UNIVERSITY OF WISCONSIN-GREEN BAY
VERTEBRATE ANIMAL USE PROTOCOL

1. Project Title: **Assessment of larval lake sturgeon production and drift behavior in the Oconto River, Wisconsin**

2. Principal Investigators and contact information:

PI	Department and campus address	Campus phone #	Emergency phone #	Institution from which animal care certification was obtained
[redacted]	[redacted]	[redacted]	[redacted]	

3. Project Period:
March 1, 2015 – December 31, 2015

4. Funding Agency Department or Unit (include grant numbers if appropriate):
This project is funded by Oconto Electric Cooperative and has been endorsed by the Fisheries Division of the Wisconsin Department of Natural Resources. Project related activities including field sampling will be conducted jointly.

5. Personnel working with animals (all personnel must be animal care certified or trained on the ethical care and use of vertebrate animals by the PI):
Personnel working with animals will be trained on all aspects of this project by the PI

Name	Animal care certified?	If not certified, trained by PI?	Campus phone #	Emergency phone #
Term Employee (TBD)				
Term Employee (TBD)				
Undergrad Assistant (TBD)				
Undergrad Assistant (TBD)				

Person(s) responsible for animal husbandry: *Does not apply*

Name	Animal care certified?	If not certified, trained by PI?	Campus phone #	Emergency phone #

6. Veterinarian to be notified when veterinary care is needed: *Will not be needed*

Name	Office address	Office phone #

7. Animals to be used:

Lake Sturgeon (Acipenser fulvescens) is the animal central to this study. Note that this is not first time lake sturgeon sampling will be conducted on the Oconto River AND this is not the first time this research has received IACUC approval. However, there is still no way of knowing at this time how many larvae will be collected, released at the collection site and kept (euthanized) for later analysis. However, my research team will adhere to restrictions provided by WDNR collaborations as sampling takes place.

Species	**# of individuals**	**Source**
Lake Whitefish	*TBD*	*Oconto River*

8. Location where animals will be housed:

Animals will not be housed.

9. Briefly state the goal of the research.

Project justification: *A large proportion of adult lake sturgeon in Lake Michigan currently originate from Green Bay tributaries, and thus research and management activities of Green Bay's lake sturgeon are vital to the restoration of the lake's population as a whole. However, estimates of larval recruitment for many tributaries including the Oconto, Menominee, and Fox rivers are limited or lacking despite the need to understand the reproductive status of these lake sturgeon populations (Holey et al 2001). Equally important, no study has attempted to correlate recruitment with stream physical features (flow and temperature, etc.) and describe these effects on the timing and synchrony of reproduction and of abundance and timing of dispersing larvae. The Oconto River specifically has potential to contribute significantly to the total Lake Michigan sturgeon population because of high quality spawning habitat upstream and larval and juvenile rearing habitat available downstream. Recent surveys conducted by the FWS on the Oconto River support this assumption by showing the presence and long-term residency of juvenile lake sturgeon late in the summer (Rob Elliott, personal communication) However, few attempts have been made to assess the level of larval production and timing of larval drift in this system. This lack of information ultimately impedes our ability to 1) determine if annual recruitment of lake sturgeon to the larval stage for the Oconto River is comparable with and consistent across Green Bay tributaries or across Lake Michigan tributaries (i.e., good year for one population is a good year for all), 2) determine whether larval numbers are related to the estimated population size of spawners, and 3) predict whether the timing of larval dispersal and potentially larval abundance are associated with environmental features, including those that vary as a function of dam operations.*

Project goal: *The baseline purpose of this project is to assess the number of larval lake sturgeon in drift and the timing of drift activity in relation to water temperature and river flow on the Oconto River during the 2015 field season. Data collected on the Oconto River will then be compared to information collected simultaneously on other Green Bay tributaries (e.g., lower Menominee River) to establish if there are commonalities that can be used to predict recruitment in all basin systems, and target regional-level risks to population viability.*

10. Describe all non-surgical manipulations or procedures involving the animals (e.g., drug administration, blood collection, behavioral assessment, capture, recapture, banding, diet change). Specify the drug(s), dose, and route of administration or other methods used. If more room is needed, attach statement.

Larval sturgeon will be collected using D-frame drift nets (bottom width = 76 cm, height = 53 cm, length = 3.4 m, mesh size = 1.6 mm). Five nets will be used and set on the bottom across a transect perpendicular to the river flow a short distance below the spawning grounds. The exact location of the sampling transect will be moved up or down river as needed based on flow rates. Ideal transects are just below the spawning grounds where nets can be set across the entire river at depths approximately equal to but not significantly greater than the depth of the nets (approx 2 feet). Nets will be anchored to the bottom using lines running upriver to standard trap net anchors.

Typical field season will be mid-May to mid-June. Larval drift typically starts 10-24 days post spawning depending on temperature. Cumulative daily water temperature units (CTUs; Kempinger 1988) will be used to predict expected larval emergence dates based on observed spawning events. Sampling will occur nightly throughout the period when larvae are expected to be drifting. Sampling will be conducted every other night up until and after the first and last larvae are captured and nightly during the drift period. Larval drift nets will be fished from 21:00-01:00, the expected peak hours of larval drift. Nets will be set before dark and then lifted hourly to remove the contents of the cod-end buckets and then reset. Lift time may be extended to 1.5 hrs if numbers of larval sturgeon are low and the amount of drift material (plant matter) will not cause mortality of the larvae. Larvae from each net lift will be counted, photographed, and measured for total length (nearest mm) prior to release.

After nets have been lifted and reset, the contents in the sample buckets will be examined for any captured lake sturgeon larvae as time permits between intervals when the nets need to be lifted. If there is not time to completely pick through every sample before the nets are to be pulled again, samples will be kept in buckets and worked up after sampling is competed for that night. Biological data on captured lake sturgeon will be recorded and will include species, total number, total length (nearest mm), and disposition. The same will be recorded for all fish species and other major aquatic species. Numbers and sizes of fish species other than sturgeon will be estimated when abundant (approx 100, 150, thousands, 5-10 mm, 10-15 mm, etc.). All individual lake sturgeon will be counted, measured, and released/housed according to DNR permits. At the end of each nights sampling, all gear including nets, anchors and buoys will be removed from the river, rinsed off and spread out to dry in a secure area and out of the sunlight so they are ready to use the next evening. Data sheets will be copied following each nights sampling with one copy being retained by UWGB in a secure location and the additional copies given to funding source and agencies (USFWS, WDNR and MDNR) as soon as practical.

11. Where will these procedures be performed?

Oconto Rivers, Wisconsin. On the campus of UWGB.

12. Is there potential for discomfort or pain as a result of the procedures (eg., tumor or ascites induction, prolonged restraint, nutrient restriction, toxic or infectious agents causing illness, aversive stimulus)?

No

13. If yes, what will be done to relieve discomfort? Include drugs and dosages, point at which animal will be killed, mechanical devices, etc.

N/A

14. Is surgery to be performed on the animal(s)?

No

15. Will the animal(s) be allowed to recover from surgery?

Surgery will not be performed

16. Identify the personnel who will perform the surgery:

N/A

17. Location where the surgery will be performed:

N/A

18. Briefly describe the surgical procedures:

N/A

19. Will more than one surgery be performed on the same animal?

N/A

20. If yes, give justification:

N/A

21. Describe the anesthetic method including all drugs, dosages, routes of administration and supplementation schedules:

N/A

22. Describe the post surgical monitoring and care procedures including all drugs and dosages. Describe measures designed to alleviate postoperative pain or discomfort:

N/A

23. Describe the method of euthanasia at the conclusion of the project. Include agents, dosages, routes of administration:

Based on similar sampling efforts for other fish species, we assume that some mortality will occur during sampling or during transportation. Mortalities of individuals occurring during sampling will be kept preferentially. A proportion of live larval whitefish collected (guided by the WDNR) will be euthanized with MS-222 in the laboratory. MS-222 is a fine white crystal that is highly soluble in water and is related to Novocain, procaine, and benzocaine. MS - 222 in this situation will be applied using a bath, i.e., the fish will be immersed in the anesthetic solution. The MS-222 solution will be prepared in a 5 gallon pail, or other suitable, well-labeled container. The container size will be large enough to easily contain the entire fish. MS -222 is absorbed rapidly via gill diffusion or by coupling to specific enzyme systems (Summerfelt and Smith 1990). In modern fishes, MS-222 is bio-transformed in the liver and probably the kidney (Harms and Bakal 1994). MS-222 is excreted in fish urine within 24 hours and tissue levels

decline to near zero in the same amount of time (when used for surgery or non-invasive tissue sampling for example).

In the United States, TMS (otherwise known as MS-222) is the only legal anesthetic for use on a limited number of food fish (FDA 2006). The limitations on TMS use with food fish include a 21-day withdrawal period before harvesting, and use is restricted to the families Ictaluridae (catfishes), Salmonidae (salmon, trout, char, whitefish, and grayling),Esocidae (pike and pickerel), and Percidae (perch,walleye, ruffe, and darters) (FDA 2006). For other species, the drug should be limited to hatchery or laboratory applications in which fish will not be released into the wild or consumed. In the United States, MS-222 is the only legal anesthetic for use on a limited number of food fish (FDA 2006).

Induction is rapid (especially for larval fish) and can take as little as 15 seconds in most cases. However, the concentration required to induce complete induction varies rapidly depending on fish species (see table below). We will use minimum concentration/dosage required for induction (i.e., 40/50 mg/L) and a minimum soak time for the fish to minimize tissue uptake. At the point of induction (opercular or breathing activity completely ceases), larval fish will then be placed in a stock solution of ethanol or formalin with the difference depending on the specific data analysis to be conducted (e.g., otolith microchemistry or genetic identification). This protocol was invoked by the Canadian Council on Animal Care with regard to using fish in research, teaching and testing.

Note that regulations or policies regarding the disposal of MS-222 or disposal of fish carcasses vary widely by state/province, or institution. Per my discussion with UWGB safety manager, after use, the MS-222 solution will be flushed down the drain to a sanitary sewer with excess water. In the case where disposal of euthanized fish is necessary for some reason (not preserved in ethanol or formalin), carcasses will be placed in two sealed plastic bags, frozen, and placed in a dumpster on the day of trash pick-up for disposal in a licensed landfill. Any residual concentration of MS-222 is not a concern. Fish that have been collected and shipped to project collaborators for analyses will be disposed of per that institution's policies.

Table 2. Dose rates of major anesthetic drugs, evaluated experimentally, for a number of commonly cultured fish species.

Anesthetic	**Atlantic salmon** *Salmo salar*	**Rainbow trout** *Onchorhychus mykiss*	**Common carp** *Cyprinus carpio*	**Channel catfish** *Ictalurus punctatus*	**Nile tilapia** *Oreochromis niloticus*	**Striped bass** *Morone saxatilis*
MS-222	40-50 mg/L	40-60 mg/L	100-250 mg/L	50-250 mg/L	100-200 mg/L	100-150 mg/L
Benzocaine	40 mg/L	25-50 mg/L	ND	ND	25-100 mg/L	50-100 mg/L
Quinaldine	25-40 mg/L	ND	10-40 mg/L	25-60 mg/L	25-50 mg/L	25-40 mg/L
2-Phenoxyethanol	100-200 mg/L	100-200 mg/L	400-600 mg/L	ND	400-600 mg/L	ND
Metomidate	2-10 mg/L	5-6 mg/L	ND	4-8 mg/L	ND	7-10 mg/L
Clove oil	10-50 mg/L	40-120 mg/L	40-100 mg/L	100 mg/L	ND	60 mg/L
Aqui-S™	10-50 mg/L	20 mg/L	ND	20-60 mg/L	ND	ND

ND = not determined. Only MS-222 is approved in the U.S. at the time of this publication.

24. If the project utilizes hazardous agents (e.g., infection agents, carcinogens, toxic chemicals, radioisotopes) briefly outline the procedures for handling and disposal:

The solution Tricaine-S (MS 222) will not be handled on the water. It will be carefully maintained with sealed containers and used only under controlled conditions. That notwithstanding, MS-222 will be treated as a hazardous chemical. Protective clothing, gloves, and goggles will be used when handling MS-222 powder. Gloves will also be worn while handling animals that have been exposed to MS-222. It is recommended by the FDA that MS-222 waste be flushed into a sewer drain with plenty of water and we will adhere to these guidelines. MS-222 will never be disposed near conveyances to natural (or otherwise untreated) bodies of water. When used in a facility setting, Material Safety Data Sheets will be readily accessible.

25. Classification of Research Animal Use (see classification at end of document and indicate highest category applicable):

Experiments which are expected to cause only minimal discomfort or none. Experiments carried out on anesthetized animals which do not recover

26. If the project requires the use of hazardous substance, has the campus Safety & Risk Manager been contacted?

Yes

27. Additional comments:

28. If any federal or state licenses are required for either the collection or experimentation with the particular species of animal being used or for work with a particular toxic agent, then submit copies of them with this form.

Copies of my scientific collectors permit are attached.

Research Animal Use Classification

Category	Procedure	Example
0	No invasive procedure or intrusion into the normal life stream of animal.	Simple observation
1	Experiments which are expected to cause only minimal discomfort or none.	Injections, blood sampling, tube feeding, behavior experiment without significant restraint, etc.
2	Experiments carried out on anesthetized animals which do not recover	Removal of organs for histological, biochemical or transplant studies
3a	Experiments with painful stimulation of awake animals, which cause momentary light pain	Behavioral experiments with flight or avoidance reactions.
3b	Surgery with anesthesia from which the animal will awaken or experience the cessation of analgesia	Biopsies, implantation of chronic catheters, gonadectomy, any survival surgery, etc.
4	Experiments on awake animals of whom some can be expected to become seriously ill or be caused significant pain or distress.	Toxicity testing, production of radiation sickness, infections or tumors, nutrient restrictions, stress or shock treatments, chronic restraint, etc.
5	Painful experiment on un-anesthetized animals with or without the use of muscle paralyzing agents	Certain physiological and pharmacological experiments on the nervous system, research on pain, etc.