A NANFA CONSERVATION RESEARCH GRANT REPORT

Life History Study and Population Structure of Vermilion Darter (*Etheostoma chermocki*) from Turkey Creek, Jefferson County, Alabama, and Warrior Darter (*E. bellator*) from Gurley Creek, Blount County, Alabama

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The Vermilion Darter (*Etheostoma chermocki* Boschung, Mayden & Tomelleri) is endemic to Turkey Creek in the Black Warrior River system in the Mobile Basin in Alabama (Fig. 1). Clabaugh et al. (1996) provided evidence using allozyme data to support the separation of *E. chermocki* from other Warrior snubnose darter species, which was consistent with morphological criteria (Boschung et al., 1992). Furthermore, this biochemical study revealed that *E. chermocki* may be closely related to the Warrior Darter (*E. bellator* Suttkus & Bailey), but their relationship was not clearly resolved. The study on historical ecology of *E. chermocki* and *E. bellator* in the Mobile Basin, Black Warrior River system offered evidence that supported the hypothesis that these two species are sister taxa (Blanco, 2001). This sister-species relationship was corroborated with DNA sequence data by Porter et al. (2002), specifically between *E. chermocki* and *E. bellator* from Gurley Creek, a system adjacent to Turkey Creek.

Etheostoma chermocki occurs as several potentially fragmented populations throughout 11.6 km of the upper mainstem reaches of Turkey Creek and the lower reaches of two tributaries in Jefferson County, Alabama (Blanco et al., 1995). This species primarily inhabits areas with moderate flows and cobble/gravel substrates. It also may be found in emergent and submerged aquatic vegetation, such as *Potamogeton*, *Ceratophyllum* and *Myriophyllum*. At present, the diet and reproductive biology of this species has not been adequately studied (Boschung and Mayden, 2004).

NANFA's Conservation Research Grant is made possible by the members of NANFA.

It has been suggested that anthropogenic activities threaten the health and viability of *E. chermocki* populations. Altered riparian vegetation, modified flow patterns, increased silt loading and other pollutants, and the loss of aquatic vegetation have interacted to decrease the amount of suitable habitat available to this species (Blanco, 2001; Blanco and Mayden, 1997). Sampling efforts documented a decline in catch-per-unit effort between 1995 and 1997 (Blanco et al. 1995, 1996; Blanco and Mayden, 1997). Due to the Vermilion Darter's restricted range and the threats to its habitat in the Turkey Creek system, this species was listed as endangered by the U.S. Fish and Wildlife Service in 2001 (FWS, 2001).

Restoration of *E. chermocki* populations will require a more thorough understanding of life history characteristics, especially aspects of reproduction. Because it was impractical to harvest additional specimens of this endangered species, museum-preserved specimens were used to determine *E. chermocki* life history characteristics including length-weight

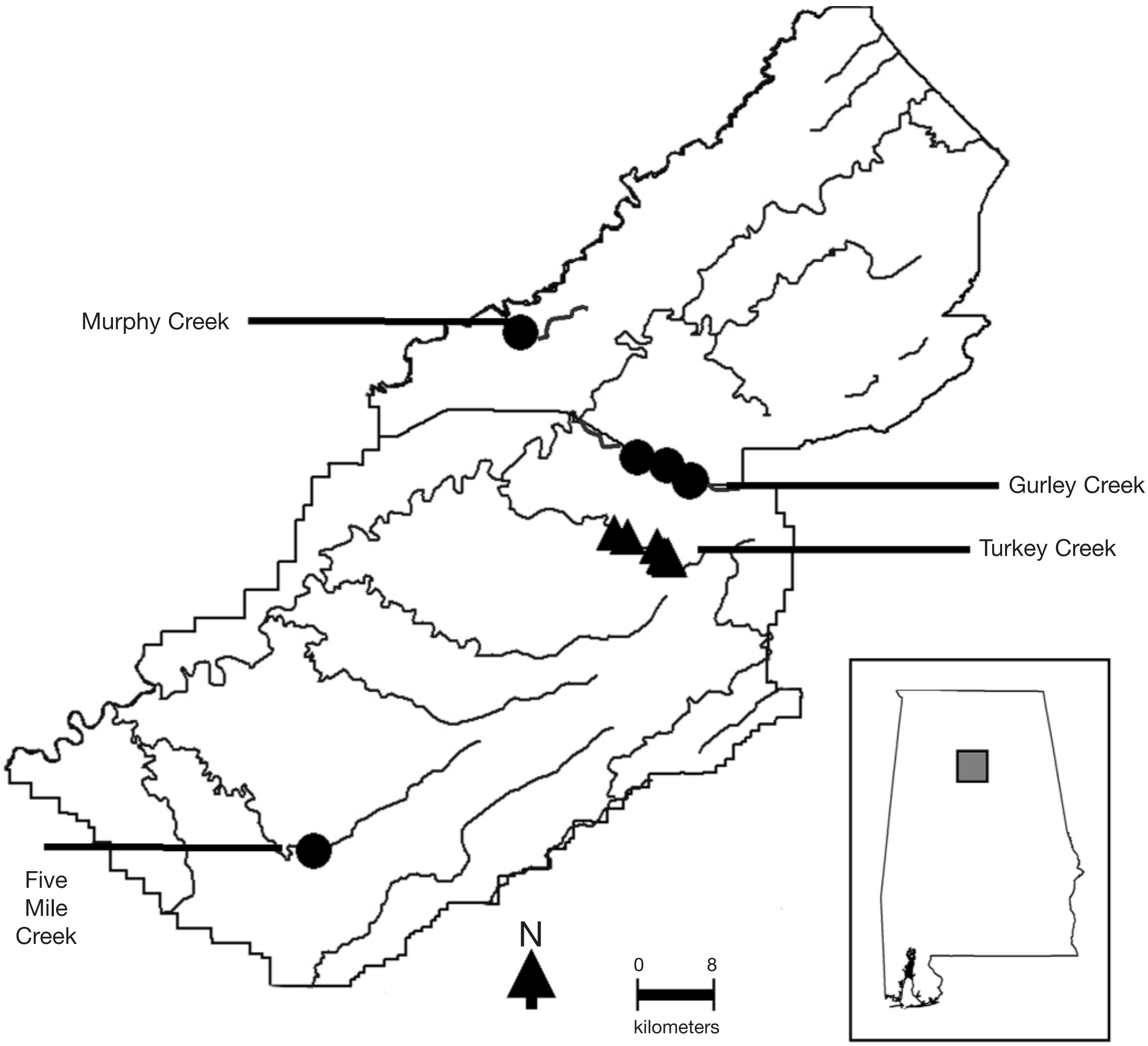

Fig. 1.
Distribution and sampling locations of *Etheostoma chermocki* and *E. bellator*.
▲= *E. chermocki* location. ● = *E. bellator* location.

relationship, population age structure and sex ratio, reproductive season, fecundity, and feeding habits.

Life History Characteristics of the Vermilion Darter (*E. chermocki*)

Collections of specimens obtained from October 1969 to January 2000 were evaluated to determine life history characteristics (Khudamrongsawat et al., 2005). Standard length was significantly correlated with body mass, gonad mass and clutch size. Sex ratio (2:1) was in favor of females. Length frequency distribution and enumeration of otolith annuli revealed four different age classes (0+ to 3+). Vermilion Darters matured at the end of the first year of life. Gonadosomatic index indicated reproduction occurred from March to June. Mean clutch size was 65 oocytes per female, and mean oocyte diameter was 1.14 mm. The Vermilion Darter is an opportunistic benthic invertivore, predominantly consuming larval chironomids, tipulids and hydropsychids. Diet breadth was greatest during warmer months and least during colder months.

Many questions remain regarding the ecology and life history of *E. chermocki*, especially those related to spawning habitats, minimum effective population size and movement dynamics. When investigations will likely result in mortality of individuals, research will be conducted on the sister species, *E. bellator*, in Gurley Creek, which represents an ideal surrogate for use in such studies (Blanco, 2001; Porter et al., 2002).

Life History Characteristics of the Warrior Darter (*E. bellator*)

The Warrior Darter (*Etheostoma bellator*) is endemic to the Black Warrior River drainage above the Fall Line in Alabama. *Etheostoma bellator* is a species complex, with a common and widespread nominal form and a population in Gurley Creek in the lower Locust Fork in Blount County and Jefferson County. Based on morphology and genetics, this population of Warrior Darter has been identified as a potential surrogate for the endangered Vermilion Darter (*E. chermocki*), which is restricted to Turkey Creek (adjacent to Gurley Creek). Fresh collections of Warrior Darters from November 2004 to June 2006 and museum collections were evaluated to determine life history characteristics (Khudamrongsawat and Kuhajda, 2007). *Etheostoma bellator* possesses several reproductive attributes—sex ratio, diet choices and selectivity, and age class structure—similar to *E. chermocki*.

This study indicates that life history characteristics are highly conserved among these two snubnose darter species regardless of differences in their abundance. Based on current information, *E. bellator* appears to be the ideal surrogate for *E. chermocki* because both species possess similar life history characteristics. When necessary characteristics of an endangered species are lacking and obtaining additional information may harm the imperiled species, a surrogate species can be studied and this information can then be directly applied back to the endangered species (Brooks et al., 1992a, 1992b). For instance, the length of the spawning season of *E. chermocki* is not known for certainty due to the lack of specimens for the entire year. By studying *E. bellator*, the length of the spawning season of *E. chermocki* can be estimated to extend into mid-June depending on water temperature.

Numerous other life history traits need to be examined to determine if there are potential differences between *E. chermocki* and *E. bellator*. This includes a true measurement of fecundity, which includes not only clutch size, but also the number of clutches laid by a female within a spawning season; darters are multiple clutch spawners (Page, 2000). But given the similarities in other life history traits between these two species, anthropogenic activities are likely the cause behind the decline of *E. chermocki* populations (Blanco, 2001; Blanco and Mayden, 1997) rather than large fecundity differences. Urbanization not only affects adult fishes, but also other life history stages including the development of embryos, availability of appropriate habitat for yolk-sac larvae, and presence of proper food and habitat for feeding larvae and juveniles. *Etheostoma bellator* can be used in laboratory and field studies to determine the requirements of all life stages, and these data can hopefully guide the management of *E. chermocki* and its habitat in Turkey Creek in the right direction.

Movement of snubnose darters is highly localized, so it is unlikely that individuals have the same ability as adults to migrate from one population to another (Paine, 1990; Page, 2000). For *E. chermocki*, the reestablishment of any extirpated population is even more difficult because there are no large or isolated source populations within its small range in Turkey Creek. Due to these limitations, there are also concerns regarding the genetic health of *E. chermocki*.

Analysis of Population Genetic Structure of the Endangered Vermilion Darter

Nine microsatellite DNA loci were used to examine genetic diversity and population structure of *E. chermocki* (Khudamrongsawat et al., 2007). Genetic variation was relatively high despite evidence of declining census size. Genetic differentiation among groups of *E. chermocki* was not significant as supported by low percent variation among groups and no significance of divergence among groups. It could be assumed that *E. chermocki* is composed of one single population, which corresponds to its distribution. Because high level of genetic diversity of this species is maintained by high gene flow within the species, fragmentation of a single large population can potentially decrease the level of genetic variation. Genetic bottlenecking was not observed in *E. chermocki*. It is possible that the effect of bottleneck may be gradual and could not be detected by current methods.

Estimates of an effective population size (N_e) were 1,174 individuals using the heterozygosity-based method and 393 individuals using the maximum-likelihood simulation. The N_e was relatively low but still reasonable considering the estimate population abundance of 1,847 and 3,238 individuals (Blanco and Mayden, 1999). The estimate of an ancestral population size of 10,645-11,485 individuals was fairly high for a population that only occurs in one stream. However, this estimate may not be unrealistic because high density of populations of darters in a small stream have been observed (Powers, 2003). A 2005 survey of *E. chermocki* found 13 individuals in a spring-fed stream and 15 individuals within approximately 50 m in an unnamed tributary of Beaver Creek, the latter representing an atypical habitat for *E. chermocki* in that it has a silty bottom and slow current (pers. obs.). It is possible that Turkey Creek may have been able to sustain as many as 10,000 individuals of *E. chermocki*. If this hypothesis was true, then the reduction of the population size would match the estimated effective population size, indicating substantial decline of the *E. chermocki* population.

Similarly, a high level of genetic variation was also observed in the surrogate species *E. bellator*. Unlike *E. chermocki*, populations of *E. bellator* appear to be fragmented as supported by significant genetic differentiation among populations of this species. This implies that *E. bellator* may not exist as a single population but as at least three different populations, which is consistent with its geographic distribution in three tributaries: Gurley Creek, Murphy Creek and Five Mile Creek. Furthermore, evidence of bottleneck was detected in *E. bellator*. Because they were originally considered the same species, the populations should have been connected to one another, although evidence of this is lacking. The development of metropolitan Birmingham and Bessemer may have extirpated some of the populations resulting in isolation of *E. bellator* populations. The effective population size (N_e) calculated from the heterozygosity-based method was comparable to the estimate using maximum-likelihood method, but much lower than the ancestral population size coinciding with the evidence of bottleneck. Although current populations of *E. bellator* are abundant (pers. obs.), they may have been much larger and less isolated.

According to Porter et al. (2002), mitochondrial DNA indicated a close relationship between *E. chermocki* and *E. bellator* from Gurley Creek. Then, by excluding populations from Murphy Creek and Five Mile Creek, *E. bellator* from Gurley Creek displayed similar genetic structure to *E. chermocki* by exhibiting high genetic variation and gene flow throughout the system. Thus it may be assumed that the population of *E. bellator* in Gurley Creek exists as one single population. Although there are some barriers within the stream, these barriers do not appear to interrupt movement of individuals.

Although populations of both species were undergoing great reductions in census size based on the estimate of the

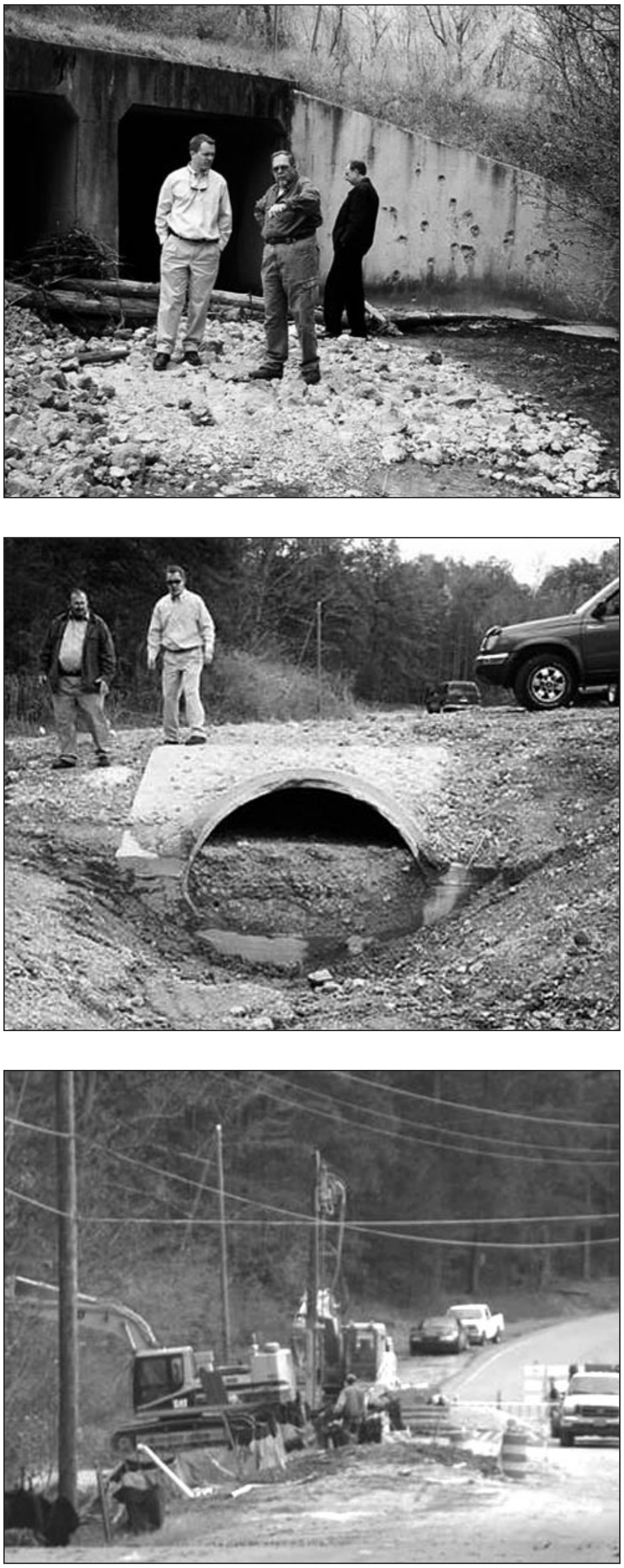

Fig. 2.
Turkey Creek, Jefferson County, Alabama, in 2004.

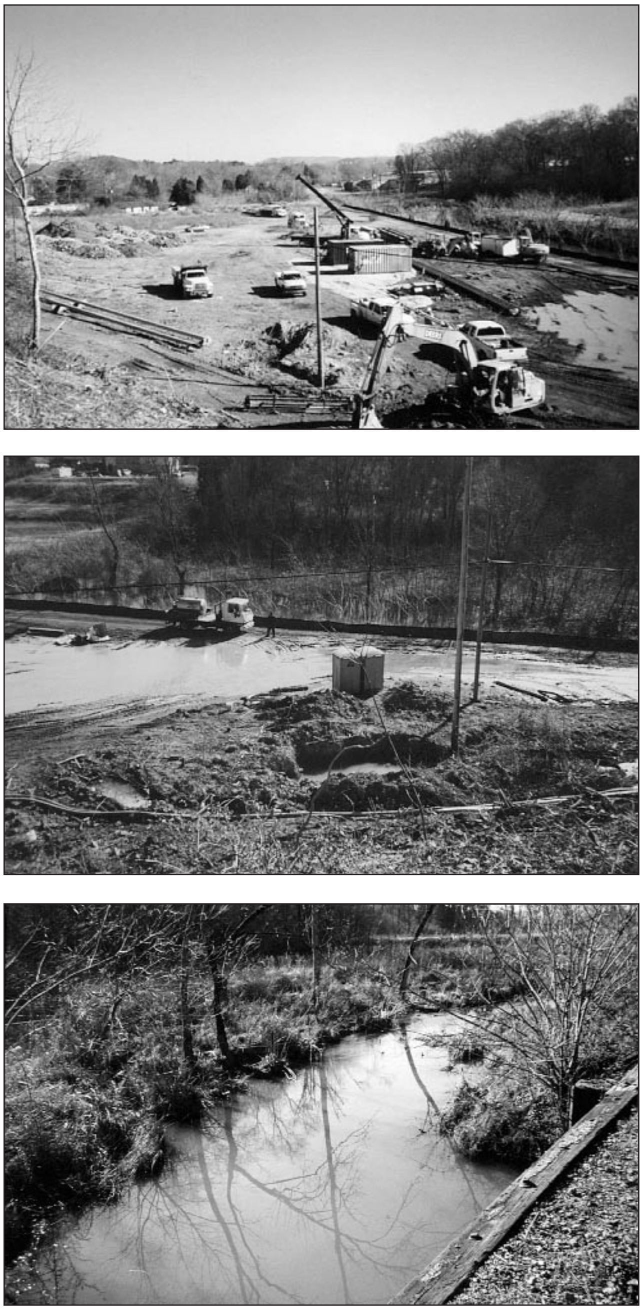

Fig. 3.
Turkey Creek, Jefferson County, Alabama, in 2005.

ancestral population size, evidence of bottleneck was detected in populations of *E. bellator* but not *E. chermocki*. This does not mean that *E. chermocki* does not experience the effect of recent population disturbances or will not be affected by any disturbances. Since the decline of its population occured in recent years, it may take some time until severe bottleneck could be observed. On the other hand, the evidence of bottleneck does not necessarily suggest that populations of *E. bellator* are imperiled. It only implies that this species is undergoing population isolation. These isolated populations differ genetically and should be treated as different units. Future monitoring of population structure of both species is essential to track the evolution of these endemic species.

Conservation Strategy for *E. chermocki*

General conservation approaches include protecting habitat and maintaining as many individuals of the species as possible in order to avoid the effect of small population (Maitland, 1995). Small populations are at risk of losing their genetic variation due to demographic stochasticity, random drift and inbreeding, all of which influence their persistence in the future (Caughley and Gunn, 1996). Although these approaches are necessary, they are occasionally inadequate in conservation. The abundance of a species is an insufficient indicator of health and viability of a species (Abrams, 2002). Genetic diversity within a species and its population structure should be examined because it also influences the existence of a species.

The evidence is clear that *E. chermocki* is now rare. A good conservation plan should involve the protection of its habitat. Turkey Creek has been highly disturbed in recent years. Sedimentation and removal of riparian vegetation has reduced the amount of breeding and feeding grounds (Figs. 2 and 3). In contrast, all populations of *E. bellator* inhabit a healthier and less-disturbed habitat (Fig. 4).

Captive breeding may be necessary for rebuilding populations of certain species to their known historical abundance (Rakes et al., 1999; Shute et al., 2005). However, for *E. chermocki*, it is not necessary because the species is naturally rare. Its habitat could only sustain a small number of individuals despite the large estimate of the population's ancestral size. By propagating a lot of individuals in captivity and releasing them into their natural habitat, the species may not be able to persist long term because the habitat may not be able to support so many individuals. Furthermore, these individuals may not survive because they are exposed to different selective pressures. In addition, there is the risk that inbreeding depression may occur and reduce the long-term fitness of the species once it is released in the wild (Osborne et al., 2006).

When native habitats of a species are severely degraded, translocation of a species may be an option (Maitland, 1995). However, this strategy should not be practiced when other options are still available to avoid any evolutionary consequences.

Fig. 4.
Gurley Creek, Blount County, Alabama, from 2005-2007.

In the case of *E. chermocki*, relocation from Turkey Creek to another location, particularly nearby Gurley Creek, is not suggested considering that these are allopatric species and there is no evidence that they had co-occurred. Hybridization may become a new problem. Since the divergent time has not been long, these species still share many phenotypic and genotypic components.

Under the popular Biological Species Concept, one may speculate that females of these species have developed sexual selection so that they only recognize the males of their own species. Nevertheless, there is no evidence of such a claim. It may also be possible that females cannot distinguish males of the sister species because they have not been exposed to such choices. The Biological Species Concept cannot be used to describe every species, particularly sister species that do not occur together, but the fact that *E. chermocki* and *E. bellator* are distinct species is supported by many pieces of evidence (Blanco, 2001; Clabaugh et al., 1996; Porter et al., 2002). In addition, these sister species do occupy the same niche as indicated by life history studies. If they were to inhabit the same areas, they would compete with each other for the same resources. The competition may drive one or both species to extinction. Therefore, relocation of *E. chermocki* to Gurley Creek will harm both *E. chermocki* and *E. bellator*.

The appropriate conservation plan for *E. chermocki* should include protection of its habitats, maintenance of connections throughout the stream, and regular monitoring of population structure and genetic variation. Because the stream is located in urban areas, anthropogenic activities are unavoidable. The best strategy may include prevention of stream run-off by establishing riparian zones and better management on storm-water runoff (B. R. Kuhajda, pers. comm.). Construction within streams should provide an outlet for flow throughout the stream. This can be done properly without severely disturbing movement of the species as seen in Gurley Creek. Regular monitoring of genetic diversity over time would be necessary to detect changes or consequences as a result of population declining. Current molecular techniques allow monitoring of genetic structure of *E. chermocki* without sacrificing live individuals.

Acknowledgments

I truly appreciated field assistance from the following people: Micah Bennett, Heath Howell, Michael Sandel, Kathryn Perez, Gregory Hubbard, Phillip Hegji, Anurach Poloek and Justin Bagley. I am very grateful to Dr. Phil Harris and Dr. Wallace Holznagel for sharing their expertise in molecular techniques. I am most indebted to Dr. Bernard R. Kuhajda for field assistance, research expertise, and his generosity during my graduate student career.

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