



# MYSTIC AQUARIUM

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OFFICE OF THE PRESIDENT

December 1, 2020

Ms. Donna Wieting  
Director  
Office of Protected Resources  
National Marine Fisheries Service  
National Oceanic and Atmospheric Administration  
Via Electronic Mail: [donna.wieting@noaa.gov](mailto:donna.wieting@noaa.gov)

Dear Donna:

We appreciate the tremendous effort that you and your team have extended to Mystic Aquarium in issuing Permit No. 22629.

Enclosed are the following documents:

1. Revised "Prevention of Breeding Plan"
2. Appendix to the "Prevention of Breeding Plan" in answer to NMFS memorandum and questions of November 25, 2020
3. Amendment to Change Whales provided in the format indicated by NMFS in our communication with staff on this subject
4. Signed Permit No. 22629

Our hope is that we can now move forward with final approval to execute the approved permit. Thank you for your diligence and concern for beluga whales.

Sincerely,

Stephen M. Coan, PhD  
President and CEO



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Dear Ms. Wieting:

Thank you for clarifying and amplifying Condition III.B.6.e in Marine Mammal Protection Act (MMPA) Permit No. 22629. On this basis we can comply with the condition and submit the following plan "to provide safe and effective contraception or other means to prevent breeding of the five subject beluga whales." This letter formally presents Mystic Aquarium's plan to prevent breeding by and among the five subject animals.

We continue to have questions and concerns regarding the policy rationale and implications of various aspects of the Condition. However, your clarifications and acknowledgement of the constraints imposed on us point to a clear path to compliance with the condition, even while uncertainties about its purpose remain.

With your acknowledgment that it is the responsibility of the licensee to comply with the Animal Welfare Act while at the same time adhering to the conditions of the Permit under MMPA, we will continue to direct our attending veterinarians to always act in the best interest of the health and well-being of individual animals.

As stated in our Permit Application, Mystic Aquarium adheres to the highest standards of veterinary practice and animal husbandry. This commitment has raised our practices for animal wellbeing and care above the minimum required by law and to the higher level necessary to maintain accreditations with the Association of Zoos and Aquariums, Association of Marine Mammal Parks and Aquariums, and American Humane. This commitment – which extends from the purposes of the MMPA, Animal Welfare Act, Animal Medicinal Drug Use and Clarification Act, and State Veterinary Practice Act and other laws governing veterinary medicine in the State of Connecticut – requires us to carry out Condition III.B.6.e within all legal and practical methods available to us, according to federal and state law, with the attending veterinarian, as stipulated under federal law, reserving the right to final judgment with regard to the best care of any one animal at any time with regard to their health and safety.

As our application amply demonstrates, and as NMFS has acknowledged, our scientific program is also defined by rigorous standards. Accordingly, to complete the permitted research and publish reliable results as required by MMPA requires that our compliance with the permit condition not interfere with the approved studies.

Under these commitments and with our new understanding that compliance does not require full-time contraceptive use or full-time physical separation, we propose a breeding prevention plan based on seasonal physical separation, as deemed appropriate by the Attending Veterinarian. This plan is to apply for the five-year term of the permit and has been developed by ACZM board certified veterinarians who are specialists in the care of belugas - Allison Tuttle, DVM, DACZM and Jennifer Flower, DVM, MS, DACZM. Consultation and peer review with numerous colleagues and specialists with and external to the beluga community also informed this plan. References utilized in the development of this plan appear at the end of the document.

Respecting our mutual agreement that the social nature of beluga whales allows them to thrive in group settings, we will limit separation to peak reproductive readiness of mature female belugas in the population as determined by ultrasound analyses explained further below. Mystic Aquarium will secure solid acrylic gates throughout the separate pools in the beluga habitat to prevent breeding amongst separated animals.

Veterinarians will conduct biannual physical exams on the belugas (January and July, annually) and utilize ultrasound to monitor follicular growth in females of reproductive age (age 7 and beyond) during the breeding season. Weekly ultrasound monitoring of each reproductively mature female beluga will begin in January of each year to ensure any early follicular development is identified. In February of each year, ultrasound frequency will increase to twice weekly (corresponding with the start of breeding season). Once a developing follicle (i.e. a follicle > 3mm) is observed on ultrasound, the female beluga will receive ultrasounds a minimum of 3x weekly to monitor follicular growth to ensure physical separation prior to when the follicle reaches pre-ovulatory size. As pre-ovulatory follicles in beluga average 2.9cm (range 2.4-4.2cm), precautionary physical separation of females will occur when a developing follicle measures  $\geq$  1.8cm. This will ensure physical separation well in advance of a follicle reaching pre-ovulatory size. Once physically separated, reproductive ultrasounds will continue at a minimum of 2x weekly until ovulation or resorption occurs. Once ovarian cycling is confirmed complete via ultrasound the females can rejoin the social group. The attending veterinarian may also use blood sampling for hormone analysis as needed for monitoring health. The additional reproductive sampling that had been proposed in Study 7 of the permit application will not occur, as Study 7 was not authorized.

The ultrasounds will occur under behavioral control utilizing a layout behavior performed parallel to the beach or in-water utilizing shallow water. All female whales intended for import have layout behaviors. That said, the medical pool at Mystic Aquarium is equipped with a beluga lift to facilitate ultrasound in a safe and efficient manner if a beluga will not perform the behavioral layout required for ultrasound. Ultrasound without behavioral cooperation will be obtained by behaviorally gating/shifting the beluga to the medical pool and using the hydraulic lift. Use of a hydraulic lift system is a key component to providing high quality medical care to cetaceans with shortened restraint time and minimal impact. This common technique is used by many zoological and aquarium facilities to ensure safe handling of whales and dolphins. Shifting belugas into the medical pool will be performed under behavioral control and the false bottom floor will be raised in 45-60 seconds for brief shallow or out-of-water restraint, based on behavior of the beluga. The utilization of the lift system for reproductive ultrasound monitoring typically requires

a handling time of less than 5 minutes, after which time the false bottom floor is lowered and the beluga is once again free-swimming. Experienced staff will be present for gentle hands-on restraint as needed and an attending veterinarian is always present when the lift is operated. No adverse reactions to the lift are expected; however, the false bottom can be immediately lowered as a contingency if the attending veterinarian deems it necessary to stop the procedure.

The habitat design allows gating of various groups between the pools. Belugas will be shifted under stimulus control during sessions utilizing positive reinforcement training, which makes it possible to move whales as needed. At arrival most training sessions will occur in the holding and medical pools so there will be an emphasis on positive reinforcement of these areas. This practice has been used previously and successfully at Mystic Aquarium to ensure successful gating and lift behaviors. All female belugas proposed for import have a gating behavior and we expect quick recovery of that behavior during acclimation at Mystic Aquarium.

In the highly unlikely instance that a reproductively mature female beluga were to repeatedly refuse behavioral ultrasound layouts and utilization of the beluga lift was not feasible or not deemed appropriate at that time by the attending veterinarian, the beluga would be physically separated from mature male belugas (age 10 and beyond) to prevent breeding until an ultrasound could be safely performed and lack of follicular development confirmed.

The priority is to maintain social groups that are compatible while adhering to the breeding prevention plan. This means incompatible animals will not be mixed. We have been able to train animals such that they can be socialized when supervised and separated when not supervised, yielding over time a successful acclimation into the group. Slow, steady, and planned supervised introductions utilizing positive reinforcement while animals are under stimulus control has worked in the past. Belugas, by nature, are a docile species and we have confidence in this technique for establishing the needed groupings to manage the required physical separations.

Thirty-three husbandry staff plus 4 veterinary staff will be trained to implement this plan. The husbandry staff will be under the direction of the Director of Marine Mammals and Birds. The training process includes a checkoff sheet which tracks detailed separation guidelines and tasks critical to implement the prevention of breeding. Each staff member will have a check sheet for training and demonstrating proficiency in carrying out each identified task. Once an individual has been signed off on the checklist and approved, they can then execute this portion of the prevention of breeding plan. Oversight will be provided by the Director of Marine Mammals and Birds and the Chief Clinical Veterinarian.

Detailed health records and reports from physical examinations and ultrasound monitoring shall be kept in the Mystic Aquarium database.

In the highly unlikely situation that oral contraceptives become necessary, a contraceptive plan would be developed in consultation with appropriate specialists and submitted to NMFS for approval, prior to use. This plan would include type of contraception utilized, timing/duration of contraceptives, monitoring protocols, anticipated adverse reactions, mitigation measures utilized, and emergency management of possible adverse drug reactions. Oral contraceptive use would occur only if deemed by the attending veterinarian to be legal, ethical, consistent with best practices for care and research methods, and in the best interest of any animal.

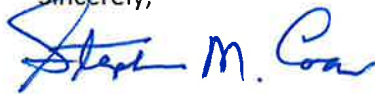
Mystic Aquarium shall establish a Beluga Health Committee reporting to the Institutional Animal Care and Use Committee. The Beluga Health Committee shall review, annually by October 1st of every year, the breeding prevention plan in place for the five beluga whales referenced in the permit, and, incorporating the latest scientific literature and developments in law with regard to this issue, make recommendations to the Institutional Animal Care and Use Committee (IACUC) for the prevention of breeding. The Beluga Health Committee shall consist of at least two marine mammal veterinarians board certified by the American College of Zoological Medicine, one representative of the general public, and shall be chaired by a member of the Institutional Animal Care and Use Committee. The entire IACUC will annually review the breeding prevention plan.

Standard operating procedures regarding prevention of breeding shall be overseen and monitored continuously by the attending veterinarian. Staff will be sufficiently trained to assist in the breeding prevention plan and animal care supervisors shall be trained and authorized to take appropriate steps to prevent breeding (i.e. physical separation) in the instance that a female beluga continues to refuse behavioral ultrasound layouts for reproductive monitoring. In all cases, veterinary and animal care decisions shall be based on informed opinions and expertise of attending veterinarians experienced in marine mammal medicine.

Mystic Aquarium agrees to maintain accreditation by the Association of Zoos and Aquariums (AZA) and to adhere to standards for Cetacean Care and Welfare set by the AZA. Mystic Aquarium also agrees to maintain accreditation by the Alliance of Marine Mammal Parks and Aquariums regarding standards of care for cetaceans. Mystic Aquarium's required annual reports to NOAA NMFS will detail (1) medical condition of the beluga whales; (2) learnings from and effectiveness of breeding prevention (3) research progress and findings related to the beluga whales.

We are grateful for the guidance provided by you and NMFS on the Permit Condition. We submit this plan with renewed commitment to the importance of bringing this cohort of beluga whales to Mystic Aquarium for advancement of conservation and research.

Sincerely,



Stephen M. Coan, PhD  
President and CEO

#### References:

O'Brien JK, KJ Steinman, T Schmidt, and TR Robeck. 2008. Semen collection, characterization, and artificial insemination in the beluga (*Delphinapterus leucas*) using liquid stored spermatozoa. *Reproduction, Fertility, and Development* 20: 770-783.

Robeck ER, O'Brien JK, Atkinson S. 2018. Reproduction. In Frances M. D. Gulland, Leslie A. Dierauf, Karyl L. Whitman (Eds.) *CRC Handbook of Marine Mammal Medicine*. 3rd Edition. CRC Press (Taylor & Francis), Boca Raton, Florida, USA. pp. 169 – 207.

Robeck TR, KJ Steinman, GA Montano et al. 2010. Deep intrauterine artificial inseminations using cryopreserved spermatozoa in beluga (*Delphinapterus leucas*). *Theriogenology* 74: 989-1001.

Robeck TR, TL Schmidt, and S Osborn. et al 2015. Development of predictive models for determining fetal age-at-length in belugas (*Delphinapterus leucas*) and their application toward in-situ and ex situ population management. *Marine Mammal Science* 31:591-611.

Robeck, TR, KJ Steinman, M Yoshioka et al. 2005. Estrous cycle characterization and artificial insemination using frozen thawed spermatozoa in the bottlenose dolphin (*Tursiops truncatus*). *Reproduction* 129: 659-674.

Robeck TR, SL Monfort, PP Calle, et al. 2005. Reproduction, Growth, and Development in Captive Beluga (*Delphinapterus leucas*). *Zoo Biology* 24:29-49.

Steinman KJ, KJ Obrien, SL Monfort, and TR Robeck. 2012. Characterization of the estrogen cycle in female beluga (*Delphinapterus leucas*) using endocrine monitoring and transabdominal ultrasound: Evidence of facultative induced ovulation. *General and Comparative Endocrinology*. 175: 389-397.

NMFS Comments on Breeding Prevention Plan and Amendment to Permit No. 22629  
November 25, 2020

#### Comments on Breeding Prevention Plan

##### *Summary*

To evaluate the safety and effectiveness of your breeding prevention plan (hereafter the 'plan') we require additional information, including the timing of reproductive monitoring, physical separation, and contraceptive use; methods, impacts, and mitigation; and the identities of the whales involved. Please indicate whether the plan is for one year or for the full five-year duration of the permit, and tailor your revision accordingly.

This plan will be for the full five-year duration of the permit.

##### *Monitoring Reproductive Cycles and Preventing Breeding through Physical Separation*

On the November 20th call, you mentioned any whales that are not trained would have ultrasound (and presumably other sampling) performed in the medical pool utilizing the hydraulic lift.

- Please provide a clear, step-by-step description of how you will use the medical pool, hydraulic lift, and light restraint to perform ultrasound or other sampling.

- Voluntary Ultrasound: Ultrasound will be behaviorally trained using a layout behavior performed parallel to the main beach or in-water utilizing the shallow wading area of the holding pool. With the beluga in a voluntary layout, the ultrasound probe is presented on lateral, dorsal, or ventral sides of the beluga's body so that the reproductive organs can be scanned by an ACZM board certified veterinarian.
- Ultrasound without behavioral cooperation of the beluga will be obtained by behaviorally gating/shifting the beluga from the holding pool to the medical pool. Once in the medical pool the samples will be collected utilizing the hydraulic lift. If the beluga is not behaviorally shifting into the medical pool, the beluga will be guided from the holding pool to the medical pool. Once in the medical pool the samples will be collected utilizing the hydraulic lift.

##### Guiding a Beluga from Holding Pool to Medical Pool

A specifically designed weighted net that spans the entire holding pool will be used as a visual barrier to guide a beluga from the holding pool to the medical pool. It will be placed in the holding pool away from the medical pool and slowly moved toward the animal as a moving wall to help guide the animal to the medical pool gate opening. Once the animal shifts freely into the medical pool, the person positioned on the gate will immediately close the gate and the net is removed. Typically, there is no contact between the net and the beluga, as belugas tend to swim away from the net. Since the net is weighted there is no possibility of entanglement or rolling into the net.

##### Operating the Hydraulic Lift

Baffles are inserted into medical pool gates. Once all baffles are in place, the valves in the control box are maneuvered to raise and lower the lift. Lift occurs slowly over a period of 45 to 60 seconds. Once lift is raised to the desired position, the valves are put in the neutral position to maintain the desired position. Once finished, the lift is lowered, and the beluga can free swim.

##### Beluga Whales Light Restraint Utilizing the Beluga Lift

The beluga lift is raised by 1 foot. Staff experienced with beluga restraint will enter the water and remain at set locations along the pool edges. When directed by supervisory staff, the in-water staff will move slowly towards the animal from both sides of the lift, narrowing the beluga's swim space. Staff will place hands on the beluga for light restraint; this is generally sufficient for belugas to cease movement. Beluga is then gently guided to desired location. The lift is then slowly raised to desired water depth. Staff remain around beluga to prevent injury. Once finished, staff will move away from animal and exit pool letting the animal return to free swimming position and lift is lowered to resting position.

- Explain how this will occur while managing physical separation of multiple animals.

- Upon arrival at Mystic Aquarium, all imported belugas will begin learning to shift between the Medical Pool and the Holding Pool to allow for separations.
  - Once a beluga is successfully shifting between back holding and medical pools, allowing for separations utilizing gates, the beluga will be introduced to Mystic Aquarium's current population of beluga whales.
  - Initially, most training sessions will be conducted in the holding pools to provide a continued emphasis on positive reinforcement of these areas, expanding to other areas only when trainers are confident in the beluga's shifting.
  - Trainers will focus on appropriate group separations, allowing for comfort in various social groups, to create a solid foundation for positive association with novel areas of habitat.
- Explain how use of the hydraulic lift and light restraint to conduct these procedures will not result in an increased risk of adverse impacts to the subject whales.

Use of a hydraulic lift system is a key component to providing high quality medical care to cetaceans with shortened restraint time and minimal impact to the animal. This common technique is used by many zoological and aquarium facilities to ensure safe handling of whales and dolphins. Shifting belugas into the medical pool is performed under behavioral control and the false bottom floor is raised in 45-60 seconds for brief shallow or out-of-water restraint. The utilization of the lift system for reproductive ultrasound monitoring typically requires a handling time of less than 5 minutes, after which time the false bottom floor is lowered and the beluga is once again free-swimming.

At time of arrival, all imported belugas will be released into the medical pool with the lift in the "up" position, approximately 3.5' – 4.0' of water. This process ensures the familiarity of the medical pool for each animal and aids in the rapid training and comfort of the belugas in the medical pool. Through the process of training the belugas to shift from the holding pool to the medical pool, positive reinforcement will be used which in turn will ensure the medical pool a positive place for the belugas.

Once the animal is comfortable in the Medical Pool for training sessions, the same positive reinforcement training for lift raising will begin. Through positive reinforcement history and training, the belugas will have positive associations with gating and riding the hydraulic lift minimizing risk and any adverse impacts to the whales.

This practice has been used previously and successfully at Mystic Aquarium to ensure successful gating and lift behaviors.

- Include monitoring protocols, anticipated adverse reactions, and mitigation measures you will use if whales have an adverse reaction during any point during the use of the lift. For example, if a whale shows signs of stress from any aspect of the procedure, will it be stopped and the floor dropped so they can swim freely? If so, when will ultrasound/sampling next be performed? If not, what will be done?

Although no adverse reactions are expected, in the highly unlikely instance that a beluga were to show adverse reaction during a lift procedure, the false bottom would be immediately lowered such that the whale can swim freely. If the ultrasound was unable to be completed prior to the lowering of the lift, the female beluga would remain physically separated from reproductively mature male belugas until an ultrasound could be safely performed and lack of follicular development confirmed. An ACZM board certified veterinarian and husbandry specialist trained in the use of the beluga lift and in monitoring beluga behavior are always present during use of the beluga lift ensuring animal safety.

Positive reinforcement training minimizes risk for any adverse reactions. These techniques have been used successfully to train Mystic Aquarium's current beluga collection for all behaviors described. Our prior experience with training belugas in this manner allows confidence in our ability to train the new belugas these behaviors, successfully separate, and monitor reproductive parameters to prevent breeding as required by the permit and as proposed in our breeding prevention plan.

- Describe your contingency plan if a whale continues to have an adverse reaction or if a whale that is trained to voluntarily allow ultrasound/sampling refuses (for both short- and long-term refusals). During these intervals, would the whales be separated by sex in order to eliminate chances for breeding?

Although no adverse reactions are expected, in the highly unlikely instance that a female beluga were to repeatedly show adverse reaction during lift procedures or were to repeatedly refuse behavioral ultrasound layouts, the female beluga would be physically separated from reproductively mature male belugas until an ultrasound could be safely performed and lack of follicular development confirmed.

As we stated in our decision issued on August 27, 2020, beluga whales are seasonal breeders, with periods of peak fertility in captive whales typically between February and May. Therefore, separating males from females during these seasonal reproductive windows may aid in preventing breeding without significant disruption to social groups. Based on your submitted plan, and our call on November 20, 2020, it seems you are proposing physical separation over only a few days during “peak reproductive readiness” of the female beluga whales, stating that once a follicle approaches “ovulatory size,” male and female beluga whales would be physically separated “until the observed follicle ovulates or resorbs,” at which point they will rejoin the social group. A more conservative approach, employing a longer period of separation to provide a sufficient buffer before and after estrous to account for uncertainty in the timing of reproductive readiness, may be more effective to ensure compliance with the requirements of the permit.

Also as stated in our decision, the permit authorizes reproductive monitoring as part of normal husbandry to allow you to monitor the reproductive status of the animals whether they are managed using physical separation or contraception (see Appendix 1, Table 1 of the permit). This includes video monitoring, biological sampling (blood, breath, and vaginal swabs), and ultrasound before, during and after the breeding season.

- Please explain how you will precisely identify when ovulation starts and ends and how you will determine the precise day that physical separation must occur to effectively prevent breeding given that it may not be possible, or desirable, to monitor the whales for reproductive readiness on a daily basis. For example, when will ultrasound begin, how frequently will it be performed prior to, during, and after peak reproductive readiness? How will you precisely monitor follicular growth and ovulation/resorption?

Weekly ultrasound monitoring of each reproductively mature female beluga (7 years and older) will begin in January of each year, to ensure any early follicular development is identified. In February of each year, ultrasound frequency will increase to twice weekly (corresponding with the start of breeding season). Once a developing follicle (i.e. a follicle > 3mm) is observed on ultrasound, the female will receive ultrasounds a minimum of 3x weekly to monitor follicular growth to ensure physical separation prior to the follicle reaching pre-ovulatory size. As pre-ovulatory follicles in beluga average 2.9cm (range 2.4-4.2cm), precautionary physical separation of females will occur when a developing follicle measures  $\geq 1.8$ cm. This will ensure physical separation well in advance of a follicle reaching pre-ovulatory size.

Once physically separated, reproductive ultrasounds will continue at a minimum of 2x/weekly until ovulation or resorption occurs. Once ovarian cycling is confirmed complete via ultrasound, the mature females will rejoin the social group.

In the highly unlikely instance that a reproductively mature female beluga were to repeatedly refuse behavioral ultrasound layouts and utilization of the beluga lift was not feasible or not deemed appropriate at that time, the animal would be physically separated from mature male belugas (age 10 and older) to prevent breeding until an ultrasound could be safely performed and a lack of follicular development definitively confirmed.

Once breeding season has finished for the year as indicated by month (June, annually) as well as lack of ovarian follicular activity confirmed via ultrasound, ultrasound schedules will return to what is necessary for routine preventative health monitoring (typically once weekly) as determined by the attending veterinarian.

- Please explain if you will include video monitoring and biological sampling in addition to ultrasound, and whether these procedures will be done at the same frequency as described in the permit application for Study 7. Do you plan to monitor testicular size in the males (including the existing male at Mystic and the male proposed for import)? Will you be taking blood and breath to monitor reproductive hormones, and vaginal swabs? If not, please explain why this sampling will not be utilized.

Study 7 was not authorized in the permit to allow such sampling. As such, we will focus on female reproductive monitoring with ultrasound as described above for the purposes of monitoring health and preventing reproduction. Female hormone monitoring (from blood) of mature female belugas may be elected by the attending veterinarians as part of the routine health management.

- If you are proposing a different time period and frequency of ultrasound measurements and sampling from what was described in the permit application, please explain why.

Ultrasound monitoring of reproductively mature female belugas will occur under behavioral control at a frequency as described in previous responses in order to adhere to the breeding prevention plan. In short:

- weekly ultrasound monitoring during the month of January of each year
- twice weekly ultrasound monitoring beginning in February of each year
- minimum of 3x weekly ultrasound monitoring once a developing follicle ( $>3\text{mm}$ ) is observed
- once physically separated, ultrasound monitoring a minimum of 2x weekly until ovulation or resorption occurs
- outside of breeding season, routine ultrasounds scheduled as necessary for routine preventative health monitoring (typically once weekly under behavioral control) as determined by the attending veterinarian

For biannual physical exams:

- Describe what samples will be taken to monitor reproductive status and what reproductive parameters will be measured (e.g., reproductive hormones in blood and other samples as described in your permit application).

Study 7 was not authorized in the permit.

We will utilize ultrasound for reproductive monitoring in combination with hormone analysis (from blood) as determined to be necessary by the attending veterinarian for health and reproductive monitoring and management in accordance with the breeding prevention plan.

- Include what months of the year physicals will be conducted.

Physical examinations will occur in January and July of each year and will be performed by an ACZM board certified veterinarian.

For gating animals:

- Describe the methods for gating animals and securing the acrylic gates throughout the separate tanks in the beluga habitat. For example, when gating animals from one pool to another, describe the steps required to open and close the acrylic gates, how many personnel are required, how quickly it can be done, and how it will be accomplished with both trained and untrained whales.

- All belugas proposed for import except for Havok have a gating behavior; therefore, we expect a quick recovery of these behaviors post transport. Gating from holding pool to medical pool will be the primary

focus during the initial acclimation period and animals will receive a high percentage of reinforcement (food) for shifting to the medical pool.

- During all gating procedures, all animals are under stimulus control (at a station with a trainer 1:1 ratio trainer: animal).
  - If a gate needs to be opened/closed one trainer is designated to perform the appropriate duty.
  - The trainer of the animal designated to separate makes all calls of when to open or close gates.
  - Once all animals are under stimulus control the trainer communicates to the gate staff to open the gate that the animal will be shifting through.
  - The animal is then point followed through the gate to the desired location and the gate staff is directed by the trainer moving the animal through the gate to close the gate once the animal is completely through. Stimulus control is maintained at the new station in the desired location.
  - Opening/closing a gate takes approximately 3-5 seconds.
  - Gates are manually pulled by a handle and on a roller, making opening and closing dependent on the person moving the animal and can be sped up or slowed down as necessary for animal safety.
  - Due to the high success rate of training this behavior with Mystic Aquarium's current collection, we are certain this approach will work with the new belugas and that Havok can be trained accordingly.
- Describe contingency plans if whales refuse to gate.

Planned approach that will be used:

- The social setting will be changed through gating other whales to get the desired separations. Being social animals, the non-gating whale will be "buddied" with a great shifting animal that is identified in the desired separated social group. This can be achieved through either gating the desired group to the holding pool, and separating the alternate group to the main pool, or vice versa, until the desired separation is achieved. This is a successful alternative plan and has been and is used successfully at Mystic Aquarium to achieve gating goals.

#### *Contraceptive Use*

You state there may be a need to "supplement this plan with contraceptives," which would be done "on a seasonal basis." Explain what you mean by "should the need arise," and what parameters would be used to determine whether contraceptives are needed. Please provide the following information (*if necessary to ensure a safe contraceptive plan is developed in consultation with the appropriate specialists, specific protocols may be submitted at a later date for approval*):

- The type of contraceptive that would be used (drug name; dosage; route, frequency and duration of administration).
- The anticipated timing and duration of use of contraceptives.
- Monitoring protocols, anticipated adverse reactions, and mitigation measures you will use if whales have an adverse reaction including the veterinary plan for treatment if a whale exhibits an adverse reaction to the drug.

As stated in our submitted breeding prevention plan, physical separation of mature male and female belugas during periods of 'peak reproductive readiness' will be the primary method in which breeding will be prevented in the 5 permitted belugas. In the highly unlikely instance that oral contraceptives become necessary, a contraceptive plan would be developed in consultation with appropriate specialists and submitted to NMFS for approval. This plan would include type of contraception utilized, timing/duration of contraceptives, monitoring protocols, anticipated adverse reactions, mitigation measures utilized, and emergency management of possible adverse drug reactions.

#### *Emergency Protocols to Prevent Breeding*

- Describe protocols for when staff may need to take "appropriate steps to prevent breeding in the case of an emergency."

Staff will be sufficiently trained to assist in the breeding prevention plan and animal care supervisors will be trained and authorized to take appropriate steps (i.e. gating of animals into non-breeding groups) to prevent breeding (i.e.

pursue physical separation) when necessary. The term ‘emergency’ in this context was meant to describe a highly unlikely scenario in which a female beluga continues to refuse behavioral ultrasound layouts for reproductive monitoring.

- What are those emergency circumstances (e.g., if mating is occurring)? As stated above, the whales should be physically separated during an appropriate time frame to allow a sufficient buffer prior to and after the breeding season and minimize the need for any emergency interventions.

As stated in our submitted breeding prevention plan, physical separation during periods of peak reproductive readiness (monitored via ultrasound) will be the primary method in which breeding will be prevented in the 5 permitted belugas. The term ‘emergency’ in this context was meant to describe a highly unlikely scenario in which a female beluga continues to refuse behavioral ultrasound layouts for reproductive monitoring.

In the highly unlikely instance that a reproductively mature female beluga were to repeatedly refuse behavioral ultrasound layouts and utilization of the beluga lift was not feasible or not deemed appropriate at that time, the animal would be physically separated from mature male belugas to prevent breeding until an ultrasound could be safely performed and lack of follicular development confirmed.

#### *Identifying Whales in the Plan*

A table(s) may be helpful to summarize the following information:

- List the whales (by name, age, and sex) that would be subject to ultrasound, physical separation, and contraception.

Name	Sex	Age	Subject to:
Kharabali	F	6	Ultrasound, physical separation beginning at age 7
Sahara	F	6	Ultrasound, physical separation beginning at age 7
Jetta	F	6	Ultrasound, physical separation beginning at age 7
Havana	F	5	Ultrasound, physical separation beginning at age 7
Havok	M	5	Separated from mature female belugas listed above in their peak reproductive periods beginning at age 10

- For physical separation, please provide the composition of anticipated animal groupings including the whales currently at Mystic.
  - For the first several weeks the 5 imported belugas will remain in the holding and medical pool for gating training and hydraulic lift training and acclimation.
  - All animals but Havok have a gating behavior, so, we anticipate quick recovery of these behaviors once at Mystic.

- All 3 whales presently at Mystic Aquarium gate to the medical pool reliably and the medical pool will be used to separate animals during introductions.
- Once imported belugas are gating from holding pool to medical pool reliably, social introductions will begin:
  - 1<sup>st</sup> social grouping: 3 Mystic Aquarium whales in main pool; 5 imported whales in holding pool and medical pool
  - 2<sup>nd</sup> social grouping: Havok to medical pool and then to main to meet 3 Mystic Aquarium whales; 4 imported female belugas in holding pool and medical pool.
  - 3<sup>rd</sup> social grouping: Mystic Aquarium male gated to medical pool, all other gates open to allow mixing of the 2 Mystic Aquarium females, and 5 imported belugas; then bringing the 2 Mystic Aquarium belugas one at a time to holding pool so all Mystic Aquarium belugas are gated to medical and holding pools while imported belugas are all on main pool.
  - Population can be mixed as desired for short-term separations and management through this means of voluntary gating. Whales are not given access outside of where trainers are certain they will gate.
- To facilitate flexibility under circumstances for animal welfare and safety, please describe your decision-making process and protocols for unforeseen events, and how preventing breeding will be maintained under such circumstances via different animal groupings. This should include planning for contingencies in the event whales are not socially compatible or if health issues arise requiring isolation or regrouping.
- The priority is to maintain social groups that are compatible while adhering to the breeding prevention plan. This means incompatible animals will not be mixed. We have been able to train animals such that they can be socialized when supervised and separated when not supervised, yielding over time a successful acclimation into the group. Slow, steady, and planned supervised introductions utilizing positive reinforcement while animals are under stimulus control has worked for incompatible social groups/individuals in the past. Belugas, by nature, are a docile species and we have confidence in this technique for establishing the needed groupings.

#### *Training Whales*

- Describe the training level of the whales for the procedures proposed. List which animals will require use of the hydraulic lift in the medical pool for ultrasound and any other sampling.

The only sample collection that would require a beluga to be put on the hydraulic lift would be for the ultrasound behavior to monitor for follicular activity and the prevention of breeding as required in the permit if a beluga will not do the ultrasound behaviorally. All other research samples will be collected from the animals behaviorally as described in the research permit.

All the female whales intended for import will do a ventral layout which could allow ultrasound; Havana also has a dorsal layout behavior and ultrasound is possible in this position as well. All the female whales intended for import, except Kharabali, are currently in training for the side layout. We would start Kharabali's side layout training as a priority behavior upon arrival. All are presently learning to accept an ultrasound probe placed on their body.

- For those not trained, describe your plan to train them including when you propose to start training relative to this plan, and when you anticipate voluntary ultrasound and other sampling will be achievable for each whale.

Each beluga will have 2 primary trainers, each assigned voluntary sampling behaviors to train. These behaviors will be prioritized and include, but not limited to the following:

- 1) Behavioral layouts, rolls, manipulation
- 2) Fluke presents for blood collection
- 3) Medical Pool gating/separations
- 4) Medical Pool hydraulic lift desensitization

For those animals not fully trained in each behavior, primary trainers will develop training plans to recover established progress with each behavior upon their arrival at Mystic Aquarium. Training for these behaviors will begin immediately, with an emphasis on gating/separations as the primary focus for each animal. Recovery rate of established behaviors is subjective dependent upon several factors including, but not limited to the following:

- 1) Established training history of behavior
- 2) Acclimation progress in new environment/social group

Recovery of all established behaviors and training of unestablished behaviors should progress over a period of 2-3 months if all whales acclimate according to anticipated timeline based on Marineland training staff input and historical data. Separations will be trained over the course of the first month and will be prioritized to ensure breeding will not occur.

#### *Training Staff*

- Describe how many staff will be trained to implement the plan.

33 Staff will be trained:

- Director of Marine Mammals and Birds
- Assistant Curator of Marine Mammals
- 1 Supervisor
- 4 Assistant Supervisors
- 6 Senior Trainers
- 11 Trainers
- 9 Assistant Trainers

- Describe what “sufficiently trained” means for the day-to-day methods for monitoring reproductive readiness, maintaining physical separation, and administering contraceptives (as appropriate) as well as in taking appropriate steps to prevent breeding in the case of an emergency.

The Director of Marine Mammals and Birds will oversee and provide a written plan to which all staff will adhere. All staff will be trained in the following areas:

- A subset of 12 individuals from the list above will be trained to work with all belugas for separations and gating.
- All 33 staff listed above will be trained to operate and assist with manipulating and operating gates.
- 19 of the above staff will be trained to assist with feeding/working with belugas for successful separations.
- All Supervisors will be trained in the beluga separation plans and carry authority to make decisions to separate animals to prevent breeding.
- Supervisors are authorized to administer any medications prescribed by the Attending Veterinarian.

#### **Training Process**

- 1) A checkoff sheet, created by the Assistant Curator of Marine Mammals, tracks detailed separation guidelines and tasks critical to implement the prevention of breeding plan.
- 2) Each staff member will have a check sheet for training and demonstrating proficiency in carrying out each identified task of the written plan.
- 3) The Director of Marine Mammals and Birds, Assistant Curator of Marine Mammals and an Assistant Supervisor will be responsible for the training of each staff member and signing off when the staff has demonstrated successfully implementing the task.
- 4) Once an individual has been signed off on the checklist and approved, they then can execute or implement any portion of the prevention of breeding plan on which they have been trained.

- 5) Daily oversight of the prevention of breeding plan is the responsibility of the Director of Marine Mammals and Birds, Assistant Curator of Marine Mammals, Supervisor, and Assistant Supervisors.

#### *Consulting with Specialists*

- Please include a statement in the plan confirming you have consulted with the attending veterinarian(s) and other specialists experienced in beluga whale reproductive husbandry on the development of the contraceptive plan.
- Provide the names and qualifications of those other specialists.

As you know from correspondence submitted to NMFS, the specialists we have consulted all object to the risks run by imposition of the condition to prevent reproduction.

The beluga breeding prevention plan was created by American College of Zoological Medicine (ACZM) board certified veterinary specialists Dr. Allison Tuttle (DVM, Dipl. ACZM) and Dr. Jennifer Flower (DVM, MS, Dipl. ACZM) and included input and peer review from a number of colleagues inside and outside of the beluga community.

#### References include:

- O'Brien JK, KJ Steinman, T Schmidt, and TR Robeck. 2008. Semen collection, characterization, and artificial insemination in the beluga (*Delphinapterus leucas*) using liquid stored spermatozoa. *Reproduction, Fertility, and Development* 20: 770-783.
- Robeck ER, O'Brien JK, Atkinson S. 2018. Reproduction. In Frances M. D. Gulland, Leslie A. Dierauf, Karyl L. Whitman (Eds.) *CRC Handbook of Marine Mammal Medicine*. 3rd Edition. CRC Press (Taylor & Francis), Boca Raton, Florida, USA. pp. 169 – 207.
- Robeck TR, KJ Steinman, GA Montano et al. 2010. Deep intrauterine artificial inseminations using cryopreserved spermatozoa in beluga (*Delphinapterus leucas*). *Theriogenology* 74: 989-1001.
- Robeck TR, TL Schmidt, and S Osborn. et al 2015. Development of predictive models for determining fetal age-at-length in belugas (*Delphinapterus leucas*) and their application toward in-situ and ex situ population management. *Marine Mammal Science* 31:591-611.
- Robeck, TR, KJ Steinman, M Yoshioka et al. 2005. Estrous cycle characterization and artificial insemination using frozen thawed spermatozoa in the bottlenose dolphin (*Tursiops truncatus*). *Reproduction* 129: 659-674.
- Robeck TR, SL Monfort, PP Calle, et al. 2005. Reproduction, Growth, and Development in Captive Beluga (*Delphinapterus leucas*). *Zoo Biology* 24:29-49.
- Steinman KJ, KJ Obrien, SL Monfort, and TR Robeck. 2012. Characterization of the estrogen cycle in female beluga (*Delphinapterus leucas*) using endocrine monitoring and transabdominal ultrasound: Evidence of facultative induced ovulation. *General and Comparative Endocrinology*. 175: 389-397.

#### *Timing of Reviews*

- If the breeding season begins in February, annual review of the breeding prevention plan needs to allow sufficient time for deliberation and approval by the Office Director of any changes to the plan before January 1, when reproductive monitoring should begin, according to your permit application.
- We suggest the plan be reviewed off-season, for example in late summer or early fall, to allow the appropriate deliberations for the committee as well as NMFS' review and approval of any changes prior to the beginning of each breeding season.

The breeding prevention plan will be reviewed each year by October 1.

#### Comments on Amendment Request to Substitute Whales

To request an amendment to the permit to substitute the whales to be imported please provide the following information.

##### *Purpose of Amendment*

You mentioned on the November 20th call that three whales (identified in your email as Frankie, Mira, and Qila) were diagnosed with health problems during pre-transport health exams that included but may not be limited to (1) septic gastritis; (2) a candida infection; and (3) gastric ulceration, anemia, and low alkaline phosphatase.

- Briefly explain these conditions and why the animals are unsuitable to be imported

Frankie – Health concerns include esophagitis and gastric ulcerations with ongoing active regurgitation. Pre-shipment diagnostic testing showed elevated erythrocyte sedimentation rate (ESR), low alkaline phosphatase (ALP), high globulins, and low hematocrit. Pre-shipment fecal examination revealed high growth of *Clostridium* sp. This poses health concerns for safe and successful transport and acclimation.

Mira – Health concerns include a high level of *Candida* sp. from respiratory tract culture and elevated globulins on pre-shipment diagnostic testing. On pre-shipment physical examination, active *Candida* sp. lesions (fungal plaques) were observed in the blowhole. This poses health concerns for safe and successful transport and acclimation.

Qila – Health concerns include a diagnosis of septic gastritis with ongoing active regurgitation, an elevated ESR, and high globulins on pre-shipment diagnostic testing. This poses health concerns for safe and successful transport and acclimation.

- Indicate if they have any infectious disease that is potentially transmissible to other whales.

Respiratory *Candida* sp. infection is not typically considered to be highly transmissible to other belugas that are otherwise healthy and immunocompetent.

Gastrointestinal *Clostridium* sp. infection is potentially transmissible to other belugas via environmental contamination with infected feces.

Septic gastritis is not typically considered to be highly transmissible to other belugas that are otherwise healthy and immunocompetent.

- Include when the three ill animals were last examined, when they were diagnosed with these conditions, and when they were last examined prior to diagnosis.

Frankie – Pre-shipment examination and samples were collected on 10/28/20. Last bloodwork performed prior to pre-shipment examination was on 6/24/20.

Qila – Pre-shipment examination and samples were collected on 10/22/20. Last bloodwork performed prior to pre-shipment examination was on 6/4/20.

Mira – Pre-shipment examination and samples were collected on 10/22/20. Last bloodwork performed prior to pre-shipment examination was on 6/3/20.

##### *Information on the New Whales*

- Add the sex of each animal to the list you provided.

Havok- Male

Sahara- Female

Jetta- Female

- To determine the MMPA status of the whales, provide the location in Russia of captures from the wild of each parent of the new whales including all potential sires other than those for which we already have data (Andre and Kodiak). Could genetic testing and determination of stock(s) for each animal be done prior to importation?

It is not possible to conduct genetic testing of these animals prior to importation. There is no definitive information on the location of collection in Russia within records examined. We understand this means that the whales will be determined to be depleted by default.

- Indicate if and when these new whales have been examined. Given that, under the Animal Welfare Act regulations, whales must be accompanied by a health certificate signed by the attending veterinarian stating that each animal was examined within the prior 10 days and found to be in acceptable health for transport, what will you do if the whales proposed are not deemed in acceptable health prior to transport?

Havok, Sahara, and Jetta were examined most recently on November 23, 2020.

If belugas are not found to be healthy for transport, transport cannot occur.

Steve M. Car  
12/01/2020

## Amendment to Change Whales

Three of the five belugas intended for import per our permit have been diagnosed with health issues that prevent their transport as detailed below:

- Frankie – Health concerns diagnosed on 10/28/2020 include esophagitis and gastric ulcerations with ongoing active regurgitation. Pre-shipment diagnostic testing showed elevated erythrocyte sedimentation rate (ESR), low alkaline phosphatase (ALP), high globulins, and low hematocrit. Pre-shipment fecal examination revealed high growth of *Clostridium* sp. This poses health concerns for safe and successful transport and acclimation of this individual whale and gastrointestinal *Clostridium* sp. infection is potentially transmissible to other belugas via environmental contamination with infected feces.
- Mira – Health concerns diagnosed on 10/22/2020 include a high level of *Candida* sp. from respiratory tract culture and elevated globulins on pre-shipment diagnostic testing. On pre-shipment physical examination, active *Candida* sp. lesions (fungal plaques) were observed in the blowhole. This poses health concerns for safe and successful transport and acclimation of this individual whale. Respiratory *Candida* sp. infection is not typically considered to be highly transmissible to other belugas that are otherwise healthy and immunocompetent.
- Qila– Health concerns diagnosed on 10/22/2020 include a diagnosis of septic gastritis with ongoing active regurgitation, an elevated ESR, and high globulins on pre-shipment diagnostic testing. This poses health concerns for safe and successful transport and acclimation of this individual. Septic gastritis is not typically considered to be highly transmissible to other belugas that are otherwise healthy and immunocompetent.

These three belugas last received health checks prior to identification of the above issues in June of 2020.

As we detailed in our permit that only healthy whales would be transported, it is important these whales be replaced with three belugas that are of healthy status.

The below substitutions of whales with the same background and sexes as those approved in the permit are proposed.

### Havok (in lieu of Frankie)

Sex: M

DOB: August 10, 2015

Dam: Secord

Origin of Dam: Wild - Imported from Russia 12/06/2008

Potential Sires: Tuktoyaktuk, Orion

Origin of Sires: Wild - Both Imported from Russia 06/25/2005

\*\*Information on exact location of Russian origin of parents is not available and it is not possible to complete genetic testing prior to transport. We understand this will mean they are considered Depleted Status.

Sahara (in lieu of Mira)

Sex: F

DOB: July 23, 2014

Dam: Acadia

Origin of Dam: Wild - Imported from Russia 12/06/2008

Potential Sires: Andre, Kodiak, Orion

Origin of Sires: Wild - Imported from Russia 10/02/1999 (Andre), 06/25/2005 (Kodiak/Orion)

\*\*Information on exact location of Russian origin of parents is not available and it is not possible to complete genetic testing prior to transport. We understand this will mean they are considered Depleted status.

Jetta (in lieu of Qila)

Sex: F

DOB: July 17, 2014

Dam: Skyla

Origin of Dam: Wild, imported from Russia 6/23/2005

Potential Sires: Andre, Kodiak, Orion, Tuktoyaktuk

Origin of Sires: Wild - Imported from Russia 10/02/1999 (Andre), 06/25/2005 (Kodiak/Orion/Tuktoyaktuk)

\*\*Information on exact location of Russian origin of parents is not available and it is not possible to complete genetic testing prior to transport. We understand this will mean they are considered Depleted Status.

The above listed belugas were examined most recently on November 23, 2020 and found to be in good health. If the health of these belugas declines or belugas are not found to be healthy for transport, transport would not occur and an alternate animal would be selected.

*Step M. Con*  
*12/01/2020*

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UNITED STATES DEPARTMENT OF COMMERCE  
**National Oceanic and Atmospheric Administration**  
NATIONAL MARINE FISHERIES SERVICE  
1315 East-West Highway  
Silver Spring, Maryland 20910

Permit No. 22629

Expiration Date: August 31, 2025

Reports Due: November 30, annually

## PERMIT TO IMPORT AND TAKE PROTECTED SPECIES<sup>1</sup> FOR SCIENTIFIC PURPOSES

### I. Authorization

This permit is issued to Mystic Aquarium, 55 Coogan Boulevard, Mystic, Connecticut, 06355 (hereinafter "Permit Holder;" Responsible Party: Stephen M. Coan, Ph.D.), pursuant to the provisions of the Marine Mammal Protection Act of 1972 as amended (MMPA; 16 U.S.C. 1361 *et seq.*) and the regulations governing the taking and importing of marine mammals (50 CFR Part 216).

### II. Abstract

The objectives of the permitted activity, as described in the application, are to contribute knowledge and inform management and recovery of beluga whale (populations in the wild including the endangered Cook Inlet beluga whale distinct population segment and the depleted Sakhalin Bay-Nikolaya Bay-Amur River beluga whale stock. Research authorized includes the following Studies: 1) Neuroimmunological response to environmental and anthropogenic stressors; 2) Development of novel non-invasive techniques to assess health in free-ranging, stranded and endangered beluga whales; 3) Hearing and physiological response to anthropogenic sound; 4) Photogrammetry body condition studies; 5) Diving physiology; 6) Microbiome; and 8) Testing of prototype telemetry and imaging devices before deployment on wild beluga whales. This permit does not authorize Study 7 (Behavioral and reproduction studies) including breeding of any of the imported beluga whales but reproductive monitoring may be conducted as part of husbandry activities.

### III. Terms and Conditions

The activities authorized herein must occur by the means, in the areas, and for the purposes set forth in the permit application, and as limited by the Terms and Conditions specified in this permit, including appendices. Permit noncompliance constitutes a violation and is grounds for permit modification, suspension, or revocation, and for enforcement action.

<sup>1</sup>"Protected species" include species listed as threatened or endangered under the ESA, and marine mammals.





A. Duration of Permit

1. Personnel listed in Condition C.1 of this permit (hereinafter “Researchers”) may conduct activities authorized by this permit through August 31, 2025. This permit may be extended by the Director, National Marine Fisheries Service (NMFS) Office of Protected Resources or the Chief, Permits and Conservation Division (hereinafter “Permits Division”), pursuant to applicable regulations and the requirements of the MMPA.
2. Researchers must immediately stop permitted activities and the Permit Holder or Principal Investigator must contact the Chief, NMFS Permits and Conservation Division within two business days for written permission to resume:
  - a. If serious injury or mortality<sup>2</sup> of the subject beluga whales occurs (see Condition B.6.k).
  - b. If an imported female becomes pregnant or the imported male impregnates any female (not just one of the imported females), and again if a progeny is born, delivered stillborn, or miscarried (see Condition B.6.l).
  - c. If authorized take<sup>3</sup> is exceeded (i.e., the animals are taken in a manner not authorized by this permit or the number of takes is exceeded).
  - d. Following incident reporting requirements at Conditions B.6.k, B.6.l, and E.2.

B. Number and Kinds of Protected Species, Locations and Manner of Taking

1. The tables in Appendix 1 outline the authorized species and stocks; number of animals to be imported and taken; and the manner of take, locations, and time period.
2. Researchers working under this permit may collect images (e.g., photographs, video) and audio recordings in addition to the photo-documentation authorized in

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<sup>2</sup>This permit does not allow for unintentional serious injury and mortality caused by the presence or actions of researchers. This includes, but is not limited to: deaths resulting from infections related to sampling procedures; and deaths or injuries sustained by animals during transport/handling/restraint, or while attempting to escape restraint. Note that for marine mammals, a serious injury is defined by regulation as any injury that will likely result in mortality.

<sup>3</sup>By regulation, a take under the MMPA means to harass, hunt, capture, collect, or kill, or attempt to harass, hunt, capture, collect, or kill any marine mammal. This includes: the collection of parts by sampling; the restraint or detention of a marine mammal, no matter how temporary; or any negligent or intentional act which results in disturbing or molesting a marine mammal.




Appendix 1 as needed to document the permitted activities, provided the collection of such images or recordings does not result in takes.

3. The Permit Holder may use visual images and audio recordings collected under this permit, including those authorized in Tables 1 and 2 of Appendix 1, in printed materials (including commercial or scientific publications) and presentations provided the images and recordings are accompanied by a statement indicating that the activity was conducted pursuant to NMFS MMPA Permit No. 22629. This statement must accompany the images and recordings in all subsequent uses or sales.
4. The Chief, Permits Division may grant written approval for personnel performing activities not essential to achieving the research objectives (e.g., a documentary film crew in areas outside of normal incidental public display practices) to be present, provided:
  - a. The Permit Holder submits a request to the Permits Division specifying the purpose and nature of the activity, location, approximate dates, and number and roles of individuals for which permission is sought.
  - b. Non-essential personnel/activities will not influence the conduct of permitted activities or result in takes of protected species.
  - c. Persons authorized to accompany the Researchers for the purpose of such non-essential activities will not be allowed to participate in the permitted activities.
  - d. The Permit Holder and Researchers do not require compensation from the individuals in return for allowing them to accompany Researchers.
5. Researchers must comply with the following conditions related to the manner of research taking:
  - a. The Permit Holder must ensure that the authorized research has been reviewed and approved by the appropriate Institutional Animal Care and Use Committees (IACUC) in accordance with Animal Welfare Act regulations, and that the IACUC protocols are consistent with the research methods approved by this permit.
  - b. The animals undergoing research must be closely monitored to determine if research activities are having an adverse effect on the individuals. The attending veterinarian must be available for emergencies, illnesses, and for treating any health problems associated with the research procedures.



- c. The Researchers must halt and re-evaluate research should the animals exhibit signs of stress, pain, or suffering resulting from the authorized activities. If the animals are showing adverse reactions or are at risk of injury during the research, Researchers must immediately discontinue the activities.
  - d. For voluntary research procedures, the animals must be able to exit the research session at any time.
  - e. All research activities must be conducted in a humane manner (i.e., that which involves the least possible degree of pain and suffering), and, to the maximum extent possible, concurrent with the routine care and husbandry of the animals.
  - f. For masking hearing studies: Researchers must test the subject animals' hearing as soon as possible after each masking session for full recovery to ensure temporary threshold shift (TTS) has not occurred. If at any point TTS occurs and full recovery is not observed, researchers must discontinue further exposure until recovery to pre-testing levels is observed.
  - g. For blood sampling: Only an attending veterinarian or experienced, qualified personnel trained by and with appropriate oversight from an attending veterinarian may conduct blood sampling. Researchers must use sterile needles for blood sampling and minimize the number of needle insertions per blood collection site (e.g., no more than 2 insertions per site).
6. Personnel must comply with the following conditions related to methods of importation, supervision, care, and transportation:
- a. The marine mammals imported under the authority of this permit must be imported in a humane manner and in compliance with the MMPA and any applicable foreign law.
  - b. The importation of marine mammals is subject to the provisions of 50 CFR Parts 14 and 23 (i.e., the import of marine mammals must be conducted in accordance with the U.S. Fish and Wildlife Service regulations for the importation, exportation, and transport of wildlife and no marine mammals may be imported without the required CITES [Convention on the International Trade in Endangered Species of Wild Fauna and Flora] permits).

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- c. The Permit Holder must transport and maintain marine mammals used in captive research in U.S. Department of Agriculture, Animal and Plant Health Inspection Service (APHIS) registered research facilities and/or licensed public display facilities; and, marine mammals must be held and transported in compliance with the provisions of the Animal Welfare Act and its implementing regulations "Specifications for the Humane Handling, Care, Treatment, and Transportation of Marine Mammals" (9 CFR Part 3, Subpart E).
- i. A current copy of the APHIS research registration and/or license for any facility to be used must be attached to this permit. All medical records must accompany the animals to the destination facility.
  - ii. Prior to transport, Mystic Aquarium must have the travel plan documented at the receiving facility, and the animals must be accompanied by a health certificate
- d. The Permit Holder cannot import any marine mammal that is pregnant or lactating at the time of import.
- e. This permit does not authorize breeding of the five subject beluga whales. Prior to importation, the Permit Holder must submit a plan to provide safe and effective contraception or other means to prevent breeding of the five subject beluga whales, for approval by the Office Director. Any contraceptive plan must be developed in consultation with the licensed attending veterinarian(s) and other specialists experienced in beluga whale reproductive husbandry.
- f. To the maximum extent possible, the beluga whales must be trained for voluntary participation in husbandry and medical procedures.
- g. Any public display of the beluga whales authorized by this permit must be incidental to and not interfere with the research. Such incidental public display may only occur as part of an educational program. A portion of this program must describe the research activities; identify the status of the species and its endangered and depleted stocks; and, provide information regarding their natural history, distribution, population status, and threats.
- h. The beluga whales authorized by this permit must not be trained for performance or included in any interactive program with the public.



Public demonstrations in which the whales perform trained husbandry, medical, research-related, and natural behaviors are authorized.

- i. The beluga whales authorized by this permit may not be released into the wild unless such a release has been authorized under an amendment to this permit or a separate scientific research or enhancement permit issued for that purpose.
- j. Disposition: The Permit Holder shall not transport, transfer, export or otherwise dispose of the marine mammals authorized by this permit except with the approval of the Director, Office of Protected Resources, and subject to such Terms and Conditions as the Director may prescribe<sup>4</sup>.
- k. In addition to requirements of Condition A.2, in the event a beluga whale authorized by this permit dies, the Permit Holder must:
  - i. Contact the NMFS Marine Mammal Health and Stranding Response Program (nmfs.mmhsrp.hq@noaa.gov) and follow any recommended necropsy and sampling protocols.
  - ii. Within two weeks, submit an incident report as described in Condition E.2. Gross necropsy findings should be included as part of an incident report. Final necropsy results (e.g., gross findings, histology, and other analyses) must be submitted when complete.
- l. In addition to requirements of Condition A.2, the Permit Holder must, within two weeks, submit an incident report as described below and in Condition E.2:
  - i. In the event that a female becomes pregnant or the male impregnates any female (not just one of the imported females). This report must identify the whales by NOAA ID and when and how mating proceeded (if observed). In addition, the report must include information on the exact methods used to prevent conception, why the particular methods are hypothesized to have failed, what measures will be taken to prevent future pregnancies, and the plan for pre-natal care of the dam and for birth; and
  - ii. Again when the pregnancy ends, either when the progeny is born, delivered stillborn, or miscarried. This report must include a summary of how the birth proceeded, the status of the calf, and

<sup>4</sup>This includes transport of any of the imported whales to Georgia Aquarium and disposition of the whales at the termination of research.



current management of the dam and calf. The disposition of live progeny shall be determined by the Office Director.

7. The Permit Holder must comply with the following conditions, and the regulations at 50 CFR 216.37, for biological samples<sup>5</sup> acquired<sup>6</sup> or possessed under authority of this permit.
- a. The Permit Holder is ultimately responsible for compliance with this permit and applicable regulations related to the samples unless the samples are permanently transferred per Conditions at B.7.d (below).
  - b. Samples must be maintained according to accepted curatorial standards and must be labeled with a unique identifier (e.g., NOAA ID number) that is connected to on-site records with information identifying the following:
    - i. Animal ID, species, age, and sex;
    - ii. Date of collection;
    - iii. Type of sample (e.g., blood, saliva);
    - iv. Origin (i.e., where collected); and
    - v. Legal authorization for original sample collection.
  - c. For temporary transfers:
    - i. The Permit Holder may transfer samples to Authorized Recipients (ARs) identified in Appendix 2 and may designate additional ARs for analysis and curation of samples related to the permit objectives. The Permit Holder must maintain a record of the transfer including the following:
      - 1. Name and affiliation of the AR;
      - 2. Address of the AR;
      - 3. Types of samples sent (NOAA ID, species, tissue type);

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<sup>5</sup>Biological samples include, but are not limited to: carcasses (whole or parts); and any tissues, fluids, or other specimens from live or dead protected species; except feces, urine, and spew collected from the water or ground.

<sup>6</sup>Authorized methods of sample acquisition are specified in Appendix 1.

*for*

4. Type of analysis; and
  5. Whether samples will be consumed in analysis, returned to the Permit Holder, curated, or destroyed.
- ii. The Permit Holder must provide a written copy of the AR designation and the permit (per Condition D.3) when transferring samples to the AR.
  - iii. Samples remain in the legal custody of the Permit Holder while in the possession of ARs. The Permit Holder remains responsible for the samples, including any reporting requirements.
- d. If the Permit Holder wishes to permanently transfer marine mammal samples (i.e., relinquish custody), recipients must have separate authorization pursuant to 50 CFR 216.37 (e.g., a permit or regional authorization letter) prior to transfer.
  - e. Samples cannot be bought or sold.
  - f. After meeting the permitted objectives, the Permit Holder may continue to possess and use biological samples acquired under this permit, including after permit expiration, without additional written authorization. The samples must be maintained as specified in the permit and a copy of the permit must be kept with the samples forever.

C. Qualifications, Responsibilities, and Designation of Personnel

1. At the discretion of the Permit Holder, the following Researchers may participate in the conduct of the permitted activities in accordance with their qualifications and the limitations specified herein:
  - a. Principal Investigator – Tracy Romano, Ph.D.
  - b. Co-Investigators – See Appendix 2 for list of names and corresponding activities.
  - c. Research Assistants – Personnel identified by the Permit Holder or Principal Investigator and qualified to act pursuant to Conditions C.2, C.3, and C.4 of this permit.

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2. Individuals conducting permitted activities must possess qualifications commensurate with their roles and responsibilities. The roles and responsibilities of personnel operating under this permit are as follows:
  - a. The Permit Holder is ultimately responsible for activities of individuals operating under the authority of this permit. Where the Permit Holder is an institution/facility, the Responsible Party is the person at the institution/facility who is responsible for the supervision of the Principal Investigator.
  - b. The Principal Investigator (PI) is the individual primarily responsible for the taking, import, export and related activities conducted under the permit. This includes coordination of activities of all personnel working under the permit. The PI must be on site during activities conducted under this permit unless a Co-Investigator named in Condition C.1 is present to act in place of the PI.
  - c. Co-Investigators (CIs) are individuals who are qualified to conduct activities authorized by the permit, for the objectives described in the application, without the on-site supervision of the PI. CIs assume the role and responsibility of the PI in the PI's absence.
  - d. Research Assistants (RAs) are individuals who work under the direct and on-site supervision of the PI or a CI. RAs cannot conduct permitted activities in the absence of the PI or a CI.
3. Personnel involved in permitted activities must be reasonable in number and essential to conduct of the permitted activities. Essential personnel are limited to:
  - a. Individuals who perform a function directly supportive of and necessary to the permitted activity (including operation of vessels or aircraft essential to conduct of the activity),
  - b. Individuals included as backup for those personnel essential to the conduct of the permitted activity, and
  - c. Individuals included for training purposes.
4. Persons who require state or Federal licenses or authorizations (e.g., veterinarians) to conduct activities under the permit must be duly licensed/authorized and follow all applicable requirements when undertaking such activities.

5. The Permit Holder cannot require or receive direct or indirect compensation from a person approved to act as PI, CI, or RA under this permit in return for requesting such approval from the Permits Division.
6. The Permit Holder or PI may add CIs by submitting a request to the Chief, Permits Division that includes a description of the individual's qualifications to conduct and oversee the activities authorized under this permit. If a CI will only be responsible for a subset of permitted activities, the request must also specify the activities for which they would provide oversight.
7. The Responsible Party may request a change of PI by submitting a request to the Chief, Permits Division that includes a description of the individual's qualifications to conduct and oversee the activities authorized under this permit.
8. Submit requests to add CIs or change the PI by one of the following:
  - a. The APPS system at <https://apps.nmfs.noaa.gov>;
  - b. An email attachment to the permit analyst for this permit; or
  - c. A hard copy mailed or faxed to the Chief, Permits Division, Office of Protected Resources, NMFS, 1315 East-West Highway, Room 13705, Silver Spring, MD 20910; phone (301)427-8401; fax (301)713-0376.

D. Possession of Permit

1. This permit cannot be transferred or assigned to any other person.
2. The Permit Holder and persons operating under the authority of this permit must possess a copy of this permit when:
  - a. Engaged in a permitted activity.
  - b. A protected species is in transit incidental to a permitted activity.
  - c. A protected species imported or taken under the permit is in the possession of such persons.
3. A duplicate copy of this permit must accompany or be attached to the container, package, enclosure, or other means of containment in which a protected species or protected species part is placed for purposes of storage, transit, supervision or care.



E. Reporting

1. The Permit Holder must submit incident and annual reports containing the information and in the format specified by the Permits Division.
  - a. Reports must be submitted to the Permits Division by one of the following:
    - i. The APPS system at <https://apps.nmfs.noaa.gov>;
    - ii. An email attachment to the permit analyst for this permit; or
    - iii. A hard copy mailed or faxed to the Chief, Permits Division (see Condition C.8).
  - b. You must contact your permit analyst for a reporting form if you do not submit reports through the APPS.
  - c. Additional information on reports can be found at <https://www.fisheries.noaa.gov/national/reports-protected-species-permits>.
2. Incident Reporting
  - a. If a serious injury or mortality occurs, authorized takes have been exceeded, upon diagnosis of an unauthorized pregnancy, or following the end of pregnancy (i.e., birth, stillbirth, or miscarriage of progeny), as specified in Condition A.2, the Permit Holder must:
    - i. Contact the Permits Division by phone (301-427-8401) as soon as possible, but no later than two business days of the incident;
    - ii. Submit a written report within two weeks of the incident as specified below; and
    - iii. Receive approval from the Permits Division before resuming research. The Permits Division may grant authorization to resume permitted activities based on review of the incident report and in consideration of the Terms and Conditions of this permit.
  - b. The incident report must include 1) a complete description of the events, and 2) identification of steps that will be taken to reduce the potential for additional incidents (see Conditions B.6.k for deaths, B.6.l.i for



unauthorized pregnancy, and Condition B.6.l.ii for a birth, stillbirth, or miscarriage).

3. Annual reports describing activities conducted during the previous permit year (from September 1 to August 31) must:
  - a. Be submitted by November 30 each year for which the permit is valid, and
  - b. Include a tabular accounting of takes and a narrative description of activities and their effects.
4. A joint annual/final report including a discussion of whether the objectives were achieved must be submitted by November 30, 2025, or, if the research concludes prior to permit expiration, within 90 days of completion of the research.
5. Research results must be published or otherwise made available to the scientific community in a reasonable period of time. Copies of technical reports, conference abstracts, papers, or publications resulting from permitted research must be submitted the Permits Division upon request.

F. Notification and Coordination

1. Inventory Reporting:
  - a. Inventory reports and notifications must be submitted to the Permits Division by one of the following:
    - i. Email (NIMM.Inventory@noaa.gov); or
    - ii. Mail (NMFS Permits and Conservation Division (F/PR1), 1315 East-West Hwy, Silver Spring, MD 20910).
  - b. Upon completion of the importation from Canada, Mystic Aquarium must complete the attached Marine Mammal Data Sheets (MMDS) for the subject animals and submit as specified above in Condition F.1.
  - c. In the event of a death of a beluga whale held under the authority of this permit, the Permit Holder must submit an updated MMDS within 30 days. A copy of the necropsy report, histopathology, and any other relevant reports must be submitted when available (see Conditions B.6.k and E.2).
  - d. If an unauthorized pregnancy results in a live birth, the Permit Holder must submit an MMDS for the calf concurrently with the incident report



(see Condition B.6.1.ii and E.2). A blank MMDS can be obtained at <https://www.fisheries.noaa.gov/webdam/download/70834590>.

- e. The Permit Holder must provide written notification of any authorized transfers or transports (pursuant to Condition B.6.j), in the requested format (<https://www.fisheries.noaa.gov/webdam/download/70834588>), at least 15 days prior to the authorized transport/transfer. Authorized transfers/ transports must be verified within 30 days by submitting an updated MMDS.
  - f. The Permit Holder must review and verify the accuracy of its Marine Mammal Inventory upon request.
2. Researchers must coordinate permitted activities with activities of other Permit Holders conducting the same or similar research on beluga whales. Contact the Permits Division to obtain contact information for coordinating with other Permit Holders.

G. Observers and Inspections

- 1. NMFS may review activities conducted under this permit. At the request of NMFS, the Permit Holder must cooperate with any such review by:
  - a. Allowing an employee of NOAA or other person designated by the Director, NMFS Office of Protected Resources to observe and document permitted activities; and
  - b. Providing all documents or other information relating to the permitted activities.

H. Modification, Suspension, and Revocation

- 1. Permits are subject to suspension, revocation, modification, and denial in accordance with the provisions of subpart D [Permit Sanctions and Denials] of 15 CFR Part 904.
- 2. The Director, NMFS Office of Protected Resources may modify, suspend, or revoke this permit in whole or in part:
  - a. In order to make the permit consistent with a change made after the date of permit issuance with respect to applicable regulations prescribed under Section 103 of the MMPA;



- b. In a case in which a violation of the terms and conditions of the permit is found;
  - c. In response to a written request<sup>7</sup> from the Permit Holder; and
  - d. If NMFS determines that the application or other information pertaining to the permitted activities (including, but not limited to, reports pursuant to Section E of this permit and information provided to NOAA personnel pursuant to Section G of this permit) includes false information.
3. Issuance of this permit does not guarantee or imply that NMFS will issue or approve subsequent permits or amendments for the same or similar activities requested by the Permit Holder, including those of a continuing nature.

I. Penalties and Permit Sanctions

- 1. A person who violates a provision of this permit, the MMPA, or the regulations at 50 CFR 216 is subject to civil and criminal penalties, permit sanctions, and forfeiture as authorized under the MMPA and 15 CFR Part 904.
- 2. The NMFS Office of Protected Resources shall be the sole arbiter of whether a given activity is within the scope and bounds of the authorization granted in this permit.
  - a. The Permit Holder must contact the Permits Division for verification before conducting the activity if they are unsure whether an activity is within the scope of the permit.
  - b. Failure to verify, where the NMFS Office of Protected Resources subsequently determines that an activity was outside the scope of the permit, may be used as evidence of a violation of the permit, the MMPA, and applicable regulations in any enforcement actions.

J. Acceptance of Permit

- 1. In signing this permit, the Permit Holder:
  - a. Agrees to abide by all terms and conditions set forth in the permit, all restrictions and relevant regulations under 50 CFR Part 216 and all restrictions and requirements under the MMPA;

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<sup>7</sup>The Permit Holder may request changes to the permit related to: the objectives or purposes of the permitted activities; the species or number of animals taken; and the location, time, or manner of taking or importing protected species. Such requests must be submitted in writing to the Permits Division in the format specified in the application instructions.

- b. Acknowledges that the authority to conduct certain activities specified in the permit is conditional and subject to authorization by the Office Director; and
- c. Acknowledges that this permit does not relieve the Permit Holder of the responsibility to obtain any other permits, or comply with any other Federal, State, local, or international laws or regulations.

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Donna S. Wieting  
Director, Office of Protected Resources  
National Marine Fisheries Service

Date Issued



Stephen M. Coan, Ph.D.  
President and CEO, Mystic Aquarium  
Responsible Party

12/01/2020  
Date Effective

## Appendix 1: Tables Specifying the Kinds of Protected Species, Locations, and Manner of Taking

Table 1. Importation and captive maintenance of five beluga whales for over the duration of the permit. Importation from Marineland of Canada, Inc. (Niagara Falls, Ontario, Canada) to Mystic Aquarium (Mystic, Connecticut) pursuant to MMPA Section 104 for scientific research (see Table 2). Captive maintenance pursuant to MMPA Section 112(c) (captive maintenance) and Section 104 (scientific research) at Mystic Aquarium. Transport from Mystic Aquarium to Georgia Aquarium (Atlanta, Georgia) under the same authorities for captive maintenance and scientific research if approved by the Office Director per Condition B.6.j. Captive maintenance includes husbandry, health assessments, and medical sampling; treatments as warranted by the attending veterinarian<sup>1</sup>; and, humane euthanasia if warranted for medical reasons, and necropsy. Beluga whales may be displayed to the public incidental to research.

Species	Stock <sup>2,3</sup>	Origin	Current Life Stage	Sex	No. Animals	Activities Procedures	Details
Whale, beluga	Offspring of dam Aurora (wild capture, Sea of Okhotsk, Russia); sire Kodiak (wild capture, Sea of Okhotsk, Russia)	Captive born	Juvenile; Born 07/20/14	Female	1	Import; Captive, maintain permanent; Transport	KHARABALI; NOA0010671
Whale, beluga	Offspring of dam Oceanna (wild capture, Sea of Okhotsk, Russia); sire Andre (wild capture, Barents or White Sea, Russia)	Captive born	Adult; Born 07/13/09	Female	1	Import; Captive, maintain permanent; Transport	MIRA; NOA0010672
Whale, beluga	Offspring of dam Isis (wild capture, Sea of Okhotsk, Russia); sire definitively unknown, but thought to be Andre (wild capture, Barents or White Sea, Russia)	Captive born	Adult; Born 6/6/10	Female	1	Import; Captive, maintain permanent; Transport	QILA; NOA0010673
Whale, beluga	Offspring of dam Sierra (wild capture, Sea of Okhotsk, Russia); sire Andre (wild capture, Barents or White Sea, Russia)	Captive born	Juvenile; Born 6/11/12	Male	1	Import; Captive, maintain permanent; Transport	FRANKIE; NOA0010674
Whale, beluga	Offspring of dam Kelowna (wild capture, Sea of Okhotsk, Russia); sire Andre (wild capture, Barents or White Sea, Russia)	Captive born	Juvenile; Born 07/23/15	Female	1	Import; Captive, maintain permanent; Transport	HAVANA; NOA0010675

<sup>1</sup>This includes reproductive monitoring (described in Study 7 in the permit application and including behavioral observations [video monitoring], biological sampling [blood, breath, and vaginal swabs], and ultrasound) and contraception or other means (e.g., physical separation) to prevent breeding.

<sup>2</sup>NMFS considers the parents captured in the Sea of Okhotsk to be from the depleted Sakhalin Bay-Nikolaya Bay-Amur River Stock, and Kharabali is thus considered to be a member of that depleted stock. For the purposes of this permit, NMFS is treating the whales with mixed-stock parentage as members of the depleted stock.


<sup>3</sup>The permit application stated that Andre was collected from the Barents Sea. However, there is evidence suggesting Andre may have been collected from the White Sea and that the Barents and White Sea populations may be of one stock.

**Table 2. Authorized annual research takes at Mystic Aquarium (or Georgia Aquarium if approved by the Office Director per Condition B.6.j) for the five beluga whales identified in Table 1. No unintentional serious injury or mortality caused by the research activities is authorized.**

Line	Species	Life Stage	Sex	No. Animals	Takes Per Animal <sup>11</sup>	Procedure	Details
1	Whale, beluga	Adult and Juvenile	Male and Female	5	83	Sample, blood	<p>Routine blood samples: 2x month x 12 months/per year = 24 samples/whale/year (24 takes/whale/year) for use in characterization of the nervous and immune systems, reagent and assay development (Study 1); to help validate measurements from other tissue matrices (Study 2, Study 5); and for in vitro diving studies (Study 5)</p> <p>Blood samples for diurnal variation assessment (Study1): 4 time points per day x 4 days/per year (one in each season) = 16 samples/whale/year (4 takes/whale/year)</p> <p>Blood samples before and after transport (Study 1): 4 time points (1 baseline, 1 upon arrival, 2 post-transport - under behavioral control) = 4 samples/whale/transport (3 takes/whale/year)</p> <p>Blood samples for OWEs associated with being lifted out of the water via a hydraulic lift for weights or veterinary examination (Study 1): 5 time points x 4 OWE (one in each season) = 20 samples/whale/year (8 takes/whale/year)</p> <p>Blood samples for novel training exercises and/or novel social interactions (Study1): 2 samples (1 before and after training session) x 12 sessions (6 control and 6 experimental) x 3 novel training exercises = 72 samples/whale/year (36 takes/whale/year)</p> <p>Blood samples for diving physiology (Study 5): 2 dive activities (1 stationary dive, 1 active dive) x 2 durations x 2 blood samples (1 before and after the dive) x 2 repetitions = 16 samples/whale/year (8 takes/whale/year)</p>

Line	Species	Life Stage	Sex	No. Animals	Takes Per Animal <sup>9</sup>	Procedure	Details
2	Whale, beluga	Adult and Juvenile	Male and Female	5	222	Sample, exhaled air (i.e., breath)	<p>Breath samples for gene expression, immune components, hormone measurements (Study 2): 2x per week x 50 weeks/year x 3 plates per session = 300 samples/whale/year (100 takes/whale/year)</p> <p>Breath samples for diurnal variation assessment (Study 2): 4 time points per day x 4 days/year (one in each season) = 16 samples/whale/year (4 takes/whale/year)</p> <p>Breath samples before, during and after transport (Study 2): 13 time points (1 baseline, 2, 4, 6, 8, 10 hour during transport, 1 upon arrival, 1, 2, 4, 6, 12, 24 hour post transport = 13 samples /whale/year (3 takes/whale/year)</p> <p>Breath samples for out of water events associated with being lifted out of the water via a hydraulic lift for weights or veterinary examination (Study 2): 9 time points x 4 OWE (one in each season) = 36 samples/whale/year (12 takes/whale/year)</p> <p>Breath samples for novel training exercises/interactions (Study 2): 2 samples (1 before and after training session) x 12 sessions (6 control and 6 experimental) x 3 novel training exercises/interactions = 72 samples/whale/year (36 takes/whale/year)</p> <p>Breath samples for transition to collect breath on wild whales (Study 2): 8 samples x 3 replicates = 48 samples (6 takes) plus 6 breaths x 2 methods = 12 samples (12 takes) for a total of 60 sample/whale/year (18 takes/whale/year)</p> <p>Breath samples for diving physiology (Study 5): 2 dive activities (1 stationary dive, 1 active dive) x 2 durations x 3 breath samples x 2 repetitions = 24 breath samples /whale/year (8 takes/whale/year)</p> <p>Breath samples for hearing and physiological response to anthropogenic sound (Study 3): Baseline = 5 sessions year (1 breath sample before and after) = 10 breath samples/whale/year (5 takes/whale/year)</p> <p>Breath samples for masked hearing sessions (Study 3): up to 3 sessions per day, 6 days per week, 2 weeks per month, for 3 months per year = 108 sessions/year x 2 breath samples (1 before and after) = 216 breath samples/whale/year (36 takes/whale/year)</p>

Line	Species	Life Stage	Sex	No. Animals	Takes Per Animal <sup>9</sup>	Procedure	Details
3	Whale, beluga	Adult and Juvenile	Male and Female	5	155	Sample, saliva	<p>Other = Saliva samples for development, validation, and measurement of hormones (Study 2): 2x per week x 50 weeks/year = 100 samples/whale/year (100 takes/whale/year)</p> <p>Saliva samples for diurnal variation assessment (Study 2): 4 time points per day x 4 days/per year (one in each season) = 16 samples/whale/year (4 takes/whale/year)</p> <p>Saliva samples before and after transport (Study 2): 8 time points (1 baseline, 1 upon arrival, 1, 2, 4, 6, 12, 24 hour post transport = 8 samples/whale/ transport (3 takes/whale/year)</p> <p>Saliva samples for out of water events associated with weights or veterinary examinations: 8 time points x 4 OWE (one in each season) (Study 2) = 32 samples/whale/year (12 takes/whale/year)</p> <p>Saliva samples for novel training exercises and/or social interactions (Study 2): 2 samples (1 before and after training session) x 12 sessions (6 control and 6 experimental) x 3 novel training exercises/interactions = 72 samples/whale/ year (36 takes/whale/year)</p>
4	Whale, beluga	Adult and Juvenile	Male and Female	5	115	Sample, feces	<p>Fecal samples for development, validation, and measurement of hormones (Studies 2 and 7): 2x per week x 50 weeks = 100 samples/year (100 takes/whale/year)</p> <p>Fecal samples before and after transport (Study 2): 6 time points (1 baseline, 6, 12, 24, 36, 48 hours post transport = 6 samples/whale/ transport (3 takes/whale/year)</p> <p>Fecal samples for out of water events associated with weights or veterinary examinations: 5 time points x 4 OWE (one in each season) = 20 samples/whale/ year (12 takes/whale/year)</p>
5	Whale, beluga	Adult and Juvenile	Male and Female	5	200	Sample, skin	Skin scrapes for development, validation, and measurement of gene expression (Study 2): 4x per week x 50 weeks = 200 samples/year (200 takes/whale/year)
6	Whale, beluga	Adult and Juvenile	Male and Female	5	100	Sample, swab	Skin swabs for microbiome (Study 6): 2x per week x 50 weeks = 100 samples/whale/year (100 takes/whale/year)
7	Whale, beluga	Adult and Juvenile	Male and Female	5	100	Sample, swab	Blowhole swabs for microbiome (Study 6): 2x per week x 50 weeks = 100 samples/whale/year (100 takes/whale/year)
8	Whale, beluga	Adult and Juvenile	Male and Female	5	100	Sample, swab	Anal swabs for microbiome (Study 6): 2x per week x 50 weeks = 100 samples/whale/year (100 takes/whale/year)



Line	Species	Life Stage	Sex	No. Animals	Takes Per Animal <sup>9</sup>	Procedure	Details
9	Whale, beluga	Adult and Juvenile	Male and Female	5	100	Sample, swab	Oral swabs for microbiome (Study 6): 2x per week x 50 weeks = 100 samples/whale/year (100 takes/whale/year)
10	Whale, beluga	Adult and Juvenile	Female	4	100	Sample, swab	Vaginal swabs for microbiome (Study 6): 2x per week x 50 weeks = 100 samples/female whale/year (100 takes/whale/year)
11	Whale, beluga	Adult and Juvenile	Male and Female	5	360	Photo-grammetry	Photogrammetry (Study 4) 30 photographs/month x 12 months/year = 360 takes/whale/year
12	Whale, beluga	Adult and Juvenile	Male and Female	5	12	Measure	Morphometric measurements (Study 4): 1 set of measurements/month x 12 months = 12 takes/whale/year
13	Whale, beluga	Adult and Juvenile	Male and Female	5	4	Weigh	Weights (Study 4): 4 weights per year = 4 takes/whale/year (Study 4)
14	Whale, beluga	Adult and Juvenile	Male and Female	5	150	Instrument, suction-cup tag	Testing suction-cups for animal borne imaging (ABI) systems and tags (Study 8): 3 sessions per week x 50 weeks = 150 takes/ whale/year
15	Whale, beluga	Adult and Juvenile	Male and Female	5	41	Auditory brainstem response test	Hearing and physiological response to anthropogenic sound (Study 3): Baseline hearing sessions: 5 sessions/whale/year (5 takes/whale/year)  Masked hearing sessions: up to 3 sessions per day, 6 days per week, 2 weeks per month, for 3 months per year = 108 sessions/whale/year (36 takes/whale/year).
<sup>11</sup> All research procedures conducted in a single day for a given row are counted as 1 take per day per animal.							



## Appendix 2: NMFS-Approved Personnel for Permit No. 22629.

Table 1. The following individuals are approved personnel pursuant to the terms and conditions under Section C (Qualifications, Responsibilities, and Designation of Personnel) of this permit.

Name/Affiliation	Role	Authorized Activities
Tracy Romano, Ph.D., Mystic Aquarium, Mystic, CT	Principal Investigator and Authorized Recipient	Supervise and perform all activities under the permit
Allison D. Tuttle, DVM, Dipl. ACZM, Mystic Aquarium, Mystic, CT	Co-investigator and Authorized Recipient	Oversee the beluga whale husbandry and veterinary program at Mystic Aquarium; oversee beluga whale health and sampling procedures for all activities.
Jennifer Flower, DVM, MS, Dipl. ACZM, Mystic Aquarium, Mystic, CT	Co-investigator and Authorized Recipient	Oversee beluga whale health and sampling procedures for all activities
Laura Thompson, Ph.D., Mystic Aquarium, Mystic, CT	Co-investigator and Authorized Recipient	Conduct breath and swab sampling and analyze samples
Ebru Unal, Ph.D., Mystic Aquarium, Mystic, CT	Co-investigator and Authorized Recipient	Conduct breath and swab sampling and analyze samples
Maureen Driscoll, Ph.D., Mystic Aquarium, Mystic, CT	Co-investigator and Authorized Recipient	Conduct breath and swab sampling and analyze samples
Manuel Castellote, Ph.D., University of Washington and Alaska Fisheries Science Center, NOAA Fisheries, Seattle, WA	Co-investigator	Conduct auditory evoked potentials (AEPs)/hearing studies
Aran Mooney, Ph.D., Woods Hole Oceanographic Institution, Woods Hole, MA	Co-investigator	Conduct AEPs/hearing studies
Greg Marshall, Marshall Innovation, Washington DC	Co-investigator	Conduct Critter Cam studies (external camera attachment via suction cups)
Justin Richard, Ph.D., University of Rhode Island, Kingston, RI	Authorized Recipient	Analyze breath samples
Dennis Christian, Georgia Aquarium, Atlanta, GA	Co-investigator	Oversee beluga whale husbandry and veterinary program at Georgia Aquarium (if whales are moved there, per Condition B.6.j)