Bubbling Ponds Hatchery Development for Native Fishes

Protocol for Collection, Transport, Quarantine, Maintenance, Propagation and Repatriation of Native Fish Species

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PROTOCOL

Most future work dealing with translocation, repatriation or introduction of native fish species within Arizona will consist of a simple collection of fish from a source population, and delivery of said fish to a new locality. The fish will typically not be held in captivity, except during transport. However, if a refuge population is to be established, or propagation is necessary due to low numbers of wild fish in the source locality, the following protocol should be followed.

Collection

When collecting wild fish for the purpose of establishing a refuge population or broodstock, the techniques used to collect fish should be very carefully selected and implemented. Bias should be minimized when collecting wild fish. For example, if seine mesh is too large for the species being targeted, perhaps only the largest adults will be captured in seine hauls. In the case of loach minnow, such size bias could result in a skewed sex ratio of collected fish, since loach minnow exhibit strong sexual dimorphism, with males sometimes twice as large as females. If sex ratios in collections are not even, the effective population size of collected fish is immediately reduced. In addition, size bias would result in a bias towards larger-than-average adults of both sexes, again resulting in lost genetic diversity of collected fish. Since most collections of rare species will be done with seines, a wise approach would be to use the smallest mesh size practicable for the species and habitat being sampled. Finally, when fish are being retrieved from the seine, there must be no bias in the size or appearance of these fish. The minimum recommendation is to collect 100 adult fish, and they should be the first 50 males and females found in the wild, that show no signs of visible deformities, parasitic disease, or infection. If fewer than 100 fish are collected, then collection events should be repeated over time (at least every other year, annually preferred) to develop a minimum captive population size of 100 adults. In either situation, enough new adults should be captured from the source stock to replace the captive population at least once per generation (i.e. to replace senescent adults) and to augment genetic variability of the captive population.

Transportation

Proper transport of collected fish is a critical step in establishing a captive adult population, because it is a high-risk procedure that can result in partial-to-complete loss of collected fish. The hauling tank should be filled with pathogen-free water, aerated (or oxygenated, if available), and devoid of sharp objects and hardware cloth (where fish can gill themselves). An insulated transport container is recommended, especially during periods of extreme heat or cold. The water should contain a 0.6% salt solution (unionized NaCl), and Stress Coat® at the manufacturer's recommended dosage. Also, the transport vehicle should always carry backup O_2. Temperature acclimation will be necessary if the hauling tank and source water differ by more than 3^{o}C. This can be achieved by placing collected fish in a plastic bag containing source water within the hauling tank, and allowing water temperature in the bag and the tank to equilibrate. Fish can be released once temperatures are the same. A copy of appropriate field data (species, collection location, date, number of individuals, etc.) plus any necessary permits should accompany the fish Finally, the transport vehicle should be stopped at least every two hours during transport, to check fish health, temperature, and oxygen levels.

Quarantine

Upon arrival at the refuge, transported fish should be acclimated to water temperature and pH of the quarantine facility. The quarantine tank(s) should be fully labeled (species, source population, collection date, number of individuals, etc., as should all aquaria, tanks, or other containers used to hold fish), physically separated from other tanks, raceways, or ponds at the refuge facility, and all outflowing water should be diverted away from the refuge. Prophylactic treatment for external pathogens should begin immediately, and 24-h treatments should be conducted for 3 consecutive days, at a water temperature of 18-20^{o}C. The preferred treatment is a mixture of malachite green, nitromersol, and acriflavine (Ich Guard®, Jungle Laboratories Corp.). If fish carry the internal pathogen Asian tapeworm (*Bothriocephalus acheilognathi*), they should be treated for this as well, prior to moving them into the refuge. This parasite can be treated with the chemical Praziquantel (Sigma Chemical Corp., Saint Louis, Missouri) at 0.67 ppm for 24 h.

Maintenance

Most adult small-bodied fish can be fed a combination of Tetramin® flake and freeze-dried bloodworms. This is true for both loach minnow and spikedace, but condition and behavior of other cyprinids held in the refuge building should be closely monitored to ensure that this diet is adequate. Large-bodied species, such as chubs and suckers, can be maintained on a diet of commercial trout chow (M. Childs, pers. obs.), but two other diets have been developed that appear to have merit, especially with YOY of these species. One diet, developed at the Bozeman Technology Center and sold through Nelson and Sons, Inc., was developed as a "razorback sucker diet." This feed has been shown to reduce developmental deformities in young suckers (R. Barrows, Bozeman Technology Center, pers. comm.). Rangen, Inc. has developed another commercial feed ("catfish diet") that incorporates *Spirulina* and krill, and also appears to reduce developmental deformities in chubs and suckers (F. Agyagos, AGFD, pers. comm.). Again, exceptions may occur, and information available in the literature and directly from southwestern hatcheries should be reviewed to ensure the best available diet for each species held at the facility. Other factors to consider are substrate and cover. Most species, both large and small-bodied, seem to do quite well without being provided any form of cover. This is likely not the case for loach minnow and speckled dace *Rhinichthys osculus*, which are bottom dwelling and secretive in nature. Captive chubs of the genus *Gila* occupy shaded areas when available, and also are notorious for jumping, and thus should be provided with appropriate cover and protection.

Fish should be fed *ad libitum* once or twice each day. Since the tanks in the refuge are flow-through, care must be taken to ensure fish are allotted enough time to eat introduced food before it is washed out of each tank. One option to consider would be employment of automatic feeders, but this is a relatively expensive alternative, and is more likely to result in over-feeding, if too much food is provided. A second alternative would be shutting off inflowing water to tanks for 30-60 min, to allow fish time to feed before flow-through is resumed.

Each tank in the refuge building should be supplied with its own broom (=brush) for cleaning, and its own dip net, for capture and movement of fish. This will help prevent disease transfer from tank to tank within the facility. In addition, each tank

should be associated with a 19 L bucket containing a standard hatchery cleaning agent, such as Parasite-S® (Western Chemical, Inc.), for sterilization of brooms and nets. Once per month, fish in each tank should be transferred to a clean tank, and used tanks should be pressure-washed to remove algae and hard water deposits.

Mortalities, when found, will likely be of inadequate quality for museum records, due to bacterial decomposition. Therefore, dead fish should be preserved in 95% absolute ethanol (for possible future genetic analysis), and an appropriate label should be added to the collection jar to identify the species, source locality, date of capture, and date of death. This information should also be entered into a daily logbook and into a spreadsheet program, so that mortality rate can be tracked for each species held in captivity.

Propagation and Rearing

For loach minnow and spikedace, propagation techniques have already been studied and determined (Childs 2004, Parmenter and Platania 2004). Because redundancy within the refuge is highly desirable, each local population (wild stock) held in captivity will be encouraged to spawn, and eggs will be incubated (if necessary, e.g. loach minnow) in the existing wetlab adjacent to the refuge. When loach minnow reach the juvenile lifestage, they will be moved back into the refuge facility, in tanks adjacent to the source adult fish. Spikedace tanks will be lined with pea gravel to allow spawned eggs to settle and thus avoid predation by adults, and larvae will be dip-netted from the surface and moved to an adjacent 1900 L tank. As many as 2000 F1 progeny will be reared to adulthood, if space and resources permit. Rearing techniques for loach minnow and spikedace have also already be determined, and will follow Childs (2004) and Parmenter and Platania (2004).

If species other than loach minnow or spikedace are brought into the refuge facility, techniques used for spawning and rearing these fish will either be determined from the literature, or developed over time. Regardless of species, all F1 progeny produced will be held in separate tanks, to avoid backcrossing with adults and to build redundancy for the captive adult population.

Repatriation

Repatriation of captive adult fish or their progeny will be considered on a case-by-case basis. Prior to the introduction, reintroduction, or translocation by AGFD of any native or listed species, resource managers (conservation biologists and sportfish managers), land owners (state, federal and private), and grazing permittees must be consulted and provide concurrence with the proposed introduction. In addition, environmental assessment by both AGFD and any federal agency involved in the operation must be completed and must show that the introduction will have no negative consequences for either the species or the environment. In most cases, the groundwork for establishing concurrence and environmental compliance will be initiated at the same time, or perhaps prior to, establishing a captive population.

A minimum of 100 adult fish (1:1 sex ratio) should be the goal to directly replicate a wild population to a protected stream. If the wild source population is too small to sustain such a direct translocation, F1 progeny from the captive population can be used either alone or in combination with wild fish. A minimum of 500 F1 progeny should be stocked to support an initial repatriation, and this number should be augmented over time with either wild fish from the source stream (preferred) or with additional F1 progeny derived from augmented captive broodstock. Follow-up monitoring will assess short- and long-term success of stocking and advise recommendations for supplemental stocking.

LITERATURE CITED

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