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Captive Propagation of Tangerine Darters for Re-introduction in the Pigeon River, Tennessee

Craig Lee Phillips
University of Tennessee - Knoxville

To the Graduate Council:

I am submitting herewith a thesis written by Craig Lee Phillips entitled "Captive Propagation of Tangerine Darters for Re-introduction in the Pigeon River, Tennessee." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Wildlife and Fisheries Science.

J. Larry Wilson, Major Professor

We have read this thesis and recommend its acceptance:

Richard J. Strange, Patrick Rakes

Accepted for the Council:
Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

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CAPTIVE PROPAGATION OF TANGERINE DARTERS FOR RE-INTRODUCTION IN THE PIGEON RIVER, TENNESSEE

A Thesis
Presented for the
Master of Science Degree
The University of Tennessee, Knoxville

Craig Lee Phillips
May 2007

Dedication

This thesis is dedicated to my family; my wife Emily, Mom, and Dad; who continually encourage and support me.

"To cherish what remains of the Earth and to foster its renewal is our only legitimate hope of survival."

Wendell Berry

Acknowledgements

I would like to thank Dr. Larry Wilson, my major advisor, for giving me the opportunity to further my education by accepting me into the graduate program. His guidance and encouragement were invaluable. I would also like to thank my committee members, Dr. Richard Strange, and Pat Rakes for their patience, advice, and superior knowledge.

I want to thank the entire staff at Conservation Fisheries Incorporated for allowing me to use their space, time, and permission to use their photographs throughout this thesis. Thanks to all the graduate students in the Department of Forestry, Wildlife and Fisheries for helping me get on my feet and staying on track. Thanks to Joseph Zimmerman and Misty Huddleston for their friendship, help, and patience with me. I also want to thank Brac Salyers for his advice and support.

A special thanks to the University of Tennessee Department of Forestry, Wildlife and Fisheries and the Tennessee Wildlife Resource Agency for providing the funding for this project. Without their support the project could not have existed.

Abstract

The Pigeon River suffered major water quality degradation from 1908 through the 1980's from paper mill effluent which resulted in the extirpation of many native fish species. Mill modifications have cleaned the effluent to the degree where some native species are recolonizing many areas of the river. In 2001, the Pigeon River Restoration Project was initiated to re-introduce native non-game species which have been unable to return of their own accord. In addition to relocation of selected suitable species, captive production of the tangerine darter (*Percina aurantiaca*) has been attempted since current translocation methods have proven impractical due to the small number found in the Pigeon River system. It is anticipated that, through hatchery propagation, sufficient numbers of tangerine darters might be produced for re-introduction. This method has seen limited success with other *Percina* species.

Using brood stock of tangerine darters collected from the Pigeon River above the paper mill, three attempts to spawn and propagate tangerine darters were conducted at the Conservation Fisheries Incorporated (CFI) facility in Knoxville, TN. In the first trial, no eggs were spawned; the second year produced approximately 290 eggs and larvae but relatively few survived. The third attempt produced approximately 331 eggs and larvae, resulting in approximately 85 juveniles, but grow-out was problematic; future propagation efforts will target optimum grow-out densities as well as determine the nutrition requirements for larval and juvenile tangerine darters.

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CHAPTER I

Introduction

Flowing out of the mountains of western North Carolina at an elevation of 803 meters (m) above mean sea level and ending in eastern Tennessee at an elevation of 305 m above mean sea level, the Pigeon River (PR) is a headwater tributary of the Tennessee River System (Saylor et al. 1993). For descriptive purposes in this paper, the accepted method for referencing a specific site or location on a river will be “river mile” instead of “river kilometer”. For example, a sample site at mile 30 on the Pigeon River will be notated as PRM30 (Pigeon River Mile 30). In Haywood County at the confluence of the West Fork Pigeon River and the East Fork Pigeon River in North Carolina, the river winds in a northwestern direction through mountainous terrain for approximately 70 river miles before connecting with the French Broad River (FBR) in Cocke County, Tennessee, below Newport near FBRM 73 (Figure 1). The headwaters of the Pigeon River are located in the Pisgah National Forest, approximately 32 km southwest of Asheville, North Carolina (Bartlett 1995). The watershed for the Pigeon River is located in Hydrological Unit Code (HUC) 06010106 and encompasses an area of approximately 1803 km² with 74.6% located in North Carolina and 23.6% located in Tennessee (Environmental Statistics Group 2003). The river has small farms and communities interspersed along its path. The current recreational use for the Pigeon River is primarily white water rafting.

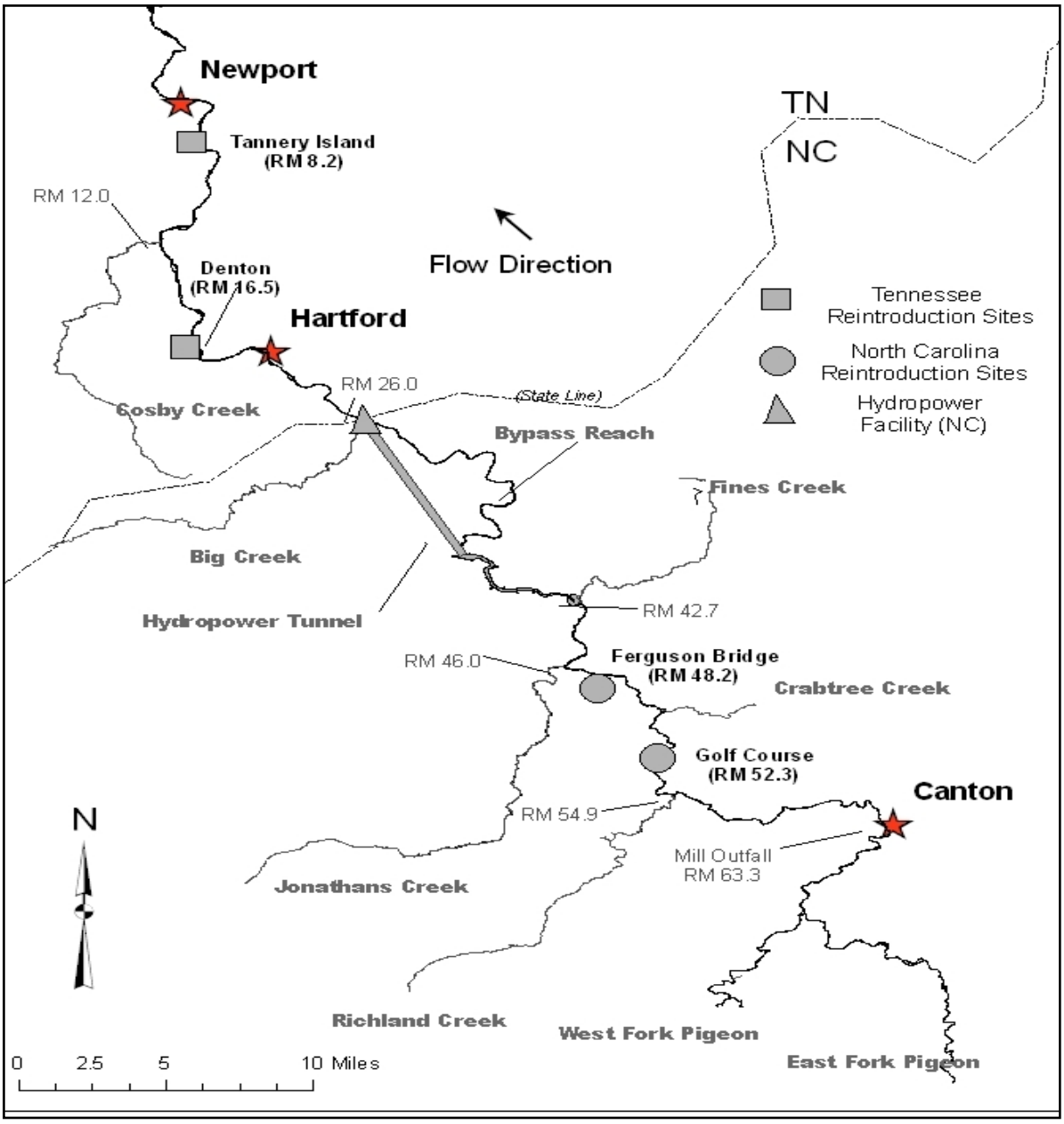

Figure 1: The Pigeon River flowing out of North Carolina showing the paper mill (PRM 63.3) and Denton reintroduction site (PRM 16.5).

In 1908, the Champion Fiber company (Champion International) constructed a paper mill on the river in Canton, North Carolina. Although the plant provided much needed jobs for the people of this Appalachian community, the paper production process at that time had a significantly detrimental effect on the water resources. In 1908, when the paper mill began operations the river quickly turned black, odorous, and was covered with foam which often exceeded one meter in height. Dead fish were reported being collected by the wagon-loads in the bends of the river (Bartlett 1995). Cool water taken from the river into the mill for the pulp-into-paper process was significantly warmer when discharged and carried a cornucopia of pollutants. Large amounts of chlorine, sodium carbonate, sodium hydroxide, sodium sulfate, sodium chlorate, sulfur, aluminum sulfate, titanium dioxide, lignin, tannin, dioxin, chloroform, and furans were discharged daily into the river (Bartlett 1995). Due to the mill,s effluents, much of the aquatic life of the river was extirpated.

The Environmental Protection Agency (EPA) was slow to impose strict regulations on Champion because of the economic and social benefits that the paper mill brought to the region (Saylor et al. 1993). In 1982, local environmental groups and Tennessee state officials started increasing pressure on Champion to clean up the river. The EPA took action in 1997 under the Clean Water Act and ordered an economic analysis of the downstream impacts resulting from the Champion discharge (Bartlett 1995). Finally in 1997, an agreement was made to strengthen North Carolina's permit requirements that required Champion to reduce discharge pollutants by 50 percent (Barlett 1995).

The Tennessee Water Quality Control Act of 1977 under Section 69-3-102(a) and Section 69-3-102(b) required that the state take action to preserve and restore polluted waters. A joint meeting between federal and state agency personnel was held in 2001 to determine if reintroducing native fish back into the Pigeon River was possible. Blue Ridge Paper, Inc. of Canton, NC, Tennessee, Department of Environment and Conservation (TDEC), Conservation Fisheries, Inc. (CFI), Tennessee Wildlife Resources Agency (TWRA), Tennessee Valley Authority (TVA), the University of Tennessee, Knoxville (UTK), U.S. Geological Survey (USGS), and the U.S. Fish and Wildlife Service (USFWS) joined to form the Pigeon River Recovery Project (PRRP) in an attempt to restore selected native species back into the river

The PRRP has been responsible for the reintroduction through translocation of 12 fish species, six snail species, and nine mussel species. A species reintroduction can be considered successful when the population becomes self-sustaining (Griffifth el al. 1989). A darter species that the PRRP is interested in reintroducing to the river is *P. aurantiaca* (Cope) (Figure 2). However, sufficiently large populations of *P. aurantiaca* do not exist in the Pigeon River and associated tributaries to be translocated in significant numbers. The need to obtain adequate numbers of individuals to stock prompted the TWRA, CFI, and World Wildlife Fund (WWF) to fund the tangerine propagation project. The objective of this study was to determine if a small sample of *P. aurantiaca* could be collected from the Pigeon River and spawned in captivity, and thereby producing juveniles that later could be reintroduced into the Pigeon River

Figure 2: Photograph of an adult male tangerine darter by Bill Roston.

CHAPTER II

Literature Review

Captive propagation has been around for many years. The use of captive propagation as a management technique with fish was first perfected with sport fish. Perhaps the most commonly recognized fish species associated with captive propagation is trout. Year after year thousands of trout are raised from eggs in hatcheries across the country for "put and take" management techniques. In recent years the use of captive propagation for the purpose of recovering endangered species has increased (Snyder et al. 1996). Supportive breeding, through captive propagation, is a technique in which a fraction of the wild population is taken into captivity for reproduction, and the offspring are reared and released where they mix with the wild population for the purpose of increasing the census population without introducing exogenous genes (Ryman and Laikre 1991; Wang and Ryman 2001).

It is generally assumed that self-sustaining populations of endangered species can be easily established (Snyder et al. 1996). This misconception has resulted in a lack of extensive research in using captive propagation for non-game fish. Similarly, only a small percentage of invertebrate and vertebrate species have been successfully bred in captivity (Conway 1986; Rahbek 1993; Snyder et al. 1996). The spawning behavior of fish is poorly known for many non-game species and propagation techniques for darters are not extensively

documented (Etnier and Starnes 1993; Mattingly et al. 2003). The few that have been well documented include the fountain darter, *Etheostoma fonticola* (Brandt el al. 1993 and Bonner et al. 1998), Pearl darter, *Percina aurora* (Schofield et al. 1999), channel darter, *P. copelandi,* (Ross et al. 1998 and Schofield et al. 1999), goldline darter, *P. aurolineata* (Rakes and Shute 2002), boulder darter, *E. wapiti* and bloodfin darter, *E. sanguifluum* (Rakes et al. 1999), longnose darter, *P. nasuta* (Anderson et al. 1998), and niangua darter, *E. nianguae* (Mattingly et al. 2003). Darters are best described by Forbes (1880) when he said "Not-withstanding their trivial size, they do not seem to be dwarfed so much as concentrated fishes – each carrying in its little body all the activity, spirit, grace, complexity of detail and perfection of finish to be found in a perch or a "wall-eyed pike"." Yet darters have received little attention from fisheries managers, often being referred to as a "trash" species.

P. aurantiaca is found in large to moderate size clear head water tributaries of the Tennessee River between 259 to 550 meters above mean sea level (Howell 1971; Greenberg 1991; Etnier and Starnes 1993; Jenkins and Burkhead 1994; Leftwich et al. 1997). Howell (1971) described breeding males as one of the most colorful freshwater fish in the United States. Members of *Percina* and *Hadropterus* (the tangerine darter's subgenus) are the only species of darters that have not lost their air bladder and, because of this, they are considered primitive darters among taxonomists (Bailey 1951; Winn 1958). The tangerine inhabits a variety of habitats depending on the season and age.

Darters can be grouped into four reproductive behavior categories according to egg placement: buriers, attachers, clumpers and cluster (Page 1985; Mattingly et al. 2003). *P. aurantiaca* is considered a burier. Spawning occurs over gravel and is typical of the genus (Hankinson 1932; Winn 1953; Howell 1971; Etnier and Starnes 1993). *P. aurantiaca* females will produce approximately 400 to 1100 ova (Etnier and Starnes 1993). Temperature is believed to be the trigger that initiates spawning behavior (Hubbs and Strawn 1957; Howell 1971; Etnier and Starnes 1993). The male will straddle the female's nape using his pelvic fins while resting upon her back. The male's peduncle is positioned along the female's body so that the genital openings are adjacent to each other. Females extrude eggs into the gravel or sand and are fertilized by milt from the male while quivering (Etnier and Starnes 1993) (Figure 3). This general behavior is considered common among all members of *Percina* (Etnier and Starnes 1993).

Larvae and young-of-the-year (YOY) inhabit shallow pools along the shoreline near the transition zone of riffle to pool habitat (Howell 1971). Adults inhabit riffles from around July to October and then move through the transition zone before over-wintering in deep pools by late October (Howell 1971).

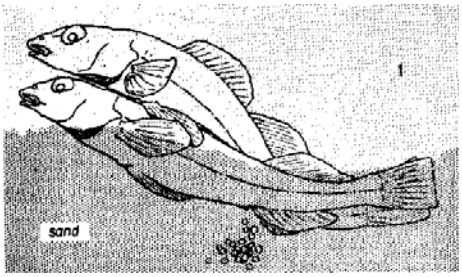

Figure 3: Modified illustration of log perch (*Percina caprodes*) spawning in sand substrate from Winn (1958).

Figure 4 modified from Winn (1958) shows the seasonal movements of most darters. Kessler et al. (1995) suggested that stream-dwelling benthic fish, such as darters, have a strong relationship between habitat use and body morphology. The long typical "cigar" shape of most darters allows them to inhabit the swift currents of riffles. Adults emerge from the pools to spawn in the transition zone from April to June (Howell 1971; Etnier and Starnes 1993). Long migration does not occur according to Howell (1971). This differs from Winn's (1958) suggestion that darters have a definitive reproduction migration.

Forbes (1880) compared the diet of 15 species of darters. His study showed that the diet of *P. caprodes* was comprised of crustaceans, Entomostraca and the smalles of our Amphipoda, *Allorchestes dentata.* The Entomostraca were cladocera that included *Daphnia, Eurycercus* and *Daphnella*.

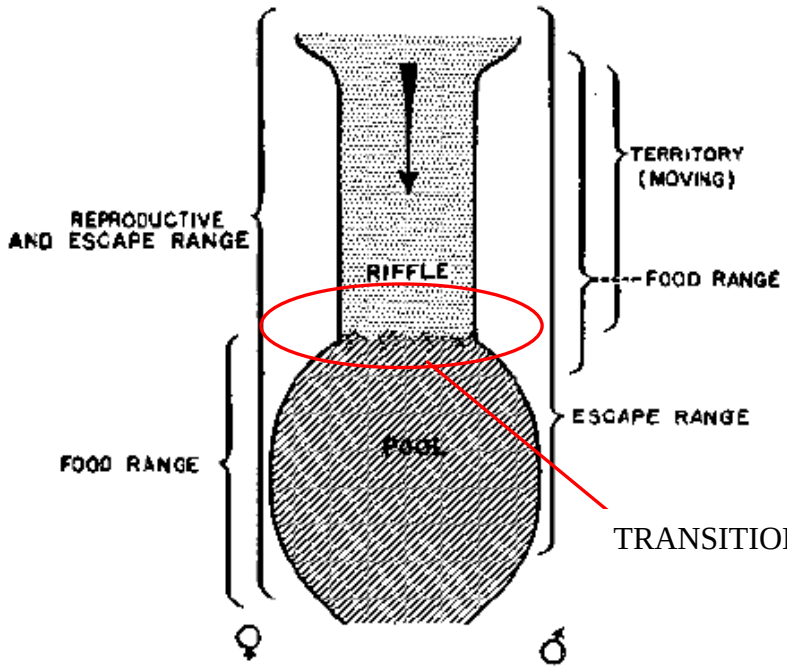

Figure 4: Modified illustration from Winn (1958) showing seasonal movements of most darters

He also concluded that YOY of nearly all species of our fresh-water fishes compete for entomostracans and larvae of minute diptera for food.

Howell (1971) found that many organisms in the diet of the tangerine darter were associated with *Podostemum ceratophyllum* (riverweed). Juveniles primarily consumed baetid mayflies and dipterans. The mayflies were mostly from the subfamily Caeninae and were from two genera, *Tricorythodes* sp*,* *Caenis* sp; the dipterans consumed were primarily tendipedids. Adults primarily consumed caddisflies, which were mostly *Hydropsyche* sp*;* however, Howell (1971) believed that *P. aurantiaca* are opportunistic feeders on any available immature insects. The smallest individual he examined (31 mm) had ingested one cladoceran, *Chydorus* sp, 75 *Eucyclops agilis* and a baetid mayfly nymph.

CHAPTER III

Early Attempts at Propagation

2004 Trial

The first attempt at tangerine propagation was undertaken by Conservation Fisheries Incorporated (CFI) in 2004, along with Brac Salyer from the University of Tennessee. Five tangerine darters (one large male, one small male, and two females) were collected from the Pigeon River above the paper mill in Canton, NC, and placed in a 379-L (100-gallon) glass aquarium. A mixture of coarse gravel and fine sand was provided in the center of the tank with abundant cover to stimulate a transition zone substrate. Water temperature (Figure 5) and photoperiod was manipulated to mimic the natural temperatures and day length by using the ventilation system at the CFI facility and lights that were on a timer. Maximum number hours of light were reached in late May at 15 hours and were maintained until October. On 3 July spawning still had not occurred, so some of the adults were replaced with other fish that CFI had previously collected. This resulted in two males and three females in the study tank. Unfortunately spawning did not occur. It was not clear why spawning did not occur but was believed to have been associated with the acclimation process of wild fish to captivity (Conservation Fisheries, Inc 2004). Another factor may have been the sensitivity of the brood stock to movements. The glass tank did not afford suitable “security”. In succeeding years, brood stock was housed in an opaque fiberglass tank.

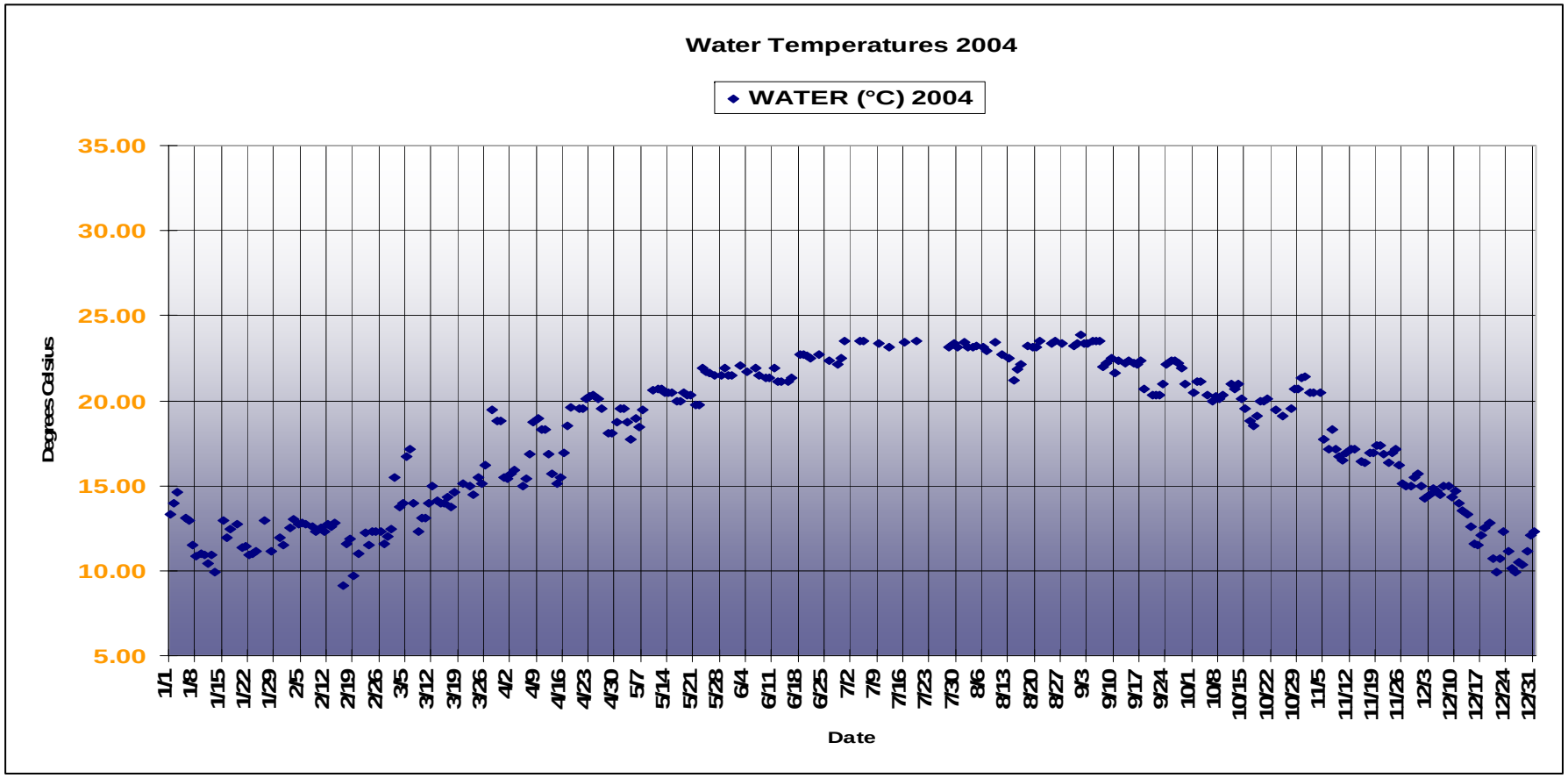

Figure 5: Water temperatures in the tangerine darter spawning tank for 2004.

2005 Trial

CFI again attempted to spawn tangerine darters in captivity in 2005. A 341-L (90-gallon) fiberglass tank was set up to house the adult fish to reduce stress that might inhibit spawning. The substrate was the same as the 2004 trial. On 26 April, spawning occurred with six older adults at 17.1° C. However, many of the larvae were lost in early May while trying to determine food and habitat requirements. It was eventually determined that larvae preferred dark containers (rubber tubs were used) with both current and slack water areas.

The photoperiod and water temperatures were controlled the same as in 2004. However, the 2005 photoperiod differed from 2004. The photoperiod was advanced so that by April there was 14 hours of light versus only 12 hours of light in 2004. Maximum hours of light were reached in June at 16 hours and were maintained until late September.

Interestingly, observations of the pelagic larvae suggested that they were surface or periphyton feeders at first before feeding within the water column (Conservation Fisheries, Inc 2005). The spawning continued sporadically until June. Approximately 290 eggs and larvae were collected but the resulting larvae experienced high mortality during rearing protocol development. Ultimately, only three offspring survived, and these were abnormal or stunted, although they were maintained at the CFI facility until April 2007. The total number of eggs and larvae that survived was divided by the total number of eggs and larvae collected to get a survival rate of 0.01 %.

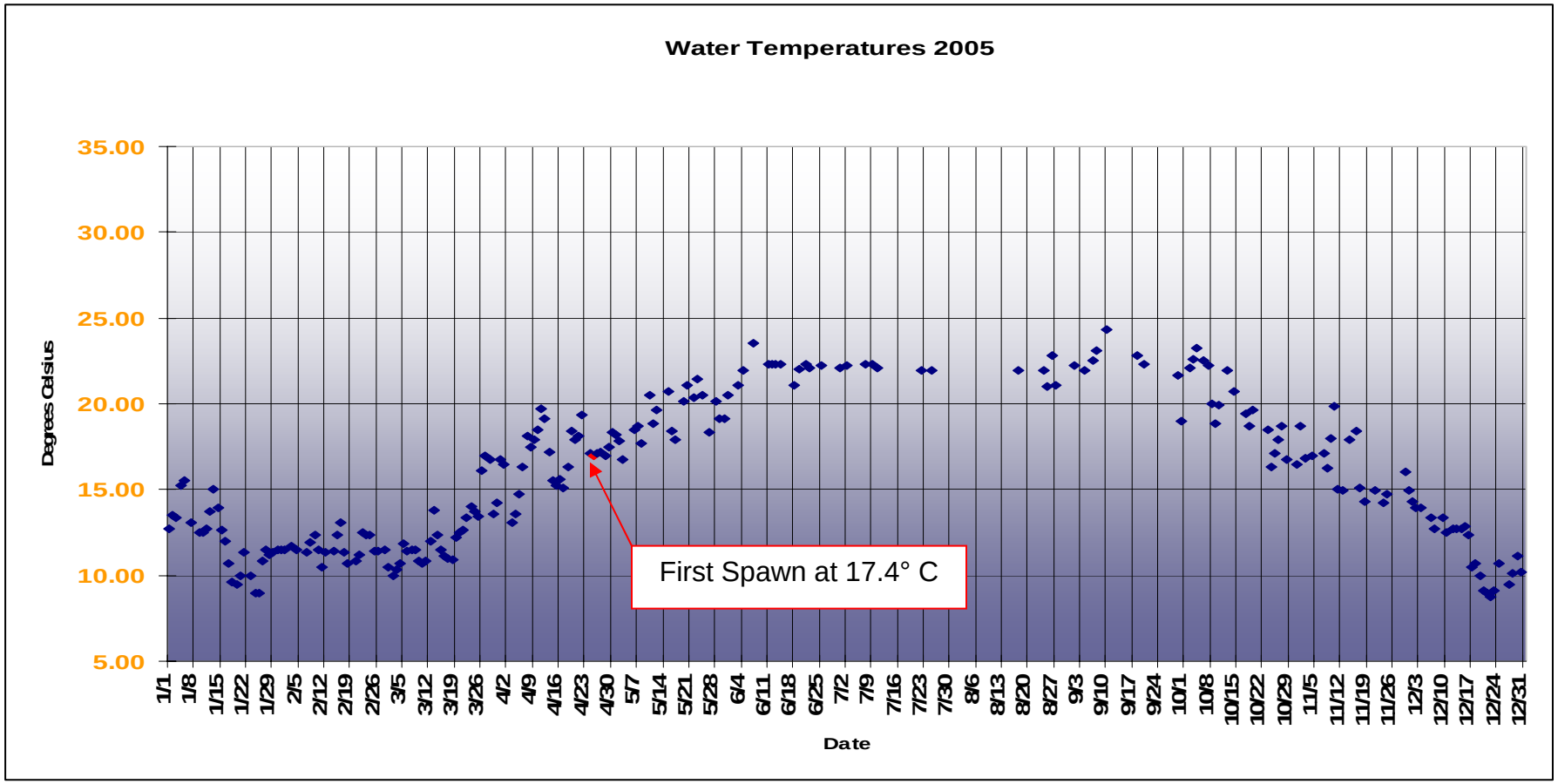

Figure 6: Water temperatures in tangerine spawning tank for 2005 showing first spawn on 24 April.

CHAPTER IV

Present Study Methods

System Design

Six adults from CFI's brood stock were used with two large older males, measuring approximately 130-140 mm total length (TL), one large older female measuring approximately 125 mm TL, and three younger females measuring approximately 100 mm TL. All adults were collected from the Pigeon River above Canton, NC, in 2004.

The adult diet consisted of whole blackworms from California-based Aquatic Foods, Inc. and chopped earthworms collected on the grounds of the CFI facility, fed to satiation several times a day.

A 341-L fiberglass tank was placed on the second shelf of a steel pallet rack at the CFI facility (Figure 7). Three 356 mm x 508 mm (14" x 20") plastic trays filled with sand to an average depth of approximately 51 mm (2") were placed in the tank to provide spawning habitat (Figure 8). Large cobble and pieces of slate were arranged within the trays to provide escape cover and optimal spawning sites based on field and 2004 study observations (Rakes personal communication 2007) (Figure 9). Gravel was dispersed across the bottom of the tank to cover the fiberglass tank bottom (Figure 10). This approach was modified on 8 May 2006. The spawning trays were removed and the sand was poured in one half of the tank to a depth of 51 mm and course gravel on the other end to create substrate that closely resembled a pool to riffle transitional

zone (Figure 11). Cobble and pieces of slate were arranged along the bottom of the tank in both sand and coarse gravel to provide diverse spawning locations rather than presumed optimal locations and to simulate a transitional zone. The tank was drained by a stand pipe into a 57-L round rubber tub with a finely screened standpipe drain to capture any larvae that might escape the tank. A powerhead was installed in the adult tank for water circulation (Figure 12). Once the water was turned on and everything was verified to be operating correctly, four female and two male tangerine darters were released into the tank after being allowed to acclimate for approximately 30 minutes (Figure 13).

Water Temperature

Water temperature was manipulated by the same system at CFI that was used in the previous two trials. The water was coldest on 2 February at 9.2° C then gradually increased to 23.7 °C on 15 July before leveling off. The temperature was then gradually decreased from 23.9 °C on 2 November to 11.2 °C on 28 December (Figure 14).

Photoperiod

The photoperiod was controlled by the same method as in 2004 and 2005. The day length closely followed the 2005 study but reached a maximum of 16.6 hours of light by June and was maintained until September.

A Micro Video MVC2000 – WP – LED digital lipstick camera was placed in the adult tank in an attempt to document the tangerine's courtship and spawning behavior (Figure 15).

Figure 7: 341-L (100 gal) fiberglass tank used to house the adults for spawning.

Figure 8**:** Spawning trays with 51mm (2 in) average depth of sand.

Figure 9: Slate being used to provide cover in spawning trays.

Figure 10: Original setup of adult tank prior to filling with water.

Figure 11: View of spawning tank with sand substrate after removing trays.

Figure 12: Powerhead being used to create circulation.

Figure 13**:** Adults in bags acclimating to the new tank.

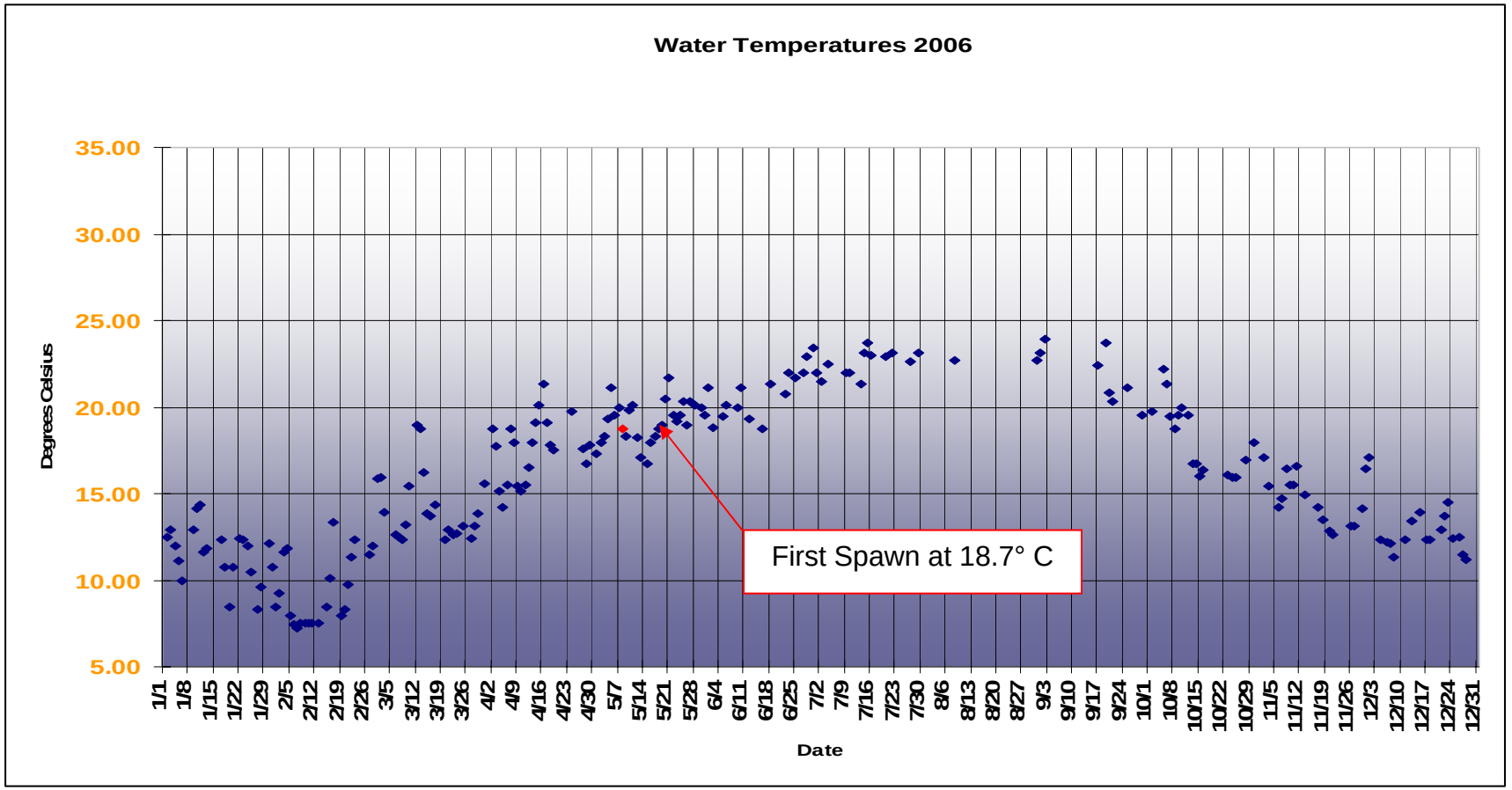

Figure 14: Water temperatures in spawning tank for 2006 with first spawn on 8 May.

It was positioned on the bottom of the tank at different locations directed under the rock slabs.

Egg Collection

Once a week the trays of sand in the tank were vacuumed for any eggs (Figure 16). The water from the vacuuming process was captured in buckets and the particulate matter was allowed to settle. This water was then poured into another bucket until approximately 90% of the water was gone (Figure 17). The remaining 10% of water was poured into a plastic container along with any sand and organic material that was in the bottom of the bucket. The container was taken back to a table were a light could be directed from underneath.

Figure 15: Micro Video MVC2000 – WP – LED digital camera being lowered into position

A pipette was used to pick through the organic matter (Figure 18). Once an egg or larva was identified, it was removed from the plastic container and placed into a Petri dish with just enough water to keep it submerged (Figure 19). The remaining contents of the plastic container were discarded once all eggs and larvae had been collected; the process was repeated until the entire adult tank was examined.

All eggs and larvae that had been collected were examined under a Nikon dissecting microscope to determine stages of development or deformities. Since damaged larvae have a low probability of survival, they were removed. Figure 20 shows an undamaged yolk sac larva which is a few days post-hatch. All eggs with fungus growth were removed immediately and discarded to prevent contamination of the other eggs and larvae. After the eggs and larvae had been examined under the microscope, the Petri dish was submerged in a 178 mm x 356 mm (7” x 14”) plastic tray for incubation of the eggs and to provide the yolk sac larvae refuge during yolk sac absorption (Figure 21). An air stone was placed in the tray to ensure oxygenated water for the larvae and eggs.

Pelagic Stage

A larva was considered pelagic when it had absorbed its yolk sac and spent most of the time within the water column.

Figure 16: Substrate being vacuumed for eggs and larvae.

Figure 17: Excess water decanted from vacuumed substrate.

Figure 18: Eggs/larvae being collected from vacuumed organic matter.

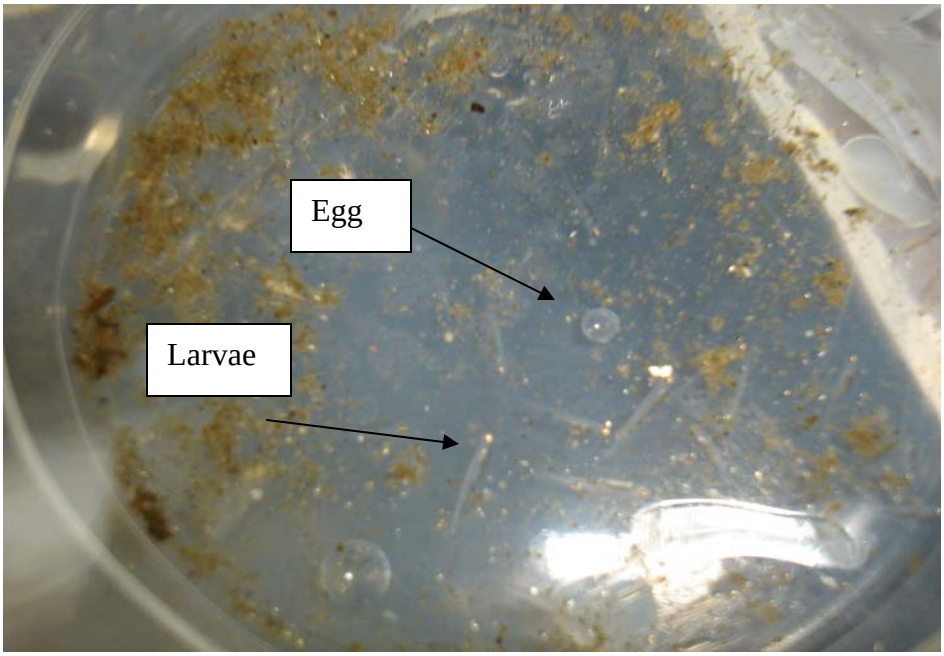

Figure 19: Petri dish with harvested eggs and larvae.

Figure 20**:** Healthy yolk sac larva under microscope.

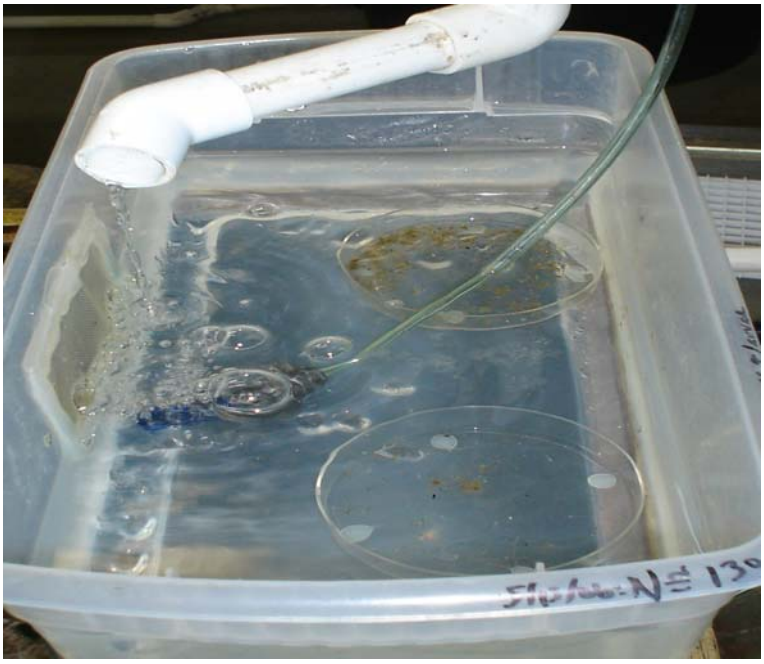

Figure 21: Incubation tray with Petri dishes holding eggs and larvae.

Pelagic larvae were removed and placed in one of three round black 57-L (15-gallon) rubber tubs (Figure 22). Sand was loosely scattered on the bottom to provide substrate and medium cobble was added to provide vertical structure and resting areas for the larvae. A 15-L (4-gallon) automatic liquid feeder was installed to operate from 0900 until 1900 hours every day. The feeder delivered liquid every odd hour for 7 minutes, providing a mixture of rotifers, *Ceriodaphnia dubia*, and first instars of *Daphnia pulex, and Daphnia magna* (Figure 23) to the pelagic larvae. Ocean Star International (OSI) Artificial Plankton - Rotifer (APR) (~150 microns in size), and Ziegler AP100 Larval Shrimp Diet (100, 100-150, 150-250, and 250-450 micron sizes) dry feed was also provided by hand several times per day, transitioning gradually to the larger microparticle sizes as the larvae grew. A powdered Spirulina alga was also mixed in, along with *Nannochloropsis* phytoplankton (greenwater) from concentrate. Adult *Ceriodaphnia* and *Artemia* (brine shrimp) (Brine Shrimp Direct) nauplii were also provided as soon as the larvae were large enough to consume them (Rakes personal communication).

Benthic Stage

A larva was considered benthic once it spent the majority of its time resting on the bottom instead of moving about in the water column. Once the pelagic larvae were benthic they were moved to one of nine 76-L (20-gallon) glass aquaria for grow-out. Sand mixed with coarse gravel was placed in each tank for substrate and medium cobble were arranged to provide escape cover and territory (Figure 24). An air stone and bubble sponge was added to each

grow-out tank to ensure adequate oxygenation. The benthic juvenile diet changed as they developed. The juveniles were first given chopped black worms from California-based Aquatic Foods, Inc. three times daily. The larger juveniles were given whole black worms and chopped frozen bloodworms (Bio-Encapsulated with multi-vitamins-Hikari). The smaller juveniles were also fed brine shrimp *nauplii* for as long as they would accept them.

Weekly visual observations were taken for each tank at all stages of development and recorded. These observations ranged from 30 minutes to 1 hour and only one to two tanks were observed on the same day. A dark colored shirt was worn each time to help prevent being noticed by the juveniles. The tank to be observed was approached very cautiously. Once in position, the observer would remain as still as possible for the duration of the observation and then record behavior before moving to the next tank.

Figure 22: 57-L round rubber tub used to house the pelagic larvae.

Figure 23**:** 15-L auto feeder with green mixture used for the pelagic larvae.

Figure 24: Visual of the substrate in a benthic grow out tank with pen for size reference.

CHAPTER V

Results and Discussion

On 8 May 2006, the first larvae were collected from the pelagic tub via passive live capture. All spawning trays were vacuumed and yielded nothing. The spawning trays were then removed and the scattered coarse gravel was vacuumed and resulted in one more larva. The spawning trays were removed and the entire tank reset with all coarse gravel at one end of the tank and all sand at the opposite end. The decision to remove the trays was made because the females appeared to be reluctant to swim into the trays despite sufficient cover and the spawning had occurred outside the trays.

On 15 May 2006, the entire tank was vacuumed again and approximately 130 eggs and larvae were collected from the side of the tank with the coarse gravel and with no cover. This was surprising because it was believed that spawning would occur on the side of the tank with the sand substrate and under the cover of rock slabs. However, on 18 May 2006, only two larvae were found in the course gravel; the other 24 eggs and 10 larvae were collected on the side of the tank with sand and under a rock slab.

The MVC2000 – WP – LED digital camera was unsuccessful at capturing spawning activity. The tangerines appeared to be very timid, so the presence of the camera may have deterred spawning from occurring in its vicinity. On 29 May 2006, a total of 37 larvae and one egg were collected on the side of the tank

with the coarse gravel and under a rock slab. It appeared that the female tangerine darters did not have a preference between coarse gravel and sand under the present lab conditions. This could simply be the result of the dominant females taking the preferred spawning sites and the subordinate females being forced to spawn in the less desired spawning sites. Spawning continued until 10 July 2006 when the last egg was collected. A total of 331 eggs and larvae were collected. Mortality consisted of 102 eggs lost during incubation, 142 larvae lost during the pelagic stage, one larva lost due to handling, and 37 juvenile deaths during grow-out. This resulted in a 14% overall survival rate.

Observations of the benthic juveniles showed interesting behavior. They would rest on top of the small cobble in the tanks with as many as 5 juveniles of various sizes occupying the same rock. Other juveniles would be staged nearby and quickly replaced any left the rock. This may be important in keeping a watch out for predators and potential food items. This behavior was observed in all grow-out tanks. No territorial aggression was observed within any of the tanks.

Water Temperature

Water temperature is believed to be an important trigger for the onset of spawning (Howell 1971; Etnier and Starnes 1993). In both 2005 and 2006, spawning was first noticed around 17.8 °C. Spawning ended in 2006 at 21.9 °C in July. Howell (1971) observed spawning at 14 °C, which was well past the spawning peak in the natural habitat; he associated this delay to the constant cold water temperature in his raceway. Hubbs and Strawn (1957) stated that the reproduction rate was controlled by temperature and the condition of the fish in

E. lepidum (greenthroat darter). Theoretically under laboratory conditions spawning could be initiated by slowly raising the water temperature to approximately 17.8 ° C regardless of the season.

Photoperiod

Although temperature is believed to be the trigger that initiates spawning in darters, photoperiod should not be ignored. CFI used a timer system with a dawn and dusk feature to imitate the natural regime. This system allowed for CFI to control the hours of "daylight". The three attempts differed slightly. In 2004 the hours of light were kept constant at ten hours until 31 January. An incremental increase in hours occurred until 24 May at 15 hours. This stayed constant until 11 October when it dropped to 12 hours. In 2005 the photoperiod was gradually increased from 10 hours on 13 January to 16 hours on 3 June. This stayed constant until 5 October when it decreased to 14 hours. In 2006 the photoperiod was gradually increased from 10.75 hours on 18 January to 16.75 hours on 19 June. This stayed constant until 1 September when it gradually dropped to 10.5 hours by 31 December. Figure 26 compares the photoperiod of all three trials. An insufficient photoperiod in 2004 may have played a role in the adults not spawning. This may suggest that a minimal threshold exists that, along with temperature, induces the tangerines to spawn.

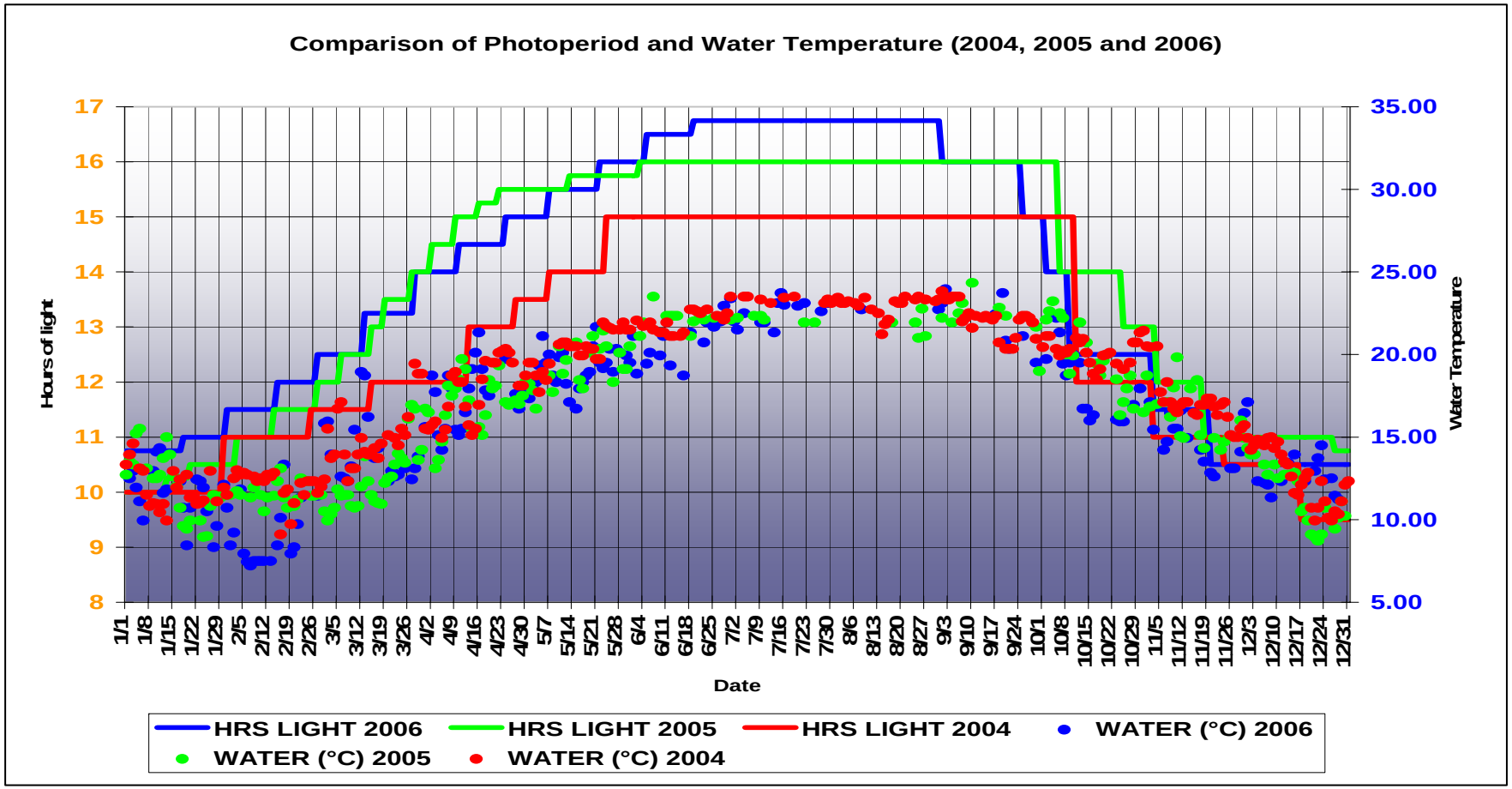

Figure 25: Comparison of 2004, 2005 and 2006 water temperature and photoperiod.

Stunting

Evidence of stunting in juvenile tangerines was quickly noticed in the 75-L glass grow-out tanks. The numbers of juveniles in some tanks were reduced from 15 to nine by moving the smaller stunted juveniles to one of the other nine grow-out tanks. Due to the lack of space in the system, this was later repeated so the number per tank was reduced from nine to five. This resulted in one tank having 20 individuals that had suffered the most stunting. These were isolated together to test whether they were simply "failing to thrive" or suppressed by dominance interactions. Medium rocks were added to the tanks to try and create more territory surface area but this appeared to have no effect on growth. Ultimately two size classes of tangerine juveniles were found. The largest size class ranged from approximately 50.8 mm – 76.2 mm (2" – 3") TL by April 2007(Figure 27). Individuals within this size class were very robust with the distinctive orange color and had a black lateral band beginning to become more evident. However, field observations of wild tangerine darters of the same 2006 year class revealed that they were somewhat larger than the average individuals captive-reared under lab conditions (P. Rakes personal communication). This indicated that intraspecific competition or some other factor such as diet, water temperature, habitat conditions, etc within the grow-out tanks resulted in slightly slower growth rates. The smaller size class was extremely stunted. Individuals ranged from 12.7 mm – 25.4 mm (0.5" – 1") TL with slightly larger disproportioned heads and emaciated bodies.

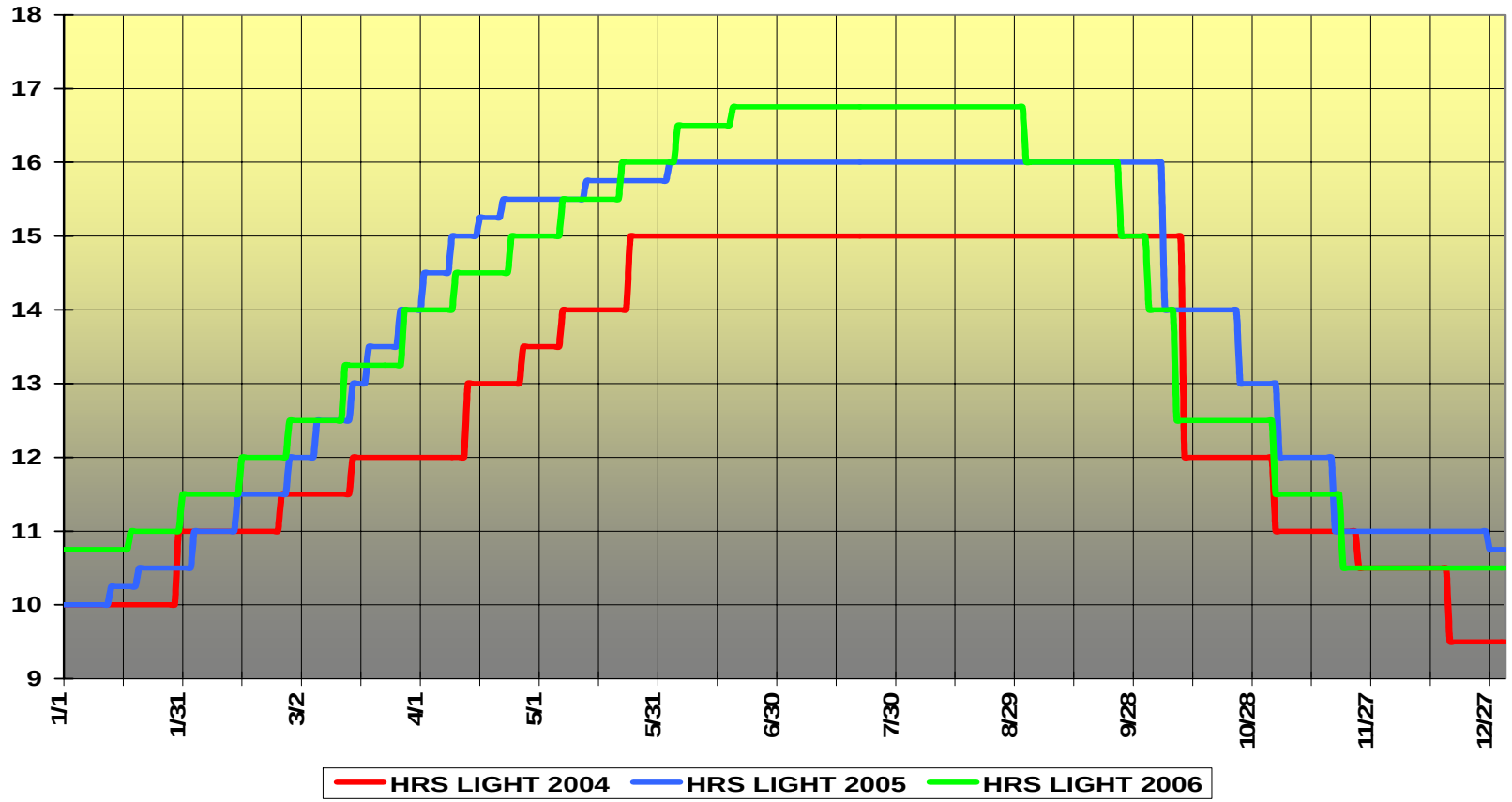

Figure 26: Comparison of all three trials photoperiod.

Their color ranged from gray to milky with black lateral blotches (Figure 28). If this stunting resulted from intraspecific competition, it could explain why *P. aurantiaca* typically occur in low densities only. Nutrition could also have had some effect on stunting due to inappropriate food types or quantities at certain stages of development. Water temperatures or details of habitat, such as cover or substrate availability may have also contributed. It is also suspected that the 57-L black rubber tubs may have exposed the pelagic larvae to sublethal toxins that impaired proper mouth development and resulted in stunting (Rakes, personal communication).

Reintroduction

Of the 85 tangerine darter juveniles, approximately 44 % were stunted or deformed. The other half appeared healthy and robust. Gradual die-offs occurred among the smaller stunted group until only 48 of the robust group and six of the stunted group were left (plus the three from the 2005 study). Four severely stunted individuals were sent to the Warm Springs lab and two severely stunted individuals were sent to the University of Tennessee's School of Veterinary Medicine OLAC lab to determine if deformities observed were bacterial or pathogen related. On 6 April 2007, forty-five juveniles from the 2006 study were reintroduced into the Pigeon River at the Denton site (PRM16.5). The juveniles were allowed approximately thirty minutes to acclimate by gradually adding water from the Pigeon River before being released (Figure 29). The juveniles did not immediately seek cover when released, but instead remained completely still in the open (Figure 30).

Figure 27: Large tangerine juvenile (76 mm).

Figure 28**:** Severely stunted tangerine darter juvenile (25.4 mm).

Figure 29: Juvenile tangerine darters acclimating before release at Denton (PRM 16.5)

Figure 30: Released juvenile tangerine darter in the Pigeon River at Denton (PRM 16.5) adjusting to the new environment.

CHAPTER VI

Summary and Recommendations

Attempts at captive propagation of *P. aurantiaca* were completed in the spring and summer of 2004, 2005, and 2006 for the purpose of reintroducing wild populations to the recovering lower Pigeon River. Although the first two attempts resulted in limited success, valuable information was gained. The results of those three studies are as follows:

1. Spawning was unsuccessful in 2004
2. Spawning was successful in the 2005 study but the resulting larvae experienced high mortality.
3. Spawning was successful in the 2006 study with approximately 85 juveniles produced. The count upon release was 51.
4. Observations in the 2005 and 2006 studies indicated that early pelagic larvae may be surface or periphyton feeders. This behavior, if verified, would be very different from other pelagic larvae.
5. Diet as well as preferred habitat used in the 2006 study was based upon the results of the 2004 and 2005 studies.
6. Observations further show that the benthic larvae were instinctively timid.
7. The digital camera was unsuccessful at documenting spawning behavior.
8. Each consecutive study has resulted in a significant increase in the survival rate from 0% in 2004, 0.01% in 2005, to 14% in 2006.

9. Forty-five juveniles were reintroduced into the Pigeon River at the Denton site (PRM16.5) on 6 April 2007 (Figure 1).
10. Stunting was the biggest problem encountered in the 2006 trial. Stunting was documented in the grow-out tanks and may have occurred as early as the pelagic stage.

It is recommended that future research be conducted to determine what the optimal stocking density of the grow-out tanks should be. This might drastically reduce stunting, resulting in a more robust, viable cohort of tangerines available for release into the Pigeon River.

It is recommended that a study be conducted to ensure the larvae are getting adequate nutrition. Techniques developed to culture macroinvertebrates commonly found in natural streams would be of great benefit. The author would also recommend that eggs be allowed to hatch in the spawning tank and the larvae collected by a passive live capture system. A passive live capture system has shown to be beneficial with other darters at CFI and could be modified for the tangerines. This would eliminate the eggs from being disturbed and potentially damaged. An increase in survivorship and a decrease in deformities could possibly be achieved through this method.

It is important to remember that captive propagation is not a typical experiment that has concrete methods that can be replicated from year to year. This project is still in the early stages and is fluid by necessity. The methods and techniques change and evolve as lessons are learned and our knowledge of how tangerine darters interact in captivity grows. We can simply provide a general

protocol that must be adapted to encompass the current circumstances at hand. With each successful spawn, more information is gained and the process adjusted. In spite of the difficulties encountered thus far, the progress made suggests that we can anticipate production of significant numbers of tangerine darters will soon.

In short, captive propagation is a valuable resource for managers. It can be used to restore populations of extirpated species, which might otherwise be unable to recolonize due to natural or man made barriers, and possibly to augment existing populations.

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