UNIVERSITY OF WINDSOR ANIMAL CARE COMMITTEE

ANIMAL UTILIZATION PROJECT PROPOSAL

All research and/or teaching projects conducted at the University of Windsor with live non-human vertebrate animals must be covered by a corresponding Animal Utilization Project Proposal (AUPP) which is approved by the Animal Care Committee (ACC) **prior to** the acquisition of any animals for the project. Approval of an AUPP indicates that the ACC is satisfied that humane practices and proper animal care standards will be used, in accordance with the requirements of the Canadian Council on Animal Care (CCAC) and the *Ontario Animals for Research Act*.

Notes to the Applicant:

1. Send **one electronic copy with all electronic signatures and dates** to the Animal Care Coordinator at acc@uwindsor.ca
2. Any changes in experimental procedures must be reported to the ACC by submitting a Request to Revise.
3. AUPPs are valid for **four years** from the date of approval. Ongoing research projects or teaching projects **must be reviewed annually** but they may be **renewed for four consecutive years** by using the Progress Report form.

Today's Date: 21/04/2025 AUPP # 21-05

Title of Research Project/Course: Ocean Tracking Network – Arctic: Baffin Bay, Svalbard and Norway

Principal Investigator/Instructor: Nigel Hussey **Department:** Integrative Biology

Research Project/Course Status: ☐New Project/Course ☒Renewal of AUPP #: 21-05

Anticipated Start Date: June 1st 2025 Anticipated Completion Date: December 1st 2029

Emergency Contact Person: Nigel Hussey After Hours Phone Number: 519 990-5497

Email: ne

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A. ANIMAL HANDLING EXPERIENCE AND RELATED TRAINING

A.1. Briefly describe the qualifications and experience of personnel concerning the procedures they will perform on animals. If personnel will not have direct contact with the animals, please describe their role in this protocol.

Nigel Hussey has extensive experience handling live animals for the application of telemetry and marker tags (internal and external), working on large fish in the Arctic, Red Sea, Mozambique, Ascension Island, the Chagos Islands, and off the coast of South Africa over the last 20 years. Nigel completed the University of Manitoba animal care course and a specifically designed course for animal handling and surgical procedures. He has also worked alongside leading people in the field of animal telemetry, for example Dr. Samuel Gruber.

Student researchers and all co-investigators have completed the University of Windsor Animal Care Committee training. All the listed team members have experience handling live animals for the application of tags in the Arctic. For new students/staff joining the cruise – surgical implantation training will be conducted on bycatch/fish that are used for dissection and sampling. Students will receive training in all surgical protocols detailed below on dead fish and observed by a senior team member with extensive experience (e.g. Hussey, Hedges, Madigan, Barkley, Ste Marie, Hollins, Davidson, Stamp). Number of repetitions will depend on individual performance (minimum of 10). Following this and once the senior person deems required level of competence (correct and precise incision, correct suturing and time to suture is reasonable) – the person will be closely monitored during first surgeries on live fish for release. Our team will closely observe and advice during the first 20 surgeries. Following that point, observation of surgeries by all team are observed by others to ensure high standards. For trainees, if after 20 fish, continued close observation (i.e. one to one) is required – this will continue until both trainee and senior person deem they are of the appropriate standard.

Application of satellite tags/biologgers to sharks and crabs will only be conducted by senior personnel with extensive experience of these procedures in the Polar/temperate/tropical climates (Hussey, Madigan, Hedges, Barkley, Ste Marie, Davidson, Stamp).

A.1.a. In addition to mandatory training, have personnel performed all procedures described in the AUPP previously? If yes, please indicate how recently. If no, please indicate how the individual will be trained and how competency will be assessed. <u>Competency must be addressed</u> before the commencement of

any procedure. Please refer to the guidelines in SOP# TR01 Measuring Competency of Animal Care Users and Staff.

Yes. All personnel have completed procedures listed in the AUPP. Last active survey work was August 2024

A.2. What is the chain of command for reporting observations to supervisors?

All student researchers will work directly with co-investigators, and report directly to PI Hussey

B. RESEARCH PROJECTS/COURSE INFORMATION

B.1. Purpose of Project: ☒ Research ☐Pilot Study ☐ Other ☐ Teaching **Course Number:** Enter text.

B.2. Level of Research Project/Course: ☒Faculty ☒ Post-doctoral ☒ Graduate ☒ Undergraduate

B.3. Does this work involve field work and wild animals? ☒**Yes** ☐**No**

If **yes**, you will need to complete all applicable sections of this form and the Fieldwork Form (can be under Forms on our webpage: https://www.uwindsor.ca/animal-care-committee/forms

C. SUMMARY OF EXPERIMENTAL PROCEDURES

Describe concisely (in 250 words or less), as if you are explaining your work to a group of Grade 9 students or for a media release, the experimental procedures of the project, including *the rationale and/or the objectives, the anticipated potential benefits to scientific knowledge, or to human and/or animal health and the procedures*.

Fish stocks have been vastly depleted worldwide, with many species driven to commercial extinction. The global Ocean Tracking Network (OTN Global), aims to revolutionize the way oceans are observed and understood, and thereby contribute to more sustainable use of ocean resources. This project specifically focuses on the development of sustainable Arctic fisheries through a tiered approach; whereby commercially and ecologically important species are tracked to determine movements relative to management boundaries and spatio-temporal interactions that may increase bycatch. The next level focuses on predator-prey interactions through food-webs to determine ecological structures in the changing environment of a warming Arctic. Lastly, the foundational level of this project involves oceanographic monitoring to relate the changing and dynamic ocean state to the species interactions and distributions seen above. Studies will primarily focus on the movement of Greenland halibut, a commercial species in the Arctic, and the primary bycatch species in this industry, the Greenland shark and Arctic skate. To understand food web interactions, we will sample a broad array of species that span all trophic levels from zooplankton to predators. Tracking will be undertaken through the use of external marker tags for survival and fishery harvest rates, acoustic tags for observations of long term (multiyear) movements in coastal and offshore regions, biologgers to derive extremely high resolution behavioural data combined with satellite tags for survivorship studies and long distance movements and habitat selectivity. Fish will primarily be captured using long-lines from a fishing vessel, and bycatch species that are not targeted for movement studies will be euthanized and sampled for food-web analysis through the collection of muscle, liver, fin and blood. This research will create the foundation to

developing sustainable fisheries on a global scale with the focus of ecosystem level integration in management.

Keywords: Provide 6 to 8 keywords to identify this project.
***(Refer to Appendix A, below, after the Signing Page, for Keywords)*.**

Research, field work, arctic, long-line, acoustic tagging, minor surgery, satellite tagging, biologging

D. DESCRIPTION OF ENDPOINT MONITORING PROCEDURES

The CCAC guidelines recommend the use of endpoints. Endpoint is the point at which an experimental animal's pain and/or distress is terminated, minimized, or reduced, by taking actions such as killing the animal humanely, terminating a painful procedure, or giving treatment to relieve pain and/or distress. Death of an animal is not an acceptable endpoint. Since the endpoint for each animal may vary depending on the treatments given to the animal, all animal care personnel should consult the appropriate ACC-approved SOP endpoint document when working with animals. (Please refer to ***ACC SOP AH25A Endpoint for Aquatics, AH26A Endpoint for Mouse, AH26B Endpoint for Rat or AH34 Endpoint for Avian****)*

D.1. Indicate who is responsible for monitoring the condition of the animals, the frequency of the monitoring, and how this frequency was determined. *(Note: All non-surgical animals must be observed at least once per day; monitoring must be initially more frequent in post-surgical animals).*

This is primarily a field study, where participants present in the field will be responsible for monitoring the animals. However, there will always be a highly experienced researcher in the field, either Hussey, Hedges, Lowen, Barkley or Stamp who all have more than 5 years of field experience working with these animals. Participants conducting field-work will evaluate field capture techniques as they are being implemented to minimize by-catch and pain and suffering. Following surgery, fish will be monitored in an holding tank, with constant water flow and high aeration, for a 15-minute period to ensure they have adequately recovered before release. This 15-minute period was chosen as it allows sufficient time for the fish to regain equilibrium in the holding tank, while minimizing the stress induced by holding the fish on the boat for long periods of time.

D.2. What are the "indicators" of pain and/or distress that are evident as a result of the experimental manipulation(s) (i.e., clinical conditions, behavioural changes, and abnormalities)? How were these indicators chosen? Could a pilot study help you to determine this?

Internal acoustic tags:

Information on endpoints, as well as evidence of pain and distress for tag insertion and surgical procedures are detailed below. In general, fish will be held before and after the tag insertion and will be monitored closely for signs of stress. The signs of stress include: non-reactive behaviour, rolling onto its side or back, or bleeding. These stressors were chosen because the majority of fish will be held for a very short time (<1-hour total) thus, physical changes in the fish, such as colour changes, may not be apparent.

External attachment of satellite tags:

Excessive bleeding from the attachment site, failure of the fish to orient properly with respect to the tag and "flashing" of the fish against the bottom or sides of the holding tank indicating irritation caused by the tag. Any of these indicators will result in removal of the tag and euthanasia of the fish. For crustaceans, an indicator is when animals do not 'stand' upright in the water along with minimal or lack of movement of the mouthparts.

Other indicators that may be apparent upon capture and/or release of the fish or crustaceans include:
Inability to attain equilibrium or ability to swim upon release.
Inability to initiate an avoidance response to the animal handler at release.
Ensure that gills are not damaged (no bleeding) during the capture
Ensure that scales are intact at capture

These indicators were chosen as common markers of pain and distress in fish. Our work regularly records fish condition and survival upon release which enable us to continuously refine techniques.

D.3. What procedures will be used to treat any adverse effects and/or complications?

Fish/crustaceans that show poor recovery post-surgery/handling will be held for an additional 15 minutes with constant water flow and high aeration to allow extra time for recovery. Any adverse effects and/or complications such as failure to recover within a reasonable time (30 + minutes) will result in euthanasia.

D.4. Specify what health condition(s) or other criteria would trigger a decision to humanely euthanize an animal. If this is necessary, when might this occur during the experiment?

Fish/crustaceans that become non-reactive will be euthanized and used for the other component of the project (chemical, stable isotope/fatty acid analysis of tissues). Target animals and non-target animals exhibiting signs of moderate to severe damage during capture will be euthanized. Moderate and severe damage includes skin ulceration, fin tearing, gill bleeding, deep lacerations and deformations resulting from suspected spinal cord damage. This will most likely occur during the capture process, if the longline hook is located in a poor position for easy removal (such as being swallowed or hooked on the gills), or if the fish has experienced predation while hooked. It is also possible that the fish may become un-responsive post-surgery in which case it will be euthanized and the tag removed.

D.5. Animals that die unexpectedly or are euthanized may be submitted for post-mortem examination by the ACC veterinarian. Are there any special instructions for sample collection?

☒**Yes** ☐**No**

If yes, explain.

Fish and crustaceans are collected on a boat in the Arctic, if a post-mortem is required we will need to be informed in advance to bring samples back to the University.

D.6.1. Specify what health condition(s) or other criteria would trigger the decision to terminate the experiment.

Capture of endangered or excessive non-target species will result in moving the sampling location to a different part of the study areas. If excessive non-target captures persist at alternate locations the study will be terminated at that site. High levels of bycatch are not anticipated in the study, however in the event that bycatch exceeds 20% of the total catch by number, the decision will be made to relocate the fishing activities. Continued bycatch in excess of 20% of total catch in all attempted fishing areas will result in the termination of the study.

Both trawl and longline capture techniques have been successfully used in the past to capture live, healthy fish for tagging. However, in the event that a specific capture technique results in the majority of the fish (> 20 %) coming to the surface in poor condition and unsuitable for tagging (as described above), alternative fishing techniques will be explored. If no other technique is capable of capturing healthy animals, the study will be terminated.

D.6.2. What is the expected baseline mortality of each species/strain during the duration of the protocol? Please provide an explanation. Note: The baseline mortality includes animals euthanized due to adverse health issues, mortalities inherent during standard breeding of animals, strain-specific deaths, and mortalities due to infectious diseases. Any excess mortalities that occur within a rolling 7-day period beyond this mortality may result in a Reportable Animal Welfare Incident (RAWI). For guidance in establishing species-specific baselines please refer to SOP AD22 and consult with the university Veterinarian and/or the ACC to establish baseline mortalities.

Nigel Hussey has been conducting tagging studies on the species listed within several AUPP since 2010. Expected mortality of each species is expected to be extremely low (<5%), and will not exceed that experienced by these species under natural conditions e.g. predation or human/fishing pressure.

E. EXPERIMENTAL DESIGN

Describe in sequence all experimental procedures to be done on the animals, including surgical procedures, behavioural manipulations, physiological assessments, restraint procedures (type and duration), etc. Procedures that could or will potentially impact the well-being of the animal(s) must be clearly outlined. The use of a flow chart is encouraged. Please attach additional pages if necessary.

As part of this fieldwork, there are three possible procedures that will be conducted on each animal: (1) fish/crustaceans captured and euthanized for chemical tracer and stomach content work; (2) fish/crustaceans captured and equipped with an external marker tag, an internal acoustic tag and/or external satellite tag or biologging package and (3) respirometry studies. Further methodological details provided in F1.1.

THE THREE R'S- REPLACEMENT, REDUCTION, REFINEMENT

REPLACEMENT ALTERNATIVES TO ANIMAL USE

Replacement refers to methods that avoid or replace the use of sentient animals in a study where they would otherwise have been used. This includes both absolute replacements (i.e., replacing animals with non-sentient systems, such as computer programs or cell cultures) and relative replacements (i.e. replacing sentient animals with animals that current scientific evidence indicates have a significantly lower potential for pain perception, such as some invertebrates).

In the space provided please explain why the study can't be conducted via the use of available non-animal models or animals of a low or lower sentience. You should consider whether there are any in vitro techniques that could replace the use of animals. **Additionally,** have any computer simulations been developed that relate to the study? Please note that if the scientific objectives of the study can be achieved by using available non-animal models or animals of low sentience, the ACC may consider this as justification for rejection.

Very little is known about the movement and biology of Arctic marine fish and this can only be determined by studying the fish in their natural environment. This study also requires sampling animals in their natural habitat and subject to normal environmental variation in diet and habitat choices. No suitable non-sentient system would therefore serve as a viable substitute.

REDUCTION IN ANIMAL USE

Reduction refers to any strategy that results in fewer animals being used to obtain sufficient data to answer a research question while maximizing the information obtained per animal. This potentially limits or avoids the subsequent use of additional animals although it is important that this be done without further compromising any individual animal's lifetime welfare.

Please explain how the number of animals requested was determined. Show calculations.

The research activities associated with the current AUPP are focussed on the development of sustainable Arctic fisheries. This primarily relies on the tracking of commercially and ecologically important species to determine movements relative to management boundaries, spatio-temporal interactions that may increase bycatch, and identification of population spatial extent based on movement characteristics. Unlike laboratory studies where the number of animals and associated environmental conditions can be controlled, survey work associated with this AUPP will be conducted across expansive areas of arctic seas. Within this environment fish are capable of moving 1000s km. Furthermore, their movement characteristics are likely to vary at an individual level, and in relation to dynamic oceanographic and climatic/weather conditions which will vary in both time and space.

Due to this inherent variability and size of our study system (Baffin bay, Svalbard and Norway), a formal power analysis to identify the minimum sample size is statistically challenging. However, a large review of the biologging literature conducted by sequira *et al*. (2019) however reported that within similar study systems i.e. large expansive areas with animals with highly dispersive behaviour, large sample sizes of >100 individuals are required to assess movement behaviour between populations. Therefore, prior experience within the PI's team will be used to identify appropriate sample sizes required to obtain sufficient data to answer the research questions.

Is there information on the proposed model that might allow the use of fewer animals? For example, could in vitro methods be incorporated into the protocol in any way to reduce the number of animals used (i.e., for early screening)? Explain.

N/A

Is the proposed experiment or test duplicative? If yes, please explain why the current proposal is required

N/A

Please provide statistical evidence on why the proposed number of animals to be utilized was requested. Could this number be reduced?

N/A

REFINEMENT

Refinement refers to any modifications to husbandry or experimental procedures that minimize pain and distress for an animal. Because it is essential to consider the entire lifetime experience of the animal and not just its time spent during a procedure, refinement also refers to welfare-enhancing changes made to the animal's living area. These changes are typically referred to as environmental enrichment, and scientists constantly work to implement effective enrichment strategies to realize the Three Rs.

A) Is there information on the proposed model that might reduce any pain experienced or the level of invasiveness in the animals or allow a few animals to undergo these procedures? Please explain.

We will tag a variety of species with either internal or external tracking tags. Expert judgement will be used at the time of surgery to select the most appropriate tag and provide the highest long-term welfare outcomes for the individual animal.

For teleost fish e.g. Greenland halibut (*Reinhardtius hippoglossoides*), the most likely tag attachment method will be via internal implantation of small acoustic telemetry tags within the peritoneal cavity. This method allows the tracking tag to sit benignly within the peritoneal cavity for the duration of the study. When conducting studies which utilize satellite telemetry to track animal movement e.g. Greenland shark (*Somniosus microcephalus*), these tags are larger and not suitable for internal implantation. These will instead be attached to the animal via externally anchoring the tag within subcutaneous or muscle tissue. All external attachment of transmitters will be conducted in the most minimally invasive method available.

The selection of external vs internal attachment and/or attachment site of tracking tags will be based on expert opinion, the species and environmental conditions at the time of tagging. The physiological condition of the animal will always be maintained at the highest possible level, by minimizing the time the animals are held during the tagging procedure and conducting physiological assessments of each individual at multiple stages throughout the procedure.

B) Please provide information on how the welfare and level of pain of the animals will be assessed. Is this information known? Please provide specifics.

Assessment of pain and distress will be visually assessed at regular time points both before, during and after a procedure is conducted on a fish. This includes visually assessing for external symptoms e.g. bleeding or scale loss. Further methods include assessing the presence/absence and intensity of key reflexes. This includes the following:

- Physical stimulation to get an escape response e.g. Tail pinching (Relevant for elasmobranchs & Teleost fish)
- Assessing the ability of the animal to orientate itself (equilibrium maintenance). This will be conducted by rotating the fish upside down, if the fish's reflexes are not impaired the individual will right itself. (relevant for teleost)

F.1. PROCEDURES

F1.1 List all procedures where pain, distress, discomfort, and/or suffering could occur in the animals. Indicate what measures will be taken to alleviate or minimize these effects. Include post-operative care; specify F1.2 analgesics and F1.3 anesthetics with dosages and routes of administration and special procedures used and F1.4 if any other drugs/medications are given
*****Distress levels may be nil, low, medium, or high.

F1.1 List All Procedures:

As part of this fieldwork, there are three scenarios that will result in distress: (A) fish/crustaceans captured and euthanized for chemical tracer and stomach content work; and (B) fish/crustaceans captured and equipped with an external marker tag, an internal acoustic tag and/or external satellite tag or biologging package and (C) respirometry studies.

A) Capture and euthanized: Distress is expected only during the capture and initial handling of the fish/crustaceans. We will be sub-sampling fish and crustaceans caught on a longline or in a trawl net or gillnet for food web studies (chemical analyses) and to record population parameters which require lethal sampling to collect gonads and/or livers. All fish/crustaceans will be euthanized as soon as they are brought on the boat. Distress level for this process is expected to be medium.

B) Tag and Release: Fish captured for short/long term tracking studies will experience distress from capture as well as surgery when the tag is inserted into the body cavity. The distress caused by these processes is high, however reducing holding and handling time is key to minimizing the stress caused to these species. The overall goal is to return them to the ocean as quickly as possible in a strong enough condition to swim back to the benthic environment. The procedures for the tag insertion are outlined below:

Surgical/Internal tags
Prior to starting surgery all devices to be implanted and surgical equipment will be immersed in non-diluted Betadine solution (a minimum of 15 minutes of soaking is required) for sterilization.

2. Wet paper towels for moisture and cushioning will be placed on the table and the fish placed on the paper towels with their ocular side facing down (for flatfish). The surgical area is wiped with sterile gauze to remove excess mucous, gently swabbed with a dilute Betadine (25%) solution and then draped with a sterile non-absorbing material. Greenland sharks will be turned on their back while in the water to induce tonic immobility and secured to the side of the zodiac. Blood will be drawn from the caudal vein (needle size: 16g; 30mL syringe; draw 10 mL blood per individual) and a fin clip (for genetics/stable isotopes; 50mm long x 5 mm wide) taken from the trailing edge of the pelvic fin.

3. All small fish (<100cm) will be scanned with a portable ultrasound to determine sex, and blood drawn from the caudle peduncle (needle size: 22g; syringe size 1mL; volume of blood drawn 0.5mL) at the same time as the

surgery to minimize operation time/time out of the water. Fin clips (for genetics/stable isotope work) will be taken from the trailing edge of the pelvic fins. Fresh sea-water will be regularly sprayed over the gills to irrigate.

4. For sharks, the initial incision is through the skin, about 1.0 cm off the abdominal midline (left side of fish) towards the posterior, but anterior to the anus. For Greenland halibut and other similar sized fish, the incision is made on the non-occular side just below the pectoral fin. The length of the incision depends on tag size. For example, a 4 cm acoustic tag requires an opening about 1-2 cm. Scalpel blade size 11 is used in all cases.

5. The acoustic tag (transmitter) is removed from the Betadine solution and rinsed with sterile saline before being placed in the abdominal cavity. The tag is pushed through the incision and advanced cranially.

6.The incision is closed with simple interrupted sutures using the appropriate needle for the thickness of the fish skin and underlying muscle layers. For example, a 3-0 Vicryl coated suture and cutting needle is adequate for most fish > 400 grams. Sutures will be placed at 0.5 cm intervals to close the incision. Surgeon knots (the thread is passed twice through the first loop) may be used due to the higher tension and knots should have 4 throws to ensure stability. Coated VICRYL* Plus Antibacterial (polyglactin 910) Sutures will be used as to avoid infection from the braided material.

7. Finally, an external marker tag will be placed on the dorsal (ocular side for flatfish) side of the fish. Spaghetti t-bar tags (Floy tags: http://www.floytag.com/) are inserted using a sterilized gun between the pterygiophores of the fish. For large fish/sharks, a dart tag is inserted at the base of the dorsal fin using a hand held tagging pole.

The overall distress level for surgical practices will be medium.

External Tags (Marker tags, Satellite tags, Biologging packages)

For mark-recapture studies, fish will be weighed, measured and a spaghetti/Floy tag inserted on the dorsal (ocular side for flatfish) side of the fish (as described above) and immediately released to minimize handling time.

For satellite tags, Greenland sharks and other smaller fish will be equipped with standard pop-up archival satellite tags and/or mark-report tags (Wildlife computers - http://www.wildlifecomputers.com/products.aspx?ID=7). Sharks will be held next to the boat using ropes (as described above). To attach the satellite tag - the tag disc attachment is first placed on the dorsal fin and using a needle, puncture holes are made for the bolts – the tag is then secured through the fin using these bolts. The bolts are designed to degrade when the tag has stopped transmitting so the tag and bolts release from the animal. Smaller fish will be handled as described above for acoustic tags, however the satellite tag will be attached using a dart sterilized in betadine solution imbedded in pterygiophores, on the dorsal side (or ocular side of flatfish). In certain instances (for post release survival studies following capture in trawl fishing gear) – satellite tags will also be attached externally using a sterilized dart.

Satellite tags applied to crustaceans will be attached using a ‘back-pack’ method, where soft nylon cords are tied around the body of the animal to hold the satellite tag in place. Since crustaceans go through a yearly moulting process, the ropes will shed from the animal within a year. Crustaceans will be monitored for 15 minutes in a holding take to ensure proper orientation before release.

Biologging packages
‘Packages’ equipped with any combination of satellite tag, radio tag, accelerometer, depth/temperature/salinity sensor, video camera, hydrophone and/or VMT (vemco mobile transceiver) will be attached to either large teleosts (~100 cm) or Greenland sharks. For teleosts, all equipment will be imbedded into a dense float with a dart and attached in the same way as satellite tags (as described above). To attach the package to Greenland sharks, the skin anterior to the head of the shark is pinched and pierced shallowly using

a metal needle, and a plastic cable (4 mm in width) is passed through the hole and around the data package to secure it firmly against the animal. The cable is connected to a timed-release mechanism by an insulated wire set to break via electric charge after a pre-determined number of days after the release of each shark.

The overall distress level for external tags will be medium.

Post-operative care
All fish/crustaceans that are tagged with any of the above methods will be checked for opercular movement/gill movement/proper orientation in a holding tank directly after handling and a 15 minute post-surgery follow up will be conducted to ensure it is upright and swimming/orienting normally. If the fish/crustacean is not swimming/standing upright monitoring will continue every 5 minutes and opercular movement noted. If the fish does not recover within an additional 15 minutes it will be euthanized and sampled for chemical tracer work. If the fish are moving normally at 15 minutes they will be released back to the ocean.

For Greenland shark, we will turn the fish over (right the body), and monitor the movement of the shark while still holding it against the boat. If the shark displays signs of active swimming (strong tail-beats) while orienting its nose downward, it will be released and post release behaviour monitored.

All tag materials and tools required will be immersed in non-diluted Betadine solution (a minimum of 15 minutes of soaking is required) to sterilize.

(C) Respirometry

Respirometry will be conducted on both large (>100cm – Greenland shark) and small fish/crustaceans. Animals will be brought on-board the fishing vessel and held in individual tanks with a constant flow of fresh sea-water for a minimum of 1-hour to acclimate to the holding tank. Once the acclimation period is over, a cover will be placed on the tank (often black plastic – placed directly on the water surface) and the flow of seawater halted. Oxygen levels in the tank will be monitored until they reach 70% saturation, as this level is not low enough to cause distress to the animal. Once this level is reached, the cover will be removed and fresh seawater will be added to the tank to bring the oxygen level back up to 100%. This process may be repeated up to 5 times on the same individual. Once the trial is over, the animal may be euthanized and samples collected, or tagged and released. The respirometry procedure will cause a medium level of distress due to handling and holding times on the vessel.

F1.2 ANALGESICS:

Are analgesics needed/used for this study? ☐Yes ☒No

Please continue if your answer to the above was yes. If it was no, you may leave this section blank.

If yes, are they administered pre-operative? ☐Yes ☐No

If yes, include parameters monitored, duration and frequency, name of Analgesic, dosage, route, and frequency analgesic(s) given.

If yes, are they administered post-operative? ☐Yes ☐No

If yes include parameters monitored, duration and frequency, name of analgesic, dosage, route, and frequency analgesic(s) given.

If you answered No to either, please explain why Analgesics are not administered pre or postoperatively.

F1.3 ANAESTHETICS:

Are anesthetics needed/used for this study? ☒**Yes** ☐**No**

Please continue if your answer to the above is yes. If it was no, you may leave this section blank.

When filling out this section please refer to SOP# AD09, Animal Care and Use Records.

Please list the anesthetic(s) agent administered.

Tricaine methanesulfonate (MS-222) 1g/L will be administered if euthanization is required for teleost fish.

Which parameters are monitored during the surgery/procedure?

N/A

How often will these parameters be monitored?

N/A

For rodents-how will hypothermia be prevented?

N/A

What is the frequency of post-surgery/procedure monitoring?

Following surgery, fish will be monitored continuously in a holding tank for a 15-minute period to ensure they have adequately recovered before release. This 15-minute period was chosen as it allows sufficient time for the fish to regain equilibrium in the holding tank, while minimizing the stress induced by holding the fish on the boat for long periods of time.

F1.4 OTHER DRUGS/MEDICATIONS:

F.1. Include Name of Drug/Medication, Dosage, Route, Duration and Frequency.

N/A

F1.5 RATIONALE FOR ANIMAL USE:

F.2. Explain the scientific hypothesis(es) to be investigated in this project.

1) Greenland halibut are resident within inshore/coastal areas along the Baffin Bay coast, however they perform a yearly migration offshore.
2) Greenland halibut are migrating to the Davis Strait, south of Baffin Bay for spawning.
3) The offshore movements of Greenland halibut are predictable with connectivity between multiple regions
4) The presence of Greenland halibut in designated conservation areas does not overlap with the presence of other species of interest (i.e. Narwhal)
5) Greenland sharks, Arctic skate, porcupine crabs and other benthic species display high spatial overlap with Greenland halibut, resulting in fishery interactions that can be detrimental to their populations.
6) Arctic skate are highly resident species, rarely migrating far from tagging regions.
7) Greenland shark display seasonal patterns in movement, with greater presence in the coastal regions during summer/ice free months, and moving offshore in the ice covered season.
8) Porcupine crabs display both resident and migratory behaviours, with little evidence of coordinated group behaviour.
9) Food webs show a latitudinal gradient, particularly with a stark contrast between Baffin Bay and the Davis Strait, with higher species diversity displayed in the more southern Davis Strait as part of the northern Atlantic Ocean and Baffin Bay more resemblant of Arctic diversity.

10) As a result of their cold water habitat, arctic species have some of the slowest metabolisms, most notably, the large arctic Greenland shark, which enables species to live for exceptionally long periods of time.

F.3. Explain what new information is expected from the conduct of this project and its anticipated value.

Although the amount of literature available for Arctic species continues to grow, they remain a relatively understudied assemblage compared to temperate and tropical species. However, the pressure imposed by fishing activities in the region has undoubtably resulted in population and diversity loss, and the threat of fishing will only continue to grow in the future as ice reduction because of climate change will lengthen the access to fishing areas. This project is specifically aimed to address questions relevant to fisheries sustainability and management. Understanding the movement of all species of interest (both commercial and bycatch) will allow for informed development of quotas and regional fishing closures, while studies of fish biology (food web and metabolism) will help understand the interdependencies between species and the ecosystem to identify threats presented by a changing Arctic ecosystem.
The anticipated value of this data is extremely high as it allows for the development of community Inuit fisheries, providing socio-economic benefits to the coastal communities and the broader long-term sustainable development of Arctic fisheries. Through this work we can help develop a model system of sustainable development in the Canadian Arctic/Baffin Bay where resource allocation favours both the small-scale Inuit fisheries and larger offshore vessels for applications on a global scale. To this effect, both the social and economic value of this work is extremely high and far-reaching.
It is important to note that this project involves diverse stakeholders from Inuit community members, the fishing industry, non-governmental organizations and resource managers (DFO)

F.4. Explain why it is necessary to use live animals in this project and what alternatives have been considered (i.e., mathematical models, computer simulations, or in vitro systems).

Very little is known about the movement and biology of Arctic marine fish and this can only be determined by studying the fish in their natural environment. This study also requires sampling animals in their natural habitat and subject to normal environmental variation in diet and habitat choices.

F.5. Explain the rationale for the choice of species.

This project targets the benthic and deep water pelagic species assemblage in the Arctic/north Atlantic ocean as these species will be primarily impacted by the developing/expanding fishing industry for Greenland halibut in the region.

G. FUNDING INFORMATION FOR RESEARCH PROJECTS

G.1. Is this project currently funded? ☒ **Yes** ☐ **No**

Title of the funded project: Turbot Fisheries – Baffin Region

Agency Name: The Government of Nunavut

Business Unit: 816376

Period of funding from: 01/01/2025 – 01/01/2028

G.2. Is funding being sought? ☐ **Yes** ☒ **No**

Title of The Funded Project: Click here to enter text.

Agency Name: Click here to enter text.

Business Unit: Click here to enter text.

Period of Funding From: Click here to enter text. **To** Click here to enter text.

G.3. Does this proposal involve a collaborative project with other institutions? ☒ **Yes** ☐ **No**

If yes, please attach documentation indicating that this proposal has been reviewed by the other institutions' ACC.

H. PEER REVIEW FOR SCIENTIFIC MERIT FOR RESEARCH PROJECTS

H.1. Has this project already been peer-reviewed for scientific merit? ☒ **Yes** ☐ **No**

If yes, please provide details.

Name of Granting Agency: The Government of Nunavut / the Nunavut Fisheries Association and the Department of Fisheries and Oceans

Other: Click here to enter text.

H.2. **If no**, the project must be peer-reviewed before the commencement of the research.

All non-funded and non-externally peer-reviewed research projects involving animals must undergo prior review for scientific merit as per *SOP AD04 Assessment of Research Protocols in the Absence of Peer Reviews*. This can be found at www.uwindsor.ca/acc. This includes pilot research and contract or grant research.

The ACC itself does not conduct formal scientific or educational merit reviews for non-funded, non-externally reviewed projects. This is done by the VP, Research and Innovation at the request of the Principal Investigator or Chair of the ACC. Once a research proposal has received positive external reviews, the applicant will be invited by the VP, Research and Innovation to submit an AUPP to the ACC.

I. PEDAGOGICAL MERIT REVIEW FOR TEACHING PROTOCOLS

All non-funded and non-externally peer-reviewed teaching protocols involving animals **must** undergo prior review for pedagogical merit. Please refer to SOP AD05 Review and Assessment of Teaching Protocols to aid you in completing this section of the AUPP. Once you've submitted your teaching AUPP to the ACC, the ACC coordinator will forward your AUPP to an independent referee within the University of Windsor community to evaluate pedagogical merit.

I.1. Has this teaching protocol already been reviewed for pedagogical merit? ☐ **Yes** ☐ **No**

If yes, please provide a completed form **SOP AD05 Review and Assessment of Teaching Protocols.**

I.2. **If no,** please fill out form **SOP AD05D Pedagogical Merit Review Form for Instructors.**

Please note that the AUPP will not be reviewed by the ACC until we have approval for pedagogical merit.

J. FOR COURSES WITH LABORATORIES INVOLVING THE USE OF ANIMALS

NEW: Please note that the CCAC strongly advocates that the use of animals for teaching purposes should be limited wherever possible. Additionally, in the rare case where a protocol may be approved, all students must take our mandatory training i.e., the online course, the veterinarian's seminar, and any additional training that is required. Since there is a cost associated with the veterinarian's time there will be charges for this.

**** For the following, please indicate if not applicable (N/A) by checking the box where appropriate)**

J.1. What on-site supervision will be provided for the students in this course? ☒**N/A**

J.2. What training is provided to Teaching Assistants to ensure they are adequately prepared for their role in this course? ☒**N/A**

J.3. Explain why the use of live animals is required in this project and identify what alternatives to live animals you have considered (i.e., demonstration, film, videotape, etc.). ☒**N/A**

J.4. What are students expected to learn from this project that justifies using live animals? ☒N/A

J.5. What is the student-to-animal ratio in this project? ☒N/A

J.6. What alternative will be provided to any student that does not wish to participate in animal-based teaching either with the AUPP as a whole or with individual procedures within the AUPP? ☒N/A

L. USE OF ANIMALS AND SUPPLIERS

L.1. Indicate the number of animals in each species required for a one-year period and the source/suppliers. Please provide an explanation of when Transgenic/mutant/knockout strains are used. If you require more space, please include on a separate sheet.

Species	Commercial Supplier & Address	Number Purchased	Number Used in Procedures
Enter text.	Enter text.	Enter text.	Enter text.
Enter text.	Enter text.	Enter text.	Enter text.

Species	Own breeding stock	Number Bred on site	Number Used in Procedures
Enter text.	Enter text.	Enter text.	Enter text.
Enter text.	Enter text.	Enter text.	Enter text.

Species	Source of donation	Number Donated	Number Used in Procedures
Enter text.	Enter text.	Enter text.	Enter text.
Enter text.	Enter text.	Enter text.	Enter text.

Total Number of Animals Required	
Species Used	Total Numbers to be used in Procedures of each Species

Greenland halibut (Reinhardtius hippoglossoides)	1500
Arctic skate (Amblyraja hyperborea)	150
Greenland shark (Sominosus microcephalus)	80
Arctic cod (Boreogadus saida)	200
Fourhorn sculpin (Myoxocephalus quadricornis)	50
Arctic flounder (*Pleuronectes glacialies)*	20
Eelpout (*Lycodes seminudus*)	50
Roughhead grenadier (*Macrourus berglax*)	100
Porcupine crab (*Neolithodes grimaldii*)	50
Black dogfish (*Centroscyllium fabricii*)	10
Blue Hake (*Antimora rostrata*)	10
Rabbit fish (*Chimaera monstrosa*)	10

L.2. Outline how the number of animals to be used was determined (i.e., number of groups, replicates, etc.).

Species	Commercial Supplier & Address	Number Purchased	Number Used in Procedures
Enter text.	Enter text.	Enter text.	Enter text.
Enter text.	Enter text.	Enter text.	Enter text.

Species	Own breeding stock	Number Bred on site	Number Used in Procedures
Enter text.	Enter text.	Enter text.	Enter text.
Enter text.	Enter text.	Enter text.	Enter text.

Species	Source of donation	Number Donated	Number Used in Procedures
Enter text.	Enter text.	Enter text.	Enter text.
Enter text.	Enter text.	Enter text.	Enter text.

L.3. If this is a continuation from a previous AUPP, what was the actual number of animals used during the previous year?

Total Number of Animals Required	
Species Used	**Total Numbers to be used in Procedures of each Species**
Greenland halibut (Reinhardtius hippoglossoides)	1500 (200/1120/ 30/150)
Arctic skate (Amblyraja hyperborea)	150 (80/0/20/50)
Greenland shark (Sominosus microcephalus)	80 (60/0/10/10)
Arctic cod (Boreogadus saida)	200(0/0/50/150)
Fourhorn sculpin (Myoxocephalus quadricornis)	50 (0/0/20/30)
Arctic flounder (*Pleuronectes glacialies)*	20 (5/0/5/10)
Eelpout (*Lycodes seminudus*)	50 (0/0/20/30)
Roughhead grenadier (*Macrourus berglax*)	100 (0/0/0/100)
Porcupine crab (*Neolithodes grimaldii*)	50 (30/0/10/10)

Black dogfish (*Centroscyllium fabricii*)	10 (0/0/5/5)
Blue Hake (*Antimora rostrata*)	10 (0/0/5/5)
Rabbit fish (*Chimaera monstrosa*)	10 (0/0/0/10)

L.4. Explain any change in numbers between those requested in L.1 and the number approved for use under the last year of the previous AUPP. (Details of any animals bred from purchased animals should also be included here).

M. HOUSING INFORMATION:

Please note that the information requested here is important in determining whether the Central Animal Care Facilities have the capacity to house the animals as requested.

M.1. Housing. Does this project require the housing of animals? ☐**Yes** ☒**No**
If yes, indicate the areas which will be used. ☐CACF ☐GLIER ☐LaSalle **Room No.:** Enter text.

M.1a Does the species to be housed require any of the following: this information will be used by the ACC and Facilities Manager for planning and to determine whether the animal care facilities have the capacity and the ability to house the required animals.

i)	**Rodents:**	☐**Yes**	☐**No**
	Standard large cages (capacity 10 adult mice or 2 adult rats)	☐**Yes**	☐**No**
	Standard small cages (capacity 4 adult mice)	☐**Yes**	☐**No**
	Vented cages (capacity 4 adult mice)	☐**Yes**	☐**No**
	Barrier level 2 containment (capacity 6 adult mice)	☐**Yes**	☐**No**
	Barrier level 2a containment (viral) (capacity 6 adult mice)	☐**Yes**	☐**No**
	Specialized cages (rats) (capacity 4-5 adult rats)	☐**Yes**	☐**No**
	Specialized light cycles	☐**Yes**	☐**No**

Note: Please refer to **ACC SOP MA01 Animal Housing Densities** for the number of animals housed per cage or tank.

Please indicate the duration of the light cycle i.e., 12 hours light:12 hours dark.

Specialized food or feeding schedule ☐**Yes** ☐**No**

If yes, please elaborate. Indicate, the type of food, form (i.e., pellets), irradiated, adlib, or if under restricted feeding.

Based on the total number of animals to be housed please indicate the <u>maximum</u> number and type(s) of cages that would be required daily.

Are there any additional requirements for the housing of these rodents? If yes, please explain.

ii. Aquatic Species (Fish/Frogs): ☐Yes ☒No

Please note that the daily care and the setup of tanks and systems must be completed by the aquatic animal care technician as indicated by CCAC policy.

For reference, please refer to **SOP MA01 Number of Animals Housed Per Cage or Tank.**

static tanks	☐Yes	☐No
flow through system	☐Yes	☐No
specialized light cycles	☐Yes	☐No

Please indicate the duration of the light cycle ex) 12 hours light:12 hours dark.

the use of chillers	☐Yes	☐No
the need for heaters	☐Yes	☐No
Indicate the required temperature range of the system		
high flow rate	☐Yes	☐No
large capacity tanks (FREC)	☐Yes	☐No
specialized tanks (zebrafish)	☐Yes	☐No

Please indicate the number and size of tanks required.

Feeding requirements ☐Yes ☐No

If yes, please elaborate. Indicate, the type of food, form (i.e., pellets or flakes) number of times fed per day.

Are there any additional requirements for the housing of the fish/frogs? ☐Yes ☐No

If yes, please explain.

M.2.1 Environmental enrichment is an important aspect of stress reduction for most animals, therefore all animals housed in the University facility must be provided with environmental enrichment elements such as group housing, nesting materials, hiding places, etc. Please describe the type of environmental enrichment that you will provide your animals. Please refer to SOP AH31- Laboratory Animal Environmental Enrichment. **Additional information can be found on the CCAC website listed by species:** **https://ccac.ca/en/training/modules/animals-housed-in-vivaria-stream/environmental-enrichment.html**

M2.2 Single housing -If animals are to be housed alone, please provide justification below. Include enrichment supplied or justification if it will not be provided Will the animal(s) receive visual, olfactory, or auditory contact with the same species?

N/A

M.3. Experiments. Are experiments going to be performed in a facility? ☒**Yes** ☐**No**
If **yes**, indicate the areas which will be used. ☐CACF ☐GLIER ☐LaSalle **Room No.:** In the field

M.4. Surgery. Are surgical procedures involved in this project? ☒**Yes** ☒**No**
If **yes**, indicate the areas which will be used. ☐CACF ☐GLIER ☐LaSalle **Room No.:** In the field

N. INVASIVENESS CATEGORY

What is the Invasiveness Category of this project? *(Refer to Appendix B, below, for examples of Categories of Invasiveness.)*

☐ ***Category A (Low):*** Experiments on most invertebrates or on live isolates.

☐***Category B (Nil-Low):*** Experiments that cause little or no discomfort or stress.

☐ ***Category C (Low-Moderate):*** Experiments that cause minor stress or pain of short duration.

☒ ***Category D (Moderate-High):*** Experiments that cause moderate to severe distress or discomfort.

☐ ***Category E (High Pain or Severe):*** Procedures that cause severe pain near, at, or above the pain tolerance threshold of anesthetized conscious animals.

O. CLASSIFICATION CATEGORY

☐ ***Acute:*** Any study involving euthanasia of an animal upon receipt or shortly after a brief period of housing.

No manipulations or experiments are to be performed on conscious animals (i.e., animals euthanized for tissues, or anesthetized and not allowed to recover from anesthesia).

☐ ***Chronic:*** Any study that involves the recovery of an animal from anesthesia or maintenance of animals in University facilities for more than 2 days.

☒ ***Other:*** Include explanation:

We select OTHER given we categorize field practices as resulting in both acute (euthanization of fish/crabs for dissection and sampling) and chronic (fish/sharks/crabs are held for a short period post tagging and then released)

P. DISPOSAL OF ANIMALS

NOTE: *Researchers are advised to consult the most recent CCAC Guidelines, which can be found at https://ccac.ca/en/guidelines-and-policies/the-guidelines/.*

*** In the case of physical methods of euthanasia, attach a sheet explaining the reason for selecting this method and detailing the relevant training of personnel undertaking this procedure.**

****Please note that the ACC requires justification if the method of euthanasia proposed is not listed within the CCAC guidelines.**

What will be the disposal of the animals upon completion of the study? (Check all that apply)

☐ Kept for future use (specify project): Click here to enter text.

☐ Humanely euthanized by (specify method):

☒ Anaesthetic overdose (specify agent and dose): Click here to enter text.

☐ C02 (***CO_2 euthanasia should be followed by another form of physical euthanasia.) (Please consult ACC SOP AH23 Carbon Dioxide Rodent Euthanasia Including Isoflurane Anesthetic and Physical Methods, subsection 15)***

☒ Cervical dislocation*

☐ Decapitation*****

☐ Exsanguinations

☐ Other (Specify and Explain):

800-1000mg/L MS222 as recommended by the CCAC guidelines (note for euthanization require 250-480 mg/L MS222 for Atlantic halibut [Malmstroem et al. 1993])

Q. HAZARDS TO HANDLERS OR ANIMALS

Q.1. Will any of the following agents be used? Note: Approval from the appropriate oversight committee must be obtained before the project commences.

Radioactive	☐Yes	☒No	**Specify isotope and Dose**: Click here to enter text.
Biological	☐Yes	☒No	**Specify Agent and Dose:** Click here to enter text.
Infectious	☐Yes	☒No	**Specify Agent and Dose:** Click here to enter text.
Chemical/Noxious*	☒Yes	☒No	**Specify Agent and Dose:** MS-222 1g/L

I acknowledge by checking this box that I am responsible for the chemical(s) utilized during the process of this research. ☒

NOTE: A copy of each SDS/drug insert is required for application review. Please attach.

Q.2. Describe the safeguards that will be used to protect other animals.

Field collection methods for fish will involve the use of benthic long lines and bottom trawls. We will set lines at intervals to minimize bycatch and move sampling sites in the event of excessive non-target species bycatch. Bottom trawls are limited to 30 minute tows (based on scientific experimental work).

All chemical agents will be kept in a separate area from holding tanks. All bins where anaesthetics are being used will be kept separate, and will not be used for any other purpose aside from administering the anaesthetics to fish to prevent any contamination.

Q.3. Describe the safeguards that will be used to protect handlers including protective equipment.

All researchers handling fish and MS222 will wear surgical gloves (changed between fish), protective eyewear and we will have an eye wash station available during the work. When not being used, MS222 anaesthetic will be stored in a sealed container in an appropriate dry chemical storage cabinet. All researchers will wash hands after handling fish or MS-222. Disposal of MS-222 will occur in the field; consequently the volume (water with MS-222) will be diluted continuously with marine water from a hosepipe as it is slowed poured down the vessel drain.

For surgeries on larger fish (Greenland sharks) over the side of the boat all researchers handling fish will wear surgical gloves (changed between fish) and protective eyewear. Disposable blades and needles will be used and all other equipment will be sterilized before and after the surgical procedure. All sharps (scalpel blades/needles etc) will be disposed of in a dedicated sharps disposal bin and transported back to the University of Windsor following fieldwork for correct disposal.

For dealing with porcupine crabs, handlers will use work gloves during all contact to minimize damage from spike protrusions from the shell.

All team members will wear life jackets when operating on smaller vessels from the main commercial fishing/research vessel (i.e. for working up sharks and transit to/from the vessel)

Q.4. Please provide a copy of the appropriate documentation from the Research Safety Committee if your protocol requires biosafety or radiation safety approval. Attach to the end of the AUPP form.

☐ Biosafety ☐Radiation Safety
☐Chemical Control Approval ☐ Chemical/Noxious Use Approval

CERTIFICATE #: Click here to enter text.

R. PURPOSE OF ANIMAL USE

This information is required by the Canadian Council on Animal Care. Choose the item below that best describes the purpose of animal use:

☐ 0. Breeding colony/stock that has not been assigned to a particular research, teaching, or testing protocol.
☒ 1. Studies of a fundamental nature in sciences relating to essential structure or function (i.e., biology, psychology, biochemistry, pharmacology, physiology, etc.)
☐ 2. Studies for medical purposes, including veterinary medicine, that relate to human or animal diseases or disorders.
☐ 3. Studies for regulatory testing of products for the protection of humans, animals, or the environment.
☐ 4. Studies for the development of products or appliances for human or veterinary medicine.
☐ 5. Education and training of individuals in post-secondary institutions or facilities.

These appendices are attached below (following the signing page) for your information. You do not need to include them with this AUPP.

Appendix A CCAC List of Keywords
Appendix B CCAC Categories of Invasiveness in Animal Experimentation

RESEARCHER'S/COURSE INSTRUCTOR'S DECLARATION

1. I believe that the proposed animal use conforms to my stated objectives, will advance knowledge, and will employ the best methods on the smallest number of animals to obtain valid information.
2. I believe that, wherever possible, all procedures having the potential to cause pain or stress have been refined and/or reduced to minimize animal discomfort.
3. I confirm that the experimental method accurately describes ALL the proposed animal use. I accept responsibility for procedures performed on animals in this project. All procedures will be carried out by, or under the guidance of, trained and competent personnel using recognized techniques.
4. All animals in this project will be used in compliance with the regulations of Ontario's *Animals for Research Act*, the guidelines of the Canadian Council on Animal Care, and the policies and procedures of the University of Windsor.
5. I am aware that the data provided in this protocol will be entered into the Animal Research Protocol Management System and submitted to the Canadian Council on Animal Care.
6. I will ensure that any individual who will perform any procedure(s), as described in this protocol, **will be familiar with the contents of this document**.

X _____ X _____

Primary Investigatory Co-Investigator/Student Investigator

FOR THE OFFICE OF RESEARCH SERVICES USE ONLY

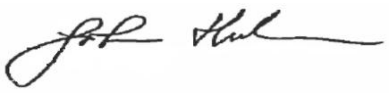
INTERIM APPROVAL
(To be reviewed at the next formal ACC meeting)

_____ _____

ACC Chair Approval Date

FINAL APPROVAL

This AUPP has received ACC approval and is valid for a period of twelve months from the approval date. It is the responsibility of the Principal Investigator to ensure that all procedures are conducted in the manner described and approved in this application.



May 28, 2025

_____ _____

ACC Chair Approval Date

APPENDICES

APPENDIX A – KEYWORDS

The CCAC highly recommends the use of the following keywords:

General

- Research, teaching, and testing;
- Regulation (are the experiments performed directly in relation to testing regulations in force in Canada and/or the US (FDA, EPA, etc.) and/or elsewhere, type of testing (i.e., cosmetic testing);
- Fieldwork, behavior observation, environmental protection study, fauna conservation;
- Development of techniques, the study of the effectiveness of a product (drugs, others) or a method (spectroscopy, others);
- Breeding, breeding colony, sentinel program;
- Antibody production (monoclonal, polyclonal);
- Palatability test, digestibility test, reinforcement/motivation, staged behavioral encounters;
- Primary cell culture, tissue/organ collection, graft, transplants;
- Species, transgenic animal;
- Validation of nonanimal model (*in vitro* test, computational methods...).

Procedures

- Trapping/netting, marking/tagging;
- Injection (intravenous, subcutaneous, intramuscular, intraperitoneal);
- Blood sampling/testing (small volume), blood removal (large volume);
- Gavaging, physical restraint, infection induction, whole-body radiation, physical euthanasia;
- Food deprivation, water deprivation, special diet;
- Altered environmental exposure, and physical restraint (duration).

Agents

- Radioisotope administration, chemical exposure, infectious agents;
- Immunogenic or inflammatory agents, Freund’s complete adjuvant.

Surgery

- Major surgery, minor surgery, stereotaxic surgery, survival surgery, multiple surgeries, cannulation.

APPENDIX B – CATEGORIES OF INVASIVENESS

Cephalopods and some other higher invertebrates have nervous systems as well developed as in some vertebrates, and may therefore warrant inclusion in Category B, C, D, or E.

The following list of categories provides possible examples of experimental procedures which are representative of each category:

Category A (Low) *- Experiments on most invertebrates or live isolates*.

Possible examples: the use of tissue culture and tissues obtained at necropsy or from the slaughterhouse; the use of eggs, protozoa, or other single-celled organisms; experiments involving containment, incision or other invasive procedures on non-cephalopod invertebrates.

Category B (Nil-Low) - *Experiments that cause little or no discomfort or stress.*

Possible examples: domestic flocks or herds being maintained in simulated or actual commercial production management systems; the short-term and skillful restraint of animals for purposes of observation or physical examination; blood sampling; injection of material in amounts that will not cause adverse reactions by the following routes: intravenous, subcutaneous, intra-muscular, intraperitoneal, or oral, but not intrathoracic or intracardiac (Category C); acute non-survival studies in which the animals are completely anesthetized and do not regain consciousness; approved methods of euthanasia following rapid unconsciousness, such as anesthetic overdose, or decapitation preceded by sedation or light anesthesia; short periods of food and/or water deprivation equivalent to periods of abstinence in nature.

Category C (Low-Moderate) - *Experiments that cause minor stress or pain of short duration.*

Possible examples: cannulation or catheterization of blood vessels or body cavities under anesthesia; minor surgical procedures under anesthesia, such as biopsies, and laparoscopy; short periods of restraint beyond that for simple observation or examination, but consistent with minimal distress; short periods of food and/or water deprivation which exceed periods of abstinence in nature; behavioral experiments on conscious animals that involve short-term, stressful restraint; exposure to non-lethal levels of drugs or chemicals. Such procedures should not cause significant changes in the animal's appearance, physiological parameters such as respiratory or cardiac rate, fecal or urinary output, or social responses.

Note: *During or after Category C studies, animals must not show self-mutilation, anorexia, dehydration, hyperactivity, increased recumbency or dormancy, increased vocalization, aggressive defensive behavior, or demonstrate social withdrawal and self-isolation.*

Category D (Moderate-High) - *Experiments that cause moderate to severe distress or discomfort.*

Possible examples: major surgical procedures conducted under general anesthesia, with subsequent recovery; prolonged (several hours or more) periods of physical restraint; induction of behavioral stresses such as maternal deprivation, aggression, predator-prey interactions; procedures which cause severe, persistent or irreversible disruption of sensorimotor organization; the use of Freund's Complete Adjuvant (see *CCAC policy statement on acceptable immunological procedures*).

Other examples include induction of anatomical and physiological abnormalities that will result in pain or distress; the exposure of an animal to noxious stimuli from which escape is impossible; the production of radiation sickness; and exposure to drugs or chemicals at levels that impair physiological systems.

Note: *Procedures used in Category D studies should not cause prolonged or severe clinical distress as may be exhibited by a wide range of clinical signs, such as marked abnormalities in behavioural patterns or attitudes, the absence of grooming, dehydration, abnormal vocalization, prolonged anorexia, circulatory collapse, extreme lethargy or disinclination to move, and clinical signs of severe or advanced local or systemic infection, etc.*

Category E (High Pain or Severe) - *Procedures that cause severe pain near, at, or above the pain tolerance threshold of unanesthetized conscious animals.*

This Category of Invasiveness is not necessarily confined to surgical procedures, but may include exposure to noxious stimuli or agents whose effects are unknown; exposure to drugs or chemicals at levels that (may) markedly impair physiological systems and which cause death, severe pain, or extreme distress; completely new biomedical experiments which have a high degree of invasive-ness, behavioral studies about which the effects of the degree of distress are not known; use of muscle relaxants or paralytic drugs without anesthetics; burn or trauma infliction on unanesthetized animals; a euthanasia method not approved by the CCAC; any procedures (e.g., the injection of noxious agents or the induction of severe stress or shock) that will result in pain which approaches the pain tolerance threshold and cannot be relieved by analgesia (e.g., when toxicity testing and experimentally-induced infectious disease studies have death as the endpoint).

From CCAC - Revised September 2023