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Confirmation of Potential Cyprinid Hosts for a State Threatened Freshwater Mussel of East Texas

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CONFIRMATION OF POTENTIAL CYPRINID HOSTS FOR A STATE THREATENED FRESHWATER MUSSEL OF EAST TEXAS

by

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Abstract

CONFIRMATION OF POTENTIAL CYPRINID HOSTS FOR A STATE THREATENED FRESHWATER MUSSEL OF EAST TEXAS

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Freshwater Unionid mussels exhibit a unique life cycle in which their larvae, called glochidia, are ectoparasites on the gills or fins of an obligate host-fish species. The attachment to a suitable host which can range from only one to several species of fish, is required for their development into a juvenile. The juveniles eventually release from the host-fish to continue development into adulthood. In East Texas, six of the 37 mussel species are listed as state threatened: Texas pigtoe (*Fusconaia askewi*), Southern hickorynut (*Obovaria arkansasensis*), Sandbank pocketbook (*Lampsilis satura*), Triangle pigtoe (*Fusconaia lananensis*), Louisiana pigtoe (*Pleurobema riddelli*), and the Texas heelsplitter (*Potamilus amphichaenus*). Of these, only the Sandbank pocket book has a confirmed host-fish on record. Understanding the fish and mussel interactions and their community structures is important in developing effective conservation practices for these highly imperiled species. This study investigated previously suggested Cyprinid host-fish for the state threatened Texas pigtoe (*Fusconaia askewi*) and Louisiana pigtoe (*Pleurobema riddelli)*. The methodology for the current study allowed for the natural development and natural drop off of juveniles from fish that were infested in the wild.

This methodology was used to validate the previously suggested hosts. The juveniles were molecularly identified with amplification of mitochondrial DNA (mtDNA) using the ND1 gene. The DNA was sequenced and compared to the NCBI database and cross-referenced with an adult molecular key that was created for all 37 mussel species that occur in East Texas.

All 15 juveniles that could be sequenced were identified as *F. askewi* despite having very little genetic variation with *Fusconaia lananensis*. All juveniles were identified as *F. askewi* based on the range of *F. askewi* and *F. lananensis* and the locations from where the infested fish were collected. The Red shiner (*Cyprinella lutrensis*) as a previously suggested host for *F. askewi* was confirmed, and in addition the Blacktail shiner (*Cyprinella venusta*) was also a confirmed host for *F. askewi*. The Bullhead minnow was also previously suggested as a host for *F. askewi*, but was rejected as a suitable host in this study.

The level of infestation of these target fish species during May, June, and July of 2014 was similar to the previous reports with the exception of a large peak in infestation or glochidial release in August from an additional site. Estimated dates of glochidial release by *F. askewi* were made based on the date the infested fish were collected from the wild and the length of time the glochidia or juveniles of *F. askewi* were attached to the fish in the lab. This time frame was also used to estimate the metamorphosis rate for *F. askewi*.

The rate of metamorphosis was found to be associated with the water temperature during collection from the wild. Juveniles released from fish that were collected from warmer waters (mean= 28^{o} C) within an average of three days. Juveniles released from fish that were collected in colder waters (mean= 25^{o} C) within an average of 8.4 days. *F. askewi* uses two species of fish of the Cyprinid family. Further investigation of other families of fish as potential hosts should be tested to confirm that they do not utilize multiple families of fish. Other driving factors for the

state threatened status of *F. askewi* other than being a host-fish specialist should be identified. Specifically, its separation from *F. lananensis* should be further investigated to confirm or revise the conservation status of this species.

Chapter One

Freshwater Unionid Mussels: Their significance, imperilment, and life history

Ecosystem services and Imperilment

One of the most ecologically important groups of organisms that aids in sustaining freshwater ecosystems is the Unionid freshwater mussels. The biological activities of freshwater mussels directly affect the water quality and the benthic substrate, the distribution and abundance of other benthic and planktonic organisms, and facilitate algal growth (Spooner and Vaughn, 2006). Unionids are filter feeders that cycle nutrients and gases, and provide organic matter that supports the feeding requirements of surrounding organisms. The biological activities of mussels are thus significant ecosystem services for freshwater systems (Spooner and Vaughn, 2006). Along with their life style, their presence alone is significant in freshwater systems as surface area for algal growth, while also providing stability for benthic sediment much like trees keep river-banks intact. Freshwater mussels have also been suggested as "indicator species" because of their vulnerability and sensitivity to pollution and climate change (Gillis, 2011). A major decline in freshwater mussel abundance and diversity is an indication of a negative change in their environment, and may also indicate a decrease in abundance of other aquatic organisms that are dependent on their presence (Williams et al., 1993).

Unionid mussels are distributed in fresh waters throughout the world; the highest diversity is found in North America, where they are among the most imperiled group of organisms (Strayer and Smith, 2003). For example, of the estimated 300 species native to North America, 37 that were extant in the 19th century are now extinct, and 105 species are currently at risk or critically imperiled (Master et al., 2000). This severe imperilment is mostly a result of

anthropogenic disturbances. Historically, several species of freshwater mussels have gone extinct or were near extinction because of over-harvesting and exploitation for their pearls, meat, and shells (Strayer, 2008). Their uses and exploitation dates back to the early settlements of Native Americans (Howells et al., 1996).

Threats

Currently, habitat destruction from building dams and bridges, channel modifications, ATV crossings over mussel beds, siltation, and also polluted runoff water are all unrelenting sources of the decline of freshwater mussels (Williams et al., 1993; Haag, 2012). In addition to human disturbances, other biota negatively affect freshwater mussel habitats and survivorship. For example, the zebra mussel (*Dreissena polymorpha*) was introduced from Europe around 1985 and has been spreading and taking over native mussel habitat. Zebra mussels and other invasive bivalves such as quagga mussels (*Dreissena rostriformis bugensis)* cause high mortality of native mussels. These invasive bivalves attach to native mussels and suffocate them while also starving the water of available microalgae and nutrients that Unionids feed on (Drake and Bossenbroek, 2004; Orlova et al., 2005). Conversely, the presence of other biota such as specific species of freshwater fish, are vital for mussels' growth and developmental stages, continued reproduction, and survival. Almost all Unionid freshwater mussels exhibit a unique life-cycle that is dependent on a fish as a host for its ectoparasitic larvae called glochidia. These glochidia encyst on the gills or fins of its host-fish where they develop into juveniles. As many of these fish-mussel relationships are obligate, the absence of certain fish species can have a negative effect on mussel survivorship. There are only a few exceptions to this parasitic requirement. Some long-term brooding species of mussels hold their glochidia within the marsupial gills for development into the juvenile state before they are released (O'Dee and

Watters, 1998; Bauer and Watchtler, 2001). For example, Sphaeriidae is a family of Unionids that use internal development and do not exhibit the life cycle that is dependent on a host-fish (Watchtler et al., 2001).

Life Cycle

Dioecious freshwater mussels reproduce sexually through broadcast spawning. Males that are upstream from females will release their sperm into the water column. Females can then intake the sperm through their incurrent syphon to fertilize and brood the eggs in the marsupial gill tissue making the females gravid (Bauer, 1987; Bauer and Watchtler, 2001). The brooding process allows the fertilized eggs to develop into larval glochidia (Strayer, 2008). Some species of freshwater mussels will spawn in the autumn months and brood their young over the winter months as long-term brooders or bradytictic species. As the water temperature warms in the spring, female mussels begin to release their glochidia through the spring and summer to attach to their suitable host-fish. Other species of mussels will spawn in the spring and brood over the summer months as short-term tachytictic species. Before the water temperatures become too cold again, the females release their glochidia in the late summer or early fall months (Bauer and Watchtler, 2001; Gillis, 2011).

Gravid female mussels will use various mechanisms to attract fish-hosts or to ensure that their glochidia come into contact with a fish. Some Unionids (e.g. *Lampsilis* species) use their mantle as a lure to attract fish that will see it as a prey item (Zanatta and Murphy, 2006). Some species of mussels, like the Snuffbox (*Epioblasma triquetra)*, will clamp down on the fish's head to purge the glochidia into the gills of the fish. Other mechanisms such as conglutinates, or a mucus net of thousands of glochidia, are released into the water column to also look like a food item for fish to ingest (Bauer and Watchtler, 2001).

The essential part of a glochidia's continued development and survival occurs when it is attached to its obligate host-fish where it metamorphoses into a juvenile. The duration of metamorphosis to the juvenile state on a host-fish can range from several days to weeks depending on the species of mussel and environmental conditions (Strayer, 2008; Taeubert et al., 2013). When glochidia encyst on their proper host-fish species, they are able to metamorphose into fully developed juveniles with the protection and nutrients of the fish gills (O'Connell and Neves, 1999; Bauer and Watchtler, 2001). When a glochidia makes an encystment in the gill tissue of an unsuitable host, the glochidia is rejected by an immune response exhibited by the fish. The glochidia then release or is actively rejected from the encystment before metamorphosing into a juvenile and is then unable to survive (Watters and O'Dee, 1996; Haag, 2012). The attachment to unsuitable hosts or 'accidental infestations' frequently occur in nature. Some Unionids are host-fish specialists that are only able to utilize one species or one specific family of fish as suitable hosts. Thus, the likelihood that their glochidia attach to their obligate host as opposed to an improper host, is low compared to those that are host-fish generalists. Generalists can utilize several species or two or more different families of fish (Neves et al., 1985). Generalists may have more success in proper attachment to obligate host-fish in the wild than host-fish specialists.

Freshwater Mussels of East Texas

Because of this co-evolutionary relationship and dependence of mussels on their obligate host fish species, it is extremely important to know the distribution of fish in freshwater ecosystems and their roles as hosts. With this, we can understand the distribution and successful reproduction of freshwater mussels. In particular, it is important to understand host-fish interactions of mussel species that are threatened or endangered. Unfortunately, only 47% of the

estimated 51 species in the state of Texas have known host-fish species with very few of those being threatened or listed species of mussels (Howells et al., 1996; Winemiller et al., 2010; Marshal, 2014). In East Texas, there are 37 species of freshwater mussels and six of them are listed as state threatened: Texas pigtoe (*Fusconaia askewi*), southern hickorynut (*Obovaria jacksoniana*), sandbank pocketbook (*Lampsilis satura*), triangle pigtoe (*Fusconaia lananensis*), Louisiana pigtoe (*Pleurobema riddelli*), and the Texas heelsplitter (*Potamilus amphichaenus*).

The ranges of these species in Texas are restricted to the Neches, Red, Sabine, and Trinity River drainages (Howells, et al. 1996). The host-fish for freshwater mussels of East Texas including state threatened species such as the Texas pigtoe and Louisiana pigtoe, have previously been investigated (Marshall, 2014). These two listed species of mussels have been targeted in this study to confirm their suggested host-fish species or to identify additional obligate host-fish.

Host-Fish Testing

There are multiple approaches or methods in testing for host-fish species for freshwater mussels. Although each method has provided to be useful in understanding fish to mussel interactions, some of these methods have disadvantages. Some studies as well as propagation facilities use artificial infestations in the lab. The artificial method involves either feeding conglutinates to the fish or extracting glochidia from a gravid female mussel and introducing them to a proposed host-fish. The fish are typically infested in an artificial habitat with induced water circulation to allow glochidia to attach to the fish. These infested fish are then housed in holding tanks in a lab where the glochidia or metamorphosed juveniles of the mussel species can drop off of the fish and be collected (O'Dee and Watters, 1998). In some instances, the particular fish to mussel relationship that is created in the lab can result in successful juveniles (Zale and

Neves, 1982; O'Dee and Watters, 1998). This can be useful in indicating the suitability of the fish species as a host. The disadvantage of this method is that it is notably artificial and not a representation of an event that would occur naturally. In other words, this method does not account for the success glochidial attachment to the particular fish species in the wild. Environmental pressures as well as changes in the composition of fish and mussel populations that may occur overtime will also alter these proposed fish-mussel pairings (Haag and Warren, 1998). Mussels that are generalists will sometimes have a temporal change in their use of fish-hosts in relation to the season, environment, or host-fish availability (Haag and Warren, 1998; Bauer and Watchtler, 2001). In addition, lab infestations only provide a limited amount of mussel to fish pairings to test. A larger variety of mussels and fish species co-occur in the wild than can be tested in the lab. Thus, further study of other predicted fish-hosts should be tested for as well as an account for the natural availability of the predicted host-fish that is represented in the wild and during particular seasons.

Other methods include collecting fish from the wild that have been naturally infested with glochidia. The fish are examined for glochidial encystment on their gills or fins. Any glochidia that are found can then be removed and identified to species through genomic DNA extraction and sequencing (Marshall, 2014). This is a more natural approach than other methods as the infestation event has happened in the wild. This method has provided evidence of the natural mussel to fish interactions, a timeline of estimated glochidial release events, and the identification of possible host-fish (Marshall, 2014). However, although some fish may be highly infested with a particular species of mussel, it is not enough evidence to state that it is a host-fish. As accidental infestations frequently occur in the wild, evidence of the metamorphosis of glochidia into successful juveniles is required to confirm a fish as a suitable host (Neves et al.,

1985; O'Dee and Watters, 1998). Fish that are not suitable hosts have a natural immune response within the gill tissue, which rejects the incompatible glochidia within approximately three to 11 days after infestation (Neves et al., 1985;Watters and O'Dee, 1996). In addition, fish that are continuously infested with glochidia have the ability to develop an acquired immune response thus preventing the fish from being a suitable host (Neves et al., 1985; Watters and O'Dee, 1996).

A host-fish identification study was completed at the University of Texas at Tyler in efforts to identify suitable host-fish species for freshwater mussels of East Texas by Marshall (2014). For this study, naturally infested fish from the wild were collected and examined for glochidial encystement. A molecular identification dataset was created and used to identify the glochidia to species. Sequences of all 37 mussels that occur in East Texas were obtained using adult tissue samples of these mussels with the amplification of mitochondrial DNA (mtDNA) using the ND1 gene. This key was used to compare the sequences of the glochidia that were collected from the fish gills to the adult sequences to accurately identify them.

The results of this study have shown that the most highly infested fish species of the Texas pigtoe and Louisiana pigtoe were the Red shiner (*Cyprinella lutrensis*), Blacktail shiner (*Cyrpinella venusta*), and the Bullhead minnow (*Pimephales vigilax*) (Marshall, 2014). These fish species were also infested to a degree with various other species of mussels that inhabit the Sabine and Neches Rivers such as the Three-ridge (*Amblema plicata*) and the Bleufer (*Potamilus purpuratus*) (Marshall, 2014). With knowledge of this community of fish and mussel populations in East Texas Rivers, these three species of fish were targeted in this study as hosts for the Texas pigtoe and the Louisiana pigtoe, or possibly as generalist hosts of various

freshwater mussels in these drainages. However, modified methods have been used to validate these fish as suitable hosts or to possibly eliminate them as suitable hosts.

Purpose and Objectives

The target fish species: Red shiners, Blacktail shiners, and Bullhead minnows, were collected from the same sites on the Sabine River and Neches River that were visited by Marshal (2014) with the addition of a third site on Lake Fork Creek off of Highway 80 which is also inhabited by the Texas pigtoe (Figure 1). The fish were then housed in the lab to examine the natural drop off of glochidia or fully metamorphosed juveniles. Successfully metamorphosed juvenile mussels that released from fish, were identified to species through DNA sequencing. The sequences were then compared to those provided in the NCBI database as well as with the adult molecular key that was created by Marshall (2014). The purpose of this study was to confirm or reject previously suggested hosts by Marshall (2014) in providing a more accurate representation of the fish-mussel interactions and the juvenile development that occurs in nature. Additionally, the current study aims to provide continued or short-term monitoring data of the fish and mussel interactions and in East Texas.

Chapter Two

Determining naturally occurring host-fish for threatened Unionids of East Texas

Introduction

Freshwater Unionid mussels are one of the most widespread and diverse groups of aquatic organisms. They are found throughout North and Central America, Europe, Asia, and Africa with an estimated species richness of 707 species (Strayer, 2008). Freshwater mussels are most abundant and diverse in North America- which is inhabited by 300 species worldwide (Winemiller et al., 2010). However, despite their diversity, they are also the most imperiled groups of organisms in North America (Master et al., 2000; Strayer and Smith, 2003). Historically, freshwater mussels were over-harvested for their pearls, shells, and meat. The exploitation of mussels had induced the start of their decline resulting in several species going extinct before the 21st century (Howells et al., 1996; Strayer, 2008). Anthropogenic disturbances continue to be the main contributors to their decline. Specifically, the building of damns and bridges, channel modifications, siltation, polluted runoff water, and the introduction of invasive species have increased freshwater mussel imperilment (Williams et al., 1993; Howells et al., 1996; Drake and Bossenbroek, 2004; Haag, 2012).

Because of these anthropogenic disturbances, approximately 71% of freshwater mussels in North America are considered endangered, threatened, or of special concern, and approximately only 23% of mussel species are considered stable (Williams et al., 1993; Neves, 1997). Despite the widespread distribution and diversity of freshwater mussels, they received very little attention until the late 1990's (Howells et al., 1997; Winemiller et al., 2010). Freshwater mussel conservation is now one of the most significant fields of study in understanding the conservation of aquatic ecosystems (Spooner, 2006; Haag, 1998; Haag, 2012).

Freshwater mussels are considered 'indicator species' in that they can provide evidence of a negative change in their environment. They also provide valuable ecosystem services that influence the diversity and abundance of other benthic and planktonic organisms (Gillis, 2011). Freshwater mussels are filter feeders that cycle nutrients and gasses, produce organic matter that is required and utilized by contiguous organisms, facilitate healthy algal growth, and provide stability to the benthic substrate (Spooner and Vaughn, 2006).

In comparison to the understanding of freshwater mussels' ecological role, the co-evolutionary relationship between freshwater Unionid mussels and fish is poorly studied. This relationship, or life history strategy, is unique to almost all Unionid freshwater mussels and is a requirement for their survival and continued reproduction. The life cycle involves an ectoparasitic stage where the larvae (glochidia) attach to the gills or fins of their obligate host-fish species. When a glochidia attaches and makes and encysts in the tissue of its proper host-fish, it develops into a juvenile within days to several weeks. The metamorphosis rate can highly vary depending on the water temperature, water chemistry, or health of the host-fish (Steingraeber et al., 2007; Taeubert et al., 2013). Juveniles then release from the fish and continue to develop into reproductive adults. However, accidental infestation events occur frequently in the wild. When glochidia attach to an unsuitable host, they are rejected by an immune response exhibited by the fish. As a result, the glochidia release from the encystment before they can metamorphose into a juvenile and are then unable to survive (Watters and O'Dee, 1996; Haag, 2012).

There are various approaches in testing for host-fish species. However, each methodology has several disadvantages in producing reliable host-fish data. As a result, there is very little knowledge of the mussel to host-fish relationships that occur in nature and thus an

insignificant amount of successful conservation efforts. These methods include artificial infestations in the lab or the sampling of infested fish from the wild with the identification of encysted glochidia using morphology or molecular genetics (Zale and Neves, 1982; O'Dee and Watters, 1998; Martel and Lauzon-Guay, 2005; Marshall, 2014).

Although each of these methods have provided a baseline understanding of the Unionid life cycle, reliable host-fish data is still lacking. The sampling of naturally infested fish from the wild does not provide evidence of the required metamorphosis into to the juvenile state after naturally attaching to a host in the wild. This methodology fails to recognize the highly infested fish species as a result of accidental infestations that occur in the wild. Artificial infestations in the lab are not capable of incorporating the fish and mussel communities and environmental conditions that occur in nature and change over time. In lab infestations do not incorporate the possibility of freshwater mussels as host-fish generalist that may use an abundance and diversity of fish species or families of fish during specific seasons and environmental conditions (Neves et al., 1985; Bauer and Watchtler, 2001; Gillis, 2011) As a result of host-fish studies, 300 host-fish species have been suggested for various Unionids. However, only one-third of these suggested host-fish have been confirmed with evidence of glochidial metamorphosis into juveniles (O'Dee and Watters, 1998).

More importantly, very few studies have confirmed viable host-fish for species of concern. Texas is inhabited by an estimated 51 species of freshwater mussels (Winemiller et al., 2010). Only 47% of these species in Texas have confirmed host-fish on record with very few of those being among the 15 state threatened species (Howells et al., 1996; Marshall, 2014). In East Texas alone, there are 37 freshwater mussel species, six of which are listed as state threatened: Texas pigtoe (*Fusconaia askewi*), Southern hickorynut (*Obovaria jacksoniana*), Sandbank

pocketbook (*Lampsilis satura*), Triangle pigtoe (*Fusconaia lananensis*), Louisiana pigtoe (*Pleurobema riddelli*), and the Texas heelsplitter (*Potamilus amphichaenus*). These six species are restricted amongst the Neches, Red, Sabine, and Trinity River drainages of East Texas (Howells et al., 1996).

The host-fishes for these state threatened mussel species and others that co-occur have previously been investigated by Marshall (2014). Fish that were naturally infested in the wild were sampled from the Sabine and Neches Rivers that are inhabited by an abundance and diversity of the state threatened mussels in East Texas (Marshall, 2014). A molecular identification data set was then created for all of the 37 mussel species that occur in East Texas (Marshall, 2014). The sequences for the dataset were obtained using tissue samples from adult mussels with the amplification of the ND1 gene. All tissue samples were obtained from adult mussels that inhabit the same rivers that fish were sampled from. The use of this molecular identification key thus provides more accurate results in identifying glochidia or juvenile mussels to species (Marshall, 2014). The dataset also incorporated sequences from the National Center for Biotechnology Information database (http://www.ncbi.nlm.nih.gov), for a total of 180 sequences within the 37 mussel species located in East Texas. The glochidia were collected from the fish gills or fins and were identified to species by comparing their sequences to the adult molecular key (Marshall, 2014). The Red shiner (*Cyprinella lutrensis*), Blacktail shiner (*Cyrpinella venusta*), and the Bullhead minnow (*Pimephales vigilax*) were among the most infested fish out of the 23 other species of fish that were infested. These three fish species were densely infested with glochidia of the state threatened Texas pigtoe and Louisiana pigtoe (Marshall, 2014).

The purpose of this study is to confirm or reject these previously suggested host-fish species for two state threatened freshwater mussels of East Texas. This study also aimed to provide subsequent data of freshwater mussels and their relationship with fish in East Texas river drainages. This study uses a modified approach from previous host-fish testing methods by housing the naturally infested fish in the lab in Aquatic Habitat Tank units or AHAB units. The objective in using this methodology was to validate or reject hosts by providing evidence of the natural development and release of fully metamorphosed juveniles or the rejection of glochidia. To continue the investigation of the mussel-fish relationships in East Texas, a short-term pattern or possible change in the major glochidial release events between the 2013 and 2014 sampling years was to be compared. In addition, the possible temporal change in the use of fish-hosts for any identified juvenile mussels was to be analyzed. Lastly, the duration of metamorphosis to the juvenile state was also estimated for any identified juvenile mussels.

Materials and Methods

Field Sites and Sampling Dates

Red shiners, Blacktail shiners, and Bullhead minnows were collected from the Sabine River near Highway 14, the Neches River near Highway 294, and from Lake Fork Creek off of Highway 80 in East Texas (Figure 1). These sites were chosen based on previous mussel survey data that found an abundance of several state threatened mussel species and an abundance of fish infested with glochidia in the spring and summer of 2013 (Marshall, 2014; Winnemiller et al., 2010; Ford, 2013). The peak time of glochidial release is typically in May from mussel species that brood over the winter months, and again in October for mussel species that brood in the summer months (Gillis, 2011; Marshall, 2014). Red shiners, Blacktail shiners, and Bullhead minnows, among other fish that inhabit the Sabine and Neches rivers, were the most highly infested with glochidia during late April to early May and early June to late July of 2013 (Marshall, 2014). Fish were collected in the spring and continued throughout the summer into early fall of 2014 from the Sabine and Neches Rivers (Table 1). A site on Lake Fork Creek was sampled on August 4, 2014 as an additional site to increase the sample sizes of target fish-host species during times of high flow on the Sabine and Neches Rivers.

Table 1. The 2014 sampling dates for infested fish from the Sabine River, Neches River, and Lake Fork Creek

Date	Site
May 29th	Sabine
June 3rd	Sabine
July 10th	Sabine
July 11th	Neches
August 4th	Lake Fork Creek
August 7th	Neches
October 23rd	Sabine
October 24th	Neches

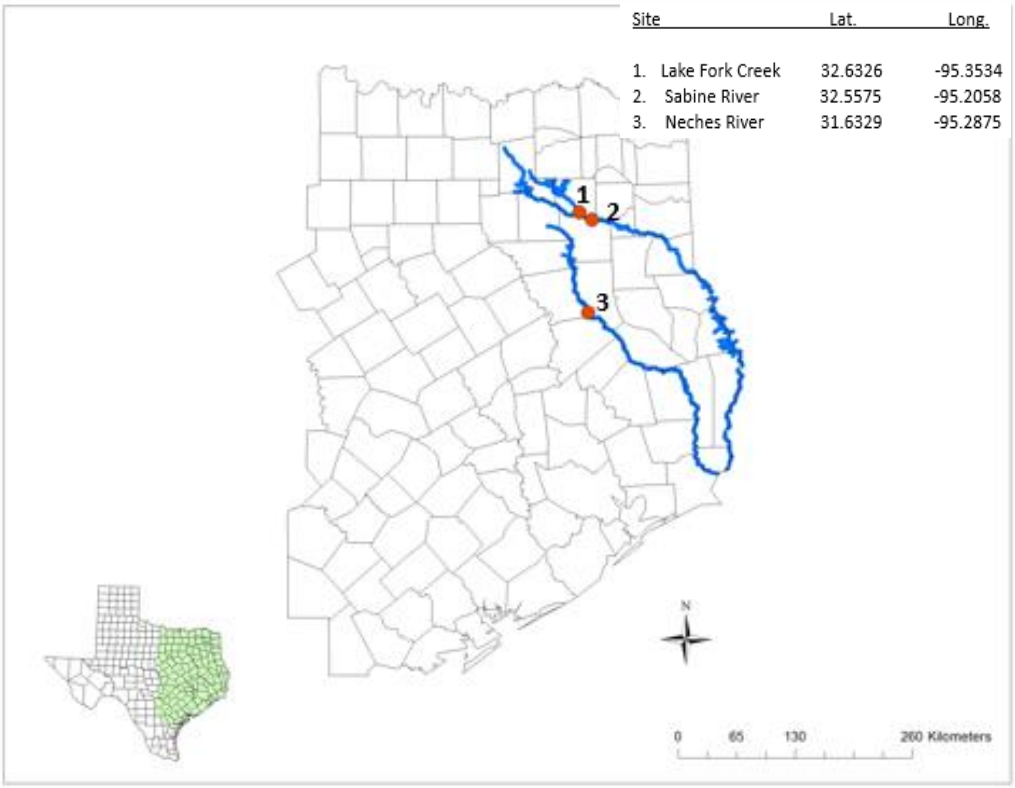

Figure 1. Locations from the 2013 (Marshall, 2014) and 2014 sampling season for collection of Red shiners, Blacktail shiners, and Bullhead minnows as targeted host-fish species of state threatened freshwater mussels that co-occur at these sites.

Fish Collection Methods

All fish were collected from each field site over a 150m reach within range of mussel beds using a 7.5m long bag seine net. Electrofishing was not used as a fish collection method to avoid any mortality or stress of the fish that may cause the release of any encysted glochidia. The target sample size of each fish species was set at 20 individuals of varying sizes. Water quality parameters such as temperature, pH, and conductivity were measured using a YSI multi-probe meter for each sampling event.

In-Lab Fish Housing

The fish collected from the field were brought back to the aquatics lab at the University of Texas at Tyler Biology Department. The fish were placed in individual 3L tanks of a 20 tank

Aquatic Habitat Tank unit (AHAB) by Pentair Aquatics. The fish were separated by species, the date they were collected, and the site from where they were collected. No more than seven fish were placed in each tank to avoid stress and overcrowding. If fish were larger than 3cm, only two to three fish of this size were held in a single tank. In this complex tank system, water consistently flows from the main sump tank and through various filter mechanisms and through each individual tank. On the backs of the tanks where water consistently flows through, juvenile and glochidia capturing structures that have been called "juvenile catchers" were placed (Barnhart, 2006). Juvenile catchers were 3.5cm length PVC pipe segments with 118 micron mesh netting on one end to act as a net for collecting glochidia or juvenile mussels (Barnhart, 2006). This size mesh was chosen based on the estimated size range of glochidia or juveniles for Texas pigtoes or Louisiana pigtoes (Howells et al., 1996). The water quality of the tank system was monitored using a multi-probe YSI meter to measure the water temperature, pH, and conductivity. Water chemistry kits were also used to measure the levels of nitrates and ammonia. Water quality and chemistry monitoring occurred every other day to at least once a week to keep consistent with that of the river sites where the fish were collected. All desired water quality parameters were manually achieved through the addition of buffers, sea salt, nitrifying bacteria, or water release methods with the addition of D.I. water.

Glochidia and Juvenile Collection

The juvenile catchers were removed every other day for the first two weeks of captivity and then more sporadically over the duration of each trial. The catchers were examined for glochidia or juveniles under an Olympus SZ dissection microscope (Figure 2). The number of glochidia or juveniles were recorded for each tank. If an abundance of roughly >100 glochidia or juveniles were present, an estimated number was recorded. Sub samples of at least 20 individuals

were collected to be used for genetic identification. If less than 20 individuals were found in a catcher, at least 10 individuals were collected to be used for identification. Each individual was placed in separate 1.5 mL centrifuge tubes with 95% ethanol and stored at -20^{o} C. All remaining glochidia or juveniles of each catcher were collected together and preserved. The gills of any deceased fish were also examined for glochidial encystment. The glochidia from fish gills were also counted and collected to be included in the level of infestation for each fish species. After fish were held in captivity for approximately 3-6 weeks, half of the first trial was removed and anesthetized to make more room for additional trials of fish from another sampling date or site. The gills of these removed fish were also examined for glochidial encystment to predict the probability of the other half of the trial of fish to still be infested with glochidia. In the event that glochidia were encysted on the removed fish, the remaining fish were kept in the tanks at least one week longer.

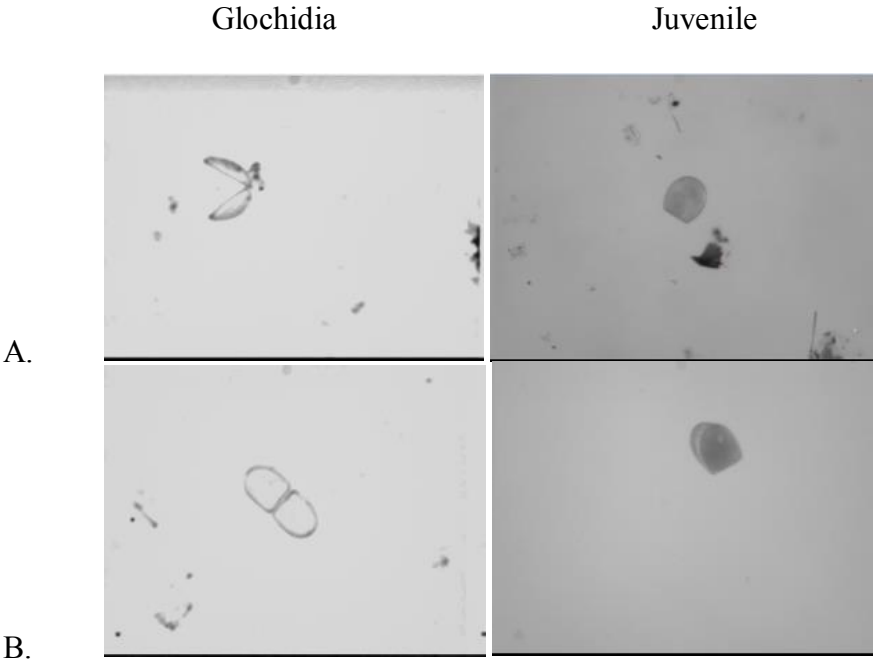

Figure 2. Comparison of glochidia versus metamorphosed Unionid juveniles. (A.) Glochidia and a juvenile that had naturally dropped off of Bullhead minnows. (B.) Glochidia and a juvenile that had naturally dropped off of Red shiners.

DNA Sequencing and identification

Genomic DNA was extracted from individual juveniles and glochidia using a Chelex double-stranded DNA extraction protocol (Casquet et al. 2011) conducted in Dr. Placyk's molecular ecology lab at the University of Texas at Tyler. The protocol described in Casquet et al. (2011) uses a 1:15 ratio of Proteinase K to 10% solution of Biotechnology Grade Chelex 100 resin solution. This protocol is specific for a small quantity of ethanol-stored tissue. However, a slight modification was made by adding 50µL instead of 150µL of the 1:15 solution to each individual to avoid diluting the genomic DNA that is extracted from glochidia and juvenile mussel tissue versus the tissue from small spiders that was used in Casquet et al. (2011). The denaturation step in DNA extraction is removed in this protocol to create a one-step method to decrease the amount of handling of the tissue sample as well as to yield double-stranded DNA compared to other classic Chelex extraction protocols that produce single-stranded DNA

(Casquet et al. 2011). Extracted DNA was stored at -20° C until use in polymerase chain reactions (PCRs). The primers Leu-uurF (TGGCAGAAAAGTGATCAGATTAAAGC) and LoGlyR (CCTGCTTGGAAGGCAAGTGTACT) were used to amplify mitochondrial (mtDNA) NADH dehydrogenase (ND1) gene (Serb et al., 2003). PCR reactions used for amplification of the ND1 gene consisted of 20 µL: 6.7 µL purified H_2O, 0.1 µL TopTaq PCR buffer (Qiagen), 0.4 µL dNTPs, 2.0 µL 10X Coral Load (Qiagen), 4.0 µL Q-Solution, 1.0 µL of each primer, 0.4 µL bovine serum albumin (BSA), and 2.4 µL of DNA (~150 ng/µL). An extra 10% of the PCR reaction was created to provide a negative control with each PCR. An Eppendorf Mastercycler gradient thermal cycler with a heated lid was used to amplify the reactions. The reaction settings for amplification of double-stranded DNA were as follows: 94° C for 5 minutes, 30 cycles of 94° C for 45 seconds, 54° C for 60, and 72° C for 60 seconds followed by a final extension of 72° C for 5 minutes. Gel electrophoresis was used to test the quality of amplification. The successfully amplified PCR products were purified using and E.Z.N.A. cycle pure kit (Omega bio-tek, Norcross, GA) following the protocol with an additional 30 µL of purified water for re-suspension. Purified DNA was concentrated at 17-20 ng/ µL with a 260/280 ratio around 1.8 to 2.0 as recommended by Eurofins MWG Operon where reactions were shipped to for sequencing using BigDye Terminator v 3.1 Cycle Sequencing kits (Applied Biosystems). Sequences were edited with the Sequencher 5.2.4 program and then initially compared with freshwater mussel sequences available on the National Center for Biotechnology Information database (http://www.ncbi.nlm.nih.gov). The edited sequences were also cross-referenced with an adult molecular key that provides sequences for all the 37 freshwater mussel species that occur in East Texas (Marshall, 2014). The tissue samples from the mussels used to create the molecular key included adult mussels collected from the same fish sampling sites on the Sabine River (HWY

14) and Neches River (HWY 294) in this study. ClustalX2.0.11 was used to generate an alignment file of the juvenile sequences with the adult sequences of the molecular key. The alignment file from ClustalX2.0.11 was then uploaded into Mesquite (version 2.75) to provide ocular observation of the alignment with the sequences of the molecular key.

Estimating Glochidial Release Dates and Metamorphosis Rates of Juveniles

The total number of glochidia and juvenile mussels that released in the lab, as well as the number of encysted glochidia on the gills of deceased fish, were combined for a measurement of total infestation for each target host-fish species. These data were used to compare the total level of infestation between each fish species from each sampling month of 2014 as well as to estimate the major glochidial release events in these rivers in the spring and summer of 2014. The amount of time for metamorphosis into the juvenile state for identified juvenile mussel species was estimated using the number of days the identified juveniles were encysted on their host-fish since the date the fish were collected. The mean number of days before juveniles released in the lab since fish were collected was compared between the sampling months to estimate variable metamorphosis rates between the spring and late summer or early fall months.

Results

Abundance and Temporal Levels of Infestation on Wild-Caught Fish

A total of 114 Red shiners, 87 Blacktail shiners, and 46 Bullhead minnows were collected from either the Sabine River, Neches River, or Lake Fork Creek over the sampling period from May 29, 2014 to October 24, 2014. The 46 Bullhead minnows were on average the most infested species with a mean of 14.3 glochidia, the 114 Red shiners were infested with a mean of 6.97 glochidia, and the 87 Blacktail shiners were infested with a mean of 2.46 glochidia.

The Bullhead minnows were infested with a total of 658 individual freshwater mussels from May 29, 2014 through August 18, 2014. Of these, two were juveniles that naturally

dropped off in the lab, and 656 were glochidia that naturally dropped off in the lab (Figure 3). No glochidia were found to be encysted on the gills of deceased Bullhead minnows. The highest peak in infestation was from three Bullhead minnows that were collected from the Sabine River on July 10 with a total of 405 glochidia (Figure 4). The second and only other peak of infestation was from two Bullhead minnows that were collected from the Sabine River on June 3 (Figure 4). The two juveniles that were collected were also from these two Bullhead minnows collected on June 3 from the Sabine River.

The Red shiners were on average the second most infested target fish species. Amongst the 114 Red shiners there were collected, a total of 243 juveniles had naturally dropped off in the lab, and a total of 584 glochidia had naturally dropped off in the lab (Figure 3). The total number of glochidia that were still encysted on the gills of deceased Red shiners was 73. In total, Red shiners were infested with 795 freshwater mussel individuals from May 29 until August 18th. The highest peak in infestation of Red shiners was from 46 individuals that were collected on May 29 from the Sabine River (Figure 4). A total of 219 mussel individuals were collected from these Red shiners from May 29 until June 7. Of these, 111 were juveniles and 205 were glochidia that naturally dropped off of the fish. A total of three glochidia were found to still be encysted on deceased Red shiners from this sampling date. An additional peak in the infestation level for Red shiners occurred in August from 11 Red shiners that were collected on August 4 from Lake Fork Creek. These Red shiners were infested with a total of 213 individuals of freshwater mussels. Of these, 13 were juveniles, 172 were glochidia, and 33 were glochidia that were still encysted in the gills of deceased Red shiners. In terms of successful juvenile release from Red shiners, a relatively large amount of juvenile mussels also naturally dropped off of 26

Red shiners collected on July 10 from the Sabine River (n=45), and from 15 Red shiners collected on August 7 from the Neches River (n=67).

Among the 87 Blacktail shiners there were collected, the total number of juveniles that had naturally dropped off of Blacktail shiners was 68 and the total number of glochidia that naturally dropped off was 79 (Figure 3). A total of 67 glochidia were found still encysted on the gills of Blacktail shiners. A total of 214 freshwater mussel individuals were infested on Blacktail shiners from June 3 until August 18. Similarly to the Red shiners, Blacktail shiners had a large peak in their infestation level from 23 Blacktail shiners that were collected on August 4 from Lake Fork Creek (Figure 4). A total of 135 freshwater mussel individuals were collected from these Blacktail shiners from August 4 until August 18. Of these, 18 were juveniles and 57 were glochidia that had naturally dropped off of fish, and a total of 60 glochidia were found still be encysted on the gills of the deceased Blacktail shiners. A large amount of juvenile mussels also dropped off of 22 Blacktail shiners that were collected on August 7 from the Neches River (n=25).

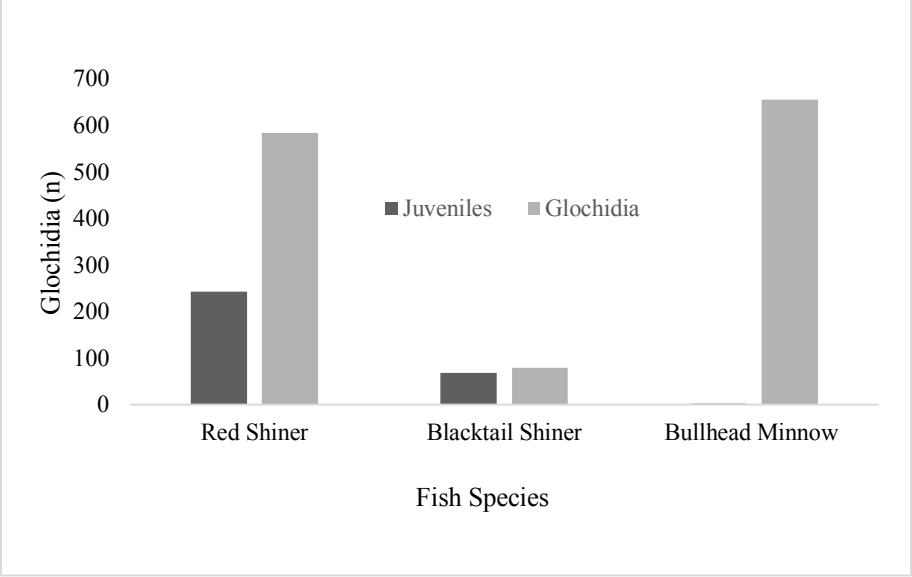

Figure 3. A comparison of the total number of glochidia and the total number of juvenile mussels that naturally dropped off of each target fish species over the entire sampling period in the Spring, Summer, and Fall of 2014

The level of infestation or the amount of glochidial release from mussels in the Sabine River, Neches River, or Lake Fork Creek, were the largest in the months of May from the Sabine River, late June and early July from the Sabine and Neches Rivers, and in early August from Lake Fork Creek (Figure 4). In comparing the infestation levels between each fish species during each sampling month, there are similar peaks or relatively the same amount of infestation in July, August, and October when all three of the target fish species were able to be collected and compared (Figure 4). From July to October, 62 Red shiners were infested with an average of 9.29 glochidia, 58 Blacktail shiners with 3.39 glochidia, and three Bullhead minnows with 135 glochidia. None of these fish species were infested to any degree in October.

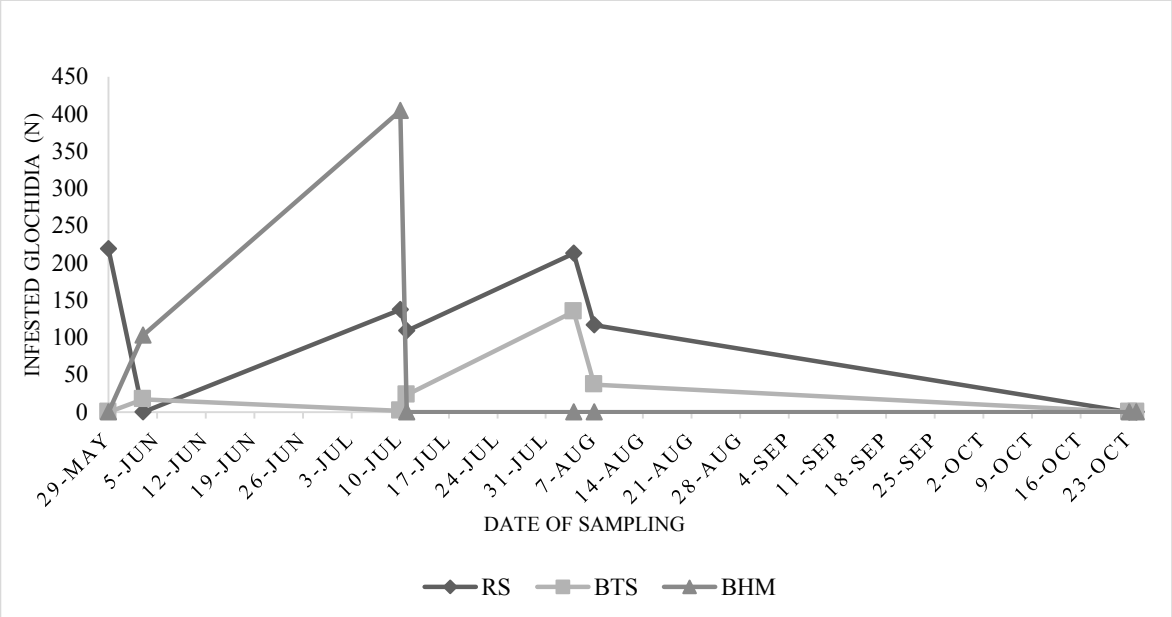

Figure 4. The degree to which each fish species (RS: Red shiner, BTS: Blacktail shiner, and BHM: Bullhead minnow) were infested with glochidia on each sampling date in the Spring, Summer, and Fall of 2014 is compared. The level of infestation is the combined number of juveniles and glochidia that naturally dropped off of each fish species collected on each date, as well as the number of glochidia still ecysted on the gills of the fish collected on each date. This is also indicative of estimated glochidial release times.

Molecular Identification of Glochidia and Juvenile Mussels

Sub samples from the juveniles and glochidia that were caught in the juvenile catchers were selected for genetic identification to species. These sub samples included juveniles that dropped off of Red shiners and Blacktail shiners that were collected from the Sabine River, Neches River, and Lake Fork Creek as well as glochidia and the two juveniles that dropped off of Bullhead minnows that were collected from the Sabine River from various sampling dates in the spring and summer. DNA was extracted for a total of 127 juveniles and 36 glochidia. A total of eight juveniles that were encysted on Red shiners, and seven juveniles from Blacktail shiners were succesfully amplified, sequenced, and identified. The two juveniles and glochidia that dropped from Bullhead minnnows were not succesfully amplified in this study.

All of the succesfully sequenced juveniles were 96-99% identical to NCBI sequences to both the Triangle pigtoe (*Fusconaia lananensis*) and the Texas pigtoe (*Fusconaia askewi*) and 14 of these were an exact match to the *F*. *lananensis* and *F*. *askewi* sequences previously generated from East Texas individuals by Marshall (2014). Only one sequence represented a haplotype not previously detected in East Texas, but this sequence was still consistent with *F*. *lananensis* and *F*. *askewi*. Previous investigations of *F*. *lananensis* and *F*. *askewi*'s distribution and relatedness has shown that they are ecologically and geographically distinct within the river drainages that they occur, yet they are genetically very simliar (Howells et al., 1996; Marshall, 2014; Burlakova, 2012). Thus, for this study, these juveniles were distinguished as being either *F*. *lananensis* or *F*. *aswewi* by their ranges in East Texas rivers and from the location where they were collected.

Red Shiner as a Viable Host for *F. askewi*

Six juveniles that were positively identified genetically as either *F. lananensis* or *F. askewi* naturally dropped off of Red shiners that were collected from the Sabine River in May and July. Because *F. lananensis* does not occur in the Sabine River and *F. askewi* is among the most abundant of the state threatened mussels in the Sabine River, these juveniles have been identified as *F. askewi* (Ford, 2013; Winnemiller et al., 2010). One juvenile naturally dropped off of one of the Red shiners that was collected from the Neches River on August 7th. Both *F. lananensis* and *F. askewi* occur within the Neches River. However, their distribution within the Neches River is not closely associated and these two species have not been found to co-occur within the Neches (Howells et al., 1996; Ford, 2013). The site on the Neches River off of Highway 249 where the Red shiners were collected is several miles North of where *F. lananensis* occurs and is not a site on the Neches where live *F. lananensis* have been found. (Howells et al., 1996; Ford, 2013) Thus, this juvenile collected from the Neches River was also identified as *F. askewi*.

One juvenile had naturally dropped off of one of the Red Shiners that were collected from Lake Fork Creek on August 4. *Fusconaia lananensis* does not inhabit Lake Fork Creek and *F. askewi* is the only state threatenend mussel species that occurs in Lake Fork Creek (Howells et al., 1996).Thus, this juvenile was identified as *F. askewi*. Despite the genetic relatedness of *F. lananensis* and *F. askewi*, all eight successfully sequenced juveniles that have dropped off of Red shiners have been identified as *F. askewi* based on the locations they were collected from and their similar morphologies.

Blacktail Shiner as a Viable Host for *F. askewi*

The seven juveniles that were sequenced and identified that naturally dropped off of Blacktail shiners were also collected from the same sites as the Red shiners and were thus also determined to be *F. askewi* and not *F. lananensis*. Two of the seven *F. askewi* juveniles naturally dropped off of Blacktail shiners that were collected from the Sabine River in June. Three *F. askewi* juveniles naturally dropped off of Blacktail shiners that were collected from the Neches River in August. Two *F. askewi* juveniles dropped off of Blacktail shiners that were collected from Lake Fork Creek in August.

Rejecting The Bullhead Minnow as a Viable Host

Although the Bullhead minnow was on average the most infested target fish species, only two out of 658 (0.3%) infested glochidia naturally dropped off as metamorphosed juveniles. The two juveniles that dropped off were among 251 glochidia that also dropped off from the same trial of fish collected from the Sabine River on June 3. Beacause of the lack of juvenile production, compared to the large sum of undeveloped glochidia that dropped off of 46 Bullhead minnows from different rivers, the Bullhead minnow is not a viable host for these species of mussels that infested them and were rejected as glochidia. The suitability of the Bullhead minnow as a host for the two juveniles that released was not determined as these juvniels were not genetically identified.

Encystment Length and Date of Glochidial Release for *F. askewi*

The 15 juveniles that were identified as *F. askewi* were on average, encysted on either Red shiners or Blacktail shiners for 5.46 days since the fish were collected. In other words, on average, these individuals stayed attached to the fishs' gills for approximately 5 ½ days after the

fish were collected before releasing as juveniles. The longest amount of time *F. askewi* juveniles were encysted was for eight to nine days. Of these, five *F. askewi* juveniles were encysted on Red shiners that were collected from the Sabine on May 29, and dropped off on June 7 and one *F. askewi* juvenile was encysted on a Blacktail shiner that was collected from the Sabine River on June 3 and dropped from the fish on June 11 (Table 2). The shortest amount of time *F. askewi* was encysted was for one to two days since the fish were collected. One *F. askewi* juvenile was encysted on a Blacktail shiner that was collected from the Neches River on August 7 and released from the fish on August 8. One *F. askewi* was encysted on a Red shiner that was collected from the Sabine River on July 10 and released from the fish on July 12 (Table 2).

The glochidia release events by *F. askewi* have shown to be persistent from the spring months through the mid and late summer months amongst all three rivers with no selected or preferred time of the year within the sampling months of this study. Using the number of days before excystment as juveniles, specific dates of release as glochidia were estimated. Those juveniles that relased after eight or nine days since the fish were collected are assumed to have been recently released as a glochidia from a gravid female since the infested fish were collected. For example, The five *F. askewi* juveniles that dropped from Red shiners on June 7 that were collected from the Sabine River nine days prior on May 29, are estimated to have been released as glochidia and attached to a host on or before May 29. One *F. askewi* that released from a Blacktail shiner after eight days that was collected from the Sabine River on June 3, was estimated to be released and attached to the fish on or before June 3 (Table 2).

F. askewi juveniles that released from fish after only one to two days since collection are then estimated to have been released as glochidia and attached to a host at least nine days prior to the collection of the infested fish. For example, a Blacktail shiner that was collected from the

Neches on August 7 had one *F. askewi* juvenile that released within one day on August 8. This juvenile is estimated to have been released as a glochida and attach to a host-fish at least nine days prior or on or before July 29. One juvenile released from a Red Shiner on July 12 that was collected from the Sabine River on July 10. This juvenile is estimated to have been released as a glochidia and attach to a host-fish on or before July 1 (Table 2).

Table 2. The number of days before all 15 *F. askewi* juveniles excysted from host-fish in the lab since the fish were collected in the field. This data was used to estimate glochidial release dates and metamorphosis rates for *F. askewi*.

Juveniles (n)	Site	Host-Fish	Sampling Date	Excystment Date	Encystment length (d)
1	Neches R.	Blacktail Shiner	Aug 7	Aug 8	1
1	Sabine R.	Red Shiner	Jul 10	Jul 12	2
2	Lake Fork Ck.	Blacktail Shiner	Aug 4	Aug 6	2
1	Neches R.	Red Shiner	Aug 7	Aug 11	4
2	Neches R.	Blacktail Shiner	Aug 7	Aug 11	4
1	Lake Fork Ck.	Red Shiner	Aug 4	Aug 8	4
1	Sabine R.	Blacktail Shiner	Jun 3	Jun 9	6
1	Sabine R.	Blacktail Shiner	Jun 3	Jun 11	8
5	Sabine R.	Red Shiner	May 29	Jun 7	9

Discussion

Molecular Identification of Juvenile Mussels

Of the three target fish species, Bullhead minnows were the most highly infested, following the Red shiner, and then the Blacktail shiner. All 15 juvniles that naturally fell off of Red shiners and Blacktail shiners, were almost 100% identical to sequences of the Texas pigtoe (*Fusconaia askewi*) and the Triangle pigtoe (*Fusconaia lananensis*). This close relationship was identified when the juvnile sequences were compared to those in the NCBI database, as well as the sequences of the molecular identification key with the exception a single haplotype that was not previously detected in the creation of the adult molecular key. The use of the ND1 gene has been suggested to be more effective in species identification than other genes such as the mitochondrial gene cytochrome oxidase c subunit I (COI) for the wider gap in intra- and interspecific genetic differences it provides (Boyer et al., 2011; Marshall, 2014). Thus, this gene provides the best option in identification of closely related organisms. It has recently been suggested that because of their low interspecific variation, *F. lananensis* is not a valid species and that only one *Fusconaia* species (*F. askewi*) is present in East Texas (Burlakova et al., 2012). These two species also have not been found to co-occur within the same locality in the Neches River using mostly morphology of adults for identification (Howells et al., 1996; Ford, 2013). While it is not widely accepted yet, it is probable, that either of these are not separate species and the morphological differences are polymorphisms. In contrast, there could have been a very recent split of the two species. Because the sites that the fish were collected from were areas where *F. lananensis* is not found to occur and in areas of high abundance of *F. askewi,* all juveniles were determined to be Texas pigtoes (*Fuscuonaia askewi*) despite the close genetic relationship and discrepency of their understanding as a species.

Further genetic analyses should be performed to either confirm the separation of these two species or to be able to eliminate *F. lananensis* as a species and identify all as *F. askewi*. This would be important in understanding their realistic distribution and conservation status. In addition, this would be important in understanding their relationship with fish hosts. Currently, *F. askewi* and *F. lananensis* as separate species means the host-fish for the state threatened *F. lananensis* is still to be determined. However, if these two species were to be combined into one, then their conservation status would need to be reconsidered and the confirmation of their known host-fish species from this study should be applied in the management of this species.

Confirming Two Previously Suggested Host-Fish for *Fusconaia askewi*

Identifying *F. askewi* juveniles that naturally dropped off of Red shiners and Blacktail shiners is evidence that *F. askewi* is possibly a host-fish specialist in terms of using two species of fish that are both in the same Cyprinid family and genus. However, additional species of fish of other famillies would need to be tested as possible hosts to truly identify *F. askewi* as a host-fish specialist or possibly a host-fish generalist. For instance, it is possible that *F. askewi* is a host-fish generalist as it has been found to be encysted on species of fish outside of the Cyprinid family (Marshall, 2014). The 15 juveniles of *F. askewi* had used both Red shiners and Blacktail shiners in all three rivers that were sampled. *Fusconaia askewi* juveniles had also persistently released from both fish species that were collected in May, June, July, and August. The consistency of *F. askewi* juveniles releasing from both species of fish from various sites and seasons provides evidence of the Red shiner and Blacktail shiner as reliable host-fish species. Although only 15 juveniles that released from Red shiners and Blacktail shiners were identified as *F. askewi*, the morphology of these juveniles were similar or not easily distinguishable

amongst the combined 311 juveniles that fell off Red shiners and Blacktail shiners. It is likely that a majority of these juveniles were also *F. askewi*.

Suitability of Host-Fish and Importance as Hosts

On average, Red shiners were the second most infested fish of the three target fish species with a total of 795 encysted individuals. Although a large percentage of these encysted individuals were rejected glochidia (70.6%) 38% of these glochidia released from Red shiners within one day of being captured. This is assumed to be related to the stress and the aggressive behavior the Red shiners exhibited from being removed from the wild and held in laboratory tanks. It is expected that because the Red shiner had a large amount of juvenile mussels succesfully release, a majority of the infested glochidia would also have been able to stay encysted and developed into fully metamorphosed juveniles without the induced stress to the fish. A majority of those glochidia are predicted to have been *F. askewi*. In addition, each time after a sampling event, the fish collection bucket was emptied of fish and seived through a juvenile catcher and an estimated >500 glochidia were found. Blacktail shiners were on average the least infested target fish species that had produced a total of 68 (46.3%) juveniles and had released 79 (53.7%) glochidia. Although, Blacktail shiners were not as infested as the Red shiner, the amount of juveniles in comparison to glochidia that naturally dropped off is still indicative of their ability to be a suitable host. Red shiners are also more likey to have higher infestation levels than Blacktail shiners because of their dominance over Blacktail shiners and other fish species in their communities (Walters et al., 2008). Red shiners are able to outcompete other minnows in foraging and eating prey items such as conglutinates that are full of glochidia

which *F. askewi* may use to attract host-fish (Haag and Warren, 1999; Bauer and Watchtler, 2001; Walters et al., 2008).

Previous investigations of these fish as hosts indicated that glochidia of Louisiana pigtoe (*Pleurobema riddellii*) were also found on the Blacktail shiners and that other species of *Fusconaia* have been found to use various Cyprinid species as hosts (Williams et al., 2008; Marshall, 2014). Cyprinids like Red shiners and Blacktail shiners may be viable hosts to mussel species that can utilize both of them like *F. askewi*, for the fact that they are closely related in the same genus, but additionally because they may hybridize (Thomas et al., 2007; Walters et al., 2008). If juvenile mussels other than *F. askewi* had dropped off of Red shiners or Blacktial shiners, it would confirm that these Cyprinids are generalist hosts or hosts to several species of freshwater mussels in these rivers. As hosts for a state threatened freshwater mussel species and possibly other co-occuring species, Red shiners and Blacktail shiners should be recognized as very important fish species in East Texas rivers.

An Unsuitable Host-Fish Species

The Bullhead minnow was also one of the most highly infested fish particularily with *F. askewi* glochida when previously investigated as a host (Marshall, 2014). However, because 656 glochidia had been rejected by the Bullhead minnows suggests that the Bullhead minnow is not a viable host-fish specifically for the species of glochidia that were rejected. In addition, only two glochidia were able to metamorphose and release as juveniles from Bullhead minnows. Although the glochidia were not able to be identified, Bullhead minnows were collected from the same sites as the Red shiners and Blacktail shiners where *F. askewi* occurs in abundance. In addition, Bullhead minnows were suggested hosts for *F. askewi* because of their high level of infestation

by *F. askewi* glochidia (Marshall, 2014). It is probable that a large amount of the glochidia that were rejected by Bullhead minnows were also *F. askewi*. However the current study is not enough evidence to eliminate the Bullhead minnow as a host for *F. askewi*, but is still indicative of their suitability as a host in these rivers.

Multiple physiological mechanisms have been suggested to be involved in the compatiability or unsuitability of fish to mussel interactions (Neves et al., 1985). There are studies that have identified humoral components (anti-body production) as the major factor in salmonids being able to reject glochidial infestation (Meyers et al., 1980). Others have suggested the properties of the serum in the blood of fish to be a contributing factor in the compatability or unsuitability of fish-hosts (Neves et al., 1985).These physiological interactions are still to be fully understood and studied. It is also not well known whether it is the fish or the glochidia that is responsible for the rejection or excystment before development. However, it is understood that the recognition of unsuitability occurs within the first few days of attachment (Neves et al., 1985). This is relative to the 83.8% (n= 550) of glochidia that released from Bullhead minnows within no longer than three days after the fish were collected from the wild. These glochidia had been rejected or unable to stay attached to the Bullhead minnows because these fish either could not provide a sufficient amount of the requried nutrients for development, or the Bullhead minnows exhibited a natural immune response that these glochidia are not resistent to and thus were actively rejected by the fish from their tissues (Neves et al., 1985). It is probable that a large sum of the glochidia had released within the first few days of captivity because of the stress of the fish being removed from the wild, much like with the Red shiners. The Bullhead minnow is also a less robust and hearty Cyprinid species than the Red shiners or Blacktail shiners (Gould and Irwin, 1962). However, the other 16.2% (n=106) of glochidia had still released without any

development several days after the Bullhead minnows had become acclimated and did not exhibit aggressive or stressed behavior. In addition, none of the 46 Bullhead minnows that were examined after death for glochidial encystment were infested to any degree with glochidia.

Fish that are repeatedly infested are able to develop an aquired immunity as well as develop scar tissue from the multiple encystments that does not allow the succesful attachment or encystment of glochidia (Neves et al., 1985). Bullhead minnows are bottom-dwellers that spend most of their time close to the benthic substrate, where they are easily and frequently able to be infested by gravid female mussels (Parker, 1964). It is possible that Bullhead minnows have simultaneously developed an aquired immunity from multiple infestations and have also developed scar tissue on their gills preventing succesful glochidial attachment or development to the juvenile state. However, at least two juveniles were able to naturally drop off of Bullhead minnows that were collected in June from the Sabine River. It is likely that they dropped off of younger Bullhead minnows that had not been infested yet. The success of metamorphosis has been found to decrease after only the second infestation attempt in host-fish lab trials and propagation efforts (Barnhart et al., 2010). The Bullhead minnow has not been confirmed as a host for any freshwater mussel species in East Texas with evidence of metamorphosis. It is possible that these two juveniles are a species of mussel that occurs in the Sabine River that uses the Bullhead minnow as a host. Although the two juveniles were not able to be identified, their morphology was distinct and dissimilar to the *F. askewi* juveniles that were released from Red shiners (Figure 2). However, morphological evidence is not enough to say that the two juveniles that released from Bullhead minnows were not *F. askewi*.

Investigations of Glochidial Release and Metamorphosis Time on Host-Fish for *F. askewi*

The certainty of when freshwater mussels of the Sabine River, Neches River, and Lake Fork Creek release their glochidia would require the sampling of multiple fish species for several consecuative days from all seasons. However, this study still provides evidence of the largest levels of infestations or in other words, the major glochidial release events in 2014. These occurred in May, late June, early July, and early August in the Sabine River, Neches River, or Lake Fork Creek (Figure 3). This is consistent with previous sampling data of infested fish from the same sites from spring and summer of 2013 by Marshall (2014). There was one exception of a relatively large peak in glochidial release in August 2014 in comparison to August of 2013. This peak may have been detected because infested fish were collected from Lake Fork Creek in August, which was not a site included in the 2013 sampling season (Marshall, 2014).

Fusconaia askewi may possibly be a bradytictic species that broods its larvae over the winter months and releases their glochidia in the spring and throughout the summer months (Howells et al., 1996; Bauer and Watchtler, 2001; Bakken, 2013). The juveniles of *F. askewi* dropped off of both Red shiners and Blacktail shiners that were collected from each sampling month (May, June, July, and August) except October amongst all three rivers. Five of those *F. askewi* juveniles dropped off from their hosts that were collected in May, two dropped off from their hosts that were collected in June, one juvenile had dropped from its host that was collected in July, and seven juveniles dropped off from their hosts that were collected in August. There was no glochidial encystment or juveniles that released from any of the fish collected from the Sabine and Neches Rivers in October. These juvenile drop off dates suggest that there is no selectivity between which spring or summer month *F. askewi* most likely releases. It is possible that no glochidia of *F. askewi* were fond encysted on fish hosts from October as *F. askewi* may

have been spawning at this time to brood over the winter. More fish of various species would need to be collected from each sampling month to determine the largest or most significant time of glochidial release between May, June, July, or August for *F. askewi*. Fish should also be sampled beyond October when water temperatures in East Texas start to significantly drop to further confirm that *F. askewi* do not still release glochidia in late fall or early winter (Gillis, 2011).

Although the exact date of glochidial release by gravid *F. askewi* was unable to be determined, it has been estimated from the date the infested fish were collected and the amount of time before the juveniles had released from their host. Based on the results, it is suggested that *F. askewi* released their glochidia on or before May 29 in the Sabine River for those juveniles that released nine days later on June 7. Thus, those juveniles that released within only one to two days since fish collection on July 10 were also estimated to have been released as glochidia at least nine days or an estimated one to two weeks prior to July 10 in the Sabine River and approximately one to two weeks prior to August 7 in the Neches River. Other release dates are estimated to have occured approximately one week prior to August 7 in the Neches River for those juveniles that released four days later on August 11, and approximately one week prior to June 3 in the Sabine River for those juveniles that released six days later on June 9.

Fusconaia askewi individuals were atttached to the fish in the lab for an average of 5.46 days since the fish were collected. It is understood that temperature plays a major role in the overall success and the rate of metamorphosis to the juvenile state (Pandolfo et al., 2010). A study has shown that temperatures ranging from 0-21^{o} C influenced significantly slower rates of the development of glochidia to juveniles than in warmer temperatures for the Winged mapleleaf (*Quadrula fragosa*) (Steingraeber et al., 2007).

The temperature of the Sabine River on July 10 when fish were collceted was 29° C. The average temperature of the Neches River on the July and August sampling dates was 30° C, and the temperature of Lake Fork Creek on August 4 when fish were collected was 25° C. The juveniles of *F. askewi* that were collected in July or August were encysted for an average of three days since the fish were collected. In contrast, the average temperature of the Sabine River in May and June was 25° C and *F. askewi* juveniles that were collected in May or June from the Sabine River were encysted for an average of 8.4 days since the fish were collected. *F. askewi* may exhibit a slower rate of metamorphosis in the Spring or in colder water temperatures. The only acception to this pattern is for the Lake Fork Creek site with a water temperature of 25° C in August with juveniels that released in the lab after only a mean of 2.4 days since the infested fish were collected. The metamorphosis rate of *F. askewi* may be a local adaption to the spring and summer temperatures at the Lake Fork Creek site where 25° C is near the maximum temperature in August. However, the date all fish were collected is only an estimate of the date that glochidia attached to these fish. The juveniles that released after an average of only three days from the warmer sampling months may be because of when they were released as glochidia at the estimtaed one to two weeks before the fish were collected. Their release dates may be indivitive of peak development and ecsystment time as juveniles and not dependent on the current water temperature.

The time of drop off of glochidia and juveniles in the lab are assumed to be as close to the duration of encystment that would have occurred in the wild. All of the fish in this study were held in similar water temperature and water chemistry conditions in the AHAB units that were measured in the field. Specifically, the average water temperature of the tanks was 27° C. The number of days before juveniles released from hosts since they were collected varied in

relation to the current water temperature they were collected in, but also was highly variable among individuals of *F. askewi*. There was not a consistent metamorphosis rate for all individual juveniles that released in the tanks from either Red Shiners or Blacktail Shiners. This is an indication that the encystment or drop off time of *F. askewi* is not dependent on the host-fish they were attached to or influenced by the water quality or slightly higher or lower water temperature of the AHAB tank units. The metamorphosis rate of glochidia to juveniles can widely vary between unionid individuals of the same species that are in the same conditions (Taeubert, 2013). Taeubert (2013) had shown that under constant water temperature, the endangered freshwater pearl mussel (*Margaritifera margaritifera* L.) had shown highly variable development times between individual glochidia. The only proposed influence in the possible disruption of the natural development and drop off time for *F. askewi* juveniles in the lab is the stress or health of the fish while in captivity. Thus, the estimated glochidial release dates and estimated metamorphosis rates are accepted here.

Conclusions

The life-history and co-evolutionary relationship of freshwater mussels with fish can be more fully understood through the testing and identification of their obligate host-fish species. In addition, conservation efforts towards the recovery and continued existence of the highly imperiled Unionids can be implimented when a host-fish species is known. In identifying the Red shiner and Blacktail shiner as host-fish for *Fusconaia askewi*, much more is understood about this state threatened species. *Fusconaia askewi* can be evolutionarily considered an opportunistic species. Firstly, *F. askewi* is able to utilize two stable species of fish as hosts. Secondly, this species has also shown to frequently and consistently release glochidia throughout the spring and summer months. However, as a result, *F. askewi* may infest several species of fish

that are not suitable hosts such as the Bullhead minnow that has repeatedly been found to be highly infested with *F. askewi* glochidia (Marshall, 2014). This is energetically costly and may be attributed to their imperiliment and glochidial mortality. In testing for host-fish, the major glochidial release events and metamorphosis rates can also be investigated. This information can also be useful in conservation and propagation efforts in identifying the critical months of the year for succesful reproduction and juvenile development for this species. Because both hosts for *F. askewi* are resilient and abundant in East Texas rivers, other driving factors of their state threatened status should be investigated and identified. Most importantly, their current identification as a separate species from *F. lananensis* should be contested or further confirmed using multiple genetic markers and phylogenetic analyses. If *F. askewi* and *F. lananensis* are later all described and accepted as *F. askewi*, the conservation status of *F. askewi* would need to be re-considered to determine if the species as a whole is still of concern or at risk. Future directions in host-fish testing should identify why and how certain species or families of fish are suitable hosts on a physiological and evolutionary level. In addition, unsuitable fish to mussel relationships should be further investigated to understand which organism plays the largest role in rejection or if it is a combination of each organisms' immunity resistence or immune response.

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Apendix A. Raw unedited data of the nucleotide alignment of the ND1 gene with sequences collected for *F. askewi* and *F. lananensis* by Marshall (2014). Sequences generated for this study are denoted with the identifier "Erin". * Where non-variable sites occur.

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                1                                                          60
71_3            ------------------------------------------NNNNNNNNNNNNNNNNNNNN
71_6            ------------------------------------------NNNNNNNNNNNNNNNNNNNN
76_5            ------------------------------------------NNNNNNNNNNNNNNNNNTNNC
69_3            -----------------------------------------NNNNNNNNNNNNNNNNNNNNNN
71_8            ------------------------------------------NNNNNNNNNNNNNNNNNNNN
69_2            ------------------------------------------NNNNNNNNNNNNNNNNNNNTN
90_6            ------------------------------------------NNNNNNNNNNNNNNNNNTNNN
90_5            ------------------------------------------NNNNNNNNNNNNNNNNNNNN
71_5            ------------------------------------------NNNNNNNNNNNNNNNNNNNN
76_4            -----------------------------------------TNNNNNNNNNNNNNNNNNNNN
11b2_Erin       GGGNNNNANNNNNTNNNNNNNNNNNNNNNNNNNNNNNNGACTNNNNNNNNNNNNNNNNNNN
2h_Erin         GGGNNNNANNNNNTNNNNNNNNNNNNNNNNNNNNNNNNGACTNNNNNNNNNNNNNNNNNNN
17d_Erin        ------------------------------------------------------------
6c21_Erin       ------------------------------------------------------------
9a2_7_Erin      ------------------------------------------------------------CTC
9a_Erin         ------------------------------------------NNNNNNNNNNNNNNNNNNCTN

6a_Erin         ------------------NNNTNATTGNAANNNNNNTNNNANNNNNNNNNNNNNNNNNNN
16d_Erin        ------------------------------------------------------------
2e_22_Erin      ------------------------------------------NNNNNNNNNNNNNNNNNTN
76_3            ----------------------------------------NNNNNNNNNNNNNNNNNNNNNTC
71_4            ----------------------------------------NNNNNNNNNNNNNNNNNNNNNNN
6b27_Erin       ------------------------------------------------------------
8d25_Erin       ------------------------------------------------------------
2d22_Erin       ------------------------------------------------------------
Sab1_4          ------------------------------------------NNNNNNNNNNNNNGNNNNNNN


71_3            NNNANNNNNNNTCANNCCCCCACA-TAACCT--CCACACTTAT-TACATACCTTCTAATC
71_6            NNNNNNNNNNANTCATCCCCCACA-TAACCT--CCACACTTAT-TACATACCTTCTAATC
76_5            NNNNNNNNNNNNTCATCCCCCACA-TAACCT--CCACACTTAT-TACATACCTTCTAATC
69_3            NNNNNNNNNNNNNCATNCCCCACA-TAACCT--CCACACTTAT-TACATACCTTCTAATC
71_8            NNNNNNNNNNNNNNATCCCCCACA-TAACCT--CCACACTTAT-TACATACCTTCTAATC
69_2            NNNNNNNNNNNNTCATCCCCCACA-TAACCT-C-CACACTTAT-TACATACCTTCTAATC
90_6            NNNNNNNNNNNNTCATCCCCCACNATAACCT-CGCACACTTAT-TACATACCTTCTAATC
90_5            NNNNNNNNNNNNNCATCCCCCACATAAGCCTGCGCACACTTAT-TACATACCTTCTAATC
71_5            NNNNNNNNNNNNNNNNNNCCCACA-TAACCT--CCACACTTAT-TACATACCTTCTAATC
76_4            NNNNNNNNNNNNNTCTCCCCCACATTAACCT--CCACACTTAT-TACATACCTTCTAATC
11b2_Erin       NNNNNNNNNNNWTCATCCCCCACA-TAACCT--CCACACTTAT-TACATACCTTCTAATC
2h_Erin         NNNNNNNNNNNWTCATCCCCCACA-TAACCT--CCACACTTAT-TACATACCTTCTAATC
17d_Erin        ------------GNCCCCCCACA-TAACCT--CCACACTTAT-TACATACCTTCTAATC
6c21_Erin       ---------AATCAT-CCCCCACA-TAACCT--CCACACTTAT-TACATACCTTCTAATC
9a2_7_Erin      CCCTCCACTAATCATTCCCCCACA-TAACCT--CCACACTTAT-TACATACCTTCTAATC
9a_Erin         NNNNNNNNTAATCATTCCCCCACA-TAACCT--CCACACTTAT-TACATACCTTCTAATC
17c_Erin        ------------TCATCCCCCACA-TAACCT--CCACACTTAT-TACATACCTTCTAATC
6a_Erin         NNNNNNNNNNNNNNNATCCCCCACA-TAACCT--CCACACTTATGTACATACCTTCTAATC
16d_Erin        ------------------------------------------------------------
2e_22_Erin      NNNNNNNNNNNANTCATCCCCCACA-TAACCT--CCACACTTAT-TACATACCTTCTAATC
76_3            NNGNNNNNNNNNATCATCCCCCACA-TAACCT--CCACACTTAT-TACATACCTTCTAATC
71_4            NNNNNNNNNNNNNNNNTCNCCCCACA-TAACCT--CCACACTTAT-TACATACCTTCTAATC
6b27_Erin       ----------------------------CCTCCACACTTAT-TACATACCTTCTAATC
8d25_Erin       ------------------------------------------------------TCTAATCTTA
2d22_Erin       ------------------------------------------------------------
Sab1_4          NNNNNNNNNNNNNTCGTCCCGCATA-CAATCT--CCACCTTTAC-CACATACCTTCTAATC
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71_3            TTACTAGGCGTAGCATTCTTTACCCTTCTTGAACGCAAAGCTTTAG--GGTAC-TTTCAA
71_6            TTACTAGGCGTAGCATTCTTTACCCTTCTTGAACGCAAAGCTTTAG--GGTAC-TTTCAA
76_5            TTACTAGGCGTAGCATTCTTTACCCTTCTTGAACGCAAAGCTTTAG--GGTAC-TTTCAA
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Appendix A (Continued)

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69_3            TTACTAGGCGTAGCATTCTTTACCCTTCTTGAACGCAAAGCTTTAG--GGTAC-TTTCAA
71_8            TTACTAGGCGTAGCATTCTTTACCCTTCTTGAACGCAAAGCTTTAG--GGTAC-TTTCAA
69_2            TTACTAGGCGTAGCATTCTTTACCCTTCTTGAACGCAAAGCTTTAG--GGTAC-TTTCAA
90_6            TTACTAGGCGTAGCATTCTTTACCCTTCTTGAACGCAAAGCTTTAG--GGTAC-TTTCAA
90_5            TTACTAGGCGTAGCATTCTTTACCCTTCTTGAACGCAAAGCTTTAG--GGTAC-TTTCAA
71_5            TTACTAGGCGTAGCATTCTTTACCCTTCTTGAACGCAAAGCTTTAG--GGTAC-TTTCAA
76_4            TTACTAGGCGTAGCATTCTTTACCCTTCTTGAACGCAAAGCTTTAG--GGTAC-TTTCAA
11b2_Erin       TTACTAGGCGTAGCATTCTTTACCCTTCTTGAACGCAAAGCTTTAG--GGTAC-TTTCAA
2h_Erin         TTACTAGGCGTAGCATTCTTTACCCTTCTTGAACGCAAAGCTTTAG--GGTAC-TTTCAA
17d_Erin        TTACTAGGCGTAGCATTCTTTACCCTTCTTGAACGCAAAGCTTTAG--GGTAC-TTTCAA
6c21_Erin       TTACTAGGCGTAGCATTCTTTACCCTTCTTGAACGCAAAGCTTTAG--GGTAC-TTTCAA
9a2_7_Erin      TTACTAGGCGTAGCATTCTTTACCCTTCTTGAACGCAAAGCTTTAG--GGTAC-TTTCAA
9a_Erin         TTACTAGGCGTAGCATTCTTTACCCTTCTTGAACGCAAAGCTTTAG--GGTAC-TTTCAA
17c_Erin        TTACCCGGCGTAGCATTCTTTACCCTTCTTGAACGCAAAGCTTTAG--GGTAC-TTTCAA
6a_Erin         TTACTAGGCGTAGCATTCTTTACCCTTCTTGAACGCAAAGCTTTAG--GGTAC-TTCCAA
16d_Erin        -----------AGCATTCTTTACCCTTCTTGAACGCAAAGCTTTAG--GGTAC-TTTCAA
2e_22_Erin      TTACCCGGCGTAGCATTCTTTACCCTTCTTGAACGCAAAGCTTTAGCCGGTACATTTCAA
76_3            TTACTAGGCGTAGCATTCTTTACCCTTCTTGAACGCAAAGCTTTAG--GGTAC-TTTCAA
71_4            TTACTAGGCGTAGCATTCTTTACCCTTCTTGAACGCAAAGCTTTAG--GGTAC-TTTCAA
6b27_Erin       TTA-TANGCNGAGNANTCTTTACCCTTCTTGAACGCAAAGCTTT--AGGGTAC-TTTCAA
8d25_Erin       NNN-NNNGNNNNNNNTTCTTTACCCTTCTTGAACGCAAAGCTTT--AGGGTAT-TTTCAA
2d22_Erin       --------------TTCTTTTACCCTTNTTGNACGCAAAGCTTNTAAGGGTAC-TTNNNN
Sab1_4          CTACTGGGGGTAGCATTTTTTACTCTACTCGAACGTAAAGCCCTTG--GCTAT-TTTCAA
                                    ***** **  * * *** *****       * **  **

71_3            ATCCGAAAA-GGCCCAAACAAAGTTGG-AATTATAGG-AATCCCACAACCACTAGCAGAC
71_6            ATCCGAAAA-GGCCCAAACAAAGTTGG-AATTATAGG-AATCCCACAACCACTAGCAGAC
76_5            ATCCGAAAA-GGCCCAAACAAAGTTGG-AATTATAGG-AATCCCACAACCACTAGCAGAC
69_3            ATCCGAAAA-GGCCCAAACAAAGTTGG-AATTATAGG-AATCCCACAACCACTAGCAGAC
71_8            ATCCGAAAA-GGCCCAAACAAAGTTGG-AATTATAGG-AATCCCACAACCACTAGCAGAC
69_2            ATCCGAAAA-GGCCCAAACAAAGTTGG-AATTATAGG-AATCCCACAACCACTAGCAGAC
90_6            ATCCGAAAA-GGCCCAAACAAAGTTGG-AATTATAGG-AATCCCACAACCACTAGCAGAC
90_5            ATCCGAAAA-GGCCCAAACAAAGTTGG-AATTATAGG-AATCCCACAACCACTAGCAGAC
71_5            ATCCGAAAA-GGCCCAAACAAAGTTGG-AATTATAGG-AATCCCACAACCACTAGCAGAC
76_4            ATCCGAAAA-GGCCCAAACAAAGTTGG-AATTATAGG-AATCCCACAACCACTAGCAGAC
11b2_Erin       ATCCGAAAA-GGCCCAAACAAAGTTGG-AATTATAGG-AATCCCACAACCACTAGCAGAC
2h_Erin         ATCCGAAAA-GGCCCAAACAAAGTTGG-AATTATAGG-AATCCCACAACCACTAGCAGAC
17d_Erin        ATCCGAAAA-GGCCCAAACAAAGTTGG-AATTATAGG-AATCCCACAACCACTAGCAGAC
6c21_Erin       ATCCGAAAA-GGCCCAAACAAAGTTGG-AATTATAGG-AATCCCACAACCACTAGCAGAC
9a2_7_Erin      ATCCGAAAA-GGCCCAAACAAAGTTGG-AATTATAGG-AATCCCACAACCACTAGCAGAC
9a_Erin         ATCCGAAAA-GGCCCAAACAAAGTTGG-AATTATAGG-AATCCCACAACCACTAGCAGAC
17c_Erin        ATCCGAAAA-GGCCCAAACAAAGTTGG-AATTATAGG-AATCCCACAACCACTAGCAGAC
6a_Erin         ATCCGAAAA-GGCCCAAACAAAGTTGG-AATTATAGG-AATCCCACAACCACTAGCAGAC
16d_Erin        ATCGGAAAA-GGCCCAA-CAAAGTTGG-AATTATAGG-AATCCC---CGCCCTAGCAGAC
2e_22_Erin      ATCCGAAAA-GGCCCAAACAAAGTTGG-AATTATAGG-AATCCCACAACCCCTAGCAGAC
76_3            ATCCGAAAA-GGCCCAAACAAAGTTGG-AATTATAGG-AATCCCACAACCACTAGCAGAC
71_4            ATCCGAAAA-GGCCCAAACAAAGTTGG-AATTATAGG-AATCCCACAACCACTAGCAGAC
6b27_Erin       ATCCGAAAA-GGCCCAAACAAAGTTGG-AATTATAGG-AATCCCACAACCACTAGCAGAC
8d25_Erin       ATCCGAAAAAGGCCCAAACAAAGTTGG-AATTATAGGGAATCCCACAACCACTAGCAGAC
2d22_Erin       NTCNGAAAANGGCCCAAACAAAGNTNGGAATTATAGGAATCCCCACAACCACTAGCAGAC
Sab1_4          ATCCGAAAA-GGCCCAAATAAAGTCGG-AATAATTGG-AATCCCACAACCGTTAGCAGAT
                 ** ***** *******  ****   * *** ** ** *  ***     *  *******

71_3            GCCCTAAAA-CTTTTTGTGAAAGAATGAGTAATACCCACATCCTCAAACTACTTACCA-T
71_6            GCCCTAAAA-CTTTTTGTGAAAGAATGAGTAATACCCACATCCTCAAACTACTTACCA-T
76_5            GCCCTAAAA-CTTTTTGTGAAAGAATGAGTAATACCCACATCCTCAAACTACTTACCA-T
69_3            GCCCTAAAA-CTTTTTGTGAAAGAATGAGTAATACCCACATCCTCAAACTACTTACCA-T
71_8            GCCCTAAAA-CTTTTTGTGAAAGAATGAGTAATACCCACATCCTCAAACTACTTACCA-T
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69_2            GCCCTAAAA-CTTTTTGTGAAAGAATGAGTAATACCCACATCCTCAAACTACTTACCA-T
90_6            GCCCTAAAA-CTTTTTGTGAAAGAATGAGTAATACCCACATCCTCAAACTACTTACCA-T
90_5            GCCCTAAAA-CTTTTTGTGAAAGAATGAGTAATACCCACATCCTCAAACTACTTACCA-T
71_5            GCCCTAAAA-CTTTTTGTGAAAGAATGAATAATACCCACATCCTCAAACTACTTACCA-T
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Appendix A (Continued)

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76_4            GCCCTAAAA-CTTTTTGTGAAAGAATGAGTAATACCCACATCCTCAAACTACTTACCA-T
11b2_Erin       GCCCTAAAA-CTTTTTGTGAAAGAATGAGTAATACCCACATCCTCAAACTACTTACCA-T
2h_Erin         GCCCTAAAA-CTTTTTGTGAAAGAATGAGTAATACCCACATCCTCAAACTACTTACCA-T
17d_Erin        GCCCTAAAA-CTTTTTGTGAAAGAATGAGTAATACCCACATCCTCAAACTACTTACCA-T
6c21_Erin       GCCCTAAAA-CTTTTTGTGAAAGAATGAGTAATACCCACATCCTCAAACTACTTACCA-T
9a2_7_Erin      GCCCTAAAA-CTTTTTGTGAAAGAATGAGTAATACCCACATCCTCAAACTACTTACCA-T
9a_Erin         GCCCTAAAA-CTTTTTGTGAAAGAATGAGTAATACCCACATCCTCAAACTACTTACCA-T
17c_Erin        GCCCTAAAA-CTTTTTGTGAAAGAATGAATAATACCCACATCCTCAAACTACTTACCAAT
6a_Erin         GCCCTAAAA-CTTTTTGTGAAAGAATGAGTAATACCCACATCCTCAAACTACTTACCA-T
16d_Erin        GCCC-AAAA-CTTTTTGTGAAAGAATGAGTAATACCCACATCCTCAAACTACTTACCA-T
2e_22_Erin      GCCCTAAAA-CTTTTTGTGAAAGAATGAGTAATACCCACATCCTCAAACTACTTACCA-T
76_3            GCCCTAAAA-CTTTTTGTGAAAGAATGAGTAATACCNNNNTCCTCAAACTACTTACCA-T
71_4            GCCCTAAAA-CTTTTTGTGAAAGAATGAGTAATACCCACATCCTCAAACTACTTACCA-T
6b27_Erin       GCCCTAAAA-CTTTTTGTGAAAGAATGAGTAATACCCACATCCTCAAACTACTTACCA-T
8d25_Erin       GCCCTAAAA-CTTTTTGTGAAAGAATGAGTAATACCCACATCCTCAAACTACTTACCA-T
2d22_Erin       GCCCTAAAAACTTTTTGTGAAAGAATGAGTAATACCCACATCCTCAAACTACTTACCA-T
Sab1_4          GCATTAAAA-CTTTTCGTAAAAGAATGAGTAATACCCACCTCCTCAAACTACCTACCT-T
                **   **** ***** ** ********* *******    ************ ****  *

71_3            TTATTTTAACCCC-AACAATCATATTAATTTTAGCACTT-AGGCTATGACAG-CTATTCC
71_6            TTATTTTAACCCC-AACAATCATATTAATTTTAGCACTT-AGGCTATGACAG-CTATTCC
76_5            TTATTTTAACCCC-AACAATCATATTAATTTTAGCACTT-AGGCTATGACAG-CTATTCC
69_3            TTATTTTAACCCC-AACAATCATATTAATTTTAGCACTT-AGGCTATGACAG-CTATTCC
71_8            TTATTTTAACCCC-AACAATCATATTAATTTTAGCACTT-AGGCTATGACAG-CTATTCC
69_2            TTATTTTAACCCC-AACAATCATATTAATTTTAGCACTT-AGGCTATGACAG-CTATTCC
90_6            TTATTTTAACCCC-AACAATCATATTAATTTTAGCACTT-AGGCTATGACAG-CTATTCC
90_5            TTATTTTAACCCC-AACAATCATATTAATTTTAGCACTT-AGGCTATGACAG-CTATTCC
71_5            TTATTTTAACCCC-AACAATCATATTAATTTTAGCACTT-AGGCTATGACAG-CTATTCC
76_4            TTATTTTAACCCC-AACAATCATATTAATTTTAGCACTT-AGGCTATGACAG-CTATTCC
11b2_Erin       TTATTTTAACCCC-AACAATCATATTAATTTTAGCACTT-AGGCTATGACAG-CTATTCC
2h_Erin         TTATTTTAACCCC-AACAATCATATTAATTTTAGCACTT-AGGCTATGACAG-CTATTCC
17d_Erin        TTATTTTAACCCC-AACAATCATATTAATTTTAGCACTT-AGGCTATGACAG-CTATTCC
6c21_Erin       TTATTTTAACCCC-AACAATCATATTAATTTTAGCACTT-AGGCTATGACAA-CTATTCC
9a2_7_Erin      TTATTTTAACCCC-AACAATCATATTAATTTTAGCACTT-AGGCTATGACAG-CTATTCC
9a_Erin         TTATTTTAACCCC-AACAATCATATTAATTTTAGCACTT-AGGCTATGACAG-CTATTCC
17c_Erin        TTATTTTAACCCC-AACAATCATATTAATTTTAGCACTT-AGGCTATGACAG-CTATTCC
6a_Erin         TTATTTTAACCCC-AACAATCATATTAATTTTAGCACTT-AGGCTATGACAG-CTATTCC
16d_Erin        TTATTTTAACCCC-AACAATCATATTAATTTTAGCACTT-AGGCTATGACAG-CTATTCC
2e_22_Erin      TTATTTTAACCCC-AACAATCATATTAATTTTAGCACTT-AGGCTATGACAG-CTATTCC
76_3            TTATTTTAACCCC-AACAATCATATTAATTTTAGCACNT-AGGCTATGACAG-CTATTCC
71_4            TTATTTTAACCCC-AACAATCATATTAATTTTAGCACTT-AGGCTATGACAG-CTATTCC
6b27_Erin       TTATTTTAACCCCCAACAATCATATTAATTTTAGCACTT-AGGCTATGACAGGCTATTCC
8d25_Erin       TTATTTTAACCCC-AACAATCATATTAATTTTAGCACTT-AGGCTATGACAG-CTATTCC
2d22_Erin       TTATTTTTAACCCCAACAATCATATTAATTTTAGCACTTTAGGCTATGACAG-CTATTCC
Sab1_4          TTGTTTTAACTCC-AACTATTATACTAATCCTAGCTCTA-AGACTTTGACAG-TTATTCC
                ** **** *  ** *** ** *** ****  **** *   ** ** *****   ******

71_3            C-ATCCTTTATACTCTCATTT-CAAATAACCCTAGG-AATACTCATATTCTTATGTATTT
71_6            C-ATCCTTTATACTCTCATTT-CAAATAACCCTAGG-AATACTCATATTCTTATGTATTT
76_5            C-ATCCTTTATACTCTCATTT-CAAATAACCCTAGG-AATACTCATATTCTTATGTATTT
69_3            C-ATCCTTTATACTCTCATTT-CAAATAACCCTAGG-AATACTCATATTCTTATGTATTT
71_8            C-ATCCTTTATACTCTCATTT-CAAATAACCCTAGG-AATACTCATATTCTTATGTATTT
69_2            C-ATCCTTTATACTCTCATTT-CAAATAACCCTAGG-AATACTCCTATTCTTATGTATTT
90_6            C-ATCCTTTATACTCTCATTT-CAAATAACCCTAGG-AATACTCATATTCTTATGTATTT
90_5            C-ATCCTTTATACTCTCATTT-CAAATAACCCTAGG-AATACTCATATTCTTATGTATTT
71_5            C-ATCCTTTATACTCTCATTT-CAAATAACCCTAGG-AATACTCATATTCTTATGTATTT
76_4            C-ATCCTTTATACTCTCATTT-CAAATAACCCTAGG-AATACTCCTATTCTTATGTATTT
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11b2_Erin       C-ATCCTTTATACTCTCATTT-CAAATAACCCTAGG-AATACTCCTATTCTTATGTATTT
2h_Erin         C-ATCCTTTATACTCTCATTT-CAAATAACCCTAGG-AATACTCCTATTCTTATGTATTT
17d_Erin        C-ATCCTTTATACTCTCATTT-CAAATAACCCTAGG-AATACTCATATTCTTATGTATTT
6c21_Erin       C-ATCCTTTATACTCTCATTT-CAAATAACCCTAGG-AATACTCCTATTCTTATGTATTT
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Appendix A (Continued)

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9a2_7_Erin      C-ATCCTTTATACTCTCATTT-CAAATAACCCTAGG-AATACTCCTATTCTTATGTATTT
9a_Erin         C-ATCCTTTATACTCTCATTT-CAAATAACCCTAGG-AATACTCCTATTCTTATGTATTT
17c_Erin        C-ATCCTTTATACTCTCATTT-CAAATAACCCTAGG-AATACTCATATTCTTATGTATTT
6a_Erin         C-ATCCTTTATACTCTCATTT-CAAATAACCCTAGG-AATACTCCTATTCTTATGTATTT
16d_Erin        C-ATCCTTTATACTCTCATTT-CAAATAACCCTAGG-AATACTCATATTCTTATGTATTT
2e_22_Erin      C-ATCCTTTATACTCTCATTT-CAAATAACCCTAGG-AATACTCCTATTCTTATGTATTT
76_3            C-ATCCTTTATACTCTCATTT-CAAATAACCCTAGG-AATACTCCTATTCTTATGTATTT
71_4            C-ATCCTTTATACTCTCATTT-CAAATAACCCTAGG-AATACTCCTATTCTTATGTATTT
6b27_Erin       CCATCCTTTATACTCTCATTT-CAAATAACCCTAGGGAATACTCCTATTCTTATGTATTT
8d25_Erin       C-ATCCTTTATACTCTCATTT-CAAATAACCCTAGG-AATACTCCTATTCTTATGTATTT
2d22_Erin       CATCCTTTAATACTCTCATTTTCAAATAACCCTAGG-AATACTCCTATTCTTATGTATTT
Sab1_4          C-ATCATTTATATTATCATCC-CAAATAATCTTAGG-TATATTTTTATTCCTGTGTATTT
                *   * ** *** * ****   ******* * ****  *** *  ***** * *******

71_3            CTTCTTTAACCGTCTACA-CAACCTTAATAGCAGGTTGGGCCTCAAACTCGAAGTATGCT
71_6            CTTCTTTAACCGTCTACA-CAACCTTAATAGCAGGTTGGGCCTCAAACTCGAAGTATGCT
76_5            CTTCTTTAACCGTCTACA-CAACCTTAATAGCAGGTTGGGCCTCAAACTCGAAGTATGCT
69_3            CTTCTTTAACCGTCTACA-CAACCTTAATAGCAGGTTGGGCCTCAAACTCGAAGTATGCT
71_8            CTTCTTTAACCGTCTACA-CAACCTTAATAGCAGGTTGGGCCTCAAACTCGAAGTATGCT
69_2            CTTCTTTAACCGTCTACA-CAACCTTAATAGCAGGTTGGGCCTCAAACTCGAAGTATGCT
90_6            CTTCTTTAACCGTCTACA-CAACCTTAATAGCAGGTTGGGCCTCAAACTCGAAGTATGCT
90_5            CTTCTTTAACCGTCTACA-CAACCTTAATAGCAGGTTGGGCCTCAAACTCGAAGTATGCT
71_5            CTTCTTTAACCGTCTACA-CAACCTTAATAGCAGGTTGGGCCTCAAACTCGAAGTATGCT
76_4            CTTCTTTAACCGTCTACA-CAACCTTAATAGCAGGTTGGGCCTCAAACTCGAAGTATGCT
11b2_Erin       CTTCTTTAACCGTCTACA-CAACCTTAATAGCAGGTTGGGCCTCAAACTCGAAGTATGCT
2h_Erin         CTTCTTTAACCGTCTACA-CAACCTTAATAGCAGGTTGGGCCTCAAACTCGAAGTATGCT
17d_Erin        CTTCTTTAACCGTCTACA-CAACCTTAATAGCAGGTTGGGCCTCAAACTCGAAGTATGCT
6c21_Erin       CTTCTTTAACCGTCTACA-CAACCTTAATAGCAGGTTGGGCCTCAAACTCGAAGTATGCT
9a2_7_Erin      CTTCTTTAACCGTCTACA-CAACCTTAATAGCAGGTTGGGCCTCAAACTCGAAGTATGCT
9a_Erin         CTTCTTTAACCGTCTACA-CAACCTTAATAGCAGGTTGGGCCTCAAACTCGAAGTATGCT
17c_Erin        CTTCTTTAACCGTCTACA-CAACCTTAATAGCAGGTTGGGCCTCAAACTCGAAGTATGCT
6a_Erin         CTTCTTTAACCGTCTACA-CAACCTTAATAGCAGGTTGGGCCTCAAACTCGAAGTATGCT
16d_Erin        CTTCTTTAACCGTCTACA-CAACCTTAATAGCAGGTTGGGCCTCAAACTCGAAGTATGCT
2e_22_Erin      CTTCTTTAACCGTCTACA-CAACCTTAATAGCAGGTTGGGCCTCAAACTCGAAGTATGCT
76_3            CTTCTTTAACCGTCTACA-CAACCTTAATAGCAGGTTGGGCCTCAAACTCGAAGTATGCT
71_4            CTTCTTTAACCGTCTACA-CAACCTTAATAGCAGGTTGGGCCTCAAACTCGAAGTATGCT
6b27_Erin       CTTCTTTAACCGTCTACAACAACCTTAATAGCAGGTTGGGCCTCAAACTCTAAGTATGCT
8d25_Erin       CTTCTTTAACCGTCTACA-CATCCTTAATAGCAGGTTGGGCCTCAAACTCGAAGTATGCT
2d22_Erin       CTTCTTTAACCGTCTACA-CAACCTTAATAGCAGGTTGGGCCTCAAACTCGAAGTATGCT
Sab1_4          CCTCCCTAGCCGTTTACA-CAACTCTTATAGCAGGCTGAGCCTCAAACTCTAAGTATGCC
                * **  ** **** **** ** *  * ******** ** ********** ********

71_3            CTACTAGGAGCCATTCGAGCCATGGCCCAAACCATCTCATATGAAGTAACAATAACACTA
71_6            CTACTAGGAGCCATTCGAGCCATGGCCCAAACCATCTCATATGAAGTAACAATAACACTA
76_5            CTACTAGGAGCCATTCGAGCCATGGCCCAAACCATCTCATATGAAGTAACAATAACACTA
69_3            CTACTAGGAGCCATTCGAGCCATGGCCCAAACCATCTCATATGAAGTAACAATAACACTA
71_8            CTACTAGGAGCCATTCGAGCCATGGCCCAAACCATCTCATATGAAGTAACAATAACACTA
69_2            CTACTAGGAGCCATTCGAGCCATGGCCCAAACCATCTCATATGAAGTAACAATAACACTA
90_6            CTACTAGGAGCCATTCGAGCCATGGCCCAAACCATCTCATATGAAGTAACAATAACACTA
90_5            CTACTAGGAGCCATTCGAGCCATGGCCCAAACCATCTCATATGAAGTAACAATAACACTA
71_5            CTACTAGGAGCCATTCGAGCCATGGCCCAAACCATCTCATATGAAGTAACAATAACACTA
76_4            CTACTAGGAGCCATTCGAGCCATGGCCCAAACCATCTCATATGAAGTAACAATAACACTA
11b2_Erin       CTACTAGGAGCCATTCGAGCCATGGCCCAAACCATCTCATATGAAGTAACAATAACACTA
2h_Erin         CTACTAGGAGCCATTCGAGCCATGGCCCAAACCATCTCATATGAAGTAACAATAACACTA
17d_Erin        CTACTAGGAGCCATTCGAGCCATGGCCCAAACCATCTCATATGAAGTAACAATAACACTA
6c21_Erin       CTACTAGGAGCCATTCGAGCCATGGCCCAAACCATCTCATATGAAGTAACAATAACACTA
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9a2_7_Erin      CTACTAGGAGCCATTCGAGCCATGGCCCAAACCATCTCATATGAAGTAACAATAACACTA
9a_Erin         CTACTAGGAGCCATTCGAGCCATGGCCCAAACCATCTCATATGAAGTAACAATAACACTA
17c_Erin        CTACTAGGAGCCATTCGAGCCATGGCCCAAACCATCTCATATGAAGTAACAATAACACTA
6a_Erin         CTACTAGGAGCCATTCGAGCCATGGCCCAAACCATCTCATATGAAGTAACAATAACACTA
16d_Erin        CTACTAGGAGCCATTCGAGCCATGGCCCAAACCATCTCATATGAAGTAACAATAACACTA
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Appendix A (Continued)

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2e_22_Erin      CTACTAGGAGCCATTCGAGCCATGGCCCAAACCATCTCATATGAAGTAACAATAACACTA
76_3            CTACTAGGAGCCATTCGAGCCATGGCCCAAACCATCTCATATGAAGTAACAATAACACTA
71_4            CTACTAGGAGCCATTCGAGCCATGGCCCAAACCATCTCATATGAAGTAACAATAACACTA
6b27_Erin       CTACTAGGAGCCATTCGAGCCATGGCCCAAACCATCTCATATGAAGTAACAATAACACTA
8d25_Erin       CTACTAGGAGCCATTCAAGCCATGGCCCAAACCATCTCATATGAAGTAACAATAACACTA
2d22_Erin       CTACTAGGAGCCATTCGAGCCATGGCCCAAACCATCTCATATGAAGTAACAATAACACTA
Sab1_4          CTTTTAGGGGCTATTCGAGCCATAGCTCAAACTATTTCCTACGAGGTAACAATAACACTA
                **  **** ** **** ****** ** ***** ** ** ** ** ***************

71_3            ATTATCATTTTCTACCTATTCTTGATTATACAAATAGACATAGTAACAATCCGCTCGGTT
71_6            ATTATCATTTTCTACCTATTCTTGATTATACAAATAGACATAGTAACAATCCGCTCGGTT
76_5            ATTATCATTTTCTACCTATTCTTGATTATACAAATAGACATAGTAACAATCCGCTCGGTT
69_3            ATTATCATTTTCTACCTATTCTTGATTATACAAATAGACATAGTAACAATCCGCTCGGTT
71_8            ATTATCATTTTCTACCTATTCTTGATTATACAAATAGACATAGTAACAATCCGCTCGGTT
69_2            ATTATCATTTTCTACCTATTCTTGATTATACAAATAGACATAGTAACAATCCGCTCGGTT
90_6            ATTATCATTTTCTACCTATTCTTGATTATACAAATAGACATAGTAACAATCCGCTCGGTT
90_5            ATTATCATTTTCTACCTATTCTTGATTATACAAATAGACATAGTAACAATCCGCTCGGTT
71_5            ATTATCATTTTCTACCTATTCTTGATTATACAAATAGACATAGTAACAATCCGCTCGGTT
76_4            ATTATCATTTTCTACCTATTCTTGATTATACAAATAGACATAGTAACAATCCGCTCGGTT
11b2_Erin       ATTATCATTTTCTACCTATTCTTGATTATACAAATAGACATAGTAACAATCCGCTCGGTT
2h_Erin         ATTATCATTTTCTACCTATTCTTGATTATACAAATAGACATAGTAACAATCCGCTCGGTT
17d_Erin        ATTATCATTTTCTACCTATTCTTGATTATACAAATAGACATAGTAACAATCCGCTCGGTT
6c21_Erin       ATTATCATTTTCTACCTATTCTTGATTATACAAATAGACATAGTAACAATCCGCTCGGTT
9a2_7_Erin      ATTATCATTTTCTACCTATTCTTGATTATACAAATAGACATAGTAACAATCCGCTCGGTT
9a_Erin         ATTATCATTTTCTACCTATTCTTGATTATACAAATAGACATAGTAACAATCCGCTCGGTT
17c_Erin        ATTATCATTTTCTACCTATTCTTGATTATACAAATAGACATAGTAACAATCCGCTCGGTT
6a_Erin         ATTATCATTTTCTACCTATTCTTGATTATACAAATAGACATAGTAACAATCCGCTCGGTT
16d_Erin        ATTATCATTTTCTACCTATTCTTGATTATACAAATAGACATAGTAACAATCCGCTCGGTT
2e_22_Erin      ATTATCATTTTCTACCTATTCTTGATTATACAAATAGACATAGTAACAATCCGCTCGGTT
76_3            ATTATCATTTTCTACCTATTCTTGATTATACAAATAGACATAGTAACAATCCGCTCGGTT
71_4            ATTATCATTTTCTACCTATTCTTGATTATACAAATAGACATAGTAACAATCCGCTCGGTT
6b27_Erin       ATTATCATTTTCTACCTATTCTTGATTATACAAATAGACATAGTAACAATCCGCTCGGTT
8d25_Erin       ATTATCATTTTCTACCTATTCTTGATTATACAAATAGACATAGTAACAATCCGCTCGGTT
2d22_Erin       ATTATCATTTTCTACCTATTCTTGATTATACAAATAGACATAGTAACAATCCGCTCGGTT
Sab1_4          ATTATTATTTTCTACCTATTCCTAATAATAAAAATAGACATAGTAATAATTCGTCTAACT
                ***** *************** * ** *** *************** *** **      *

71_3            AACACCTCTATACCAACCTTTGCCCTCTCCGCACCATTAGCCATTATGTGAACTGTTGTC
71_6            AACACCTCTATACCAACCTTTGCCCTCTCCGCACCATTAGCCATTATGTGAACTGTTGTC
76_5            AACACCTCTATACCAACCTTTGCCCTCTCCGCACCATTAGCCATTATGTGAACTGTTGTC
69_3            AACACCTCTATACCAACCTTTGCCCTCTCCGCACCATTAGCCATTATGTGAACTGTTGTC
71_8            AACACCTCTATACCAACCTTTGCCCTCTCCGCACCATTAGCCATTATGTGAACTGTTGTC
69_2            AACACCTCTATACCAACCTTTGCCCTCTCCGCACCATTAGCCATTATGTGAACTGTTGTC
90_6            AACACCTCTATACCAACCTTTGCCCTCTCCGCACCATTAGCCATTATGTGAACTGTTGTC
90_5            AACACCTCTATACCAACCTTTGCCCTCTCCGCACCATTAGCCATTATGTGAACTGTTGTC
71_5            AACACCTCTATACCAACCTTTGCCCTCTCCGCACCATTAGCCATTATGTGAACTGTTGTC
76_4            AACACCTCTATACCAACCTTTGCCCTCTCCGCACCATTAGCCATTATGTGAACTGTTGTC
11b2_Erin       AACACCTCTATACCAACCTTTGCCCTCTCCGCACCATTAGCCATTATGTGAACTGTTGTC
2h_Erin         AACACCTCTATACCAACCTTTGCCCTCTCCGCACCATTAGCCATTATGTGAACTGTTGTC
17d_Erin        AACACCTCTATACCAACCTTTGCCCTCTCCGCACCATTAGCCATTATGTGAACTGTTGTC
6c21_Erin       AACACCTCTATACCAACCTTTGCCCTCTCCGCACCATTAGCCATTATGTGAACTGTTGTC
9a2_7_Erin      AACACCTCTATACCAACCTTTGCCCTCTCCGCACCATTAGCCATTATGTGAACTGTTGTC
9a_Erin         AACACCTCTATACCAACCTTTGCCCTCTCCGCACCATTAGCCATTATGTGAACTGTTGTC
17c_Erin        AACACCTCTATACCAACCTTTGCCCTCTCCGCACCATTAGCCATTATGTGAACTGTTGTC
6a_Erin         AACACCTCTATACCAACCTTTGCCCTCTCCGCACCATTAGCCATTATGTGAACTGTTGTC
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16d_Erin        AACACCTCTATACCAACCTTTGCCCTCTCCGCACCATTAGCCATTATGTGAACTGTTGTC
2e_22_Erin      AACACCTCTATACCAACCTTTGCCCTCTCCGCACCATTAGCCATTATGTGAACTGTTGTC
76_3            AACACCTCTATACCAACCTTTGCCCTCTCCGCACCATTAGCCATTATGTGAACTGTTGTC
71_4            AACACCTCTATACCAACCTTTGCCCTCTCCGCACCATTAGCCATTATGTGAACTGTTGTC
6b27_Erin       AACACCTCTATACCAACCTTTGCCCTCTCCGCACCATTAGCCATTATGTGAACTGTTGTC
8d25_Erin       AACACCTCTATACCAACCTTTGCCCTCTCCGCACCATTAGCCATTATGTGAACTGTTGTC
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Appendix A (Continued)

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2d22_Erin       AACACCTCTATACCAACCTTTGCCCTCTCCGCACCATTAGCCATTATGTGAACTGTTGTC
Sab1_4          AACTTCCTTATACCTACCATCACTCTTTCATTACCGTTAGCCATTATATGAATAACAGTT
                ***  *  ****** *** *  * ** **   *** *********** ****     **

71_3            ATCTTAGCAGAAACAAACCGGGCCCCA-TTTGACTTTGCTGAAGGGGAATCAGAA-CTAG
71_6            ATCTTAGCAGAAACAAACCGAGCCCCA-TTTGACTTTGCTGAAGGGGAATCAGAA-CTAG
76_5            ATCTTAGCAGAAACAAACCGAGCCCCA-TTTGACTTTGCTGAAGGGGAATCAGAA-CTAG
69_3            ATCTTAGCAGAAACAAACCGAGCCCCA-TTTGACTTTGCTGAAGGGGAATCAGAA-CTAG
71_8            ATCTTAGCAGAAACAAACCGAGCCCCA-TTTGACTTTGCTGAAGGGGAATCAGAA-CTAG
69_2            ATCTTAGCAGAAACAAACCGAGCCCCA-TTTGACTTTGCTGAAGGGGAATCAGAA-CTAG
90_6            ATCTTAGCAGAAACAAACCGAGCCCCA-TTTGACTTTGCTGAAGGGGAATCAGAA-CTAG
90_5            ATCTTAGCAGAAACAAACCGAGCCCCA-TTTGACTTTGCTGAAGGGGAATCAGAA-CTAG
71_5            ATCTTAGCAGAAACAAACCGAGCCCCA-TTTGACTTTGCTGAAGGGGAATCAGAA-CTAG
76_4            ATCTTAGCAGAAACAAACCGAGCCCCA-TTTGACTTTGCTGAAGGGGAATCAGAA-CTAG
11b2_Erin       ATCTTAGCAGAAACAAACCGAGCCCCA-TTTGACTTTGCTGAAGGGGAATCAGAA-CTAG
2h_Erin         ATCTTAGCAGAAACAAACCGAGCCCCA-TTTGACTTTGCTGAAGGGGAATCAGAA-CTAG
17d_Erin        ATCTTAGCAGAAACAAACCGAGCCCCA-TTTGACTTTGCTGAAGGGGAATCAGAA-CTAG
6c21_Erin       ATCTTAGCAGAAACAAACCGAGCCCCA-TTTGACTTTGCTGAAGGGGAATCAGAA-CTAG
9a2_7_Erin      ATCTTAGCAGAAACAAACCGAGCCCCA-TTTGACTTTGCTGAAGGGGAATCAGAA-CTAG
9a_Erin         ATCTTAGCAGAAACAAACCGAGCCCCA-TTTGACTTTGCTGAAGGGGAATCAGAA-CTAG
17c_Erin        ATCTTAGCAGAAACAAACCGAGCCCCA-TTTGACTTTGCTGAAGGGGAATCAGAA-CTAG
6a_Erin         ATCTTAGCAGAAACAAACCGAGCCCCA-TTTGACTTTGCTGAAGGGGAATCAGAA-CTAG
16d_Erin        ATCTTAGCAGAAACAAACCGAGCCCCA-TTTGACTTTGCTGAAGGGGAATCAGAA-CTAG
2e_22_Erin      ATCTTAGCAGAAACAAACCGAGCCCCA-TTTGACTTTGCTGAAGGGGAATCAGAA-CTAG
76_3            ATCTTAGCAGAAACAAACCGAGCCCCA-TTTGACTTTGCTGAAGGGGAATCAGAA-CTAG
71_4            ATCTTAGCAGAAACAAACCGAGCCCCA-TTTGACTTTGCTGAAGGGGAATCAGAA-CTAG
6b27_Erin       ATCTTAGCAGAAACAAACCGAGCCCCATTTTGACTTTGCTGAAGGGGAATCAGAA-CTAG
8d25_Erin       ATCTTAACAGAAACAAACCCGAACCCCATTTGACTTTGCTGAAGGGGAATCAGAA-CTAG
2d22_Erin       ATCTTAGCAGAAACAAACCGAGCCCCA-TTTGACTTTGCTGAAGGGGAATCAGAAACTAT
Sab1_4          ATTATAGCAGAAACAAACCGAGCCCCA-TTTGATTTTGCCGAAGGGGAATCAGAA-CTAG
                **  ** ************    ***  ***** ***** ************** ***

71_3            TCTCTGGATTTAATATTGAGTACGGCGGAGCCGG-CTTTGCTTTCCTCTTTATAGCCGAA
71_6            TCTCTGGATTTAATATTGAGTACGGCGGAGCCGG-CTTTGCTTTCCTCTTTATAGCCGAA
76_5            TCTCTGGATTTAATATTGAGTACGGCGGAGCCGG-CTTTGCTTTCCTCTTTATAGCCGAA
69_3            TCTCTGGATTTAATATTGAGTACGGCGGAGCCGG-CTTTGCTTTCCTCTTTATAGCCGAA
71_8            TCTCTGGATTTAATATTGAGTACGGCGGAGCCGG-CTTTGCTTTCCTCTTTATAGCCGAA
69_2            TCTCTGGATTTAATATTGAGTACGGCGGAGCCGG-CTTTGCTTTCCTCTTTATAGCCGAA
90_6            TCTCTGGATTTAATATTGAGTACGGCGGAGCCGG-CTTTGCTTTCCTCTTTATAGCCGAA
90_5            TCTCTGGATTTAATATTGAGTACGGCGGAGCCGG-CTTTGCTTTCCTCTTTATAGCCGAA
71_5            TCTCTGGATTTAATATTGAGTACGGCGGAGCCGG-CTTTGCTTTCCTCTTTATAGCCGAA
76_4            TCTCTGGATTTAATATTGAGTACGGCGGAGCCGG-CTTTGCTTTCCTCTTTATAGCCGAA
11b2_Erin       TCTCTGGATTTAATATTGAGTACGGCGGAGCCGG-CTTTGCTTTCCTCTTTATAGCCGAA
2h_Erin         TCTCTGGATTTAATATTGAGTACGGCGGAGCCGG-CTTTGCTTTCCTCTTTATAGCCGAA
17d_Erin        TCTCTGGATTTAATATTGAGTACGGCGGAGCCGG-CTTTGCTTTCCTCTTTATAGCCGAA
6c21_Erin       TCTCTGGATTTAATATTGAGTACGGCGGAGCCGG-CTTTGCTTTCCTCTTTATAGCCGAA
9a2_7_Erin      TCTCTGGATTTAATATTGAGTACGGCGGAGCCGG-CTTTGCTTTCCTCTTTATAGCCGAA
9a_Erin         TCTCTGGATTTAATATTGAGTACGGCGGAGCCGG-CTTTGCTTTCCTCTTTATAGCCGAA
17c_Erin        TCTCTGGATTTAATATTGAGTACGGCGGAGCCGG-CTTTGCTTTCCTCTTTATAGCCGAA
6a_Erin         TCTCTGGATTTAATATTGAGTACGGCGGAGCCGG-CTTTGCTTTCCTCTTTATAGCCGAA
16d_Erin        TCTCTGGATTTAATATTGAGTACGGCGGGCCCGG-CTTTGCTTTCCTCTTTATAGCCGAA
2e_22_Erin      TCTCTGGATTTAATATTGAGTACGGCGGAGCCGG-CTTTGCTTTCCTCTTTATAGCCGAA
76_3            TCTCTGGATTTAATATTGAGTACGGCGGAGCCGG-CTTTGCTTTCCTCTTTATAGCCGAA
71_4            TCTCTGGATTTAATATTGAGTACGGCGGAGCCGG-CTTTGCTTTCCTCTTTATAGCCGAA
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6b27_Erin       TCTCTGGATTTAATATTGAGTACGGCGGAGCCGGGCTTTGCTTTCCTCTTTATANCCGAA
8d25_Erin       TCTCTGGATTTAATATTGAGTACGGCGGAGCCGG-CTTTGCTTTCCTCTTTATAGCCGAA
2d22_Erin       TCTCTGGATTTAATATTGAGTACGGCGGAGCCGG-CTTTGCTTTCCTCTNNNNNNNCGAA
Sab1_4          TCTCAGGGTTTAATGTAGAATACGGCGGAGCCGG-CTTTGCTTTCCTCTTCATAGCTGAA
                **** ** ****** * ** ********  **** **************        ***

71_3            TACAGTAACATC-TTAATAATAA-GACTCC-TTACTGCC-TGTATACTAA-CAGGCACCC
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Appendix A (Continued)

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71_6            TACAGTAACATC-TTAATAATAA-GACTCC-TTACTGCC-TGTATACTAA-CAGGCACCC
76_5            TACAGTAACATC-TTAATAATAA-GACTCC-TTACTGCC-TGTATACTAA-CAGGCACCC
69_3            TACAGTAACATC-TTAATAATAA-GACTCC-TTACTGCC-TGTATACTAA-CAGGCACCC
71_8            TACAGTAACATC-TTAATAATAA-GACTCC-TTACTGCC-TGTATACTAA-CAGGCACCC
69_2            TACAGTAACATC-TTAATAATAA-GACTCC-TTACTGCC-TGTATACTAA-CAGGCACCC
90_6            TACAGTAACATC-TTAATAATAA-GACTCC-TTACTGCC-TGTATACTAA-CAGGCACCC
90_5            TACAGTAACATC-TTAATAATAA-GACTCC-TTACTGCC-TGTATACTAA-CAGGCACCC
71_5            TACAGTAACATC-TTAATAATAA-GACTCC-TTACTGCC-TGTATACTAA-CAGGCACCC
76_4            TACAGTAACATC-TTAATAATAA-GACTCC-TTACTGCC-TGTATACTAA-CAGGCACCC
11b2_Erin       TACAGTAACATC-TTAATAATAA-GACTCC-TTACTGCC-TGTATACTAA-CAGGCACCC
2h_Erin         TACAGTAACATC-TTAATAATAA-GACTCC-TTACTGCC-TGTATACTAA-CAGGCACCC
17d_Erin        TACAGTAACATC-TTAATAATAA-GACTCC-TTACTGCC-TGTATACTAA-CAGGCACCC
6c21_Erin       TACAGTAACATC-TTAATAATAA-GACTCC-TTACTGCC-TGTATACTAA-CAGGCACCC
9a2_7_Erin      TACAGTAACATC-TTAATAATAA-GACTCC-TTACTGCC-TGTATACTAA-CAGGCACCC
9a_Erin         TACAGTAACATC-TTAATAATAA-GACTCC-TTACTGCC-TGTATACTAA-CAGGCACCC
17c_Erin        TACAGTAACATC-TTAATAATAA-GACTCC-TTACTGCC-TGTATACTAA-CAGGCACCC
6a_Erin         TACAGTAACATC-TTAATAATAA-GACTCC-TTACTGCC-TGTATACTAA-CAGGCACCC
16d_Erin        TACAGTAACATC-TTAATAATAAAGACTCCCTTACTGCC-TGTATACTAA-CAGGCACCC
2e_22_Erin      TACAGTAACATC-TTAATAATAA-GACTCC-TTACTGCC-TGTATACTAA-CAGGCACCC
76_3            TACAGTAACATC-TTAATAATAA-GACTCC-TTACTGCC-NGTATACTAA-CNGGCACCC
71_4            TACAGTAACATC-TTAATAATAA-GACTCC-TTACTGCC-TGTATACTAA-CAGGCACCC
6b27_Erin       TACAGTANCATC-TTAATAATAN-GACTNCCTTACTGCNCTGTATACTAM-CAGGCACCC
8d25_Erin       TACAGTAACATCCTTAATAATAA-GACTCCCTTACTGCC-TGTATACTAA-CAGGCACCC
2d22_Erin       TACAGNAACATC-TTAATAATAA-GACTNCCTTACTGCC-TGTATACTAAACAGGCACCN
Sab1_4          TACAGAAATATC-TTAATAATGA-GACTAC-TTACCGCC-AGTATATTAA-CGGGTACCT
                ***** *  *** ********   **** * **** **   ***** **  * ** ***

71_3            -TACTGATA-TCAACCCCAGT-AGCAATCATTTT-CCTTTGAGCACGA-GCCACCC-TCC
71_6            -TACTGATA-TCAACCCCAGT-AGCAATCATTTT-CCTTTGAGCACGA-GCCACCC-TCC
76_5            -TACTGATA-TCAACCCCAGT-AGCAATCATTTT-CCTTTGAGCACGA-GCCACCC-TCC
69_3            -TACTGATA-TCAACCCCAGT-AGCAATCATTTT-CCTTTGAGCACGA-GCCACCC-TCC
71_8            -TACTGATA-TCAACCCCAGT-AGCAATCATTTT-CCTTTGAGCACGA-GCCACCC-TCC
69_2            -TACTGATA-TCAACCCCAGT-AGCAATCATTTT-CCTTTGAGCACGA-GCCACCC-TCC
90_6            -TACTGATA-TCAACCCCAGT-AGCAATCATTTT-CCTTTGAGCACGA-GCCACCC-TCC
90_5            -TACTGATA-TCAACCCCAGT-AGCAATCATTTT-CCTTTGAGCACGA-GCCACCC-TCC
71_5            -TACTGATA-TCAACCCCAGT-AGCAATCATTTT-CCTTTGAGCACGA-GCCACCC-TCC
76_4            -TACTGATA-TCAACCCCAGT-AGCAATCATTTT-CCTTTGAGCACGA-GCCACCC-TCC
11b2_Erin       -TACTGATA-TCAACCCCAGT-AGCAATCATTTT-CCTTTGAGCACGA-GCCACCC-TCC
2h_Erin         -TACTGATA-TCAACCCCAGT-AGCAATCATTTT-CCTTTGAGCACGA-GCCACCC-TCC
17d_Erin        -TACTGATA-TCAACCCCAGT-AGCAATCATTTT-CCTTTGAGCACGA-GCCACCC-TCC
6c21_Erin       -TACTGATA-TCAACCCCAGT-AGCAATCATTTT-CCTTTGAGCACGA-GCCACCC-TCC
9a2_7_Erin      -TACTGATA-TCAACCCCAGT-AGCAATCATTTT-CCTTTGAGCACGA-GCCACCC-TCC
9a_Erin         -TACTGATA-TCAACCCCAGT-AGCAATCATTTT-CCTTTGAGCACGA-GCCACCC-TCC
17c_Erin        -TACTGATA-TCAACCCCAGT-AGCAATCATTTT-CCTTTGAGCACGA-GCCACCC-TCC
6a_Erin         -TACTGATA-TCAACCCCAGT-AGCAATCATTTT-CCTTTGAGCACGA-GCCACCC-TCC
16d_Erin        -TACTGATA-TCAACCCCAGT-AGCAATCATTTT-CCTGTAAGCACGAAGCCACCCCTCC
2e_22_Erin      -TACTGATA-TCAACCCCAGT-AGCAATCATTTT-CCTTTGAGCACGA-GCCACCC-TCC
76_3            -TACTGATA-TCAACCCCAGT-AGCAATCATTTT-CCTTTGAGCACGA-GCCACCC-TCC
71_4            -TACTGATA-TCAACCCCAGT-AGCAATCATTTT-CCTTTGAGCACGA-GCCACCC-TCC
6b27_Erin       -TACTGATA-TCAACCCCAGTTANCAATCATTTTTCCTTTGAGCACGA-GCCACCC-TCC
8d25_Erin       -TACTGATA-TCAACCCCAGTTAGCAATCATTTTTCCTTTGAGCACGA-GCCACCC-TCC
2d22_Erin       CTACTGATNATCAACCCCAGN-ANCAATCATTTT-CCTTTGAGCACGA-GCCANCC-TYC
Sab1_4          -TGTGAATA-TCAACCCCAGT-AGCCATCGTCTT-TCTGTGGACACGA-GCAACTC-TAC
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71_3              CCCGATATC-GATATGACCTACTGAT-AGCAATAGCCTGAAAATCTTTTCTCCCAGTAAG
71_6              CCCGATATC-GATATGACCTACTGAT-AGCAATAGCCTGAAAATCTTTTCTCCCAGTAAG
76_5              CCCGATATC-GATATGACCTACTGAT-AGCAATAGCCTGAAAATCTTTTCTCCCAGTAAG
69_3              CCCGATATC-GATATGACCTACTGAT-AGCAATAGCCTGAAAATCTTTTCTCCCAGTAAG
71_8              CCCGATATC-GATATGACTTACTGAT-AGCAATAGCCTGAAAATCTTTTCTCCCAGTAAG
69_2              CCCGATATC-GATATGACCTACTGAT-AGCAATAGCCTGAAAATCTTTTCTCCCAGTAAG
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Appendix A (Continued)

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90_6              CCCGATATC-GATATGACCTACTGAT-AGCAATAGCCTGAAAATCTTTTCTCCCAGTAAG
90_5              CCCGATATC-GATATGACCTACTGAT-AGCAATAGCCTGAAAATCTTTTCTCCCAGTAAG
71_5              CCCGATATC-GATATGACCTACTGAT-AGCAATAGCCTGAAAATCTTTTCTCCCAGTAAG
76_4              CCCGATATC-GATATGACCTACTGAT-AGCAATAGCCTGAAAATCTTTTCTCCCAGTAAG
11b2_Erin         CCCGATATC-GATATGACCTACTGAT-AGCAATAGCCTGAAAATCTTTTCTCCCAGTAAG
2h_Erin           CCCGATATC-GATATGACCTACTGAT-AGCAATAGCCTGAAAATCTTTTCTCCCAGTAAG
17d_Erin          CCCGATATC-GATATGACCTACTGAT-AGCAATAGCCTGAAAATCTTTTCTCCCAGTAAG
6c21_Erin         CCCGATATC-GATACGACCTACTGAT-AGCAATAGCCTGAAAATCTTTTCTCCCAGTAAG
9a2_7_Erin        CCCGATATC-GATACGACCTACTGAT-AGCAATAGCCTGAAAATCTTTTCTCCCAGTAAG
9a_Erin           CCCGATATC-GATACGACCTACTGAT-AGCAATAGCCTGAAAATCTTTTCTCCCAGTAAG
17c_Erin          CCCGATATC-GATATGACCTACTGAT-AGCAATAGCCTGAAAATCTTTTCTCCCAGTAAG
6a_Erin           CCCGATATC-GATACGACCTACTGAT-AGCAATAGCCTGAAAATCTTTTCTCCCAGTAAG
16d_Erin          CCCGATATC-GATATGACCTACTGAT-AGCAATAGCCTSWAAATCTTTTCTCCCAGTAAG
2e_22_Erin        CCCGATATC-GATACGACCTACTGAT-AGCAATAGCCTGAAAATCTTTTCTCCCAGTAAG
76_3              CCCGATATC-GATATGACCTACTGAT-AGCAATAGCCTGAAAATNNTTTCTCCCAGTAAG
71_4              CCCGATATC-GATACGACCTACTGAT-AGCAATAGCCTGAAAATCTTTTCTCCCAGTAAG
6b27_Erin         CCCGATANM-GANNCNACCTACNNNNTNNNANTAGCCNGNAAATCTNNNCNNNYNNNNGA
8d25_Erin         CCCGATATCAGATACGACCTACTGATTAGCAATAGCCTGAAANNNNTTTCNNCNCNNNNN
2d22_Erin         CCCGATATN-NNNACGACCNACNGAT-AGCAATAGCCTGAAANTCTTTTTCTCCNANNNA
Sab1_4            CACGATACC-GGTACGACCTATTAAT-AGGAATAGCATGAAAATCTTTCCTTCCAGTCAG
                  * *****         **  *         * ****    **

71_3              AT-TAATTATTCT-ACTAGCATCCACCCCACTTATGTTTATCATAAGNNNNNNNNNNAAN
71_6              AT-TAATTATTCT-ACTAGCATCCACCCCACTTATGTTTATCATAANNNNNNNNNNNNAA
76_5              AT-TAATTATTCT-ACTAGCATCCACCCCACTTATGTTTATCATAANNNNNNNNNNNAAN
69_3              AT-TAATTATTCT-ACTAGCATCCACCCCACTTATGTTTATCATANGNNNNNNNNNAAN
71_8              AT-TAATTATTCT-ACTAGCATCCACCCCACTTATGTTTATCATCCNNNNNNNNNNAAAA
69_2              AT-TAATTATTCT-ACTAGCATCCACCCCACTTATGTTTATCNNNNNNNNNNNNNNNNNN
90_6              AT-TAATTATTCT-ACTAGCATCCACCCCACTTATGTTTATNNNNNNNNNNNNNNNNNN
90_5              AT-TAATTATTCT-ACTAGCATCCACCCCANNTANNTNTATCNNNNNNNNNNNNNNNNN
71_5              AT-TAATTATTCT-ACTAGCATCCACCCCACTTATGTTTATCATNANNNNNNNNNNNANN
76_4              AT-TAATTATTCT-ACTAGCATCCACCCCACTTATGTTTATCATCCNNNNNNNNNNNNNN
11b2_Erin         AT-TAATTATTCT-ACTAGCATCCACCCCACTTATGTTTATCATAAGATAACTCATAACG
2h_Erin           AT-TAATTATTCT-ACTAGCATCCACCCCACTTATGTTTATCATAAGATAACTCATAACG
17d_Erin          AT-TAATTATTCT-ACTAGCATCCACCCCACTTATGTTTATCATAAGATAACTCATAACG
6c21_Erin         AT-TAATTATTCT-ACTAGCATCCACCCCACTTATGTTTATCATAAGATAACTCATAACG
9a2_7_Erin        AT-TAATTATTCT-ACTAGCATCCACCCCACTTATGTTTATCATAAGATAACTCATAACG
9a_Erin           AT-TAATTATTCT-ACTAGCATCCACCCCACTTATGTTTATCATAAGATAACTCATAACG
17c_Erin          AT-AAATTATTCT-ACTAGCATCCCCCCCACTTATGTTTATCATAA--------------
6a_Erin           AT-TAATTATTCT-ACTAGCATCCACCCCACTTATGTTTATCATAAGATAACTCATAANN
16d_Erin          AT-TAATTATTCW-ACTAGCATCCACCCCACTTATGTTTAT-------------------
2e_22_Erin        AT-TAATTATTCT-ACTAGCATCCACCCCACTTATGTTTATCATAAGNNNNNNNNNNNNN
76_3              ANTNANTTATTCT-ACTAGCATCCNCCCCNCTTATGNTNATCNNMNGANNNNNNNNNNAN
71_4              AT-TAATTATTCT-ACTAGCATCCACCCCACTTATGTTTATCATAAGACTNNNNCNNNAA
6b27_Erin         NATTNATNANTCNNNMTANNNTCCNACNCCCACCTTANGNTTWTMNNNNNNNNNNNNANNN
8d25_Erin         NATTANNTAWTCTANNANNNATCCCNCNCNNN--TNATGNNNTANCATAA----------
2d22_Erin         GNTTANTNANTNYTACTAGCATCCACC---------------------------------
Sab1_4            TT-TAGCTATCCT-ATTATTATCTATCCCATTAATATTTATTATAGTATAAGCANNNNNN
                         *           **   *

71_3              NNNNNANNNNNNNNNNNNNCAAGTACACNNNNNNNNNNCNNNNNNNNNGNN--------
71_6              NANNNATNNANNNNNNNNNNN---------------------------------------
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76_5            NNNNCATNNNNNNNNNNNNNNNNN-----------------------------------
69_3            NNNNATNNNNNNNNNNNNNNNNAGTACACTTGCNTTNNCNANNCANGGNN-----------
71_8            NNNNNCANNNNNNNNNNNN--------------------------------------------
69_2            NNNNNNNNNNNNNNNNNNNNN-----------------------------------------
90_6            NNNNNNNNNNNNNNNNNNNNNNNNNNACA-CTTGCTTTCCAANNNGGANNN-----------
90_5            NNNNNNNNNNNNNNNNNNNNNNNNTACAC-TTGCCTTTCCAAGCAGGNNN------------
71_5            MGNNNNNNNNNNNNNNNNNNNNNNNNNNACACTTGCCTTCCAAGCNNGGN-------------
76_4            NNNNCNNNNNNNNNNNNNNNNN-----------------------------------
11b2_Erin       TTAATGCTGGAAGTATATCAAGTACACTTGCCNTTNCCAAGCAGGNA-------------
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Appendix A (Continued)

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2h_Erin         TTAATGCTGGAAGTATATCAAGTACACTTGCCNTTNCCAAGCAGG---------------
17d_Erin        TTAATGCTGGAAGTATNNN---NGTACNTGCCNTTNCCAAGCAG----------------
6c21_Erin       TTAATGCTGGAAGTATATCAAGT-------------------------------------
9a2_7_Erin      TTAATGCTGGAAGTATATCAAGTANN-TNGCCNTTNCCNNNNAGANA-------------
9a_Erin         TTAATGCTGGAAGTATNTCAAGTANNT---------------------------------
17c_Erin        ------------------------------------------------------------
6a_Erin         NTNANNNNNNNNNNGNNNNNCAAGNANCTTGCCNNNNNNNCAAGGAANANNNNNNNNNNN
16d_Erin        ------------------------------------------------------------
2e_22_Erin      ANNNNNNNNNNNNNNNNNN-----------------------------------------
76_3            NNCATNNNNNNNNNNNNNNNNNN------------------------------------
71_4            NRNTNNNNNNNNNNNNNNNNNNNNACACTTGNNTTCAANANGN-----------------
6b27_Erin       N-----------------------------------------------------------
8d25_Erin       ------------------------------------------------------------
2d22_Erin       ------------------------------------------------------------
Sab1_4          NNNRNNNNNNNNNNNNNNNNNNNNNNNNNNTACACTTGNNNNNNNNNANNNNNNNNN----
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71_3            --------------
71_6            --------------
76_5            --------------
69_3            --------------
71_8            --------------
69_2            --------------
90_6            --------------
90_5            --------------
71_5            --------------
76_4            --------------
11b2_Erin       --------------
2h_Erin         --------------
17d_Erin        --------------
6c21_Erin       --------------
9a2_7_Erin      --------------
9a_Erin         --------------
17c_Erin        --------------
6a_Erin         CNAGCCCNAAAANN
16d_Erin        --------------
2e_22_Erin      --------------
76_3            --------------
71_4            --------------
6b27_Erin       --------------
8d25_Erin       --------------
2d22_Erin       --------------
Sab1_4          --------------
```

Appendix B. The total level of infestation on each target fish species per site and fish sampling date

Red shiner	N	Date	Site	# Juveniles	# Gloch.	# Encysted	Total
	46	29-May	SBN	111	205	3	219
	26	10-Jul	SBN	45	75	17	137
	10	11-Jul	NCHS	7	87	15	109
	11	4-Aug	LKFRC	13	172	33	213
	15	7-Aug	NCHS	67	45	5	117
	3	23-Oct	SBN	0	0	0	0
	3	24-Oct	NCHS	0	0	0	0
TOTAL:	114			243	584	73	795

Blacktail shiner	N	Date	Site	# Juveniles	#Gloch	# Encysted	Total
	14	3-Jun	SBN	16	0	1	17
	6	10-Jul	SBN	0	0	2	2
	7	11-Jul	NCHS	9	14	0	23
	23	4-Aug	LKFRC	18	57	60	135
	22	7-Aug	NCHS	25	8	4	37
	0	23-Oct	SBN	0	0	0	0
	15	24-Oct	NCHS	0	0	0	0
TOTAL:	87			68	79	67	214

Bullhead minnow	N	Date	Site	# Juveniles	# Gloch.	# Encysted	Total
	1	29-May	SBN	0	0	0	0
	2	3-Jun	SBN	2	251	0	253
	3	10-Jul	SBN	0	405	0	405
	14	23-Oct	SBN	0	0	0	0
	26	24-Oct	NCHS	0	0	0	0
TOTAL:	46			2	656	0	658

Appendix C. Water quality data for the Aquatic Habitat Tank Units throughout the duration of the study

Date:	Water Supply	pH	Conductivity (µS)	Temp. C	DO mg/L	NO2 (ppm)	NO3 (ppm)	NH+4 (ppm)
May 21st	Reservoir	8.28	606	25.8				
May 21st	Tanks	8.3	670	26.4			5.00	3.00
May 22nd	Tanks	8.3	680	27				1.0
May 23rd	Tanks	8.3	680	26.7			0.0-5.0	3.00
May 27th	Tanks	8.54	714	27.5			10.0	0.00
May 27th	Reservoir	8.2	622	26.9				
May 28th	Tanks	8.34	698	26.2			10.00	0.00
May 30th	Tanks	8.39	714	26.8			10.0	0.00
May 31st	Tanks	8.4	726	28			10.00	0.00
May 31st	Reservoir	8.1	632	25				
June 1st	Tanks	8.35	725	28			10.00	0.00
June 2nd	Reservoir	8.3	642	26.6			10.00	0.00
June 2nd	Tanks	8.2	715	28.4			10.0	0.00
June 2nd	Reservoir	8.14	615	25			5.00	0.00
June 3rd	Tanks	8.37	720	27				
June 6th	Tanks	7.64	735	27.9	7.79		10.00	0.00
June 6th	Reservoir	7.85	647	26	5.5		10.00	0.00
June 7th	Tanks	8.02	750	28.6	5			
June 9th	Tanks	8.38	755	29	5.53		40.00	0.00
June 9th	Reservoir	8.3	602	27	3.54		10.00	0.00
June 10th	Tanks	8.23	747	26.98	5.27		40.00	0.00
June 11th	Tanks	8.16	716	27.9	5.25		40.00	0.00
June 11th	Reservoir	8.2	512	25.47	3.24		5.00	
June 12th	Tanks	8.2	712	27	4.36		40.00	
June 12th	Reservoir	8.38	511				5.00	
June 13th	Tanks	8.28	715	27.46	4.5			
June 13th	Reservoir	8.1	426	24				
June 16th	Tanks	8.12	686	27.98	3.74		40.00	0.00
June 16th	Reservoir	8.45	471	26.33	3.8			
June 17th	Reservoir	8.14	556	26	3.41		5.00	
June 17th	Tanks	7.9	690	29	2.46		40.00	0.00
June 18th	Tanks	8.2	691	29.26	4.78		40.00	0.00
June 18th	Reservoir	8.13	560	26.5	2.51		0.0-5.0	
June 19th	Tanks	8.22	417	28.36	5.08		40.00	0.00
June 19th	Reservoir	8.11	561	26.12	2.87		5.00	0.00
June 19th	Tanks	7.81	787	28.22	2.3		40.00	0.00
June 23rd	Tanks	7.99	775	27.54	7.14		0.00	0.00
June 23rd	Reservoir	7.98	562	25.45	2.78		5.00	0.00
June 23rd	Tanks	7.66	767	27.15	35.9		20.00	0.25
June 26th	Tanks	7.76	744	27.25	7.5		20.0	0.25
June 26th	Reservoir	7.77	511	24.4	7.75		0.0-5.0	0.25
June 27th	Tanks	7.75	742	27.69	8.22			0.25
June 29th	Tanks	7.89	727	28	12			0.25
June 30th	Tanks	7.68	725	28.88	12.06		40.00	0.00
July 3rd	Tanks	7.57	728	29.24	11.15		40.00	0.25
July 3rd	Reservoir	7.8	425	29.08	11.09		0.0-5.0	0.10
July 4th	Tanks	7.63	709	28.92	11.28		40.00	0.25
July 4th	Reservoir	7.57	423	27.61	11.5		5.00	0.10
July 6th	Tanks	7.92	718	29.4	11.09		40.00	0.25
July 6th	Reservoir	7.84	428	27.53	12.25		5.00	0.10
July 9th	Tanks	7.6	720	28.65	15.45		40.0	0.00
July 9th	Reservoir	7.69	445	25.89	16.3		0.0-5.0	0.00

Appendix C. (Continued)

Date:	Water Supply	pH	Conductivity (µS)	Temp. C	DO mg/L	NO2 (ppm)	NO3 (ppm)	NH+4 (ppm)
July 12th	Tanks	7.72	665	27.38	14.4		40.00	0.25
July 12th	Reservoir	7.8	445	26.2	15		0.0-5.0	0.00
July 14th	Tanks	7.85	690	28	13.11		40.00	0.25
July 14th	Reservoir	7.92	450	26.67	14.06		0.00	0.00
July 14th	Reservoir	7.67	430	25	13.11			
July 14th	Tanks	7.45	638				20.0	
July 15th	Tanks	7.7	658	28	13		20.00	
July 15th	Reservoir	7.65	435	26	12.15			
July 20th	Tanks	7.96	606	26.56	9.46		40.00	0.25
July 20th	Reservoir	7.87	443	25.09	9.51		0.0-5.0	0.00
July 22nd	Tanks	7.97	560	27.31	8.29		20.00	0.00
July 22nd	Reservoir	7.96	391	26.37	8.52		0.00	0.00
July 22nd	Tanks		625					
July 28th	Tanks	8.5	639	28.43	2.35		80.00	0.25
July 28th	Reservoir	7.92	362	26.84	3.04		0.0-5.0	0.00
July 28th	Tanks	7.85	509	27.52	2.36		20.0	
July 28th	Reservoir	7.91	332	26.65	3.06			
July 28th	Tanks		670				20.00	0.00
July 28th	Reservoir		425					
July 29th	Tanks	7.83	684	27.5	2.59		10.00	0.00
July 29th	Reservoir	7.83	423	26.7	2.19		0.00	
July 30th	Tanks	8.02	688	27.39				
July 31st	Tanks	8.05	690	26.7			20.0	
July 31st	Reservoir	8.06	487	25.5				
Aug 2nd	Tanks	8.2	634	26			20.0	
Aug 2nd	Reservoir	8.17	513	25				
Aug 5th	Tanks	8.15	615	27.5			40.00	0.00
Aug 5th	Reservoir	8.1	504	26.75			0.00	
Aug 6th	Tanks					0.00	10.0	
Aug 9th	Tanks	7.95	635	27			10.00	0.00
Aug 9th	Reservoir	7.9	436	26.5				
August 12th	Tanks	8.2	647	27.68		0.00	10.00	0.25
August 12th	Reservoir	8.1	440	26.4				
August 17th	Reservoir	8.16	381	27.5				
October 22nd	Tanks	7.22	468	24.8	3.14	0.00	5.00	0.00
October 22nd	Reservoir	7.22	330	23.9	2.29	0.00	0.00	0.00
October 28th	Tanks	8.32	529	25.91	3.57	0.00	0.0-5.0	0.00
October 28th	Reservoir	8.34	248	24.67	1.66			
Average:		8.0089773	593.010989	26.97069767	7.7859649	0.00	20.63	0.17

Appendix D. Water quality conditions of each field site at the time of fish collection

Date	Site	Water Temp C	pH	Conductivity (μS)
May 29th	Sabine River	24	7.9	313
June 3rd	Sabine River	26	8.5	340
July 10th	Sabine River	29	7.9	364
July 11th	Neches River	30.5	7.7	228
August 4th	Lake Fork Creek	25	8.3	201
August 7th	Neches River	29.6	8	200
October 23rd	Sabine River	18.21	8.9	350
October 24th	Neches River	19.6	7.85	205