I. Name: Jason Keagy
II. Host Lab: Dr. Victoria Braithwaite and Dr. Thomas Neuberger, Pennsylvania State University
III. Dates of visit: November 6-9, 2012 and December 4-7, 2012
IV. Title of protocol: Specimen preparation for magnetic resonance imaging (MRI) of stickleback (or other) fish.
V. Rationale and background: This protocol describes steps taken to prepare fish samples for MRI of their brains. A separate protocol will be submitted to describe how to process the image data produced, using Avizo (version 6.3, VSG Inc., Burlington, MA, USA).
VI. Protocol:

Specimen Preparation for MRI

1. Fish were euthanized using an overdose of anaesthetic (MS-222, Tricaine Methane Sulfonate). Dosage will vary depending on the species and the size of the fish. Fish should stop moving in a minute or less.
2. They were then photographed (standard length and other size information can later be computed from this photograph) with a label with the date, lake and species (i.e, benthic, limnetic, or undifferentiated).
3. Each fish was rinsed with water.
4. The caudal fin and a large section of the tail was cut off by pressing down with a razor blade. This section of tissue was put in a small vial (5mL) of ethanol. This was done to preserve DNA.
5. The rest of the fish was fixed in a 10% neutral buffered formalin solution for 72 hours. (I fixed fish in 15mL falcon tubes).
6. The fish were then prepared for imaging by immersion in a 2% Magnevist (Bayer HealthCare) phosphor-buffered solution for 1 week and stored at 4C. Magnevist is a contrast agent allowing for faster imaging. It may be possible to get unused Magnevist that is slated to be disposed of from human imaging facilities. (I also used 15 mL falcon tubes for this step).
7. All vials at each stage were labeled on the outside with information about the date of each step (i.e., capture, euthanization, Magnevist) and the lake and species. This information was also written in pencil on waterproof paper and included in the vial.

Imaging

1. Most fish were too large to fit in the sample chamber, and so were cut in two using a razor just behind the operculum (gill cover).
2. The fish heads were put in a sample vial (originally made by cutting the bottom off of a falcon tube). To inhibit movement of the specimen in the vial, cotton wool soaked in Fluorinert Electronic Liquid FC-43 (3M) was packed around them. To prevent the fish heads from drying out and to minimize magnetic susceptibility artifacts during scanning, the entire vial was filled Fluorinert.
3. In order to reduce artifacts in the images, air bubbles were carefully dislodged from the surface of the specimen by gently tapping the outside of the vial or by using tweezers.
4. The vial top was wrapped with parafilm and a small amount of Fluorinert injected just prior to being completely sealed to remove the rest of the air.

5. All specimens were scanned at the High Field MRI Facility, Pennsylvania State University, in a vertical 14.1 tesla Agilent imaging system using a home built saddle coil. Scan time was between 4-8 hours for a resolution of at least 20µm (Dr. Thomas Neuberger or his graduate student did this step).
6. After imaging, the fish head was removed from the sample vial and returned to its original vial for use in later histological work if needed.

Image Post-processing

1. Matlab (The Math Works) was used for post-processing (Dr. Thomas Neuberger did this step). This step may not be necessary depending on the MRI setup and the needs of the researcher.
2. Volume of olfactory bulbs, telencephalon, cerebellum, optic tectum and whole brain were measured using 3D data visualization and reconstruction software (Avizo version 6.3, VSG Inc., Burlington, MA, USA). Protocols/tutorials for using this software will be submitted separately.