

ONTARIO, PRAIRIE and ARCTIC ANIMAL CARE COMMITTEE **ANIMAL USE PROTOCOL**

Protocol Number (ACC use): OPA-ACC-2022-XX

1. Project Title: Movement and genetics of whales in the Canadian Arctic using satellite telemetry and tissue biopsies

2. Keywords:

5-10 keywords that focus on animal use procedures rather than research objectives or results

See: <http://ccac.ca/Documents/Assessment/Interpretation-bulletin-AUDF.pdf> page 14

research, field work, belugas, *Delphinapterus leucas*, narwhal, *Monodon monoceros*, biopsies, sampling, tags, remote attachment

3. This application is a/an: (double click to activate a square)

- a) **New AUP** ☒
- b) **Renewal** 1 ☐ 2 ☐ 3 ☐
- c) **Amendment to the original** ☐

4. What type of work is this protocol for?

Research ☒ Monitoring ☐ Regulatory ☐ Breeding Colony ☐

5. Proposed Starting Date: July 1, 2022 Finishing Date: October 30, 2022

All research, regulatory and breeding colony AUPs expire December 31st and must be renewed if the project is to be carried over into the new year. Monitoring AUPs may be valid for up to 3 years, provided that the methods are identical from year to year. Annual reporting is required for all AUPs.

6. Contact Person for AUP: Cortney Watt

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Project Authority: Cortney Watt

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7. Names, Affiliations and Descriptions of the Qualifications* of the Project Members Who Will Be Handling Animals

**List any technical training/relevant experience/courses in ethical use and handling of animals in research. Please refer to CCAC training modules <https://www.ccac.ca/en/training/> and online training courses such as: <https://www.upei.ca/professional-development/certificates/experimental-fish-canadian-aquaculture-institute>*



Ricky Kilabuk, Pangnirtung, Cumberland Sound, Nunavut. Ricky Kilabuk is an experienced boat driver and guide who has been working on bowhead whale boat-based research with DFO since 2008, has been collecting biopsies from bowhead whales for over a decade, and has experience and training tagging killer whales with crossbow in Cumberland Sound. His expertise as a hunter and previous experience in bowhead whale biopsy sampling and killer whale tagging will be relied on to collect biopsies and tag beluga whales in 2021.

Cortney Watt, Fisheries and Oceans Canada. Cortney has conducted research on beluga whales in the Canadian Arctic for the last 12 years and has experience live capturing, handling, and tagging narwhals in the Canadian Arctic.

Gregory O'Corry Crowe, Florida Atlantic University. Greg conducted his postdoc on beluga whales, and designed and led the Arctic program at NOAA Fisheries (SWFSC) for 15 years. He also designed and leads the Wildlife Evolution and Behavior program at Florida Atlantic University for the past 13 years. He has experience establishing remote field camps, running small boats around marine mammals, collecting tissue samples from belugas, and conducting research in the Alaskan, Canadian, and Russian Arctic.

Maha Ghazal, Fisheries and Oceans Canada. Maha has worked on marine mammal field and research programs in the Canadian Arctic for the last eight years. She has helped to collect biopsies and other samples, supported whale tagging, and collected photograms for whale identification. Species include beluga, narwhals, bowhead, killer whales, and ringed seals.

Jeremiah Young, Fisheries and Oceans Canada. Jeremiah has 10 years of field experience working on a variety of research programs in the Canadian Arctic. Jeremiah has experience in biological sampling; the operation of outboard motors and the live capturing and handling of narwhal for the purpose of satellite tagging.

Justine Hudson, Fisheries and Oceans Canada. Justine has nine years of experience studying animals, including invertebrates, small mammals, and marine mammals, in both a field and laboratory setting. Justine has experience using non-invasive research techniques, such as collecting respiratory vapour and eDNA, and collecting drone footage to study free-ranging marine mammals in the Canadian Arctic. Justine is also a current member of DFO's Animal Care Committee.

8. Location of the Study:

Cumberland Sound, Nunavut: 65°33' N, 65°83' W

Creswell Bay, Nunavut: 72°46' N, 94°5' W

Nelson River, Manitoba: 57°6' N, 92°20' W



9. a) Category of Invasiveness: A ☐ B ☐ C ☒ D* ☐ E* ☐

See Appendix 2, and/or 'CCAC guidelines on: the care and use of wildlife & CCAC Categories of Invasiveness

<https://www.ccac.ca/Documents/Standards/Guidelines/Wildlife.pdf>

https://www.ccac.ca/Documents/Standards/Policies/Categories_of_invasiveness.pdf

b) Detailed Scientific Justification

Only necessary if category of invasiveness falls into D or E, or if CCAC Guidelines are not being followed)

c) Purpose of Animal Use: 1 ☒ 2 ☐ 3 ☐ 4 ☐ 5 ☐ (See Appendix 3)

10. Lay Summary/Objectives of the Study

Describe why this work should be done and how the environment and public will benefit from the research findings. This must be written in plain language. Spell out all acronyms and explain / define scientific terms

Biopsy (skin) samples will be collected from narwhal and beluga whales to study stock discrimination using genetics and diet using stable isotopes. Satellite tags will be deployed to evaluate movements to determine distribution and migratory patterns. This information will provide us with a better understanding of narwhal and beluga populations and movements and assist with sustainable management of these subsistence hunted species in Canada.

11. Species to be used (include scientific names):

Beluga whales (*Delphinapterus leucas*)

Narwhal (*Monodon monoceros*)

12. Maximum number of animals:

-to be handled/released/unharmred: up to 10 whales will be satellite tagged in each location, and up to 30 biopsies will be collected in each location for each species (narwhal are not found in Nelson River, and unlikely to be found in Cumberland Sound).

-to be euthanized: None

13. Supplier OR source of animals:

- a) If laboratory experiment, please list
- i) their location (FWI or CCIW):
 - ii) fish batch number:

- b) For commercially available animals, please list:
- i) the name of supplier:



- ii) and their location:
- iii) method of transportation:

c) For wild animals, please describe:

- i) location of capture: Animals will not be captured but will be tagged/biopsied remotely from boats in Cumberland Sound, Creswell Bay and Nelson River.
- ii) method of capture: Tags and biopsies will be shot with a crossbow.
- iii) restraints used (either physical or chemical): No physical or chemical restraints will be used.
- iv) method of transportation: NA
- v) housing of animal: NA
- vi) potential mortality or injury that may occur during capture and/or transportation: There is potential for minor injury if the biopsy is taken near the eye or blowhole, or if the satellite tag is deployed close to the head. To avoid this injury, the biopsy sampler/tagger will only sample/tag adult animals that are deemed to be in healthy condition (no visible injuries, rounded rather than concave dorsal ridge). Adult whales will be assessed based on their colour (adult belugas are white and narwhal are white and black mottled, while young juveniles and calves are grey in colour) and size. If an adult whale displays visible wounds, from hunting attempts or other injuries, the animal will not be biopsied or tagged. The biopsy sampler/tagger will aim to take biopsies/samples only on the back half of the animal while parallel to the animal. No shots will be taken from the front of the animal or near the head. If a shot did hit the blow hole or head area, it would likely be a minor injury and would be extremely unlikely to result in mortality. More common would be a small nick or mark, from which there could be bleeding for a short time.

14. Permit for field capture *(indicate if you have applied and to whom you have applied:*

- a) For License to Fish for Scientific Purposes (LFSP):
DFO Permit Office: XCNA-NT-NUpermit@dfo-mpo.gc.ca
DFO Fisheries officer:
License Delivery Officer
Fisheries and Oceans Canada
Northern Operations
Central & Arctic Region
Fisheries Management
P.O. Box 358
Iqaluit, NU
X0A 0H0
Telephone: (867) 979-8005
- b) Indicate any additional permits needed for this study:
(e.g., OMNR, Species at Risk, etc)
Species at Risk for Cumberland Sound beluga

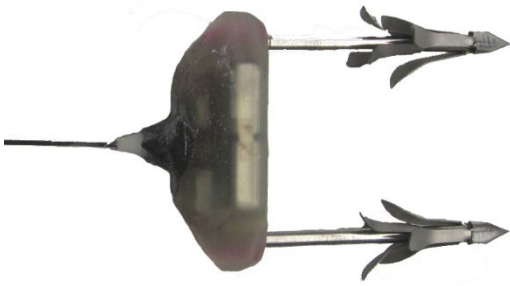
15. Summary of Project



Include methods, including a detailed description of the procedures to be used involving live animals. If this project is a renewal, please describe any significant changes to the species used, methods or sampling protocol. Significant changes may include additional types of sampling, changes in gear being used for field capture, changes to surgeries being done or tags being used.

Small motorized boats and zodiacs will be used to travel and locate whales. Boats will be operated by experienced boaters, who are knowledgeable on boating around marine mammals. As the boat approaches the animal the biopsy sampler/tagger will assess the distance of the selected animal from the vessel during a parallel approach. As the vessel moves into position, the operator will landmark anywhere on the posterior back half of the animal to be biopsied. Aiming for the back half of the animal is a necessary safety measure to avoid accidental contact with the blowhole and eyes. The operator is responsible for the final decision as to whether the biopsy will be attempted. Only adult animals that appear healthy will be biopsied or tagged. Adult whales will be assessed based on their colour (adult belugas are white and narwhal are mottled with black and white, while young juveniles and calves are grey in colour) and size. If an adult whale displays visible wounds, from hunting attempts or other injuries, the animal will not be biopsied or tagged. Also if adult animals have a concave dorsal ridge, which may indicate poor body condition, they will not be tagged or biopsied. Darting and tagging attempts will cease if the target animal displays active avoidance of the research vessel, or if a sample has not been obtained after 20 minutes of approaching; this will be the maximum approach time. Animals will be approached at low speed, parallel to the animal's direction of travel. The animal will not be "chased" as a tag or biopsy sample needs to be positioned/taken parallel to the animal and not from forward or behind the animal. Once a pod of whales is approached in a parallel direction, a healthy adult will be targeted for biopsy or tag deployment. If a biopsy dart/tag strikes or completely misses an animal, further attempts may be made on the same day, provided the individual shows no evidence of changed/evasive behaviour (i.e., no flinch or other reaction). On particularly hot days (>25 °C), when heat stress could be a concern, darting and tagging attempts will cease if a sample has not been obtained after 10 minutes of approaching.

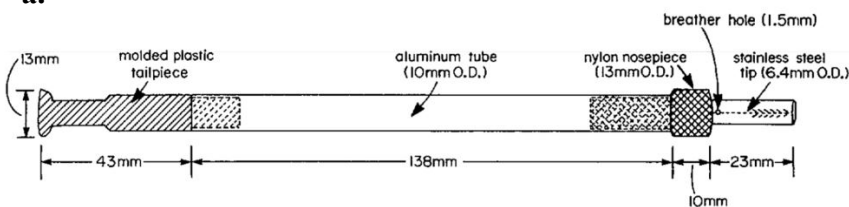
Satellite transmitters will be affixed to whales through use of a cross-bow or CO₂ gun. Transmitters will be attached using 6cm metal barbs that penetrate dermal/blubber tissue. Metal barbs used to affix satellite tags will be sterilized using isopropyl alcohol and then encased in plastic tubes for storage until deployment. Isopropyl alcohol (or a mixture of 1 part bleach to 9 parts water, if isopropyl alcohol is unavailable in the field due to issues with shipment of dangerous goods), will also be on board the boat to sterilize any darts that come into contact with water. Satellite tags are maximum 121 mm long, 66 mm wide, and 51 mm high, and weigh less than 300 g.



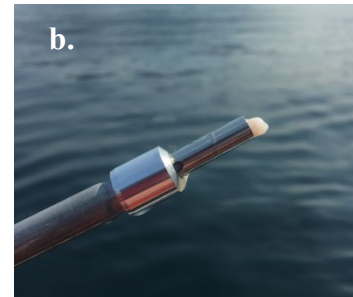
Standard 68-mm titanium darts with two rows of three petals used to affix satellite tags onto whale dorsal ridge.

Biopsies will be collected by cross bow or CO₂ gun. The darting system consists of commercially available DNA and biopsy remote biopsy darts (Pneu-Dart <https://www.pneudart.com/products/rdddevices/specialty-darts/>). The dart body consists of an aluminum tube with a molded plastic tailpiece and flight stabilizers on one end, and a threaded nylon nosepiece at the other. The nylon end acts as a stop to limit the depth of penetration. Embedded in the nylon is a multi-barbed

a.



b.



broach designed to facilitate retention of the biopsy sample within the dart. A biopsy tip screws onto the nylon nosepiece. The biopsy tip consists of a stainless-steel tube with a honed internal bevel at one end and an external thread at the other. Stainless steel biopsy tips are autoclaved and sealed until use. Any that need to be re-used are thoroughly cleaned using detergent and water, and then sterilized using isopropyl alcohol (or, in the case of unavailability, the bleach mixture described above for satellite tag darts). Any biopsy tips that come into contact with seawater (e.g. when a shot is missed) will be re-sterilized.

Darts are fired from a pneumatic variable-power dart projector with a maximum muzzle velocity of 450 feet/second. Pneu-Dart Inc. specializes in the production of wildlife biopsy equipment (<https://www.pneudart.com>). Upon release of the trigger, the firing pin comes in contact with the cartridge rim and ignites the primer, resulting in ignition of the propellant powder. The gas produced during the ignition from the power load is channeled into the dart chamber, propelling the dart through the barrel. The velocity at which the dart leaves the barrel is related to the weight of the projectile, the selected power load, and the valve setting that restricts the amount of gas pressure entering the dart barrel. This CO₂ gun offers a benefit over the crossbow as the power can be adjusted depending on how close the whale is to the boat. The power settings can be adjusted in real time, and will be decided based on field tests before locating



whales. The least power needed to achieve successful sample collection will be used to minimize disturbance to the animals. Preferentially the CO₂ gun will be used for biopsy collection, but Ricky Kilabuk in Cumberland Sound has been using crossbow in the past and so he may use the crossbow if he is more comfortable.

16. Endpoint Determination

The term "Endpoint" is defined as 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. Please see the following for more information: http://ccac.ca/Documents/Standards/Guidelines/Appropriate_endpoint.pdf

Do you expect the subject animal to experience any discomfort as a result of the treatment? **YES** ☒ **NO** ☐ *If yes, please answer the following:*

Whales will experience discomfort when the biopsy tip or tag anchor enters the skin.

i) Describe at what point the experimental treatment can be discontinued without jeopardizing the collection of important experimental observations.

Whales will be approached slowly and in parallel to the direction of travel. Darting and tagging attempts will cease if the target animal displays active avoidance of the research vessel, or if a sample has not been obtained after 20 minutes of approaching; this will be the maximum approach time. Animals will be approached at low speed, parallel to the animal's direction of travel. The animal will not be "chased" as a tag or biopsy sample needs to be positioned/taken parallel to the animal and not from forward or behind the animal. Once a pod of whales is approached in a parallel direction, a healthy adult whale will be targeted for biopsy or tag deployment. If a biopsy dart/tag strikes or completely misses an animal, further attempts may be made on the same day, provided the individual shows no evidence of changed/evasive behaviour (i.e., no flinch or other reaction). On particularly hot days (>25 °C), when heat stress could be a concern, darting and tagging attempts will cease if a sample has not been obtained after 10 minutes of approaching.

ii) Describe what will be done to relieve the discomfort of the animal.

This may include but is not limited to removing the stimulus or irritant, administering an analgesic or euthanizing the animal)

Darting and tagging attempts will cease if the target animal displays active avoidance of the research vessel, or if a sample has not been obtained after 20 minutes of approaching. No treatment will be enlisted after the biopsy has been taken or the tag has been equipped.

iii) Has the range of effects of this procedure been pre-determined by carrying out a preliminary experiment on a small number of test subjects?

This method has been used previously on beluga whales (FWI-ACC-2021-09), and killer whales in the Canadian Arctic.

iv) Describe the action to be taken if humane endpoints are reached (i.e., the animal is in undue pain and suffering) before the end of the experiment.



The biopsy sampler/tagger will aim to take biopsies/samples only on the back half of the animal while parallel to the animal. No shots will be taken from the front of the animal or near the head. If a shot did hit the blow hole or head area, it would likely be a minor injury and would be extremely unlikely to result in undue pain or suffering. More common would be a small nick or mark, from which there could be bleeding for a short time.

17. Incidental Catch

- i) Please list the potential non-target species: None
- ii) Indicate the potential for injury or mortality due to the method of capture:
- iii) How will by-catch be disposed of?

18. Anaesthetics and Analgesics used (*indicate dosage and methods*): None

19. Euthanasia:

a. Method of euthanasia (injured animals may require euthanasia):

NA

b. Provide justification for use of physical forms of euthanasia or for any methods that deviate from those described in the CCAC

Guidelines (<https://ccac.ca/Documents/Standards/Guidelines/Euthanasia.pdf>)

NA

20. If any bio-hazardous or hazardous materials including: infectious, biological/chemical agents, or radioisotopes be used during your experimentation, what materials will be used?

If so, provide an indication of institutional approval of this use.

NA

21. List relevant standard operating procedures which will be referred to while conducting this research:

SOP-FWI-ACC-01 Procedures for capture/pursuit and tagging of marine mammals

SOP-FWI-ACC-02 Procedures for biopsy and tissue sampling of marine mammals

22. Disposition of animals following termination of the project:

(check all that apply)

- ☐ Euthanasia
- ☐ Kept for future projects



☒ Released into the wild

☐ Other, specify:

23. Justification:

a. Why is it necessary to use vertebrates in this study?

The objective of the study is to specifically study genetics and movements of beluga whales and narwhals and so there is no invertebrate substitute.

b. What is the rationale for using this particular species?

The objective of the study is to specifically study genetics and movements of beluga whales and narwhals and so no other species can be substituted.

c. What is the rationale for the number of animals used?

Individual variability in movements and dive behaviour have been seen in previous tagging research on Cumberland Sound belugas. A sample size of ten will provide information on a large enough sample of belugas to make inferences on the larger population. For biopsies, skin tissues of as many beluga as possible are necessary to develop a catalogue of genetic signatures for individuals. This will allow future biopsy samples to be compared and determine if the same whales return to the same areas annually.

d. Have you considered the CCAC's "Three R Principles?"

(Please see <http://3rs.ccac.ca/en/about/> for further clarification.)

REDUCTION of number of animals required

YES ☒ **NO** ☐ If yes, explain how and if NO, explain why not.

We do not tag and biopsy all whales within a group. Once a sufficient number of animals are tagged we will switch to biopsy only. Biopsy sampling will end when a sufficient number of biopsies have been collected (likely less than 30 but that would be an ample sample size for each population).

REFINEMENT of procedures so as to reduce the pain and distress placed on animals and

YES ☒ **NO** ☐ If yes, explain how and if NO, explain why not.

The tagging and biopsy procedures used by the marine mammal program have been honed and adapted from years of experience biopsying and tagging bowhead and killer whales remotely. This project will follow the protocols that have been approved and used for these species for years.



REPLACEMENT of animals with alternatives whenever possible in the design of this project?

YES ☐ **NO** ☒ **If yes, explain how and if NO, explain why not.**

Beluga whales and narwhals are the focus on the study and the objectives of the research are to specifically evaluate genetic and geographic discreteness of these populations.

24. Provide evidence that the project involving animals has been subjected to peer review for scientific merit:

This AUP will be sent to DFO Science Senior Management to solicit two reviews from independent scientists if scientific merit is considered absent by the OPA-ACC.

If peer review was conducted on this study, have there been significant changes to the research objectives or procedures since it was reviewed? **YES** ☐ **NO** ☒
If yes, please describe below.

The methods used in this study for tagging and biopsy collection have been used widely in the marine mammal literature and within DFO. DFO has used the same remote tagging methods proposed here successfully on belugas¹ and killer whales² previously. Biopsy sampling methods for marine mammals used in the present study have also been used extensively and reviewed in the scientific literature³.

¹Lemieux Lefebvre, S., Michaud, R., Lesage, V., and Berteaux, D. 2012. Identifying high residency areas of the threatened St. Lawrence beluga whale from fine-scale movements of individuals and coarse-scale movements of herds. Marine Ecology Progress Series. 450: 243-257.

²Matthews, C.J.D., Breed, G.A., LeBlanc, B., and Ferguson, S.H. 2020. Killer whale presence drives bowhead whale selection for sea ice in Arctic seascapes of fear. PNAS. 117: 6590-6598.

³Noren, D.P., and Mocklin, J.A. 2012. Review of cetacean biopsy techniques: factors contributing to successful sample collection and physiological and behavioral impacts. Marine Mammal Science. 28: 154-199.

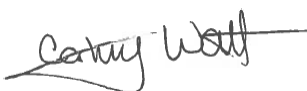
25. Source of funding: NWMB, National Geographic, Species at Risk

26. Your signature and checking all boxes below, indicates:

- ☒ That techniques employed during the execution of this project are in accordance with the Guidelines of the Canadian Council on Animal Care and that animals used in this research project will be cared for in accordance with the principles contained in the "Guide to the Care and Use of Experimental Animals,

Canadian Council on Animal Care", and the "Guidelines for the Use of Fish in Field Research"

- ☒ That alternative procedures that do not involve the use of living animals have been considered
- ☒ That the minimum number of animals consistent with the objectives of your research/teaching program will be used
- ☒ That the species proposed for use in this project have been carefully selected
- ☒ That you are required to submit an AUP report form within 30 days of completion of the project, outlining the protocol followed, changes to the protocol, the number of animals used and any unanticipated results. Report form is attached below.
- ☒ That if an incident (injury or mortality) occurs, an incident report form must be completed and sent to the ACC chair as soon as possible. You are required contact the ACC immediately if a major incident occurs, as these events must be reported to the CCAC within 10 days.
- ☒ That you may be subject to Post Approval Monitor (PAM) and may be asked to submit photos/videos of the actual procedures and methods that were done to the animals for the OPA-ACC to view AND/OR an unannounced visit may occur at any time during the length of your project.
- ☒ If the researcher wishes to appeal an adverse decision, they must appeal to the senior management responsible for animal care and use (Regional Director, Science). The Regional Director will then arrange and oversee a separate, fair and impartial appeal panel.



Project Authority

March 15, 2022

Date

For any concerns or assistance in completing the Animal Use Protocol form, contact the Ontario, Prairie and Arctic Animal Care Committee directly, at xca-fwisl-acc@dfo-mpo.gc.ca or Michelle Wetton-Salo at (204) 983-5238 or xca-fwisl-acc@dfo-mpo.gc.ca

The OPA-ACC has veterinarians on the committee. If you have questions regarding your study design or animal health, please contact Dr Charlene Berkvens at (204) 204-467-7643 or cberkvens.fwi.acc@gmail.com.

Please note; the OPA-ACC requires 3-4 weeks to review an Animal Use Protocol.

PRIVACY NOTICE STATEMENT

The information you provide on this form is collected under the authority of the *Fishery (General) Regulations* for the purpose of determining eligibility for a licence, and to create the licence and related conditions for, a licence to fish for Scientific, Experimental, Educational, Public Display or Aquatic Invasive Species control purposes. The information may be used for planning or management, reporting, safety or security purposes, audit, evaluation, statistical, research, policy development, administration or enforcement of a law, the detection, prevention, or suppression of crime, and/or investigative purposes and disclosed to Parks Canada (PC) when a licence is issued in a park under PC's jurisdiction. Failure to provide this personal information may result in your licence being denied. You have the right to the correction of, access to, and protection of, your personal information under the *Privacy Act* and to file a complaint with the Privacy Commissioner of Canada over DFO's handling of your information. Personal information collected through the processing of your application is described in the Personal Information Bank, DFO PPU 085 and can be accessed and assessed for accuracy. For more information visit Info Source www.infosource.gc.ca.



APPENDIX 1

WHEN IS AN ANIMAL USE PROTOCOL REQUIRED?

Any project that involves handling and/or euthanizing live fish or marine mammals requires an approved Animal Use Protocol to ensure that the relevant project methods conform to the guidelines established by the Canadian Council on Animal Care (CCAC).
(<https://www.ccac.ca/en/standards/guidelines/>)

Activities requiring Animal Use Protocols include the following:

1. Holding (even for very short periods of time) of all living vertebrates, including those that are or have been genetically modified, for research, display, teaching or testing.
2. All activities that involve physical tagging or chemical restraint and/ or the taking of measurements or tissue samples.
3. All tagging/identification activities including insertion/attachment of transmitters on fish or mammals.
4. All lethal field sampling for research, teaching or testing purposes.
5. Dosing of animals and/or their habitats with toxic or hazardous chemicals, including those studies administering non-lethal concentrations or doses of analgesics or other pharmaceuticals.

The OPA-ACC advises that an Animal Use Protocol should be submitted for projects involving behavioural observations of marine mammals by drone or boat.

Activities not requiring Animal Use Protocols include the following:

1. Projects involving fish eggs or larvae that have not yet reached the first life cycle in which reasonable survival is expected or developed beyond exclusive reliance on their own yolk nutrients.
2. Hatchery fish reared for release, unless specifically used in experiments.
3. Lethal sampling of fish required for regulated or legislated routine scheduled monitoring of contaminant/toxin burdens, disease, abundance and other population parameters by government agencies such as Canadian Food Inspection Agency, Health Canada, DFO, Environment Canada, and provincial or territorial departments.
4. Fish already killed in the course of an established aquaculture industry.
5. Sampling from commercial operations or subsistence fisheries where the animals are already dead as a result of standard commercial practices or subsistence catch.
6. Pets or display animals (often fish) kept in offices or public areas, unrelated to teaching or research.

If research or teaching animals are involved in community outreach activities, an "Oversight of Animal-Based Community Outreach Activities Form" must be completed and forwarded to the OPA Animal Care Committee. Please contact the chairperson for more information.



APPENDIX 2

Categories of Invasiveness in Fish Experiments Ontario, Prairie and Arctic Animal Care Committee (OPA-ACC)

Note: If more than one procedure is to be used, please indicate the **highest** category of invasiveness on the AUP form. Protocols must be submitted to the OPA-ACC for all studies which involve the use of vertebrates in Categories B through E.

Category A – Non-invasive, e.g.:

- collection of tissues post-mortem from fish caught by recreational or commercial fishers (e.g. otoliths, scales, fin rays)
- use of eggs (terminated prior to hatching)
- behavioural observations in the wild without capture or interference (e.g. visual surveys of spawning, migration)

Category B - Experiments which cause little or no discomfort or stress, e.g.:

- short-term skillful restraint of fish without anesthesia (to be live-released) for length and weight measurements, physical examination
- capture of fish with seines, dip nets, or live traps (e.g. Gee minnow traps, trap nets, fyke/hoop nets)
- lethal sampling with approved methods of euthanasia:
 - rapid unconsciousness and anesthetic overdose by immersion in tricaine methanesulfonate (=“TMS”, “MS-222”, or “Finquel”)
 - a stunning blow to the head followed by pithing or cervical dislocation and severing the gills.
- transport of live fish from supplier to lab
- maintaining fish in the laboratory for breeding and culturing purposes
- holding fish for use in approved future experiments (e.g. pre-trial acclimation, grow-out, behavioural trials)
- maintaining captive fish at study sites, e.g. in mesh cages, mesocosms, or simulated cage aquaculture operations
- blood sampling (small volume, non-lethal) with anesthesia
- subcutaneous, intramuscular, or intraperitoneal injection of material in amounts that will not cause adverse reactions

Category C - Experiments which cause minor stress or pain of short duration, e.g.:

- minor surgical procedures under anesthesia (biopsies, fin clips, scale removal)
- external tagging (e.g. visible implant, floy, spaghetti tags)
- non-lethal chemical exposure studies
- gill netting – constant monitoring by watching with a go pro or watching the set buoy on the surface and checking every hour to two hours

Category D - Experiments which cause moderate to severe distress or discomfort, e.g.:

- major surgical procedures under general anesthesia, with subsequent recovery
- insertion of internal tags/intraperitoneal implants
- cannulation or catheterization of blood vessels or body cavities
- capture of fish using longlines
- rapid retrieval of fish from depths causing decompression trauma
- prolonged periods of physical restraint



- procedures which cause severe, persistent or irreversible disruption of sensorimotor organization
- exposing fish to noxious stimuli from which escape is impossible
- exposing fish to drugs or chemicals at levels that impair physiological systems
- capture of fish using gill netting over a 2 hour soak time up to 12 hours set time, electro fishing, or benthic trawling

Category E - Procedures which cause severe pain near, at, or above the pain tolerance threshold of unanesthetized conscious animals. This category is not necessarily confined to surgical procedures, but may include:

- gillnetting if over 12 hour soak time
- 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 invasiveness
- behavioral studies about which the effects of the degree of distress are not known
- 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).

APPENDIX 3

PURPOSE OF ANIMAL USE

Choose the item (1-5) below that best describes the purpose of animal use (determined by ACC and investigator):

- 1 Studies of a fundamental nature in sciences relating to essential structure or function (e.g., biology, psychology, biochemistry, pharmacology, physiology, etc.).
- 2 Studies for medical purposes, including veterinary medicine, that relate to human or animal disease
- 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