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Causes of Reproductive Failure in North Atlantic Right Whales: New Avenues of Research

Report of a Workshop Held 26-28 April 2000
Falmouth, Massachusetts

by

Randall R. Reeves, Rosalind Rolland, and Phillip J. Clapham, Editors

November 2001

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EXECUTIVE SUMMARY

Despite its protected status, the western North Atlantic right whale (*Eubalaena glacialis*) population remains critically endangered, numbering about 300 individuals. This population's recovery has been hindered by at least three factors: mortality from ship strikes, mortality from entanglement in fishing gear, and, in recent years, a significant decline in reproductive success. A workshop was held in Falmouth, Massachusetts on 26-28 April 2000 to examine the possible causes of reproductive dysfunction in this population (including survival and recruitment of calves) and to develop a feasible research strategy to investigate these factors.

An international group of participants attended the workshop, including 35 scientists representing a broad range of disciplines including pathology, toxicology, reproductive biology, nutrition, reproductive endocrinology, chemistry, marine biology, large whale biology, genetics and veterinary medicine. The workshop was designed to be multi-disciplinary, bringing together a wide range of expertise with experience studying a variety of taxa to provide a comparative dimension to the discussion.

The workshop focused on five factors as potential contributors to reproductive dysfunction in North Atlantic right whales: 1) environmental contaminants/endocrine disruptors; 2) body condition/nutritional stress; 3) genetics; 4) infectious diseases; and 5) marine biotoxins. On the first day of the workshop plenary presentations reviewed the current status of the population and summarized relevant research and necropsy data. On day two there was a comparative review of causes of reproductive failure in mammals in general and a review of reproduction in the North Atlantic right whale, especially focused on where in the reproductive cycle impairment or failure might be occurring. Each of the five factors was discussed in detail; for each factor, discussions included a review of the existing knowledge of effects on reproduction (in any species), a review of the available data for effects in North Atlantic right whales, an evaluation of the likelihood that the factor is affecting right whale reproduction, and development of testable hypotheses. Consideration was also given to additional factors (such as habitat alteration or loss) that could be impacting reproduction. The final day of the workshop was devoted to designing a prioritized research program to test the hypotheses that had been developed and to a discussion of appropriate "control" population(s). Based upon discussions with participants both at and after the workshop, a problem-based approach was developed as a framework for the research program. An alternative hypothesis-driven approach was also developed by one of the participants subsequent to the workshop. Both approaches are included in this report.

The workshop concluded that if calf production and recruitment do not recover from the low levels observed in recent years, the population of North Atlantic right whales is unlikely to recover, even if known anthropogenic causes of mortality are reduced to zero. Therefore, the participants emphasized that it is important to determine the cause(s) of the reproductive dysfunction as soon as possible, and strongly recommended implementation of the proposed research program. The workshop concluded that none of the five factors considered could be eliminated as a possible contributor to the observed reproductive dysfunction, and that interaction among two or more factors (possibly over different time scales) is more likely than a single cause. It was stressed that the research program should consider all factors and be interactive and multi-disciplinary in nature, and the workshop recommended development of a steering committee to provide central coordination of the research. The workshop further recommended development of a comprehensive database linked for all whales across all research programs to allow for multivariate analyses using different data sets. Finally, the workshop recognized the importance of the long-term right whale research program and the photo-identification catalogue and recommended full continuing support for these activities.

1. INTRODUCTORY ITEMS

The Workshop was held in Falmouth, Massachusetts, on 26-28 April 2000. The Workshop steering committee was chaired by Roz Rolland of the Center for Conservation Medicine, Tufts University, and included Phil Clapham, Scott Kraus, Michael Moore, Teri Rowles, Bruce Russell, and Greg Silber. Participants included scientists from a variety of disciplines such as pathology, toxicology, nutrition, reproductive endocrinology, chemistry, marine biology, large whale biology, genetics, and veterinary medicine (Appendix 1). The expectation was that this broad range of expertise would provide a useful comparative approach to the issue.

Michael Sissenwine, Science and Research Director of the Northeast Fisheries Science Center, welcomed participants to the Woods Hole-Falmouth area and briefly summarized the main elements of the Northeast Center/Northeast Region right whale research program. This program focuses on three topics of particular concern: ship strikes, entanglement in fishing gear, and reproductive dysfunction. The ship strike issue is being addressed through a surveillance and early warning effort and by an attempt to develop an acoustic method for ships to detect (and thus avoid) right whales. The entanglement problem is being addressed by seeking to develop alternative fishing gear and methods, and by supporting disentanglement efforts. The problem of reproductive dysfunction is not yet being addressed in a systematic way, and Sissenwine expressed hope that the workshop report would provide guidance in this regard.

Sissenwine acknowledged the financial contributions to the Workshop from the International Fund for Animal Welfare and the Island Foundation, as well as from the National Marine Fisheries Service's Recover Protected Species program. Clapham, the local convenor, acknowledged the hard work of Sara Wetmore, Cheryl Kitts and Deb Depunte in helping with the Workshop logistics.

The Workshop was chaired by Peter Best. Randall Reeves acted as rapporteur, with assistance from Greg Donovan.

1.1. Objectives of the workshop

The Workshop had been prompted by evidence that reproductive dysfunction may be a contributory factor to the failure of the western North Atlantic right whale (*Eubalaena glacialis*) population to recover (IWC, in press a; in press b). The Workshop goals were to identify factors potentially affecting reproduction (including calf survival and recruitment) and to develop an appropriate and feasible research strategy to investigate these factors. The strategy was to include: (a) development of testable hypotheses, (b) drawing a distinction between those factors that can be studied with existing research techniques and those that require new techniques, and (c) developing prioritized recommendations for new areas of research. Prior to the Workshop, five major potential factors had been identified: (1) environmental contaminants/endocrine disruptors; (2) body condition/nutritional stress; (3) genetics; (4) pathology/infectious diseases; and (5) biotoxins. Finally, the Workshop was to consider the importance of its conclusions and recommendations in the broader context of the conservation requirements of western North Atlantic right whales.

The North Atlantic right whale population (estimated at around 300 animals, IWC in press b) has been studied intensively for the past 20 years. Almost the entire population is believed to be represented in a photo-identification catalogue maintained at the New England Aquarium in Boston. A large proportion of the population (over 250 individuals) has been biopsied for studies of genetics, demographics, and skin and blubber characteristics (including contaminant levels). Estimates of life history parameters are available from the observational and photo-identification work, and some material is available from necropsies of stranded right whales. These extensive databases provide a powerful framework for examining factors potentially associated with reproductive dysfunction.

In addition to directed studies of North Atlantic right whales, the Workshop was to consider the potential for populations of southern right whales (*E. australis*) which are increasing in abundance (and are thus presumably healthy) to act as control populations. Also, the closely related bowhead whale (*Balaena mysticetus)* may be a useful model species because the Bering-Chukchi-Beaufort Seas population is large and robust, and fresh specimen materials are available from the Eskimo hunt.

Although the focus of the Workshop was to be on the North Atlantic right whale population, it was hoped that the results would have relevance to the conservation of other highly endangered populations as well.

1.2 Adoption of agenda

The agenda developed by the steering committee was adopted with only minor changes (Appendix 2).

The first day was devoted to invited presentations. Each plenary presentation was followed by a brief discussion. Summaries of the presentations and subsequent discussions are included under the appropriate agenda item in the body of this report.

The second and third days consisted of discussions of each agenda item, with the afternoon of Day 3 devoted to the development of a research program outline. Most of the work was conducted in plenary, although small *ad hoc* working groups met informally between sessions.

The workshop report was prepared by the rapporteur and a small steering group (Best, Rolland, Clapham and Donovan) following the meeting. A draft was circulated to all participants for review, then revised by the rapporteur in consultation with the steering group, and finalized by the report editors.

2. OVERVIEW OF REPRODUCTIVE BIOLOGY AND REPRODUCTIVE FAILURE

2.1 General review of mammalian reproduction and causes of reproductive failure

Munson provided an overview of mammalian reproduction and noted the difficulty of determining the precise cause(s) of reproductive failure or dysfunction in wild animals. There are numerous potential sites, mechanisms and critical time periods for reproductive problems, including the hypothalamic-pituitary-gonadal axis, the gonads, the uterus, foetal development, and post-partum survival. In addition to permanent reproductive failure, animals may experience reduced reproductive success or temporary reproductive failure. Because reproductive function is expendable to the individual, it can be suppressed under many circumstances, e.g. nutritional or environmental stress, systemic disease.

Loss of integrity of the hypothalamic-pituitary-gonadal axis can result in (1) absence of or decrease in hormone secretion (GnRH, LH or FSH), or (2) disruption in the essential timing of hormone release. Gonadal inactivity or lesions (i.e. abnormalities) can be caused by many factors including genetic defects, infectious disease, degenerative changes, neoplasia or aging (senescence). In addition, gonadal problems can be secondary to other primary problems such as nutritional or environmental stress, systemic infection, central nervous system disease or toxins. Abnormal genital tract structure can be the result of developmental defects (genetic, disease- or toxin-induced) or acquired abnormalities due to hormone deficiencies or excesses, toxic exposure or infection. Foetal development or survival can be impaired by genetic defects, nutritional deficiencies or excesses, toxic exposure or infection. Post-partum neonatal death can be caused by inherited or congenital defects, poor nutrition, environmental stress or infectious disease.

Infectious agents can cause reproductive dysfunction through a variety of mechanisms, including direct effects on the central nervous system, damage to the genital tract and damage to the foetus. For example, canine distemper virus and other morbilliviruses can cause systemic problems and disrupt the hypothalamic-pituitary-gonadal axis, infect the gonads directly, or cross the placenta and damage the foetus (resulting in abortion). Neonatal infections can affect the survival of newborns, as occurred in red wolves (*Canis rufus*) (Acton et al. 2000).

Toxins and genetic defects can also operate in a number of different ways to impair reproductive performance. For example, environmental pollutants can act at the central nervous system, pituitary or gonadal level to inhibit or over-stimulate (i.e. disrupt) endocrine function (e.g. PCBs) or cause direct damage to the genital tract (e.g. exogenous progestins and estrogens) or the foetus. Genetic defects can be manifest by abnormal genital tract development [e.g. cryptorchidism in Florida panthers (*Felis concolor*) (Cunningham et al. 1999)], abnormal germ cell development [e.g. decreased spermiogenesis in Ngorongoro Crater lions (*Panthera leo*) (Munson et al. 1996) and reduced primordial follicles and minimal folliculogenesis in vaquitas (*Phocoena sinus*) (Munson, pers. comm.)], predisposition to the development of genital tract tumours [e.g. ovarian, endometrial and mammary gland cancers in jaguars (*Panthera onca*) (Munson 1994) and ovarian dysgerminomas in maned wolves (*Chrysocyon brachyurus*) (Munson and Montali 1991)] or embryo-lethal effects.

Reproductive dysfunction in captive cheetahs (*Acinoynx jubatus*) was long thought to be related to the high incidence of abnormal sperm produced by the small, degenerative testes in these animals. However, this dysfunction is now attributed to hypercortisolemia from chronic stress (Wildt et al. 1993, Munson 1993), with corticoids acting as hormone disruptors inhibiting gonad function. The etiology of this discovery included the following observations: (1) normal genital anatomy and responsiveness to exogenous hormones, (2) suboptimal spermatogenesis and folliculogenesis, (3) high prevalence of adrenal cortical hyperplasia, and (4) faecal corticoids approximately four times the levels found in wild cheetahs.

Degenerative changes can occur in the reproductive tract of animals that are nulliparous or reproductively inactive for long periods of time. Old female elephants in captivity can become infertile and exhibit leiomyomas and endometrial hyperplasia. Vaquitas exhibit ovarian calcified bodies and early senescence.

The North Atlantic right whale is the only baleen whale for which reproductive dysfunction has been documented (see Item 2.2). Despite the fact that other mysticete populations (e.g. gray whales (*Eschrichtius robustus*) in the western North Pacific, right whales (*Eubalaena japonica*) in the eastern North Pacific, bowheads in the eastern North Atlantic Arctic, and blue whales (*Balaenoptera musculus*) in several areas) have been reduced to equally low (or even lower) abundance levels, there is no conclusive evidence of reproductive problems in these populations. This may simply reflect the fact that these stocks have been less well studied than the North Atlantic right whale population, or that the lack of recovery in several of these depleted stock is indeed a striking indication of reproductive impairment. Clapham noted that there have been no confirmed observations of right whale calves in the eastern North Pacific for at least 100 years.

DeGuise summarized the situation of white whales (belugas, *Delphinapterus leucas*) in the St. Lawrence River, probably the most familiar example of an odontocete population thought to be experiencing some kind of reproductive dysfunction. The evidence was based upon an age-structured population model using data from stranded animals to determine what proportion of the living population would have to be immature (gray-colored) to replace annual mortality (Béland et al. 1988). The results indicated that the proportion of immature whales was lower than required to offset mortality and therefore that the population was declining. DeGuise also reported that per capita calf production in the St. Lawrence population was less than in the Arctic. However, this conclusion remains controversial (see Lesage and Kingsley 1998), as does the hypothesis that the population is declining (see Kingsley 1998).

DeGuise reported that detailed post-mortems carried out on stranded St. Lawrence white whales did not reveal any reproductive lesions. Stranded mature males appeared to have normal spermatogenesis, although the lack of fresh samples of viable sperm precluded examination of sperm morphology and motility. Although stranded adult females exhibited a short-term shutdown of ovarian activity, this is expected of ill animals. The St. Lawrence white whale population lives downstream of a basin heavily contaminated by industrial pollutants and appears to have an unusually high incidence of cancer (Martineau et al. 1999).

Oftedal highlighted the differences in reproductive patterns between mysticetes and odontocetes from a nutritional perspective, and noted that odontocetes were probably not very good models for right whales. Female mysticetes store immense quantities of lipids that can be mobilized and transferred rapidly to their offspring, and in this sense are more like phocids than odontocetes. Although balaenids have a more protracted lactation cycle than balaenopterids, they are still probably more like phocids than like odontocetes (see Oftedal 1997).

Gray remarked that there is nothing unique about either the endocrinology or reproductive physiology of mysticetes and therefore findings from other, more easily studied mammals could be used to make inferences about right whales. In particular, studies of other species living in the same habitat would be useful.

The difficulty of isolating a causal mechanism for observed reproductive dysfunction was reiterated. A good example is the mass mortality of striped dolphins (*Stenella coeruleoalba*) in the Mediterranean Sea (Aguilar 2000). In addition to the mortality of individuals, the population was affected by the loss of normal fecundity, manifest in the high number of abortions and the presence of luteinized ovarian cysts in many adult females (Munson et al. 1998). The latter could have been caused either by morbillivirus infection or by high PCB levels (or a combination of both). If the reproductive disorder was caused by the viral epidemic, restoration of reproductive function and population recovery would be expected during the typical period of immunity following the epidemic. If, however, the primary cause was PCB toxicity, continued reproductive problems would be likely and this would affect long-term population recovery (Munson et al. 1998).

2.2. Review of North Atlantic right whale reproduction, especially indicating where in the cycle impairment or failure may be occurring, and development of testable hypotheses

Right whales migrate between feeding grounds in cool northern waters (summer) and breeding grounds in lower latitudes (winter). Some feeding occurs in areas between the two ends of the migration, particularly in Cape Cod Bay and in the Great South Channel during late winter and early summer. Although the location of one calving ground (southeastern US coastal waters - December to March) is known, the location of the mating ground(s) is unknown.

Kraus and Hatch (in press) discuss mating strategies in the western North Atlantic right whale population, based largely on observations of surface active groups engaged in what appears to be courtship behavior (although if a gestation period of approximately 12 months is assumed/accepted, summer mating would not result in conception, unless a mechanism such as delayed implantation occurred). Females do not appear to actively select males but instead create conditions that lead to competition among males for opportunities to copulate. Sperm competition is also very likely given the immense size of the testes (Brownell and Ralls 1986).

Appendix 3 summarizes the available information on North Atlantic right whale reproductive parameters (see Kraus et al. in press). In the context of this Workshop, the main points are as follows:

(1) Annual calf production per female is highly variable. Since 1990, the total number of calves observed (believed to reflect the true total in the population) has been about half what would have been expected from comparison with Southern Hemisphere females. From 1998-2000, annual calf production has been lower in absolute terms than in all but one of the preceding 17 years.

(2) In recent years, calf production has been largely from cows that do not take their calves to the Bay of Fundy in summer. This 'non-Fundy' component consists of only about one-third of the 70 'reproductively active' females (i.e. those older than 9 years that have given birth at least once) in the population.

(3) Calving interval has increased significantly from the 1980s through the 1990s and now averages more than 5 years. This increase is apparent in cows of all ages.

(4) The survival rate of calving females shows a significant decline over time (Caswell et al. 1999).

(5) The survival rate of immature females shows no significant decline.

(6) Age at first reproduction is similar to that in Southern Hemisphere animals.

(7) The rate of population increase is significantly lower than those of several Southern Hemisphere right whale populations, and it may have been negative since 1990 (IWC in press a, b).

A number of points were made in the general discussion, mainly concerning the increase in the mean calving interval. The Workshop agreed that the assumption previously made (IWC in press b), that the peak in 5-year intervals (the 'normal' 3 plus 2) was the result of late prenatal or neonatal mortality, required further consideration before being accepted.

The lengthening of the mean calving interval had been suggested in the early 1990s (Knowlton et al. 1994) but was only confirmed to be significant in the mid-1990s. Kraus commented that had data on the apparently complete cessation of calving by some Fundy females been included in the calving interval analyses, the average would be much greater than 5 years. The likelihood model of Cooke and Glinka (1999) takes account of this. However, while the mean calving interval has increased significantly, individual calving histories reveal that the intervals have not increased for every female. Examination of individual calving histories could provide valuable insight into which factors most affect calving intervals.

From experience with other species, it is clear that reproductive failure can occur at several stages in the reproductive cycle, as summarized below:

(1) Failure to ovulate. Ovulation in right whales is assumed to be spontaneous as in other cetaceans, and conceptions occur primarily during the winter season, i.e. probably November-February in the Northern Hemisphere (see Best 1994). While it may be possible to diagnose ovulation from samples of feces or blowhole exudates, this requires knowledge of relative hormone levels at various stages in the reproductive cycle and longitudinal sampling of individual females.

(2) Failure to mate at a time when the female is fertile. Since the locality of the mating ground(s) is unknown, there is little prospect of addressing mating failure using courtship observations as an index of sexual activity. Based on assumptions about the gestation period, Kraus suggested that the peak mating period should occur between November and the end of February. Courtship groups ('surface-active groups'), usually comprising 1-10 individuals, are seen on and near the calving grounds in the South Atlantic (Best and Schaeff, in prep.). However, such groups are not seen off the southeastern United States in the winter, when most conceptions are thought to occur. The courtship groups observed on the feeding grounds in the western North Atlantic are often larger than 10 individuals, but Marx commented that small courtship groups are seen occasionally in Cape Cod Bay in February and March.

(3) Lack of adequate sperm production. Addressing this possibility will require reasonably fresh samples, which in practice are available only from stranded or ship-struck adult males. Munson noted that if right whales are seasonally polyestrous (like felids), inferior sperm production could well cause reproductive dysfunction. Munson suggested looking for a point source of pollution (for example in Cape Cod Bay) which through short-term exposure to the whales might affect the availability, production or quality of sperm.

(4) Failure to implant (pregnancy loss). This possibility will be difficult to study among living animals in the right whale population (see item 1).

(5) Abortion or neonatal mortality. If caused by infectious disease, this is not likely to be repetitive in nature because immunity acquired by the population would lessen the impacts over time. Genetic factors might also be implicated in abortions (see Item 3.1.1). Death of the mother is obviously a potential cause of neonatal mortality.

The Workshop **agreed** that none of the above could be ruled out, *a priori*, as not being relevant to the observed reproductive dysfunction in North Atlantic right whales.

Sampling for gonadal steroid hormone analysis from the free-swimming population is constrained by field logistics, timing (except for parturient females, adult whales are available primarily in the northern feeding areas between spring and fall, i.e. during what is presumed to be the non-breeding season), and the need to avoid or at least minimize invasive procedures. Full use needs to be made of opportunities to sample feces and blowhole exudate (if steroid hormones can be detected in respiratory excretions), and if possible to obtain time series of samples from individual females. Relative levels of gonadal steroid hormone metabolites from feces or blowhole exudate could provide clues to reproductive status. Unfortunately, at this stage, it does not appear to be feasible to obtain even minute blood samples with biopsy darts. Rolland suggested that the bowhead could provide a useful model for testing gonadal steroid hormone levels in the feces and blowhole exudate of pregnant and non-pregnant females, as well as males. The assays could be validated in bowhead whales through the collection of feces, blowhole exudate, serum and urine from individual dead whales whose reproductive tracts could be examined directly. Southern mentioned that work was underway in her laboratory to identify a genetic marker to assess pregnancy in white whales using frozen skin samples. It was **agreed** that a skin test for pregnancy would be a useful tool in studying reproductive dysfunction in right whales.

Munson recommended the following two-step, multi-disciplinary approach to studying reproductive dysfunction in North Atlantic right whales (see Appendix 4 for more details):

A. Conduct pathologic analysis on all available tissues, i.e.:
   Collect all genital tracts that become available, regardless of state of autolysis
   Conduct complete gross and histopathologic analyses
   Compare lesions between historic and current populations
   Compare lesions between Northern and Southern Hemisphere populations

B. Link genital pathology to other data, i.e.:
   Other pathologic findings (overall disease in the animal)
   Faecal analysis for gonadal and adrenal steroid hormone metabolites
   Evidence of breeding or fertility
   History of breeding behavior/success
   Nutritional status
   Environmental contaminant and biotoxin levels

Ovaries of right whales seem to be exceptionally friable, making them difficult to locate and collect from beach-cast carcasses. There is little prospect of meaningful comparisons of lesions in the historic and current populations, as 'historic' specimen materials and information are almost entirely lacking.

Munson urged that social and behavioral factors should be taken into account when considering possible reasons for reproductive dysfunction. The low density of individuals on the mating grounds could mean that the frequency of contacts is suboptimal for the species, given its reproductive strategy (cf. the Allee effect). Rowntree noted that females with calves off Argentina form groups and may derive some kind of selective advantage from this behavior. The low number of mothers in any one year in the North Atlantic right whale population probably precludes extensive 'peer group' interaction.

3. CONSIDERATION OF POSSIBLE CAUSES OF REPRODUCTIVE FAILURE

3.1. Genetic factors (e.g. loss of diversity, inbreeding, effective sex ratio)

3.1.1. Review of existing knowledge of effects of these factors on reproduction

The possible role of genetic factors in preventing small populations from recovering has been widely discussed. Low genetic variation can lead to reduced population fitness, manifest as the expression of deleterious alleles or the reduction of heterozygosity at loci that confer immuno-competence. The term 'inbreeding depression' refers to a condition in which a population's reproduction and recruitment are correlated with inbreeding. This can be expressed as reduced fertility and fecundity, decreased foetal and neonate survival, impaired development or lowered disease resistance. Inbreeding depression is extremely difficult to detect or verify in natural populations. In one of the few examples involving natural populations, inbreeding was found to have a significant detrimental effect on the survivorship of white-footed mice (*Peromyscus leucopus noveboracensis*) derived from a wild population and reintroduced into a natural habitat (Jimenez et al. 1994). In another example, a depleted snake population in the UK with symptoms of reproductive failure showed improved reproduction once snakes from an external source were introduced into the inbred population (Madsen et al. 1999).

Of particular relevance to ongoing genetic studies of North Atlantic right whales are examples in which traits linked to the Major Histocompatibility Complex (MHC), or Human Leukocyte Antigen (HLA), have been shown to be associated with reproduction, growth and development. In humans, increased embryonic cleavage, neural tube defects, recurrent spontaneous abortions and unexplained infertility have been associated with the structure of MHC. Extensive studies of an isolated Hutterite community in South Dakota have shown that parental sharing of the 16-locus HLA haplotype is associated with foetal loss (Ober et al. 1998). It is hypothesized that foetuses that are genetically identical to the mother have a lowered survival probability. In addition to its implication in reproductive success, MHC diversity generally provides the body with a better chance of mounting a successful immune response to antigens.

However, low genetic diversity, including specifically low MHC polymorphism, does not necessarily indicate an unhealthy population (e.g. the northern elephant seal, *Mirounga angustirostris*, which is large and growing).

3.1.2. Review of data on these factors in North Atlantic right whales

Multi-locus DNA fingerprinting has shown that North Atlantic right whales have significantly less genetic variation than right whales in the South Atlantic (Schaeff et al. 1997), and a lower allelic diversity than any other baleen whale population studied to date. Half-siblings in the South Atlantic population are equivalent to the most distantly related individuals in the North Atlantic (White, pers. comm.). This low genetic variation has elicited concern because of the possibility that the population is suffering from the effects of inbreeding (Knowlton et al. 1994, Schaeff et al. 1997). Preliminary analyses of DNA from bone recovered at 16th century Basque whaling sites in Labrador suggest that the greatest loss of genetic diversity occurred about four centuries ago. As most deleterious recessive alleles would have therefore been purged from the population long ago, the recent decline in reproductive success is unlikely to be solely a genetic problem.

About 70% of the 94 females in the right whale photo-identification catalogue are estimated to be older than 9 years, still alive, and have given birth at least once (IWC in press b). Paternity assignments made by White and colleagues at McMaster University suggest an unexpectedly broad mating success rate, with about 70 different males having sired at least one calf. Effective population size is therefore on the order of 100-130, and the estimated loss of heterozygosity per generation ($1/2N_e$) is approximately 0.5%. Of 221 calves documented to have been born between 1980 and 1999, 214 are believed to still be alive; of these, 121 were taken by their mothers to the Bay of Fundy in the summer (see Appendix 5). There are 160 known mother-calf pairs (some females are represented more than once) in the catalogue, and 85 of these pairs have been genotyped (72 Fundy, 13 non-Fundy). Work is underway to isolate additional microsatellite loci to better assess population structuring and develop detailed genealogies.

Low observed genetic variation at MHC Class II loci in North Atlantic right whales has been hypothesized as contributing to a higher-than-normal incidence of abortion. While this hypothesis cannot explain any of the recent changes in reproductive parameters, it may indicate (if true) that the maximum rate of growth of the North Atlantic population is intrinsically lower than in Southern Hemisphere populations. This, in turn, may partially explain the lack of recovery of this population despite its long period of protection.

Rosenbaum and collaborators are also studying changes in the genetic diversity of right whales in relation to trends in exploitation and abundance.

3.1.3. Evaluation of likelihood that genetic problems may be affecting North Atlantic right whale reproduction and development of testable hypotheses

White explained that his group would continue to investigate the hypothesis that low genetic variation at MHC Class II loci is leading to MHC haplotype sharing between parents, which in turn increases the similarity at MHC loci between mothers and foetuses and increases the probability of spontaneous abortion. This will be accomplished by assessing whether the transmission of a given MHC haplotype from father to calf is significantly different from random. The conclusiveness of the results depends on the exclusionary power of the microsatellite markers, and thus more of these are being developed.

The Workshop **agreed** that it is unlikely that intrinsic genetic factors alone are responsible for the reproductive dysfunction in North Atlantic right whales. However, genetic factors could be interacting with extrinsic factors including poor nutrition and exposure to toxic chemicals and/or pathogens. Unfortunately, the relationship of MHC to disease susceptibility is not amenable to a hypothesis-testing approach as the lack of genetic diversity in the population predisposes it to the effects of other stressors. Reduced diversity of cellular detoxification genes could be contributing to an immune response problem, impairing the ability of the whales to deal with oxidative stress. It was suggested that one potential approach would be to look for correlations between DNA profiles of individuals and the presence or absence of lesions, reproductive success, et cetera.

The Workshop noted that the topic of whether, and how, genetic factors are affecting reproduction in North Atlantic right whales was to be explored in greater detail at the proposed International Whaling Commission (IWC) Workshop on Right Whale Genetics. The Workshop offered its support for this initiative.

3.2. Nutrition

3.2.1. Review of existing knowledge of effects of nutrition on reproduction

The links between good nutrition (in terms of both quality and quantity) and reproductive success are well known. Nutrition can affect many stages of the reproductive process in both sexes. In males, it can influence the age at which an animal attains sexual (and, where pertinent, social) maturity; sperm production, quality and viability; and the likelihood of successful copulation with a receptive female. In females, nutrition plays a role in the age at which sexual maturity is reached; ovulation and fertility; quality and quantity of milk production; and the time between offspring. Nutrition affects calf survival and growth, and influences the time to weaning.

In most animal species, the energetic costs of reproduction are high (up to 3-5 times the maintenance requirements). In mammals, the lactation period is especially demanding. This is particularly true for those marine mammals that undergo long periods of fasting (or very low food intake) (see Lockyer 1984; Oftedal 1997). In fin whales (*Balaenoptera physalus*), for example, the energetic cost of lactation can be double that of gestation (Lockyer, 1984).

Given the high energetic cost, and that reproduction is a non-essential activity (at least at the individual level), reproduction is often suppressed during periods of nutritional stress and resumes only after the return of more favourable nutritional conditions.

In addition to the more obvious direct effects (e.g. Lockyer, 1987, found a strong correlation between body fat and reproductive success in female fin and sei whales, *Balaenoptera borealis*), poor nutrition can affect reproduction in other ways. For example, it can make the individual more susceptible to disease or cause mobilization of fat reserves that contain high levels of chemical contaminants (see Items 3.5 and 3.3, respectively, below).

3.2.2. Review of data on nutrition in North Atlantic right whales

As noted under Item 2, right whales feed in the northern part of their range (Bay of Fundy and the Scotian Shelf) primarily from June to October, with some feeding in the winter and spring in Cape Cod Bay, and in the Great South Channel in the late spring and early summer. Although *Calanus finmarchicus* is the most commonly observed prey species, North Atlantic right whales feed on other species including barnacle larvae. Patch density may be the critical factor in eliciting feeding behavior. Lack of information on where animals are when they are not in known feeding or calving grounds makes it difficult to estimate how long the 'fasting season' lasts but it is probably around 3 months (for those individuals that migrate to lower latitudes in the winter). In 1993, right whales appeared to abandon the Roseway Basin habitat for unknown reasons.

There have been a number of studies on right whale feeding requirements (see Kenney and Wishner, 1995; IWC In press b) and on whether changes in right whale habitat have led to a shortage of food thereby threatening the population. Successful right whale feeding depends on the presence of extremely dense zooplankton patches (Wishner et al., 1995). Simply measuring the average zooplankton density in an area will not provide sufficient information to judge whether feeding conditions exist for right whales. The main factors influencing prey density are physical (i.e. advection of prey and its concentration along density discontinuities). Kenney (in press) concluded that the absence of right whales in the

Great South Channel in 1992 was due to a large reduction in *Calanus* abundance and a shift in zooplankton dominance induced by hydrographic changes.

Any habitat studies investigating whether nutritional factors (i.e. prey availability) are involved in reproductive dysfunction in North Atlantic right whales will need to examine a number of factors including: availability and abundance of patches of prey of suitable size and density; the quality of the prey; and the abundance of any competitors for the prey. These investigations should also take cognizance of ongoing studies of physical forcing (e.g. the North Atlantic Oscillation). This is discussed further in the following section.

Studies of blubber thickness of living animals have shown that North Atlantic right whales have significantly thinner blubber than right whales off South Africa (Miller and Moore, in prep.). These findings are consistent with necropsy measurements and observations that North Atlantic right whales lack the typical post-blowhole fat rolls evident on right whales in the Southern Hemisphere. A number of possible explanations for these differences have been advanced including: inadequate sample size, bias from differences in body length or sex, genetically determined differences in morphology, adaptation to different habitat temperature regimes, seasonal difference in sampling periods, and differences in plane of nutrition. The Workshop **agreed** that a difference in the nutritive regime between the two populations was a plausible explanation that merited further investigation.

Based on their analyses of blubber thickness in North Atlantic right whales, Moore and Miller reported that: (1) there was no significant difference between males (n=17) and females (n=16); (2) a significant correlation existed between age and blubber thickness for females (n=16) but not males (n=17); and (3) blubber thickness in females was significantly correlated with time since last calving, regardless of age (n=6). The last of these observations might help explain the recent increase in calving interval. Miller and Moore noted that a female might lose her foetus if a certain threshold of maternal body condition was not met at a critical time during pregnancy.

Although the Workshop **agreed** that obtaining blubber thickness data is valuable (particularly when such data are combined with other biological information from the same individual whales), it recognized that blubber thickness alone may not be an adequate measure of body condition. For example, in fasting seals, the type of lipid and fatty acid in the blubber is more relevant in assessing nutritional condition than the actual mass of blubber. Moore and Miller indicated that a more comprehensive index of body condition could be developed for right whales, which would include (at least) blubber thickness, blubber lipid content, body length and body girth.

The Workshop also recognized the need to: (1) obtain increased sample sizes from right whales of various sex, age and reproductive classes and (2) investigate the significance of taking measurements at various sites on the body. Studies on dead animals have revealed large differences in blubber thickness at different body sites.

The Workshop noted the potential value of obtaining time series of blubber measurements from individual females to track blubber changes in relation to pregnancy, calving and lactation. A marked change in blubber thickness (as well as in lipid content) is likely to occur during lactation. Meaningful estimates of lipid content would probably be very difficult to obtain, although detailed examination of ultrasound traces may be of some value. Miller indicated that there is more connective tissue in northern than southern right whale blubber, implying lower lipid content in the former.

3.2.3. Evaluation of likelihood that nutritional problems may be affecting North Atlantic right whale reproduction and development of testable hypotheses

The Workshop agreed that inadequate nutrition could be a factor in the poor reproductive performance of the North Atlantic right whale population in recent years, and recommended several avenues of research be continued or initiated.

(1) Investigation of food resources and diet

- Review the literature and unpublished data for information on factors contributing to the formation of dense copepod patches and on the spatial and temporal distribution of such patches.
- Encourage studies of factors affecting nutritional composition of right whale prey, including seasonal, geographic, ontogenetic, nutritional (phytoplankton) and long-term climatic influences.

- Apply new methodologies, including use of isotopes of carbon, nitrogen and other elements as well as fatty acid protocols. Skin/blubber biopsies should be evaluated as primary data sources and the effects of seasonal, geographic, ontogenetic, nutritional (phytoplankton) and other factors on prey signatures should be investigated.
- Encourage maximal use of traditional sampling protocols (e.g. faecal sampling, necropsy sampling, observations of behavior) and remote telemetry (e.g. satellite tags, time-depth recorders, critter cams) in obtaining date on feeding habits.

(2). Assessment of body condition

- Encourage continued development /application of ultrasonic techniques to measure blubber-depth to evaluate changes in condition of reproductive-aged females before, during and after reproduction. These efforts should include evaluation of various body measurement sites, and validation against necropsy samples (including data from control species such as the bowhead whale). The research should attempt to: (a) normalize acoustic blubber data for measurement location using a model of right whale blubber topography so that single-point *in situ* measurements form different body locations can be compared, and (b) determine lipid composition of blubber using ultrasound.
- Continue development of morphometric and photogrammetric approaches for estimating body condition (e.g. estimating girth and other body parameters using stereo videogrammetry), particularly for reproductive-aged females.
- Evaluate biopsy samples as indicators of lipid stores. Do they reveal seasonal or reproductive trends and are they correlated to analyses of blubber from necropsy specimens?
- Analyze necropsy samples for chemical (nutritional) constituents and biomarkers that may indicate condition. Characterize lipid deposition and mobilization patterns in blubber.

(3) Indicators of reproductive performance

- Analyze variability in the number of right whale births and calving interval with respect to climatic pattern, fluctuations in food supply, use of foraging areas and female age/size (especially primiparous individuals).
- Obtain systematic photogrammetric information on calf size, on both the nursing and feeding grounds, for evaluating growth performance.
- Conduct repeated sampling of blubber depths in relation to the lactational cycle.
- If a lactating female becomes available for necropsy, collect milk samples and data on mammary gland mass and anatomy.

The Workshop also identified the role of leptin in right whale body condition and reproduction as worthy of further research. Leptin is a hormone produced by adipose tissue and secreted into the bloodstream. In other mammals, leptin affects feeding behavior, metabolism, reproduction and immunology, and may regulate overall energy balance. Leptin production increases as energy stores increase. Leptin alters nutrient partitioning through metabolic actions on muscle and adipose tissue. It may influence the onset of puberty through actions in the central nervous system and in the ovaries, and it may promote development of the immune system.

Leptin expression in dermal biopsies and blubber samples could be analyzed using immunohistochemical, biochemical and molecular methods. The initial aims of such analyses should be to: (a) characterize patterns at different body sites; (b) compare blubber and serum levels; and (c) evaluate leptin levels and biological information from known animals in the photo-identification catalogue (including age, sex, reproductive history and habitat use). If possible, leptin levels should be compared in northern and southern right whale populations.

3.3. Chemical contaminants

3.3.1. Review of existing knowledge of effects of contaminants on reproduction

An extensive body of literature on the effects of contaminants on mammalian reproduction (including in marine mammals) has recently been reviewed by O'Shea (1999), O'Shea et al. (1999) and Reijnders et al. (1999). A great deal of information exists on the traditional suite of elements and organic compounds found in mammal tissues (with emphasis on persistent organic pollutants), but little data exist on dose-response effects for even the better-known contaminants. Although the IWC Scientific Committee concluded that sufficient data were available on the adverse effects of pollutants on terrestrial species and non-cetacean marine mammals to warrant concern for cetaceans, the Committee indicated that significant fundamental research was needed to adequately assess the effects of pollutants on cetaceans. To this end, the Committee developed the multi-national multi-disciplinary research program POLLUTION 2000+ (Reijnders et al. 1999).

Gray provided a concise summary, with a few examples, of mechanistic studies on laboratory animals. Endocrine-disrupting toxicants (e.g. antiandrogenic pesticides, xenoestrogens, TCDD, some PCB congeners) affect development, puberty, sexual differentiation and fecundity in rats (see the extensive tables on reproductive and endocrine effects on rodents prepared by Gray and Rolland in O'Shea et al. [1999: their Tables 4-6]). PAHs (of concern for right whales, see below) are known to affect ovarian function and DNA synthesis, and potentially cause permanent infertility. In Gray's view, *in vivo* or *in vitro* studies using well-characterized surrogate species can enhance our understanding of the effects of endocrine disruptors on species, such as right whales, that will never be amenable to controlled studies. Notwithstanding this, the Workshop recognized that extrapolations fro one species to another must be made with caution.

Ross briefly summarized the effects of toxic contaminants on pinnipeds, and noted that contaminants have been implicated in, or associated with, impaired reproduction in pinnipeds, as identified via reduced reproduction rate, reproductive tract pathologies, and complete reproductive failure. Persistent organicpollutants, notably PCBs and the DDT-type compounds, have been implicated in the failure of depleted Baltic Sea seal populations to recover (Helle 1980; Bergman 1999). Uterine lesions were found in 42% of reproductive-aged female ringed seals in the Baltic Sea in the 1970s, suggesting a possible pathogenesis for a toxic effect (Helle 1980). PCBs are thought to have affected reproductive success in harbour seals in the Wadden Sea (Reijnders 1980). A captive feeding study in the Netherlands found that harbour seals fed fish from the more contaminated western Wadden Sea had greatly reduced reproductive success when compared with seals fed fish from the less contaminated eastern Wadden Sea (Reijnders 1986). The results of marine mammal studies, all of which have involved exposure to complex mixtures rather than single contaminants, have been consistent with mechanistic studies of laboratory animals that document a range of reproductive effects. DeGuise pointed out that there is much confusion in the literature regarding contaminant mixtures as the effects of particular chemicals in combination can be additive, antagonistic or synergistic.

3.3.2. Review of data on contaminant levels in North Atlantic right whales

Total PCB fractions in North Atlantic right whale blubber are an order of magnitude lower than those in seals and odontocetes (Weisbrod et al. 2000). Seasonal trends suggest that lipid-stored organochlorines may be released during winter when whale food intake is reduced and body fat becomes depleted. Concentrations of some metabolizable PCBs and trans-chlordane increase with age in males. Comparisons of organochlorine accumulations in biopsy tissues and feces with those in prey species have been interpreted as indicating that right whales consume different prey or forage in different localities during their annual migration cycle (Weisbrod et al. 2000). Deshpande and colleagues found very low levels of PCBs in zooplankton sampled from Cape Cod Bay.

Because right whales occupy a relatively low position in the food chain, feed at least occasionally in the sea surface microlayer, and inhabit feeding grounds near regional sources of pollutants, they may be exposed to certain 'non-traditional' contaminants.

Moore reported a study which measured cytochrome P450 1A (CYP1A) in the dermal vascular endothelia of right whales, using biopsies obtained from various locations in the western North Atlantic (Bay of Fundy, Cape Cod Bay, Georgia/Florida coast) and in the Southern Hemisphere (South Africa, Argentina, Auckland Islands, South Georgia). CYP1A induction occurs in response to the presence of Ah receptor agonists, and it is therefore a sensitive biomarker for potential toxicological impacts from dioxin-like compounds. Marked differences were detected in CYP1A among areas, ranging from a mean of 4.0 (SD=2.6) in the Bay of Fundy to 0.8 (SD=1.3) in the Auckland Islands (New Zealand). Within the western North Atlantic, PAH concentrations in copepods were relatively high in the Bay of Fundy and Cape Cod Bay (both well-known feeding areas for right whales), but much lower on Georges Bank where right whales are not known to congregate to feed. Moore views these findings as suggestive of a link between exposure to non-persistent and non-bioaccumulated chemicals and reproductive dysfunction in right whales.

The Workshop recognized the importance of controlling for confounding variables (e.g. age, sex, reproductive state, et cetera; see Aguilar et al. 1999) when comparing samples.

Castellini stressed the importance of differentiating between starvation and fasting as these are separate biochemical events (with different characteristics relative to energy mobilization). During winter when right whales reduce their food intake, they are fasting not starving. Clapham pointed out that there is no evidence of right whales in the North Atlantic fasting for a full six months and that, except for parturient females, animals forage at least occasionally throughout winter and spring. However, females that are simultaneously lactating and fasting may mobilize and circulate toxic chemicals stored in their blubber during late autumn when they are at the southern end of their migration.

Southern noted that CYP1A can be induced by stressors other than chemical contaminants, referencing ice-entrapped white whales and humans with HIVas examples. Moore responded that even in these cases the induction may still be due to toxins if the stressors cause fat mobilization, thereby releasing toxins into circulation.

3.3.3. Evaluation of likelihood that chemical contaminants may be affecting North Atlantic right whale reproduction and development of testable hypotheses

The Workshop **agreed** that right whales are routinely exposed to a wide array of xenobiotic chemicals, some of which generate toxic effects on mammalian reproductive and immune systems. Thus, even though most of the fat-soluble persistent compounds usually associated with reproductive dysfunction and impaired immuno-competence seem to occur at relatively low levels in right whale tissues, chemical contamination may be partly responsible for the observed reproductive problems in the stock.

A number of questions were raised regarding the search for potentially relevant types of contaminants:

(1) Are there chemicals whose release into the environment is correlated either spatially or temporally with the decline in reproductive success of right whales?
(2) What are the levels of xenobiotic substances in right whale prey species?
(3) Are there unique avenues of exposure that might be affecting right whales?

With regard to the last of the three questions, it was noted that the North Atlantic right whale population forages downstream of a large metropolis that dumps sewage treatment effluent into nearby waters, creating a high probability of exposure to estrogenic chemicals and other pharmaceuticals. The whales also feed in and near major shipping lanes where they may be exposed to aromatic hydrocarbons (oil leaks and discharges) and organotins (leaching from hulls). Marx pointed out that the component of the population that migrates to the calving grounds in the southeastern USA must swim through paper mill effluent and is in close proximity to military activities. Since defecation has occasionally been observed in these waters, the whales may feed opportunistically in this area.

Appendix 6 lists examples of contaminants of possible concern with regard to North Atlantic right whales. The list is based on three considerations: (1) likely exposure because of the right whale's trophic level and prey selection; (2) a recent general increase in usuage of the chemical concerned; and (3) a regional source of contaminant supply is known to exist. There are several reasons why 'non-traditional' chemicals are included on this list. Flame retardants and plasticizers were used extensively during the 1980s and 1990s. Use of alkylphenols in surfactants (and as additives in many commercial products) has also increased since the 1980s. Emissions of pharmaceuticals, including synthetic estrogen, have accelerated, as has the use of anti-fouling agents. Proliferation of aquaculture since the 1980s has meant the introduction of antibiotics and pesticides directly into the sea. Finally, inputs of methyl mercury via atmospheric deposition have increased in both New England and the Canadian Maritimes in recent years.

Ross summarized the elements of toxic risk assessment as follows:

(1) identifying the chemicals - types, history of production and use, transport pathways and fates;

(2) characterizing the chemicals - solubility, bioaccumulation pathways, persistence in the environment and levels in prey species;

(3) pharmacology/toxicology of the chemicals - metabolizable or not, formation of toxic metabolites and immunological or endocrine effects;

(4) quantification of exposure - feeding locations, prey selection et cetera;

(5) consideration of species sensitivity.

As obtaining direct, conclusive evidence concerning contaminant effects on right whale reproduction will be difficult, Ross advocated that a 'weight-of-evidence' approach be used to assess the risks of such effects (see Ross 2000). This would have at least six components, as follows:

(1) characterize and eliminate all possible confounding factors, e.g. age, sex and condition;

(2) conduct comprehensive, congener-specific contaminant analyses (concentrations and patterns) using blubber biopsies (Deshpande noted that current biopsies, which contain considerable amounts of connective tissue, may not represent the lipid contents deep within the blubber and that therefore deeper biopsy samples may be necessary);

(3) conduct similar analyses on right whale prey species to help identify contaminant sources, chemical classes of concern and trophic pathways;

(4) examine biomarkers of exposure and effect (e.g. CYP1A, as discussed above; petroleum biomarker compounds such as triterpanes and steranes);

(5) explore linkages between toxicological endpoints measured in right whales and the more comprehensive multi-compartment studies of harbor seals, with a view to making appropriate inferences about effects in right whales; and,

(6) use all available information to evaluate the risks to right whales.

Ideally such studies should take place in a tiered fashion and follow the U.S. Environmental Protection Agency's guidelines for ecological risk assessment. This would involve identifying potential hazards and exposure pathways, completing a toxicity assessment, and then characterizing risk, using a weight-of-evidence approach.

Moore emphasized the importance of giving closer attention to non-bioaccumulative contaminants rather than being fixated on the 'traditional' problem chemicals.

Southern expressed a dissenting view with regard emphasizing the detection of chemicals rather than impacts (physiological responses). In her opinion, it is important to investigate the molecular evidence of stress response in right whales, as well as to catalogue toxin levels.

Muir pointed out that differences in types or levels of contaminants in tissues can sometimes be used to distinguish populations. He suggested that a comparison of contaminant profiles in Fundy and non-Fundy right whales could help establish the location of the non-Fundy 'nursery' area.

3.4. Biotoxins

3.4.1. Review of existing knowledge of effects of biotoxins on reproduction

In recent years, the frequency, distribution and diversity of harmful algal blooms have increased along the east coast of North America, as well as globally. Five major classes of biotoxins are associated with these algal blooms : saxitoxins (responsible for paralytic shellfish poisoning, or PSP), brevetoxins (neurotoxic shellfish poisoning, NSP), domoic acid (amnesic shellfish poisoning, ASP), okadaic acid and dinophysistoxins (diarrhetic shellfish poisoning, DSP), and ciguatoxins (ciguatera fish poisoning, CFP). Four of these classes have been implicated in mortality events involving marine mammals; saxitoxins and brevetoxins are the two groups that most often occur in the distributional range of North Atlantic right whales. Domoic acid (ASP) events also occur off the northeastern United States, but such events appear to have declined since the late 1980s.

Most toxins of algal origin are neurotoxic. Saxitoxins and domoic acid are water-soluble compounds that function as sodium channel blockers and as glutamate agonists, respectively. Brevetoxins are lipid-soluble and act as sodium channel agonists. The water-soluble toxins, while capable of attaining high levels during acute exposure, do not biomagnify, but the lipid-soluble toxins bioaccumulate.

It is unclear how biotoxins affect reproduction, *per se*. Doucette noted that mice exposed *in utero* to domoic acid during the middle embryonic period experienced severe reorganization of the hippocampus within three weeks of birth (Dakshinamurti et al. 1993), and that neonatal rats exhibited a 40-fold increase in sensitivity per body weight to domoic acid (Xi et al. 1997). Acute exposure of California sea lions (*Zalophus californianus*) to toxigenic diatoms (via trophic interactions) produced seizures and mortality of adult animals, as well as abortions (Scholin et al. 2000). Brevetoxins are immuno-suppressive *in vitro* and may cause hemolytic anemia. A mass mortality of manatees in Florida was likely due to prolonged respiratory exposure to brevetoxins (Bossart et al. 1998). Saxitoxins cause loss of equilibrium and respiratory distress, with possible implications for feeding efficiency. Acute trophic exposure via contaminated mackerel was associated with a mass mortality of humpback whales in New England (Geraci et al. 1989). However, very little is known about the sublethal, chronic effects of exposure to saxitoxins.

3.4.2. Review of data on biotoxin levels in North Atlantic right whales

The algae producing brevetoxins are most prevalent in warm waters (22-28˚C), and are thus largely outside the southern migratory range of right whales, which is limited primarily to waters cooler than 18-20˚C; Anonymous, 1997). However, direct respiratory exposure of adult females and young calves to biotoxins in Florida and Georgia nearshore waters during winter cannot be ruled out.

Exposure to saxitoxins is a concern with regard to North Atlantic right whales. The dinoflagellate genus *Alexandrium*, which produces saxitoxin, overlaps in space and time with right whales, and toxicity levels generally increase from southern New England northwards, e.g. to the Bay of Fundy. Peak dinoflagellate blooms typically occur in July and August, concurrent with the presence of right whales in the Gulf of Maine/Bay of Fundy region. Exposure of right whales to saxitoxins most likely occurs via trophic transfer. Laboratory studies indicate that various copepod species (including *Calanus finmarchicus*) largely avoid *Alexandrium* cells but still ingest enough to acquire measurable toxicity levels (Turriff et al. 1995). In this regard, Doucette noted that an ECOHAB regional study in the Gulf of Maine should soon provide information on biotoxin loading in right whale prey species. He also cited a recent study in Massachusetts Bay that showed preferential saxitoxin accumulation in large copepods, including *Calanus finmarchicus* and *Centropages typicus* (Turner et al. in press). Based on the LD_{50}s (i.e. the dose level at which 50% of treated animals die) of saxitoxin for terrestrial mammals (200-600µg/kg), the estimated daily food consumption of right whales (ca. 4% of body weight/day) (see Anderson and White,1989), and typical toxin loads in larger copepods (100-200µg STX equiv./g wet weight; Turner et al. in press), Doucette estimated that right whales could receive a lethal dose of saxitoxin in a day.

3.4.3. Evaluation of likelihood that biotoxins may be affecting North Atlantic right whale reproduction and development of testable hypotheses

If biotoxins impair reproduction of right whales, this likely occurs via acute toxicity to pregnant females or neonates. The central nervous system may be affected during foetal or post-natal development, with consequent long-term effects on regulation of the reproductive system by the hypothalamic-pituitary axis. However, given rapid renal clearance rates in right whales for water-soluble saxitoxins, it is unlikely that *in utero* exposure represents a high risk to foetuses. Geraci et al. (1989) suggested that cetacean diving adaptations (which channel blood into the toxin-sensitive heart and brain and away from the organs responsible for detoxification (liver and kidney)), could make right whales susceptible to the neurotoxic effects of biotoxins. Historical data series of biotoxin activity are available from long-term monitoring efforts of PSP toxicity in the Gulf of Maine (since 1957) and the Bay of Fundy (since 1944) that could be compared with temporal trends in right whale calf production. White (1987) proposed a 18.6 yr cycle in maximum toxicity in the Bay of Fundy, and his analysis predicted a peak in the late 1990s. Jennifer Martin (DFO, St. Andrews, New Brunswick), has maintained the data set initiated by White, thereby allowing comparisons to be made with the most recent calving data.

Determination of toxin levels in zooplankton size fractions containing *C. finmarchicus* or *Centropages* spp. would be useful (frozen samples are best for such analyses), as would assays of right whale feces, body tissues and fluids for biotoxins (particularly saxitoxins). The Marine Biotoxins Program at the NOAA/NOS Charleston laboratory is currently diagnosing PSP toxins using dried blood spot cards, and Doucette suggested that this technique might be useful if blood was available from biopsies. Brown reported, however, that very little, if any, blood is present in most biopsy samples. Although exposure by right whales to brevetoxins on the winter calving grounds in the southeastern U.S. coast is unlikely, detection of these toxins (most likely acquired via inhalation of aerosols) may be possible by testing blowhole exudates.

A key issue is whether a causative link can be established between the presence of toxins in the feces, body tissues, or fluids of right whales and the occurrence of acute or subacute toxicity. In this regard, Doucette pointed out that in bullfrogs a special protein (saxiphilin; Mahar et al. 1991) is synthesized that binds with saxitoxin with sub-nanomolar affinity, thereby conferring 'resistance' to the toxin. Based on this, if right whales have been historically exposed to saxitoxin, they may have developed a similar detoxification mechanism. Nonetheless, measuring PSP toxins in both right whales and their primary prey is an important first step in assessing the potential impact of biotoxins on these mammals.

3.5. Disease

3.5.1. Review of existing knowledge of effects of disease on reproduction

Munson provided an overview of how disease can affect reproduction. When an animal suffers from any chronic (non-infectious) systemic disease, reproductive activity generally diminishes or shuts down entirely. Infectious systemic disease in adults can similarly inhibit reproductive activity although this can be reversible, depending on recovery. Neonates that contract an infectious systemic disease either die or suffer long-term developmental damage. Neonatal deaths are frequently observed in marine mammals but the cause usually remains undiagnosed. If a pregnant female contracts a reproductive tract infection, e.g. in the uterus, she may fail to carry the foetus to term. Reproductive diseases can be acutely lethal (e.g. infection), recurrent (e.g. associated with environmental conditions that recur on a seasonal basis) or chronic causing permanent damage (e.g. uterine occlusions in Baltic seals).

Hall provided a broad review of epidemiological principles, highlighting the need to view disease exposure and susceptibility in an ecosystem context. Hall noted that it is important to consider interactions between pathogens, as well as the possible involvement of multiple pathogens, in a given disease syndrome. In some instances, the ultimate cause of infection may be an antecedent event, condition or characteristic which 'sets the stage' for the proximate disease occurrence. In other words, there may be no single cause but rather a series of components which, acting together, comprise the causal mechanism.

A number of viral, bacterial and protozoal diseases affecting reproduction have been documented in marine mammals. These include: leptospirosis, salmonellosis, campylobacteriosis, brucellosis, toxoplasmosis, morbillivirus, papillomavirus, adenovirus, influenza virus, herpes virus, nasitremosis, stenurosis and halocerosis. *Brucella* sp. has been found in marine mammals but marine species of *Brucella* have yet to be definitely linked with abortions in the wild. Rowles, however, called attention to the fact that a *Brucella* isolate from a seal in Puget Sound caused abortions when inoculated into cattle. Morbillivirus has been implicated in harbor seal abortions.

3.5.2. Review of data on disease in North Atlantic right whales

Little is known about disease in North Atlantic right whales. Moore reviewed necropsy data from 25 of the 45 right whales known to have died in the western North Atlantic since 1970. Trauma, mainly from ship strikes, was detected in12 cases. One or more bones were salvaged from 14 carcasses, and one or more soft-tissue samples from nine. On only eight occasions were gonads found. Tissue autolysis has generally been a major impediment in histological investigations of right whales. Munson emphasized that even when the cause of death is attributable to trauma based on gross findings, it is important to strive for histological analyses that could reveal an underlying pathological condition. Southern added that it would be useful to test grossly normal biopsy tissues for virus antigens.

Marx described three types of skin lesion observed on North Atlantic right whales: circular, blister or crater, and swath. Skin lesions, mainly circular, have been detected on more than 100 individuals in the right whale populationsince 1980, and have significantly increased over time ($p=0.004$). During 1980-1996, the average percentage of identified whales with skin lesions was 5.06% (sd=5.86), with a peak of 24% in 1995. Regressions for females that should have calved but did not (as determined by calving history) and for whales with lesions both showed significant increases ($F=23.3$, $p<0.01$ and $F=32.6$, $p<0.01$, respectively). The two classes were themselves significantly correlated ($r=0.768$, $p<0.01$). De Guise noted that the death of an adult female in 1999 (caused by a ship strike) provided an opportunity for histological examination of the skin lesions. The lesions on this animal were papilloma-like, exhibiting some acute and chronic inflammation in the dermis. Electron microscopic studies did not reveal an etiological agent, but further analyses of the lesions are underway. Given that skin lesions have increased coincident with the decline in the whale population's reproductive success, there was considerable discussion concerning a possible etiological link between the two trends. It is not clear whether the lesions are primary and thus limited to the whale's skin, or a secondary manifestation of an underlying systemic health problem.

Skin lesions of the kind observed on North Atlantic right whales have not been observed on North Atlantic humpback whales. Rowntree noted that blister-like lesions are seen on right whales in Argentina but the type of lesions seen on North Atlantic right whales are generally not observed on southern right whales.

Ross noted that vitamin A plays an important role in skin maintenance. He suggested that a possible link involving a lack of vitamin A, or alternatively a contaminant-induced breakdown in normal circulatory transport of vitamin A, should be explored as a cause of the skin lesions. Southern pointed out that a molecular stress response could be involved, as either a cause or an effect of vitamin A deficiency, and that causation would be difficult to establish. Munson indicated that skin lesions similiar to those on right whales had been observed on black rhinoceroses and that vitamin A involvement had been ruled out as a causative agent. Moore stated that the only right whale tissue presently available for vitamin A assays is a frozen sample in the care of Robert Bonde, US Geological Survey, Sirenia Project, in Florida.

Marx noted that the distribution, abundance, and other characteristics of cyamids are used as a crude index of a right whale's overall health status. She and Rowntree explained that orange cyamids (*Cyamus erraticus*) occur primarily in body folds or recesses (e.g., in the genital and axillary regions) but quickly invade and colonize wounds. In debilitated animals, cyamids also occur in the mouth area and blowholes. The sloughed skin that cyamids eat, as well as the cyamids themselves, are less likely to be 'washed off' on whales moving more slowly than normal, leading to the orange appearance.

3.5.3 *Evaluation of likelihood that disease may be affecting North Atlantic right whale reproduction and development of testable hypotheses*

Workshop participants agreed that skin lesions were a high priority for further research. It was suggested that individual sighting histories could be informative if skin lesions could be related to reproductive events using the photo-identification database. No opportunity should be missed to obtain biopsy or necropsy samples of skin lesions. Hamilton explained that lesions tend to be more focal than general, suggested that photographs or video of the whole bodies of living whales would be useful in characterizing the lesions. Skin diseases are often classified according to the distribution of lesions on the body. Brown noted that permits were being procured for the use of a critter-cam for studying North Atlantic right whales and that this equipment might be useful in documenting the distribution of body lesions.

It was generally agreed that obtaining fresh tissue samples as quickly as possible from carcasses was a high priority. Munson urged that necropsies examine all organs for lesions and that necropsy reports not be limited only to diagnosing the cause of death. Moore responded that existing necropsy reports could be scrutinized further for this kind of ancillary information.

House and DeGuise cautioned that the presence of a disease agent does not necessarily indicate a pathologic outcome and should, therefore, be interpreted in context. Recognizing that sample sizes for North Atlantic right whales would almost certainly be too small to support meaningful epidemiological analyses, Hall indicated that case control studies were suited to small sample sizes and therefore could be useful.

3.6 Other potential factors (e.g. habitat loss/disturbance) and multi-factorial processes

Right whales no longer occupy their full historic range in the North Atlantic. This range contraction could mean that formerly occupied areas are no longer suitable to support right whales, or that the cultural memory for finding or using those areas has been lost to the population. Reproduction could be affected if calving habitat has been reduced (e.g. loss of Delaware Bay) or foraging habitat no longer used (e.g. Gulf of St. Lawrence, Strait of Belle Isle, the Labrador coast). Stormy Mayo is said to have data showing a decline since 1987 in the availability and quality of prey resources in Cape Cod Bay. Participants hoped that Mayo's analyses of these data would be completed and published soon. It was also noted that Kenney (in press) provides documentation for a significant alteration in habitat use over time. Participants discussed the importance of finding out where breeding (= mating) occurs and where non-Fundy females take their calves in the summer. It was recognized that there may not be a single non-Fundy nursery area, or a single breeding ground. Apart from the use of satellite telemetry to locate the 'missing' areas, it was suggested that useful insights might be gained from further analyses of individual sighting histories, particularly those of adult females seen on or near the Florida/Georgia calving grounds unaccompanied by calves.

4. DESIGN OF RESEARCH PROGRAM TO TEST ONE OR MORE OF THE ABOVE HYPOTHESES, BASED ON RELATIVE LIKELIHOOD OF OCCURRENCE AND FEASIBILITY OF RESEARCH ACHIEVING STATED OBJECTIVES, INCLUDING DISCUSSION OF APPROPRIATE 'CONTROL' POPULATION(S)

A table of research topics was drafted during the workshop and discussed during the last hours of the third day. Each research problem - stated as either a hypothesis, an objective, or a task - was prioritized based on its relevance and the likelihood of it being successfully addressed. Requirements for biological specimens and data were also identified, as was the need for 'control' species or populations. In some instances, other whale species (e.g. bowhead whales or Southern Hemisphere right whales) were identified as appropriate models or surrogates. For various analytical purposes, it was considered appropriate to subdivide the North Atlantic right whale population, for example into 'treatment' (e.g. Fundy) and 'control' (non-Fundy) groups.

Munson offered an alternative framework for organizing the presentation on a problem-by-problem basis (see Appendix 4). After the workshop, Rolland took the lead in developing this approach in consultation with workshop participants.

The following research program outline was developed from the a table of research topics discussed at the workshop. It should be read in the context of both the main report text and Appendix 4, the latter being a more hypothesis-based approach prepared after the workshop by Munson. Priorities are indicated in brackets for each item listed as a research need.

PROBLEM: DECLINING CALF PRODUCTION

NEED:

To investigate the incidence of females without calves in the southeastern US and relate to individual calving histories (HIGH).

To establish baseline information on reproductive physiology using gonadal and adrenal steroid hormone metabolite levels in feces and blowhole exudate to monitor reproductive status (HIGH).

To determine if extremely reduced haplotypic and allelic diversity in the major histocompatibility complex (MHC) has increased the risk of disease and probability of abortion (HIGH).

To investigate the potential value of molecular analysis of stress response in right whale skin (including enzyme assays) (LOW TO MODERATE).

ACTION:

Continue collection of photo-identification and sighting histories of individual whales.

Analyze existing catalogue data on an individual basis to determine if non-calving females are clustered by age (suggesting development) or region (suggesting genetics, biotoxins, contaminants, disease or nutritional stress).

- Compare BOF vs. non-BOF females.
- Conduct geographic and temporal analysis of non-calving vs. calving females.
- Conduct age-based analysis.
- Include nulliparous females over 9 years.

Continue biopsies for genetic testing and to develop additional genealogies.

- Increase sample coverage of the population.
- Conduct genetics-based analyses of the paternity of calves during critical and baseline periods to assess if there is regional (BOF vs. non-BOF) or temporal male infertility.
- Determine which males are breeding and their habitat preferences through paternity analysis.
- Determine inbreeding coefficients for breeding/non-breeding whales.

Determine MHC class II genetic profile of more females and calves.

- To determine if reduced diversity could increase risk of early abortion.

Develop assay for gonadal steroid hormone metabolites in feces and validate with samples from bowhead whales.

- To determine if pregnancy is occurring and being lost.
- To determine if females are cycling.
- To determine if unsuccessful males are producing androgens.
- To determine if an underlying endocrine problem is related to the reproductive dysfunction.
- To determine if non-breeding whales have elevated levels of glucocorticoid hormones suppressing reproductive function.

Investigate use of blowhole exudates to assay for gonadal steroid hormones using samples from bowhead whales (similar to saliva assay).

Investigate analysis of stress proteins in skin to determine if elevated stress impairs reproductive health.

- Include control groups, e.g. southern hemisphere right whales, BOF vs. non-BOF right whales.

PROBLEM: APPARENT DETERIORATION IN BODY CONDITION

NEED:

To determine if variability in calf production is associated with variations in prey abundance (MODERATE TO HIGH).

To determine if variability in calf production is associated with variations in the condition of prey organisms (e.g. lipid content) and in diet quality (HIGH).

To determine if variability in calf production is related to variations in whale body conditions (HIGH).

To evaluate calf growth rates (MODERATE).

To publish studies of historical prey availability in Cape Cod Bay (HIGH).

To develop a comprehensive necropsy protocol and rapid response capability (HIGH).

To establish a centralized necropsy database including all data (not just cause of death) (HIGH).

ACTION:

Collate and analyze existing data on prey abundance and environmental variables.

- Review prey patch formation, history of spatial/temporal distribution and environmental variables (e.g. oceanographic factors, North Atlantic Oscillation).
- Encourage studies on factors affecting the nutritional composition of major and minor prey species, including seasonal, geographic, ontogenetic, nutritional and long-term climatic effects.

Apply new methodologies to the study of North Atlantic right whale diets.

- Study nutrient composition of prey.
- Apply new techniques, e.g. stable isotopes of carbon and nitrogen, fatty acids, retinoids.
- Evaluate diet composition and feeding habits using feces and behavioral observations.

Establish a comprehensive index of right whale body condition, which includes estimates of blubber thickness, quality and composition, and estimates of body girth and length.

- Continue collecting acoustic blubber data with amplitude mode ultrasound, and validate using necropsy samples and controls (e.g. bowheads).
- Develop methods for determining lipid composition of blubber from ultrasound data and characterize lipid mobilization and deposition patterns.
- Calibrate blubber measurements with regard to position on body.
- Compare blubber thickness in BOF vs. non-BOF females, calving vs. non-calving females and Northern vs. Southern Hemisphere right whales.
- Determine if incidence and type of skin lesions are related to blubber thickness.
- Determine if whales that are ship-struck are thinner than other whales.
- Analyze leptin expression in dermal biopsies and necropsy samples of blubber using immunohistochemical, biochemical and molecular methods.

_ Assess biopsy and tissue samples as indicators of lipid stores, for nutritional constituents and for biomarkers indicating condition.

Analyze photos of calves to determine if growth rates can be deduced from length and girth measurements.

_ Assess need for dedicated photogrammetric program.

_ Review available data on calf growth rates from southern hemisphere right whales, bowheads.

If lactating females are necropsied, collect milk samples and data on mammary gland mass and anatomy.

Establish a comprehensive necropsy protocol.

_ Improve methods for necropsy to acquire more data on population health.

_ Improve tissue harvesting methods and archiving of tissues.

_ Develop capability and protocol for sampling right whale carcasses at sea.

_ Conduct pathologic assessment of fertility, collect reproductive tracts, assess gonadal activity, and perform gross and histopathological assessment of reproductive tracts.

_ Identify and characterize lesions, and their possible causes.

Collate all available pathology information in a central database.

_ Further review/examine existing necropsy reports.

PROBLEM: INCREASED NUMBER OF WHALES WITH SKIN LESIONS

NEED:

To determine if different types of skin lesions affect individual reproductive success (HIGH).

To determine if different types of skin lesions impair reproductive success (MODERATE).

To better assess the gross character, distribution and histopathology of skin lesions (LOW).

ACTION:

Analyze existing photo-identification catalogue data for skin lesions and reproductive history of individual females and males.

_ Determine if occurrence of skin lesions correlates with females without calves.

_ Conduct a temporal and habitat preference analysis of whales with skin lesions.

Obtain biopsies of skin lesions to determine their cause.

_ Conduct histopathology and electron microscopy on all biopsies.

_ Conduct viral culture on cetacean cell lines.

_ Conduct PCR for pox and herpes viruses using genetic primers.

Integrate the assessment of the distribution of skin lesions in ongoing studies measuring blubber thickness, body length and girth.

PROBLEM: INCREASED EXPOSURE TO BIOTOXINS (PSP) FROM HARMFUL ALGAL BLOOMS MAY BE AFFECTING REPRODUCTION.

NEED:

To determine if an association exists between the occcurence/prevalance of harmful algal blooms (especially saxitoxins) and annual right whale calf production (MODERATE TO HIGH).

To examine biotoxins in zooplankton (*C. finmarchicus* and *Centropages*) (MODERATE).

To analyze bio-toxins in tissues, fluids and feces from North Atlantic right whales (MODERATE TO LOW).

ACTION:

Analyze available data (going back to the 1940s) on biotoxin events in the Bay of Fundy.

Collect zooplankton samples in close proximity to feeding right whales and analyze samples for biotoxins.

Analyze fresh material (e.g. blood) from stranded whales and feces from free-swimming and dead whales for presence biotoxins.

Investigate diagnosis of biotoxins using dried blood spot cards.

Develop baseline data on tissue and fluid samples from controls and measure background toxin levels.

PROBLEM: EXPOSURE TO CONTAMINANTS MAY BE AFFECTING REPRODUCTION.

NEED:

To review available information on the types and sources of contaminants in the areas where right whales feed (HIGH).

To analyze dead whales (esp. ship strikes) for a targeted list of chemicals (HIGH).

To assay right whale prey, sympatric species, and co-predators for chemicals of concern (based on the preceding two items), possibly using historical samples (HIGH).

To design a biomarker approach based on exposure data (e.g. CYP1A, DNA adducts, retinoids, leptins, triterpanes, steranes) (LOW TO MODERATE).

To extract contaminant mixtures from right whale tissues (and/or right whale prey) for bioassays, *in vitro* studies or gene expression assays (HIGH).

ACTION:

Perform literature review on sources and types of chemicals and pharmaceuticals occurring in right whale habitats (including POPs and non-persistent chemicals) to develop a targeted list of chemicals that may impact right whale reproduction (see Appendix 6).

- Review the chemical list from O'Shea et al. (1999).

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

- Analyze samples of copepods, herring, sandlace, and co-predator species for chemicals of concern.
- Assess availability of historical samples for comparative analysis.

Analyze tissues from biopsies, stranded whales, and archived samples for chemicals of concern.

- Relate concentration of persistent chemicals in biopsies to concentrations in blubber.

Apply and develop suitable biomarkers of effects to determine if there is a physiological response to the chemicals of concern (e.g. CYP P450, DNA adducts, retinoids, leptins).

Re-analyze existing contaminant data (e.g. Weisbrod et al. 2000; Westgate et al. unpublished data; Moore et al. 2000, draft manuscript).

- Compare reproductively successful vs. non-successful whales and habitat preferences.
- Apply PAH toxic equivalency factors (TEQs) and dioxin TEQs to assess toxicity in right whales.

Conduct further congener-based analysis of PCBs and other 'dioxin-like' chemicals and calculation of TEQs.

Compare levels of contaminants of concern in calving and non-calving females (BOF vs. non-BOF) and successful vs. unsuccessful males.

Investigate the use of chemical 'fingerprints' to assess habitat preference and reproductive history.

Extrapolate results to better-studied species to assess toxicity thresholds and potential risk.

Standardize sample collection protocols and inter-laboratory analysis techniques.

Use a weight-of-evidence approach to assess risk from toxic chemicals to northern right whale reproduction.

5. SUMMARY AND CONCLUSIONS IN THE CONTEXT OF THE OVERALL CONSERVATION REQUIREMENTS OF NORTH ATLANTIC RIGHT WHALES

The most immediate conservation priority for the North Atlantic right whale population is to reduce anthropogenic mortality to zero (see IWC, in press b: Report of the Workshop on Status and Trends of Western North Atlantic Right Whales). However, the Workshop noted that, if calf production does not increase from the low levels observed during 1998-2000, even the complete removal of anthropogenic mortality may be insufficient to allow the population to recover. It is therefore critical that the reasons for the reproductive dysfunction of the population be established as soon as possible. To this end, the Workshop **recommended** that the research program proposed above be supported to the fullest extent possible.

The Workshop concluded that genetics, nutrition, contaminants, biotoxins and disease could each/all cause reproductive dysfunction in North Atlantic right whales. It is likely that no one factor is entirely responsible, and that two or more factors could be interacting, possibly over different time scales. For example, reduction in genetic diversity might have been caused a loss of population resilience over several centuries, while in recent decades, exposure by right whales to contaminants, biotoxins, and/or adverse environmental conditions may have resulted in poor body conditions and a greater incidence of disease. It is therefore important that the proposed research program be interactive and multi-disciplinary in nature and have strong central coordination. As such, the Workshop **recommended** that a steering committee be established to develop protocols, review results and progress, and provide nececessary revisions to the research program proposed in this report. The steering committee should consist of researchers possessing expertise in the various topics of concern (e.g. contaminants, pathology, nutrition et cetera). The steering committee has responsibility for selecting an appropriate coordinator for the research program, and this coordinator's position should be fully funded.

The Workshop further **recommended** the development of a comprehensive database (coordinated through the North Atlantic Right Whale Catalogue) linked for all whales across all research programs. Such a database would allow for multivariate analyses using data from photo-identification studies, health assessments, genetic studies, pathology results, contaminant levels and biomarker studies, biotoxin levels, and blubber thickness/composition studies.

The Workshop noted that the North Atlantic Right Whale catalogue (and its associated sighting history data base) has provided most of the data used in determining the status of the right whale population. The catalogue (and the sightings database) are integral components of the proposed research program and will be essential for determining reproductive performance in the future. The Workshop therefore **recommended** that the photo-identification program be fully supported on a continuing basis.

The Workshop agreed that the multi-disciplinary nature of the meeting and the interactive nature of the discussions had been extremely valuable, and commended the steering group for their efforts in setting up the meeting.

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APPENDIX 1

Causes of Reproductive Failure in North Atlantic Right Whales: New Avenues of Research

April 26-28, 2000

Falmouth, Massachusetts

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Appendix 2

Cause of Reproductive Failure in North Atlantic Right Whales: New Avenues of Research
April 26-28, 2000
Holiday Inn, Falmouth, Massachusetts

PLENARY PRESENTATIONS
WEDNESDAY, APRIL 26TH

0830 Welcome and Workshop Introduction
Michael Sissenwine
Phil Clapham
Roz Rolland

0845 Workshop Organization and Rules
Peter Best, Workshop Chair

0900 *North Atlantic Right Whale History and Status* - Scott Kraus
0945 Discussion

1000 *Blubber Thickness in Atlantic Right Whales, E. glacialis and E. australis: Relationship to Age, Gender, Reproductive Condition and Location* - Carolyn Miller
1020 Discussion

1030 Break

1050 *A Review of Necropsy Data for Northwest Atlantic Right Whales (Eubalaena glacialis): 1970-1999* - Michael Moore
1105 Discussion

1115 *Cytochrome P450 1A in Dermal Endothelia of Northern and Southern Right Whales, and Organic Contaminants in Right Whale Dermis and Gulf of Maine Zooplankton Prey* - Michael Moore
1135 Discussion

1145 *Skin Lesions in North Atlantic Right Whales: 1980-1996* - Marilyn Marx
Histopathology of Skin Lesions in a North Atlantic Right Whale - Sylvain DeGuise
1200 Discussion

1230 Lunch

1330 *Risk of Environmental Contaminant-Related Toxicity in Northern Right Whales* - Peter Ross
1400 Discussion

1415 *Reproductive Failure in North Atlantic Right Whales: The Marine Biotoxins Perspective* - Greg Doucette
1435 Discussion

1445 Break

1505 *Infectious Disease as a Factor Affecting Reproduction and Offspring Survival in the Northern Right Whale* - Ailsa Hall
1525 Discussion

1535 *Reproductive Dysfunction in Wild Animals: What the Tissues Tell Us* - Linda Munson
1605 Discussion

1615 General discussion

WORKSHOP AGENDA
THURSDAY, APRIL 27 & FRIDAY, APRIL 28

1. Introductory items
   1.1 Objectives of workshop
   1.2 Adoption of agenda

2. Overview of reproductive biology and reproductive failure
   2.1 General review of mammalian reproduction and causes of reproductive failure
   2.2 Review of North Atlantic right whale (NARW) reproduction, especially indicating where in the cycle impairment or failure may be occurring and development of testable hypotheses.

3. Consideration of possible causes of failure
   3.1 Genetic factors (e.g. loss of diversity, inbreeding, effective sex ratio)
      3.1.1 Review of existing knowledge of effects of these factors on reproduction
      3.1.2 Review of data on these factors in NARW
         Presentation: *Summary of Relevant Genetic Data on Northern Right Whales* - Brad White
      3.1.3 Evaluation of likelihood that genetic problems may be affecting NARW reproduction and development of testable hypotheses.
   3.2 Nutrition
      3.2.1 Review of existing knowledge of effects of nutrition on reproduction
      3.2.2 Review of data on nutrition in NARW
      3.2.3 Evaluation of likelihood that nutritional problems may be affecting NARW reproduction and development of testable hypotheses.
   3.3 Chemical contaminants
      3.3.1 Review of existing knowledge of effects of contaminants on reproduction
      3.3.2 Review of data on contaminant levels in NARW
      3.3.3 Evaluation of likelihood that chemical contaminants may be affecting NARW reproduction and development of testable hypotheses.
   3.4 Biotoxins
      3.3.4 Review of existing knowledge of effects of biotoxins on reproduction
      3.3.5 Review of data on biotoxin levels in NARW
      3.3.6 Evaluation of likelihood that biotoxins may be affecting NARW reproduction and development of testable hypotheses.
   3.5 Disease
      3.5.1 Review of existing knowledge of effects of disease on reproduction
      3.5.2 Review of data on disease in NARW
      3.5.3 Evaluation of likelihood that disease may be affecting NARW reproduction and development of testable hypotheses.
   3.6 Other potential factors (e.g. habitat disturbance/loss)

4. Design of research programme to test one or more of above hypotheses, based on relative likelihood of occurrence and feasibility of research achieving stated objectives, including discussion of appropriate "control" population(s).

5. Summary and conclusions in the context of the overall conservation requirements of NARW.

6. Adoption of report.

Appendix 3

SUMMARY OF AVAILABLE INFORMATION ON NORTH ATLANTIC RIGHT WHALE REPRODUCTION

Reproductive parameter	**Result/Conclusion**	**Caveats**	**Source**	**Cf Southern Hemisphere**
Calving interval	Regression of observed calving intervals against year shows significant increase from 1985 to 1998. Mean 3.67 yrs (1980-1992), over 5 yrs (1996-98).	Observed intervals only. Some of longer intervals undoubtedly represent unobserved calvings (12/20 or 60% of 6-7 yr intervals). But intervals between sightings of adult females similar in 1980s and 1990s.	Kraus et al (in press)	
	Mean calving interval (likelihood model) 3.28 ± 0.24 yrs in 1980s, 4.44 ± 0.43 yrs in 1990s. Distribution of calving intervals shifted from 3-year intervals in 1980s to 5+ intervals in 1990s, 4-year intervals unchanged.	Annual sighting probabilities estimated to have been close to 100% in recent years. Shift in calving interval distribution consistent with increased pre- or neonatal mortality in 1990s (2-yr intervals).	Cooke & Glinka (SC/O99/RW1)	3.26 ± 0.14 yrs in 1970s, 3.43 ± 0.14 yrs in 1980s (Cooke & Glinka)
		Analysis does not include mature females that have calved only once or not at all, so actual intervals are likely to be even longer.	SC/O99/Report	
%age of mature females that are reproductively active	70%	Potentially biassed low due to missed calvings and some females reaching sexual maturity after 9 years of age.	SC/O99/Report	
Average annual calf production rate per mature female	12.94%	Refers to 1990s B may well have been higher in 1980s but not possible to estimate. Fluctuates substantially from year to year.	SC/O99/Report	More than double that in N Atlantic (SC/O99/Report)
	Average annual calf production about 40% of expected.	Derivation unclear.	White et al (SC/O99/RW6)	
Mean age at first calving	Mean age of first observed calvings = 9.53 ± 2.32 (SD) yrs	Assumes all first calvings observed.	Kraus et al (in press)	
	Likelihood model indicates 10.1 ± 0.5 years in 1990s	Too few known-age animals to test for temporal trend	Cooke & Glinka (SC/O99/RW1)	9.8 ± 0.6 yrs in 1980s (Cooke & Glinka)

Survival rate of calving females	0.94 in 1980, declining to 0.63 in 1995	Stage-structured model indicates that this is the primary component of the decline in population growth rate and survival	Fujiwara et al (SC/O99/RW7)	
	0.982 ± 0.017 in 1980s, declining to 0.955 ± 0.067 in 1990s		Cooke & Glinka (SC/O99/RW1)	0.984 ± 0.005 in 1970s and 1980s (Cooke & Glinka)
Survival rate of females from birth to first calf	Overall rate 0.85 ± 0.29 in 1990s	Implied rate required to account for estimated rate of population increase B may be too high because increase rate over-estimated?	Cooke & Glinka (SC/O99/RW1)	1.01 ± 0.17 in 1980s (Cooke & Glinka)
	No significant trend in annual survival rate of female calves or immature females from 1980 to 1995		Fujiwara et al (SC/O99/RW7)	
Annual population growth rate	+3% in 1980 shifting to B2% in 1995; overall rate 1.3% (95% CI 0.1, 2.5%)	Decline mainly due to vital rates of females with calves	Fujiwara et al (SC/O99/RW7)	
	4.4% ± 2.8% for 1980-97	Likelihood model B estimate very imprecise and may not be significantly different from zero	Cooke & Glinka (SC/O99/RW1)	7.1 ± 1.4 % for 1971-90 (Cooke & Glinka)

SUMMARY

- Annual calf production per female highly variable and, since 1990, about half that expected from comparison with Southern Hemisphere (*Eubalaena australis*) females: from 1998 to 2000, lower in absolute terms than in all but one of the preceding 17 years
- Calf production now largely from cows not taking their calves to the Bay of Fundy nursery ground
- Calving interval has increased significantly from 1980s to 1990s, now averaging 5 years
- This increased calving interval apparent in cows of all ages
- Shift in distribution of calving intervals consistent with increased pre- or neonatal mortality
- Survival rate of calving females shows significant decline over time
- Survival rate of immature females shows no significant decline
- Age at first parturition similar to that in Southern Hemisphere animals
- Population increase rate significantly lower than in Southern Hemisphere, and since 1990 may be negative

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).**

Appendix 5

ASSESSMENT OF CURRENT REPRODUCTIVE DATA FOR THE WESTERN NORTH ATLANTIC RIGHT WHALE

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Since 1980, the birth of a total of 221 right whale calves has been documented in the western North Atlantic. Of these, seven are known to have died and 54 were not photographically identified (Table 1). An analysis of sighting data shows that 57% of the calves were brought to the Bay of Fundy by their mothers (Fundy calves, Table 1) and 43% were not taken to the Bay of Fundy (Non-Fundy calves, Table 1). The mothers of non-Fundy calves use a yet unknown summering and nursery location (Malik et al, 1999). Since 1987, genetic analyses have been used to assess population structure and reproduction in the North Atlantic right whale. Eighty-five mother-calf pairs have been sampled and genetically analyzed (Total genotyped, Table 2). These 85 pairs comprise 52% of the total mother-calf pairs for which the calf was photo-identified (Table 2). Of the mother-calf pairs that have been sampled, 60.0% of those seen in the Bay of Fundy have been sampled, and 32.5% of those not seen in the Bay of Fundy have been sampled (Table 3). Genetic analyses of these samples, using both mitochondrial DNA (mtDNA) and nuclear DNA, have shown that there is significant population substructuring between calves that are brought to the Bay of Fundy and calves that are not brought to the Bay of Fundy in their first year by their mothers (Malik et al, 1999, Waldick 1999). This substructuring is the result of site fidelity to a specific summer habitat area on the part of right whale mothers. They bring their calves either to the Bay of Fundy (Fundy calves) or to an unknown summer area (Non-Fundy calves). Offspring tend to show the same site fidelity as their mothers. This pattern of differential habitat use means that some lineages use the Bay of Fundy in summer and others use an unknown area. Genetic studies show that there is also a degree of reproductive isolation between these two groups, suggesting that they could be using different mating areas and that they are therefore more distinct than previously thought (Waldick 1999).

The population substructuring described above is of increasing interest when considering reproduction over the past three years, during which time no mothers from the Fundy subgroup have produced offspring (Table 1, plus data from the calving season of 2000; Phil Hamilton, pers. comm.). This skew in reproductive performance could indicate that there are recent and crucial differences in other aspects of the two subgroups, such as habitat quality and mortality due to anthropogenic factors. Such differences between subpopulations within the North Atlantic right whale population should be of primary consideration in all future studies of this population as conservation efforts are often futile when population structure is not taken into account (Taylor and Dizon 1999).

References

Malik, S., M.W. Brown, S.D. Kraus, A.R. Knowlton, P.K. Hamilton, and B.N. White. 1999. Assessment of mitochondrial DNA structuring and nursery use in the North Atlantic right whale (*Eubalaena glacialis*). *Can. J. Zool.* 77:1-6.

Taylor, B.L. and A.E. Dizon. 1999. First policy then science: why a management unit based solely on genetic criteria cannot work. *Molecular Ecology* 8: S11-S16.

Waldick, R.C. 1999. Assessing the status of the endangered North Atlantic right whale using genetic and demographic data. Ph.D. thesis, McMaster University. 172 pp.

Table 1: Numbers of right whale calves photographed from Fundy and non-Fundy population. $=calves which did not die in the calving ground. *Calves for which photos are inadequate to permit individual identification.

Year	Calves born	Calves alive$	Fundy calves	Non-Fundy	Unknown calves*
1980	5	5	3	2	3
1981	8	8	7	1	0
1982	12	11	6	5	2
1983	9	9	4	5	2
1984	12	12	11	1	1
1985	11	11	5	6	3
1986	13	13	6	7	2
1987	11	11	7	4	0
1988	8	7	4	3	0
1989	19	16	11	5	1
1990	12	12	9	3	2
1991	17	17	9	8	5
1992	12	12	4	8	4
1993	8	6	4	2	1
1994	8	8	3	5	3
1995	7	7	3	4	4
1996	21	21	14	7	7
1997	19	19	11	8	7
1998	5	5	0	5	3
1999	4	4	0	4	4
Total	221	214	121	93	54

Table 2: Genotyped mother-calf pairs from Fundy and non-Fundy populations

Year	Possible Pairs*	Total Genotyped	Fundy	Non-Fundy
1980	2	0	0	0
1981	8	3	3	0
1982	9	4	2	2
1983	7	2	2	0
1984	11	6	6	0
1985	8	2	1	0
1986	11	5	3	2
1987	11	6	5	1
1988	7	3	3	0
1989	15	11	9	2
1990	10	4	4	0
1991	12	8	8	0
1992	8	7	4	3
1993	5	3	3	0
1994	5	2	1	1
1995	3	2	2	0
1996	14	9	9	0
1997	12	7	7	0
1998	2	2	0	2
1999	0	0	0	0
Total	160	86	72	13

Table 3: Percentage of genotyped cow-calf pairs from Fundy and non-Fundy population

Year	Identified Fundy	Genotyped Fundy	%-Fundy	Identified Non-Fundy	Genotyped Non-Fundy	%-Non-Fundy
1980	2	0	N/A	0	0	N/A
1981	7	3	42.8571	1	0	N/A
1982	6	2	33.3333	3	2	66.66667
1983	4	2	50	3	0	N/A
1984	11	6	54.5455	0	0	N/A
1985	5	1	20	3	0	N/A
1986	6	3	50	5	2	40
1987	7	5	71.4286	4	1	25
1988	4	3	75	3	0	N/A
1989	11	9	81.8182	4	2	50
1990	9	4	44.4444	1	0	N/A
1991	9	8	88.8889	3	0	N/A
1992	4	4	100	4	3	75
1993	4	3	75	1	0	N/A
1994	3	1	33.3333	2	1	50
1995	3	2	66.6667	0	0	N/A
1996	14	9	64.2857	0	0	N/A
1997	11	7	63.6364	1	0	N/A
1998	0	0	N/A	2	2	100
1999	0	0	N/A	0	0	N/A
Total	120	72	60	40	13	32.5

Appendix 6

EXAMPLES OF CONTAMINANTS OF POSSIBLE CONCERN WITH REGARD TO NORTH ATLANTIC RIGHT WHALES.

(See O'Shea *et al.* (1999) and Reijnders *et al.* (2000) for more detailed lists and discussion).

Chemical Class	**More specific compounds or examples**
'TRADITIONAL'	
Persistent organic pollutants	PCBs, PCDDs, PCDFs
	DDT family
	Chlordanes (including Toxaphene)
	HCH
	Other pesticides
Non-bioaccumulative pollutants	PAHs
'NON-TRADITIONAL'	
Flame retardants	PBDEs (polybrominated diphenyl ethers) and other brominated flame retardants
Plasticizers	Phthalate esters
Surfactants	Alkylphenol ethoxylates (e.g. NPEO – nonylphenoletoxylates)
New-era pesticides and herbicides	
Municipal and industrial effluents	Endocrine disrupting compounds (e.g. synthetic estrogens, natural hormones, pulp byproducts)
Anti-fouling agents	Organotins (e.g. TBT - tributyltin) and replacement compounds
Dielectric fluids	PCB replacements (e.g. PCNs – polychlorinated naphthalenes; PBBs – polybrominated biphenyls)
Aquaculture-related chemicals	Antibiotics
	Pesticides
Metals	Methyl mercury (MeHg)
Radionuclides	

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