GENERAL INFORMATION AND INSTRUCTIONS

**ANIMAL SUBJECTS REVIEW FORM**

**IMPORTANT:**

**Allow a minimum of 4-6 weeks for protocol approval.**

**SUBMIT ONE ORIGINAL WITH ALL SIGNATURES TO:**

**Office of Research Compliance**

**115 Ramsay Hall Basement**

**Wilmore Drive**

**Auburn, University 36849**

**Phone: 334-844-5966**

**Or scan/email to:** [**IACUCadmin@auburn.edu**](mailto:IACUCadmin@auburn.edu)

**IACUC Website:** [**https://cws.auburn.edu/OVPR/pm/compliance/iacuc/home**](https://cws.auburn.edu/OVPR/pm/compliance/iacuc/home)

University policy requires that all research, teaching, production/maintenance, and demonstration activities involving vertebrate animals be approved by the Auburn University Institutional Animal Care and Use Committee (IACUC) prior to initiation of the project. The *Auburn University Policies and Procedures for the Care and Use of Live Vertebrate Animals* is available on the IACUC website. This policy is in accordance with federal regulations and guidelines.

When submitting the original, the General Information and Instructions and the Additional Information sections should be omitted.

The IACUC meets the first and third Thursdays of each calendar month. Protocols received at least seven days prior to a scheduled meeting date (e.g. by 11:30 a.m. on Thursday of the week prior to a scheduled Thursday p.m. meeting) will be placed on the agenda. Approved protocols will be assigned a PRN (protocol review number). Approved Animal Subjects Review Forms will remain in the official files of the University for not less than three years beyond the completion of the project.

Annual review of all protocols is required. An Annual Review Form will be sent to the Principal Investigator approximately 30 days prior to the Annual Review Due Date.

Animal users are required to become familiar with all guidelines and regulations pertaining to the care and use of animals in research and teaching by visiting the Animal Welfare Information Center (AWIC) website: <http://www.nal.usda.gov/awic/>

An Animal Subjects Review Form may be obtained by downloading it from the IACUC website. Only the current version ASRF 04/2012 will be accepted.

Complete this form by providing **BOLD TYPED** answers in the text boxes in each item. All acronyms must be spelled out upon first use. If an item is not applicable, please indicate NA. The attached REQUIRED Checklist must be completed and included with the original protocol.

**ALL SIGNATURES are required for the protocol to be eligible for placement**

**on the IACUC meeting agenda.**

### ANIMAL SUBJECTS REVIEW FORM

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| **PRINCIPAL INVESTIGATOR:** | | | | | | **Matthew J. Catalano** | | | | | | |
| RANK/TITLE: | | | | Assistant Professor | | | | | | |  | |
| DEPARTMENT: | | | | Fisheries, Aquaculture, and Aquatic Sciences | | | | | | |  | |
| COLLEGE/SCHOOL: | | | | Agriculture | | | |  | |  | | |
| CAMPUS ADDRESS: | | | | 203 Swingle Hall | | | | | CAMPUS PHONE #: | | | 334-844-7366 |
| E-MAIL: | | [Mjc0028@auburn.edu](mailto:Mjc0028@auburn.edu) | | | | | | | FAX #: | | | 334-844-9208 |
|  | **Check if PI will serve as faculty advisor to the Lead Graduate Student or Resident associated with this activity.** | | | | | | | | | | | |
| **LEAD GRADUATE STUDENT/RESIDENT:** | | | | | | |  | | | | | |
| RANK/TITLE: | | |  | | | | | |  |  | | |
| DEPARTMENT: | | |  | | | | | | CAMPUS PHONE #: | | |  |
| EMAIL: | | |  | | | | | | FAX #: | | |  |
| **CO-INVESTIGATOR:** | | | | |  | | | | | | | |
| RANK/TITLE: | | |  | | | | | |  |  | | |
| DEPARTMENT: | | |  | | | | | | CAMPUS PHONE #: | | |  |
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|  | **Check box if this protocol has more than one co-investigator. Additional co-investigators should be listed on page 2.** |

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| --- | --- | --- | --- | --- | --- |
| **PROJECT TITLE:** | **Shoreline rotenone to reduce largemouth bass recruitment in Alabama ponds** | | | | |
| **STARTING DATE:** | **5-8-2017** | | **EXPIRATION DATE:** | **5-7-2020** | |
| *(Must not be prior to IACUC approval)* | |  | *(Must not exceed three years)* | |  |

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| **Is any part of the funding from a U.S. Public Health Service Agency:** | **YES** |  | **NO** | **X** |

**REQUIRED SIGNATURES**

The information contained on this form provides an accurate description of the animal care and use protocol which will be followed. I agree to abide by governmental regulations and university policies concerning the use of animals. I will allow veterinary oversight to be provided to animals showing evidence of pain or illness. If the information provided for this project concerning animal use should be revised, or procedures changed, I will so notify the committee of those changes in writing, and no proposed changes will be implemented until full IACUC approval has been granted.

**X**

# Principal Investigator Date

Medical care for animals will be available and provided as indicated by a qualified veterinarian. By accepting this responsibility, the veterinarian is providing assurance that any personal interest he/she might have in the project will not conflict with his/her responsibility for the provision of adequate veterinary care for the animals. Furthermore, the veterinarian provides assurance of review and consultation on the proper use of anesthetics and pain relieving medications for any painful procedures.

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|  |  | **X** |
| **Project Veterinarian Name (print or type)** |  | **Project Veterinarian Signature Date** |
|  |  | **X** |
| **Unit Veterinarian Name (print or type)** |  | **Unit Veterinarian Signature Date** |
|  |  | **X** |
| **Departmental Chairperson Name (print or type)** |  | **Departmental Chairperson Signature Date** |
| **X** |  | **X** |
| **Lead Graduate Student/Resident signature Date** |  | **\*IACUC Chair Signature Date**  \*IACUC Chair signs the protocol after IACUC approval has been granted |

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**PLEASE TYPE IN BOLD FONT AND COMPLETE THE FOLLOWING FORM IN FULL.**

**IMPORTANT: Allow a minimum of 4-6 weeks for protocol approval.**

1. Will the animals be used in:

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| A. | Teaching |  | If Teaching, give the course number(s): |  |
| Research | **X** |  |  |
| Demonstration |  |  |  |
| Production |  |  |  |

B. If Teaching, complete the following chart:

|  |  |  |  |
| --- | --- | --- | --- |
| Number of Students in the Class | Number of Students per animal | Number of Animals per Lab | Number of Labs per year |
|  |  |  |  |

1. A.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Animal Common Name | Total Used1 | Sex | Approximate Weight | Source2 | Housing Location3 |
| **Largemouth bass fry/juveniles**  **(young-of-year; YOY)** | **24,000** | **Both**  **50/50** | **0.5 - 3 g** | **Existing wild natural population** | **AU Fisheries Department pond S-3, AU E.V. Smith Ponds (n=2), private ponds (n=5)** |
| **Largemouth bass** | **240** | **“** | **100 - 2,500g** | **“** | **“** |
| **Bluegill fry/juveniles (YOY)** | **48,000** | **“** | **0.5 -2 g** | **“** | **“** |
| **Bluegill** | **480** | **“** | **10 - 400 g** | **“** | **“** |

1The number(s) listed in this column must match the total number of animals described in Question #7.

2 If reusing animals from another protocol, please provide the protocol number and assurance statement that the animals’ well-being has not been compromised by previous research and that the animals exhibit normal physiologic function. Please state how well-being and normal physiologic function

was determined for these animals (i.e. physical exam prior to accepting animals for use in protocol).

**SOURCE NOTE: Fish in the AU Fisheries ponds were previously stocked under PRN 2012-2086 and/or are currently under PRN 2015-2708. The origin of populations in the other ponds is unknown and may be the result of stocking decades ago or natural colonization from feeder streams. Few if any of the originally stocked fish are still alive in these ponds due to natural mortality over the years. The ponds are now populated by wild progeny of these originally stocked fish. These fish exist under a wild and natural state of well-being in which they are free to reproduce at will, consume others, or be consumed as part of a natural food web. Thus these fish require no care or maintenance because they are living in a natural pond environment. They reproduce annually via natural processes in the pond and are completely wild populations. Protocol 2017-3048 covers annual sampling of these wild populations.**

**Pond S-3 was chosen for this study because no other studies were ongoing in that pond and therefore our study would not be confounded by another manipulation nor would another study be disrupted by ours. It also could be paired with an ideal nearby control pond AE-1. A nearby control pond is essential to estimating the effectiveness of the treatment.**

3 Please state the housing facility as well as the area (ft2 or m2) allocated per animal in cages, stanchions, floor pens, etc. and the reference used to determine the area i.e. *Ag Guide* (2010) or *Guide* (2011).

B. Select pain/distress category relevant to the use of animals in this study.

(*See Item 2B of Additional Information at the end of this form.)*

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| B |  | C |  | D |  | E | **X** |

3. Will animals be maintained for a period of 12 or more consecutive hours in a location other than the housing location mentioned in Item 2? (*See Item 3 of Additional Information at the end of this form.*)

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| Yes |  |  | No | **X** |

If Yes, specify the location and reason:

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If Yes, how will the animals be transported to that location, by whom, and was this vehicle inspected and approved by IACUC?

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4. PERSONNEL QUALIFICATIONS (*See Item 4 of Additional Information at the end of this form.*)

IACUC-required CITI training and Occupational Health & Safety Program (OHSP) Enrollment for all individuals listed in section 4.A and 4.B must be completed prior to protocol approval.

1. Indicate who will provide daily care and maintenance of the animal(s). Indicate name(s) or identify the particular unit staff.

|  |
| --- |
| **Matthew Catalano - No daily care will be necessary. All fish populations are self sustaining. Periodically, water quality (dissolved oxygen, algal biomass) and water levels will be checked. Daily care and maintenance of the AU fish is covered under PRN 2015-2708, but there is no daily care and maintenance required because these are wild fish populations that exist in a natural state. See Source Notes in 2A, above.** |

1. List the names of all individuals who will conduct procedures involving animals on this protocol. Any individual not identified by name prior to protocol review will not be approved to conduct procedures. To add personnel after IACUC review and approval of the protocol, a *Personnel Modification Form* must be submitted and approved by the IACUC. These personnel must complete the CITI training and OHSP enrollment.

|  |
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| **Matthew Catalano** |

1. Principal Investigator Certifications

My signature on page 1 of this form certifies that:

1. Individuals performing animal procedures on this protocol are or will be qualified to perform their particular animal related duties through training and/or experience (individuals will be supervised until adequate training has occurred). Training and/or experience must encompass the following: \*biology, handling, and care of the species; aseptic surgical methods and techniques (if applicable); the concept, availability, and use of research or testing methods that limit the use of animals or minimize distress; the proper use of anesthetics, analgesics, and tranquilizers (if applicable); and procedures for reporting animal welfare concerns. Informative links regarding training resources can be found on the IACUC website.
2. All individuals working with animals, animal tissues, or animal products on this protocol will be informed of relevant \*occupational health and safety issues prior to performing their duties. \* Informative links have been provided for assistance in this and other areas as needed on the IACUC website.

5. State HOW or WHY you selected the species to be used in this project.

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| **Largemouth bass are an important sport fish stocked by private pond owners throughout the USA. Bluegill serve as prey for largemouth bass and reach a size large enough to serve as sport fish. If left unmanaged, largemouth bass populations become too dense due to high reproductive rates. High densities lead to poor growth and condition (plumpness), which may lead to disease outbreaks and undesirable fish sizes for anglers to catch. Bluegill growth, condition, and reproductive rates vary in response to largemouth bass population structure and pond conditions. Identifying effective methods to reduce largemouth bass population density is an ongoing effort for pond managers and would thus be of great value so that these populations can be managed to improve growth and condition, and reduce the incidence of disease outbreaks.** |

6. STUDY/ACTIVITY JUSTIFICATION AND OBJECTIVES:

1. Justify your animal use in one or two brief paragraphs:

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| **The Fisheries Department at Auburn University has an internationally-known research program to improve sportfish and fishing in small ponds and lakes. Largemouth bass and bluegill are the most important fish species in these systems. Achieving satisfactory fish growth, body condition are central goals of small pond fishery management. If left unmanaged, largemouth bass populations become too dense due to naturally high reproductive rates, which outstrips the capacity of the pond to provide natural food (small bluegill) for these bass. When there is not enough food for the bass, their growth and body condition suffers and the fish can become stunted and very thin, which lead to disease outbreaks and is undesirable for anglers that wish to catch large fish. Thus management actions usually must be taken to prevent high largemouth bass densities from occurring. The most common method for thinning the bass populations is to remove fish by hook and line fishing, however this approach often targets larger adult fish that have already established themselves and lived in the pond for several years. In practice, pond owners and anglers often toss these fish onto the shoreline alive to perish out of the water or be consumed by terrestrial animals. Moreover, this approach is undesirable because it does not address high densities of small, young (< 1 year old) bass that are overly-abundant and capable of consuming more prey (small bluegill) than the pond can produce. Thus we are interested in evaluating methods that can selectively exterminate these wild populations of these overly-abundant young (fry/juveniles) bass.**  **One such approach to exterminating overly-abundant young-of-year (YOY; 1 month 1-2 inches long) bass is to apply (spray and/or inject into the water) the botanical pisicicde rotenone in a narrow band along the shoreline of a pond, at a time of year when recently hatched YOY bass are hiding very close to shore to avoid predation by larger fish. This approach is very selective and has the potential to exterminate populations of young bass without substantial unintended deaths of larger adult bass because it is applied in such shallow water close to shore where large fish rarely venture. These larger fish are also able to easily swim to deeper water to escape the pisciciede when disturbed by the application process.**  **Rotenone is an essential piscicide for fisheries management that is derived from the roots of plants in the family Leguminosae. Rotenone can be used safely and effectively to exterminate undesirable fish populations. The American Fisheries Society, the pre-eminent professional fisheries organization in the North America, states in their standard operating procedures for the use of rotenone (**[**http://www.fisheriessociety.org/rotenone/rot.pdf**](http://www.fisheriessociety.org/rotenone/rot.pdf)**) that “Rotenone continues to be a valuable tool in fisheries, without which many management options will be lost. Fisheries managers rely on a wide variety of tools for the management and assessment of fish populations to maintain diverse and productive aquatic ecosystems and quality recreational fisheries. Piscicide application is the only method other than dewatering that can consistently eradicate undesirable fish communities or sample a portion of the entire fish population, including all species.”**  **Shoreline rotenone is often applied by state natural resource management agencies to reduce bass recruitment, particularly in the southeastern US where conditions are favorable for high very high largemouth bass reproductive rates the thus population densities. For example, the Alabama Department of Conservation and Natural Resources typically treats 3-5 large 50-100 acre lakes each year with shoreline rotenone. Unfortunately, the effectiveness of these treatments has not been thoroughly evaluated. There is only one published study on the effectiveness of shoreline rotenone (McHugh 1990), and it was somewhat inconclusive and had a low sample size. Thus we would like to conduct a study to assess the effectiveness of shoreline rotenone. If shoreline rotenone proves effective at eliminating the undesirable over-abundance of YOY largemouth bass then continued use of this method may be justifiable to maintain adequate largemouth bass growth and would eliminate the need to cull adult bass from these populations. Conversely, if our study suggests that shoreline rotenone is ineffective, then these agencies could be persuaded to stop the practice.**  **Literature Cited:**  **Finlayson, B., R. Schnick, D. Skaar, J. Anderson, L. Demong, D. Duffield, W. Horton, and J. Steinkjer. 2010. Planning and standard operating procedures for the use of rotenone in fish management—rotenone SOP manual. American Fisheries Society, Bethesda, Maryland.**  **James J. McHugh (1990) Responses of Bluegills and Crappies**  **to Reduced Abundance of Largemouth Bass in Two Alabama Impoundments,**  **North American Journal of Fisheries Management, 10:3, 344-351.** |

1. What are the main objectives of your study:

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| **The main objective of this study is to evaluate the effectiveness of shoreline rotenone application to exterminate undesirable and overly-abundant young (fry/juveniles; YOY) largemouth bass to reduce population density and improve growth and body condition.** |

7. A. SUMMARY OF PROPOSED ACTIVITY: USE LAY TERMS to give a description of the proposed

activity. *From reading this section it should be possible for a non-scientist to determine exactly how*

*animals will be used in the context of the proposed activity.*

This section should include a clear description of the EXPERIMENTAL DESIGN (research protocols) or activities involving animals (teaching, demonstration, or production/maintenance protocols). This section should also include a brief description of each phase of activities involving animals and should make it possible to account for all animals requested in Item 2. Tables may be helpful to show animal numbers. Justification for animal numbers is required to assure that only the necessary number of animals is being used. If applicable, include the technique, location and volume of blood drawn. If applicable, describe method of transportation and/or method of restraint. (*See Item 7 of Additional Information at the end of this form for guidance in providing the appropriate information.)*

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| **To evaluate the effectiveness of shoreline rotenone application at exterminating unwanted and overly abundant young largemouth bass, we plan to conduct a controlled study by applying rotenone in some ponds and comparing the post-treatment abundance of YOY largemouth bass at these treated ponds with untreated control ponds that will be sampled in the same way. We will also evaluate whether reduced bass population density results in improved largemouth bass growth and body condition.**  **In this study we will annually treat 8 of the 29 ponds listed under our IACUC protocol PRN 2017-3048 with shoreline rotenone. We will compare these 8 treated ponds with another 8 untreated controls that will be selected from the same pool of 29 ponds. Sampling to make the comparison has been previously described in PRN 2017-3048. Here we will focus on the rotenone application for the extermination of wild populations of YOY largemouth bass that were recently hatched in the pond via natural processes. Statistical power analyses suggest that eight treated and eight untreated ponds will be sufficient to detect effects of rotenone application on largemouth bass density at 80% power.**  **Liquid 5% rotenone solution will be applied by boat at a rate of 1 pint per 100 yds of shoreline dissolved in the appropriate amount of water calibrated to sprayer rates and boat speed. Half of the rotenone/water solution will be applied via surface spray in a narrow band within 5 feet of the shoreline, and the other half will be injected below the surface into the same area. The boat will move along the shoreline at 1-2 miles per hour as the rotenone is applied to the entire perimeter of the pond. The application will be done in late May and early June because that time period comes after the majority of largemouth bass reproduction but before much of the reproduction of bluegill. This rotenone will be of high enough concentration along the shoreline area to kill fish but will rapidly dilute into the rest of the pond and breakdown via natural processes such that it will be effectively inactive after 10 minutes - 20 minutes. Applicators of rotenone will wear approved protective gear including masks, gloves, suits, and goggles as recommended by the American Fisheries Society Rotenone SOP (Finlayson et al 2010).**  **Prior experience suggests that less than 10 adult largemouth bass and 20 adult bluegill per pond will unintentionally succumb to the rotenone. These fish rise to the surface via rapid loss of equilibrium if exposed to rotenone and will be netted and euthanized in a 300 mg/l solution of MS-222 then placed on ice and transported to the disposal facility. Young largemouth bass and bluegill will be vulnerable to the rotenone and rapidly succumb. These fish will be left in the pond as there is no practical method available for their removal considering their large numbers, small size (0.5 – 2 inches) and the large spatial scale. The young bluegill that are inadvertently exterminated by rotenone will have no appreciable effect on the population because these fish spawn continuously during the summer and thus many more bluegill will be produced after the rotenone application. However, bass reproduce for only one month per year and their young are vulnerable to extermination by rotenone as no reproduction will occur during the summer immediately after rotenone application.**  **We will use shoreline seine hauls and electrofishing to assess whether rotenone application resulted in reduced YOY largemouth bass density and recruitment to age 1. Seine hauls will be conducted the day of and just prior to rotenone application and then again the following day at both the treated lakes and control lakes. Electrofishing will be conducted once per year in spring to assess the relative abundance of age-1 largemouth bass over time before and after rotenone treatment. These seining and electrofishing activities are covered under protocol PRN 2017-3048 and thus fish captured by these sampling efforts are not requested under this new protocol. Here we request only those fish that would be potentially impacted by the rotenone application.**  **Summary of Animals Used:**  **Largemouth bass young (fry/juveniles, 1 month old)**  **1,000 per pond\*8 ponds\*three years = 24,000**  **Largemouth bass adults euthanized**  **10 per pond\*8 ponds\*3 years = 240**  **Bluegill young (fry/juveniles, <1 month old)**  **2,000 per pond\*8 ponds\*3 years = 48,000**  **Bluegill adults euthanized**  **20 per pond\*8 ponds\*3 years = 480** |
|  |
|  |

B. For experiments regarding food and/or fluid restriction:

1. Describe animal health monitoring procedures and frequency (e.g. body weight, blood urea

nitrogen, urine/fecal output, food/fluid consumed):

|  |
| --- |
| **N/A** |

1. Describe methods of ensuring adequate nutrition and hydration during the regulated period:

|  |
| --- |
| **N/A** |

C. If this research involves production of genetically modified animals or is a pilot study and has the potential to result in unexpected outcomes, please address the following:

1. New phenotypes or other unanticipated results which may affect animal health and well-being.

|  |
| --- |
| **N/A** |

1. Method for monitoring and managing unexpected outcomes to assure animal health and well-being.

|  |
| --- |
| **N/A** |

1. Procedure for reporting unexpected outcomes to the IACUC.

|  |
| --- |
| **N/A** |

D. If exterior windows are present within the animal housing or procedure areas, describe how this may affect temperature and photoperiod control, as well as potential security risks.

|  |
| --- |
| **N/A** |

E. If this is a field study involving observation or use of a non-domesticated vertebrate species, please respond to the following:

1. What is the potential impact on the wild population of the species to be studied?

|  |
| --- |
| **The rotenone application has the potential to eliminate the wild population of YOY largemouth bass, which is the goal of the application, but will have minimal impact on bluegill and adult bass. Adult fish have low vulnerability to the shoreline application of rotenone and bluegill reproduction occurs mostly later in the year, after our rotenone application. Bass and bluegill are the only two species that inhabit the ponds we have selected for rotenone application.** |

1. How might the study compromise health of either animals or persons e.g. zoonoses?

|  |
| --- |
| **We do not anticipate negative health effects of animal or people. Following the product label and standard operating procedures will create minimal risk to persons or animals other than the YOY fish targeted by the treatment. Rotenone application using recommended rates and procedures results in very low risk to other animals such as birds and mammals due to low environmental concentrations and the fact that rotenone degrades quickly in the presence of water and sunlight and does not accumulate in biota (Finlayson et al. 2010).** |

1. Describe the final disposition of the animals being studied (i.e. return to wild population, preserve in museum collection, etc).:

|  |
| --- |
| **Young largemouth bass and bluegill killed by rotenone will remain in the pond. Adults of both species that appear to have unintentionally succumbed to the rotenone will be netted, euthanized and their carcasses will be disposed of at the North Auburn Fisheries Station by composting per PRN 2015-2708, the Standard Operative Procedures protocol for the Auburn Fisheries Research Unit.** |

F. Humane endpoints:

1. If pain/distress category D/E or food/fluid restriction is applicable to this protocol, please define humane endpoints:

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| --- |
| **We anticipate that a few adult largemouth bass and bluegill in each pond will be unintentionally affected by the rotenone application. We will visually monitor the pond for these moribund fish. They will be easily identified by their rapid loss of equilibrium and rise to the surface. These moribund fish will be netted at the surface and euthanized by immersion in a 300 mg/L solution of MS-222 for at least 10 minutes post cessation of operculum movement.** |

1. If research is novel and little or no information is available in the literature, state who you have collaborated with to define humane endpoints; please define humane endpoints resulting from your collaboration; state how you will periodically communicate with the IACUC to ensure the well-being of the animal(s) on this protocol:

|  |
| --- |
| **N/A** |

1. For category C and all other protocols where the potential exists for weight loss and/or other

parameters that could be potentially harmful to the animals, state the humane end points:

|  |
| --- |
| **N/A** |

G. Environmental Enrichment: *(See Bloomsmith et al. Lab Anim. Sci. 41:372-377); also the Ag Guide*

*(2010) has a good discussion on this topic by species in Chapter 4*.

1.) Social enrichment: Please describe direct or indirect animal contact (visual, olfactory, auditory)

with conspecifics or humans.

|  |
| --- |
| **N/A** |

2.) Occupational enrichment: Please describe any devices that provide animals with control or

challenges (psychological enrichment); enrichment that encourages exercise.

|  |
| --- |
| **N/A** |

3.) Physical enrichment: Please describe alteration of the size or complexity of the animal’s

enclosure which may include objects, substrate or permanent structures (e.g. nestboxes, rocks

and hiding places in an aquatic environment).

|  |
| --- |
| **N/A** |

1. Sensory enrichment: Please describe visual stimuli (television); auditory stimuli (music, vocalizations); olfactory, tactile, taste stimuli.

|  |
| --- |
| **N/A** |

1. Nutritional enrichment: Please describe presentation of varied or novel food types; changing the method of food delivery.

|  |
| --- |
| **N/A** |

1. Other types of enrichment: Please describe any other types of enrichment that do not fit the categories above.

|  |
| --- |
| **N/A** |

1. No Enrichment: Please justify the decision to provide no environmental enrichment if you have not responded to #7. G. 1-6 above.

|  |
| --- |
| **No enrichment will be provided because these animals are completely wild and live their entire lives in a natural pond environment and will not be held captive for any length of time. They live in close contact with thousands of other fish and experience daily natural challenges such as avoiding predation; they inhabit an extremely complex physical environment that contains many rocks, sticks, and aquatic plants; they receive continuous stimuli that they must monitor closely at all times to avoid predation and to find natural food items; they actively forage for food resources that are naturally present in the wild pond environment. Therefore they do not require environmental enrichment.** |

1. If category D or E was chosen in Question 2B, please complete the following. (*See Item 8A of*

*Additional Information at the end of this form.)*

1. Database(s) searched or other sources consulted to determine the availability of alternatives.

|  |  |  |  |
| --- | --- | --- | --- |
| Database Searched | **X** | Date of Search | Years Covered |
| Medline |  |  |  |
| Agricola |  |  |  |
| CABA |  |  |  |
| Altweb |  |  |  |
| Other (describe) | **X** | **May 11, 2017** | **1970-present** |

1. Scientifically relevant terminology (e.g. keywords) and search strategy used when considering alternatives to the painful or distressful procedure(s):

|  |
| --- |
| **Google scholar search terms: shoreline rotenone, largemouth bass recruitment, fish density reduction, culling, rotenone alternatives. I searched the first 100 citations for each search term.** |

1. A succinct written narrative based on results of the database search, that will permit the IACUC to readily assess whether the search topics were appropriate and whether the search was sufficiently thorough. This narrative must address the following:

Reduction:

|  |
| --- |
| **We cannot reduce the number of fish used because doing so would compromise the statistical validity of the study. We are already working with a relatively small number of ponds. Further reduction will not provide enough statistical power to detect differences. There is no feasible way to reduce the number of fish used in each pond because we want to apply the rotenone using the same methods as are used by state agencies so that we can do a valid assessment of its effectiveness. State agencies attempt to eliminate as many bass as possible with their treatments.** |

Replacement:

|  |
| --- |
| **There is no other taxon or approach that would be appropriate to replace the species requested in this protocol. Any use of other species or approaches would become irrelevant to the question, which is whether continued use of shoreline rotenone by state agencies to kill YOY largemouth bass is effective at achieving the stated purpose of reducing the density of YOY bass and improving growth and body condition of this species. No other model system or experimental approach could realistically mimic the complex suite of conditions and variation under which these treatments take place and which undoubtedly influences the effectiveness of the treatment in unknown ways.** |

Refinement:

|  |
| --- |
| **Refinement of methods is also not possible in this case. We are already applying the rotenone only in a small space along the perimeter of the pond and at a very specific time of year carefully calibrated to maximize the elimination of YOY largemouth bass while killing very few adult bass and bluegill and also minimizing the deaths of YOY bluegill as much as possible. Specifically we will treat after largemouth bass begin to use very shallow shoreline habitats but before most of the annual bluegill reproduction has occurred. We are also using small ponds (<10 acres) to evaluate shoreline rotenone which use more typically used by state agenceis at 50-100 acre lakes. Thus we have scaled down as much as possible already. Using any smaller a scale would require the use of an unnatural environment which would not provide relevant results for natural lakes.** |

1. If alternatives are available but will not be used, provide a justification.

|  |
| --- |
| **There are no alternatives to rotenone for large scale elimination of unwanted fish populations. Moreover, we have no alternative in our study because we are evaluating the effectiveness of shoreline rotenone application. Thus by definition we must use it. If we use some other product then our study would not be relevant for concluding whether it is an effective practice or whether it should be discontinued due to ineffectiveness.** |

1. If pain/distress category E is to be employed, provide a justification for withholding pain and/or distress relieving drugs.

|  |
| --- |
| **It is not feasible to provide pain/distress relieving drugs to the YOY bass and bluegill in this particular case. Products that could be used to relieve pain and distress such as ms-222 or clove oil are not labeled for large scale application in a natural pond environment for this purpose. These fish are extremely small and there will be large numbers of them and they live in a very complex natural habitat with many small spaces among and within patches of aquatic vegetation and woody debris in the pond. Thus these fish will not even be fully visible to be identified and removed from the pond because they will become stuck in these complex habitats and will not be accessible to sampling teams that may endeavor to capture them. Moreover, the rate of these fish succumbing will far exceed the maximum rate at which a crew could feasibly collect these fish before they succumb so that they could be euthanized. Thus it will not be possible to remove these tiny fish from the pond with any available fish sampling technique.**  **That said, we will euthanize large adult fish that not targets of the treatment but may inadvertently be killed by the rotenone. We anticipate that these fish will be few and thus will be feasible to collect and euthanize them.** |

1. SURGERY:

Will surgery be performed?

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Yes |  |  | No | **X** |

If yes, please address the following, as applicable:

1. Non-survival surgery - Describe all surgical procedures, including surgical preparation. Indicate where surgery will be performed (building and rooms). Identify the person(s) and DESCRIBE their qualifications for performing the particular surgical procedure(s).

|  |
| --- |
| **N/A** |

1. Survival surgery - Describe all surgical procedures, including surgical preparation. Indicate that aseptic technique will be followed if the procedure is a survival surgical procedure. Indicate where surgery will be performed (building and rooms). Identify the person(s) and describe their qualifications for performing the particular surgical procedure(s).

|  |
| --- |
| **N/A** |

1. Post-surgical Care - Describe POST-SURGICAL CARE including, who will be providing it (qualifications), what it will consist of, and where it will be provided (bldg., rooms).

|  |
| --- |
| **N/A** |

1. Administration of analgesics, anesthetics, tranquilizing drugs, and/or neuromuscular blocking agents (Indicate generic name, dose, route of administration and frequency; if by inhalation, method of scavenging waste anesthetic gases.)

|  |
| --- |
| **NA** |

1. A. Administration of reagents, cells, drugs (other than anesthetics or analgesics), infectious agents, carcinogens, recombinant DNA, etc. (Indicate generic name, dose, route of administration and frequency, anticipated side effects, monitoring protocol.)

|  |
| --- |
| **5% rotenone diluted in water to achieve an application rate of 1 pint per 100 yards of shoreline. It will be applied once per year in late May or early June around the entire pond perimeter. Anticipated side effects could be the unintended mortality of a few adult fish as indicated in section 7A above. We will visually monitor the shoreline areas for these fish and euthanize them if present.** |

If using cells, what is the source? Provide proper documentation to show that they are free from any infectious animal or human pathogens?

|  |
| --- |
| **N/A** |

1. If a non-pharmaceutical grade compound or chemical is being used, the following criteria must be addressed:
2. Provide a rationale for using less than pharmaceutical grade compounds. Cost savings alone do not adequately justify the use of non-pharmaceutical grade compounds in animals.

|  |
| --- |
| **N/A** |

1. Describe any expected side effects:

|  |
| --- |
| **N/A** |

1. Discuss the methods to be used to ensure sterility and storage of the drugs (e.g., sterile 0.22 micron filters, sterile diluent, storage in sterile vials, etc.):

|  |
| --- |
| **N/A** |

1. ASSURANCES:
2. Provide a brief statement to confirm that proposed activities involving animals do not duplicate previous experiments unnecessarily. If your protocol is a continuation of a previously approved project, include the PRN for the previous protocol and provide a brief statement summarizing previous work to justify study continuation.

|  |
| --- |
| **One gap in the pond fisheries research is thorough evaluation of the performance of shoreline rotenone to eliminate overly abundant young largemouth bass. Thus conducting this study will fill a gap in the literature.** |

1. My signature on page 1 of this form certifies that exercise of caged dogs will be accomplished according to the Animal Welfare Act (AWA) or cage size provides adequate space for exercise to meet AWA requirements. Alternatively, explain why an exception should be approved by the IACUC.

|  |
| --- |
|  |

1. Will wild caught or endangered animals be utilized?

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Yes | **X** |  | No |  |

If yes, the investigator is responsible for obtaining and maintaining valid permits (if required) for collecting, purchasing, transporting, and holding of these animals. List applicable federal and/or state permit numbers including expiration dates and attach copies of the permits to the protocol. Copies of active collection permits must be provided prior to protocol approval.

|  |
| --- |
| **Largemouth bass, and bluegill are common and abundant fish, and are not listed under any Federal or State protected species list. Fish collection permits are not required from the State of Alabama to sample private waters or university property.** |

1. HAZARDOUS AGENTS

Use of hazardous agents in animals may require approval of the appropriate institutional committee. Contact the Department of Risk Management and Safety (844-4870) for specific information.

Copies of an approval letter from the IBC along with the approved BUA must be provided prior to protocol approval.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Hazardous Agent | Yes | No | Agent | Date of Committee Approval & BUA # |
| Radioisotopes |  | **X** |  |  |
| Biological Agents |  | **X** |  |  |
| Hazardous Chemicals or Drugs | **X** |  | 5% rotenone | IBC approval not required |
| Recombinant DNA |  | **X** |  |  |
| Physical Agent (UV, Laser, Noise, Magnetic fields, etc.) |  | **X** |  |  |

Describe the practices and procedures required for the safe handling and disposal of contaminated animals and material associated with this study. Also describe methods for removal of hazardous waste and, if applicable, the monitoring of hazardous waste.

|  |
| --- |
| **Applicators will follow label directions and will wear protective gear including rubber gloves, goggles, suits, and masks as recommended in the American Fisheries Society SOP (Finlayson et al 2010). No contaminated animals or materials will be produced via rotenone application due to rapid breakdown in the presence of water and sunlight, as well as low environmental concentrations, low concentrations in affected fish, and lack of bioaccumulation (Finlayson et al 2010). No hazardous waste will be created or need to be disposed of.** |

1. What will be the disposition of the animals at the termination of the project? If euthanasia is to be performed, what will be the method of carcass disposal?

|  |
| --- |
| **Fish carcasses will be disposed of at the North Auburn Fisheries Station by composting per PRN 2012-2094, the Standard Operative Procedures protocol for the Auburn Fisheries Research Unit.** |

1. All protocols must include the method of euthanasia that will be used during the normal course of the protocol or in the event of unforeseen circumstances resulting from illness or injury. Please specify the method, agent, dose, and route of administration. The euthanasia method must be consistent with the *AVMA Guidelines for the Euthanasia of Animals: 2013 Edition* or justification for deviation should be indicated.

This document is available here: <https://www.avma.org/KB/Policies/Documents/euthanasia.pdf>

|  |
| --- |
| **Fish that will be sacrificed will be euthanized by immersion in a solution of 300 mg/L of MS-222 for at least 10 minutes post cessation of operculum movement. This follows the protocol listed in the AVMA Guidelines for the Euthanasia of Animals: 2013 Edition section (page 70).** |

**REQUIRED CHECKLIST: *MUST be completed by the PI and attached to the original protocol submission.***

**Documentation of all items identified in Items II – VII is required prior to protocol approval.**

|  |  |  |  |
| --- | --- | --- | --- |
| **PI:** | **Mathew J. Catalano** | **Department:** | **Fisheries** |

**Project Title:**

|  |
| --- |
| **Shoreline rotenone to reduce largemouth bass recruitment in Alabama ponds** |

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| I. Is this protocol a continuation of or similar to a project/ SOP / activity previously  approved by the IACUC? | | |  | Yes |  | No |
| **X** |  |
|  |  |
| If yes, include PRN of previous protocol. | PRNs: | **PRN 2017-3048** | | | | |

II. List all individuals (PI, co-PI, Lead Graduate Student, and Other Individuals listed in Question #4B) who

will conduct procedures involving animals on this protocol.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Project Personnel | CITI | OHS | Project Personnel | CITI | OHS |
| Dr. Matthew J. Catalano (PI) |  |  |  |  |  |
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| --- | --- |
|  | Additional Individuals are Listed on the Next Page. |

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| III. Will wild caught or endangered animals be utilized for this project? | **X** | Yes |  | No |
| If yes, are copies of active federal and/or state permits attached? |  | Yes | **X** | No |
| IV. Does the protocol involve Hazardous Agents or activities for which  IBC or other approvals are required? |  |  |  |  |
|  | Yes | **X** | No |
| If yes, are approval letters and BUA’s attached? |  | Yes |  | No |
| V. Does the protocol involve the use of privately owned animals? | **X** | Yes |  | No |
| If yes, is an owner consent form attached? | **X** | Yes |  | No |
| For CVM PIs, is the CRRC approval letter attached? |  | Yes | **X** | No |
| VI.Will animals used for this protocol be transferred to or from another institution? |  | Yes | **X** | No |
| If yes, is a copy of the institution’s IACUC attached? |  | Yes |  | No |
| VII.Does this protocol involve the use of Investigational New Animal Drugs (INAD)? |  | Yes | **X** | No |
| If yes, is the approved INAD attached? |  | Yes |  | No |

VIII. Please check all of the following that apply to this project:

|  |  |  |  |
| --- | --- | --- | --- |
|  | Food and/or Fluid Restriction |  | Multiple Survival Surgeries |
|  | Survival Surgery |  | Variation from Exercise/Enrichment |
|  | Prolonged Physical Restraint |  | Variation from Euthanasia Guidelines |
|  | Variation in Blood Volume Limits |  | ”E” Pain Category |
|  | Unexpected Outcomes |  | Use of Freund’s Complete Adjuvant |
|  | Genetically Modified Animals Used |  | Variation From Housing Guidelines |

INFORMED CONSENT

for a Research Study entitled

“Evaluating the effectiveness of shoreline rotenone application to reduce largemouth bass recruitment in small impoundments”

You are invited to allow your pond to be treated as part of a research study to evaluate the effectiveness of shoreline rotenone to reduce largemouth bass recruitment in small impoundments. The study is being conducted by Dr. Matthew J. Catalano, in the Auburn University School of Fisheries, Aquaculture, and Aquatic Sciences. You were selected as a possible participant because your pond fits criteria for inclusion in this study. The reason for the rotenone treatment is to reduce the density of baby bass in the pond, which may increase food availability thereby increasing bass growth and body condition (plumpness). The effectiveness of this treatment method has not been thoroughly studied, which underscores the need for our study and the importance of your pond in the study.

If you decide to participate in this research study, you will be asked to allow the investigators to apply the botanical piscicide rotenone along the shoreline of your pond on \_\_\_\_\_\_ \_\_\_\_\_\_ \_\_\_\_\_(year, month,day). Rotenone is a natural fish poison derived from the roots of several species of plants in the family Leguminosae (the bean/pea family) and will kill fish that encounter it. We will apply it at low concentrations in shallow water along the edge of the pond and thus the application method will kill baby bass and bluegill but have minimal impact on adult fish. We will also need to access your pond one day after rotenone treatment to pull a 15’X4’ net along the shoreline to assess reductions in baby bass (1-2 month old fish). We will also need to access your pond to conduct an electrofishing survey in spring to evaluate changes in the number of largemouth bass reaching age-1 the following spring. Upon completion of the sampling, we will provide you with a professionally done pond fish population report to communicate to you the status of the fish population in your pond (lengths, weights, body condition, and relative abundance) and also to report on any changes that we documented following the shoreline rotenone treatment.

Are there any risks to your pond? The risks associated with participating in this study are that it is possible that a small number of adult largemouth bass and bluegill will be inadvertently killed by the rotenone application. To minimize these risks, we will apply the rotenone very close to shore in shallow water and after bass reproduction has ceased but before substantial bluegill reproduction has begun. The low rates of rotenone that we will apply will create no risks for wildlife and/or humans in the area. Out of an abundance of caution, we recommend that the pond and surrounding shoreline be avoided the day of treatment and up to 48 hours after treatment to minimize contact with treated water and to not interfere with our follow-up fish netting activities.

Are there any benefits to your pond? If you participate in this study, your pond fish population may achieve a more desirable size structure consisting of lower densities of large bass that are of improved body condition and should be less susceptible to disease outbreaks. However, I cannot promise you that your pond will receive any or all of the benefits described. You will receive no compensation for participating but there will be no costs to you.

If you change your mind about participating, you can withdraw at any time during the study. Your participation is completely voluntary. If you choose to withdraw, your data can be withdrawn as well. Your decision about whether or not to participate or to stop participating will not jeopardize your future relations with Auburn University School of Fisheries.

Participant’s initials \_\_\_\_\_\_

Page 1 of 2

Your privacy will be protected. Any information obtained in connection with this study will remain anonymous (or confidential). If you have questions about this study, please ask them now or contact Dr. Matthew Catalano at 334-844-7366 or mjc0028@auburn.edu. A copy of this document will be given to you to keep.

If you have questions about your rights as a research participant, you may contact the Auburn University Office of Research Compliance (334)-844-5966.

HAVING READ THE INFORMATION PROVIDED, YOU MUST DECIDE WHETHER OR NOT YOU WISH TO PARTICIPATE IN THIS RESEARCH STUDY. YOUR SIGNATURE INDICATES YOUR WILLINGNESS TO PARTICIPATE.

\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Participant's signature Date Investigator obtaining consent Date

\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Printed Name Printed Name

\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Co-Investigator Date

\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Printed Name

Page 2 of 2

Additional space for listing individuals who will conduct procedures involving animals on this protocol:

|  |  |  |  |  |  |
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| Project Personnel | CITI | OHS | Project Personnel | CITI | OHS |
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**ADDITIONAL INFORMATION**

***THIS PAGE NEED NOT BE INCLUDED WHEN SUBMITTING FORM FOR REVIEW***

**Question 2B** USDA promulgated PAIN/DISTRESS CATEGORIES - Please use the following categories when categorizing the pain/distress level.

B Pain or Distress – None

Animals being bred, acclimatized, or held for use in teaching, testing, experiments, research or surgery BUT NOT YET USED for such purposes. Some examples would include:

* + - 1. Animals used in the Animal Production/Maintenance such as routine farm animal production operations and transgenic animal core facility breedinga.
      2. Animals being bred or housed without any research manipulation, prior to euthanasia or transfer to another protocol
      3. Animals used for demonstration purposes in teaching and outreach.
      4. Animals being held under an “administrative protocol” for reasons determined by the unit, project, or program veterinarian.
      5. Observation of animal behavior in the wild without manipulating the animal or its environment.

aThis does not include tail snips in mice used in genotyping. This is then a research use and puts it in higher pain/distress category.

C Pain or Distress - None or Minor

These include studies that DO NOT involve surgery; induction of painful or stressful disease conditions, or pain or distress in excess of that associated with routine injections or blood collection. Included are induction or transplantation of tumors in animals (as long as the tumors do not cause pain and the animals are terminated prior to becoming ill), administration of mildly toxic substances or pathogenic agents that cause no significant disease or distress, polyclonal antibody production (antigen inoculations and blood collection) as long as significant disease does not result, mild food restriction, and, typically, the collection of animals from the wild or from experimental units (i.e. fish in earthen ponds) for minor procedures. NOTE: If blood is to be collected via the retro-orbital or intracardiac methods, then anesthesia is required and Pain/Distress D must be selected. Also, if *in vivo* monoclonal antibody production is to be performed, the pain category D must be selected.

D Pain or Distress Relieved by Appropriate Measures

A major concern of the reviewers of these protocols is the degree of pain and/or distress imposed on the animals in the studies, and the methods the investigators will use to prevent, relieve, or minimize such pain or distress.

Following is a partial list of procedures known to involve significant pain and/or distress:

* + - 1. Surgical procedures such as biopsies, gonadectomy, exposure of blood vessels, chronic catheter implementation, laparotomy, or laparoscopy

2. Administration of any chemical or organism that would be expected to produce pains or distress but which will be alleviated by analgesics

3. Intracardiac or retro-orbital blood collections

4. Monoclonal antibody production (ascites method)

5. Other procedures which would be painful or distressful to the animal if performed without the benefit of anesthesia, analgesic, and/or tranquilization (e.g., exsanguination).

E Pain or Distress without Anestheia, Analgesia or Tranquilizers

If the nature of the study prohibits the use of pain and/or distress relieving drugs, or if unavoidable and unalleviated pain or distress will be produced, you must provide a written justification. (Include this in your response to Item 8, B, 5.) Such procedures include: direct stimulation of central nervous system pain tracts, nociceptor stimulation by physical or chemical means that cause severe pain (e.g., corneal abrasions), or any potentially painful procedure if performed without chemical relief of pain.

**Question 3** The IACUC is required to inspect animal housing areas and laboratories (at least twice per year) where animals are housed for 12 or more hours.

**Question 4** PERSONNEL QUALIFICATIONS:

Federal regulations require institutions to ensure that people caring for or using animals are qualified to do so through documented training or experience. This training is to include investigators, technical personnel, trainees, visiting investigators, and any other individuals who may perform animal husbandry, anesthesia, surgery, or other experimental manipulations involving animals.

**Question 7** Please use this procedure list for guidance in providing the necessary information. Please note that this is not meant to be an exhaustive list, but only a guide.

* **Body fluid sampling** (e.g. blood, cerebrospinal fluid, ascites, urine —describe method of collection, amount, frequency).
* **Antibody production** (indicate route of administration, volume administered per site, number of sites, adjuvant use and frequency, consideration of alternatives to Freund’s adjuvant, anticipated side effects, monitoring protocol).
* **Ascites method for monoclonal antibody production**. Auburn University requires adherence to the Office for Laboratory Animal Welfare (OLAW) policies concerning the production of monoclonal antibodies using the mouse ascites method. Please refer to the OLAW document <http://grants.nih.gov/grants/olaw/references/dc98-01.htm> Use of the ascites method requires justification as to why in vitro systems cannot be used.
* **Special diets** (describe any anticipated nutritional deficit or other health concerns).
* **Indwelling catheters or implants** (describe type, maintenance/monitoring protocol).
* **Restraint of an unanesthetized animal** other than that associated with brief routine procedures such as for the collection of blood (describe method, duration, frequency).
* **Tumor transplantation** (describe any anticipated functional deficit to the animal, monitoring protocol, endpoint).
* **Food or fluid restriction** (e.g. greater than that associated with pre-anesthetic procedures — describe, include justification and monitoring protocol.)
* **Special housing, equipment, animal care** (e.g. describe special caging, water, feed, waste disposal, etc.)
* **Experimental endpoint criteria** (list the criteria to be used to determine when euthanasia is to be performed. Death as an endpoint must always be scientifically justified.)
* **For experiments regarding new genotypes** that may result in unanticipated phenotypes, or other research involving unanticipated results, these results must be identified, interpreted, and reported to IACUC.

**Question 8A** The Animal Welfare Act (AWA) requires that the Principal Investigator (PI) consider alternatives and provide a written narrative of the sources consulted to determine whether or not alternatives exist to procedures which may cause pain or distress.

According to the Animal Welfare Information Center (AWIC) of the U.S. Department of Agriculture (USDA), an alternative to procedures that may cause more than momentary pain or distress to animals is any procedure which results in REDUCTION in number of animals used, REFINEMENT of techniques to alleviate such pain or distress, or REPLACEMENT of animals (e.g. with an insentient model such as might be accomplished through use of cell culture or computer simulation).

To explore a variety of resources for evaluating alternatives investigators may consult the following website: [http://www.aaalac.org/resources/links.cfm#alternatives](http://www.aaalac.org/resources/links.cfm%23alternatives)