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Proceedings of the workshop on the St. Lawrence Estuary beluga - review of carcass program

Compte rendu de l'atelier sur le béluga de l'estuaire du Saint-Laurent – Revue du programme des carcasses

November 14 – 17, 2005, Mont-Joli

14 au 17 novembre 2005, Mont-Joli

Lena Measures

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TABLE OF CONTENTS

SUMMARY

The beluga (*Delphinapterus leucas*) is a medium-sized toothed whale found in Arctic and sub-Arctic waters. Of seven populations of beluga in Canada the St. Lawrence Estuary (SLE) beluga is the southernmost population. The SLE beluga lives in an estuarine habitat that is part of an international waterway, the St. Lawrence Seaway, with daily marine traffic and input of various contaminants including industrial effluents, chemical and biological waste from agricultural runoff, municipal and ship wastewater and ballastwater. This population has been protected from hunting since 1979. It currently numbers around 1,000 animals. The SLE beluga population was designated endangered in 1983 by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC) and down-listed to threatened in 2004 and protected as such by the Species at Risk Act (SARA). Concerns have been raised about the role of chemical contaminants causing certain pathological changes that may be hindering recovery of this population. Over the last two decades various measures to improve habitat quality and further protect this population have been undertaken by governmental and non-governmental organisations. However, the SLE beluga population has failed to show significant signs of recovery. The reasons for this are still not understood.

Belugas are long-lived, high trophic level predators and are exposed to a variety of anthropogenic and natural contaminants. The SLE beluga is highly visible, charismatic and emblematic in Quebec, Canada and the world. The Department of Fisheries and Oceans Canada (DFO) has the legal responsibility to conserve and protect this population and promote recovery through the development and implementation of a species recovery strategy and action plan as stipulated in by SARA. Since 1982 a program to monitor mortalities of the SLE beluga population with necropsy and sampling of beach-cast carcasses has been supported by DFO, Parks Canada and others [Institut National d'Écotoxicologie du Saint-Laurent (INESL), Les Industries Filmar, Université de Montréal, Faculté de medicine vétérinaire (FMV), World Wildlife Fund (WWF)]. The objective of this beluga carcass program was to determine the cause of death of beach-cast animals using adapted standard veterinary pathological and diagnostic methods. Results in publications were often used as a scientific basis for implementation of conservation measures and in decisions made by resource and habitat managers including creation of the Saguenay-St. Lawrence Marine Park and the proposed St. Lawrence Estuary Marine Protected Area, both of which, in part, are designed to protect the SLE beluga and its habitat.

The SLE carcass program consists of three components: monitoring, sampling and necropsy (post-mortem examination). After 22 complete years (1983 – 2004) of this program, DFO and Parks Canada considered it worthwhile to conduct a critical evaluation of this program by organising a workshop with various scientists or managers involved with the program and or using its results in conservation from DFO and Parks Canada. External experts were asked to provide critical evaluations of the program. The workshop had four themes, namely, Cause of mortality and diseases; Contaminants and toxic effects; Biology and demography; Conservation. Participants were also asked to provide an evaluation of the consequences for the carcass program of four options (elimination, reduction, maintenance of status quo or modification) with respect to their particular field of research or interest.

The SLE beluga has become a worldwide symbol of environmental awareness and protection. Knowledge gained from the carcass program has contributed to conservation efforts to preserve and protect this population of beluga. Monitoring has documented mortalities. Sampling has documented exposure to certain toxic contaminants. Necropsies and sampling have documented lesions, anomalies such as cancer, opportunistic infections, and biomarkers such as DNA adducts suggesting toxic effects, possible endocrine disruption and immunosuppression due to contaminants which may, at the population level, be affecting growth, survival, recruitment, reproduction and increase mortality rate. In this respect the carcass program has been valuable in identifying potential threats to recovery of the population. The etiology or cause of certain lesions and the role of toxic contaminants, pathogens or both are still unknown. Limitations of the carcass program include: the small number of carcasses found with unknown biases such as under-representation of juveniles in the stranding data base; problems aging animals, particularly old individuals; advanced decomposition of some

carcasses and other factors which result in a significant percentage of carcasses with unknown cause of death; few tissue samples of sufficient quality to test hypotheses on the role of pathogens or contaminant levels and toxic effects or to detect annual trends; poor data base management; and no integration of contaminant data with pathologic or biomarker data in order to demonstrate any causal link between certain lesions and the presence of certain toxic contaminants. Integration of these data including data from other research (i.e. aerial surveys, diet studies, etc.) would greatly increase the power of analyses and help to understand why this population is not recovering. Confounding problems include the longevity of beluga and the decline of some toxic contaminants and the discovery of new toxic contaminants in the marine environment and beluga tissues. Four key recommendations concerning the carcass program resulted from workshop discussions and are as follows:

Recommendation 1: The SLE carcass program, a world-recognized case study, should continue in order to monitor the state and recovery (detect improvement or deterioration) of this population. The carcass program provides valuable information on strandings (monitoring component); pathologies, cause of death (necropsy component), and provides tissue samples for further assessment such as age at death, pathogens, genotyping, contaminants, physiological and reproductive states and diet (sampling component). With long-lived animals data must be collected over a long time period in order to detect changes in contaminant loads and toxic effects and to evaluate responses to management efforts to help this population recover. What is needed now is that the necropsy data base be updated, validated, better organized, and accessible to certain researchers. With this large time series of data, integration of existing data linking information from various aspects of SLE beluga research including monitoring, necropsy, sampling, contaminant analyses, genetic data, aerial surveys, photographic identification, tagging, etc. should begin. Hypotheses could be proposed and tested using existing data, and this will provide a valuable resource platform on which to design special projects financed with special funding.

Recommendation 2: A consortium should be created and all available information integrated. A consortium of participating researchers studying the SLE beluga, with DFO as the lead agency, would be an efficient way to manage and share information, enhance collaborations, identify research needs and facilitate financing of special projects. Objectives of the consortium should include: 1) establish and document protocols for all components of the carcass program; 2) improve data sharing and promote research collaborations on the SLE beluga; 3) advise on research direction, priorities and mitigation measures to protect the population and facilitate recovery; 4) inventory, maintain, manage and co-ordinate use of archived tissue banks and data bases; 5) explore potential sources of funding to conduct special research projects; 6) assist in developing a contingency plan to detect and respond in a timely manner to an unusual stranding event, mortality event or immediate threat to the population; 7) maintain an inventory of all publications related to the program and communicate results to other scientists, managers and the public. Funding to help organize and co-ordinate activities of the consortium would be required.

Recommendation 3: The core components of the SLE beluga carcass program (monitoring, necropsy, sampling) should continue with adequate funding since it constitutes the foundation upon which essential data required in managing, recovering and conserving this population are based. However, this "core" program is not adequately financed at present. Intrinsic funding (from DFO and Parks Canada) has thus far proved insufficient. Almost all parts of the "core" program have been partially financed by extrinsic funding (funds from outside of the carcass program, volunteers, etc) and are unpredictable with no long term financial commitment.

Recommendation 4: There should be special research projects funded by special funds from outside the "core" program, i.e. NSERC, FCAR, non-governmental funds. Special projects financed by special extrinsic funds will benefit from and enhance the "core" program, and will answer specific questions posed by results from the "core" program as well as serve specific management needs. The "core" program has collected sufficient data thus far to generate a number of testable hypotheses that can be examined as special projects which may or may not be supported by DFO (depending on the mandate and priorities). Some examples of special projects were suggested.

Successful conservation of the St. Lawrence ecosystem and recovery of its endemic and emblematic beluga population depends on sound scientific data. Current evidence shows that the beluga population is not declining, but at the same time it does not appear to be increasing. Given that this population is protected from hunting there is a need to identify and quantify factors that may be limiting recovery – valuable data obtained from the carcass program can help identify these factors but these data must be integrated with data from other research on this population and close collaboration of all interested researchers and managers be facilitated and developed further. The successful protection and recovery of the SLE beluga population falls under one of DFO's key strategic outcomes - healthy and productive aquatic ecosystems - for all Canadians.

SOMMAIRE

Le béluga (*Delphinapterus leucas*) est un odontocète (baleine à dents) de taille moyenne des eaux arctiques et subarctiques. Des sept populations de bélugas au Canada, la population de l'estuaire du Saint-Laurent (ESL) est celle qui se trouve le plus au sud. Le béluga de l'ESL vit dans un habitat estuarien faisant partie d'une voie navigable internationale, à savoir la voie maritime du Saint-Laurent, où le trafic maritime est quotidien et où divers contaminants sont introduits, notamment des effluents industriels, des résidus chimiques et biologiques provenant du ruissellement des terres agricoles, des eaux usées des villes et des navires et des eaux de ballast. Cette population est protégée de la chasse depuis 1979. Elle compte actuellement environ 1 000 animaux. En 1983, elle a été désignée comme étant en voie de disparition par le Comité sur la situation des espèces en péril au Canada (COSEPAC) et a été inscrite en 2004 dans une catégorie de moindre risque, à savoir menacée; elle est protégée à ce titre par la *Loi sur les espèces en péril* (LEP). Des préoccupations ont été soulevées quant au rôle des contaminants chimiques qui causent certaines modifications pathologiques pouvant empêcher le rétablissement de cette population. Au cours des deux dernières décennies, diverses mesures visant à améliorer la qualité de l'habitat et à protéger davantage cette population ont été prises par des organismes gouvernementaux et non gouvernementaux. Cependant, la population de béluga de l'ESL n'a toujours pas montré de signes importants de rétablissement. On n'en comprend toujours pas les raisons.

Les bélugas sont des prédateurs longévifs de niveau trophique supérieur et sont exposés à divers contaminants anthropiques et naturels. Le béluga de l'ESL est très visible et charismatique et fait figure d'emblème au Québec, au Canada et dans le monde. Le ministère des Pêches et des Océans du Canada (MPO) a la responsabilité légale de conserver et de protéger cette population ainsi que d'en favoriser le rétablissement par l'élaboration et la mise en œuvre d'un programme de rétablissement de l'espèce et d'un plan d'action tels que stipulés dans la LEP. Depuis 1982, le MPO, Parcs Canada et d'autres organismes (Institut national d'écotoxicologie du Saint-Laurent [INESL], Industries Filmar, Faculté de médicine vétérinaire [FMV] de l'Université de Montréal, Fonds mondial pour la nature [WWF]) appuient un programme permettant le suivi des mortalités au sein de la population de béluga de l'ESL ainsi que la nécropsie et l'échantillonnage des carcasses échouées. L'objectif de ce programme des carcasses de bélugas est de déterminer la cause de décès des animaux échoués au moyen de méthodes pathologiques et diagnostiques vétérinaires qui sont adaptées et normalisées. On a souvent employé les résultats publiés dans la littérature comme fondement scientifique sur lequel repose la mise en œuvre de mesures de conservation et dans la prise de décisions par les gestionnaires des ressources et des habitats, y compris la création du parc marin du Saguenay-Saint-Laurent et le projet de la zone de protection marine (ZPM) Estuaire du Saint-Laurent, tous les deux étant conçus, en partie, pour protéger le béluga de l'ESL et son habitat.

Le programme des carcasses de bélugas de l'ESL comporte trois volets : suivi, échantillonnage et nécropsie (examen *post-mortem*). Après 22 années complètes (1983-2004), le MPO et Parcs Canada ont considéré qu'une évaluation critique de ce programme méritait d'être réalisée et ont organisé un atelier auquel prendraient part divers scientifiques ou les gestionnaires du MPO et de Parcs Canada qui sont associés au programme ou, encore, qui emploient ses résultats pour la gestion de la conservation. Des spécialistes externes ont été invités à fournir des évaluations critiques du

programme. L'atelier comptait quatre thèmes, à savoir les causes de mortalité et les maladies, les contaminants et les effets toxiques, la biologie et la démographie ainsi que la conservation. Les participants ont également été invités à fournir une évaluation des conséquences des quatre options présentées pour le programme des carcasses (élimination, réduction, maintien du statu quo ou modification) selon leur champ particulier de recherche ou d'intérêt.

Le béluga de l'ESL est devenu un symbole mondial de la sensibilisation à l'environnement et à la protection. Les connaissances acquises dans le cadre du programme des carcasses ont contribué aux efforts de conservation visant à préserver et à protéger cette population de béluga. Le volet sur le suivi a permis de documenter les cas de mortalité. Le volet sur l'échantillonnage a permis de documenter l'exposition à certains contaminants toxiques. Les volets sur les nécropsies et l'échantillonnage ont permis de documenter des lésions, des anomalies comme le cancer, des infections opportunistes et des biomarqueurs comme les adduits de l'ADN dont la présence laisse sous-entendre des effets toxiques, une possible perturbation endocrinienne et une immunosuppression attribuables à des contaminants qui peuvent, à l'échelon de la population, avoir une incidence sur la croissance, la survie, le recrutement et la reproduction ainsi qu'accroître le taux de mortalité. À cet égard, le programme des carcasses s'est révélé important dans la détermination des menaces potentielles pesant sur le rétablissement de la population. L'étiologie ou la cause de certaines lésions ainsi que le rôle des contaminants toxiques et des agents pathogènes, ou des deux, demeurent encore inconnus. Les limites du programme des carcasses incluent : le petit nombre de carcasses trouvées, dont les biais sont inconnus, comme la sous-représentation des jeunes bélugas dans la base de données sur les échouements; les problèmes liés à la détermination de l'âge des animaux, particulièrement pour les vieux individus; l'état de décomposition avancée de certaines carcasses et d'autres facteurs qui donnent lieu à un pourcentage important de causes de décès inconnues. Au rang des limites du programme, mentionnons aussi le faible nombre d'échantillons de tissus d'assez bonne qualité pour permettre de vérifier des hypothèses relatives au rôle des agents pathogènes ou des concentrations de contaminants et de leurs effets toxiques ou, encore, de détecter des tendances annuelles; la mauvaise gestion de la base de données; enfin, l'absence d'intégration des données sur les contaminants avec des données sur les affections ou les biomarqueurs, intégration dont on a besoin pour démontrer tout lien causal entre certaines lésions et la présence de certains contaminants toxiques. L'intégration de ces données, y compris des données d'autres recherches (relevés aériens, études sur le régime alimentaire, etc.) permettrait d'augmenter grandement la puissance des analyses et nous aiderait à comprendre les raisons du non-rétablissement de cette population. Parmi les problèmes qui sont sources de confusion figurent : la longévité du béluga et le déclin de certains contaminants toxiques ainsi que la découverte de nouveaux contaminants toxiques dans le milieu marin et dans les tissus du béluga. Les discussions en ateliers ont permis la formulation de quatre recommandations clés concernant le programme des carcasses.

<u>Recommandation 1</u> – On doit maintenir le programme des carcasses de bélugas de l'ESL, une étude de cas reconnue à l'échelle mondiale, afin de suivre l'état et le rétablissement (détecter l'amélioration ou la détérioration) de cette population. Ce programme fournit des données importantes sur les échouements (volet sur le suivi), les affections et la cause de décès (volet sur la nécropsie) de même que des échantillons de tissus dont on a besoin pour approfondir les évaluations portant, par exemple, sur l'âge au décès, les agents pathogènes, le génotypage, les contaminants, les états physiologiques et reproducteurs ainsi que le régime alimentaire (volet sur l'échantillonnage). Dans le cas des animaux longévifs, il faut recueillir des données sur une longue période pour détecter les changements dans les charges de contaminants et les effets toxiques et pour évaluer les réactions aux efforts de gestion visant à favoriser le rétablissement de cette population. Il est maintenant nécessaire que la base de données sur la nécropsie soit mise à jour, validée, mieux organisée et rendue accessible à certains chercheurs. Avec cette grande série chronologique de données, on doit débuter l'intégration des données actuelles qui reliera l'information concernant divers aspects de la recherche sur le béluga de l'ESL, notamment le suivi, la nécropsie, l'échantillonnage, les analyses des contaminants, les données génétiques, les relevés aériens, l'identification photographique, l'étiquetage, etc. On pourrait proposer et évaluer des hypothèses à l'aide des données actuelles, et ces hypothèses serviront de plateforme

de ressources utiles sur laquelle on peut concevoir des projets spéciaux financés par des fonds spéciaux.

Recommandation 2 **–** Il y aurait lieu de créer un consortium et d'intégrer toute l'information disponible. Un consortium regroupant des chercheurs participants qui étudient le béluga de l'ESL, avec le MPO à titre d'organisme responsable, serait une manière efficace de gérer et de partager l'information, d'améliorer la collaboration, de déterminer les besoins en matière de recherche et de faciliter le financement de projets spéciaux. Les objectifs du consortium devraient inclure les points suivants : 1) établir et documenter les protocoles de tous les volets du programme des carcasses; 2) améliorer le partage des données et favoriser les travaux réalisés en collaboration, et portant sur le béluga de l'ESL; 3) formuler des avis sur l'orientation à donner aux recherches, les priorités et les mesures d'atténuation en vue de protéger la population et de faciliter le rétablissement; 4) effectuer l'inventaire des banques de tissus et des bases de données archivées, les mettre à jour, les gérer et coordonner leur utilisation; 5) explorer les sources possibles de financement afin de mener des projets de recherche spéciaux; 6) aider à l'élaboration d'un plan d'urgence pour détecter un échouement inhabituel, une mortalité inhabituelle ou une menace immédiate pour la population et y réagir en temps opportun; 7) tenir un inventaire de toutes les publications liées au programme et communiquer les résultats à d'autres scientifiques, à des gestionnaires et au public. Il faudrait obtenir des fonds pour faciliter l'organisation et coordonner les activités du consortium.

Recommandation 3 – Les volets de base du programme des carcasses de bélugas de l'ESL (suivi, nécropsie, échantillonnage) doivent se poursuivre et bénéficier d'un financement adéquat puisqu'ils sont nécessaires à l'obtention des données essentielles à la gestion, au rétablissement et à la conservation de cette population. Cependant, ce programme de « base » ne bénéficie pas à l'heure actuelle d'un financement adéquat. Le financement interne (du MPO et de Parcs Canada) s'est révélé jusqu'ici insuffisant. Presque tous les volets du programme de « base » ont été partiellement financés par des fonds externe (provenant de l'extérieur du programme des carcasses, de bénévoles, etc.) et leur avenir demeure imprévisible, faute d'un engagement financier à long terme.

Recommandation 4 – On doit établir des projets de recherche spéciaux financés par des fonds spéciaux à l'extérieur du programme de « base », c.-à-d. des subventions du Conseil de recherches en sciences naturelles et en génie (CRSNG), du Fonds pour la formation de chercheurs et l'aide à la recherche (FCAR) et d'organismes non gouvernementaux. Les projets spéciaux financés par des fonds externes spéciaux bénéficieront du programme de « base » et l'enrichiront. Ils permettront de répondre à des questions précises soulevées par les résultats du programme de « base » de même qu'à des besoins de gestion particuliers. Le programme de « base » a recueilli suffisamment de données jusqu'ici pour permettre la formulation d'un certain nombre d'hypothèses vérifiables qui peuvent être examinées en tant que projets spéciaux appuyés ou non par le MPO (selon le mandat et les priorités). Certains exemples de projets spéciaux ont été proposés.

La réussite de la conservation de l'écosystème du Saint-Laurent et du rétablissement de sa population endémique et emblématique de béluga repose sur des données scientifiques solides. Selon des preuves actuelles, la population ne diminue pas, mais ne semble pas non plus s'accroître. Étant donné qu'elle est protégée de la chasse, il faut relever et quantifier les facteurs qui peuvent limiter le rétablissement. Des données utiles obtenues du programme des carcasses peuvent aider à déterminer ces facteurs, mais elles doivent être intégrées à celles d'autres recherches portant sur cette population, et une collaboration étroite entre les chercheurs et les gestionnaires intéressés doit être facilitée et accrue. La réussite de la protection et du rétablissement de la population de béluga de l'ESL figure parmi les résultats stratégiques clés du MPO – le maintien d'écosystèmes aquatiques sains et productifs – pour tous les Canadiens.

INTRODUCTION

The beluga (*Delphinapterus leucas*) is a medium-sized ice-adapted odontocete (toothed whale) found in Arctic and sub-Arctic waters in the Holarctic. Of seven populations of beluga in Canada the St. Lawrence Estuary (SLE) beluga is the southernmost population occurring in the St. Lawrence Estuary, Saguenay River, and occasionally the Gulf of St. Lawrence. The summer distribution of this population is primarily centered at the confluence of the St. Lawrence and Saguenay Rivers (Michaud, 1993). Winter distribution is poorly known but there is some evidence of SLE beluga entering the Gulf (Kingsley, 1998). This population is likely a marine post-glacial relict, having been isolated from arctic populations at the end of the last Wisconsin glaciation about 11,000 years ago (Harington, 1977; Sergeant, 1986; Brown Gladden et al., 1997), and is most genetically similar to populations from Eastern Hudson Bay (de March and Postma, 2003).

The SLE beluga lives in an estuarine habitat that is part of an international waterway, the St. Lawrence Seaway with daily marine traffic. The St. Lawrence ecosystem receives considerable natural freshwater runoff from precipitation and snowmelt via the St. Lawrence and Saguenay Rivers, their various tributaries and other rivers within the watershed. Atmospheric deposition of natural, industrial and municipal particulates and other contaminants, industrial effluents, chemical and biological wastes from agricultural runoff, municipal and ship wastewater and ballastwater, which may include chemical contaminants, pathogens and exotic invasive species, are daily inputs into the St. Lawrence ecosystem (St. Lawrence Centre, 1996; Gagnon and Bergeron, 1997).

This population has been protected from hunting since 1979 at which time the population was given full protection under the Beluga Protection Regulations of the Fisheries Act. The SLE beluga population was designated as endangered in 1983 by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC) and recently re-evaluated as threatened (Pippard, 1983; Lesage and Kingsley, 1997; COSEWIC, 2004). Various surveys have been conducted to determine the estimated population size and about 1000 animals are believed to remain (Gosselin et al., 2001) with no to low apparent growth (DFO, 2005). It has been estimated that this population may have been as large as 5,000 to 6,000 animals in 1885 (Reeves and Mitchell, 1984). Re-analyses suggest the pristine population in the 1800s (before the commercial hunt) may have been 10,100 (DFO, 2005). Despite protection from hunting, regulation of some environmental contaminants and new whale-watching and environmental regulations, the SLE beluga population has failed to show significant signs of recovery.

Beluga are long-lived, high trophic level predators (Lesage et al. 2001) and are, to some extent, indicators of the health of the St. Lawrence ecosystem including that of human communities in the area. They are exposed to a variety of anthropogenic and natural contaminants present in the air, water, sediment and their food which includes fish and some invertebrates. The St. Lawrence ecosystem has a fluvial, estuarine and marine faunal diversity and endemicity of great economic value. The SLE beluga is highly visible, charismatic and emblematic in Quebec, Canada and the world. Overharvesting led to severe population declines until its protection by DFO in 1979. Under the Species at Risk Act (SARA), the DFO, as the responsible body for aquatic species such as the beluga, has the legal responsibility to conserve and protect this population and promote recovery through the development and implementation of a species recovery strategy and action plan. A recovery plan was published by DFO in 1995 and some 56 recommendations have been proposed to aid in the recovery of the SLE beluga (Bailey and Zinger, 1995). Many of these recommendations are in various stages of implementation (Anonymous, 1998). Assessment of populations such as the SLE beluga is required through SARA and DFO monitors the population through regular surveys and by supporting the carcass program. The Oceans Act, passed in 1996, defines Canada's responsibility to protect and preserve the marine environment and its Oceans Management Strategy is based on the precautionary principle. A number of initiatives by DFO (Oceans-Marine Protected Areas) and Parks Canada (Saguenay-St. Lawrence Marine Park) serve to protect beluga habitat.

Since 1982 a program to monitor mortalities of the SLE beluga population with necropsy and sampling of beach-cast carcasses has been supported by DFO (through various funding programs), Parks Canada and others [Institut national d'écotoxicologie du Saint-Laurent (INESL), Faculté de médicine vétérinaire de l'Université de Montréal (FMV), World Wildlife Fund (WWF)]. The objective of this carcass program was to determine the cause of death of beach-cast beluga using adapted standard veterinary pathologic and diagnostic methods. Over the years a variety of lesions, anomalies and infections have been documented in these carcasses. Methods, protocols, techniques and analyses have evolved over time due to development of veterinary expertise, and experience and advances in technology, scientific knowledge and interest. This program, which involved a number of veterinary pathologists, students and various collaborative researchers in government, universities and other non-governmental groups, has led to numerous publications (100 being primary publications, see Annex 1) involving cetacean anatomy, histopathology, microbiology, parasitology, toxicology, biology, demographics, etc., all of which have been generated from necropsies and opportunistic sampling of beach-cast carcasses. These publications have contributed scientific advice used as a scientific basis for implementation of conservation measures and in decisions made by resource and habitat managers (Bailey and Zinger, 1995; Anonymous, 1998; Measures, 2004).

The SLE carcass program consists of three components: a monitoring component whereby the mortality of a beluga is documented and verified and minimal data collected (i.e. date, location); a sampling component whereby beluga tissues or other samples and data are collected on the beach or in the laboratory with or without a complete necropsy; a necropsy component whereby a post-mortem examination of a beluga is conducted to determine cause of death and detect new or unusual lesions.

After 22 years (1983 – 2004) DFO and Parks Canada considered it worthwhile to conduct a critical evaluation of this program by organising a workshop with scientists who have or are benefiting from this carcass program. Scientists with expertise in various disciplines as well as managers from DFO (Science, Oceans), and Parks Canada were invited to give presentations in a similar format indicating the value of this program with respect to their particular field of research or interest. Presentations were organised under four themes, Cause of mortality and diseases; Contaminants and toxic effects; Biology and demography; Conservation. Participants were also asked to evaluate the consequences of four options for the carcass program with respect to their particular field of research or interest. These options included eliminating, reducing, maintaining the status quo or continuing the program with modifications. A brief history of the SLE beluga carcass program was provided as background information as well as a brief overview of aerial survey methodology and photographic identification of beluga (the latter two overviews are not included in this proceedings but data are available (Gosselin et al., 2001; DFO, 2005) on the Canadian Science Advisory Secretariat website (www.dfo-mpo.gc.ca/csas/). Four scientists with relevant expertise but not involved with the SLE beluga program were invited as external experts and asked to provide critical evaluations of the program. An invited presentation on similar programs for different marine mammal species was also given for comparative purposes.

This proceedings includes abstracts and cited publications of each presenter, the final workshop decision table of options and consequences on the SLE beluga carcass program, general discussion during the workshop, conclusions and recommendations from workshop participants as well as a list of publications coming from the carcass program since its beginning in 1982.

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TERMS OF REFERENCE AND OBJECTIVES

1. Determine the value of the monitoring/sampling/necropsy program (1983 – 2004) on the St. Lawrence Estuary beluga population with respect to 4 principal themes:

a. Cause of mortality and diseases	c. Biology and demography
b. Contaminants and toxic effects	d. Conservation

2. Examine four possible options (eliminate, reduce, maintain status quo or continue the program with modifications) on the monitoring/sampling/necropsy beluga program with a cost/benefit analysis for each option and define general and specific objectives for each.

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AGENDA

Day 1 - November 14, 2005 Monday – A-554 Salle St-Laurent

08:45 **Welcome to workshop participants**
Ariane Plourde [DFO Science Director, Maurice Lamontagne Institute (MLI), Mont-Joli] and Serge Gosselin, Director, Fish and Marine Mammal Division)

Objectives & Terms of reference
Lena Measures (MLI, Mont-Joli) Chair

Introduction and format of workshop, Introduction of workshop participants
Mike Hammill (MLI, Mont-Joli) moderator

09:05 **History of the beluga carcass recovery program in the St. Lawrence Estuary (1982 – 2004)**
Pierre Béland (Institut National d'Écotoxicologie du Saint-Laurent, Montréal)

09:35 **Question period**

Theme 1. Cause of mortality and diseases

09:45 **Necropsy program: health monitoring of the St. Lawrence Estuary beluga population using post-mortem examination of stranded carcasses**
Stéphane Lair (Université de Montréal, Faculté de medicine vétérinaire, St-Hyacinthe)

10:45 **Question period**

10:55 – 11:10 – Break

11:10 **Parasitic and infectious diseases of the St. Lawrence Estuary beluga**
Lena Measures (MLI, Mont-Joli)

11:40 **Question period**

Theme 2. Contaminants and toxic effects

11:50 **Exposure of the St. Lawrence Estuary beluga to contaminants**
Michel Lebeuf (MLI, Mont-Joli)

12:20 **Question period**

12:30 – 13:30 - Lunch

13:30 **Toxic effects of contaminants in St. Lawrence beluga**
Catherine Couillard (MLI, Mont-Joli)

14:00 **Question period**

Theme 3. Biology and demography

14:10 **The value of the St. Lawrence Estuary beluga carcass program for solving ecological questions**
Véronique Lesage (MLI, Mont-Joli)

14:40 **Question period**

14:50 **Demographic information from the St. Lawrence Estuary beluga stranding data base**
Solange Brault (University of Massachusetts Boston, USA)

15:20 **Question period**

15:30 – 15: 45 – Break

Theme 4. Conservation

15:45 **The Saguenay-St. Lawrence Marine Park: its role in the recovery of the St. Lawrence Estuary beluga**
Sylvain Paradis, Suzan Dionne, Nadia Ménard (Parks Canada, Quebec City and Tadoussac)

16:00 **Question period**

16:05 **The beluga carcass program: from a "wake-up call" for conservation to the St. Lawrence Estuary Marine Protected Area (MPA)**
Guy Cantin, Jean-Yves Savaria and Luci Bossé (MLI, Mont-Joli)

16:20 **Question period**

16:25 **End of day one**

18:00 **Casual evening supper/reception**

Day 2 – November 15, 2005 Tuesday - A-554 Salle St-Laurent

08:45 **Value of a marine mammal strandings response program in central California**
(invited speaker)
Martin Haulena (The Marine Mammal Center, Sausalito, California)

09:15 **Question period**

09:25 **Explanation of how consultation and roundtable discussion will proceed**
Lena Measures/Mike Hammill

Roundtable Discussion

Mike Hammill (moderator)

09:30 **Discussion - Theme 1. External experts, invitees, other participants**

10:30 – 10:45 – Break

10:45 **Continue Discussion on Theme 1**

12:30 – 13:30 – Lunch

13:30 **Discussion - Theme 2. External experts, invitees, other participants**

15:15 – 15:30 Break

15:30 **Continue Discussion on Theme 2**

16:30 **End of day 2**

Day 3 – November 16, 2005 Wednesday - A-554 Salle St-Laurent

Roundtable Discussion

Mike Hammill (moderator)

08:45 **Discussion on Theme 3. External experts, invitees, other participants**

10:00 – 10:15 – Break

10:15 **Continue Discussion on Theme 3**

11:45 **Discussion on Theme 4. External experts, invitees, other participants**

12:30 – 13:30 – Lunch

13:30 **Continue Discussion on Theme 4**

14:30 **Development of option/decision table with recommendations and suggested modifications to the program integrating themes**

15:00 – 15:15 Break

15:15 **Development of option/decision table with recommendations and suggested modifications to the program integrating themes**

16:00 **End of day 3**

18:00 - **Supper at restaurant**

<u>Day 4 – November 17, 2005 Thursday - A-554 Salle St-Laurent</u>

	Finalizing option/decision table with recommendations and suggested modifications to the program
	Mike Hammill (moderator)
08:45	**Discussion integrating all themes**
	10:00 – 10:15 – Break
10:15	**Continue discussion**
	12:30 – 13:30 – Lunch
13:30	**Continue discussion**
	15:00 – 15:15 Break
17:00	**End of workshop.**
17:30	**Transport to hotel or airport.**

ABSTRACTS OF PRESENTATIONS

History of the beluga carcass recovery program in the St. Lawrence Estuary (1982-2005).

Pierre Béland, Institut national d'écotoxicologie du Saint-Laurent, Montréal. Research scientist.

Introduction

I examine a possible relationship between catch, the number of beluga carcasses retrieved during each of the 22 complete years of the program and effort. Based on the agency under which the program was carried out, I have identified four different sub-periods during which some components of effort may have varied, namely, budgetary aspects, management issues, publicity around the program, the team involved in locating and in retrieving the carcasses, and public interest in the program.

Although there have been changes in policy and direction with respect to the main source of funding to the program (i.e. DFO), the team actually carrying out the field work has been unchanged since 1984. Obviously, over such a large territory, they rely on the general public, more specifically shore-residents, tourists, specific occupational workers (such as commercial fishermen), and government officials (police officers, fisheries officers, park wardens) to report cases. With the exception of 5 years, ad hoc publicity was carried out every year. Indirect publicity and the need to dispose of carcasses has helped in reporting. Over the period considered, the interest of the public in general has not failed, while the team has remained essentially unchanged and dedicated from the beginning to this day. However, there have been large variations in funding and spending for this program, as well as minor changes in other factors that might have influenced effort.

Nevertheless, the catch in terms of number of carcasses retrieved is not related to spending, and shows no conspicuous trend. Between 10 and 21 carcasses were found in any year, with an overall average of 15. A three-year moving average would perhaps suggest a slight increase in the number of cases recorded between 1989 and 1994. This does not mean that all belugas that died in any given year were seen, reported and entered into the record. The territory is immense, and is accessible at most during 9 months: no carcasses have been reported in January and February, and only 6 in March. The carcasses reported in any year are those that float or are stranded within a restricted area of the overall territory, which is defined broadly as those waters and shores that are frequented more or less assiduously by humans; not unexpectedly, 64% of the carcasses are reported during the warmer season, from May to August. Yearly variations in the catch within this core area are likely due to factors other than effort, such as actual differences in the death rate, changes in the sites and dates where and when individuals die (the Estuary and Gulf widen dramatically toward the less populated east), seasonal variations in ice cover, storm events, and others. It is not straightforward, if at all possible, to estimate overall effective coverage and the actual number of deaths that may occur in one year or whether the actual death rate varies from month to month. Nevertheless, however limited a sample the catch happens to be, I would suggest that the data are comparable from year to year.

Necropsy program: health monitoring of the St. Lawrence Estuary beluga population using post-mortem examination of stranded carcasses.

Stéphane Lair. Université de Montréal, Faculté de medicine vétérinaire, St-Hyacinthe. Veterinary pathologist.

Theme 1. Cause of mortality and diseases

Between 1983 and 2004 a total of 318 beluga carcasses from the St. Lawrence Estuary (SLE) population were reported stranded (=beach-cast) or drifting. A total of 166 of these carcasses (85 males, 79 females and 2 intersexes) were collected and transported to pathology laboratories for postmortem examination. During the first four years necropsies were performed by Daniel Martineau under the guidance of André Lagacé, professor in anatomic pathology at the Faculté de médecine vétérinaire. Subsequently, five pathologists, three of them directly trained by either D. Martineau or A. Lagacé, have conducted these examinations.

Standard necropsies were conducted on each carcass using an evolving but relatively uniform protocol. A set of 22 morphometric measurements, including total weight, estimation of the blubber weight, total length, girth, and blubber thicknesses at three different anatomical sites, has been taken routinely since almost the beginning of the program (Table 1). Since 1995, most organs, including the epaxial musculature from L1 to L12, have been weighed. Age was estimated in most of the cases by counting dentine growth layers on longitudinal sections of teeth.[1] Degree of autolysis has been estimated using carcasses classification codes proposed by Geraci and Lounsbury (1993)[4] either at the time of the examination or retrospectively based on the postmortem report. Samples for routine histopathology and bacteriology have been taken on each carcass.

Table 1. Measurements and sampling conducted on 166 beluga carcasses examined in a necropsy room setting.

	Number of carcasses	Comments
Morphometric measurements		
Total length	163	From snout to tail fluke fork
Body circumferences	106	Axillary, maximum and "anal"
Blubber thickness	116	Ventral, lateral and dorsal (axillary)
Other measurements	74 to 114	Set of 15 measurements
Weight		
Total weight	138	
Blubber weight	105	Half blubber adjusted to ratio skin/blubber
Epaxial muscle weight	79	One side epaxial musculature from L1 to L12
Other organs	11 to 87	Set of 23 organs
Age	163	
Carcass autolysis code	162	Geraci and Lounsbury (1993)[4]

Beluga carcasses were often poorly preserved; 41% of the carcasses were given decomposition codes of 2.5 or higher (decomposed to advanced decomposition).[4] Failure to identify a presumed cause of stranding increased as the degree of decomposition increased; cause of stranding was not determined in 15%, 34% and 57% of the relatively fresh carcasses (codes 1 and 2), moderately decomposed carcasses (codes 2.5 and 3), and markedly decomposed carcasses (codes 3.5 and 4) respectively. The percentage of cases for which a presumed cause of stranding was determined increased throughout the years from 58% in the first 4 years to 85% in the last 4 years. Presumed causes of stranding were determined by the pathologist based on his/her interpretation of postmortem findings. Table 2 presents the presumed causes of stranding for four different age groups.

Table 2. Presumed causes of stranding in St. Lawrence beluga whales from 1983 to 2004.

Presumed causes of stranding - n (%)	**Age groups[a]**				**TOTAL**
	< 1yr	**1yr – 7yr**	**8yr - 20yr**	**> 20yr**	
Not determined	-	6 (26.1%)	17 (27.4%)	24 (37.5%)	47 (28.3%)
Perinatal mortality	9 (64.3%)	-	-	-	9 (5.4%)
Infectious – bacterial	2 (14.3%)	4 (17.4%)	13 (21.0%)	12 (18.8%)	32 (19.3%)
Infectious – parasitic	2 (14.3%)	12 (52.2%)	11 (17.7%)	5 (7.8%)	31 (18.7%)
Neoplasia	0 (0%)	0 (0%)	10 (16.1%)	15 (23.4%)	25 (15.1%)
Trauma	0 (0%)	1 (4.3%)	5 (8.1%)	5 (7.8%)	11 (6.6%)
Degenerative	0 (0%)	0 (0%)	2 (3.2%)	2 (3.1%)	4 (2.4%)
Dystocia	-	0 (0%)	3 (4.8%)	0 (0%)	3 (1.8%)
Other	1 (7.1%)	0 (0%)	1 (1.6%)	1 (1.6%)	4 (2.4%)
Total number of carcasses	14	23	62	64	166

[a] Age not determined in 3 of the 166 beluga.

Overall, infectious diseases were the most common causes of stranding. Verminous pneumonia (either caused by the nematodes *Halocercus* sp. or *Stenurus arctomarinus*)[14] was the presumed cause of death in 15% of the overall cases, with higher prevalence in juvenile animals (1-7yr) in which 49% was the presumed cause of stranding. Other fatal parasitic diseases include 4 cases of nematode-associated perforated ulcers of the gastro-intestinal tract and 2 cases of toxoplasmosis.[15] Presumably fatal bacterial infections, such as abscesses, bacterial pneumonia and septicaemia, were diagnosed in 19% of the sampled belugas. The bacterial isolates associated with these fatal bacterial infections included: *Aeromonas* sp., *Clostridium* sp., *Edwardsiella tarda*, *Escherichia coli*, *Klebsiella oxytoca*, *Morganella morganii*, *Nocardia* sp., *Pseudomonas putefaciens*, *Streptococcus* sp. and *Vibrio* sp.

A total of 31 malignant neoplasms were diagnosed in 28 of the 126 adult (>7yr) beluga examined (mean estimated age [range] = 21 [11 – 30]yr). Several of these cases have been published in the peer-reviewed literature.[3,5,7,8,10-13,16] The prevalence at death of malignant neoplasms in adult animals (>7yr) was 20%. These cancers are believed to have caused the death of 25 of the examined belugas. Eighteen phenotypes of malignant neoplasms have been diagnosed, with gastro-intestinal adenocarcinomas being the most prevalent cancers with 11 cases (8.7% of adult belugas). Three cases of malignancies were also observed in tissues closely associated with the gastro-intestinal tract (one salivary gland adenocarcinoma, one bile duct carcinoma and one hepatocellular carcinoma). Mammary gland carcinomas and ovarian malignancies were seen in 4 and 3 females respectively.

Benign tumours, such as haemangiomas (6 cases), intestinal leiomyomas (5 cases), genital tract leiomyomas[17] (4 cases), schwannomas (3 cases) and lipomas (4 cases), were seen in 15 belugas with estimated ages between 14 and 31 yr.

Hyperplasic and degenerative changes have been commonly observed in the thyroid and adrenal glands. The occurrence and severity of these changes, which have been described elsewhere,[6,18] increase with age.

Traumatic lesions, such as vertebral fractures, deep skin lacerations and pulmonary lacerations, were seen in 11 animals. Although the causes of these traumatic lesions could not be determined with certainty in all cases, they are believed to be most likely associated with boat strikes. Interestingly, 9 of the 11 cases of fatal traumatic injuries were seen after 1994.

Three cases of dystocia and nine cases of mortality of new-born calves were seen over the 22 years of this study. Other interesting findings include two cases of dissecting aneurysms of the pulmonary trunk,[9] and two cases of intersexes (one true hermaphroditism[2] and one male pseudohermaphroditism).

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Parasitic and infectious diseases of the St. Lawrence Estuary beluga.

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Theme 1. Cause of mortality and diseases

As part of the parasitology research program established at the Maurice Lamontagne Institute (MLI) in 1989 a collaborative study of parasites of the St. Lawrence Estuary (SLE) beluga was initiated using parasites previously collected and preserved from 38 beach-cast or stranded carcasses examined from 1984 to 1993. The objective was to document the helminth fauna of stranded SLE beluga, comparing it with that of belugas studied elsewhere. Sampling was not systematic nor quantitative so prevalence and mean intensity data were not available for most helminths. Using standard parasitological methods 11 helminths were identified (Table 1). Histopathologic data in necropsy reports indicated the pathogenicity of some of these parasites which was, until recently, not fully appreciated in terms of effect on specific age classes of stranded beluga due to lack of analysis of the data base (see below).

Table 1. Helminth parasites of St. Lawrence beluga with an asterisk denoting those of pathogenic interest[1].*

Hadwenius seymouri	*Stenurus arctomarinus**
Bolbosoma sp.	*Halocercus monoceris**
Corynosoma cameroni	*H. taurica*
Diphyllobothrium sp.	*Pharurus pallasii**
Pseudoterranova sp. (likely *P. decipiens*)	*Anisakis simplex**
Contracaecinea sp. (*Contracaecum* sp. or *Phocascaris* sp.)	*Crassicauda* sp.

Quantitative study was required to relate parasite infection levels and host parameters, stratifying age and sex class, with lesions and mortalities from a larger sample of stranded carcasses to validate field observations and corroborate hypotheses in the laboratory if possible. Additional studies with testable hypotheses were undertaken, primarily with graduate students using DFO (A- and B-base) and NSERC funding, to investigate further certain parasites of pathogenic interest including studies of transmission. Additional beluga organ and tissue samples were collected from the necropsy/sampling program and other field and laboratory data were used to corroborate data from carcasses. Age (GLGs), sex, length, girth, weight, blubber weight and description of lesions were needed for analyses with the knowledge that carcasses are biased towards the sick and old.

The nematode *Anisakis simplex* present in 28 of 38 (74%) stranded SLE beluga at intensities up to 3,158, was found predominantly in the first chamber of the stomach[1]. Beluga acquire infections probably from feeding on capelin and herring[2] which in turn acquire infections from krill[3]. Histopathologic evidence indicated that this stomachworm, common in cetaceans worldwide, may be involved in at least 4 deaths due to gastro-intestinal tract perforations and ulcers leading to peritonitis in SLE beluga by creating portals of entry for opportunistic viral or bacterial pathogens. Unfortunately, nematodes are seldom found or identified in these cases. Pseudaliid nematodes of cetaceans, are poorly understood and are particularly pathogenic[6]. The pseudaliid *Pharurus pallasii*, inhabiting cranial and ear sinuses of beluga, infected 88% of 32 adult, 72% of 11 juvenile beluga and no fetuses or calves (N=9). Mean intensities are relatively low (419 and 179 in adults and juveniles, respectively)[5]. Gross lesions associated with infections appear relatively minor and there was no relationship between intensity of infection and body condition of beluga. Histological evidence supports this but identification of parasites did not complement histopathology. Thus the hypothesis that these sinus worms cause strandings due to negative effects on diving performance, navigation, foraging or physical condition of infected beluga could not be supported. There was no evidence of transplacental or transmammary transmission and experimental work using live material from fresh beluga at necropsy suggested that a fish intermediate host is involved in the life cycle of *P. pallasii*[4]. Reports of adult *P. pallasii* in the lungs are misidentifications[5].

The most pathogenic pseudaliid lungworm in stranded SLE beluga appears to be the minute parenchymal *Halocercus monoceris* found predominantly in small bronchioles and alveoli of the lungs and has been implicated in the majority of mortalities of juvenile beluga due to verminous pneumonia. Prevalence of infection in a systematic study of the lungs of 25 stranded SLE beluga was 25% of 4 calves (mean intensity or MI = 246), 100% of 3 juveniles (MI=2242) and 100% of 18 adults (MI=1944). Intensities have been estimated as high as 12,561 worms. (Measures, unpub. data). The large bronchial *Stenurus arctomarinus*, may also be responsible for some fatal verminous pneumonia in stranded SLE beluga but infections are less prevalent and at low intensities. Histological evidence supports the pathogenicity of some of these lungworms in mortalities but identification of parasites was either incorrect, not performed, or did not complement histopathologic analyses until recently. There is some evidence from histopathologic data that lung infections may be acquired by transplacental or transmammary transmission. Histopathological analysis of pneumonia in beluga became more thorough as of 1993 when 18 samples were routinely collected from both lungs (cranial, caudal, median: superficial, middle, deep). A collaborative project is in progress to quantify lung infections and lesions from previously examined material using archived histopathologic material and parasitologic data in a complementary manner to evaluate the importance of these parasites in beluga and understand their transmission and pathogenicity.

In 1996 a marine mammal health program was initiated at MLI with a mandate to understand diseases in marine mammal populations and provide advice to the Minister relative to the conservation and protection of marine mammals. Serology, classical parasitology, immunohistochemistry, molecular biotechnology, transmission electron microscopy (TEM) and virus isolation were used to analyse various samples including complementary use of histopathology.

Serological studies using blood or serum indicated that 27% of 22 stranded SLE beluga were exposed to the protozoan parasite, *Toxoplasma gondii;* however, only two deaths have been attributed to acute clinical toxoplasmosis[7] which does not support the hypothesis that SLE beluga are immunologically incompetent, at least to *T. gondii*. Serological studies indicate that 16% of 25 stranded SLE beluga have been exposed to the bacterium *Brucella*[8], none of 31 to cetacean morbillivirus[9,10] and 46 - 58% of 13 to an alphaherpesvirus[10]. The latter may be involved in oesophageal ulcers and necrotizing dermatitis seen in stranded SLE beluga. A bacterium (*Dermatophilus*-like) was later reported in the skin of SLE beluga[11]. The absence of lesions in SLE beluga attributable to *Brucella* as observed in narwhal is uncertain and may reflect the poor state of preservation of carcasses and inability to isolate and detect *Brucella* using standard microbiological methods. The lack of exposure of SLE and arctic beluga as well as narwhal to cetacean morbillivirus, the most important viral disease of marine mammals, is puzzling and perhaps worrisome in terms of their possible susceptibility to a potential epizootic, especially since morbillivirus is enzootic in other marine mammal populations in the Estuary and Gulf of St. Lawrence. Serology has limitations however; most methods have not been not validated in marine mammals, serology indicates exposure only, antibodies can cross-react and decomposing carcasses may restrict use or influence results).

Some viruses have been reported in SLE beluga including an intestinal adenovirus[12], gastric papillomavirus[13, 14], a dermal herpes-like virus[13] and a dermal pox-like virus but have not been identified nor studied further. In some cases TEM has been used to attempt to identify acidophilic intracytoplasmic structures that appear to be inclusion bodies seen on histology. The role of viruses as etiologic agents or co-factors of specific lesions observed in beluga including neoplasia are poorly investigated. Bacterial infections, mostly opportunistic[15], have caused abscesses, bacteremia, septicaemia and death based on histopathological and bacteriological analyses. However, in many cases bacteria cannot be isolated, identified or associated with many lesions due to the poor state of preservation of most carcasses. Two bacteria that warrant special attention are *Brucella* and *Helicobacter* because of the former`s potential effect on reproduction of SLE beluga and the latter`s potential as an etiological agent or co-factor in gastrointestinal neoplasia. Greater emphasis on study of infectious etiologies would be instructive including use of more sensitive bacteriological and virological methods. Infectious etiologies of neoplasia must be eliminated as an alternate hypothesis to resolve the debate concerning the role of contaminants including whether the SLE beluga population is immunosuppressed either due to infectious agents or contaminants or both.

The value of the monitoring/necropsy/sampling program in studies of parasites and other infectious agents of the SLE beluga as a complement to histopathology and toxicology cannot be dismissed particularly in recovery of a species at risk. Histopathologic analyses, the gold standard in diagnostic work, have been used to describe lesions associated with certain parasites and infectious agents that infect SLE beluga and suggest that similar pathogens may play a significant role in other marine mammal populations, i.e. lungworms. The controversy arises in interpretation and ascribing causal relationships. Results obtained from the necropsy program have permitted advice to the Minister on protecting the SLE beluga (from municipal and industry waste, disturbance, marine traffic, rehabilitation) and its habitat. The present SLE beluga stranding data base including histopathological, microbiological, parasitological, toxicological, morphometric and demographic data has not been adequately archived, validated, nor analyzed in an integrated manner to test various hypotheses proposed over the years, a great disservice considering work performed. New methodologies (PCR) could be employed to overcome some of the limitations of poor carcass preservation in terms of isolating and identifying viral and bacterial pathogens. Laboratory work may address specific questions. The bias of stranded carcasses cannot be overcome but should be recognized during analyses and caution used when comparing data from arctic beluga populations which are also biased but differently due to hunting.

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Exposure of the St. Lawrence Estuary beluga to contaminants.

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Theme 2. Contaminants and toxic effects

Located downstream from the Great Lakes – St. Lawrence River, the St Lawrence estuary (SLE) receives contaminants from a highly populated and industrialized area. The resident population of beluga inhabiting the SLE has been and is still continuously exposed to contaminants. These long-lived animals are prone to accumulate elevated levels of a variety of persistent contaminants during their life time. In addition, females transfer substantial loads of contaminants to their offspring during gestation and through lactation, continuing the contamination between generations.

Exposure of SLE beluga to contaminants constitutes a potential health risk for the population. That risk can only be assessed through a thorough characterization of their environment, including their diet which constitutes their primary source of contaminants. The distribution of contaminants within animals needs to be examined to identify storage tissues. Concentrations of contaminants that are very resistant to biodegradation are expected to increase with time in belugas creating an even more critical situation. Because not all contaminants bioaccumulate in beluga, it is also necessary to understand mechanistically how beluga transform and/or eliminate contaminants. Ultimately, exposure of SLE beluga to contaminants has to be translated into direct and indirect consequences and effects on individuals and at the population level.

Over the last 23 years (1983 – 2005), tissues of belugas found stranded on the shores of the SLE have been collected through the SLE beluga monitoring/necropsy/sampling program. Samples were collected either directly at the site of stranding or in the laboratory during necropsy. About half of the stranded beluga carcasses was either too decomposed or inaccessible for transport to the laboratory for necropsy. Two tissues have been preferentially collected for contaminant analyses, namely blubber and liver. Occasionally, other tissues such as brain, lung and kidney have been sampled and analysed for contaminants. Slight changes in the sampling protocol have occurred over the years, including the collection of larger samples especially for blubber where an integrated sample from the skin to the muscle is now collected.

Exposure of beluga to contaminants has been primarily assessed by measuring contaminant levels in beluga tissues. It must be stated, however, that the program has been limited to the collection of samples and chemical analyses have always been conducted with external sources of funds. Complementary information on stranded animals such as age, sex or length are collected within the program and commonly used to interpret contaminant data and to better understand the transfer of contaminants from mother to offspring. Contaminant analyses have changed toward more specific and reliable measurements making comparison with old data more difficult. Samples collected through the program have also been useful to assess temporal trends of some persistent contaminants by reanalysing samples using state of the art methodologies and analytical instruments. Beluga samples have been particularly valuable to explore the presence of bioaccumulative chemicals in the SLE.

Slightly more than 20 peer-reviewed scientific papers reporting contaminant levels in SLE beluga whales have been published so far. Most of the efforts have been directed toward the analysis of persistent organic contaminants currently regulated, which include polychlorinated biphenyls (PCBs) and several organochlorine pesticides such as chlordanes, hexachlorocyclohexane (HCHs), toxaphene, chlorobenzenes (CBs) and mirex. Other families of contaminants, including polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs), polychlorinated paraffins (PCPs), polychloro-n-alkanes (PCAs) and polybrominated diphenyl ethers (PBDEs) have been found in SLE beluga. More recently, contaminant degradation products such hydroxylated, methoxylated and methylsulfonated organohalogens, along with other halogenated chemicals of unknown origins have been detected in SLE beluga. Organometallic compounds such as tributyltin (TBT) and its degradation products have been also reported in SLE beluga. Surprisingly, only one study has examined metal contamination in SLE beluga. Despite the fact that the SLE beluga is probably the most thoroughly characterized species for contaminants within the SLE, our knowledge regarding the exposure of beluga to contaminants remains quite limited.

Beluga samples obtained as part of the monitoring/necropsy/sampling program have been beneficial in characterizing, to a limited extent, the exposure of beluga to bioaccumulative contaminants. Such characterization is not only relevant for the beluga population itself but because they rapidly accumulate high levels of persistent contaminants, beluga whales have served as a sentinel species for emerging contaminants in the SLE. Obviously, a wide range of chemicals, known to be present in the Great Lakes – St. Lawrence River ecosystem and expected to accumulate in SLE beluga have not been examined yet, neither in the beluga nor in the SLE ecosystem.

Unfortunately, the monitoring/necropsy/sampling program suffers from important limitations. For instance, certain tissues such as blood or bile are difficult to collect from stranded animals. Rarely are code 1 (alive or moribund) animals available. This prevents the characterization of hydrosoluble contaminants and certain metabolites including a variety of phenolic compounds. A complete characterization of beluga exposure to contaminants requires examining contaminants that are not accumulated in beluga tissues, including those readily biotransformed by beluga such as polycyclic aromatic hydrocarbons (PAHs) or those not persistent in the environment such as many currently used pesticides. For these chemicals, tissues of stranded animals are of little value and assessment has to be done in their environment, especially in their diet. Levels of contaminants in tissues of stranded beluga may not be representative of the free-ranging population. For instance, important changes in contaminant concentrations may occur during illness and few die of traumatic injuries or accidents or sudden death. In addition, *post mortem* changes may occur in certain tissues undergoing decomposition. It is not clear, however, to what extent these two factors bias contaminant concentrations measured in beluga tissues compared to live-sampled animals.

An effort has been initiated to model the exposure of SLE beluga to certain contaminants. However, intensive work is needed to acquire contaminant data in beluga prey and in beluga tissues before the fate of contaminants within beluga can be modelled. Characterisation of beluga exposure to contaminants in its habitat represents the only way, in complement with toxicological evaluations, to assess the effect of contaminants on the health of the SLE beluga population.

Monitoring and sampling components of the present program are essential in assessing the exposure of beluga to contaminants. Despite clear improvements over the years, especially in the management of monitoring data, there is room for additional modifications in these program components. For example, collected samples should be systematically archived for further studies and managed by a small committee to avoid duplication of research efforts. The program should provide financial support directed to analyses of contaminants to insure continuity in the assessment of temporal trends. Research efforts seeking to link levels of contaminants with biological effects should also be encouraged and financially supported.

The present program has major limitations in assessing some important aspects of exposure of beluga to contaminants. For instance, there is a major need to identify and quantify the presence of contaminants in beluga prey and in abiotic components (water and sediments) in beluga habitat. A thorough assessment of the exposure of beluga to contaminants will necessitate sampling live animals with non-invasive techniques or hunting some animals.

The necropsy component of the present program is of little help in determining the exposure of beluga to contaminants. In consequence, the utility of this component of the current program, which drains about half of the funds, has to be assessed in the context of other objectives, especially toxicological assessments.

There is still a lot of work to be done before an adequate characterisation of the exposure of beluga to contaminants can be provided. Gathering monitoring data on stranded beluga, collecting samples from those animals and determining contaminant levels provide relevant information, especially for bioaccumulative contaminants expected to reach toxic levels. Although incomplete, such information is essential to toxicological assessments.

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Toxic effects of contaminants in St. Lawrence beluga.

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Theme 2. Contaminants and toxic effects

Effects of toxic contaminants on St. Lawrence Estuary (SLE) beluga need to be assessed because they may have an effect on survival, growth and reproduction and threaten the population. For ethical and practical reasons, it is difficult to capture and sample live animals or to conduct in vivo toxicity experiments in this species. Thus, beluga tissue samples must be obtained either by skin biopsy in free-ranging belugas or from beach-cast or stranded whales found in variable states of decomposition. Different types of toxic effects are of concern in beluga whales exposed to a complex mixture including organohalogens, heavy metals and polycyclic aromatic hydrocarbons (PAHs): genotoxicity and carcinogenicity, immunotoxicity, endocrine disruption and reproductive effects, developmental toxicity and neurological and behavioural toxicity. The first three categories have been investigated in SLE beluga. In addition, several studies have been conducted to evaluate their metabolic capacity.

The main objectives of ecotoxicological studies conducted on SLE beluga are:

1. To assess if toxic chemicals have or are likely to have adverse effects on SLE belugas.
2. To provide information for managing the risk of toxic effects on SLE belugas.

Several approaches have been used to evaluate the toxic effects of contaminants on SLE beluga:

1. epidemiological studies comparing biomarker responses in SLE beluga and less contaminated arctic beluga.
2. in vitro studies exposing beluga cells to single chemicals or complex mixtures of chemicals.
3. in vivo studies in model species exposed to single chemicals or complex mixtures of chemicals.

The SLE beluga monitoring/sampling/necropsy program provided histopathological data and tissue samples from stranded SLE beluga for biomarker analyses. Biomarker responses, contaminant levels and data on sex and age were compared between SLE and arctic beluga using an epidemiological approach. At the beginning of the program, the objective of histopathological analyses was to identify the cause of death. As knowledge developed about lesions possibly related to toxic contaminants, some tissues were systematically examined and some histopathological lesions were used as biomarkers. Very few studies measured biochemical biomarkers in SLE beluga because they require tissues from live animals or must be collected immediately after death. Necropsy of stranded SLE beluga revealed high prevalence of gastrointestinal tumors indicating possible exposure to genotoxic contaminants. DNA adduct levels were measured as an index of exposure to genotoxic PAHs in SLE beluga brain and liver using two different techniques: ^{32}P-postlabelling measuring total DNA adducts and HPLC-fluorescence measuring specifically benzo[a]pyrene diolepoxide. The HPLC-fluorescence technique demonstrated exposure of SLE beluga whales to a genotoxic PAH, benzo[a]pyrene. The ^{32}P-postlabelling revealed presence of relatively high levels of DNA adducts of unknown origin in both the SLE beluga and arctic beluga. The relationship between the levels of DNA adducts in brain and liver and the observed neoplastic lesions in the gastrotintestinal tract is still unknown. The possible role of other contaminants in the diet and of infectious agents should also be considered.

Liver samples were collected from stranded SLE beluga for characterization of cytochrome P450 and phase II xenobiotic-metabolizing enzymes. Catalytic activities were absent in the tissues of stranded SLE beluga as a consequence of tissue degradation after death. It was possible to characterize cytochrome P450 protein by immunochemistry but the intensity of the protein bands was lower in the most autolysed samples. In another study, the expression of cytochrome P4501A was demonstrated by immunohistochemistry in various tissues from stranded SLE beluga. These techniques could possibly be used to relate the intensity of cytochrome P4501A expression and contaminant burdens.

Observation of high prevalence of infections with opportunistic pathogens in stranded SLE beluga led to in vitro immunotoxicity tests using arctic beluga cells exposed to heavy metals or to organochlorines. Observation of high prevalence of neoplasia led to in vitro genotoxicity tests using arctic beluga cells exposed to mercury, organochlorines, single PAH and PAH mixtures. These studies indicate that genotoxic and immunotoxic effects could be induced in arctic beluga cells exposed to concentrations of contaminants similar to those measured in SLE beluga tissues. It is not possible, however, to directly extrapolate results from in vitro toxicity tests to in vivo toxicity because exposure conditions differ markedly. Blubber samples collected from one stranded SLE beluga were incorporated into the diet of mice, rat and mink for in vivo studies evaluating effects on reproductive and immunological systems.

With respect to evaluation of toxic effects of contaminants on SLE beluga, the monitoring/sampling/necropsy program has obvious limitations: small and biased samples, not representative of the free-ranging SLE beluga population (old, stressed, diseased individuals that have not been eating for several days). Various degrees of autolysis of the tissues may affect the biomarker responses. Stranded beluga tissues are of little use to assess exposure to non persistent toxic compounds and current exposure to persistent compounds. There might be a 15-25 year delay between exposure to contaminants and development of pathological lesions such as neoplasia thus pathological lesions in stranded beluga are not a good indicator of the actual state of contamination of the habitat. Because very few neonates are found stranded, the program cannot be used to assess effects on this highly sensitive life stage.

The SLE beluga carcass program does not cover costs for analyses of biochemical biomarkers or for quantitative evaluation of histopathological lesions used as biomarkers (i.e. thyroid adenomatous hyperplasia). External sources of funding are also required for sampling a less contaminated population of beluga which is essential to the interpretation of biomarker responses. Exposure assessment in beluga is essential to the interpretation of epidemiological and laboratory toxicity studies and it is not part of the program. Unfortunately, the program does not cover the cost of studies monitoring chemical and biological effects in sentinel species which are essential to provide information on current exposure to toxic contaminants and advice in risk management. As a consequence of these limitations, the program cannot, on its own, provide an assessment of toxic effects of contaminants on SLE beluga and recommendations for risk management.

Despite these limitations, the program plays the crucial role of providing observational data on pathological lesions and tissue samples for exposure and biomarker assessments. These data were used to generate hypotheses tested in laboratory toxicity experiments and field studies which will contribute to the demonstration of a cause and effect relationship. In the SLE, beluga may be unique in their vulnerability and sensitivity to environmental contaminants. Therefore, it is essential to have data on potential effects of contaminants on the beluga itself since exposure or effects may not occur in surrogate species. Observation of increased prevalence of pathologies or deformities is often the first observed sign of the presence of a new toxic contaminant in a wildlife population. The SLE beluga program may also provide samples and data for comparative physiology and toxicology studies needed to select model species.

Several improvements may be suggested for the SLE beluga program.

- Periodic evaluation of the responses of histopathological and biochemical biomarkers in arctic beluga and assessment of exposure to contaminants in SLE and arctic beluga populations should complement the current program.
- Presence of viruses and bacteria should be systematically investigated in gastrointestinal proliferative lesions since viruses or bacteria may be involved in the pathogenesis of these lesions.

- QA/QC systems and standardized protocols should be developed for all biomarkers measured at different times and in different laboratories. Techniques could be developed to assess quantitatively the degree of autolysis and the effect of autolysis on responses of various biomarkers.
- Sampling and archiving of skin samples for biomarker analyses in parallel with existing sampling of these tissues for chemical analyses should be conducted. It would provide data useful in the interpretation of studies conducted on skin biopsies from free-ranging belugas.
- Collection of plasma and milk samples from relatively fresh (Code 1 or 2) stranded beluga should be attempted and these samples should be archived for use in laboratory toxicity studies.

In conclusion, the SLE beluga monitoring/sampling/necropsy program is essential but is not sufficient by itself to evaluate the toxic effect of contaminants in SLE beluga and to provide information for management of the toxic risk. Epidemiological studies using biomarkers and measurement of chemical exposure, laboratory toxicity studies, field monitoring studies in free-ranging beluga or in other aquatic species are all needed to demonstrate cause and effect relationships and to provide information that may be used to manage the risk of toxic effects on SLE beluga.

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The value of the St. Lawrence Estuary beluga carcass program for solving ecological questions.

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Theme 3. Biology and demography

Ecology is the study of interactions of individuals with their biological and physical environments. In this section, ecological questions will refer specifically to distribution, movements, habitat use and diet. Only questions related to distribution and diet were addressed under the current St. Lawrence Estuary (SLE) beluga monitoring / necropsy / sampling program (MNS program).

The study of distribution represents the first step towards an understanding of the ecology of populations and of their potential interactions with other species for space and food resources. Distribution can be studied at the level of the population to delimit boundaries, but also at smaller scales to define for example summering or wintering areas of a population or one of its components. The MNS program contributed samples that allowed the examination of SLE beluga population genetic make-up and discreteness relative to other beluga populations. The main approach used to study these questions was the analysis of genetics biomarkers (Patenaude et al. 1994; Brennin et al. 1997; Brown Gladden et al. 1997; 1999; de March and Postma 2003). However, a comparison of contaminant levels among the different populations was used in some instances to identify the origin of extralimital beluga observations (e.g., Béland et al. 1992; see also Kingsley 2002). The tissue used for genetic analyses was usually the skin, and the method of preservation was, until recently, freezing (-20°C). Since 1996, R. Michaud (GREMM) specifically requested that a sample be preserved in DMSO (20% v, 0.25M EDTA saturated with NaCl) for genetic analyses in a social context (ongoing research). Studies based on contaminants (POPs) used blubber stored at -20°C. In addition to samples, the MNS program provided information on stranding date and location and on the sex, age and morphometrics of individuals. Results obtained using genomic DNA and mitochondrial DNA indicates that the SLE beluga is more genetically distinct than other beluga populations in the Canadian Arctic, having a haplotype not found in any other population. Genetic studies also suggest that SLE beluga and Eastern Hudson Bay beluga might have originated from the same inland sea (Lakes Agassiz and Ojibway), an Atlantic refugium during the last glaciation (Brennin et al. 1997; Brown Gladden et al. 1997; 1999; de March and Postma 2003).

The understanding of foraging ecology, and particularly diet, is central to the identification of sources of contaminants and of factors potentially limiting population growth such as food shortage or competition with other species, habitat loss, etc. In SLE beluga, the issue of diet is particularly important given that the existing information is out of date (late 1930s), is biased towards males, and was collected during summer only and from areas no longer intensively frequented by belugas (Vladykov 1946). Diet can be studied using a variety of methods, which all require access to tissues of marine mammals. Samples were obtained through the MNS program and were used to study diet using four approaches: 1) digestive tract contents, 2) stable isotope ratios, 3) contaminant profiles, and 4) fatty acid composition. Digestive tracts were not analysed using standard protocols for dietary studies and were opened during necropsies mainly to identify potential pathologies. Muscle and blubber samples were collected from most if not all of the beluga. Until the early 1990s, blubber and muscle samples were preserved in glass or plastic jars and frozen at -20°C. Blubber samples were archived as three separate layers. Afterwards, blubber samples used for contaminant and diet analyses were preserved as a block, including the skin, blubber and underlying muscle, in hexane-rinsed aluminium paper and frozen (-20°C). Muscle samples were put directly in un-treated aluminium and frozen (-20°C). In addition to samples, the MNS program also provided information on the date of stranding, location, sex, age and morphometrics of individuals. The four approaches varied in their capacity to provide information on diet. Digestive tract analysis provided direct evidence of recent ingestion of particular prey through the identification of otoliths and other prey hard parts (e.g., bones, vertebrae). However, only 4–5 individual stranded beluga had identifiable prey remains in their digestive tracts, thereby limiting the usefulness of this approach (Béland et al. 1995; Lesage, unpublished data). Stable isotopes through the analysis of muscle tissue of beluga provided insights mostly into the variability of trophic position and carbon sources exploited by beluga between years and genders (Lesage et al. 2001). Models can be developed to estimate the relative contribution of different prey to the diet provided that some basic knowledge of potential prey species exists and that food sources are not too numerous. However, given the probably varied nature of beluga diet (according to historical data), these models have limited applications with this species. Contaminants were used to determine the probability of ingestion of certain prey species, but did not provide information on diet composition per se (Hickie et al. 2000; Law et al. 2003). Only the approaches using digestive tracts and fatty acid composition have the potential to provide information on diet composition. The analysis of blubber fatty acids provided some insights into the diet of beluga by indicating the larger classes of prey species (pelagic vs benthic, marine vs estuarine) that were most likely ingested (C. Nozères, submitted). However, in order to obtain information on diet composition from fatty acid analyses, complementary information must be obtained through captivity trials (Iverson et al. 2004).

The methodology for the preservation of samples (freezing at -20°C) was satisfactory, although not ideal for genetics analyses, and yet, satisfied objectives. The lack of a protocol for the systematic collection of skin samples resulted in some animals not being sampled and thus failure to fully exploit the data base. Similarly, sample freezing at -20°C permitted stable isotope, fatty acid and contaminant analyses. However, a deterioration of the quality of samples through time was noted, which reduced the number of samples available for some types of analysis, such as fatty acid analyses. The lack of a systematic examination of digestive tracts using an accepted protocol precludes any firm conclusions as to the complete absence of prey remains in stranded SLE belugas.

Standardization of the location of tissue collection and diverse methods of preservation would be highly desirable to permit full exploitation of the data base and enlarge the number of analytical techniques or scope of ecological questions that could be addressed by the program. In addition, a tissue/data bank managed by a committee should be put in place to insure that standard methods of collection are followed, and that tissues are distributed according to priorities, scientific contributions and without duplication. Specifically, two sets of samples (skin, blubber, muscle, liver, etc.) should be collected from each individual, archived at -80°C and -20°C; one or the other should be used depending on the level of preservation necessary for the specific study. A skin sample should also systematically be archived and preserved in DMSO at -4°C for genetic purposes. A set of teeth preserved dry instead of chemically should be archived to permit studies of long-term diet using, for example, trace elements, metals or stable isotopes through microprobing. Feces should also be frozen at -80°C and -20°C for use with alternative methods such as molecular scatology and polyclonal antibodies (Pierce and Boyle 1991; Sheppard and Harwood 2005; Deagle et al. 2005). Suggested modifications to protocols, particularly the -80C freezing, would permit long-term preservation of samples and thus, more flexibility in the time schedule of sample analysis and in the type of ecological questions that can be addressed.

None of the ecological issues addressed so far under the program require a complete necropsy similar to those currently performed at Ste-Hyacinthe for interpretation of results. Samples can be obtained directly on the beach. A program reduced to sampling of superficial tissues such as skin, blubber, muscle and jaw (for age determination) would continue to permit addressing questions related to the distribution and diet using most of the techniques presented above. A systematic examination of digestive tracts for diet determination would require a more complete necropsy on the beach. A reduction of the program to the monitoring of calls without sampling would result in the non-attainment of any of the objectives related to ecological issues. In the case of studies related to distribution using genetic or contaminant levels, complete elimination of the program could be mitigated by alternative methods such as biopsy sampling. Techniques such as satellite telemetry could also be used to determine the extent of beluga movements, but live-captures in the St. Lawrence Estuary have been, to date, unsuccessful. Diet using stable isotopes could also be examined through biopsy sampling given that the skin provides an image over a period of time similar to that of muscle. However, the most promising technique to examine diet in St. Lawrence beluga, the fatty acid composition approach, relies entirely on access to stranded individual carcasses. Blubber biopsy sampling in this species is not a viable option because the blubber of odontocetes is highly stratified. The internal layer is the most representative for diet, whereas the external layer contains mostly structural biosynthesized fatty acids. Alternative techniques using feces are also not viable with free-ranging beluga given that the observation of defecation at the surface is an extremely rare event.

The MNS program permitted collection of a sufficient number of samples (intrinsic) and associated information (date, location, sex, age, morphometrics). External funding was necessary to conduct genetic and chemical analyses (extrinsic). None of these funding sources is stable. Although some expertise and lab equipment exist in-house to perform a small number of analyses per year, any project on a larger scale requires external funding.

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Demographic information from the St. Lawrence Estuary beluga stranding data base.

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<u>Theme 2. Biology and demography</u>

My field of research is the study of population dynamics, with a special interest in conservation biology. I am presently involved in constructing a model of the St. Lawrence Estuary (SLE) beluga population. I have no role in collecting or using samples from the carcass program, but rather, I am a "secondary consumer" of the data from the carcass program. The proposed model will hopefully be used as an aid in the assessment of its recovery status. However, in the current context it could serve as a blueprint of sorts to represent and organize the various data sets, and possibly to formulate better the connections that may be made among them. This approach has worked well in considering research needs of a cross-boundary population of geese, linking scientists across several agencies and countries (Brault et al. 1994). Other models were formulated in the past for the SLE beluga population (Béland et al., 1988; Kingsley 1998), and for other beluga populations (Bourdages et al. 2002, Hudson's Bay; Burns and Seaman 1985, Alaska).

The objective of the current modeling project is to make use of all available demographic data from the SLE beluga population, and from other populations if necessary, in a Bayesian framework, i.e., where uncertainty about each data source is used as information. At a minimum, demographic models require **estimates of reproductive rate and survival rate** for the whole population - obtaining such estimates and their uncertainties (or confidence intervals) by age and/or reproductive stage is the *nec plus ultra* for any wild population. This is possible for the SLE beluga, but it requires analysis of at least three data sets integrating each with the other: carcass data, aerial surveys by DFO personnel and previous researchers, and photographic identification data from the GREMM.

Basic **data types** needed from the carcass program are the age, sex, reproductive stage, and size of individuals. Of these, age and perhaps reproductive state are the more problematic. Age estimation from sectioned teeth is a difficult task requiring an experienced reader. Readers have changed over the 23–year carcass sampling program, but the extent of cross-calibration among readers is unclear to me. Reproductive state data were collected under different conditions of carcass preservation (Code 1 to 4) and by different people, with protocols varying in their degree of detail.

Limitations from variable data quality are that analysis can mostly use them at their lowest level of definition. For example, reproductive stage data may include number of *corpora albicans* for some females, but these are of limited utility if not collected or processed using a consistent standardized protocol. Although not all carcasses can yield high-quality data, it is possible to "index" data value by carcass coding within the Bayesian modeling framework, if sampling is consistent to that state. Similarly, errors in tooth-aging primarily due to wear (teeth from older animals are more difficult to read, some layers are worn away from use or age) as well as known reader bias can be quantified in demographic analysis: ironically, the more we know about our uncertainty, the clearer our view of current knowledge.

Age at death data, if cross-checked using the other data sets, may be useable as a measure of survivorship at age, on a relative scale, and for several sampling years combined. As such, these data are of great value, and extremely rare in marine mammal biology, because they could represent "natural" mortality, that is, the cause of death is likely to affect the whole population – but this may be an item to discuss. By contrast, an extensive carcass recovery and necropsy program exists for the Florida manatee (Anon. 2003), but age data from it could not be used for survival estimation: a large fraction of the animals are killed by boat collision, which likely happens in unmeasured biases with regard to age, size and level of activity.

Reproductive status data are also valuable, again because it provides an overall snapshot of age at first reproduction, age-specific pregnancy rate for females, and reproductive maturity for males.

Although the photographic identification program has been going for more than 15 years, this does not cover the SLE beluga's entire lifespan based on tooth-aging. Information about fertility of older adults, for example, can currently only come from the carcass data.

Other types of data, such as **lesions, parasites or contaminant loads**, etc. can be analyzed in relation to demographic parameters, to determine their potential influence on reproductive output or survival rate. This would truly bring demographic studies of this population up a notch by allowing the formulation of hypotheses with regard to the effects of external agents on the life history of this particular population of belugas. An obvious requirement is that data sets from various researchers, agencies and NGOs be linked, or cross-referenced.

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The Saguenay-St.Lawrence Marine Park: its role in the recovery of the St. Lawrence Estuary beluga.

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<u>Theme 4. Conservation</u>

The why and how of the creation of the Saguenay-St.-Lawrence Marine Park, its involvement in managing whales in a park context and in the recovery of the St. Lawrence Estuary (SLE) beluga population (through the monitoring / necropsy / sampling program) is presented in this brief overview.

In 1977 an analysis by Parks Canada was made of the marine region of the St. Lawrence Estuary and Gulf and in 1981 an area at the confluence of the Saguenay River and the St. Lawrence Estuary was identified as a potential marine park site. In 1986 a workshop with community representatives and interest groups on the proposed Marine Park was chaired by Leone Pippard, a SLE beluga researcher. Then, in 1988, a coalition for the creation of the Marine Park was formed during the International Forum for the Survival of the Beluga. A government-based initiative, the St. Lawrence Action Plan, was launched in 1998 with one of its objectives being the creation of a Marine Park. Consultations and discussions between federal and provincial governments led to the Marine Park being officially created and recognized by both governments in 1998.

As one of Canada's first marine protected areas and due to its unique nature, the Marine Park is managed under a law different from the National Parks Act. There is a participatory management structure – with the provincial and federal governments involved - and the Park is managed in accordance with existing legal mandates as in other governmental agencies.

The objective of the Marine Park is to increase the level of protection of a representative portion of the Saguenay River Fjord and the St. Lawrence Estuary marine ecosystem for conservation purposes, while encouraging educational, recreational and scientific uses.

Why this location? Although the limits of the Marine Park were defined during public consultations in 1993, they are linked, in part, to the summer distribution of the SLE beluga population. The inclusion of the head of the Laurentian Channel within these limits was also an important decision.

Because SLE beluga show a certain site fidelity to this area which has easy public access and a high diversity of other marine mammals, the Saguenay-St. Lawrence Marine Park is now an intensive whale-watching site with an important tourist-based industry valued at $42.5 million Can per year in direct and indirect economic activity (D. Gosselin, Parks Canada, pers. comm.). Initially problems were uncontrolled growth of the whale-watching industry and lack of a management framework to ensure protection of marine mammals including the SLE beluga and sustainability of observation activities at sea. Activities are still not always compatible with a "protected area experience".

Presently Marine Park management has addressed these issues by using various approaches, including those in the SLE Beluga Recovery Plan namely,

- education and public awareness at marinas
- development of land-based whale watching
- law enforcement, regulations on harassment, increased surveillance during the tourist season, adoption of regulations concerning whale-watching activities (observation activities at sea), excluding belugas as a "target" species for whale-watching cruises through regulation
- stakeholder participation
- habitat and site protection (through the ecosystem conservation plan and development of a Marine Park zoning plan that is currently in development)

- developing an environmental emergency plan in the event of an oilspill
- support of the SLE beluga necropsy program and research on live beluga through biopsy sampling
- helping to decrease the level of toxic contaminants that may affect the beluga through education and leverage
- participation in SLE beluga population monitoring and census
- working with Transport Canada and DFO to modify guidelines for maritime traffic through the Park to reduce collisions between whales and ships

Research activities also occur in the park and are concerned with prey distribution, "behavioural ecology" of whale-watching ships and boats, impact of whale-watching activities on large whales and more specifically on SLE beluga, namely,

- impact of noise on SLE beluga
- use of Baie Ste-Marguerite and Baie Ste-Catherine by SLE beluga and various vessels (2002-2004-2005)
- spatial distribution of whale-watching activities (1985-2002 and ongoing)

The SLE beluga is a charismatic symbol for conservation issues in Quebec, Canada and the world and this program has high public interest. Furthermore, the SLE beluga monitoring/necropsy/sampling program is relevant to Park conservation issues and species recovery. It permits us to evaluate the health of stranded animals from a population that is a highly visible indicator of the state of the ecosystem, to obtain data on population dynamics, causes of mortalities and presence of contaminants in whales and the ecosystem in general, and to maintain an archived tissue databank that could be used to evaluate potential new contaminants or threats to the SLE beluga population and St. Lawrence ecosystem and to identify and develop solutions.Scientific information is essential to help us better identify and understand current and emerging issues. This information is not always readily accessible and or used to determine management guidelines but it is essential to support and maintain partnerships and communication between scientists and managers. Although disease causality remains unclear in some stranded SLE beluga at present, it is vital to maintain this type of monitoring. There is a great need to obtain data from many different fields of research and various sources to assist managers in making conservation decisions. It is noteworthy that significant progress has been achieved in the last 10 years and further activities should continue in the future.

The beluga carcass program: from a "wake-up call" for conservation to the St. Lawrence Estuary Marine Protected Area (MPA).

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Theme 4. Conservation

Over the years the beluga carcass program has revealed high levels of contaminants in the body tissues of these whales, bringing our attention to the threat that contaminants pose as one of the main factors that could hinder the beluga population's recovery. Gradually, all sectors of society, including scientists, governments and the public at large, have become aware of the legacy of toxic substances that has resulted from industrialization and the impact of these substances on the St. Lawrence River and its wildlife. The beluga is an endearing species whose plight quickly captured the interest and concern of the general public. Originally listed as an endangered population in 1983, the St. Lawrence Estuary (SLE) beluga has become an emblem for the recovery of species at risk in Quebec and Canada as well as a symbol that transcends our national boundaries.

Public education and awareness have likely contributed to the increase that has occurred in marine observation activities over the past 25 years, particularly in the area around the mouth of the Saguenay River. Although the Marine Mammal Regulations, passed under the Fisheries Act, make it an offence to disturb marine mammals, they have proven inadequate to the task of managing mammal-watching and other activities at sea that have the potential to disturb marine mammals. The notion of "disturbance" is hard to prove and easy to contest in court. The Regulations are currently being revised with a view to overcoming these deficiencies.

Awareness of the potential impact of high levels of contaminants in beluga whales in the St. Lawrence has certainly prompted concerted government efforts to clean up the river and maintain biodiversity. These efforts took concrete form in 1988, with the implementation of the St. Lawrence Action Plan, among other measures. Under this five-year plan (1988–1993), action was directed at reducing discharges of persistent contaminants by the most polluting industrial plants; excluding beluga from marine observation activities through the adoption of a code of ethics in 1993; and getting the federal and provincial levels of government to agree on the boundaries of the Saguenay–St. Lawrence Marine Park (SSLMP). Two subsequent phases of the St. Lawrence Action Plan, both known as St. Lawrence Vision 2000 (SLV 2000), were carried out from 1993 to 1998 and from 1998 to 2003.

It was during the second phase of SLV 2000 that the *St. Lawrence Beluga Recovery Plan* and the *St. Lawrence Beluga Recovery Implementation Plan 1996-1997* were established and disseminated. The recovery plan was prepared by the recovery team set up in 1994 under the leadership of the World Wildlife Fund and Fisheries and Oceans Canada. Since the promulgation of the Species at Risk Act in 2003, the status of the SLE beluga has undergone a review, leading to its down-listing, in July 2005, to threatened status. This status automatically created a legal obligation to prepare or, in the case at hand, to revise and adapt the existing recovery plan in keeping with the requirements of the new legislation. One of the recommendations of the revised recovery plan, which is still under development, is to create a marine protected area, or MPA.

Marine protected areas represent one of the tools that the Oceans Act offers for providing enhanced protection to marine species and habitats while respecting existing jurisdictions. The concept of adaptive management is very important in the context of MPAs. The adaptive management approach provides a framework for adjusting management measures over time as new knowledge is acquired through scientific programs such as the beluga carcass program.

The goal of the St. Lawrence Estuary MPA project is to ensure the conservation and long-term protection of marine mammals that live year-round or, for part of the year, in the St. Lawrence Estuary as well as their habitats and food resources. The proposed management measures are based on the best scientific information available. When this information is insufficient, the precautionary approach is applied, which involves erring on the side of caution when making decisions about vulnerable marine resources and habitat. The problem arises in that industry wants to be given “proof” to justify management measures imposed by government, but this kind of proof is often difficult or even impossible to provide. The level of industry support for a given management measure is directly proportional to the amount of information that exists about the causality (“proof”). In this context, it is therefore paramount to encourage specific research programs, like the beluga carcass program, that are aimed at identifying causal relationships.

From a conservation standpoint, scientific knowledge is essential in order to convince the industry and to justify management measures. This knowledge is also crucial for developing coherent and effective measures for protecting marine mammals, while minimizing the negative impact on the industry. The acquisition of scientific knowledge is necessary to permit the intelligent review of management measures with a view to applying adaptive management principles.

The first need we have with respect to the SLE beluga carcass program is to determine the primary cause of mortality for every beluga carcass that is found. Second, it is important to evaluate the health status of these whales to determine secondary or contributory causes of death (e.g. noise-induced deafness that prevents an animal from avoiding collision). In our view, it is also crucial to identify threats that may not necessarily be lethal for beluga (e.g. contaminant burdens leading to cancer or other illnesses; deafness that impedes communication between beluga). In short, the necropsy aspect of the program needs to be given priority so that the primary and secondary causes of mortality can be determined and the most appropriate protective measures put in place.

Carcass sampling should be maintained at a level providing sufficient samples to enable scientists to 1) determine contaminant burden trends in belugas; 2) detect “new” contaminants; 3) guide research on contaminants with a view to evaluating associated toxic effects; 4) indirectly evaluate the quality of beluga habitat; and finally 5) continue efforts to raise public awareness. The monitoring aspect of program alone, despite its value in terms of furnishing a minimum of information on dead individuals (obvious cause of mortality and number of mortalities), appears to fall short of our conservation needs.

The beluga carcass program has played an instrumental role in helping to justify the creation of two contiguous protection zones, namely the Saguenay–St. Lawrence Marine Park and the St. Lawrence Estuary MPA. This linkage of two federal initiatives with some common objectives—such as measures to conserve the SLE beluga, a species at risk, and its habitat—is unique in Canada. Information collected through this program has provided impetus and considerable support for the implementation of acts, regulations and government programs. In view of the foregoing, adequate funding to secure the program’s future is essential.

Value of a marine mammal stranding response program in central California.

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Examination of stranded marine mammals provides valuable insight into the types of problems that threaten individuals or populations. Long-term data collection improves our understanding of the relative importance of specific diseases in different marine mammal populations, and of ecosystem health in general. A knowledge of enzootic pathogens (pathogens known to occur continuously or in cycles in a population) may help to differentiate natural disease outbreaks (epizootics) from unusual mortality events. Some pathologic processes may be directly or indirectly related to human activity (i.e gunshot trauma in California sea lions (*Zalophus californianus*) or introduction of the parasitic protozoan, *Toxoplasma gondii* to the southern sea otter (*Enhydra lutris*). Documenting and investigating these disease processes improve our understanding of the human impact on ecosystems and its health, and may influence management decisions. Conversely, marine mammals may be infected by or transmit pathogens or agents that are of human health concern. Recent examples of such agents include domoic acid toxin, *Mycoplasma* sp., West Nile Virus, and marine *Brucella* sp. Banking of specimens is essential for retrospective studies into the significance of newly-discovered pathogens.

The Marine Mammal Center (TMMC) is a private, non-profit marine mammal rehabilitation center located in Sausalito, California near San Francisco. The Center primarily responds to live-stranded pinnipeds along approximately 850 km of the central California coastline; from Mendocino to San Luis Obispo county. Every year, TMMC rescues approximately 650 to 1000 marine mammals. Approximately 50-60% of live-stranded animals are released after rehabilitation. This access to a large number of animals provides a unique opportunity for data collection. The staff includes approximately 45 permanent positions including 4 veterinarians, and approximately 650 volunteers. The annual budget is approximately $4.3 million (USD) with 35, 000 members worldwide.

In general, sampling of marine mammals at TMMC is conducted for diagnostic purposes, collection of data for specific TMMC projects, sample collection for outside researchers, and for specimen banking. Examples of current projects conducted by TMMC staff include: identification of novel pathogens, investigation of carcinoma associated with a gamma herpesvirus in California sea lions, the epidemiology of leptospirosis in California sea lions, identifying the long-term effects of domoic acid toxicity in California sea lions, and investigation into the pathogenesis of skin disease in northern elephant seals (*Mirounga angustirostris*). There are currently over 40 specific sample requests from different outside researchers. Requested samples vary from simple single tissue requests such as serum or skin to more complex sampling protocols involving various tissues and tissue processing for a *Brucella* sp. survey. Tissues are stored in a specimen bank for access by future researchers. Currently, these tissues include serum, DNA, urine, feces, and toxicology samples (blubber, muscle, liver and kidney). These tissues have been used for studies such as a retrospective West Nile Virus and *Brucella* sp. survey.

As part of its operating budget, TMMC conducts routine necropsies, collects samples for diagnostic purposes, for research projects, and maintains a tissue bank. Some specific projects are funded by competitive research grants such as Prescott and Oceans and Human Health. Outside researchers are asked to provide funding for storage and shipping of requested samples and to provide materials for more complex sampling protocols. Examples of protocols for California sea lion (CSL) necropsies and sample collection are provided in Appendix 2 and a list of publications by TMMC staff from the last 10 years are provided in Appendix 3.

A stranded marine mammal response program provides the opportunity for access to marine mammal tissues for a variety of purposes. Responding to live-stranded animals provides access to fresh tissues and allows trained professionals to observe clinical signs of a disease that may be difficult to diagnose post-mortem. Maintenance of a facility that responds to marine mammals in distress provides trained individuals, materials, and storage equipment that are available to carry out required protocols. This facility also allows for training of students and individuals from other facilities or other countries. If the program continues to run status quo it will result in continued access to specimens by TMMC staff and outside researchers. A strong research program and publication of results is and will be supported. Monitoring of animals for diseases of zoonotic potential, cataloguing potential threats to each species examined, and banking tissues for future studies are considered valuable components of the stranding program. Running the program with modifications may increase costs and may not be attainable with current staffing levels.

Reducing the scope of the program may reduce the number of personnel required and the costs involved with running the program but will result in a decreased number of animals examined, a decrease in samples, fewer students trained, and less publication. Currently, however, there is increasing outside demand for samples, increasing numbers of students interested in participating in TMMC programs, and increasing complexity of sampling protocols.

Discontinuing the program will result in loss of access to important data and reduced opportunities for learning. There is likely no other way of collecting the data and specimens as outlined above. Currently, there is no legal hunting of most of the species that TMMC responds to thus eliminating this activity as a possible source of samples. Some information could be potentially collected from free-ranging animals but sampling enough animals under field conditions may be more costly and more difficult logistically. However, sampling of apparently healthy, free-ranging animals is often necessary to adequately describe the role of specific pathogens in a population as animals within a rehabilitation setting likely represent a biased sample of the free-ranging population.

GENERAL DISCUSSION

After presentations and question periods, which were confined directly to points of clarification for each presentation, the external experts, presenters and other participants were asked for their overall opinion on the SLE beluga carcass program. This was followed by general discussion of each theme with external experts providing their evaluation and comments first. A summary of these discussions is presented below followed by overall recommendations from workshop participants.

Workshop participants recognized the three integrated components of the SLE beluga carcass program, namely monitoring, necropsies and sampling and their importance in understanding the present situation of the SLE beluga population. Sampling of carcasses on the beach was considered key to demonstrate exposure to contaminants and necropsy to show that contaminant exposure could have some toxic effects. Necropsies provided insight on some of these toxic effects (such as cancer, opportunistic infections, biomarkers such as DNA adducts, possible endocrine disruption and immunosuppression) which could have effects at the population level by impairing growth, survival and reproduction and increasing mortality rate. These negative effects could impair recovery of this threatened population and its long term conservation for future generations of Canadians. The value and limitations of the current program were recognized and various options for the future of this program were discussed (also see Table 1).

Theme 1. Cause of mortality and diseases.

The SLE beluga carcass program has received international recognition as a case study evaluating cause and effect relationships of exposure to chemical contaminants in marine mammal populations. Workshop participants were encouraged to examine Reijnders et al. (1995), MMC (1999) and Vos et al. (2003) for additional guidance and recommendations pertaining to this species with respect to contaminants, health and future research priorities. The beluga carcass program started 23 years ago in 1982 with a limited number of interested scientists (i.e. D. Martineau, P. Béland, R. Plante and later R. Michaud) who collaborated closely, has now increased to a larger group of participants with varying expertise and somewhat less collaboration leading to, in some instances, redundant or repetitive research. This group would benefit from coordination that could be done through the establishment of a formal partnership or consortium which could help manage samples, provide access to data and facilitate collaboration as well as integrate analyses in multidisciplinary studies. The field and necropsy data base requires updating, better organization and validation. For example, errors in use of morphometric units (i.e. metric vs standard), errors in transcription of data collected in the field or necropsy room and changes in interpretation of lesions and causes of death have been documented. Completion and submission of necropsy reports are behind schedule (i.e. backlogged to 2003). Methodology in aging of teeth needs standardization and validation using new technologies. Some of these problems are understandable and efforts to resolve them are underway with support from DFO and the Canadian Co-operative Wildlife Health Center (CCWHC). The field and necropsy data base is not accessible to researchers limiting its usefulness in analyses by collaborators. Collaborations, data integration and standardized procedures, with results undergoing general review and acceptance, perhaps through an internal DFO peer review process, could be used to formulate a united strong message about results and evaluation of the overall health status of the SLE beluga population. In addition this formal group could support and provide leverage for access to various sources of funding, internal or external to the federal government, including funds from institutions, corporations and industries (through conscience funds) or the public. Private support, however, depends very strongly on single, consistent messages with positive feedback through websites, newsletters, etc. The Québec Ministry of Sustainable Development, Environment and Parks, as an example, could be a potential partner in a continuing program, especially on an emblematic species such as the SLE beluga.

Assessment of threatened populations such as the SLE beluga is required through the Species at Risk Act and DFO monitors the population through regular surveys. As in commercial species, monitoring of beluga carcasses is similar, in some respect, to monitoring of catches in years between stock or population evaluations. It could also serve as environmental monitoring in ecosystem assessment and research, providing information on a high trophic level predator.

Monitoring is the basis of all that is done in the carcass program and it provides an index of mortality rate that is important in evaluating the status of the population despite some limitations and uncertainties. Monitoring needs to be continuous to maintain its usefulness as an alarm system, and to avoid losing valuable information as a small number of samples becomes more important when collecting demographic information of a small threatened population. Monitoring of marine mammal strandings cannot be reduced to only monitoring species at risk such as the SLE beluga since maintaining reporting and volunteer involvement and promoting public awareness requires that every stranding call receive adequate response and that results are publicised. This is particularly important for volunteers as this may represent their only reward. Training of staff and volunteers answering stranding calls is important and use of new ways to rapidly transmit information from the field to experts (i.e. digital photography through the internet) allows quick identification of species and facilitates informed rapid decisions on actions to take as well as helps reduce expenses. This would avoid errors in reporting carcasses that are not beluga and is also important during unusual events involving endangered or threatened populations or species.

Although established initially and specifically for the SLE beluga population, the monitoring component of the carcass program has value with respect to other marine species that strand or are reported dead (marine fish, turtles, birds and mammals). In some cases dead fish or birds may provide some insight concerning the etiological (causal) agent if the mortality of these species precedes those of marine mammals.
Information from other species could also provide valuable data and serve to identify potential risks to the threatened SLE beluga population [e.g. assess the risk of exposure of the SLE beluga population to cetacean morbillivirus from pilot whales entering the estuary]. This is particularly critical for species with similar life histories to beluga. Furthermore, extending the program to other species could also extend the geographic area of interest, i.e. regionally, nationally or internationally for species with trans-boundary distribution. This could permit access to new sources of support or funding although this may constrain the resources (manpower and funds) for the SLE beluga even further.

In the current marine mammal monitoring program run by Groupe de recherche et d`éducation sur les mammifères marins (GREMM) collection of data begins as each stranding call is handled and an identification number is assigned to each stranding event which should remain through all phases of analysis and integration. Information is distributed to investigators almost in real time on a website, which is essential to detect unusual mortality events. Codifying the quality of the information received from the field (i.e. experience of the person identifying species and evaluating carcass condition) has also been developed. Archiving of data requires further refinement. Information from other research on the SLE beluga population (e.g. photographic identification of live beluga as conducted by GREMM) should be integrated with data from the carcass program (similar systematic photographs are taken of each carcass prior to necropsy or sampling) as this provides added value to research programs. The data base of the CCWHC, of which the University of Montreal, FMV at St-Hyacinthe is a member, is still under development but is an example of a web-queriable data base that will be widely accessible and adaptable through addition of modules.

All current and past procedures of all aspects of the program (from questions to ask when answering calls, to the detailed protocol of necropsies at University of Montreal, FMV, St Hyacinthe should be documented, deposited centrally or distributed to everyone involved in the program to assure continuity, which is the essence of monitoring. Some detailed protocols are documented in published articles, but some simple procedures essential to new research may not have been documented and are not readily available to new participants. As the program also relies on a network of observers in the field (public, volunteers, fisheries officers, etc), procedures used to inform the public and to provide responses in order to maintain observer awareness and interest are needed and, therefore, should also be documented. This documentation should also include standardised training of people participating in the program, including volunteers in the field, people answering calls, carcass evaluations, transporting and sampling carcasses. This could involve an updating of the protocols and methods in Béland et al. (1987). In other words, how each carcass is handled and changes in protocols over time need to be documented.

The carcass program could assist in unusual or rare mortality events by helping develop protocols with a team approach, provision of adequate necropsy equipment, laboratory and storage facilites and participate in necropsies as required if funding is available. Unusual mortality events may include mass strandings of beluga or individuals of a species that is not at risk or of special concern. Rare events could be single individuals in the case of the endangered blue whale or endangered northern right whale or exotic species not endemic to the estuary (striped dolphin, common dolphin, pygmy sperm whale). Rare events may also include live strandings of moribund or orphaned beluga neonates that could be kept in captivity for a short period of time to assess condition or collect data not usually available from carcasses (e.g. haematology and biochemistry). This would be subject to Canadian Council on Animal Care guidelines as well as Marine Mammal Regulations and be cognizant of public and community values.

Necropsies, as currently conducted, determine causes of mortality important in conservation of the SLE beluga. Necropsies are also an important surveillance system, screening for new diseases. At present, necropsies screen for expected diseases but protocols could be improved in surveillance of potential new pathogens or biomarkers. Necropsies should be directed by an expert in marine mammal pathology in a laboratory setting allowing extensive post-mortem examinations including access to diagnostic laboratories (bacteriology, virology, etc.). Necropsy protocols have been consistent through the years, although some methods, techniques and analyses have evolved; however, standardised protocols should be documented to assure continuity within the program and also to allow external investigators to evaluate and demonstrate objectively the quality of the data and samples collected especially when external funding is sought. These standardised protocols should include objective measures of autolysis of the different organs. External funding can support special research projects but will unlikely support longterm “core” operations such as monitoring and necropsies. Support of the “core” program (necropsy) could be done through national DFO support of the CCWHC which could also examine other species at risk. If “core” funding of necropsies is available, specific research activities which depend on necropsies can then be funded through special grants.

The efficacy of conservation measures or efforts to mitigate human activities implemented due to conclusions from necropsy results may require re-assessment. In this respect archived tissues for comparative or retrospective purposes may prove valuable in assessments of data and review of management decisions. As a consequence archiving of the necropsy data base and samples has to be organised and tracking records have to be centralised to assure perenniality and facilitate integration by different investigators. Identifying or establishing storage policies may become an important issue in the program especially that of frozen tissues due to limitations of storage space (dedicated freezers required for often bulky samples), deterioration of samples over time (oxidation, dehydration, etc.) rendering some tissues useless for some types of analyses and security of samples required in legal cases (maintaining a chain of custody) with controlled and restricted access to storage areas. Three types of tissues have been conserved: paraffin blocks, histologic slides, fixed tissues and frozen tissues. Storage facilities are becoming limited, especially for the latter two. Solving the storage problem should be a priority considering potential legal issues. But also from a scientific point of view, this tissue collection is likely the largest of any beluga population in the world and it cannot be replaced. If selective archiving of tissues becomes the only solution, priority should be given to those important in supporting management decisions, those of unique diagnosis or those that are well preserved (Code 1, 2). Two archival systems are in place at the University of Montreal, FMV – one is administered by the University’s diagnostic services, the other by the CCWHC. Despite the fact that these samples are not managed within the SLE beluga carcass program per se, these samples could stay at the University. Formalin-fixed tissues can be problematic in terms of storage space due to quantity and bulk and long term usefulness (e.g. the longer samples are in formalin, the less useful they may become for certain analyses). There is an increasing demand to analyze tissues with molecular technologies (i.e. PCR) and DNA/RNA in soft tissues is best preserved in 100% alcohol

(1 part tissue, 5 parts alcohol). Keeping duplicate samples in the freezer, in formalin and in alcohol is recommended.

The number of mortalities of SLE beluga documented per year is small (9 – 21). Of these 3 to 12 are transported to the University of Montreal, FMV annually and examined at necropsy to determine cause of death. Due to decomposition and other factors the cause of death of these transported carcasses cannot be determined in almost 30% of all cases. Once the remaining 70% of cases are stratified by sex and age small sample size limits analyses of annual trends and interpretation of lesions and cause of death at age. However, there is little that can be done to increase sample size through changes in monitoring effort and reducing the number of necropsies as a cost-saving measure is counter-productive. Furthermore, any anticipated cost reduction by cutting the number of necropsies performed would not be proportional to the reduction in numbers of carcasses examined because it would not reduce baseline costs. It is not possible to reduce the number of samples collected based on their past value in evaluating cause of death or disease, as it is obviously impossible to predict new diseases and threats which the program is meant to identify.

Publication in the primary literature provides recognised objective evaluation of results that may form the basis for management decisions, and it demonstrates the value of the program internationally. The necropsy program, however, needs to use new up-to-date analytical techniques and approaches to assure that it will remain on the leading edge of research in the field and maintain high standards for publication.

Sampling without necropsy may be useful in collecting certain demographic and biological data for descriptive purposes (i.e. contaminants) or to determine prevalence of specific diseases if there is gross evidence but it does not provide a cause of death that can be used in epizootiological studies. Sampling a carcass destined for necropsy by opening the body cavity (i.e. on the beach to collect tissues that deteriorate rapidly post-mortem) can compromise results of the subsequent necropsy due to potential contamination.

Condition of samples is highly variable due to autolysis (decomposition). Except for some neonates who may strand alive, carcasses of juvenile and adult beluga may drift for days with wind, currents and tides before being beach-cast and are usually in a moderate (Code 3) to advanced (Code 4) state of decomposition. Carcasses may sink, become trapped in bottom debris, drift into the Gulf of St. Lawrence and be consumed by scavengers and consequently are never found or reported. During the summer months, which is when most carcasses are found, the rate of decomposition is high due to ambient temperatures, especially when carcasses are on the beach and during transportation to the necropsy laboratory. It is difficult to assess the degree of autolysis of a carcass based solely on its outward appearance. The present "coding" of marine mammal carcasses developed by Geraci and Lounsbury (1993 and refined in 2005) in warm climates can be difficult to apply to carcasses in cold climates. Generally, decisions to transport carcasses for necropsy are based on outward appearance only. Geraci and Lounsbury' s (1993, 2005) coding system includes characterisitics of external and internal tissues and organs and field evaluation of beluga carcass condition could be improved with additional training. Histologic or biochemical evidence of autolysis should be used to complement Geraci and Lounsbury's coding system which only relates to the gross or macroscopic level.

Theme 2: Contaminants and toxic effects.

Monitoring of SLE beluga mortalities is necessary in the context of contaminant studies in order to have available information such as sex, age, morphometrics, or photographic identification useful in interpreting contaminant data. However, monitoring alone is not useful in documenting exposure to contaminants without sampling and analyses. In addition, data on exposure to contaminants is needed in order to relate contamination with toxic effects. Without monitoring, the power to detect mortality events is lost, and with it, the information that would help in responding to an event. Monitoring is not only useful in detecting acute mortalities due to contaminants, but also in detecting effects of chronic exposure to contaminants on survival rate. Long-term monitoring is necessary because beluga are long-lived animals and it will take a long time series of data stratified by age and sex class to detect changes in mortality rate especially with only a few mortalities observed each year (i.e. detection power).

In addition to discussions in Theme 1 concerning monitoring that facilitates detection of mortality events, it was further discussed here that monitoring could also help detect mortalities above an expected baseline mortality rate (see Theme 3), that could be associated with toxic algal blooms, chemical spills, oilspills, etc. A contingency plan with required emergency resources (manpower and funds) should be developed to co-ordinate collection of appropriate samples that would allow identification of the causative agent and document its effects and permit a rapid and efficient response to the event. Clusters of carcasses or moribund animals either temporally or spatially may suggest a specific etiology, i.e. infectious or toxic in nature. However, it is important to relate observed mortalities to the life history of marine mammals as spatial and temporal clusters of dead animals could be simply related to the natural number of animals in an area seasonally, during migration or perhaps due changes in weather, climate, oceanographic events or prey availability.

Necropsies are necessary for the interpretation of potential toxic effects of contaminants (xenobiotics) and detection of new contaminants through observation of new or unusual lesions and evaluation of mitigation measures (e.g. reduction in prevalence of gastrointestinal tract cancer following reduction of emissions of PAHs), especially at the population level. Data would be incomplete without necropsies as these provide information on diseases and other disorders (e.g. reproductive disorders). A list of contaminants in various tissues does not provide much information, especially from a conservation viewpoint as it appears that all marine mammals are contaminated with xenobiotics to some extent. Not all toxic contaminants accumulate in tissue since some are metabolized and excreted (e.g. PAHs) but may nevertheless cause irreversible lesions. Biomarkers may be used to detect exposure to some toxic contaminants that do not accumulate in tissues. How contaminants affect their health is one of the more important questions to answer as well as to determine how beluga are exposed to these contaminants (i.e. via air, water, sediment, prey). Answers can be obtained and hypotheses tested if necropsies are performed on the same animals as those for which we have contaminant and biomarker data. For example, we know from the literature that certain types of gastro-intestinal neoplasms (=cancer) in some animals are indicative of exposure to certain types of contaminants (i.e. PAHs) and that, to support this hypothesis, we should look for evidence of exposure to PAHs such as presence of DNA adducts in beluga tissues.

Necropsies may serve to test alternative hypotheses: Can infectious agents be involved as etiological agents or co-factors in the development of gastro-intestinal tract cancers observed in SLE beluga? Do infectious agents (and contaminants) have synergistic effects on the development of cancer or other diseases? Are SLE belugas immunosuppressed, and if so, by what [contaminants, infectious agents such as bacteria (*Helicobacter*), viruses (cetacean or phocine morbilliviruses, herpesvirus), age, genetic predisposition, etc]? What is the significance of opportunistic infections seen in SLE beluga carcasses? Necropsies cannot provide all the answers, but they provide a starting point for further investigation.

Necropsies may reveal lesions indicating exposure to some contaminants that occurred decades ago; however, long time frames should not deter us from continued investigations. Toxicological investigations are often initiated based on observations of specific lesions and deformities (biomarkers) acting as “red flags” (e.g. cormorants with deformed beaks, skeletal deformities or feminization in fish) that provide an “early warning” sign that something is going wrong in the environment.

Although the recovery of the SLE beluga population appears slow and reasons for this are not fully understood, it is important to acknowledge efforts made so far by industries, municipalities, governments and non-governmental organizations to eliminate, reduce or mitigate pollution and sources of contaminants, as well as recognize positive effects of mitigation (i.e. successes) which have led to reduction of many pollutants and contaminants in the environment. For management purposes, we have to know where the PAH or other contaminants are coming from, if it is still present in the environment, how beluga are exposed to the contaminant and be able to provide sound scientific advice for appropriate actions to be taken by authorities in order to meet conservation objectives, etc. We need to examine the environment more closely. New scientific evidence (from specific research studies on contaminants and toxic effects) will assist conservation efforts by the Saguenay-St. Lawrence Marine Park and the proposed St. Lawrence Estuary Marine Protected Area to identify and implement conservation measures in the recovery of the SLE beluga population.

Necropsies need to be complemented by additional studies on contaminants and on toxic effects of contaminants (e.g. *in vitro* and *in vivo* laboratory toxicity tests in surrogate species), and by comparisons with other less contaminated populations of beluga in the Arctic. Furthermore, there is a need to test hypotheses developed in the case of the SLE beluga using other populations of beluga to avoid false interpretations (e.g. fluorosis in SLE beluga). This could be done opportunistically since DFO already conducts studies on arctic beluga populations. These specific studies will support necropsy data and assist managers in making decisions. Monitoring, necropsies and sampling of SLE beluga carcasses are all useful, but have limitations as does the current program as a whole. External funding is necessary to conduct studies which are essential in interpreting results from the carcass program with respect to management and effects of contaminants in SLE beluga.

There was extensive discussion on sampling with respect to this theme and some key elements are presented in Table 1. Although many points overlap with other themes most are included here to reflect the discussion and for the purpose of facilitating organization of the issues. Sampling whether done in a limited manner on the beach or during necropsy, is of paramount importance yet is problematic and is of relevance to all four themes. Important points are summarized here by specific topics discussed.

<u>Types of samples:</u> Hypotheses generated from the carcass program cannot be answered within the present program due to the types and condition of samples available. Other tissues such as blood (i.e. from live animals) are better biomarkers of recent exposure to contaminants and their toxic effects on target cells, tissues and organs. The present program provides few opportunities for collection of these types of samples except, in rare instances, fresh blood and tissues have been obtained from abandoned neonates. Samples from neonates, however, may not indicate the distribution of contaminants in tissues of juvenile or adult animals. Other opportunities to sample live animals could include biopsies of skin and the superficial blubber layer from free-ranging belugas. This is currently done by GREMM for genetic studies but biopsy sampling is outside the carcass program. In order to relate biomarker responses in skin samples to changes in internal organs or animal health, biomarker analyses on biopsy samples collected on beach-cast beluga carcasses (using a technique similar to that used in free-ranging animals) are needed. Sampling skin on relatively fresh carcasses as quickly as possible on the beach may be necessary. There is also a need to sample other populations of beluga (i.e. in the Arctic) for comparative purposes and to determine the significance of necropsy findings and biopsy findings from SLE beluga. For example, dental lesions (fluorosis) were documented in SLE belugas and an anthropogenic source of fluorine was suspected in the SLE but discounted when this chemical was observed to occur naturally in arctic beluga populations as well. Nevertheless, the available samples and corresponding data need to be analyzed for completeness, validity and trends.

<u>Condition of samples:</u> Nearly all samples for contaminant analyses have been obtained through the carcass program (sampling on the beach or from necropsies). Cold water can preserve the outer tissue layers of a beluga that died several days earlier yet the internal organs may be very decomposed as discussed in Theme 1 and of little value in sampling for contaminants and to study toxic effects. In addition, autolysis does not occur at the same rate in all tissues - valuable tissues such as the brain, liver, and gastrointestinal tract become rapidly autolysed post-mortem, partly due to autogenous enzymes, thus limiting their usefulness in studies of contaminants and toxic effects. Although robust tissues, which are less susceptible to rapid autolysis than fragile tissues, could theoretically be used as indicators of toxicological processes, their usefulness really depends on the target tissue (of the contaminant) and the question being asked (e.g. a contaminant or its biomarker may act or be found locally in the intestine and it is, therefore, not appropriate nor desirable to select a surrogate tissue for analysis). The degree of autolysis of tissues obtained through the SLE beluga

carcass program should be taken into account if results are to be compared to other beluga populations, from which samples are often obtained “fresh” by hunting, in order to avoid confounding effects. Further studies are needed to develop reliable quantitative indices of autolysis of various tissues and to determine precisely which autolysis state is suitable for different contaminant or biomarker analyses.

Archiving of samples: Archived samples are extremely valuable, especially in retrospective studies, because hypotheses can be tested using new knowledge and advances in technology. For example, are DNA adducts, as revealed by recently developed technology and biomarkers, present in samples collected 20 years ago? Due to small annual sample sizes it is imperative to have access to samples collected over a long time period in order to increase sample size in toxicological and other studies (perhaps by pooling data from a few years), determine and detect trends and increase the power of analyses. It is proposed that the new toxicology analytical centre at the Maurice Lamontagne Institute (DFO Science) in Mont-Joli be the archival depository of frozen beluga samples. However, due to changing governmental policies and priorities over time, long term storage of samples could still be problematic. It was suggested that the task of caring for and managing archived samples be a shared responsibility so that the archives do not become orphaned as may happen if a single archival manager leaves or is re-assigned.

Integration of data: A question arose concerning the limited number of necropsies performed each year on SLE beluga (i.e. about 3 to12 per year) and whether these provide enough information for management purposes (i.e. prevalence of cancer versus contaminant levels). It was stated that with the current monitoring system a fair proportion of SLE beluga carcasses are found which provide a representative sample size for each known cause of death and this has already proven useful in influencing management and conservation priorities. What is required is the integration of these data which could greatly increase the power of analyses. However, there is no escaping the unknown biases in beach-cast carcasses, the fact that juvenile animals are under-represented in the stranding record, advanced decomposition of some carcasses precluding definitive determination of cause of death, and no causal link between certain lesions or cause of death and the presence of certain toxic contaminants. It was now considered time, after some 22 years of collecting data from necropsies, toxicological analyses, demographic studies, etc, to integrate all information and correlate contaminant data with pathologies observed at necropsy by age and sex class. Integration of information is the basis of good epizootiological data, which are needed to account for variability (i.e. it may not be the most contaminated whales that develop cancer, etc). This also supports the need for archived tissues required in retrospective analyses. With increasing knowledge and advances in technology, there is probably a necessity to return to archived tissues for re-analyses, to test old and new hypotheses and to help identify what is most needed to help this population recover. To date, present knowledge of contaminants comes mostly from blubber samples and most data concerns persistent organic pollutants (POPs) with little data on non-POPs. The high prevalence of gastro-intestinal cancers in SLE beluga suggests that efforts should also target other contaminants (PAHs) and metabolites in other tissues and to use biomarkers to detect exposure to non-bioaccumulative compounds.

Ownership of samples and access to data: “Ownership” and management of archived samples and access to data will have to be determined by collaborating parties and program participants in a formal arrangement. Access to data should be improved to facilitate collaborations and multidisciplinary studies. Conflicting use of archived tissues can be expected to occur occasionally. Questions that need to be addressed by collaborating parties and program participants include: who has access to archived tissues, who has priority to request and obtain tissues and how much tissue is needed, who will manage archived tissues, should access or utilization fees be established to defay costs of handling and storage, if fees are charged, how much should they be, how will outside requests for tissues and data be managed?

Theme 3: Biology and demography

Concerns have been raised about different biases when using data from stranded carcasses to assess biological or demographic parameters of the SLE beluga population. Systematic effort to search for and collect carcasses could be used to decrease time in locating and collecting carcasses, increase the number of carcasses recovered and estimate the efficiency of reporting methods used in the past. Existing drift models could also be used to estimate the geographic area within the range of SLE beluga that is efficiently sampled from coastal zones where carcasses have been observed. As in any monitoring program, when a change in the sampling regime occurs, an analysis of overlap of independent sampling methods could be conducted in order to calibrate both methods.

The SLE beluga carcass program contributes information on the biology of this population. In addition, this population is an indicator of the state of the ecosystem only if we have good information on the biology of the population.

It is relatively easy to characterise the water, sediment or potential prey of beluga in terms of contaminant levels, but it is hard to link the former with contaminant levels observed in beluga. For example, to use beluga as an indicator of a contaminant that is ingested, beluga diet information is essential. We need to identify the feeding link through studies of diet since inference from contaminant or indicator levels such as DDE for DDT in beluga tissues may be affected by the pathway and speed by which these contaminants present in the physical environment are metabolised by prey and beluga before being deposited in their tissues.

Diet composition of the SLE beluga can no longer be estimated through traditional methods such as analysis of stomach and intestinal contents from large samples of hunted animals as done in the 1930's by Vladikov (1946). Alternative methods identified to date have different limitations. Although none of these methods provide a definitive answer, each one contributes to our knowledge as part of a multidisciplinary approach to determine diet composition of beluga in the St. Lawrence Estuary.

To date the SLE beluga carcass program provides limited information on diet composition using traditional methods as stomach and intestinal contents rarely contain identifiable parts of prey items. However, samples collected from carcasses may be instructive if alternative methods are used. Some parasites with specific intermediate or transport hosts could be used as biological markers to help identify some consumed prey. For example, the parasitic stomachworm, *Anisakis simplex*, in beluga indicates consumption of pelagic prey such as herring in which 95-99% carry larval forms of this parasite. Alternative chemical analytical methods using tissues from carcasses or live animals (biopsies) can provide information only on generalities with respect to food chain components (i.e. pelagic vs benthic, marine vs freshwater; carbon isotopes), trophic level (nitrogen isotopes), or possible prey groups with limited quantitative data on each prey item to species level (fatty acid analysis). Studies using fatty acid analysis require baseline data on qualitative species identification of potential prey available to beluga. Such a data base or library of potential prey items is currently being assembled for the SLE. But to complete the quantitative aspect of this technique, experiments with captive animals could be conducted to estimate the metabolic factor of different fatty acids. Molecular scatology, both qualitative (identification of prey species) and quantitative (contribution of different prey items) which have not yet been used with gastrointestinal tract contents from stranded beluga from the SLE, could be developed. All these alternative methods could be used on both live animals (biopsies) and samples from stranded carcasses to build a diet composition data base.

Geographical and temporal changes in diet composition will not be captured by the above mentioned methodologies. The diet composition of beluga is unlikely to be a simple average, as beluga elsewhere have been shown to be opportunists with individuals specialising on certain prey items (as reported in harp seals) and as prey composition varies throughout their geographical range and during the year. Tracking movements, diving activity and feeding events (through the use of satellite transmitters; time, depth and velocity recorders; acoustic tracking of echolocating animals; “critter-cams”) could also provide indirect information when compared to the temporal and geographic distribution of potential prey.

The SLE beluga carcass program will not provide complete answers for demography, but will complete and complement information provided through surveys and photographic identification methodologies in order to estimate other population parameters needed in modelling.

The different components (monitoring, necropsy, sampling) of the program provide increasing levels of detail about mortality that is important in demographic modelling. Monitoring provides the number of stranded carcasses found which is an index of mortality rate of the population with unknown bias. Sampling of stranded carcasses provides teeth, which are used in age determination, and reproductive tracts, which are used to estimate reproductive status, and these data could further clarify mortality in a stepwise process. Parasite loads, contaminant levels and causes of death determined from stranded carcasses examined at necropsy, while more or less biased, could be used to explain mortality and to determine different risks of mortality at different life stages and nutritional conditions. The additional information provided from necropsies and sampling, even if cause of death is not determined, could provide covariates for mortality in population modelling. However, this needs to be examined, because in other studies there have been biases which have limited the ability of using age at death data from stranded carcasses.

Theme 4: Conservation

Various human activities occur in the Saguenay-St. Lawrence Marine Park (SSLMP) and the proposed St. Lawrence Estuary Marine Protected Area (MPA). Both of these protected areas are designed, in part, to protect the SLE beluga. The most important activities include commercial shipping and whale-watching activities, but fishing and other human activities are allowed within the SSLMP and MPA boundaries. With the current approach, these activities will not cease, but they can be managed to meet objectives of conservation on the basis of sound scientific knowledge and advice. For instance, it may not be advisable to modify shipping lanes to protect the SLE beluga because several other species of marine mammals occupy various parts of the Estuary. Modifying commercial shipping lanes could have an effect on these other species by increasing the risk of ship collisions and by compromising maritime security (i.e. accidents). However, for conservation purposes, nautical speed of commercial ships and other vessels (e.g. cruise ships, zodiacs) could be regulated, if necessary. Regulations in the SSLMP are in place to protect whales from harassment (e.g. safe distances must be respected for whale-watching vessels, beluga are excluded from whale-watching activities, restrictions on number of permits for whale-watching vessels, etc). The SSLMP and MPA are also, for the most part, located downstream and downwind of industrialized areas, consequently exposing the SLE beluga population to PAHs, persistent organic pollutants (POPs), municipal sewage, agricultural runoff, and other contaminants. All these human activities located inside and outside the SSMLP and MPA may have serious direct and indirect negative effects on the SLE beluga population and its recovery.

Human activities may kill beluga either directly (ship collisions) or indirectly (chemical contaminants may cause cancer and or immunosuppression and increased susceptibility to enzootic, novel or exotic pathogens). Only the necropsy component of the SLE beluga carcass program can provide scientists and managers with information on cause of death and determine if there is a relationship with human activities. This information is essential to develop efficient and effective conservation and management measures.

The monitoring component of the program can provide some estimate of minimum mortality rate, although not all carcasses are found nor verified as beluga. Monitoring can also be used to detect trends (unusual mortalities and events), and provide location data where the mortality occurred in some cases (currents, tides, winds can influence carcass drift) but monitoring does not give information on cause of death. However, monitoring is essential for other components of the program (necropsy and sampling) which are important in providing data upon which sound management decisions for the recovery of the SLE beluga is based.

Necropsies revealed a number of traumas (a physical injury or wound caused by external force or violence which may occur during agonal death or be caused by human activities, conspecifics or predators) in SLE beluga since 1983. These traumas included cutaneous lacerations, internal hemorrhage and fractured or broken bones**.** Evidence of *ante mortem* trauma (trauma occurring while a beluga is still alive) suggestive of a collision with a ship or boat (i.e. multiple fractured bones, hemorrhage, obvious propeller wounds) was observed in 9 SLE beluga carcasses from 1983 to 2002 (2 additional cases in 2003 may be added once final necropsy reports are received). In one of the 9 cases, underlying pathologies may have led to ship collision (i.e. floating moribund animal) and in another may have been due to dystocia – difficult birth. In 2 of the 9 cases and in potentially another trauma case not included in the 9 cases, definitive diagnoses could not eliminate underlying pathologies due to incomplete necropsies or advanced decomposition. Two beluga received traumatic wounds from a harpoon and gunshot but these animals were floating moribund and had serious pathologies (pulmonary abscess, chronic peritonitis, probable pneumonia) responsible for their weakened condition.

Only complete necropsies conducted in a laboratory can provide quality data on contributing factors despite some limitations (i.e. diseased animals could be predisposed to collisions). For instance, underwater noise of human origin is perceived as a potential problem for whales (e.g. ship traffic, and especially sonar and seismic surveys). Hearing-impaired whales may not be aware of approaching ships thus, increasing the chance of trauma and death. However, evidence of hearing loss in a whale would be very difficult to detect with a regular necropsy because of the state of decomposition of carcasses and confounding factors. Carcasses are usually not fresh enough to detect subtle changes in the inner ear structure (rapid loss of the ciliated epithelium of the inner ear), and necropsies often reveal numerous parasites in surrounding structures (lungworms in cranial and ear sinuses), or other infectious processes could account for localized inflammation of the ear. Advanced technology (CT scan) is expensive and may be more appropriate in studying hearing loss in freshly dead beluga, but the information obtained remains controversial at present. To study hearing acuity would require physiological studies (evoked potentials) on live animals.

Necropsies remain valuable if we want to assess the toxic effects of contaminants (i.e. population effects) whether they are cancer or evidence of immunosuppression (beluga dying from opportunistic pathogens or parasitism). Necropsies may also be helpful in testing alternative hypotheses of tumour formation and immunosuppression (i.e. infectious causation and or synergistic interactions of pathogens and contaminants). Although a direct cause and effect relationship between cancer and various contaminants in beluga may not be obtained directly from necropsies, necropsies can generate testable hypotheses and provide epizootiological data that could provide “weight of evidence” that beluga are being harmed by contaminants and or pathogens.

Sampling is an essential component of the program providing samples and generating information on exposure of SLE beluga to contaminants and their toxic effects, and obtaining basic demographic information (morphometrics, teeth for aging, photographic identification) required to interpret all data. However, the carcass program should also support analyses of samples (i.e. contaminants, biomarkers).

In conclusion, conservation measures must be supported by good data, i.e. scientific proof or inferences based on “weight of evidence”. These data can be used to identify threats, establish priorities, define and justify specific measures and regulations needed to help this population recover and evaluate effects of mitigation. Much of this data or proof are obtained from the necropsy program in addition to supporting information from other program components (monitoring, sampling).

CONCLUSIONS AND RECOMMENDATIONS

- The SLE beluga population was severely depleted by commercial hunting. It was expected that protection from hunting instituted in 1979 would allow the SLE beluga population to recover which it did not. Concerns were raised about the role of chemical contaminants causing certain pathological changes and impairing reproduction and possibly hindering recovery of this population. Over the last two decades various measures to improve habitat quality and further protect this population were undertaken by governmental and non-governmental organisations. The continuing apparent lack of recovery of this population is not understood.
- The SLE beluga carcass program was initiated more than two decades ago by a small team of dedicated people (D. Martineau, P. Béland, R. Plante, and later R. Michaud) who wished to know causes of mortality of SLE beluga and to reduce some of these causes. Beluga, as high trophic level predators, reflect, to some extent, the health status of the St. Lawrence ecosystem with its diverse and endemic fauna, and serve as sentinels of the health of various marine organisms as well as that of human communities in the area. However, for a long-lived animal like the beluga temporal biological resolution is low. The carcass program (a community led and involved initiative) was unique and prompted governments to address issues of SLE beluga conservation and environmental protection.
- The SLE beluga has since become a worldwide symbol of environmental awareness and protection. Conservation efforts to preserve and protect the most southern population of beluga in its ecosystem have been made possible through knowledge gained from the monitoring, necropsy and sampling program. Some of that knowledge has been used to promote recovery of the SLE beluga population and protect its habitat through programs and entities such as the St. Lawrence Action Plan, St. Lawrence Vision 2000, the St. Lawrence Beluga Recovery Plan, Saguenay-St. Lawrence Marine Park, and the proposed St. Lawrence Estuary Marine Protected Area. Successful conservation of the St. Lawrence ecosystem and recovery of its endemic and emblematic beluga population depends on sound scientific data. With no evidence that the beluga population is recovering, there is a need to identify and quantify factors that may be limiting its recovery. The successful protection and recovery of this population falls under one of DFO's key strategic outcomes - healthy and productive aquatic ecosystems - for all Canadians.
- The current SLE beluga carcass program should be seen in its entirety rather than an assemblage of its component parts i.e. monitoring, necropsy, sampling. Monitoring has documented mortalities. Sampling has documented exposure to certain toxic contaminants. Necropsies and sampling have documented lesions, anomalies such as cancer, opportunistic infections, and biomarkers such as DNA adducts suggesting toxic effects, possible endocrine disruption and immunosuppression due to contaminants. Negative effects at the population level may be affecting growth, survival, recruitment, reproduction and increase mortality rate threatening recovery of this threatened population and its long term conservation. In this respect the carcass program has been valuable in identifying potential problems but has not determined why this population is not recovering. The etiology or cause of certain lesions and the role of toxic contaminants, pathogens or both are still unknown. Limitations of the carcass program include: the small number of carcasses found with unknown biases such as under-representation of juveniles in the stranding data base; problems aging animals, particularly old individuals; advanced decomposition of some carcasses and other factors which result in a significant percentage with unknown cause of death; few tissue samples of sufficient quality to test hypotheses on the role of pathogens or contaminant levels and their toxic effects or detect annual trends; poor data base management; and no integration of contaminant data with pathologic or biomarker data in order to demonstrate any causal link between certain lesions and the presence of certain toxic contaminants. Some of these limitations are inherent in dealing with beach-cast marine mammals. However, integration of data would greatly increase the power of analyses. Confounding problems include the longevity of beluga and the decline of some toxic contaminants and the discovery of new toxic contaminants in the marine environment and beluga tissues. Four key recommendations concerning the carcass program resulted from workshop discussions and are as follows:

Recommendation 1: The SLE carcass program should continue in order to monitor the state and recovery (detect improvement or deterioration) of this population, identify and quantify factors that may be limiting recovery and aid in assessment of population size and mortality rate. Twenty-three years (1983 – 2005) of data have now accumulated in a world-recognized study of a population of a medium-sized cetacean with unique characteristics (non-exploited with an effective absence of predators, essentially closed population with a restricted geographic range, small population size, readily accessible and close to centers of research, with a stranding network of observers resulting in a relatively good recovery of carcasses, long-term tissue bank and data base, etc). More specifically, the carcass program provides valuable information on strandings (monitoring component); pathological changes, pathogens, age at death, cause of death, demographics, genotyping, contaminants, physiological and reproductive states and diet (necropsy and sampling components). Other data obtained from SLE beluga research such as aerial surveys (estimated population size and trends, demographics and distribution), mark-recapture (i.e. photographic identification of individuals, biopsies, habitat utilization), tagging (time-depth recorders), etc. are all useful in monitoring this population. Unfortunately, none of these data in isolation indicates why the population has failed to recover but integration and analysis of the data together may be instructive. These data could be described as forming four main necessary elements or pillars in recovery efforts on this population: necropsy, contaminants, abundance estimates, and mark-recapture. Beluga are long-lived animals and data must be collected over a long time period in order to detect changes in population size, contaminant loads and their toxic effects as a response to management efforts to help this population recover. What is needed now is that this field and necropsy data base be updated, validated, better organized, accessible to certain researchers and integrated with other data bases. With the large time series of data available, it is now time to begin to integrate existing data linking information from various aspects of SLE beluga research including monitoring, necropsy, sampling, contaminant analyses, genetic data, aerial surveys, photographic identification, tagging, etc. With a complete inventory of data bases available, hypotheses could be proposed and tested using existing data, and will provide a valuable resource platform on which to design special projects financed with special funding.

Recommendation 2: A consortium should be created and all available information integrated. A consortium of participating researchers studying the SLE beluga would be an efficient way to manage and share information, enhance collaborations, identify research needs and facilitate financing of special projects. The consortium could include working groups involving three themes - pathology and infectious diseases, contaminants and their toxic effects, and biology and demography of the SLE beluga. Consortium members and working groups would include representatives from DFO, Parks Canada, INESL, GREMM and CCWHC who would bring different knowledge and expertise of benefit to the consortium and help in the recovery of the SLE beluga. Thus organization and collaboration are paramount and should be initiated immediately, with DFO as the lead agency. In this respect funding to help organize and co-ordinate activities of the consortium would be required.

Objectives of the consortium should include:

1) Establish and document protocols of all components of the carcass program.
2) Share data and ideas on the status of the population including health, health-related or other biological aspects of the SLE beluga.
3) Provide advice, perhaps through an internal DFO peer review process, on research direction, research priorities and mitigation measures to protect the SLE beluga (i.e. control threats from novel, exotic or introduced pathogens) and facilitate recovery of the population.
4) Maintain and manage archived tissue bank and data base. Conduct an inventory of existing tissues and data bases available. Co-ordinate requests for tissues, data and collaborations.
5) Explore potential sources of funding to conduct special research projects.
6) Develop a contingency plan to respond quickly and efficiently to an unusual stranding event (i.e. live neonates), unusual mortality event (i.e. epizootics) or immediate threat to the population (i.e. toxic algal bloom, chemical or oil spill).
7) Communicate results of the program to the public and managers with a `common voice`.

Recommendation 3: The core components of the SLE carcass program (monitoring, necropsy, sampling) should be adequately funded. The SLE beluga carcass ("core") program should continue with adequate funding since it constitutes the foundation upon which essential data required in managing, recovering and conserving this population are based. Results from the "core" program also stimulate further studies in the form of special projects upon which future management decisions are made. However, this "core" program is not adequately financed at present. Intrinsic funding (from DFO and Parks Canada) has thus far proved insufficient. Almost all parts of the "core" program have been partially financed by extrinsic funding (funds from outside of the carcass program, volunteers, etc) and are unpredictable with no long term financial commitment. Without a continuation of the "core" program new projects will not be considered for extrinsic funding. It was proposed that the "core" program be managed by the CCWHC, which would receive funding direct from DFO and Parks Canada (other provincial and federal governments including Environment Canada already contribute to the CCWHC).

Recommendation 4: There should be special research projects funded by special funds from outside the "core" program. Special projects financed by special extrinsic funds will benefit from and enhance the "core" program, and will answer specific questions posed by results from the "core" program as well as serve specific management needs. The "core" program has collected sufficient data thus far to generate a number of testable hypotheses that can be examined as special projects which may or may not be supported by DFO (depending on the mandate and priorities). For example, NSERC, FCAR or non-governmental funding may support research on specific questions posed by data generated from the core program. Some examples of special projects include:

1) Etiology of gastric and intestinal adenocarcinoma (cancer of the gastrointestinal tract). The hypothesis proposed is that this type of cancer is due to exposure to polycyclic aromatic hydrocarbons (PAHs) presumably via ingestion of contaminated benthic prey. New advanced technologies including biomolecular techniques are required to reject alternative hypotheses such as infectious causation (bacteria, viruses, parasites). Laboratory toxicity studies with surrogate species are required to evaluate the toxic potency of various potential benthic prey collected in the SLE beluga habitat and evaluate trophic transfer of PAHs and their metabolites. Characterization of diet and habitat use by SLE beluga is required to evaluate the risk of exposure to PAHs or other contaminants in the Saguenay-St. Lawrence ecosystem. Genetic studies can examine genetic predispositions to this type of cancer especially if linked with studies of age- and sex-matched beluga from populations in the Arctic. Further studies can examine certain biomarkers of exposure to carcinogenic compounds (cytochrome P4501A induction, DNA adducts), variability in diet and contaminant load between affected (those with cancers or opportunistic infections) and unaffected animals (those dead of trauma with no underlying contributory pathologies).
2) Are SLE beluga immunosuppressed? The hypothesis proposed is that SLE beluga are immunosuppressed by contaminants, stress from disturbance and general decline in habitat quality making them more susceptible to infectious diseases and development of abnormalities and cancers. The status of the immune system of the SLE beluga population should be determined using blood and other tissues from orphaned, biopsied and live-captured beluga and compared to that of age- and sex-matched beluga from populations in the Arctic. In vitro and in vivo toxicity studies may be used to relate observed contamination levels in tissues and altered immune function.
3) Poor reproductive success and recruitment to the population has been proposed as an hypothesis explaining the lack of recovery of this population. This could be examined using demographic studies from aerial and boat surveys, photographic dentification, genetic studies, tagging as well as evaluation of reproductive tissues and other biomarkers of reproductive activity or endocrine disruption in carcasses and biopsied live animals including comparisons with age- and sex-matched beluga from populations in the Arctic. Histopathological, microbiological, parasitological, haematological and biochemical data need to be included in assessing reproductive health. Assessment of exposure to endocrine disrupting contaminants is also needed.

TABLE 1. DECISION TABLE OF OPTIONS AND CONSEQUENCES ON SLE BELUGA MONITORING/SAMPLING/NECROPSY PROGRAM (THEMES 1 - 4).

Theme	Consequence if program component eliminated, maintained at status quo or modified with cost and benefit of each option.
Definitions	**Monitoring component**: documentation and verification of the mortality of a beluga with collection of minimal data (date, location). No necropsy or sampling involved. **Sampling component**: collection of beluga tissues or other samples for research or other purposes. Samples collected on the beach or in the laboratory with or without a complete necropsy to determine cause of death. Usually other data (morphometric, sex, photos, teeth for aging, etc) collected as well depending on objectives. **Necropsy component**: post-mortem examination of a beluga carcass, usually to determine cause of death, performed on the beach or in the laboratory. Data and samples usually collected but may be limited due to condition of carcass.
1. Cause of mortality and diseases	Elimination of monitoring is not an option unless the entire program is cancelled as necropsy &/or sampling requires knowledge of a mortality and carcass location - knowing the number of carcasses is the only index of the number of deaths. The mean of 15 carcasses reported per year is likely an under-representation of all deaths as effort on the vast Quebec coastline is limited by low density of human observers and winter conditions. The monitoring system seems to work well based on low variability in mean number of mortalities over 22 years. Reducing monitoring will result in loss of valuable data on number of mortalities. Monitoring can be improved by: 1) standardizing reporting protocol; 2) timely verification of the identification of beluga carcasses on the beach or drifting through the use of digital photos and electronic transmission of images, use of experienced observers and data base maintenance; better evaluation of carcass condition to assist in deciding to transport carcass for necropsy or sample on the beach (this would reduce costs and result in better quality necropsy data). However, it is recognized that it is best to err in rating carcass condition (Code 3 vs Code 4) to avoid loss of important data even if the carcass appears in poor condition on the beach and to train field personnel. For example, Code 4 carcasses provide less reliable diagnoses on cause of death but can provide data on specific lesion frequency (i.e. intestinal adenocarcinoma). 3) rapid and appropriate response to reported carcasses will encourage further participation by those reporting carcasses.

1. Cause of mortality and diseases (cont'd)	Sampling is a key component of the program for data acquisition and cannot be reduced or eliminated, however, sampling can be improved by: 1) Code 1 cases (live animals) should be held temporarily according to CCAC guidelines and complete haematological / biochemical / immunological / toxicological / microbiological / parasitological clinical data collected before or following euthanasia due to negative prognosis and unreleaseability; 2) Establish and document a standardized sampling protocol for all carcasses that is comparable to the standardized necropsy protocol. Samples should have consistent and clear labelling and inventories of archived samples be maintained; 3) Replicates of tissues should be collected from all carcasses for multiple analyses, and as an archive for retrospective analyses; 4) Assure appropriate archiving of sampled tissues (paraffin blocks, histologic slides, fixed tissues and frozen tissues stored at -80C) which would allow better leveraging for external funds in future research proposals; 5) Sampling of gross lesions and "normal" tissues including routine bacterial/viral swabs of lesions and "normal" tissues including the gastro-intestinal tract at standardized locations (even in the absence of gross lesions) should be included in routine histopathology and microbiology analyses; 6) Data integration and validation. For example, validation of aging data (1 or 2 GLGs per year?) obtained from sampled teeth collected from carcasses compared to animals aged by photographic identification. Retrospective analyses of teeth using new techniques and methodologies should be conducted; 7) Analyses of pathogens and lesions with respect to body condition, specific age- and sex-classes, contaminant loads and temporal trends for the entire data set are required.
1. Cause of mortality and diseases (cont'd)	Eliminating necropsies would result in: 1) loss of one of the longest uninterrupted monitoring programs on the cause of mortality, health assessment and biology of a threatened marine mammal population, in this case a highly visible "flagship" species for conservation, a population of beluga that is no longer exploited, and for which an expected rate of recovery has not been detected; 2) inability to identify human activities (contaminants, ship traffic, introduced pathogens) and natural processes (infectious and non-infectious diseases, oceanographic changes) including emerging diseases or new threats as possible causes of morbidity and mortality; 3) loss of a means to evaluate the efficacy of management decisions or policies aimed at reducing human or natural mortality, promoting conservation and monitoring recovery of this population at risk; 4) loss of a local high level of expertise in marine mammal pathology developed as a result of the necropsy component of the carcass program; 5) saving financial resources currently allocated to this program as there would be no need for transport of carcasses. By reducing the number of necropsies performed, transport costs would be reduced but this would not necessarily reduce necropsy costs which are partly independent of the number of carcasses examined. Furthermore, the problem of small sample size inherent in monitoring small populations at risk, which is a recognized limitation, would be aggravated by reducing the number of necropsies performed. By maintaining a relatively standard sampling protocol with collection of minimal data (e.g. indicators of specific lesions, diseases or pathogens) in place for over 20 years this would continue a valuable time-series data base permitting investigation of temporal trends. The current level of federal (DFO and Parks Canada) funding which is granted annually covers less than 50% of real costs associated with each necropsy and does not allow exhaustive analyses to determine possible causation (etiology) using new methods or technologies. This program also depends on non-governmental organizations such as the University of Montreal, Faculty of Veterinary Medicine (FMV), the Institut national d`écotoxicologie du Saint-Laurent, Les Industries Filmar and the Groupe de recherche et d'éducation sur les mammifères

marins (GREMM). Lack of resources also affects time to complete necropsy reports, which are currently backlogged to 2003. DFO should directly support the Canadian Cooperative Wildlife Health Center (CCWHC), of which the University of Montreal is a member, at the national level which would enable the development and maintenance of an on-going comprehensive coast to coast surveillance program on health, diseases and causes of death of marine mammals. This would secure and institutionalize the expertise of Canadian veterinarians in surveillance of marine mammal diseases which is currently depending on the good will of a few interested individuals. Necropsies should continue to be directed by a board-certified veterinary pathologist with marine mammal expertise in a location allowing intensive post-mortem examinations including access to diagnostic laboratories (bacteriology, virology, etc.). The University of Montreal, FMV is such a location and also has numerous veterinary students who assist in necropsies and analyses (i.e. low cost, educational benefits).

The necropsy component of the program can be improved by: 1) increased effort to isolate and identify potential pathogens such as viruses and parasites especially those causing recurrent lesions; 2) formation of a multidisciplinary team or committee including a microbiologist (bacteriologist/virologist), parasitologist, pathologist, biologist, epidemiologist, toxicologist and statistician to meet periodically (in person, conference call, virtually) to evaluate necropsy reports and data collected and set future questions and research priorities including requests for collaborations, tissues and funding; 3) centrally located and archived tissue bank and data base in an accessible and adaptable format for different investigators who wish to collaborate, consult, contribute expertise, share or integrate data, or perform analyses e.g. the CCWHC data base that is being developed may satisfy these needs; 4) appropriate documentation of archived tissue samples would support proposals for external funding of focused investigations with clear objectives and hypotheses designed to answer specific questions related to pathology, epizootiology and population dynamics in specified time frames, and would promote research in the field of marine mammal health in Canada; 5) standardized protocols using modern analytical methods; 6) specific hypotheses and methodologies need to be proposed a priori and re-evaluated periodically; 7) the present data base should be analyzed in its entirety for various temporal trends including an analysis of cause of death and disease prevalence by age- and sex-class, characterization of age-specific lesions, correlations of pathologies with contaminant burdens, pathogens, nutritional condition, i.e. useful in risk analysis. 7) development of life expectancy tables and risk of death by age, sex, contaminant burdens, pathologies, nutritional condition and pathogens. 8) a re-analysis of all histologic material collected since 1983 in light of new scientific knowledge and using new techniques could be particularly informative with respect to interpreting lesions and assigning cause of death as well as determining etiology.

2. Contaminants and toxic effects

Monitoring is necessary to detect changes in mortalities associated with, for example, infectious agents possibly related to exposure to immunotoxic contaminants and to detect toxic spill events or toxic algal blooms.

Data collected from monitoring and sampling is presently minimal and cannot be reduced.

Monitoring can be improved by:

1. systematically centralizing and validating monitoring data gathered within the program and making it available to collaborators and partners.
2. developing an adequate contingency plan to detect and react to an unusual mortality event in a timely manner. Without such a plan, application of mitigation measures may be delayed, causing additional mortalities.
   A. The number of stranded carcasses and mortality rate should be examined by a scientist or panel of scientists as soon as new data is collected in order to detect, in a timely manner, any unusual mortality event.
   B. Mortality rates should be assessed separately for neonates, juveniles and adults (i.e. stratify data by age and sex classes). Since generally very few dead neonates are found, the contingency plan should be implemented more quickly if there are an unusual number of dead neonates.
   C. Develop criteria to define what is considered a significant increase in mortality rate requiring sampling and investigations in the field.
   D. If a sudden increase in mortality rate is detected, a protocol should be developed to react immediately with water samples and tissue samples being collected quickly for toxicological analyses including algal toxins and infectious agents.
3. Assuming that the sampling component of the program continues, then the monitoring component should include collection of data such as morphometrics, sex, photos and teeth (to determine age). These data are necessary in interpreting contaminant data.

2. Contaminants and toxic effects (cont'd)

If necropsies are eliminated:

1. data essential in evaluating toxic effects of contaminants (risk management) in SLE beluga will no longer be available. Necropsies are an important tool in detecting new lesions or anomalies possibly associated with a new contaminant in the habitat.
2. a useful tool to detect a positive response to toxic substance management (ex. decrease in tumor rate in response to reduction of PAH emissions) would be lost. Furthermore, it would not be possible to relate chemical burden and cellular and tissue biomarker responses to responses at higher levels of organisation such as organs and whole body. This information is essential to relate biomarker responses to population effects.
3. few hypotheses will be generated (from observations of pathologies possibly associated with contaminants) in order to design in vitro and in vivo toxicity studies that could demonstrate cause and effect relationships.
4. it would be insufficient from a management perspective to have contaminant data only (this only indicates exposure). Assessment of possible toxic effects and population effects are directly or indirectly obtained from necropsies.

Reduction in number of necropsies conducted is not an option – the number of stranded SLE beluga is minimal and reducing necropsies would limit interpretation of data.

If necropsies are not improved:

1. The necropsy program is focused on determination of the cause of death. If it is maintained this way, the interpretation of temporal and spatial changes of histopathological biomarkers will be hindered without development of standardized procedures for the collection, preparation and examination of tissues from all individuals.
2. The absence of quantitative information on the state of autolysis of tissues will introduce a bias in interpretation of various biomarkers.
3. If the presence of viruses, bacteria and parasites is not systematically investigated in cases of proliferative gastrointestinal lesions, then the role of these agents in the pathogenesis of these lesions cannot be eliminated as alternative or contributing hypotheses to causation. Infectious agents may also contribute to immunosuppression, either alone (morbilliviruses) or by acting synergistically with contaminants.
4. Periodic necropsies of Arctic beluga (stratifying and matching data by age and sex class) should be part of the program since it is essential for epizootiological evaluation of the risk associated with natural or toxic factors.

2. Contaminants and toxic effects (cont'd)

If sampling was eliminated it would not be possible to:

1. relate lesions seen in necropsies to exposure to contaminants and it would reduce information available for risk management of toxic effects,
2. to conduct chemical analyses in order to determine exposure to persistent and bioaccumulative toxic contaminants, including new contaminants, and to measure responses of chemical biomarkers to assess the effects of such exposure on, for example, growth, survival or reproduction.

The sampling component of the program is essential in assessing exposure to contaminants, including new contaminants, and cannot be eliminated. Samples collected exclusively on the beach will satisfy the need for assessment of exposure of beluga to bioaccumulative contaminants only. Sampling of tissues for assessment of exposure and toxic effects is important in the design and interpretation of in vivo and in vitro toxicity and field monitoring studies which are an essential part of investigations on the relationship between exposure to contaminants and toxic effects in SLE beluga.

Sex, age, reproductive and nutritional condition, and infectious diseases are essential factors to consider in the interpretation of epizootiological studies and can be determined more easily in stranded SLE beluga than in free-ranging SLE beluga. Furthermore, evaluation of toxic effects on internal organs is not possible using free-ranging beluga whales without live-capture and extensive haematological and biochemical sampling.

Reduction of sampling is not an option - sample size of stranded SLE beluga is already minimal and reduction of sample size would limit interpretation of toxicological data and result in loss of valuable data. Limiting sampling to a few tissues is not expected to significantly reduce the overall cost of sampling.

Modification of the sampling component should include:

1. The current program carries out sampling of specific tissues (blubber, liver, skin) to assess beluga exposure to contaminants. Additional samples are collected and distributed to other interested researchers upon request. There is presently no control nor follow-up of these samples and this has caused/led to duplication of effort, redundancy, and inefficient use of samples. As part of the sampling component, a committee should be formed to manage the distribution of samples and create a bank of samples for future use. The committee should determine who will be responsible for archived tissues, who should manage outside requests for tissues, who should get tissues, ownership of samples, etc.)
2. Currently, little or no funding is provided within the SLE beluga program to determine contaminant concentrations in and to conduct biomarker analyses of sampled tissues. The program should provide some financial support to determine contaminant concentrations and measure biomarker responses in sampled tissues. Furthermore, this will ensure continuity in the assessment of temporal trends.
3. Although fixed tissues (in paraffin blocks) can be easily archived, there is a need for dedicated freezer space for beluga tissues with restricted access in cases where legal issues may arise. A chain of custody for legal cases should be maintained. A solution for long term storage of archived tissues is needed to ensure the integrity and continuity of the program. A solution for storage of frozen tissues could come from the new toxicology analytical centre at MLI (technical and financial support would be need to acquire equipment), but this aspect needs to be defined.
4. Skin samples must be collected and archived for biomarker and chemical contaminant analyses on all carcasses examined at necropsy in order to interpret skin biopsies from free-ranging animals. Analysis of skin biopsies from carcasses for contaminants would be useful for comparative purposes to those collected from live animals.
5. Selected samples for chemical and biomarker analyses should be collected immediately on the beach and in the necropsy room, as autolysis will affect results and valuable sample sizes will be reduced. This could be achieved in the field with minimal opening of carcasses in order not to compromise transportation nor necropsy but field personnel would need specific training.
6. In the necropsy room the degree of autolysis by organ should be assessed quantitatively and the sampling strategy should be a function of the degree of autolysis by organ based on a validated quantitative approach (e.g. biochemical or cytological measurements).
7. There is a need for scientific information on the effect of autolysis on the responses of various biomarkers and on chemical analysis of contaminants.
8. Milk samples should be collected and preserved for toxicological studies in order to assess toxicity of SLE beluga milk for neonates. Plasma should also be collected from relatively fresh carcasses (Code 1 & 2) and archived for toxicological studies.
9. Quality Assurance/Quality Control (QA/QC) procedures for sampling needed in temporal and spatial

epizootiological investigations of biomarker responses and contaminant levels must be instituted to avoid biases.

3. Biology and demography	For studies of SLE beluga distribution using genetics or contaminants elimination or reduction of the monitoring component could be mitigated by alternative methods such as biopsy sampling. Techniques such as satellite telemetry could be used to determine extent of beluga movements, but live-captures in the SLE have been, to date, unsuccessful. Studies to determine diet composition of beluga would be compromised by reduction or elimination of monitoring. Diet studies using stable isotopes could also be examined through biopsy sampling given that skin provides an image over a period of time similar to that of muscle. However, the most promising technique to examine the diet of SLE beluga, the fatty acid composition approach, relies entirely on access to carcasses. Alternative techniques using feces are also not viable with free-ranging beluga given that observation of defecation at the surface is an extremely rare event. If monitoring is eliminated or reduced, there would be no way to check carcasses with the photographic identification catalogue developed by GREMM. Present monitoring is satisfactory, however, there should be a measure of monitoring effort [i.e. using man-hours of search, or some other measure of catch per unit effort (CPUE)] in the monitoring protocol. Any modification of monitoring would have little change in ecological data quality.
3. Biology and demography (cont'd)	If sampling is eliminated: 1) no chance of finding a known photographically identified individual; 2) loss of the only source of age- and sex-specific data on survival and reproduction. If sampling is reduced to collection of superficial tissues such as skin, blubber, muscle and jaw (for age determination) this would continue to address questions related to SLE beluga distribution and diet using most of the techniques indicated in the abstract by V. Lesage. However, a systematic examination of digestive tracts for diet determination would require a more complete necropsy on the beach. Reduction of sampling would decrease sample size of age- and sex-specific data. Maintaining status quo sampling would continue to address some questions related to ecology, but long-term studies require rigorous digestive tract analytical protocols using optimal methods of preservation. Sampling could be modified: to enlarge the number of techniques potentially useful in examining diet in particular; 2) to increase the reliability of results obtained using current techniques; 3) to unify sampling and measuring protocols, based on quantifying the uncertainty of each measure. No ecological issues addressed to date in the necropsy component require complete necropsy for interpretation of results. If necropsies are eliminated cause of death cannot be determined. Given the threatened status of this population, elimination of necropsies removes a unique tool for investigating causes of poor population recovery. Current necropsy results should be analyzed in the context of demographic rates which would permit a better assessment of the utility of different categories of data collected during necropsies.
4. Conservation	Reducing or eliminating monitoring is not an option - monitoring is necessary for sampling and necropsies which are of paramount importance to implement and adapt conservation measures for successful recovery of SLE beluga. Monitoring is essential but insufficient alone in sound management and conservation purposes, which requires knowledge of the relative importance of various causes of mortality. Monitoring 1. provides minimum data on number of mortalities, date, location, which permits tracking any changes in mortality

and detection of unusual mortality events, all of which furnishes basic information for management decisions and recovery planning.
2. provides some partial information on fishery- and ship-related injuries due to entrapment, entanglement with gear, propellers, and collisions, however, necropsies are required in these cases to identify ante- and post-mortem changes.
3. data is useful in developing public awareness programs and stimulating public interest and assistance in locating carcasses and seeking corporate support (e.g. pollution abatement initiatives, protection, funding).
4. should receive regular and multi-year financial support.

4. Conservation (cont'd)

Elimination of sampling is not an option. For management and conservation purposes sampling and analyses are needed to:

1. track contaminant trends in belugas (to determine efficacy of pollution reduction measures) but this also requires funds to support contaminant analyses;
2. detect new contaminants in order to direct pollution reduction efforts at these new threats;
3. determine which contaminants should be the focus of research into toxic effects and whether a particular contaminant is a threat to beluga.

This information is needed to develop public awareness programs and stimulate public and corporate support for pollution abatement initiatives. Other data collected during sampling (i.e. teeth for aging, etc) is important in interpretation of results obtained from other aspects of the program and are necessary in decision making.

Sampling could be reduced only if the above 3 objectives are met. This could be achieved by

1. taking fewer samples (frequency?), or other samples from beluga.
2. sampling other indicator (substitute) species other than beluga.
3. sampling for specific contaminants for research into their toxic effects and potential threat to the recovery of the beluga population.

However, if sample size is reduced or different samples are collected, analyses must be performed and not reduced in order to maintain contaminant monitoring as an objective. A certain minimum of data is sufficient for public awareness programs. Other data collected during sampling (i.e. teeth for aging, etc) is still important in interpretation of results and should not be reduced.

The current sampling program seems adequate in providing samples to achieve objectives mentioned above and should be maintained; however, analyses (especially for contaminants) are not done in a timely manner because there is no support from the program to do so. Unanalysed samples do not provide information to help decision making and meet conservation goals. In this respect regular and multi-year financial support is required. Other data (morphometric, sex, photos, teeth for aging, etc) collected during sampling are important in interpretation of results obtained from other aspects of the program (e.g. necropsies, demographic analyses).

4. Conservation (cont'd)

Elimination or reduction of necropsies is not an option, because necropsies support conservation. Necropsies are required to:

1. determine cause of mortality, providing data needed to support conservation and protection measures, specifically to document a) primary causes of mortality (e.g., ship collision) of every single beluga carcass found. b) secondary causes of mortality, or contributing factors (e.g., noise-induced hearing loss leading to the inability to avoid ship collisions). This information is obtained through conducting complete necropsies performed in a laboratory setting. (e.g. moribund animals may be more susceptible to ship collisions).
2. maintain pathological expertise required to determine cause of mortality.
3. collect as much data as possible from carcasses – relatively few are examined at necropsy each year but each carcass has value, therefore reducing the number of necropsies is counter-productive.
4. provide minimal information to develop public awareness programs and stimulate public and corporate support for pollution abatement initiatives.

The current necropsy component provides very important information on causes of mortality (i.e. ship collisions, etc) and on health status that could be linked to human activities (i.e. cancer and immunosuppression due to chemical pollution, other diseases due to pathogen pollution). The necropsy component represents an effort which should not be eliminated or reduced because it supports conservation. Managers need scientific data obtained from necropsies to implement conservation measures and develop regulations as well as monitor the efficacy of such measures and regulations in order to promote sustainable use of the Saguenay-St. Lawrence Marine Park and St. Lawrence Estuary Marine Protected Area.

The necropsy component should receive regular and multi-year financial support. Better financial support would permit use of new diagnostic tools (e.g. PCR) which could provide a more accurate assessment of cause of mortality. Subacute and sub-lethal threats [e.g. biological (pathogen) pollutants, chemical pollutants causing non-terminal cancer or other diseases including impaired hearing which could cause problems in social communication, capacity to echolocate, forage or dive] are equally important problems in conservation of marine mammals.

APPENDIX 1. PUBLICATIONS

Primary publications from the SLE beluga carcass program:

Béland, P., and Martineau, D. 1988. About carcinogens and tumors. Can. J. Fish. Aquat. Sci. 45: 1855-1856.

Béland, P., Vézina, A., and Martineau, D. 1988. Potential for growth of the St. Lawrence (Quebec, Canada) beluga whale (*Delphinapterus leucas*) population based on modelling. J. Cons. Int. Explor. Mer 45: 22-32.

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APPENDIX 2. Examples of protocols from The Marine Mammal Center

STUDY CRITERIA (check to see if your dead animal meets any of the criteria below) * Only collect samples that need to be shipped fresh on Sun, Mon, Tues, Wed

Person	Animals Needed	Animals sampled
Blaire van Valkenburgh-respiratory turbinate structure	**1 fresh CSL head**	
Tanja Zabka-helicobacter	**10 fresh CSL**	4
Larry Dunn-brucella	**35 each CSL, HS, ES, any SSL** Serum, feces, histo, frozen tissues-see protocol	
Chris Kreuder-carnitine	**CSL** **15 acute or chronic DA** **13 controls (normal hearts)** 1. atria and ventricle in histo 2. pectoral muscle and left ventricle in -80	
Alex Chow-Hg analysis	**Adult female CSL** **1 normal (DA, trauma)** **5 cancer** 1. muscle, liver, brain in whirlpaks in -20 2. take tooth for aging	
Reynolds-fatty acids	**10 Adult female CSL-acute DA** **20 Adult female CSL-non-DA** **20 Adult CSL non-DA, but seizuring** 1. liver in whirlpak in -80	
Leptospirosis protocol	**10 non lepto control CSLs**-see protocol	
Domoic acid	All fetuses-protocol coming soon	
Wise lab-cell culture	**Any fresh marine mammal, especially SSL** see protocol	
Hendrik Nollens-pox	**CSL**. If animal had pox when alive, freeze lymph nodes. Pox noted at necropsy, sample lesions and lymph nodes	
Alex Travis-sperm preservation	**Male CSL, aged from birth through puberty, with no signs of viral or bacterial infection. n=5** See protocol	
Gene Lyons-hookworm	**Female CSL within a few weeks of having given birth or lactating**	
Tiff Brookens-Hg analysis	**Male HS >1** year 1. liver (put a chunk from each lobe together in ziplock) 2. fur 3. teeth (2 upper canines) **use ziplocks without color in the zipper**	
Katie Colegrove-cancer	**All cancer cases-see protocol!**	

Sampling protocols

Person/Study	Samples to take	Storage/shipment
Gene Lyons-hookworm	1. body wall through mammary 8" x 4" x full thickness 2. milk (preferably collected through teat-please specify whether from teat or mammary tissue)	Ship fresh day of sampling or next morning
Hendrik Nollens	Excise all pox-like lesions. Split each biopsy in half. Place one half in standard 10% formalin and freeze the other half. Collect mandibular, retropharyngeal, prescapular, axillary, superficial inguinal lymph nodes, or the lymph nodes that drain the area of the pox lesion(s).	Put samples in the pox box in the left hand freezer in the garage (except for formalin fixed pox lesions)
Tanja Zabka – Helicobacter	Glycerol (-80): 1x fundus, pylorus, cecum, colon Histo GI sections: 2x fundus, 2x pylorus, 1x prox duodenum (<3cm), 1x distal duodenum, 2x jejunum, 1x ileum, 1x cecum, 1x colon. Plus regular histo.	Put parafilm around glycerol vial lids and store in -80

CSL All ages Necropsy Protocol

Complete wound sheet if external wounds			
Check Study List for samples needed and binder for protocols			
If In Study, also take:	**Full Histology, Tooth if adult (upper Left Canine)**		
If CANCER suspect switch to carcinoma protocol			
NMMTB	>45 kg, acute disease	Collect within 3 hrs of death	
see poster			
Genetics	skin	cryovial w/ DMSO	-20
Histology	No Histology if: emaciated		
	classic lepto, trauma,		
	or lungworm pneumonia		
	Full Histology if: abnormal	10% formalin	Room temp
	(including brain, if possible)		
	Adults: if taking a tooth, weigh right thyroid and adrenal and fix		
	in separate histo pot, (left thyroid and adrenal with diagnostic set)		
			Refrigerate
Microbiology	Abnormal tissue	Bacterial Swab	on shelf
(<12hr)			marked 'micro'
If pregnant	blubber, liver (mom+pup)	teflon then whirlpak	-20
	serum (mom+pup)	spun and frozen	-80
Worms	Follow parasite preservation instructions		
ANY GUNSHOT ANIMALS: No Histology			
Bullets/shot	collect,wash & dry	Seal in paper envelope with your signature across flap. Fill out Chain-of-Custody form, file in vet office.	

CSL CARCINOMA Necropsy Protocol			
Complete wound sheet if external wounds			
Blood	serum	spin 5 SST	bank at TMMC
	whole blood (2 purple tops at euthanasia)		ECD
	RNA (paxgene tube at euthanasia)		MHC
ADDUCTS-Sampled immediately after death/euthanasia-<6 hours dead			
take ~300mg of tissue. **Note:** 300 mg = ~2x2 cm2 or the size of a thumbnail	liver	whirlpak	liquid N, then -80
	bile (3ml)	dark glass vial	
	tumor	whirlpak	
	esophagus epithelium		
	Target urogenital tissue*	whirlpak or cryovial	
(if visible tumor in urogenital tract, please take samples of normal and cancerous tissue)			
If pregnant and fetus is big enough	fetal liver	whirlpak or cryovial	
	placenta		
	amniotic fluid (3 ml)		
	fetal esophagus		
	fetal bile		
	fetal urogenital tissue		
Swabs	2 dry vaginal/prepucial swabs in cryovials		-80
MHC	skin	cryovial w/70% etoh	freeze
PCR	If adult male (Beth's protocol in necropsy binder)		-80
Histology	Full set	10% formalin	Room temp
	Repro tract whole	fix flat if possible	
	adrenal & thyroid (right)		
Microbiology	Lung	small chunks in petri	Refrigerate
(<12 hr)	Liver		for Judy
	Tumor		
ECD	Blood, whole	small teflon jar	-20
	Blubber	smaller teflon jar	
	Tumor	teflon then whirlpak	
	Esophagus mucosa		
	Urinary bladder		
	proximal vagina **or** penile epithelium		
TOX	Blubber w/skin	1 samples of each in	-20

	Liver	teflon then whirlpak	
	milk (if lactating)		
Genetics	skin	cryovial w/DMSO	-20
Tumor ID/	Liver	1 samples of each in	room temp
DNA	Tumor	10% formalin	
	Liver	1 samples of each in	-80
	Tumor	whirlpak	
	Target urogenial tissue*		
Aging	Upper left canine	whirlpak full of water	room temp
If female within	body wall through	ziplock	refrigerate
few weeks of	mammary		fed-ex next day
giving birth	8"x4"x full thickness		
or lactating	Milk (preferable through teat- specify whether collected through teat or from mammary tissue)	Plastic vials	refrigerate
			fed-ex next day
Gene Lyons			
If pregnant	blubber (mom+pup)	teflon then whirlpak	-20
	serum (mom+pup)	spun and frozen	bank in lab
Worms	Follow parasite preservation instructions		
*Target urogenital tissues for female and males			
	cervix	prepucial epithelium	
	proximal vagina	penis epithelium	
	urinary bladder	prostatic/proximal urethra epithelium	

CSL Domoic Acid
Necropsy Protocol

Complete wound sheet if external wounds			
Blood	serum	spin 5 SST	bank at TMMC
	whole blood (2 purple tops -if on weightloss study)		-20
	RNA (paxgene tube - if on weightloss study)		MHC
CSF	As much as possible	cryovial	-80
Urine	2cc - DA	cryovial	-80
Bile	1 vial - DA	brown glass vial	-80
Feces	DA	whirlpak	-80
Aqueous humor	2cc - DA	cryovial	-80
If pregnant	Follow DA fetus protocol		-80
PCR -	Tonsil	Foil, place all pieces in	-80
if long term	Spleen	one whirlpak	
	Adrenal		
	Brain		
	Liver		
	Lung		
	Kidney		
	Skeletal muscle		
	Heart		
	Stomach contents		
	Tracheobronchial LN		
Histology	Full set	10% formalin	Room temp
	Brain whole		
	Heart - 2 sections, see photo		
Chow (Adult F only)	Muscle	Whirlpak	-20
	Liver		
	Brain		
Carnitine	L ventricle	Whirlpak	-80
	Skeletal muscle		-80
Swabs	2 dry vaginal/prepucial swabs in cryovials-		-80
	if on weighloss study		
TOX	Blubber w/skin	1 samples of each in	-20
	Liver	teflon then whirlpak	
	Bile	Glass vial	
Genetics	skin	cryovial w/DMSO	-20

Aging	Upper left canine	whirlpak full of water	room temp
If female within	Body wall (through mammary	ziplock	refrigerate
few weeks of	8"x4"x full thickness)		fed-ex next day
giving birth	Milk (preferable through teat-	Plastic vials	Gene Lyons
or lactating	specify whether collected		
	through test or mamary tissue)		
Worms	Follow parasite preservation instructions		

APPENDIX 3. The Marine Mammal Center Scientific Publications (1996-2005)

Bowen, L., B.M. Aldridge, R.DeLong, S.Melin, E.L. Buckles, F. Gulland, L.J. Lowenstine, J. Stott, and M.L. Johnson. 2005. An immunogenetic basis for the high prevalence of urogenital cancer in a free-ranging population of California sea lions (*Zalophus californianus*). Immunogenetics 56: 846-848.

Colegrove, K.M., L.J. Lowenstine and F.M.D. Gulland. 2005. Leptospirosis in northern elephant seals (*Mirounga angustirostris*) stranded along the California coast. Journal of Wildlife Diseases 41: 426-430.

Colegrove, K.M., D. Greig, and F. M. D. Gulland. 2005. Causes of stranding of phocids (northern elephant seals (*Mirounga angustirostris*) and Pacific harbor seals (*Phoca vitulina*)) along the central California coast, 1992 – 2001. Aquatic Mammals, 31(1): 1-10.

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Greig, D.J., F.M.D. Gulland, C. Kreuder. 2005. A decade of live California sea lion (*Zalophus californianus*) strandings along the central California coast: causes and trends, 1991-2000. Aquatic Mammals, 31(1): 40-51.

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Lander, M.E., M.Haulena, F.M.D. Gulland, J.T. Harvey. 2005. Implantation of subcutaneous radio transmitters in the harbor seal (*Phoca vitulina*). Marine Mammal Science 21(1): 154-161.

McKnight, C.A., Reynolds, T. L., Haulena, M., deLahunta, A. and F.M.D. Gulland. 2005. Congenital anomaly in a stranded Pacific harbor seal (*Phoca vitulina richardsi*). Journal of Wildlife Diseases 41: In press

Neale, J.C.C., F.M.D. Gulland, K.R. Schmelzer, J.T. Harvey, E.A. Berg, S.G. Allen, D.J. Greig, E.K. Grigg, and R.S. Tjeerdema. 2005. Contaminant loads and hematological correlates in the harbor seal (*Phoca vitulina*) of San Francisco Bay, California. J Toxicology and Environmental Health, Part A, 68:617-633.

Silvagni, P.A., L.J. Lowenstine, T. Spraker, T. Lipscomb and F. Gulland. 2005. Pathology of domoic acid toxicity in California sea lions (*Zalophus californianus*). Veterinary Pathology 42(2): 184-191.

Wise, J.P., Sr., C.E.C. Goertz, S.S. Wise, A.T. Morin, J.L. Dunn, F.M.D. Gulland, M. Bozza, S. Atkinson, and W.D. Thompson. Chromium cytotoxicity in Steller sea lion lung, skin and testes cells. Sea Lions of the World Conservation and Research in the 21st Century. In Press.

Ylitalo, G.M., J.E. Stein, T.E. Hom, L.J. Johnson, K.L. Tilbury, A.J. Hall, T. Rowles, D. Greig, L.J. Lowenstine, and F. Gulland. 2005. The role of organochlorines in cancer-associated mortality in California sea lions (*Zalophus californianus*). Marine Pollution Bulletin 50, 30-39.

Bowen L., Aldridge B.M., Gulland F., Van Bonn W., DeLong R., Melin S., Lowenstine L.J., Stott .JL., Johnson, M.L. 2004. Class II multiformity generated by variable MHC- DRB region configurations in the California sea lion (*Zalophus californianus*). Immunogenetics. 56(1):12-27.

Dailey, M.D., Kliks, M.M., Demaree, R.S., 2004. Heterophyopsis hawaiiensis n. sp. (*Trematoda: Heterophyidae*) from the Hawaiian monk seal, Monachus shauinslandi Matschie, 1905 (*Carnivora: Phocidae*). Comparative Parasitology 71(1): 9-12.

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Goldstein, T., Mazet, J.A.K., Gulland, F.M.D., Rowles, T., Harvey, J.T., Allen, S.G., King, D.P., Aldridge, B.M., Stott, J.L., 2004. The transmission of phocine herpesvirus-1 in rehabilitating and free-ranging Pacific harbor seals (*Phoca vitulina*) in California. Veterinary Microbiology 103: 131-141.

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Acevedo-Whitehouse, K., de la Cueva, H., Gulland, F.M.D., Aurioles-Gamboa, D., Arellano-Carbajal, F., Suarez-Guemes, F., 2003. Evidence of Leptospira interrogans infection in California sea lion pups from the Gulf of California. Journal of Wildlife Diseases 39(1):145-151.

Acevedo-Whitehouse, K., Gulland, F., Greig, D., Amos, W., 2003. Disease susceptibility in California sea lions: Inbreeding influences the response of these animals to different pathogens in the wild. Nature 422:35.

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Desch, C.E., Dailey, M.D., Tuomi, P., 2003. Description of a hair follicle mite (Acari: Demodecidae) parasitic in the earless seal family Phocidae (*Mammalia: Carnivora*) from the harbor seal *Phoca vitulina* Linnaeus, 1758. International Journal of Acarology 29(3):231-235.

Duffield, D. A., Barros, N. B., Espinoza, E. O., Ploen, S., Gulland, F. M. D., Heyning, J., 2003. Identifying pygmy and dwarf sperm whales (genus *Kogia*) using electrospray ionization mass spectrometry of myoglobin and hemoglobin. Marine Mammal Science 19:395-399.

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