INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE
COMPLETING AN APPLICATION

Policies & Procedures Reference #:	Section F.10 – F.13; G.2

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PROCEDURES FOR APPLYING FOR IACUC APPROVAL: STEP ONE (F.11)

Georgia Aquarium's IACUC utilizes fillable Word documents for its application to conduct research. Only the most current version of official Georgia Aquarium IACUC application documents will be accepted for review. These forms can be accessed by the Research & Conservation Team, the IACUC, or from Georgia Aquarium's website.

Prior to the completion of a research application, Principal Investigators must coordinate with Georgia Aquarium's Research & Conservation Department to discuss the feasibility of the proposed project by e-mailing the required information (as outlined in the previous module) to gairesearch@georgiaaquarum.org.

The Research & Conservation Department will determine if the project is aligned with Georgia Aquarium's Research Policy and will complete the following steps:

Projects and Partnerships Request Summary (Step 1A) (F.11.1)

The Senior Director of Research & Conservation will complete a **Projects and Partnerships Request Summary** in DocuSign, which will be routed to various departments within Georgia Aquarium, including Education, Development, and Finance. If the request is approved by these parties, the Senior Director of Research & Conservation will move on to the next step.

Animal Care Pre-Approval Codes (Step 1B) (F.11.2)

It should be noted that under federal regulation §2.31(d)(8), "proposed activities and proposed significant changes in ongoing activities that have been approved by the IACUC may be subject to further appropriate review and approval by officials". The Zoological Operations/Aquatic Sustainability and Animal Health teams have final determination of which animals participate in approved research and when such research is scheduled to take place, throughout the duration of a protocol's approval period.

To obtain this approval, the Senior Director of Research & Conservation will then assign a task to the Compliance Office planner board under the "Research Pre-Approvals" bucket and select the individuals who need to review the request, as outlined below:

- **Research & Conservation Director:** All submissions to the IACUC must have an approval code from this director and a GAQ "Projects and Partnerships Request Summary" on file.
- **Animal Care Director:** Any submission that includes observation of animals being housed at Georgia Aquarium.
- **Registrar:** Any submission that includes a request for animal or habitat records (not including wild populations).
- **Animal Heath Veterinarian or Manager:** Any submission that includes a request for archived samples from animals being housed and/or owned by Georgia Aquarium.

If an approver is unavailable for more than **one week**, the request can be elevated to a more senior official. Once all required pre-approval codes have been obtained, these must be included on the cover sheet of any application (new requests or amendments) to the IACUC. The IACUC will not review any protocols that require such review codes and does not have them at the time of submission.

In addition, the Senior Director of Research & Conservation will also help the Principal Investigator identify a Georgia Aquarium Champion, who must be listed on the protocol and is responsible for ensuring proper communication with Research & Conservation. This individual may or may not be the same as the Animal Care Point of Contact.

Selection and Completion of Forms (Step 1C) (F.11.3)

The Senior Director of Research & Conservation will then work with the requestor to complete the appropriate application form(s), based on the following categorization:

- **Level 1 Application Form**
  - Archived Sample or Data Request
  - Observational (B Pain Only)
  - Technical Expertise on External Projects
  - Animal Handling by GAQ Staff at Other Institutions (C Pain Only)
- **Level 2 Application Form**
  - Animal Handling by GAQ Staff, *In Situ* or Non-Institutional Settings
  - Animal Handling at Georgia Aquarium
  - Animal Handling by GAQ Staff at Other Institutions (D or E Pain Only)
  - Research Involving D or E Pain on GAQ-Owned Animals at Other Institutions

Instructions and information that may be helpful when completing an application form can be found under "Completing the Application Forms" in this document.

PROCEDURES FOR APPLYING FOR IACUC APPROVAL: STEP TWO (F.13)

Once all application materials have been prepared, they should be sent to iacuc@georgiaaquarium.org.

Submission Deadlines (F.13.1)

Level 1 application materials can be submitted at any time. However, all **Level 2** application materials must be submitted by the designated deadline, with consideration given to the timing of IACUC meetings, which are held quarterly. For instance, if a project is expected to begin in May, the Principal Investigator should plan to submit the application no later than January to allow for timely review.

It is strongly recommended that applications be submitted at least two review cycles in advance of the intended start date. This provides adequate time for the IACUC to request modifications, schedule additional meetings, or require presentations, helping to prevent delays in project initiation.

Official dates for submission can be found on Georgia Aquarium's website.

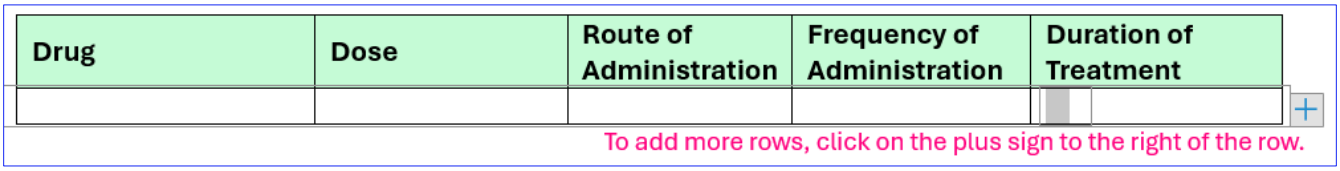
COMPLETING THE APPLICATION FORMS: GENERAL HELPFUL TIPS

Acronyms

To ensure clarity and accessibility for all readers, always spell out acronyms the first time they appear in a document, followed by the acronym in parentheses. For example: *Association of Zoos and Aquariums (AZA)*. This practice helps avoid confusion, especially for interdisciplinary teams or new staff who may not be familiar with specialized terminology. After the initial definition, you may use the acronym alone throughout the rest of the document.

Adding Rows to Tables

To add additional rows to the tables, click within the first row and you should see the "+" sign. Click on this icon as needed to add more rows:

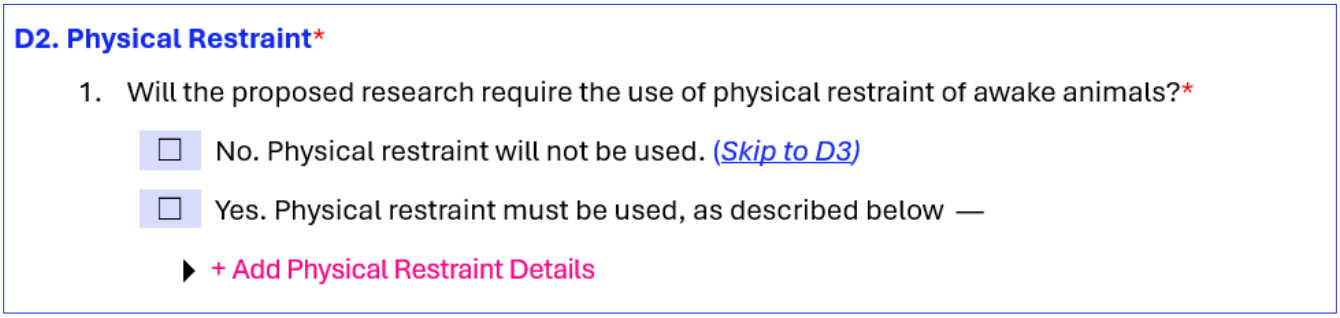


Details

Protocols should be written with enough detail that a reader can clearly visualize each step of the process. Avoid assuming familiarity with procedures that may seem routine within the zoological field (such as stimulus control). Reviewers often include members of the general public and non-scientific regulatory personnel, so clarity and completeness are essential. Every component of the methodology should be explained, including rationale, materials, timing, and contingency plans. If a step is skipped or modified, that decision should be documented and justified.

Expanding Sections

Throughout the applications there are collapsed sections. It's important that you work through the applications slowly so that you do not accidently miss a collapsed section. To expand these sections, click on the black arrow next to the section:



Scientific Language

Protocols are reviewed by individuals with varying levels of scientific training. Write with the assumption that your reader may not have a background in science, zoology, animal behavior, or veterinary medicine. Use plain language where possible, define technical terms, and avoid jargon unless it is clearly explained. This approach ensures transparency, facilitates regulatory compliance, and supports broader understanding of the work being proposed.

COMPLETING THE APPLICATION FORMS: LEVEL ONE

The following information should be used for clarification and as guidelines when completing a Georgia Aquarium IACUC **Level 1** application:

Cover Sheet

The cover sheet of the Level 1 or Level 2 applications serves as a place to record the Animal Care Pre-Approval Codes and to assure that the requestor has their supervisor's support (Georgia Aquarium staff only). When Georgia Aquarium staff complete this assurance section, they must agree to **copy their supervisor** on the submission of their application to the IACUC.

Section A. Administrative

Principal Investigator (A1)

The individual who will be serving as the **Principal Investigator** should be listed here. Careful consideration should be given to whom is put in this section, as this person will be held to the following expectations, as outlined in Section F.12.1 of the IACUC's Policies and Procedures (Full Document):

Serving as a Principal Investigator in partnership with Georgia Aquarium is a leadership role that carries significant responsibility. The Principal Investigator is not only the lead researcher but also the individual ultimately accountable for the ethical conduct, regulatory compliance, and operational integrity of the entire research protocol. This includes oversight of all personnel, procedures, and activities, regardless of whether the research involves animals, humans, or environmental components.

The Principal Investigator is responsible for ensuring that every aspect of the protocol is followed exactly as approved. All team members working under the protocol must be properly trained, informed, and supervised. Accurate records must be maintained, safety standards upheld, and all required approvals confirmed as current prior to initiating any work. If changes to the protocol are necessary, the Principal Investigator must submit amendments and obtain formal approval before implementation.

The Principal Investigator serves as the primary point of contact for institutional oversight bodies and external collaborators. This includes responding to inquiries, participating in Post-Approval Monitoring (PAM) audits, and reporting any adverse events, instances of noncompliance, or unexpected outcomes in a timely manner. The Principal Investigator is responsible for the conduct of all individuals listed on the protocol and is held accountable for any deviations from approved procedures.

Active engagement and ongoing awareness throughout the life of the project are essential. Lack of knowledge regarding activities conducted under the protocol does not exempt the Principal Investigator from responsibility. Full ownership of the research and the actions of the research team is a core expectation of this role.

In summary, the role of Principal Investigator requires diligence, integrity, and a comprehensive understanding of all responsibilities. It is a position of active leadership and accountability from initiation through project completion. The individual selected to serve as Principal Investigator must fully understand these obligations and be in a position to uphold them throughout the duration of the project.

Study Type (A2)

Select the category of research that best encompasses the most expansive part of your research. For example, if you are doing observational work on some animals and animal handling work on other animals, under the same protocol, you should select one of the animal handling categories. Different categories of research are captured on a Level 1 versus a Level 2 application. If you feel that the work you are doing does not fit into any of these categories, please contact iacuc@georgiaaquarium.org.

Emergency Veterinarian Contact (A3)

Follow the instructions on the application form to complete this section. One of Georgia Aquarium's veterinarians must be used when the location in which is being done is either at Georgia Aquarium or has no dedicated veterinarian on-site. For locations with established veterinarians, their contact information must be provided.

Animal Care Point of Contact (A4)

All projects taking place onsite involving animal use at Georgia Aquarium must identify a designated point of contact from one of Georgia Aquarium's animal care teams, as outlined in Section F.12.2 of the IACUC's Policies and Procedures (Full Document). This individual will serve as a liaison between the Research Team and Georgia Aquarium's IACUC, providing an additional layer of oversight and communication.

The animal care point of contact is responsible for:

- **Protocol Access and Understanding**: Maintaining access to and a thorough understanding of all details within the approved IACUC protocol(s), including amendments, conditions, and species-specific considerations.
- **Compliance Monitoring**: Ensuring that no animal-related work is conducted outside the scope of IACUC approval. Animals must not be involved in any activities until all necessary amendments have been submitted and approved.
- **Project Closure and Annual Reporting:** Communicating with the Principal Investigator to ensure that all protocol-related reporting requirements are met. This includes notifying the IACUC when work under the protocol has concluded and submitting the Annual Report to the IACUC by November 1st each year.
- **Communication Support**: Facilitating timely and accurate communication between the Research Team and the IACUC, particularly regarding animal care logistics, protocol adherence, and emergent welfare concerns.
- **Taking Photos and Videos**: When available, taking pictures or videos of the methodology set up or in action and sending it to the IACUC is helpful.

This role is intended to strengthen institutional oversight and support operational clarity. However, the Principal Investigator retains full responsibility for ensuring protocol compliance, ethical conduct of research, and direct communication with the IACUC. The presence of an animal care point of contact is a safeguard, not a substitute, for the Principal Investigator's obligations.

Renewal Protocol (A5)

Follow the instructions on the application form to complete this section.

Section B. Research Request Scope

Scientific and Species Justification (B1)

Follow the instructions on the application form to complete this section. See "Completing the Application Forms: Level Two" for more information about the IACUC's review criteria related to the potential value of a study, selection of animal species, and justification of animal numbers.

The only pain categories authorized under a Level 1 application include category B or C. Otherwise, all other requests under a Level 1 are for non-animal handling purposes.

Archived Samples Requested / Archived Sample Handling & Shipping (B2, B3)

An **Archived Sample Request** is a request for previously collected and stored biological specimens from animals or diet/prey, or newly collected and/or stored environmental samples. If you are requesting that new samples be taken from an animal, you must complete a Level 2 application. Do not forget to include the Shipping Details (B3), if you are requesting samples.

Archived Data Requested (B4)

An **Archived Data Request** is a request for access to and/or use of animal records, photos, videos, research data, or similar materials for purposes other than the direct care and management of Georgia Aquarium's animal collection. If you are requesting that new data be collected on an animal, you must complete a Level 1 (observational) or Level 2 application.

Data and Media End Use (B5)

This section must be completed for all project types. Discussions related to publication, authorship, and presentations will be initiated by the Senior Director of Research and Conservation during the preliminary stage of the application process and may result in the completion of a Data Sharing Agreement, Memorandum of Understanding, or other similar document (if deemed necessary).

Multi-Institution Collaboration (B6)

Research involving multi-institution collaboration should be identified under this section, as outlined in Section F.12.4 of the IACUC's Policies and Procedures (Full Document). In cases of research involving multi-institution collaboration, Georgia Aquarium will take responsibility for the training and oversight of its personnel. The IACUC will establish a Letter of Understanding with the primary institution who will be taking responsibility for all other research participants.

This Letter of Understanding may include, but is not limited to:

- Designate responsibility for reviewing and monitoring the protocol.
- Identify which institution's policies will apply to the researcher and the research.

- Establish guidelines for animal care and use activities that require cooperation and coordination among the institutions, such as animal transport.
- Establish guidelines for the sharing of information regarding the research, programs, as well as issues arising from the research.
- Establish guidelines for handling non-compliance and, specifically, whether the institutions will investigate separately or jointly.

Section C. Regulatory Compliance

Permits (C1)

Follow the instructions on the application form to complete this section. Copies of all applicable permits must be in-hand and provided to the IACUC as part of your application (except for Technical Expertise requests). The IACUC will not grant approval for a project in which copies of these permits are missing.

Terms and Conditions (C2)

A Level 1 or 2 IACUC application includes a range of terms and conditions covering investigator responsibilities, personnel oversight, animal welfare monitoring, emergency veterinary protocols, duplication avoidance, occupational health and safety participation, data sharing, public information release, reporting obligations, and administrative requirements such as amendments, annual updates, de novo reviews, and project completion. It also addresses publication and media approvals, recordkeeping standards, and compliance enforcement. It is essential that these terms and conditions are read carefully and fully abided by throughout the duration of the approved work to ensure regulatory compliance and uphold ethical standards in research.

Training Requirements: (F.12.7)

Georgia Aquarium is required by federal regulations to provide training for all personnel and researchers involved in the use and/or care of vertebrate animals in research, testing and teaching[2]. As Georgia Aquarium strives to exceed requirements and industry standards, training is required of personnel and researchers involved in the use and/or care of all animals, regardless of taxa. USDA regulations require that training be made available in the following areas:

- Humane methods of animal maintenance and experimentation, including the basic needs of each species of animal.
- Proper handling and care for the various species of each animal used by the facility and proper pre-procedural and post-procedural care of animals.
- Research and testing methods that minimize the number of animals required to obtain valid results and minimize animal distress.
- Proper use of anesthetics, analgesics, and tranquilizers for any species of animals used by the facility.
- Methods whereby deficiencies in animal care and treatment are reported, including deficiencies in animal care and treatment reported by any employee of the facility.

All parties interested in conducting research with Georgia Aquarium must be adequately trained to conduct the proposed work on their protocol(s)[1]. To ensure continuous education, all participants with a training requirement described below must complete **retraining every 5 years**. More frequent and/or additional training/re-training may be requested by the Committee at any time as deemed necessary.

Training completion is verified by the Committee for all personnel proposing to work with animals, at the time of protocol application, continuing review, and when new personnel, including students, are added to the protocol. Complementary training modules from other programs may be considered to satisfy Georgia Aquarium's training requirements. In addition, Principal Investigators are responsible for providing adequate and appropriate training to team members (students, co- Principal Investigators, lab techs, etc.).

Under **Level 1 Research**, the Principal Investigator assumes full responsibility to ensure that all personnel are appropriately qualified and trained to conduct activities under their protocol.

- Georgia Aquarium personnel are required to complete the Research Basics training (Georgia Aquarium Academy) as part of their employment training responsibilities and additional training may be required as deemed appropriate.
- All Principal Investigators must also complete CITI Program's "Working With the IACUC", regardless if they will be handling animals or not.

Sponsor (C3)

Any Level 1 or Level 2 application being submitted by a Principal Investigator that is not a university faculty member, degreed research scientist, or veterinarian must have a sponsor for their project. Sponsors must be a university faculty member, degreed research scientist, or veterinarian and will serve as an advisor to the Principal Investigator during the execution of their project. You can reach out to gairesearch@georgiaaquarium.org if you need assistance with obtaining a sponsor.

COMPLETING THE APPLICATION FORMS: LEVEL TWO

The following information should be used for clarification and as guidelines when completing a Georgia Aquarium IACUC **Level 2** application:

Cover Sheet and Section A. Administrative

See "Completing the Application Forms: Level One".

Section B. Research Justification

Scientific Justification (B1)

Follow the instructions on the application form to complete this section. Activities involving live animals must be designed and performed with the reasonable expectation that such use of animals will contribute to the enhancement of human or animal health, the advancement of knowledge, or the good of society (IACUC Policies and Procedures, G.2.1).

Question 2. When conducting research on marine mammals, all research must be conducted in spaces that are compliant with the minimum space requirements outlined in the Animal Welfare Regulations. If you need to use a space that is smaller than the minimum specifications, written justification must be provided and specifically approved by the IACUC.

Species Justification (B2)

Follow the instructions on the application form to complete this section. The animals selected should be of an appropriate species and quality with the minimum number required to obtain valid results[29]. Consideration must also be made to ensure that there are no restrictions on the use of species and/or specific animals that are under Breeding and/or Exhibit loan agreements from other institutions or state/federal permits (IACUC Policies and Procedures, G.2.2).

You must provide written justification for why each species has been selected to participate in this project. Examples of written justification statements include, but are not limited to:

- "We selected *Danio rerio* (zebrafish) due to its well-characterized genome, rapid development, and transparent embryos, which allow for non-invasive observation of physiological processes. These traits make it an ideal model for studying vertebrate development and toxicological responses."
- "The use of *Xenopus laevis* is justified because its large oocytes allow for multiple experimental replicates from a single individual, reducing the total number of animals required to achieve statistically valid results."
- "We selected *Octopus bimaculoides* for this behavioral cognition study due to its demonstrated problem-solving abilities and complex nervous system, which are not present in other invertebrate models."

Target Animal Numbers and Justification (B3)

Follow the instructions on the application form to complete this section. You must select a pain category for each species of animal listed. The listed information under B3 should be in the same order as that listed under B2. For example, if you have "*Tursiops truncatus*" on line 1 under B2, this should be line 1 under B3.

B2. Species Justification*

Complete the following table for **each** species being requested for use:

Species (Scientific Name)	Written Justification For Selection
Tursiops truncatus	Bottlenose dolphins were selected due to their well-characterized visual system and cooperative behavior in managed care, which allows for reliable, non-invasive ophthalmic data collection.
Delphinapterus leucas	Beluga whales were chosen for their unique ocular adaptations to low-light environments, offering critical comparative insight into cetacean eye structure and function across ecological contexts.

To add more rows, click on the plus sign to the right of the row.

B3. Target Animal Numbers and Justification*

Complete the following table for **each** species being requested for use, listed in the same order as above.

Age / Weight Range / Sex	# of Animals For 3 Years	USDA Pain Category	Written Justification For Number of Animals
8-16 / 250-500 pounds / Males and Females	5	Category C (Slight to No Pain Procedures)	This study will utilize the 5 individuals currently in the institution's available population.
4-10 / 500-1000 pounds/ Males and Females	6	Category C (Slight to No Pain Procedures)	This study will utilize the 6 individuals currently in the institution's available population.

To add more rows, click on the plus sign to the right of the row.

Justification of Animal Numbers

A proposal to conduct an activity involving animals must contain the following: (1) Identification of the species and the approximate number of animals to be used; (2) A rationale for involving animals, and for the appropriateness of the species and numbers of animals to be used[35]. If the justification of animal numbers is noted to be based on statistical analysis, the application must include a copy of the power analysis that was performed (IACUC Policies and Procedures, G.2.3).

You must provide written justification for why you are requesting the number of animals that you would like to use for this project. Examples of written justification statements include, but are not limited to:

- "A power analysis was conducted using GPower software to determine the minimum sample size required to detect a statistically significant difference in cortisol levels between treatment groups. Assuming an effect size of 0.5, alpha of 0.05, and power of 0.8, the analysis indicated that 12 animals per group are needed. This ensures scientific validity while minimizing animal use."
- "This study is exploratory in nature and aims to identify trends in behavioral responses to novel enrichment. A small cohort of 6 individuals was selected to minimize animal use while still allowing for meaningful observational data to inform future, more robust studies."
- "Only 8 animals are required because each individual will serve as its own control in a repeated measures design. This approach reduces inter-individual variability and allows for statistically valid comparisons with fewer subjects."
- The study will utilize 14 individuals of *Siganus vulpinus* currently housed in the institution's quarantine system. This number reflects the total available population that meets the study's inclusion criteria (e.g., age, health status, and housing conditions).

Pain Categories

For purpose of this section, the following definitions apply (G.2.5)(a):

- Distress: An aversive state in which an animal fails to cope or adjust to various stressors with which it is presented.
- Nociception: The detection of certain stimuli by sensory nerves and the transmission of signals to the central nervous system.
- Painful Procedure: One that would reasonably be expected to cause more than slight or momentary pain or distress in a human to which the procedure is applied.

When reviewing this section, the following considerations apply (G.2.5)(b):

- Procedures involving animals will avoid or minimize discomfort, distress, and pain to the animals, consistent with sound research design[2].
- Procedures that may cause more than momentary or slight pain or distress to the animals will be performed with appropriate sedation, analgesia, or anesthesia, unless the Principal Investigator justifies, in writing, the scientific reasons for the procedure[5].

- The Principal Investigator shall consult with the Internal Veterinarian or authorized designee in planning such use of animals[5].
- Paralytics are not used without anesthesia[5].
- Animals that would otherwise experience severe or chronic pain or distress that cannot be relieved will be humanely euthanized at the end of the procedure, or if appropriate, during the procedure[6].
- Medical care for animals will be available and provided as necessary by a qualified veterinarian[8].

To ensure ethical oversight and compliance with federal standards, the Georgia Aquarium IACUC applies USDA pain/distress categories to all animals, regardless of taxa. These categories guide the assessment and minimization of pain or distress in proposed protocols.

- Category B
  - Animals bred, conditioned, or held for future use in research, testing, teaching, or surgery, but not yet used in any such procedures.
  - Includes breeding animals and those in holding without experimental manipulation.

- Category C
  - Animals involved in procedures not expected to cause more than slight or momentary pain or distress.
  - Pain-relieving drugs are not required.
  - Examples include:
    - Routine blood collection or subcutaneous injections
    - Behavioral observations
    - Euthanasia performed prior to any potentially painful procedure
    - Sedation or anesthesia used solely for restraint during non-painful procedures
- Category D
  - Animals that may experience pain or distress but receive appropriate anesthetics, analgesics, or tranquilizers to minimize or eliminate discomfort.
  - Examples include:
    - Surgeries with anesthesia and postoperative pain management
    - Tumor removal under local or general anesthesia
    - Terminal procedures (e.g., exsanguination) conducted under deep anesthesia
    - Any procedure where pain is anticipated and effectively managed

- Category E
  - Animals subjected to procedures that may cause pain or distress, and for which pain-relieving agents are withheld because their use would interfere with scientific objectives.
  - Examples include:
    - Pain studies where analgesics would confound results
    - Lethal dose (LD50) studies without intervention
    - Conditioning experiments involving unavoidable noxious stimuli
  - Additional considerations:
    - Category E protocols receive heightened IACUC scrutiny to ensure no less painful alternatives or humane endpoints are available.
    - Approval is limited strictly to the procedures described, justified, and approved within the protocol.
    - Approval does not grant unrestricted authority to cause pain or distress.
    - Research teams must uphold professional animal welfare standards and promptly report any unanticipated pain or distress.

Section C. Research Request Scope

Detailed Animal Use Narrative (C1)

Follow the instructions on the application form to complete this section, making sure to incorporate the tips provided under "Completing the Application Forms: General Helpful Tips". The following information below may also be helpful to use when completing this section:

Pilot Study Policy (F.10)

Principal Investigators may be required to conduct a pilot study with a subset of their original requested animals to test their methods and or treatments, prior to receiving IACUC approval on the full, original protocol. Principal Investigators may also submit protocols whose scope only includes a pilot study where methods or treatments need refinement prior to expansion and/or species numbers cannot be justified with statistics[19].

Procedure Bank and Journal Referencing (F.12.3)

When completing a protocol that describes a commonly used procedure, the Principal Investigators may reference one of the IACUC's approved procedure descriptions from its Procedure Bank rather than typing out the entire procedure within the protocol itself.

The Principal Investigator must note that the procedure follows that of an IACUC Banked Procedure and note the procedure number[10].

The Principal Investigator may also cite peer-reviewed research articles which thoroughly describe methodology of procedures that will be used for their research, rather than typing out the entire procedure within the protocol itself. The Principal Investigator must appropriately cite the research article and provide a copy.

Industry Standard Procedures (G.2.13)

Proposed activities on protected species must follow all applicable research technique guidelines (e.g., NOAA's Sea Turtle Research Techniques Manual). In addition, animal care and welfare standards established by the Association of Zoos and Aquariums (AZA) and the Alliance of Marine Mammal Parks and Aquariums (AMMPA) must serve as the model for industry best practices where applicable. For species not covered under federal regulations, Georgia Aquarium applies internal welfare standards through its **NON-USDA REGULATED POLICY**, which outlines expectations for the care and welfare of these animals, ensuring ethical practices even in the absence of federally mandated oversight.

Specimen Collection (C2)

If you are requesting sample collection, please follow the instructions on the application form to complete this section. You are encouraged to provide a range for the number of samples needed, from minimum to maximum, to allow flexibility. Specifying an exact number may lead to protocol non-compliance in dynamic research environments where adjustments are often necessary.

Animal Identification (C3)

Follow the instructions on the application form to complete this section.

Archived Samples or Data (C4 to C8)

See "Completing the Application Forms: Level One".

Schematics, Diagrams, Photos and/or Videos (C9)

Applicants are strongly encouraged to include schematics, diagrams, photos, and/or videos of all research components, such as equipment, devices, and placement within enclosures or facilities. While not required, these visuals significantly aid IACUC review, support USDA inspections, and may be requested later during protocol evaluation or site visits. Providing them upfront can streamline the review process and reduce follow-up questions.

Section D. Special Use of Animals

Controlled Substances (D1)

Follow the instructions on the application form to complete this section.

Physical Restraint (D2)

Follow the instructions on the application form to complete this section. Physical restraint must be limited to the least restrictive method and shortest duration necessary to achieve approved research or veterinary objectives. Prolonged restraint is only permitted when scientifically justified and approved by the IACUC (IACUC Policies and Procedures, G.2.7).

Requirements:

- Restraint devices are not considered standard housing and must not be used for convenience.
- Animals must be trained, when feasible, to voluntarily participate in procedures or to acclimate to restraint equipment.
- Animals that do not adapt to restraint must be removed from the procedure or study.
- Animals under restraint must be monitored at intervals appropriate to the species and procedure.
- Veterinary care must be provided if injury, illness, or behavioral distress is observed. These conditions may require removal from restraint.
- The purpose and duration of restraint must be clearly documented and communicated to all involved personnel.

Examples of prolong restraint may include:

- Using a stretcher to gently restrain a dolphin during voluntary participation in a metabolic or physiological study.
- Holding a small elasmobranch (e.g., bamboo shark or cownose ray) in a padded trough for non-invasive tagging or sampling.
- Using a soft wrap or towel to restrain a penguin during feather sampling or microchipping.
- Briefly isolating a fish in a clear acrylic tube for behavioral observation or biometric measurements.

Secondary Research (D3)

This section must be completed if any research activities not covered under this IACUC protocol will occur concurrently with the work described in this protocol. Examples include procedures conducted under NOAA permits or other regulatory frameworks. Completion of this section does **not** authorize the simultaneous execution of multiple IACUC protocols. Such actions are strongly discouraged, as they may unintentionally result in non-compliance with the approved protocol if not explicitly described in the methodologies. Please provide sufficient detail to clarify how secondary research will be separated or integrated without compromising compliance.

Alternative Husbandry Requirements (D4)

Follow the instructions on the application form to complete this section. Living conditions of animals are appropriate for their species and contribute to their health and comfort. Living conditions that are different from how animals are typically managed can be requested with justification. However, no living conditions will be approved that are in violation of the Animal Welfare Regulations (IACUC Policies and Procedures, G.2.8).

Section E. Research with D or E Pain

Literature Search for Alternatives (E1)

Follow the instructions on the application form to complete this section. In accordance with 9 CFR §2.31, all Principal Investigators must consider alternatives to procedures that may cause more than momentary or slight pain or distress to animals(IACUC Policies and Procedures, G.2.6).

This includes:

- Evaluating whether non-animal methods (e.g., computer models, in vitro systems) can be used in place of live animals (Replacement).
- Designing studies to use the minimum number of animals necessary to achieve valid results (Reduction).
- Modifying procedures to minimize pain, distress, or lasting harm (Refinement).

A written narrative must be included in the IACUC application describing the methods and sources used to determine that no suitable alternatives are available. Investigators must also justify the use of animals, explain why non-animal models are insufficient, and provide a rationale for the species selected.

The IACUC will not approve protocols involving potentially painful or distressing procedures without adequate documentation of this review. Investigators are encouraged to consult with the Attending Veterinarian or IACUC staff for guidance during protocol development.

Survival Surgical Procedures (E2 to E10)

Follow the instructions on the application form to complete this section. For purpose of this section, the following definitions apply (G.2.10)(a):

- Survival Surgery: Survival surgery refers to any surgical procedure in which an animal regains consciousness after anesthesia and is expected to recover and survive the intervention.
- Non-Survival Surgery: Non-survival surgery refers to any surgical procedure in which an animal is euthanized while still under anesthesia and does not regain consciousness. This type of surgery may be performed on a wide range of taxa, including both vertebrates and invertebrates, and may take place in laboratory, clinical, or field settings.
- Major Surgery: Major surgery refers to any surgical procedure that involves significant physical intervention, such as penetrating and exposing a body cavity (for example, the thoracic, abdominal, or cranial cavities), or that results in substantial physical or physiological impairment. Examples of major surgery include procedures like laparotomy, thoracotomy, craniotomy, limb amputation, and organ removal. In invertebrates or smaller-bodied animals, surgeries that disrupt major organ systems, significantly impair mobility or function, or involve prolonged recovery may also be considered major.

- Minor Surgery: Minor surgery refers to surgical procedures that are less invasive and do not involve entry into major body cavities or cause significant physical or physiological impairment. These procedures typically involve minimal tissue disruption, have a low risk of complications, and often require shorter recovery times. Examples of minor surgery include the implantation of subcutaneous devices such as osmotic pumps or telemetry units, placement of indwelling catheters, superficial wound closures, and small biopsies. In invertebrates or small-bodied animals, procedures that do not disrupt major organ systems or significantly impair function may also be considered minor.

Requirements: (G.2.10)(b)

- Aseptic technique is the standard for minimizing infection risk after surgery.
- In laboratories or veterinary clinics, full aseptic technique is required, including sterilized instruments, gloves, and a sterile surgical field [10].
- In field settings, where sterile conditions may be limited, researchers must use clean and disinfected instruments as much as possible [10].
- The surgical site should be prepared by removing hair or feathers and disinfecting with antiseptics such as chlorhexidine or iodine.
- Hands and gloves should be kept clean, and contamination should be minimized during the procedure.
- For non-survival surgeries, full sterility is not required, but basic hygiene must be maintained.
- At minimum, wear gloves, prepare the surgical site, and use clean instruments and a clean work area.
- In the field, use the cleanest materials available and take reasonable steps to reduce contamination.
- Animals should not undergo more than one major survival surgery unless scientifically justified in writing by the Principal Investigator or required for veterinary care [10, 11, 37].
- All survival surgeries must use aseptic procedures, including sterile gloves, masks, instruments, and techniques [10, 37].
- Major surgeries (excluding birds) must be performed in dedicated, aseptic facilities [10].
- Surgeries on birds must still follow aseptic procedures [10].
- Field surgeries do not require dedicated facilities but must still use aseptic technique [10].
- Multiple major survival surgeries on one animal are discouraged and must be scientifically justified and approved by the IACUC.
- Cost savings alone is not a valid reason for performing multiple major surgeries.
- Animals cannot be passed between investigators solely to reduce costs.

- Use of an animal in more than one protocol involving major survival surgery requires USDA approval for regulated species.
- The Institutional Official must submit the request to the USDA Animal Care Regional Director.
- Provide appropriate postoperative care after survival surgeries [10].
- Monitor animals to ensure proper recovery.
- Assume a procedure is painful for an animal if it would be painful for a human, unless there is evidence to the contrary.
- Provide pain relief unless the IACUC has approved withholding it.
- Discuss the analgesic agent, dose, route, frequency, and duration with a veterinarian, ideally during the planning phase.

Section F. In Situ (Wild Populations) Research

Follow the instructions on the application form to complete this section.

Section G. Research Completion

Endpoints (G1)

Follow the instructions on the application form to complete this section. For purpose of this section, the following definitions apply (G.2.12)(a):

- Experimental Endpoint: Occurs when the scientific aims and objectives have been reached.
- Humane Endpoint: The point at which pain or distress is prevented, terminated or relieved in an experimental animal.

Euthanasia Plan (G2)

Any projects that involve animal handling **must** complete this section, even if euthanasia is not planned. Follow the instructions on the application form to complete this section.

Requirements: (G.2.11)

- Methods must follow the AVMA Guidelines for the Euthanasia of Animals (2020), unless a scientifically justified deviation is approved in writing by the Principal Investigator. [12, 16 & 33]
- The goal of euthanasia is rapid and humane death, beginning with loss of consciousness, followed by cardiac or respiratory arrest, and ultimately, loss of brain function.
- Animal handling and euthanasia methods must minimize distress prior to unconsciousness.
- Personnel must be trained and competent in the selected euthanasia method before performing the procedure.

- Euthanasia methods are categorized as:
  - Pharmacologic: Use of drugs to induce unconsciousness and death.
  - Physical: Use of trauma or blood loss (e.g., cervical dislocation, decapitation, captive bolt, exsanguination).
- AVMA classifications include:
  - Acceptable: Consistently humane when used alone.
  - Acceptable with Conditions: Require specific conditions or secondary methods to ensure humane death.
  - Unacceptable: Inhumane under any condition (e.g., strychnine, nicotine, pesticides, solvents).
- **Adjunctive methods** (e.g., potassium chloride) must not be used alone and are only appropriate when the animal is deeply anesthetized or in combination with an approved method.

Final Disposition Plan (G3)

You must select how animals will be dispositioned following the conclusion of research. Options to chose from include:

Transfer to Another Protocol (G.2.12)(b)

As all animals under the care of Georgia Aquarium are held for exhibition purposes, with the addition of research opportunities, the preferred and most likely alternative endpoint will be for animals to stay within the exhibit collection and be available for future research protocols.

Transfer to Another Facility (G.2.12)(c)

Another alternative endpoint for animals that can no longer be returned to exhibit or otherwise held at Georgia Aquarium includes being transferred to another partner facility, in accordance with all applicable laws and accreditation requirements.

Release to Wild (Field Studies) (G.2.12)(d)

Upon completion of sample collection or completion of the protocol, the animal is released back to the wild (for Field Studies).

Section H. Animal Handling Personnel

Multi-Institution Collaboration (H1)

See “Completing the Application Forms: Level One”.

Research Participation List (H2)

Who Needs to Be Listed:

All personnel who will be handling the animals, their parts, or their samples must be listed. Whereby, "animal handling" includes any research activity involving physical contact, behavioral manipulation, or environmental interference with animals. This includes, but is not limited to, capturing, restraining, sampling, tagging, conditioning, or applying stimuli that influence the animal's behavior, movement, or physiology (F.12.5).

Exemption: If the project includes multi-institutional collaboration, only Georgia Aquarium personnel need to be accounted for under this section.

Section(s) to Complete:

There are two sub-sections under H2. One is for Georgia Aquarium personnel, in which you must select any team(s) that will be assisting with the animal handling for a project. These personnel do not have to be listed individually, unless they are on a team that is not already captured on the application.

The IACUC will cross-reference the list of personnel currently on those team(s) and their training(s) and will provide a list with any approval letter of individuals who are **not** authorized to assist with the project.

The second section is for any other personnel who will be assisting with animal handling under the project to be listed. The "Qualifications Exemption" box will tell you which people on that list need to complete a Level 2 Personnel Qualifications Form.

Research Participant Qualifications (H3)

Level 2 Personnel Qualifications Form:

All non-exempt individuals (i.e., Georgia Aquarium Personnel Acting Outside Position Scope or individuals from another U.S. institution), that are listed on the Research Participation List, must complete a protocol-specific **Level 2 Personnel Qualifications form** and include them with a Level 2 Application Form.

The following individuals are considered exempt from this section, unless they are serving as the Principal Investigator: Georgia Aquarium personnel acting within their employment position scope, international colleagues, or Native Peoples.

All individuals completing the Level 2 Personnel Qualifications form are required to read the Terms and Conditions in full and ensure they understand the expectations associated with participating in research activities. By signing this form, participants acknowledge that they will be held accountable to the Terms and Conditions outlined.

The Principal Investigator holds ultimate responsibility for ensuring that all personnel involved in the project are familiar with the approved protocol, as well as all applicable policies, procedures, and regulatory requirements governing the work. It is the responsibility of each participant to seek clarification if any aspect of the protocol or expectations is unclear prior to engaging in research activities.

Training Requirements: (F.12.6)

See "Completing the Application Forms: Level One" for general information on training requirements.

Under **Level 2 Research,** the IACUC requires that the following training modules be completed[3]:

Research Group	Required Training or Forms
Animal Handling - Georgia Aquarium Personnel	1. Working with the IACUC 2. Species Appropriate Module(s)* 3. Research Basics **(GAQ Academy)** 4. Wildlife Research **(for *In Situ* projects)**
Animal Handling -All Other Researchers	1. Working with the IACUC 2. Species Appropriate Module(s)* 3. Wildlife Research **(for *In Situ* projects)**
No Animal Handling – Principal Investigators	1. Working with the IACUC
Any Proposal – International Colleagues or Native Peoples	Exempt unless serving as Principal Investigator – Approved for Conditional Animal Handling Only

* "Species Appropriate Module(s)" include, but are not limited to, Working with Fish in a Research Setting, Working with Amphibians in a Research Setting, Working with Reptiles in a Research Setting, and Working with Birds in a Research Setting. For species appropriate training for mammals or invertebrates, please contact the IACUC.

Animal Welfare Monitor (H4)

Follow the instructions on the application form to complete this section.

Section I. Regulatory Compliance

Permits (I1)

See "Completing the Application Forms: Level One".

Internship Project (I2)

Follow the instructions on the application form to complete this section.

Terms and Conditions (I3)

See "Completing the Application Forms: Level One".

Sponsor (I4)

See "Completing the Application Forms: Level One".