The risks and benefits of an invasive technique, biopsy sampling, for an endangered population, the St. Lawrence beluga (*Delphinapterus leucas*)

by

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Preface

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Abstract

Research can conflict with conservation when invasive techniques are used on protected animal species. We developed a decision framework including the research question, the choice of technique, and the recommended course of action following the evaluation of the risks and benefits. This evaluation includes biological risks and benefits and considerations linked to the perception of resource users. We applied this framework *a posteriori* to a case study, the use of biopsy sampling on St. Lawrence belugas. We monitored the biological risks and benefits over four field seasons using behavioural and physiological indices and reports on the work in progress. We evaluated the risks as "low" and the benefits as "medium". For benefits to outweigh risks, procedures to minimise risks, publication of the work, and formulation of recommendations for conservation are essential. Researchers should be prepared to discuss with stakeholders the potential conflicts between their projects and conservation.

Résumé

La recherche peut entrer en conflit avec la conservation si des techniques "invasives" sont utilisées sur des espèces animales protégées. Nous avons développé un cadre décisionnel incluant la question scientifique, le choix de la technique et la décision suivant l'évaluation des risques et des bénéfices. Cette évaluation inclut les risques et bénéfices biologiques et ceux liés à la perception des utilisateurs. Nous avons appliqué ce cadre décisionnel *a posteriori* à une étude de cas, la prise de biopsie sur les bélugas du Saint-Laurent. Nous avons contrôlé les risques et bénéfices biologiques au cours de quatre saisons de terrain, à l'aide d'indices comportementaux et physiologiques et des rapports sur les travaux en cours. Nous avons évalué les risques comme "bas" et les bénéfices comme "moyens". Pour que les bénéfices l'emportent sur les risques, il est essentiel d'appliquer les procédures pour minimiser les risques, de publier les travaux et de formuler des recommandations pour la conservation. Les chercheurs devraient être prêts à discuter avec les intervenants des conflits potentiels entre leurs travaux de recherche et la conservation.

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General Introduction

Conservation and management of natural systems often require biological research so that decisions may be based on scientific evidence (Tracy & Brussard 1996). Most biological research with applications to conservation implies fieldwork. Fieldwork on animals uses a variety of techniques ranging from benign, such as simple observation, to more invasive, such as blood sampling (Cuthill 1991). Invasive techniques can potentially interfere with the system studied, however, and alter it in ways that are at variance with the goals of the conservation programme (Cuthill 1991). It is essential, therefore, to balance the risks and benefits of research applied to conservation.

As technology progresses and becomes more cost-effective, more techniques, often invasive, are readily available for the study of animals in the wild. For example, various invasive techniques to obtain samples for genetic analyses are now widely used, especially on protected species (e.g. fish : Bernatchez et al. 1991, Sheffer et al. 1997; snake : Gibbs et al. 1998; bird : Haig & Avise 1996; terrestrial mammal : McGowan & Davidson 1994, Taylor et al. 1997; marine mammal : Kretzmann et al. 1997, Schaeff et al. 1998). In all of these cases, there is no detailed rationale in the published reports on the decision to use an invasive technique on a protected species.

A few recent publications have discussed the short and/or long-term effects of techniques liable to disturb animals studied in the wild (e.g. Cuthill 1991, Hoysack & Weatherhead 1991, Trites 1991, Laurenson & Caro 1994, Weller et al. 1997, Schneider et al. 1998). The authors of these studies consider the validity of the research done (Hoysak & Weatherhead 1991, Trites 1991, Schneider et al. 1998) and/or weigh the risks and benefits of using field techniques (Cuthill 1991, Hoysak & Weatherhead 1991, Laurenson

& Caro 1994, Weller et al. 1997). Nevertheless, they do not propose a framework in which to assess the risks and benefits of invasive techniques in the context of conservation biology. For example, when field researchers apply for government and university approval to study a protected species, application criteria and review procedures are rigorous, but based on a qualitative assessment of the case rather than a formal, point-by-point decision framework (J. Dravenak, National Marine Fisheries Services of the United States of America, J.-M. Nadeau, Department of Fisheries and Oceans Canada, L. Lefebvre, McGill University, pers. comm.).

There is thus an obvious need for a formal framework in assessing risks and benefits of invasive research in conservation biology. The goal of the present thesis is to propose such a framework and to apply it *a posteriori* to a case study, biopsy sampling on the endangered St. Lawrence beluga (*Delphinapterus leucas*). I will present the decision framework in Chapter 1. In Chapter 2, I will apply it to the case study and present the results from a monitoring programme of the biological risks and benefits resulting from the use of biopsy sampling on St. Lawrence belugas. Two methods will be used in this case study: in part 1, I discuss in qualitative terms the risks and benefits for which no quantitative data are presently available. In part 2 of chapter 2, I provide statistical analyses of the quantitative data that are available, focusing on behavioural indicators of potential negative effect of biopsy sampling.

Chapter 1: Development of a Formal Framework for the Use of an Invasive Technique on a Protected Animal Species

Introduction

If we survey the literature, two approaches from areas outside conservation offer interesting analogies for the case of protected species. One is the risk-benefit analysis used in clinical medicine to decide whether or not a treatment should be used on a particular patient (Pochin 1982, Forrow et al. 1988). The risks and benefits are assessed in probabilistic terms. Their balance takes into consideration issues beyond physical health such as quality of life and subjective levels of importance given by the patient to different risks and benefits.

The second approach is a decision cube for the use of animals in laboratory work on behaviour (Bateson 1986, Driscoll & Bateson 1988). This framework has three dimensions : animal suffering, quality of research, and its applicability to human benefit. The qualitative evaluation of these three aspects provides criteria for deciding whether a given laboratory project should be done or not.

Using these analogies, I developed a flow chart to formalise the decision on whether or not a research project on a protected animal species or population should proceed. This flow chart includes an evaluation table of the risks and benefits of an invasive technique when the use of a non-invasive technique is not possible. The evaluation table takes into account biological risks and benefits as well as other considerations linked to the perception of the resource users. Consideration of aspects beyond the biology of the system was adapted from the medical approach described above. The assessment of the biological benefits in the evaluation table was adapted from two dimensions of the decision cube discussed above, one being the quality of the research and the second being its applicability, applied in this case to conservation and not strictly to human benefit.

An important ultimate consideration in conservation is population size. Therefore, ideally, the risks and benefits of research in conservation should be balanced in terms of demographic parameters like recruitment and population growth. In practice, however, the available data rarely allow this type of risk evaluation to be made (but see Gill et al. 1996), and potential benefits are also hard to predict in terms of demographic parameters. To resolve this difficulty, indices for the evaluation of biological risks need to have potential significance in terms of reproductive success and/or survival of individuals in the population, even though these indices might not be able to measure one or the other directly. As for the benefits, we assume that conservation measures ultimately aim for positive effect on demographic parameters. Therefore, if the knowledge we gain leads to more adequate conservation measures, it should normally have a positive effect on demographic parameters.

Another important aspect of conservation is the sustainability of the use of the resource. If an invasive research technique had the potential to disrupt behaviour critical to the use of the resource, it could bring about a serious conflict between research and conservation. When evaluating the overall levels of risks, this possibility must therefore be taken into account, along with the potential effects on population size.

Decision framework for the use of an invasive research technique on a protected animal species

The first element of the flow chart I developed (Figure 1) is the formulation of a research objective involving the study of a protected population or species. This step implies potential conflicts between research and conservation and requires an examination of the following factors.

The first item in the flow chart concerns the type of technique to be used. I define two broad categories of techniques : invasive and non-invasive. I propose the following definitions :

Invasive technique : when sample collection is liable to disrupt the behaviour or physiology of animals in the population studied. This definition includes, but is not restricted to, cases when it is required to touch, manipulate, capture, or take tissue samples of the animals.

Non-invasive technique : all cases not covered by the above. When sample collection (in statistical terms, i.e. a sample can be an observation, a photograph, faeces, etc.) is not liable to disrupt the behaviour or physiology of animals in the population studied.

Non-invasive techniques, when available, should always be favoured since they virtually eliminate potential conflicts between research and conservation. If an invasive technique is chosen, there is a need to investigate the risks and benefits it implies using the evaluation table (Table 1), after having reviewed the status of the species (or population) as well as the objectives and the methods of the invasive technique.

The evaluation table has three main considerations : immediate biological effects, delayed biological effects, and the perception of the resource users. Conceivably, there could be no immediate biological benefits of the research for the animals studied, and the immediate biological risks could include risks of injury and disturbance.

Delayed biological risks include risks of disease or infection and risks of generalisation learning taking place if the animals associated unpleasant or painful stimuli to a particular place or situation (Domjan 1993). Generalisation learning can have long-term effects on certain behaviours such as habitat use or approachability by humans (e.g. Duffus 1996).

Delayed biological benefits of a research programme applied to conservation are assessed as the applicability of the knowledge sought to the design of adequate conservation measures. The applicability of the knowledge gained depends on the objectives and limitations of the project. It also supposes high quality research, since poor science could result in erroneous conclusions and would obviously not benefit the conservation programme.

Other potential risks and benefits of using an invasive technique to study a protected species or population are linked to the perception of the resource users. The risks result from a potential perceived paradox between the status of the species (or population) studied and the use of an invasive technique. This perceived paradox could exacerbate the discontent, where it exists, of the resource users, and consequently change their interactions with the species (or population) studied. Nevertheless, if there is good communication between the researchers and the resource users, research efforts and new knowledge can help raise awareness and increase commitment to the protection of the system studied (e.g. McDonald 1997). Research can help raise the general public's awareness as well, a tool used in many conservation programmes (Tangley 1997).

Each of the risks and benefits presented above are assessed in terms of the potential ultimate effects on population size and on important behaviours for the resource users. The assessment of these effects can be based on a literature review and discussions with other researchers and major stakeholders, and it can also involve the monitoring of a pilot project. Then the risks and benefits are evaluated as a whole. I propose a scale with three levels : low, medium, and high. Note that, in accordance with the precautionary principle, there are no cases using invasive techniques where risks could be evaluated as non-existent.

Risks: - **low** : no impact on the reproductive success or the survival of animals in the population studied and no disruption of a behaviour critical to users of the protected species (e.g. ecotourism, limited harvesting).

- **medium** : suggestion of potential effects on the reproductive success or the survival of animals in the population studied, but with no detectable effects on the population size; or, suggestion of behavioural disruption of some individuals, but with no critical effects for the resource users.

- **high** : suggestion of potential effects on population size through effects on reproductive success or survival of an important proportion of the population; or suggestion of large-scale disruption of a behaviour critical to users of the protected species.

Benefits: - **low** : the knowledge sought has no evident links to the conservation of the population studied or the research is of poor quality.

- **medium** : research quality is high, and the knowledge sought has potential applications for the conservation of the population studied, but these applications are not critical to the conservation of the population.

- **high** : research quality is high, and the knowledge sought can help essential decision-making for the conservation of the population studied to the point where not doing the research might threaten the conservation of the population.

A course of action is chosen based on the relative levels of risks and benefits assessed in the evaluation table (see Figure 2). If the decision to proceed with the research involving the invasive technique is made, the implementation of a monitoring programme of the risks and benefits assessed in the evaluation table is recommended. The results of this monitoring programme can be used to redirect the course of the project. They can also be used to formulate recommendations for future projects, either on the same species (or population) with different invasive techniques, or on other protected species. The strengths and limits of this proposed decision framework will be discussed in the General Conclusions.

Chapter 2: Case Study : the Decision Framework Applied to Biopsy Sampling St.

Lawrence Belugas

Introduction

I applied the decision framework described in chapter 1 to a case study : the use of biopsy sampling on the St. Lawrence beluga (*Delphinapterus leucas*). This population has been studied since the 1960's using non-invasive research techniques, such as aerial surveys (e.g. Pippard 1985, Kingsley & Hammill 1991), photo-identification (e.g. Michaud 1995), behavioural observation from the shore (e.g. Chadenet 1997), and necropsy (e.g. Béland et al. 1993). Biopsy sampling started in 1994 and was the first invasive technique used on this population. It addresses genetic and toxicological questions. Projects have been proposed and pilot studies have been conducted using other invasive techniques such as capturing belugas for blood sampling. It was therefore important to document the risks and benefits of an invasive research technique used on this animal and the criteria that should be taken into consideration when deciding if an invasive technique should be used or not.

To reach these objectives and as an example of the application of the decision framework, I simulated *a posteriori* the steps that lead to the decision of using biopsy sampling to study the St. Lawrence beluga. I documented background information on the population and on the application of the invasive technique under evaluation, and applied the decision flowchart presented in Figure 1. The decision made was to proceed with the research programme and to monitor the risks and benefits of the project. The results of the monitoring programme are presented as part of the case study.

Background

Status of the Population

The beluga is an arctic odontocete species that has been present in the St. Lawrence estuary and Saguenay fjord area (Canada, Québec) since the end of the last glaciation (about 10 000 years ago; Harington & Occhietti 1988). This southernmost population of belugas, isolated from northern populations, was attributed "Endangered" status in 1983 by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC; Pippard 1985). Massive hunting ended in the first half of this century after having depleted the population to about 10% of pre-exploitation levels (Reeves & Mitchell 1984). Currently, the population size is thought to be stable (St. Lawrence Beluga Recovery Committee 1998). The latest index yields a population size of between 600 and 700 individuals (Lesage & Kingsley 1998). Another limiting factor for this population is its small, restricted summer range (Michaud 1993, Lesage & Kingsley 1998).

Several plans were designed to promote the recovery of this population of beluga. In 1988, the Department of Fisheries and Oceans Canada (DFO) and Environment Canada designed the Interdepartmental Plan for the Survival of the St. Lawrence beluga (DFO & Environment Canada 1988, Prescott & Gauquelin 1990). This plan was applied within the St. Lawrence Action Plan and its phase 2, the St. Lawrence Vision 2000 plan, and focused mainly on the contaminants affecting belugas (St. Lawrence Beluga Recovery Team 1995). Following up on this plan, the St. Lawrence Beluga Recovery Plan was produced by a team of experts supervised by DFO and the World Wildlife Fund (WWF) in 1995, proposing five strategies and 56 recommendations favouring the recovery of this population (St. Lawrence Beluga Recovery Team 1995). The five strategies are:

- To achieve an overall reduction in levels of toxic contaminants
- To reduce disturbances
- To prevent ecological catastrophes
- To monitor the state of the population
- To investigate other potential obstacles to recovery

The St. Lawrence Beluga Recovery Committee works on the promotion and the implementation of the St. Lawrence Beluga Recovery Plan. It comprises six members from three areas of activity, namely conservation, the private sector, and government (St. Lawrence Beluga Recovery Committee 1998).

A portion of the summer range of this beluga population is encompassed by the Saguenay-St. Lawrence Marine Park (Figure 3). An agreement between the governments of Québec and Canada was reached in 1990 to create this conservation park. The provincial and federal Acts creating the Marine Park were promulgated in the spring of 1998 (Saguenay-St. Lawrence Marine Park 1998). Within this Marine Park there is a rapidly growing whale-watching industry (Michaud & Gilbert 1993). DFO put together a set of guidelines in 1988 to inform whale-watch tour operators as well as private boat owners on how to approach whales and minimise disturbance of the animals (M. Breton, pers. comm.). These guidelines give a special status to the St. Lawrence beluga, excluding it from whale-watching activities.

Biopsy Sampling : Objectives, Methods, and Measures of Success

Biopsy sampling was used to provide samples for two research projects conducted in collaboration by two private non-profit organisations, the *Groupe de recherche et d'éducation sur le milieu marin* (GREMM) and the St. Lawrence national institute of ecotoxicology (SLNIE). Collection of skin and blubber samples from identifiable individuals was designed for DNA and toxicological analyses to determine 1) sex, 2) kinship among individuals, and 3) contaminant levels in the blubber (Michaud 1995). Sex identification with genetic techniques is interesting since it is difficult to identify the sex of belugas swimming in the wild (Hughes & Pippard 1986).

In the first project, sex and kinship information contributes to the study of the social and sub-population structures of this population of belugas. This research project will also use information from the long-term photo-identification programme of the St. Lawrence beluga population. Individual belugas are photographed and catalogued according to natural marks such as scars and deformities. The identification of individual animals allows the monitoring of group associations and of habitat use (Michaud 1995).

In the second project, contaminant levels measured in the blubber portion of the biopsy samples are compared to levels that have been measured in St. Lawrence beluga carcasses since 1983, as part of a long-term monitoring programme (Béland et al. 1993). This project's objective is to verify if toxicological studies done on carcasses accurately reflect the contamination levels of the population and are not biased by sampling dead animals that could have lost weight, thereby potentially changing contaminant dynamics in the blubber. This project uses contamination information in combination with information about the sex of the individual (obtained through the genetic analyses) and about its age-class (obtained through the photo-identification history of individual belugas).

Biopsy sampling was conducted aboard the research vessel *Le Bleuvet* based out of Tadoussac (Québec). When observing belugas, the research boat approached parallel to their swimming direction and kept a constant speed. This type of approach is thought to minimise change in behaviour for the St. Lawrence beluga (Blane 1990, Michaud 1995). In 1994, the research team conducted a pilot study and tested two types of sampling systems : darts fired with a rifle (calibre .22, Pneu-Dard Model 191) and bolts fired with a crossbow (model Wildcat by Barnett, 23 kg in 1994, 1995, and 1996, 34 kg in 1997). The crossbow system was used for the following field seasons because its success rate was higher. Skin and blubber samples were obtained using the crossbow and a bolt fitted with a sterile stainless steel dart (diameter : 6.3 mm, length : 23 mm) adapted from previous tests and designs by Barrett-Lennard et al. (1996) and Patenaude and White (1995). When a beluga with easily recognisable marks was within 15 m of the boat and perpendicular to the archer, the bolt was fired at the animal. The sample was recovered from the floating bolt and the blubber was separated from the skin.

Of the 141 biopsy attempts performed during the four studied field seasons (May to October 1994, 1995, 1996, and 1997), 67 skin and blubber samples were obtained from 66 individuals (one individual was mistakenly sampled twice). Table 2 shows the outcome of biopsy attempts for each field season. Biopsy attempts were classified as a "missed attempt" when the bolt did not touch the animal, as a "hit" when the bolt touched the animal, and as a "successful biopsy" when a sample could be retrieved. The percentage of fired bolts that were hits was 56 % and the percentage of hits that yielded samples was 85 %. The hitting rate was within the lower range of studies published on biopsy sampling of cetaceans, which varied from 42 to 89 % (Whitehead et al. 1990, Brown et al. 1991, Weinrich et al. 1991, Clapham & Mattila 1993, Brown et al. 1994, Barrett-Lennard et al. 1996, Weller et al. 1997). Aiming at a beluga is probably more difficult than aiming at larger species such as the humpback whale (*Megaptera novaeangliae*, more than three times longer) or the killer whale (*Orcinus orca*, about twice as long). The team involved in the fieldwork was highly experienced with approaching St. Lawrence belugas and with using crossbows.

The other measure of success, the percentage of hits that yielded samples, was within the higher range when compared to values in published studies, which varied from 62 to 90 % (Whitehead et al. 1990, Brown et al. 1991, Weinrich et al. 1991, Clapham & Mattila 1993, Brown et al. 1994, Barrett-Lennard et al. 1996, Weller et al. 1997). The pilot study in 1994 had a low sample yield mainly because of difficulties linked to the use of the rifle, where the dart frequently failed to bounce off the back of the target beluga, detached later, and consequently got lost. Increased experience of the archers as well as slight modifications to the sampling equipment contributed to increasing the overall success rate over the four field seasons.

The blubber samples were stored on ice and transferred as rapidly as possible to a freezer. Sub-sampling was done to achieve the minimal number of blubber samples sufficient to address the questions asked. Samples were selected on the basis of sex and age-class of individuals to achieve a representative sample. About half of the samples were then sent in a cooler to the toxicology laboratories of D. C. G. Muir (Department of Fisheries and Oceans Canada, Freshwater Institute) and R. J. Norstrom (Environment

Canada, National Wildlife Research Centre). These are the same laboratories that regularly analyse the samples from the beluga carcasses (Béland et al. 1993). All standard quality assurance and control procedures were followed for the toxicological analyses (Muir et al. 1996a).

The skin samples were stored in 20 % DMSO, 0.25 M EDTA, and NaCl saturated solution and kept at 4 °C. They were then sent in a cooler to Dr. B. White's laboratory (McMaster University). The DNA extracted from the skin samples was of good quality for all but one sample, which was only considered average (Michaud 1996, R. Michaud, pers. comm.). The DNA extracted was suitable for PCR amplification, analysis of the Zinc Finger gene for sex identification, and analysis of microsatellite markers and mitochondrial DNA to address questions about genetic variability and population structure. So far, seven microsatellite loci have been analysed, and additional loci developed for belugas will be added to the analysis (Michaud 1996, R. Michaud pers. comm.).

Flow Chart Applied to Biopsy Sampling St. Lawrence Belugas

The researchers decided in 1994 to proceed with biopsy sampling St. Lawrence belugas. They made this decision according to several considerations, but they had no formal framework in which to organise these considerations. The following section is an *a posteriori* simulation of the application of the proposed framework to this case study. The goal of this simulation is to test the applicability and the usefulness of my proposed framework.

Formulate questions linked to the conservation of the St. Lawrence beluga

- What is the genetic nature of the different levels of associations observed between identified individual belugas?
- What are the contaminant loads in the blubber of living St. Lawrence belugas?

Examine the possible non-invasive techniques available to answer these questions

Non-invasive techniques were not available to address these questions. Skin sampling is less invasive than any other tissue sampling in cetaceans. Sloughed skin has been used to study the genetics of living cetaceans (e.g. Whitehead et al. 1990), but this technique is not an option with the St. Lawrence beluga because sloughed skin was never observed (R. Michaud, pers. comm.). As for blubber sampling, carcasses washed up on the shore are already collected and analysed for toxicology. Biopsy sampling is the only technique available to obtain blubber and skin from living belugas.

Analyse the risks and benefits of biopsy sampling, since no non-invasive technique is available to address the questions formulated

A literature review enabled the identification of potential risks and benefits of biopsy sampling St. Lawrence belugas, following the outline given in Table 1. The background information on this case study, needed in order to make an adequate assessment of the risks and benefits, was presented above.

Biological Risks

Three pieces of information indicated to the researchers that the potential biological risks of biopsy sampling St. Lawrence belugas were probably "low" (see definitions above) :

1) Several studies on the behavioural reaction of cetacean species to biopsy sampling concluded that the risks were minimal in terms of injury and disturbance, and that there were only short-term effects of biopsy sampling on animals studied (Whitehead et al. 1990, Brown et al 1991, Weinrich et al. 1991, Clapham & Mattila 1993, Brown et al. 1994, Barrett-Lennard et al. 1996,Weller et al. 1997).

2) Brennin (1992) conducted a pilot study on the effects of biopsy sampling on belugas in the Arctic and concluded that if the belugas were sampled opportunistically, without pursuing the animals, biopsy sampling was likely to result in minimal disturbance.

3) Patenaude and White (1995) conducted tests on carcasses to study the type of wounds caused by different types of sampling equipment. They recommended types of darts and crossbows where wounding appeared minimal while sample retrieval was good.

Biological Benefits

1) Research Quality

The scientific quality of the two research projects using biopsy sampling will be evaluated through the peer-review process. The first project is the study of the social structure of the St. Lawrence beluga, a Ph.D. thesis conducted by Robert Michaud under the supervision of Hal Whitehead, at Dalhousie University (Nova Scotia, Canada). For the second project, the toxicological analyses are performed by a laboratory that participated in an interlaboratory comparison of PCB analysis and reported results within acceptable limits (De Boer et al. 1996, Westgate et al. 1997). The team of senior scientists involved in the toxicological work regularly publishes its work on the toxicology of belugas and other aquatic mammals (e.g. Béland et al. 1993, Muir et al. 1995, Muir et al. 1996*a*, Muir et al. 1996*b*, Weis & Muir 1997, Westgate et al. 1997). Therefore the scientific quality of the research will be evaluated when Robert Michaud defends his thesis and when the toxicology papers are submitted for publication. This is a mechanism widely used to ensure the scientific quality of research (Driscoll & Bateson 1988). Therefore, the research linked to biopsy sampling belugas is likely to be of high quality, provided the work is completed and published.

2) Genetic Analyses and their Applicability to Conservation

Genetics and its applicability to conservation is a matter of debate in recent scientific literature. Lande (1988) compared the relative importance of genetics and demography for conservation, where genetics only implied genetic variability as a result of inbreeding and genetic drift. Genetic variability is important for long-term survival of a population or species because it is required for adaptation to a changing environment. Furthermore, inbreeding can have a negative impact on average individual fitness. Demographic parameters such as social structure, life history variation, and patterns of dispersal can have a short-term effect on population dynamics. Lande's conclusions were that demography was of more immediate importance to conservation than genetic variability. In fact, genetic variability and the way in which it relates to the risks of extinction of a population is still unclear (e.g. Caro & Laurenson 1994, May 1995, Avise 1996). Nevertheless, many authors consider there are important links between inbreeding and reduced individual fitness (e.g. Charlesworth & Charlesworth 1987, Roelke et al. 1993, Jiménez et al. 1994, Frankham 1995, Lacy 1997).

Studies of genetic variability can be useful for the management of hunting of Arctic belugas by allowing stock identification (e.g. Brown et al. 1993, Helbig et al. 1989). Nevertheless, it is unclear how they can contribute to the conservation of the St. Lawrence beluga population. Contrary to what is sometimes stated in the primary literature (e.g. Patenaude et al. 1994), showing that one population has a low genetic variability compared to another does not imply that inbreeding is a problem for that particular population (Buckley 1987, Avise 1994a).

Nevertheless, genetic analyses have more to offer than a portrait of genetic variability (Avise 1994b, Milligan et al. 1994, Hedrick et al. 1996). Genetic markers can provide a more accurate picture of population structure, effective population size, and gene flow in metapopulations, provided ecological and demographic information is also used (Milligan et al. 1994, Nichols 1996, Steinberg & Jordan 1998). These demographic parameters can be critical in establishing relevant management units (Moritz et al. 1996) and useful criteria for recovery, such as minimum viable population size (Parker et al. 1996).

The genetic information on St. Lawrence belugas will be combined with long-term monitoring of habitat use, grouping associations, and recruitment of females to provide insight into questions on social structure (R. Michaud, pers. com.). It is difficult to predict what type of recommendations for conservation could follow such a study (Steinberg & Jordan 1998). For example the study could provide evidence for the existence of sub-populations which would call for a change of scale of the conservation strategy currently in effect (Michaud 1995). Therefore, this work could be viewed as exploratory research to test certain assumptions in conservation approaches on the

St. Lawrence beluga. Furthermore, it could provide a new model using both genetic and ecological information, a recommended combination that is not always available in research applied to conservation (Steinberg & Jordan 1998). This could enhance theoretical work and apply to the conservation of other protected beluga populations or other protected species.

In summary, it is difficult to anticipate the contribution of genetic work to conservation strategies on the St. Lawrence beluga. Genetic studies have a greater potential for making a meaningful contribution to the management and recovery of protected species if the scientists are involved in the recovery process as members of multidisciplinary teams including researchers and managers (Moritz et al. 1996). The principal investigator of the project on the social structure of the St. Lawrence beluga population, Robert Michaud, is an active member of the multidisciplinary St. Lawrence Beluga Recovery Committee (St. Lawrence Beluga Recovery Committee 1998). For this case study, genetic information has the potential to make a significant contribution to the conservation and management of the St. Lawrence beluga.

3) Toxicological Analyses and their applicability to conservation

The toxicological analyses done on the biopsy samples have the potential to provide useful data to be compared to the existing data from the St. Lawrence beluga carcasses (Béland et al. 1993). Validating the work done on the carcasses will strengthen the evidence on contaminant accumulation in individuals, as well as trends over time in the population (Béland et al. 1993, Muir et al. 1996b). The toxicological analyses performed on the biopsy samples have other potential applications that have not yet been explored, but are under discussion by the scientists involved in the project. One of these applications would be to try to relate the condition of individuals, as assessed by behavioural descriptors, to known levels of contamination in individuals sampled for biopsy. This could provide evidence of sub-lethal effects of pollution on the health of St. Lawrence belugas (P. Béland, pers. comm.).

4) Other Uses of the Biopsy Samples

The biopsy samples obtained were not completely used up for genetic and toxicological analyses; additional questions, judged to be important in the future, may therefore be answered using these conserved samples instead of targeting more animals. For genetic analyses, a portion of the skin sample of every animal sampled was put in long-term storage (R. Michaud, pers. comm.). Toxicological analyses of blubber samples unfortunately involve destructive techniques. Nevertheless, sub-sampling was performed to reach the minimal number of samples necessary for valid comparison with the carcasses. Therefore, the stored, unused blubber samples could be used for other studies, such as fatty acid or stable isotope analyses to help answer questions about the diet of the St. Lawrence beluga (e.g. Hobson et al. 1994, Borobia et al. 1995, but see Grahl-Nielsen & Mjaavatten 1991). Diet has been identified as a critical research area for the recovery of the St. Lawrence beluga (St. Lawrence Beluga Recovery Committee 1998). Overall, the possibility of conducting more research on the same samples increases the delayed biological benefits of biopsy sampling belugas.

Other Risks and Benefits

1) Perceived Paradox

One of the critical aspects of the St. Lawrence beluga Recovery Plan is to minimise disturbance to belugas by controlling commercial and recreational whale-watching activities (St. Lawrence Beluga Recovery Team 1995). The principal investigator at the GREMM receives a scientific permit delivered by the Department of Fisheries and Oceans Canada (DFO) that allows the field team to approach belugas in order to do research, including biopsy sampling. This situation can potentially encourage whale-watch tour operators or private boat owners to also approach belugas, despite the code of ethics precluding intentional approaches of belugas.

It is possible to control this potential risk by increasing communication between the researchers and the whale-watching industry. For example, for another research project involving the tagging of fin whales in the same whale-watching area, the researchers organised presentations and meetings with the whale-watch tour operators before, during, and after the project to explain the goals, the techniques, and the results of the project. In addition, communication between researchers and whale-watch tour operators was frequent in the field during the tagging and tracking of fin whales. This communication favoured advance resolution of contentious issues, making the whale-watch tour operators feel part of the project, and furthering their knowledge of the resource they exploit (Giard 1996).

2) Raising Awareness

Research can help raise awareness of the status of a protected species and its recovery plan when the researchers devote part of their time to making their science available to the general public (Shrader-Frechette 1996, Tangley 1997). An important part of the GREMM's activity is directed towards educational projects related to conservation (P. Corbeil, pers. comm.). For example, the GREMM manages the Interpretation Centre on Marine Mammals (CIMM) in Tadoussac. The research using biopsy sampling was included in this educational strategy. There is an exhibit in the CIMM presenting biopsy sampling. Biopsy sampling is also explained in a documentary, *Encounters with whales*, co-produced by the GREMM. The documentary was locally and widely distributed and broadcast on Canadian national television in French and in English (P. Corbeil, pers. comm.). Therefore, the research linked to biopsy sampling contributes to raising awareness about the St. Lawrence beluga, both for the resource users and the general public.

Overall Evaluation of the Risks and Benefits

Overall, the potential risks of biopsy sampling St. Lawrence belugas were assessed as "low" (see definitions above). There were no indications in the literature of potential effects of biopsy sampling on the reproductive success, the survival, or critical behaviours of sampled whales. Furthermore, biopsy sampling is not likely to cause important problems linked to a paradox perceived by the resource users.

The potential benefits were assessed as "medium" (see definitions above). The research projects using biopsy sampling are for the moment presumed to be of high quality. The knowledge sought is likely to provide important, but not critical, information for the monitoring of the state of the population and the investigation of other potential obstacles to the recovery of the St. Lawrence beluga. The St. Lawrence Beluga Recovery Committee (1998) also reached this conclusion in rating the priority level of different recommendations involving research : none of the projects using biopsy sampling were considered top priority, but they were all listed as important or required. Furthermore, the research team demonstrated an ability to use their research to promote awareness of the conservation needs of belugas with the general public and the resource users.

Design measures to minimise risks and maximise benefits

Several measures were taken to minimise the potential risks and maximise the potential benefits of biopsy sampling, either at the start of the project or during the project. These measures are summarised in Table 3.

Proceed with biopsy sampling and monitor the risks and benefits

Following the recommended course of action described in Figure 2, a monitoring programme was designed to evaluate the biological risks of biopsy sampling (Michaud 1995), and four indices were developed. The biological benefits were reviewed after four field seasons by using reports written by the researchers on the work in progress. The results of this monitoring programme and the corresponding recommendations for the continuation of the research projects using biopsy sampling, as well as for the use of other invasive techniques on this population, are presented in the following section.

Monitoring Risks and Benefits in Four Field Seasons

Materials and Methods

Immediate Biological Risks

1) Immediate Reaction to Biopsy Attempts

The immediate reaction following a biopsy attempt was noted on standardised data sheets and filmed on video whenever possible (54 attempts filmed out of a total of 141). The reaction was either "no detectable reaction" or "crash diving", a behaviour where the animal interrupts its breathing sequence to dive quickly, producing a splash of water with an abrupt movement of the peduncle or a tail slap (Blane 1990). The frequency of "crash diving" was compared for different types of biopsy attempts (missed attempts and hits) and for target belugas as well as their group members using Yates corrected Chi-square tests (Statistica 4.3).

2) Behaviour Pre/Post Biopsy Attempts

When a third team member was present on the research boat, the behaviour of a focal animal or group was recorded on every surface bout by using a tape recorder. A group was defined as individuals synchronised in their breathing behaviour, swimming at less then a body length from each other, and in the same direction. A surface bout is a sequence of breaths taken at the water surface and separated by short (< 30 s), shallow dives. The information was transcribed later on standardised data sheets. Ventilation data was recorded either as the time (hour : minute : second) of every breath of the focal animal or as the time of the first and last animal to surface in a surface bout of the focal group.

The ventilation descriptor retained for analysis was the duration of the dive cycle, a long dive followed by a surface bout. The behavioural descriptors retained for analysis were group size (it ranged from 1 to15 belugas) and activity level. Activity level was classified into four ordinal categories (low, medium, high, and very high) according to the speed of the belugas and the vigour of their movements. In addition, the type and distance of the approach performed by the research boat were noted for every surface bout of the focal animal or group. If the observer were unable to reliably locate the focal animal or group at every surface bout, the interruption in tracking was noted.

The activity level was compared before and after a biopsy attempt by taking the mean for two to four surface bouts before and two to four surface bouts after a biopsy attempt. The differences were tested using a t-test for dependent samples (Statistica 4.3).

Group dynamics was assessed as the change in group size between two consecutive surface bouts. There were three possible events : group size increased, diminished, or stayed the same. Group dynamics was evaluated between the surface bout just prior to the biopsy attempt and the surface bout of the biopsy attempt. This value was compared to the group dynamics between the surface bout of the biopsy attempt and the surface bout just after. The distribution of the three possible events (size increased, diminished, or did not change) was compared before and after the biopsy attempt using a Yates corrected Chi-square test (Scherrer 1984).

Sixteen trackings with up to four breathing sequences before and after the biopsy attempt were used to monitor the possible change in behaviour described above. They were the treatment trackings. Trackings before and after a biopsy attempt differed in the pattern of approaches performed by the boat : the boat tended to approach the belugas more during the surface bouts prior to the biopsy attempt. Therefore, 10 trackings where there was no biopsy attempt, but that were similar to the treatment trackings in the pattern of approaches performed by the research boat, were selected as controls. The mean duration of the dive cycle of two to four surface bouts was compared for pre/post effects in the treatment and the control trackings using a 2-way ANOVA with a between-group and repeated-measures design (Statistica 4.3). The distance between the boat and the belugas was compared the same way to test if indeed the control trackings had an approach pattern similar to the treatment trackings. This allowed a distinction between an effect of the biopsy attempt and an effect of the approach associated with a biopsy attempt.

Delayed Biological Risks

1) Number of Sightings Before and After Biopsy Sampling

A sighting was defined as a series of photographs of an individual from a given encounter. An encounter was defined as an episode limited to three hours where the research team proceeded to collect data on a herd, a herd being composed of one to several groups of belugas. The distance between the research boat and an individual beluga affects the possibility of obtaining photographs from this individual. If biopsy sampling was associated with the presence of the research boat by belugas, sampled animals might become warier of the research boat through generalisation learning. We compared the number of sightings per 100 encounters the seasons before and after the biopsy attempt on a given individual to assess its approachability (t-test for dependent samples, Statistica 4.3). This was an indicator of a long-term change in behaviour relative to the research boat following the biopsy attempt.

We selected animals that had been identified and catalogued for at least one season before the season of their biopsy. We analysed only belugas that were sampled for biopsy in 1994 and 1995 because sighting data of the season 1997, i.e. of the post-biopsy season for beluga sampled in 1996, were not available.

2) Scarring of Sampled Animals

Careful observation during the biopsy attempt and close examination of video recordings when available allowed us to document the exact location of the biopsy. An effort was made to obtain photographs of the sampled animal in the few minutes following the biopsy. The photographs obtained in the months following the biopsy allowed us to monitor changes in the appearance of the wound. We selected only photographs of superior quality for this monitoring because the type of mark left by a biopsy is very subtle. Therefore, we did not have a record of the evolution of the wound resulting from the biopsy for all individuals. For selected individuals, we monitored the time it took for the biopsy wound to become invisible on high-quality photographs.

Results and Discussion

Immediate Biological Risks

1) Immediate Reaction to Biopsy Attempts

The typical reaction of belugas to biopsy attempts was "crash diving". Target belugas reacted as frequently to hits as they did to missed attempts (Yates corrected Chi-square = 1.93, $p = 0.165$), but group members reacted more often to hits on the target beluga than they did to missed attempts (Yates corrected Chi-square = 4.37, $p = 0.037$).

"Crash diving" does not appear to be stimulus specific and is best described as a startle response followed by a fleeing response. Belugas have been seen "crash diving" in response to a change in the regime of the boat (Blane 1990; R. Michaud, pers. comm.). They have also been observed "crash diving" when apparently surprised by other belugas (R. Michaud, pers. comm., and personal observation).

St. Lawrence belugas responded more systematically than any other cetacean for which there is published information on immediate reactions to biopsy sampling (Table 4). It was the only species studied where the startle response was systematic for successful biopsy attempts and was almost as frequent for missed attempts and for other members of the group. During a pilot project in the Arctic, a beluga was sampled three times using a biopsy sampling system similar to the one used in this study. It reacted only once and it exhibited the typical "crash diving" behaviour associated with biopsy sampling in this study (Brennin 1992).

If this avoidance reaction implied potential biological risks, these risks were approximately equal for both successful and missed attempts, for the target beluga and for other individuals in its group. This suggests potential cumulative effects of biopsy attempts on an individual. These cumulative effects could not be evaluated here, but were likely to be minimal at the level of sampling that occurred during this study.

In summary, the immediate reaction seems to indicate a startle response to an unexpected stimulus, painful or not, followed by a fleeing response. It probably has no long-term consequences on the survival or the reproductive success of the target individual or members of its group.

2) Behaviour Pre/Post Biopsy Attempts

Activity level was not significantly different before and after a biopsy attempt (t-test for dependent samples, $p = 0.333$, $n = 16$); this suggests that the type of activity that the belugas were engaged in did not change. Similarly, group dynamics did not change following a biopsy attempt (Yates corrected Chi-square, $p > 0.75$, $n = 12$).

The dive cycle was on average 43 % (110 s) longer after a biopsy attempt when comparing mean duration of the dive cycle prior and after biopsy attempts (2-way ANOVA, $F = 12.829$, post-hoc using Tukey HSD test : treatment effect $p = 0.0003$, control effect $p = 0.994$). Furthermore, there was no significant difference between the approach distance pattern in the treatment and in the control (2-way ANOVA, $F = 3.773$, $p = 0.0644$). The low p value was due to the fact that the approach distance in the post part of the control trackings tended to be greater than in the treatment trackings. This inflated difference between the pre/post of the control compared to the treatment would predict a greater increase in the duration of the dive cycle for the control if the approach by the boat were causing the increase in the duration of the dive cycle. This was not observed, and it suggests that the increase in the duration of the dive cycle observed for the treatment trackings did not come from a difference in boat behaviour prior to and after a biopsy attempt, but was due to the biopsy attempt itself.

Blane (1990) noted that an increase in the duration of the dive cycle was a possible avoidance strategy for the St. Lawrence beluga in response to disturbance stimuli. The observed increase in the duration of the dive cycle could therefore be an extension of the fleeing response characteristic of the immediate reaction to biopsy sampling. Nevertheless, this prolonged fleeing response might not be systematic. For example, after 8 out of the 141 biopsy attempts the field team noted that the targeted animal continued or started to approach the boat, to pass under it, and to circle around it, a behaviour known as "investigation" (Blane 1990).

There is theoretical work (Kramer 1988, Houston & Carbone 1992) and field and laboratory work (e.g. Dolphin 1987, Dolphin 1988, Carbone & Houston 1994) showing that aquatic air breathers tend to optimise the time spent underwater when foraging. A change in the duration of the dive cycle may represent a departure from this optimum and might therefore have physiological costs to the target individuals and other individuals in the group. These risks are probably small if the biopsy attempt is considered as an isolated event, an acceptable assumption at the level of sampling that occurred during this study. Furthermore, the belugas targeted for biopsy sampling were selected for ease of sampling when swimming in a directional and predictable pattern. This type of behaviour is probably associated not with foraging but with travelling (Blane 1990), and there might be little physiological costs to changing the duration of the dive cycle in these circumstances.

In summary, the fact that the duration of the dive cycle was found to be longer after a biopsy attempt is likely to have consequences limited in time. It is not likely to affect the survival or the reproductive success of target individuals or group members.

Delayed Biological Risks

1) Number of Sightings Before and After Biopsy Sampling

Four animals sampled in 1994 and 19 sampled in 1995 were selected for this analysis, based on their photo-identification history. The number of sightings per 100 encounters did not diminish the season after the biopsy. In fact, there was a non-significant trend in the other direction : on average, 41 % more sightings per 100 encounters were obtained the season following the biopsy for the 23 monitored animals (t-test for paired samples, $p = 0.0749$). Therefore, this index does not suggest long-term avoidance of the research boat by belugas exposed to biopsy sampling.

The lack of long-term avoidance detected with this index does not imply that there is no long-term effect of biopsy sampling on St. Lawrence belugas. This index was based on the assumption that a beluga would associate the stimuli accompanying biopsy attempts to the presence of the research boat. The lack of long-term avoidance could result from the mild nature of the stimuli or from belugas failing to associate the stimuli with the presence of the boat (Barrett-Lennard et al. 1996). Future research using the photo-identification history of individual belugas will involve a detailed study of habitat use and associations (Michaud 1995). This study could verify that no changes in habitat use or associations occurred as a result of biopsy sampling.

In summary, it is unlikely that there are long-term negative effects on the survival, the reproductive success or the behaviour of belugas resulting from a generalisation of the stimuli involved in biopsy sampling, but more studies should be conducted to confirm this conclusion.

2) Scarring of Sampled Animals

We selected 15 animals for the monitoring of the wound inflicted by biopsy sampling. For the 13 animals that we could monitor during the season of the biopsy, the wound stayed visible for the rest of the season (13 to 60 days). We were able to monitor the wound the seasons following the biopsy for eight animals. For four animals (50 %), the wound was not visible on photographs taken the season following the biopsy. For one individual, the wound was still visible the season following the biopsy, but disappeared during the season due to another mark that masked the biopsy wound. For two animals (25 %), the wound was still visible the season following the biopsy, but was no longer visible the next season. In one case, the dart took a sample through the dorsal ridge and left a notch that will probably be a permanent mark. Notches in the dorsal ridge are a common type of marks in St. Lawrence belugas (R. Michaud, pers. comm.).

In all 15 animals monitored there was no sign of infection or disease associated with the wound. Furthermore, the wound became invisible within the timeframe of natural surface wounds observed on belugas (R. Michaud, pers. comm.) and of biopsy wounds monitored on the bottlenose dolphin, *Tursiops truncatus*, (Weller et al. 1997). St. Lawrence belugas are often seen with wounds and scars that are believed to come from bites by other belugas, rubbing on rocks, or scraping on ice during the winter (R. Michaud, pers. comm.). The wound associated with biopsy sampling is probably less traumatic than these natural wounds, as judged by the size of the wound inflicted and the speed with which the scar became invisible on high-quality photographs (Figure 4).

In summary, it is unlikely that the wound associated with biopsy sampling has an impact on the reproductive success or the survival of sampled individuals.

Biological Benefits

1) Genetic Analyses

Studies pertaining to the genetic variability of the St. Lawrence beluga have already been published, using data from biopsy samples and/or carcasses. Murray et al. (1995) reported that the St. Lawrence beluga population is not different from six Arctic populations when compared at a Major Histocompatibility Complex (MHC) locus. Furthermore, St. Lawrence belugas were not different from the eastern Hudson Bay population when compared for variation in mitochondrial DNA (Brennin et al. 1997). Nevertheless, compared to Beaufort sea belugas, St. Lawrence belugas had a lower genetic variability, as assessed by DNA fingerprinting with 3 minisatellite probes (Patenaude et al. 1994) and by restriction site polymorphism of mitochondrial DNA (White et al. 1991). From these studies, it is clear that different markers give different answers concerning the relative level of genetic variability in the St. Lawrence beluga population, further emphasising the difficulty of using measures of genetic variability to formulate recommendations for the conservation of this population. Furthermore, data from stranded carcasses should provide sufficient information for analyses of genetic variability. St. Lawrence beluga carcasses have been shown to be genetically representative of the living population, at least for two MHC loci (Murray 1997).

The analysis of the Zinc Finger gene allowed certain identification of the sex of sampled individuals. In most cases, it confirmed the presumed sex of the animals, assessed using criteria available to identify the sex of wild free-swimming belugas (Hughes & Pippard 1986). This analysis yielded a strong male bias, with 92 % of the 50 samples analysed at this point being from males (R. Michaud, pers. comm.). This bias can compromise the answers proposed for certain questions. Nevertheless, it should not decrease the delayed biological benefits predicted for the project since this bias has already started being corrected, and since important questions about the social structure of the St. Lawrence beluga can still be addressed with male-biased samples. Increasing biopsy sampling effort towards presumed females can be done by spending more time in areas where groups of presumed females segregate (Michaud 1993). Changes to the protocol might be necessary to increase the hitting rate in these areas (R. Michaud, pers. comm.).

These preliminary results do not change the assessment presented in the application of the decision framework to the case study: genetic analyses are likely to provide important, but not critical, information for the conservation of the St. Lawrence beluga population.

2) Toxicological Analyses

So far, the comparison of the organochlorine contaminant loads between biopsy samples and carcasses has not led to clear interpretations (Béland et al. 1996). Concentrations of contaminants are different in biopsy samples and in carcasses, but these differences do not follow a clear pattern. Observed differences could be linked to the sampling year, sample size, age of sampled individuals, and different dynamics of contaminants in different blubber layers (Béland et al. 1996, and see Lockyer et al. 1984, Aguilar and Borrell 1990). Therefore, it is not yet possible to conclude whether or not carcasses give a good representation of contaminant levels in living animals. Further work on carcasses is projected to tease out confounding variables thought to prevent a direct comparison between data from carcasses and from biopsy samples (P. Béland, pers. comm.).

Toxicological research done with biopsy samples has the potential to contribute important information to the conservation of the St. Lawrence beluga, providing that future research allows direct comparison between data from carcasses and from biopsy samples.

Risk of a Perceived Paradox

Considerations linked to the perception of the resource users were presented in the *a posteriori* application of the framework to this case study. The possibility of a perceived paradox by whale-watch tour operators between the status of the St. Lawrence beluga and the use of an invasive research technique was discussed. This risk could not be assessed formally, but I report here facts that occurred during the four-year study period and that are relevant to this risk.

The St. Lawrence Beluga Recovery Team (1995) noted that the whale-watching industry showed a reduction in voluntary compliance to the code of ethics. A multitude of factors probably caused this situation, and the role of the perceived paradox between the status of the St. Lawrence beluga and the use of an invasive research technique could not be determined within this study. There are indications of tensions between researchers and at least some members of the whale-watching industry, in part due to misconceptions about the role of research in conservation (R. Michaud pers. comm., and personal observation). However, problems related to a potential perceived paradox between research and conservation were not discussed by representatives of the whale-watching industry in a workshop on the management of the whale-watching activities in the spring of 1998 (Gilbert & Saguenay-St. Lawrence Marine Park 1998). Therefore, research has potential indirect risks if it contributes to increase exposure of belugas to whale-watching activities; the potential perceived paradox does not seem to play a major role, however, in the attitude of the whale-watching industry towards belugas.

Recommendations and Conclusions

Overall, the risks of biopsy sampling St. Lawrence belugas can be evaluated as "low" (see definitions above). None of the indices I studied reveal potential risks of reduced reproductive success or survival, or of long-term behavioural changes for St. Lawrence belugas as a result of biopsy sampling. Furthermore, the potential perceived paradox does not seem to play a major role in the attitude of the whale-watching industry towards belugas. This does not mean that there are no effects of biopsy sampling on St. Lawrence belugas. However, we can conclude that the biological risks were limited to short-term effects on individuals. This confirms the conclusions of studies that looked at the effects of biopsy sampling on other cetacean species (Whitehead et al. 1990, Brown et al 1991, Weinrich et al. 1991, Clapham & Mattila 1993, Brown et al. 1994, Barrett-Lennard et al. 1996,Weller et al. 1997). The evaluation of the biological risks to belugas included hits and/or missed attempts on presumed or confirmed females. There were no indications that the risks were different for males and for females.

The benefits of biopsy sampling St. Lawrence belugas were evaluated as "medium" using a literature review (see definitions above), and preliminary results from the genetic and the toxicological analyses did not change the evaluation presented in the *a posteriori* application of the framework.

In conclusion, the monitoring programme confirms the pre-assessment of the levels of risks and benefits of biopsy sampling St. Lawrence belugas. This conclusion is valid for the conditions at the time of the study. The following recommendations are critical for the benefits to continue to outweigh the risks until the completion of the research linked to biopsy sampling. The research team should :

1) Continue applying the procedures to minimise the potential risks associated with biopsy sampling (see Table 3).
2) Continue improving hit rate, since my study suggests that missed attempts have similar immediate potential risks compared to actual hits ("crash diving" and increased duration of the dive cycle); missed attempts, however, do not contribute to the biological benefits, i.e. do not provide samples for analysis. Furthermore, constant improvement of the success rate of the technique could help prevent potential problems with the resource users.
3) Confirm the absence of long-term effects of biopsy sampling on habitat use and associations among individuals.
4) Consider maintaining the monitoring programme, especially if there are changes to the sampling protocol in an effort to obtain more samples from

females. The male bias in the current biopsy data could for instance be caused by a deliberate attempt on the part of females, particularly when they are accompanied by offspring, to avoid approaching boats; if this is the case, an increased research and pursuit effort selectively directed at females could possibly increase stress levels on them.

5) Re-evaluate every year the objectives of the projects using biopsy samples, as well as the progress in sample collection, in order to plan the minimum number of samples for answering research questions, and ensure that the questions continue to address important issues for the conservation of the St. Lawrence beluga.
6) Resolve the ambiguity in the interpretation of the toxicological analyses of the blubber samples obtained from living animals.
7) Produce work of publishable quality so that data obtained from samples will be available to the widest possible biological community.
8) Formulate clear and scientifically sound recommendations for the conservation of the St. Lawrence beluga.
9) Continue being active in the recovery plan of the St. Lawrence beluga.
10) Maintain good communication with the resource users, i.e. whale-watch tour operators (captains and crews, owners, and naturalists).

Future research projects on this population of beluga should adhere to the formal decision framework presented in this study. Furthermore, the recommendations resulting from the application of the decision framework to the case study of biopsy sampling St. Lawrence belugas may have a general applicability to all research done on this population. The recommendations in this thesis should be considered, adapted if necessary, and followed for all projects using invasive research techniques to study the St. Lawrence beluga population.

General Conclusions

The framework presented in this study proved to be a useful tool for the evaluation of an invasive research technique on a protected population. We believe it can be easily generalised to other case studies especially since there exists no formal decision framework for this problem in conservation biology. Our decision framework can help identify the reasons for choosing an invasive technique, as well as other potentially sensitive variables in the proposed research project. It is a desirable exercise to prepare when a project is presented to funding agencies, animal care committees, and stakeholders involved in the conservation of the protected animal species or population.

The framework does not eliminate the inherent subjectivity of the decision-making process, but it helps clarify the issues and organise discussions among researchers, policy makers, resource users, and other stakeholders. Subjectivity plays a role at three points in the framework : 1) the evaluation of the technique as invasive or non-invasive, 2) the evaluation of the risks as "low", "medium", or "high", and 3) the evaluation of the benefits as "low", "medium", or "high". Nevertheless, the framework minimises subjectivity at the crucial stage when risks and benefits are weighed to decide on a course of action.

Partly due to the subjective aspect of the evaluation, it would be important to have a multidisciplinary team discuss the validity of the project, using the formal framework; this would at the very least ensure that all important issues have been considered.

Our proposed framework did not take into considerations the possibility that alternative research projects could be proposed, nor the frequent need to choose between different options because of limited financial resources. Although having to choose between options is a reality in conservation biology (Maguire 1997), funding is rarely transferable from one project to another. Nevertheless, our framework could be adapted to choose between projects when financial resources are limited, balancing the evaluation of the risks and benefits of the different projects with their relative financial implications.

The application of the decision framework to a case study was a good test of its usefulness. The framework formalised the steps towards decision-making. It allowed a clear listing of the important risks and benefits of an invasive technique used on a protected animal species (or population). We recommend that scientists studying protected animal species (or populations) use this framework to help avoid conflicts between their research and conservation.

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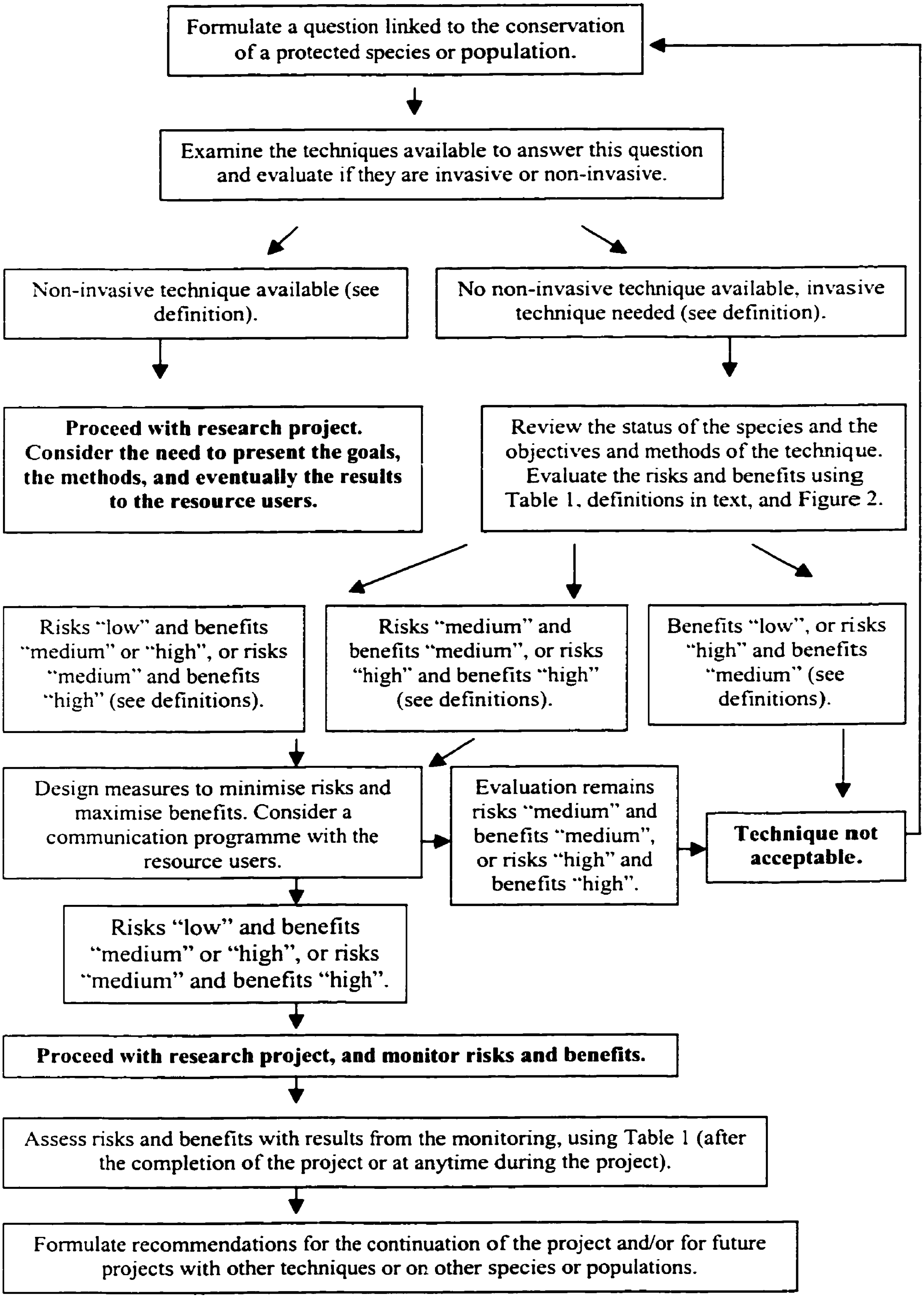
Figure 1. Flow chart to guide the decision of whether or not a research project on a protected animal species or population should proceed.


Formulate a question linked to the conservation of a protected species or population.
Examine the techniques available to answer this question and evaluate if they are invasive or non-invasive.
Non-invasive technique available (see definition).
No non-invasive technique available, invasive technique needed (see definition).
Proceed with research project. Consider the need to present the goals, the methods, and eventually the results to the resource users.
Review the status of the species and the objectives and methods of the technique. Evaluate the risks and benefits using Table 1, definitions in text, and Figure 2.
Risks "low" and benefits "medium" or "high", or risks "medium" and benefits "high" (see definitions).
Risks "medium" and benefits "medium", or risks "high" and benefits "high" (see definitions).
Benefits "low", or risks "high" and benefits "medium" (see definitions).
Design measures to minimise risks and maximise benefits. Consider a communication programme with the resource users.
Evaluation remains risks "medium" and benefits "medium", or risks "high" and benefits "high".
Technique not acceptable.
Risks "low" and benefits "medium" or "high", or risks "medium" and benefits "high".
Proceed with research project, and monitor risks and benefits.
Assess risks and benefits with results from the monitoring, using Table 1 (after the completion of the project or at anytime during the project).
Formulate recommendations for the continuation of the project and/or for future projects with other techniques or on other species or populations.

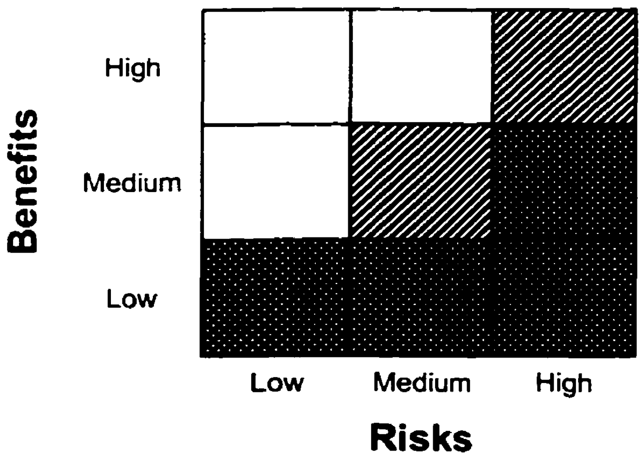


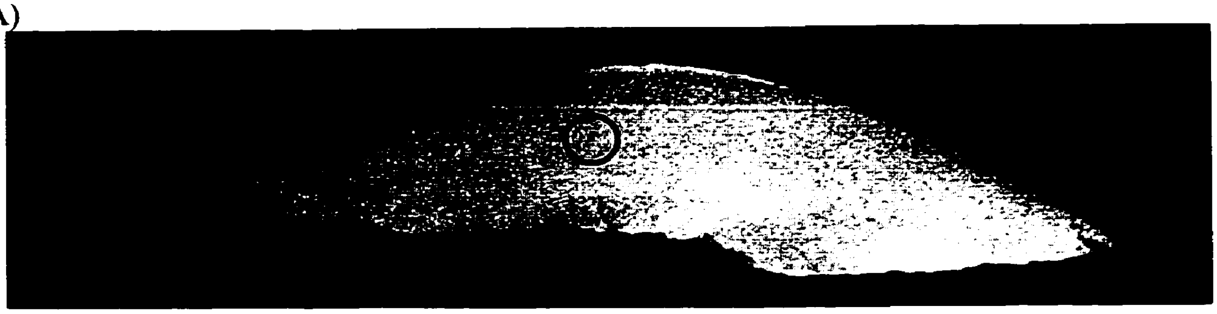
Figure 2. Chart relating the different levels of risks and benefits and coding the recommended course of action. White: design measures to minimise risks and maximise benefits, then proceed with research, and monitor the risks and benefits, hatched: re-evaluate risks and benefits after designing measures to minimise risks and maximise benefits, double-hatched: technique not acceptable.

Figure 3. Summer distribution of the St. Lawrence beluga, with the limits of the Saguenay-St. Lawrence marine park included (adapted from Michaud 1993 by Michel Moisan).


ST. LAWRENCE
Ile aux Coudres

Figure 4. Photographs showing the evolution of scars resulting from A) biopsy sampling and B) natural surface wounds. The arrows and circles indentify the wound. The last photograph of each series is the first photograph where the wound is no longer visible.

A)



3 Aug. 1995

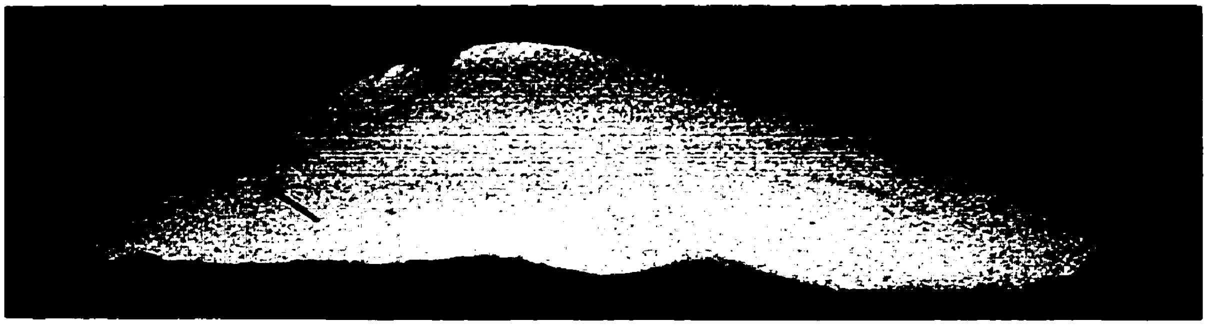
4 Aug. 1995

2 Oct. 1995

22 May 1996

B)

24 Jul. 1995



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13 Jun. 1996

Table 1. The risks and benefits of using an invasive technique to study protected animal species and populations. The evaluation should be preceded by a review of the status of the species as well as the objectives and the methods of the research using the invasive technique under evaluation. It supposes non-invasive techniques have been explored. The risks should be evaluated with the precautionary principle in mind. The evaluation of overall risks and overall benefits follows the definitions presented in the text.

Considerations	*Risks*	*Benefits*
Immediate Biological (behavioural and physiological)	Disturbance Injury	None
Delayed Biological (behavioural and physiological)	Generalisation learning Disease, infection	Knowledge can lead to more adequate conservation measures (effects on reproductive success and/or survival). The evaluation of these benefits must include the evaluation of the quality of the research as well as its applicability to conservation.
Others	Perceived paradox between conservation status and the use of an invasive technique could affect the actions of the stakeholders (resource users, policy makers, protection agencies, general public, etc.)	Research efforts and new knowledge can help raise awareness. Could be an important factor in certain conservation plans.

Table 2. Biopsy attempts from the four field seasons

Year	*Successful biopsies*	*Missed attempts*	*Hits without samples* *	*Total*	*% hitting success*	*% sample yield success*
1997	17	13	0	30	57	100
1996	24	21	5	50	58	83
1995	22	19	2	43	56	92
1994	4	9	5	18	50	44
Total	67	62	12	141	56	85

* *No sample either because the dart did not penetrate the skin or because the arrow was lost*

Table 3. Measures applied between 1994 and 1998 to minimise risks and maximise benefits of biopsy sampling St. Lawrence belugas.

Considerations	*Measures minimising risks*	*Measures maximising benefits*
Immediate Biological (behavioural and physiological)	**Disturbance** : slow, parallel approaches; groups with calves avoided; use of tethered line abandoned after the pilot project, following recommendations in the literature (e.g. Barrett-Lennard et al. 1996). **Injury** : dart, bolt, and crossbow chosen to minimise injury, following work by Patenaude and White (1995). Slight modifications to the sampling equipment allowed to reduce the weight of bolts and increase their accuracy.	N/A
Delayed Biological (behavioural and physiological)	**Generalisation mechanisms** : none **Disease, infection** : darts sterilised in autoclave.	**Increase likelihood of applicability to conservation :** 1) High standards for the quality of science. 2) Multiple uses of the sample obtained. 3) The principal investigator is a member of the St. Lawrence Beluga Recovery Committee.
Others	**Reduction of the perceived paradox** : nothing specific to the biopsy programme was done involving the resource users. The resource users (whale-watch tour operators and naturalists) are regularly invited by the GREMM to presentations and discussions about their research.	**Raising awareness** : integration of the biopsy programme (objectives and methods) in the education programme of the CIMM and in the documentary *Encounters with whales*.

Table 4. Immediate reaction of six cetacean species to biopsy attempts. Reactions were startle responses.

Species	*Location*	*Reaction to successful biopsies and hits, % (n)*	*Reaction to missed attempts, % (n)*	*Reaction of group members to biopsy attempts, % (n)*	*Reference*
Megaptera novaeangliae (humpback whale)	South-West Pacific (East Australia)	41 (203)	16 (77)	n. a. *	Brown et al. 1994
Megaptera novaeangliae (humpback whale)	South-East Atlantic (Dominican Republic)	56 (565)	12 (427)	n. a. *	Clapham and Mattila 1993
Megaptera novaeangliae (humpback whale)	North-East Atlantic (Gulf of Maine)	89 (118)	36 (28)	n. a. *	Weinrich et al. 1991
Eubalaena glacialis (right whale)	North-East Atlantic (Bay of Fundy-Scotian Shelf)	19 (206)	3 (89)	n. a. *	Brown et al. 1991
Physeter macrocephalus (sperm whale)	North-East Atlantic (Nova Scotia)	100 (8)	55 (11)	n. a. *	Whitehead et al. 1990
Orcinus orca (killer whale)	North-East Pacific (British Columbia)	81(72)	53 (19)	1 (91)	Barrett-Lennard et al. 1996
Tursiops truncatus (bottlenose dolphin)	Gulf of Mexico (Galveston Bay)	100 (8)	40 (5)	n. a. *	Weller et al. 1997
Delphinapterus leucas (beluga)	St. Lawrence River and Saguenay fjord (Quebec)	100 (79)	95 (62)	88 (98)	This study

* *Either the sampled animals were solitary or the reaction of the group was not systematically reported.*