SICKLEFIN REDHORSE PROPAGATION PLAN
Warm Springs NFH

Male Sicklefin Redhorse, *Moxostoma species*, Photo Credit: S. Fraley

Department of the Interior
U.S. Fish and Wildlife Service

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January 17, 2019
Introduction

Sicklefin Redhorse are in the *Moxostoma* genus, their status as a distinct species remains under review. Sicklefin Redhorse (sicklefin) are listed by the State of North Carolina as a Threatened species and by USFWS in 2016 as a candidate species (for elevated listing) throughout its entire range. Habitat fragmentation and alteration have contributed to lengths of waterways within historical ranges being depopulated of the species. Sicklefin are important not only for their place in the ecosystem but also for their significant role in preserving a part of Cherokee culture.

The Sicklefin Redhorse Research Partnership was developed in part to restore and recover populations in these locations fragmented by dams and other forms of habitat alteration. Warm Springs National Fish Hatchery (WSNFH) began sicklefin propagation efforts during 2013 in cooperation with conservation partners. Sicklefin at that time were confined to tributaries of the Hiwassee and Little Tennessee rivers of the upper Tennessee River basin as reported in *Propagation and Reintroduction of Sicklefin Redhorse (Moxostoma sp.) to the Tuckasegee and Oconaluftee Rivers, North Carolina: Final Report for 2013* by: M.A. Petty, P.L. Rakes, C.L. Ruble, and J.R. Shute, Conservation Fisheries, Inc. February 25, 2014.

Relevant management guidelines are included in *Strategy for the Conservation and Recovery of Southeastern Imperiled Fishes and the Fishery Management Plan of the*
Eastern Band of Cherokee Indians, Qualla Boundary (Swain, Jackson, Cherokee, and Graham Counties, North Carolina). A Candidate Conservation Agreement for sicklefin was finalized in December 2015 by the Partnership for continued conservation work that includes use of propagated fish. Current sites recommended for distribution of propagated sicklefin includes sites within the Oconaluftee River Watershed located in Swain and Jackson Counties, North Carolina and within the Eastern Band of Cherokee Indian Reservation.

![Map of the Oconaluftee River Watershed](image)

**Background**

This plan provides propagation guidelines used at WSNFH to meet sicklefin fry production and distribution tasks identified by the Partnership. Propagation undertaken at Warm Springs is in cooperation with co-located fisheries programs at Warm Springs, the Eastern Band of Cherokee Indians, USFWS Ecological Services (ES), Ashville, NC, Conservation Fisheries Inc. (CFI), North Carolina Wildlife Resource Commission (NCWRC), Duke Power, Western Carolina University, and others.

This plan concludes with rearing of larger sicklefin (with stronger swimming abilities) for research needs, optional tagging opportunities and distribution.

To that end, these guidelines draw upon previous experience, other facilities’ information, and techniques developed for spawning and producing Robust Redhorse through the 1990’s and 2000’s. Many of the techniques reported by Greg Looney in his 1997 *Robust Redhorse Spawning Protocol* are incorporated where they have proven applicable to production of sicklefin.
The scope of this propagation plan is limited to spawning procedures involving ripe females and flowing males at streamside collection sites in North Carolina, egg incubation, and fry and fingerling culture techniques at Warm Springs NFH. It does not include long term holding of adults, conditioning potential brood fish through use of hormone injections, or temperature regulation to condition captive brood fish.

Biosecurity Plan Components

1. Watersheds from which brood sicklefin are collected and spawned in North Carolina do not overlap with that of Warm Springs National Fish Hatchery. Therefore, biosecurity elements of this plan cover collecting gametes and tissue samples from a watershed outside of the drainage basin at Warm Springs and rearing fish which are not native to this basin for extended periods of time. The protocols also cover distributing fish reared at Warm Springs into another watershed.

2. Significant pathogens or those that under ideal conditions may result in outbreaks are described in this section. They include both the watersheds in which sicklefin gametes are collected and the spring water source at Warm Springs in which eggs are incubated and fish are reared.

Pathogens known to cause issues in recirculated or closed system culture of Moxostoma species at Warm Springs are common and widespread aquatic fungus species, bacteria, and protozoans that are not unique to this watershed but can cause issues in confined culture systems. Known pathogens from work with robust redhorse and sicklefin include the following external protozoans: ich (Ichthyophthirius multifilis), costia (Ichtiobodo), epistylis (Epistylis) and trichodina (Trichodina). Also, a bacterial infection commonly known as columnaris (Flexibacter columnaris) has been reported.

The watersheds surrounding WSNFH may have amphibians carrying fungus commonly known as chytrid (Batrachochytrium dendrobatidis). Asian clams are also known to be within the watershed, but neither chytrid nor asian clams have been identified in the waters from South Spring, the protected artesian source of water used for sicklefin culture at WSNFH.

3. Vectors which could possibly be transported in river water and fertilized eggs from North Carolina watersheds of the Lower TN River and Tuckasegee / Oconaluftee could include juvenile asian clams, undocumented viruses, fungus,
protozoans, and bacteria. In 2018, a myxosporidian, *Myxobulus* species, collected from Sicklefin Redhorse brood stock was brought to the station.

4. Disinfectants suitable for cleaning equipment used to produce fertilized eggs and rear Sicklefin Redhorse include the product Virkon Aquatic. Virkon contains oxone (potassium peroxymonosulfate), sodium dodecylbenzenesulfonate, sulfamic acid, and inorganic buffers. Soaking equipment in a 1.0% solution (184 grams per 5 gallons water) for a minimum of 10 minutes is effective against bacteria, fungi, and viruses. Also, this product is used in footbaths leading to all intensive culture facilities at WSNFH. Footbath solutions are changed as needed at least once a week. Disinfectants containing benzalkonium chloride, such as Roccal, can also be used as an alternative in waters of high hardness and heavy soil loading conditions at a dilution rate of ½ ounce per gallon. Tabletops and other objects that cannot be submerged (meters, electronics) are sprayed with 70% isopropyl alcohol. It is effective against many potential pathogens, but it is reported to be ineffective against non-lipid coated viruses or bacterial spores. *Guideline for Disinfection and Sterilization in Healthcare Facilities (2008).*

5. Sterilization of waste products that may contain viable eggs or fry and untreated waters from out of basin sources is conducted using chlorine solutions before disposal. Commercial products such as clorox or HTH can be used to produce a 300 ppm solution for this purpose.

6. Egg disinfection following brood stock spawning or receipt of transferred eggs is mandatory before eggs are transferred to our facilities at WSNFH. A 100 ppm solution of iodine over a 10 minute period is a proven method of egg disinfection for a wide variety of species including Sicklefin Redhorse, *Iodophor Disinfection of Fish Eggs, FWS.* This process is described in detail later in the document.

7. Receipt of transferred fry from other production facilities may occur on occasion. Fish received in this situation should be isolated in culture systems independent of other fish on station. Samples should be examined for ecto-parasites upon arrival. Salt treatments followed by formalin at standard rates and at lower water temperatures may be required. Other products such as potassium permanganate and Chloramine-T may also be utilized but require testing on small numbers of fish first. Alkalinity, hardness, temperature, and pH of water to be treated will influence treatment levels and durations as well as the relative health of the fish prior to treatment. Culture
systems will utilize ultraviolet disinfection to reduce transfer of pathogens within the system.

8. Distribution of sicklefin off station requires a sample of fish be checked for ectoparasites prior to distribution. Fish will not be transferred off station until any identified health issues are resolved.

Collection and Selection of Brood Fish

Successful collection and spawning of ripe Sicklefin Redhorse requires skilled and knowledgeable fisheries personnel to identify and capture brood fish. Collecting conditions through the habitat areas to be sampled are challenging with multiple sucker and redhorse species present in fast flowing and shallow draft conditions. Electrofishing gear used in conjunction with shallow draft boats such as Zodiac’s and/or those with jet drive outboards are often used to access, collect, and transport potential brood fish to the centralized marking and spawning sites. Fyke nets and other brood fish collection methods are being evaluated for their efficiency under variable rapidly changing water conditions. Successful boat collection of ripe brood fish requires a combination of good visibility, availability of collection crews, optimum water levels for boat access, and a temperature history conducive for the fish to be in a ripe spawning condition. Appendix 1 correlates (USGS 03512000) Oconaluftee River’s Birdtown gauge’s discharge and temperature profiles by dates (depicted by arrows) of gamete collections for 2015, 2016, and 2018. Temperature profile data was not available for 2014 collection efforts on the Little TN River, and no eggs were collected during 2017. Staging, sampling, and egg collection efforts range from late April through May. Although ripe males can be collected over a wider range of temperatures, freely flowing eggs were generally collected from females as water temperatures gradually warmed over three or more continuous days from 12°C to 15°C or higher during the second and third weeks of May.

![Typical spawning habitat. Credit: FWS](image-url)
Additional habitat, life history, and spawning information is included in the Candidate Conservation Agreement for the Sicklefin Redhorse, Dec. 2015.

Collected Sicklefin Redhorse are transported in aerated (compressed oxygen cylinder with Micro Pore ceramic diffuser or similar equipment) hauling tanks back to the collection site for evaluation, sampling, and spawning. A suggested list of supplies for spawning fish is included in Appendix 2: Sicklefin Redhorse Spawning Supplies and Gear List.

Staging, handling, and spawning of Sicklefin Redhorse is comparable to that described in the 2001 document, Culture Protocol for Robust Redhorse, (Moxostoma robustum). Differences between the protocols include the fact that Sicklefin spawn at lower temperatures than Robust Redhorse, hormone injections are not currently utilized, and the fish are not put under anesthesia during collection, spawning, and sampling.

Genetic Consideration

In order to maximize genetic diversity in a multi-year propagation program where brood fish are returned to the river following spawning, all fish will be checked for prior use by scanning for Passive Integrated Transponder (PIT) tags. Hatchery production summaries and a brood stock electronic record database can be checked for prior history with any recaptured fish in hand. For newly collected fish found without tags, a tissue sample via fin clip is collected and assigned a tissue sample number. Fish are then implanted with a PIT tag. Sampling and tagging of female fish should be done after gamete collection. Optimally, collected eggs from a single female are divided and fertilized separately with 3 to 5 males. All new tissue samples collected from brood fish will be transferred, processed, and stored at the Fish Technology Center’s Genetic Lab in Warm Springs.
Moyers 2009 described additional sicklefin genetic management strategies for a hatchery stocking program in the Tuckasegee River Basin.

![David Matthews (TVA) and Carlos Echevarria collecting genetic tissue sample. Credit: FWS](image)

**Brood Fish Spawning**

Stream side setup of tanks, tables and supplies should be completed before collection boats return with fish for evaluation and processing. Bankside crews handle sampling, tissue collection, tagging, data recording, gamete collection, spawning, and releasing of brood fish. Brood Sicklefin Redhorse are typically sampled and spawned without use of anesthesia. Ideally, all people handling the fish should wear gloves to limit handling induced injuries of the fish. Fish delivered in the boat hauling tank are easily differentiated by sex. All fish may release gametes upon handling or transfer to the staging tank. Female Sicklefin Redhorse should be transferred by hand from the boat to staging tank rather than using a net, and personnel should place a finger over the vent to prevent loss of eggs during the transfer. The process of stripping eggs and sperm
requires at least two people to secure brood fish, remove water, and position containers to collect gametes.

Dave Matthews and Carlos Echevarria implanting a PIT tag on a brood fish. Credit: FWS

Haile Macurdy handling fish wearing gloves. Credit: FWS

Care is required while collecting gametes in order to avoid contamination by water and feces. Before striping brood fish, collect river water for water hardening and rinsing
ahead of time. Suspend the container in the river or other means to maintain this container at the river’s temperature.
Also add a several teaspoons of Fullers Earth to a second container for use during the water hardening and de-adhesion phase following fertilization. Maintain this container at river temperatures. Once gametes have been collected from the brood fish, they are transferred to a holding tank, or a floating cage in the river to recover prior to their release.

Jason Mays, Asheville ES, FWS holding male Sicklefin Redhorse in spawning condition, tubercles on snout, anal, and caudal fins. Credit: FWS

Milt is collected from a minimum of five males prior to collection of a ripe, flowing female. Ripe males are easily identified by spawning tubercles present on head and fins. Position a finger over the vent while second person dries the pelvic fin area with a dry towel to remove water that could get into collection vial and activate sperm. Approximately 3 to 5 milliliters of sperm per male is collected. Collect sperm in sample vials labeled with PIT tag number. Avoid contaminates such as feces getting into the
sample tube. Store away from sunlight, inside a cooler with small amount of ice and a test tube rack. The temperature in the cooler should be near 4°C. Collect up to five different male samples before handling and spawning a female.

Sperm samples may be stored for longer periods of time using extenders such as Hanks solutions, if required. If possible, flush tube containing sperm with compressed oxygen then cap and store in Ziploc bag. Label either bag or vial with tissue sample or PIT tag number with grease pencil/marker. Store away from sunlight, inside cooler with small amount of ice.

Collect eggs into a dry aluminum, or plastic pan. Try to keep the pan away from sun or direct light. Do not allow the pan to heat up from exposure to sunshine. If ovarian fluid samples are being collected for fish health assessment purposes, use small disposable pipet or dial titrator to collect the clear fluid within the pan from around the edges of the collected eggs. Cap the sample tube and label with the tissue number of the female. Decant as much fluid from eggs prior to fertilizing with sperm.

Transfer eggs into a volumetric flask to estimate total volume of green eggs collected. Based on 2018 sampling, the average number of eggs per milliliter is 51 to 54. Use this information to divide eggs between partners if fertilizing with different males or to aid in dividing eggs into three to five smaller metal bowls, plastic bowls, or plastic bags for fertilizing with individual males. Handle the smaller containers to avoid water contamination and temperature extremes prior to adding eggs.

Use disposable pipets to transfer sperm from desired male and mix with egg lot as best as possible by swirling or using a dry feather. Do not reuse pipets to avoid water contamination. Activate with river water held at same temperature as the river. After several minutes of gently mixing, slowly rinse the water and sperm mixture off the eggs. Add river water and Fuller’s earth mixture to prevent adhesion of eggs, slowly stir with a turkey feather, and allow the eggs to water harden out of sunlight. Maintain temperatures by replacing the river water and Fuller’s earth solution occasionally. Let eggs water harden for approximately 20 minutes with as little disturbance as possible. Eggs will begin to become adhesive in this process if no Fuller’s earth or turbid river water is used. It is not necessary to sample count green eggs at this point in time as they will be inventoried prior to transfer to hatching jars. Eggs will also be expanding as they water harden. Conduct a final rinse before gently transferring river water and eggs into shipping bags. Maintaining a small amount of Fullers earth in the water of the transport bags will help prevent clumping during transportation. A tablespoon or less of Fuller’s earth per two liters of water will suffice. Flush and oxygenate the bag containing
the fertilized eggs with oxygen, double bag, and add two bags of ice within an insulated container for transport. Keep the box out of direct sunshine during transport and contact with hot surfaces such as over exhaust systems of transport vehicles.

**Sicklefin Egg & Fry Management**

The hatching jar and fry culture system consists of two banks of aquariums and hatching jars with 16 units per side. Each aquarium provides approximately 11 gallons of water when used with the existing standpipes. Each aquarium has a screened internal stand pipe which drains discharge through a common collection line into a sump. This outflow is filtered into a containment bag while entering the sump. Water collected in the sump is processed through a bio-filter, ultraviolet light disinfection, and sand filter. The system also has independent heating and chilling capacity. Spring water supplying the culture system arrives at a consistent 17° to 19°C temperature and may be used in part to maintain cooler water temperatures of the system. Recirculating water through the system’s equipment will gradually increase water temperatures unless sufficient replacement water is added or if a chiller is set to limit upper temperatures. Prior to adding water to the aquariums, the sump is filled with water approximately three weeks ahead of system use. The bio-filter and sand filters are conditioned by adding ammonium chloride then later with bacteria to seed the bio-filters per established protocols.

Tempering, treating, and sampling (if splitting boxes or sample count is not done during spawning) of the eggs is preformed upon arrival at Warm Springs.

Eggs may stick to each other and hatching jar without use of Fuller’s Earth during fertilization process.

*Credit: FWS*
Check temperature and oxygen in bag of incoming eggs, and record observations on quality of eggs received, clumping, cloudy, evidence of overripe eggs, etc.

Eggs are tempered by slowly adding water with a beaker used only for this purpose to the bag until the desired temperature of the water is reached. Work in the wetlab adjacent to the incubation system away from sunlight. Discard water on occasion to the waste container and bleach solution with a separate beaker. Do not contaminate the incubation system with anything used to temper the eggs before iodine disinfection. The primary objective of treating the eggs with iodine is to protect the station from the introduction and/or release of disease and pathogens. The hatchery is committed to maintaining a biosecure facility. Warm Springs has instituted the following biosecurity protocols for egg treatment upon arrival to the station.

After tempering for 30 minutes or so, decant most of the water into the waste container. Transfer less than 2000 mL eggs and water to a 3000 mL capacity beaker or volumetric flask in order to treat with a 100 ppm solution of iodine for 10 minutes. Gently transfer eggs and remaining water from the bag into the 3,000 mL container (which is sitting inside the secondary containment box or tray in the event of a spill). Bring water volume in flask up to an even mark (1000 or 2000 mL); then add iodine with a dial titrator with the needed amount. Gently swirl the eggs and solution and mark the time of treatment on the egg transfer sheet or jar record. After 10 minutes, decant and rinse the eggs several times to remove the iodine solution. Continue to transfer decanted water to waste container. Measure the total volume of eggs at this point following the 10 minute treatment and rinsing if sample counts were done at the river and the bag is not being split. Transfer the eggs to a volumetric flask and allow them to settle for approximately 30 seconds. After measuring the egg volume, they are ready for transfer to hatching jars. Do not add more than 250 mL of eggs per hatching jar; split eggs into additional jars to limit the amount of water needed to roll eggs.
Eggs are very fragile, more so than sturgeon eggs. Water flows required to move them will not be anything like those used with sturgeon. Eggs will be moving slowly in the jars. The above chart shows flow rates used throughout sicklefin egg incubation from 2014 through 2018. Flow rates are reduced as eggs continue to water harden and increase in size or numbers are reduced due to die off; so, daily monitoring and adjustment will be required.

After all eggs have been transferred to hatching jars, collect a sample of eggs from the hatching jar using a sampling pipette. While holding a finger over one end, lower the pipette into the hatching jar, then remove the finger to allow eggs and water to flow into the pipette as air escapes. Return a finger over one end while transferring the collected sample to a graduated cylinder for measurement in order to keep from losing eggs and water from the pipette. Collect 7 to 12 mL of eggs for counting purposes. Record milliliters collected after allowing the eggs to settle in the graduated cylinder for 30 seconds. Place a petri dish over the top of the graduated cylinder, invert, and transfer the eggs and water to the petri dish for counting. Do all transfers while working under secondary containment. Use soft pipettes to remove excess water from the eggs so they do not freely float during the counting process, then count eggs. Return eggs to the hatching jar after sampling.

Eggs should be maintained in the hatching jars at temperatures within a degree or two above the average river temperatures observed during that time of year, estimated to be somewhere in the 18.0° to 21.0°C range. Keep overhead lights off. Maintain a 100 microns filter bag at the discharge into the sump from aquariums, and keep screens in place inside each aquarium. Also, maintain a block of filter floss at the sump’s overflow.
pipe that discharges water into the wetlab’s pit drain in case of escapement. Dispose of all waste siphon tailings into a bleach solution. Sicklefin are not native to the ACF River Basin, and measures to prevent escapement must be utilized.

Any egg die-off should occur by or during day five post fertilization. Fry should begin hatching day five through seven depending on water temperatures. During the die off phase, dead eggs and shells will float above the good eggs. Water flows can be temporarily increased up to 4,000 mL per minute to check for fungus clumps then reduced or shut off for a minute to siphon. Use a siphon to remove shells during this process multiple times a day. If available, place small mesh nets at the jar’s overflow into the aquarium to catch egg shells before they enter the aquarium. Removal of dead eggs before they breakdown lessens the ammonia load placed on the incubation system. Once the die off slacks off, eggs should be re-sampled (eggs per mL count) and the total volume of remaining eggs remeasured to determine the number of viable eggs at hatch. This measurement will be the value used to maintain tank inventories throughout the program. The egg per mL count will probably be different than it was at or before water hardening. Use pipets to capture a sample of 10 to 12 mL of eggs for counting. Allow eggs to settle in a graduated cylinder, and then measure volume of eggs. Transfer to petri dish lid for counting and calculation of eggs per mL. The eggs should be examined under magnification to establish percent of viable eggs remaining which should be close to 100% at this point of culture. The number of viable eggs per mL multiplied by total egg volume will equal fry hatch and also provides percent hatch data when compared to number of eggs stocked into the jar.

If eggs are returned to hatching jars, maintain close watch for fry hatching in the jars over the next day or two. They will be down in the bottom of the jar under the eggs. Eggs in the jar should be immediately released into the aquarium once fry are observed in order to allow the eggs to hatch out over the next day or two while on the bottom of the aquarium. This is a key step that prevents the fragile fry from being killed in the hatching jar. They should not be flushed out or allowed to swim out of a hatching jar. The eggs may move around and pile up in the aquariums based on currents created by air stones and water flows. Periodic swirling of the eggs out of piles and limiting the use of air will aid during this time. **Appendix 3: Sicklefin Egg Development over Time, Hatch Fry** provides photos of egg development through one week post fertilization, along with eggs released into an aquarium and a picture of newly hatched fry.

Record all actions including counting any removed deformed fry or dead eggs after transfer into the aquariums. Use standard daily tank inventory logs to update aquarium inventories. Record cleaning, siphoning, general observations, sampling, and release information on the corresponding jar record until the jar is released into an aquarium.
Once a jar or multiple jars have been released or combined into an aquarium, the individual jar records will then be attached to the new aquarium record. It is probable that a single jar will go into a single aquarium, but the above process will ensure proper tracking of individual lots.

Water Management

Care must be taken to avoid creating surges of air through the water supply lines while eggs are in hatching jars. Surges created by air passing through water supply lines submerged in hatching jar diffusion tubes may flush eggs out of the jars onto the floor with this incubation system. If water is stopped for any reason, the overhead supply line to the jars will drain and result in air becoming trapped in the line. Restarting a pump following backwashing can produce this effect. Feed lines can be moved out of the jars and into the aquariums for a few minutes while water flows are slowly returned to individual supply lines. A bypass line is also plumbed directly into the sump and should be moved to a wide open position following pump maintenance, or backwashing. Slowly restrict water flow though this bypass line and thus slowly redirect water flows back through the header pipe. This will slowly purge air out of the line. Empty tank lines can also be opened to facilitate bleeding air out of the system. Maintain jar covers in place as a safety measure during jar incubation. Water quality of incoming replacement spring water is typically maintained at 80 to 100 ppm hardness, 40 to 60 ppm alkalinity, and 6.7 to 7.0 pH. Buildup of ammonium should not be a problem if replacement water is used to maintain water temperatures, but it should be monitored and recorded along with the other listed water quality parameters.

Pre-swim up

Fry will be bright yellow in appearance and will scoot around on the bottom of the tank after several days post hatch. Initially, they will move to corners of the aquariums. Shade cloth is placed to block directional light from overhead fluorescent lights. Yolk absorbance and development prior to feeding will take place approximately 12 days from fertilization. Suspected deformed fry will exhibit different behavior in comparison to those developing normally; this behavior includes circular swimming upon disturbance or notably slower transformation to freely swimming fry. These fish should be siphoned out before they swim up and if desired moved to an empty aquarium for further processing. Late swimming is not the only criteria to a deformed fish, but it is an effective means of separating obvious fry with problems. Feeding is not necessary until fry have begun swimming up in the water column. Once fry begin to swim freely in the water column, directional cover from lighting can be removed.
Fry prior to swimming up in the water column. Credit: FWS

**Fry Feeding and Care**

**Feeding**

Brine shrimp should be hatched prior to the first fish swimming up in the water column. Once fry are swimming, brine shrimp can be fed up to 4X per day. At the same time brine shrimp are being introduced, imprinting using commercial rations is also begun. The key at this point is to ensure the fish are eating and to facilitate the transition to a diet that will eventually ensure good growth beyond what feeding brine shrimp can provide. Brine shrimp can be fed spirulina supplements ahead of feeding them to the sicklefin as a means of increasing their nutritional value.

Sicklefin fry respond vigorously to the commercial Zeigler AP rations, and this brand is the feed of choice for start feed and early culture. Zeigler Larval Diet AP100 diets range up from particle sizes ranging from 50 to 450 microns. The formula has highly digestible mixture of marine and animal proteins, vegetable protein, yeast, vegetable starches, fish and vegetable oils, vitamin and mineral premixes, pigments, and antioxidants with a minimum protein content of 50.0%.
The following table (Table 1) is a general guideline to transition between feeds as fish grow.

Fingerling sicklefin are transitioned to larger feeds custom manufactured by Zeigler and include the Rio Grande Slivery Minnow Ration and Razorback Sucker ration. General fry and fingerling feeding directions are to first shut off water flow into each aquarium, while leaving aeration on. Feed several passes of dry feed at approximately 10 minute intervals followed by a final feeding of live brine shrimp. Reset and check water flows for each aquarium following a feed event. Sicklefin will take other fine particle feeds, but care should be given not to provide exceedingly high fat and protein feeds at higher temperatures. There are some indications from previous experiences of metabolic problems occurring with larger fingerlings continually fed high protein and fat feeds. Silvery Minnow and Razorback rations are available in larger pellet sizes for use producing larger sicklefin. Currently these pellets are also top coated with spirulina.

**Table 1: Sicklefin Redhorse Feed Schedule for 2018.**

<table>
<thead>
<tr>
<th>Feed</th>
<th>Size</th>
<th>Days of Culture (post hatch)</th>
<th>Fish Length (mm) at diet introduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brine Shrimp</td>
<td>n/a</td>
<td>8-35</td>
<td>8-9 mm</td>
</tr>
<tr>
<td>Zeigler AP 1</td>
<td>&lt; 50 um</td>
<td>8-22</td>
<td>12-15 mm</td>
</tr>
<tr>
<td>Zeigler AP 2</td>
<td>50 – 100 um</td>
<td>22-30</td>
<td>15-18 mm</td>
</tr>
<tr>
<td>Zeigler AP 3</td>
<td>100 - 150 um</td>
<td>22-44</td>
<td>15-22 mm</td>
</tr>
<tr>
<td>Zeigler AP 4</td>
<td>150 - 250 um</td>
<td>33-81</td>
<td>22-30 mm</td>
</tr>
<tr>
<td>Zeigler AP 5</td>
<td>250 – 450 um</td>
<td>44 +</td>
<td>25-45 mm</td>
</tr>
<tr>
<td>Spirulina</td>
<td>Powder</td>
<td>38 +</td>
<td>30 mm +</td>
</tr>
<tr>
<td>Silvery Minnow</td>
<td>#1, #2 crumbles</td>
<td>38 +</td>
<td>30 mm +</td>
</tr>
<tr>
<td>Razorback Diet</td>
<td>0.8 mm, 1.0mm</td>
<td>100 +</td>
<td>46 mm +</td>
</tr>
</tbody>
</table>
Sicklefin Redhorse growth through the first 300 days of culture is lower than that achieved for Robust Redhorse over a similar period of time. Lower culture temperatures used for sicklefin and higher stocking densities are thought to be contributing factors.

Observed growth of sicklefin fry and fingerlings over time is shown in the following graph.

Once sicklefin reach lengths of 1.0 inch or more, they may be transferred to circular tanks. These tanks are equipped with perforated stainless steel bottom drains and provide a means to challenge the fingerlings with a current and greater swimming space. This is a method to condition and exercise sicklefin for swimming against a current upon distribution. Rations fed to the sicklefin are fed by hand up to three to four times per day and also via belt feeders stationed over each tank. Water temperatures may be adjusted to maximize growth or condition the fish to targeted distribution sites.

Larger sicklefin will have greater swimming abilities and may be tagged with internal tags such as Passive Integrated Transponders (PIT) tags, or acoustic tags for research purposes as identified by the Partnership.

Cleaning

Utilize siphons and sponges to maintain the aquariums. Siphon inside standpipe screens daily. Exchange screens periodically for cleaning. Count all removed eggs or fry after stocking the aquariums; this provides an accurate number of fish in the aquariums. Disinfect cleaning instruments in Roccal, Virkon, or similar solution. Rinse all equipment thoroughly before reuse. Spray hands with alcohol before and after cleaning.
Disease Prevention and Treatment

Post hatch, a sample of live fry will be provided to Fish Health personnel to check for protozoan and other disease organisms. Adult redhorse suckers were known to be subject to “Ich” infections during the spawning cycle because they lose protective mucus coating during spawning. Costia, like protozoans, have been found in some years among the robust redhorse fry and among larger sicklefin. Therefore, preventive assessments will be made to check for any issues. Healthy fry should not die for unknown reasons. If mortalities are observed and the cause is from disease vectors, the window of opportunity to treat or correct the situation is very narrow, and serious losses may occur over a period of a few days.

Formalin can be used at standard recommended treatment rates. Salt may be used with caution at up to 5,000 ppm. Treatment rates over this 0.5% rate should be tested on a small lot fish ahead of treating larger groups of fish. Healthy sicklefin have been tested to 7,500ppm, but stressed fish may not tolerate treatments at similar levels or durations. Pennox 343 brand oxytetracycline antibiotic may be used at 21 to 35 ppm for bacterial infections and requires a prescription for use and multiple days of treatment. Chloramine T is an alternative for treating external bacterial infections over several daily 1 hour treatments. This product must be tested on small lots prior to use. While labeled for use at up to 20 ppm, the recommended 1 hour doses for treatment of bacterial infections should start at 5 ppm.

Marking and Distribution

To evaluate the survival of stocked fish in the river, we utilized oxytetracycline immersion as skeletal marking technique. The current marking process is under evaluation as a technique of choice for mass marking fry and fingerling size fish. Pennox 343 brand oxytetracycline (OTC) has an approved INAD (Investigational New Animal Drug) for finfish marking. A veterinarian must prescribe this prior to purchase and use of this product. The product has been successfully used at up to 600 ppm with striped bass at Warm Springs NFH in prior years. The product is typically buffered with sodium di-phosphate at two times the amount of Pennox 343 used in order to stabilize pH of the water. Aquariums are treated a day or two ahead of distribution. A subset of treated fish should be held back for marking efficiency.

Use of OTC for skeletal marking requires otoliths to be removed, sectioned and examined with a fluorescing microscope. Fielder, 2002 describes marking walleye with OTC, and otolith removal preparation in detail.
Appendix 4: Protocol for skeletal marking Sicklefin Redhorse using Pennox 343 oxytetracycline provides detailed steps to treat sicklefin at 500 ppm in the incubation system at Warm Springs NFH.

Preparing individual aquariums for distribution involves lowering water levels to concentrate the fish. Use aquarium dip nets to remove larger fingerlings into double bagged shipping boxes. Smaller fish may be siphoned using a ½ diameter hose into the shipping box after the aquarium is blocked up on one end to further concentrate fish. Lift aquariums, and rinse out remaining fish into the shipping box with a beaker of water. The process works better with two employees.

Sicklefin are sampled to determine lengths and size prior to distribution. A sample of fish is moved to tared containers of water for weighing and counting. Individual fish less than 1.5 inches in length are normally not measured for weight. A small representative sample of fish is measured to determine average lengths in millimeters. As the number of fish in the aquarium should be known, the sampling provides weights of distributed fish by multiplying size by inventoried numbers of fish in the aquariums.

Sicklefin are tempered to targeted stream water chemistry and temperatures prior to release. If fish are transported by shipping boxes, bags are floated in the river or stream and water slowly added over a 30 minute period into the bags. Many targeted streams and rivers in watersheds identified for recovery work have hardness levels below 40 ppm, pH at 7.0 or slightly lower.
Tempering two month old fingerlings to adjust temperatures prior to distributing. Credit: FWS

Tempering four month old fingerlings during distribution in August. Credit: FWS
Carlos Echevarria tempering Sicklefin Redhorse prior to release. Credit: FWS
References:

**Albanese, B.**, Georgia DNR Species Account, Sicklefin Redhorse, June 2008


**Looney, G., Young, D.**, Robust Redhorse (*Moxostoma robustum*), Culture Protocols, Warm Springs Regional Fisheries Center Internal Document, June 19, 2001


Sicklefin Redhorse Research Partnership, Candidate Conservation Agreement for the Sicklefin Redhorse (Moxostoma sp.), Dec. 2015

USFWS, Iodophor Disinfection of Fish Eggs, Journal of Fish and Wildlife Management, Jan. 2004
Appendix 1: Discharge and Temperature Profiles Correlated with Sicklefin Egg Collection 2015 – 2018
Appendix 2: Sicklefin Redhorse Spawning Supplies and Gear List:

- Holding tank, external overflow pipe
- Oxygen tank, regulator and air stone, nozzle (aeration of fertilized eggs, use with holding fish.
- Small oxygen bottle 40 cu. Ft. if available and extra regulator
- Honda generator, air pump and air stone, water pump and hose to circulate water in holding tank.
- Recovery cage, stream for post spawning or sampling work.
- Bucket
- Long handled dip net for brood transfer to recovery cage, plastic coated netting.
- Digital or laser temperature gauge, thermometer ... D.O. meter
- Items used by State when conducting weight & length sampling, tagging and tissue sampling.
- Measuring board, tripod or rope to support hanging scale, bucket for weighing.
- Tissue kit (scissors, disinfecting solutions, tissue vials with pre-labeled numbers).
- Pit tags and log book.
- Stud list of previously spawned fish which contributed off spring to restocking.
- Three metal bowls, egg collection, up to five smaller bowls – plastic for making individual male crosses, one for each male cross.
- Sperm vials and caps to collect and hold sperm, markers, disposable pipets at least 30.
- Quart sized plastic baggies, one per sperm vial (water barrier while in ice chest) or use vial rack in larger plastic bag.
- Ice chest just for ice, ice chest or shipping box with ice and shipping bag for insulation to hold sperm samples, maintain metal pans at temperature.
- Tropical fish bags for small fertilized egg batches, double bagged.
- Shipping bags, double bagged, rubber bands and banding tool for sealing bags.
- Three empty shipping boxes.
- Duct tape
- Cotton towels for drying sicklefin vent and fins prior to stripping eggs or collecting milt, drying pans, etc.
- Fullers earth and plastic bottle (river water and fullers earth mix) prepared and maintained at temperature. Attach bottle to string maintain in river water.
- Paper towels, adjustable wrench (regulator), knee boots, wading gear or change of clothes.
- Fish health kits for fluid samples from females spawned.
- Tool box, twine or rope (hanging scale, securing hoses
- Alcohol and scissors, disinfection tray per Nathen’s protocol for tissue collection samples
- Nathen’s data form
- Brood stock forms, clipboard
- Volumetric flash, large size to measure and split green eggs prior to fertilization.
- Smaller volumetric flasks
- Graduated cylinders at least 100 ml.
- Folding table
- Canopy Tent
Appendix 3: Sicklefin Egg to Hatch Fry Development Over Time

Sicklefin Redhorse Eggs 44 Hrs., 2 Days post spawn

Eggs at 76 Hours, 3 Days post spawn

124 Hrs, 5 Days post spawn

176 hrs, 7 Days Pre Hatch

Eggs released into Aquarium Day 7

Hatched Sicklefin Fry, Pre swimup
Appendix 4: Protocol for Skeletal Marking Sicklefin Redhorse Using Pennox 343 oxytetracycline

Background:

Skeletal marking is a technique that provides biologists an assessment tool to help identify hatchery reared fish from wild fish during sampling operations. Fish immersed in oxytetracycline solutions absorb amounts sufficient to be detected using fluorescing microscopy examination of skeletal tissues. Thus, immersion marking of hatchery reared fish provides an economic tool for stocking program assessment. Additional marking techniques also include genetic analysis, fin clipping, and coded wire tagging among others.

Pennox 343 brand oxytetracycline (OTC) is the approved product for marking fish. It is produced by Pharmgate Animal Health, 14040 Industrial Road, Omaha, Nebraska 68144. The product is commonly sold in 677.6 gram packages, containing 512 grams of OTC. The product contains OTC as 75.56% active ingredient. The product is authorized for use in marking finfish at the following levels.

“For the marking of skeletal tissues in finfish fry and fingerlings.

Immerse in 200-700mg oxytetracycline HCl (buffered)/L of water for 2-6 hours. Solution should be tested on a small number of fish before full-scale use. See Residue Warnings.”

This product has been used previously at Warm Springs NFH for marking striped bass fingerlings at 500 to 600 ppm for 6 hours. Our water chemistry during treatment has hardness maintained at levels between 75 to 100 ppm, alkalinity at 48 ppm to 64 ppm, and pH ranging from 6.7 to 7.5.

OTC products can lower pH of treated waters. Our experience using Pennox in the past has shown that buffering the treated waters by addition of sodium diphosphate buffer at a rate of 2X the weight of Pennox used will maintain a neutral pH.

Methods:

Fingerling Sicklefin Redhorse are reared in aquariums equipped with the ability to recirculate waters. As the sicklefin grow, densities in the aquarium are maintained by dividing the fish into new aquariums. Treatment is to occur in the aquariums. Each aquarium maintains on average 11.05 gallons of water. The total number of aquariums to be used for marking will range between 7 and 14. Prior to treating all aquariums, a trial treatment in one aquarium with a subsample of sicklefin will be conducted to evaluate the process. Survival will be assessed at 24 and 48 hours post treatment before the remaining tanks are treated.
OTC marking at a 500 ppm level will be conducted. Water flow to the aquariums will be shut off at time of treatment. Temperatures will be lowered from 21° to 20°C prior to treatment. 1000 mL of aquarium water will be removed for mixing in the OTC and buffer. This solution will be added to the aquarium in 200 mL aliquots at 15 minute intervals to slowly bring treatment levels up at 100 ppm at a time until 500 ppm level is obtained.

**Calculations:**

A 500 ppm active ingredient OTC treatment in 11.05 gallons is determined as follows:

500 ppm x .0038 grams / gallon x 11.0528 gallons x 1.3234 correction for less than 100% A.I = 27.8 grams Pennox per aquarium. This amount is added to 1000 mL of aquarium water along with 2X weight of Pennox = 55 grams sodium diphosphate buffer.

This solution is added slowly back into the aquarium to bring the final treatment level to 500 ppm.

A maximum amount of 4 mL of NO-FOAM or similar de-foaming product is added after the oxytetracycline solution is added, equaling a maximum of 100 ppm application to prevent excessive foam buildup caused by the treatment products.

Fish are monitored during the treatment period and water quality parameters, pH, O2, and temperature will be monitored through the process.

**Disposal of treatment waters:**

The OTC solution at the end of the treatment period must be removed from the aquariums without recirculation into the bio-filter and sand filter. The solution also cannot be discharged into overflows leading to streams.

The aquariums have a common collection drain line that can be redirected into a 150 gallon portable stock tank setup to collect the discharge waters. The OTC water will be flushed into this alternative collection sump. The water will then be pumped by submersible pump and garden hose into a portable hauling tank mounted on work carts and moved to a proper disposal location. Following flushing of the aquariums, the common drain line will be redirected back to the recirculation sump containing the bio-filter.

We have a sediment collection pond set aside for disposal of treatment chemicals.
Appendix 5:

Sicklefin Redhorse HACCP Plan
(Hazard Analysis Critical Control Point)

1. Activity Description
2. Potential Hazards
3. Flow Diagram
4. Hard Analysis Worksheet
5. HACCP Plan Form

1) Activity Description

<table>
<thead>
<tr>
<th>Facility: Warm Springs National Fish Hatchery</th>
<th>Site: Warm Springs National Fish Hatchery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Project Coordinator: Carlos Echevarria</td>
<td>Activity: Sicklefin Redhorse culture</td>
</tr>
<tr>
<td>Site Manager: Carlos Echevarria</td>
<td></td>
</tr>
<tr>
<td>Address: 5308 Spring Street</td>
<td></td>
</tr>
<tr>
<td>Warm Springs, GA 31830</td>
<td></td>
</tr>
<tr>
<td>Phone: (706) 655-3382 #1224</td>
<td></td>
</tr>
</tbody>
</table>

Project Description
i.e. Who; What; Where; How; Why

Warm Springs NFH staff working in cooperation with members of the Sicklefin Redhorse Research Partnership are propagating sicklefin redhorse in part to restore and recover populations in locations fragmented by dams and other forms of habitat alteration. Sicklefin redhorse are limited to tributaries of the Hiwassee and Little Tennessee rivers of the upper Tennessee River basin. The species is currently classified as a Candidate for future listing actions by the USFWS. Warm Springs NFH staff travel to spawn brood Sicklefin Redhorse on river sites where they are captured by electrofishing for spawning. Gametes are stripped from fish and fertilized eggs are then boxes and transported back to WSNFH. Eggs are transported in oxygenated river water, boxed in standard shipping boxes. Eggs upon arrival at WSNFH are disinfected, sampled and transferred to an egg incubation and fry culture system located in secure facilities equipped with anti-escapement barriers. Hatchings are cultured for periods of time ranging from several weeks to a year or more to meet propagation tasks of the Partnership. Prior to distribution the fish are sampled and inspected by Fish Health biologists. Current sites recommended for distribution of propagated sicklefin includes sites within the Oconaluftee River Watershed located in Swain and Jackson Counties, North Carolina and within the Eastern Band of Cherokee Indian Reservation.

2) Identify Potential Hazards

<table>
<thead>
<tr>
<th>Hazards: Species which may potentially be moved/introduced</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vertebrates: None</td>
</tr>
<tr>
<td>Invertebrates: Larval mussels, non-native zooplankton</td>
</tr>
<tr>
<td>Plants: non-native and out-of-basin fungus</td>
</tr>
<tr>
<td>Other Biologies: non-native or out-of-basin bacteria and viruses</td>
</tr>
<tr>
<td>Others: None</td>
</tr>
</tbody>
</table>

3) Flow Diagram For Wetlab Culture Only

<table>
<thead>
<tr>
<th>Step 1</th>
<th>Collect gametes and fertilize eggs, use river water for water hardening and egg transport to Warm Springs NFH.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Step 2</td>
<td>Temper, sample and transfer eggs from transport bags to water and incubation system at Warm Springs NFH.</td>
</tr>
<tr>
<td>Step 3</td>
<td>Conduct microscopic exam for protozoans, monitor fish for deformities &amp; mortalities,</td>
</tr>
<tr>
<td>Step 4</td>
<td>Maintain culture system filtration, anti-escapement, water quality and environmental control systems.</td>
</tr>
</tbody>
</table>
### Step 5
Subsample fry for a fish health diagnostic examination and growth, sample & mark before distribution.

### Step 6
Fish are readied for shipping → Lower Tennessee River, Tuckasegee / Oconaluftee river basins, NC

#### 4) Hazard Analysis Worksheet cont’d (Wetlab Culture Only)

<table>
<thead>
<tr>
<th>(1) Harvest or Aquaculture Step</th>
<th>(2) Identify potential ANS hazards introduced or controlled at this step (1)</th>
<th>(3) Are any potential ANS hazards significant? (Yes/No)</th>
<th>(4) Justify your decisions for column 3</th>
<th>(5) What control measures can be applied to prevent the significant hazards</th>
<th>(6) Is this step a critical control point? (Yes/No)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) Collect gametes and fertilize eggs, use river water for water hardening and egg transport to Warm Springs NFH.</td>
<td>Fish/other vertebrates: none</td>
<td>No</td>
<td>ANS vertebrates possible in incoming fish bags as eggs from river water source</td>
<td>Inspect water containers used during fertilization &amp; water hardening process for eggs. Avoid using mud from river bank for de-adhesion process.</td>
<td>No</td>
</tr>
<tr>
<td>Invertebrates, microscopic forms, mussels, protozoans, zooplankton</td>
<td>Yes</td>
<td>ANS invertebrates or out of basin species possible in incoming fish bags containing river water.</td>
<td>Do not allow untreated water in shipping boxes to enter drainage system at Warm Springs.</td>
<td></td>
<td>Yes</td>
</tr>
<tr>
<td>Plants: Unknown fungus or other out of basin organisms.</td>
<td>No</td>
<td>ANS plants possible in incoming fish bags</td>
<td>Inspect bags and remove any larger detritus before sealing bags for transport to Warm Springs.</td>
<td></td>
<td>No</td>
</tr>
<tr>
<td>Other biologics: viruses and bacteria out of basin</td>
<td>Yes</td>
<td>ANS biologics possible in incoming fish bags on or in fish eggs and or in river water used for transport of eggs to Warm Springs.</td>
<td>Maintain quarantine protocols for handling eggs and river water until completing egg disinfection and river water sterilization following step 2.</td>
<td></td>
<td>Yes</td>
</tr>
<tr>
<td>(2) Temper, sample and transfer eggs from transport bags to water and incubation system at Warm Springs NFH</td>
<td>Sicklefin Redhorse egg escapement</td>
<td>Yes</td>
<td>Sicklefin are not a native species in ACF watershed</td>
<td>Maintain secondary containment basins during transfer / treatment process to avoid losing eggs.</td>
<td>Yes</td>
</tr>
<tr>
<td>Invertebrates, microscopic forms, mussels, protozoans, zooplankton</td>
<td>Yes</td>
<td>ANS invertebrates or out of basin species possible in incoming fish bags containing river water</td>
<td>Use 100 ppm iodine egg disinfection protocol for all eggs and water entering incubation system. Sterilize all other discarded water in bleach, disinfect all utensils and beakers. Use secondary containment for every transfer / tempering process.</td>
<td></td>
<td>Yes</td>
</tr>
<tr>
<td>Plants: Unknown fungus or other out of basin organisms</td>
<td>No</td>
<td>None that are expected to be out of basin or remaining on fish eggs.</td>
<td>Use 100 ppm iodine egg disinfection protocol for all eggs and water entering incubation system. Sterilize all other discarded water in bleach, disinfect all utensils and beakers. Use secondary containment for every transfer / tempering process.</td>
<td></td>
<td>No</td>
</tr>
<tr>
<td>Other biologics: viruses and bacteria out of basin</td>
<td>Yes</td>
<td>Unknown status of out of basin pathogens.</td>
<td>Yes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Use 100 ppm iodine egg disinfection protocol for all eggs and water entering incubation system. Sterilize all other discarded water in bleach, disinfect all utensils and beakers. Use secondary containment for every transfer / tempering process.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| (3) Conduct microscopic exam for protozoans, monitor fish for deformities & mortalities. | Fish/other vert: none | No | No ANS present in culture water source | No |
| Inspect aquariums for anything that is not a sicklefin redhorse |

| Yes | Possible out of basin invertebrates attached to the fishes skin, fin or gill tissues. | Yes |
| Skin scrapings, gill tissue examinations to find any out of basin invertebrates or those that have been found onsite. |

| Plants: Unknown fungus or other out of basin organisms | Yes | Presence of fungus would most likely be common species but need to verify. | Yes |
| Skin scrapings, gill tissue examinations. |

| Yes | Sicklefin Redhorse mortalities will increase rapidly in intensive culture systems if bacterial or viral infections are a cause | Yes |
| Observer fish behavior, feeding activity, daily mortalities. Conduct microscopic exams to determine other causes such as protozoan infections or nutritional issues. If necessary send samples off for viral assessment. |

| (4) Maintain culture system filtration, antiescapeament, water quality and environmental control systems. | Fish/other vert: none | Yes | Necessary to prevent escapement of Sicklefin Redhorse to watershed and maintain health of the cultured fish. | Yes |
| Clean filter bags and inspect for fish. Rinse into secondary containment then bleach. |

| Yes | Necessary to keep common and / or ANS species at low levels while culturing fish in intensive culture system. | Yes |
| Clean aquariums, siphoning internal tank screens, removing dead fish, wiping tanks, backwashing sand filter. |

| Plants: Unknown fungus or other out of basin organisms | Yes | Necessary to keep common and / or ANS species at low levels while culturing fish in intensive culture system | Yes |
| Clean aquariums, siphoning internal tank screens, removing dead fish, wiping tanks, backwashing sand filter. |

| Other biologics: viruses and bacteria out of basin | Yes | Necessary to keep common and / or ANS species at low levels while culturing fish in intensive culture system | Yes |
| Clean aquariums, siphoning internal tank screens, removing dead fish, wiping tanks, backwashing sand filter. |

| (5) Subsample fry periodically for a fish health diagnostic examination and growth, sample before distribution. | Fish/other vert: none | No | No ANS species will remain in culture system at this stage. | No |
| Inspect for other fish species that may be in culture system, arriving through water supply lines. |

| Yes | Final microscopic exam for common fish pathogens that may be present. | Yes |
| Skin scrapings, gill tissue examinations to find any out of basin invertebrates or those that have been found onsite |

<p>| Plants: Unknown fungus or other organisms that may not be in NC watersheds. | Yes | Necessary prior to transfer of fish to NC watersheds, preventing transfer of any fungus species not found there. | Yes |
| Skin scrapings, gill tissue examinations. Examine history of fish mortalities to date. |</p>
<table>
<thead>
<tr>
<th>Other biologics: viruses and bacteria that may not be in NC watersheds.</th>
<th>No</th>
<th>Exams conducted in previous steps, not necessary if no significant mortalities from unknown cause are occurring.</th>
<th>Examine history of fish mortalities to date.</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>(6) Fish are readied for shipping → Lower</td>
<td>Fish/other vert: none</td>
<td>No</td>
<td>No ANS species will remain in culture system at this stage</td>
<td>Inspect for other fish or vertebrate species that may be in culture system while boxing or loading tanks for distribution.</td>
</tr>
<tr>
<td>Tennessee River, Tuckasegee / Oconaluftee river basins, NC</td>
<td>Invertebrates, microscopic forms, mussels, protozoans, zooplankton</td>
<td>Yes</td>
<td>No ANS present in culture water source but tempering at discharge site requires equipment disinfection upon return to Warm Springs.</td>
<td>Check for abnormal fish behavior during transfer, distribution and release. Utilize bleach spray on transport equipment; disinfect any portable equipment such as beakers or nets used during release.</td>
</tr>
<tr>
<td>Plants: Unknown fungus or other out of basin organisms</td>
<td>Yes</td>
<td>No ANS present in culture water source but tempering at discharge site requires equipment disinfection upon return to Warm Springs</td>
<td>Utilize bleach spray on transport equipment; disinfect any portable equipment such as beakers or nets used during release.</td>
<td>Yes</td>
</tr>
<tr>
<td>Other biologics: viruses and bacteria that may be found in NC watersheds.</td>
<td>Yes</td>
<td>No ANS present in culture water source but tempering at discharge site requires equipment disinfection upon return to Warm Springs</td>
<td>Utilize bleach spray on transport equipment; disinfect any portable equipment such as beakers or nets used during release.</td>
<td>Yes</td>
</tr>
</tbody>
</table>

### 5) ANS-HACCP Plan Form

<table>
<thead>
<tr>
<th>(1) Critical Control Point (CCP)</th>
<th>(2) Significant Hazard(s)</th>
<th>(3) Limits for each Control Measure</th>
<th>Monitoring</th>
<th>(8) Evaluation and Corrective Action(s)</th>
<th>(9) Supporting Documentation (if any)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) Sicklefin Redhorse eggs are received and stocked directly into Wetlab at WSNFH</td>
<td>Non-native plants, non-native fish, non-native invertebrates, other biologics</td>
<td>Visually inspect shipping bags for ANS arriving from NC watersheds. Unknown microscopic organisms in water and on eggs.</td>
<td>Visual Inspection unreliable, assume pathogens are present. Utilize full secondary containment of disposed waters. Disinfection and sterilization protocols during tempering, sampling and transfer process from shipping bags to culture system at Warm Springs.</td>
<td>Minimize water transfer from shipping bags to circular tanks. Maintain secondary containment for spills and escapement issues with sicklefin redhorse eggs. Follow iodine egg disinfection protocols; recheck calculations, time and concentrations of applied treatments. Utilize fresh solutions of bleach disinfectants for all discarded rinses during tempering and transfer process. Maintain fresh solutions of Vikron or equivalent equipment cleaners and use 70% isopropyl spray alcohol on surfaces that cannot be submerged.</td>
<td></td>
</tr>
<tr>
<td>2) Fingerlings tempered and distributed to</td>
<td>Non-native plants, non-native fish, non-native</td>
<td>Visually inspect all equipment used to temper and Unknown microscopic organisms in water and on eggs</td>
<td>Visual inspection, Utilize equipment disinfection</td>
<td>Sterilize tanks with bleach spray solutions. Submerge any equipment that is not</td>
<td>NA</td>
</tr>
</tbody>
</table>
### NC Watersheds.

- **Invertebrates, other biologics**
- **Transfer sicklefin redhorse into NC watersheds.**
- **Protocols for any container or equipment used to temper and release sicklefin redhorse into NC watersheds.**
- **Damaged by bleach in hauling container. Utilize Vikron or similar solutions for other equipment.**

<table>
<thead>
<tr>
<th><strong>Facility:</strong></th>
<th>Warm Springs National Fish Hatchery</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Address:</strong></td>
<td>5308 Spring Street</td>
</tr>
<tr>
<td></td>
<td>Warm Springs, GA 31830</td>
</tr>
</tbody>
</table>

**Activity:** Sicklefin Redhorse culture: Transporting fertilized eggs, hatching, rearing to desired sizes and distributing fish for restoration / recovery tasks within Sicklefin Redhorse historical North Carolina habitat.

**Signature:**

**Date:**

**HACCP Plan Was Followed**