ONLINE SUPPLEMENT 16.2

SAMPLE IACUC STREAM FISH PROTOCOL[1]

STUDY IDENTIFICATION

1.0 Principal Investigator:
Dr. Daniel McScience

2.0 Study Title:
A Hypothetical Survey of Fish Assemblages in Mid-Atlantic Streams

PERSONNEL & QUALIFICATIONS

1.0 Principal Investigator

List the PI's training and qualifications, including degrees earned and workshops attended, to conduct animal procedures for each species. If training is scheduled, identify who will provide the training:

Dr. McScience has 16 years of experience working with freshwater fishes in streams, rivers, and lakes throughout North America. In this time, he has worked as an academic researcher, a government employee, and an industry consultant. And he has trained approximately 20 field assistants on methods used to collect, identify, and analyze fishes in the field. Throughout the course of his career, Dr. McScience has sampled tens-of-thousands of fishes, including more than 200 species. And he has employed most types of field sampling equipment, including backpack and boat-mounted electrofishers, seines, hoop nets, and fyke nets. He has a Ph.D. in Freshwater Biology (University of Somewhere, 2007), a M.S. in Fisheries Science (Somewhere State University, 2001), and a B.A. in Biology/Geology (Nowhere University, 1997).

[1] All names of specific persons and institutions have been changed for confidentiality.

List the PI's study related years of procedure experience, per species, for all animal procedures performed by the PI:

2007-2011 – FRESHWATER FISH ECOLOGIST (National Stream Surveying Agency, Dogtown, GA): sampling and analyzing freshwater fish assemblages in streams and rivers of the Southeastern Coastal Plain. (Note – these were assemblage-level (>100 species total) surveys in which all locally occurring fish species were collected. Samples were not limited to specifically targeted species. Thus, a 'per species' list is not plausible.)

2007 – FISH ECOLOGIST (Fishes 'R' Us, Corvallis, OR): sampling native salmonid fishes [Chinook salmon (*Oncorhynchus tshawytscha*), coho salmon (*O. kisutch*), cutthroat trout (*O. clarkii*), rainbow trout (*O. mykiss*), and bull trout (*Salvelinus confluentus*)] throughout the Pacific Northwest region.

2003-2007 – FISH ECOLOGIST/DOCTORAL RESEARCHER (University of Somewhere, Pigskin, AL): sampling and analyzing freshwater fish assemblages in Southeast (AL & TN), Pacific Northwest (OR & WA), and Southwest (NM & AZ) streams. (Note – these were assemblage-level surveys. Thus, a 'per species' list is not plausible.)

1999-2002 – STREAM ECOLOGIST (National Consulting Group, Inc., Over the Bridge, WA): sampling and analyzing freshwater fish assemblages in streams and rivers throughout Oregon, Mississippi, and Pennsylvania. (Note – these were assemblage-level surveys. Thus, a 'per species' list is not plausible.)

1998-1999 – STREAM ECOLOGIST/MASTER'S RESEARCHER (Somewhere State University, Happyville, PA): sampling and analyzing freshwater fishes in streams and rivers throughout eastern Pennsylvania and northern West Virginia. Some of this work addressed complete fish assemblages (i.e., no species-specific focus), but some of it selectively addressed brook trout (*Salvelinus fontinalis*) and brown trout (*Salmo trutta*).

2.0 Study Staff

Select all study staff and indicate their role(s), procedure training, qualifications and experience:

Name – Student 1
Roles – Animal handler; Student
Training – Graduate student (M.S.) field assistant under the direct supervision of the PI. Student 1 has a B.A. (2006) in Geospatial Analysis from Another Big University. All necessary field training will be provided prior to conduction fieldwork or on-site by the PI.

Experience – Student 1 does not yet have field experience working with stream fishes, but will learn all necessary procedures under the direct supervision of the PI.

EMERGENCY CONTACT INFO

PI Contact Info: (*Name*, *Department*, *Role(s)*, *E-Mail*, *Phone*)

Study Staff Contact Info: (*Name*, *Department*, *Role(s)*, *E-Mail*, *Phone*)

SUMMARY OF RESEARCH

1.0 First, provide a brief lay summary describing the overall purpose of the study. Second, provide a clear and concise, sequential description of the procedures involving the use of animals that is easily understood by all members of the committee:

The objective of the study is to measure annual fish production rates in remote, headwater streams of the Mid-Atlantic (USA) region. To do so, fishes must be collected at repeat intervals; quarterly samples will be collected over a two year period in this study. Briefly, annual production (for a given species) is calculated as the sum of changes in population-level biomass (i.e., population abundance x average individual weight) among sampling intervals. All fish samples will be collected with a battery-powered backpack electrofisher; this is the industry standard method used to collect fishes in small to mid-sized streams. It is both effective for capturing fishes and relatively benign (i.e., very low or zero rates of injury to sampled fish; further technical information is provided below). Population size estimates will be obtained using the Zippin multiple-pass depletion method: three successive electrofishing passes will be performed within each sampled stream reach. Collected fishes from each pass will be stored in a 30-gallon holding tank until processing (i.e., identification and weighing) is complete. Immediately prior to processing and handling, fishes will be transferred in small batches (~30-50 fish per batch) from the holding tank to a 10-gallon anesthesia tank. A 25 mg/L solution of buffered Tricaine methanesulfonate (MS-222) will be used to sedate fishes in the anesthesia tank; mild sedation (sufficient for handling and processing) is normally observed in 1-2 minutes. All processed fish will then be deposited in a separate 30-gallon recovery tank and allowed to re-equilibrate for a minimum of 30 minutes prior to release back into their native stream. Population abundance for each species will then be estimated as the zero-intercept of a cumulative catch (i.e., total individuals summed among successive passes) vs. catch-per-unit-effort (CPUE) regression model, where CPUE is expected to decrease at a linear rate among successive passes.

When all quarterly samples (in a given year) have been completed, annual population growth (i.e., production) will be calculated as the accumulation of population-level biomass over the course of the year. Because annual production is a species-specific process, all locally occurring fish species in the study streams will be collected and measured (i.e., separate annual production estimates will be calculated for each species and/or group of functionally similar species). The motivation for this study is to provide the first empirical estimates of natural or baseline production rates in the remote study streams. The annual fish production estimates will subsequently be used to create a regional fish carrying capacity model.

2.0 Does this protocol involve procedures that engender a risk of morbidity/mortality to animals (e.g. tumor models, infectious diseases, vaccine challenge, pain modeling, trauma, production of monoclonal antibodies, assessment of toxicologic effects, organ or system failure, or models of cardiovascular shock): (*No*)

JUSTIFICATION

1.0 Provide the reason for the experiment or activity and a description of the benefits to be gained. Provide specific information regarding disease(s) to be studied, if applicable:

The immediate purpose of the study is to estimate annual fish production rates in streams of the remote streams of the Mid-Atlantic region. These streams have been highly impacted by human activity (e.g., mountaintop removal mining). Annual fish production estimates will then be used to build and calibrate a carrying capacity model that can in turn be used to predict maximum potential fish production rates throughout the region. The fish surveys conducted as part of the study will also provide basic natural history and species' distribution information, which will be of interest to state managers as well as the broader scientific community.

2.0 Justify 1) the selection of the proposed species and strain, and 2) animal numbers, including statistical (e.g., power analysis) or other justification for the number of animals requested for each procedure type. Note that for breeding colonies, all animals generated (not just those of the experimental sex/genotypes) must be included:

The study aims to estimate population sizes of all fish species that occur in the study streams. No particular species or age-group (juvenile vs. adult) will be targeted; rather, all locally occurring species and age-groups will be sampled. For this reason, it is difficult or impossible to specify a priori which fish species will be collected. Fish species that are common in streams throughout

the study region (southern West Virginia), and are therefore likely to be sampled, include (listed by family):

Atherinopsidae: *Labidesthes sicculus* (brook silverside)
Catostomidae: *Catostomus commersonii* (white sucker); *Hypentelium nigricans* (northern hog sucker); *Ictiobus bubalus* (smallmouth buffalo); *Moxostoma anisurum* (silver redhorse); *Moxostoma breviceps* (smallmouth redhorse); *Moxostoma duquesnei* (black redhorse); *Moxostoma erythrurum* (golden redhorse)
Centrarchidae: *Ambloplites rupestris* (rock bass); *Lepomis cyanellus* (green sunfish); *Lepomis macrochirus* (bluegill); *Lepomis megalotis* (longear sunfish); *Micropterus dolomieu* (smallmouth bass); *Micropterus punctulatus* (spotted bass)
Cyprinidae: *Campostoma anomalum* (central stoneroller); *Cyprinella spiloptera* (spotfin shiner); *Cyprinella whipplei* (steelcolor shiner); *Erimystax dissimilis* (streamline chub); *Hybopsis amblops* (bigeye chub); *Luxilus chrysocephalus* (striped shiner); *Macrhybopsis hyostoma* (shoal chub); *Nocomis micropogon* (river chub); *Notropis ariommus* (popeye shiner); *Notropis atherinoides* (emerald shiner); *Notropis blennius* (river shiner); *Notropis buccatus* (silverjaw minnow); *Notropis photogenis* (silver shiner); *Notropis rubellus* (rosyface shiner); *Notropis stramineus* (sand shiner); *Notropis volucellus* (mimic shiner); *Pimephales notatus* (bluntnose minnow); *Pimephales vigilax* (bullhead minnow); *Rhinichthys cataractae* (longnose dace); *Rhinichthys obtusus* (western blacknose dace); *Semotilus atromaculatus* (creek chub)
Cottidae: *Cottus bairdii* (mottled sculpin)
Ictaluridae: *Ameiurus natalis* (yellow bullhead); *Ictalurus punctatus* (channel catfish); *Noturus flavus* (stonecat); *Noturus stigmosus* (northern madtom)
Percidae: *Ammocrypta pellucida* (eastern sand darter); *Crystallaria cincotta* (diamond darter); *Etheostoma blennioides* (greenside darter); *Etheostoma caeruleum* (rainbow darter); *Etheostoma camurum* (bluebreast darter); *Etheostoma flabellare* (fantail darter); *Etheostoma nigrum* (johnny darter); *Etheostoma variatum* (variegate darter); *Etheostoma zonale* (banded darter); *Percina caprodes* (common logperch); *Percina copelandi* (channel darter); *Percina maculate* (blackside darter); *Percina oxyrhynchus* (sharpnose darter); *Percina sciera* (dusky darter)
Percopsidae: *Percopsis omiscomaycus* (trout perch)
Petromyzontidae: *Ichthyomyzon bdellium* (Ohio lamprey)
Salmonidae: *Salvelinus fontinalis* (eastern brook trout)

It is also impossible to specify numbers of individuals that will be collected a priori. The sampling method is designed to estimate population sizes (Zippin multiple-pass depletion) and cannot be standardized by sample size. Multiple-pass depletion (with a backpack electrofisher)

will be used because it is superior to mark-recapture methods when working with small populations (where adequate numbers of recaptures are problematic) and/or with small, cryptic species (such as the Cyprinidae and Percidae species listed above). Furthermore, the Zippin multiple-pass depletion method assumes only that CPUE (i.e., the numbers of specimens captured in a given period of time) will decline at a constant rate among successive samples (i.e., "passes"); in essence, population sizes are estimated by plotting a CPUE trajectory among samples, where the zero-intercept is used to estimate population size. That said, past experience working in eastern U.S. headwater streams suggests that "characteristic" numbers of specimens collected in a single electrofishing pass will range from approximately 100-300 individuals (distributed among all locally occurring species); subsequent electrofishing passes (i.e., passes two and three) in a depletion estimate will necessarily collect fewer individuals. Using this "first pass rule-of-thumb", the total number of individual fishes likely to be sampled during this study can be estimated as:

200 specimens (1st pass average) + 100 spec. (2nd pass) + 50 spec. (3rd pass) = 350 spec. / sampling event
x 4 study sites per season = 1400 specimens per season
x 4 sampling seasons per year = 5600 specimens per year
x 2 year study = 11,200 TOTAL SPECIMENS (approximate)

3.0 Does this experiment duplicate previous experiments (other than control data): (*No*)

DATABASE SEARCH

1.0 Date search conducted for literature related to the three "R's" (Replacement, Refinement, and Reduction): (*4/14/2013*)

2.0 Database name and publications years covered:

Name	*From Year*	*To Year*
Google Scholar	1970	2013
Web of Science	1970	2013
PubMed	1970	2013
Aquatic Sciences and Fisheries Abstracts	1978	2013

3.0 Keywords used in the search for literature for the three "R's":

fish sampling; fish population estimation; mark-recapture; multiple-pass depletion; depletion sampling; fisheries methods; stream sampling

4.0 Other sources consulted (if any):

Canadian Council of Animal Care website resources (http://3rs.ccac.ca/en/searchesand-animal-index/guide/)

Fisheries Techniques (2nd edition, 1996) by B.R. Murphy & D.W. Willis (American Fisheries Society, Bethesda, MD)

5.0 Based on the search results above and protocol planning, explain why you cannot replace your animal model with a non-animal model or less sentient species:

The primary objective for this study is to empirically estimate fish abundances and production rates within the study streams. It is therefore impossible to replace the sampled fishes with a non-animal model.

6.0 Based on the search results above and protocol planning, explain why you cannot reduce the animal numbers necessary for the experiment:

If the study objective were to measure some attribute of the fish community other than population size, such as feeding behavior or physiological response to in-stream pollution, it would likely be possible to sample fewer individual fishes. However, when measuring population size/abundance, the information that must be collected is the reduction in captured numbers of fishes per standardized sampling effort (i.e., "depletion sampling"). This means that power analysis cannot be used to identify a minimum acceptable sample size a priori.

7.0 Based on the search results above and protocol planning, explain why you cannot further minimize pain or distress in the experiment:

The standard collection method/equipment to be used (backpack electrofishing) is the "industry standard" method for fish sampling in small to mid-sized streams. While it is true that the electroshocking field causes some discomfort to fishes, the end result is narcosis: fishes are rendered immobile until they can be netted. And once netted, all fishes will be held in a 100 gallon aerated holding tank, then released unharmed. NO SURGICAL OR OTHERWISE HIGHLY MANIPULATIVE/INVASIVE PROCECURES WILL BE USED WHEN

IDENTIFYING AND MEASURING FISHES. Thus, pain and discomfort cannot be further minimized without compromising the study objective (i.e., failing to collect fish).

DOCUMENTATION

Upload files relevant to this submission:

File	*Version*	*Date Modified*	*Uploaded By*
McScience Health Letter	0.01	4/29/2013 4:47 PM	Dr. McScience
Student 1 Health Letter	0.01	4/29/2013 4:47 PM	Dr. McScience
WV Collecting Permit	0.01	6/13/2013 11:17 AM	Dr. McScience

ANIMAL GROUPS

Animal arms should only be separated by species (not pain category within species) with the exception of pain category E, which requires a separate animal group. Protocol submissions with excessive groups may be returned to the PI for modification. PIs are encouraged to minimize the number of animal groups

Group Name	*Species*	*# Yr 1*	*# Yr 2*	*# Total*	*Pain Category*	*USDA*
Stream fishes	Fish	5600	5600	11,200	D	no

GROUP DETAILS

1.0 List strain(s) in this group of animals: (*N/A - no SPECIFIC strain of fish will be handled*)

2.0 Age at final disposition (sacrifice or transfer) for this group of animals: (*N/A*)

3.0 Will this group of animals be subjected to food/fluid regulation (includes caloric restriction, scheduled access to food/water, etc.) or receive a special diet [ad lib consumption of a specialized diet (e.g., added drugs, minerals, etc.)]: (*No*)

4.0 Will this group of animals need single housing: (*No*)

5.0 Will DAR provide environmental enrichment for this group of animals: (*No*)

6.0 What amount of time, if any, will this group of animals be held outside of the vivarium: (*0 - 11 Hours*)

USDA DETERMINATION

1.0 Group Species: *Fish*

USDA regulated animals include any live or dead: Birds **EXCEPT** those bred for use in research; Cats; Dogs; Guinea pigs; Hamsters; Horses **EXCEPT** those not used for research; Mice **EXCEPT** those of the genus *Mus* and bred for use in research; Nonhuman primates; Rabbits; Rats **EXCEPT** those of the genus *Rattus* and bred for use in research.

Any farm animals, including livestock and poultry, **EXCEPT** when used or intended for use as food, fiber or in agricultural research, including: Cows, Goats; Pigs; Sheep.

Any other warm-blooded animals used for research, teaching, testing, experimentation or exhibition purposes, including: Ferrets; Gerbils; Skunks; Squirrels; Voles.

Per the definition above, is this group of animals USDA regulated: (*No*)

ENRICHMENT JUSTIFICATION

1.0 Justify withholding enrichment or provide the alternative enrichment plan:

Field-sampled fishes will be held stream-side in a 100 gallon, aerated tank for approximately 1 hour, to allow sufficient time for sample processing (i.e., identification and weighing). Artificial enrichment materials are neither standard nor commercially available for this "live well" set-up.

GROUP DRUGS/COMPOUNDS

1.0 List ALL drugs/compounds used for this group of animals:

Drug – Finquel (tricaine methanesulfonate: MS-222)
*Dose – 2*5 mg/L

Route – Immersion in water bath (holding tank)
Frequency – Single immersion
Duration – 1 minute
Hazard – N/A
Biosafety Level – N/A
Pharmacy Grade – No
Justification – Adulteration

GROUP EUTHANASIA METHODS

1.0 Select euthanasia method(s) for this group of animals: *Tricane (MS-222)*

2.0 Describe euthanasia method(s) for this group of animals and how carcasses will be disposed:

No fishes will be intentionally euthanized. 100% release of live specimens is anticipated.

GROUP STUDY TYPE(S)

1.0 Select study type(s) for this group of animals:

Field Studies/Biological Surveys
Non-Surgical Procedures Requiring Anesthesia

FIELD STUDIES/BIOLOGICAL SURVEYS PROCEDURES

1.0 List any field study/biological survey procedures that will be performed on this group of animals:

Procedure – Field Studies/Biological Surveys
Status – Active
Description – Collection with Delayed Live Release
Selected – D
Default – C

Pain Category Justification – The use of an electrical field (i.e., the backpack electrofisher) does create discomfort for sampled fishes, but the effect is momentary - just long enough to net sampled specimens. Once netted, specimens will be kept safe in a holding tank until they can be identified and weighed, then released. To facilitate ease of handling and minimal stress, a standard fish anesthetic (MS-222) will be used.

FIELD STUDIES/BIOLOGICAL SURVEYES INFORMATION

Total number of animals estimated for this group: (*11,200*)

1.0 Out of the total number of animals for this group, how many are estimated to undergo field study/biological survey procedures: (*11,200*)

2.0 Out of the total number of animals needed for this group, how many are estimated to be ordered through DAR or transferred from another protocol/institution (not captured): (*0*)

3.0 Will the study involve species on state or federal lists of threatened or endangered species: (*No*)

4.0 Are state and/or federal permits required for collections and/or the survey work: (*Yes*)

5.0 Describe the sampling methods to be used, including the kind of net or trap and whether it is a kill or live type sampling apparatus:

All fish samples will be collected with a battery-powered backpack electrofisher; this is the industry standard method used to collect fishes in small to mid-sized streams. It is both effective for capturing fishes and relatively benign (i.e., very low or zero rates of injury to sampled fish; further technical information is provided below). Population size estimates will be obtained using the Zippin multiple-pass depletion method: three successive electrofishing passes will be performed within each sampled stream reach. Population abundance for each species will then be estimated as the zero-intercept of a cumulative catch (i.e., total individuals summed among successive passes) vs. catch-per-unit-effort (CPUE) regression model, where CPUE is expected to decrease at a linear rate among successive passes.

Collected fishes from each pass will be stored in an aerated 30-gallon holding tank (for approximately 1 hour) until processing (i.e., identification and weighing) is complete. Immediately prior to processing and handling, fishes will be transferred in small batches (~30-50

fish per batch) from the 100-gallon holding tank to a 10-gallon anesthesia tank. A 25 mg/L solution of buffered Tricaine methanesulfonate (MS-222) will be used to sedate fishes in the anesthesia tank; mild sedation (sufficient for handling and processing) is normally observed in 1 2 minutes. All processed fish will then be deposited in a separate 30-gallon recovery tank and allowed to re-equilibrate for a minimum of 30 minutes prior to release into their native stream.

6.0 Will kill-type sampling devices be used: (*No*)

7.0 Will voucher specimens be kept: (*No*)

8.0 Will live trapped animals be released: (*Yes*)

9.0 Will live animals be euthanized in the field following live capture: (*No*)

10.0 Describe any additional population/strain information: (*N/A*)

11.0 Field Studies/Biological Surveys Location(s): (*To be decided*)

FIELD STUDIES/BIOLOGICAL SURVEYS – PERMIT INFO

1.0 Name the state and/or federal permits required for collections and/or survey work and give permit number, expiration date, and/or other appropriate details of your permit:

A standard Mid-Atlantic State Scientific Collecting Permit (# 12345) was issued through the Mid-Atlantic State Division of Natural Resources on April 1, 2013. It is an annual (i.e., calendar year) permit that expires on Dec. 31, 2013.

FIELD STUDIES/BIOLOGICAL SURVEYS – ANIMAL RELEASE INFO

1.0 Describe the nature of handling, sampling and marking or tagging methods that will be used when live trapped animals are released:

Collected fishes from each of the three electrofishing passes will be stored in a 30 gallon holding tank (for approximately 1 hour) until processing (i.e., identification and weighing) is complete. Immediately prior to processing and handling, fishes will be transferred in small batches (~30-50 fish per batch) from the holding tank to a 10-gallon anesthesia tank. A 25 mg/L solution of buffered Tricaine methanesulfonate (MS-222) will be used to sedate fishes in the anesthesia tank; mild sedation (sufficient for handling and processing) is normally observed in 1-2 minutes.

All processed fish will then be deposited in a separate 30-gallon recovery tank and allowed to re-equilibrate for a minimum of 30 minutes prior to release back into their native stream. No marks or tags will be applied to the collected fishes. (This is one of the advantages of a multiple-pass depletion estimate, relative to conventional mark-recapture studies.)

NON-SURGICAL ANESTHESIA PROCEDURES

1.0 List any non-surgical anesthesia procedures that will be performed on this group of animals:

Procedure – Chemical Restraint for Physical Examination (e.g., body measurements, catheter patency check).
Status – Active
Description – Animals are anesthetized per protocol description for physical examination and/or collection of specific body measurements.
Selected – D
Default – D
Pain Category Justification – N/A

NON-SURGICAL ANESTHESIA INFORMATION

1.0 Total number of animals estimated for this group: (*11,200*)

Out of the total number of animals for this group, how many are estimated to undergo non-surgical anesthesia procedures: (*11,200*)

2.0 Select pre-anesthetic agents used for non-surgical procedures: (*N/A*)

3.0 Select anesthetic and analgesic agents used for non-surgical procedures:
Drug – Finquel (tricaine methanesulfonate: MS-222)
Dose – 25 mg/L
Route – Immersion in water bath (holding tank)
Frequency – Single immersion
Duration – 1 minute
Hazard – N/A

4.0 Describe the expected duration of anesthesia:

Fish sedation in a 25 mg/L solution of MS-222 is normally observed in 1-2 minutes. Fishes will be removed from the anesthesia bath and processed as soon as sedation occurs.

5.0 Describe pre-anesthetic care:

N/A - no specific pre-anesthetic care will be necessary or administered. Captured fishes will be held in a 30-gallon tank prior anesthesia.

6.0 Will gas/inhalation anesthesia be used: (*No*)

7.0 Will paralytic drugs be used: (*No*)

8.0 How will the level of anesthesia be monitored and how often:

Fishes will remain in the anesthetic solution until sedation is observed. Monitoring will be performed by field personnel on a continuous basis.

9.0 What method will be used to prevent dehydration and hypothermia:

N/A - collected fishes will not be at risk of dehydration and/or hypothermia, given the field protocol to be used.

10.0 Describe non-surgical procedures:

Once anesthetized, all collected fishes will be identified (to species), weighed, and measured (total length). Processed fishes will then be placed in a recovery tank for 30-60 minutes, prior to release.

11.0 Describe recovery from anesthesia, including frequency and type of observations. Also list any lab tests, monitoring and management of pain when indicated, observations and management of potential experimentally related disease, parenteral fluids, special diet, etc.:

Processed fishes will be placed in a recovery tank for 30-60 minutes, prior to release.

12.0 Non-Surgical Procedures Location(s): (*To be decided*)

ANIMAL ARMS

1.0 Total Study Animals: (*11,200*)

2.0 Animal Groups:

Group Name	*Species*	*# Yr 1*	*# Yr 2*	*# Total*	*Pain Category*	*USDA*
Stream fishes	Fish	5600	5600	11,200	D	no