Life History of Small Cetaceans in the Northwest Atlantic

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Introduction

This report describes results from an integrated program of research into the life history of small cetaceans in the Northwest Atlantic Ocean. This was a multi-year, multi-investigator research effort directed at improving our understanding of the biology of porpoises, dolphins and small whales killed incidentally in commercial fishing operations within the U.S. Exclusive Economic Zone (EEZ).

This research forms an important part of efforts directed to better understand the impact of removals of small cetaceans by commercial fisheries. Most of our efforts were directed at the species most affected by these removals in the Northwest Atlantic, the harbour porpoise *Phocoena phocoena*. In addition, our work has also involved strategic stocks, including long-finned pilot whales *Globicephala melas*, common dolphins *Delphinus* sp., and beaked whales *Mesoplodon* spp. The various components of this project address different aspects of the biology of these species, but all are interrelated and interdependent. This approach has resulted in productive collaborations among the Principal Investigators involved in the project. Results of the various research components are appended separately, usually in the form of papers published by their respective Principal Investigator.

Component 1: Collection of Life History Samples

Andrew Read
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The goal of this component was to collect life history data from small cetaceans, with particular emphasis on the harbour porpoise and pelagic cetaceans taken in the Atlantic swordfish drift net fishery. Whenever possible, these tissues were obtained during necropsy workshops, in which many researchers participated (see Nicolas 1993). These workshops have proven particularly valuable to participating scientists and students, and ensure that the maximum amount of scientific benefit is obtained from each specimen. We now have a large data set of tissues, measurements and observations on the life history of these animals and are actively working on the analysis and interpretation of this material.

Component 2 : Analysis of Vital Rate Parameters

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The goal of this component was to supplement information on the age structure and reproductive biology of harbor porpoises obtained in previous iterations of this Co-operative Agreement (Read and Hohn 1995). In particular, we attempted to estimate uncertainty surrounding estimates of potential rate of

increase for the Gulf of Maine stock of harbour porpoises. A paper published in *Ecological Applications* resulting from this research effort is included as Appendix 1. In addition, we described the reproductive biology of harbour porpoises in a paper published in the *Journal of Zoology, London* and included as Appendix 2.

Component 3: Analysis of Small Cetacean Diets

James Craddock and Pam Polloni
Woods Hole Oceanographic Institution

The goal of this component was to continue our examination of the diets of small cetaceans killed in commercial fisheries. Our analysis of small cetacean diets has continued to focus on harbor porpoises and various species taken in the pelagic drift net fishery for swordfish, focusing on beaked whales and common dolphins. We concluded our analysis of harbour porpoise stomachs from the southern Gulf of Maine in autumn (September through December) and published these findings in the journal *Fishery Bulletin* (see Appendix 3). We are continuing to analyze the diets of common dolphins and beaked whales.

Component 4: Genetic Analysis of Population Structure

Patricia Rosel
College of Charleston

The goal of this component was to collect genetic data relevant to stock structure for harbour porpoises in the North Atlantic. Specifically, we examined the population structure of this species in the Northwest Atlantic using mitochondrial DNA sequences and nuclear microsatellite data. Samples from four previously proposed summer breeding populations (Gulf of Maine, Gulf of St. Lawrence, Newfoundland and western Greenland) and one wintering area (mid-Atlantic states) were used in this analysis. A full description of the results of this work is included in a paper published in the journal *Molecular Ecology* and included as Appendix 4 to this report.

Component 5: Demographic and Morphometric Analysis Of Pilot Whale Data

Solange Brault
UMass Boston

Research in this component focused on the demography of North Atlantic long-finned pilot whales, using data from the Faroes Islands to derive life history parameter values. We conducted an analysis of the effect of pod size on vital rates, based on the observation that the rate of increase of large

pods tends to be lower than that of small pods. We obtained a significant negative effect of pod size on observed pregnancy rate. Effects on other vital rates, such as survival of juveniles, were also observed. An oral presentation of this analysis was made at the annual meeting of the Ecological Society of America (August 1997, Albuquerque, NM). We have modified the population model from Sanders-Reed's Masters thesis to include the pod size effects. We present evidence of pod formation by one or a few females, which are not necessarily siblings. Pod size can be predicted by the age of the oldest female in the pod, and by the age gap between this and the next oldest female. Pod size tends to increase exponentially with age of the oldest female; it is larger if the second oldest is closer in age to the oldest. This is best explained by a simple demographic from one or a set of female founders, at a rate of about 5%. Pods do not increase forever, but appear to split as old females die off. We also worked, in collaboration with Dr. T. Smith of NEFSC and Dorete Bloch of the Faroe Islands Museum of Natural History, on a theoretical model of pod dynamics based on the above results and the long-term pilot whales catch data from the Faroe Islands. We re-examined the fetus length data from the Faroese pilot whale data set, to better understand why we could not obtain a fetal mortality estimate. In particular, we checked the assumption of equal sampling of all fetal size classes. We have found that small fetuses are absent or rare in samples from large pods (> 100 individuals), compared with those from small pods sampled during the same period of the year. It appears that, because of the limited time window available for sampling during a pilot whale drive, some of the smaller fetuses are overlooked. This produced a lower frequency than expected of small fetuses, which caused, at least partially, the mortality estimation failure. Such a bias in the sampling procedure also lowers the pregnancy rate, and may have distorted sex-ratio analysis.

Component 6: Evaluating seasonal movements of harbor porpoises

Andrew Read and Andrew Westgate
Duke University Marine Laboratory

This research involves monitoring the seasonal migrations of harbor porpoises by tracking the movements of individual animals with satellite-linked transmitters. Information on the long-term movements of harbor porpoises off the northeast U.S. coast is required for the assessment and mitigation of porpoise by-catch in the domestic sink gillnet fishery in the Gulf of Maine. This information is necessary to evaluate the potential for reducing by-catches by instituting time-area closures of the fishery; such management measures require knowledge of the pattern and variability of porpoise migration patterns in the Gulf of Maine. Such information is also critical because of continuing uncertainty about the stock structure of harbor porpoises off the east coast of North America. Monitoring long-term movements of individual porpoises will help to resolve two outstanding assessment questions: (1) do porpoises in the Gulf of Maine form a functional population unit during the summer months? and (2) do porpoises from the Gulf

of Maine mix with animals from other stocks during the winter months? The results of this work are contained in a manuscript published in the journal *Marine Biology* (Appendix 5).

Component 7: Behavior of Harbor Porpoises around Gill Nets

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Little is known about how harbour porpoises acquire their prey, and consequently when and where they are vulnerable to entanglement in bottom-set gill nets. The development of satellite-linked depth recorders (SDRs) has made it possible to collect detailed diving behavioural data from wild odontocetes. We attached three SDRs to harbour porpoises released herring weirs in the Bay of Fundy during the period covered by this Co-operative Agreement. We are continuing to collect data from SDR deployments and once a sufficient sample of animals has been tagged, we will analyze and publish the results of this work.

Component 8: Evaluating the Potential for Habituation of Harbor Porpoises to Pingers

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Acoustic alarms, or 'pingers' have been proven to reduce the bycatch of harbor porpoises in experiments conducted with the Gulf of Maine sink gill net fishery. Although the results of research trials and initial implementation have been promising, reservations to the widespread use of pingers still exist within the scientific community. Of particular concern is the possibility that the effectiveness of pingers will decrease over time as porpoises become habituated to the sounds of the devices. As part of its Consensus Plan, the Gulf of Maine Take Reduction Team recommended that research should be conducted on the potential for porpoise habituation to pingers. We conducted research to address the question of habituation in three ways, as described in a manuscript recently submitted for publication in the *Canadian Journal of Fisheries & Aquatic Sciences* (Appendix 6).

Life History of Small Cetaceans in the Northwest Atlantic

Appendix 1

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HARBOR PORPOISE AND FISHERIES: AN UNCERTAINTY ANALYSIS OF INCIDENTAL MORTALITY

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Abstract. The harbor porpoise (*Phocoena phocoena*) in the western North Atlantic is subject to mortality due to entanglement in gillnets. Such incidental mortality threatens a population if it is too large relative to the potential population growth rate. Critical values for incidental mortality have been established by the International Whaling Commission and the U.S. Marine Mammal Protection Act. As in many situations in conservation biology, use of these critical values depends on demographic calculations that are based on uncertain data. It is important to report not only estimates of demographic parameters, but also the uncertainty in those estimates. Here, we use a Monte Carlo approach to evaluate uncertainty in population size, incidental mortality, and population growth rate of harbor porpoise. To describe survival, we used model life tables derived from other mammals with similar life histories. By randomly sampling the space of model life tables and the distributions of estimated fertility and age at first reproduction, we produced a probability distribution that characterizes the uncertainty in the potential population growth rate. The median estimate for the potential annual rate of increase λ is approximately 1.10. Combining this information with the uncertainty of incidental mortality and of population size, we estimate the probability that the rate of incidental mortality exceeds the critical values established by the various management agencies; this probability ranges from 0.46 to 0.94. We conclude that recent incidental mortality rates are a threat to harbor porpoise populations. The methods developed here are applicable to other situations in which demographic analyses must be based on uncertain data.

Key words: conservation biology; harbor porpoise; incidental mortality; Marine Mammal Protection Act; matrix population models; Monte Carlo methods; Phocoena phocoena; *population growth rate; uncertainty analysis.*

INTRODUCTION

Conservation biologists often need estimates of demographic statistics for endangered or threatened species, in order to provide advice to managers and policy makers. Such statistics include population size, mortality rates, rates of increase, sensitivities and elasticities of rates of increase, and extinction probabilities (e.g., Crouse et al. 1987, Dennis et al. 1991, Crowder et al. 1994, Doak et al. 1994, Heppell et al. 1994, Fiedler and Kareiva 1998). They must be computed from data that are always uncertain, sometimes extremely so. This uncertainty in the demographic data translates into uncertainty in the estimates of population statistics. However, because of the complex calculations involved in transforming the basic data into demographic statistics, it can be difficult to estimate that uncertainty. In some cases, the data must be supplemented with estimates extrapolated from other species or drawn from the literature or expert opinion, making it even more difficult to quantify the uncertainty.

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The situation of the harbor porpoise (*Phocoena phocoena*) in the western North Atlantic is such a case. The harbor porpoise is a small (45–70 kg) cetacean, found in shallow, coastal waters of the temperate to subarctic Northern Hemisphere. In the western North Atlantic, it occurs from Labrador south to North Carolina. It is listed as a threatened species in Canada, and a petition was filed in 1993 to list it as threatened under the U.S. Endangered Species Act (Federal Register 58: 3108–3120). The Gulf of Maine and Bay of Fundy stock has been classified as ''strategic'' by the National Marine Fisheries Service, under the provisions of the Marine Mammal Protection Act (Blaylock et al. 1995, Waring et al. 1997).

Harbor porpoise are subject to incidental mortality from entanglement in sink gillnets. Whether or not this incidental mortality is a threat to the population depends on its magnitude relative to the potential rate of increase (i.e., the population growth rate at low densities). Incidental mortality that exceeds the potential rate of increase will, in the long run, drive a population to extinction. Prudence suggests that incidental mortality should be kept below some critical value, which is less than the potential rate of increase. Both the

International Whaling Commission (IWC) and the Marine Mammal Protection Act have established such critical values. In the case of the Marine Mammal Protection Act, incidental mortality in excess of the critical value has immediate management consequences.

Determining whether incidental mortality exceeds a specific critical value requires estimates of three quantities: population size, the number of animals taken as by-catch, and the potential rate of increase of the population. Each of these quantities is known only with uncertainty, and our goal is to assess how this uncertainty affects the final conclusions. In doing so, we will go considerably beyond the provisions of the Marine Mammal Protection Act, which does not consider the uncertainty in these quantities.

Approaches to measuring uncertainty

Uncertainty and its measurement are important to scientists, managers, and policy makers. Attempts to legislate the reporting of uncertainty in risk assessments are increasingly frequent (Davies 1995). For an example from the United States, see the Department of Energy Risk Management Act of 1995 (S. 333), which would have required the inclusion of ''an array of multiple estimates (showing the distribution of estimates and the best estimate) based on assumptions, inferences, or models which are equally plausible, given current scientific understanding'' in any risk assessment document.

It is an obvious mistake to ignore the uncertainty of an estimate, especially if that estimate has management implications. It is equally a mistake to use the mere existence of uncertainty as an excuse to avoid management action (for examples from the history of marine fisheries, see Smith 1994). To avoid such mistakes, it is important to quantify and document the uncertainty, and to take it into account in making policy.

Approaches to estimating uncertainty fall, more or less, into three categories. First, there are the statistically easy cases. If the quantity being calculated is simple enough and is based on samples from a known distribution, then classical statistical theory provides methods for computing standard errors and confidence intervals. Second, there are cases in which standard errors and confidence intervals can be computed by bootstrap resampling methods (Efron and Tibshirani 1993). These require no assumptions about sampling distributions, but do require that the statistic be based on a sample of some well-defined units that can be resampled to generate the bootstrap distribution.

The harbor porpoise problem falls in a third category. Even the bootstrap does not apply, because the data are fragmentary, from different sources and of different types, or are not obtained from well-defined samples at all. Such problems can be attacked using Monte Carlo methods, if the investigator can specify statistical distributions that characterize the uncertainty in each of the parameters of the problem.

The essence of this approach is as follows. Suppose that X is a random variable with a probability distribution $P_X(x)$, and that we are interested in another random variable $Y = f(X)$, which is a function of X. What is the distribution $P_Y(y)$ characterizing the uncertainty in Y?

If the distribution P_X and the function $f(X)$ are simple enough, the distribution P_Y may be calculated directly (e.g., any linear transformation of a normal random variable is also normally distributed). The Monte Carlo approach, which is independent of the complexity of P_X or $f(X)$, begins by randomly generating many values of X, by sampling from P_X. The corresponding values of Y are calculated. The empirical distribution of these values will, with probability one, converge to the true distribution of P_Y as the number of samples becomes large. Alternatively, if X takes only a few discrete values, sampling is not necessary, and a probability tree can be constructed showing the result of each possible value (e.g., Maguire et al. 1987).

In the present context, the random variable X is an estimate of some quantity (e.g., population growth rate). Its probability distribution reflects the uncertainty in the estimate. Given lots of high-quality data, P_X will be concentrated at one value of X. If the data are scarce or of poor quality, P_X will have a much broader distribution. The Monte Carlo procedure shows how this uncertainty is transmitted to the estimate of Y. For general discussions of uncertainty analysis, see Cox and Baybutt (1981) and Morgan and Henrion (1988); Hertz (1964) is a particularly early description. Although Monte Carlo uncertainty analysis has been applied to ecosystem models (Gardner et al. 1981, O'Neill et al. 1982, 1983, Suter 1993), it has only occasionally been used in demographic calculations (e.g., Goodman 1984, Barnthouse et al. 1990, Ragen 1995, Powell et al. 1996).

In this paper, we will use Monte Carlo methods to calculate uncertainty in the potential population growth rate for harbor porpoise, and to compare the rate of incidental mortality to the critical values specified by different management bodies, in a manner that accounts for the aggregate uncertainty.

The Harbor Porpoise Problem

By-catch and incidental mortality

Harbor porpoises in the Gulf of Maine and Bay of Fundy are believed to form a relatively discrete population unit that can be managed as a separate stock (Blaylock et al. 1995, Waring et al. 1997). Estimates of the number of harbor porpoises taken annually as by-catch in the U.S. sink gillnet fishery in the Gulf of Maine between 1990 and 1993 range from 1200 to 2900 porpoises/yr (Bravington and Bisack 1996; see Table 1). Trippel et al. (1996) estimate that 424 and 101 harbor porpoises were taken as by-catch in the Bay of Fundy in Canada, in 1993 and 1994, respectively.

TABLE 1. Estimated number of harbor porpoises killed as by-catch in the Gulf of Maine, with 95% bootstrap confidence intervals (from Bravington and Bisack 1996).

Year	By-catch	Confidence interval
1990	2900	1500–5500
1991	2000	1000–3800
1992	1200	800–1700
1993	1400	1000–2000

To transform these estimates of by-catch into incidental mortality rates, they must be divided by an estimate of population size. Sighting surveys using line transect methodology, conducted by the National Marine Fisheries Service in the summer of 1991 and 1992, yield an estimated total population size of 47 200 porpoises for the Gulf of Maine and Bay of Fundy (95% bootstrap confidence interval 32 500–70 600 porpoises; Smith et al. 1993, Palka 1995).

Policy implications of incidental mortality: how much is too much?

Dividing a total by-catch of about 2300 by a population size of about 47 200 yields an incidental mortality rate of approximately 5% per year. Is this cause for concern? It would be, of course, if it exceeded the potential rate of increase; this would eventually drive the population extinct. To be on the cautious side, however, incidental mortality should be maintained below some critical value strictly less than the potential rate of increase. In 1991, the Scientific Committee of the International Whaling Commission recommended that incidental mortality should not exceed half of the potential rate of increase (IWC 1991). In 1995, it added a recommendation that harvest and incidental mortality greater than one-fourth of the potential rate of increase should be considered cause for concern (IWC 1996).

Management of marine mammals in the United States (under the 1994 amendments to the Marine Mammal Protection Act) is based on the calculation of a ''Potential Biological Removal.'' The Potential Biological Removal is the maximum number of animals that can be removed from a population while still allowing it to remain at, or recover to, its ''Optimum Sustainable Population'' size, which ranges from the largest supportable population down to the population size maximizing net productivity (Barlow et al. 1995, Wade 1998). The Potential Biological Removal is the product of three quantities: one-half of the potential rate of increase, a minimum estimate of population size, and a ''recovery factor'' that ranges from 0.1 to 1.0. The minimum population size is defined as the 20th percentile of its sampling distribution, assumed to be lognormal. It is calculated as a function of the best estimate and its coefficient of variation (Wade 1998). The recovery factor reflects that status of the stock and the perceived quality of the data. A recovery factor of 0.1 is used for stocks classified as endangered or threatened under the Endangered Species Act. For other stocks, the recovery factor reflects uncertainty; the more uncertain the information about the stock, the smaller the recovery factor.

If the number of animals killed exceeds the Potential Biological Removal, the stock is classified as ''strategic.'' This classification has immediate management consequences; the Secretary of Commerce is required by law to prepare a take reduction plan, which will, within 6 mo of its implementation, reduce incidental mortality to a level judged to be compatible with recovery of the population. The plan must reduce mortality within 5 yr to ''insignificant levels approaching zero mortality.''

The Potential Biological Removal represents a removal from the population that is considered safe from a management perspective. If it is converted to a mortality rate by dividing by the population size, the Marine Mammal Protection Act requirements can be rephrased: the incidental mortality rate must not exceed the product of one-half the rate of increase, the recovery factor, and the ratio of the 20th percentile of the population size distribution to the population size estimate. In the first stock assessment conducted under the ammended Marine Mammal Protection Act, Blaylock et al. (1995) set the recovery factor for the harbor porpoise at 0.5 and the 20th percentile of the population size distribution at 0.854 of the estimated population. Thus, the critical mortality rate under that implementation of the Marine Mammal Protection Act (MMPA) is 0.2134 times the potential rate of increase. (A second stock assessment has recently been reported [Waring et al. 1997] in which the minimum population size is 0.89 of the best estimate. The recovery factor is unchanged at 0.5; this leads to a critical mortality rate of 0.22R, and would not noticeably change our conclusions.)

Thus, there exists a sequence of critical values for the incidental mortality rate. Let λ be the annual rate of increase, and define $R = \lambda - 1$ as the amount by which the population increases each year. Then the various critical values are:

Maximum possible without extinction	R
IWC 1991	$0.5R$
IWC 1995	$0.25R$
MMPA	$0.213R$.

In the next section, we estimate the potential rate of increase for the harbor porpoise, and, more important, the uncertainty of that estimate. We then combine that with the uncertainty in the estimates of by-catch and of population size to compute the probability that incidental mortality exceeds the critical values set by the various management agencies.

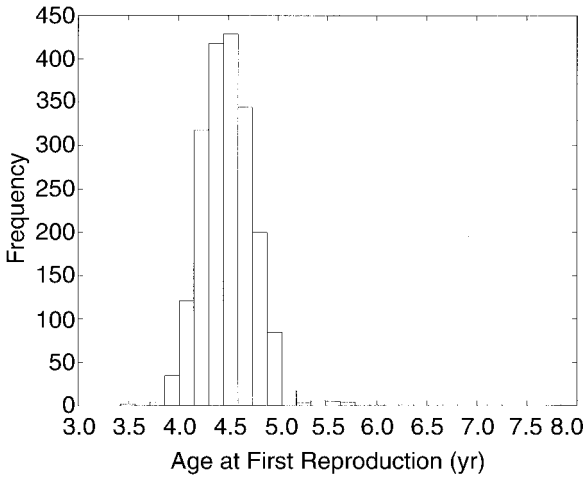

FIG. 1. The frequency distribution of age at first reproduction (AFR) for the harbor porpoise. The distribution was obtained by bootstrap sampling of the maturity data from Read (1990: Table 2). Weighted logistic regression was applied to each bootstrap sample to estimate the median age at maturity, and one year was added to obtain AFR. Sample size is 2000.

HARBOR PORPOISE VITAL RATES AND THEIR UNCERTAINTY

The harbor porpoise is one of the smallest cetaceans, and has evolved a life history that features early reproduction and relatively high fecundity (Read 1990, Read and Hohn 1995). In the Gulf of Maine, most females reach sexual maturity at three years of age and reproduce annually thereafter. Females bear a single calf in May and ovulate and conceive in late June; thus, they are simultaneously lactating and pregnant for much of the year. This intensive reproductive schedule is accompanied by relatively high rates of mortality; the oldest animals found among ~600 specimens taken in herring weirs and gillnets in the Gulf of Maine and Bay of Fundy were 17 yr old (Read and Hohn 1995).

Information on harbor porpoise demography is extremely limited. Estimates of ages and pregnancy rates are available from samples of animals killed in fisheries and from strandings, but there are essentially no data on survival.

Two previous studies have attempted to estimate the rate of increase (Barlow and Boveng 1991, Woodley and Read 1991). In the absence of data on survival, both relied on model life tables (Coale and Demeny 1966); i.e., they used life tables for other species, adapted in ways that would plausibly make them fit the harbor porpoise. Barlow and Boveng (1991) fitted a five-parameter model (Siler 1979) to survivorship curves for fur seals, old-world monkeys, and humans. They rescaled these curves by estimates of maximum longevity (operationally defined as the 99th percentile of the observed age distribution) for the harbor porpoise, and combined them with estimated reproductive rates to calculate the rate of increase λ. They obtained values of $\lambda = 0.917$, 0.989, and 1.094 for survivorship curves derived from fur seals, old-world monkeys, and humans, respectively.

Woodley and Read (1991) used a survivorship curve for the Himalayan thar (*Hemitragus jemlahicus*) as a model life table for harbor porpoise. The life histories of thar and harbor porpoises were considered similar enough that no rescaling was done. The resulting value of λ depended on the value assumed for the calf mortality rate; they found $\lambda \approx 1.04$, using the minimum calf mortality rate considered realistic.

In this study, we have also used a model life table approach, but have set more rigorous standards for how similar to the harbor porpoise a species must be to be included. We have also used more species as models, enabling us to use Monte Carlo sampling to estimate the uncertainty in this crude estimate of survival, and to combine it with the uncertainty in the other vital rates.

Maturity and fertility

The age at first reproduction (AFR) is an important quantity in our calculations, because we use it to adjust the time scale of the life cycles of other species to match that of the harbor porpoise. There are several ways of estimating age at first reproduction (DeMaster 1984). Of these, we chose to estimate age at sexual maturity as the age at which 50% of the population has matured, and AFR as the age at sexual maturity plus an approximate gestation period.

We estimated age at sexual maturity by fitting a logistic regression to the proportion $p(x)$ of individuals mature at age x:

$$\frac{p(x)}{1 - p(x)} = \exp(b_1 + b_2 x). \quad (1)$$

We estimated b_1 and b_2 by weighted least squares (Chatterjee and Price 1977: 141). The age at sexual maturity is then given by $-b_1/b_2$.

Read (1990: Table 2) reports the proportion of individuals that are mature, for ages 1–7 yr. We added 0.5 to the ages (which start at 0) to get the midpoint of the age class, and estimated b_1 and b_2 by weighted least squares. The resulting values ($b_1 = -4.7018$, $b_2 = 1.3570$) give an estimated age at sexual maturity of 3.46 yr, which agrees well with the value of 3.44 yr reported by Read (1990).

We estimated the uncertainty in our estimate of age at sexual maturity by bootstrapping the data on maturity. The sample at age x has $n_i(x)$ immature and $n_m(x)$ mature individuals in a total sample of $N(x) = n_i(x) + n_m(x)$. For each age x, we drew a bootstrap sample of size $N(x)$, with replacement, from the observed sample. We fit the logistic regression to this bootstrap sample and calculated the resulting age at sexual maturity, and repeated this for 2000 samples. Adding the gestation period (~1 yr) gives the distribution of AFR values shown in Fig. 1.

TABLE 2. Age-specific birth rate m_x for the harbor porpoise, obtained from data of Read (1990) on the age-specific pregnancy rate of animals taken as by-catch in the Gulf of Maine.

Age (x)	m_x	N
1	0	19
2	0	2
3	0.136	22
4	0.417	12
5	0.818	11
6	0.714	7
≥7	0.833	6

Read (1990) also reported age-specific pregnancy rates and sample sizes for harbor porpoises killed as by-catch in the Gulf of Maine. We divided these rates by two to estimate birth rate (m_x) one year later (Table 2). This assumes a sex ratio of unity, and that all observed pregnancies are carried to term. We describe the uncertainty in the birth rates by treating each age-specific rate as a binomial random variable with the specified sample size.

Survival

Survival can be estimated directly from repeated observations of marked individuals, or, with appropriate assumptions, from estimates of the age distribution. There are no data on marked individuals for harbor porpoise, and such limited age distribution data as exist come from samples of the by-catch in the fishery, which cannot be considered random or unbiased. Thus, we are forced to use information from other species to construct model life tables.

We assume that the harbor porpoise survival schedule is similar to that of other mammals with similar life histories (i.e., large mammals that produce only a single offspring at a time). We selected life tables from the literature for unharvested populations of such species. By limiting our selections to monovular species, we factored out the major correlation in vertebrate (and other) life histories: the correlation between high litter size and high juvenile mortality (e.g., Spinage 1972). The species we used are listed in Table 3.

Life-span varies widely among these nine species, so we needed to rescale time in translating the species life tables into a harbor porpoise model life table. This scaling potentially can be done in many ways; Barlow and Boveng (1991) used longevity, and Eakin (1994) suggested using life expectancy at birth. We chose AFR as our scaling factor, for several reasons. We have no good age distribution data from which to estimate maximum longevity, and, in any event, the use of maximum life-span in comparative life history studies has been criticized by Krementz et al. (1989). In addition, because λ is known to be most sensitive to survival at young ages (e.g., Caswell 1989), scaling by AFR focuses on the most critical period of the life cycle.

Let T_i denote the time scale for model species i. We rescale time for species i by

$$T_i^* = T_i \frac{\mathrm{AFR_H}}{\mathrm{AFR}_i} \tag{2}$$

where $\mathrm{AFR_H}$ is the age at first reproduction for harbor porpoise. Thus, for example, a single year in the life of a killer whale, with a typical AFR on the order of 15 yr, corresponds to a little less than one-third of a year in the life of a harbor porpoise with a typical AFR on the order of 4 yr.

Our estimates of AFR and its uncertainty varied from species to species, depending on the information available. The appendix details sources of the data and the calculations. The end result of these computations is a set of distributions that characterize the uncertainty in knowledge of the AFR of the species used for the model life tables. These distributions are summarized in Table 3. The coefficient of variation of AFR is on the order of 1–5% for well-documented species such as the pilot whale, the orca, and the wildebeest, and on the order of 5–10% for species such as the Dall sheep, impala, and zebra, for which little information was available.

Monte Carlo uncertainty estimation.—Fig. 3 shows an example of the rescaled survivorship curves for the nine species; they span a wide range of mortality patterns. We expect that the harbor porpoise survival schedule falls somewhere in this space of model life tables. To estimate the uncertainty in harbor porpoise survival, we sampled this space by combining the model life tables at random, according to the following algorithm (Fig. 2):

1) Select at random a value of harbor porpoise age at first reproduction, $\mathrm{AFR_H}$, from the distribution shown in Fig. 1.

2) Select at random a value of AFR_i for model species i, from the appropriate distribution.

3) Rescale the time for model species i, according to Eq. 2 to obtain T_i^*, and generate a rescaled survivorship curve $l(x)$. From this curve, calculate the survival probabilities $P_j^{(i)} = l(j)/l(j-1)$ for each age class j of species i, assuming a birth-pulse model with post-breeding census (Caswell 1989).

TABLE 3. Species used in model life table construction, with the mean, standard deviation, and coefficient of variation of age at first reproduction (AFR).

Species	Age at first reproduction (yr)		
	Mean	1 SD	CV
Harbor porpoise	4.50	0.292	0.065
Dall sheep	2.00	0.206	0.103
Wildebeest	2.24	0.101	0.045
Elephant	13.17	0.615	0.047
Impala	2.00	0.203	0.102
Buffalo	4.00	0.204	0.051
Zebra	3.00	0.194	0.065
Orca	14.50	0.480	0.033
Ringed seal	5.86	0.377	0.064
Pilot whale	8.93	0.147	0.016

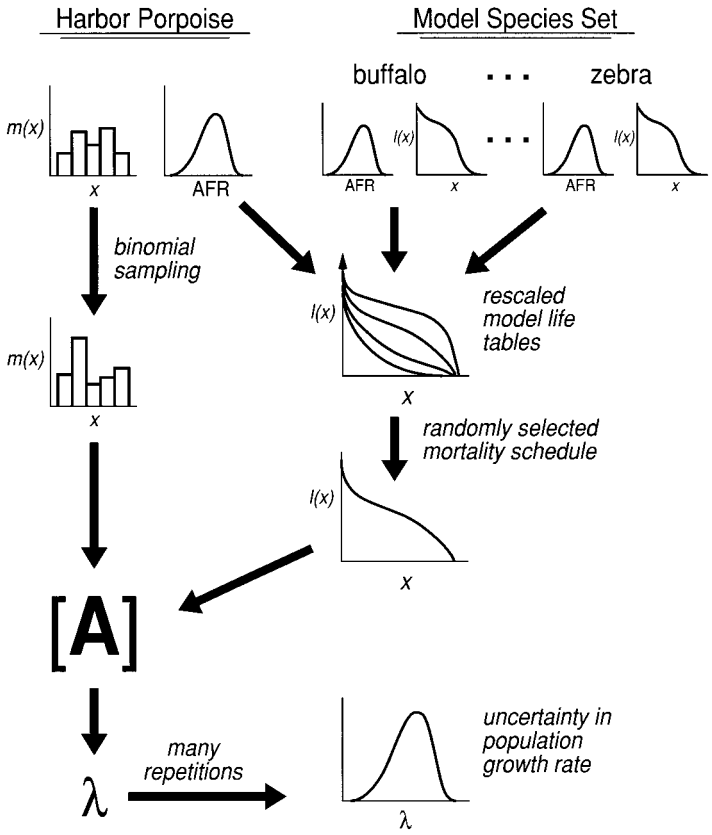

FIG. 2. The algorithm for generating the uncertainty distribution of λ or other demographic statistics for the harbor porpoise, using model life tables for other species rescaled according to age at first reproduction (AFR).

4) Repeat steps 2–3 for all nine species.

5) For each age *j*, treat the nine values of survival probability as a distribution of possible values for the harbor porpoise. Draw one of these at random and use it as the survival probability at age *j* for the harbor porpoise.

The random selection of one of the survival probabilities was based on the following rules. With probability *q*, the survival probability for age *j* was obtained from the same species used at age $j - 1$. With probability $1 - q$, survival probability at age *j* was obtained from a species chosen at random from one of the species *not* used at age $j - 1$. If $q = 1$, each life table is treated as a unit, and the sampling consists of picking a model species at random and using its life table. At the other extreme, if $q = 1/9$, survival probabilities are sampled randomly at each age, independently of the species providing the survival probability at the previous age. Thus, *q* is a way to examine the effect of correlations among age-specific survival values within species.

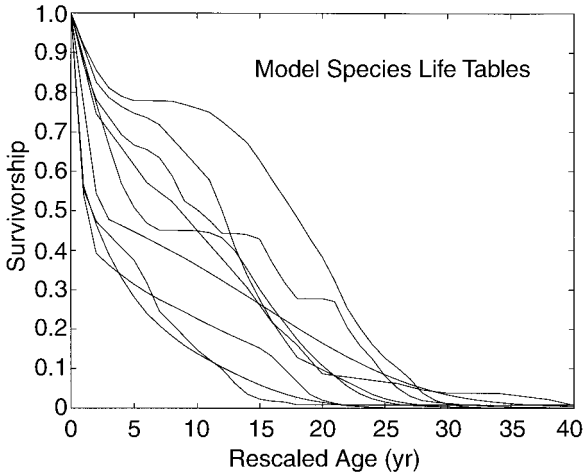

FIG. 3. A set of rescaled survivorship curves for the nine species used as model life tables for the harbor porpoise. These curves have been rescaled using the median AFR for harbor porpoise and for each of the model species.

6) Repeat steps 1–5 many times, each time generating a new harbor porpoise survival schedule from within the space of our nine model survival schedules.

Fig. 4 shows samples of the survivorship curves computed in this way. The tendency for the curves to cluster into species groups is evident when $q = 1$.

Constructing demographic models

The Monte Carlo procedure previously described generates a set of age-specific survival probabilities. These were combined, as shown in Fig. 2, with a set of reproductive output (m_i) values obtained by binomial sampling of the observed distribution of age-specific pregnancy rates. (Because the reproductive data are

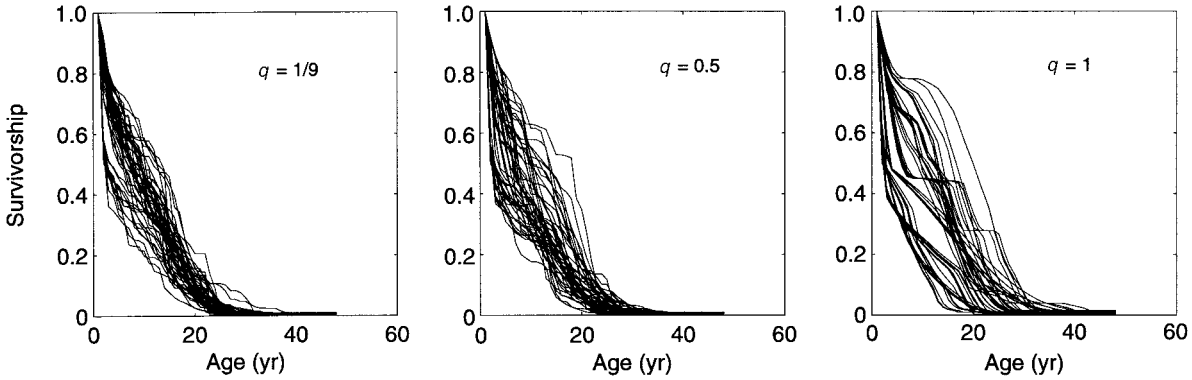

FIG. 4. A Monte Carlo sample of 50 harbor porpoise survivorship curves generated from randomly sampling the survival probabilities corresponding to the rescaled model survivorship curves. Results are shown for $q = 1/9$, $q = 0.5$, and $q = 1$.

already expressed on the harbor porpoise time scale, no rescaling is necessary.) These values were combined into an age-classified projection matrix

$$\mathbf{A} = \begin{pmatrix} F_1 & F_2 & F_3 & \dots \\ P_1 & 0 & 0 & \dots \\ 0 & P_2 & 0 & \dots \\ \dots & & \dots & \dots \end{pmatrix} \quad (3)$$

where the age-specific fertility term $F_i = P_i m_i$, assuming a postbreeding census (Caswell 1989).

Population growth rate was calculated as the dominant eigenvalue λ of the matrix **A**. This process was repeated 2000 times to give a distribution of λ incorporating uncertainty in the AFR for harbor porpoise, uncertainty in AFR for each of the species used in construction of the model life table, uncertainty in the location of the harbor porpoise survivorship schedule within the space of survivorships of other similar mammals, and uncertainty in harbor porpoise fertilities.

RESULTS

Rate of increase and its uncertainty

The uncertainty in population growth rate is shown in the probability distribution of λ (Fig. 5), for $q = 1/9$ (random selection of model species survivals at each age), $q = 1$ (each species life table treated as a unit), and an intermediate value of $q = 0.5$. The value of q does not have a large effect on the results. The distribution of λ values for $q = 1$ is more variable, because when $q < 1$ some of the interspecies variability in survival is averaged out.

The percentiles of the distribution are given in Table 4. The median values of λ range only from 1.096 to 1.111, depending on the value of q. The means are even more similar (1.0914, 1.0932, 1.0915). The median and mean values are close to the highest of the three model life table estimates (λ = 1.094) of Barlow and Boveng (1991). Woodley and Read's (1991) model life table estimate of 1.04 corresponds to about the 8th,

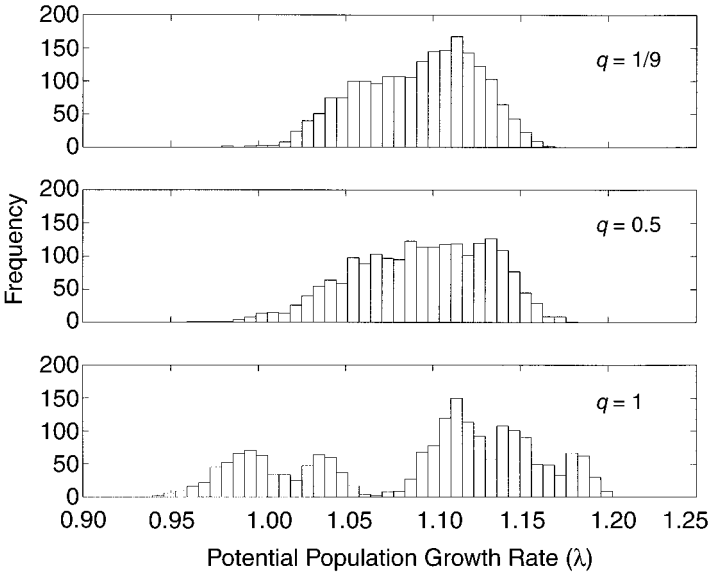

FIG. 5. The distribution of the population growth rate λ from 2000 randomly sampled population projection matrices for the harbor porpoise, for three values of q.

TABLE 4. Percentiles of the distribution of λ (rate of increase) for harbor porpoise, based on 2000 samples. Results are shown for λ under three values of *q*.

	Rate of increase, λ		
Percentile	$q = 1/9$	$q = 0.5$	$q = 1$
0.01	1.0187	1.0009	0.9598
0.05	1.0346	1.0280	0.9789
0.10	1.0438	1.0399	0.9906
0.25	1.0653	1.0646	1.0325
0.50	1.0956	1.0957	1.1108
0.75	1.1173	1.1252	1.1426
0.90	1.1326	1.1407	1.1698
0.95	1.1399	1.1489	1.1826
0.99	1.1536	1.1623	1.1916

10th, and 29th percentile, depending on the value of *q*. This is also the value of λ adopted by the National Marine Fisheries Service as a default value to be used in Potential Biological Removal calculations for cetacean stocks for which no other estimates are available (Barlow et al. 1995).

Based on these results, we conclude that, unless the vital rates for the harbor porpoise are unusual among large, monovular mammals, potential population growth rates greater than about 14–18% per year are unlikely. Values of about 10% seem much more plausible. We also note that λ is estimated with considerable uncertainty. 90% confidence intervals (the range between the 5th and 95th percentiles) are 1.03–1.14 for $q = 1/9$; 1.03–1.15 for $q = 0.5$; and 0.98–1.18 for $q = 1$.

Incidental mortality and its uncertainty

Bravington and Bisack's (1996) estimates of U.S. by-catch in the Gulf of Maine are based on a shipboard observer program and estimates of total landings in the fishery. The by-catch was significantly lower in 1992 and 1993 than in 1990; no other between-year differences were significant. They used a bootstrap procedure to generate confidence intervals for by-catch in each year.

Trippel et al. (1996) provide estimates of Canadian by-catch in the Bay of Fundy, also based on an observer program. In 1993, 424 ± 224 individuals (mean ± 1 SE) were killed. In 1994, the estimate was 101, with a 95% confidence interval from 80 to 122 individuals. They do not explain why they report a standard error in one year and a confidence interval in the other.

There are two sources of uncertainty in these estimates: variability from year to year and sampling uncertainty within the year. We combined these in our Monte Carlo procedure by treating the estimate as a stratified sampling problem. We generated a number for the U.S. by-catch by picking at random a year from the four years for which we have data (1990–1993), and then picking a value from the bootstrap distribution for that year. This is equivalent to drawing a single value from the pooled bootstrap distributions for the four years (Fig. 6). We did the same for the Canadian by-catch, randomly selecting a year and then drawing a value from a triangular distribution on the interval appropriate for that year (200–648 in 1993, 80–122 in 1994). The distribution for the Canadian by-catch is shown in Fig. 7. We add the two numbers together to get a total by-catch estimate *K*.

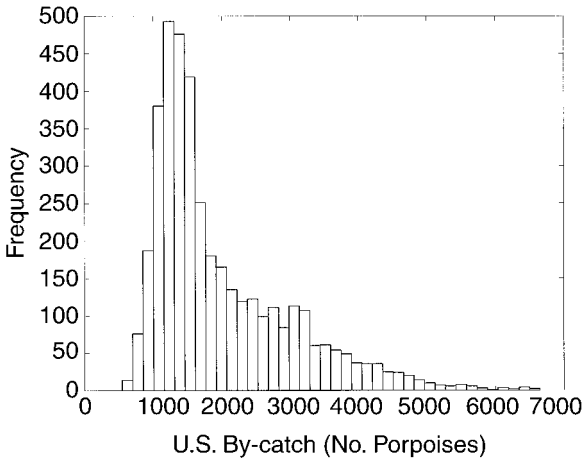

FIG. 6. The bootstrap distribution (sample size 3000) of estimates of harbor porpoise by-catch in the Gulf of Maine. Bootstrap samples are drawn from the distributions of Bravington and Bisack (1996), with all four years pooled.

The incidental mortality rate $M = K/N$ is generated by dividing the by-catch value by a population size *N*, drawn at random from the bootstrap distribution for population size (see Fig. 8) in Smith et al. (1993).

The resulting distribution for the mortality rate *M* is shown in Fig. 9. The mean of *M* is 0.0495, and the median is 0.0419, but the distribution is skewed to the right and contains considerable variability (a 95% confidence interval ranges from 0.0186 to 0.1119).

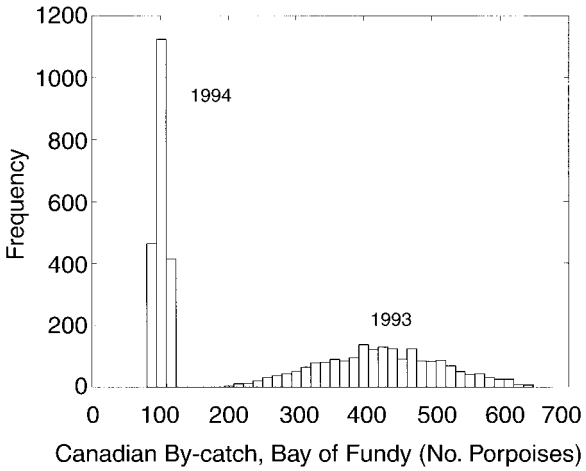

FIG. 7. The distribution of Canadian by-catch in the Bay of Fundy, based on results of Trippel et al. (1996). The two obvious modes correspond to their estimates in 1993 and 1994.

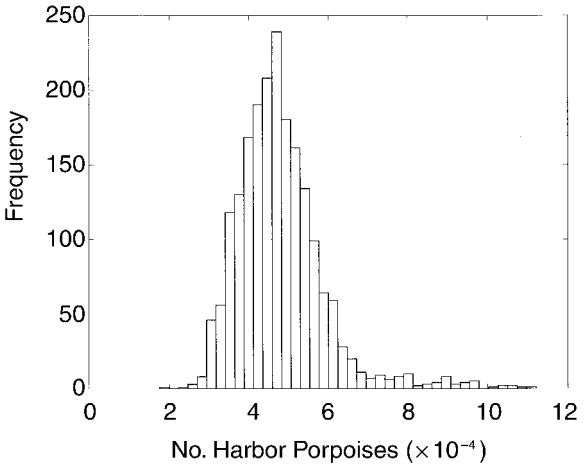

FIG. 8. The bootstrap distribution (sample size 2000) of estimates of harbor porpoise population size in the Gulf of Maine in 1992–1993. The distribution is that of a variance-weighted average of the estimates in the two years; calculations are described in Smith et al. (1993).

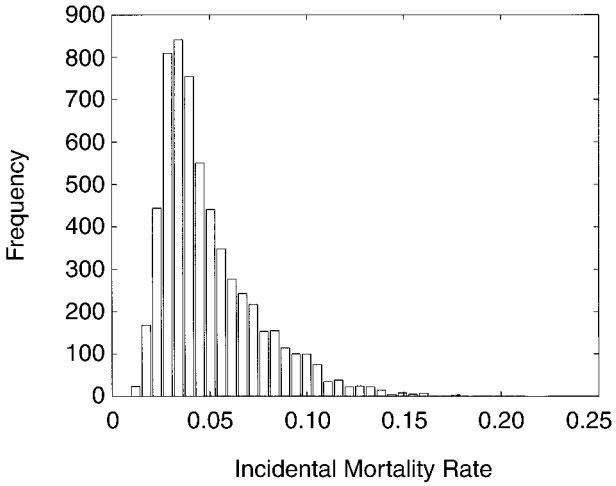

FIG. 9. The distribution (sample size 2000) of estimates of incidental mortality rate, calculated by Monte Carlo sampling from the distributions in Figs. 6–8.

Mortality compared with the rate of increase

We have, finally, two quantities: the population growth rate λ, and the incidental mortality rate *M*, each measured with uncertainty characterized by a distribution of values. We compared *M* with the yearly growth rate, calculated as $R = \lambda - 1$. Doing this repeatedly, we calculated the probability that the by-catch mortality rate *M* exceeds each of four threshold values:

1) The value *R*, which represents a theoretical upper bound to the mortality rate that the population can possibly sustain.

2) The value *R*/2, which is recommended by the International Whaling Commission (IWC 1991) as an upper limit to the combined rates of harvest and incidental mortality. This is the maximum possible critical value under the Marine Mammal Protection Act; it would result from setting the recovery factor to its maximum value (1.0) and from knowing the population size with absolute certainty.

3) The value *R*/4, recommended by the International Whaling Commission (IWC 1996) as a rate sufficient to be cause for concern about incidental mortality. This would be the Marine Mammal Protection Act critical value, given the recovery factor of 0.5, if actual population size, rather than the minimum population size, were used in the calculation.

4) The value 0.2134*R*, which is the current critical value for this stock under the Marine Mammal Protection Act, given the recovery factor of 0.5 and using the 20th percentile of a lognormal distribution as an estimate of minimum population size.

Results are shown in Table 5. They are sensitive to the value of *q*, but not dramatically so. Taking into account what is known, and acknowledging what is unknown about harbor porpoise demography, the probability that incidental mortality exceeds the International Whaling Commission recommendation of *R*/4 is at least 0.8. The probability that it exceeds the threshold for classification as a strategic stock under the Marine Mammal Protection Act ranges from 0.88 to 0.94, depending on the value of *q*. There is about an even chance (probability from 0.46 to 0.51) that it exceeds the maximum possible threshold (*R*/2) under the Marine Mammal Protection Act. The probability that it exceeds *R*, leaving no margin for safety at all, is at least 0.166.

DISCUSSION

Critical values of incidental mortality of the harbor porpoise range downward from one-half the potential rate of increase (IWC 1991) to one-fourth that rate (IWC 1996) to roughly one-fifth that rate under the Marine Mammal Protection Act. The probabilities that these critical values are exceeded range from about 0.5 to 0.95 (Table 5). Thus, it is very likely under international standards, and almost certain under U.S. standards, that the level of harbor porpoise by-catch in this population is too high. We conclude that the available evidence, fragmentary and uncertain as it is in some areas, indicates that the harbor porpoise is at risk from recent levels of incidental mortality in sink gillnet fisheries in the Gulf of Maine and the Bay of Fundy.

TABLE 5. Probabilities (Pr) that incidental mortality exceeds critical values, defined relative to the population growth rate. Values are given for $q = 1/9$, $q = 0.5$, and $q = 1$. $R = \lambda - 1$ denotes the yearly growth rate.

Probabilities	Probability of exceeding critical value		
	$q = 1/9$	$q = 0.5$	$q = 1$
Pr ($M \geq R$)	0.1660	0.1895	0.3030
Pr ($M \geq R/2$)	0.5055	0.4820	0.4640
Pr ($M \geq R/4$)	0.8850	0.8705	0.7990
Pr ($M \geq 0.2134R$)	0.9400	0.9245	0.8795

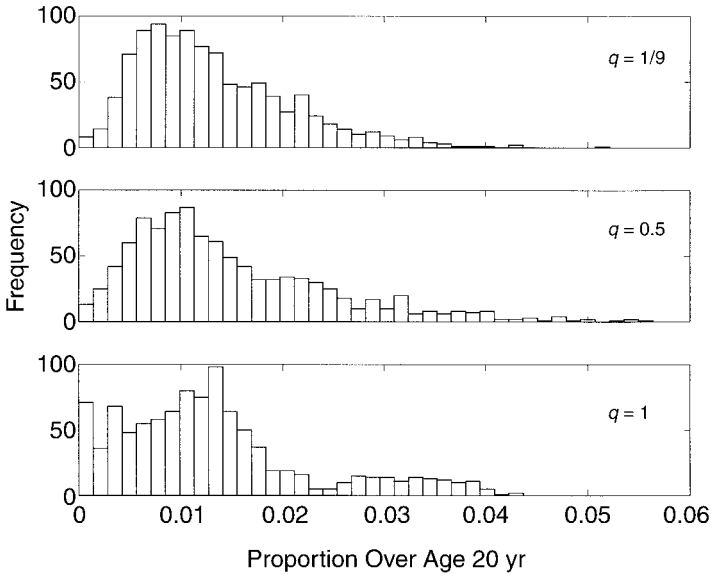

FIG. 10. The distribution of the proportion of the stable population >20 yr old, for three values of *q*.

Incidental mortality rates for the harbor porpoise have also been estimated in other parts of the world. Hammond (1995) reports rates of 3–5% per year in the North Sea and the Celtic Shelf, and Carlstrom and Berggren (1995) report a rate of 2.9% in the Skagerrak Sea off Sweden. These rates are comparable to the values in the Gulf of Maine. We have not carried out uncertainty calculations for these populations, because we have no information on the uncertainty in the estimates of by-catch and population size. However, because we expect the biology of harbor porpoises to be similar in these populations, we believe that there is cause for concern in these populations as well.

The results of our analysis are probabilities, not yes-or-no answers. This raises the question of how high such a probability must be before it warrants management action. The most optimistic of the results in Table 5 is that incidental mortality exceeds *R*, without any safety margin, with a probability of 0.166. Is this a dangerously high probability, or a reassuringly low one? Note that this is also the probability of losing a round of Russian roulette. Most people would say that accepting these odds and pulling the trigger is reckless behavior, because the consequences are so extreme. The consequence of incidental mortality that exceeds the potential growth rate is eventual extinction.

Of course, this analogy cannot be pursued too far. The consequences of losing at Russian roulette are immediate and irreversible, whereas extinction takes time. Nonetheless, the high probabilities that incidental mortality exceeds all the criteria considered, even that without a safety margin, are cause for immediate concern.

Sources of uncertainty

Much of our effort in this paper is directed at estimating λ, because the lack of data on survival of the harbor porpoise has forced us to a novel use of model life tables. Given this situation, our estimate of λ has much more uncertainty than that estimated for the killer whale by Brault and Caswell (1993), based on the best set of demographic data for any cetacean. But, how does the uncertainty in λ compare with that in the other quantities (*N* and *K*) appearing in our final calculations? One way to quantify the relative precision of an estimate is the coefficient of variation (CV; the ratio of the standard deviation to the mean). The CV of λ, depending on the value of *q*, ranges from 0.031 to 0.061. This is much less than the CV of by-catch (0.470) or of population size (0.230; based on the bootstrap distribution) or of the incidental mortality rate (0.521). Thus, the aspect of this problem about which we know the least is not harbor porpoise demography, but incidental mortality rate, and within that, the amount of by-catch.

Parameter estimation is not the only source of uncertainty. Our results also depend on our underlying conjecture that the survival probabilities for the harbor porpoise lie somewhere in the space spanned by the survival schedules of other large mammals with similar life histories. If this conjecture is incorrect—if, unbeknownst to us, the harbor porpoise were unique among mammals in having the survivorship curve of a clam, for example—our results would not capture anything like the true value of λ for the harbor porpoise.

One way to assess the quality of our description of harbor porpoise demography is to compare the age distributions predicted by the model with the (admittedly limited) age distribution data. Read and Hohn (1995) conclude that ''harbor porpoises are clearly capable of living to ages of 20 yr or more, but individuals seldom reach such advanced age.'' The oldest animals found in over 600 individuals from the Gulf of Maine and Bay of Fundy were two 17-yr-olds. In a sample of 200 individuals from California, the oldest individual was 24 yr old.

Fig. 10 shows the proportion of the population over 20 yr old predicted by the stable age distribution from a sample of 1000 randomly generated sets of harbor porpoise demographic parameters. Regardless of the value of q, the median proportion is about 0.012. These stable age distributions are calculated assuming no incidental mortality. Incidental mortality, even if independent of age, will result in even fewer old individuals. Thus, the observed oldest animals are not inconsistent with the age distributions implied by our use of model life tables.

The implementation of management strategies can also introduce uncertainty. Rosenberg and Brault (1993) studied the effects of reduced fishing mortality on stock recovery in a model of yellowtail flounder. Uncertainty in the impact of regulations on the actual fishing mortality changed the pattern of stock recovery and the probability that the intervention would meet its goals of reducing yield.

The interpretation of uncertainty

Our approach treats uncertainty in the same way that classical statistics treats sampling error; it yields the uncertainty of an estimate of λ, where λ is viewed as a fixed, but unknown, parameter. If we knew more (having larger samples or better data), the uncertainty would be less, just as the standard error of the estimate of a mean decreases with increasing sample size.

As for most statistical calculations, there exists a Bayesian alternative to this interpretation, in which the parameters are treated as random variables whose distribution depends on prior subjective knowledge. Bayes' formula is used to update the distribution as additional data are obtained. To a Bayesian, our distribution of λ would be a posterior distribution, the result of applying our information on the harbor porpoise (the data on AFR and reproductive rates, the characteristics of the porpoise life history, the available life tables for mammal species with similar life histories) to a non-informative prior distribution expressing our ignorance about the vital rates of the harbor porpoise. There is currently considerable interest in (Raftery et al. 1995, Taylor et al. 1996) and controversy about (Dennis 1996) Bayesian methods in ecology, but we leave the development of a Bayesian version of our results as an open problem.

Uncertainty in an estimate should not be confused with stochastic variation in demographic rates over time. We do not consider effects of stochastic temporal variation here, because we have no information on such variation. If we did, we could calculate stochastic population statistics (e.g., long-term growth rates, extinction probabilities; see Tuljapurkar 1990). These statistics would be uncertain because of uncertainty in the parameters of the stochastic models, and that uncertainty could be addressed using the methods we adopt here.

We have shown that it is possible to incorporate uncertainty into even complicated demographic calculations, and to map that uncertainty into the results of those calculations. In the case of the harbor porpoise, we provide strong support for the conclusion that incidental mortality rates exceed the levels recommended by national and international management agencies. It remains to be seen what actions will be taken to ameliorate the situation.

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APPENDIX

AFR and Survivorship Calculations for Model Life Table Species

This Appendix summarizes the sources of data and analytical procedures used to estimate age at first reproduction and survivorship for each of the nine species used for model life tables. We have tried to be sufficiently precise in our descriptions that the interested reader could repeat the calculations.

In this appendix, ASM denotes age at sexual maturity, and AFR age at first reproduction.

Pilot whale (Globicephala melas).—Bloch et al. (1993) estimated the median age at sexual maturity as 8.1 yr from a sample of 283 female pilot whales (aged 4–12 yr) taken in the Faroese drive fishery. They do not provide any information on sample sizes for individual ages, so we divided the 283 individuals evenly among the eight age classes. We estimated the proportions of mature whales from Bloch et al. (1993: Fig. 11). Fitting a logistic regression to these data gives $b_1 = -10.968$, $b_2 = 1.3637$, ASM = 8.042, which is in close agreement with the values reported by Bloch et al. (1993; $b_1 = -10.070$, $b_2 = 1.249$, ASM = 8.1). We generated a distribution of values for AFR by the bootstrap procedure described for the harbor porpoise, adding a gestation period of 326 d (Bloch et al. 1993) to the ASM values.

The survivorship curve for the pilot whale was based on a nonparametric fit to the age distribution data in Bloch et al. (1993) (Sanders-Reed 1996). It gives values similar to the Siler model fit described in Bloch et al. (1993: Table 6), but gives a significantly better fit.

Killer whale (Orcinus orca).—Olesiuk et al. (1990: Table 6) give sample sizes and the number of individuals that have reproduced for ages 10–18 yr. These data refer to first reproduction, not sexual maturity, so there is no need to add the gestation time to the estimate. Olesiuk et al. (1990) report a median AFR of 14.40 yr. Our logistic regression gives $b_1 = -13.177$, $b_2 = 0.916$, and AFR = 14.38 yr. We calculated the uncertainty in this estimate by the bootstrap procedure described for the harbor porpoise. The survivorship curve for the killer whale was obtained from Olesiuk et al. (1990: Table 14).

African elephant (Loxodonta africana).—The age at first reproduction in the elephant is sensitive to density (Laws 1973). Because we are trying to generate a potential population growth rate for the harbor porpoise, we chose data from Laws (1973: Fig. 1) for a low-density population (Mkomasi). This gives a median age at sexual maturity of 12.3 yr (with a 95% confidence interval of [10.62, 13.33]). We added 1 yr to obtain an AFR of 13.5 yr. To describe the uncertainty in the estimate, we used a triangular distribution with support on the 95% confidence interval; i.e., from 11.62 to 14.33 yr. Survivorship information for the African elephant was obtained from the figures for females in Table 2 and Figure 6 of Laws (1969); these data are from an unharvested population.

Wildebeest (Connochaetes taurinus).—Watson (1970) reports that 26 out of 70 cows reproduced at age 2 yr; 40 out of 48 reproduced at age 3 yr; and 86 out of 90 reproduced at ages $\geq$4 yr. No 1-yr-old cows reproduced, but Watson does not report the sample size, so we assumed a sample size of 50 as a value similar to that for the other ages. Logistic regression gives $b_1 = -3.5030$, $b_2 = 1.5733$, and AFR = 2.227 years. We calculated the uncertainty in this estimate by the bootstrap procedure described for the harbor porpoise. Survivorship data for wildebeest were taken from Watson (1970: Table 2).

Dall sheep (Ovis dalli).—Simmons et al. (1984: Table 2) give reproductive output figures as a function of age. By age 2 yr, reproduction has reached about 50% of its maximum value, which suggests using 2 years as a median AFR. In the absence of any information on which to calculate uncertainty, we used a triangular distribution on the interval [1.5, 2.5]. Survivorship data for Dall sheep were taken from Simmons et al. (1984: Table 2).

Ringed seal (Phoca hispida).—Smith (1975: Table 55) gives sample sizes and proportion of individuals mature for ages 4–10 yr. We fit a logistic regression to these data and obtained $b_1 = -3.793$, $b_2 = 0.7713$, and ASM = 4.92 years. We calculated the uncertainty in age at sexual maturity using the bootstrap procedure described for the harbor porpoise, and added 1 yr to the resulting figure to obtain a distribution of AFR. Survivorship data for the ringed seal were taken from the composite life table in Smith (1975: Table 61).

Impala (Aepycero melampus).—Western (1979) gives the age at first reproduction as 2 yr. In the absence of any other information, we used a triangular distribution on the interval [1.5, 2.5] to describe the uncertainty in AFR. Survivorship data for the impala were taken from the female life table in Spinage (1972: Table 4).

Zebra (Equus burchelli).—Western (1979) gives the mean age at first reproduction as 3 yr. In the absence of any other information, we used a triangular distribution on the interval [2.5, 3.5] to describe the uncertainty in AFR. Survivorship data for the zebra were taken from the female life table in Spinage (1972: Table 6).

African buffalo (Syncerus caffer).—Western (1979) gives the mean age at first reproduction as 4 yr. In the absence of any other information, we used a triangular distribution on the interval [3.5, 4.5] to describe the uncertainty in AFR. Survivorship data for the African buffalo were taken from the female life table in Spinage (1972: Table 5).

Life History of Small Cetaceans in the Northwest Atlantic

Appendix 2

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Seasonal regression in testicular size and histology in harbour porpoises (*Phocoena phocoena*, L.) from the Bay of Fundy and Gulf of Maine

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Abstract

Seasonal regression of testes and epididymides is described for 161 mature harbour porpoises *Phocoena phocoena*, L. from the Bay of Fundy and Gulf of Maine from June to December 1984–1995. Based on histological appearance and size of gonads, testes are fully active from late June until at least the end of July, spanning the estimated period of conceptions. During testicular regression, spermatocytes and round spermatids disappeared first from the lumina of seminiferous tubules, followed by the gradual disappearance of spermatozoa. Ultimately, all signs of spermatogenesis were absent, but tubules retained an alternating lining of Sertoli cells and spermatogonia. Testicular and epididymal mass, testicular length and seminiferous tubular diameter decreased approximately 3.5, 1.5 and 1.5 times, respectively, from peak production to full regression and this decrease was best described by a quadratic function. During early July when most females are ovulating, all males had active testes; variation in the degree of regression among males increased as the season progressed. This may reflect a trade-off between the costs of maintaining active testes at 4% body mass and the probability of successful fertilization. Testes are completely regressed during the winter, suggesting that few reproductive opportunities exist during this season. Unlike some other odontocete species, testicular mass of porpoises is a good indicator of breeding season.

Key words: seasonality, reproduction, testes, harbour porpoise, *Phocoena phocoena*

INTRODUCTION

Harbour porpoises *Phocoena phocoena* (L.) are known to reproduce seasonally (Møhl-Hansen, 1954; Fisher & Harrison, 1970; Gaskin *et al*., 1984; Read, 1990; Sørensen & Kinze, 1994; Read & Hohn, 1995). For the population of porpoises inhabiting the Bay of Fundy and Gulf of Maine, most parturitions occur in late May (Read, 1990), although some births probably occur from March to August (Gaskin & Blair, 1977). Observations from female harbour porpoises in this population suggests a breeding season confined to a few weeks (Read, 1990; Read & Hohn, 1995) or months (Gaskin *et al*., 1984). This pattern of reproductive seasonality probably enables lactating females to take advantage of rich food resources in the summer and autumn (Read, 1990). The peak reproductive activity of male mammals is usually constrained to periods when females are in oestrus (Lincoln, 1992). In harbour porpoises, seasonal changes in testicular size and activity have been used to infer or corroborate mating seasons in this species (Gaskin *et al*., 1984; Read, 1990; Sørensen & Kinze, 1994; Lockyer, 1995; Read & Hohn, 1995).

Male harbour porpoises have a large ratio of testicular mass to body mass: up to 4% during the breeding season (Gaskin *et al*., 1984). Maintenance of such large testes likely requires a considerable energetic investment during this period. Seasonal regression in testicular mass and function is therefore expected to occur over the remainder of the year and evidence for this has been provided in various descriptive accounts (Gaskin *et al*., 1984; Read 1990; Sørensen & Kinze, 1994; Lockyer, 1995; Read & Hohn, 1995), although this process has not been examined in detail for this species.

Prior studies on the testicular activity of harbour porpoises in the Bay of Fundy have been restricted to samples collected between May and September (Gaskin *et al*., 1984; Read, 1990) because most animals leave the Bay during autumn (Gaskin, 1977). Read & Hohn

* Died, September 1998.

(1995) obtained testicular samples in the autumn and winter from the Gulf of Maine, but their analysis was restricted to seasonal variation in gonadal mass. Seasonal changes in the histology of seminiferous tubules of the testes of harbour porpoises have been noted by Gaskin *et al.* (1984) and Sørensen & Kinze (1994), but no detailed description of this process has been published. Likewise, regressive changes and density of spermatozoa in epididymides of harbour porpoises have been largely ignored. In this study, we collected data and samples from 161 mature male porpoises from the Bay of Fundy/Gulf of Maine from June to December, to provide a comprehensive account of seasonal changes in the testes and epididymides of harbour porpoises and to evaluate the use of testicular activity as an indicator of breeding season.

Table 1. Temporal distribution of sample collection of mature male harbour porpoises from the Bay of Fundy and Gulf of Maine from 1984 to 1995

Month	Total no. of porpoises collected	No. of porpoises with data on gonad size (mass and/or length)	No. of samples suitable for histological examination[a]
June	2	2	1
July	23	22	16
August	85	83	44
September	15	15	9
October	13	13	–
November	18	17	–
December	4	4	–
Unknown	1	1	–
Total	161	157	70

[a] See Materials and methods.

MATERIALS AND METHODS

Sample collection

Samples and data were available from 161 mature male harbour porpoises collected from June to December to examine testicular regression (Table 1). Maturity status was determined through histological examination of testes (see below). Samples of gonadal tissue were unavailable for 7 porpoises determined to be mature, based on gonadal mass or age (Neimanis, 1996). Specimens were killed incidentally in gill nets ($n = 146$) or herring weirs ($n = 10$), or stranded ($n = 5$) from 1984 to 1995. No abnormalities were noted on necropsy or on histology of testes. We therefore assumed that these animals were representative of the normal, healthy population. Satellite telemetry (Read & Westgate, 1997), contaminant levels (Johnston, 1995; Westgate, 1995), life history (Gaskin *et al.*, 1984; Read & Hohn, 1995) and genetic evidence (Wang, Gaskin & White, 1996) suggest that porpoises from the Bay of Fundy and Gulf of Maine comprise a single subpopulation (Palka *et al.*, 1996), therefore porpoises from both areas were pooled for this study. Carcasses from the Bay of Fundy ($n = 122$) and the Gulf of Maine ($n = 39$) were examined during standard necropsies (American Society of Mammalogists, 1961). Not all information could be collected from each carcass, so sample sizes vary between parameters. Body length ($n = 161$) and body mass ($n = 149$) were recorded and teeth were collected for estimation of age ($n = 148$). Testes and epididymides were excised and weighed ($n = 151$). The length, width and depth of each testis was measured ($n = 157$) and samples of testis and epididymis were fixed in 10% neutral buffered formalin ($n = 154$). Gonadal mass included testes and epididymides. Neimanis (1996) found bilateral symmetry in the testicular mass of porpoises, therefore mean mass was used if data from both gonads were available. If only 1 gonad was examined, the mass of the single organ was used. For 10 animals, only testicular mass without epididymides was available. We estimated the epididymal mass of these animals using a regression equation of testicular *vs* epididymal mass of 104 male porpoises: $y = -0.5994 + 0.2935x$, $r^2 = 0.80$ (Neimanis, 1996). Ages of 148 animals were estimated from counts of growth layer groups in decalcified and stained tooth sections as detailed in Bjørge *et al.* (1995).

Histological processing

Samples of formalin- or Bouin's fluid-fixed tissue were dehydrated in graded ethyl alcohol, cleared in xylene and embedded in paraffin at 60 °C; 5 μm sections were cut, stained with hematoxylin and eosin and mounted in Cytoseal®, a permanent medium. The protocol for Harris's hematoxylin and eosin procedures followed Prophet *et al.* (1992). All histological preparations were performed by the Histology Laboratory in the Department of Pathobiology, Ontario Veterinary College, University of Guelph, Guelph, Ontario.

Histological examination

One testicular section per animal was examined at magnifications of up to 600× under a light microscope. All changes in spermatogenesis were assumed to be the result of seasonal regression. Sexual maturity was confirmed by the presence of spermatogenesis. A tubular diameter between 111 and 225 μm and a well-developed lumen with little interstitial tissue area confirmed maturity (Neimanis, 1996). Time of death was not known for most of the carcasses collected, and autolysis of the testes in the specimens ranged from mild to severe. To determine the point at which autolysis significantly affected histological appearance, a mature testis was collected from a fresh carcass and allowed to autolyse, with samples taken at intervals over 48 h. Each histological slide in this study was compared to the series of slides of post-mortem autolysis. Only tissues that were not significantly affected by autolysis were included in the present analysis ($n = 70$). Freezing also significantly

affects gonadal tissue of porpoises (Neimanis, 1996). All testes from specimens collected later than 18 September were frozen and/or severely autolysed and were not included in examination of histological detail (Table 1). Germ cells, however, were still recognizable in these specimens and seasonal regression could be observed.

Three random fields of view were examined per slide at 200 × magnification. Co-ordinates of each field of view were determined using a random number generator. The percentage of tubules containing elongate spermatids/spermatozoa and spermatocytes/round spermatids was determined for each field. Epididymides were available for histological examination from 58 of the 70 non-autolysed specimens. The amount of spermatozoa present in epididymal sections was classified as copious (spermatozoa occupying half or more of the luminal area), sparse (intermediate content) or none (no spermatozoa seen). The quantity of spermatozoa in the epididymis could be classified in an additional 38 specimens, even though degradation of testicular tissue was severe.

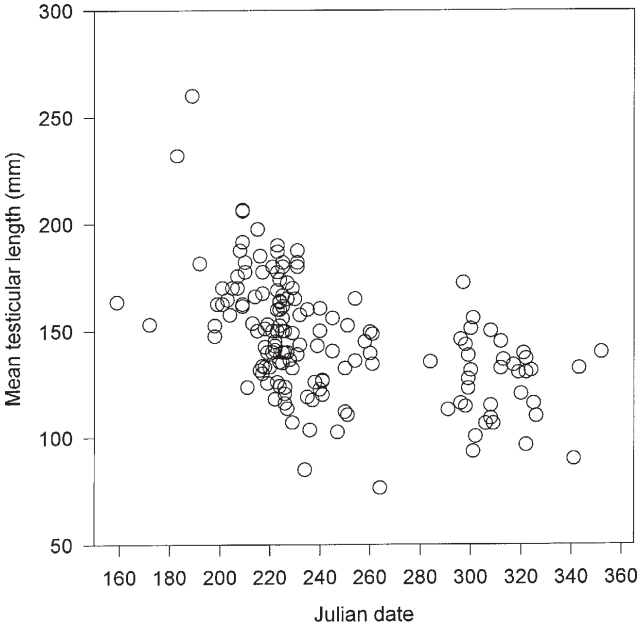

Fig. 1. Mean length of testes from mature harbour porpoises collected from the Bay of Fundy and Gulf of Maine (1984–1995) *vs* time of year.

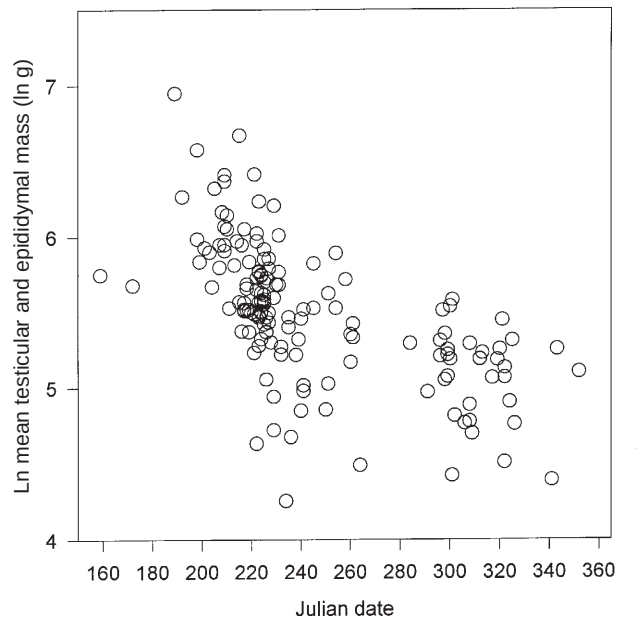

Fig. 2. Mean testicular and epididymal mass of mature harbour porpoises collected from the Bay of Fundy and Gulf of Maine (1984–1995) *vs* time of year.

Morphometric analysis

To ensure that post-mortem degradation did not affect the diameter of seminiferous tubules, only slides acceptable for histological examination were included ($n = 70$). Twenty random, circular seminiferous tubules containing a lumen were measured per specimen. An image of each tubule was projected on a computer monitor and the smallest diameter was measured using Sigma Scan Pro v. 2.0 (Jandel Scientific Software, 1993–1995). The smallest diameter was selected to ensure that the cross-section of the longitudinal axis was being measured at 90°. In slightly autolysed tissues, some areas of the basement membrane are detached from the seminiferous epithelium, but the diameter of tubules does not differ significantly from fresh tissue (Neimanis, 1996). Therefore, all measurements were made from the basement membrane.

Statistical analysis

All analyses were performed using SAS for Windows (SAS Institute Inc., Cary, NC, 1991). Models to describe the seasonal decrease in testicular length, testicular and epididymal mass and mean seminiferous tubular diameter were generated using PROC GLM. Testicular size varies with body size (Neimanis, 1996), so body length was included in the model. Testicular mass was regressed against body length cubed to maintain dimensionality. Based on testicular histology (see below), testes from an animal collected on 21 June were undergoing recrudescence (i.e. a renewal of spermatogenesis) rather than regressing. Although no histological sample was available for a male collected 8 June, the length and mass of this animal's testes suggested that these gonads were also recrudescing (Figs 1 & 2). These samples were therefore excluded from the statistical analysis of testicular regression.

Each regression model included Julian date (serial day from 1 January following Read & Hohn, 1995), Julian date squared, body length, body length squared and the interaction term of Julian date × body length. Parameters were considered significant and retained within the model if their P-value was < 0.10. Normality of residuals was assessed using PROC UNIVARIATE. To ensure that the addition of parameters did not

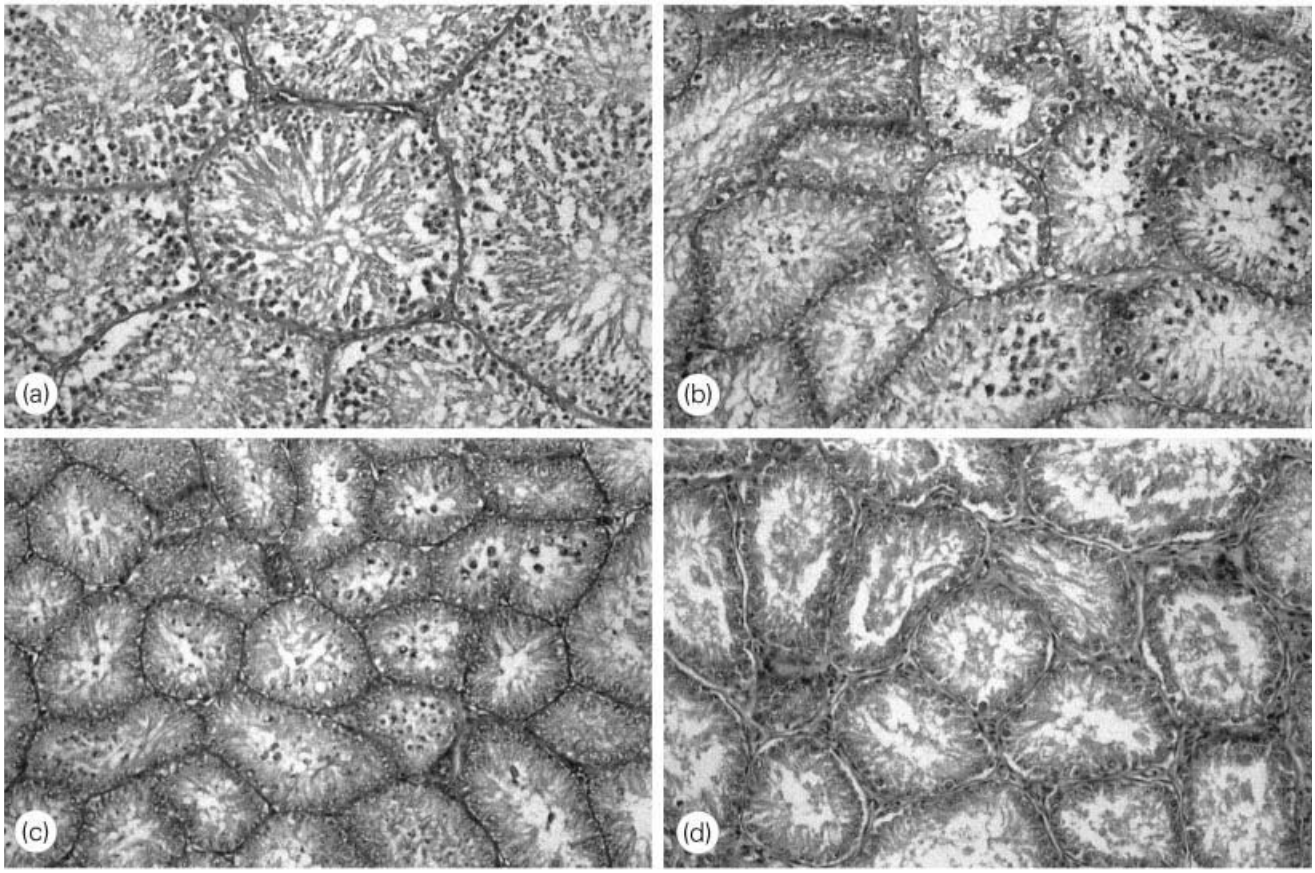

Fig. 3. Seasonal regression in testes of mature harbour porpoises collected from the Bay of Fundy and Gulf of Maine (1984–1995). (a) Full production. (b–d) Increasing degrees of seasonal regression. All micrographs were taken at the same magnification, $\times 200$.

artificially inflate r^2, an adjusted value was calculated (Neter, Wasserman & Kutner, 1985). Distribution of data for copious, sparse and no spermatozoa by Julian date were compared using PROC ANOVA and a Fischer's least significant difference test (LSD) at a significance level of 0.05. A log transformation of Julian date was required before comparison.

RESULTS

Histological examination and morphometric analysis

Full testicular activity, in which spermatogenesis was evident in every seminiferous tubule, was observed in specimens collected between 3 July and 29 August. Because many tubular cross-sections contained more than one stage of spermatogenesis, spermatogenesis was assumed to proceed in helical waves along seminiferous tubules (Neimanis, 1996). For example, at least two stages of spermatogenesis can be seen in the central tubule in Fig. 3a.

The earliest specimen to display histological signs of seasonal regression was collected on 18 July. As the season progressed, spermatocytes and round spermatids disappeared from randomly scattered tubules and degenerated spermatogenic cells were observed in tubular lumina (Fig. 3a–d). This was followed by a decrease in numbers of spermatozoa in tubular lumina. Eventually, spermatocytes and round spermatids were absent from seminiferous tubules, although some spermatozoa persisted in the lumen (Fig. 3c). Ultimately, all signs of spermatogenesis, including spermatozoa, disappeared from the tubules (Fig. 3d). Tubules retained an alternating lining of Sertoli cells and spermatogonia. Regressive changes in the presence of spermatogenic cells were accompanied by an increase in interstitial tissue area up to 1.5 or 2 times, and a decrease in seminiferous tubular diameter. In regressed testes with decreased tubular size, basement membranes were almost twice as thick as those in fully active testes.

The maximum mean diameter of seminiferous tubules in a mature male was 225 μm, from a specimen collected on 8 July and in peak activity. The smallest mean tubular diameter (111 μm) was from a mature male collected on 18 August. The second smallest (117 μm) was from a collection on 8 September. Tubular diameters of all other mature testes fell within this range.

Only one histological sample was available before the period of full testicular activity to examine recrudescence. In this specimen collected on 21 June, germ cells

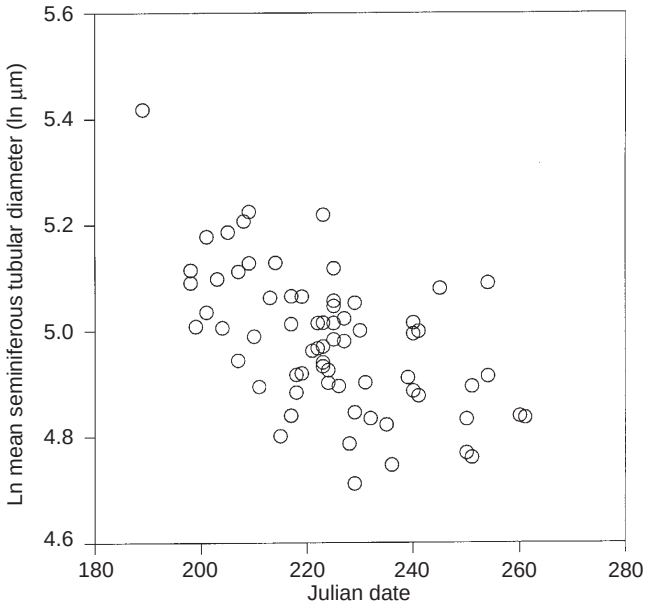

Fig. 4. Mean seminiferous tubular diameter of testes of mature harbour porpoises collected from the Bay of Fundy and Gulf of Maine (1984–1995) *vs* time of year.

were still distinguishable, although autolysis of this testis was advanced. Spermatocytes and round spermatids filled the tubules as in fully active specimens, but there were only half as many elongate spermatids and/or spermatozoa present. This specimen seemed to be in the final stages of recrudescence.

Seasonal changes were also observed in the epididymides. At and just after full production (3 July–14 September), the lumina were packed with spermatozoa and the epithelial lining was thick and well-developed. During seasonal regression, spermatozoal density decreased and degenerating spermatogenic cells, such as round spermatids, were observed in the epididymal lumen. Lumen size and thickness of epithelial lining decreased. A slight delay occurred between reduction of spermatocytes and round spermatids and spermatozoa in the seminiferous tubules and in the epididymis. All samples at peak production contained copious spermatozoa in the epididymis and the majority of testes displaying regressive changes had sparse or no spermatozoa. Testes in the early stages of regression (when all or most tubules contained spermatocytes and round spermatids, but their numbers were reduced) were accompanied by epididymides still packed with spermatozoa.

Table 2. Bimonthly means of seminiferous tubular diameter of mature harbour porpoises collected from the Bay of Fundy and Gulf of Maine from 1984 to 1995

Date	Mean seminiferous tubular diameter (µm)	Sample size	SE
1–15 July	225.3	1	–
16–31 July	161.6	15	4.07
1–15 August	147.3	29	2.48
16–31 August	133.9	15	3.57
1–15 September	136.1	7	7.11
16–30 September	126.1	2	0.21

Statistical analysis of seasonal changes in gonadal parameters

Seasonal changes in testicular length, testicular and epididymal mass and seminiferous tubular diameter are presented in Figs 1, 2 & 4. Bimonthly means of tubular diameters are presented in Table 2. Regressions of testicular and epididymal mass and seminiferous tubular diameter required log transformation. For all three regressions, body length squared and the interaction term were not significant and were eliminated from the models. For tubular diameter, body length was not significant and was eliminated from the model. Regressions of all measures of gonadal size were highly significant ($P = 0.0001$) and the decrease of each parameter after mating season was best described by a quadratic rather than linear function (Table 3).

The animal with the largest testes (combined mass = 2085g) was collected on 8 July and testes of two porpoises collected 8 June and 21 June appeared to be

Table 3. Summary of regression results of various gonadal parameters of mature harbour porpoises collected from the Bay of Fundy and Gulf of Maine from 1984 to 1995 *vs* time of year. All regressions were highly significant ($P = 0.0001$)

Parameter	Intercept (SE)	Julian date (SE)	Body length (SE)	Julian date squared (SE)	Equation[a]	Adjusted r^2
Mean testicular length (mm)	373.725 (96.731) $P = 0.0002$	−2.948 (0.677) $P = 0.0001$	137.886 (24.132) $P = 0.0001$	0.005 (0.001) $P = 0.0002$	$y = 373.725 - 2.948x + 137.886b + 0.005x^2$	0.434
Mean testicular and epididymal weight (g)	12.748 (1.470) $P = 0.0001$	−0.056 (0.011) $P = 0.0001$	0.287[b] (0.065) $P = 0.0001$	$9.081*e^{-5}$ ($2.088*e^{-5}$) $P = 0.0001$	$\ln(y) = 12.748 - 0.056x + 0.287c + 9.081*e^{-5}x^2$	0.521
Mean seminiferous tubular diameter (µm)	10.704 (2.023) $P = 0.0001$	−0.046 (0.018) $P = 0.012$	–	$9.177*e^{-5}$ ($3.948*e^{-5}$) $P = 0.023$	$\ln(y) = 10.704 - 0.046x + 9.177*e^{-5}x^2$	0.367

[a] x = Julian date, b = body length (m), c = body length cubed (m^3).
[b] Body length cubed (see Materials and methods).

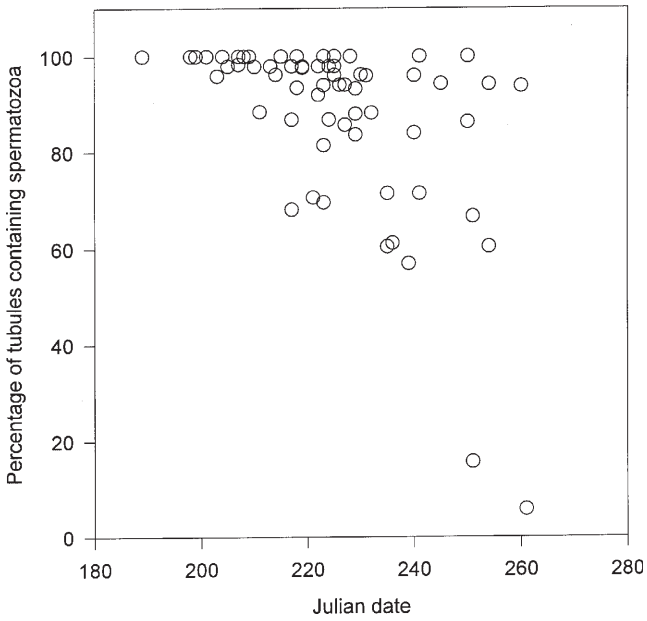

Fig. 5. Percentage of seminiferous tubules containing spermatozoa *vs* time of year for testes of mature harbour porpoises collected from the Bay of Fundy and Gulf of Maine (1984–1995).

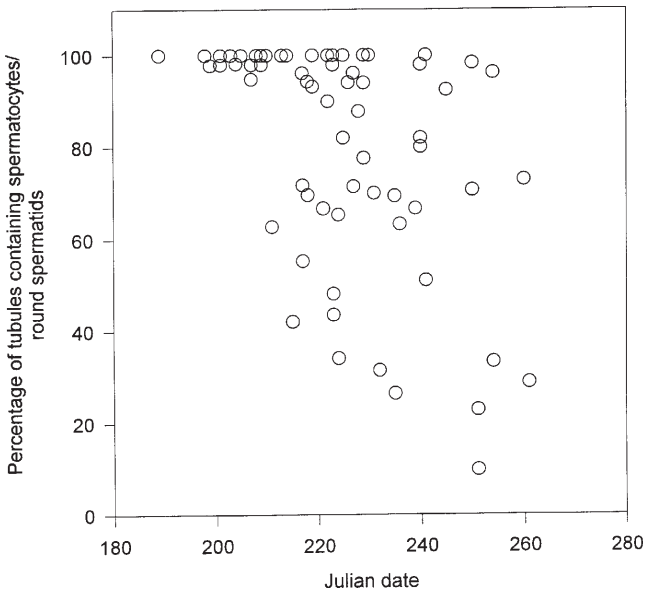

Fig. 6. Percentage of seminiferous tubules containing spermatocytes and/or round spermatids *vs* time of year for testes of mature harbour porpoises collected from the Bay of Fundy and Gulf of Maine (1984–1995).

recrudescing. Testicular length, mass and tubular diameter decreased approx. 1.5, 3.5 and 1.5 times, respectively from peak production to full regression. Other variation in testicular size could be partially attributed to body size (i.e. body length), but size of the animal had no significant effect on the size of tubular diameters.

The percentage of tubules containing spermatozoa or spermatocytes/round spermatids had a declining seasonal trend (Figs 5 & 6). Inter-individual variation in this parameter increased as Julian date increased. No specimens collected after 7 September contained spermatozoa or spermatocytes and round spermatids in 100% of the tubules.

Seasonal variation in the relative abundance of spermatozoa in the epididymides is presented in Fig. 7. Mean dates for copious (217.6 ± 14.7; 6 August), sparse (226.9 ± 10.2; 15 August) and no spermatozoa observed (244.2 ± 28.6; 1 September) were significantly different ($P = 0.0001$).

DISCUSSION

The testes of harbour porpoises undergo annual fluctuations in size and activity. Peak size and activity of testes is presumed to correspond with the oestrus cycle of females (18 June–1 July; Read, 1990), but based on spermatozoa densities in the epididymides, most males appear to be capable of fertilization until at least the end of July. Seasonal changes observed in histological sections suggest that harbour porpoise testes are inactive during the winter months.

All males had copious spermatozoa in their epididymides from 3 July to 22 July (Fig. 7). No epididymides were available before these dates, so the beginning of the mating season could not be determined accurately. Matings should occur when epididymides are filled with spermatozoa, because in mammals, spermatozoa mature in the epididymis and are stored there before copulation (Amann, Hammerstedt & Veeramachaneni, 1993). Using data from female porpoises, Read (1990) derived two estimates of mating season: ovarian activity suggested that conception spanned from 18 June to 1 July and a back-calculation from mean birth date yielded a mean conception date of 29 June. Data from male porpoises support this timing of events, particularly the latter estimate, but males appear to be active for somewhat longer. Spermatogenetic activity in the testes and quantity of spermatozoa in epididymides indicate that most male porpoises were active until the end of July, and one-quarter of the individuals were still active in mid-August, while copious spermatozoa were still seen in the epididymis of one male collected on 14 September. Clearly, most males are capable of fertilization into August and beyond, and Gaskin *et al.* (1984) reported observing mating behaviour from May to September. The foetal growth curve presented by Read & Hohn (1995) suggests that successful conceptions do not occur later than the second week in July.

Temporal variation in conception data presumably can be described by a normal distribution (Fig. 8). By definition, testicular activity must encompass the entire span of successful conception and marks the boundaries of the mating season (i.e. tails of the curve). Selection should favour maximal testicular activity for the entire duration of the conception period, in the event that

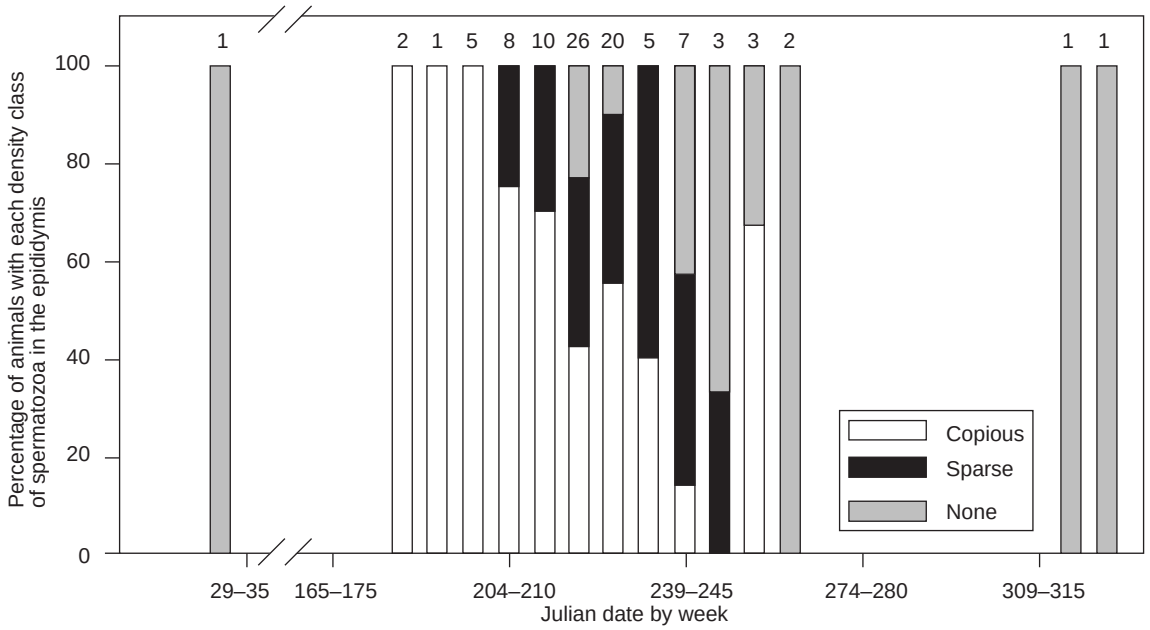

Fig. 7. Weekly frequencies of various densities of spermatozoa in the epididymides of harbour porpoises collected from the Bay of Fundy and Gulf of Maine from 1984–1995.

some females ovulate early or late in the breeding season or other females fail to conceive during their first oestrus cycle. The foetal growth curve ($n = 48$) presented by Read & Hohn (1995) includes two or three outliers that would correspond to early or late conceptions. Gaskin & Blair (1977) also reported the occasional capture of neonates in August, implying that in a few cases, conception occurs as late as September, as the duration of gestation is approx. 10.6 months (Read, 1990). The shape of the left side of the curve in Fig. 8 could be symmetrical or skewed, but cannot be accurately represented until testicular samples are collected from February to June. The mating season for porpoises in the Bay of Fundy/Gulf of Maine therefore spans from mid-June (and possibly earlier), until mid-August, but most conceptions occur from late June to mid-July. Testes of other cetaceans such as long-finned pilot whales (Desportes, Saboureau & Lacroix, 1993) and spotted dolphins (Hohn, Chivers & Barlow, 1985) never regress to the degree seen in the harbour porpoise, in concordance with the female reproductive cycle. For example, in spotted dolphins, there are two distinct calving seasons and some calves are born year-round (Hohn *et al.*, 1985). A few females may ovulate at any given time throughout the year, so year-round spermatogenetic activity in males is expected.

The degree of testicular regression varies between individual harbour porpoises collected at the same time of year (Figs 5 & 6). This has also been observed in long-finned pilot whales (Desportes *et al.*, 1993). Some of this variation could arise from annual differences in the timing of the onset of seasons. In a 4-year study by Read (1990), foetuses were consistently detected *in utero* by 6–12 August, and no foetuses were observed before this, suggesting a fairly constant date of conception each year. Little variation between individual animals was observed during the first 3 weeks of July, when most females were ovulating. At this period, over 95% of tubules of all males contained spermatozoa and spermatocytes/round spermatids (Figs 5 & 6). However, as the summer progressed, variation observed in the stage of regression increased. This suggests that the differences observed between individual males have evolved in response to a trade-off; the costs of maintaining active testes and associated behaviours *vs* the probability of successful fertilization. Gaskin *et al.* (1984) remarked on the large energetic expense required to maintain testes of 4% total body mass. The chances of successfully fertilizing a female after the second week in July decrease as the season progresses, so some males may be unable to continue to expend the energy required to remain reproductively active. This hypothesis could be tested by examining the body condition of individual males and determining whether porpoises with greater energy stores are able to prolong their period of reproductive activity.

Changes in testicular dimensions mirror changes in testicular histology; testicular mass and length are at a maximum during peak spermatogenetic activity and then decrease as tubules regress. Thus, testicular mass is a good indicator of breeding season. In harbour porpoises, testicular mass decreases 3.5 times from peak activity to the regressed state, whereas only a 1.5-fold change was reported in long-finned pilot whales (Desportes *et al.*, 1993). The testes of spotted dolphins (Hohn *et al.*, 1985) and striped dolphins (Miyazaki, 1977) do not fully regress either.

Spermatogenesis in harbour porpoises proceeds in helical waves along the seminiferous tubules. Each segment of the wave represents a different stage of germ

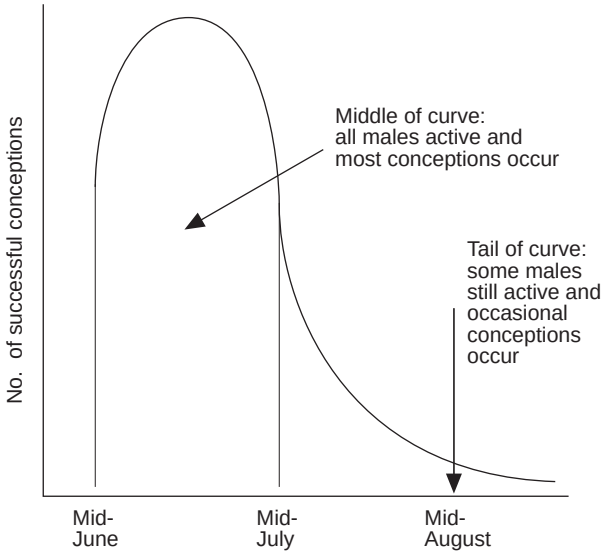

Fig. 8. A schematic representation of the distribution of successful conceptions in harbour porpoises from the Bay of Fundy and Gulf of Maine.

cell maturation (see Neimanis, 1996). In histological sections of testes in various stages of regression, the pattern of the disappearance of germ cells is consistent with the wave-like nature of spermatogenesis. Once spermatogonia stop dividing or disappear, spermatocytes, spermatids and spermatozoa can no longer develop from these cells. However, germ cells in various stages of spermatogenesis from previously initiated waves persist and may continue to differentiate. In concordance with this hypothesis, examination of regressed testes revealed that spermatocytes and round spermatids disappeared first and spermatozoa persisted the longest. A few spermatozoa also persisted in the epididymis, but eventually all spermatocytes, spermatids and spermatozoa disappeared. In porpoises, it is not known how the cessation of spermatogenesis is achieved, but several events co-ordinated by hormonal cues probably contribute to this phenomenon. For example, Hsueh *et al.* (1996) found that in seasonally breeding hamsters, testicular regression was accompanied by an increase in testicular cell apoptosis, or programmed cell death. In a review article, Heindel & Treinen (1989) discuss the structural and sustentacular roles of Sertoli cells for developing germ cells. Spermatogenesis may therefore be halted directly via germ cell apoptosis and/or hormonal cues that prevent spermatogonia from dividing. Alternatively, spermatogenesis may be stopped indirectly if Sertoli cells cease to maintain their supportive role for germ cells.

Although regressive changes have been described in the structure and size of testes, the physiological mechanisms driving these seasonal changes have not been examined in the harbour porpoise. These mechanisms are probably similar to those operating in other temperate zone mammals. Spermatogenesis in mammals is regulated by gonadotrophins (luteinizing hormone (LH) and follicle stimulating hormone (FSH)), as well as androgens (testosterone) (Russell *et al.*, 1990). Seasonal breeders in temperate latitudes receive external cues (primarily photoperiod) that synchronize endogenous reproductive cycles (Martinet, Ortavant & Courot, 1984; Lincoln, 1992). For many species (e.g. deer, Lincoln, 1992; sheep, Almeida & Lincoln, 1984), photoperiod affects melatonin levels that directly influence the pulsatile secretion of LH releasing hormone (LHRH) (Lincoln, 1992). LHRH is responsible for controlling gonadotrophin concentration. As pulsatile secretion of LHRH decreases, testicular activity decreases (Lincoln & Short, 1980). Mammals do not require constant exposure to daylight, but only need to sample photoperiod occasionally throughout the day, as does a porpoise surfacing for air (Sørensen & Kinze, 1994).

Finally, the question of whether seasonal breeding may contribute to the evolution of large testes of harbour porpoises should be addressed. Although mating behaviour has only been observed occasionally in this species (Gaskin & Blair, 1977), the reverse sexual dimorphism and large ratio of testicular mass to body mass suggests that male porpoises are sperm competitors and multiple mating by females is common (Neimanis, 1996; Read & Tolley, 1997). Males with larger testes are believed to be favoured in these systems because they can produce proportionately more sperm. This increase in sperm production allows them to either increase their number of inseminations or dilute semen in female reproductive tracts from previous matings. If testes are active for only a relatively brief period (e.g. a few months *vs* year-round), males can invest considerable energy to maximize testicular size and activity during this time, at levels that would be costly year-round. The complete regression of testicular activity and considerable decrease in testicular size following the breeding season suggests that seasonal reproduction may contribute to the large testicular size of harbour porpoises.

To examine seasonal reproduction in harbour porpoises in more detail, future studies should focus on the period of testicular recrudescence and the physiological mechanisms underlying seasonal changes in testes. Descriptions of recrudescence will require access to samples from mature males during the spring and early summer. Few samples are available from porpoises killed in commercial fisheries in the Bay of Fundy or Gulf of Maine during this period. Changes in testicular dimensions can be monitored via ultrasound, but this requires access to males in captivity over an extended period. Blood samples are needed to evaluate hormone concentrations for elucidation of physiological mechanisms of seasonal reproduction, but they must be longitudinal samples because of the pulsatile nature of gonadotrophins (Kirby, 1990). Porpoises temporarily housed in captivity for rehabilitation provide an excellent opportunity to examine these parameters and rehabilitators should take advantage of such situations in the future.

Acknowledgements

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Life History of Small Cetaceans in the Northwest Atlantic

Appendix 3

Autumn food habits of harbor porpoises, *Phocoena phocoena*, in the Gulf of Maine

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Abstract.—This study describes the stomach contents of 95 harbor porpoises (*Phocoena phocoena*) killed in groundfish gill nets in the Gulf of Maine between September and December, 1989–94. The importance of prey was assessed by frequency of occurrence, numerical proportion, and proportion of ingested mass. Atlantic herring (*Clupea harengus*) was the most important prey, occurring in 78% of noncalf porpoise stomachs and contributing 44% of ingested mass. Pearlsides (*Maurolicus weitzmani*), silver hake (*Merluccius bilinearis*), and red and white hake (*Urophycis* spp.) were common prey items. There were no significant differences among diets of sex and maturity groups, but the calf diet differed significantly from adults in number of Atlantic herring eaten and the total mass of food consumed. At four to seven months of age, calves were eating pearlsides, small silver hake, and euphausiids (*Meganyctiphanes norvegica*) while still nursing.

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Harbor porpoises (*Phocoena phocoena*) from the Bay of Fundy and Gulf of Maine are believed to comprise a single population, hereafter referred to as the Gulf of Maine population (Palka et al., 1996; Wang et al., 1996). To date, studies of the food habits of this population have been restricted to samples collected in the Bay of Fundy during summer, where porpoises feed primarily on Atlantic herring (*Clupea harengus*; Smith and Gaskin, 1974; Recchia and Read, 1989; Smith and Read, 1992). Many porpoises leave the Bay of Fundy in fall, moving southward into the Gulf of Maine (Gaskin, 1977; Gaskin, 1984; Read and Westgate, 1997). During winter, a portion of the population disperses over the continental shelf from New England to North Carolina (Polacheck et al., 1995; Read et al., 1996).

Because of their small size and limited energy stores, harbor porpoises must remain close to food resources to avoid starvation (Koopman, 1994). Moreover, their unusual life history incurs high energetic costs; most females attain sexual maturity at three years of age and give birth to a calf each year (Read and Hohn, 1995). Lactation lasts for at least eight months; thus mature females spend most of their lives simultaneously pregnant and lactating. This intensive reproductive schedule requires calves to become nutritionally independent at a relatively early age, usually before the end of their first year (Smith and Read, 1992).

Large numbers of harbor porpoises are killed each year in gill nets in the Bay of Fundy, Gulf of Maine, and Mid-Atlantic Bight (Read and Gaskin, 1988; Read et al., 1993; Bravington and Bisack, 1996). For the Gulf of Maine, the estimated average annual harbor porpoise bycatch for 1990 to 1995 was 1800 (Bisack[1]). Little is known about the process by which porpoises become entangled in gill nets, and thus efforts are hampered in mitigating this conservation problem. Porpoises may become entangled because they feed on fish species targeted by the fish-

[1] Bisack, K. 1996. Harbor porpoise bycatch estimates in the U.S. Gulf of Maine sink gillnet fishery: 1994 and 1995. Paper presented to the International Whaling Commission Scientific Committee Meeting in Aberdeen, Scotland, June 1996 (in review).

ery or because they feed on the same prey as the target species.

In this paper, we examine the stomach contents of harbor porpoises in the Gulf of Maine during autumn and investigate dietary differences amongst various sex and maturity categories. Our main objectives were to elucidate seasonal changes in the harbor porpoise diet and expand our knowledge of the dynamics between porpoises and their prey that may be responsible for entanglement of porpoises in gill nets.

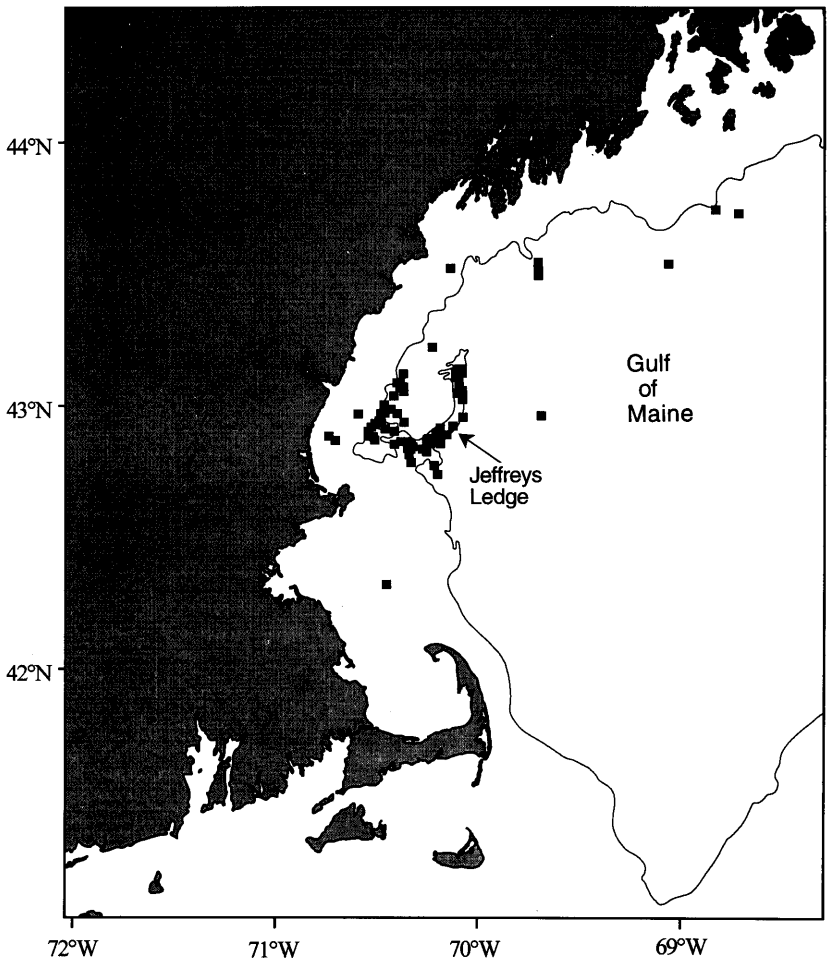

Figure 1

Capture locations of harbor porpoises taken during the autumn (1989–94) in the Gulf of Maine sink gillnet fishery and used in this analysis of food habits. The isobath shown is 91.4 m (50 fathoms).

Methods

Sample collection

The sample consisted of 95 porpoises killed in gill nets during autumn (1 September– 31 December) of 1989 and 1991–94. All porpoises were captured in bottom tending gill nets set for groundfish, principally cod (*Gadus morhua*), pollock (*Pollachius virens*), goosefish (*Lophius americanus*), and several species of flatfish. Most porpoises were taken in the vicinity of Jeffreys Ledge in the west central Gulf of Maine, at water depths between 35 and 185 m (Fig. 1). All samples were obtained by fisheries observers working onboard gillnet vessels. Observers were instructed to retain whole porpoise carcasses whenever possible, but when sea conditions or other factors prevented retention of carcasses, observers excised stomachs in the field. Carcasses and excised stomachs were frozen after the vessels returned to shore (usually 12–48 hours post mortem) for later examination.

On the basis of age (determined from dentinal growth layers and body length; see Read and Hohn, 1995) and reproductive condition (determined by examination of gonads and mammary glands; see Read and Hohn, 1995), porpoises were classified to the following sex, maturity, and reproductive categories: porpoises were considered calves (less than one year of age, not fully weaned), juveniles (older than one year but sexually immature), or sexually mature. The sex and maturity composition of the sample was as follows: (males and females combined) calves = 13; female juveniles = 12; male juveniles = 18; female mature adults = 10; male mature adults = 34; and unknown sex or maturity = 8. Because sample sizes were small, pregnant (n=4), simultaneously pregnant and lactating (n=5), and resting adult females (n=1) were pooled in the "mature female" group for statistical analyses. However, to facilitate comparisons with the findings of Recchia and Read (1989), data for lactating and nonlactating mature females are also presented separately.

Prey identification

The contents of all three stomach chambers were examined in the laboratory. Intact prey were removed first, then loose flesh was decanted. The remaining stomach contents were poured through a 1-mm metal sieve to separate hard parts from liquefied digesta. Solid prey remains used for identification were separated from other skeletal remains by hand. Structures used to identify partially digested food items included sagittal otoliths, dentary bones, and skulls

of teleosts; lower mandibles ("beaks") from cephalopods; tooth cusp plates ("combs") from agnathans; and exoskeletons and eyes from crustaceans. Prey items were identified with the aid of a laboratory reference collection and published guides, including those of Bigelow and Schroeder (1953), Clarke (1986), Harkonen (1986), and Scott and Scott (1988).

Prey importance

Relative food importance in the autumn diet of the harbor porpoise was determined by 1) frequency of occurrence, 2) proportion of numerical abundance, and 3) proportion of total ingested mass. Frequency of occurrence is the percentage of porpoise stomachs containing a particular food type. Proportion of numerical abundance is the number of individuals of a prey species recovered from all stomachs, divided by the total number of all prey from all stomachs. The number of individuals from each fish species in each stomach was determined by summing the number of intact fish and half the number of free otoliths. The number of either upper or lower beaks (whichever were more abundant) from each species was used to determine the number of squid present.

Proportion of prey mass is the percentage of total prey mass in the stomach at the time of death that was represented by a particular species. Reconstituted mass, or the mass of prey prior to ingestion, rather than the existing mass of partially digested prey, was used in this calculation. Reconstituted prey masses were estimated from body lengths of intact prey and the lengths of otoliths or cephalopod beaks (Table 1). If a stomach contained more than 25 otoliths from the same species, all otoliths from that species were counted, and a subsample of 25 was randomly selected and measured. Otoliths were scored on a scale from 0 (undamaged otoliths re-

Table 1

Equations used to estimate length and mass of harbor porpoise prey. ML = mantle length; H = hood length; M = mass; FL = fork length; OL = otolith length; LRL = lower rostral length; and SL = standard length. Length is in millimeters and mass is in grams.

Prey species	Equations	Source
Bathypolypus arcticus	$ML = 15.4 + 12.28\,H$	Clarke, 1986
(North Atlantic octopus)	$\ln M = 1.06 + 2.55 \ln H$	Clarke, 1986
Clupea harengus	$FL = 69.23\,OL - 27.48$	Recchia and Read, 1989
(Atlantic herring)	$\log M = 3.12 \log FL - 5.41$	Recchia and Read, 1989
Gadus morhua	$\ln(FL/10) = 3.3138 + 1.6235 \ln(OL/10)$	Hunt, 1992
(Atlantic cod)	$M = 0.0124\,(FL/10)^{2.93}$	Bowen and Harrison, 1994
Illex illecebrosus	$\ln M = 1.773 + 2.4 \ln LRL$	Clarke, 1962
(Northern short-fin squid)		
Loligo pealei	$\log ML = 1.767 + 1.4 \log LRL$	Gannon et al., 1997b
(Long-fin inshore squid)	$M = 0.25662\,(ML/10)^{2.1582}$	Lange and Johnson, 1981
Maurolicus weitzmani[1]	$FL = 9.82 + 28.75\,OL$	Harkonen, 1986
(Weitzman's pearlsides)	$M = 0.3737\,OL^{2.503}$	Harkonen, 1986
Merluccius bilinearis	$FL = 20.9\,L - 0.41$	Recchia and Read,1989
(Silver hake)	$\log M = -2.26 + 3.08 \log(FL/10)$	Kohler et al., 1970
Peprilus triacanthus[2]	$SL = -9.15919 + 25.01871\,OL$	Present study (r^2=0.983)
(Butterfish)	$\log M = -0.67576 + 3.222 \log OL$	Present study (r^2=0.924)
Pollachius virens	$\ln(FL/10) = 3.251 + 1.6251 \ln(OL/10)$	Harkonen, 1986
(Pollock)	$M = 0.0134\,(FL/10)^{2.94}$	Bowen and Harrison, 1994
Scomber scombrus	$FL/10 = 7.33\,OL + 0.37$	Recchia and Read, 1989
(Atlantic mackerel)	$M = 0.00756\,(FL/10)^{3.082}$	Kulka and Stobo, 1981
Sebastes spp.[3]	$FL = 16.165\,L^{1.224}$	Harkonen, 1986
(Rockfish)	$M = 0.0741\,OL^{3.295}$	Harkonen, 1986
Urophycis spp.[4]	$FL/10 = 1.525\,OL^{1.1456}$	Clay and Clay, 1991
(Red and white hake)	$M = 0.003998\,(FL/10)^{3.1718}$	Clay and Clay, 1991

[1] Taxonomy of the genus *Maurolicus* has been revised recently (Parin and Kobylansky, 1996). The equations used to estimate *M. weitzmani* size are those given by Harkonen (1986) for *M. muelleri*.
[2] Standard length range: 49–153 mm; weight range: 3–104 g; n = 44.
[3] Equations given by Harkonen (1986) for *S. marinus*.
[4] Equations given by Clay and Clay (1991) for *U. tenuis*.

trieved from skulls) to 5 (severely degraded, free otoliths) following the methods of Recchia and Read (1989). Otoliths categorized as 3 or higher were not used in size estimations, unless no undamaged otoliths were present. When only damaged otoliths from a particular prey species were present in a porpoise stomach, the available skeletal structures were measured; consequently the reconstituted prey mass for that stomach may have been underestimated (see Jobling and Breiby, 1986; Sekiguchi and Best, 1997)

These three measures of prey importance were applied to data from the 82 noncalf porpoises as a group and to each sex and maturity class. Food habit studies in which different methods are used can yield widely disparate results, making it difficult to draw comparisons between studies (Gannon et al., 1997a, 1997b). Because one of the primary objectives of this research was to obtain information on seasonal changes in the diet, it was important for these data to be treated in a manner similar to those of Recchia and Read (1989) and Smith and Read (1992).

Results

Overall sample

Table 2 lists the numbers and mean sizes of 15 prey taxa recovered from the 95 porpoise stomachs. Atlantic herring (78%), silver hake (*Merluccius bilinearis*, 68%), pearlsides (*Maurolicus weitzmani*, 38%), and red and white hake (*Urophycis* spp., 29%) occurred most frequently in the stomachs of the 74 noncalf porpoises (Table 3). Atlantic herring represented only 7% of the food by proportion of numerical abundance but accounted for 44% of ingested mass. Pearlsides accounted for 67% of food by proportion of numerical abundance but only 3% by ingested mass, owing to their small size. The unknown fish present in porpoise stomachs may have been alewives (*Alosa pseudoharengus*) but this could not be determined with certainty. Both red and white hake (*Urophycis chuss* and *U. tenuis*) were present; however it is difficult to differentiate between small, eroded otoliths from red and white hake, therefore all *Urophycis* otoliths were grouped together. Atlantic hagfish (*Myxine glutinosa*) and euphausiids (*Meganyctiphanes norvegica*) were included in analyses of frequency of occurrence only because the numerical abundance and mass of these two species were difficult to estimate. To allow comparisons to be drawn with the summer diet, data from Recchia and Read (1989) are also given in Table 3.

Figure 2 shows length-frequency distributions for the three most abundant prey: pearlsides, silver hake, and Atlantic herring. On average, Atlantic herring was the largest prey consumed by length (254 mm ±36 SD) with a range from 159 to 339 mm. The average fork length

Table 2

Number and mean sizes of food items present in the stomachs of harbor porpoises sampled in the Gulf of Maine during autumn. ML = mantle length, FL = fork length, and SL = standard length. Present = present in porpoise stomach contents but numerical abundance not determined.

Food item	*n*	Length measurement	Mean length ± SD (mm)	Mean mass ± SD (g)
Bathypolypus arcticus	1	ML	52	48
Clupea harengus	507	FL	254 ± 36	133 ± 56
Gadus morhua	5	FL	241 ± 133	137 ± 201
Illex illecebrosus	18	ML	—	55 ± 22
Loligo pealei	8	ML	129 ± 30	68 ± 29
Maurolicus weitzmani	5898	FL	50 ± 4	0.9 ± 0.2
Meganyctiphanes norvegica	present	—	—	—
Merluccius bilinearis	1605	FL	164 ± 96	65 ± 88
Myxine glutinosa	present	—	—	—
Peprilus triacanthus	38	SL	97 ± 12	24 ± 7
Pollachius virens	76	FL	195 ± 101	136 ± 130
Scomber scombrus	15	FL	224 ± 53	127 ± 91
Sebastes spp.	47	FL	37 ± 3	0.7 ± 0.2
Urophycis spp.	474	FL	159 ± 146	111 ± 172
Unknown fish	4	—	—	—
Milk	present	—	—	—

Table 3

Relative food importance, measured by frequency of occurrence (%FO), numerical proportion (%Num), and proportion of total mass (%Mass), in the diet of noncalf harbor porpoises during autumn in the Gulf of Maine (present study) and summer in the Bay of Fundy (Recchia and Read, 1989).

	Gulf of Maine			Bay of Fundy		
Prey	%FO	%Num	%Mass	%FO	%Num	%Mass
Alosa pseudoharengus	0	0	0	3	<1	—
Bathypolypus arcticus	1	<1	<1	3	<1	<1
Clupea harengus	78	7	44	88	44	64
Gadus morhua	4	<1	<1	14	14	14
Illex illecebrosus	10	<1	<1	6	1	<1
Loligo pealei	4	<1	<1	1	<1	<1
Macrozoarces americanus	0	0	0	2	<1	—
Maurolicus weitzmani	38	67	3	0	0	0
Meganyctiphanes norvegica	12	—	—	—	—	—
Merluccius bilinearis	68	16	22	41	33	19
Myxine glutinosa	7	—	—	—	—	—
Peprilus triacanthus	12	1	1	0	0	0
Pollachius virens	7	1	2	0	0	0
Pleuronectes americanus	0	0	0	<1	<1	—
Scomber scombrus	9	<1	1	6	1	2
Sebastes spp.	11	<1	<1	0	0	0
Urophycis spp.	29	7	26	13	3	2
Unknown fish	1	<1	<1	26	4	—

for silver hake was 163 mm (±95 SD), with the length-frequency distribution showing a strong peak between 30 and 55 mm and another peak between 180 and 205 mm. The mean length of pearlsides was 50 mm (±4 SD), ranging from 40 to 62 mm.

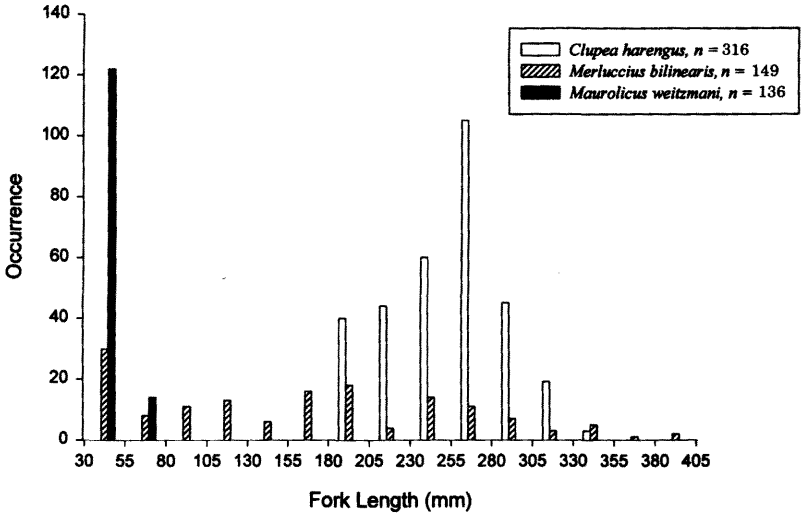

Figure 2

Length-frequency distributions of *Clupea harengus* (Atlantic herring), *Merluccius bilinearis* (silver hake), and *Maurolicus weitzmani* (pearlsides) eaten by harbor porpoises during autumn (1989–94) in the Gulf of Maine.

Diet of sex and maturity categories

The stomach contents of calves differed substantially from those of nutritionally independent porpoises. Pearlsides, silver hake, and euphausiids each occurred in more than half (7/13) of the calf stomachs (Table 4). Pearlsides (72%) and silver hake (26%) were the most numerous prey in calf stomachs and accounted for 53% and 27% of the calf diet by proportion of total mass, respectively. Only 11% of the ingested mass in calf stomachs comprised Atlantic herring (<1% of numerical abundance). Although euphausiids and milk were common in the calf diet, they were excluded from the analyses of

Table 4

Relative food importance, measured by frequency of occurrence (%FO), numerical proportion (%Num), and proportion of total mass (%Mass), in the autumn harbor porpoise diet. Numbers in parentheses refer to frequency of occurrence values found by Smith and Read (1992) for the summer calf diet of the same population in the Bay of Fundy portion of their range.

	Calves (n=13)			Juvenile males (n=18)			Juvenile females (n=12)			Mature males (n=34)			Mature females (n=10)		
Food items	%FO	%Num	%Mass	%FO	%Num	%Mass	%FO	%Num	%Mass	%FO	%Num	%Mass	%FO	%Num	%Mass
Bathypolypus arcticus	0 (0)	0	0	0	0	0	0	0	0	3	<1	<1	0	0	0
Clupea harengus	15 (4)	<1	11	89	8	44	75	38	66	79	6	66	70	20	35
Gadus morhua	0 (0)	0	0	0	0	0	0	0	0	6	<1	1	10	1	1
Illex illecebrosus	0 (0)	0	0	0	0	0	17	1	<1	3	<1	<1	20	1	<1
Loligo pealei	0 (0)	0	0	11	<1	1	0	0	0	3	<1	1	0	0	0
Maurolicus weitzmani	54 (0)	72	53	39	42	1	17	3	<1	41	87	7	30	7	<1
Meganyctiphanes norvegica	54 (63)	—	—	22	—	—	17	—	—	9	—	—	0	—	—
Merluccius bilinearis	54 (0)	26	27	78	38	31	67	50	32	62	4	19	70	65	37
Myxine glutinosa	8 (0)	—	—	0	0	0	8	—	—	3	—	—	40	—	—
Pandalus montagui	0(4)	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Peprilus triacanthus	15 (0)	<1	2	11	1	1	8	1	<1	21	1	1	0	0	0
Pollachius virens	0 (0)	0	0	6	<1	1	0	0	0	9	1	2	20	4	10
Scomber scombrus	0 (0)	0	0	6	<1	1	8	1	<1	15	<1	2	0	0	0
Sebastes spp.	23 (0)	1	<1	17	3	<1	25	1	<1	6	<1	<1	0	0	0
Urophycis spp.	15 (0)	1	7	39	7	21	33	6	1	24	1	1	20	2	18
Unknown fish	0(8)	0	0	0	0	0	0	0	0	0	0	0	10	2	—
Milk	23 (29)	—	—	0	0	0	0	0	0	0	0	0	0	0	0

numerical and mass proportions because it was not possible to quantify their contributions. To facilitate comparisons between seasons, Table 4 also contains data from Smith and Read (1992) on the summer diet of calves from the Bay of Fundy.

Significant differences in stomach contents existed among the five sex and maturity groups regarding the mass proportion of Atlantic herring (no. of cases=87, df=4, K [the Kruskal-Wallis test statistic] =16.077, P=0.003), the number of Atlantic herring present (Table 5; K=18.313, P=0.001), and the existing mass of all stomach contents (K=11.594, P=0.021). The stomach contents of calves were the most divergent of these three categories and when the Kruskal-Wallis tests were repeated with calves excluded, none of the results were significant (Atlantic herring mass proportion: no. of cases=74, df=3, K= 4.284, P=0.232; number of herring: K=1.739, P=0.628; existing mass of stomach contents: K=0.270, P=0.855). No other significant dietary differences were noted between any of the sex and maturity groups at the α = 0.05 level.

Qualitative comparisons between lactating and nonlactating mature females revealed that the former had higher frequencies of occurrence for most prey (Table 6). The proportion of total reconstituted mass represented by herring was much higher in nonlactating females. The mass proportions of silver hake and red and white hake were higher in lactating females. It is also interesting to note that three of five lactating females ate hagfish, a frequency far greater than that of any other sex and maturity group.

Discussion

Atlantic herring was the most important prey of harbor porpoises in the Gulf of Maine during autumn; silver hake, red and white hake, and pearlsides were of secondary importance. Although herring was the most significant prey for porpoises in autumn, it was not as dominant as in the summer diet in the Bay of Fundy (Recchia and Read, 1989). Recchia and Read (1989) found Atlantic herring in 88% of noncalf porpoise stomachs, contributing 64% of ingested prey mass; we found herring in 78% of stomachs from noncalves, contributing 44% of prey mass. The relative importance of silver hake, of red and white hake, and of pearlsides was greater in the autumn than in the summer. For example, pearlsides occurred in 38%

Table 5

Prey consumption by harbor porpoises of different maturity and reproductive conditions caught incidentally in Gulf of Maine sink gill nets during autumn 1989–94 (mean ± standard deviation). *Clup.* = *Clupea harengus*, *Maur.* = *Maurolicus weitzmani*, *Mer.* = *Merluccius bilinearis*, and *Uroph.* = *Urophycis* spp.

Porpoise groups	Average mass of individual prey				Average no. of individual prey				Average mass of stomach contents		Average no. of prey taxa
	Clup.	*Maur.*	*Mer.*	*Uroph.*	*Clup.*	*Maur.*	*Mer.*	*Uroph.*	Existing	Reconstituted	
Calves	57 ±40	0.92 ±0.16	19 ±30	42 ±59	0.3 ±0.9	106.5 ±269.6	38.2 ±74.2	1.5 ±5.0	33 ±23	209 ±327	2.4 ±1.4
Juvenile males	131 ±35	0.95 ±0.20	51 ±56	108 ±105	4.2 ±5.0	21.3 ±46.8	19.1 ±38.7	3.6 ±10.8	284 ±288	1304 ±1036	3.2 ±1.3
Juvenile females	140 ±37	0.95 ±0.01	82 ±78	23 ±41	6.9 ±7.5	0.6 ±1.7	9.0 ±19.5	1.0 ±1.8	363 ±356	1389 ±1166	2.8 ±2.4
Mature males	125 ±30	0.95 ±0.05	73 ±103	50 ±46	8.1 ±10.6	117.0 ±372.3	5.6 ±9.5	0.6 ±1.6	274 ±285	1506 ±1526	2.9 ±1.5
Mature females	107 ±28	0.87 ±0.13	77 ±65	339 ±360	4.4 ±7.6	1.6 ±3.2	14.3 ±35.0	0.5 ±1.3	343 ±371	1378 ±1996	2.8 ±1.4

of porpoise stomachs in the autumn, representing 67% of numerical abundance and 3% of food mass but were absent from the summer diet. Recchia and Read (1989) found 11 prey taxa in the stomachs of 127 noncalf porpoises; we found 15 taxa in 82 noncalf stomachs. These results suggest that the diet of this population becomes more diverse as porpoises move out of the Bay of Fundy and into the Gulf of Maine. At the present time, we do not know whether these changes reflect seasonal differences in prey availability, interannual variability in prey populations, or choice on the part of foraging porpoises. Nevertheless, Atlantic herring remains the single most important prey of harbor porpoises in the Gulf of Maine during the autumn.

The size range of prey in the noncalf porpoise diet is larger in fall than in summer (Recchia and Read, 1989). Porpoises continue to eat large prey during autumn, such as adult herring and silver hake, but also eat a substantial number of smaller herring, silver hake, pearlsides, and red and white hake. The large standard deviations in Tables 2 and 5 reflect the wide range of prey sizes eaten.

With the exception of calves, the diet of porpoises did not vary significantly with age or sex. None of the comparisons of forestomach content mass, individual prey mass, or numbers of prey among the four noncalf categories yielded significant differences. Although previous studies of other marine mammal species have found measurable dietary differences between lactating and nonlactating adult females (Bernard and Hohn, 1989; Cockcroft and Ross, 1990; Cheal and Gales, 1991; Kastelein et al., 1993; Young and Cockcroft, 1994; Hobson et al., 1997; Robertson and Chivers, 1997), small sample sizes in this study prevented detailed investigation of potential dietary changes associated with changes in female reproductive condition. Therefore, the findings on diets of lactating and nonlactating mature females should be viewed with caution.

Table 6

Relative food importance, measured by frequency of occurrence (%FO), numerical proportion (%Num), and proportion of total mass (%Mass), in the autumn diets of lactating and nonlactating mature female harbor porpoises.

Prey	Lactating (n=5)			Nonlactating (n=5)		
	% FO	% Num	% Mass	% FO	% Num	% Mass
Clupea harengus	80	7	14	60	52	71
Gadus morhua	20	1	1	0	0	0
Illex illecebrosus	40	1	<1	0	0	0
Maurolicus weitzmani	0	0	0	60	25	<1
Merluccius bilinearis	80	87	52	60	13	10
Myxine glutinosa	60	—	—	20	—	—
Pollachius virens	20	1	5	20	11	19
Urophycis spp.	40	3	28	0	0	0

At four to seven months of age (Read and Hohn, 1995), calves eat a variety of solid foods and continue to supplement their diet by nursing. The large standard deviations for calves in Table 5 may be an indi-

cation that some porpoise calves begin weaning sooner than others. The species composition found in the stomachs of calves in autumn begins to resemble that of older animals. However, the proportions of prey types and sizes of prey differ from those of adults. In autumn, calves eat a greater proportion of pearlsides and euphausiids than do older animals, and the sizes of Atlantic herring and silver hake are smaller than those eaten by older porpoises. Pearlsides, euphausiids, juvenile silver hake, juvenile herring, and juvenile red and white hake appear to be important in the "transitional diet" of calves, as they learn to forage independently. Calves eat a larger quantity and greater diversity of solid food in autumn than in the summer (Smith and Read, 1992). Our observations support and extend the findings of Smith and Read (1992), who suggested that porpoise calves eat euphausiids while their mothers are feeding on other euphausiid predators.

Although harbor porpoises prey on some of the groundfish species targeted by the sink gillnet fishery in the Gulf of Maine, these species contribute just a small fraction of the overall diet. Furthermore, the size range of groundfish consumed by porpoises is much smaller than that targeted by the gillnet fishery because porpoises feed on only the juvenile age classes of those commercial species. The prey that represent the bulk of the porpoise diet (i.e. Atlantic herring, silver hake, and pearlsides) are important forage items for groundfish targeted by the sink gillnet fishery (Langton, 1982). These dietary similarities may lead to overlap between the distributions of groundfish and porpoises, leading both to be caught in the same nets. Silver hake found in porpoise stomachs were highly digested (only 0.1% of silver hake were intact), indicating that they had been consumed some time prior to entanglement. In contrast, herring were often found in a relatively undigested state (15.8% were intact), indicating that many porpoises had been feeding on herring at, or just before, the time of entanglement.

Several potential biases should be kept in mind when interpreting these results. First, all the porpoises we examined had been killed in gill nets anchored to the ocean floor. This capture method may have led to a bias towards demersal prey and against pelagic prey. Without comparable samples collected near the surface, it is not possible to fully address this potential bias. The samples of Recchia and Read (1989) and Smith and Read (1992) may be similarly biased because both studies also obtained samples from porpoises killed in sink gill nets. Second, differential digestion and retention of hard parts are unavoidable in studies of marine mammal stomach contents. Consequently, the importance of species that are resistant to digestion, or that accumulate in porpoise stomachs, will be overestimated. Without empirical data on digestion times for each prey species, it is not possible to evaluate this potential bias fully.

A third potential source of bias arises from the difficulty in discriminating between primary prey (consumed by porpoises) and secondary prey (consumed by porpoise prey). For example, it is possible that small organisms, such as pearlsides, euphausiids, and juvenile silver hake, were secondarily introduced into the porpoise stomach contents. Careful examination of species co-occurrences in porpoise stomachs can provide insights into whether these small organisms were actually eaten by the porpoises. Because many porpoise prey are euphausiid predators (Bigelow and Schroeder, 1953; Langton, 1982; Scott and Scott, 1988), it is difficult to evaluate the likelihood of secondary consumption of euphausiids. However, two calves had euphausiid remains but no other solid food in their stomachs, indicating that they had consumed the euphausiids directly. One calf had pearlsides remains and a herring in its stomach; herring are not considered predators of pearlsides (Bigelow and Schroeder, 1953; Scott and Scott, 1988). Five calves had remains of pearlsides together with juvenile red, white, and silver hake less than 57 mm in length, too small to be predators of pearlsides. We interpret the co-occurrence of pearlsides and juvenile gadiforms in stomachs of calves as an indication of their preference for small prey, rather than as the presence of predators and secondary prey in their stomachs. Among older porpoises, one individual had pearlsides with no other food remains; four had pearlsides and herring; one had 13 pearlsides (totaling 16 grams), a 14-gram butterfish, and a herring; and one had 1100 pearlsides (1052 g) and one butterfish (6 g). Therefore, it is apparent that porpoises do indeed prey directly on euphausiids, pearlsides, and juvenile gadiforms.

In conclusion, the seasonal movements of harbor porpoises are accompanied by changes in diet. Seasonal movements of porpoises may, in fact, be driven by their need to maintain proximity to sufficient concentrations of prey. Assuming that there have not been any major shifts in prey availability between the previous study in the Bay of Fundy (Recchia and Read, 1989) and the present study, the diet of harbor porpoises in the Gulf of Maine during autumn appears to be more diverse than that of harbor porpoises in the Bay of Fundy during summer. The winter ecology of this population probably differs also because many porpoises are believed to leave the Gulf of Maine and Bay of Fundy region during this season. Further information on the diet of this popula-

tion in the winter and spring is required before we can fully assess the ecological relations between harbor porpoises and their prey in this system. We also suggest that further investigation of the ecological relations among Atlantic herring, groundfish, and harbor porpoises may provide information that will allow improved understanding of the causes of porpoise entanglement in gill nets and that will perhaps offer some insight into measures that may mitigate this problem.

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Life History of Small Cetaceans in the Northwest Atlantic

Appendix 4

Molecular Ecology (1999) **8**, S41–S54

Genetic structure of harbour porpoise *Phocoena phocoena* populations in the northwest Atlantic based on mitochondrial and nuclear markers

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Abstract

The harbour porpoise, *Phocoena phocoena*, experiences high levels of nonnatural mortality owing to interactions with commercial fisheries throughout its range. To accurately evaluate the significance of this bycatch, information on population structure is required. We have examined the population structure of this species in the northwest Atlantic Ocean using mitochondrial DNA (mtDNA) sequence and nuclear microsatellite data. Samples from four previously proposed summer breeding populations—the Gulf of Maine, eastern Newfoundland, the Gulf of St Lawrence and West Greenland—were analysed. Control-region sequences revealed a significant partitioning of genetic variation among most of these summer populations, indicating that northwest Atlantic harbour porpoises should not be considered one panmictic population. Analysis of females alone yielded the highest levels of population subdivision, suggesting that females are more philopatric than males. At least three management units may be defined for harbour porpoises in the northwest Atlantic based on these data. Analysis of six microsatellite loci failed to detect significant population subdivision. Male-mediated gene flow may maintain homogeneity among nuclear loci, while female philopatry is sufficient to produce a signal of population subdivision in the maternally inherited mtDNA genome. mtDNA analyses also indicate that winter aggregations of harbour porpoises along the US mid-Atlantic states comprise animals from more than one summer breeding population.

Keywords: bycatch, control region, management, microsatellite, stocks

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Introduction

Management policies and practices for marine mammals in US waters differ significantly from the management methods based on evolutionarily significant units (Waples 1991) and are more akin to those based on management units (Moritz 1994). In the USA, the Marine Mammal Protection Act of 1972 requires that each population of a marine mammal species present in US waters be maintained at a population size between the maximum net productivity level and carrying capacity (see Wade 1998 for details). The Marine Mammal Protection Act also mandates that 'population stocks' be maintained such that they remain a 'significant functioning element in the ecosystem of which they are a part' where a 'population stock' is defined as: 'a group of marine mammals of the same species or smaller taxa in a common spatial arrangement that interbreed when mature'. Thus, the Marine Mammal Protection Act provides for, and in fact mandates, management of marine mammals at levels below that of the species, but provides only vague direction as to how such management units are to be defined. Determining directly whether individuals in a group of marine mammals are interbreeding is difficult in the wild. Furthermore, we have a limited understanding of what barriers to

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movement and gene flow may be encountered by highly mobile creatures, such as porpoises. Thus, it is difficult to determine where one population boundary ends and the next one begins.

The harbour porpoise, *Phocoena phocoena*, is a small, delphinoid species found throughout north temperate and subarctic waters of the world. This species is primarily restricted to coastal waters, particularly during the breeding season. Throughout its range, this species experiences a high degree of incidental mortality, primarily as a result of entanglement in gillnets (Jefferson & Curry 1994). In US waters of the Gulf of Maine in the northwest Atlantic, the minimum estimated bycatch averaged 1833 animals per year between 1990 and 1995 (Bravington & Bisack 1996; Bisack 1997). This level of bycatch exceeds that allowable under federal law and exceeds the International Whaling Commission's (IWC) maximum recommended removal rate (IWC 1993). The high bycatch rate has raised considerable concern over the sustainability of this Gulf of Maine population. At one time, the US federal government was petitioned to list the population as threatened under the Endangered Species Act. The IWC has also expressed concern and has requested that member states reduce bycatch of this species (IWC 1991, 1992, 1993). In Canada, similar problems exist and the species is listed as threatened in the northwest Atlantic by the Committee on the Status of Endangered Wildlife (Gaskin 1992).

Management efforts for harbour porpoises in the northwest Atlantic are complicated by the fact that the species crosses international boundaries. In addition to US waters of the Gulf of Maine, during summer months harbour porpoises are common in coastal Canadian waters: in the Bay of Fundy, the Gulf of St Lawrence and around eastern Newfoundland, as well as in coastal waters of West Greenland. Bycatch occurs in all of these regions. Gaskin (1984) defined each of these four geographical regions in the northwest Atlantic—the Gulf of Maine/Bay of Fundy, the Gulf of St Lawrence, Newfoundland-Labrador and Greenland (western and southeastern)—as separate 'subpopulations'. These areas contain the highest density of porpoises in the northwest Atlantic during the summer months. The presence of porpoises in these regions is highly seasonal. Breeding is also highly seasonal and occurs during a relatively short period of time in the spring or summer. Female porpoises attain sexual maturity at ≈ 3.5 years and the majority breed every year (Read 1999). Calves remain with their mothers from 8 to 18 months, certainly for their first summer season (Gaskin 1992), and it is unlikely that a juvenile born in the Gulf of Maine, for example, would be found off Newfoundland that same summer. Therefore, biological and ecological evidence suggest that these four regions may serve as core areas in the northwest Atlantic where harbour porpoises forage and reproduce during the summer months. These population subdivisions have formed the basis for the majority of management discussion over the last decade.

The degree of mixing of animals from these four regions is unknown. Satellite telemetry data suggest that porpoises in the Bay of Fundy/Gulf of Maine region are relatively restricted in their movements (Read & Westgate 1997). However, harbour porpoises leave most of the summer breeding areas during winter months and it is unclear where they go. Significant increases in the number of porpoise strandings along the mid-Atlantic states in late winter and early spring (Polachek *et al.* 1995) suggest that at least some animals migrate south along the coast, but whether these are animals from the Gulf of Maine and/or from Canadian waters is unknown. The mid-Atlantic animals also experience incidental mortality in gillnets (Haley & Read 1993). There is a critical need to know from which summer population these animals originate, in order to accurately estimate the level of incidental mortality affecting the population(s).

Mandates to accurately quantify the 'biological significance' of bycatch have led to a critical need for an accurate picture of the population structure of harbour porpoises in the northwest Atlantic. Wang *et al.* (1996) conducted a restriction fragment length polymorphism (RFLP) analysis of mitochondrial DNA (mtDNA) isolated from harbour porpoises from three putative populations in the northwest Atlantic, including the Gulf of St Lawrence, Newfoundland and the Bay of Fundy. Their results suggest the presence of a weak cline in mtDNA genotype frequencies from the Bay of Fundy north to Newfoundland. The strongest support for population subdivision was present when females were analysed separately, suggesting some degree of philopatry by female porpoises (Wang *et al.* 1996). The purpose of this research was to further examine the validity of the four proposed subpopulations of harbour porpoises in the northwest Atlantic using genetic markers with a higher resolving power than mtDNA RFLP analysis, namely mtDNA control-region sequences and microsatellite markers, with the aim to augment baseline data available for management of the species.

Materials and methods

Samples

Tissue samples from 253 porpoises were collected from the four proposed summer breeding populations in the northwest Atlantic: the Gulf of St Lawrence, eastern Newfoundland, within the Gulf of Maine/Bay of Fundy, and West Greenland; and from a presumed wintering population along the mid-Atlantic United States (New Jersey to North Carolina) (Fig. 1). All summer population samples were collected from incidentally entangled animals, thereby eliminating complications (e.g. dilution of genetic signal)

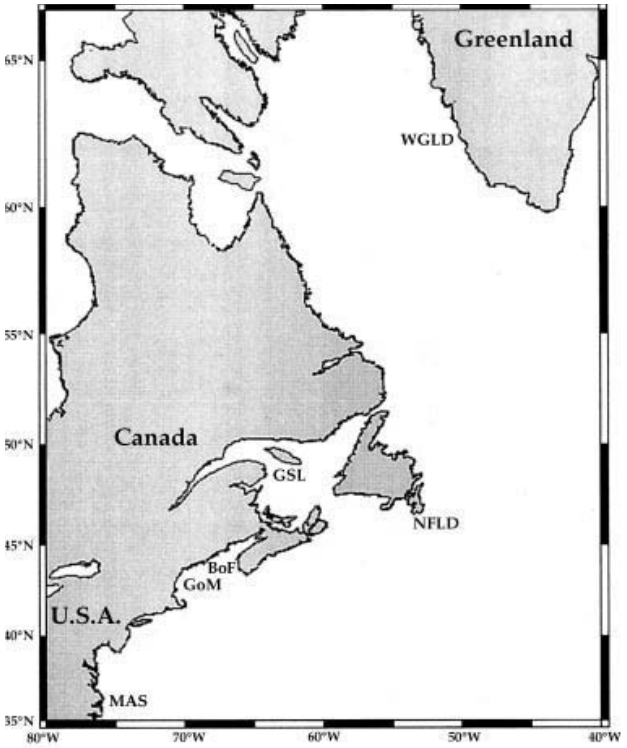

Fig. 1 Northwest Atlantic showing the five areas where harbour porpoise samples were obtained. BoF, Bay of Fundy; GoM, Gulf of Maine; GSL, Gulf of St Lawrence; NFLD, East Newfoundland; WGLD, West Greenland; and MAS, mid-Atlantic states.

arising from samples collected from stranded animals that may have floated in from elsewhere. Samples collected from the mid-Atlantic states were from stranded animals; however, as there is no other known source of animals in this area during winter, we feel it is safe to assume that they are representative of the mid-Atlantic states' aggregation. DNA was extracted following standard proteinase K digestion and phenol–chloroform extraction, as described in Rosel & Block (1996).

Mitochondrial control-region sequences

A 450-bp region of the 5′ end of the highly variable control region and flanking tRNAs was amplified using the polymerase chain reaction (PCR) and the primers L15824 (5′-CCTCACTCCTCCCTAAGACT-3′) and H16265 (5′-GCCCGGTGCGAGAAGAGG-3′) (Rosel *et al.* 1999), with positions defined based on the complete mtDNA sequence of the fin whale (Árnason *et al*. 1991). Genomic DNA (50–250 ng) was added to a 50-μL PCR reaction mix (Saiki *et al*. 1988) containing 10 mM Tris-HCl, pH 9.0, 50 mM KCl, 15 pmol of each primer, 150 μM of dNTPs and 1.5 U of *Taq* DNA polymerase. The cycling profile consisted of an initial denaturation at 95 °C for 30 s, followed by 30 cycles of 1 min at 94 °C, 1 min at 55 °C and 1 min at 72 °C, and was performed in a Perkin-Elmer thermocycler (model 480). Five microlitres of the product was screened on a 1% agarose gel to determine the quality of the reaction, and the remaining 45 μL of double-stranded product was gel purified and digested with 5–10 U of agarase (Sigma). A sample (3.5–8.5 μL) of this digestion mix was used in a cycle-sequencing reaction using fluorescently labelled dideoxy terminators and Amplitaq FS, according to the manufacturer's recommended conditions (Applied Biosystems) and loaded onto an ABI 373A automated DNA sequencer. All samples were sequenced in both directions with the primers used in the amplification. Alignment of the resultant sequences was performed by eye.

Nucleotide and haplotypic diversity were estimated for all populations (Nei 1987) using the program ARLEQUIN (Schneider *et al*. 1996). An analysis of molecular variance (AMOVA; Excoffier *et al.* 1992) was conducted to detect concordance between DNA sequences and geographical location. The AMOVA calculates Φ_{ST}, corresponding to Wright's F_{ST} (Wright 1978), a measure of population subdivision. Φ_{ST} incorporates information on both the degree of genetic distance between haplotypes and the frequencies of haplotypes in each population. A distance matrix of gamma distances (Tamura-Nei model of evolution, $\alpha = 0.5$, as recommended for control-region sequences; Kumar *et al*. 1993) was generated using the computer program MEGA (Kumar *et al.* 1993) for use in ARLEQUIN. The AMOVA was also run using the option of utilizing haplotype frequency data only, i.e. not incorporating the degree of genetic distance between haplotypes, resulting in an estimate of Wright's F_{ST} Recently, O'Corry-Crowe *et al.* (1997) have suggested that this latter method of analysis may be a better estimate of population differentiation in situations where many very closely related haplotypes exist and little phylogeographical structure is observed in the data. These properties are often present in recently separated populations, where sufficient time has not elapsed to allow for sorting of mtDNA lineages into the separate populations. However, haplotype frequencies can respond more quickly to a reduction in genetic exchange, and so haplotype frequencies may differ significantly among populations before phylogeographical partitioning is evident. In these situations, estimates of F_{ST} may more accurately reflect the degree of population subdivision, while Φ_{ST} may be biased downward. In pairwise population comparisons, F_{ST}, rather than Φ_{ST}, is presented.

The AMOVA was first performed excluding the mid-Atlantic States, as this was a winter sample and it is unknown from which summer breeding population or populations it originates. However, to determine whether we could identify the source of these wintering animals, they were included in a subsequent analysis. In all cases, analyses were conducted using both sexes and then repeated on each sex separately. In addition, to determine

whether the sample from the mid-Atlantic states may have been derived from just one of the summer populations, pairwise tests for homogeneity of haplotype frequencies between each summer sample and the winter sample were performed using contingency tests. Monte Carlo methods implemented in the program RXC of Miller (1997) were utilized to determine significance levels (*P*-values) of these tests. Furthermore, under the assumption that the winter sample comprised a mixture of the summer populations, the mtDNA data were analysed using methods of standard likelihood mixture models (Pella & Milner 1987). Relative contributions of each summer stock were estimated using a conditional maximum-likelihood approach with bootstrapping for precision, as implemented in the program CONSQRT (Masuda *et al.* 1991). To test whether the data contained sufficient signal to determine the source of the mid-Atlantic states sample had it come from a single summer population, four sets of summer + winter samples were generated using the program SIMULATR (kindly provided by J. Pella and M. Masuda, and available at ftp://wwwabl.afsc.noaa.gov/sida/mixture-analysis/) and processed using the conditional maximum-likelihood mixture-analysis approach. For each of the four simulations, the simulated data for the winter 'mixture' was taken from just one of the summer populations and sample sizes were identical to those present in the original data.

Finally, a minimum spanning network of mtDNA haplotypes was constructed using the program MINSPNET (Excoffier & Smouse 1994) to visually examine relationships among the haplotypes.

Microsatellite isolation

Harbour porpoise-specific microsatellites were isolated following the procedure of Pulido & Duyk (1994). Genomic DNA from two northwest Atlantic harbour porpoises was pooled and digested with the restriction enzyme *Alu*I. The digested DNA was size selected (300–800 bp), modified with *Bst*XI adapters, ligated into the phagemid cloning vector pJCP1 (provided by G. Duyk, Harvard Medical School) and transformed into the *dut-ung-* *Escherichia coli* strain JMG1 (provided by G. Duyk, Harvard Medical School). This constituted the primary library. The primary library was infected with the M13 helper phage M13K07 (Promega) and single-stranded circular phagemid DNA was recovered. This DNA was used as a template for primer extension using a $(CA)_{10}$ oligonucleotide probe. The double-stranded primer-extension products were transformed into pBluescript (Stratagene). Colony lifts were screened using a ^{32}P end-labelled $(CA)_{10}$ oligonucleotide probe according to standard procedures (Sambrook *et al.* 1989). Plasmid DNA was purified from positive clones using a Wizard miniprep kit (Promega) and was sequenced on an ABI 373A automated sequencer, following the manufacturer's instructions. PCR primers to unique loci containing perfect microsatellites of 13 or more repeat units were designed using the computer program PIPELINE (Resnick & Stein 1995). Primers for each locus were synthesized commercially with a fluorescent phosphoramidite dye attached to the 5′ end of one primer of each pair. Eight of the nine loci were polymorphic.

Microsatellite data collection and analysis

Harbour porpoise samples from the northwest Atlantic were genotyped using these eight loci. Amplifications were conducted in 25-μL reaction volumes containing 10 mM Tris-HCl, pH 9.0, 50 mM KCl, 150 μM of dNTPs, 7.5 pmol of each primer, 0.75 U of *Taq* DNA polymerase and 10–50 ng of genomic DNA. The cycling profile consisted of an initial denaturation at 95 °C for 30 s, followed by 25 or 27 cycles (see Table 6) of 94 °C for 30 s, 50 °C or 55 °C for 30 s and 72 °C for 30 s, and was performed in a Perkin-Elmer thermocycler (model 480 or 9600). Amplified products were mixed with a size standard (Genescan-500 TAMRA) and loaded onto an ABI 373A automated sequencer (ABI). Sizing of allele fragments using the Genescan Analysis software (ABI) was automated and relied on the use of the internal lane standards (Ziegle *et al.* 1992). Because we isolated a dinucleotide repeat, allele sizes should differ by 2 bp owing to the mutational processes that produce the length variation (Tautz & Renz 1984; Levinson & Gutman 1987). In practice, however, because of the influence of base composition and charge on the mobility of these DNA fragments and the size standard in a gel matrix, 2-bp increments among alleles were not always achieved. As a result, it was necessary to bin fragments into discrete allele categories. This was accomplished by sorting all the alleles at a locus by size. Inspection of this graphical representation of all the alleles clearly showed the cut-off points between each successive allele size.

Genetic diversity was characterized by observed heterozygosity (H_O), expected heterozygosity (H_E) and the number of alleles per locus (A). The analysis package GENEPOP version 3.1 (Raymond & Rousset 1995) was used to perform a variety of statistical tests. Deviations from Hardy–Weinberg equilibrium (HWE) were examined for each population at each locus and for each locus at each population using Fisher's exact test. *P*-values were estimated using a Markov chain model (Guo & Thompson 1992). Tests for differences in genotypic distributions among populations were also performed. GENEPOP default parameters were used for the Markov chain tests (dememorization, batches, iterations). Sequential Bonferroni corrections (Rice 1989) were made to adjust significance levels for

Table 1 Northwest Atlantic harbour porpoise control-region haplotypes by region

Haplotype	GOM	GSL	NFLD	WGLD	MAS	Total
1	1					1
2	3	1	1	2		7
3	31	4	1	4	5	45
4	1					1
5	1					1
6	1			3	1	5
7	1					1
8	1			1		2
9	1		1			2
10	2	1		2	1	6
11	1	3		1	1	6
12	1					1
13	1					1
14	9	5	15	5	8	42
15	1					1
16	1			2	1	4
17		2			1	3
18	1	2	1	4	1	9
19		1			1	2
20	1	2		2	1	6
21	3	2	3	4	3	15
22		1				1
23		1				1
24		2		2		4
25		1				1
26		2	2		1	5
27		1			1	2
28	1	1	1			3
29		1	1	2	1	5
30		2	1	1	1	5
31		1				1
32		1				1
33	2	1				3
34		1			1	2
35		1	1			2
36			1			1
37	1		1			2
38			2	2		4
39			1			1
40			1	1	1	3
41			1			1
42	1		1		1	3
43			1			1
44			1			1
45			1			1
46			1			1
47	1		1			2
48	1		1			2
49	1					1
50	2					2
51	1				1	2
52	1					1
53	1					1
54	1					1
55	1			3		4
56	1					1
57	1					1
58	1					1
59				1		1
60				1		1
61				1		1
62				1		1
63				1		1
64				1	1	2
65				1		1
66				1		1
67				1		1
68					1	1
69					1	1
70					1	1
71					1	1
72					1	1
73					1	1
74					1	1
75					1	1
Total	80	40	42	50	41	253

GOM, Gulf of Maine; GSL, Gulf of St Lawrence; NFLD, Newfoundland; WGLD, West Greenland; MAS, mid-Atlantic states.
The most common haplotypes, 1 and 14, were submitted to GenBank with accession nos AF152570 and AF152571.

multiple tests. The AMOVA was used to test for correlations between geographical collection location and microsatellite DNA diversity. With microsatellite data, the Φ_{ST} estimator incorporates variance in allele size and distribution of alleles in each population. Finally, Slatkin's R_{ST}, an analogue of F_{ST} that assumes a stepwise mutation model rather than an infinite alleles model (Slatkin 1995), was also estimated. Owing to differences in sample sizes among the different populations, Goodman's (1997) unbiased estimate of R_{ST} was obtained using the program RSTCALC 2.2.

Results

Mitochondrial control-region sequences

We resolved 342 bases of the mitochondrial control region from 253 west Atlantic harbour porpoises, including 50 from West Greenland, 42 from Newfoundland, 40 from the Gulf of St Lawrence, 80 from the Gulf of Maine and 41 from the mid-Atlantic states winter sample. There were 61 variable positions defining 75 unique haplotypes (Table 1). Several common haplotypes were shared across

Location	N	Haplotype diversity	Nucleotide diversity
Gulf of Maine	80	0.839 ± 0.039	0.009 ± 0.005
Gulf of St Lawrence	40	0.967 ± 0.014	0.011 ± 0.006
Newfoundland	42	0.872 ± 0.049	0.012 ± 0.007
West Greenland	50	0.967 ± 0.010	0.013 ± 0.007
Mid-Atlantic states	41	0.950 ± 0.023	0.012 ± 0.007

Table 2 Genetic diversity estimates based on mitochondrial DNA (mtDNA) control-region sequences for northwest Atlantic harbour porpoise populations

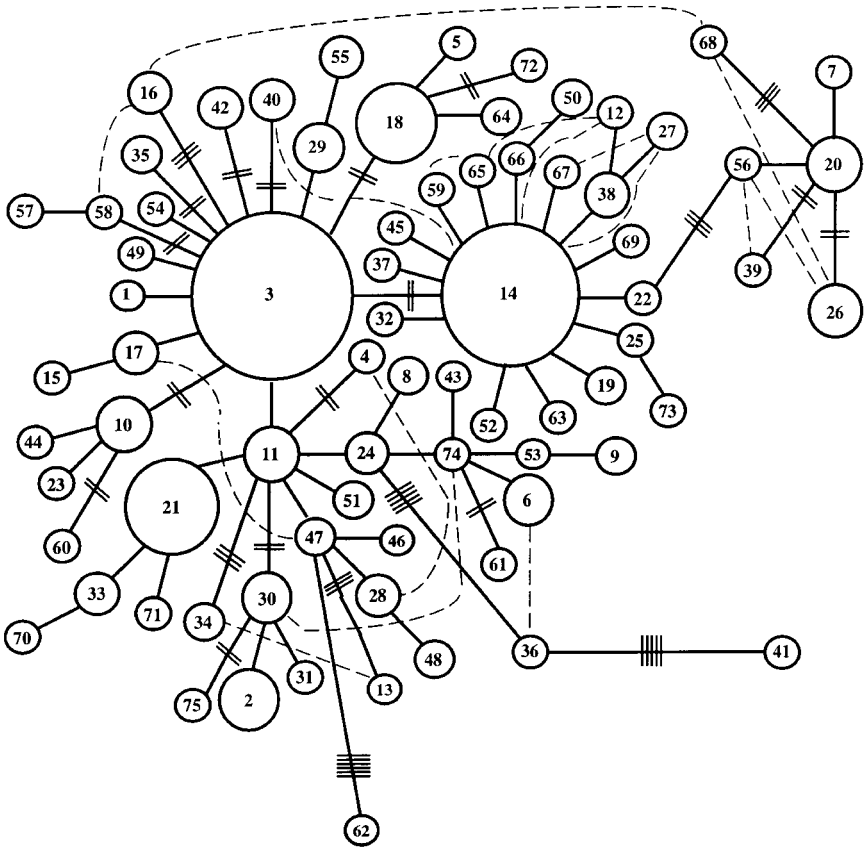

Fig. 2 Haplotype network showing the relationships among 75 harbour porpoise mitochondrial DNA (mtDNA) control-region haplotypes. Haplotype numbers correspond with the numbers in Table 1. The diameter of the circle is approximately proportional to the number of individuals bearing that haplotype. All haplotypes are separated by at least one substitution. Multiple substitutions between haplotypes are indicated by hash marks. Alternative connections between haplotypes (dotted lines) indicate homoplasy in the DNA sequence data.

most populations, but each region was also characterized by rarer, unique haplotypes.

Haplotypic diversity estimates ranged from 0.84 in the Gulf of Maine population to 0.97 in the Gulf of St Lawrence and West Greenland populations, with an overall average of 0.93. Nucleotide diversities ranged from 0.99% in the Gulf of Maine to 1.26% in West Greenland, with an overall average of ≈ 1.1% (Table 2). A minimum-spanning network (Fig. 2) consisted of two common haplotypes from which radiated multiple, rare haplotypes. A clear pattern of haplotype and geographical locale was not detected. The most commonly occurring haplotypes, 3 and 14, were found in all sampling locations, but with differing frequencies, and each has given rise to a number of closely related haplotypes, including five singly occurring haplotypes, unique to West Greenland, originating from haplotype 14, and five singly occurring haplotypes, unique to the Gulf of Maine, arising from haplotype 3. Extensive homoplasy in the data is evident in the large number of possible alternative connections in the network.

The AMOVA results indicated the presence of population subdivision among the summer breeding populations. Whether the AMOVA analysis was performed using genetic distance and frequency information (Φ_{ST}), or using haplotype frequencies alone (F_{ST}), the results using both sexes together indicated that a significant amount of the molecular variance could be accounted for by differences among populations (Table 3). As with previous studies, F_{ST} values were higher and P-values lower when only haplotype frequency information was utilized (O'Corry-Crowe *et al.* 1997). This data indicates that the West Greenland, Gulf of St Lawrence, Newfoundland and Gulf of Maine populations are not panmictic. Analysing the sexes separately produced different patterns. The

Table 3 Results of analysis of molecular variance (AMOVA) on mitochondrial DNA (mtDNA) control-region sequences

	% Variance among populations	% Variance within populations	Φ_{ST}/F_{ST}	*P*-value
A				
Excluding mid-Atlantic states				
Both sexes	1.08	98.92	0.011	≤0.03
Females only	2.30	97.70	0.023	≤0.04
Males only	0.27	99.73	0.0026	≤0.349
Including mid-Atlantic states				
Both sexes	0.54	99.46	0.0054	≤0.13
Females only	1.4	98.60	0.014	≤0.11
Males only	0.0	100.00	0.00	≤0.65
B				
Excluding mid-Atlantic states				
Both sexes	4.57	95.43	0.046	≤0.001
Females only	5.58	94.42	0.056	≤0.001
Males only	4.01	95.99	0.040	≤0.002
Including mid-Atlantic states				
Both sexes	3.41	96.59	0.034	≤0.0001
Females only	4.37	95.63	0.044	≤0.0001
Males only	2.92	97.08	0.029	≤0.003

A, AMOVA using genetic distance and haplotype frequency information; B, AMOVA using haplotype frequencies only.

results for females alone differed significantly from zero using either of the AMOVA analysis methods (Table 3). For males only, the analysis of haplotype frequency alone yielded a significant F_{ST} value (Table 3).

For both sexes pooled together, pairwise population comparisons of F_{ST} values showed a significant partitioning of the molecular variance, after sequential Bonferroni correction, for all pairs except the Gulf of St Lawrence to West Greenland comparison when using haplotype frequency data (Table 4). For females alone, the Gulf of Maine population differed significantly from all other summer breeding populations. For males, four of six pairwise comparisons yielded significant F_{ST} values; the Gulf of Maine to Gulf of St Lawrence and the Gulf of St Lawrence to West Greenland comparisons were not significant.

Inclusion of the mid-Atlantic states samples into the analysis decreased overall Φ_{ST} and F_{ST} values in all three cases: both sexes analysed, females alone and males alone (Table 3). Pairwise comparisons between the mid-Atlantic states and all other populations yielded F_{ST} values that did not differ significantly from zero, except the Gulf of Maine to mid-Atlantic comparison using females. This may stem from the fact that the number of females in the Gulf of Maine sample was very small.

Contingency table analysis rejected homogeneity of haplotype frequencies between the Gulf of Maine and the mid-Atlantic states winter sample ($P < 0.06$), but found no significant differences in haplotype frequencies between the winter sample and any of the remaining summer samples ($0.39 < P < 0.99$). Pooling of haplotypes into six categories to reduce the risk of problems associated with many rare haplotypes did not alter this conclusion. On the other hand, the conditional maximum-likelihood mixture analysis demonstrated that the winter sample

Table 4 Population pairwise F_{ST} and significance values for northwest Atlantic harbour porpoise summer breeding populations estimated from analysis of molecular variance (AMOVA) using mitochondrial DNA (mtDNA) haplotype frequency information

	GOM	GSL	NFLD	WGLD
Both sexes				
GOM	—	0.001	0.001	0.001
GSL	0.042	—	0.020	0.767
NFLD	0.095	0.024	—	0.004
WGLD	0.049	0.000	0.032	—
Females				
GOM	—	0.001	0.001	0.001
GSL	0.115	—	0.379	0.764
NFLD	0.131	0.001	—	0.428
WGLD	0.069	0.00	0.00	—
Males				
GOM	—	0.162	0.005	0.008
GSL	0.011	—	0.007	0.227
NFLD	0.062	0.051	—	0.003
WGLD	0.047	0.008	0.050	—

F_{ST} below diagonal, *P*-values above. *P*-values ≤ 0.008 are significant after Bonferroni correction at $\alpha = 0.05$.
GOM, Gulf of Maine; GSL, Gulf of St Lawrence; NFLD, Newfoundland; WGLD, West Greenland; MAS, mid-Atlantic states.

Table 5 Results of mixture analysis using the conditional maximum-likelihood approach indicating relative contributions of harbour porpoises from the summer populations to the mid-Atlantic winter sample

Population	Point estimate	Standard error	95% Confidence intervals
Gulf of Maine	0.19	0.21	0.00–0.50
Gulf of St Lawrence	0.40	0.34	0.00–0.66
Newfoundland	0.18	0.20	0.00–0.49
West Greenland	0.24	0.25	0.00–0.57

is probably a mixture of more than one of the summer populations as none of the confidence intervals for the contributions of the four summer populations included 100% (Table 5). However, the mtDNA haplotype frequencies of these four summer populations were not sufficiently distinguishing to accurately determine the relative contribution of each population to the winter mid-Atlantic sample (Table 5). Finally, use of the program SIMULATR indicated that the stock mixture analysis could have distinguished the sole summer source of the mid-Atlantic states sample if it existed (data not shown).

Microsatellite isolation

Screening of ≈ 1500 colonies resulted in 265 (18%) positive clones of which 60 were sequenced. The nucleotide sequences were examined for duplicates using the computer program STS PIPELINE (Resnick & Stein 1995), which also simultaneously designs primer pairs for each locus. We found that 23 of 45 (51%) clones were considered to be duplicates by this program. Upon closer examination, we discovered that they were not identical sequences because the repeat region and flanking regions of most of these clones were different. However, they all contained a common sequence of ≈ 100 bp either upstream or downstream of the cloned microsatellite region, causing the computer program to consider them duplicates. When compared with sequences in the GenBank DNA database, this common sequence showed 74% identity with a region of a cosmid-derived microsatellite sequence cloned from the cow, *Bos taurus* (Y. Zhang *et al.* 1995; GenBank accession no. X86815). We chose to eliminate these clones from further analysis because it seemed probable that they might be associated with a larger satellite DNA sequence present in the genome.

Primers designed by STS PIPELINE (Resnick & Stein 1995) were synthesized for nine of the isolated microsatellite markers. Each locus was screened for variation using seven porpoise samples. Eight of the nine loci amplified the appropriately sized DNA fragment were polymorphic (Table 6). However, as we continued genotyping all samples for all alleles, we encountered difficulties in scoring alleles for locus PPHO102. We therefore discontinued use of this microsatellite locus.

Microsatellite analysis

Summary statistics for microsatellite variation are shown in Table 7. As expected, microsatellite variation within populations was high compared with other nuclear markers (e.g. allozymes), with the number of alleles per

Table 6 Characterization of eight harbour porpoise microsatellite loci

Locus	Primer pairs (5′–3′)	Annealing temperature/no. of cycles	Repeat	Fragment size
PPHO104	F: CCTGAGGTGTGTAGTCA R: GACCACTCCTTATTTATGG	57 °C/25x	$(CA)_{19}$	164
PPHO110	F: ATGAGATAAAATTGCATAGA R: ATCATTAACTGGACTGTAGACCTT	50 °C/27x	$(CA)_{22}$	124
PPHO130	F: CAAGCCCTTACACATATG R: TATTGAGTAAAAGCAATTTTG	50 °C/27x	$(CA)_{25}$	192
PPHO131	F: GTTAGGTACCAGCCTCC R: CTAGTTATCATGCAGGGAGT	57 °C/25x	$(CA)_{13}$	186
PPHO133	F: AGGGGTTTCTGAAGTGA R: CCTTAATCACACCTTGG	50 °C/27x	$(CA)_{18}$	186
PPHO137	F: CAGGGCGGCCATGTACAGTTGAT R:GAGTTTGGCTCCCTCTCCAG	57 °C/25x	$(CA)_{26}$	123
PPHO142	F: GAAGGCTCAGGGTATTG R: CAGTTACTTTCCTCGGG	50 °C/27x	$(CA)_{22}$	152
PPHO102	F: CCTATCAACACCCTGGAGTTATGC R: GGGGCTGCACCTGTTCCT	57 °C/27x	$(CA)_{18}$	128

F, forward; R, reverse.
Repeat size and fragment length refer to the original clone from which primers to each locus were designed.
GenBank accession nos of the cloned loci are AF151785-AF151792.

Table 7 Summary statistics for *Phocoena phocoena* microsatellite loci

Region	Locus	PPHO110	PPHO130	PPHO131	PPHO137	PPHO142	PPHO104	PPHO133	Mean all loci	Mean w/o PPHO133
Gulf of Maine										
	N	80	80	79	80	80	80	78	79.57	79.83
	R	101–127	166–200	182–198	102–140	131–161	146–184	173–203		
	A	9	15	9	18	16	17	14	14.00	14.00
	H_E	0.78	0.90	0.81	0.90	0.89	0.90	0.89		
	H_O	0.65	0.88	0.79	0.86	0.86	0.84	0.69*	0.80	0.81
Gulf of St Lawrence										
	N	47	47	47	47	47	47	47	47.0	47.0
	R	107–127	174–200	182–196	104–132	127–159	150–180	173–201		
	A	9	13	8	15	15	15	14	12.71	12.50
	H_E	0.80	0.91	0.83	0.92	0.90	0.87	0.89		
	H_O	0.83	0.87	0.81	0.98	0.83	0.85	0.66*	0.83	0.86
Newfoundland										
	N	48	48	48	48	48	48	48	48.0	48.0
	R	107–127	166–200	182–198	94–132	131–159	134–188	177–199		
	A	10	14	9	15	15	16	11	12.86	13.17
	H_E	0.84	0.89	0.83	0.89	0.87	0.89	0.86		
	H_O	0.85	0.92	0.81	0.94	0.85	0.92	0.54*	0.83	0.88
West Greenland										
	N	50	50	50	49	50	50	49	49.7	49.8
	R	105–125	166–196	182–198	94–128	133–159	148–192	177–201		
	A	9	14	9	15	14	16	12	12.71	12.83
	H_E	0.81	0.91	0.84	0.91	0.86	0.89	0.87		
	H_O	0.76	0.98	0.84	0.96	0.90	0.90	0.65*	0.86	0.89
Mid-Atlantic states										
	N	49	51	50	50	50	50	49	49.9	50.0
	R	101–129	174–202	182–196	104–132	127–157	146–184	173–203		
	A	12	15	8	14	15	16	13	13.29	13.33
	H_E	0.85	0.92	0.83	0.91	0.89	0.90	0.87		
	H_O	0.94	0.96	0.84	0.96	0.88	0.86	0.53*	0.85	0.91

Number of individuals (*N*), range of allele sizes (*R*), number of alleles (*A*), and expected (H_E) and observed (H_O) heterozygosities are given for each locus in each population.
Mean values of *N*, *A* and H_O across loci within each population are given for all loci, and for all loci except PPHO133 (see the text).
*Indicates a significant heterozygote deficit ($P < 0.0001$).

locus ranging from eight to 16 and H_O values ranging from 0.53 to 0.96. All but one locus (PPHO133) conformed to HWE in all populations. Significant heterozygote deficiencies were observed in all populations at locus PPHO133. We chose to eliminate this locus from further analysis. For the remaining six loci, H_O values within populations ranged from 0.65 to 0.96.

We examined the microsatellite data for evidence of population structure. An analysis of the distribution of genotypes among populations at each locus revealed significant differences in 10 of 60 pairwise comparisons ($P < 0.05$); however, after a Bonferroni correction for multiple comparisons, only the Newfoundland vs. the Gulf of St Lawrence comparison at locus PPHO104 was significant ($P = 0.002$).

Most of the variation in genetic diversity was found within populations. The analysis of population subdivision attributed less than 0.5% of the genetic variance to among-population variation, which was not significantly greater than 0 ($F_{ST} = 0.18\%$, $P = 0.052$; $R_{ST} = 0.24\%$, $P = 0.181$). Among pairwise population comparisons, only a single value was marginally significant (Gulf of Maine vs. Newfoundland, $F_{ST} = 0.62\%$, $P = 0.005$); all pairwise R_{ST} values were not significant. Estimates of pairwise *Nm* values ranged from 16.1 to infinity.

Discussion

Genetic diversity

The northwest Atlantic populations of *Phocoena phocoena* show substantially higher levels of mtDNA diversity than populations present in the northeast Atlantic (Tiedemann *et al*. 1996; Walton 1997; Wang & Berggren 1997; Rosel

et al. 1999), and similar or slightly lower levels than those of the northeast Pacific (Rosel *et al.* 1995). Walton (1997) sequenced the homologous section of the control region of 327 harbour porpoises from the northeast Atlantic and found only 24 unique haplotypes. In this study, 253 porpoises from the northwest Atlantic revealed 75 unique haplotypes. Likewise, nucleotide diversity in the northwest Atlantic sample was nearly twice that estimated for the northeast Atlantic. These differences suggest that the northwest and northeast Atlantic populations of harbour porpoises experience limited genetic exchange (Rosel *et al.* 1999).

The average H_O value at six microsatellite loci ranged from 0.81 to 0.91. As expected for these loci with high mutation rates, the values are substantially higher than those estimated from allozyme data. Andersen (1993) surveyed 31 allozyme loci in 262 harbour porpoises from the northeast Atlantic and West Greenland and found that only two were polymorphic. Average H_O values estimated from her data for these two loci were 0.328 and 0.387, respectively. Andersen *et al.* (1997) later collected microsatellite data from three loci in these same populations: two loci were isolated from pilot whales (Schlötterer *et al.* 1991) and one was developed from sequence from cows and pigs (Kirkpatrick 1992). Two of these loci showed significant deviations from HWE in the West Greenland sample, and the authors suggested that this may have resulted from inbreeding or sampling of multiple populations. Our West Greenland samples, collected in the same areas and time periods, showed no evidence of deviation from HWE (with the exception of locus PPHO133, which showed a significant heterozygote deficiency in all populations) and hence do not support the presence of inbreeding or a mixed sample. It may be that a null allele was present at these two loci, as they were derived from evolutionarily divergent taxa.

The mean H_O value for harbour porpoise microsatellites was higher than that found in other cetacean species using loci isolated from the species studied. A world-wide sampling of humpback whales (Valsecchi *et al.* 1997), using three loci, produced a mean H_O value of 0.79 with an average of 15.3 alleles per locus. Using five microsatellite loci, Richard *et al.* (1996) found an average of 10.2 alleles per locus and a mean H_O of 0.79 in sperm whales, *Physeter macrocephalus*. A survey of 15 loci from beluga whales, *Delphinapterus leucas*, revealed the lowest diversity levels, with an average of 8.6 alleles per locus and a mean H_O of 0.65 (Buchanan *et al.* 1996).

Geographic variation

Examination of harbour porpoise control-region sequences revealed small, but significant, differences in the spatial distribution of genetic variation among summer breeding populations in the northwest Atlantic. Analyses based both on mtDNA control-region haplotype frequencies alone (F_{ST}) and haplotype frequencies coupled with the degree of genetic divergence between haplotypes (Φ_{ST}), indicated that there is significant partitioning of genetic variability among the four populations. The Gulf of Maine population was differentiated from all other summer populations. Not surprisingly, this population is the most geographically isolated of the four populations. Satellite telemetry data gathered from nine porpoises tagged in the Bay of Fundy/Gulf of Maine also indicate that this population is disjunct. None of the tagged porpoises left the area, leading the authors to conclude that this population is restricted in their movements (Read & Westgate 1997). The Newfoundland population also showed significant differentiation from the other populations. The habitat used by porpoises off eastern Newfoundland is also fairly well isolated from the other areas by both intervening land masses and deep water. Finally, the Gulf of St Lawrence and West Greenland populations could not be discriminated from one another. These results are congruent with an analysis of organochlorine contaminant levels in juvenile harbour porpoises, which revealed significant geographical variation in contaminant levels among the Gulf of St Lawrence, Newfoundland and Bay of Fundy/Gulf of Maine populations (Westgate & Tolley 1999).

Although the degree of partitioning of genetic variability among the four summer populations was small, it differed significantly from zero, indicating that these four populations are not panmictic. Many of the present day summer feeding areas, including the Gulf of St Lawrence, the Bay of Fundy/Gulf of Maine, western Greenland and at least the coastal waters around eastern Newfoundland, were covered with ice during the last glacial periods 17 000–21 000 years (Williams *et al.* 1998) and hence did not provide suitable habitat for harbour porpoises. Thus, the Gulf of St Lawrence, West Greenland and Newfoundland summer habitats contain relatively young populations and it is probable that there has not been sufficient time to effect significant mtDNA lineage sorting among them. The estimates of genetic exchange rates may thus be biased upwards, a signature of evolutionarily recent fragmentation of a refugial population, rather than of gene flow among ancient populations.

Harbour porpoises are small and difficult to see in the water, and they tend to avoid boats. Individuals bear few marks that could be used for individual identification as can be done, for example, with humpback whales and bottlenose dolphins. These characteristics make the study of behaviour in wild populations very difficult. However, one study of porpoises in the Bay of Fundy suggested that some females return to the area annually (Gaskin & Watson 1985). Whether site fidelity was a behaviour

common in harbour porpoises or unique to these particular females remains to be tested. Wang *et al.* (1996) published the first genetic study of harbour porpoises in the region. Using mtDNA RFLP analysis, they concluded that female porpoises are more philopatric than males, supporting the previous study. To test this hypothesis using higher resolution mtDNA control-region sequences, we subdivided our samples into males and females and reanalysed the data set using an AMOVA. Analysis of females alone produced the highest levels of genetic variance attributable to between-population comparisons, while analysis of males alone produced a lower overall F_{ST} value and a correspondingly higher estimate of *Nm*, although, interestingly, significant population subdivision was detected among males when haplotype frequencies only were used in the analysis. These data support the hypothesis that throughout the northwest Atlantic, females show stronger site fidelity than males, a behaviour that would be difficult to quantify in the field.

However, males may also show site fidelity to a lesser degree. Although the overall degree of population subdivision measured in males was lower than in females (Table 3), suggesting that male movement dilutes the differentiation of populations, several pairwise population comparisons using males differed significantly from zero (Table 4). Analyses of contaminant levels in male porpoises from the Gulf of St Lawrence and Newfoundland also revealed significant differences between these two populations, suggesting that movement of males between these areas is limited (Westgate & Tolley 1999). Analysis of contaminant loads provides information on an ecological timescale, while genetic analyses provide information on an evolutionary timescale. The relatively recent (evolutionarily) separation of these populations, coupled with low levels of male movement between them, may limit the ability of genetic data to differentiate the males, while contaminant analysis was better able to detect the differences.

In contrast to the mtDNA data, the six harbour porpoise-specific microsatellite loci, while highly variable, detected no population differentiation among the northwest Atlantic populations surveyed. Although nonsignificant, the trends seen in the microsatellite data did mirror the results of the mtDNA data, e.g. the Gulf of Maine population differed most from all others. One possible conclusion to draw from this result is that male-mediated gene flow is sufficiently high to maintain near homogeneity among these loci, while stronger female philopatry results in significant geographical heterogeneity in the maternally inherited mtDNA sequences.

Mid-Atlantic states

While the summer range of harbour porpoises in the northwest Atlantic is well defined, where animals spend the winter is not well known. When water temperatures drop and/or ice begins to form in the northern regions, porpoises appear to migrate out of these areas (Gaskin 1992). While the species is common in the Bay of Fundy in the summer, winter abundance is much lower (Gaskin 1992). Stranding records along the mid-Atlantic states (New York to North Carolina) document an increase in harbour porpoises during late winter and early spring (Polachek *et al.* 1995), suggesting that some proportion of the northwest Atlantic populations move south along the US coast during the winter. However, the source of these animals is unknown. Are they simply animals from the Gulf of Maine that have followed the coastline south, or does the mid-Atlantic region provide a wintering area for animals from other summer populations such as the Gulf of St Lawrence, Newfoundland or even West Greenland?

In 1993, 50 harbour porpoises stranded along the mid-Atlantic states, the majority of which were less than 1 year old (Haley & Read 1993). An additional 124 animals were stranded between 1994 and 1996 (inclusive) along the states of Maryland, Virginia and North Carolina (A. Read, personal communication). Some of these animals exhibited signs of human interactions, most commonly entanglement in fishing gear. In order to manage harbour porpoise populations effectively, it is critical that the bycatch be attributed to the correct population stock. Thus, there is a substantial need for determining where these winter animals originate. To analyse this question, we repeated the AMOVA analysis on the mtDNA sequences with the mid-Atlantic states sample included. Inclusion of this sample reduced overall F_{ST} and Φ_{ST} values for comparisons of females, males and both sexes combined. This is the pattern expected if the mid-Atlantic states sample comprises mixed stocks. If the mid-Atlantic states aggregation consisted solely of animals from the Gulf of Maine, we would have expected it to show the same pattern as the Gulf of Maine sample, i.e. significant divergence from all other summer populations. In fact, in pairwise comparisons involving the mid-Atlantic states, the only significant F_{ST} value obtained was that between the Gulf of Maine vs. mid-Atlantic states samples. In addition, haplotype diversity estimates for the Gulf of Maine (0.839 ± 0.04) were significantly lower than any other population, including the mid-Atlantic states (0.95 ± 0.02). Some haplotypes unique to the Gulf of St Lawrence or West Greenland summer populations appeared in the winter mid-Atlantic states sample. In fact, eight of the 28 haplotypes present in the winter sample were unique to that sample, suggesting that either not all source populations were surveyed or, more likely, we did not have sufficiently large sample sizes from the source populations to have surveyed all of the diversity present within them. In order to account for the presence

of other haplotypes, we concluded that the mid-Atlantic states winter aggregation comprised more than just Gulf of Maine animals. The contingency table analysis of mtDNA haplotype frequencies supports this conclusion. However, this test could not reject any of the other populations (Gulf of St Lawrence, Newfoundland, West Greenland) as being the sole source of the mid-Atlantic states sample. This result seems at first to be contradictory to the conditional maximum-likelihood analysis, which indicated that more than one summer population was present in the winter sample. However, because the contingency test analysis could not distinguish between the winter sample and any of the three (non-Gulf of Maine) summer populations, it probably could not distinguish between a winter sample comprising a mixture of the summer populations and any one of the summer populations either. Thus, together these analyses demonstrate that the mid-Atlantic states winter sample probably comprises porpoises from more than one summer population, that any of the four summer populations could contribute to the winter sample and, finally, that the relative contributions of any of the summer populations is very imprecisely determined by the mtDNA data.

Management implications

Genetic data collected globally from harbour porpoises reflects the existence of at least two probable evolutionarily significant units, as defined by Moritz (1994); one in the northeast Pacific and one in the North Atlantic (Rosel *et al.* 1995; Wang *et al.* 1996). These two porpoise populations exhibit reciprocal monophyly in mtDNA sequences, but no nuclear data is available, and so the definition of evolutionarily significant units for these populations has not been fully tested. Within the North Atlantic there is no evidence for reciprocal monophyly or unique diagnosable groups among any populations (Rosel *et al.* 1999). Thus, both the evolutionarily significant unit concept and the phylogenetic species concept (Vogler & DeSalle 1994) would support pooling of all North Atlantic porpoises into one conservation unit. Defining units for management of exploited marine fish and mammal species differs, however, from defining units for conservation of most rare or endangered terrestrial species where the evolutionarily significant unit and phylogenetic species concepts are often applied. Management of exploited marine species must be able to predict and incorporate the effects of harvesting and/or bycatch on the sustainability of any given population stock. Pooling of all North Atlantic populations into one management unit means that the overall quota of allowable porpoise bycatch for the North Atlantic, which could number upwards of 5000 animals, could conceivably occur within one small geographical region. The biological impact of such a removal of porpoises from any given geographical locale is not known; even if there is gene flow into this region from other areas would it be sufficient to maintain the population in the face of substantial incidental mortality? For a risk-averse strategy of management of many cetacean species, the use of evolutionarily significant units or phylogenetic species concept criteria is too restrictive (see also Baker & Palumbi (1997)).

However, in the northwest Atlantic, the mtDNA data do support a significant partitioning of genetic variation among the four defined summer populations. Thus, can we define management units (Moritz 1994) in this region? The Gulf of Maine population revealed significant divergence in mtDNA sequences and frequencies when compared with the other three populations. This population can then be considered a management unit. The Newfoundland sample was also significantly differentiated from the Gulf of St Lawrence and West Greenland. Thus, the Newfoundland population may also be considered a management unit. This then leaves only the Gulf of St Lawrence–West Greenland pair undifferentiated. The lack of distinction between these two populations could be a result of gene flow between them, or an artefact of insufficient power to detect differences given the relatively recent repopulation of these regions following the retreat of the glaciers. Finally, the genetic analysis suggests that there is probably a mixed stock occurring off the mid-Atlantic states in winter. Allocating winter bycatch to the appropriate summer population is the next step in fulfilling management needs for this species in the northwest Atlantic.

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Life History of Small Cetaceans in the Northwest Atlantic

Appendix 5

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Monitoring the movements of harbour porpoises (*Phocoena phocoena*) with satellite telemetry

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Abstract The movements of nine harbour porpoises, *Phocoena phocoena* (L.), in the Bay of Fundy and Gulf of Maine were tracked using satellite telemetry. Transmitters were attached to the porpoises in August 1994 and 1995 after they were captured near Grand Manan Island at the mouth of the Bay of Fundy. Tracking periods ranged from 2 to 212 d (mean 50 ± 65 d). Porpoises exhibited a high degree of individual variation in movement patterns; five moved out of the Bay of Fundy into the Gulf of Maine. The porpoise with the longest tracking period moved extensively throughout the Gulf of Maine. These data suggest that seasonal movement patterns of individual harbour porpoises are discrete and are not temporally coordinated migrations. Porpoises that moved out of the Bay of Fundy into the Gulf of Maine did so following the 92 m isobath, which may represent an important movement corridor. The movement of porpoises from the Bay of Fundy into the Gulf of Maine supports the hypothesis that harbour porpoises from these two regions comprise a single population at risk of entanglement in both Canadian and US fisheries.

Introduction

Harbour porpoises (*Phocoena phocoena*) are distributed throughout coastal waters of the temperate northern hemisphere. The general distribution of the species is known from sighting data, strandings, and incidental catches in commercial fisheries (IWC 1996). Little is known, however, about the daily movements of individual porpoises or of the seasonal movements of porpoise populations.

An understanding of the scale, pattern and variability of movements is of both fundamental and applied interest to biologists studying the ecology of this species. Information on distribution and movement patterns is required to understand the relationships between porpoises, their prey, and abiotic factors such as temperature. Such information is also necessary for the conservation of this species. Throughout their range, harbour porpoises are vulnerable to entanglement and mortality in gillnets (Jefferson and Curry 1994). Mortality in these gillnets may threaten the viability of affected populations (IWC 1996). For example, recent estimates of harbour porpoise mortality in gillnet fisheries in the Bay of Fundy and Gulf of Maine range from 2.7 to 4.3% of the total population size (Bravington and Bisack 1996; Trippel et al. 1996). These removals are unlikely to be sustainable (Woodley and Read 1991), which has prompted conservation action, such as time-area restrictions on commercial fisheries, in both Canada and the United States (Palka et al. 1996). For conservation strategies intended to reduce porpoise mortality in commercial fisheries to be effective, information is required on the movement patterns of porpoises and the distribution of fishing effort.

Knowledge of the movements of harbour porpoises has been limited because they are difficult to study at sea due to their small size, subtle individual markings (Koopman and Gaskin 1994) and the limited time they spend at the surface (Westgate et al. 1995). To overcome these logistical difficulties, VHF radio transmitters have been placed on harbour porpoises in the Bay of Fundy, allowing researchers to follow the movements of individuals for periods up to 22 d (Gaskin et al. 1975; Read and Gaskin 1985; Westgate et al. 1995). Despite the potential of this technique, these telemetry studies were hampered by short periods of contact with tagged porpoises due to the difficulty of tracking at sea. This radio telemetry approach is not feasible for long-term studies

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of the movements of marine mammals because of the logistical difficulty tracking individuals at sea for months at a time.

The use of satellite-linked telemetry (Fancy et al. 1988) has revolutionized the study of marine mammals. It is now possible to obtain long-term data on the movements and behaviour of tagged individuals via computer uplink to the laboratory. Satellite-linked transmitters have been successfully deployed on several species of cetaceans (Mate 1989; Martin and Smith 1992; Mate et al. 1992, 1994, 1995; Martin et al. 1993, 1994; Davis et al. 1996; Watkins et al. 1996), but their use on smaller species, such as harbour porpoises, has been restricted because the transmitters were too large. Recent advances in tag miniaturization have made satellite-linked telemetry appropriate for use with harbour porpoises for the first time.

In this paper we describe the long-term movements of nine harbour porpoises in the Bay of Fundy and Gulf of Maine using data obtained from satellite-linked telemetry. Our objectives were twofold: to improve our understanding of the scale over which porpoises travel on a seasonal basis; and to better understand their seasonal movements in relation to large-scale patterns of gillnet fishing effort in these areas.

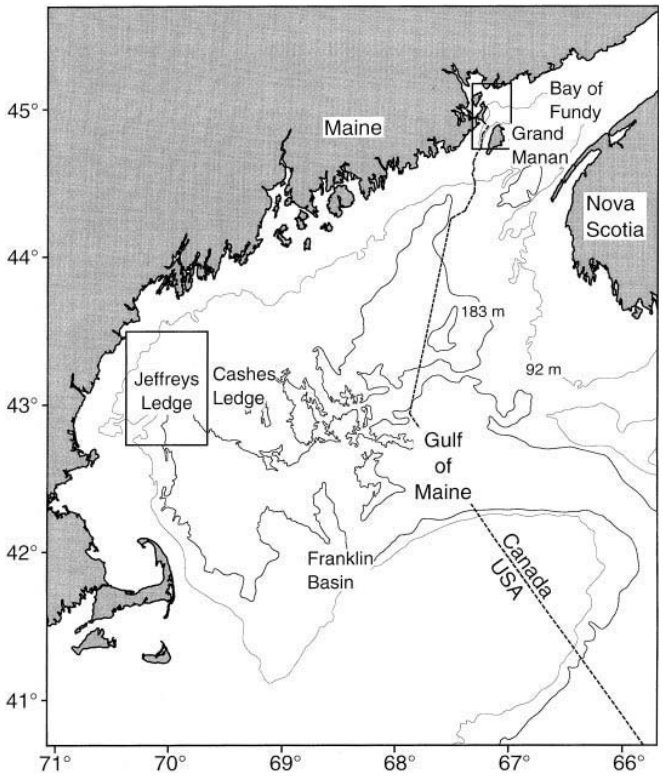

Fig. 1 Map of study area including the lower portion of the Bay of Fundy and the Gulf of Maine. *Boxes* indicate the principal areas of harbour porpoise bycatch in bottom-set gillnets determined from Canadian (Trippel et al. 1996) and US (Bravington and Bisack 1996) fisheries observer programs

Materials and methods

Porpoise capture

Satellite-linked transmitters were placed on nine harbour porpoises, *Phocoena phocoena* (L.), released from herring weirs around Grand Manan Island, New Brunswick, Canada (44°45′N; 66°45′W), in August 1994 and 1995 (Table 1; Fig. 1). Porpoises were removed from weirs with a seine net, placed on a closed-cell foam pad and sponged with sea water. Body mass was measured with a spring balance or estimated from regressions using length and girth as predictive variables (Read and Tolley 1997) (Table 1). The blood chemistry and hematology of seven porpoises indicated that they were healthy, based on values of Koopman et al. (1995). The two adult females exhibited elevated levels of progesterone and were likely in the first trimester of pregnancy; one of these females (No. 1) was also lactating and accompanied by a calf.

Transmitters

Satellite-linked transmitters, or platform transmitter terminals (PTTs), were attached to the dorsal fin of each porpoise. Prior to attachment, the dorsal fin was cleaned with a topical antiseptic and the tagging site was injected with 1 cc of lidocaine HCL 2%, epinephrine 1:100 000 at the location of each attachment pin. There were two PTT configurations (Telonics, Mesa, Arizona, USA): front-mount (n = 5) and side-mount (n = 4). The front-mount design used a stacked-board ST-10 PTT. This transmitter was encased in a steel cylinder and attached to a thin, neoprene-lined, polyethylene saddle which provided a firm base to secure the cylinder to the dorsal fin. The plastic saddle wrapped around the front of the dorsal fin and extended caudally approximately 3.5 cm; the transmitter was mounted on the leading edge of the saddle. The saddle was attached to the dorsal fin using three 8.0 mm diameter high density polyethylene or Delrin pins secured with steel lock nuts. The PTT was 15 cm long with a 17-cm whip antenna. The entire cylindrical PTT package, including saddle, weighed approximately 300 g in air. The side-mount configuration consisted of

Table 1 *Phocoena phocoena*. Data on harbour porpoises equipped with satellite transmitters in 1994 and 1995 in the Bay of Fundy, Canada

	Ident. no.	Sex	Standard length (cm)	Body mass (kg)	Period of contact	Configuration	Tracking period (d)
1994	1	F	141	53[a]	11 Aug–12 Aug	Front/VHF	2
	2	M	145	53[a]	17 Aug–22 Aug	Front/VHF	6
	3	M	140	46	24 Aug–4 Sep	Front/VHF	21
1995	4	M	142	48	13 Aug–3 Sep	Front	21
	5	M	147	51	13 Aug–2 Sep	Front	19
	6	F	147	56	16 Aug–18 Sep	Side	33
	7	M	141	48	21 Aug–20 Mar (96)	Side/VHF	212
	8	M	151	54[a]	21 Aug–26 Oct	Side	66
	9	M	140	47	21 Aug–27 Oct	Side	67

[a] Body mass estimated by predictive equations using length and girth (Read and Tolley 1997)

a flat-board ST-10 mounted in a low profile, rectangular, lexan box. These tags were attached directly to the side of the dorsal fin using three 6.5 mm Delrin pins. The backing plate on the transmitter housing provided attachment points for the pins. The pins passed through the backing plate and dorsal fin and were secured on the opposite side of the fin with steel lock nuts backed with small (30×1.5 mm) Delrin washers. Both backing plate and washers were lined with open cell foam. This tag had a 17-cm whip antenna, measured $11 \times 5 \times 2$ cm, and weighed approximately 150 g in air.

To minimize the size of the PTT packages, we only used one environmental sensor, a surface time counter, which provided a cumulative record of the time the tag was above the water surface. The value of the surface time counter was transmitted twice during each signal, allowing us to detect transmission errors. Each tag also incorporated a salt-water switch, which prevented transmission when a porpoise was submerged. To further conserve battery life, we used a duty cycle of 8 h d^{-1}. The PTTs were powered by two 2/3 A lithium cells which, under these operating conditions, were predicted to provide several months of battery life.

We also attached standard Model 2 VHF transmitters (ATS, Ipsanti, Minnesota, USA) on four porpoises fitted with PTTs. These tags transmitted at frequencies in the 148 MHz range at 110 pulses min^{-1}, without a salt-water switch or duty cycle. VHF transmitters had life expectancies of > 50 d. Each tag was attached to a livestock ear tag (Jumbo roto-tag, Dalton Supplies, Nettlebed, England) which we applied to the trailing edge of the dorsal fin. VHF tags had 33-cm-long whip antennae, measured $1.1 \times 2.5 \times 5.5$ cm and weighed 15 g. VHF transmitters had an effective range of ~5 km at sea level, with greater ranges for receivers on cliff tops or in airplanes.

Data analysis

Location and sensor data from each porpoise were obtained from Service ARGOS, Inc. (Landover, Maryland, USA) in ASCII format on magnetic media. In addition to the location and surface time data, Service ARGOS provided information on the quality of the estimated location. Location quality depends on the number of transmissions received from a PTT during a satellite overpass, the time elapsed between these receptions, movement of the PTT, and the stability of the transmitter oscillator. Each location was classified into one of four categories: Class 3 (at least six uplinks received in a single satellite pass, position accuracy better than 150 m), Class 2 (five uplinks received in a single satellite pass, position accuracy within 350 m), Class 1 (four uplinks received in a single satellite pass, position accuracy within 1 km), Class 0 (less than four uplinks received in a single satellite pass, position accuracy greater than 1 km). In addition, location estimates in Classes A (three uplinks received) and B (two messages received) were provided. The location estimates of these latter two classes were of unknown quality. All estimated locations were filtered using a speed plausibility check; consecutive positions resulting in an average travel speed of greater than 7.5 km h^{-1} were excluded. This filter value was selected based on published travel speeds (Gaskin et al. 1975; Westgate et al. 1995) and field observations of wild harbour porpoises. We tested each tag on shore prior to deployment, and obtained position estimates from known locations allowing us to ground-truth the accuracy of the location classes as provided by ARGOS.

Analysis of movement data was performed using Arcview Geographic Information System (GIS) (ESRI 1994). We included only the best position obtained per day from each porpoise to avoid bias associated with multiple daily positions. Mean daily distance travelled was calculated by summing the distance (km) between the best position received each day for all days of the deployment and then dividing this value by the number of days of the deployment. Mean rate of travel was calculated by dividing the distance (km) between sequential best daily positions by the time (h) that had elapsed between those positions. Mean distance from shore (km) was calculated by averaging the distance from the best daily position to the nearest mainland shoreline (including Grand Manan Island). The proportion of time spent in various water depths was estimated by assigning best daily positions to one of three bathymetric brackets: 0–92 m, 92–183 m, and > 183 m. Depth was estimated using bathymetry contours on digitized National Ocean Service (USA) and Canadian Hydrographic Service marine charts. The proportion of time spent at the surface was estimated using telemetered data from the surface time counter.

Results

Data were obtained from tagged harbour porpoises (*Phocoena phocoena*) for periods ranging from 2 to 212 d. A total of 1334 locations were received on 447 tracking days. The mean number of positions per day for all location classes ranged from 1.9 ± 0.8 to 3.9 ± 0.9. Reliable location classes (0, 1, 2, 3) accounted for 53.8% of all position estimates (Table 2).

We used conventional radiotracking techniques to locate two of the four VHF tagged porpoises after release. The other two porpoises carrying VHF transmitters moved quickly out of range after release, making it impossible to relocate them. Porpoise No. 1 was relocated on the day following release and again 4 d later, after we had lost satellite contact with the PTT. From visual observation and photographs taken on this day, we noted that the polyethylene attachment pins had sheared and the entire PTT package had been lost. This observation led us to employ more robust Delrin attachment pins for the remaining deployments. We continued to monitor VHF radio signals from No. 1 until

Table 2 *Phocoena phocoena*. Data on movements and surface behaviour calculated from tracking harbour porpoises equipped with satellite transmitters

Ident. no.	Mean no. uplinks d^{-1}	Mean daily distance travelled (km)	Mean rate of travel (km h^{-1})	Mean distance from shore (km)	Proportion of time spent at surface
1[a]	NA	NA	NA	NA	NA
2	3.5	58.5	2.3 ± 1.3	27.3 ± 20.3	NA[a]
3	3.8	13.9	0.6 ± 0.4	11.5 ± 4.7	NA[b]
4	1.9 ± 0.8	34.1	1.6 ± 1.2	23.4 ± 20.9	0.05 ± 0.01
5	2.1 ± 0.7	22.6	1.1 ± 1.1	16.0 ± 15.4	0.07 ± 0.04
6	3.6 ± 0.7	18.1	0.8 ± 0.7	6.6 ± 5.1	0.04 ± 0.01
7	2.6 ± 1.2	28.1	1.2 ± 1.0	81.4 ± 44.8	0.03 ± 0.01
8	3.7 ± 1.0	15.0	0.6 ± 0.4	18.5 ± 6.4	0.05 ± 0.01
9	3.9 ± 0.9	17.6	0.7 ± 0.5	26.0 ± 26.1	0.04 ± 0.01

[a] Due to the short duration summary statistics were not calculated
[b] Due to an attachment failure that affected the salt-water switch, surface data were not calculated

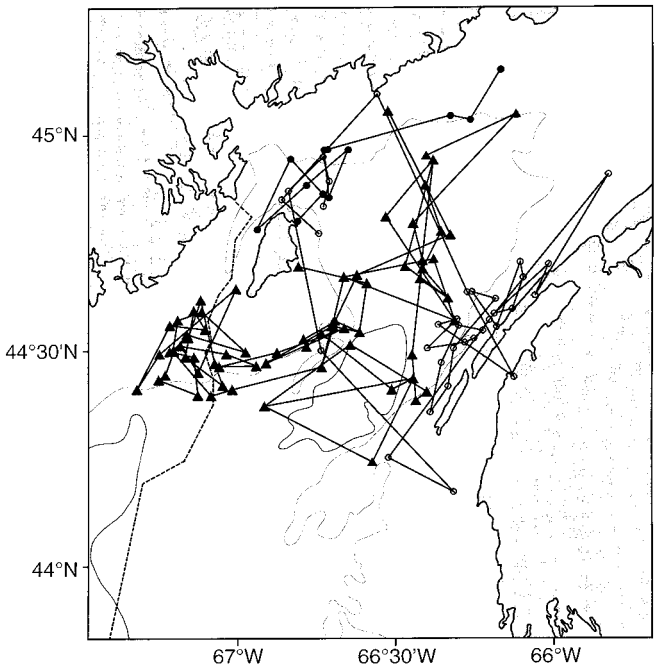

Fig. 2 *Phocoena phocoena.* Tracks of three harbour porpoises in the lower Bay of Fundy obtained from satellite telemetry. Only best daily positions are shown. Due to the brief tracking period of No. 1, movements of this porpoise are not shown (● No. 3; ○ No. 6; ▲ No. 8)

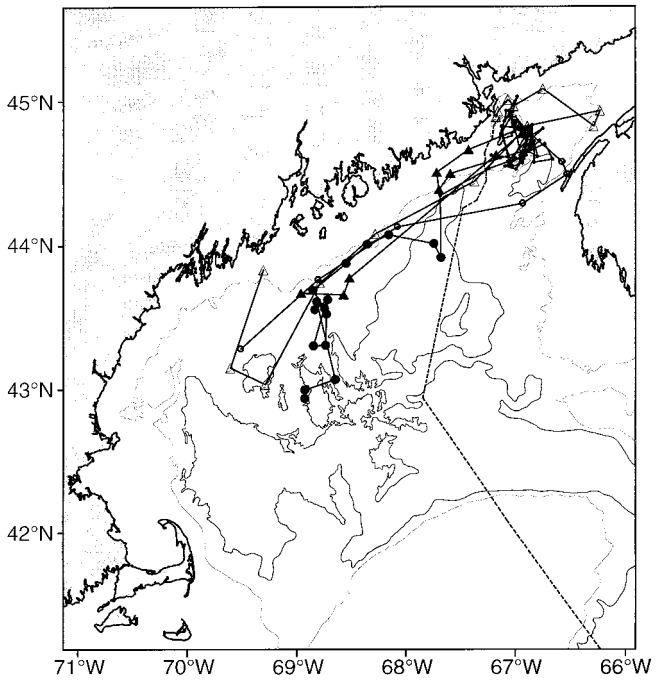

Fig. 3 *Phocoena phocoena.* Tracks of four porpoises in the Bay of Fundy and Gulf of Maine obtained from satellite telemetry. Only best daily positions are shown (○ No. 2; △ No. 4; ▲ No. 5; ● No. 9)

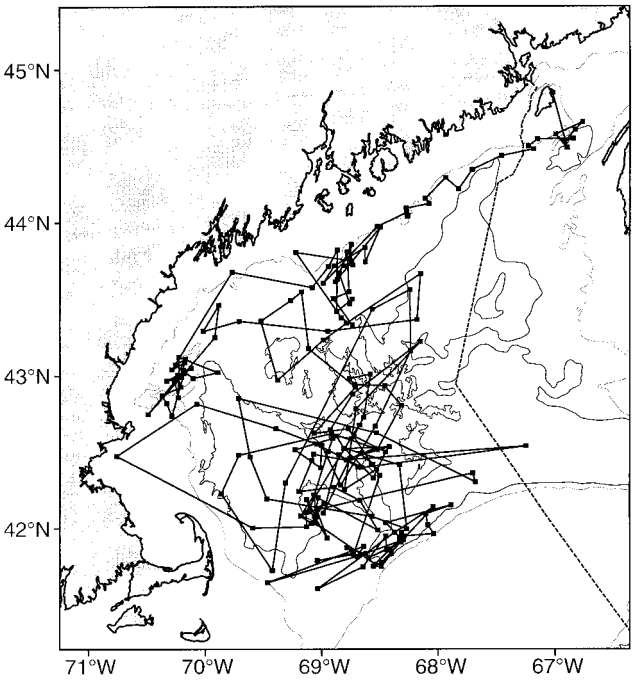

Fig. 4 *Phocoena phocoena.* A 212-d track of a single harbour porpoise (No. 7) obtained from satellite telemetry. Only best daily positions are shown

31 August, visually relocating her and her calf on four other occasions. We also located No. 3, 7 d after release in a large group of feeding porpoises east of Grand Manan Island. In all of these sightings, tagged porpoises were swimming normally, usually in the company of other porpoises.

Tagged harbour porpoises displayed considerable variability in their movement patterns. Four (Nos. 1, 3, 6 and 8) remained in the Bay of Fundy throughout their tracking periods (2 to 66 d) (Fig. 2). These porpoises did not remain in the deep, central portions of the Bay for extended periods, although several traversed the deep water (>200 m) between Grand Manan and Nova Scotia. One porpoise (No. 8) spent several weeks to the southwest of Grand Manan. Tagged porpoises rarely moved further northeast into the Bay of Fundy than the northern tip of Grand Manan Island. All of the individuals that remained in the Bay of Fundy spent at least some time in the primary areas of Canadian gillnet fishing effort, located to the northeast and southwest of Grand Manan Island (Fig. 1).

Five tagged porpoises (Nos. 2, 4, 5, 7, 9) left the Bay of Fundy and did not return during their tracking periods (6 to 212 d) (Figs. 3, 4). None of these porpoises left the Gulf of Maine. When porpoises left the Bay of Fundy they moved southwest along the 92 m isobath. One of these porpoises, No. 7, had the longest tracking period (212 d) and moved extensively throughout the Gulf of Maine (Fig. 4). After release, this porpoise moved from the Bay of Fundy to Cashes Ledge in the Gulf of Maine, covering 300 km in 21 d. Porpoise No. 7 stayed in this area for approximately 1 month before travelling south to Jeffreys Ledge, where it remained during the height of the US autumn gillnet fishery. In mid-November, No. 7 moved directly east to the

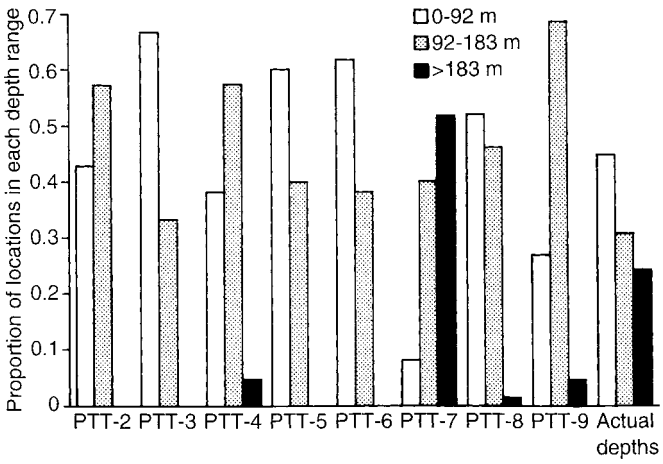

Fig. 5 *Phocoena phocoena.* Histogram showing the proportion of time spent in various water depths by porpoises as determined by satellite telemetry. Depths were calculated by assigning the best daily positions to one of three bathymetric brackets: 0–92 m, 92–183 m, or >183 m. Actual depths of the entire study area are shown in the column at far right

Franklin Basin and remained there through December. Between January and mid-March this porpoise travelled throughout the central Gulf of Maine.

Individual variability in porpoise movements was exemplified by the tracks of three adult male porpoises released from the same weir on 21 August 1995. One of these porpoises (No. 8) spent the entire 66-d tracking period in the Bay of Fundy (Fig. 2). The second porpoise (No. 9) remained southeast of Grand Manan Island in the Bay of Fundy for 25 d before moving southwest to Cashes Ledge in the Gulf of Maine (Fig. 3). The last porpoise (No. 7) immediately left the Bay of Fundy and spent the entire tracking period in the Gulf of Maine (Fig. 4).

Estimates of daily distance travelled were similar for all porpoises (13.9 to 28.1 km) with the exception of No. 2 with a mean daily distance of 58.5 km. Mean rates of travel ranged from 0.6 to 2.3 km h^{-1}, but the rates of travel in longer tracking periods (>30 d) were similar (Table 2). Mean distances from shore ranged from 6.6 to 81.4 km with an overall mean of 50.2 ± 46.2 km. Porpoises spent between 3 ± 1 and 7 ± 4% of their tracking periods at the surface (Table 2). Tagged porpoises were most frequently (55% of locations) in water depths of 92 to 183 m and least frequently (12%) in depths >183 m (Fig. 5).

Discussion

Ecological significance

The movements of harbour porpoises (*Phocoena phocoena*) monitored by satellite telemetry can be interpreted at several scales. Individual porpoises often spent periods from days to weeks in fairly restricted areas. Many of these areas, particularly in the waters to the southeast of Grand Manan Island (Fig. 3), the western shore of the Digby Peninsula (Fig. 2) and Jeffreys Ledge (Fig. 3), are known, from sighting surveys and aggregations of incidental catches in commercial fisheries, to be important habitat for this species (Gaskin 1984; Palka 1995; Palka et al. 1996). Other areas, like the waters to the southwest of Grand Manan Island (Fig. 2) and the Franklin Basin (Fig. 4) were not previously considered important porpoise habitat. Harbour porpoises are small endotherms (ca. 50 kg) which inhabit temperate waters (< 10 °C) and are suspected to have a limited energy storage capacity (Koopman 1994). Given these energetic constraints, it may be advantageous for porpoises to maintain close proximity to aggregations of prey. Many areas that harbour porpoises frequented are known to support seasonal concentrations of Atlantic herring (*Clupea harengus*) (Stephenson et al. 1993), the primary prey of harbour porpoises in the Bay of Fundy and Gulf of Maine (Recchia and Read 1989). The periods of restricted movements recorded with satellite telemetry were consistent with previous findings from VHF telemetry studies of porpoises in the Bay of Fundy (Gaskin et al. 1975; Read and Gaskin 1985; Westgate et al. 1995).

Porpoises also made fairly rapid point-to-point excursions that lasted from several hours to several days. This directed travel was seen most frequently by porpoises exiting the Bay of Fundy along the 92 m isobath (Figs. 3, 4). This area may represent an important movement corridor connecting the Bay of Fundy and lower Gulf of Maine. The impetus for such short-term movements is unclear. Porpoises may undertake such movements in response to changes in local prey availability, the presence of predators, or to social factors. These rapid long-distance movements were not captured in previous VHF telemetry studies (Gaskin et al. 1975; Read and Gaskin 1985; Westgate et al. 1995) because of the limited range of the transmitters, the relatively small areas that could be effectively surveyed and the fact that porpoises were not tracked at sea for extended periods.

When the movements of tagged individuals are examined at their largest scale, it is clear that the home range of harbour porpoises occupies most of the Gulf of Maine and is much larger (ca. 50 000 km^2) than the 210 km^2 previously estimated (Read and Gaskin 1985). Tagged porpoises moved throughout the Bay of Fundy and Gulf of Maine, covering hundreds of kilometers in a relatively short time. The mobility of these small porpoises was surprising and has forced us to reassess our concept of the scale at which they use their habitat on a seasonal and annual basis. In particular, the movements of Porpoise No. 7 illustrate the extensive use of the Gulf of Maine by these marine mammals. Prior to this study, our knowledge of the habitat use of harbour porpoises in the autumn and winter was limited to observations of incidental catches in gillnets. We can now better appreciate the dynamic nature of habitat utilization during this period.

The movements of porpoises from the Bay of Fundy into the Gulf of Maine, but not around the southwestern tip of Nova Scotia, supports Gaskin's (1984) hypothesis that harbour porpoises in the Bay of Fundy and Gulf of Maine comprise a single population. This finding corroborates other evidence from mtDNA studies (Wang et al. 1996), life history parameters (Read and Hohn 1995), and organochlorine profiles (Westgate et al. 1997). The current management strategy, based on the assumption of a single population in this region, is appropriate. These data also show that the seasonal movements of individual porpoises are discrete. The seasonal decline in harbour porpoise density in the Bay of Fundy during autumn (Gaskin 1984) is not the result of a coordinated migration, but a gradual net movement of porpoises into a wider geographic region. Records from strandings and incidental catches in commercial fisheries indicate that the winter distribution of harbour porpoises extends as far south as North Carolina (Gaskin 1984; Read et al. 1996). The movements of Porpoise No. 7, however, show that some individuals do not leave the Gulf of Maine during winter.

The values for the mean daily distance and mean rate of travel represent minimum estimates for harbour porpoises. The scale at which we measured these parameters, based on best sequential positions per day, misses much of the fine-scale movement exhibited by free-ranging porpoises (Westgate et al. 1995). The congruence between the mean daily distance and mean rate of travel among the discrete movements recorded from Porpoises Nos. 7, 8 and 9 (Figs. 2, 3, 4) reflects the coarseness of this scale. These values may therefore be more indicative of average porpoise movements within restricted areas (as discussed above) rather than between such areas. This is illustrated by the record from Porpoise No. 2, which had a much greater daily travel distance (58.5 km), a consequence of its relatively straight movement during the short tracking period.

Knowledge of the proportion of time a harbour porpoise spends at the surface is important for the design and analysis of abundance surveys. Data from the present study reflect the actual proportion of time (3 to 7%) that the salt-water switch, and hence the dorsal fin, was above the water surface. These values are very close to the true time that a harbour porpoise would be visible to observers aboard a survey vessel, and much less than estimates of the time porpoises spend in the upper 2 m of the water column (33 to 60%) (Westgate et al. 1995).

Conservation significance

The movements of harbour porpoises from areas of gillnet fishing effort in the Bay of Fundy to similar fishing grounds in the Gulf of Maine indicate that individuals in this population are at risk of entanglement during several periods of the year. For example, Porpoise No. 7 travelled from the most concentrated area of Canadian gillnet fishing effort in August to an area of intense US gillnet activity later in the fall. These movements emphasize the trans-boundary nature of the Bay of Fundy/Gulf of Maine population.

These data allow us to evaluate, albeit in preliminary fashion, the efficacy of time-area fishery closures as a management strategy for reducing the level of incidental mortality. Our data indicate that porpoises exhibit a high degree of individual variability in their movements, suggesting that effective closures will have to be extensive in time and space. The fishing industry has proposed the use of trigger mechanisms, so that fishery closures would be tied to the appearance of harbour porpoises in particular areas, thus minimizing disruptions to fishing activity. Our results suggest that the movement patterns of individual harbour porpoises are extremely variable and are not currently predictable on a scale that would serve as a useful trigger mechanism.

The bathymetric analysis showing that in the Bay of Fundy and Gulf of Maine, harbour porpoises are found most frequently in areas where depths range between 92 and 183 m are consistent with observations of high rates of incidental catches in this depth range (Bisack and Northridge 1993). It is unclear why porpoises use these waters to a greater extent than other areas, but it may be related to the distribution of their prey. This is an area of study that may offer considerable insight into the nature of entanglement and factors that contribute to its risk. We hope to obtain detailed information on harbour porpoise foraging behaviour in relation to prey distribution and bottom topography in the future.

The high degree of individual variability in the movements of these porpoises has important consequences for the practice of applying telemetry data to both basic ecology and conservation problems. It is clear that large data sets are required to capture the full extent of variation among individuals of different ages and sexes. Our results are limited by the relatively small number of porpoises we studied and the brief tracking periods of some porpoises. A larger data set might reveal other areas of the Bay of Fundy and Gulf of Maine that constitute important habitat for these porpoises. Two such regions identified in the present study are the area southwest of Grand Manan used by Porpoise No. 8 and the Franklin Basin used by No. 7 during the late fall and winter.

Finally, we note that this technology offers great promise for the study of the ecology, behaviour, and conservation of small cetaceans. Our observations represent the first satellite telemetry data obtained from harbour porpoises and the longest period of satellite contact yet obtained from a cetacean (212 d). Despite the limited time that porpoises spend at the surface, it was possible to obtain reliable location estimates from most porpoises on most days. It was not possible to make any objective assessment of the effects of PTT packages on tagged porpoises. Over the course of the longest deployment (212 d, No. 7) we noted a significant increase ($p < 0.01$) in the average daily distance travelled and a significant decrease ($p < 0.01$) in the pro-

portion of time spent at the surface. Neither of these findings are consistent with trends one might expect from a sick individual, suggesting that any effects were within the tolerance limits of harbour porpoises. Utilization of two tags (VHF and PTT) enabled us to relocate porpoises after release and to modify and improve the tag design after identifying a problem. The side-mounted design holds more promise as a long-term attachment configuration. We believe that the short longevity of the front-mount design resulted from increased drag, as measured from mock tags in wind tunnel testing (Brad Hansen, National Marine Mammal Laboratory, Seattle, Washington, personal communication).

These data provide new insights into the movement patterns of harbour porpoises. Porpoises do not confine their movements to the Bay of Fundy during the summer but also utilize extensive parts of the Gulf of Maine. Generally, porpoises made relatively fast linear movements between apparently productive habitats where they then remained for extended periods. There was a high degree of individual variability in porpoise movements and there was no evidence that porpoises engaged in a coordinated migration out of the Bay of Fundy/Gulf of Maine during the autumn. The movements of porpoises from Canadian to US gillnet fishing areas shows that they are at risk of entanglement for a significant portion of the year. These movements underscore the trans-boundary nature of this population and emphasize the need for co-ordination between management agencies in Canada and the USA in resolving conflicts between porpoises and gillnet fisheries.

Acknowledgements This paper is dedicated to the memory of Frank G. Carey, a pioneer in the study of free-ranging pelagic animals. This research was supported by the US National Marine Fisheries Service, Northeast Fisheries Science Center (NEFSC), the US Office of Naval Research, the Endangered Species Recovery Fund of World Wildlife Fund Canada, and the Canadian Wildlife Service. D. Gaskin (University of Guelph) contributed funds, field equipment and logistical support. D. Potter (NEFSC) made this work a reality through his consistent support and encouragement. T. Cox (Duke University) deserves special recognition for her patient and thorough analysis of these data. We also thank: K. Anderson and H. Koopman (Duke University); K. Murray and D. Palka (NEFSC); D. Gannon, T. Howald, T. Spradlin and W. Watkins (Woods Hole Oceanographic Institution); M. Scott (Inter-American Tropical Tuna Commission); W. McLellan and A. Pabst (University of North Carolina, Wilmington); the Grand Manan Whale and Seabird Research Station; and especially the weir fishermen of Grand Manan, without whose dedicated assistance this work would not have been possible. Insightful comments from A. Martin and one anonymous reviewer improved the manuscript greatly. This work was carried out under Permit 1995-168 (Department of Fisheries and Oceans, Canada) and University of Guelph Animal Utilization Protocol 94-R082.

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Life History of Small Cetaceans in the Northwest Atlantic

Appendix 6

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Harbour Porpoises (*Phocoena phocoena*) Habituate to Pingers

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ABSTRACT

Large bycatches of harbor porpoises (*Phocoena phocoena*) occur in gillnet fisheries throughout the northern hemisphere. Several mitigation measures, including acoustic deterrent devices or 'pingers', have been used to reduce this bycatch. The potential exists for harbor porpoises to habituate to pingers, thus reducing their effectiveness over time. We conducted a field experiment to test the hypothesis that porpoises will habituate to the sound produced by pingers. We monitored porpoise echolocation and tracked porpoise movements around a mooring equipped with a pinger (Dukane NetMark 1000) for three months in summer 1998 in the Bay of Fundy. Using a mean-shift model, we estimated that porpoises were initially displaced 151m from the pinger ($p = .05$), but this displacement diminished by 50% within 5 days ($p = .02$). Echolocation rate ($p < .001$) and occurrence ($p < .001$) were significantly reduced in the vicinity of the pinger. These results indicate that porpoises will habituate to pingers and that porpoises are not alerted to echolocate in the presence of nets by pingers.

INTRODUCTION

Large numbers of dolphins and porpoises die in gillnets worldwide, posing serious threats to several populations and species (Jefferson and Curry 1994; Perrin et al. 1994). Acoustic alarms or 'pingers' are currently used in several fisheries to reduce these bycatches (Kraus et al. 1997; Gearin et al. 1996; Cameron 1998; Trippel et al. 1999). As the use of pingers spreads, concerns have been raised about their long-term effectiveness (Dawson et al. 1998).

One of the most intensive efforts to reduce small cetacean bycatch has occurred in the Gulf of Maine. Between 1992 and 1996, an average of 2100 harbor porpoises (*Phocoena phocoena*) died annually in gillnets – approximately 4% of the estimated population of 54,300.

This mortality greatly exceeded allowable removal levels set under US legislation (Waring et al. 1999). Kraus et al. (1997) demonstrated that pingers caused a significant reduction in the bycatch rate of harbor porpoises in the Gulf of Maine. Fishermen have taken an active role in the development and testing of pingers and are supportive of their widespread use in this fishery. Consequently, the use of pingers was recommended as an integral component of the management plan designed to reduce incidental mortality to sustainable levels (Federal Register 1998).

In addition to recommending the use of pingers in the Gulf of Maine, the management plan recommended that research be conducted on several aspects of their use, including the potential for habituation. Habituation is defined as "the relatively permanent waning of a response as a result of repeated stimulation which is not followed by any kind of reinforcement" (Thorpe 1966). Participants at a workshop sponsored by the US National Marine Fisheries Service and the Marine Mammal Commission also noted the possibility that the effectiveness of pingers could decline due to habituation (Reeves et al. 1996). As more and more pingers are used in the Gulf of Maine, the response of harbor porpoises to these pingers could wane, reducing the efficacy of this management tool.

The purpose of this study was to evaluate the potential for porpoises to habituate to pingers. This experiment, conducted in the summer of 1998, is part of a larger research program designed to address the question of habituation. Another important aspect of this overall program will be to monitor the observed bycatch rate of porpoises over time in areas where pingers are used, to determine whether or not habituation is occurring. In the field experiment described here, we used a technique developed by Koschinski and Culik (1997), in which shore-based observers used a theodolite, or surveyor's transit, to track the movements of porpoises in the vicinity of active pingers. In a short-term study of six days duration, Koschinski and Culik

noted that porpoises avoided an experimental net equipped with pingers. Similar findings have been reported by Kastelein et al. (1997) for porpoises in a captive setting. We monitored patterns of harbor porpoises in relation to pingers over longer periods to assess the potential for habituation.

METHODS

Study Area and Experimental Design

We observed porpoises from a cliff on Grand Manan Island, New Brunswick, Canada between June 26, 1998 and September 20, 1998. This area has a very high density of harbor porpoises during the summer months (Waring et al. 1999). We attached a single Dukane NetMark 1000 pinger 10m below the surface to a mooring at 44°47.7'N, 66°48.2'W (Figure 1). The mooring was approximately 1000m offshore and was set in 75m of water. The Dukane NetMark pinger emits a broad-band signal with a fundamental frequency of 10kHz and a sound pressure level of approximately 132dB re 1μPa at 1m. During an initial two-week training period, we tracked porpoises to become comfortable with the theodolite (see below). We then tracked porpoises for two weeks around the mooring while the pinger was attached but not turned on (Control 1) (Table 1). On July 11, we turned the pinger on and tracked porpoises for four weeks (Experimental Trial 1). On August 7, we turned the pinger off, and began tracking again on August 19 (Control 2). At this time we also attached a porpoise echolocation detector, POD (see below). On September 2, we turned the pinger back on, and tracked for four weeks (Experimental Trial 2).

Pinger sound pressure level and frequency decrease with decay in battery voltage (Trippel et al. 1999), so we changed the pinger batteries once a week, and tested the voltage of the batteries after they were removed.

Tracking

Two researchers tracked porpoises using a Geodolite 404 total station and a Husky FS/GS data collector from a 100m cliff approximately 1000m from the mooring. The observational area encompassed a 500m radius around the mooring. One researcher, the *surveyor*, used Fujinon 7x50 binoculars to scan the observational area for porpoises. The surveyor looked in concentric circles around the mooring, extending out to 500m. This individual reported sightings of porpoises to the *tracker*, the researcher stationed at the theodolite. The tracker used the theodolite to track surfacings of the lead porpoise in a group, until one of the following situations occurred: 1) the animals left the study area or 2) the tracker lost sight of the porpoises or could not confirm that it was the same group. The tracker then began tracking the next group of porpoises identified by the surveyor.

Echolocation

On August 20, 1998, we attached a POD to the mooring. The POD continuously logged the number of echolocation clicks in 10s intervals. We programmed the POD to record several channels of echolocation clicks of varying duration and frequency. The frequencies were fixed at 50 kHz, 93 kHz, and 132 kHz. Because porpoises produce distinctive narrow band sonar clicks from 110-150kHz (Møhl and Andersen 1973), we only used clicks at 132 kHz in our

analysis. Single click durations for harbor porpoises are typically 100μs (Møhl and Andersen 1973). Thus, we programmed the POD to capture any click that lasted up to 400μs in duration.

Response Variables

From the results of previous studies, we expected to detect a change in porpoise behavior when the pinger was first activated. Then, if habituation occurred, we expected a gradual waning of this response over the experimental period. We examined three variables that have direct relevance to entanglement: the *point of closest approach* to the pinger, *echolocation rate,* and *echolocation occurrence.* We defined the point of closest approach as the minimum distance between the pinger and a surfacing porpoise during a track. Echolocation rate was defined as the number of clicks recorded per unit time. Echolocation occurrence was expressed as the proportion of 10 second intervals in which clicks were detected.

Sound Field

We mapped the sound field produced by the pinger on September 26, 1999. The day was overcast, and the Beaufort Sea State was 2, diminishing to 1. Researchers drifted past the mooring in a small boat while the position of the boat was recorded from shore using the theodolite. The observers in the boat monitored the sound produced by the pinger with a Bruel and Kjaer 8100 calibrated hydrophone and a 2635 charged amplifier, which emitted a reference signal at 160Hz, 174 dB re1μPa@1m, connected to a Sony TCD-D8 DAT recorder. We then ran a Fast Fourier Transform to estimate the sound pressure level of the pinger in relation to the reference signal.

Analysis

We used a mean-shift model to test the hypothesis that porpoises were initially displaced from the pinger and then gradually moved closer to the pinger:

$$E(Y_i) = m + d^{(-g(t_i - t_o))} I_o(t_i)$$

Where:

Y_i is the closest approach distance for group i (i = 1, 2, 3, ..., n)

m is the control mean

d is the mean shift due to the pinger

g is the rate at which the pinger effect decays to 0

$I_o(t_i) = 1$ if $t_i > t_o$, otherwise $I_o(t_i) = 0$

t_o is the day the pinger was turned on.

The time after t_o at which the mean shift has been reduced by 50% can then be defined as

$$T_{50} = -\log 0.5 / g$$

Because we had a small sample size, we pooled the two trials and fit the model to the combined observations by least squares. To test whether there was an initial response when the pinger was turned on, we tested the null hypothesis H_o: d = 0 against the one-sided alternative hypothesis H_1: d > 0. To test whether there was a significant waning of response over time, we tested the null hypothesis H_o: g = 0 against the one-sided alternative H_1: g > 0. This involved comparing the goodness of fit of the null model:

$$E(Y_i) = m + d\ I_o(t_i)$$

Significance of both null hypotheses were tested using 200 random permutations, in which the values of Y_i were permuted and the full model fitted to the permuted values. P-values were estimated by the proportion of these permutations for which $T > 1.70$. For the second null hypothesis, only those values of Y_i for which $t_i \geq t_o$ were permuted.

We used a univariate factorial analysis of variance to examine variation in echolocation rate as a function of the state of the pinger (on or off) and time of day. Day was defined as occurring between 0700 and 1859 and night occurred between 1900 and 0659 (Westgate et al. 1995). We also used a Chi-squared test to compare the proportion of 10s intervals in which echolocation clicks occurred when the pinger was off and on. Means are presented with their associated standard deviations.

RESULTS

The closest observed approach of the porpoises to the active pinger decreased exponentially over time (Figure 2). Under the mean-fit model, the control mean (m) was 223m (n = 54), the mean shift due to the pinger (d) was 151m (n = 82), and the rate at which the pinger effect decayed to 0 (g) was .14. This estimate of g corresponds to an estimate of t_{50} of 5.0 days. The mean shift due to the pinger was significantly different from 0 (p = .05). The rate at which the pinger effect decayed was also significantly different from 0 (p = .02).

We chose 30 minutes as the unit of time for our analysis of echolocation rate, because only one group of porpoises remained in the area for more than this period (31 minutes). Therefore, we assumed independence among measurements of the number of echolocation clicks per half hour. Echolocation rate for the control (516 ± 2062; n = 288) was significantly greater than when the pinger was active (82 ± 366; n = 496) (p < .001). In addition, echolocation rate

was higher at night (377 ± 1699; n = 432) than in the day (75 ± 409; n = 352) ($p < .001$) for both control and active periods. The proportion of 10 second intervals in which clicks were detected decreased after we activated the pinger (control = .174; experimental = .041) ($\chi^2 = 9241$; $p < .001$).

Received sound pressure levels decreased to 20 dB above ambient at approximately 150m from the pinger (Figure 3). Ambient noise level varied from 95dB to 115dB re 1μPa@1m. Battery voltage averaged 1.46 ± .06V when removed from the pingers.

DISCUSSION

Habituation

Our statistical analysis indicates that porpoises habituated to the pinger. The initial displacement distance of 151m we observed was similar to the displacement of 133m and 125m observed by Koschinski and Culik (1997) and Laake et al. (1998), respectively. Porpoises initially reacted by avoiding the pinger, but that response began to wane almost immediately. Thus porpoises habituated rapidly and approached the pinger more closely over time.

Demonstration of habituation typically relies on repeated observations of known individuals (Richardson et al. 1995). It was not possible to identify individual porpoises as we tracked their movements with the theodolite. However, previous studies of the movements of porpoises in the Grand Manan area using satellite and VHF telemetry have shown that individual animals are present in particular areas for weeks or months (Read and Westgate 1997). For example, we tracked a porpoise tagged with a satellite-linked radio transmitter around the mooring on September 1, 1998. This porpoise had been in the area for several weeks (Figure 4).

Thus, individual porpoises likely experienced multiple exposures to the pinger over the course of the experiment.

Our experimental protocol involved only a single pinger on a mooring, so we cannot say with certainty that porpoises will habituate to pingers attached to a gillnet. In fact, even if habituation occurs, it may not lead to an increase in bycatch rate if there is enough residual effect to keep porpoises away from nets. Thus, a monitoring program is necessary to ensure bycatch does not rise as porpoises habituate to the pinger.

Elucidating the mechanism by which pingers work will further aid in determining if porpoises will habituate to pingers on gillnets (see below). For example, if the sound of pingers is aversive to porpoises, they are likely to habituate to it. However, if porpoises are using ambient noise imaging to detect the net (Potter 1997), then they are less likely to habituate. Ambient noise imaging is the use of scattered sound to detect objects. The sound from a pinger may scatter around a gillnet, making it detectable to the porpoise, and thus making the porpoise aware of a barrier. Under this scenario, a porpoise would associate pingers with barriers and not habituate to their presence.

Echolocation

Kraus et al. (1997) hypothesized that pingers might stimulate porpoises to echolocate, and thus detect a gill net. We tested this hypothesis by examining echolocation rate of porpoises in relation to the moored pinger. The reduction in echolocation rate (number of clicks per unit time) when the pinger was activated demonstrated that porpoises were either echolocating less frequently in the vicinity of the pinger or using shorter click trains. If porpoises were using a similar number of shorter trains we would have expected the proportion of 10s intervals

containing clicks to be similar in control and experimental nets. However, the proportion of 10s intervals in which echolocation events occurred was significantly reduced when the pinger was activated, suggesting that porpoises echolocate less frequently in the vicinity of an active pinger.

It is possible, and perhaps likely, that many porpoises were displaced from the pinger, and the POD did not detect their clicks. Preliminary studies estimate the range of the POD to be 50-100m (unpub. data). This distance is considerably greater than the distance (2 to 9 m) at which porpoises can detect nets with floatlines using echolocation (Hatakeyama and Soeda 1990). Thus, we can reject the hypothesis of Kraus et al. (1997) that pingers stimulate porpoises to echolocate, as the echolocation frequency of porpoises around the pinger did not increase when the device was activated.

Even during the control period, echolocation clicks were recorded only 17% of the time. We tracked porpoises around the mooring at this time, and three times porpoises were oriented towards the mooring within 50m, but no echolocation clicks were recorded. Thus, it is likely that porpoises are not echolocating constantly. This finding has relevance for the development of other acoustic means of reducing bycatch, particularly those which rely on a passive approach.

Because Trial 2 was truncated due to poor weather conditions, we were unable to monitor changes in echolocation response to the pinger over time. Future studies should monitor echolocation rate and frequency as additional response variables that could wane over time. Investigating these responses over time would further elucidate the potential for porpoises to habituate to the presence of a pinger.

Conclusion

Our results show that the effects of habituation must be considered when pingers are used to reduce the bycatch of small cetaceans. Long-term monitoring of bycatch should take place to ensure the effectiveness of pingers in gillnet fisheries. Our study was not designed to test hypotheses of the mechanism by which pingers reduce harbor porpoise bycatch, but we were able to reject the hypothesis that pingers stimulate harbor porpoises to echolocate and thus detect a gillnet. Monitoring harbor porpoise echolocation around gillnets equipped with pingers could further elucidate the mechanism by which pingers reduce bycatch.

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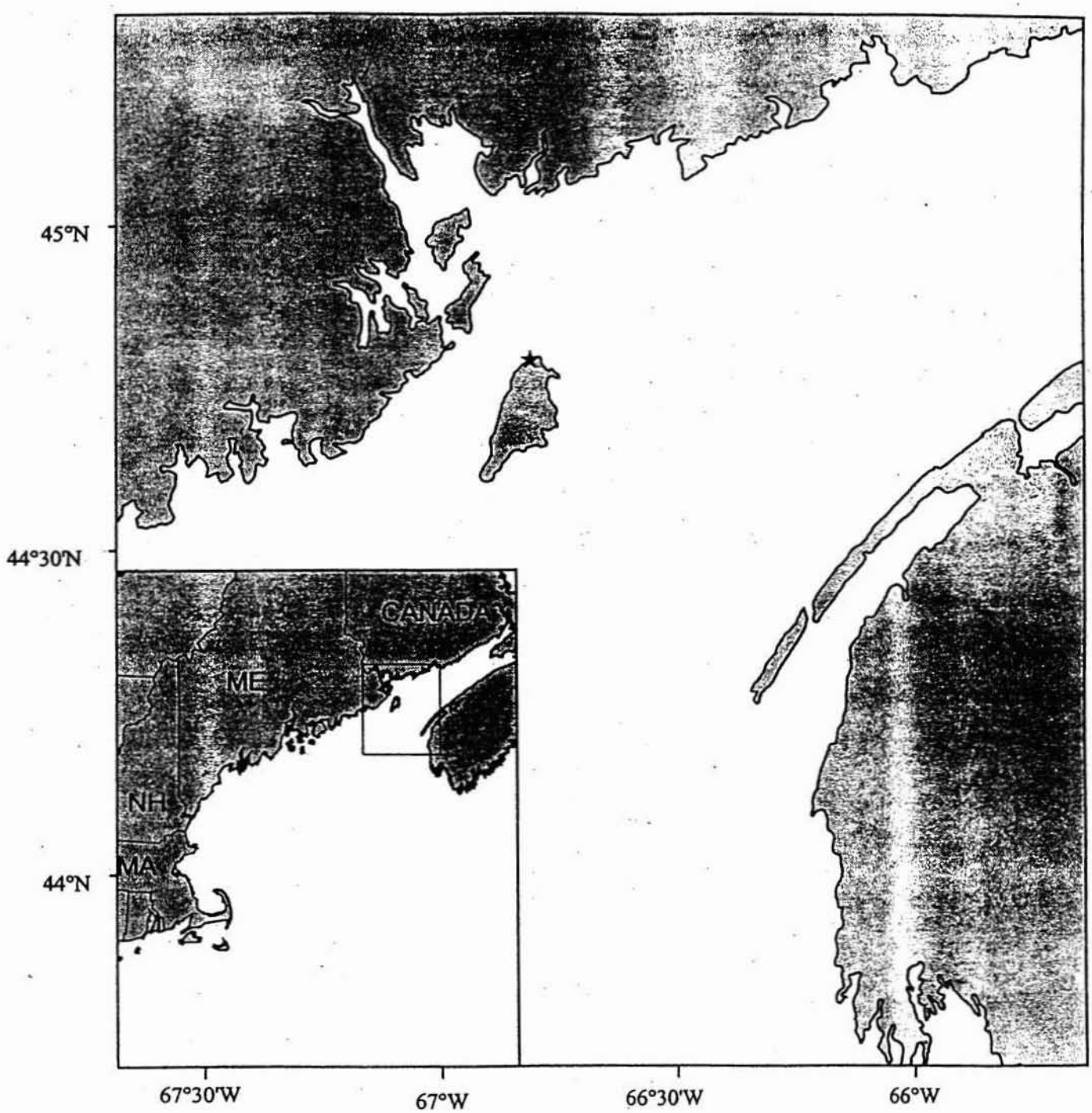
Table 1. Timing of habituation trials. Trial 2 was terminated early due to poor weather conditions.

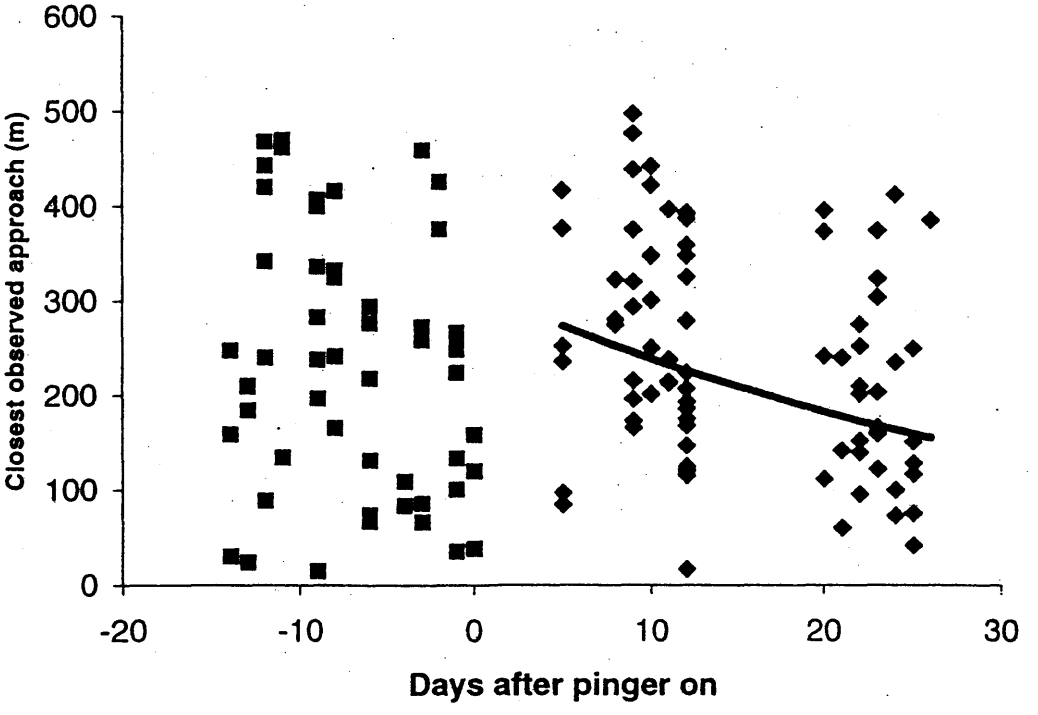
	Begin	End	Pinger	POD
TRAINING	6 June 1998	22 June 1998	OFF	-
TRIAL 1				
Control	26 June 1998	10 July 1998	OFF	-
Experimental	11 July 1998	7 August 1998	ON	-
TRIAL 2				
Control	19 August 1998	1 September 1998	OFF	ON
Experimental	2 September 1998	27 September 1998	ON	ON

List of Figures

Cox
Figure 1



Cox
Figure 2


600
500
400
300
200
100
0
Closest observed approach (m)
-20
-10
0
10
20
30
Days after pinger on

Cox
Figure 3

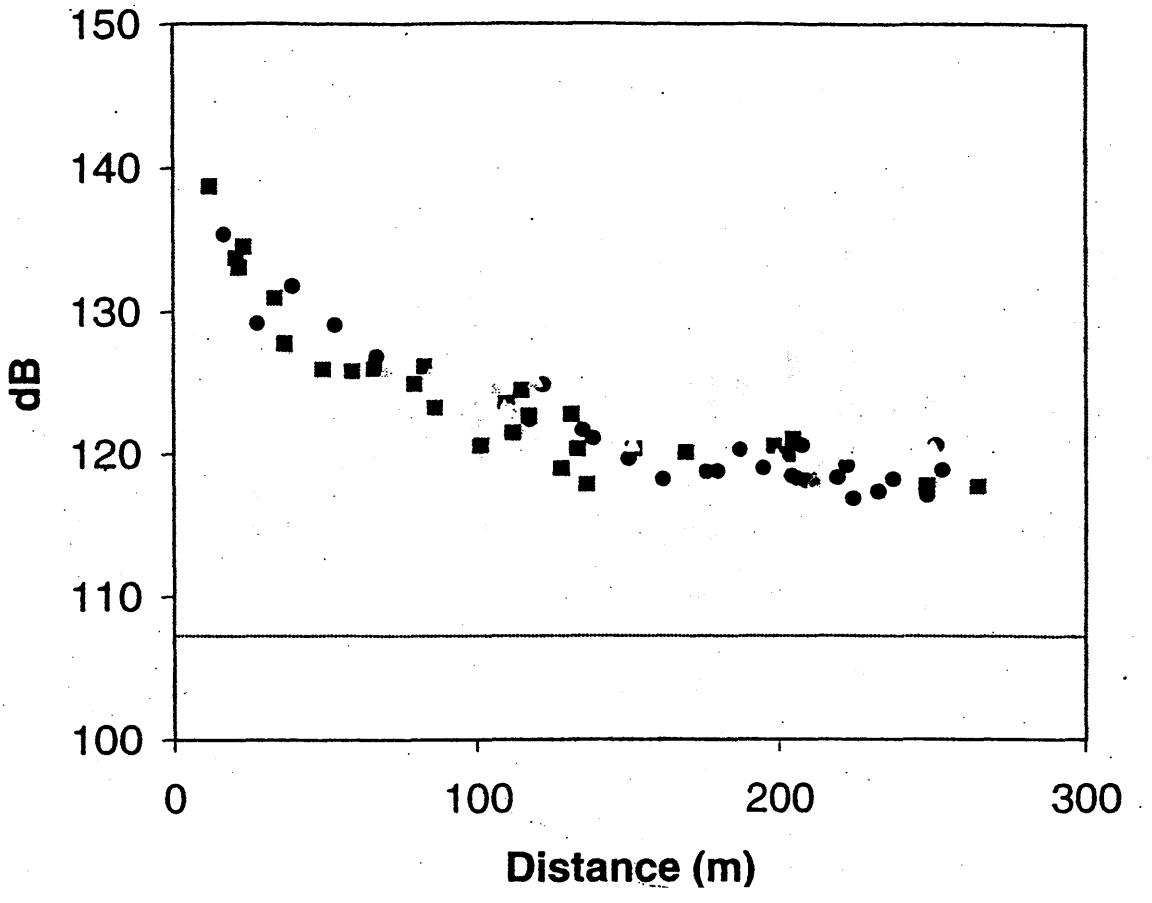


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Figure 4