A suggested protocol for collecting cutthroat trout tissues for subsequent genetic analysis

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Overview

A recurring frustration among field biologists is the lack of a consistent protocol on methods to acquire fin tissues usable for subsequent genetic analysis. Labs that perform genetic analyses have slightly different recommendations on how to collect tissues, and these approaches are often not conveyed to the field technicians conducting the collections. When over 80 biologists and 10 geneticists convened at a recent symposium devoted to cutthroat trout taxonomy in Denver, Colorado, an opportunity to develop some consistency in collection protocols presented itself. The group discussed methods that would be both amenable to biologists, and ensure that the tissues they collect would provide usable DNA to any lab conducting PCR based DNA analyses (e.g. mtDNA, microsatellites, PINEs, BIAMs, AFLPs and SNPs) but not allozyme work. Geneticists were solicited to provide written responses to several sample collection questions prior to the symposium. Their responses were condensed into a single recommendation for each topic, and then presented to the entire group to make sure it was agreeable to all parties. The consensus for each step is presented here with a brief rationale.

Collecting samples

Range and number

Animals targeted for investigation should be acquired from **across the range of a population**, as barriers (not necessarily obvious ones) can dramatically alter the genetic composition of a population along the stream gradient, and fish collected from the same location are more likely to be related. UTM coordinates (and projection) should be recorded wherever tissues are collected.

While comfortable with relatively small sample sizes in the past, it has become apparent that to obtain accurate assessments of introgression or address certain phylogenetic questions, that more samples are needed. Rather than making repeated sampling trips to a site when new questions arise, biologists should strive to acquire **30 samples** during a visit. A quick screening for flagrant introgression with rainbow trout

can be run on a portion of the samples, with the remaining samples to be run if the potential for a pure population exists.

Tissues to collect

Since sampling of fish should be non-lethal, fins are the recommended target for acquiring tissue samples for genetic analysis. Adipose fins have been popular in the past, but pose several problems. The tissue does not regenerate, so the fish is left without an adipose fin for the remainder of its life - a fin that is used in mate selection at least in brown trout. In addition, some labs have observed that the high lipid content of the adipose fin can complicate the isolation of DNA.

In an effort to allow for repeated genetic tests on the same piece of fin tissue, the group recommended acquiring **a piece of tissue 1 cm^2**. Adipose and pelvic fins are very small on small trout, making the caudal fin a more appealing target. Since the bottom of the caudal fin is used for digging redds, the **top of the caudal fin** was selected as the preferred tissue to collect.

Sampling tools

Scissors or side-cutters are usually all that is necessary to acquire tissue samples (serrated fly-tying scissors are ideal). It should be noted that if samples are to be collected from multiple populations, sampling tools should be disinfected between sites. An 80% EtOH bath should be adequate to eliminate the potential for disease transfer.

Contamination between samples does not appear to be much of a problem when acquiring fresh tissues, as there is so much DNA available. This is not the case with museum specimens where contamination is a major concern.

Sample storage

Collection tubes

Storing collected fin tissues in individual vials is recommended, as it allows one to record covariates such as UTM coordinates for where the fin was collected, different forks of a stream that are part of the same population, fish length, or photographs of fish that can be later used to link phenotypic traits to results of molecular analyses. By having a single tube for each sample, problems with fins splitting in transit (thereby looking like two samples) are eliminated. In addition, if only small fish are available, multiple fins can be collected from the same individual to arrive at the 1 cm^2 tissue size target. Finally, storing the collection in multiple vials spreads the risk of desiccation.

In an effort to ensure proper tissue preservation (making sure the ethanol is not diluted by fluid in the tissue sample) the use of larger 15 mL centrifuge tubes is encouraged (Corning 15 mL conical centrifuge tubes, VWR catalog #21008-678, or #20171-024, or equivalent). Polypropylene tubes are best, and polystyrene or polyethylene (PET) tubes should be avoided. Make sure to get “plug-seal” caps to reduce potential for desiccation.

Preservation

Considerable expense has been devoted to purchasing the highest-grade ethanol possible to preserve specimens. In some situations that can actually be counterproductive as benzene is often used to achieve that high purity, and can negatively affect the DNA. **Mid-range** (denatured reagent grade ethanol, VWR catalog #3609 or equivalent) ethanol is adequate. Low-grade reagents may contain contaminants that may compromise the DNA over longer-term storage.

Past collections have been stored in dilutions of ethanol ranging from 70% to virtually pure. Higher concentrations tend to desiccate the tissue more, making extraction of DNA mildly more difficult, whereas lower concentrations may inadequately preserve large amounts of tissue that are placed in small volumes of diluted ethanol. A compromise was reached, and all parties agreed that an **80% dilution** would be adequate, particularly if larger storage tubes are used to guarantee high ethanol:tissue ratios. Note: **do not dilute the ethanol with chlorinated tap water**, as this will denature the DNA. Distilled water is recommended. Keep in mind that DNA does degrade over time so if long term storage is desired, samples should be removed from ethanol and stored at -70°C.

Labeling samples

Label each tube with a unique identifier so that it can be linked to specific covariates such as collection location, fish length, phenotypic characteristics, photographs, etc. If writing on the sample tube directly, one should use a special purpose marker (VWR Lab Markers, VWR catalog #52877-310 or equivalent) since most standard markers are very soluble in the ethanol that the fins are stored in

Some labs prefer that you not place sample information inside the tube as is common for museum collections. Labels can be difficult to extricate, usually must be removed to be read allowing for contamination to occur, and may contain bleaching agents that can inhibit detection of the target DNA.

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