Population genetics of harbour porpoise in Swedish waters

– a literature review
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Preface

This report is aimed at reviewing current knowledge on the genetic population structure of harbour porpoise (Phocoena phocoena) in Swedish and adjacent waters (here defined to include the Baltic, Kattegat, and Skagerrak Seas). The review was initiated by the Swedish Environmental Protection Agency to provide conservation and management guidelines for the harbour porpoise.

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Summary

This report is aimed at reviewing current knowledge on the genetic population structure of harbour porpoise (*Phocoena phocoena*) in Swedish and adjacent waters (here defined to include the Baltic, Belt, Kattegat, and Skagerrak Seas). Population genetic information is necessary for effective conservation and management of the species, and one of the key questions refers to how many genetically distinct populations of porpoises that exist in this region.

The review was conducted within the framework of ongoing efforts of the Swedish Environmental Protection Agency to provide conservation and management guidelines for the harbour porpoise. No new data has been collected; the review is based exclusively on previously published information.

The harbour porpoise is regarded as threatened in most parts of its distribution range, which includes the three major geographic areas of the North Pacific Ocean, the North Atlantic, and the Black Sea/Sea of Azov. The global threat status of the species is "vulnerable". In Sweden, the abundance has declined during the second half of the 20th century, and the population reduction is considered particularly dramatic within the Baltic Sea, where the census size is estimated to only a few hundred individuals.

We collected previously published scientific studies that directly or indirectly address questions concerning the genetic population structure of the harbour porpoise through search of literature databases. We summarize and discuss the conclusions of those studies focusing on papers that are based on clearcut genetic data obtained by means of various molecular techniques. Several workers address the issue of population differentiation through studying characters for which the genetic basis is not fully understood (e.g., skull or tooth morphology, time for reproduction, etc.), and it is therefore not clear to what extent the variation of such characters reflect divergence on the gene level. Some of those studies are referred to in this report, but our discussion is based exclusively on the results of the "direct" genetic studies.

The report also includes background sections on harbour porpoise biology and population status, as well as basic information on biological diversity on the gene level (genetic diversity), processes that affects this diversity, and methods for studying population genetic variability patterns.

Studies of population structure

We found 50 publications dealing with population differentiation in harbour porpoises. Those papers include all the 16 reports using "direct" genetic data that we have been able to identify. They also include all the 17 papers using morphological data that we found, 16 examples of studies using various types of ecological information, and one study using biogeography/distribution data (Table 1).

The majority (11 out of 16) of the genetic studies have analyzed genetic variation of the mtDNA, only seven peer reviewed studies use nuclear DNA markers (microsatellites and allozymes). Thus, most of the present knowledge of the genetic population structure of harbour porpoise on a global scale is based on variation of mtDNA that is maternally inherited and provides no information on the composition of the nuclear genome. Of the
16 studies based on "direct" molecular genetic markers, seven include samples from Swedish and adjacent waters.

On a worldwide geographic scale, the mtDNA differences among porpoises from the three major distribution basins (North Pacific, North Atlantic, and Black Sea/Sea of Azarov) are so large that it might be justified to classify those groups as different subspecies. There is also genetic heterogeneity within major basins with statistically significant allele or haplotype frequency differences among many, or most, of the regions sampled within basins. The amount of genetic differentiation observed between areas within major basins is generally quite small. For the North Atlantic, pairwise Fst-values between regions such as West Greenland, Norway, Ireland, and "IDW" are typically around 0.01 or less for nuclear genes. The overall genetic population structure of the species is by no means resolved.

**Number of populations in Swedish and adjacent waters**

There are seven reports on population genetic structure of harbour porpoises in Swedish and adjacent waters. Two studies are based on mtDNA (Tiedemann et al. 1996; Wang & Berggren 1997), three on microsatellites (Andersen et al. 1995, 1997, 2001), two use allozymes (Andersen 1993, Andersen et al. 1997), and one study deals with RAPD variation (Stensland 1997). Five of the seven studies report significant genetic differences among sampling areas within Swedish and adjacent waters, although the sampling locations and/or the patterns of differentiation are not necessarily the same.

There are considerable difficulties to compare information from the seven studies. First, the definition of geographic areas varies among authors, frequently reflecting a general lack of strictness of the nomenclature used for various parts of the water bodies surrounding Sweden and Denmark. Second, in several cases the exact location for sample collection is not clearly presented; the geographic origin of analyzed specimens is sometimes given only as an ambiguous location name rather than as geographic coordinates. Further, some studies are based on rather small sample sizes (tens of individuals), and the statistical evaluation and/or presentation of the results is in some cases incomplete or misleading.

Five studies report small but statistically significant genetic differences between harbour porpoises of the North Sea and Skagerrak on one hand and the Kattegat/Belt/Baltic area (east of Skagerrak) on the other. Only two studies include samples that make it possible to address the issue of genetic heterogeneity within the Kattegat/Belt/Baltic area east of the North Sea, and neither of those studies provide unambiguous evidence for the existence of a substructure within this region.

On the basis of presently published information there seems to be consensus on the existence of a minimum of two populations in Swedish and adjacent waters. The area around Skagerrak appears to hold a population of porpoises (that may extend into the North Sea, and perhaps into Kattegat) that is genetically distinct from porpoises further east.

With respect to the question of the existence of more than two populations, it appears that this issue cannot be settled from presently reported information. There are indications of genetic heterogeneities within the area comprising the Baltic, Belt, and Kattegat/Skagerrak Seas, but the underlying biology reflected by these heterogeneities is not clear.
We do not think that further sampling represents the most urgent line of action, rather, it appears that additional statistical evaluation of available genetic data may be an easier way of providing further information on the population genetic structure within Swedish and adjacent waters. For example, the question of isolation by distance should be examined, e.g. through testing for associations between geographic and genetic distance.

Conclusions
The main conclusions of this review include:

- A minimum of two porpoise populations appear to inhabit Swedish and adjacent waters. Porpoises in the Skagerrak area, are genetically different from those further east.
- Genetic heterogeneity appears to occur within the Kattegat, Belt, and Baltic Seas, but local populations have not been identified.
- There are shortcomings, particularly with respect to the statistical treatment, in several of the published studies on population genetic structure in Swedish and adjacent waters.
- Inconsistent definitions of geographic areas among authors confuse interpretation of results.
- Important questions that have not been addressed in sufficient detail include:
  - Is the genetic structure of the harbour porpoise characterized by discrete subpopulations, or are there "clines" of continuous genetic change without distinct boundaries?
  - Do the observed genetic heterogeneities reflect true spatial variation, or do they only reflect "evolutionary noise" that is caused by e.g. differences among age classes or by temporal allele frequency change resulting in differences between samples collected several years apart?

- Until it has been clarified whether the area east of the North Sea and Skagerrak consists of multiple distinct subpopulations, one homogenous population, or a cline of continuous genetic change the precautionary principle implies the following:
  1. The North Sea and Skagerrak harbour porpoises should be treated as genetically distinct from those of the Kattegat/Belt/Baltic regions east of the North Sea and Skagerrak.
  2. The area east of the North Sea and Skagerrak should be managed as if comprising multiple subpopulations.
  3. The Baltic should be treated as if representing a distinct population.
1 Introduction

This report is aimed at reviewing current knowledge on the genetic population structure of harbour porpoise (*Phocoena phocoena*) in Swedish and adjacent waters. The review was conducted within the framework of ongoing efforts of the Swedish Environmental Protection Agency to provide conservation and management guidelines for the species. No new data have been collected for the present review that is based exclusively on previously published information. Swedish and adjacent waters are defined here to include the Baltic, Belt, Kattegat, and Skagerrak Seas (including the Kiel and Mecklenburg Bays).

The harbour porpoise is regarded as threatened in most parts of its distribution range (e.g. Berggren 1994; IUCN 1996; Hammond et al. 2002), primarily due to fisheries by-catches (Perrin et al. 1994). In Sweden the abundance has declined during the second half of the 20th century, and the species is currently classified as "vulnerable" in Sweden as well as globally (Ahlén & Tjernberg 1996; www.redlist.org). The total population size in Swedish waters and adjacent waters is approximated to about 36 000 individuals (Hammond et al. 2002). The population decline is considered particularly dramatic within the Baltic Sea, where the census size some years ago was estimated to only 599 individuals (Hiby & Lovell 1996), and a more recently conducted survey indicates a considerably smaller population (Dr. Per Berggren, pers. comm.).

Information on the genetic population structure of the harbour porpoise is crucial for effective management and conservation. For instance, it is necessary to know whether one or more genetically distinct population exist within Swedish and adjacent waters. Lack of such knowledge may result in severe reduction, and even extinction, of individual populations. Similarly, information on the amount of genetic exchange between potential populations, as well as the rate of inbreeding and genetic drift within those populations, is of great importance for the development of conservation management strategies.

We have collected previously published scientific studies that directly or indirectly address questions concerning the genetic population structure of the harbour porpoise through search of literature databases. Here, we summarize and discuss the conclusions of those studies focusing on papers that are based on clearcut genetic data obtained by means of various molecular techniques (studying variation at e.g., mitochondrial DNA, allosyme, or microsatellite loci). Several workers address the issue of population differentiation studying characters for which the genetic basis is not fully understood (e.g., skull or tooth morphology, time for reproduction, etc.), and it is therefore not clear to what extent the variation of such characters reflect divergence on the gene level. Some of those studies are summarized in this report, but our discussion is based exclusively on the results of the "direct" genetic studies.

1.2 The organization of this report

Some sections of elementary background material included in this report provide basic information on the harbour porpoise (2) and on biological diversity on the gene level and basic conservation genetic issues (3). Readers familiar with those topics may skip these parts. The methods and results are presented in sections 4-6. Seven genetic studies
including samples from Swedish and adjacent waters have been identified in the literature search, and the main observations of those studies are summarized separately in Figures 3a-g and Table 2, as well as in the text (5.2).
2 The harbour porpoise – brief background

There are six species of porpoises (order Cetacea, suborder Odontoceti - toothed whales, family Phocoenidae), and the harbour porpoise (*Phocoena phocoena*) is one of three species of the genus *Phocoena* (Read 1999; MacDonald 2001; www.iwcoffice.org). As indicated by the name, the harbour porpoise is found primarily in relatively shallow coastal zones, and it exists in much of the temperate zone of the Northern Hemisphere (Gaskin 1984; Rosel et al. 1995; Read 1999). The species is the only cetacean regularly occurring in the Swedish parts of the Skagerrak, Kattegat and Baltic Seas (Berggren & Arrhenius 1995a).

An adult harbour porpoise is typically around two meters long and weighs around 50 kg (Read 1999). It becomes sexually mature at an age of 3-4 years and seldom lives longer than 15 years (Berggren 1995; IWC 2000). The female typically gives birth to a single calf each or every second year (Read 1990), and mortality among newborn calves is high (Lindahl et al. 2003). Females with calves are usually fairly stationary during the summer season, whereas males and young individuals may migrate long distances (Wang et al. 1996).

The harbour porpoise preferably feeds on small fish (10-30 cm). The prey composition varies between areas, seasons, years, reproductive status, ontogeny, and sex (Read 1999 and references therein; Lockyer & Andreasen 2004). In the Baltic Sea harbour porpoises feed mainly on clupeoids, such as herring and sprat (Lindroth 1962; Read 1999), whereas the most important prey in the Skagerrak and Kattegat are gadids (cod-type fish) and gobids (Lockyer & Andreasen 2004), as well as herring and Atlantic hagfish (Börjesson et al. 2003). It is not known to what degree the porpoise is opportunistic with respect to prey selection (Koschinski 2002), but Lockyer & Andreasen (2004) suggest that there has been a dietary switch in the Baltic Sea because of changed prey availability, with cod-type fish now being the most important prey. Porpoises feed on both pelagic and bottom living fish; in the latter case they compete for food with, e.g., cod, haddock, and whiting (Berggren 1995).

2.1 Distribution

The harbour porpoise occurs in three major geographical areas, namely the North Pacific Ocean, the North Atlantic, and the Black Sea/Sea of Azov (Figure 1), but it appears that it has practically disappeared from the Sea of Azov (Gaskin 1984). There is strong evidence that no, or minor, genetic exchange occurs between these three major basins, and this conclusion is based on biogeographic, morphological, as well as mitochondrial DNA (mtDNA) data (e.g., Gaskin 1984; Yurick & Gaskin 1987; Rosel et al. 1995, 1999b; Wang et al. 1996). No observations of porpoises have been made in the Mediterranean Sea during the past 15 years, further indicating that the Black Sea/Sea of Azov population is currently isolated (Read 1999).

In the North Pacific Ocean harbour porpoises are found in the Bering's Strait, in the coastal waters of northern Japan and from Alaska to southern California. The North Atlantic basin comprises two different areas holding harbour porpoises, namely the western and eastern North Atlantic (Figure 1; Gaskin 1984).
In northern European waters the harbour porpoise occurs around Iceland, Great Britain (but not in the Channel), along the German coast, in Danish waters including the Belt Seas, and along the western and northern coasts of Norway. In Sweden the porpoise now exists along the western and southern coasts, and in the Baltic up to the Gotland area, and occasionally even further north (Gaskin 1984; Berggren & Arrhenius 1995a; IWC 1996; Wang & Berggren 1997; Read 1999; Hammond et al. 2002).

2.2 Population status

There is a general lack of information on the status and size of most harbour porpoise populations (Berggren & Arrhenius 1995a; Read 1999). Information on global abundance is presented in Figure 1, and estimates for Swedish and adjacent waters are given in Figure 2a (in cases where more than one estimate of population size is available for a particular area, all estimates are presented).

There is a general notion that the harbour porpoise population of the Baltic Sea has declined dramatically during the past century and that population sizes during the early part of the 1900s were considerably larger than presently (e.g. Andersen 1982; Berggren & Arrhenius 1995a). Strict population estimates from that period are lacking, however. The number of sightings of harbour porpoises in the Baltic Sea is currently very small (Berggren & Arrhenius 1995b).

2.3 Intrinsic growth rate

Population growth rate is low for whales in general. For the harbour porpoise there is an obvious lack of demographic information, and only few studies report estimates of population growth rates. Woodley and Read (1991) estimated the intrinsic population growth rate to be in the order of 5%, while Barlow and Boveng (1991) arrived at an estimate of 9.4% under more optimistic conditions. The ASCOBANS (Agreement on the Conservation of Small Cetaceans of the Baltic and North Seas) Working Group of the International Whaling Commission (IWC) has agreed to consider 4% as the maximum rate of intrinsic growth for the harbour porpoise (IWC 2000) to avoid that the potential rate of increase is overestimated.

2.4 Threats to harbour porpoise populations

The harbour porpoise is considered to be strongly affected by human activities (Tolley et al. 2001). The species is classified as "Vulnerable" on a global scale (IUCN criterion VUA1cd; IUCN 1996; www.redlist.org).

In Sweden the porpoise was included in the national list of protected species in 1973 (Ahlén & Tjernberg 1996). Despite the listing, no increase of the number of porpoises has been reported during the past decades (Berggren & Arrhenius 1995a). The porpoise population of the Baltic Sea is regarded particularly sensitive, and in 1996 the World Conservation Union (IUCN) classified it as a "vulnerable geographical population" (ASCOBANS: Jastarnia Plan 2002). The Red Data Book of the Baltic Region also classifies the species as "Vulnerable" (Ingelög et al. 1993)
Potential threats in Swedish waters

Historically, there appears to have been an extensive hunting of harbour porpoises in the Baltic and adjacent waters (Andersen 1982; Berggren 1994; Kinze 1995). Porpoises were caught primarily for the blubber, which was used as oil for lighting (Andersen 1982). During the hunts, pods of porpoises were driven into shallow areas where they were enclosed by nets and pulled ashore (Møhl-Hansen 1954; Kinze 1995). The average annual take in Danish waters may have comprised 1000-2000 individuals (Andersen 1982; Kinze 1995). There are no records on porpoise hunting in Swedish waters (Berggren 1994; www.scb.se), and it is not clear to what extent hunting occurred in Sweden that lacks the type of shallow bays typically used for porpoise harvesting (Dr. Håkan Westerberg, pers. comm.).

Hunting pressure, in combination with four winters with particularly severe ice conditions in the Baltic during the first half of the 20th century, has been suggested to be the primary cause for the population decline in the Baltic area (Skora et al. 1988). Heavy ice cover may result in porpoises drowning (Møhl-Hansen 1954) or migrating long distances to ice free waters (Kinze 1985). Presently, the main threats to the porpoise in Sweden are thought to include by-catch, pollution, vessel traffic/tourism, diseases/parasites, demographic and genetic stochasticity, and possibly food depletion.

By-catch: By-catch in commercial fisheries is currently considered the primary global threat to the harbour porpoise (e.g. Berggren & Arrhenius 1995a; Carlström & Berggren 1996; Wang et al. 1996; Wang & Berggren 1997), and the occurrence of by-catch has also been confirmed in Swedish waters (Berggren 1995). According to Berggren et al. (2002) current levels of by-catch exceed the limit for sustainable anthropogenic mortality.

Pollution: We have found no studies demonstrating a detrimental impact of chemical pollutants on cetaceans. However, high levels of organochlorines/contaminants are reported for Swedish and other Scandinavian waters (Kleivane et al. 1995; Berggren et al. 1999), and those substances have been shown to cause reproduction problems in other mammalian Baltic top predators such as the grey seal (*Halichoerus grypus*; Helle et al. 1976). It appears likely that such pollutants may also be detrimental to porpoises (Gaskin 1984; Aguilar & Borrell 1995; Kleivane et al. 1995).

Vessel traffic and tourism: Increasing vessel traffic and tourism may have negative effects on the porpoises, and are regarded as potential threats (Berggren 1995).

Diseases and parasites: The harbour porpoise has a relatively large parasite fauna (Møhl-Hansen 1954; Tomilin 1967). Most adult porpoises carry many parasites without any apparent health problems (Read 1999), but diseases and heavy loads of parasites may have a negative influence on harbour porpoise populations (Lindahl et al. 2003).

Food depletion: Commercial fisheries for several of the species that the harbour porpoise feed on have increased dramatically over the past century, and decreasing fish stocks may have a negative effect on porpoise survival (Börjesson & Berggren 1997). Overall biomass production has increased in the Baltic over the past decades, however, and it therefore appears unlikely that harbour porpoises in that area would lack food (Dr. Sture Hansson, pers. comm.). The issue of whether food depletion constitutes a threat to porpoises is addressed only briefly in the literature, and information appears to be lacking.

Demographic and genetic stochasticity: Demographic and genetic stochasticity are potentially severe threats towards harbour porpoise populations. If individual populations are small (as indicated by i.e., the population size estimates for the Baltic) pure chance
events affecting mortality and reproduction of individual specimens may have a significant effect on population persistence. Similarly, if populations are isolated, inbreeding and genetic drift may lead to loss of genetic diversity and reduced population viability (see below).

2.5 International agreements

Several political measures have been taken in response to the increased awareness of the need for conservation and sustainable use of natural resources, and some of those measures directly concern small cetaceans such as the harbour porpoise. Sweden, together with seven other countries (Belgium, Denmark, Germany, Netherlands, Finland, Poland, and the United Kingdom), has ratified an agreement about small cetaceans in the Baltic and the North Seas (ASCOBANS: Agreement on the Conservation of Small Cetaceans of the Baltic and North Seas) under the UN Bonn Convention in 1994. The aim of ASCOBANS is "to restore and/or maintain biological or management stocks of small cetaceans at the level they would reach when there is the lowest possible anthropogenic influence". The temporary goal for ASCOBANS is "to restore populations to, or maintain them at, 80% or more of carrying capacity".

The population structure of harbour porpoise in Swedish and adjacent waters has been addressed in several studies (e.g. Koschinski 2002; see also Andersen 2003 for an extensive review). On the basis of some of those studies, the IWC-ASCOBANS Working Group (IWC 2000) suggested the existence of five local stocks in the Baltic and North Seas: 1) Baltic Sea, 2) Kattegat, inner Danish waters, and German Baltic Sea, 3) northern North Sea, 4) central and southern North Sea, and 5) Celtic Shelf. ASCOBANS clearly states, however, that it is difficult to define potential local populations within this area (ASCOBANS: Jastarnia Plan 2002).
3 Biological diversity on the gene level – brief background

Biological diversity is frequently referred to as variation on the three levels of ecosystems, species, and genes (e.g., the UN Convention on Biological Diversity, Rio de Janeiro 1992). In reality, however, there are no strict "borders" between these levels - they rather represent points along a continuum that originates in differences at the gene level.

Intraspecific genetic variation is based on sequence differences of the DNA molecule. Particular parts of a DNA molecule have specific functions - they contain the sequence information for individual genes (so-called loci). For a specific gene (locus), the DNA sequence may differ somewhat between different copies of the gene - the nucleotide building blocks do not always follow exactly the same order - resulting in different variants of the same gene (so-called alleles). This variation on the gene level constitutes the basis for the biological evolution on our planet.

Most higher plants and animals are diploid - they have two copies of each gene. One was inherited from the mother and one from the father, i.e., each parent provides half the genes to each of its offspring.

Nuclear and mitochondrial DNA

The DNA molecule is located in the nucleus of every cell, i.e., nuclear DNA (nDNA). In addition, a small amount of DNA also exists in the mitochondrial organelles in the cell cytoplasm outside the nucleus (mitochondrial DNA or mtDNA). MtDNA has several characteristics that differ from those of the nuclear DNA. MtDNA consists of a single, circular DNA-molecule, implying that the mitochondrial genome is haploid, i.e., there is only one copy of each gene. The genes of the mtDNA molecule are inherited together. Differences in DNA sequence in the mitochondrial genome result in the occurrence of different haplotypes (cf. alleles of the nuclear genome). Because only the eggs contain cytoplasm (not the sperms) - and thus mitochondrial organelles - mtDNA is maternally inherited. The maternal inheritance implies that the observed genetic structuring using mtDNA markers reflects the evolutionary forces acting on the female segment of the population(s).

Selection, migration, mutation

The genetic diversity of a species is distributed within populations - as differences between individuals of the same populations with respect to the variants of a specific gene they carry - and between populations. Different alleles may, or may not, occur in all populations of a species, and when occurring, their frequency may differ between populations. The presence of genetic variation within species is essential for their potential to survive and for successfully evolving in response to both short-term and long-term environmental changes.

In a particular environment a specific variant of a gene (allele) may be more or less advantageous for the individual carrying it. Through the process of natural selection individuals that carry advantageous alleles in a specific environment are "favored", i.e.,
they are more likely to survive and reproduce than individuals that do not carry these particular alleles.

A population acquires new alleles primarily through immigration of individuals from surrounding populations (i.e., gene flow or genetically effective migration), and through the process of random change of the DNA sequence (mutation). In addition to these two processes, the number of alleles and their frequency is determined by the size of the population and the selective forces that particular alleles are subjected to. Mutations typically occur very seldom, and this process for recreating genetic variation that has been lost can usually be ignored within the time frames typically surveyed by human activities (tens or hundreds of years). From an evolutionary perspective (thousands of years), however, mutations are very important, and the only way in which new genetic variability can be created.

The genetic composition of natural populations can be studied by the use of different laboratory techniques which can identify alleles at particular loci. A brief presentation of some of the methods for distinguishing such genetic markers is given in section 3.2.

3.1 Loss of genetic diversity

Contrary to biodiversity on the levels of ecosystems and species, diversity on the gene level may be more difficult to recognize directly. The reason is that the genes themselves are "invisible" to the human eye. Typically, only a small fraction of them have been visualized by means of advanced molecular genetic techniques. The loss of genetic diversity may therefore go undetected, unless coupled with loss of diversity on the levels of species or ecosystem.

Three phenomena cause loss of genetic variation, namely population extinction, hybridization, and limited population size. Population extinction results in loss of within species diversity if the population that went extinct was genetically different from other populations of the same species. Hybridization may occur both between species and between genetically divergent populations within a species. The process of hybridization does not necessarily imply the loss of individual alleles but may result in rearrangements of previously existing combinations of alleles at different loci. This may, in turn, result in breakdown of adaptations to particular environmental conditions, and, in a next step, to population decline.

Populations of restricted size always lose genetic variation due to chance alone; not all alleles carried by the individuals of a particular generation are transferred to the next. The process of random genetic change of allele frequencies due to a restricted population size is called genetic drift; the smaller the population, the larger the temporal frequency shifts become. Similarly, the rate of inbreeding (i.e., matings between close relatives) is associated with the size of the population. If a population is small and isolated, inbreeding is inevitable. In many species inbreeding is coupled with reduced viability and reproduction, and increased occurrence of diseases and defects (so-called inbreeding depression). To our knowledge, the effects of inbreeding has not been studied in cetaceans - but virtually all other mammals that have been studied suffer from inbreeding depression in one way or another (e.g., Ralls & Ballou 1983; Ralls et al. 1988; Lacy et al. 1993; Laikre 1999).
Effective population size

The rate of genetic drift and inbreeding is not determined by the actual, census, population size, but by a parameter denoted effective population size or \( N_e \). Effective population size is one of the most important concepts in population genetics with respect to conservation biology, and it refers to the size of an "ideal" population that would have the same rate of drift and inbreeding as the observed, actual population.

The effective population size of a natural population is typically much smaller than the census size and depends on factors such as sex ratio, variance in family size (i.e., variability on numbers of offspring per individual), temporal fluctuations in the number of breeding individuals, the degree to which generations overlap, etc. Minimum effective population sizes of 50 to 5000 per generation have been suggested by various workers as being necessary to avoid significant loss of genetic variability over various periods of time (Allendorf & Ryman 2002).

3.2 Methods for studying genetic variation

During the past few decades the development of molecular genetics has been rapid, and many laboratory techniques are now available for the accumulation of population genetic data on harbour porpoise and other species.

**Allozymes:** Electrophoresis of allelic variants at protein coding loci of the nuclear DNA, so called allozymes (e.g. Utter et al. 1987; May 1992) is a widely used method for studying genetic population structure of natural animal and plant populations. However, for harbour porpoises only two protein coding loci showing variation have been reported (Andersen 1993; Andersen et al. 1997).

**Microsatellites:** Microsatellite loci consist of non-coding regions of the genome where very short DNA segments are repeated in so-called tandem repeats (e.g., Estoup & Angers 1998; Goldstein & Schlötterer 1998). Different alleles at microsatellite loci differ with respect to the number of tandem repeats, and thus in size. This type of loci are generally highly variable and each locus typically exhibits many more alleles than e.g., allozyme loci. A total of 24 microsatellite loci have so far been identified and analyzed in the harbour porpoise (Andersen et al 1995, 1997, 2001; Rosel et al. 1999a; Chivers et al. 2002; Duke 2003).

**Mitochondrial DNA:** Analyses of genetic variation of the mtDNA is conducted on the total mtDNA genome or on particular parts of the mtDNA molecule which are analyzed for DNA sequence differences, using a set of restriction enzymes (so-called Restriction Fragment Length Polymorphism or RFLP analysis) or through determining the exact sequence (so-called sequencing). MtDNA is so far the most frequently used genetic marker in harbour porpoise studies. As stated above, mtDNA variability patterns does not necessarily reflect that of the nuclear genome (that represents the vast majority - 99% or more - of the genetic information of an individual; e.g., Avise 1994).

**Selective neutrality:** The genetic markers that have been employed to study the genetic structure of the harbour porpoise (as well as most other species) are considered to be selectively neutral. Selective neutrality implies that the fitness of an individual is independent of its genotype at that particular locus. The genetic structure observed using selectively neutral loci is expected to be affected by genetic drift and migration, but not by selection. Therefore, studies using neutral markers do not provide information on the
extent to which genetically distinct populations are adapted to local environmental conditions.

*Genetic differentiation:* Genetic data obtained through either of the above type of methods may be used to address issues of, for example, the amount of genetic similarity/difference between harbor porpoises collected in different areas. Various types of statistical techniques may be applied in this context. A frequently used measure of genetic differentiation within species is $F_{ST}$ (Nei 1987; Weir 1996), which is estimated among two or more samples. $F_{ST}$-values may range from 0-1 (negative values are interpreted as zero), and an $F_{ST}$ of zero indicates no genetic differentiation between samples (identical allele frequencies at all loci). An $F_{ST}$ of 1 typically implies that the samples are perfectly genetically differentiated, and fixed for alternate alleles (but see Hedrick 1999 for further discussion of this topic). The statistical significance of $F_{ST}$-values may be evaluated using a variety of different procedures.

There are several ways of computing/defining $F_{ST}$, but the difference between those approaches are typically quite small or negligible. For most species the majority of $F_{ST}$-values recently presented in the scientific literature were obtained using the method of Weir & Cockerham (1984; see also Weir 1996). This is also the technique that we have used when computing $F_{ST}$-values and associated significance levels from published allele and/or haplotype frequencies in cases were such measures are not presented in the original paper (using the software GENEPOP version 3.3; Raymond & Rousset 1995).
4 Literature review

We searched the literature for publications on harbour porpoise population structure using the BIOSIS computerized database. We also collected references from the papers retrieved which were not obtained through BIOSIS.

The publications found have been numbered and are listed in Table 1. They include studies that are based exclusively on molecular genetic markers as well as reports using other types of data (i.e., morphological and ecological data; Table 1). For addressing the present question of genetic population structure we consider studies based on "direct" genetic information most appropriate; the results/conclusions from "indirect" data are included for comprehensiveness, but they are only discussed briefly.

We found 50 publications dealing with population differentiation in harbour porpoises. Those papers include all the 16 reports using "direct" genetic data that we have been able to identify (Table 1: 1-8, 26-33). They also include all the 17 papers using morphological data that we found (Table 1: 9-19, 34-39), 16 examples of studies using various types of ecological information (Table 1: 20-25, 40-49), and one study using biogeography/distribution data (Table 1: 50). There are additional studies that consider the present issue of population divergence from some kind of ecological perspective (e.g., accumulation of pollutants, time for reproduction), but we have not included that entire body of non-genetic literature in this presentation.

Half (Table 1: 1-25) of the 50 papers include porpoise samples from the geographic area targeted in the present review. Of the 16 studies based on "direct" molecular genetic markers, seven include samples from the target area addressing the issue of population differentiation within this area (Table 1: 1-4, 6-8; Table 2). The majority (11 out of 16) of the genetic studies have analyzed genetic variation of the mtDNA (Table 1: 5, 7, 8, 26-33), only seven peer reviewed studies use nuclear DNA markers (microsatellites and allozymes; Table 1: 1-4, 26, 27, 29). Thus, most of our present knowledge of the genetic population structure of harbour porpoise on a global scale is based on variation of mtDNA that is maternally inherited and provides no information on the composition of the nuclear genome.

4.1 Genetic divergence among major distribution areas

Two genetic studies address the issue of differentiation among the three major basins in which the harbour porpoise occurs (the North Pacific Ocean, the North Atlantic, and the Black Sea/Sea of Azov). Both these studies are based on mtDNA and indicate that no haplotype overlap occurs among the three basins (Rosel et al. 1995; Wang et al. 1996). Wang et al. (1996) estimate that the mtDNA lineages they observe in the eastern North Pacific and the western North Atlantic were separated 1-5 million years ago. This degree of mtDNA divergence is large for conspecific populations, and may justify a basis for addressing the systematic status of the porpoises of these three major basins; should they really be considered the same species? Unfortunately, there appear to be no studies comparing the three basins using nuclear genetic markers such as allozymes or microsa-
tellites, so information is lacking on the amount of differentiation at loci potentially reflecting levels of gene flow also for the male segment of the population.

The results of the seven studies based on morphology or ecology are congruent with the notion of substantial differentiation among the basins (Table 1: 15, 19, 34, 35, 37-39), and several workers discuss the porpoises of the three basins in terms of three different subspecies *P. p. relicta* (Black Sea/Sea of Azov), *P. p. phocoena* (North Atlantic), and *P. p. vomerina* (North Pacific; Tomilin 1967; Amano & Miyazaki 1992; Rosel et al. 1995; Wang et al. 1996).

### 4.2 Population differentiation within major distribution areas

We have identified seven studies on population differentiation within the North Pacific (Table 1: 15, 26, 28, 34, 37, 43, 50), two of which are based on genetic data (Table 1: 26, 28). For the North Atlantic the corresponding number of studies are 43 and 14, respectively (Table 1: 1-25, 27, 29-34, 36, 40-42, 44-50; 1-8, 27, 29-33). We found no studies looking into subdivision of the isolated population of the Black Sea/Sea of Azov. Collectively, the genetic studies within the Atlantic and Pacific Oceans indicate that there is genetic differentiation within ocean basins. The amount of divergence ($F_{ST}$) among locations are typically not very large, however, suggesting that migration among populations may be substantial.

A review by Gaskin (1984) of the sub-structure within the Pacific Ocean and the North Atlantic has largely influenced the current view of harbour porpoise population differentiation. Gaskin's work was based on biogeography and distribution data, and did not include genetic information. Within the Pacific, Gaskin identified three sub-populations: Bering Sea, Sea of Okhotsk, and Gulf of Alaska to Los Angeles harbour.

For the North Atlantic Gaskin (1984) suggests 14 regional sub-populations, and revision of this work by the International Whaling Commission (IWC) in 1996 resulted in provisional identification of 13 sub-populations in the North Atlantic, four of which belong to the Western North Atlantic (Gulf of Maine-Bay of Fundy, Gulf of St. Lawrence, Newfoundland and Labrador, and Greenland), and nine to the Eastern North Atlantic (Iceland, Faroe Islands, North Norway and Barents Sea, North Sea, Kattegat and adjacent waters, Baltic Sea, Ireland and western United Kingdom, Iberia and Bay of Biscaya, and Northwest Africa). Recent studies, however, stress the need for a revision of this division (e.g., Walton 1997; Andersen et al. 2001, Andersen 2003).

*North Pacific Ocean*

Neither of the two genetic studies focusing on genetic population differentiation within the North Pacific Ocean (i.e. Rosel et al. 1995; Chivers et al. 2002) have tested the genetic justification of identifying three sub-populations within this area as suggested by Gaskin (1984). Both studies focus on samples collected along the North American Pacific west coast (i.e. within Gaskin's third subgroup - Gulf of Alaska to Los Angeles harbor) and report significant heterogeneity among sampling localities on the basis of the variation of mtDNA and at microsatellite loci. It may be mentioned that three papers using morphological data have compared samples from two of Gaskin's proposed three groupings within the North Pacific, and they report significant differences (Miyazaki et al. 1987; Amano & Miyazaki 1992; Koopman & Gaskin 1994).
North Atlantic

Three genetic studies (based on mtDNA or two allozyme loci) concern differentiation between the eastern and western North Atlantic (Andersen 1993; Rosel et al. 1999b; Tolley et al. 2001) and the results indicate differentiation between these two areas. Two estimates of $F_{ST}$ between these two regions are available, and both are based on mtDNA data. Tolley et al. (2001) present an $F_{ST}$ of 0.04, whereas an estimate calculated from the haplotype frequencies presented by Rosel et al. (1999b) yields an $F_{ST}$ of 0.13, and the latter value appears to reflect considerable divergence between the eastern and western North Atlantic. Significantly lower levels of mtDNA variation are detected within the eastern part of the North Atlantic than in the western one (Wang & Berggren 1997; Rosel et al. 1999b).

Western North Atlantic: Three genetic studies deal with population structure within the western North Atlantic (Wang et al. 1996 (mtDNA); Rosel et al. 1999a (mtDNA and microsatellites); Tolley et al. 2001 (mtDNA)). Rosel et al. (1999a) analyzed samples from four of the western North Atlantic sub-groupings proposed by Gaskin/IWC, and found differences among most of them. Tolley et al. (2001) sampled the same four areas as well as two additional ones arriving at a similar result, as did Wang et al. (1996) who analyzed data from three of the areas.

None of these three studies provide overall quantitative measures of the amount of divergence among the areas sampled. For two of the studies the data presented permit calculation of $F_{ST}$-values, and based on the mtDNA information of Rosel et al. (1999a) we computed an average $F_{ST}$ among five areas of 0.03 (P<0.01). Similarly, mtDNA data from Wang et al. (1996) yield an overall $F_{ST}$ estimate of 0.02 (P<0.001).

Eastern North Atlantic including the Baltic Sea: Eleven (Table 1: 1-4, 6-8, 27, 30, 32, 33) of 30 studies (Table 1: 1-4, 6-14, 16-25, 27, 30, 32-33, 40-41, 46) on population structure in the eastern North Atlantic are based on genetic marker data. Half of these studies use mtDNA, and half microsatellites or allozymes (reflecting variation at the nuclear genome), the most comprehensive one (Andersen et al. 2001) being based on microsatellites.

There appears to have been no systematic attempts to confirm genetically the existence of the nine Gaskin/IWC suggested subpopulations within the Eastern North Atlantic including the Baltic Sea, but all the eleven studies include samples from at least two of the nine areas. We have tried to interpret and summarize how these eleven studies refer to the nine Gaskin/IWC subpopulations (Table 2). There were several difficulties associated with this procedure. For example, the definitions of the Gaskin/IWC subgroupings are unclear. Further, the exact geographic location of samples collected by various investigators are frequently not presented. Our interpretation of how specific sampling localities should be classified with respect to the Gaskin/IWC subgroupings may therefore deviate from the view of the respective authors. The confusion and lack of consistency with respect to the definition of geographic areas is particularly pronounced for the Baltic Sea and the waters around Denmark and southern Sweden.

As indicated in Table 2, it appears that the porpoises of the North Sea and the Kattegat and adjacent waters are, by comparison, relatively well studied genetically, whereas three of the Gaskin/IWC areas (Iberia and Bay of Biscaya, Northwest Africa, and Faroe Island) do not seem to have been studied separately. Other areas such as the waters around Iceland, Ireland and western UK, as well as the Baltic Sea are relatively poorly examined.
Our impression is that the information is scattered with respect to both the regions studied and the techniques applied, and that a general synthesis/consensus is lacking regarding the overall population genetic structure among and within regions of the eastern North Atlantic. Most studies, however, report statistically significant heterogeneity both among and within regions representing the different Gaskin/IWC groupings. Thus, the existence of genetic substructuring within the eastern North Atlantic is generally recognized, but the details of this structure are not clear. With respect to the present target area, however, it appears that most investigators consider the porpoises of the North Sea to be genetically distinct from those inhabiting the Baltic Sea and the areas around the Belt and Kattegat Seas (see below).

Although most studies report significant genetic heterogeneity among locations, the amount of divergence is generally quite small, and $F_{ST}$-values are typically in the range of 0.002-0.01 (Andersen et al. 2001; Duke 2003), and 0.02-0.08 (Tiedemann et al. 1996; Walton 1997; Wang & Berggren 1997; Tolley et al. 1999, 2001) for estimates based on nuclear genes and mtDNA, respectively. Estimates based exclusively on mtDNA are generally expected to produce $F_{ST}$-values that overestimate the amount of divergence for the genome as a whole, because the rate of mtDNA drift is determined by the female effective size rather than by the total effective size (cf. Laikre et al. 1998). For porpoises the observation of larger $F_{ST}$-values for mtDNA is also in line with the notion that female porpoises have a tendency to migrate over shorter distances than males (Andersen et al 1997; Walton 1997; Tolley et al. 1999).
5 Population genetic structure in Swedish and adjacent waters

As mentioned above, there are seven reports on population genetic structure of harbour porpoises in Swedish and adjacent waters (Table 3), defined to include the Baltic, Belt, Kattegat, and Skagerrak Seas. Two studies are based on mtDNA (Tiedemann et al. 1996; Wang & Berggren 1997), three on microsatellites (Andersen et al. 1995, 1997, 2001), two use allozymes (Andersen 1993, Andersen et al. 1997), and one study deals with RAPD variation (Stensland 1997).

Five of the seven studies (Table 1: 1, 3-4, 7-8) report significant genetic differences among sampling areas within Swedish and adjacent waters, although the sampling locations and/or the patterns of differentiation are not necessarily the same. There are considerable difficulties to compare information from these studies. First, the definition of geographic areas varies among authors, frequently reflecting a general lack of strictness of the nomenclature used for various parts of the water bodies surrounding Sweden and Denmark. Second, in several cases the exact location for sample collection is not clearly presented; the geographic origin of analyzed specimens is frequently given only as an ambiguous location name rather than as geographic coordinates. A special problem in this context refers to the samples collected as "stranded" porpoises because it is unclear where these porpoises actually originated. Further, some studies are based on rather small sample sizes (tens of individuals), and the statistical evaluation and/or presentation of the results is in some cases incomplete or misleading.

5.1 Inconsistent definitions of geographic areas

The following water bodies are inconsistently defined in the literature.

The Baltic Sea: The critical question when defining the Baltic in the present context refers to the western border. Some authors use the strict hydrogeographic definition of the Limhamn and Darss under-water ridges (Figure 2b; Dr. Annika Tidlund, pers. comm) as the western border line, whereas others also seem to include parts of the Belt Seas and/or the Kattegat when referring to the Baltic. We recommend that the strict definition of the Limhamn and Darss under-water ridges is applied to define the Baltic.

Inner Danish Waters (IDW): This concept is not strictly defined geographically and does not appear on general maps. However, the term is frequently used in the literature on the harbour porpoise, but it is not always clear exactly what water bodies are included. Further, there seems to be inconsistencies in the application of this term between different publications. For example, in some cases IDW appears to include the Øresund, the Kattegat, and the Belt Seas, whereas in other cases IDW is said to include the Kattegat, Belt, and Baltic Seas (cf. Figure 2b).
The Kattegat/Skagerrak: The assignment of sampling locations to the Kattegat or Skagerrak Seas sometimes appears ambiguous.

5.2 Population genetic studies

Because of the inconsistencies regarding the definition of particular geographic areas within Swedish and adjacent waters we have summarized each of the seven population genetic reports that include samples from this region in separate figures (Figures 3a-g), and discuss briefly the findings of each of these publications.

Andersen 1993 (Figure 3a) genotyped a total of 185 porpoises representing IDW (here including the Öresund, Belt, and Kattegat Seas) and the North Sea using two polymorphic allozyme loci. No statistically significant difference could be found when analyzing the total material (F\textsubscript{ST}=0), but when the samples were classified with respect to season of collection (summer vs. winter), significant differences were observed between the IDW and the North Sea samples within both seasons (F\textsubscript{ST}=0.06 and F\textsubscript{ST}=0.04, respectively).

Andersen et al. 1995 (Figure 3b) conducted a preliminary study using two microsatellite loci, including samples from the North Sea, IDW, and West Greenland (total n=143). They detected no allele frequency differences between harbour porpoises from the IDW (area not defined in the original publication) and the North Sea, but reported an overall genetic heterogeneity when including the sample from Greenland.

Tiedemann et al. 1996 (Figure 3c) report genetic differences of mtDNA haplotype frequencies between the Baltic and the North Sea when analyzing two fairly small samples (n=20 and 19, respectively). F\textsubscript{ST} is estimated to 0.08.

Andersen et al. 1997 (Figure 3d) used two allozyme and three microsatellite loci and observed significant allele frequency differences between the IDW (here defined to include Öresund, the Belt and Kattegat Seas; n=53), and the North Sea (n=33) when comparing porpoise samples collected during summer. No significant heterogeneity was observed when combining summer and winter samples, however.

Stensland 1997 (Figure 3e) found no significant difference between porpoises from the Swedish Baltic Sea (n=24) and the Skagerrak Sea (n=24) using a RAPD technique. It is not clear how Stensland defines the western border of the Baltic, nor is the exact location of her samples.

Wang & Berggren 1997 (Figure 3f) compared samples from the Swedish Baltic (n=27; defined according to the above mentioned under-water ridges), the Kattegat-Skagerrak area (n=25), and the Norwegian west coast (n=13) using mtDNA RFLP analysis. They report statistically significant differences of haplotype frequency distributions for all pairwise comparisons. For the comparison between the Baltic and the Kattegat-Skagerrak samples, however, recalculation does not fully support the notion of a statistical significance; the appropriate P-value for this comparison is about 0.095 (exact calculation), rather than 0.035 as reported by Wang & Berggren (see Appendix). The difference is not very large, and strict application of the 0.05 limit for defining statistical heterogeneity should be exercised with caution. Nevertheless, the result of the statistical re-analysis implies that this set of data does not lend strong support to the idea of a Baltic population that is genetically distinct from that of the Kattegat-Skagerrak area. We calculated an overall F\textsubscript{ST} of 0.04 from the haplotype frequencies presented (P < 0.01), including their Baltic, Kattegat/Skagerrak, and Norwegian west coast samples.
Andersen et al. 2001 (Figure 3g) is the most comprehensive population genetic study of harbour porpoises in Swedish and adjacent waters (as well as world wide). It comprises a total of 807 individuals, and 12 microsatellite loci were scored. The authors classify their samples into six different regions (IDW n=169, Danish North Sea n=151, British North Sea n=131, Ireland n=105, Norway n=49, West Greenland n=150), and in addition they analyze a sample from the Netherlands (n=52). They report statistically significant allele frequency heterogeneity for all pairwise comparisons (the Netherland sample not included), the pairwise $F_{ST}$-values ranging in the interval 0.002-0.014.

Andersen and co-workers refer all their porpoises from the Baltic, the Belt, and the Kattegat Seas to the region IDW (Inner Danish Waters), but their conclusion regarding the degree of population structuring within this area is not perfectly clear. On one hand they state that "samples within regions showing no substantial evidence of heterogeneity were pooled in the subsequent tests among regions", implying that the IDW exhibits no substantial evidence of substructuring.

On the other hand, they report significant deviations from Hardy-Weinberg expectations (heterozygote deficiency) within the IDW. They present $F_{IS}$-values for each of the twelve loci (their Table 4), and for the IDW they report heterozygote deficiency at nine of these loci, two of them being statistically significant (no overall $F_{IS}$ is presented, and it cannot be calculated from the information provided). As noted by the authors, heterozygote deficiency is a potential indicator of population subdivision, but they leave this observation with the general statement that "these results suggest that deviations from HWE are likely minor and they likely result from a Wahlund effect or non random mating, though we cannot exclude the possibility that they may reflect inbreeding or the presence of null alleles". Further, when comparing allele frequencies within their IDW region they find statistically significant differences among some of their subsamples. Andersen et al. (2001) have 32 porpoises from the Baltic Sea, 27 of which are identical to the ones examined by Wang & Berggren (1997; Dr. Per Berggren, pers. comm.).

Andersen and co-workers do not, however, report results from comparisons of allele frequencies between their Baltic sample and other locations (in all the tests they present, the Baltic sample has been pooled with one or more other locations).
6 Discussion and conclusions

Genetic structure

It is obvious that genetic structuring exists in the harbour porpoise. On a world wide geographic scale, the mtDNA differences among porpoises from the three major distribution basins (North Pacific, North Atlantic, and Black Sea/Sea of Azarov) are so large that it might be justified to classify those groups as different subspecies (Amano & Miyazaki 1992; Rosel et al. 1995, Wang et al. 1996) or maybe even species, but studies of the nuclear genome are necessary to further clarify this issue.

There is also genetic heterogeneity within major basins with statistically significant allele or haplotype frequency differences among many, or most, of the regions sampled within basins. The overall genetic population structure for the species is by no means resolved, however. For instance, the North Atlantic sub-population structure suggested by Gaskin (1984) and IWC (1996) has not been unambiguously verified by genetic studies. Similarly, the existence of the five local stocks as proposed by the IWC-ASCOBANS Working Group (IWC 2000) has not yet been confirmed genetically.

The amount of genetic differentiation observed between areas within major basins is generally quite small. For the North Atlantic, for example, pairwise F_{ST}-values between regions such as West Greenland, Norway, Ireland, and "IDW" are typically around 0.01 or less for nuclear genes (Andersen et al. 2001).

Low F_{ST}-values at nuclear loci have also been observed for some other marine mammals such as the grey seal (Halichoerus grypus; Allen et al. 1995), whereas considerable divergence has been reported for, e.g., the Eastern Atlantic harbour seal (Phoca vitulina vitulina; Goodman 1998). In fishes, there is a general tendency for marine species to exhibit considerably less differentiation (smaller F_{ST}-values) than freshwater resident and anadromous ones, and the common explanation to this phenomenon is that geographic barriers are frequently lacking in the marine environment, thereby permitting gene flow over large geographic distances (e.g. Hauser & Ward 1998). The low F_{ST}-values observed for harbour porpoise are by no means incompatible with the potential existence of separate populations or of a more continuous genetic change over geographic space (so-called isolation by distance), but they indicate that the amount of divergence may not be very large, potentially reflecting considerable gene flow.

Number of populations in Swedish and adjacent waters

Five studies (Andersen 1993; Andersen et al. 1997, 2001; Tiedemann et al. 1996; Wang & Berggren 1997) report small but statistically significant genetic differences between harbour porpoises of the North Sea and Skagerrak on one hand and the Kattegat/Belt/Baltic area (east of Skagerrak) on the other. Only two studies (Wang & Berggren 1997; Andersen et al. 2001) include samples that make it possible to address the issue of genetic heterogeneity within the Kattegat/Belt/Baltic area east of the North Sea, and neither of those studies provide unambiguous evidence for the existence of a substructure within this region.

On the basis of presently published information, how many genetically distinct populations occur in Swedish and adjacent waters? There seems to be consensus on the
existence of a minimum of two populations, although there appears to be some disagree-
ment on how to classify the geographic areas inhabited by these two populations. As
mentioned above, Andersen et al. (2001) consider the Baltic, Belt, and Kattegat Seas to
be inhabited by one population which is distinct from that of the North Sea, and they
include Skagerrak in their definition of the North Sea. In contrast, Wang & Berggren
(1997) consider the Baltic porpoises to represent one population separated from a distinct
group in the Kattegat/Skagerrak area (although recalculation using their published data
does not provide statistical significance). Thus, the area around Skagerrak appears to hold
a population of porpoises (that may extend into the North Sea, and perhaps into Kattegat)
that is genetically distinct from porpoises further east.

With respect to the question of the existence of more than two populations, it appears
that this issue cannot be settled from presently reported information. There are indications
of genetic heterogeneities within the area comprising the Baltic, Belt, and Katte-
gat/Skagerrak Seas, but the underlying biology reflected by these heterogeneities is not
clear.

We do not think that further sampling currently represents the most urgent line of
action to separate among the above alternatives. Rather, it appears that additional
statistical evaluation of available genetic data may provide further information on the
population genetic structure within Swedish and adjacent waters. For example, the
question of isolation by distance should be examined, e.g. through testing for associations
between geographic and genetic distance. Of course, analysis of more samples is always
valuable, but it is important and cost effective that as much information as possible is
generated from the material already available.

Important questions that currently published information does not appear to address
include:

- Is the genetic structure of the harbour porpoise characterized by discrete subpopula-
tions, or are there "clines" of continuous genetic change without distinct boundaries?
- Do the observed genetic heterogeneities reflect true spatial variation, or do they only
reflect "evolutionary noise" that is caused by e.g. differences among age classes or by
temporal allele frequency change resulting in differences between samples collected
several years apart?

Similarly, it may be informative to test for spatial heterogeneity after "re-grouping" part
of the material. For example, most of the samples analyzed for mtDNA variation by
Wang & Berggren (1997) were also included in the microsatellite study by Andersen et
al. (2001). As mentioned above, Wang & Berggren (1997) use a "strict" definition of the
Baltic Sea, whereas Andersen et al. (2001) lump the same samples with those from the
Belt and Kattegat Seas and refer them to "IDW". Thus, Wang & Berggren address the
question of the existence of a distinct Baltic population corresponding to the suggestion
from Gaskin/IWC, whereas Andersen et al. (2001) state that they have tested for genetic
differentiation within the Kattegat/Belt/Baltic area, but that they did not find any
statistical significances (data not presented in the publication). At the same time,
however, they report several indications of heterogeneity within the area (see above).
Using the data of Andersen and co-workers to test exactly the same hypothesis as that of
Wang & Berggren might shed some additional light on the issue of the possible existence
of a genetically distinct Baltic population. Similarly, with respect to the samples that were used by both Wang & Berggren and by Andersen et al. it appears that the genetic information has not been used to its full capacity. For the individuals included in both studies there is genotypic data on both mtDNA and microsatellites, but the analyses reported so far utilize these data sets separately, without exploiting the potential of combining them.

Estimates of population size in Swedish and adjacent waters indicate that the number of porpoises in the Baltic Sea is only about 500 or less, whereas about 30 000 individuals appear to exist in the area of the Belt, Kattegat, and Skagerrak Seas. Because there are no apparent geographic barriers preventing migration into the Baltic, the large difference in density almost provides the impression of some other form of biological "fence", but we have seen no discussions of this issue in the literature. It seems to us that one possible explanation for such a "fence" could be that the Baltic environment is perceived as so "unattractive" that porpoises typically avoid to enter the region. Alternatively, the Baltic requires genetic adaptation to particular environmental conditions. If the latter is correct, there may be a genetically distinct Baltic population that is currently on the verge of extinction. A third possibility implies continuous immigration into the Baltic, but that mortality rates (e.g. due to by-catches) are so high that the population size does not increase.

Implications for management

Clearly, there are a number of issues that are not unambiguously resolved with respect to the genetic structure of the harbour porpoise in Swedish and adjacent waters. Until it has been clarified whether the area east of the North Sea and Skagerrak consists of several distinct subpopulations, one homogenous population, or a cline of continuous genetic change the precautionary principle implies the following:

1. The North Sea and Skagerrak harbour porpoises should be treated as genetically distinct from those of the Kattegat/Belt/Baltic regions east of the North Sea and Skagerrak.

2. The area east of the North Sea and Skagerrak should be managed as if comprising multiple subpopulations.

3. The Baltic should be treated as if representing a distinct population.
7 References


Berggren, P., Ishaq, R., Zebühr, Y., Näf, C., Bandh, C., and Broman, D. 1999. Patterns and levels of organochlorines (DDTs, PCBs, non-ortho PCBs and PCDD/Fs) in male harbour porpoises (Phocoena phocoena) from the Baltic Sea, the Kattegat-Skagerrak Seas and the west coast of Norway. Marine Pollution Bulletin, 38: 1070-1084.


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www.scb.se (2003-09-30)
Table 1 (a and b). Studies concerning population differentiation in the harbour porpoise. All the studies using genetic and/or morphologic data that were found are included, as well as examples of studies based on ecological data. The studies are separated with respect to genetic, morphologic, and ecological data. \( n \) = number of animals. Table 1a presents reports including Swedish and/or adjacent waters, whereas Table 1b refers to studies not including Swedish and/or adjacent waters.

Table 1a. Studies including Swedish and/or adjacent waters.

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<th>No.</th>
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<th>( n ) (total)</th>
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<td>Allozymes</td>
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1) IDW not defined. Unclear if porpoises from the Baltic Sea are included.
2) The Baltic Sea sample may include specimens from the Belt Seas.
Table 1b. Studies not including Swedish and/or adjacent waters.

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<td>27</td>
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<td>Iceland, Ireland, Norwegian waters</td>
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Table 2. Genetic studies providing information on subpopulations within the eastern North Atlantic suggested by Gaskin/IWC. Allozyme = allozyme data, Micros. = microsatellite data, mtDNA = data based on mtDNA, RAPD = study dealing with RAPD variation. (?) refers to problematic area definition; see text for comments. Note that neither of the three subpopulations Faroe Island, Iberia and Bay of Biscaya, and Northwest Africa have been investigated separately.

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Table 3. Genetic studies concerning population structure in harbour porpoises in Swedish and adjacent waters. n = number of animals. 

F_{ST} - and P-values not given by the authors have been calculated with GENEPOP using allele/haplotype frequencies presented in the original publication; those values are given in italics.

<table>
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<th>Reference</th>
<th>n (total)</th>
<th>n (Baltic Sea)</th>
<th>Number of areas</th>
<th>Material</th>
<th>Marker</th>
<th>Number of loci</th>
<th>Number of alleles / haplotypes</th>
<th>Overall heterogeneity</th>
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<td>Stranded</td>
<td>Allozymes</td>
<td>2</td>
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<td>Andersen et al.</td>
<td>143</td>
<td>57 3) (IDW)</td>
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<td>Stranded</td>
<td>Microsat.</td>
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<td>Stranded</td>
<td>Microsat.</td>
<td>2/3</td>
<td>5/32</td>
<td>0.031</td>
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<td>12</td>
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<td>0.002-0.014 6)</td>
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<td>11</td>
<td>0.044</td>
<td>Baltic Sea, Kattegat-Skagerrak Seas, Norwegian westcoast</td>
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</table>

1) F_{ST} or equivalent.  
2) Refers to IDW vs. North Sea. (IDW(summer) vs. North Sea(summer): F_{ST} = 0.057, P = 0.003. IDW(winter) vs. North Sea(winter): F_{ST} = 0.037, P = 0.01.)  
3) IDW not defined. Unclear if animals from the Baltic Sea are included.  
4) No significant differentiation in pairwise comparison between the Baltic Sea and the North Sea.  
5) No significant difference between the Baltic and Kattegat Seas. Significant difference between IDW and Danish North Sea-Skagerrak.  
6) No overall F_{ST}-estimate is presented. Values refer to pairwise F_{ST}-values between six regions.  
7) No significant difference between the Baltic and Kattegat Seas. Significant differentiation between IDW and Danish North Sea-Skagerrak. Deviation from Hardy-Weinberg expectations (heterozygote deficiency) and/or allele frequency differences occur within IDW and the Danish North Sea-Skagerrak.  
8) The Baltic Sea sample may include specimens from the Belt Seas.  
9) Re-calculation yields P>0.05 (non-significant) for the pairwise comparison between the Baltic Sea and the Kattegat-Skagerrak Seas.
Fig. 1. Geographic distribution of harbour porpoise in the early part of the 1900s. Black areas: known consistent occurrence. Stippled areas: occasional, peripheral, or probable range. Note that during recent years the species has reduced in abundance or disappeared from several of these areas, including the English Channel, the Mediterranean, the west coast of Spain, and the east coast of Greenland (Read 1999). \( n \) = abundance (from Gaskin (1984), Ivashin & Borodin (1992), Donovan & Bjørge (1995), Hammond et al. (2002), and references therein). See Fig. 2a for abundance estimates around Scandinavia. This figure is a modification of Figure 4 in Gaskin (1984) and is presented with kind permission from the IWC Office.
**Fig. 2a.** Harbour porpoise abundance around Scandinavia. For some areas more than one estimate is available.
Fig. 2b. Detail of Swedish and adjacent waters constituting the target area of the present report. The Limhamn and Darss under-water ridges (A and B) are frequently used to define the western border of the Baltic Sea.
Fig. 3a. Sampling areas; Andersen 1993. Here IDW refers to Öresund and the Belt and Kattegat Seas. $F_{ST}$ and P-values are not given in the original paper, but were calculated from allele frequencies reported in the original publication. In addition to the 185 porpoises from the North Sea and IDW, the study also included specimens from West Greenland (n = 66), Canada (n = 12), and from the Dutch coast (n = 40).
Fig. 3b. Sampling localities; Andersen et al. 1995. Here, the area of IDW is not defined.

Andersen et al. (1995)

n = 143
2 microsatellite loci
n (IDW) = 57
IDW vs. North Sea vs. West Greenland P<0.05
IDW vs. North Sea P>0.05
Overall F_{ST} = 0.008 (P<0.007)
**Fig. 3c.** Sampling localities; Tiedemann *et al.* 1996. The Baltic Sea sample may include specimens from the Belt Seas. $F_{ST}$ and $P$-values are not given in the original paper, but were calculated from haplotype frequencies reported in the original publication.
Fig. 3d. Sampling localities; Andersen et al. 1997. Here, IDW refers to the Öresund and the Belt and Kattegat Seas. Sampling area specifications are not presented in the original publication, but were given by Koschinski (2002).
Fig. 3e. Sampling localities; Stensland 1997. RAPD markers are dominant, and “$F_{ST}$” refers to a variance component calculated by means of AMOVA technique.
Wang and Berggren (1997)

- n = 65
- 1 mtDNA locus; 11 alleles/haplotypes

- n (Baltic Sea) = 27

**Baltic Sea vs. Kattegat and Skagerrak Seas**
P < 0.05<sup>a</sup>

**Baltic Sea vs. Norway** P < 0.05

Overall heterogeneity \( F_{ST} = 0.044 \) (P = 0.01)

<sup>a</sup> Re-calculation yields P > 0.05

**Fig. 3f.** Sampling localities; Wang and Berggren 1997. Note that the statistical significance (P < 0.05; Baltic Sea vs. Kattegat/Skagerrak Seas) reported in the original paper cannot be confirmed when checking the calculation. \( F_{ST} \) - and P-values are not given in the original paper, but were calculated from haplotype frequencies reported in the original publication.
Andersen et al. (2001)
n = 807
12 microsatellite loci

\[
n (\text{Baltic Sea}) = 32; n (\text{IDW}) = 169
\]

- IDW vs. Skagerrak/Danish North Sea \( F_{ST} = 0.005 \) (P<0.05)
- IDW vs. British North Sea \( F_{ST} = 0.009 \) (P<0.05)
- IDW vs. Ireland \( F_{ST} = 0.012 \) (P<0.05)
- IDW vs. Norway \( F_{ST} = 0.014 \) (P<0.05)
- IDW vs. West Greenland \( F_{ST} = 0.01 \) (P<0.05)

Fig. 3g. Sampling localities; Andersen et al. 2001. Here, IDW refers to the Kattegat, Belt, and Swedish Baltic Seas. The overall \( F_{ST} \)-value cannot be calculated from the data given in the original publication. No estimate of \( F_{ST} \) is provided for IDW vs. Netherlands in the original paper, and it cannot be calculated from the data presented.
Appendix

Wang and Berggren 1997 compared samples from the Swedish Baltic (n=27), the Kattegat-Skagerrak area (n=25), and the Norwegian west coast (n=13) using mtDNA RFLP analysis. They report statistically significant differences of haplotype frequency distributions for all pairwise comparisons using the Monte program of the Restriction Enzyme Analysis Package (REAP) version 4.1 (McElroy et al. 1992). Based on the data presented in their Table 2 we re-analyzed the haplotype frequency difference between the Swedish Baltic area on one hand and the Kattegat-Skagerrak area on the other, where Wang and Berggren report a significant P-value of 0.035. They observed the following haplotype frequencies (modified from their Table 2, excluding haplotypes that were not found in the Baltic or the Kattegat-Skagerrak):

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>Baltic Sea</th>
<th>Kattegat-Skagerrak Seas</th>
</tr>
</thead>
<tbody>
<tr>
<td>AAAABAAAAA</td>
<td>2</td>
<td>24</td>
</tr>
<tr>
<td>AAAAAAEEA</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>HAAAAACAAA</td>
<td>53</td>
<td>0</td>
</tr>
<tr>
<td>AABCAADBH</td>
<td>54</td>
<td>0</td>
</tr>
<tr>
<td>AAABAAQA</td>
<td>55</td>
<td>0</td>
</tr>
<tr>
<td>AAAABAAD</td>
<td>56</td>
<td>1</td>
</tr>
<tr>
<td>AAAAAANAA</td>
<td>58</td>
<td>2</td>
</tr>
</tbody>
</table>

We used several statistical softwares (including REAP’s Monte, version 4.1) applying both exact calculations and simulation approaches, but never obtained a P-value of less than 0.05. The null hypothesis tested is that the haplotype frequency distribution is the same in the Baltic and the Kattegat-Skagerrak Seas. We accept this null hypothesis if, under the null hypothesis, the probability of obtaining an observation as likely as, or less likely than, the present one exceeds 0.05.

GENEPOP (version 3.1c, subprogram STRUC, 100,000 iterations; Raymond & Rousset 1995) provides P=0.09087 (S.E. 0.00326). REAP’s (McElroy et al. 1992) Monte Carlo simulation performed by Monte (200 simulations with 10,000 iterations per simulation) yields P=0.08121. StatXact-3 (CYTEL 1997) calculates an exact P of 0.0956, and a Monte Carlo-estimate implemented in the same software also results in P=0.0956 (1,000,000 simulations). A "regular" contingency chi-square test (e.g. Sokal & Rohlf 1981) yields $\chi^2=8.299$, df=6, and P=0.2170. In no case did we obtain a P-value less than the 0.05 significance limit, and we therefore accept the null hypothesis of identical haplotype frequencies in the Baltic and the Kattegat-Skagerrak Seas.
This is a review of the current knowledge on the genetic population structure of harbour porpoise (Phocoena phocoena) in Swedish and adjacent waters. Information on the population genetics of the species in other geographic areas is also included.

On the basis of presently published information there seems to be general consensus on the existence of a minimum of two populations in Swedish and adjacent waters. The area around Skagerrak appears to hold a population of porpoises (that may extend into the North Sea, and perhaps into Kattegat) that is genetically distinct from porpoises further east.

With respect to the question of the existence of more than two populations, it appears that this issue cannot be settled from presently reported data. There are indications of genetic heterogeneities within the area comprising the Baltic, Belt, and Kattegat/Skagerrak Seas, but the underlying biology reflected by these heterogeneities is not clear. There are no unambiguous indications of a genetically distinct Baltic population. More in depth statistical analyses of available genetic information might provide further information on the genetic structure within this geographic region.