Successful Out-of-Season Spawning of Westslope Cutthroat Trout Males

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The present range of westslope cutthroat trout (*Onchorhynchus clarki lewisi*) is greatly reduced compared to the historic distribution of the subspecies. Degradation of the environment and competition with introduced species are considered major causes of the reduction (Liknes and Graham 1988). In response to declining populations of westslope cutthroat trout across Montana, the Montana Department of Fish, Wildlife and Parks developed a captive broodstock in the early 1980s to assist in conservation and restoration efforts. The broodstock is held at the Washoe Park trout hatchery in Anaconda, Montana.

In October 2002, we initiated a project to spawn wild males with captive broodstock females the following spring to relieve the broodstock of any genetic effects of prolonged hatchery rearing. However, synchronized availability of wild males and captive broodstock females was a concern.

Protocols for the project required collection of genetic and health samples from wild fish in possible donor streams. During health examinations, we found some males to have secondary sexual characteristics, i.e., kype/compressed body, and enlarged gonads. Additionally, we expressed milt from males not sacrificed for sampling. Because these collections took place in early October, months after the normal spring spawn timing of the species, the viability of the milt was unknown, but of interest.

Our objective was to determine if we could use sperm collected from out-of-season males to successfully fertilize eggs. If westslope cutthroat males successfully fertilize eggs several months away from the typical spawn time, wild westslope cutthroat males might be usable during captive broodstock spawning, just weeks before the normal spawn time. This information may also be valuable to biologists attempting to spawn wild fish in the field where asynchronous maturation or low numbers of spawners may be hindering efforts.

Eggs were collected from a fall spawning rainbow trout (*Oncorhynchus mykiss*) at the Jocko River trout hatchery in Arlee, Montana, on 19 November 2002. A random sample of eggs (~ 1500) from a pooled group of four females was transferred in a hard plastic container on ice to the Washoe Park hatchery (2 hr) and stored unfertilized in a refrigerator at 4 °C overnight. The following day, fish from 3-year-old westslope cutthroat broodstock were anaesthetized with tricaine methanesulfonate (MS-222). We found two males exhibiting secondary sexual characteristics from which milt could be freely expressed by gently squeezing the abdominal area. We used milt from these males to fertilize approximately one-half the eggs. Approximately 2 min post-fertilization, eggs were rinsed in fresh water, water-hardened in an iodine solution at 100 ppm for 20 min and placed into a partitioned heath tray for incubation at 13 °C.

Next, we found two males that exhibited secondary sexual characteristics but from which we could express little or no milt. The two males were lethally

anaesthetized with MS-222 and transferred into the hatchery laboratory. One teste was surgically removed from each fish. The teste was placed on a sterile cutting board, cut into small pieces with a scalpel and the liquid/gel material poured onto the remaining eggs. After 2 min, eggs were rinsed in fresh water and handled in an identical manner as the previous group. We did not attempt to replicate groups and no control was utilized. Results represent a documented event rather than a controlled experiment. Therefore, we performed no statistical analyses.

Eye-up occurred at 14 days post-fertilization. Average eye-up for both groups of eggs was 69 percent. No difference in eye-up occurred between the group of eggs fertilized with sperm from live fish (68.7%) and eggs fertilized with sperm collected post mortem (69.2%). While the resulting fry would have been of interest to observe, we disposed of all eggs after evaluating eye-up due to the background of the contributing parents (rainbow x cutthroat).

Out-of-season spawning of other fish species is quite common when the rearing environment is manipulated. Kelly and Kohler (1996) induced channel catfish (*Ictalurus punctatus*) to spawn out-of-season using photothermal and hormonal manipulation. Mischke and Morris (1997) were able to induce sunfish (*Lepomis* spp.) to spawn over a 6-month period by manipulating temperature and photoperiod in a laboratory setting. It is noteworthy, however, that we made no attempt to manipulate any part of the environment of the westslope cutthroat males used in this experiment.

An additional incident of out-of-season spawning was observed at the Washoe Park hatchery. The hatchery has a visitor center with a living trout stream display that contains westslope cutthroat trout, arctic grayling (*Thymallus arcticus*), and a single rainbow trout. In November 2002, the rainbow trout female was observed building a redd in a riffle portion of the stream. During this time, we observed a westslope cutthroat male from the captive broodstock in typical courtship displays with female rainbow. Approximately one month later, we observed and photographed numerous sac fry in the gravel underneath the redd. Because no other fall spawning species were present in the display stream, we assumed that any offspring produced would be from that male or another westslope cutthroat male—grayling males were never observed in the area.

Our results and observations document two successful out-of-season spawning events of westslope cutthroat trout males. We are not aware of any other literature documenting successful out-of-season spawning of any strain of cutthroat trout. Because westslope cutthroat males successfully produced progeny so far from the typical spawning time, we believe that we can successfully collect viable milt from wild males during spring for spawning with captive broodstock.

In addition to benefiting captive broodstock, the fact that these males were fertile over an extended period may benefit biologists attempting to collect eggs from wild trout where asynchronous maturation is occurring, or where there is an attempt to fertilize eggs from neighboring populations. Using the techniques described here, a biologist should be able to use sperm from any known male to fertilize eggs. Additionally, sperm from one teste could be used immediately while the other teste could be transported to a neighboring population to fertilize other females, potentially doubling its use.

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