

Appendix 4

PROPOSED RESEARCH PROGRAM TO ADDRESS DECLINING CALF PRODUCTION IN NORTH ATLANTIC RIGHT WHALES

Prepared (post-workshop) by Linda Munson, University of California, Davis

OVERVIEW: This tiered approach to investigating the cause(s) of declining calf production begins by assessing whether male or female infertility (or both) is most likely. It then proposes more in-depth studies of males and females involving comparisons of subpopulations of fertile and infertile animals. The focus of these studies is reproductive failure, so worthwhile projects that focus on other important issues that may be affecting right whale survival (e.g. nutrition, health, toxin levels, etc) would be included only if they were first shown to be associated with reproductive failure. More in-depth studies are outlined in the main report text under agenda item 4.

HYPOTHESIS 1: FEMALES ARE INFERTILE. Studies need to determine, first, whether the increased calving intervals in the population are due to infertility of individuals, infertility of females in some subpopulations (e.g. BOF vs non-BOF), or decreased fertility in the population as a whole. This step is necessary for the design and focus of subsequent studies.

Methods:

ANALYZE EXISTING CATALOGUED DATA ON AN INDIVIDUAL BASIS TO DETERMINE IF NON-CALVING FEMALES ARE CLUSTERED BY AGE (SUGGESTING A PROBLEM WITH DEVELOPMENT) OR BY REGION OR TIME-PERIOD (SUGGESTING A PROBLEM RELATED TO GENETICS, BIOTOXINS, CONTAMINANTS, DISEASE OR NUTRITIONAL STRESS).

Increase direct-observation and photo-identification records to determine which animals are calving.

IF SOME FEMALES ARE FOUND TO BE INFERTILE, THEN:

SUB STUDY I: Investigate the reproductive status of infertile females.

Exp. 1: **Determine if infertile females are cycling.** This step is necessary to determine if ovarian cyclicity is normal.

If females that had calves are no longer cycling, then causes of ovarian quiescence, such as inadequate nutrition, stress, toxins, or infectious diseases, can be investigated.

If nulliparous females are not cycling, then genetic causes could be added to the above list.

If females are cycling but not becoming pregnant, then male fertility, lack of access to males, or uterine disease should be investigated.

Methods:

Determine ovarian activity by ovarian steroid analysis.

Validate blowhole exudate method so that enough sequential samples to determine cyclicity can be acquired even during periods of fasting (not feasible with fecal steroid analysis). This validation also is necessary for Experiment 2.

Increase observations of whales in estrus/breeding activity.

Conduct complete gross and histopathologic analyses on all ovaries and uteri available from carcasses to assess folliculogenesis, ovulation, and presence of any diseases. Compare findings between fertile and infertile females, if possible.

Exp.2: Determine if females are becoming pregnant, but subsequently losing their calves (abortion or neonatal deaths)

Methods:

Measure progesterone levels in feces or blowhole exudate during estimated mid-gestation, then follow the same females to determine calf production and survival.

INCREASE OBSERVATIONS ON THE CALVING GROUNDS.

SUB STUDY II. Determine if there are differences in health status between fertile and infertile females:

The design of these studies is contingent on there being two identifiable populations of whales, 1) infertile animals and 2) fertile animals. 'Infertility' should be defined from what is known concerning fecundity for this species, combined with some level of proof that the animals had the opportunity to breed. These two study populations would be compared for all subsequent nutritional and toxicologic studies. If two populations cannot be defined, it will hamper interpretation of significance of toxin levels, stress indicators, and body condition.

Exp.1: Determine if there is evidence of poor health in infertile females

Methods:

Compare blubber thickness, blubber quality, or other body condition indices between fertile and infertile animals. *If infertile females are in poorer condition, then conduct studies to determine if nutrition or underlying disease is the cause:*

DETERMINE HABITAT PREFERENCES OF FEMALES WITH LOWER SCORES.

CONDUCT NUTRITIONAL ANALYSIS OF FOOD SOURCES TO ASSESS DIET QUALITY IN HABITATS OF ANIMALS WITH HIGH AND LOW BODY CONDITION INDICES.

REVIEW PREY PATCH FORMATION, HISTORY OF SPATIAL/TEMPORAL DISTRIBUTION AND ENVIRONMENTAL VARIABLES.

Determine character of skin lesions and compare prevalence of skin lesions between fertile and infertile females. *If higher prevalence in infertile females, then:*

Assess if lesions indicate a primary skin disease or are a secondary manifestation of systemic disease or poor nutritional status.

ANALYZE TEMPORAL TRENDS AND HABITAT PREFERENCES OF WHALES WITH SKIN LESIONS.

Compare prevalence of other lesions (available from necropsy data) between fertile and infertile females.

Compare levels of cell stress indicators between fertile and infertile females.

Exp 2. Determine if infertile females have higher levels of toxins than fertile females. THESE STUDIES SHOULD FOCUS ON TOXIC CHEMICALS THAT ARE KNOWN TO IMPAIR REPRODUCTION OR GENERAL HEALTH IN OTHER SPECIES.

Methods:

Re-analyze existing contaminant data, comparing fertile and infertile females and their habitat preferences.

Analyze tissues, fluids and feces for biotoxins in fertile and infertile females. *If levels are higher in infertile females, then:*

Conduct in-depth studies on zooplankton sources and temporal and spatial distribution.

Analyze tissues and fluids of fertile and infertile females for a targeted list of contaminants. *If levels are higher in infertile females, then:*

Use a 'food-web'-based approach to determine exposure.

Analyze biomarkers of toxic exposure (e.g. Cyp1A, DNA adducts, retinoids, leptins) and compare levels in fertile and infertile females.

Exp 3. Determine if infertile females have higher levels of stress than fertile females. Cortisol measurements would probably provide the most reliable indications of stress as cortisol affects reproduction. It can suppress ovarian cycling and is usually elevated with acute and chronic stress.

Methods:

Validate and measure cortisol metabolites in blowhole exudate or feces and compare levels between fertile and infertile animals.

SUB STUDY III. Determine if there are genetic differences between fertile and infertile populations.
Because lack of genetic diversity does not necessarily affect reproduction or health, this study is important to assess potential genetic effects.

Exp. 1: Determine inbreeding coefficients for whales with and without reproductive success

Methods:

Pedigree analysis based on existing observational and molecular genetics data.

HYPOTHESIS 2: MALES ARE INFERTILE

Because of the competitive mating strategy of this species, a single infertile male would not be expected to affect calf production. However, infertility in groups of males partitioned by region or social structure could affect calf production. 'Infertility' implies that an animal had the opportunity to breed, so evidence of contact with females would be important before considering a male infertile. These first analyses would determine if there is any evidence of male infertility. The population could be divided into two groups, 1) fertile males and 2) infertile males, for subsequent comparative studies.

Methods:

ESTABLISH PATERNITY OF CALVES BORN DURING CRITICAL AND BASELINE PERIODS TO ASSESS WHETHER THERE IS EVIDENCE OF REGIONAL (BOF VS. NON-BOF) OR TEMPORAL MALE INFERTILITY.

Analyze existing catalogued data on individuals to determine if breeding activity has been observed.

ANALYZE EXISTING CATALOGUED DATA ON AN INDIVIDUAL BASIS TO DETERMINE IF INFERTILE MALES ARE CLUSTERED BY AGE (SUGGESTING A PROBLEM WITH DEVELOPMENT) OR BY REGION OR TIME-PERIOD (SUGGESTING A PROBLEM RELATED TO GENETICS, BIOTOXINS, CONTAMINANTS, DISEASE OR NUTRITIONAL STRESS.

IF SOME MALES ARE FOUND TO BE INFERTILE, THEN:

SUB STUDY I: Investigate the reproductive status of infertile males.

Exp. 1: Determine if infertile males have normal testosterone levels.

Methods:

Validate fecal and blowhole exudate methods of measuring testosterone.

MEASURE TESTOSTERONE LEVELS IN FERTILE AND INFERTILE MALES AT DIFFERENT TIMES OF THE YEAR (RE: POSSIBLE SEASONAL VARIATION).

Conduct complete gross and histopathologic analyses on all testes available from carcasses to assess spermatogenesis and presence of diseases. Compare findings between fertile and infertile males during the same time of year, if possible.

SUB STUDY II. Determine if there are differences in health status between fertile and infertile males

Exp.1: **Determine if there is evidence of poor health in infertile males**

Methods:

Compare blubber thickness, blubber quality or other body condition indices between fertile and infertile animals.

Determine habitat preferences of males with lower scores.

Conduct nutritional analysis of food sources to assess diet quality in habitats of animals with high and low body condition indices.

Review prey patch formation, history of spatial/temporal distribution and environmental variables.

Determine character of skin lesions and compare prevalence of skin lesions between fertile and infertile males. *If higher prevalence in infertile males, then:*

Assess if lesions indicate a primary skin disease or are a secondary manifestation of systemic disease or poor nutritional status.

Analyze temporal trends and habitat preferences of whales with skin lesions.

Compare prevalence of other lesions (available from necropsy data) between fertile and infertile males.

Compare levels of cell stress indicators between fertile and infertile males.

Exp 2. **Determine if infertile males have higher levels of toxins than fertile males.** These studies should focus on toxic chemicals that are known to impair reproduction or general health in other species.

Methods:

Re-analyze existing contaminant data comparing fertile and infertile males and their habitat preferences.

Analyze tissues, fluids and feces for biotoxins in fertile and infertile males. *If levels are higher in infertile males, then:*

Conduct in-depth studies on zooplankton sources and temporal and spatial distribution.

Analyze tissues and fluids of fertile and infertile males for a targeted list of contaminants. *If levels are higher in infertile males, then:*

Use a 'food-web'-based approach to determine exposure

Analyze biomarkers of toxic exposure (e.g. Cyp1A, DNA adducts, retinoids, leptins) and compare levels in fertile and infertile males.

Exp 3. Determine if infertile males have higher levels of stress than fertile males. Cortisol measurements would probably provide the most reliable indications of stress as cortisol affects reproduction. It can suppress testicular function and is usually elevated with acute and chronic stress.

Methods:

Validate and measure cortisol metabolites in blowhole exudate or feces and compare levels between fertile and infertile animals.

SUB STUDY III. Determine if there are genetic differences between fertile and infertile populations. Because lack of genetic diversity does not necessarily affect reproduction or health, this study is important to assess potential genetic effects.

Exp. 1: Determine inbreeding coefficients for whales with and without reproductive success

Methods:

Pedigree analysis based on existing observational and molecular genetics data.

HYPOTHESIS 3 : ABORTIONS AND NEONATAL DEATHS ARE OCCURRING.

If females are determined to be pregnant (Hypothesis 1, substudy I, Exp. 2), then the cause of abortion or neonatal deaths should be investigated. There are multiple causes of abortion and neonatal death. The most common causes are *in utero* infections, poor nutritional condition, genetic defects, stress, and problems at calving. Determining the cause in a given instance requires complete necropsy with ancillary microbial and genetic testing on aborted calves. As this is usually not feasible, indirect measures will be needed to compare aborting and successfully calving females.

Exp. Determine if aborting females have evidence of poor health

Methods:

Compare blubber thickness, blubber quality, or other body condition indices between calving and aborting animals.

Assess blowhole exudate [or feces?] for viruses that target the fetus.

Assess calf birth weights and growth rates as indirect measures of maternal nutritional status.

Exp. Determine if aborting females have higher levels of toxins than calving females.

Exp. Determine if aborting females have higher levels of stress than calving females

Exp. Determine if there are genetic differences at MHC loci between mothers and calves in calving females (and if possible in aborting females).