

THE EFFECTS OF AN ARTIFICIALLY ELEVATED THERMAL ENVIRONMENT AND
SEASONAL ACCLIMATIZATION ON THE THERMAL TOLERANCE
OF THE WESTERN MOSQUITOFISH, *GAMBUSIA AFFINIS*

by

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ABSTRACT

THE EFFECTS OF AN ARTIFICIALLY ELEVATED THERMAL ENVIRONMENT AND SEASONAL ACCLIMATIZATION ON THE THERMAL TOLERANCE OF THE WESTERN MOSQUITOFISH, *GAMBUSIA AFFINIS*

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Gambusia affinis, the western mosquitofish, is a very hardy, live-bearing fish that has invaded freshwater habitats worldwide. The success of the western mosquitofishes' invasion is due to its reproductive capabilities and ability to tolerate a wide range of temperatures. The mosquitofish can inhabit waters as low as 0°C and higher than 40°C. Because of this species' eurythermicity, questions have been raised regarding the impact of artificially heated environments on its thermal tolerance limits and whether its thermal tolerance limits are consistent across seasons. Past studies have shown that mosquitofish populations inhabiting hot ponds receiving thermal effluents at steam-electric power stations had higher upper thermal tolerance limits than populations inhabiting the associated lake's main reservoirs. This higher thermal tolerance limit was found to be heritable, pointing to fitness differences between two populations. This led to the suggestion that directional selection was occurring in the population exposed to thermal effluents, leading to a more thermally tolerant population of moquitofish. This study examined whether the previously documented increase in upper thermal tolerance of

the mosquitofish population affected by thermal effluents has continued to increase over the past five years, supporting the hypothesis of directional selection and whether a continued increase in the population's upper thermal tolerance has impacted its lower thermal tolerance limits as well. In order to determine whether thermal effluents and seasonal acclimatization affects the thermal tolerance limits of *G. affinis*, the upper and lower thermal tolerance limits were established for two populations and compared to previous research; temperature tolerance polygons were established to examine whether thermal effluents influence the degree of eurythermicity of this species; and seasonal comparisons of the upper and lower thermal tolerance limits were assessed to determine whether seasonal acclimatization has influenced the thermal tolerance limits of this species. The upper thermal tolerance limits were found to be inconsistent. Depending on the season, the population exposed to thermal effluents did not always exhibit a higher thermal tolerance than the population exposed to ambient lake waters. The lower thermal tolerance limits were more consistent, showing that the population exposed to thermal effluents were consistently less cold tolerant than the population exposed to ambient lake waters. Only female individuals exposed to thermal effluents exhibited an increase in upper thermal tolerance compared to individuals tested five years previously. Due to the inconsistency in the upper thermal tolerance limits and the continuously varying upper and lower thermal tolerance temperatures among seasons, seasonal acclimatization was shown to heavily influence the thermal tolerance limits of *G. affinis*. Temperature tolerance polygons demonstrated that the population exposed to thermal effluents and the population exposed to ambient lake waters had similar areas of thermal tolerance, suggesting that these populations generally inhabit the same thermal niche. These polygons also supported the fact that *G. affinis* has a wide range of thermal tolerance.

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CHAPTER 1

INTRODUCTION

1.1 Physiological Adaptation to the Environment

It is a well established fact that temperature is not evenly distributed across the globe. Organisms exhibit adaptations that best suit their thermal environment. Normal life processes occur for most animal species within a range of 0 to 40°C (Prosser 1993). Although many species have a relatively broad range of thermal tolerance, factors such as competition and resource availability may limit the actual range of temperatures in which they occur. This constricted temperature range is known as a species' realized thermal niche, while the broader tolerance range is known as its fundamental niche (Magnuson et al 1979).

The fundamental niche of an organism is bound by its physiological tolerances and has an effect on its ability to establish populations in areas at or near its tolerance limits. These tolerance limitations can be expanded by natural selection and associated adaptations to extreme thermal environments. In theory, more thermally tolerant individuals would survive a thermal selection event, resulting, over time, in a more thermally tolerant population, as genes from the survivors are passed on to subsequent generations (Darwin 1859). The capacity to physiologically adapt to extreme temperatures differs among species. A greater capacity to adapt to thermal extremes may allow a species to expand its range along a latitudinal gradient more so than another less adaptable species.

The ability to tolerate or adapt to diverse environmental conditions is a fundamental trait that contributes to many species' invasiveness. A broad thermal tolerance, in particular, allows potentially invasive species to disperse across a larger latitudinal gradient. This associated broad thermal tolerance range has been implicated as one of the two most important characteristics in predicting non-native fish invasiveness in California (Marchetti et al 2004).

Marchetti et al. (2004) specifically notes the western mosquitofish, *Gambusia affinis*, as an extremely hardy fish that can tolerate conditions beyond that of many other fish species.

1.2 *Gambusia affinis* (Baird and Girard), Western Mosquitofish

One of the most studied live-bearing fish species is *Gambusia affinis* (Baird and Girard). Commonly known as the western mosquitofish, *G. affinis* is a small, viviparous fish found in shallow freshwater habitats at or near the surface (Meffe & Snelson 1989) (Fig 1.1). The popularity of mosquitofish for biological research is partially due to their hardiness in both the wild and the laboratory, and their ease of collection. Once caught, they are easy to maintain in the laboratory. *G. affinis* can reach reproductive maturity in approximately 30 days. Females can have two to six broods per reproductive season, with 14 to 218 embryos per brood (Pyke 2005).

Although the native habitats of western mosquitofish are subtropical areas (Central America, Mexico, and southeastern United States), this species is currently the most widely distributed freshwater fish in the world, inhabiting every continent except Antarctica (Lloyd, 1986; Pyke 2005). Such a wide distribution is likely due to the introduction of *G. affinis* for mosquito control, which was later found to be largely ineffective (Courtenay & Meffe, 1989). Although Prosser (1993) notes that environmental temperature will limit the distribution of many organisms, the broad distribution of *G. affinis* illustrates their tolerance of a wide range of environmental conditions. Some of the most commonly researched aspects of these tolerance limits are their reaction to chemical substances, salinity, dissolved oxygen, and water temperature.

Along with its generalist diet, hardiness, and reproductive capacity, this species' ability to tolerate a wide range of temperatures has allowed it to successfully invade habitats outside of its native range, leading to *G. affinis* being named one of the world's 100 worst invasive species by the Invasive Species Specialist Group of the World Conservation Union (IUCN) (Lowe et al.

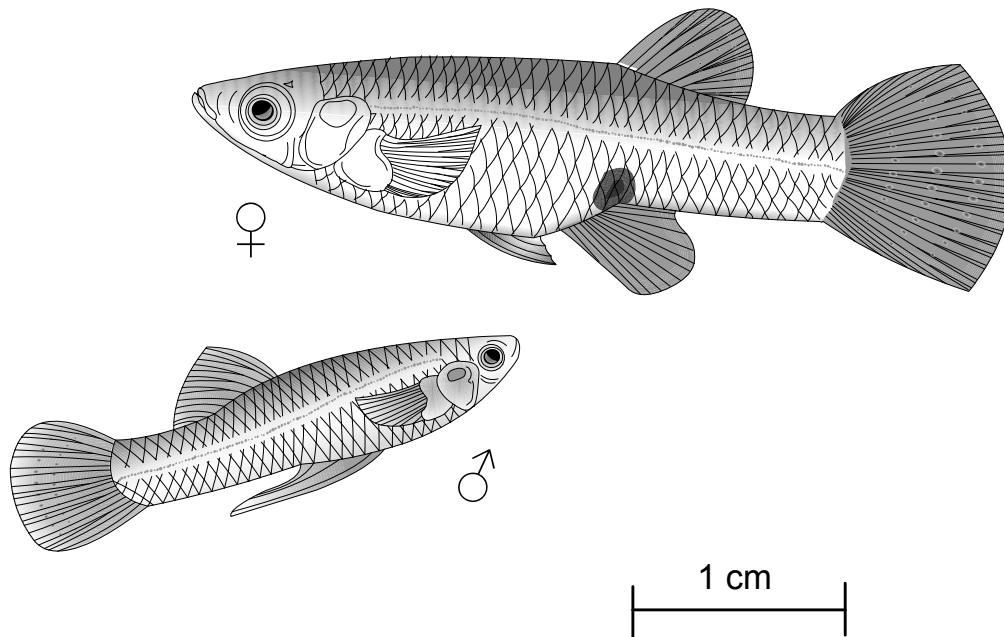


Figure 1.1 External features of *Gambusia affinis* (Britton 2005).

2004). Populations of *G. affinis* prefer water temperatures between 31°C to 35°C, however populations of this species occur in waters of much broader temperature ranges (Pyke 2005). *G. affinis* has been found in power-station cooling ponds where water temperatures exceed 40°C (Dean 1981; Britton 2005). Krumholz (1944) observed specimens of *G. affinis* swimming beneath ice in ponds where water temperature approached 0°C.

1.3 The Study Site, Big Brown Power Station

The Big Brown Steam-Electric Power Station, located in Freestone County, Texas (31°48'05.36" N, 96°03'35.26"W), draws raw water from Lake Fairfield for steam-condenser and service-water cooling. This steam-turbine power station burns lignite, creating heat in order to boil water into steam, driving the turbines to generate electricity. After leaving the turbine, the

steam is condensed by cooling with raw water from the main reservoir of Lake Fairfield in order to pump it back to the boilers. Water from the main reservoir at Lake Fairfield is continuously pumped through the steam condenser of the power station, where it discharges through an “upper” canal and into a cooling pond (also known as a hot pond). Partially cooled effluents exit the hot pond and then run down a “lower” canal before emptying into the main reservoir. The effluent canal system was designed to allow heated water to cool in the hot pond before entering the main reservoir (Fig 1.2).

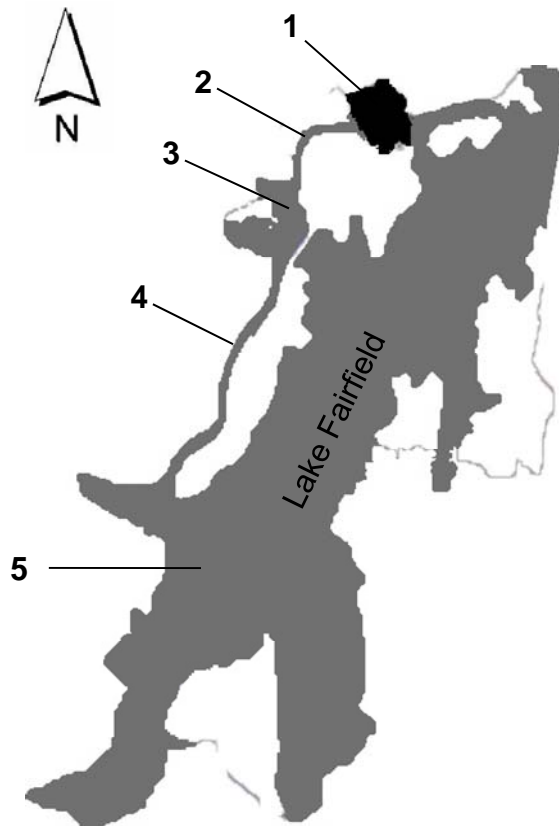


Figure 1.2 An illustration of the water flow at Lake Fairfield located in Freestone County, Texas. Raw water from Lake Fairfield is taken in Big Brown Power Station (1), then discharges through an upper effluent canal (2) and into the hot pond (3). Effluents exit the hot pond and travel down the lower effluent canal (4) before emptying into the main reservoir (5).

Temperatures in the main reservoir are typically cooler compared to the hot pond (Fig. 1.3 and Fig. 1.4). The lowest temperature recorded in the main reservoir was in January 2008 at 11.8°C (Table 2.1), whereas the lowest temperature recorded in the hot pond was in January 2007 at 19.1°C (Table 2.2). The highest temperatures recorded in both the main reservoir and the hot pond were in August 2007, with the main reservoir reaching 38.5°C (Table 2.1) and the hot pond reaching 43.7°C (Table 2.2). However, both bodies of water maintain a relatively limited range of temperatures every month, between 11.9°C (October 2007) and 6.5°C (July 2007) in the main reservoir and 11.6°C (November 2007) and 6.7°C (September 2007) in the hot pond.

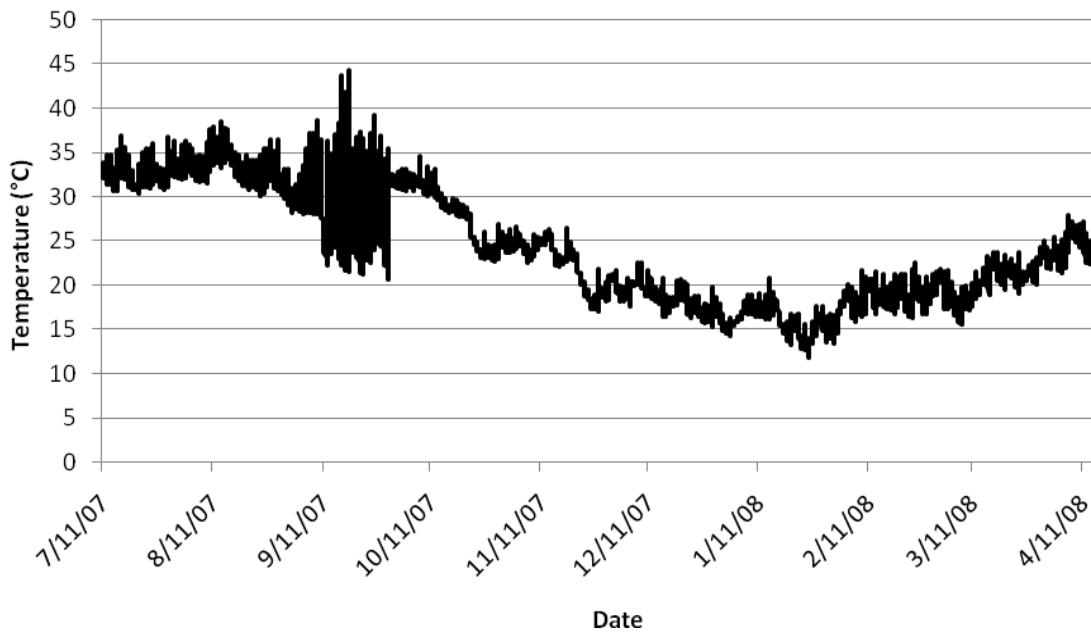


Figure 1.3 Daily surface water temperatures of the main reservoir at Lake Fairfield, Texas.

Table 1.1 The low monthly temperature, high monthly temperature, and mean monthly temperature of the main reservoir at Lake Fairfield (in °C). The dashes (-) indicate no data collected.

Month	Low Monthly Temperature (°C)	High Monthly Temperature (°C)	Mean Monthly Temperature (°C)
July-07	30.4	36.9	32.8
August-07	29.8	38.5	33.5
September-07	20.6	-	-
October-07	22.7	34.6	28.6
November-07	17.1	26.7	22.7
December-07	15.3	22.6	18.7
January-08	11.8	20.8	16.0
February-08	13.4	22.5	18.5
March-08	15.6	25.0	20.5
April-08	21.4	29.0	24.6

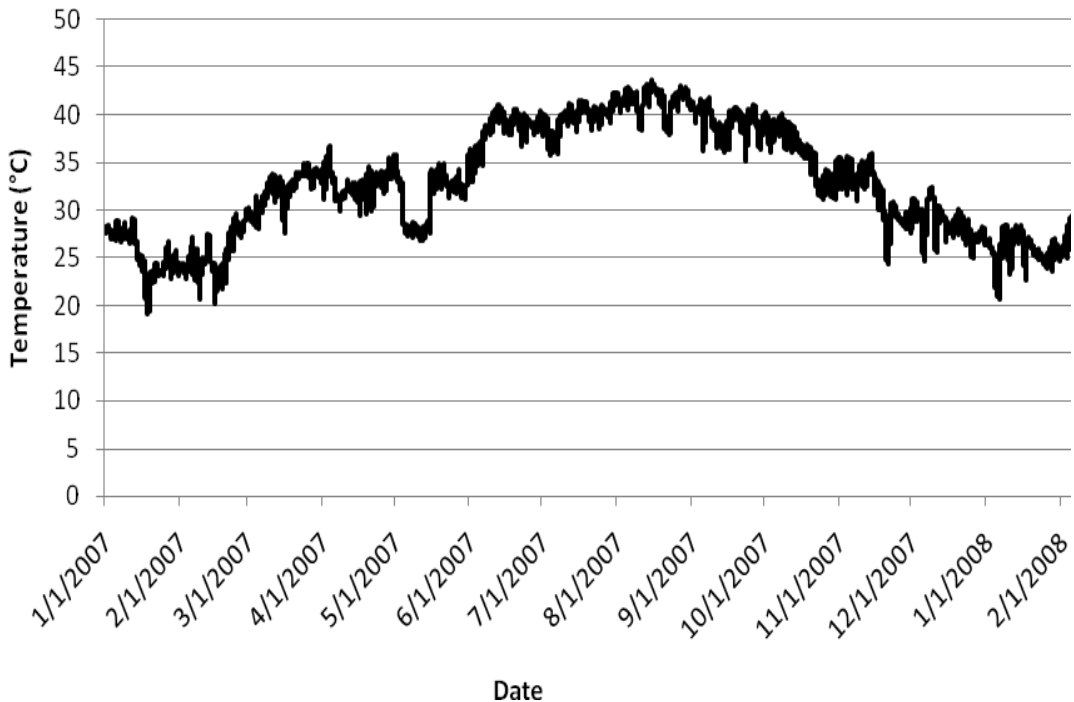


Figure 1.4 Daily surface water temperatures of the hot pond at Lake Fairfield, Texas.

Table 1.2 The low monthly temperature, high monthly temperature, and mean monthly temperature of the hot pond at Lake Fairfield (in °C).

Month	Low Monthly Temperature (°C)	High Monthly Temperature (°C)	Mean Monthly Temperature (°C)
January-07	19.1	29.2	25.4
February-07	20.2	30.0	25.3
March-07	27.7	34.9	32.1
April-07	29.4	36.7	32.9
May-07	26.8	35.7	31.2
June-07	33.0	41.1	38.5
July-07	35.8	42.3	39.8
August-07	37.9	43.7	41.4
September-07	35.1	41.8	39.3
October-07	31.1	40.2	36.1
November-07	24.3	35.9	31.6
December-07	24.7	32.4	28.6
January-08	20.7	28.4	25.6
February-08	24.0	30.7	28.0

1.4 Historical Studies of Thermal Biology in *G. affinis*

1.4.1 Work at Big Brown Power Station

Hot-pond temperatures in Lake Fairfield usually remain below the upper thermal limits of the resident mosquitofish population. However, during mid-summer, surface water temperatures of the hot pond often exceeded the resident mosquitofish population's upper thermal limit of 42.1°C (males), and 42.5°C (females) (Britton 2005). Despite this, many individuals of *G. affinis* residing in the hot pond survive (Dean 1981; Britton 2005). One explanation for this was offered by Dean (1981) and Britton (2005), who both worked on the impact of artificially heated waters at the Big Brown Power Station on the upper thermal tolerance of mosquitofish. Both authors suggested that selection pressures due to artificially heated waters may only favor the most thermally tolerant individuals, leading to the development of a thermally tolerant race over time in the hot pond (Dean 1981; Britton 2005).

Dean (1981) originally studied upper thermal tolerance and adaptation in *G. affinis* populations from the hot pond and the main reservoir at Lake Fairfield by using an acute-

exposure, or critical thermal, method (described in the Methods section). The critical thermal method was originally performed on desert reptiles by Cowells and Bogert (1944) and later first studied in fish by Heath (1963). In this acute-exposure method, a random sample of fish is subjected to a constant linear increase or decrease in water temperature until a sub-lethal endpoint is reached. Depending on whether the critical thermal maximum (CT_{Max}) or critical thermal minimum (CT_{Min}) is being tested, the sub-lethal endpoint will vary (described in the Methods section). Advantages of the critical thermal method include that it is fast, requires a relatively small sample size, and directly measures temperature as a response variable.

Dean (1981) found that the hot-pond mosquitofish population was able to tolerate slightly higher temperatures ($37.0^{\circ}C$) than the main-reservoir moquitofish population ($36.7^{\circ}C$). Dean (1981) also found allozyme frequency differences between the two populations suggesting that they were genetically different, but was unable to correlate thermal tolerance differences with these genetic differences (Dean 1981). Dean (1981) suggested that adaptation of thermal tolerance and gene frequency changes were due to selection pressures driven by thermal effluents.

Britton (2005) expanded on the upper thermal tolerance and adaptation work previously performed by Dean (1981). Britton (2005) tested key elements required for supporting the hypothesis that artificially induced selection has lead to evolution of increased thermal tolerance in the hot-pond mosquitofish population. Britton (2005) re-examined heat tolerance in the two populations at Lake Fairfield and found that the upper thermal tolerance limits had increased to $39.0^{\circ}C$ (males) and $39.3^{\circ}C$ (females) in the hot-pond population, and to $38.0^{\circ}C$ (males) and $38.7^{\circ}C$ (females) in the main reservoir population. This led to the observation that the upper thermal tolerance limits of the two populations had increased over the two decades since Dean's (1981) observations.

Britton (2005) also investigated two essential requirements for attributing the increase in the upper thermal tolerance limits of the two mosquitofish populations to selection. Without

heritable variation and differential fitness, selection cannot occur (Darwin 1859). Britton (2005) demonstrated that the upper thermal tolerance limits in individuals of *G. affinis* from Lake Fairfield were heritable by showing that second generation offspring had similar levels of heat tolerance compared to their ancestral parents, after controlling for external environmental influences in the laboratory. Britton (2005) also demonstrated differential survival in the field by conducting reciprocal transplant experiments between individuals from the hot pond and main reservoir. At mid-summer temperatures, Britton (2005) found that mosquitofish from the main reservoir transferred to the hot pond exhibited 100% mortality whereas 33-42% of hot pond fish survived over the same exposure period. Conversely, there was essentially no mortality in either group when held in the cooler main reservoir. Britton's (2005) observations from these two experiments, along with the increase in the upper thermal tolerance limits of both the hot-pond and main-reservoir *G. affinis* populations over two decades supported Dean's (1981) suggestion that selection was the basis for the elevated thermal tolerance of hot-pond individuals and that directional selection continued to influence the thermal tolerance of the hot-pond population. However, the impact of thermal selection on the lower thermal tolerance limits of this population had not been studied.

1.4.2 Lower Thermal Limits

Despite the extensive research performed on the upper thermal limits of *G. affinis* (Hart 1952; Hagen 1964; Otto 1973; Dean 1981; Al-Habbib and Yacoob 1993; Britton 2005), very few studies have been carried out on lower thermal limits of this species. The three studies of lower thermal limits of *G. affinis* have been done using chronic-exposure methodology (Hart 1952, Otto 1973, Al-Habbib and Yacoob 1993). With chronic-exposure methods (which is also known as static or incipient lethal temperature methods), groups of fish are removed from various acclimation temperatures and plunged into a series of static test temperatures and monitored until an endpoint is reached for each fish (Fry 1947). The endpoint for this method is typically death, but the estimated time for which 50% of the fish reach mortality (LT_{50}) is often used as

the response variable. The incipient lower lethal temperature (ILLT) and the incipient upper lethal temperature (IULT) are reported for the chronic-exposure method (Fry 1947). These temperatures are the highest (IULT) or lowest (ILLT) temperatures reached where no further mortality is observed within a predetermined amount of time (usually 24 to 96 hours). The IULT and ILLT are typically lower than those reported for CT_{Max} and CT_{Min} .

One of the first studies on the lower thermal limits of *G. affinis* was done by Hart (1952). Hart (1952) found that the IULT and the ILLT of specimens of *G. affinis* were 35.4°C and 1.5°C, respectively, for individuals acclimated to 15°C; 37.3°C and 5.5°C for individuals acclimated to 20°C; and 37.3°C and 14.5°C for individuals acclimated to 35°C. The temperature range between upper and lower thermal limits in the three acclimation groups varied from 33.9°C and 22.8°C for the 15°C and 35°C acclimated individuals, respectively. Of the various species of fish tested by Hart (1952), *G. affinis* had the highest IULT at each acclimation temperature, along with the largest temperature range at each acclimation temperature.

Otto (1973) also tested IULT and ILLT of *G. affinis*, using a warm-adapted population from Arizona and a cold-adapted population from Utah. Otto (1973) found that the warm-adapted population was more heat tolerant at the expense of being less cold tolerant. For the cold-adapted population, he found an equivalent trend, except that this population was more cold tolerant at the expense of being less heat tolerant.

Al-Habbib & Yacoob (1993) recorded the IULT and ILLT of *G. affinis* acclimated to three different temperatures (10°C, 20°C, and 30°C). Results showed that increasing acclimation temperature from 10°C to 30°C had a significant effect on both the upper and lower lethal temperatures of mosquitofish (Al-Habbib & Yacoob 1993). The IULT and ILLT recorded were 32.3°C and 1.1°C, respectively, when acclimated to 10°C; 36.6°C and 2.0°C when acclimated to 20°C; and 38.7°C and 5.1°C when acclimated to 30°C (Al-Habbib & Yacoob 1993). Thus, the temperature range between upper and lower thermal limits in the three acclimation groups only

varied from 31.2°C when acclimated to 10°C to 34.6°C when acclimated to 20°C (Al-Habbib & Yacoob 1993).

1.4.3 Temperature Tolerance Polygons

By examining at the upper and lower incipient lethal temperatures at different acclimation temperatures, Hart (1952), Otto (1973) and Al-Habbib & Yacoob (1993) calculated an area of thermal tolerance for *G. affinis*. The area of thermal tolerance (or zone of thermal tolerance) was first developed by Fry et al. (1942) who defined it as the area enclosed by a species' upper and lower lethal ranges, relative to acclimation temperature. When plotted (with acclimation temperatures plotted on the x-axis and critical or lethal temperatures corresponding to the acclimation temperatures plotted on the y-axis), these upper and lower thermal tolerances are represented by a polygon shape, therefore representing a temperature tolerance polygon (Bennett and Beitinger 1997; Fig. 1.5). Temperature tolerance polygons give an approach to qualitatively compare a species' tolerance to a wide range of temperatures. Therefore, this area defines the degree of eurythermicity of individual species of organisms (Fry et al. 1942; Beitinger et al. 2000). Temperature tolerance polygons can be calculated using either the chronic-exposure method (static temperature tolerance polygon) or the acute-exposure method (dynamic temperature tolerance polygon) (Bennett and Beitinger 1997).

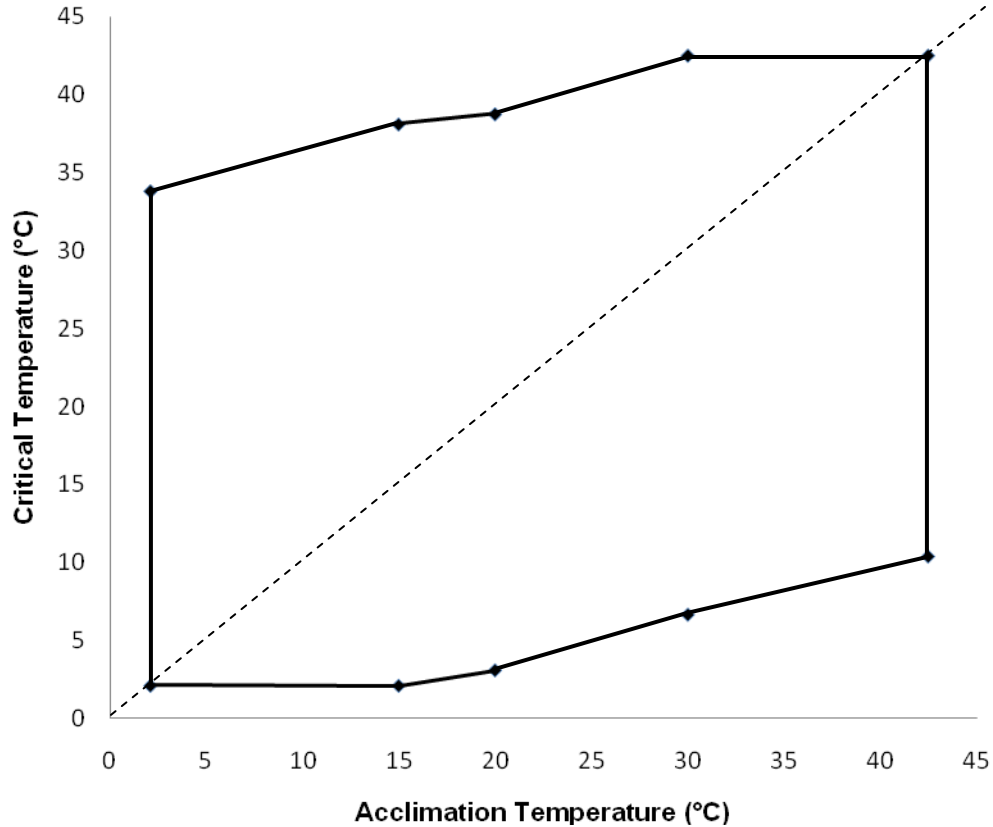


Figure 1.5 An example of a dynamic temperature tolerance polygon.

Hart (1952) calculated the first temperature tolerance polygon of *G. affinis*, finding the area of thermal tolerance to be 1110°C^2 . This area was one of the highest reported among 23 different species of fish tested (Brett 1956), which in turn would make *G. affinis* one of the most eurythermic fish species researched to date. Otto (1973) also reported areas of thermal tolerance for two populations of *G. affinis*, finding that a warm-adapted population had a slightly higher area (1065°C^2) than a cold-adapted population (1033°C^2). Finally, Al-Habbib and Yacoob (1993) found the area of thermal tolerance to be 633.3°C^2 in their study of *G. affinis*.

1.5 The Effect of Season on Thermal Tolerance

Schmidt-Nielson (1997) addressed the issue of seasonal effects on the temperature tolerance of animal species in natural environments. The thermal tolerance of the same

species can differ between seasons. An individual experiencing winter conditions will be able to tolerate low temperatures that would otherwise be lethal to an individual experiencing summer conditions. The same is true in the opposite situation- an individual experiencing summer conditions will be able to tolerate higher temperatures that would otherwise be lethal to an individual experiencing winter conditions. This ability for an individual to tolerate different temperatures under different seasonal climates in the natural environment is known as acclimatization (Schmidt-Nielson 1997).

Hart (1952) addressed how thermal tolerance can be affected by season. After testing the lethal temperatures of seven different species of fish and comparing these temperatures between winter and summer, he found that the winter-collected fish were not able to withstand temperatures as high as summer-collected fish. However, Hart (1952) found discrepancies with different acclimation temperatures. Two species of fish showed similar lethal temperatures between winter and summer at a 20°C acclimation temperature, but at a 25°C acclimation temperature, the lethal temperature between winter-collected and summer-collected individuals were different. Hart (1952) suggested that incomplete acclimation could be an explanation for these discrepancies in thermal tolerance. Since acclimatization involves multiple and often unknown factors, it can overshadow the effects of acclimation, which involves only one controlled factor (in this case, temperature) (Prosser 1993). Thus, the effects of acclimatization cannot always be controlled for, or acclimated away, in the laboratory.

Ingersoll and Claussen (1984) found different results when testing the critical thermal maximum (CT_{Max}) of two species of darters. When comparing the CT_{Max} between winter-collected and summer-collected fish, *Etheostoma flabellare* (fantail darters) showed essentially no difference between seasons (31.1°C for winter; 31.3°C for summer). The same results were reported with *E. nigrum* (johnny darters) as well. The CT_{Max} found for the winter-collected fish (30.9°C) was not different from that of summer-collected fish (30.5°C). The sensitivity of a fish species' CT values to seasonal climate changes may, therefore, vary among species and must

be addressed on a species to species basis. This phenomenon has yet to be described in *Gambusia affinis*.

1.6 Specific Aims

If temperature adaptation impacts both the upper and lower thermal limits of a species, as Otto (1973) found, then both should be examined in any study of species thermal adaptation. If the hot-pond population of *Gambusia affinis* found at Lake Fairfield has adapted to become more tolerant of elevated temperatures, as described above, then it needs to be determined whether this population has become less tolerant of cold temperatures, which may affect the range of thermal environments this population could inhabit. Upper thermal tolerance limits have been investigated extensively in *G. affinis*, yet this species' lower thermal limits have been largely ignored. This is an area that deserves attention in order to develop a better understanding of the physiological ecology of this species and will provide a more complete examination of how a highly invasive, ectothermic organism responds to anthropogenic habitat alteration.

1.6.1 Thermal Tolerance

In order to assess whether exposure to thermal effluents has caused a continued directional selection response in the upper thermal tolerance limits of the hot-pond population of *G. affinis* at Lake Fairfield, critical thermal maximum (CT_{Max}) temperatures were established for both the hot-pond and main-reservoir populations. These findings were compared with those of Britton (2005) to determine whether the previously documented increase in heat tolerance of *G. affinis* populations from the hot pond of Lake Fairfield has continued over the past five years. Critical thermal minimum (CT_{Min}) temperatures were also established in order to address the question of whether exposure to thermal effluents has lead to an expansion of the thermal tolerance range in the hot-pond population of *G. affinis*, or if it has simply shifted the upper and lower thermal tolerance limits upward without a change in temperature tolerance range, allowing the hot-pond mosquitofish population to tolerate warmer waters, but simultaneously making this

population less tolerant of colder waters. These findings for the hot-pond and main reservoir populations at Lake Fairfield were compared to upper and lower thermal tolerance limits determined for an additional population of *G. affinis* not exposed to thermal effluents.

Due to the increase in upper thermal tolerance limits of the hot-pond and main-reservoir populations of *G. affinis* found over the two decades between Dean's (1981) and Britton's (2005) studies, I hypothesized that an additional, yet smaller, increase would be found in both the hot-pond and main-reservoir populations. If an additional increase in upper thermal tolerance limits is found in the hot-pond population, it will support the suggestion that directional selection is driving the upper limits of thermal tolerance higher for the hot-pond population found at Lake Fairfield. Since Dean (1981) and Britton (2005) both reported that the hot-pond mosquitofish population demonstrated a difference in upper thermal tolerance limits compared to the main-reservoir population, similar results were expected. An increase in the lower thermal tolerance limits of the hot pond population were also expected, congruent to that of Otto (1973) who found that an increased upper thermal limit was coupled with an increased lower thermal limit in a warm-adapted population of *G. affinis*. An increase in the upper thermal tolerance limit associated with the hot-pond population were compared with the corresponding lower thermal tolerance limit to see if this reported trend holds true for this population of *G. affinis*.

1.6.2 Seasonal Effects

Pilot studies suggested that thermal tolerance differences were not consistent across seasons. In April of 2007, lower thermal tolerance experiments were performed on *G. affinis* collected from the main reservoir and hot pond of Lake Fairfield. A significant difference in the lower thermal tolerance limits was found between the hot-pond population and the main-reservoir population when acclimated at 14°C. In June of 2007, the lower thermal tolerance experiments were repeated on the main-reservoir and hot-pond populations. The findings of this experiment showed no statistical difference between the two populations, but the mean CT_{Min} temperatures were different than those found in April 2007. Britton (2005) demonstrated

that thermal history can have a considerable impact on adult thermal tolerance in *G. affinis*. The short generation time in western mosquitofish can lead to substantial differences in thermal history experienced between individuals born during different seasons.

Because of the findings in the pilot study, I hypothesized that seasonal acclimatization may present an explanation for these differences. By investigating the upper and lower thermal tolerance limits of mosquitofish caught in spring, summer, fall, and winter, the effects of seasonal acclimatization on thermal tolerance were assessed. These findings allowed for a better understanding and interpretation of the data and accounted for the variation present due to seasonality.

1.6.3 Eurythermicity

Gambusia affinis has been shown to have a broad range of tolerance to temperatures relative to other fish (Hart 1952). This broad range of temperature tolerance, in theory, corresponds to a broad fundamental thermal niche and a high degree of eurythermicity (Bennett and Beitinger 1997). Therefore, the species with the largest thermal tolerance range has an advantage over other fish species in a thermally stochastic environment. Furthermore, fish with a higher upper thermal tolerance limit can persist at temperatures above the physiological limitations of potential competitors. While sampling in the main reservoir of Lake Fairfield, at least one other species of similar size fish (*Menidia beryllina*) was often noted in the same microhabitat. However, *G. affinis* was the only fish of that size found in any season in the hot pond. This observation supports the hypothesis that *G. affinis* has a higher upper thermal limit than other similarly sized species. The extent of the eurythermicity of three studied mosquitofish populations were established by construction of dynamic temperature tolerance polygons following the methods of Bennett and Beitinger (1997).

If an upward shift in both the upper and lower thermal tolerance limits was exhibited in the hot-pond population (as previously predicted in subsection 1.6.1), then the temperature tolerance polygon associated with this population was expected to remain similar in size relative

to that of the main-reservoir population, even though upper and lower thermal limits had increased. However, if the upper thermal tolerance limit of the hot-pond population was found to vary independently of its lower thermal tolerance limit, then the hot-pond temperature tolerance polygon was expected to exhibit an increased or decreased area relative to that of the main-reservoir temperature tolerance polygon.

CHAPTER 2

METHODS

2.1 Thermal Tolerance

2.1.1 Collections for Thermal Tolerance Experiments

Specimens from three different populations of *Gambusia affinis* were collected for this study. Thermal tolerance experiments included samples from two populations in Lake Fairfield, Freestone County, Texas. One of these populations was collected from the hot pond, an isolated area receiving artificially heated water from the Big Brown Steam-Electric Power Station (Fig. 2.1). A second population was collected from the main reservoir of Lake Fairfield, an area indirectly exposed to the heated effluent only after it has cooled while flowing through the hot pond and discharging through the lower effluent canal into the lake proper (Fig. 2.1).

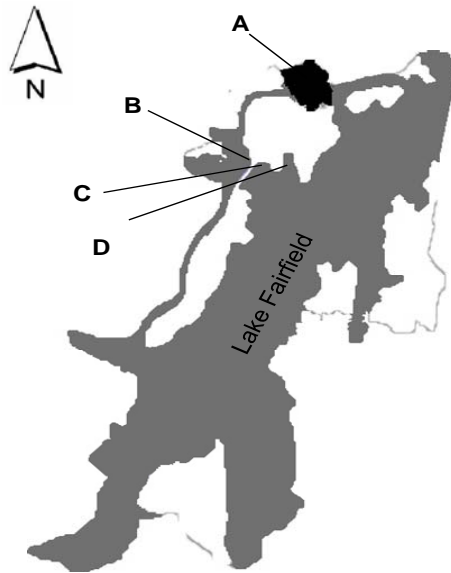


Figure 2.1 Lake Fairfield, Freestone County, Texas. Site "A" is the location of the Big Brown Power Station. Site "B" is the location of the hot-pond population collection. Sites "C" and "D" are the locations of the main-reservoir population collection.

A third population was collected from a pond in Deaver Park located in Arlington, Texas (32°39'14.20" N, 97°10'41.11" W), an area completely isolated from artificially heated waters. Deaver Park pond is a small body of water, therefore exhibiting extremes in water temperature (Fig. 2.1). The lowest temperature recorded was in December 2007 at 3.9°C, whereas the highest temperature recorded was in August 2007 at 42.0°C (Table 2.1). This body of water has a large range of temperatures each month, with the largest range of 30.2°C seen within 8 days in April 2008.

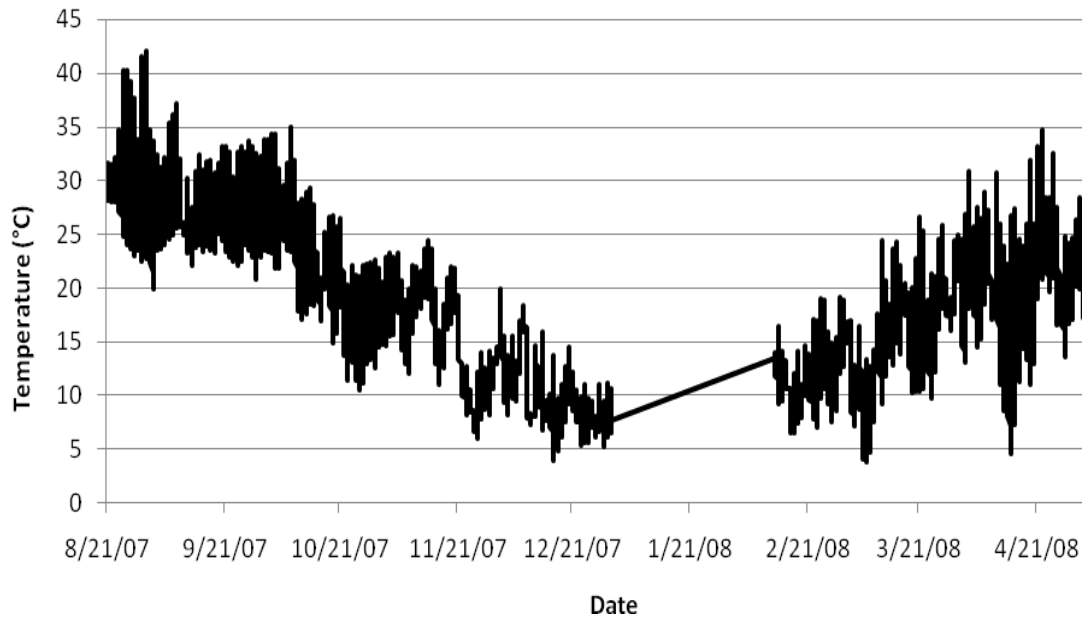


Figure 2.2 Daily surface water temperature of the Deaver Park pond in Arlington, Texas.

Table 2.1 The low monthly temperature, high monthly temperature, and mean monthly temperature (in °C) of Deaver Park pond located in Arlington, Texas. The dashes (-) indicate no data collected.

Month	Low Monthly Temperature (°C)	High Monthly Temperature (°C)	Mean Monthly Temperature (°C)
August-07	22.4	42.0	28.5
September-07	19.9	37.2	26.5
October-07	10.5	34.9	20.7
November-07	6.0	24.4	15.4
December-07	3.9	19.9	9.8
January-08	-	-	-
February-08	6.5	19.1	11.4
March-08	3.8	26.6	15.2
April-08	4.5	34.7	19.2
May-08	16.7	32.7	22.4

For the thermal tolerance experiments, each collection included approximately 120 fish each from the hot pond and main reservoir at Lake Fairfield, and Deaver Park pond in Arlington, Texas. Fish were collected by dip netting around the margins of each water body near emergent vegetation. In order to compare thermal tolerance differences between seasons, collections were made in May 2007 (Spring-tested fish), August 2007 (Summer-tested fish), November 2007 (Fall-tested fish), and February 2008 (Winter-tested fish).

Fish were returned to the laboratory at The University of Texas at Arlington and divided randomly into treatment samples within stock tanks (380-L, polyethylene tanks) filled with continually aerated, dechlorinated tap water (City of Arlington, Texas municipal supply). Tank media was continuously passed through ≈19-L sand/gravel-filled biological filters. Water quality parameters, measured as ammonia, nitrate and nitrite concentrations, hardness, alkalinity and pH, were tested every five days with commercial testing kits. A 20% water change was performed on tank media every five days or more frequently if warranted by water quality testing. The water temperature in each stock tank was initially set to correspond to the field ambient water temperature at collection.

Tank water temperature was then adjusted at a rate of 1°C per day until the target acclimation temperature of 24°C or 14°C ($\pm 0.1^\circ\text{C}$) was met after which it was held constant for at least three weeks prior to experimental trials. The 24°C acclimation temperature was chosen to correspond to that used by both Dean (1981) and Britton (2005) in their studies of the upper thermal tolerance limits of hot-pond mosquitofish. This common acclimation temperature of 24°C allowed comparison of the upper thermal limit data from the present study with the data of Dean (1981) and Britton (2005). The 14°C acclimation temperature was chosen for convenience, because it was 10°C lower, yet still high enough to ensure survival. Temperature was maintained using either an Arctica Titanium DBE-200[®] in-line water chiller for 14°C or a Marine Metal[®] submersible water heater for 24°C. Water temperature was monitored with digital thermometers and HOBO[®] data loggers. Fish were fed daily with commercial flake fish food *ad libitum* during the holding and acclimation periods.

2.1.2 Determination of Critical Thermal Maxima and Minima for Thermal Tolerance Experiments

In order to determine the upper and lower thermal limits of samples from the three populations of *G. affinis*, experiments measuring acute-exposure tolerance to cold temperatures (CT_{Min} or critical thermal minimum) and warm temperatures (CT_{Max} or critical thermal maximum) were performed on and compared among specimens in the collected samples. These tests allowed estimation of the thermal-tolerance range for the populations examined. The upper and lower thermal limits were tested and compared over each season (Spring, Summer, Fall, and Winter) in order to determine the effects of seasonality on the thermal tolerance of *G. affinis*.

For determination of either CT_{Max} or CT_{Min} , approximately 120 specimens of *G. affinis* were used for each experiment from each of the three collection sites: 1) the hot pond at Lake Fairfield (HP), 2) the main reservoir at Lake Fairfield (MR), and 3) Deaver Park Pond (DP). Specimens collected from each population were divided equally between the two acclimation temperatures (i.e., 14°C and 24°C). Following acclimation, mosquitofish from each population were split into two groups in order to test their CT_{Min} and CT_{Max} . In order to test CT_{Min} , approximately 60 fish from each population site (HP, MR, and DP) and each acclimation

temperature (24°C and 14°C) were placed in 1-L plastic-mesh containers inside 25-L insulated test tanks. Testing tank temperatures were controlled using a Techne Tempette[®] circulating heater and one or more Haake[®] chillers with a submersible wand. Temperatures were continuously monitored with a HANNA[®] K-thermocoupler digital thermometer with the probe immersed in the water common to the test subjects.

For testing CT_{Min} of *G. affinis* individuals, temperature within test tanks were lowered 1°C every five minutes by adjusting the water circulator until all sampled fish displayed signs of attaining the CT_{Min} endpoint identified by no response to gentle prodding with a net (Ford and Beitinger 2005). The temperature at which each fish reached the CT_{Min} endpoint was recorded. On attaining CT_{Min} , each fish was removed from the test tank and immediately placed in individually labeled plastic containers filled with 500 ml of room temperature water for 24 hours to allow recovery.

Britton's (2005) approach to testing CT_{Max} in *G. affinis* was followed allowing for relative comparison with his results. Samples of *G. affinis* were divided and treated as described above for the CT_{Min} test. For testing CT_{Max} , fish samples were held in insulated tanks as described above and subjected to a 1°C increase in temperature every three minutes until all sampled fish displayed signs of attaining the CT_{Max} endpoint, at which equilibrium and a righting response was lost (Paladino et al. 1980; Meffe et al. 1995). The temperature at which each fish reached the CT_{Max} endpoint was recorded and the fish was removed from the test tank and immediately placed in individually labeled plastic containers of room temperature water for 24 hours to allow recovery.

After all fish were allowed to recover for 24 hours, gender, total length, and mass were recorded. Gender was determined by locating a gonopodium, which is a modified anal fin on male mosquitofish that is used for insemination (Pyke 2005) (Fig. 1.1). Absence of the gonopodium on a fish >15mm indicated that a specimen was female. The total length (TL) of each individual was measured to the nearest 0.01 mm from the snout to the posterior extremity

of the caudal fin, using Mitutoyo[®] Solar Digimatic Calipers. The wet mass of each individual was measured to the nearest 0.001 g using an Ohaus[®] digital scale.

2.1.3 Statistical Analysis of Thermal Tolerance Experiments

Models were built and tested using cross-validation. This was done by grouping data by strata (population/season/acclimation temperature/gender) and using a random number generator to assign a unique identifier (tag number) to each data point. Data points within strata were then sorted by tag number and divided into two subsamples across the median. This resulted in two datasets that contained approximately equal numbers of randomly assigned data points from each of the strata categories. One dataset was then used to construct a model that regressed the predictor variables on CT_{Max} or CT_{Min} . The other dataset was used to validate the model.

Two models (one to test CT_{Max} and a second to test CT_{Min}) with all linear terms and interactions (up to 5-way interactions), a quadratic term (length) and two-way interactions with the quadratic term, and cubic term (length) and two-way interactions with the cubic term were proposed as a maximal (saturated) model to compare reduced model fit. Each model was reduced by both stepwise forward and stepwise backward variable selection using $\alpha = 0.2$, allowing a more inclusive than exclusive fit. Additional terms were removed starting with high-order interaction, cubic and quadratic terms, until the R^2 value was reduced by ~5%. This resulted in more manageable models while maintaining a relatively high explanation of the variance (Appendix Table A1 and A2). Analysis of residual plots indicated that the length term required transformation ($1/\ln$) in order to reduce heteroscedasticity in both the CT_{Max} and CT_{Min} models. Residual plots and normal probability plots indicated that the regression assumptions were met.

The validation data set was then analyzed and the R^2 value was compared with the R^2 of the dataset used to construct the model. If the value dropped by less than 10%, the model was assumed to be adequate. The R^2 value did not decrease by more than 5%, validating the

model. Residual plots and normal probability plots indicated that regression assumptions were met.

The complete dataset was then analyzed. Outliers were identified and removed (less than three from each model) based on large departures from expected values. In all cases, individual fish represented by outliers had succumbed to experimental treatment well before a majority of the other test animals, indicating that these specimens may have been injured, sick, infested with parasites, or were otherwise unhealthy. Additional statistical outliers (studentized residual >2 , high leverage for some) that did not obviously deviate from the expected values were not removed from the model because these data points could have been true treatment responses. The large sample size associated with this dataset should have negated the effect of such data points. Model fit was assessed as were residual plots and normal probability plots to test for normality and heteroscedasity. These plots satisfied the assumptions for the fit of the model.

As expected from data with relatively absolute bounds, the datasets were skewed. Animals in the CT_{Max} dataset were bound by an absolute upper thermal limit that constrained how far to the right (higher CT_{Max} values) animals could deviate from a majority of the samples above the predicted CT_{Max} , with a lesser constrained lower bound. This resulted in the CT_{Max} dataset having a left skew. The opposite condition existed in the CT_{Min} dataset such that a right skew was present. In both models, transformations were applied to the response variable but were abandoned due to their inability to completely correct the skew and because transformation of the response variable would have made interpretation much more difficult. Therefore the final analysis was performed on a non-transformed response variable although some skew was present.

A table of all strata, including all combinations of population, season, gender, and acclimation temperature, was created and the model based statements for those strata were defined using model terms. Specific hypothesis tests were performed using additive and subtractive methods of model defined strata to prepare SAS estimate statements so that

significance of specific hypothesis tests could be assessed. For these tests, the length covariate was controlled to the overall median (26.4 mm) for the entire dataset. In some cases, this median length was not indicative of the median for the specific combination of population/season/gender as lengths differed among population samples, sexes, and seasons. Controlling to an overall median length allowed hypothesis tests to be assessed regardless of the effects of different sizes among the strata.

The regression model was used to predict values for strata defined categories that were subsequently used to produce graphical summaries of the data. Hypothesis tests were performed on the effects of population, acclimation temperature, and gender. Statistical significance was determined with $\alpha = 0.05$.

2.1.4 Comparisons of CT_{Max} with Britton (2005)

In order to compare the current CT_{Max} data with Britton's (2005) data collected in 2002, the raw data was acquired from David Britton, Ph.D., and compared to the current data with a two sample t-test for each gender between the hot-pond population and main-reservoir population. Statistical differences were determined with $\alpha = 0.05$.

2.1.5 Determining Shifts between the Hot-Pond and Main-Reservoir Mosquitofish Populations

The hot-pond population's relationships of upper and lower thermal tolerance limits for each season were compared to that of the main-reservoir population's corresponding seasonal upper and lower thermal tolerance limits. Data from the previous analysis (Subsection 2.1.3) was used for comparison. If the hot-pond population's CT_{Max} (or CT_{Min}) was significantly higher compared to the main-reservoir population's CT_{Max} (or CT_{Min}), it was considered an increase and indicated with an upward arrow. If the hot-pond population's CT_{Max} (or CT_{Min}) was significantly lower compared to the main-reservoir population's CT_{Max} (or CT_{Min}), it was considered a decrease and indicated with a downward arrow. If the hot-pond population's CT_{Max} (or CT_{Min}) was not significantly higher or lower compared to the main-reservoir population's CT_{Max} (or CT_{Min}), it was considered that there was no difference in the two values and indicated with a line.

2.2 Seasonal Effects

2.2.1 Statistical Analysis of Seasonal Effects

Statistical analysis of the seasonal effects on the upper and lower thermal tolerance limits of *Gambusia affinis* were performed using the temperature tolerance data mentioned in subsections 2.1.1 and 2.2.2 of the Methods section. Specific hypothesis tests were performed using additive and subtractive methods of model-defined strata to prepare SAS estimate statements so that significance of specific hypothesis tests could be assessed. For these tests, the length covariate was controlled to the overall median (26.4 mm) for the entire dataset. Hypothesis tests were performed on the effects of season within populations. Comparisons were made among populations within seasons, as well as among seasons within population. Statistical significance was determined with $\alpha = 0.05$.

2.3 Eurythermicity

2.3.1 Collections for Temperature Tolerance Polygons

Specimens for determination of temperature tolerance polygons were collected from the three sample sites previously described for the thermal tolerance experiment. Approximately 60 fish were collected from the hot pond and main reservoir at Lake Fairfield, and Deaver Park pond in Arlington, Texas, by dip netting around the margins of each water body, near emergent vegetation. Fish were returned to the laboratory at The University of Texas at Arlington and divided randomly into treatment samples in stock tanks (380-L, polyethylene tanks) filled with continually aerated, dechlorinated tap water (City of Arlington, Texas municipal supply). Stock tank media, temperature, and feeding were maintained as previously described.

The temperature in each stock tank was initially set to correspond to the field ambient water temperature at collection. Temperature was then adjusted at a rate of 1°C per day until the target acclimation temperatures of 30°C, 20°C, and 15°C were met, after which they were held constant ($\pm 0.1^\circ\text{C}$) for at least three weeks prior to experimental trials. Temperatures were maintained and monitored as per the methods previously described for thermal tolerance testing (Subsection 2.1.2).

2.3.2 Determination of Critical Thermal Maxima and Minima for Temperature Tolerance

Polygons

Determination of CT_{Min} and CT_{Max} allowed construction of polygons for temperature tolerance for the three populations of *G. affinis*. For determination of either CT_{Max} or CT_{Min} , 60 specimens of *G. affinis* were used from each of the three collection sites described previously for the thermal tolerance experiments (i.e. HP MR, and DP). Following acclimation, mosquitofish from each population acclimation group (30°C, 20°C, and 15°C) were divided into two groups, one for testing CT_{Min} , and the other for testing CT_{Max} . CT_{Min} and CT_{Max} methodology was followed as previously described (Subsection 2.1.2) using 15 fish from each population site (HP, MR, and DP) and each acclimation temperature (30°C, 20°C, and 15°C). After each CT_{Min} and CT_{Max} endpoint was recorded, individual fish were placed in individually labeled plastic containers filled with 500 ml of room temperature water for 24 hours to allow for recovery. Following recovery, gender, total length, and mass were recorded as described previously in subsection 2.1.1.

2.3.3 Construction and Calculation of Temperature Tolerance Polygons

Temperature tolerance polygons were constructed following the methods of Fry et al. (1942), Brett (1952) and Bennett and Beitinger (1997) (Beitinger pers. comm.). After CT_{Min} and CT_{Max} were determined, the arithmetic mean of the CT_{Min} and CT_{Max} temperatures were calculated for each acclimation temperature from each population site. The mean values for CT_{Min} and CT_{Max} were then plotted on a graph with axes of acclimation temperature (horizontal) and CT temperature (vertical). A line of equal temperatures was then fitted so that one degree acclimation temperature corresponded to one degree CT temperature along the line. Brett (1952) described the line of equal temperatures as a line that is representative of all values where the CT temperature and acclimation temperature are equal. The absolute upper and lower calculated mean CT temperatures were then plotted on this line. To complete the polygon, least-squares, linear regressions were performed on the upper and lower calculated mean CT values at each acclimation temperature, and a point was plotted along this line at the

value where the acclimation temperature (x) equaled the highest and lowest calculated mean CT temperatures. The formula for the area of a polygon, $A = \frac{1}{2} \sum_{i=1}^n (x_i y_{i+1} - x_{i+1} y_i)$ (Bockman 1989), was used to calculate the area of temperature tolerance for each population in °C².

2.3.4 Quantitative Comparison of Temperature Tolerance Polygons

Due to the nature of this data, there is no established means by which to statistically compare temperature tolerance polygons (Bennett and Beitinger 1997). Therefore, a conservative approach was applied to compare the areas of the polygons for this study. A maximum-size polygon was constructed using the upper 95% confidence interval for the mean CT_{Max} value at each acclimation temperature, and the lower 95% confidence interval for the mean CT_{Min} value at each acclimation temperature. To construct a minimum-size polygon, the same procedures were used with the lower 95% confidence interval for the mean CT_{Max} value at each acclimation temperature, and the upper 95% confidence interval for the mean CT_{Min} value at each acclimation temperature. The areas of these polygons were calculated as described in subsection 2.3.3 to represent a crude confidence interval for comparison.

CHAPTER 3

RESULTS

3.1 Thermal Tolerance

3.1.1 Comparisons of Critical Thermal Limits among Populations

The constructed regression model demonstrated that the hot-pond population (HPP) was overall less cold tolerant than both the main-reservoir population (MRP) and the Deaver Park population (DPP) at both acclimation temperatures (HPP-MRP 14°C: $t = 8.9$, $p = <0.001$; HPP-MRP 24°C: $t = 4.73$, $p = <0.001$; DPP-HPP 14°C: $t = -10.01$, $p = <0.001$; DPP-HPP 24°C: $t = -3.12$, $p = 0.002$) (Table 3.1). The HPP was more heat tolerant than the DPP at both acclimation temperature (DPP-HPP 14°C: $t = -2.51$, $p = 0.012$; DPP-HPP 24°C: $t = -2.58$, $p = 0.01$) (Table 3.2). However, the HPP was more heat tolerant than the MRP at only the 14°C acclimation temperature (HPP-MRP 14°C: $t = 6.07$, $p = <0.001$; HPP-MRP 24°C: $t = 0.48$, $p = 0.629$) (Table 3.2).

Table 3.1 ANOVA table for CT_{Min} model

Source	df	Sum of Squares	Mean Square	F-Value	Pr > F
Model	41	4998.22	121.91	151.15	<0.0001
Error	1449	1168.67	0.8065		
Corrected Total	1490	6166.89			

Table 3.2 ANOVA table for CT_{Max} model

Source	df	Sum of Squares	Mean Square	F-Value	Pr > F
Model	46	4121.04	89.588	154.3	<0.0001
Error	1327	770.451	0.5806		
Corrected Total	1373	4891.49			

3.1.2 Comparisons of Critical Thermal Maximum with Britton (2005)

The comparison of the present data with Britton's (2005) data collected in 2002 revealed no consistent trend (Fig. 3.1). There were no significant differences noted between the main-reservoir males ($t_{21} = 0.90$, $p = 0.378$), main-reservoir females ($t_{39} = 0.14$, $p = 0.682$), or hot-pond males ($t_{22} = 0.17$, $p = 0.864$). Only hot-pond females exhibited a detectable increase in CT_{Max} ($t_{35} = 2.77$, $p = 0.009$).

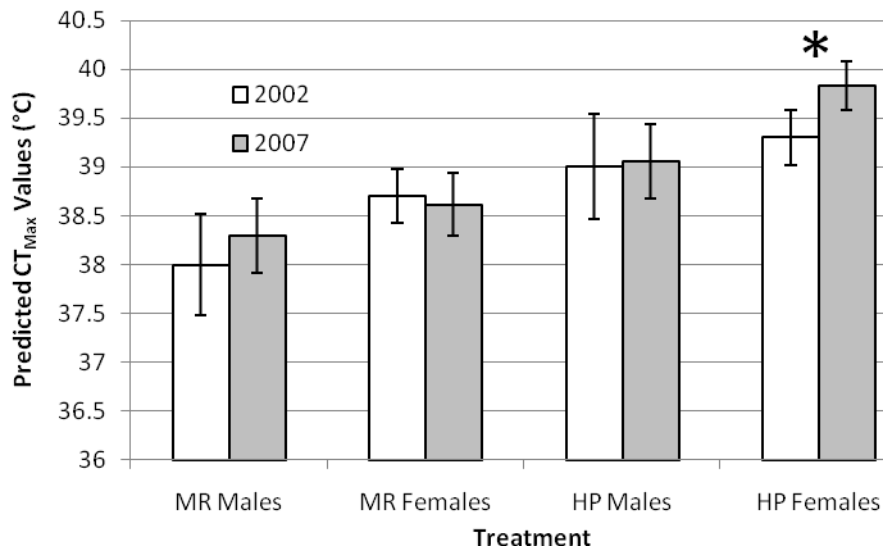


Figure 3.1 Critical thermal maximum [$\pm 2SE$] comparisons between the present study and that of Britton (2005). Asterisk denotes statistical significance $\alpha = 0.05$. Dates in legend represent actual collection dates and not publication dates.

3.1.3 Shifts in Thermal Tolerance between the Hot-Pond and Main-Reservoir Populations

There was no consistent trend regarding the relationship of CT_{Max} and CT_{Min} between populations across seasons (Fig. 3.2 and Fig. 3.3). When compared to main reservoir values, the hot-pond values were not always consistently higher. Results depended largely on season and gender. In some cases, the temperature tolerance range of the HPP was shifted upward relative to that of the MRP (indicted with an upward arrow in CT_{Max} as well as an upward arrow in the corresponding CT_{Min}). In other cases, the HPP showed a constricted thermal tolerance range relative to the MRP (indicted with a downward arrow in CT_{Max} with a dashed line in the corresponding CT_{Min}). Season/gender combinations that showed an upward shift in thermal

tolerance range as evidenced by corresponding increases in both CT_{Max} and CT_{Min} (HPP vs. MRP) were winter-collected females and males at the 14°C acclimation temperature, spring-collected females at the 24°C acclimation temperature, and fall-collected females at the 24°C acclimation temperature. Various season/gender combinations (HPP vs. MRP) exhibited a constricted range of temperature tolerance as a result of a decrease in CT_{Max} without a change in CT_{Min} . In no situation did an increase in CT_{Max} correspond to a decrease in CT_{Min} , which would have suggested an expanded range of temperature tolerance.

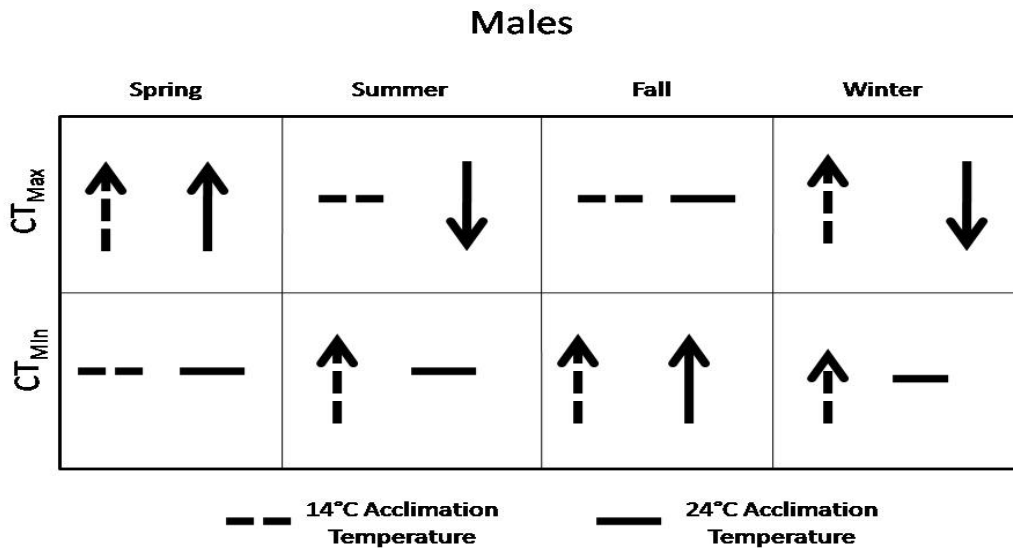


Figure 3.2 Relationships of hot pond critical thermal limits to main reservoir critical thermal limits in males. Upward pointing arrows indicate that the CT_{Max} or CT_{Min} of the hot-pond sample was significantly greater than that of the main-reservoir sample. Downward pointing arrows indicate that the CT_{Max} or CT_{Min} of the hot-pond sample was significantly less than that of the main-reservoir sample. A horizontal line indicates no significant difference between the CT_{Max} or CT_{Min} of the hot-pond and main-reservoir samples.

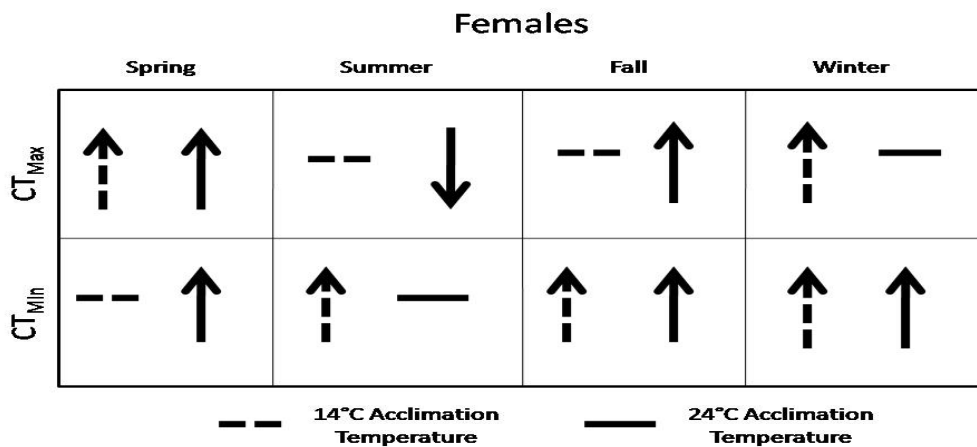


Figure 3.3 Relationships of hot pond critical thermal limits to main reservoir critical thermal limits in females. Upward pointing arrows indicate that the CT_{Max} or CT_{Min} of the hot-pond sample was significantly greater than that of the main-reservoir sample. Downward pointing arrows indicate that the CT_{Max} or CT_{Min} of the hot-pond sample was significantly less than that of the main-reservoir sample. A horizontal line indicates no significant difference between the CT_{Max} or CT_{Min} of the hot-pond and main-reservoir samples.

3.2 Seasonal Effects

3.2.1 Comparisons among Populations within Seasons

When examining critical thermal minimum (CT_{Min}), there were generally few significant differences for males collected in the spring (Figure 3.4 and Figure 3.5; all t-stats and p-values are presented in Appendix Table B1). Males from the DPP were less cold tolerant than males from both the HPP and MRP at the 24°C acclimation temperature. Females collected in the spring exhibited a similar trend, except a significant difference was detected between the HPP and the MRP at the 24°C acclimation temperature, where the HPP exhibited less cold tolerance than the MRP. For both males and females collected in the summer, the HPP exhibited less cold tolerance than the DPP at both acclimation temperatures. The summer-collected HPP for both males and females was only significantly less cold tolerant than the MRP at the 14°C acclimation temperature. For both males and females collected in the fall, the HPP exhibited less cold tolerance than the DPP and MRP at the 14°C acclimation temperatures. The fall-

collected HPP was significantly less cold tolerant at the 24°C acclimation temperature between both genders with the exception of DPP males. For both males and females collected in the winter, the HPP was less cold tolerant than both the DPP and the MRP at the 14°C acclimation temperature. At the 24°C acclimation temperature, the winter-collected HPP females were significantly less cold tolerant than the MRP females.

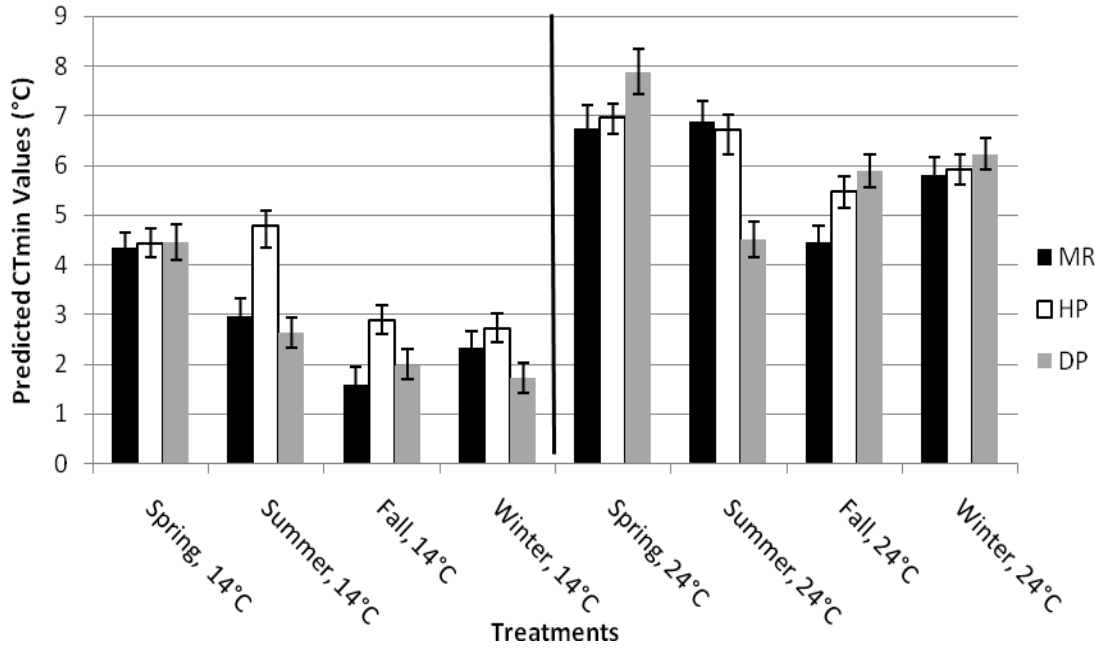


Figure 3.4 Predicted critical thermal minimum values [$\pm 2SE$] derived from the regression model for male *Gambusia affinis* across seasons for each population.

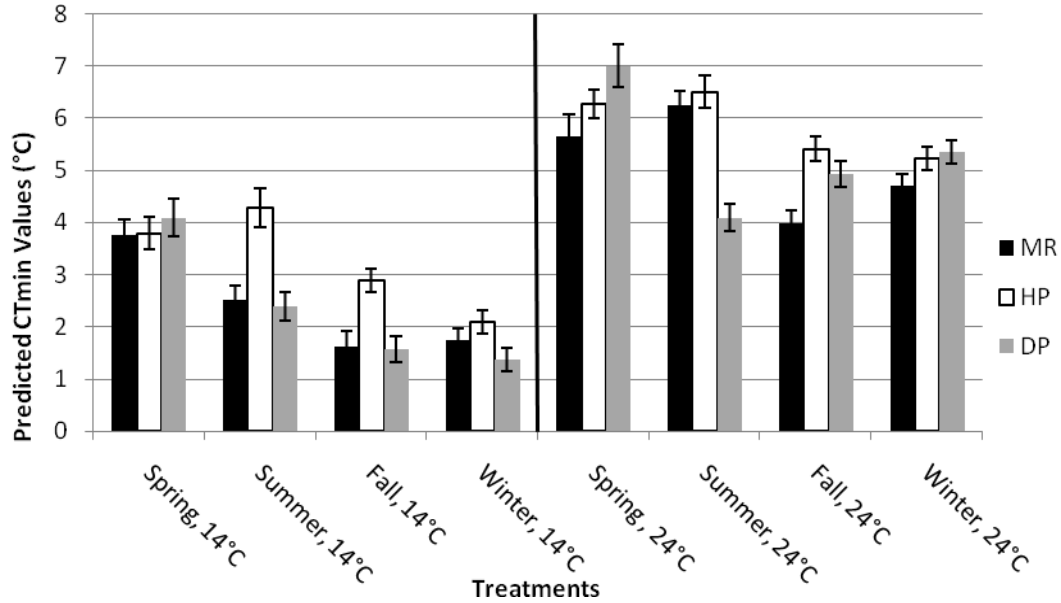


Figure 3.5 Predicted critical thermal minimum values [$\pm 2SE$] derived from the regression model for female *Gambusia affinis* across seasons for each population.

When examining critical thermal maximum (CT_{Max}), the general trends as found with CT_{Min} were not similar (Figure 3.6 and Figure 3.7; all t-statistics and p-values are presented in Appendix Table B2). Among spring-collected populations, all comparisons were significantly different across genders for both acclimation temperatures except males from the HPP and the DPP at the 24°C acclimation temperature. The HPP in spring was consistently more heat tolerant than the MRP, yet the DPP was significantly more heat tolerant than the HPP and MRP for both males and females at the 14°C acclimation temperature. For the summer-collected fish at the 24°C acclimation temperature, the MRP was more heat tolerant than the HPP for both genders. Only fall-collected females acclimated to 24°C from the HPP were more heat tolerant than the MRP. The DPP in fall was consistently less heat tolerant than the HPP and the MRP. For both males and females collected in the winter, the HPP was more heat tolerant than the DPP and the MRP at the 14°C acclimation temperature. However, at the 24°C acclimation temperature for winter-collected males, the MRP was more heat tolerant than the HPP. The DPP were consistently more heat tolerant than the HPP at 24°C acclimation temperature for

both genders, yet this population was consistently less heat tolerant than the HPP at the 14°C acclimation temperature for both genders.

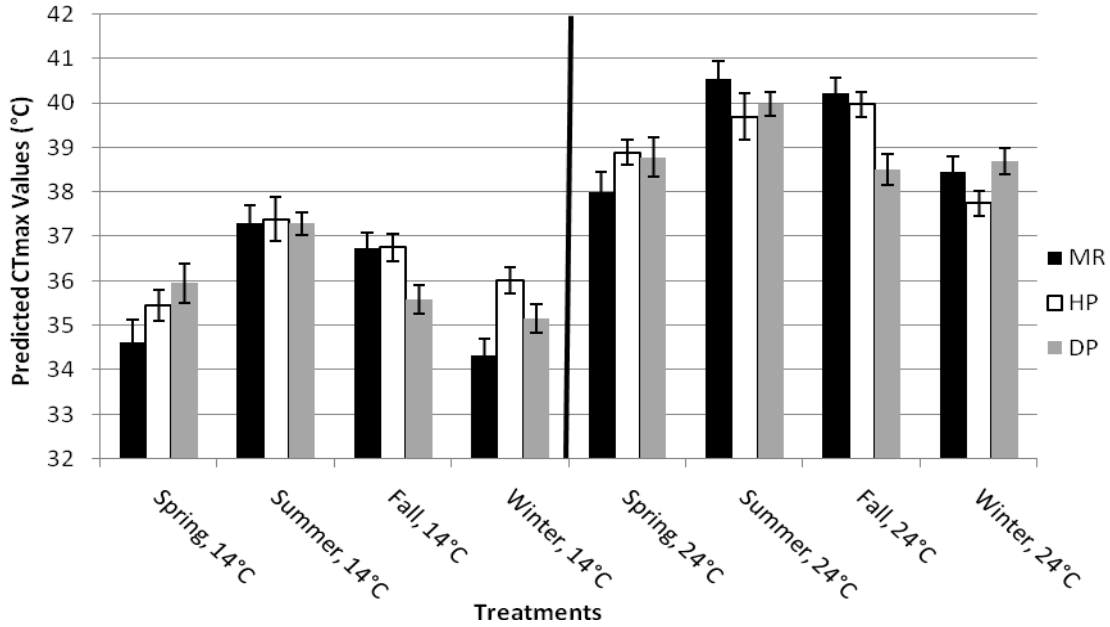


Figure 3.6 Predicted critical thermal maximum values [$\pm 2SE$] derived from the regression model for male *Gambusia affinis* across seasons for each population.

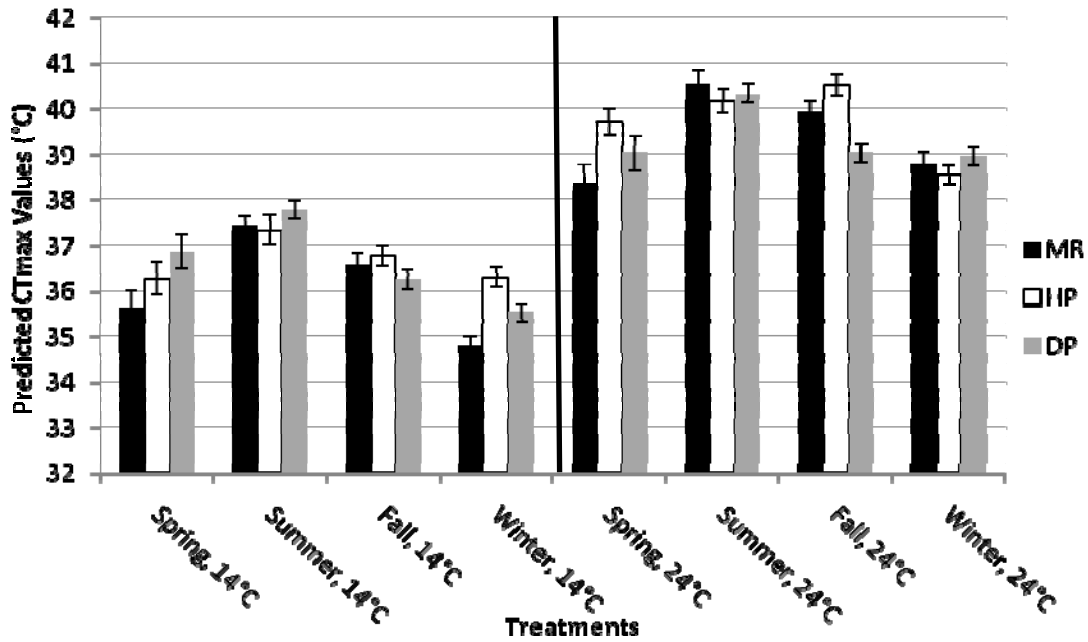


Figure 3.7 Predicted critical thermal maximum values [$\pm 2SE$] derived from the regression model for female *Gambusia affinis* across seasons for each population.

3.2.2 Comparisons among Seasons within Populations

Significant differences in CT_{Min} among seasons were noted for each population (Fig.3.8). The hot-pond population females acclimated to 24°C exhibited their highest degree of cold tolerance in the fall (5.41°C, SE = ±0.12, n = 44) and winter (5.22°C, SE = ±0.11, n = 43), while the females acclimated to 14°C exhibited their highest degree of cold tolerance in the winter (2.09°C, SE = ±0.11, n = 41). The hot-pond male population exhibited a similar trend, with their highest degree of cold tolerance occurring in the fall (5.48°C, SE = ±0.16, n = 17) at the 24°C acclimation temperature and in the fall (2.90°C, SE = ±0.15, n = 21) and winter (2.72°C, SE = ±0.14, n = 22) at the 14°C acclimated temperature. For both acclimation temperatures and genders, the main-reservoir population exhibited its highest degree of cold tolerance in the fall (MR 24°C Females = 3.98°C, SE = ±0.12, n = 40; MR 14°C Females = 1.63°C, SE = ±0.14, n = 44; MR 24°C Males = 4.46°C, SE = ±0.16, n = 20; MR 14°C Males = 1.59°C, SE = ±0.18, n = 14) with a similar winter value for females acclimated to 14°C (1.75°C, SE = ±0.11, n = 54). The Deaver Park population acclimated to 24°C exhibited its highest degree of cold tolerance for both genders in the summer (Females = 4.09°C, SE = ±0.13, n = 44; Males = 4.50°C, SE = ±0.18, n = 18). Both genders exhibited their highest degree of cold tolerance at 14°C acclimation during the fall and winter (Fall females = 1.56°C, SE = ±0.12, n = 42; Winter females = 1.37°C, SE = ±0.11, n = 47; Fall males = 1.99°C, SE = ±0.15, n = 26; Winter males = 1.73°C, SE = ±0.15, n = 16).

Significant differences among seasons were also noted for CT_{Max} (Fig. 3.9). However, the season showing the highest CT_{Max} was more consistent. All populations and genders reached their highest CT_{Max} during the summer (HPP 14°C Males = 37.39°C, SE = ±0.24, n = 18; HPP 14°C Females = 37.33°C, SE = ±0.16, n = 59; MRP 14°C Males = 37.30°C, SE = ±0.21, n = 10; MRP 14°C Females = 37.44°C, SE = ±0.10, n = 47; MRP 24°C Females = 40.57°C, SE = ±0.13, n = 18; DPP 14°C Males = 37.29°C, SE = ±0.13, n = 26; DPP 14°C Females = 37.77°C, SE = ±0.10, n = 45; DPP 24°C Males = 39.98°C, SE = ±0.13, n = 25; DPP 24°C Females = 40.34°C, SE = ±0.10, n = 40), with the exception of the hot-pond population

acclimated to 24°C (Summer males = 39.70°C, SE = ±0.26, n = 4; Fall males = 39.98°C, SE = ±0.14, n = 24; Summer females = 40.17°C, SE = ±0.13, n = 52; Fall females = 40.52°C, SE = ±0.11, n = 40) and main-reservoir males acclimated to 24°C (Summer = 40.55°C, SE = ±0.20, n = 12; Fall = 40.22°C, SE = ±0.17, n = 14).

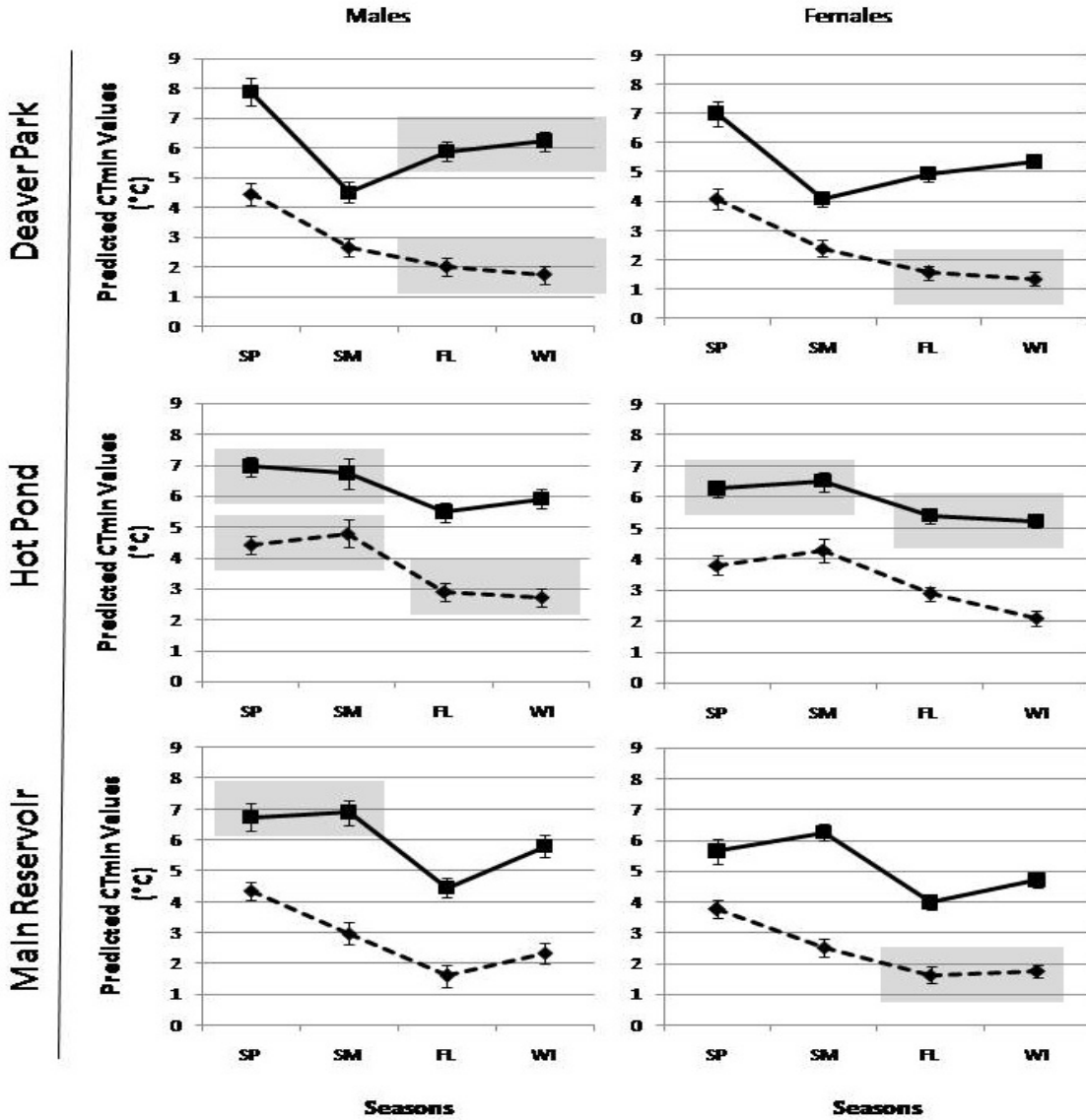


Figure 3.8 Seasonal differences in the critical thermal minimum [$\pm 2SE$] within populations for each gender. Solid lines represent the 24°C acclimation temperature and dashed lines represent the 14°C acclimation temperature. Shaded boxes indicate no statistical difference among the values contained therein.

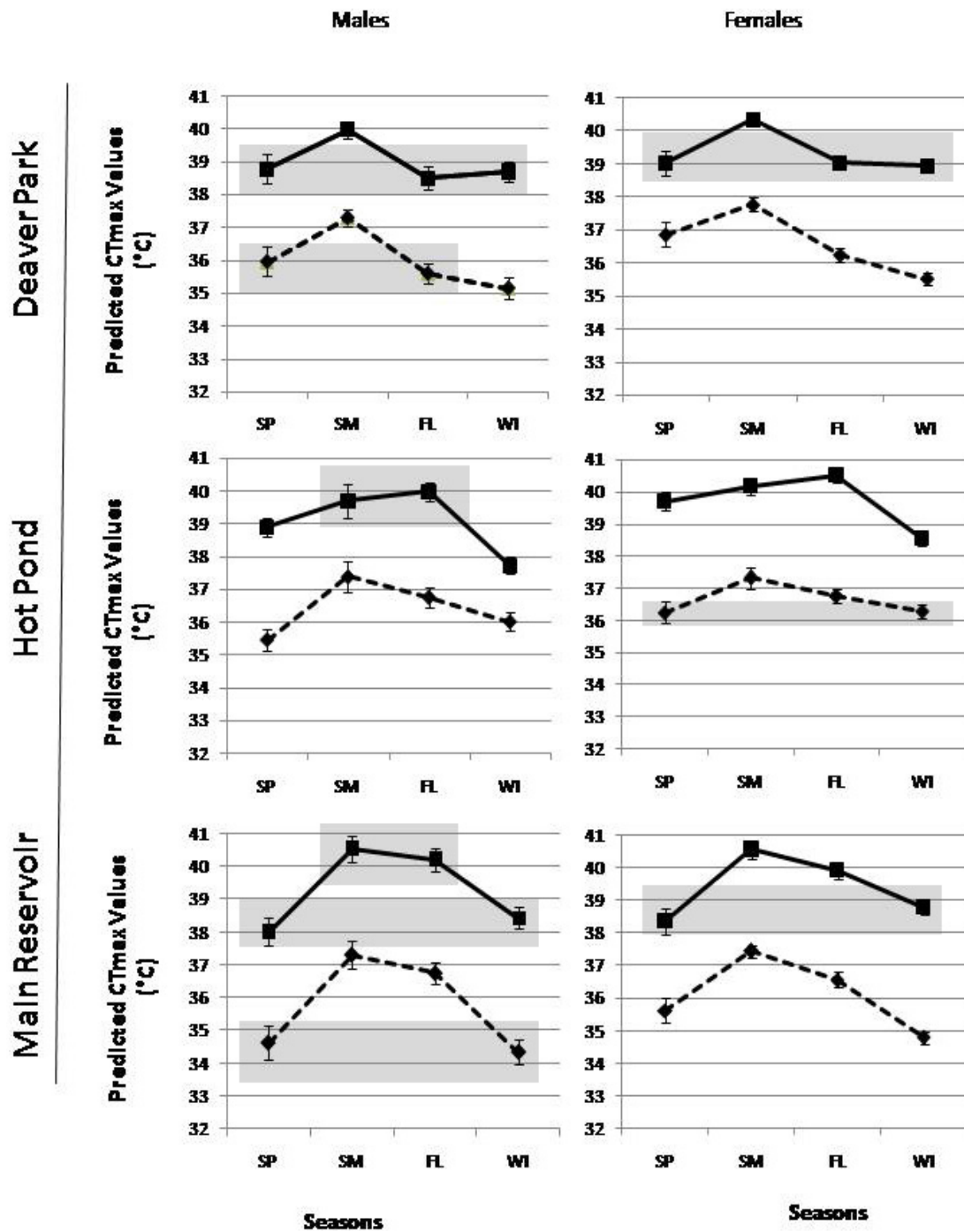


Figure 3.9 Seasonal differences in the critical thermal maximum [$\pm 2SE$] within populations for each gender. Solid lines represent the 24°C acclimation temperature and dashed lines represent the 14°C acclimation temperature. Shaded boxes indicate no statistical difference among the values contained therein.

3.3 Eurythermicity

The mean areas of the three temperature tolerance polygons differed slightly among the three populations (Fig. 3.10). Deaver Park exhibited the largest thermal area ($1393.17^{\circ}\text{C}^2$) followed by the main reservoir ($1321.89^{\circ}\text{C}^2$) and the hot pond ($1296.44^{\circ}\text{C}^2$).

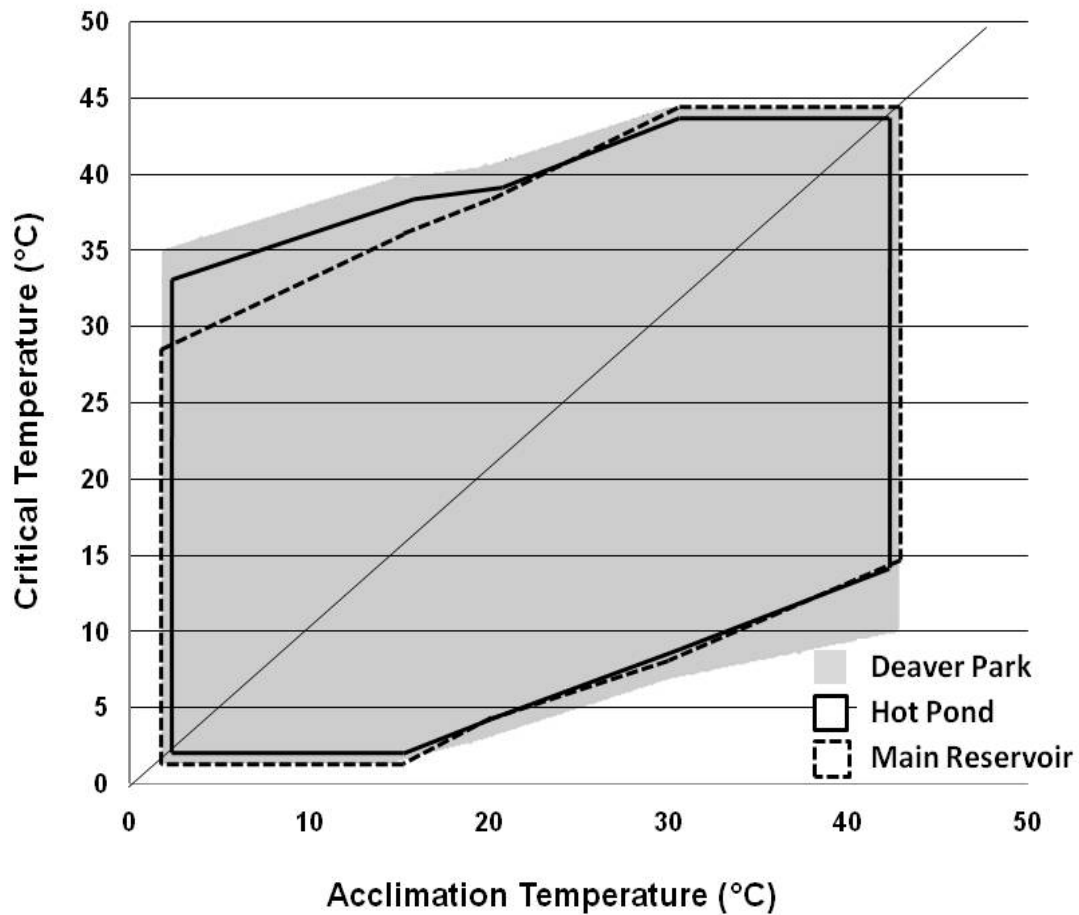


Figure 3.10 Temperature tolerance polygon comparisons among populations. The Deaver Park population exhibited the largest area of thermal tolerance ($1393.17^{\circ}\text{C}^2$), followed by the main-reservoir population ($1321.89^{\circ}\text{C}^2$), and finally the hot-pond population ($1296.44^{\circ}\text{C}^2$).

The method of comparison produced relatively wide confidence intervals for the area of each polygon. There was no difference noted between the main-reservoir and Deaver Park temperature tolerance polygons, as well as the hot-pond and main-reservoir temperature tolerance polygons. However, the hot-pond temperature tolerance polygon exhibited an overlap of less than 2°C^2 with the Deaver Park temperature tolerance polygon (Hot Pond [$1207.01^{\circ}\text{C}^2$, $1346.06^{\circ}\text{C}^2$]; Main Reservoir [$1274.39^{\circ}\text{C}^2$, $1364.64^{\circ}\text{C}^2$]; Deaver Park [$1345.00^{\circ}\text{C}^2$, $1442.08^{\circ}\text{C}^2$]) (Fig. 3.11). Perhaps if a more rigorous statistical test were devised to detect statistically significant differences in thermal polygon areas, these marginal differences could be better interpreted. The mean hot pond polygon is nested with the mean main reservoir and Deaver Park polygon with some deviation exhibited at the maximum range of the lower acclimation temperatures.

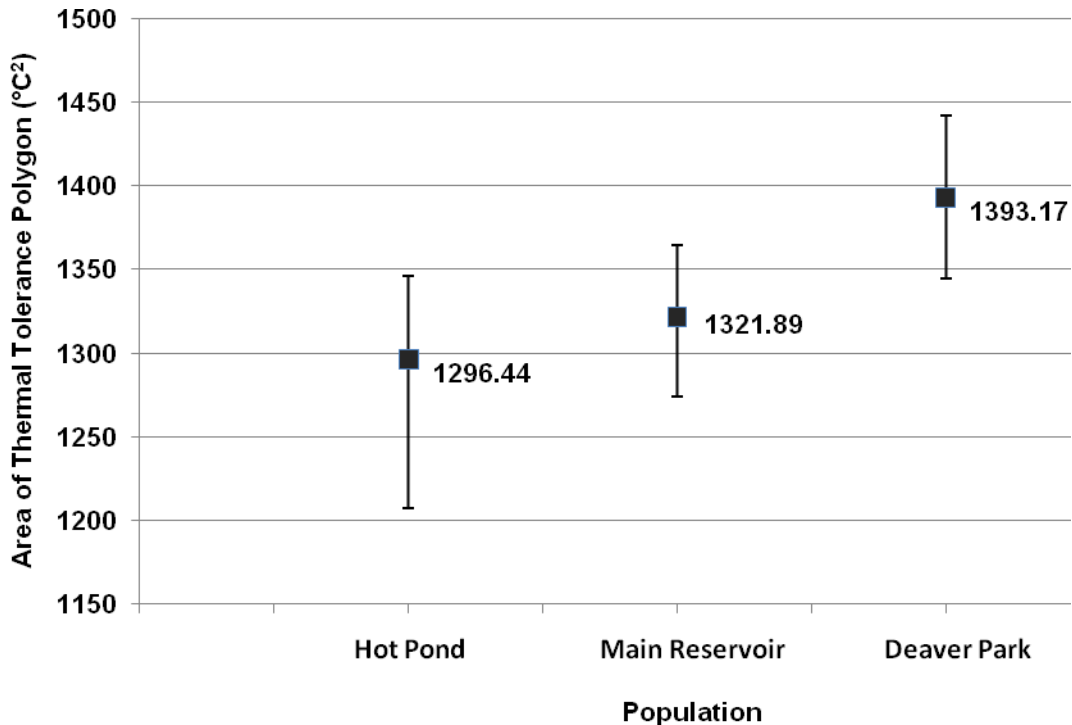


Figure 3.11 Quantitative comparison of temperature tolerance polygons for the hot-pond, main-reservoir, and Deaver Park populations.

CHAPTER 4

DISCUSSION

It would stand to reason that, when exposed to an elevated thermal regime, those individuals within that population who are physiologically more adept at those temperatures would experience higher fitness and thus produce offspring with a higher thermal tolerance, eventually resulting in an overall increase in thermal tolerance within that population. This general idea was the basis for the original hypothesis of directional selection acting upon the hot-pond mosquitofish population found at Lake Fairfield. If this occurred, then the hot-pond population, having been separated from the main-reservoir population and exposed to thermal effluents, should exhibit a higher thermal tolerance that increased sequentially with successive generations. Likewise, it was hypothesized that an increased upper thermal tolerance limit would result in a corresponding increased lower thermal tolerance limit. Such a consistent general pattern was not found in this study. The previous findings of Dean (1981) and Britton (2005) that did support such a hypothesis were only consistent within the context of the single spring season in which they were collected. Males and females often reacted to this thermal regime in very different ways and the broad range of tolerance exhibited by all populations included in this study could be explained by an overall broad thermal niche and high degree of plasticity exhibited by *Gambusia affinis* as a species.

4.1 Thermal Tolerance

Overall, exposure to thermal effluents decreased the degree of cold tolerance, but an increase in heat tolerance was not consistently found. This was not consistent with the findings of Dean (1981) and Britton (2005). Taking all data into account, the hot-pond population was

only found to be more heat tolerant than the main-reservoir population at the lower 14°C acclimation temperature. Moreover, the present study demonstrated that CT_{Max} was variable among seasons. Dean (1981) collected specimens in March, while Britton (2005) collected specimens in April. When looking only at the current spring-collected CT_{Max} values between the hot-pond population and the main-reservoir population, spring was different in that it exhibited trends consistent with that of Dean (1981) and Britton (2005) between genders and acclimation temperatures. This differed from the other seasons, which showed different relationships between gender and acclimation temperatures. Since Dean (1981), Britton (2005), and the present study show a difference in CT_{Max} between the hot-pond population and the main-reservoir population in the spring season which the mosquitofish were collected, it is assumed that the difference has been consistent over the years, and the hot-pond mosquitofish population will continue to be more heat tolerant than the main-reservoir mosquitofish population. However, if the fish were collected in any other season for all three studies, different conclusions may have been reached.

The hypothesized shifts, or correlated increase in CT_{Max} and increase in CT_{Min} , were not consistently found in the hot-pond population compared to the main-reservoir population across genders, acclimation temperatures, and seasons (Fig. 3.2 and Fig. 3.3). Therefore, there was little indication that CT_{Max} and CT_{Min} dependently co-vary among these two populations.

It has been shown that marine invertebrates that inhabit waters near their upper thermal limits exhibited a lower capacity to expand their heat tolerance through acclimation (Somero 2005). If this phenomenon is occurring within the hot-pond mosquitofish, then an increase in upper thermal tolerance associated with acclimation exhibited by the main-reservoir population would be absent or muted in the hot-pond population. The same would not be true for the lower thermal limits since these populations are not routinely subjected to long term exposure at or near their lower thermal limits. This explanation is supported by the fact that the hot-pond population exhibited a significantly higher CT_{Min} yet did not significantly differ in CT_{Max} .

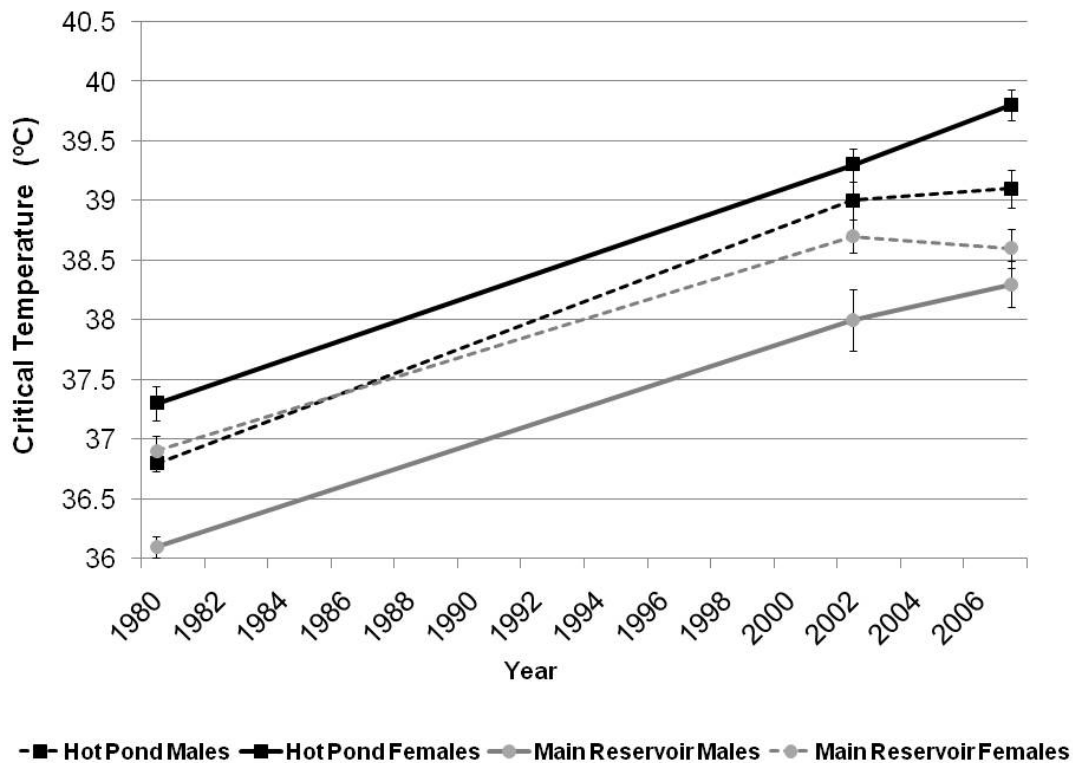


Figure 4.1 Critical thermal maximum [$\pm 1SE$] recorded over a 27 year period for hot-pond females, hot-pond males, main-reservoir females, and main-reservoir males found at Lake Fairfield, Texas. Years represent actual year of collection and not publication dates. The first CT_{Max} collection and study was in 1980 by Dean (1981), followed by Britton (2005) in 2002, and the present study in 2007.

By taking into account the lack of a significant consistent increase in the thermal tolerance of the hot-pond population, the data from the present study was unable to support that directional selection in thermal tolerance has taken place among the mosquitofish population found in the hot pond at Lake Fairfield. This appears to be in conflict with the findings of Britton (2005). However, Britton (2005) based many conclusions upon comparisons with Dean (1981). Britton (2005) was able to show an increase in CT_{Max} since Dean's (1981) observations, yet no overall increase in CT_{Max} was detected in the five year interval between Britton's (2005) and the present study (Fig. 4.1). The similarities in the CT_{Max} values between the present study and Britton's (2005) study are likely a result of greater consistency with methodology in determining

CT_{Max}. Therefore, the observed increase in CT_{Max} recorded between Dean's (1981) and Britton's (2005) study may have been due to differences in methodologies, as recognized by Britton (2005). Inconsistencies in methodology involved differences in equipment, precision of instruments, and statistical analysis. Dean (1981) used a hot plate to increase temperature as opposed to a circulating heater used in Britton's (2005) and the present study. Dean (1981) also used a mercury thermometer accurate to 0.1°C, whereas a digital thermocoupler accurate to 0.01°C was used in Britton's (2005) and the present study. When testing statistical significance, Dean used $\alpha = 0.1$ for the primary comparison, whereas $\alpha = 0.05$ was used by Britton (2005) and the present study. Furthermore, Dean (1981) ran trials with two fish, one from each population, at the same time in the same beaker. Britton (2005) and the present study used small groups of fish from the same population in each trial. Due to the similarity of methodology used between Britton (2005) and the present study, comparisons between the findings of these two studies should be considered more reliable.

In summary, the thermal tolerance data from this study did not support directional thermal selection in the hot-pond mosquitofish population in the five-year period between this study and that of Britton (2005). Although these findings are not consistent with those of Dean (1981) and Britton (2005), they offer findings more suitable for comparison to Britton (2005). However, additional tests, such as heritability and genetic differences, and determination of upper thermal tolerance limits over a period extended beyond five years must be performed in order to make a more solid conclusion.

4.2 Seasonal Effects

This study demonstrated that the values of CT_{Max} and CT_{Min} in all three *G. affinis* populations studied were not consistent across seasons. Results of the pilot study indicated that the relationships between population and CT_{Max} and CT_{Min} may not be consistent. This exhaustive study confirmed that seasonal acclimatization is an important consideration when comparing the critical thermal limits of *G. affinis*. While the findings of this study did show that

the relationships among populations vary across season with respect to critical thermal limits, there was a generally consistent relationship within seasons. While comparisons of each population and gender within seasons did not bear statistical significance, general trends in thermal tolerance were observed.

Perhaps an explanation for this discrepancy is that the thermal tolerance of the mosquitofish populations may have been heavily influenced by seasonal acclimatization and only secondarily affected by artificially elevated temperatures. If this is the case, then the prediction that seasonal comparisons will reveal trends in thermal tolerance that is generally consistent within seasons was supported by these findings. *Heteropneustes fissilis* has shown differences in both upper and lower lethal temperatures between winter-collected and summer-collected fish (Vasal and Sundararaj 2005). The lower lethal temperature for this species was reported to be 4°C for winter-collected fish and 7.9°C for summer-collected fish, along with the upper lethal temperatures of 37.7°C for winter-collected fish and 39.8°C for summer-collected fish (Vasal and Sundararaj 2005). Hart (1952) also found differences attributed to seasonal acclimatization. In the majority of fish that he studied, he found that upper thermal limits were greater in summer-collected fish compared to winter-collected fish.

Britton (2005) demonstrated that thermal history can have a considerable impact on adult thermal tolerance in *G. affinis*. It was found that mosquitofish born at 26°C or 32°C and raised at cooler temperatures (20°C) exhibited a higher thermal tolerance than those born and raised at 20°C. This could explain why, in some seasons, the main-reservoir population exhibited a higher thermal tolerance than the hot-pond population. Gene flow from the hot pond to the main reservoir in Lake Fairfield is possible. Therefore, it is possible for fish to be born in the hot pond at warmer temperatures and migrate downstream to the main reservoir, where they would be raised in cooler temperatures. Ferens and Murphy (1974) and Bennett and Goodyear (1978) both have shown that in other effluent-affected populations, female *Gambusia holbrooki* reproduce year round, whereas females found in the ambient population reproduced

mainly in the summer. They also found a higher percentage of reproductively active females in warmer water. If the same is true for *G. affinis* at Lake Fairfield, and migration is taking place, then the hot-pond population could be contributing a larger proportion of offspring to the main reservoir than previously believed. These offspring, having experienced an effluent-affected environment early in development may retain an increased thermal tolerance, resulting in homogenization of critical thermal limits among the main-reservoir *G. affinis* population.

Inconsistency across seasons in CT_{Max} and CT_{Min} must be taken into consideration when comparing these values with ones reported in previously published literature. Species must be taken into consideration as well. Hart (1952) found that the upper lethal limits of six species of fish were different between winter and summer collections. However, he also found one species that exhibited the same upper lethal limits between winter and summer collections. Another study where upper thermal tolerance limits in certain species seem to be unaffected by seasonal acclimatization is Ingersoll and Claussen (1984) who looked at two darter species (*Etheostoma flabellare*, the fantail darter, and *E. nigrum*, the johnny darter). These species showed no significant differences in CT_{Max} between summer and winter. Therefore, it cannot be assumed that a species would or would not exhibit consistent thermal tolerance limits across seasons unless indicated by previous trials. Also, more importantly, it cannot be assumed that the thermal tolerance limits recorded in one season represents the actual absolute thermal limits of the species.

4.3 Eurythermicity

Based on statistical tests extrapolated from individual point confidence intervals, the Deaver Park temperature tolerance polygon was the largest polygon ($1393.17^{\circ}C^2$), followed by the main-reservoir population ($1321.89^{\circ}C^2$), with the hot-pond population exhibiting the smallest area ($1296.44^{\circ}C^2$). One explanation for these differences in thermal tolerance areas is that Deaver Park is a shallow pond that experiences extremes and more rapid temperature fluctuations, therefore mosquitofish found in this pond may be hardened to both higher and

lower temperatures, functionally expanding the area of their temperature tolerance polygon. The hot pond and main reservoir at Lake Fairfield has shown to be a relatively stenothermic environment, where the mosquitofish are not subject to temperature extremes. Therefore, the temperature tolerance polygon for the hot pond and the main reservoir exhibited similar areas, both of which resulted in areas smaller than Deaver Park. However, since it was found that CT_{Max} was inconsistent between the hot-pond and the main-reservoir populations, but CT_{Min} was not, this could account for the slightly higher area of the main-reservoir population over the hot-pond population.

When comparing the CT_{Max} values of the hot-pond population with the main-reservoir population at the 20°C and 30°C acclimation temperatures, it appears that a rotational shift is occurring. At the 20°C acclimation temperature, the hot-pond population's CT_{Max} is slightly higher than the main-reservoir population. However, at the higher acclimation temperature of 30°C, the opposite is true- the main-reservoir population's CT_{Max} is higher than the hot-pond population. Therefore, this may be a further implication that the hot-pond population may be reaching a ceiling in their ability to expand heat tolerance through acclimation.

Regardless of these inter-population differences, *Gambusia affinis*'s temperature tolerance polygon, as a whole, is comparable to those of both *Carassius auratus* (goldfish, 1429°C²) and *Cyprinodon variegatus* (sheepshead minnow, 1470°C²), which are both considered to be a highly eurythermic species (Bennett and Beitinger 1997; Ford and Beitinger 2005). Both *C. auratus* and *G. affinis* exhibit a broad latitudinal distribution (Page and Burr 1991). Native to Asia, *C. auratus* extends from Florida well into Canada and across the United States. It, along with *G. affinis*, is implicated as an invasive species (Invasive Species Specialist Group 2004). As discussed in the introduction, this broad thermal niche may be a factor contributing to the invasiveness of both *G. affinis* and *C. auratus*.

The primary limitation inherent in establishing the temperature tolerance polygons is that no statistical evaluation is currently available. Therefore, comparisons between populations

and among other organisms are simply qualitative. The method used in the current study of constructing maximum and minimum polygons from confidence intervals lends itself to an increased probability of type II error. Once a more powerful means of statistically analyzing temperature tolerance polygons is devised, such rigorous comparisons may make them an important tool for examining thermal tolerance both inter-specifically and intra-specifically. Furthermore, as shown earlier, seasonal acclimatization affects critical thermal limits in *G. affinis*, and would thereby change the area of each polygon among seasons. In order to draw complete conclusions regarding these populations' comparative eurythermicity, seasons must be taken into account.

4.4 General Conclusions

In conclusion, there were three primary findings of this study. The first was that *Gambusia affinis*, as a species, exhibits a relatively high degree of eurythermicity, as evidenced by the large areas of temperature tolerance polygons of all three tested populations. Secondly, seasonal acclimatization plays an important role in determining the critical thermal limits of this species in particular. Thirdly, although a general trend has been previously documented (Dean 1981; Britton 2005), there was no compelling evidence that would indicate that the upper thermal tolerance limits have changed for both sexes in the thermal-effluent exposed population in the five years that elapsed since Britton's (2005) previous study.

Future studies regarding the effects of seasonal acclimatization on eurythermicity in *G. affinis* and other fish species may reveal patterns of change across seasons. Also, repetition of this study at a future date allowing a greater time interval than the five years separating this study and that of Britton (2005) may allow detection of directional selection in the thermal tolerance limits of the hot pond population that could not be detected by this study. The lower thermal tolerance limits of *G. affinis* recorded in this study have been largely ignored in thermal tolerance determination for this species. The results of this study revealed interesting patterns of seasonal variation in the lower thermal tolerance limits of *G. affinis* which appeared to be

independent of its upper tolerance limits. It also demonstrated that the lower thermal tolerance limits of the thermally influenced hot-pond population were elevated relative to both the main reservoir and the Deaver Park pond populations. These results suggest that further experimental attention to the lower thermal tolerance limits of *G. affinis* and other fish species is warranted particularly in regard to its relation to upper thermal tolerance limits relative to the impacts of seasonal acclimatization and exposure to thermally influenced environments.

APPENDIX A

ESTIMATES AND STATISTICS OF REGRESSION COEFFICIENTS

Table A1. Estimates and statistics for lower thermal tolerance of the regression coefficients.

Parameter	Estimate	Error	t Value	Pr > t
Intercept	5.8073	0.1777	32.69	<.0001
length	13.2274	4.9296	2.68	0.007
sexcode	-1.1015	0.1615	-6.82	<.0001
popcode1	0.4253	0.1931	2.20	0.028
popcode2	0.1051	0.2098	0.50	0.616
seacode1	0.9440	0.2308	4.09	<.0001
seacode2	1.0853	0.2334	4.65	<.0001
seacode3	-1.3446	0.1912	-7.03	<.0001
acccode	-3.4761	0.1831	-18.99	<.0001
pop1sea1	0.7036	0.2172	3.24	0.001
pop2sea1	0.1001	0.2779	0.36	0.719
pop1sea2	-2.8139	0.2192	-12.84	<.0001
pop2sea2	-0.2736	0.2447	-1.12	0.264
pop1sea3	0.9983	0.2490	4.01	<.0001
pop2sea3	0.9088	0.1771	5.13	<.0001
pop1acccode	-1.0260	0.1411	-7.27	<.0001
pop2acccode	0.2913	0.2314	1.26	0.208
pop1sexcode	0.2208	0.1575	1.40	0.161
pop2sexcode	0.4167	0.1930	2.16	0.031
sea1acccode	1.0723	0.2223	4.82	<.0001
sea2acccode	-0.4375	0.2946	-1.49	0.138
sea3acccode	0.6078	0.1347	4.51	<.0001
sea2sex	0.4658	0.2060	2.26	0.024
sea3sex	0.6193	0.1621	3.82	0.000
acccodesex	0.5178	0.1564	3.31	0.001
pop1length	35.553	5.2906	6.72	<.0001
pop2length	22.628	5.5128	4.10	<.0001
sea1length	18.333	5.2435	3.50	0.001
sea2length	7.4672	5.9392	1.26	0.209
sea3length	9.3905	4.3584	2.15	0.031
accodelength	21.857	4.6943	4.66	<.0001
pop1sea2acccode	3.0732	0.2826	10.87	<.0001
pop2sea1acccode	-0.4149	0.2937	-1.41	0.158
pop2sea2acccode	1.6898	0.3179	5.32	<.0001
pop1sea3sex	-0.6870	0.2562	-2.68	0.007
pop2accodesex	-0.4692	0.2306	-2.04	0.042
acccodesea2sex	-0.3428	0.2534	-1.35	0.176
pop1sea2length	-25.818	6.9484	-3.72	0.000
pop1accodelength	-36.279	5.4369	-6.67	<.0001
pop2sea1length	-10.940	6.4599	-1.69	0.091
pop2accodelength	-25.615	6.4810	-3.95	<.0001
sea2accodelength	22.848	6.3638	3.59	0.000

Table A2. Estimates and statistics for upper thermal tolerance of regression coefficients

Parameter	Estimate	Error	t-Value	Pr > t
Intercept	38.4450	0.1723	223.06	<.0001
length	-9.8674	7.1097	-1.39	0.165
sexcode	0.3580	0.1650	2.17	0.030
popcode1	0.2561	0.2091	1.22	0.221
popcode2	-0.7007	0.2107	-3.33	0.001
seacode1	-0.4335	0.2233	-1.94	0.052
seacode2	2.1060	0.2383	8.84	<.0001
seacode3	1.7752	0.2323	7.64	<.0001
acccode	-4.1155	0.1645	-25.02	<.0001
pop1sea1	0.5124	0.2137	2.40	0.017
pop2sea1	1.5848	0.2694	5.88	<.0001
pop1sea2	-0.8268	0.2800	-2.95	0.003
pop2sea2	-0.1504	0.2259	-0.67	0.506
pop1sea3	-1.9714	0.2964	-6.65	<.0001
pop2sea3	0.4598	0.2821	1.63	0.103
pop1acccode	0.5617	0.1113	5.05	<.0001
pop2acccode	2.3792	0.2363	10.07	<.0001
pop1sexcode	-0.1054	0.2064	-0.51	0.610
pop2sexcode	0.4544	0.2035	2.23	0.026
sea1acccode	0.7294	0.2551	2.86	0.004
sea2acccode	0.8672	0.1478	5.87	<.0001
sea3acccode	0.6451	0.1476	4.37	<.0001
sea2sex	-0.3394	0.2219	-1.53	0.126
sea3sex	-0.6495	0.2410	-2.70	0.007
accodesex	0.1140	0.1335	0.85	0.393
sexlength	3.2389	6.0895	0.53	0.595
pop1length	16.055	9.6700	1.66	0.097
pop2length	-19.410	6.8863	-2.82	0.005
sea1length	-14.893	5.5680	-2.67	0.008
sea2length	-23.471	5.6072	-4.19	<.0001
sea3length	-17.407	4.7803	-3.64	0.000
accodelength	-19.939	7.0292	-2.84	0.005
pop2sea1acccode	-2.4383	0.2684	-9.08	<.0001
pop2sea2acccode	-1.4396	0.2647	-5.44	<.0001
pop2sea3acccode	-2.1271	0.2410	-8.82	<.0001
pop1sea2sex	0.4492	0.2933	1.53	0.126
pop1sea3sex	0.9246	0.3277	2.82	0.005
pop2sea3sex	0.3820	0.2846	1.34	0.180
pop2accodesex	-0.6516	0.2071	-3.15	0.002
accodesea1sex	0.5333	0.2255	2.36	0.018
sexpop1length	-21.975	8.9759	-2.45	0.015
sexaccodelength	16.365	7.1838	2.28	0.023
pop1sea2length	15.635	6.5092	2.40	0.016
pop1accodelength	15.829	4.3442	3.64	0.000

pop2sea1length	30.068	8.6828	3.46	0.001
pop2sea2length	23.992	8.8391	2.71	0.007
pop2sea3length	30.456	8.8799	3.43	0.001

APPENDIX B

RESULTS OF HYPOTHESIS TESTS APPLIED TO CT_{MAX} AND CT_{MIN} MODELS

Table B1. Estimates and statistics for differences in lower thermal tolerance of treatment populations at each acclimation temperature for each season

Parameter	Estimate	Standard Error	t	Pr > t
DP/FL/24/F - HP/FL/24/F	-0.4733	0.1693	-2.80	0.005
DP/FL/24/F - MR/FL/24/F	0.9573	0.1587	6.03	<0.001
HP/FL/24/F - MR/FL/24/F	1.4306	0.1616	8.85	<0.001
DP/FL/14/F - HP/FL/14/F	-1.3214	0.1601	-8.26	<0.001
DP/FL/14/F - MR/FL/14/F	-0.0686	0.1683	-0.41	0.683
HP/FL/14/F - MR/FL/14/F	1.2528	0.1685	7.44	<0.001
DP/SM/24/F - HP/SM/24/F	-2.4161	0.2062	-11.72	<0.001
DP/SM/24/F - MR/SM/24/F	-2.1678	0.1749	-12.39	<0.001
HP/SM/24/F - MR/SM/24/F	0.2483	0.1996	1.24	0.214
DP/SM/14/F - HP/SM/14/F	-1.8808	0.2292	-8.21	<0.001
DP/SM/14/F - MR/SM/14/F	-0.1206	0.1853	-0.65	0.515
HP/SM/14/F - MR/SM/14/F	1.7602	0.2106	8.36	<0.001
DP/SP/24/F - HP/SP/24/F	0.7277	0.2457	2.96	0.003
DP/SP/24/F - MR/SP/24/F	1.3497	0.1926	7.01	<0.001
HP/SP/24/F - MR/SP/24/F	0.6220	0.2434	2.56	0.011
DP/SP/14/F - HP/SP/14/F	0.2945	0.2295	1.28	0.200
DP/SP/14/F - MR/SP/14/F	0.3237	0.1843	1.76	0.079
HP/SP/14/F - MR/SP/14/F	0.0291	0.2014	0.14	0.885
DP/WI/24/F - HP/WI/24/F	0.1242	0.1508	0.82	0.410
DP/WI/24/F - MR/WI/24/F	0.6461	0.1387	4.66	<0.001
DP/WI/24/F - HP/WI/24/F	0.5219	0.1516	3.44	0.001
DP/WI/14/F - HP/WI/14/F	-0.7239	0.1506	-4.81	<0.001
DP/WI/14/F - MR/WI/14/F	-0.3799	0.1408	-2.70	0.007
HP/WI/14/F - MR/WI/14/F	0.3440	0.1522	2.26	0.024
DP/FL/24/M - HP/FL/24/M	0.4097	0.2285	1.79	0.073
DP/FL/24/M - MR/FL/24/M	1.4236	0.2071	6.87	<0.001
HP/FL/24/M - MR/FL/24/M	1.0139	0.2014	5.03	<0.001
DP/FL/14/M - HP/FL/14/M	-0.9076	0.2089	-4.34	<0.001
DP/FL/14/M - MR/FL/14/M	0.3977	0.2135	1.86	0.063
HP/FL/14/M - MR/FL/14/M	1.3052	0.1980	6.59	<0.001
DP/SM/24/M - HP/SM/24/M	-2.2201	0.2596	-8.55	<0.001
DP/SM/24/M - MR/SM/24/M	-2.3886	0.2084	-11.46	<0.001
HP/SM/24/M - MR/SM/24/M	-0.1685	0.2570	-0.66	0.512
DP/SM/14/M - HP/SM/14/M	-2.1540	0.2483	-8.68	<0.001
DP/SM/14/M - MR/SM/14/M	-0.3414	0.2032	-1.68	0.093

HP/SM/14/M - MR/SM/14/M	1.8126	0.2381	7.61	<0.001
DP/SP/24/M - HP/SP/24/M	0.9237	0.2715	3.40	0.001
DP/SP/24/M - MR/SP/24/M	1.1289	0.2173	5.20	<0.001
HP/SP/24/M - MR/SP/24/M	0.2052	0.2666	0.77	0.442
DP/SP/14/M - HP/SP/14/M	0.0213	0.2268	0.09	0.925
DP/SP/14/M - MR/SP/14/M	0.1029	0.2040	0.50	0.614
HP/SP/14/M - MR/SP/14/M	0.0816	0.1984	0.41	0.681
DP/WI/24/M - HP/WI/24/M	0.3202	0.2029	1.58	0.115
DP/WI/24/M - MR/WI/24/M	0.4253	0.1931	2.20	0.028
HP/WI/24/M - MR/WI/24/M	0.1051	0.2098	0.50	0.616
DP/WI/14/M - HP/WI/14/M	-0.9971	0.1875	-5.32	<0.001
DP/WI/14/M - MR/WI/14/M	-0.6006	0.1882	-3.19	0.001
HP/WI/14/M - MR/WI/14/M	0.3964	0.1944	2.04	0.042

HP = Hot Pond Population, MR = Main Reservoir Population, DP = Deaver Park Population

SP = Spring, SM = Summer, FL = Fall, WI = Winter

14 = 14°C Acclimation, 24 = 24°C Acclimation

Table B2. Estimates and statistics for differences in upper thermal tolerance of treatment populations at each acclimation temperature for each season

Parameter	Estimate	Error	t	Pr > t
DP/FL/24/F - HP/FL/24/F	-1.4915	0.1563	-9.55	<0.001
DP/FL/24/F - MR/FL/24/F	-0.8960	0.1414	-6.34	<0.001
HP/FL/24/F - MR/FL/24/F	0.5955	0.1674	3.56	0.000
DP/FL/14/F - HP/FL/14/F	-0.5304	0.1540	-3.44	0.001
DP/FL/14/F - MR/FL/14/F	-0.3344	0.1432	-2.33	0.020
HP/FL/14/F - MR/FL/14/F	0.1960	0.1675	1.17	0.242
DP/SM/24/F - HP/SM/24/F	0.1698	0.1665	1.02	0.308
DP/SM/24/F - MR/SM/24/F	-0.2269	0.1460	-1.55	0.120
HP/SM/24/F - MR/SM/24/F	-0.3967	0.1843	-2.15	0.032
DP/SM/14/F - HP/SM/14/F	0.4435	0.1906	2.33	0.020
DP/SM/14/F - MR/SM/14/F	0.3348	0.1309	2.56	0.011
HP/SM/14/F - MR/SM/14/F	-0.1087	0.1877	-0.58	0.563
DP/SP/24/F - HP/SP/24/F	-0.6753	0.2340	-2.89	0.004
DP/SP/24/F - MR/SP/24/F	0.6632	0.1883	3.52	0.000
HP/SP/24/F - MR/SP/24/F	1.3384	0.2409	5.56	<0.001
DP/SP/14/F - HP/SP/14/F	0.5971	0.2291	2.61	0.009
DP/SP/14/F - MR/SP/14/F	1.2248	0.1830	6.69	<0.001
HP/SP/14/F - MR/SP/14/F	0.6277	0.2320	2.71	0.007
DP/WI/24/F - HP/WI/24/F	0.3971	0.1420	2.80	0.005
DP/WI/24/F - MR/WI/24/F	0.1507	0.1242	1.21	0.225
DP/WI/24/F - HP/WI/24/F	-0.2463	0.1536	-1.60	0.109
DP/WI/14/F - HP/WI/14/F	-0.7689	0.1377	-5.58	<0.001
DP/WI/14/F - MR/WI/14/F	0.7124	0.1183	6.02	<0.001
HP/WI/14/F - MR/WI/14/F	1.4813	0.1404	10.55	<0.001
DP/FL/24/M - HP/FL/24/M	-1.4744	0.2246	-6.57	<0.001
DP/FL/24/M - MR/FL/24/M	-1.7153	0.2213	-7.75	<0.001
HP/FL/24/M - MR/FL/24/M	-0.2409	0.2170	-1.11	0.267
DP/FL/14/M - HP/FL/14/M	-1.1648	0.2229	-5.23	<0.001
DP/FL/14/M - MR/FL/14/M	-1.1536	0.2231	-5.17	<0.001
HP/FL/14/M - MR/FL/14/M	0.0112	0.2193	0.05	0.959
DP/SM/24/M - HP/SM/24/M	0.2804	0.2873	0.98	0.329
DP/SM/24/M - MR/SM/24/M	-0.5707	0.2216	-2.58	0.010
HP/SM/24/M - MR/SM/24/M	-0.8511	0.2447	-3.48	0.001
DP/SM/14/M - HP/SM/14/M	-0.0975	0.2708	-0.36	0.719
DP/SM/14/M - MR/SM/14/M	-0.0090	0.2239	-0.04	0.968

HP/SM/14/M - MR/SM/14/M	0.0885	0.2421	0.37	0.715
DP/SP/24/M - HP/SP/24/M	-0.1155	0.2597	-0.44	0.657
DP/SP/24/M - MR/SP/24/M	0.7686	0.2429	3.16	0.002
HP/SP/24/M - MR/SP/24/M	0.8841	0.2541	3.48	0.001
DP/SP/14/M - HP/SP/14/M	0.5053	0.2456	2.06	0.040
DP/SP/14/M - MR/SP/14/M	1.3302	0.2453	5.42	<0.001
HP/SP/14/M - MR/SP/14/M	0.8249	0.2604	3.17	0.002
DP/WI/24/M - HP/WI/24/M	0.9568	0.2028	4.72	<0.001
DP/WI/24/M - MR/WI/24/M	0.2561	0.2091	1.22	0.221
HP/WI/24/M - MR/WI/24/M	-0.7007	0.2107	-3.33	0.001
DP/WI/14/M - HP/WI/14/M	-0.8607	0.2055	-4.19	<0.001
DP/WI/14/M - MR/WI/14/M	0.8178	0.2132	3.84	0.000
HP/WI/14/M - MR/WI/14/M	1.6785	0.2128	7.89	<0.001

HP = Hot Pond Population, MR = Main Reservoir Population, DP = Deaver Park Population

SP = Spring, SM = Summer, FL = Fall, WI = Winter

14 = 14°C Acclimation, 24 = 24°C Acclimation

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BIOGRAPHICAL INFORMATION

Rebbekah was born in Abilene, Texas on April 28, 1976 and moved to Youngsville, Louisiana shortly thereafter. She spent the next 10 years in Louisiana and finally moved to Texas. After graduating with honors from South Garland High School, she completed her Bachelor's degree in Biology at the University of Texas at Arlington in 2000. Rebbekah spent the next six years in the work field, working as a research assistant at various labs at the University of Texas Southwestern Medical Center for four years and teaching chemistry at local high schools for two years. It was during her short years of teaching that she discovered the importance of education and a passion to teach people, young and old, the beauty of science. She found herself back at the University of Texas at Arlington in July of 2006 to begin work on her Master's degree. Rebbekah plans to commit most of her time in the future to her family, raising her two sons. Rebbekah hopes to one day find a position in outdoor education or at a junior college which will allow her to spend time with the three things she holds most dear to her: family, science, and education.