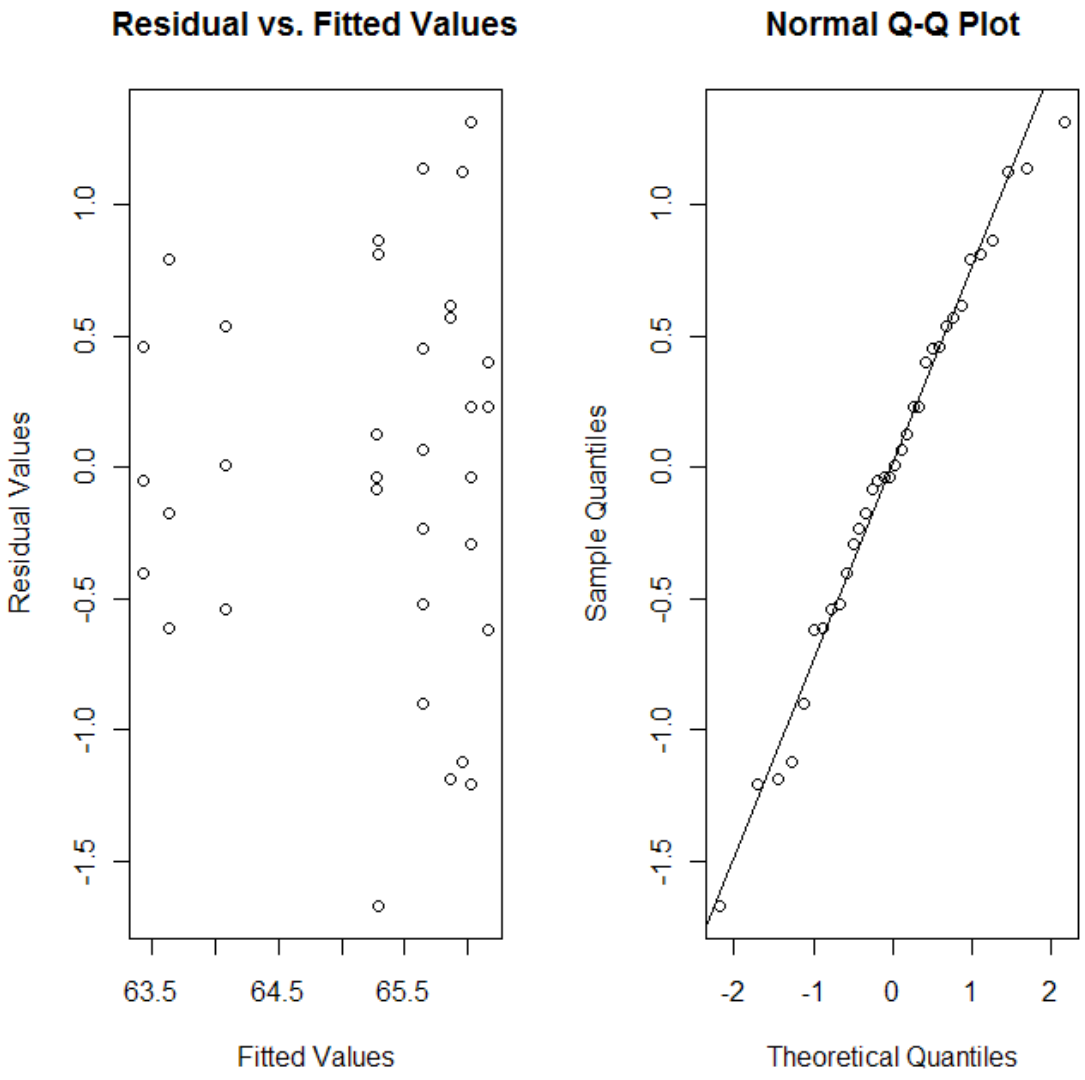

Figure 22: Residuals versus fitted values and QQ Plot for the model $\mu\{\text{Protein} \mid \text{Population, Temperature}\} = \beta_0 + \beta_1\text{Population} + \beta_2\text{Incubation Temperature} + \beta_3\text{Population} * \text{Incubation Temperature}$. The fitted versus residuals plot shows an equal variance and the QQ plot shows near normality. Therefore, the use of a two-way ANOVA is appropriate.

Table 5: Analysis of variance table for the model μ{Percent Protein | Population, Temperature}= β0 + β1Population + β2Incubation Temperature + β3Population * Incubation Temperature. No evidence of population x temperature interactions was detected.

Analysis of Variance Table					
Response: Percentage Protein					
	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Population	4	23.46	5.87	8.09	< 0.001
Temperature	1	4.14	4.14	5.71	0.03
Pop. * Temp.	4	2.81	0.70	0.97	0.44
Residuals	24	17.41	0.73		

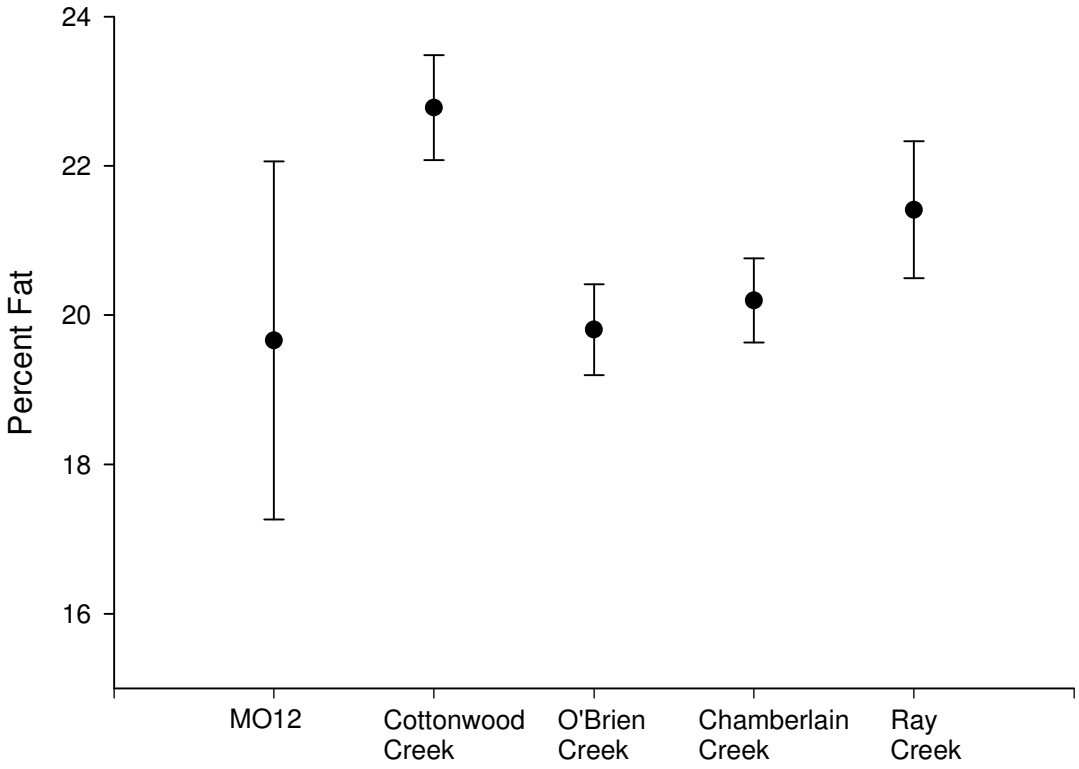

Figure 23: Percents fat of each tank of fish from each population. Each dot represents the average percent dry fat for a tank. Average 2009 summer temperatures were 6.7° (Ray Creek), 7.3° (Chamberlain Creek), 8.2° (O'Brien Creek), and 11.2°C (Cottonwood Creek).

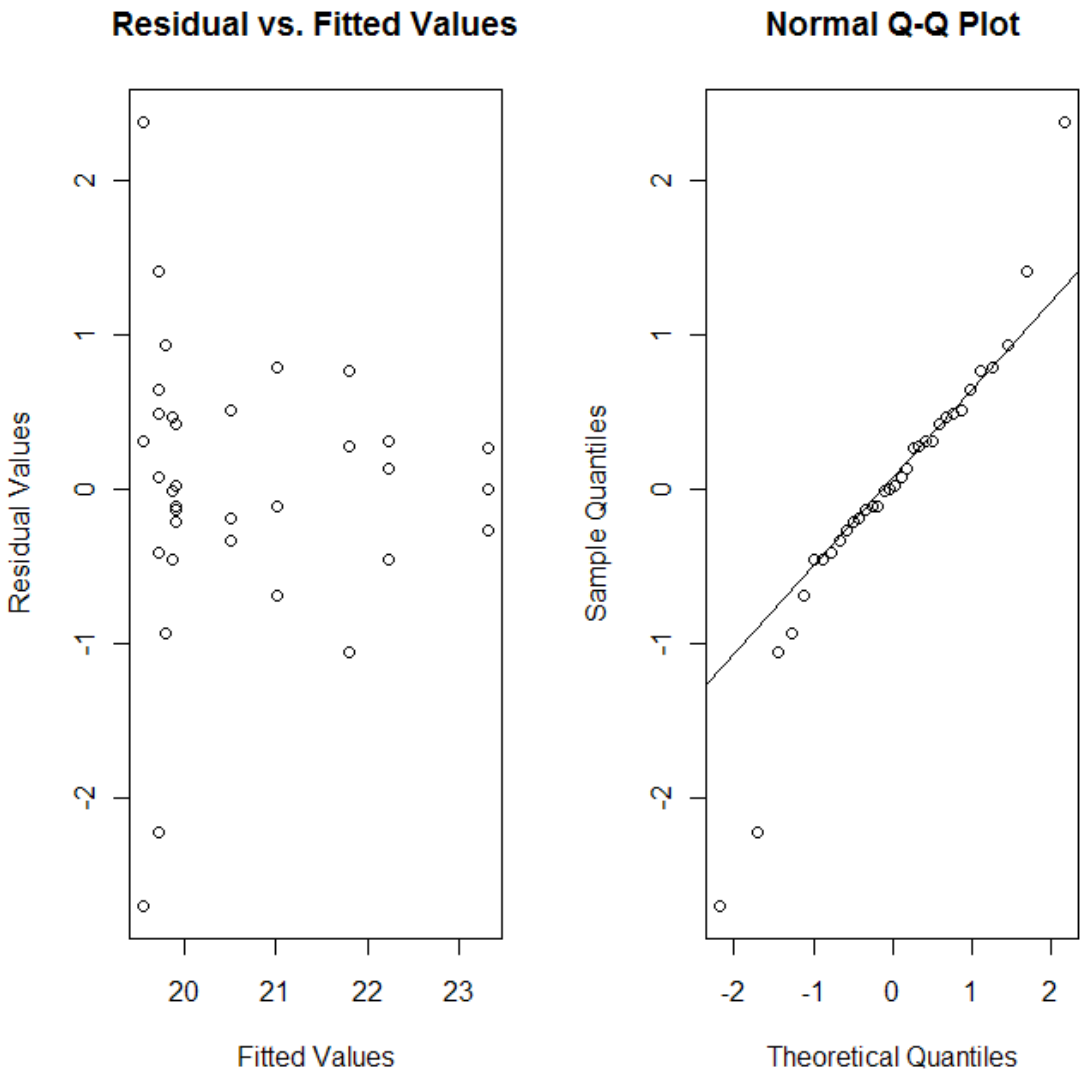

Figure 24: Residuals versus fitted values and QQ Plot for the model $\mu\{Fat \mid Population, Temperature\} = \beta_0 + \beta_1 Population + \beta_2 Incubation\ Temperature + \beta_3 Population * Incubation\ Temperature$. The fitted versus residuals plot shows an unequal variance and the QQ plot shows outliers. Therefore, the use of a two-way ANOVA is inappropriate.