The relationship between developmental stability, Ggenomic diversity and environmental stress in two cetacean species:: the Harbour Porpoise (Phocoenaphocoena) and The Bottlenose Dolphin (Tursiops truncatusl)

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The Relationship between Developmental Stability, Genomic Diversity and Environmental Stress in Two Cetacean Species: The Harbour Porpoise (*Phocoena phocoena*) and The Bottlenose Dolphin (*Tursiops truncatus*).

by

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Declaration

The material contained within thus thesis has not been previously submitted for a degree at the University of Durham or any other university. The research reported here has been conducted by the author unless indicated otherwise.

Abstract

The relationship between developmental stability, genomic diversity and environmental stress in three eastern North Atlantic populations of the harbour porpoise (*Phocoena phocoena*), and in two populations of the western North Atlantic and one from the Gulf of California of the bottlenose dolphin (*Tursiops truncatus*) was investigated. In addition, the population structure for the two species from the study areas mentioned was also assessed.

Population structure was determined using discriminant function analysis for morphological characters and a Bayesian analysis for microsatellite loci. Consistency of the results was assessed with pairwise comparisons between populations using two indices of population differentiation (F_{ST} and Rho_{ST}). For the harbour porpoises classification was made into three putative populations: Norwegian, British and Danish. For the bottlenose dolphin significant differentiation was found for the three populations studied. Population differentiation between the two western North Atlantic parapatric populations was the highest among the pairwise comparisons. This result highlights the importance of resource specialisation of bottlenose dolphins in causing population structure for parapatric populations.

Developmental stability was assessed by fluctuating asymmetry (FA) measured on morphological traits. Genomic diversity was determined by five indices (mean d^2, scaled mean d^2, multilocus individual heterozygosity, standardised heterozygosity and internal relatedness). Environmental stress was assessed by the concentration of chemical pollutants in tissues, and from the literature published for chemical pollutants, by-catch rate, parasite load and mean surface ocean temperature. Significant relationships between FA and the indices of genomic diversity were

found. The Norwegian population of harbour porpoises and the coastal population of the western North Atlantic of bottlenose dolphin showed the highest level of FA. Both populations also showed the least genetically diverse animals. However, no clarity was obtained in respect of the relationship between FA and environmental stress. British and Norwegian harbour porpoises did not show significant correlations between the concentration of several chemical pollutants in tissues and FA. In addition, the Norwegian population of harbour porpoise inhabits the least impacted areas in respect to the concentration of chemical pollutants in tissues, parasite load and by-catch rates. Environmental stress was difficult to assess on the bottlenose dolphins populations due to the scarcity of data. These results show the influence of genetic diversity on the disruption of developmental stability and they also show the importance of conservation practices in maintaining genetic diversity as an important factor for the subsistence of natural populations.

Dedicatory

I dedicate this thesis to my wife Adriana and my son Adrian. Adriana without your immense love and huge support I would not have finished this phase. Thank you very much for your unconditional love and for all your support throughout these years. You chose to venture with me in this journey and I really appreciate the fact that you are always by my side. Thank you, wife.

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Chapter 1: General Introduction.

The expansion of the human population and the consequent need to exploit more resources in the marine environment has increased the risks to marine mammal species (Reeves and Reijnders 2002). Threats include vessel collision, exposure to chemical pollutants, by-catch, noise pollution, etc. Conservation of marine mammal populations has become an important practice in recent years (Taylor 2002).

One of the major challenges in conservation biology is to understand how animal populations will respond to environmental changes created anthropogenically and to maintain the full historical range of wild species including feeding and breeding grounds (Merilä et al. 2004) by prioritising actions through a scientific analysis of risks that will lead management actions by analysing data and recommending policies (Taylor 2002).

Threatened species frequently require active management to ensure their persistence. Management of widely scattered remnant populations present difficulties for effective conservation (Zenger et al. 2005), especially when prior knowledge of population structure and a valuation of the different kind of stress that a species are subject to, are not well understood.

The potential for cetaceans to move in the marine environment makes illogical the allocation of threatened species into sub-populations or stocks based on geographical regions. However, genetic and morphometric analyses provide suitable means to assess significant population subdivisions (Lande 1991).

Morphological differences have been used to distinguish marine mammal populations as stocks for management (e.g. Börjesson and Berggren 1997, Yurick and Gaskin 1987) and recent genetic analyses have been used to define population structure in marine mammal species (e.g. Andersen et al. 2001, Hoelzel et al. 1998a, Natoli et al. 2004, Tolley et al. 1999, Tolley et al. 2001).

Population genetics has become a useful tool for management and conservation of threatened or endangered species (Dover 1991). The use of molecular markers is a relatively new technique that has a potential to provide information in respect to molecular ecology for species that are considered difficult to study such as cetaceans (Engelhaupt 2004). With the development of the polymerase chain reaction technique, small amounts of DNA from different tissues and other biological material can be replicated so it can produce a viable sample for analysis (Engelhaupt 2004).

Natural variation in DNA can be used for investigating genetic relationships within a species and to give knowledge of the variation in populations through space and time (Dover 1991). They can also be useful in the determination of population subdivision that may have management implications (Lande 1991). The differentiation of allele frequencies within and among populations can be a result of gene flow via migration of individuals or their gametes, random genetic drift, natural and sexual selection modes, mutations, and genetic recombination opportunities that have been mediated by mating systems (Avise 1994).

Genomic diversity can be measured by using molecular DNA markers (i.e. microsatellites). Three measurements have been developed and commonly applied in recent years in studies that deal with the interaction between genomic diversity and fitness (Amos et al. 2001; Balloux et al. 2004; Coltman et al. 1999; Coulson et al.

1998, 1999; Hansson et al. 2001, 2004; Overall et al. 2005, Tsitrone et al. 2001). These measures are: mean d^2, heterozygosity and internal relatedness.

Mean d^2 estimates the genetic similarity of an individual in relation with its parents based on the mean evolutionary distance between its alleles (Balloux et al. 2004, Coulson et al. 1999), and depends on long-term mutational differences (Amos et al. 2001, Pemberton et al. 1999). Heterozygosity is the proportion of typed loci for which an individual is heterozygous (Slate and Pemberton 2002). Internal relatedness is a measure based on allele sharing where the frequency of every allele counts towards the final score (Amos et al. 2001).

Reviews on the association between these metric measures of genetic diversity and fitness have found that this association is common but generally weak (Coltman and Slate 2003). Balloux et al. (2004) found that internal relatedness show higher correlations with inbreeding coefficients than heterozygosity and mean d^2. Overall et al. (2005) compared the three measures against two fitness traits (neonatal survival and birth weight) in the Soay sheep of St. Kilda. They found that none of the three genetic diversity indices explained significant variation in fitness. Slate and Pemberton (2002) using a population of red deer of the Isle of Rum found that heterozygosity was better at predicting fitness (birth weight and juvenile survival) than mean d^2. Tsitrone et al. (2001) used mathematical models to determine genotype-fitness correlations, and found that heterozygosity provided higher correlations with fitness than other molecular measures. However, studies on harbour seal (Coltman et al. 1998) and red deer (Coulson et al. 1998) found a better association of mean d^2 with juvenile survival. Hansson et al. (2001, 2004) reported that both, heterozygosity and mean d^2, were associated with juvenile survival in a population of great reed warblers.

Genetic variation within populations is necessary to allow adaptation to a changing environment (Lande 1991). Habitat fragmentation and the reduction of the effective size of a population may decrease the diversity of species subject to this kind of stress (Lens 2000a). The immediate effects related to reduction in population size and declines in genetic diversity are the loss of rare alleles as individuals are lost from the population (Prober and Brown 1994). Long term impacts of fragmentation can occur if increased isolation alters patterns of gene flow (Butcher et al. 2005).

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Environmental and genetic pressures may produce a disruption during the development of an organism (Clarke 1995). Developmental stability is a term that describes controlled processes that occur during the development of an organism, it corresponds to the development of well co-adapted genotypes under optimal environmental conditions (Graham & Felley 1985, Mather 1953, Zakharov & Yablokov 1990), and also reflects the ability to develop similar phenotypic characters and ensures that the intended endpoint is reached under a given developmental trajectory (Waddington 1957) by acting as a balance or as a buffer between two opposite forces: developmental homoeostasis, and developmental noise (Palmer 1994, Palmer 1996, Møller and Swaddle 1997, Clarke 1998).

This intended endpoint has also been called as the "target or -ideal- phenotype" (Nijhout and Davidowitz 2003). The target phenotype is the phenotype produced by an organism under predetermined genetic and environmental conditions, and it will only be achieved in the absence of any disturbance whether genetic or environmental in origin (Nijhout and Davidowitz 2003).

Nevertheless, it happens that only during certain times of development where adverse factors are present, the controlled developmental processes can produce a diversion on the developmental pathways onto another developmental trajectory (Møller & Swaddle 1997). This could be triggered by genetic –e.g. allele changes by mutation, drift, etc. - and environmental –e.g. climatic changes, pollution, etc. - disturbances. Developmental stability will struggle to maintain the homeostatic state and a disruption may occur to the buffering processes that reduce the variation resulting from developmental accidents. This diversion from the ideal phenotype is known as developmental noise or developmental instability (Møller & Swaddle 1997). Adverse environments have been suggested to affect the level of recombination, mutation, and transposition; the additive and genotypic variance, the intensity of selection, and hence the speed of micro-evolutionary change (Møller & Swaddle 1997).

Developmental instability applies its effect as development progresses (Klingenberg 2003). This disturbance does not cause death, but it worsens the state of the organism (Zakharov and Yablokov 1990). Moreover, because the development of an organism is a continuous and highly interactive process, any disturbance at any time could have serious implications later on developmental events (Klingenberg 2003).

Developmental stability has been used as an indirect indicator of fitness, and sometimes as a useful resource for conservation (e.g. Leary and Allendorf 1989,

Parsons 1992, Clarke 1995). The relationship between developmental stability and fitness is mainly due to the fact that developmentally stable organisms show higher metabolic efficiency, thus an excess of energy should be available for maintenance, growth and reproduction (Møller & Swaddle 1997).

The ability of developmental stability to predict changes in fitness assumes that changes in developmental stability will be manifest in the phenotype before any detectable change occurs in more direct component of fitness (e.g. fecundity, Clarke 1995). Developmental stability has also been used as an important population parameter, and is considered as a characteristic of population health (Zakharov & Yablokov 1990).

Another important characteristic of developmental stability is that it can be used according to Clarke (1995) as an "early warning system that could monitor the status of a species long before it has been impacted by genetic and environmental stressors".

It has been demonstrated that information on the level of developmental stability as a general characteristic of the condition of an organism can be obtained through morphological estimates (Zakharov & Yablokov 1997). It is at the level of morphological expression and function that developmental changes have consequences for the fitness of the organisms, due to the fact that natural selection of the morphological traits plays a role on the evolution of developmental mechanisms (Klingenberg 2002). Therefore, morphometrical analyses allow the detection of variation in size of a particular character in an organism, and they are increasingly used in the developmental context (Klingenberg 2002).

Fluctuating asymmetry (FA) has been used as a measure of developmental stability. Non-directional alterations from perfect symmetry for morphometric characters represent measures of fluctuating asymmetry. These small, completely random departures from bilateral symmetry, caused by random developmental errors provide a convenient measure of developmental precision: the more precisely each side develops the greater the symmetry (Palmer & Strobeck 1992, Van Valen 1962). Fluctuating asymmetry results from the inability of an organism to develop precisely along determined pathways (Leary et al. 1983). The easiest way to determine developmental stability is to measure the difference between the left and the right side of a homologous structure when in average the structures are symmetric (Zakharov and Yablokov 1990).

FA is used as an indicator of developmental stability because it is supposed that the left and right side of a bilaterally symmetric organism are separate replicates of the same structure (Klingenberg 2003). A highly conserved pathway, controlled mainly by genetic processes, organizes the development of the left-right axis of the mammalian body plan during embryogenesis (Beddington and Robertson 1999).

Even though the mammalian body plan is consistently asymmetrical about the midline (McCarthy and Brown 1998) - the stomach lies on the left, lung lobes are asymmetrical in number and so on - the left and right sides of an organism share the same genome, therefore it is expected that in the presence of a stable environment, external disturbances will exert the same effect on both sides, thus left and right sides of the body would be an exact mirror image of one another (Klingenberg 2003).

However, when the organism is subject to a given perturbation, usually it will only produce disturbances on one side of the body, and the effects of perturbations will accumulate on the developing organs on the left and right sides separately. If

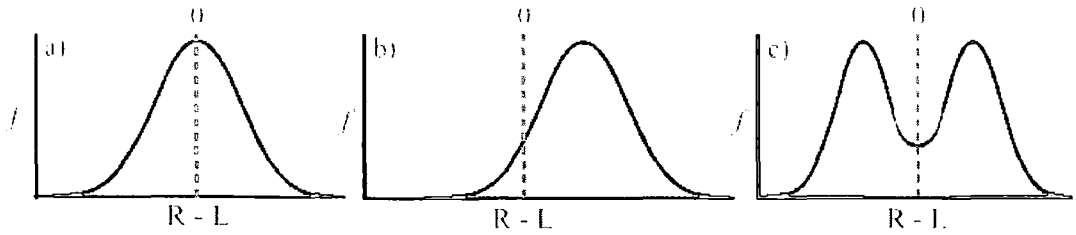

Figure 1.1 Types of asymmetry, a) fluctuating asymmetry, b) directional asymmetry (where the left side is larger than the right side), and c) antisymmetry (Taken from Palmer 1996).

compensatory mechanisms are not present, the development of both sides of an organism will deviate from each other (Klingenberg 2003). Experiments done in mice have suggested that the genetic pathway is balanced unless disturbances occur; therefore instabilities alter this conserved pathway and "insults" take place; this randomisation of the left – right axis can occur because of an absence of an intracellular motor consistent with asymmetric movement of molecules that later form the left – right axis (Beddington and Robertson 1999).

Although there are other types of asymmetry besides fluctuating (directional asymmetry, and antisymmetry) only FA has been suggested to result from genetic and environmental disturbances, and therefore to be useful as a measure of developmental stability (Baranov et al. 1997, Borisov et al. 1997, Leary & Allendorf 1989, Leary et al. 1983, Palmer & Strobeck 1986, Valetsky et al. 1997, Zakharov et al. 1997a, Zakharov et al. 1997b, Zakharov & Sikorski 1997, Zakharov et al. 1997c, Zakharov & Yablokov 1990).

Antisymmetry occurs when one side is larger than the other on average, but the larger member of a bilateral pair occurs on either the right or left side at random (Leary & Allendorf 1989, Møller & Swaddle 1997, Palmer 1994). Directional asymmetry (DA) arises when one side is larger than the other on average, and the

larger member of a bilateral pair tends to be on the same side (Leary & Allendorf 1989, Møller & Swaddle 1997, Palmer 1994). Antisymmetry and DA are developmentally controlled and are normally adaptative as asymmetries, whereas FA is reduced by natural selection and seems to be the "residuum after developmental processes in an organism tends to symmetry" (Van Valen 1962).

In practice FA can be measured by variance of minor non-directional differences between the paired structures on the left and on the right side of the body (Palmer & Strobeck 1986). It occurs when the difference between a character on the left and right sides of the body is normally distributed about a mean of zero (Leary et al. 1983). DA will also be normally distributed but its mean will be different from zero. Antisymmetry will have bimodal normal distribution about a mean of zero (Palmer and Strobeck 1986, figure 1.1).

In general, there have been numerous studies linking levels of genetic diversity and indirect indicators of fitness, such as FA (e.g. Hartl et al. 1995, Leary et al. 1983), but there have been few studies regarding developmental stability on marine mammal populations to date (but see Pertoldi et al. 2000a, Hoelzel et al. 2002a), however these populations have the potential to be impacted by factors leading to FA in significant ways.

However, FA studies have proven to be controversial as there are many published studies in the literature that have not found a direct relationship between FA and fitness. Bjorksten et al. (2000) stated that that the problems of FA as a measure of stress or quality are related to the fact that the genetic and developmental basis of FA are poorly understood and a solid theoretical basis is missing. Because of the reasons mentioned before, these authors concluded saying that FA is not a general and sensitive indicator of stress. Kruuk et al. (2003) explained that FA is a poor

indicator of developmental stability as FA did not reflect environmental and genetic stress on more asymmetrical individual. Other studies (e.g. Leung and Forbes 1996, Markow 1995) have found that the relationship between symmetry and fitness are weak, heterogeneous and equivocal (Clarke 1998a). In synthesis there have been inconsistencies in the studies of FA as an indicator of stress as there are studies that support this theory (see Møller and Swaddle 1997) but there are other studies that have not found a relationship between stress and FA (see review in Houle 1998).

In studies where the skull morphometry of toothed cetaceans are used, directional asymmetry is a major issue because odontocetes are one example in nature of species that encompass asymmetrical skulls (Ness 1967). This asymmetry appears to be a transition from symmetry to directional asymmetry as a result of adaptative and functional processes (Palmer 1996). The facial region of the skull of odontocetes is asymmetrical which is unique among mammals (Howell 1930, cited in Yurick and Gaskin 1988). Mead (1975) concluded that this sinistrad asymmetry relates with the form and function of the superimposed soft tissues in the facial region. Toothed cetaceans possess an acoustic and sound production system that is allocated in the facial region (Mead 1975). The allocation of these organs produces a DA in the skull as the result of the postrostral fraction of the right premaxillary that grows to a larger size than the left equivalent (Yurick and Gaskin 1988, Milinkovitch 1995). The simple nasal passage of most mammals has been replaced in odontocetes by a complex system of paired diverticula (nasal sacs), vestibules and nasal plugs (Yurick and Gaskin 1988).

As a result of this enlargement, the bony nares and the frontal crest are deviated from the midline of the skull (Yurick and Gaskin 1988). As the bones and muscles of the face are reshaped to accommodate the evolution of the melon and its need for complex mechanical manipulation, the sensory and motor structures are moved up

and over the orbit (Yurick and Gaskin 1988). This asymmetry in odontocetes is in accord with the necessity for an early development of the nasal sac complex and associated structures in these species, as they will become involved in sound production and/or manipulation adversely affecting ventilation as they increased in size (Mead 1975). This modification in size of the skull bones allows them to inhabit an aquatic environment in which the acoustic and echolocating systems are of supreme importance (Yurick and Gaskin 1988).

The harbour porpoise *Phocoena phocoena* is a small cetacean widely distributed in temperate and sub-arctic coastal waters in the northern hemisphere. Because the species inhabit coastal waters, they are affected by human activities. Chemical pollution, noise, ship traffic and overfishing of prey species are some of the anthropogenic activities that have created an impact on this species (Bjørge and Tolley 2002). The problem seems more complex as a significant number of porpoises are by-caught in fishing nets; even though the species is under legal protection in almost every country, it is not protected against incidental death as entanglement in fishing nets (Bjørge and Tolley 2002).

Incidental death in fishing nets has created global concern in recent years as the reduction on the numbers of the population in some areas may exceed the level considered as sustainable (Perrin et al. 1994, Bjørge and Tolley 2002). The IWC included this species in 1981 in a short list of small odontocete species to be the subject of conservation and management assessment in population size (Yurick and Gaskin 1987).

Successful management of this species must accommodate conservation of a variety of local stocks (Gao and Gaskin 1996a). In the eastern North Atlantic, both the Agreement on the conservation of Small Cetaceans of the Baltic and North Seas

(ASCOBANS) in 1994, and the International Whaling Commission (IWC) in 1995, have agreed on the necessity to research factors affecting survival of harbour porpoises (Börgesson and Berggren 1997).

The bottlenose dolphin *Tursiops truncatus* is a widely spread cetacean. It occurs from temperate to tropical coastal and offshore waters around the world. Two different ecotypes has been described, one coastal and one pelagic. Evidence of these different types has been well documented by several authors including Mead and Potter (1995) using morphological and ecological characteristics, and genetically by Hoelzel et al. (1998a).

Similar to the harbour porpoise the interaction of the species with human activities has been well documented. Along its distribution bottlenose dolphin are exposed to chemical pollution, noise and vessel traffic that could result in change of behaviour or in collision, depletion of prey numbers, etc. Incidental catches have been reported for several fisheries, some examples are tuna, sardines and anchovies (Wells and Scott 2002). High concentrations of organochlorine pollutants have been found in tissues of bottlenose dolphin (Hansen et al. 2004). As a consequence of this, immunosuppression has been documented. The incidence of epidermal diseases has found to be common in the species in several locations of the world (Wilson et al. 1999).

The changes in the habitat of harbour porpoises and bottlenose dolphins, chemical pollution, vessel traffic, noise, and low availability of prey in local areas can place both species under the effects of environmental stress. As a consequence of the environmental factors, it is expected that a depletion and consequently fragmentation in the different populations occur; therefore, it is most likely that genetic stress may arise. High levels of inbreeding and the loss of heterozygosity could occur; thus a

subsequent decrease in developmental stability of the species is also expected. That is why it is important to find if a relationship between these parameters exists. If a relationship is found, we could then infer the fate of the species by using the "early warning system" characteristic of developmental stability to recommend conservation practices for management and conservation of harbour porpoises and bottlenose dolphins.

Thesis Aims.

Few studies have attempted to relate the relationship between genetic and environmental factors in the production of disruptions in the developmental stability on cetaceans (e.g. Pertoldi et al. 2000a). Therefore, the relationship between genomic diversity and FA is examined by using microsatellite loci and morphological characters. Moreover, the relationship between environmental stress, measured as the concentration of chemical pollutants, and FA is also analysed in some of the same animals where the information on skull asymmetry and genomic diversity was available. Published results from the literature are used as indirect measures of environmental stress within populations. The classification of harbour porpoises and bottlenose dolphins into stocks or subpopulations from both a morphological and genetic perspective is analysed to provide resolution with respect to how stocks are structured. Population structure chapters precede those that deal with the relationship between developmental stability and stress in order to give a logical sequence to the study. Harbour porpoises and bottlenose dolphins had to be allocated into sub-populations first in order to compare the level of developmental stability, genomic diversity and environmental stress that the different sub-populations are facing.

*Chapter 2: Population structure in the eastern North Atlantic population of the Harbour Porpoise (*Phocoena phocoena*).*

2.1. Introduction

The harbour porpoise (*Phocoena phocoena*) is a small odontocete that inhabits cold and temperate coastal and continental shelf waters of the northern hemisphere. In the eastern North Atlantic its distribution includes the Barents Sea and west coast of Norway, around the coasts of Iceland, in the North and Celtic Seas, and around Danish waters in the Skagerrak and Kattegat seas. Possible migration routes include the Baltic Sea, the English Channel, the Bay of Biscay and the coast of Portugal and north-west Africa (IWC 1996, Rosel et al. 1999).

Although the harbour porpoise is considered common in the eastern North Atlantic (around 340,000 individuals, Hammond et al. 2002), a significant number of porpoises are being by-caught on fishing nets. Although the species is under legal protection in almost every country, it is not protected against incidental death from entanglement in fishing nets (Bjørge and Tolley 2002, table 3.15). The reduction in numbers of the population in some areas may exceed the level considered as sustainable (Perrin et al. 1994, Bjørge and Tolley 2002).

Regional management of stocks of harbour porpoises should be defined, so local governments and non-governmental organisations can recommend remedial actions to be implemented. To achieve this in recent years the population of harbour porpoise in the North Atlantic has been subject to several studies of population structure (see review in Lockyer 2003). Some of the methods that have been used include among others tagging (Berggren et al. 1996, Teilmann et al. 2003), contaminant analysis (Berrow et al. 1998, Tolley and Heldal 2002, Westgate and Tolley 1999) parasite load (Herreras et al. 1997), distribution (Hammond et al.

2002), and diet (Aarefjord 1995). However two methods have been used more extensively: morphology (Amano and Miyazaki 1992, Börjesson and Berggren 1997, Gao and Gaskin 1996a, Kinze 1985), and genetics (Andersen et al. 1997, Andersen et al. 2001, Tolley et al. 1999, Tolley and Rosel 2002, Walton 1997).

Previous studies on population structure of harbour porpoises have made comparisons between large geographic regions, e.g. North Pacific vs. North Atlantic (Amano and Miyazaki 1992, Gao and Gaskin 1996b, Yurick and Gaskin 1987). These studies have found that there are significant differences between North Pacific and North Atlantic populations of harbour porpoises, except from the study of Gao and Gaskin (1996b), where they did not detect any differentiation between the porpoises inhabiting the two ocean basins. They explained that morphological meristic characters were not useful to detect any significant differences.

Other studies have made comparisons limited only to the western North Atlantic e.g. Gao and Gaskin (1996a) using morphological skull characters, and by using mtDNA and microsatellites Rosel et al. (1999) studied the population structure of the western North Atlantic population, they found three different sub-populations: Gulf of Maine, Newfoundland, and Gulf of St. Lawrence-West Greenland. Comparisons between the western and eastern North Atlantic have also been made (e.g. Tolley et al. 2001, Tolley and Rosel 2002, Yurick and Gaskin 1997). All of them have concluded a clear distinct population inhabiting the two coasts of the North Atlantic. For morphological characters, the study of Börjesson and Berggren (1997) focused solely on the population structure of harbour porpoises in the eastern North Atlantic but was limited only to a comparison between Danish waters and the Baltic Sea. The International Whaling Commission in 1996 made an attempt to classify the eastern North Atlantic into eight different sub-populations: 1) Iceland, 2) Faeroe Islands, 3) Norway and Barents Sea, 4) North Sea, 5) Kattegat and adjacent waters,

6) Baltic Sea, 7) Ireland and Western British Isles, and 8) Iberia and Bay of Biscay. Although some regional modifications have been proposed (e.g. Walton 1997, Andersen et al. 2001), the above classification is now widely accepted.

2.1.1. Population structure around Norwegian waters.

Around Norwegian waters (fig. 2.1) harbour porpoises are distributed from northern Norway into the northern North Sea (Bjørge and Øien 1995), with an apparent absence of porpoises in the mid-coastal region (Gaskin 1984). Therefore, two putative populations were proposed: one population that inhabits the Barents Sea and another from the northern North Sea with the division around the 66°N parallel (Bjørge and Øien 1995, IWC 1996). Tolley et al. (1999) tried to confirm this apparent division but did not find any variability in the sequences of the D-loop in mitochondrial DNA (mtDNA) of porpoises from the two groups. Andersen et al. (2001) used microsatellite loci and also failed to demonstrate this division and the existence of two subpopulations along Norwegian waters.

Other studies support the proposal of a different Norwegian (NOR) putative population in the eastern North Atlantic. Wang and Berggren (1997) used restriction fragments (RFLP) on one locus of mtDNA. They found a significantly different haplotype frequency between 13 porpoises off the coast of Norway and 27 from the Swedish Baltic and 25 from the Skagerrak and Kattegat seas. Tolley et al. (2001) using mtDNA found that porpoises from the Norwegian population (n= 87) were genetically differentiated from those of Icelandic waters (n= 72). Even further in their study of 12 microsatellite loci Andersen et al. (2001) found the Norwegian sub-population the most genetically differentiated among the examined samples from

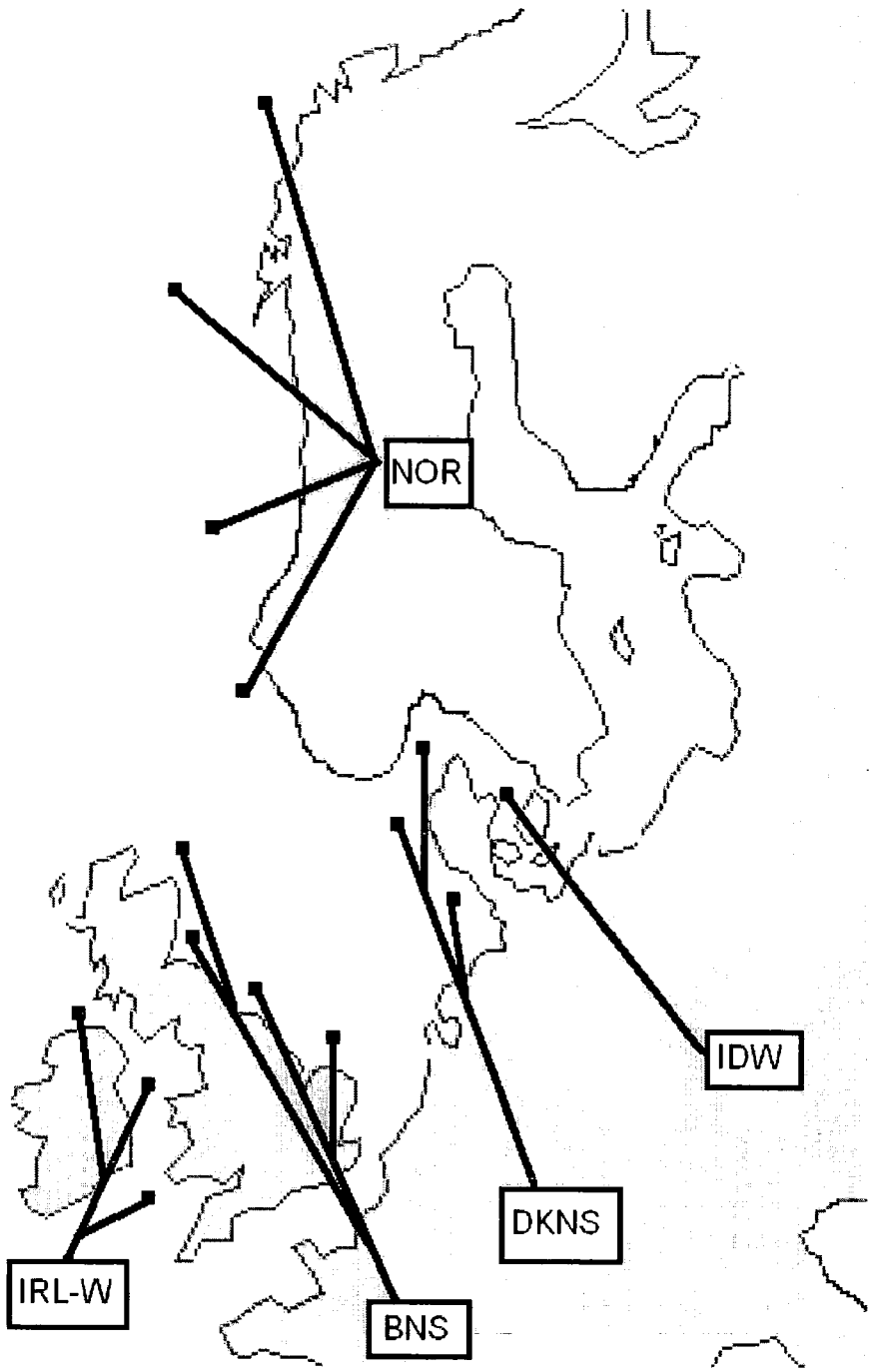

Figure 2.1. Subpopulations of harbour porpoise in the eastern North Atlantic subject to analysis in this study. NOR.- Norwegian; IDW.- Inner Danish Waters; DKNS.- Danish North Sea and Skagerrak Sea; BNS.- British North Sea: IRL-W.- Irish-Welsh.

the Inner Danish Waters, the Danish North Sea, the British North Sea, Ireland, the Netherlands and West Greenland.

2.1.2. Population structure around Danish waters.

Gaskin (1984) and the revision made by the IWC in 1996, divided the harbour porpoises that distributes around Danish waters into two separate populations, one belonging to the Danish North Sea (DKNS) and another that belongs to the inner Danish waters (IDW), i. e., the Skagerrak and Kattegat Seas, the Belts and the Øresund. This second population has been separated from the Baltic proper by several studies, e. g. Börjesson and Berggren (1997) using 17 skull characters; and Wang and Berggren (1997) using RFLP in 1 locus of mtDNA. The latter found no structuring in the Inner Danish waters population, whereas Andersen et al. (1993) using isozymes (2 loci), Andersen et al. (1997) using two isozymes and three microsatellite loci, and Andersen et al. (2001) using 12 microsatellite loci, not only have found that animals from the Skagerrak are genetically different from animals from the Kattegat, Belts and Øresund; but also that the Skagerrak porpoises are genetically similar to the DKNS porpoises. Thus they proposed that the two putative populations should be revised into the DKNS including the Skagerrak Sea and the IDW without it.

2.1.3. Population structure around British waters.

Walton (1997) studied regional differences in harbour porpoises around the British Isles by detecting variability in a 200 bp section of the control region of mitochondrial DNA of harbour porpoises around the UK. Among his findings, he reported a sub-division of the population of harbour porpoises around British waters into an Irish/west Britain (IRL-W) and the North Sea (BNS). Andersen et al. (2001) using 12 microsatellite loci, also found a genetically different population of IRL-W from porpoises originating form the BNS.

Proper management and abundance estimates could be achieved by defining population structure. An example of this is by-catch rates. The impact of estimates

of harbour porpoises killed each year in the fishery industry could be better understood if sub-populations are properly recognised (Andersen et al. 20001). In this study the population structure of the population of harbour porpoises around Norwegian, Danish and British waters was revisited based on the information of 16 morphometric characters and 12 microsatellite loci. According to the regions where the samples were obtained, and from the results of the studies mentioned above, the hypothesis of this study is to find five distinct sub-populations: Norwegian, British North Sea, Irish-Welsh, Danish North Sea-Skagerrak Sea, and Inner Danish Waters.

2.2. Methods.

2.2.1. Cranial measurements.

2.2.1.1. Sample collection.

A total of 462 skulls of harbour porpoise were measured from the collection of 4 European museums (table 2.1). The museums visited (and the number of skulls measured) were the Zoological Museum of the University of Oslo in Norway (50); the Zoological Museum of the University of Copenhagen in Copenhagen, Denmark (93); the National Museums of Scotland in Edinburgh (274), and the Natural History Museum in London (45), United Kingdom. The information regarding the classification of the skulls to a sub-population was provided by the records of each museum in respect to where the animal was by-caught or stranded. In total they were 50 for the Norwegian population, 53 for the Danish North Sea, 40 for the Inner Danish Waters, 152 for the British North Sea, and 154 for the Irish-Welsh population.

2.2.1.2. Choice of traits.

Sixteen bilateral characters were chosen for this study and described below. Traits 1, 4, 5, 10, 12,13,14,15 and 16 were taken from Perrin (1975). Traits 3, 9 and 11 were taken from Yurick and Gaskin (1987). Trait 7 was taken from Amano and

Table 2.1. Summarised information on the skulls of harbour porpoise measured (ZMUO - Zoological Museum, University of Oslo; ZMUC -Zoological Museum of the University of Copenhagen; NHM - Natural History Museum; NMS - National Museums of Scotland).

MUSEUM	SEX			AGE CLASS			SOURCE		
	Male	Female	N/A	Juvenile	Adult	N/A	Stranded	By-caught	N/A-other
ZMUO	28	18	4	14	31	5	1	46	3
ZMUC	50	43	0	65	28	-	20	61	12
NHM	12	9	24	2	26	17	-	-	45
NMS	144	127	3	152	120	2	-	-	274
Total	234	197	31	233	205	24	21	107	334

Miyazaki (1992). Trait 2 was taken from Börjesson and Berggren (1997); Trait 6 and 8 were devised in this study mainly because the majority of skulls had those bones intact. They were measured using precision callipers. The traits were measured to the nearest 0.001 cm, except for CBL, LOR, and ML that were measured to the nearest 0.01 cm. Three repeated measurements for each trait of every skull were done and the callipers were reset to zero after each measurement. The median of the three was used (Zar 1984). Measurements were taken on the left and the right side of the skull; however, because of the asymmetry present in the skull of the harbour porpoise, measurements on the left side of the skull were used on this chapter (Yurick and Gaskin 1987). No measurements were attempted on missing or worn structures; therefore there are missing data. The traits measured followed Perrin's (1975) nomenclature. They were (fig. 2.2):

1. **CBL** - Condylobasal length. Distance from the tip of rostrum to the hindmost margin of the occipital condyles.
2. **WON** – Greatest width of external nares.
3. **AOT** – Distance from the antorbital notch to the hindmost external margin of the raised suture of the post-temporal fossa.

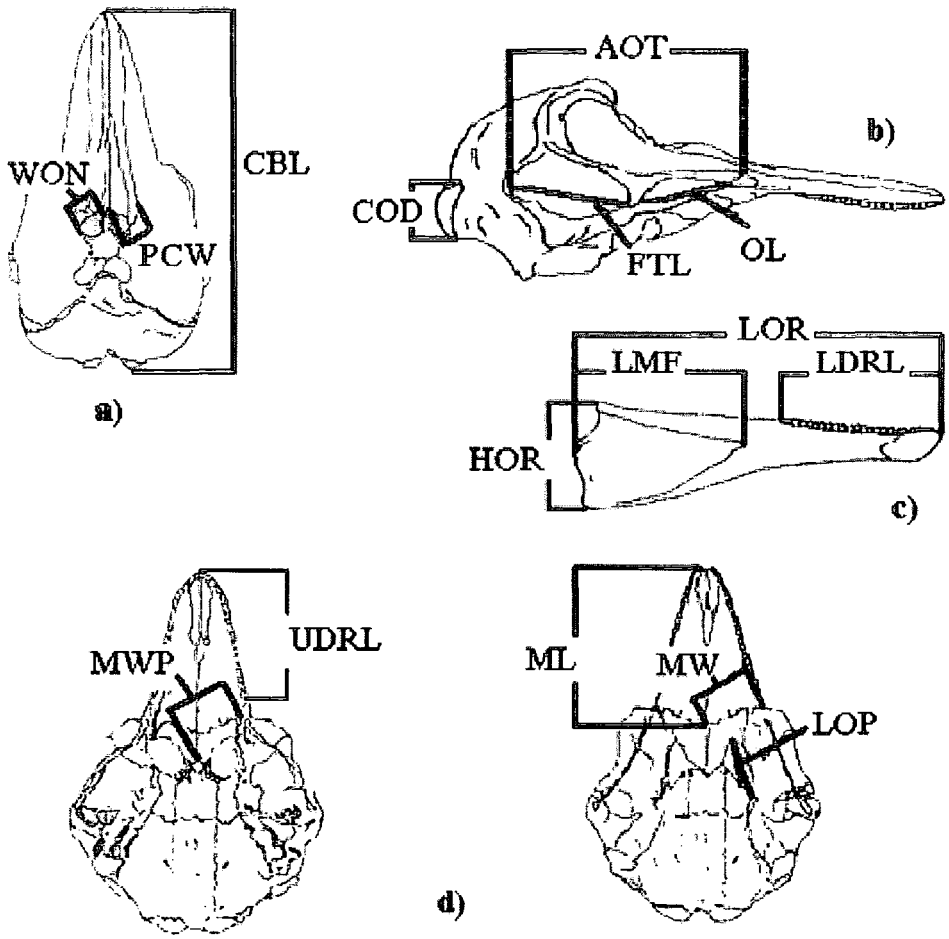

Figure 2.2. Bilateral traits measured in the skull of the harbour porpoise. a) Dorsal view. b) lateral view. c) medial view of mandible. d) ventral view.

4. **FTL** – Greatest length of the post-temporal fossa, measured to the hindmost external margin of raised suture.
5. **OL** – Length of the orbit. Distance from apex of preorbital process to the apex of postorbital process of the frontal.
6. **COD** – Greatest height of occipital condyle.
7. **ML** – Length of maxilla. Distance from the tip of rostrum to the hindmost margin of suture of maxilla with the palatine.
8. **MW** – Greatest width of maxilla. Distance from the antorbital notch to the hindmost margin of the suture of maxilla with the palatine.
9. **MWP** – Greatest width of palatine.

10. **LOP** – Greatest length of pterygoid.
11. **PCW** – Greatest width of premaxillar crest
12. **LDRL** – Length of lower dental row. Distance from the hindmost margin of the hindmost alveolus to the tip of ramus
13. **UDRL** – Length of upper dental row. Distance from the hindmost margin of the hindmost alveolus to the tip of rostrum.
14. **LOR** – Greatest length of ramus.
15. **HOR** – Greatest height of ramus.
16. **LMF** – Length of mandibular fossa, measured to mesial rim of internal surface of condyle.

2.2.2 Microsatellite analyses.

Microsatellites are a powerful molecular marker, since they show a high level of polymorphism, they can have a high number of alleles and they have high heterozygosity, thus they provide useful information in studies that focus on DNA variation for population structure and the loss of diversity to assess fitness (Pemberton et al. 1999).

2.2.2.1. Samples obtained and previously published data used.

Muscle samples were obtained for 47 of the 50 porpoises for which the skull was measured from the Norwegian sub-population. Oliver Thatcher (Unversity of Cambridge, UK) provided the genotypes for ten of the twelve microsatellite loci (GT101 and EV 96 were not provided) for 113 porpoises from the British North Sea population and 107 for the Irish-Welsh population. The genotypes for the twelve microsatellite loci for all the porpoises from the Danish North Sea and Inner Danish Waters were provided by Liselotte W. Andersen and were published in Andersen et al. (2001). Replications among labs were carried out to standardise the results, this involved sharing a few microliters of reference DNA from assorted animals from

Oliver Thatcher and Lisolette Andersen. They were amplified and measured with respect to known genotypes so datasets were made compatibles.

2.2.2.2. DNA extraction and isolation from skin samples.

Skin samples had been stored in a 20% DMSO/5M NaCl solution (Amos and Hoelzel 1991). A small sub-sample of muscle approximately a 3 mm cube, was cut from the specimen and finely chopped. Samples were digested at 37°C overnight in 500μL of digestion buffer (50 mM Tris pH 7.5, 1 mM EDTA, 100 mM NaCl, 1% w/v SDS; Milligan 1998) with 0.6 mg/mL proteinase K. Total DNA was then extracted using standard phenol/chloroform extraction followed by ethanol precipitations using sodium acetate and then lithium chloride as the monovalent cations (Sambrook et al. 1989). DNA was then stored in 1X TE buffer at an approximate concentration of 100-200 ng/μL at –20°C.

2.2.2.3. PCR amplification.

Twelve published microsatellite loci were used in this study. These loci, their primer sequences, and, the references are listed in table 2.2 along with the MgCl2 concentration and annealing temperatures used for amplification. To allow sizing of the PCR product using ABI Prism™ technology, one tenth of one of the primers of each pair in each reaction was from a primer solution in which the oligonucleotides had been labelled at the 5' end with a fluorescent ABI Prism™ dye. The primer that was labelled in each set and the dye used is noted in table 2.2.

PCR amplification was carried out in 15 μL reactions using 0.5 μL of DNA extract. Reaction conditions were 10 mM Tris-HCl pH 9.0, 50 mM KCl, 0.1% Triton® X-100, 0.2 mM each dNTP, $MgCl_2$ at the concentration specified in Table 2, 10 ng/μL of each primer and 0.3 units of *Taq* (Bioline™). Cycle conditions for the microsatellites

Table 2.2. Microsatellite loci used for harbour porpoise, their primer sequences, and the PCR conditions used. The top primer sequence of each pair was fluorescently labelled with the dye indicated.

Locus	Primer sequence	Reference	Annealing. Temp. in ºC	[$MgCl_2$] in mM	Dye
Igf-I	5'-GGGTATTGCTAGCCAGCTGGT 5'-CATATTTTTCTGCATAACTTGAACCT	Kirkpatrick 1992	50	1.5	FAM
415/416	5'-GTTCCTTTCCTTACA 5'-ATCAATGTTTGTCAA	Amos et al. 1993	40	2.0	NED
417/418	5'-GTGATATCATACAGTA 5'-ATCTGTTTGTCACATA	Amos et al. 1993	48	1.5	FAM
GT011	5'-CATTTTGGGTTGGATCATTC 5'-GTGGAGACCAGGGATATTGC	Bérubé et al. 1998	59	1.5	FAM
GT015	5'-GAGAATGGCTGGGCTCAGATC 5'-TTCCCTATTAGAGGCTCACGA	By courtesy of Palsbøll P, Bérubé M, and Jorgensen H	59	1.5	NED
GT101	5'-CTGTGCTGGTATATGCTATCC 5'-CTTTCTCCTAGTGCTCCCCGC	Bérubé et al. 2000	56	1.5	HEX
GT136	5'-AAAAAGTCTCCTCTGGACCTG 5'-GTGCACCCTGGACTGTTAGTG	By courtesy of Palsbøll P, Bérubé M, and Jorgensen H	52	1.5	NED
EV94	5'-ATCGTATTGGTCCTTTTCTGC 5'-AATAGATAGTGATGATGATTCACACC	Valsecchi and Amos 1996	48/54	1.5	HEX
EV96	5'-AAGATGAGTAGATTCACTACACGAGG 5'-CCACTTTTCCTCTCACATAGCC	Valsecchi and Amos 1996	48/54	1.5	HEX
EV104	5'-TGGAGATGACAGGATTTGGG 5'-GGAATTTTTATTGTAATGGGTCC	Valsecchi and Amos 1996	48/54	1.5	HEX
TAA031	5'-TCCAGTGGTTAGGACTTGGCG 5'-TCACTTCCTACTTTGATGAGG	Palsbøll et al. 1997	53	1.5	FAM
GATA053	5'-ATTGGCAGTGGCAGGAGACCC 5'-GGTGAGTGAGTGATGCAGAGG	Palsbøll et al. 1997	55	1.5	NED

used were: denaturation at 95°C for 3 minutes, 35 cycles of denaturation at 94°C for 1 minute, annealing temperature (see table 2.2) for 30 seconds, extension at 72°C for 10 seconds with a final extension step of 15 minutes at 72°C. Exceptions of cycle conditions were used for loci EV94, EV104 and EV 96. Cycle conditions for these were: denaturation at 95°C for 3 minutes, 7 cycles of denaturation at 93°C for 1 minute, annealing temperature (48°C) for 30 seconds, extension at 72°C for 50 seconds, followed by 25 cycles of denaturation at 90°C for 45 seconds, annealing temperature (56°C) for 1 minute, extension at 73°C for 1 minute, with a final extension step of 15 minutes at 72°C.

2.2.2.4. Interpretation of results.

Microsatellite PCR products were run, without further purification, on 6% polyacrylamide gels. DBS Genomics (University of Durham) ran them on a 377 ABI polyacrylamide slab gel automated sequencers. As mentioned in the earlier section on PCR, each product had been labelled by the use of a flourescently labelled primer, allowing the product to be detected by the sequencer. ABI Prism™ fluorescent labels of FAM, HEX, and NED were used. The PCR products were then added in specific amounts (0.2 μL for FAM dyed PCR products, 0.3 μL for the HEX dyed products and 0.4 μL for the NED dyed products) to a 1.625 μL mixture of ABI loading buffer. Sets of loci were assembled taking care not to overlap allele sizes on the same given dye before run together on the 377 ABI sequencer. Therefore, three sets were assembled: 1) FAM: D22 and TexVet 5; HEX: D08 and EV37; and NED: D18 and KWM1b; 2) FAM: TtruAAT$_{44}$ and TexVet 7; HEX: MK8 and KWM2b; and NED: KWM2a; and 3) FAM: KWM9b; and NED: KWM12a. Running of a ROX labelled DNA size ladder in each lane allowed sizing of the detected PCR products. Visualization of PCR product sizes to a resolution of 1 bp

was possible on a chromatogram produced by analysis of the output of the automated sequencer using ABI Genescan™ and Genotyper™ software.

Microsatellite alleles were considered reliable and used in the analysis if the peaks met certain criteria. First, the highest amplitude peak, used as the allele size, was only considered valid if it had an amplitude higher than 50 on the chromatogram. Most alleles, especially in skin samples, were well above this amplitude, and any peaks below 100 were duplicated before use in the analysis. Second, alleles deemed reliable had to show the expected signature structure. Each locus showed a pattern in the shape and prominence of the stutter peaks associated with an allele, and any peaks not showing this pattern were considered to be background 'noise' in the chromatogram or unspecific amplification.

2.2.3. Statistical analyses.

2.2.3.1 Morphometric characters.

To test for significant differences in skull morphology between sexes and between age classes within populations, all measurements were standardised over the total length of the skull (CBL) to control for size. This gave a relative ratio for each measurement. For each population a multivariate analysis of variance (MANOVA) was used to find differences in the relative skull characters between age classes and sex.

A MANOVA was also used to find differences in the skull morphology characters between the three populations. Discriminant function analysis (DFA) was used to classify the porpoises into one of the five putative populations based on the discriminant functions (see Tabachnick and Fidell 1996). The adequacy of the classification was determined by the percentage of correct classification, assuming that there was an equal probability (33%) of being classified into any of the three

groups by chance alone. Classification percentages substantially greater than 33% for any given group would indicate that the discriminant functions were satisfactory for predicting group membership. The Malahonibis distance was used to allocate a sample into a population by measuring the distance of the mean vector of each case from the mean vectors of each population. DFA classified each porpoise to a population based on the assumption that the shorter the distance of the mean of a case in respect to the mean of a particular population, the higher the probability the sample belongs to that particular population. The Wilks' λ test was used to determine if the classification done by the DFA into the discriminant functions was significant (Field 2005). Pairwise t-tests were performed to assess the presence of significant differences on skull morphology between populations.

2.2.3.2. Microsatellite loci analysis.

Genotyping errors caused by low quantity template DNA may result because of an allele failing to amplify, i. e. null alleles (Wandeler et al. 2003). Another cause of genotyping errors is scoring errors due to stuttering. Before proceeding with analysis, genotyping errors were investigated by using the methods described by Chakraborty et al. (1992) and Brookfield (1996) and implemented in the software Micro-checker (van Oosterhout et al. 2004). The software calculates the probabilities for the observed number of homozygotes and it looks for an excess of homozygotes that is not homogenously distributed across all loci for each population. This may be evidence of null alleles. Allele dropout is suggested when an excess of homozygotes is biased towards either extreme of the allele size distribution. Stuttering is suggested when there is a deficiency of heterozygotes with alleles differing in size by one base pair, and a relative excess of large homozygotes.

Polymorphism was estimated as the number of alleles per locus, number of private alleles per putative population, allelic richness, observed heterozygosity and expected heterozygosity. Deviation from Hardy-Weinberg equilibrium and linkage disequilibrium were tested using an analog of Fisher's exact test with a Markov-chain method (10^5 iterations, 5 x 10^3 dememorisation steps, sequential Bonferroni correction applied) as described in Guo and Thompson (1992) and calculated by ARLEQUIN 2.0 (Schneider et al. 2000).

Allelic richness for each locus and for each population was measured, using the program FSTAT 2.9.3 (Goudet 2001), by employing a rarefaction method that adjusts for sample size. A Kruskal-Wallis test was employed to test for differences in allelic richness among populations.

Linkage disequilibrium (null hypothesis: independence between genotypes at separate loci) was tested for each pair of loci using GENEPOP 3.4 (Raymond and Rousset 1995a, b) to determine whether associations existed between pairs of alleles by using a probability test (Fisher's exact test) using a Markov chain approach (Guo and Thompson 1992). For each population 10^3 dememorisations, 10^2 batches and 10^3 iterations per batch were used.

Random mating assessment was devised using Wright's F_{IS} (Wright 1951). Non-random mating (inbreeding) is a cause of a reduction of heterozygosity of an individual. F_{IS} is the correlation between homologous alleles among individuals that are part of a local population (Avise 1994). The degree of inbreeding within a population was assessed by comparing the observed and expected heterozygosity levels by using:

$$F_{IS} = \frac{H_s - H_i}{H_s}$$

where H_I is the observed heterozygosity of an individual, estimated as the mean frequency of heterozygotes averaged over all subpopulations and H_S is the expected heterozygosity of an individual in a subpopulation, calculated first for each subpopulation an then averaged. F_{IS} was calculated using the program FSTAT 2.9.3 (Goudet 2001).

Structure 2.0 (Pritchard et al. 2000, Falush et al. 2003) which uses a Bayesian based model to infer population structure, was used in assigning putative populations (*K*). The program takes allele frequencies into consideration and can be used to determine a likely number of populations existing within a group of samples and to assign individuals to these populations. The admixture model was assumed and the analysis was performed considering both the independent and the correlated allele frequency models. Burn-in length was set at 10^5 repetitions and the length of the simulation was run at 10^6 repetitions. A series of four independent runs for each value of *K* (from 1-9) were done to test the convergence of priors and the appropriateness of the chosen burn-in length and simulation length as suggested by Pritchard et al. (2000).

The levels of differentiation among populations was estimated based on the infinite allele model using F_{ST} (Weir and Cockerham 1984) and calculated using the program ARLEQUIN 2.0 (Schneider et al. 2000). It was also estimated by the stepwise mutation model by using Rho_{ST} (Michalakis and Excoffier 1996) by using the program RSTCALC 2.2 (Goodman 1997); statistical significance was calculated by permutation tests with bootstrapping to provide 95% confidence intervals with 10^3 iterations. A permutation test to assess differentiation for allele size was used for F_{ST} and for Rho_{ST} using the program SPAGeDi (Hardy and Vekemans 2002).

Table 2.3. Means and standard deviation of standardised measurements for each population. All traits are relative ratios in respect to the condylobasal length (CBL). CBL is reported in cm.

	NOR		DK		BRIT	
	Juveniles	Adults	Juveniles	Adults	Juveniles	Adults
CBL	24.0 ± 1.7	25.7 ± 1.2	24.0 ± 1.2	27.1 ± 1.2	24.2 ± 1.5	26.5 ± 1.4
WON	0.046 ± 0.03	0.049 ± 0.00	0.050 ± 0.00	0.051 ± 0.00	0.061 ± 0.01	0.059 ± 0.01
AOT	0.475 ± 0.03	0.471 ± 0.02	0.482 ± 0.02	0.468 ± 0.01	0.471 ± 0.02	0.465 ± 0.02
FTL	0.254 ± 0.02	0.249 ± 0.01	0.246 ± 0.02	0.254 ± 0.01	0.219 ± 0.02	0.228 ± 0.03
OL	0.258 ± 0.01	0.254 ± 0.01	0.222 ± 0.01	0.221 ± 0.01	0.202 ± 0.02	0.202 ± 0.03
COD	0.142 ± 0.02	0.157 ± 0.01	0.157 ± 0.01	0.157 ± 0.01	0.158 ± 0.01	0.156 ± 0.01
ML	0.310 ± 0.07	0.289 ± 0.04	0.435 ± 0.02	0.439 ± 0.02	0.444 ± 0.02	0.452 ± 0.02
MW	0.170 ± 0.02	0.155 ± 0.01	0.147 ± 0.01	0.147 ± 0.02	0.157 ± 0.01	0.160 ± 0.01
MWP	0.085 ± 0.02	0.095 ± 0.01	0.182 ± 0.01	0.192 ± 0.02	0.176 ± 0.02	0.180 ± 0.01
LOP	0.156 ± 0.01	0.114 ± 0.02	0.154 ± 0.02	0.156 ± 0.01	0.149 ± 0.01	0.151 ± 0.01
PCW	0.062 ± 0.00	0.065 ± 0.01	0.061 ± 0.01	0.064 ± 0.01	0.065 ± 0.01	0.065 ± 0.01
UDRL	0.352 ± 0.01	0.350 ± 0.02	0.364 ± 0.01	0.361 ± 0.02	0.354 ± 0.01	0.369 ± 0.02
LDRL	0.343 ± 0.02	0.346 ± 0.02	0.350 ± 0.00	0.355 ± 0.01	0.350 ± 0.01	0.347 ± 0.01
LOR	0.725 ± 0.02	0.740 ± 0.04	0.755 ± 0.01	0.770 ± 0.01	0.757 ± 0.01	0.765 ± 0.02
HOR	0.194 ± 0.01	0.201 ± 0.01	0.205 ± 0.01	0.209 ± 0.01	0.200 ± 0.01	0.204 ± 0.01
LMF	0.312 ± 0.02	0.312 ± 0.02	0.315 ± 0.02	0.315 ± 0.02	0.313 ± 0.02	0.305 ± 0.03

2.3. Results.

2.3.1. Cranial measurements.

MANOVA did not find significant differences between sexes and age classes. Therefore the data set was pooled for analysis within each population. The MANOVA that tested for differences in skull morphology among the five populations did not find significant differences. However, when the data set was pooled into three main populations: Norwegian, Danish and British, significant differences for all traits were found (all $p<0.001$, Bonferroni correction applied). Table 2.3 shows basic statistics for each trait among the three main populations.

From the results of the discriminant function analysis (DFA), the percentages of successful classification for each of the five sub-populations are presented in table

Table 2.4. Adequacy of classification results for the discriminant analysis. Left column indicates the original group while the top row indicates the predicted group. Values are as percentage. Correct classifications are italicised.

	NOR	IDW	DKNS	BNS	IRL-W
NOR	*95.2*	2.0	1.7	0.8	0.3
IDW	0.0	*98.2*	1.6	0.2	0.0
DKNS	1.5	*95.3*	0.2	2.7	0.3
BNS	5.6	*0.0*	1.2	*88.7*	4.5
IRL-W	0	3.3	15.5	*74.9*	6.3

Table 2.5. Adequacy of classification results for the discriminant analysis after re-classification into three main populations. Correct classifications are italicised.

	NOR	DK	BRIT
NOR	*98.1*	1.0	0.9
DK	0	*93.5*	6.5
BRIT	0	24.2	*75.8*

Table 2.6. Results of the Wilks' λ test.

Discriminant Function	Wilks' λ	χ^2	df	Significance
1	0.004	116.146	14	***
2	0.368	20.985	14	n.s.

*** $p<0.001$.

2.4. IDW and DKNS populations were correctly classified into one single group; the same happened for the BNS and IRL-W. They were also classified correctly into one single group. Therefore, following these results and the results of the MANOVA described above. The IDW and DKNS were pooled into one Danish population and the BNS and IRL-W were pooled into one British population. The

Table 2.7. Pooled within-groups correlations between discriminating variables and standardised canonical discriminant functions. Variables ordered hierarchically by absolute size of correlation within function. The largest absolute correlations with either discriminant function are shown in bold and italicised.

	Discriminant Function	
	1	2
MWP	***0.332***	0.233
ML	***0.227***	0.029
LOR	***0.037***	0.015
PCW	***0.026***	0.009
OL	0.216	***0.602***
FTL	0.069	***0.398***
MW	0.015	***0.345***
WON	0.051	***0.335***
LMF	0.018	***0.195***
AOT	0.054	***0.183***
HOR	0.017	***0.089***
COD	0.003	***0.084***
LDRL	0.035	***0.078***
UDRL	0.038	***0.042***
LOP	0.009	***0.016***

percentages of successful classification for each "new" group are presented in table 2.5. For each population the percentage of correct classification was much higher than the 33% expected by chance

The results from the DFA showed that the Wilks λ test was significant for discriminant function 1, but not significant for the second function among the population centroids. This suggests that the populations were distinguishable based on skull morphology only for the traits that correlated with the first discriminant function (table 2.6 and 2.7). Table 2.7 shows the structure matrix of the DFA. This matrix shows the relationship between each trait and the discriminant functions. The higher the correlation index for a skull character in

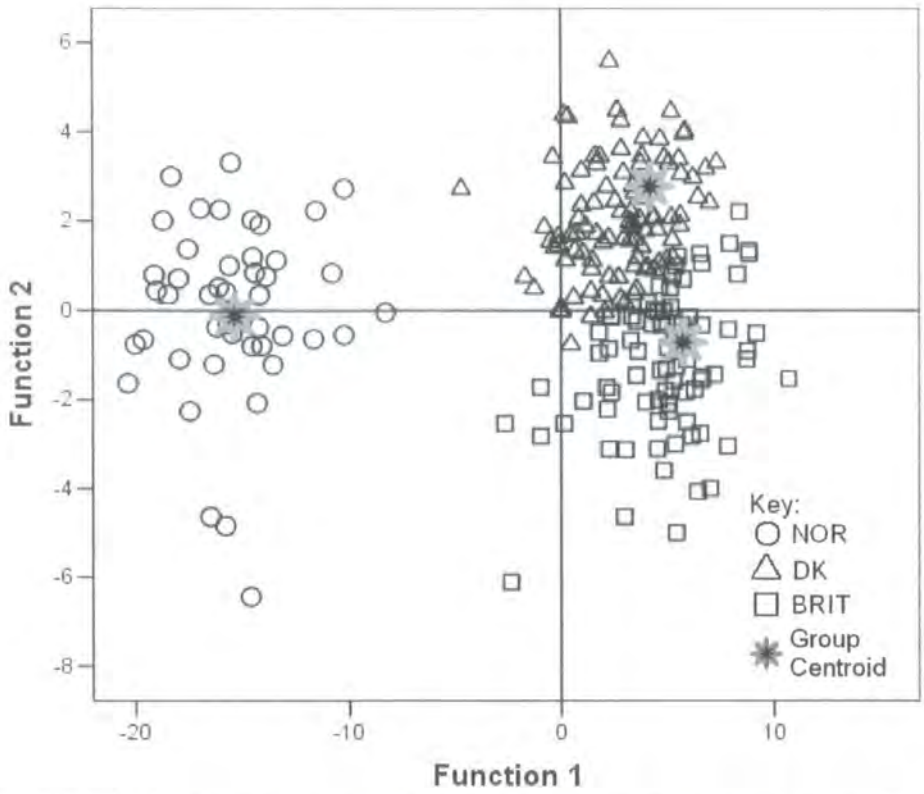

Figure 2.3. Plot of the discriminant function scores for harbour porpoises from the eastern North Atlantic based on skull morphology.

Table 2.8 Discriminant functions at population centroids.

	Function	
	1	2
NOR	-15.42	-0.13
DK	4.15	2.78
BRIT	5.70	-0.72

respect of a discriminant function, the more that trait contributed to the separation between populations. Discriminant function 1 explained 98% of the variance. The population centroid of the Norwegian population showed a value of -15.69 for the discriminant function 1, this value represented the largest degree of separation among the groups (table 2.8, figure 2.3). Table 2.9 shows the t-test values that

Table 2.9. Pairwise t-tests in the traits among the populations studied.

	NOR vs. DK			NOR vs. BRIT			DK vs. BRIT		
	t	df	Sig.	t	df	Sig.	t	df	Sig.
WON	-2.15	54	*	-5.36	162	***	-4.15	160	***
AOT	-0.72	45	n.s.	1.13	134	n.s.	2.06	133	*
FTL	-0.22	37	n.s.	4.49	117	***	4.11	112	***
OL	11.87	50	***	10.52	147	***	3.81	145	***
COD	-1.44	54	n.s.	-1.62	152	n.s.	0.56	154	n.s.
ML	-12.01	50	***	-25.56	161	***	-2.60	157	**
MW	2.65	49	**	0.10	165	n.s.	-4.56	160	***
MWP	-21.48	53	***	-27.15	163	***	2.21	162	*
LOP	-0.87	37	n.s.	0.48	112	n.s.	1.60	115	n.s
PCW	1.48	51	n.s.	-0.64	169	n.s.	-2.41	164	*
UDRL	-1.45	31	n.s.	-2.49	101	n.s.	0.16	82	n.s.
LDRL	-0.86	37	n.s.	-0.71	101	**	0.88	85	n.s.
LOR	-3.90	45	***	-5.24	94	***	-0.28	101	n.s.
HOR	-2.73	41	**	-1.62	114	n.s.	2.03	117	*
LMF	0.76	44	n.s.	0.71	118	n.s	1.34	124	n.s

Significance= * $p<0.05$; ** $p<0.01$; *** $p<0.001$ (Bonferroni correction applied).

tested for significant differences between pairwise comparisons for each trait among populations.

2.3.2. Microsatellite analysis.

2.3.2.1 Genotyping errors, test for Hardy Weinberg equilibrium and genetic diversity.

No evidence of genotyping errors was found. However, heterozygote deficiency compared against the Hardy Weinberg (HW) equilibrium expectations were found for locus 415/416 and EV96 for the Norwegian population and EV104 for the British population. Omission of the loci that depart from HW equilibrium expectations did

Table 2.10. Number of alleles (A), private alleles, allelic richness, allele size range, expected (He) and observed (Ho) heterozygosity and F_{IS} for each population at each microsatellite locus. Asterisks indicate those loci with a *p*-value <0.001 (Bonferroni correction applied) when tested for heterozygote deficiency.

		Norwegian	Danish	British
IGF-I	*N*	47	93	220
	A	14	13	16
	Private A	-	-	1
	Allelic Richness		6.82	13.03
	Size range	134-164	136-162	132-164
	H_o	0.893	0.774	0.798
	H_e	0.897	0.872	0.869
	F_{IS}	0.001	0.011	0.066
415/416	*N*	41	93	213
	A	4	8	7
	Private A	-	2	1
	Allelic Richness	4.00	6.43	5.40
	Size range	213-221	201-221	209-221
	H_o	0.425*	0.527	0.502
	H_e	0.650	0.606	0.54
	F_{IS}	0.114	0.119	-0.04
417/418	*N*	45	93	218
	A	9	6	8
	Private A	4	-	1
	Allelic Richness	8.97	5.51	6.64
	Size range	165-185	175-185	161-187
	H_o	0.680	0.624	0.569
	H_e	0.832	0.654	0.590
	F_{IS}	0.128	0.047	-0.015
GT011	*N*	47	93	220
	A	11	11	14
	Private A	1	-	2
	Allelic Richness	10.82	8.72	10.25
	Size range	107-131	103-127	101-129
	H_o	0.745	0.688	0.802
	H_e	0.837	0.732	0.816
	F_{IS}	0.111	0.055	-0.004
GT015	*N*	47	93	218
	A	21	26	25
	Private A	1	1	2
	Allelic Richness	19.48	20.55	21.28
	Size range	122-174	118-176	120-176
	H_o	0.830	0.935	0.914
	H_e	0.926	0.947	0.946
	F_{IS}	0.104	0.013	0.009
GT101	*N*	47	93	-
	A	8	8	-
	Private A	1	-	-
	Allelic Richness	6.94	6.82	-
	Size range	103 - 113	99-111	-
	H_o	0.702	0.827	-
	H_e	0.752	0.770	-
	F_{IS}	0.067	-0.081	-

GT136	*N*	47	93	220
	A	15	10	12
	Private A	3	-	-
	Allelic Richness	14.40	9.92	10.18
	Size range	105-111	87-109	87-11
	H_o	0.936	0.774	0.820
	H_e	0.858	0.824	0.835
	F_{IS}	-0.093	0.056	-0.001
EV94	*N*	47	93	222
	A	6	8	7
	Private A	1	1	-
	Allelic Richness	6.00	6.44	6.58
	Size range	196-208	198-224	198-210
	H_o	0.745	0.763	0.753
	H_e	0.834	0.777	0.795
	F_{IS}	0.108	0.014	0.046
EV96	*N*	20	93	-
	A	10	4	-
	Private A	7	-	-
	Size range	185-213	185-191	-
	Allelic Richness	10.00	2.77	-
	H_o	0.213*	0.451	-
	H_e	0.658	0.463	-
	F_{IS}	0.334	0.011	-
EV104	*N*	47	93	212
	A	8	11	13
	Private A	-	-	2
	Allelic Richness	7.83	10.25	10.50
	Size range	148-164	156-164	136-164
	H_o	0.787	0.752	0.582*
	H_e	0.863	0.858	0.874
	F_{IS}	0.081	0.124	0.289
GATA053	*N*	47	93	223
	A	4	3	4
	Private A	-	-	-
	Allelic Richness	4.00	1.84	3.38
	Size range	201-213	205-213	201-213
	H_o	0.766	0.075	0.215
	H_e	0.664	0.103	0.231
	F_{IS}	-0.165	0.195	0.054
TAA031	*N*	47	93	220
	A	7	9	11
	Private A	-	-	2
	Allelic Richness	7.00	6.88	9.58
	Size range	217-235	217-241	214-244
	H_o	0.702	0.559	0.726
	H_e	0.774	0.624	0.74
	F_{IS}	0.095	0.101	-0.004
Total	Mean A	9.33 ± 5.12	9.75 ± 5.88	11.70 ± 5.96
	Mean A. Rich. ± SD	9.41 ± 4.58	7.96 ± 4.74	9.68 ± 4.99
	Mean He ± SD	0.751 ± 0.072	0.646 ± 0.226	0.678 ± 0.215
	Mean F_{IS}	0.073	0.054	0.046

Table 2.11. Estimated posterior probabilities of *K*.

K	*P*(*K*|*X*)	lnPr(*X*|*K*)
1	~0	-14186
2	~0	-13578
3	0.999	-13228
4	~0	-13334
5	~0	-13258
6	~0	-13384
7	~0	-13494
8	~0	-13491
9	~0	-13672

not significantly change the pattern of differentiation of the populations so they were retained for the analyses. No significant heterozygote excess was observed at any locus in any population. Each pair of loci was tested for linkage disequilibrium and genotypic independence was confirmed. Allelic richness was not significantly different among populations (χ^2=1.64, df=2). The degree of random mating (F_{IS}) was based on observed and expected heterozygosity values for each locus within the three populations. Large positive values of F_{IS} at particular loci over the populations can be an indicator of homozygosity excess at that locus and is an indicator of non random mating. The Norwegian population showed the highest degree (0.073) of F_{IS}. A summary of the statistics computed on the microsatellite genotype data is shown in table 2.10.

2.3.2.2. Population structure.

The Bayesian analysis implemented in the software Structure 2.0 was run without the loci that departed from HW equilibrium expectations, and without the two loci not typed for the British population. Figure 2.4 shows a bar plot of the results of the likelihood of each individual to belong to a sub-population. The consistency among

different runs for the estimation of *P(X|K)* and the prior α was met. This indicates that the burn-in length and the lengths of the runs were appropriate. *K*=3 had the highest likelihood for *P(X|K)* considering both the independent and the correlated allele frequency models. This suggests the presence of three distinct subpopulations: Norwegian, British and Danish. Table 2.11 show the estimated posterior probabilities of *K*. Table 2.12 shows the proportion of individuals from a pre-defined population to belong to the a priori population structure. The British North Sea and the Irish-Welsh populations showed a high proportion (0.874 and 0.903 respectively) to belong to a unique population (British), the same happened with the DKNS and the IDW (0.940 and 0.895 respectively. They showed high proportion to belong to a unique population (Danish). These results support previous findings from the morphometric analysis.

Genetic differentiation among pairwise populations using F_{ST} and Rho_{ST} values are displayed in tables 2.12 and 2.13. F_{ST} and Rho_{ST} values testing the hypothesis of five putative populations suggested by Andersen et al. (2001) are presented in table 2.12 (Bonferroni correction applied). Significant differences were found in almost all the pairwise comparisons, but for more geographically closed regions, no significant results were found; i.e. British North Sea vs. Irish-Welsh (0.001 for both F_{ST} and Rho_{ST}) and Danish North Sea-Skagerrak vs. Inner Danish Waters (0.003 and 0.000 for F_{ST} and Rho_{ST} respectively). These results suggest that there are only three populations among these samples, and are consistent with the results of the morphometric and Bayesian analyses.

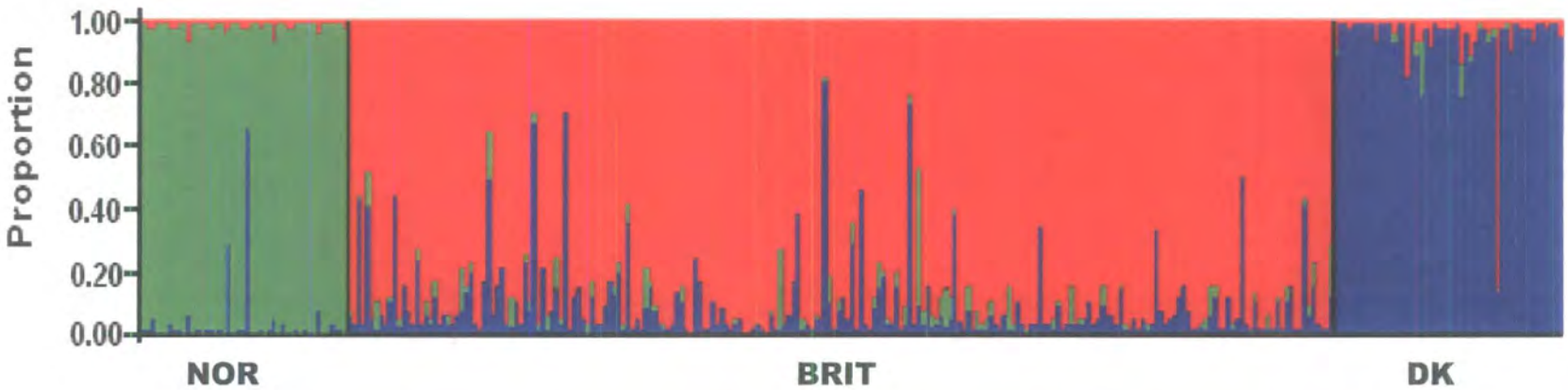

Figure 2.4. Graphic representation of the proportion of each individual to belong to a sub-population based on the coefficient of admixture. Each bar represents and individual. Green=Norwegian, red=British, blue=Danish.

Table 2.12. Proportion of individuals from the pre-defined populations allocated to the inferred clusters.

Predefined Population (Andersen et al. 2001)	Inferred Clusters		
	Norwegian	Danish	British
Norwegian	0.954	0.032	0.014
British North Sea	0.022	0.104	0.874
Irish-Welsh	0.026	0.071	0.903
Danish North Sea-Skagerrak	0.015	0.940	0.045
Inner Danish Waters	0.014	0.895	0.119

Table 2.14 shows the F_{ST} and Rho_{ST} values of the three putative populations detected in this study (Bonferroni correction applied). The highest differentiation occurred in the comparison between the British and Danish population for F_{ST} (0.180) and between the Norwegian and British population (0.237) for Rho_{ST}. A comparison between F_{ST} and Rho_{ST} to assess the role of allele size in population differentiation (after Hardy et al. 2003) indicated no significant role for allele size.

2.4. Discussion.

In this study three putative populations have been clearly defined using both morphometric and genetic approaches: Norwegian, Danish and British. All populations showed private alleles and significant differentiation among them in both the morphometric and the microsatellite analyses.

Table 2.13. Genetic differentiation among pairwise populations.

	Norwegian	British North Sea	Irish-Welsh	Danish North Sea-Skagerrak	Inner Danish Waters
Norwegian		0.126***	0.100***	0.169***	0.241***
British North Sea	0.115***		0.001NS	0.028*	0.009*
Irish-Welsh	0.107***	0.001NS		0.008*	0.007*
Danish North Sea-Skagerrak	0.113***	0.035***	0.044***		0.000 NS
Inner Danish Waters	0.110***	0.037***	0.038***	0.003NS	

RhoST values are reported above the diagonal, FST values below the diagonal (*p<0.05, ***p<0.001, NS- Not significant, Bonferroni correction applied).

Table 2.14. Genetic differentiation among pairwise populations.

	Norwegian	Danish	British
Norwegian		0.116***	0.237***
Danish	0.146***		0.009*
British	0.178***	0.180***	

RhoST values are reported above the diagonal FST values below the diagonal (*p<0.05, ***p<0.001, Bonferroni correction applied).

2.4.1. Morphometric analysis.

The traits that showed the highest correlation with the discriminant function 1 were the maximum width of the palatine (MWP, 0.332) and the length of the maxilla (ML, 0.227). Pairwise comparisons of MWP between populations showed that this trait was significantly different among populations (NOR vs. DK t=-21.48, df=53, NOR vs. BRIT t=-27.15, df=163, p<0.001, and for DK vs. BRIT t=2.21, df=62, p<0.01, Bonferroni correction applied). On average the Norwegian population show relatively smaller MWP (0.085 in juveniles, 0.095 in adults), than Danish (0.182 in juveniles, 0.192 in adults) and British porpoises (0.176 in juveniles, 0.176 in adults). The

palatine bones are situated behind the maxillae and they form the roof of the hard palate.

The second trait, ML, was also significantly different among populations (NOR vs. DK t=-12.01, df=50, NOR vs. BRIT t=-25.56, df=161, p<0.001, and for DK vs. BRIT t=2.60, df=157, p<0.01, Bonferroni correction applied). On average the Norwegian population show relatively shorter ML (0.310 in juveniles, 0.289 in adults), than Danish (0.435 in juveniles, 0.439 in adults) and British porpoises (0.444 in juveniles, 0.452 in adults). The maxillae form the roof of the mouth and they hold the upper teeth.

The third trait found in the discrimination among populations was the length of the mandible (LOR). It did not have a high correlation index (0.037) with the discriminant function 1, however it was also significantly different in pairwise comparisons between the Norwegian population and the other two (NOR vs. DK t=-3.90, df=45, NOR vs. BRIT t=-5.24, df=94, p<0.001, Bonferroni correction applied). On average the Norwegian population showed relatively shorter mandibles (0.725 in juveniles, 0.740 in adults), than Danish (0.755 in juveniles, 0.770 in adults) and British porpoises (0.757 in juveniles, 0.765 in adults).

These three traits are involved in feeding. The fact that the Norwegian population showed relatively shorter maxillae and mandibles and narrower palatines suggest that they have smaller beaks than the British and Danish porpoises. These results may suggest an adaptation to the different prey found in the environment they live on. Aarefjord et al (1995) found that porpoises from the Barents Sea and the Atlantic mid-coast of Norway prey mainly on mesopelagic and pelagic fish like capelin (*Mallotus villosus*), herring (*Clupea harengus*), saithe (*Pollachius virens*), haddock (*Melanogrammus aeglefinus*), blue whiting (*Micromesistius poutassou*) and greater

argentine (*Argentina silus*). On the other hand porpoises from the relatively more shallow North Sea and the Skagerrak seas prey mainly on benthic species like gobids, ammotydids, sprat (*Sprattus sprattus*), whiting and cod (*Gadus morhua*). Although phocoenids do not have prominent beaks, short and long beaked forms are commonly found in odontocetes (Heyning and Perrin 1994, Natoli et al. in press). Short beaked forms of delphinids are more commonly found in pelagic environments, whereas long beaked often occupy coastal areas, this is especially accentuated in river dolphin species (Natoli et al. in press). Several studies have proposed the possibility of habitat choice as an important mechanism that favours population structure in marine mammals (Hoelzel et al. 1998a, 1998b, Natoli et al. 2004, 2005, Heyninng and Perrin 1994).

2.4.2. Genetic analyses.

2.4.2.1. Norwegian population.

Several studies have found a differentiated Norwegian population of harbour porpoises in the eastern North Atlantic. Wang and Berggren (1997) found a significantly different haplotype frequency (0.007, $p<0.05$) between 13 porpoises off the coast of Norway and 27 from the Swedish Baltic, and from 25 from the Skagerrak and Kattegat seas (0.021, $p<0.05$). Tolley et al. (1999) sequenced the D-loop region of mtDNA and they found that female porpoises from Norway were genetically different from those of the British North Sea based on F_{ST} (0.06, $p<0.05$). Andersen et al. (2001) found F_{ST} values that ranged from 0.007-0.014 ($p<0.05$) when compared with the DKNS, IDW, BNS and IRL-W. In this study the F_{ST} values found (0.107-0.115, all $p<0.001$) are much higher than those reported previously by Andersen et al. (2001) for equivalent pairwise comparisons with the other four sub-populations (table 2.13). Rho_{ST} values ranged from 0.1-0.241, and were also significant (all $p<0.001$). These findings along with those from the Bayesian analyses and from the

morphological comparison discussed above support the theory of a separate Norwegian population.

The results found in this study also suggest the lack of further sub-structuring of the Norwegian population as previously suggested by Bjørge and Øien (1995), and it is in accordance with the studies made by Andersen et al. (2001), Tolley et al. (1999), Tolley et al. (2001), and Wang and Berggren (1997). Finally, the level of differentiation found for the morphological analysis and the pairwise comparisons of the genetic analyses between the Norwegian population and the other sub-populations suggests that Norwegian porpoises are the most differentiated.

2.4.2.2. Danish population.

The results found in this study to test the hypothesis of sub-structure in the Danish population are consistent. MANOVA and the percentages of correct classification of DA for the morphometric, and the Bayesian analyses, and the indexes of population differences (F_{ST} and Rho_{ST}) found only one population of harbour porpoises around Danish waters.

Andersen et al. (2001) used the same twelve microsatellite loci that were used in this study, and found data to support the subdivision of the Danish population into two, one that comprises the Danish North Sea and the Skagerrak Sea (DKNS), and a second for the Inner Danish Waters (IDW). They used assignment tests (Paetkau et al. 1995, 1997) to genetically assign the porpoises to the most likely group. The majority of the porpoises were assigned to their original sampling group. However, of the 151 DKNS porpoises only 45 (29%) were assigned to this group. For the remaining misclassified porpoises, 34 (22%) were assigned to the IDW group. In contrast, of the 169 individuals of the IDW group, 92 (54%) were correctly assigned and 17 (10%) were assigned to the DKNS. The majority of miss-assignments

occurred between contiguous populations. They explained that this occurred mainly because of the effect of isolation by distance (Andersen et al. 2001).

Andersen et al. (2001) also reported a significant ($p<0.05$) F_{ST} value (0.005) in the pairwise comparison between the DKNS and the IDW. In this study the F_{ST} value (0.003) found is similar but not significant. The low values found for F_{ST} and Rho_{ST} in this study suggest high rates of gene flow between these two populations. However, one possible reason that could explain the difference in significance between the two studies may be the effect of sample size, since Andersen et al. (2001) compared 151 porpoises of the DKNS with 169 porpoises of the IDW. In this study 53 porpoises were from the DKNS and 40 from the IDW. Another possible explanation is that the majority of the samples used in this study that came from the DKNS population are from the Skagerrak Sea (only one was from the Danish North Sea proper), and the remaining 39 were taken from porpoises that inhabit the IDW.

Nevertheless, the results presented in this study are consistent with a previous study reported by Wang and Berggren (1997) using haplotype frequencies of RFLP on mtDNA. They did not find structuring in the porpoises from around the Danish waters, and when they compared porpoises form the Skagerrak Sea vs. the Kattegat Sea, they did not find a clear separation between the two. However it is likely that this study offered a low resolution since the comparison was made only on one locus.

2.4.2.3. British population.

A similar situation to that mentioned above regarding the Danish population occurred in the case of the British population. The results from the morphometric studies along with the microsatellite analysis from this study did not detect sub-structuring in the British population. The theories so far proposed (e.g. Walton 1997 and Andersen et al. 2001) divide the British population of harbour porpoises into two separate sub-

populations: one that inhabits the North Sea (BNS) and a second located in the west coast of Britain and east coast of Ireland, or Irish-Welsh (IR-LW).

Walton (1997) found significant differences between porpoises from the North Sea (n=158) and from the Irish-Welsh sub-population (n=138) in the control region of mtDNA. Significant differences in haplotype frequencies (χ^2=56.4, p<0.001) were reported for the whole North Sea group. However, for Φ_{ST} the significant differences were only found for the southern North Sea porpoises (which also included porpoises from the Netherlands) for the sex pooled group (0.058, p< 0.01) and for females (0.079, p< 0.01). For males no significant structuring was found. Studies based on mtDNA reflect only the maternal inheritance of the mitochondria, therefore the observed sub-structuring might be caused by the philopatric behaviour of females that could reflect different family units and not necessarily imply the presence of two separate populations (Andersen 2003).

Andersen et al. (2001) also found significant differences between the British sub-populations. They used the same twelve microsatellite loci that were used in this study. Assignment tests (Paetkau et al. 1995, 1997) showed that the majority of the porpoises were assigned to their original sampling group. However, of the 105 porpoises from the IRL-W population only 28 (27%) were assigned to this group. For the remaining misclassified porpoises, 19 (18%) were assigned to the BNS group, and the same number of porpoises (19) were assigned to the DKNS. For the 131 BNS porpoises 51 (39%) were assigned to this group, and 19 (14%) were assigned to the IRL-W. As mentioned above, they explained that the occurrence of miss-assignments was because of an isolation by distance effect (Andersen et al. 2001).

The value reported for F_{ST} (0.004) in Andersen et al. (2001) for the parwise comparison between BNS vs. IRL-W was significant at $p<0.05$. In this study the F_{ST} value reported (0.001) was similar but not significant. This could be explained by the origin of the samples. Porpoises that formed the IRL-W sample in the Andersen et al. (2001) study came from Cornwall, Wales, Ireland and the Irish Sea. In the present study the IRL-W sample was formed of 107 animals from Cornwall, north Devon, Wales, north-west England and western Scotland. However, the low values of F_{ST} and Rho_{ST} found in this study suggest high gene flow between these populations.

In summary this study has found population structure in the eastern North Atlantic population of the harbour porpoise by using a morphological and a genetic approach. The results from both analyses were consistent in detecting the presence of three defined sub-populations: Norwegian, Danish and British. Therefore management considerations and conservation issues should be addressed taking into account the different sub-populations found in the eastern North Atlantic. Evidence of further sub-structuring in any of the three populations has not been found.

Chapter 3: The relationship between developmental stability, genomic diversity and environmental stress in the eastern North Atlantic population of harbour porpoise (Phocoena phocoena).

3.1. Introduction.

Reduced developmental stability in an organism has been linked to disturbances during the development *in utero* mainly due to environmental and genetic stress (e.g. Leary and Allendorf 1989). However, the exact mechanisms are still unknown. Some theories state that the organism under pressure has to allocate energy to control for developmental processes, thus increasing its vulnerability to suffer from developmental inaccuracies (Møller and Swaddle 1997). Organisms that can withstand stress could be at a selective advantage (Clarke 1995, Zakharov et al 1991). Clarke (1998b) suggested that the development of each side of a bilateral character is controlled by an identical set of genes, thus any observed difference in symmetry must be environmental in origin.

Developmental stability, when assessed as fluctuating asymmetry (FA), can be determined indirectly by subtle alterations in the symmetry of bilateral structures in an organism (Palmer & Strobeck 1986). FA is the only asymmetry present in nature that is useful as an indicator of developmental stability, the other two, directional asymmetry (DA) and antisymmetry, have a functional component (Palmer 1994).

Directional asymmetry (DA) is present in the skulls of odontocetes (see chapter 1). The skull of the harbour porpoise (*Phocoena phocoena*) has evolved this kind of adaptation to survive in an aquatic environment (Yurick and Gaskin 1988). Toothed cetaceans possess an acoustic and sound production system that is allocated in the facial region (Mead 1975). The allocation of these organs produces a DA in the skull

as the result of the postrostral fraction of the right premaxillary that grows to a larger size than the left equivalent (Yurick and Gaskin 1988, Milinkovitch 1995).

The relationship between FA, as an indicator of developmental stability, and genomic diversity have already been established in several species including marine mammals (e. g. Hoelzel et al. 2002a), but it has never been attempted in cetaceans. However, inconsistencies among studies have been found. Hartl et al. (1995) studied the relationship between heterozygosity of 13 enzyme loci and FA at both population and individual level in the brown hare (*Lepus europaeus*). Although their data included the measurement of 27 meristic and 9 metric traits on 417 hares from 17 sampling locations in Austria, they did not find a clear relationship between FA and heterozygosity. Conversely, Leary et al. (1983) found a relationship between heterozygosity of 13 enzyme loci and FA of five bilateral characters in 50 individuals of rainbow trout (*Oncorhynchus mykiss*). More recently Hoelzel et al. (2002a) found a relationship between genetic diversity on a recently bottlenecked population of the northern elephant seal (*Mirounga leonina*) and FA of a mandibular trait.

Environmental stress has also been linked with developmental stability, although again inconsistencies have been found. Sonne et al. (2005) compared the level of FA between two populations: a pre-pollution (organohalogen) group of polar bears (*Ursus maritimus*) skulls from the period 1892-1960 and a post-pollution group of skulls from the 1961-2002 period. They did not find any significant difference in the level of FA between the two groups. However, in a similar study Zakharov and Yablokov (1990) compared skulls from two populations: a pre-pollution group (before 1940) vs. skull from a post-pollution group (after 1960), and they found significant differences in the level of FA between the two groups. In a study on the Eurasian otter (*Lutra lutra*) a comparison between FA on skulls of two populations of otters collected over the past century from European countries and FA on skulls

from a presumed endangered population did not find a clear increase in FA in the otter population that was considered as endangered (Pertolid et al. 2000b). Lens et al. (2000a) found a relationship between FA and inbreeding on three populations of Taita thrushes (*Turdus helleri*) that inhabit more degraded and fragmented forests in Kenya, while in less impacted environments the relationship between FA and inbreeding was not significant.

The harbour porpoise is an odontocete that inhabits the temperate and sub-arctic coastal waters of the eastern North Atlantic. Harbour porpoises are considered to be common in the eastern North Atlantic. Recent estimates in the North Sea and adjacent waters have reported an estimated number of around 340,000 individuals (Hammond et al. 2002). In the eastern North Atlantic the population of harbour porpoise is facing a number of threats across its distribution. Chemical pollution, noise, depletion of prey stocks, and population fragmentation are just some of the main pressure factors that impact the welfare of this species (Bjørge and Tolley 2002).

In the past the species was exploited for meat and blubber (Yurick and Gaskin 1987). Kinze (1995) stated that significant numbers were killed in the Baltic Sea. Although the species is no longer a target of fisheries, a significant number of porpoises are by-caught on fishing nets. Although the species is under legal protection in almost every country, there are no legal measures to prevent incidental death by entanglement in fishing nets (Bjørge and Tolley 2002).

The issue of incidental death in fishing nets has created global concern in recent years as by-catch rates are putting the species into serious peril as the threshold level of 1% defined as sustainable by the International Whaling Commission (IWC) and the Agreement of Small Cetacean in the Baltic and North Seas (ASCOBANS)

may be exceeded in some areas (Bjørge and Tolley 2002, Perrin et al. 1994, Stenson 2003). While numerous declarations of intent have been voiced by the European Union member states to reduce by-catch and direct killing of cetaceans, practice shows that little has changed in spite of the marine mammals enjoying protection under multinational agreements such as ASCOBANS; each year, almost 8,000 harbour porpoises die in the nets of Danish and British fishermen operating in the North Sea (CEC 2002, Vinther 1999). The International Whaling Commission included this species in 1981 in a short list of small odontocete species to be the subject of conservation and management assessments in population size (Yurick and Gaskin 1987).

Modifications in fishery practices seem urgent. In some areas, knowledge of porpoise movements and habitats has helped in setting fishing regulations (Bjørge and Tolley 2002), and led to the development of deployment devices designed to help protecting this species (see Carlström et al. 2002).

The population of harbour porpoises in the eastern North Atlantic is also experiencing serious environmental pressures from the level of pollutants in some areas. Previous studies (Bennett et al. 2001, Jepson et al. 1999, Jepson et al. 2005) have suggested a relationship between the incidence of infectious disease mortality and high levels of heavy metals and polychlorinated biphenyls (PCBs). If environmental stress is important in the disruption of developmental stability in this species, these may be some of the relevant factors. Furthermore, if there is regional variability in levels of stress, then this may be reflected in regional variation in levels of FA.

Environmental stress could lead to population fragmentation and increase the chance of the species losing genetic diversity (Lens et al. 2000a). In this study a

comparison among regions of the eastern north Atlantic for the most common causes of environmental stress, and levels of genomic diversity were determined to evaluate which factors may have the greatest impact on the developmental stability of different populations of harbour porpoise of the eastern north Atlantic.

3.2. Material and Methods.

3.2.1 Determination of developmental stability.

3.2.1.1 Morphometric measurements and determination of asymmetry.

3.2.1.1.1 Collection of skulls.

The fact that the estimation of developmental stability can be achieved by the measurement of morphological characters provides an opportunity to measure skulls allocated in museum collections (Zakharov & Yablokov 1997). Museums that contain a significant amount of skulls of harbour porpoises in their collections were chosen for sampling the morphometric traits used in the determination of fluctuating asymmetry. The museums sampled, number of skulls measured and traits measured have already been provided in Chapter 2.

3.2.1.1.2. Indices of fluctuating asymmetry used and statistical analyses.

Asymmetry was recorded by calculating the absolute difference in length between right and left sides (|R-L|, index 1 of Palmer and Strobeck 1986). Each pair of measurements was repeated three times. So three estimates of asymmetry were obtained. The median of the three sets of measurements was used, as it is more useful than the arithmetic mean in asymmetric distributions (Zar 1984).

A composite index of asymmetry for each individual was calculated using the information from all the traits that showed FA. For each individual it was calculated as the average of the absolute FA values of each trait (hereafter Avg. FA). For each population, it was calculated as the average of the sum of the absolute FA values of

each trait, hereafter CFA; (Index 11 of Palmer and Strobeck 2003, Index CFA 1 of Leung et al. 2000). Correlations of FA across traits within individuals were done using the Spearman coefficient to test for independence across traits and that CFA may include added together non-independent information. A non-parametric approach was chosen since FA is not distributed normally.

Deviations from symmetry (i.e. FA) are so small, that they can be similar in magnitude to measurement error (ME, Palmer and Strobeck 2003); therefore ME could seriously inflate descriptions of FA. Three different methods were used to control for this. First, three repeated measurements for each side and for each trait of every skull was done "blindly"; that is, without previous knowledge of previous measurements, this is based in the assumption that the more reliable the estimate of ME, the more reliable will be the estimate of FA (Palmer 1994). The method involved measuring one side for the whole set of traits, then the other side was measured, this was repeated three times, and the callipers were reset to zero after each measurement. Second, for detecting the variance of FA due to ME, Palmer and Strobeck (1986) recommended a two-way (with sides as fixed and individuals as random) mixed model analysis of variance with repeated measurements of each side. Finally, the third method used was described by Palmer and Strobeck (2003). They suggested the use of the standard deviation of a set of measurements as an ideal descriptor of measurement error.

Directional asymmetry (DA) can also inflate the true underlying estimation of FA. Because the skull of odontocetes is asymmetrical, DA was treated with caution. DA was tested in three ways. The first one was by using the same two-way ANOVA described above; this tests for a significant difference between the mean of the right and left sides to the mean sides' variation. The second test used is a one-sample t-test from the departure of the mean (R-L) from an expected mean of zero. Finally a

simple visual observation was made of the frequency of observations of the subtraction of right side minus left side (R-L) in a scatter plot.

Antisymmetry (AS) is another factor that inflates the value of FA, so it also must be eliminated from the determination of FA. Using the same visual observation of frequency of R-L in a normal plot described for DA tested also for AS. AS distributes as platykurtic or bimodal curves with an expected mean of zero. And because antisymmetry is not distributed normally the Kolomogorov-Smirnov test was also used for the determination of AS.

The traits were also tested for size dependency. Variation of the magnitude of asymmetry among populations can exist due to the difference in size of a trait (Palmer 1994). To test for trait size dependency the Spearman coefficient of rank correlation between the absolute value of FA (|R-L|) vs. the average of the sum of both sides ((R+L)/2) was used because it does not assume homogeneity of variance and is not influenced by extreme observations (Palmer and Strobeck 2003).

Where traits showed FA>ME (at least two fold, Palmer and Strobeck 2003), and they did not show directional asymmetry or deviation from normality against a mean of zero, they were chosen for the assessment of fluctuating asymmetry.

Differences between the levels of FA among populations were tested using a Levene's test of homogeneity of variance (Palmer and Strobeck 2003). Finally an ANOVA was used to test for differences of the degree of FA among sexes and age classes.

3.2.2. Genetic analyses and determination of genetic diversity.

3.2.2.1. Samples obtained and previously published data used.

Details of samples obtained and the techniques used to extract and isolate DNA, the microsatellites used, the amplification and interpretation of the microsatellite data has already been provided in Chapter 2.

3.2.2.2. Genomic diversity indices used.

Measures of genomic diversity used in this study included mean d^2, scaled mean d^2, multilocus individual heterozygosity, standardised multilocus individual heterozygosity, and internal relatedness. Mean d^2 (Coulson et al. 1998) is an internal distance measure estimated from the two alleles at a locus. Mean d^2 was calculated as the squared difference in repeat units between the two alleles an individual had at a microsatellite locus, averaged over all loci for an individual (equation 1):

$$\text{Mean } d^2 = \sum_{i=1}^{n} \frac{(i_a - i_b)^2}{n} \qquad (1).$$

Scaled mean d^2 (Coulson et al. 1999) is mean d^2 calculated as above, but scaled by the variance at each locus for each specific population before scoring the average of d^2, (equation 2):

$$\text{Mean } d^2_{scaled} = \sum_{i=1}^{n} \left[\frac{\left(\frac{(i_a - i_b)^2}{\sigma_i^2} \right)}{n} \right] \qquad (2).$$

The scaling of mean d^2 over the variance controls for an effect in which highly polymorphic loci contribute more in the overall score of mean d^2, therefore it allows all loci to contribute equally to the mean d^2 score (Coulson et. al. 1999).

Multilocus individual heterozygosity (H) was calculated as the proportion of homozygous loci typed for an individual. Standardised multilocus individual heterozygosity (H_s) was used because not all individuals were typed for the same panel of microsatellites, therefore avoiding potential biases in the determination of multilocus individual heterozygosity (from here on it will be referred as "heterozygosity"), thus ensuring that the heterozygosity of all individuals was measured on an identical scale (Coltman et al. 1999). It was calculated as individual heterozygosity over mean heterozygosity at loci typed for each specific population (equation 3):

$$H_S = \frac{H}{\bar{x}H_{l_i}} \tag{3}$$

where l is loci typed at individual i.

Finally internal relatedness (IR) takes into account the allelic frequencies in the final score (equation 4), therefore, allowing homozygosity for rare alleles to be weighed more than homozygosity of common alleles (Amos et al. 2001):

$$\text{IR} = \left[\frac{\left(2H - \sum f_i\right)}{\left(2N - \sum f_i\right)}\right] \tag{4}$$

where H is the number of loci that are homozygous, N is the number of loci and fi is the frequency of the ith allele contained in the genotype.

Differences in genomic diversity among the different subpopulations were tested using an ANOVA. Correlation across indices was done in order to determine independence among each other by using the Pearson coefficient.

3.2.2.3. Evidence for historical bottleneck in the different populations sampled.

To investigate if the study populations had experienced a recent reduction in population size, two methods that are implemented in the software Bottleneck 1.2.02 (Piry et al. 1999) were used. The first takes into account the analysis of the allelic frequencies for a so-called "mode shift" that discriminates recently-bottlenecked from stable populations (Luikart et al. 1998). The second approach was done by analysing if a significant number of loci exhibit a heterozygosity excess compared with equilibrium expectations, given that allelic diversity reduces faster than heterozygosity in bottlenecked populations (Cornuet and Luikart 1996).

A second method for the detection of a historical bottleneck was used. The mean ratio of the number of alleles to the range in allele size, *M* (Garza and Williamson 2001), was also used. The magnitude of the decrease in *M* is positively related with the severity and duration of the bottleneck. It follows the ratio:

$$M = \frac{k}{r}$$

where k is the number of alleles and $r = S_{max} - S_{min} + 1$, where S_{max} is the size of the largest allele, and S_{min} is the size of the smallest allele in the sample (Garza and Williamson 2001). It is based in the principle that the loss of any allele will contribute to a reduction in k, but only a loss of the largest or smallest allele will contribute to a reduction in r, therefore k is expected to be reduced more quickly than r, thus M is expected to be smaller in recently reduced populations than in equilibrium populations (Garza and Williamson 2001).

3.2.3 Environmental stress.

Published results for the more common causes of environmental stress that porpoises in the eastern North Atlantic are facing were reviewed. This included

chemical pollutants (PCB's, DDT's, radionuclides, and heavy metals), parasite loads, mean ocean surface temperature, and by-catch rate for several regions of the eastern North Atlantic.

For the British population information on the concentration of chemical pollutants was provided for 41 harbour porpoises for which the skull was measured and for which the genotypes were available. The concentration of 12 heavy metals (Cr, Mn, Fe, Ni, Cu, Zn, As, Se, Ag, Cd, Hg, and Pb), DDT's (DDE, TDE, Dieldrin and DDT), 25 polychlorinated biphenyls (PCB) congeners, and 10 polybrominated diphenyl ethers (BDE) congeners were provided by Robin J. Law (CEFAS, UK), some of the results have already been published in Jepson et al. (1999), Jepson et al. (2005), and Law et al. (2002).

For the Norwegian population the information of the concentration of a radionuclide, ^{137}Cs, was provided for 25 harbour porpoises for which the skull was measured and for which the genotypes were available by Krystal A. Tolley and the results were published in Tolley and Heldal (2002).

3.2.4. Establishing study populations of harbour porpoise in the eastern North Atlantic for the analysis of data.

Two approaches were taken in the establishment of the sub-populations of harbour porpoise in this study. The first was based in the results from Chapter 2, where three putative populations were detected: Norwegian, British and Danish. The second was based in the presence of five sub-populations according to Andersen et al. (2001). This included a subdivision of the British sample into two sub-populations: British North Sea and Irish-Welsh. The Danish population was also subdivided in two: Inner Danish Waters and Danish North Sea-Skagerrak. The Norwegian population remained the same as in the first approach. The second approach was considered

appropriate since several studies including the IWC have suggested the presence of these five putative populations therefore the levels of FA and genetic diversity for each are worth knowing. The 5 sub-populations from the second approach from here on will be referred as "management units".

3.3. Results.

3.3.1. Determination of developmental stability.

3.3.1.1. Detecting traits that depart from ideal FA.

The two-way ANOVA (sides by individuals, Palmer 1994) for all traits showed that the between sides variation was highly significantly greater than expected due to measurement error, in all cases $p<0.001$ (Bonferroni correction applied table 3.1). In all the traits the mean of (R-L) is more than five times the standard deviation of the repeated measurements. Measurement error did not appear to differ among sub-populations or among traits, since no statistical significance was found when tested with the two-way ANOVA.

When tested for departures from normality, no trait showed the effects of antisymmetry as not a single scatter plot of traits revealed a bimodal curve; neither was platykurtosis present in the traits. However the following traits: WON, AOT, FTL, OL, PCW, UDRL, LDRL, and LMF showed statistically significant departures from normality when tested with the Kolomogorov-Smirnov test (table 3.1).

From the results of the two way ANOVA, and from the two-tailed one sample t-test against a mean of zero performed to test for directional asymmetry, DA was found in WON, AOT, FTL, OL, PCW, LDRL, and UDRL (table 3.1). Finally, no trait showed size dependence for FA when tested using the Spearman bivariate rank correlation (table 3.1) this means that the different between sides (R-L) did not depend on trait

Table 3.1 Statistical analyses for each trait in relation to ME, departures from normality, DA and size dependence. ** $p < 0.01$, *** $p < 0.001$ Bonferroni correction applied.

Effect tested	Statistic used	CBL *n*=206	WON *n*=384	AOT *n*=260	FTL *n*=224	OL *n*=354	COD *n*=343	ML *n*=244	MW *n*=377
Measurement Error	two- way ANOVA	***	***	***	***	***	***	***	***
Departures from normality	Kolomogorov-Smirnov	0.04	0.07	0.06	0.07	0.08	0.03	0.04	0.04
	Sig.	n.s.	***	*	*	***	n.s.	n.s.	n.s.
Directional asymmetry	Mean	-0.01	-0.03	-0.13	-0.15	-0.03	-0.02	0.01	0.02
	t-test *t*	0.91	-6.24	-4.58	-9.91	2.13	-0.71	1.01	0.62
	Sig.	n.s.	***	***	***	*	n.s.	n.s	n.s.
Size dependence of FA	Spearman ρ	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Effect tested	**Statistic used**	**MWP** *n*=371	**LOP** *n*=232	**PCW** *n*=435	**LDRL** *n*=212	**UDRL** *n*=147	**LOR** *n*=260	**HOR** *n*=269	**LMF** *n*=319
Measurement Error	two- way ANOVA	***	***	***	***	***	***	***	***
Departures from normality	Kolomogorov-Smirnov	0.03	0.04	0.05	0.20	0.08	0.04	0.04	0.74
	Sig.	n.s.	n.s.	*	***	*	n.s.	n.s.	***
Directional asymmetry	Mean	0.02	0.03	0.07	0.02	0.10	0.04	-0.02	0.04
	t-test *t*	0.78	1.60	0.52	0.27	-1.26	0.29	-1.93	1.81
	Sig.	n.s.	n.s.	***	***	***	n.s.	n.s.	n.s.
Size dependence of FA	Spearman ρ	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

size, (R+L)/2. Therefore the results demonstrated that the traits that showed FA were CBL, COD, ML, MW, MWP, LOP, LOR, and HOR. No further statistics were attempted on the remaining traits.

Correlation across FA traits using the Spearman coefficient showed that only the correlation between ML and MW was significant, although weak, at the $p<0.001$ level after Bonferroni correction was applied ($r^2=0.08$, n=246).

3.3.1.2. Differences in FA between sexes and age groups in each of the three subpopulations and the five management units.

The results of *F*-tests in respect to variance between sex and age groups found no statistical significance in FA of the traits measured within the three sub-populations and five management units studied. Therefore all the data was used in further analysis without considering age class or sex.

3.3.1.3. Differences in FA among the three sub-populations and the five management units of harbour porpoise in the eastern north Atlantic.

Basic statistics for each of the three sub-populations of harbour porpoise are shown in table 3.2. For the five management units they are presented in table 3.3. The results of the Levene's test for testing differences in the traits in the three sub-populations showed that FA at two traits, ML and LOR were significant at the $p<0.01$ and the $p<0.001$ level respectively. FA at CBL was significant at the $p<0.05$ level (table 3.4). For the five management units FA at CBL and LOR were significant at the $p<0.001$. FA at ML was significant at the $p<0.05$ level (table 3.4). The mean FA of both CBL and LOR is higher in the Norwegian sample, if the mean FA of the traits is used; the Norwegian sample contained the most asymmetrical animals in five out of eight traits (CBL, MWP, LOP, LOR and HOR).

For the three sub-population subdivision, the British population contained the most asymmetrical animals for the three remaining traits, especially for ML. The sub-population that contained the least asymmetrical animals was the Danish, with six out of the eight traits (table 3.3). When divided into the five management units, the Norwegian management unit contained the most asymmetrical animals for CBL, MWP, LOP and LOR and for the composite index of individual asymmetry. The Irish-Welsh had the most asymmetrical animals for COD, MW and ML.

3.3.2. Genomic diversity.

Table 3.4 summarises the results of the different indices of genomic diversity used in the three sub-populations studied, and table 3.5 summarises them for the five management units. The Norwegian population and management unit showed the lowest level of genomic diversity when measured with mean d^2 and internal relatedness, but showed the highest level of heterozygosity.

Although the three sub-populations were all very similar in the level of heterozygosity they showed (range from 0.63 – 0.73), when standardised heterozygosity is considered in either division (tables 3.5 and 3.6) the range is even smaller (0.049 and 0.028 respectively).

Differences in genomic diversity among the three sub-populations showed that mean d^2, scaled mean d^2, and heterozygosity are statistically different among the putative populations (table 3.7). However, after Bonferroni correction the difference in heterozygosity among the three sub-populations is no longer significant. However, if the Danish and British sub-populations are pooled in one group, a significant difference ($p<0.05$) between the Norwegian and Danish-British group is found (Mann-Whitney $Z = -2.0$). When the porpoises were divided into the five

Table 3.2. Basic Statistics of FA (in cm) in three sub-populations of the eastern North Atlantic.

	SUB-POPULATIONS								
	NORWEGIAN			DANISH			BRITISH		
Trait	Mean ± S.D.	Min - max	n	Mean ± S.D.	Min - max	n	Mean ± S.D.	Min - max	n
CBL	0.11 ± 0.12	0.02 - 0.50	29	0.05 ± 0.04	0.01 - 0.18	30	0.07 ± 0.10	0.00 - 0.69	147
COD	0.06 ± 0.06	0.00 - 0.26	44	0.07 ± 0.05	0.00 - 0.21	82	0.08 ± 0.07	0.00 - 0.42	217
ML	0.07 ± 0.14	0.00 - 0.75	30	0.07 ± 0.07	0.00 - 0.25	33	0.13 ± 0.13	0.00 - 0.65	181
MW	0.10 ± 0.10	0.01 - 0.49	44	0.11 ± 0.09	0.01 - 0.47	71	0.13 ± 0.11	0.00 - 0.64	262
MWP	0.14 ± 0.12	0.00 - 0.57	45	0.12 ± 0.09	0.00 - 0.40	78	0.12 ± 0.11	0.00 - 0.60	248
LOP	0.08 ± 0.06	0.01 - 0.54	27	0.07 ± 0.07	0.01 - 0.33	46	0.08 ± 0.06	0.00 - 0.30	159
LOR	0.19 ± 0.15	0.01 - 0.54	32	0.10 ± 0.08	0.01 - 0.35	70	0.12 ± 0.10	0.00 - 0.59	158
HOR	0.08 ± 0.08	0.01 - 0.31	33	0.07 ± 0.05	0.00 - 0.24	57	0.08 ± 0.07	0.00 - 0.45	79
CFA	0.58 ± 0.33	0.04 – 1.68	47	0.45 ± 0.24	0.08 – 1.27	91	0.53 ± 0.31	0.00 – 1.59	302

Table 3.3.- Basic Statistics of FA (in cm) in the five management units of the eastern North Atlantic.

	MANAGEMENT UNITS								
	NORWEGIAN			DANISH NORTH SEA - SKAGERRAK			INNER DANISH WATERS		
Trait	Mean ± S.D.	Min - max	n	Mean ± S.D.	Min - max	n	Mean ± S.D.	Min - max	n
CBL	0.11 ± 0.12	0.02 - 0.50	29	0.05 ± 0.05	0.01 - 0.18	21	0.05 ± 0.03	0.01 - 0.10	9
COD	0.06 ± 0.06	0.00 - 0.26	44	0.07 ± 0.05	0.00 - 0.21	48	0.07 ± 0.05	0.00 - 0.20	34
ML	0.07 ± 0.14	0.00 - 0.75	30	0.06 ± 0.06	0.00 - 0.22	22	0.08 ± 0.09	0.01 - 0.25	11
MW	0.10 ± 0.10	0.01 - 0.49	44	0.11 ± 0.09	0.01 - 0.47	39	0.11 ± 0.10	0.00 - 0.33	32
MWP	0.14 ± 0.12	0.00 - 0.57	45	0.12 ± 0.09	0.00 - 0.40	46	0.10 ± 0.10	0.01 - 0.26	32
LOP	0.08 ± 0.06	0.01 - 0.54	27	0.06 ± 0.07	0.01 - 0.31	27	0.07 ± 0.07	0.01 - 0.20	19
LOR	0.19 ± 0.15	0.01 - 0.54	32	0.12 ± 0.08	0.01 - 0.31	39	0.09 ± 0.08	0.01 - 0.35	31
HOR	0.08 ± 0.08	0.01 - 0.31	33	0.07 ± 0.06	0.00 - 0.24	31	0.06 ± 0.05	0.00 - 0.20	26
CFA	0.58 ± 0.33	0.04 – 1.68	47	0.46 ± 0.23	0.15 - 1.03	52	0.42 ± 0.24	0.08 - 1.26	39

	BRITISH NORTH SEA			IRISH - WELSH		
Trait	Mean ± S.D.	Min - max	n	Mean ± S.D.	Min - max	n
CBL	0.05 ± 0.05	0.00 - 0.25	74	0.09 ± 0.13	0.00 - 0.69	73
COD	0.07 ± 0.07	0.00 - 0.42	107	0.08 ± 0.06	0.00 - 0.35	110
ML	0.12 ± 0.12	0.00 - 0.58	92	0.14 ± 0.14	0.00 - 0.65	89
MW	0.11 ± 0.11	0.00 - 0.63	133	0.13 ± 0.12	0.00 - 0.64	129
MWP	0.12 ± 0.12	0.00 - 0.61	126	0.12 ± 0.11	0.00 - 0.59	122
LOP	0.08 ± 0.06	0.00 - 0.29	73	0.08 ± 0.07	0.00 - 0.30	86
LOR	0.13 ± 0.10	0.00 - 0.48	70	0.12 ± 0.09	0.00 - 0.59	88
HOR	0.07 ± 0.07	0.00 - 0.30	77	0.09 ± 0.07	0.00 - 0.45	102
CFA	0.51 ± 0.31	0.02 – 1.59	149	0.50 ± 0.07	0.05 – 1.45	153

Table 3.4.- Levene's test for homogeneity of variances to test for differences in FA among the three sub-populations and five management units.

	Sub-populations		Management Units	
Trait	**Levene Statistic**	**Significance**	**Levene Statistic**	**Significance**
CBL	3.15	*	5.79	***
COD	0.43	n. s.	1.10	n. s.
ML	4.49	**	2.56	*
MW	0.43	n. s.	0.55	n. s.
MWP	0.97	n. s.	0.62	n. s.
LOP	0.65	n. s.	0.33	n. s.
LOR	12.03	***	9.88	***
HOR	0.92	n. s.	2.35	n. s.
CFA	4.28	*	2.23	n. s.

Significance: *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$, Bonferroni correction applied.

management units all genomic diversity indices but internal relatedness were significantly different (table 3.8).

Table 3.9 shows the Pearson coefficient for the linear correlations of the five indices of genomic diversity. High correlations between the indices indicate that these are not independent from each other, especially heterozygosity and internal relatedness, where the correlation coefficients were high. Internal relatedness takes into account the heterozygosity of an individual, whereas mean d^2 depends on long-term mutational differences (Amos et al. 2001).

Table 3.5. Basic statistics of several genomic diversity indices in the three sub-populations of the eastern North Atlantic.

	n	Mean d^2	Scaled mean d^2	Heterozygosity	Standardised Heterozygosity	Internal Relatedness
Norwegian	47	40.8 ± 20.0	0.022 ± 0.017	0.727 ± 0.12	1.002 ± 0.16	0.110 ± 0.17
Danish	92	57.2 ± 35.3	0.024 ± 0.024	0.646 ± 0.13	0.994 ± 0.20	0.033 ± 0.18
British	218	63.4 ± 41.2	0.016 ± 0.014	0.679 ± 0.14	1.000 ± 0.20	0.050 ± 0.17

Table 3.6. Basic statistics of several genomic diversity indices in the five management units of the eastern North Atlantic.

	n	Mean d^2	Scaled mean d^2	Heterozygosity	Standardised Heterozygosity	Internal Relatedness
Norwegian	47	40.8 ± 20.0	0.022 ± 0.016	0.727 ± 0.12	1.003 ± 0.16	0.110 ± 0.17
Danish North Sea	53	59.2 ± 30.5	0.020 ± 0.017	0.660 ± 0.12	1.000 ± 0.18	0.026 ± 0.17
Inner Danish Waters	39	55.1 ± 41.4	0.031 ± 0.031	0.688 ± 0.12	0.990 ± 0.20	0.038 ± 0.17
British North Sea	112	65.1 ± 42.0	0.016 ± 0.016	0.677 ± 0.15	0.987 ± 0.21	0.053 ± 0.18
Irish-Welsh	106	61.7 ± 40.3	0.015 ± 0.012	0.681 ± 0.14	1.015 ± 0.19	0.047 ± 0.15

Table 3.7. ANOVA to test for differences of several genomic diversity indices among the three sub-populations.

	Mean d^2	Scaled Mean d^2	Heterozygosity	Standardised Heterozygosity	Internal Relatedness
$F_{(2,356)}$	7.07	9.127	5.80	1.99	2.48
Significance	***	***	**	n.s.	n.s.

Significance: ** $p < 0.01$, *** $p < 0.001$, Bonferroni correction applied.

Table 3.8. ANOVA to test for differences of several genomic diversity indices among the five management units.

	Mean d^2	Scaled Mean d^2	Heterozygosity	Standardised Heterozygosity	Internal Relatedness
$F_{(4,356)}$	3.69	6.80	3.24	3.05	1.76
Significance	**	***	**	**	n.s.

Significance: ** $p < 0.01$, *** $p < 0.001$, Bonferroni correction applied.

Table 3.9. Correlation between indices of genomic diversity (Pearson coefficient).

	Mean d^2	Scaled Mean d^2	Heterozy-gosity	Standardised Heterozygosity
Scaled Mean d^2	0.10n.s.			
Heterozygosity	0.15**	0.28***		
Standardised Heterozygosity	0.17**	0.26***	0.98***	
Internal Relatedness	-0.19***	-0.22***	-0.91***	-0.93***

Significance: n.s.- not significant, ** $p < 0.005$, *** $p < 0.001$, Bonferroni correction applied; n=356.

3.3.3. Detection of a recent bottleneck in the study populations.

None of the performed bottleneck tests, -mode shift and heterozygosity excess analyses-, implemented in the software Bottleneck 1.2; nor *M* (Garza and Williamson 2002) detected any sub-population or management unit to have suffered from recent reduction in size, as the *M* values are above the threshold limit (0.68)

3.3.4. Relationship between developmental stability and genomic diversity.

3.3.4.1. Within each of the three sub-populations.

Negative correlations of FA and the indices of genomic diversity were expected, except when developmental stability is correlated with internal relatedness (IR) positive correlations should be found, because negative numbers of IR will show outbred individuals, whereas positive values will show inbreeding. Individuals that score a zero value or close to it, will be those that are born to unrelated parents (Amos et al. 2001)

Table 3.10 highlights the significant correlations between FA and genomic diversity indices among individuals within the three sub-populations (Bonferroni correction

Table 3.10. Summary of significant correlations found between FA and the genomic diversity indices for each of the three sub-populations (Bonferroni correction applied).

Trait	n	Mean d^2	Scaled Mean d^2	Heterozygosity	Standardised Heterozygosity	Internal Relatedness
	NORWEGIAN					
Single Traits	47	n. s.	n. s.	n. s.	n. s.	n. s.
Avg. FA	47	n. s.	n. s.	n. s.	n. s.	n. s.
	DANISH					
HOR	58	**-0.28***	n. s.	n. s.	n. s.	n. s.
Avg. FA	92	n. s.	n. s.	n. s.	n. s.	n. s.
	BRITISH					
ML	137	n. s.	n. s.	**-0.23****	**-0.22****	**0.22***
Avg. FA	218	n. s.	**-0.15***	**-0.20****	**-0.19****	**0.18****

Values shown represent the Pearson product, * $p \leq 0.05$, ** $p \leq 0.01$.
Abbreviations are as figure 2.1.

applied). For the Danish population, FA of height of ramus (HOR) was negatively correlated with mean d^2 at the $p<0.05$ level. For the British population a similar result was found with the correlation between FA of length of the maxilla (ML) vs. internal relatedness, and the individual composite index of asymmetry (average FA) with scaled mean d^2 (table 3.10) However these correlations were no longer significant after the Bonferroni correction was applied. For all the populations pooled significant correlations included the relationships between FA of length of the maxilla (ML) and internal relatedness, FA of width of maxilla (MW) and scaled mean d^2, and FA of length of the ramus (LOR) and internal relatedness. Again, these did not meet the stringency threshold after Bonferroni correction.

Nevertheless, significant correlations after the Bonferroni correction were found (table 3.10 and figure 3.1). This included the length of the maxilla (ML) and the composite index of individual asymmetry (average FA) for the British sub-population.

Table 3.11. Summary of significant correlations found between FA and the genomic diversity indices for each of the five management units (Bonferroni correction applied).

Trait	n	Mean d^2	Scaled Mean d^2	Heterozygosity	Standardised Heterozygosity	Internal Relatedness
	NORWEGIAN					
Single Traits	47	n. s.	n. s.	n. s.	n. s.	n. s.
Avg. FA	47	n. s.	n. s.	n. s.	n. s.	n. s.
	DANISH NORTH SEA-SKAGERRAK					
Single Traits	52	n. s.	n. s.	n. s.	n. s.	n. s.
Avg. FA	52	n. s.	n. s.	n. s.	n. s.	n. s.
	INNER DANISH WATERS					
Single Traits	39	n. s.	n. s.	n. s.	n. s.	n. s.
Avg. FA	39	n. s.	n. s.	n. s.	n. s.	n. s.
	BRITISH NORTH SEA					
COD	76	n. s.	n. s.	**-0.24***	**-0.24***	n. s.
LOR	58	n. s.	**-0.27***	n. s.	n. s.	n. s.
Avg. FA	111	n. s.	**-0.20***	**-0.20***	**-0.20***	n. s.
	IRISH-WELSH					
Single Traits	106	n. s.	n. s.	n. s.	n. s.	n. s.
Avg. FA	106	n. s.	n. s.	n. s.	n. s.	n. s.

Values shown represent the Pearson product, * $p \leq 0.05$, ** $p \leq 0.01$.
Abbreviations are as figure 2.1.

The correlation between FA of ML vs. heterozygosity, and vs. standardised heterozygosity for the British population was significant. Moreover, average individual FA was correlated ($p<0.01$) with three (heterozygosity, standardised heterozygosity and internal relatedness) of the five indices of asymmetry (table 3.10, figure 3.1). However, these three indices are highly correlated and therefore they are not independent.

3.3.4.2. Within each of the five management units.

For the British North Sea population, FA of the height of the occipital condyle was negatively correlated with heterozygosity and standardised heterozygosity. The length of ramus (HOR) was negatively correlated with scaled mean d^2 at the $p<0.05$ level. (Bonferroni correction applied, table 3.11).

For the composite index of individual asymmetry (Avg. FA) only the British North Sea management unit showed significant correlations with scaled mean d^2, heterozygosity and standardised heterozygosity (table 3.11 and figure 3.2). However these correlations were no longer significant after the Bonferroni correction was applied (table 3.11). A relationship exists between an individual with low genetic diversity and FA on multiple traits. This was in some way expected, since an individual genetically challenged showed FA in multiple independent traits since a correlation between individual asymmetry (Avg. FA) and genomic diversity was established (table 3.11 and 3.12 and figure 3.1 and 3.2).

3.3.5. Environmental stress.

Published information of different kinds of environmental stress is shown in tables 3.12 through table 3.15.

3.3.5.1. Chemical pollution.

Table 3.12 shows the results of chemical pollutants in the different areas of the eastern North Atlantic. Despite the level of variability that several studies have reported on the concentration of chemical pollutants in several regions of the eastern North Atlantic, it is evident that the Norwegian population of harbour porpoises seem to live in the least impacted area as in this region the lower concentrations of ΣPCBs, ^{137}Cs and heavy metals have been reported. Total DDTs are very similar in

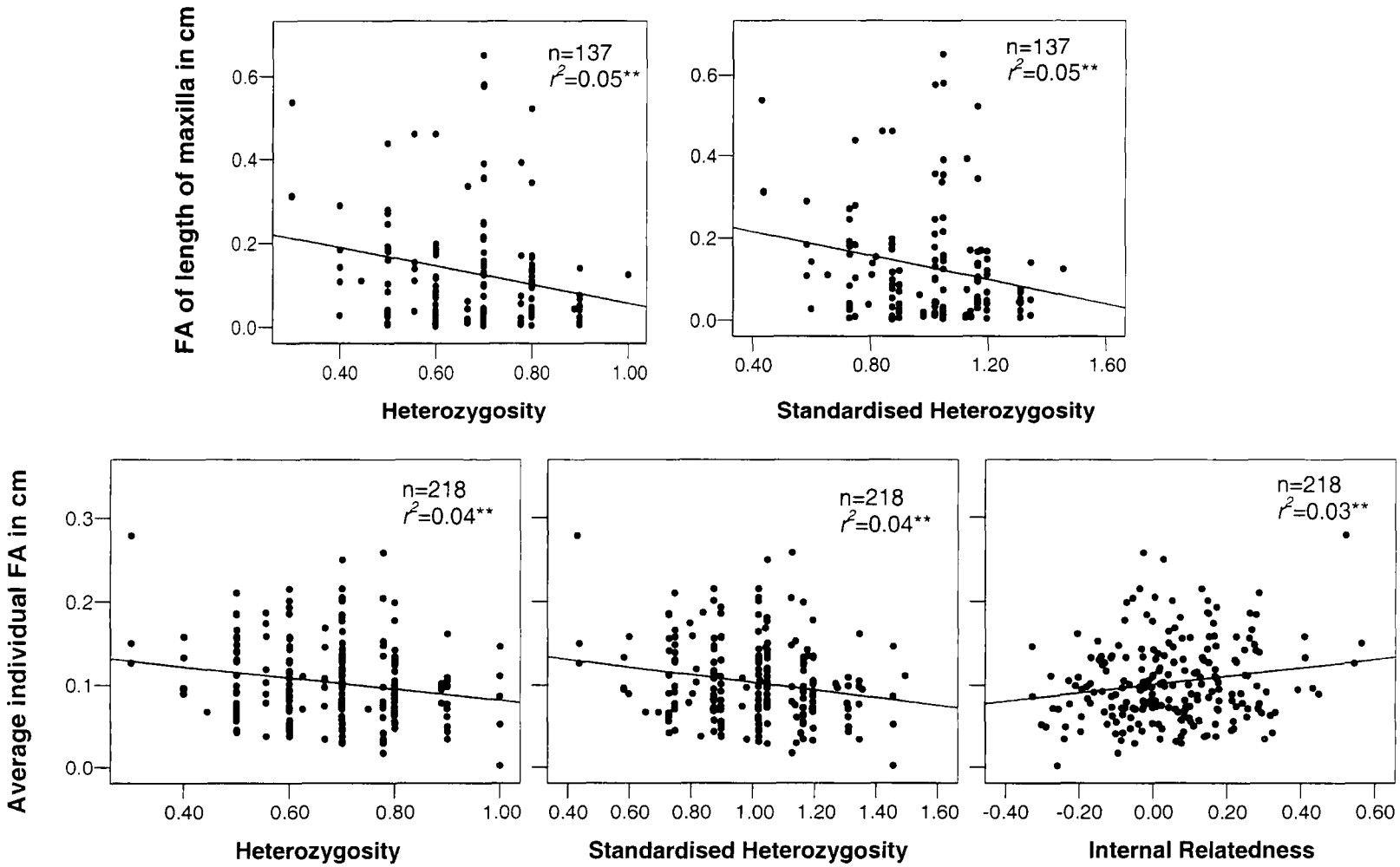

Figure 3.1. Graphic representation of the significant correlations found between FA and genomic diversity for the British population, ** $p \leq 0.01$, Bonferroni correction applied.

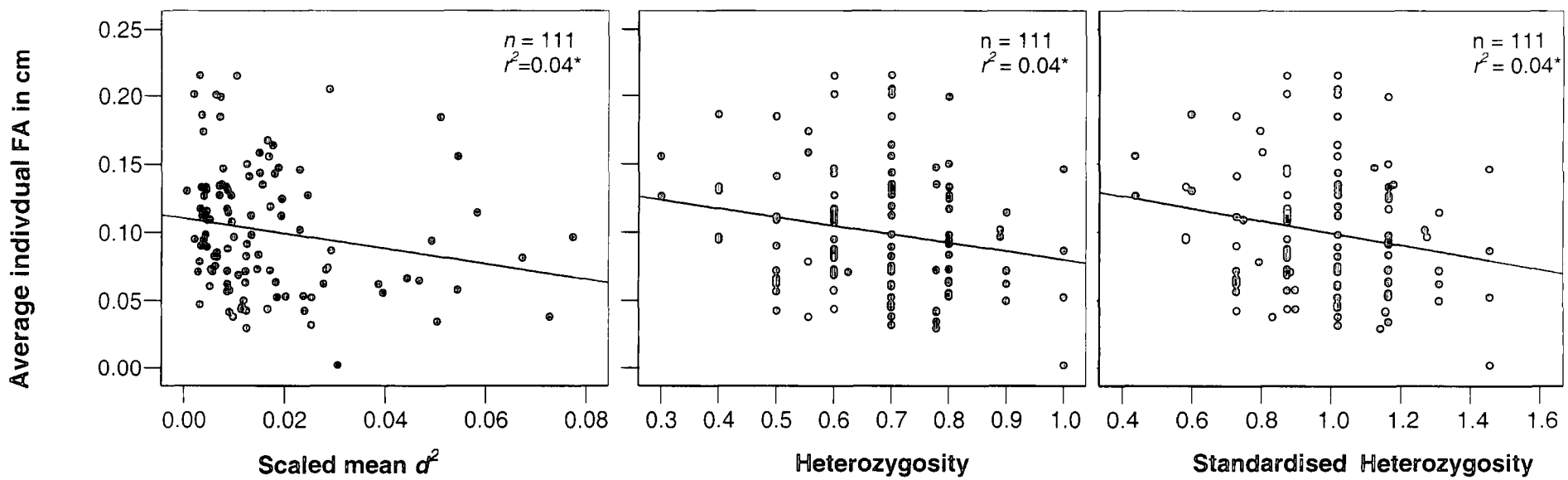

Figure 3.2. Graphic representation of the significant correlations found between average individual FA and genomic diversity for the British North Sea management unit *$p \leq 0.05$, Bonferroni correction applied.

Table 3.12. Geographical comparison of chemical pollutants in harbour porpoise.

Pollutant	Region	Mean	Min-max	Sex	n	Source
ΣPCB	Norway	15	7.2 - 33	M	8	Berggrena et al. (1999)
		24	3.7 - 54.2	Both	27	Klevaine et al. (1995)
	Denmark	25	6.7 - 22	M	7	Berggrena et al. (1999)
		17	4.5 - 39.1	Both	11	Bruhn et al. (1999)
		18	5.6 - 45	F	7	Clausen and Andersen (1988)
		116	27 - 382	M	29	Clausen and Andersen (1988)
		131	6.3 - 21	M	3	Karlson et al. (2000)
		30	10.2 - 65.3	Both	12	Klevaine et al. (1995)
	Britain	62	4.2 - 13.9	F	22	Vetter et al. (1996)
		16	-	Both	16	Vetter et al. (1996)
		16	0.6 - 44.2	M	21	Wells et al. (1994)
		7	2.7 - 32	F	15	Wells et al. (1994)
		41	0.4 - 109.5	M	23	Kuiken et al. (1994)
		19	1.6 - 87	F	25	Kuiken et al. (1994)
		26	0.1 - 109.5	M	50	Law (1994)
		18	0.1 - 138.7	F	47	Law (1994)
		61	-	M	13	Jepson et al. (1999)
		20	-	F	14	Jepson et al. (1999)
tDDT's	Norway	9	3.1 - 22	M	6	Berggrena et al. (1999)
		10	3.2 - 38.1	Both	22	Klevaine et al. (1995)
	Denmark	25	2.8 - 61	M	7	Berggrena et al. (1999)
		19	7.3 - 45.9	Both	12	Klevaine et al. (1995)
		3	1.9 - 4.8	M	3	Karlson et al. (2000)
		4	1.4 - 9.60	F	7	Clausen and Andersen (1988)
		36	7.53 - 202.3	M	29	Clausen and Andersen (1988)
	Britain	14	1.6 - 42.7	F	22	Vetter et al. (1996)
		6	1.4 - 18.8	Both	16	Vetter et al. (1996)
		5	0.8 - 12.6	M	21	Wells et al. (1994)
		3	0.9 - 10.4	F	15	Wells et al. (1994)
		11	0.6 - 33.1	M	23	Kuiken et al. (1994)
		6	0.4 - 22.9	F	25	Kuiken et al. (1994)
		7	0.04 - 33.1	M	50	Law (1994)
		5	0.06 - 34	F	47	Law (1994)

Concentrations are in mg Kg^{-1} lipid in blubber.

Table 3.12 (continued). Geographical comparison of chemical pollutants in harbour porpoise.

Pollutant	Region	Mean	Min-max	Sex	n	Source
^{137}Cs	**Norway**	0.5	0.3 – 1.1	Both	35	Tolley and Heldal (2002)
	Britain	2.4	2.2-2.7	Both	3	Berrow et al. (1998)
Cd (liver)	**Norway**	0.5	<0.05 - 21	Both	21	Das et al. (2004)
(kidney)		6	<0.05 - 16	Both	20	Das et al. (2004)
Hg (liver)		14	1 - 32	Both	21	Das et al. (2004)
(kidney)		7	1 - 43	Both	20	Das et al. (2004)
Cd (liver)	**Denmark**	0.2	<0.05 - 0.4	Both	17	Das et al. (2004)
(kidney)		1.1	0.1 - 3.5	Both	15	Das et al. (2004)
Hg (liver)		22	1 - 147	Both	17	Das et al. (2004)
(kidney)		n.d.	n.d.	Both	?	Das et al. (2004)
Cd (liver)	**Britain**	1.5	<0.02 - 30	Both	200	Courtesy of Law R.J.
Hg (liver)		38.2	0.05 - 589	Both	200	Courtesy of Law R.J.

Concentrations for ^{137}Cs are in Bq. Kg^{-1} wet wt. in muscle; for heavy metals it is in mg Kg^{-1} dry wt.

porpoises from Norwegian and British waters, whereas those from Danish waters showed the highest levels of total DDT's. The harbour porpoises from Danish waters also showed the highest concentrations of ΣPCBs. The porpoises from the British population showed the highest burden of ^{137}Cs and heavy metals.

3.3.5.2. Parasite load.

Table 3.13 shows regional differences in the prevalence of helminth fauna in harbour porpoises. Although difficult to compare because of the scarcity of available information, harbour porpoises from British waters showed the highest diversity in helminth fauna (7 species). The Norwegian population had the lowest species diversity (three species). However the prevalence for *Halocercus sp* in the Norwegian population is much higher than in the British population, while for the other two

Table 3.13. Geographical comparison of incidence of helminth fauna in harbour porpoise.

Region	Britain (*n*=173)		Denmark (*n=70*)		Norway (*n=64*)	
Reference	Gibson et al. (1998)		Herreras et al. (1997)		Balbuena et al. (1994)	
Parasite	Infected	%	Infected	%	Infected	%
Anisakis simplex	103	59.5	54	38	-	-
Pseudalius inflexus	152	88	-	-	22	34.4
Torynurus convolutus	85	49	-	-	27	42.2
Halocercus sp	4	2.4	-	-	63	98.4
Diphyllobothrium sp	7	4	4	2.9	-	-
Pholeter gastrophilus	35	20.3	4	2.8	-	-
Bolbosoma sp	1	0.6	2	1.4	-	-

Data given as % is prevalence.

species found in Norway the British porpoises had a higher prevalence than those from Norwegian waters.

3.3.5.3. Mean ocean surface temperature.

Table 3.14 shows regional differences in the mean ocean surface temperature in different regions of the eastern North Atlantic from the period of 1982-1999. A clear north to south gradient in temperatures is shown.

The Norwegian Sea showed the least mean surface temperature. In comparison with other regions where porpoises from the Danish and British population live this is very similar, whereas porpoises from Norwegian waters live in very cold waters.

3.3.5.4. By-catch rate.

Table 3.15 shows regional differences in the rate of removals by incidental catches in fishing gear of harbour porpoises in the eastern North Atlantic. High differences occur throughout this region; the highest rate of by-catch occurs in the Danish North sea

Table 3.14. Geographical comparison of mean ocean surface temperature (in °C) for the period of 1982-1999 in several regions of the eastern North Atlantic.

Region	Mean	Min-max
Norwegian Sea	4.8	1.2 - 10.2
North Sea	9.6	-0.44 - 20.53
Skagerrak Sea	9.7	1.2 - 20.6
Kattegat and the Bælts	10.0	-0.1 - 20.54
NW Scotland	10.6	7.1 - 15.1
Celtic Sea	11.7	5.2 - 19.1
English Channel	12.4	5.8 - 18.2

followed by the Celtic sea, whereas the lowest level reported occurs around Norwegian waters.

3.3.6. Relationship between developmental stability and environmental stress.

No significant relationships were found between the level of FA and the concentration of the chemical pollutants available. Figure 3.3 shows the correlations between individual average FA vs. ^{137}Cs in the Norwegian population and between FA and tDDT, ΣPCBs, ΣBDE's, and selected heavy metals (Cd and Hg) for the British sub-population. The British North Sea and Irish-Welsh management units were pooled into the British sub-population to try to increase the statistical power since none management units showed significant correlations.

3.4. Discussion.

The level of developmental stability (measured as FA) will reflect the ability of a particular genotype to develop similar phenotypic characteristics under certain environmental conditions. It is expected that high quality genotypes as well as high quality environments will produce symmetrical phenotypes (Shykoff and Møller 1999).

Table 3.15. Geographical comparison of estimates of yearly harbour porpoise catches in several regions of the eastern North Atlantic.

Region	By-catch rate (per year)	95% CI	Year	Reference
Norwegian Sea	46	-	1988-1990	Bjørge et al. (1991)
Inner Danish waters	113	53-173	1996-1997	Berggren et al. 2002
Danish North Sea	6785	-	1994-1998	Vinther (1999)
Celtic Sea	2237	-	1993	Treguenza et al. (1997)
West Scotland	120	14-365	1995-1999	CEC (2002)
British North Sea	600	351-1233	1995-1999	CEC (2002)

This study did not demonstrate differences in FA between females and males. One reason that may explain this is that since the phenomenon of developmental instability occurs *in utero* in the presence of external forces in the form of stress, and because developmental instability is a stochastic process, no difference in sex and age classes should be expected.

When the level of FA on the three sub-populations was compared, a higher degree of FA was found in the Norwegian population, followed by the British; whereas the Danish contained the least asymmetrical individuals. However, there is no much information that this approach could offer due to the lack of statistical power since there are only three independent populations (therefore, there are only two degrees of freedom).

Among the five management units similar results were found. The phenotypic differences among the three sub-populations/five management units are considered as a consequence of disturbed development, even slight difference in the level of developmental stability among the three sub-populations, will reflect a change in the

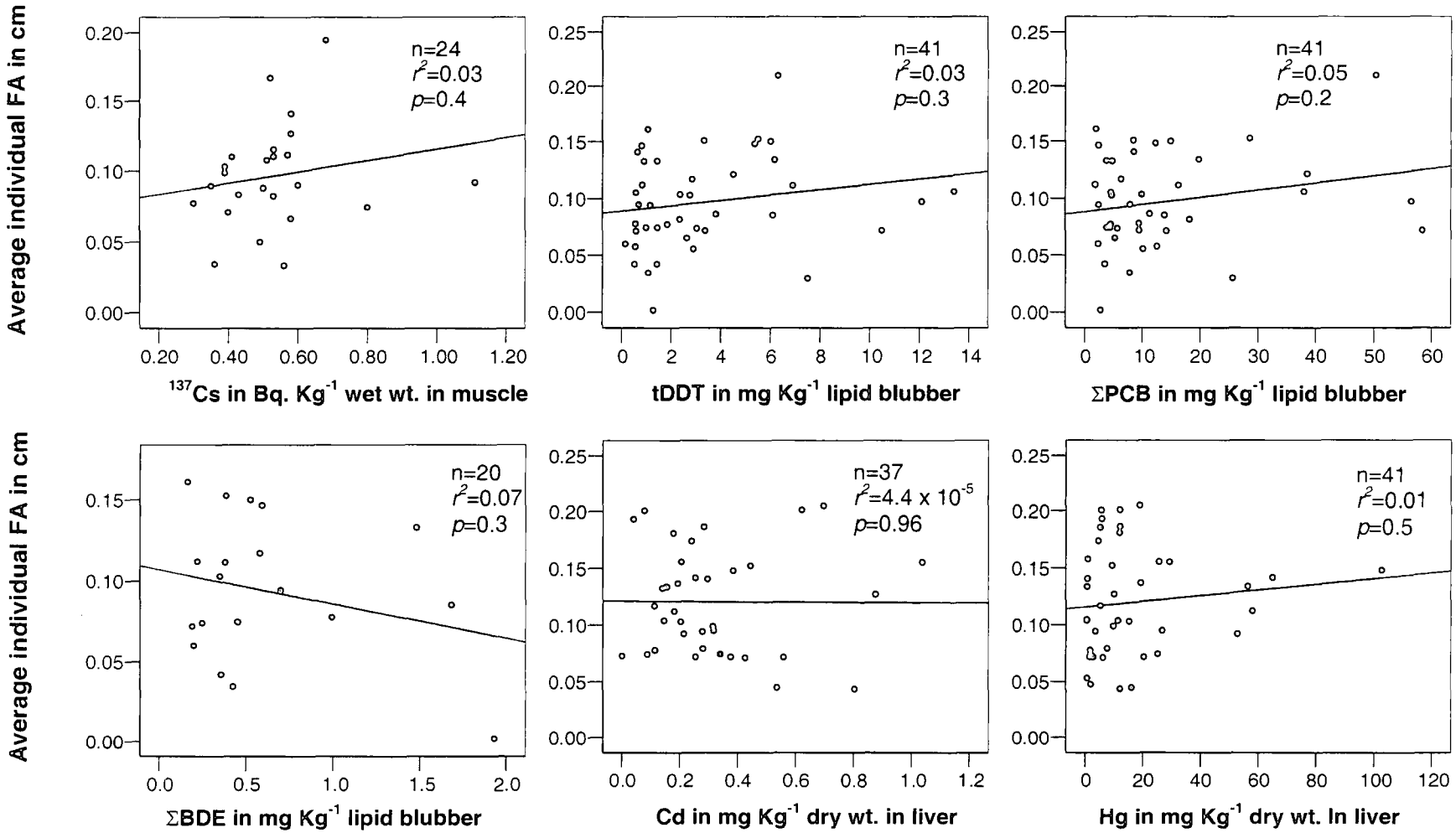

Figure 3.3. Graphic representation of the correlations between FA and chemical pollutants. ^{137}Cs corresponds to the Norwegian sub-population, the rest of the figures corresponds to the British sub-population, Bonferroni correction applied.

developmental stability and could be used to detect initial changes in the condition of a population (Zakharov and Yablokov 1990).

Significant differences in the levels of genetic diversity were found among the three sub-populations and the five management units of harbour porpoise in the eastern North Atlantic. However, it must be stressed the lack of independence across the indices of genomic diversity. Amos et al. (2001) found that standardised heterozygosity vs. internal relatedness correlate better than mean d^2 vs. both standardised heterozygosity and internal relatedness. They explained this lack of correlation on the fact that mean d^2 depends on long term mutational differences, and may be better suited for population admixture (Pemberton et al. 1999).

The Norwegian sub-population/management unit contained the least genetically diverse individuals for two of the five genetic diversity indices, mean d^2 and internal relatedness. This result is in accordance with the previous findings regarding FA. Loss of diversity in a population is related to population fragmentation. Isolated or fragmented- populations may not have the necessary amount of gene flow with other populations and also may not be mating randomly. Andersen et al. (2001) used 12 microsatellite loci to assess population structure in the eastern North Atlantic. They found that the Norwegian population is the most genetically differentiated population of all the populations studied. This finding may confirm the Norwegian population as an isolated unit from neighbouring populations, however no evidence of a recent bottleneck was found.

A significant relationship between individual fluctuating asymmetry and individual genomic diversity was found for the British sub-population. With the exception of mean d^2, all the remaining indices were significantly correlated with the composite index of individual asymmetry, measured as average FA. Slate and Pemberton

(2002) suggested that the low efficacy of mean d^2 to detect a relationship with stress may be due to the fact that certain loci contribute more than others to the overall score, that is, the squared differences between the two alleles may be greater for some loci, therefore these loci should have more influence in the overall mean d^2. One way to correct for this difference in the contribution of the overall mean d^2 score is to use scaled mean d^2. Scaled mean d^2 weighs equally the contribution of each locus to the overall score. This index also recorded a significant relationship with the composite index of FA.

The composite index of FA showed more power to detect relationships with the indices of genomic diversity than single FA traits alone. Leung et al. (2000), previously stated that the relationship between FA traits and stress are typically weak and difficult to detect, and therefore suggested the use of composite indices of FA to increase the power of FA to detect relationships with stress. Nevertheless, one single FA trait, length of the maxilla, showed significant correlations with genomic diversity.

On the other hand, no significant relationships were found when FA was compared with measures of environmental stress. The relationship between environmental stress and FA may be difficult to prove in natural populations (see Introduction) Hendrickx et al. (2003) suggested that one possible explanation for the lack of relationship between environmental stress and FA may be due to the fact that the levels of population FA may be biased downward under stressful conditions because of a high selective force against those developmentally unstable organisms, thus confounding the effects of environmental stress in producing FA.

Another explanation may be sample size. When correlations between FA and the indices of genomic diversity were done, the sample size is almost an order of magnitude higher (356 vs. 41) than those correlations done with environmental stress

for the British population. The values of r^2, although still weak, are very similar to those shown for the correlations of FA with genomic diversity, and even higher in some cases.

Nevertheless, if we compare the regional differences among the different types of environmental stress (tables 3.12 through 3.15) the British and the Danish populations/management units were the more impacted regions for chemical pollutants, parasite load and removals (by-catch rate). But they also contained the more symmetrical and the more genetically diverse animals; whereas the Norwegian porpoises showed the highest level of FA and the lowest level of genomic diversity, but they live in the least impacted area.

When mean ocean surface temperatures are compared within the eastern North Atlantic, the Norwegian Sea showed the lowest of the mean temperatures. The harbour porpoise is a small cetacean and because of its size and high basal metabolic rate (Yasui and Gaskin 1986), low environmental temperatures may trigger stressful situations that could be reflected during development. This however is very unlikely, since the harbour porpoise is a cetacean species that has to dive in order to obtain their prey; therefore porpoises from warmer waters could face colder water when they travel through the water column when feeding. Another fact to consider is that harbour porpoises are adapted to live in temperate and cold waters of the Northern hemisphere, so they have the physiological adaptations e. g. a layer of blubber to insulate them from lower temperatures to withstand this kind of stress.

Developmental stability is an indirect measure of fitness of individuals in a population. Changes in developmental stability will predict subsequent changes in fitness (Clarke 1995). The eastern North Atlantic population of harbour porpoises showed more sensitivity to changes in genetic diversity than to environmental pressures as

indicated by developmental instability. The Norwegian population has been identified in this study as a population that is subject to genetic stress, for that reason remedial action should be taken to avoid inbreeding depression in this putative population. These results suggest an overriding influence of heterozygosity on this indirect measure of fitness, and emphasize the importance for conservation management to retain genetic diversity.

*Chapter 4: Morphological and genetic comparison between two populations of bottlenose dolphin (*Tursiops truncatus*) from the western North Atlantic and one from the Gulf of California.*

4.1. Introduction.

The bottlenose dolphin (*Tursiops truncatus*) is cosmopolitan in distribution, and is found in most temperate and tropical regions in coastal and offshore waters. Along its distributional range the species shows variation in morphology and population genetic structure. Body size appears to vary inversely with water temperature in many parts of the world (Wells and Scott 2002). Variation in size and cranial characteristics associated with feeding and breathing (e.g. Mead and Potter 1995), have led to descriptions of several nominal species of *Tursiops* (Hershkovitz 1966, Rice 1998). Recent morphological (e.g. Ross and Cockcroft 1990), genetic (e. g. Hoelzel et al. 1998a, Natoli et al. 2004, Wang et al. 1999), and physiological (e. g. Duffield et al. 1983) studies suggest that a revision of the genus may be necessary to acknowledge differences between the different morphotypes and genetic lineages found around the world.

The distinction between coastal (nearshore) and pelagic (offshore) morphotypes varies among geographic regions (Hoelzel et al. 1998a, Mead and Potter 1995, Walker 1981). Walker (1981) found a clear distinction between coastal and pelagic populations of the eastern north Pacific based on skull morphology, body size, diet and parasite load. In his study the coastal population preyed mainly on fish from the Sciaenidae and Embiotocidae families, whereas the pelagic fed mainly on pelagic fish species and cephalopods (Walker et al. 1999). Taxonomical considerations at the alpha-level have been suggested for these two putative populations: *T. gilli* (Dall 1873) has been

proposed for the coastal population and *T. nuuanu* (Andrews 1911) for the pelagic form.

In the South Atlantic, Indian Ocean, and around China, *T. aduncus* (Ross 1977) a distinct coastal species has also been proposed. In recent years this distinction has been supported by phylogenetic analyses (Curry 1997, LeDuc et al. 1999, Natoli et al. 2004). Around these areas, *T. aduncus* is relatively smaller in size than the pelagic form *T. truncatus* (Gao et al. 1995). Natoli et al. (2004) further showed that the *T. aduncus* type around coastal waters around South Africa is likely to be a different species compared with the type that inhabits coastal waters around Asia.

Coastal and pelagic populations in the western North Atlantic (WNAC and WNAP figure 4.1) have been distinguished from each other by several methods. This includes feeding ecology (Walker et al. 1999), morphology, parasite load (Mead and Potter 1995), haemoglobin profile (Hersh and Duffield 1990), microsatellite and mitochondrial DNA diversity (Hoelzel et al. 1998, Natoli et al. 2004). Mead and Potter (1995) used, among several methods, skull morphological characters. They found that the relative width of the internal nares was different in the two populations, the pelagic form having relatively wider nasal bones. They suggested that this may represent a physiological adaptation when diving to deeper waters. Parasite and contaminant load was also different. Analysis of stomach contents revealed that the dolphins prey on different species. The pelagic population tend to feed mostly on pelagic fish, such as blue whiting, cod fish, and squid. Those found in coastal Atlantic waters feed on mullet, herring, smelt, capelin, catfish, eels, shrimp, and other crustaceans.

Hoelzel et al. (1998a) used five microsatellite loci and the mtDNA control region to distinguish between the two ecotypes. They found a significant differentiation between the populations for both markers. R_{ST} values averaged 0.373 for microsatellite loci. For mtDNA none of the 18 haplotypes found were shared between the two populations. The average number of nucleotide differences was D_{xy}=0.039. Finally Φ_{ST} indicated that 60.4% of the variation could be explained by differences between the two populations.

More recently Natoli et al. (2004) made a comparison between populations of bottlenose dolphin world wide, including the western North Atlantic populations. They used both nuclear DNA (9 microsatellite loci) and mtDNA (control region) to study population structure and found a clear distinction between the two western North Atlantic populations. For microsatellites, the F_{ST} value of the pairwise comparison between the two WNA populations was 0.205 and Rho_{ST} was 0.236. For the comparison between the eastern north Pacific and the WNA, F_{ST} values were 0.270 for WNAC and 0.219 for WNAP. Rho_{ST} values were 0.236 for WNAC and 0.272 for WNAP. Meanwhile for mtDNA the F_{ST} value of the pairwise comparison between the two WNA populations was 0.355 and for Φ_{ST} it was 0.647. All values were significant at $p<0.05$.

In the western North Atlantic both ecotypes of bottlenose dolphin are distributed from Nova Scotia in Canada to Florida in the USA. Abundance of the coastal population from this region is estimated mainly from two aerial surveys done in the early and mid 1990's (NOAA 2001). The first survey was conducted in 1992 in the coastal area from the south of Cape Hatteras in North Carolina to mid-Florida. Transects that were sampled randomly revealed that the estimated population of bottlenose dolphin for the coastal stock was 12,435 dolphins (CV=0.18, 95% CI= 9,684-15,967; Blaylock and Hoggard

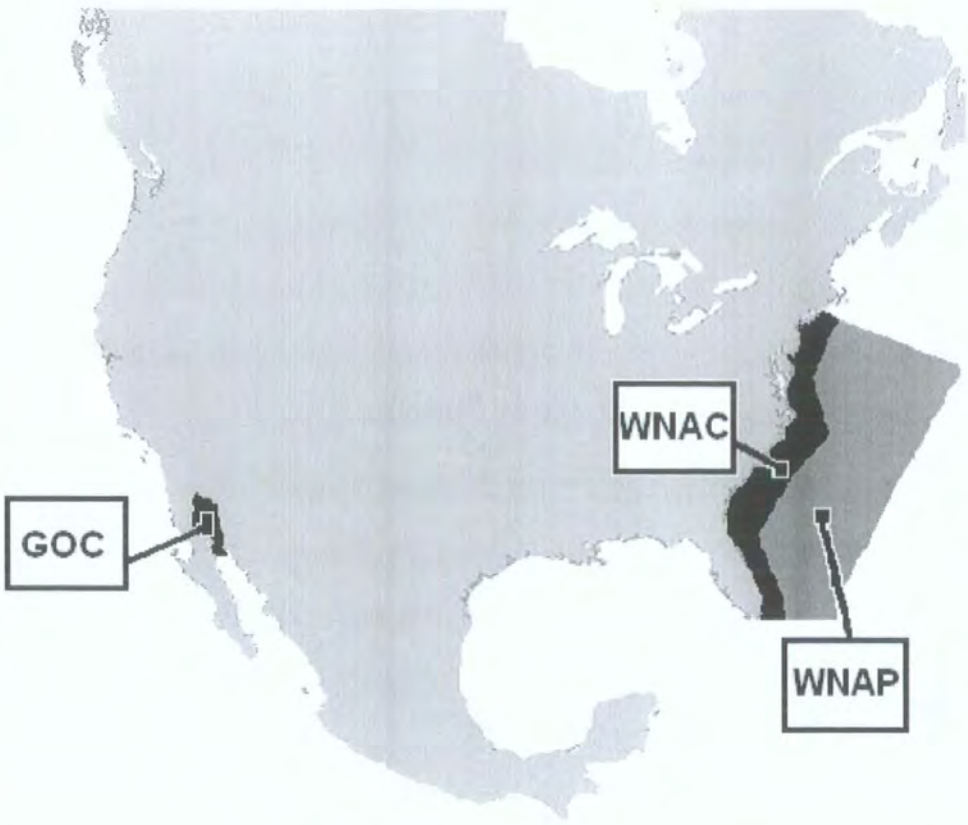

Figure 4.1. Map of the populations of bottlenose dolphin studied. Abbreviations are as in the text.

1994). The second aerial survey was conducted in 1995 and covered the same area as the first one. The abundance estimates were 21,128 dolphins (CV=0.18, 95% CI=13,815-32,312, NOAA 2001). In both surveys the chance that these estimates were inflated by incidentally including dolphins from the pelagic form was acknowledged. The coastal stock is considered depleted relative to the optimum sustainable population (OSP) level, and therefore is listed under the United States Marine Mammal Protection act as depleted.

For the pelagic form abundance is unknown. However abundance estimates are available for portions of the eastern US North Atlantic. The surveys were conducted

between 1978 and 1995. The majority comprised the area from Nova Scotia to Cape Hatteras. These surveys estimated the pelagic population size of bottlenose dolphins to range from 7,696 to 13,453 dolphins (CV=varies from 0.38-0.66, 95% CI not reported, Waring et al. 1998). The minimum population estimate is calculated to be 8,794 animals (CV=0.54, Waring et al. 1998). The status of the stock relative to the OSP is unknown; this stock is not listed as threatened or endangered under the Endangered Species Act of the US.

The distribution of bottlenose dolphins along the California and Baja California coasts depends on oceanographic events, as indicated by a change in residency patterns after the 1982-83 El Niño event (Carretta 2001). Bottlenose dolphins along the Gulf of California (GOC) are mainly distributed around estuaries and shallow sandy areas where they primarily feed (Ballance 1992). A previous study based on a genetic analysis of mtDNA (Segura-Garcia et al. 2004) found that the population of bottlenose dolphins in the Gulf of California is also structured in a coastal and a pelagic form. The abundance for this population is unknown; however the bottlenose dolphin is listed as a species subject to special protection under the Mexican Official Norm.

The pattern of morphotypic diversity is common among delphinid species, e.g. killer whales (Ford et al. 2000, Hoelzel et al. 1998b, Pitman and Ensor 2003), spinner dolphin (Perrin et al. 1991) and common dolphin (Jefferson and van Waerebeek 2002). More research is needed to determine to what extent these are polytypic species or clusters of closely related species.

It has already been mentioned that the coastal and pelagic populations of the western North Atlantic are well differentiated. However, it is not clear if the differences among

ecotypes of populations of bottlenose dolphins are a consequence of phenotypic plasticity or if they are a consequence of a real phylogenetic separation (Natoli et al. 2004). In this study greater resolution in both morphometrics and genetic analyses, and a larger sample size, were implemented than previous studies by analysing fifteen cranial characters and thirteen microsatellite loci to distinguish between both populations of the western north Atlantic. A geographically isolated outgroup (Gulf of California) was also included in the analysis to test the hypothesis that population structure has occurred as a response to evolutionary processes as a consequence of phylopatry and historical founder events; and that resource exploitation leading to adaptation to local environments (e.g. Hoelzel et al. 1998b) are also important in the separation of natural populations of bottlenose dolphin.

4.2. Methods.

4.2.1. - Cranial measurements.

4.2.1.1. Sample collection.

A total of 261 skulls were measured from the collections of the National Collection of Mammals, University of Mexico, Mexico City, Mexico (CNMA), and the National Museum of Natural History, Smithsonian Institution, Washington D. C., USA. (NMNH-SI). The museum in Mexico provided the skulls for the Gulf of California population, whereas the museum in the USA provided the skulls for the coastal and pelagic populations of the WNA (table 4.1).

The information regarding the classification of the skulls to either population of the WNA was provided by the records held in the NMNH-SI. The classification is based on geographical, ecological and/or morphological parameters (relative width of nares). In

Table 4.1. Summarised information on the skulls of bottlenose dolphin measured (CNMA – National Collection of Mammals, NMNH-SI - National Museum of Natural History, Smithsonian Institution).

MUSEUM	SEX			Age Class			Population		
	Male	Female	N/A	Juvenile	Adult	N/A	Coastal	Pelagic	N/A
CNMA	22	20	5	-	-	47	-	-	47
NMNH-SI	75	72	29	105	49	22	61	114	-
Total	97	92	34	120	67	36	61	114	47

cases of live capture or by-catch, animals could be classified by the location of the catch. Stomach contents and parasite load, as discussed above, could also be used as another form to distinguish between both populations of dolphins. A third possibility of classification includes one cranial character, the relative width of the internal nares (Mead and Potter 1995). The population origin of the skulls that came from the Gulf of California is not known. Since the majority of samples (46 out of the 47) came from the northern part of the Gulf, it is more likely that the samples belong to the coastal population. However a comparison with samples from the other population is needed to assess the exact source population of the samples used in this study.

4.2.1.2. Choice of traits.

Sixteen skull characters were used in the analysis. Traits 1, 4, 5, 8, 9, 11, 12, 13, 14, and 15 were taken from Perrin (1975). Trait 3 was taken from Yurick and Gaskin (1987). Trait 2 was taken from Börjesson and Berggren (1997). Trait 10 was taken from Mikkelsen and Lund (1994). Finally the height of the occipital condyle COD and the width of the maxilla (MW) were devised in this study, mainly because they were characters that the majority of the skulls had undamaged. The traits were measured to the nearest 0.001 cm using precision callipers except for CBL and LOR which were

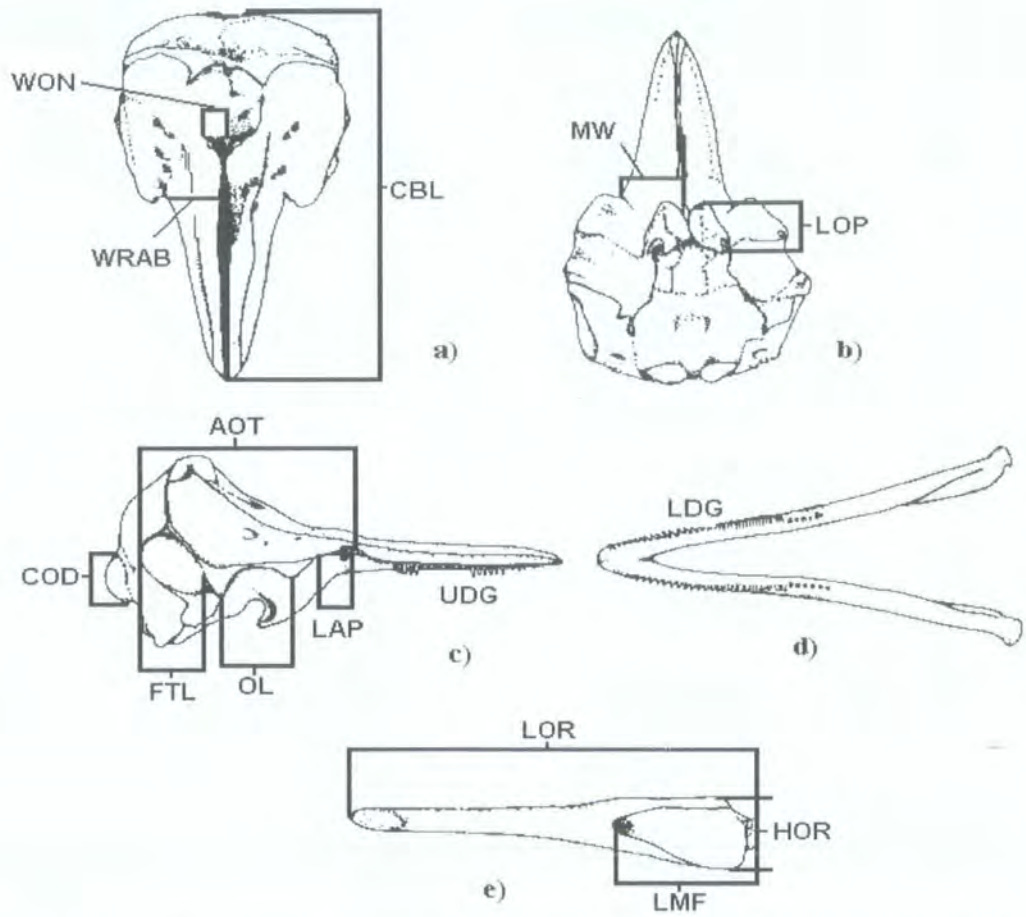

Figure 4.2. Traits measured in the skull of the bottlenose dolphin. a) Dorsal view, b) ventral view, c) lateral view, d)dorsal view of mandible, e) medial view of mandible.

measured to the nearest 0.01 cm. Three repeated measurements for each trait of every skull were done and the callipers were reset to zero after each measurement. In the case of traits 11 and 12 they were counted three times. The median of the three was used (Zar 1984). Measurements were taken on the left and the right side of the skull; however, because of the asymmetry present in the skull of the bottlenose dolphin, measurements on the left side of the skull were used on this chapter (Yurick and Gaskin 1987). No measurements were attempted on missing or worn structures;

therefore there are missing data. The traits measured followed Perrin's (1975) nomenclature. They were (Fig. 4.2):

1. **CBL** - Condylobasal length. Distance from the tip of rostrum to the hindmost margin of the occipital condyles.
2. **WON**.- Maximum width of nares
3. **AOT** – Distance from the antorbital notch to the hindmost external margin of the raised suture of the post-temporal fossa.
4. **FTL** – Greatest length of the post-temporal fossa, measured to the hindmost external margin of raised suture.
5. **OL** – Length of the orbit. Distance from apex of preorbital process to the apex of postorbital process of the frontal.
6. **COD** – Greatest height of occipital condyle.
7. **MW** – Greatest width of maxilla. Distance from the antorbital notch to the hindmost margin of the suture of maxilla with the palatine.
8. **LOP** – Greatest length of pterygoid.
9. **LAP** – Length of antorbital process of lacrimal.
10. **WRAB** – Width of rostrum at base. Along line between midline and limits of antorbital notches.
11. **UDG** – Number of teeth in the maxilla.
12. **LDG** – Number of teeth in the mandible.
13. **LOR** – Greatest length of ramus.
14. **HOR** – Greatest height of ramus.
15. **LMF** – Length of mandibular fossa, measured to mesial rim of internal surface of condyle.

4.2.2. Microsatellite analysis.

4.2.2.1. Samples obtained and previously published data used.

For some of the individuals for which the skull was measured, samples of skin were available. This included 35 animals from the pelagic population and 5 from the coastal population of the western North Atlantic. Additionally, genotypes of 24 individuals for each of 9 microsatellite loci (KWM1b, KWM2a, KWM2b, KWM9b, KWM12a, EV37, Texvet 5, Texvet 7 and D08) from dolphins of the coastal population and 21 from the pelagic population of the western north Atlantic were taken from Natoli et al. (2004). Replication among labs was not necessary as these genotypes were obtained at the University of Durham. For the dolphins from the Gulf of California bone or teeth were obtained for the 47 animals.

4.2.2.2. DNA sampling, digestion, extraction, and isolation from tooth and bone samples.

4.2.2.2.1 Sampling and digestion.

DNA sampled from teeth and bones followed standard measures recommended to avoid contamination. Sampling of teeth and bones to obtain material for DNA extraction was done in a separate laboratory located in a separate building from that used for the skin samples. This was a room where no PCR or post PCR work was performed. This laboratory was not entered if work had been carried out inside the other laboratory or if work on PCR products had been done earlier in the day. All materials brought into the lab either had never been in the skin sampling lab or had been thoroughly wiped down with an approximately 10% dilution of bleach (sodium hypochlorite) at least twice before being taken inside.

Sampling from teeth and bones was done in a laminar flow hood. All surfaces in the hood were wiped down with a 10% dilution of bleach each time before work was begun and again after the work was completed. All materials and equipment utilised were dedicated only for use on this specific laboratory and were cleaned with diluted bleach before and after use and in between use on different samples. Drill bits used for sampling were soaked in diluted bleach for several minutes after each drilling session and were also autoclaved. Water and solutions prepared were autoclaved and filtered through a 0.2 μm syringe filter. Pipette tips came certified sterile from the manufacturer in individually wrapped packages, which were always unwrapped inside the lab and only opened inside the hood. A laboratory coat dedicated for use only in this lab was used at all times, and plastic gloves were worn, which were taped to the lab coat sleeves to avoid exposure of wrist skin. Controls were carried out in parallel with all isolations and PCR reactions to monitor for contamination.

Sampling of the specimens was accomplished using a counter-drilling technique. Teeth were counter-drilled through the natural cavity in the proximal end, into the area where the dental pulp had once been located; sampling from bones were taken from the area of the bone that appeared most dense, as the densest areas of bone carry the most DNA (MacHugh et al. 2000). In preparation for drilling, the samples were treated with 10% bleach to remove any contaminating DNA that may have collected on the outer surfaces and then they were placed in a shaker for 4 hours. They were rinsed afterwards with deionised water.

The powder drilled was collected into 10 mL tubes. The first powder created by the drilling of the outermost layer was discarded to avoid including any possible contamination of the specimen surface in the extraction sample. The design of the drill

bits allowed a fairly large cavity to be created inside most specimens, from which powder was collected, while only a small outer hole was needed. It was attempted to fill the collection tube up to the 0.5 mL point with powder; however, some specimens could not yield this much powder and extraction was carried out on less, and sometimes, very minute amounts of material (down to approximately 0.1 mL).

Enzymatic digestion of the samples was completed by adding 3 mL of digestion buffer (0.425 M EDTA pH 8, 0.5% sodium dodecyl sulfate, 0.05 M Tris pH 8.5). Individual solutions used to make the digestion buffer were certified sterile by their manufacturer or autoclaved and filtered if produced in the laboratory. After mixing of the digestion buffer, the total solution was exposed to UV irradiation for 10 minutes while in a sealed container, to destroy any potential DNA contamination that occurred during the preparation.

Proteinase K was then added at a concentration of 0.5 mg/mL. Extraction controls were continued at this step by placing 3 mL of the digestion buffer and proteinase K into the tubes that had been set to the side during drilling. From this point forward, extraction controls were treated identically to all other sample digests. All digests were incubated on a shaker at 37°C for 48 hours.

4.2.2.2.2. Extraction.

Extraction was done using the recommended protocol of the QIAquick PCR Purification Kit™ method recommended in Yang et al. (1998).

4.2.2.2.3. PCR amplification.

Thirteen published microsatellite loci were used. Four loci, D18, D22, MK8 and TtruAAT$_{44}$, were additional to the nine used by Natoli et al. (2004). These loci, their primer sequences and, the references are listed in Table 4.2 along with the $MgCl_2$ concentration and annealing temperatures used for amplification in skin, bone and teeth samples. To allow sizing of the PCR product using ABI Prism™ technology, one tenth of one of the primers of each pair in each reaction was from a primer solution in which the oligonucleotides had been labelled at the 5' end with a fluorescent ABI Prism™ dye. The primer that was labelled and the dye used in each set are noted in Table 4.2.

For the skin samples PCR amplification was carried out in 15 μL reactions using 0.5 μL of DNA extract. Reaction conditions were 10 mM Tris-HCl pH 9.0, 50 mM KCl, 0.1% Triton® X-100, 0.2 mM each dNTP, $MgCl_2$ at the concentration specified in table 4.2, 10 ng/μL of each primer and 0.3 units of Taq (Bioline™). Cycle conditions were: denaturation at 95°C for 5 minutes, 35 cycles of denaturation at 94°C for 40 seconds, annealing temperature (see Table 4.2) for 1 minute, extension at 72°C for 1 minute with a final extension step of 10 minutes at 72°C. Exceptions were loci D08, D18, D22, MK8 and TtruAAT$_{44}$. For D08, D18 and D22 cycle conditions were: denaturation at 95°C for 5 minutes, 30 cycles (28 for D22) of denaturation at 94°C for 1 minute, annealing temperature (see Table 4.2) for 30 seconds, extension at 72°C for 30 seconds with a final extension step of 10 minutes at 72°C. For locus MK8 cycle conditions were: denaturation at 94°C for 3 minutes, 10 cycles of denaturation at 92°C for 30 seconds, annealing temperature (60°C) with a 1°C decrease per step for 30 seconds, extension

Table 4.2. Microsatellite loci used for bottlenose dolphin, their primer sequences, and the PCR conditions used with skin and with bone and tooth samples. The top primer sequence of each pair was flourescently labelled.

Locus	Primer sequence	Dye	Reference	Skin		Bones and teeth	
				Ann. Temp. in ºC	[MgCl2] in mM	Ann. Temp. in ºC	[MgCl2] in mM
KWM1b	5'-TAAGAACCTAAATTTGGC 5'-TGTTGGGTCTGATAAATG	NED	Hoelzel et al. 2002b	45	1.5	-	-
KWM2a	5'-GCTGTGAAAATTAAATGT 5'-CACTGTGGACAAATGTAA	NED	Hoelzel et al. 1998b	43	1.5	41	2.0
KWM2b	5'-AGGGTATAAGTGTTAAGG 5'-CAACCTTATTTGGATTTC	HEX	Hoelzel et al. 2002b	44	1.5	42	2.0
KWM9b	5'-TGTCACCAGGCAGGACCC 5'-GGGAGGGGCATGTTTCTG	FAM	Hoelzel et al. 2002b	55	1.5	-	-
KWM12a	5'-CCATACAATCCAGCAGTC 5'-CACTGCAGAATGATGACC	NED	Hoelzel et al. 1998b	46	1.5	48	2.0
EV37	5'-AGCTTGATTTGGAAGTCATGA 5'-TAGTAGAGCCGTGATAAAGTGC	HEX	Valsecchi and Amos 1996	57	1.5	54	2.0
TexVet 5	5'-GATTGTGCAAATGGAGACA 5'-TTGAGATGACTCCTGTGGG	FAM	Rooney et al. 1999	54	1.5	44	2.0
TexVet 7	5'-TGCACTGTAGGGTGTTTCAGCAG 5'-CTTAATTGGGGGCGATTTCAC	FAM	Rooney et al. 1999	57	1.5	53	2.0
D08	5'-GATCCATCATATTGTCAAGTT 5'-TCCTGGGTGATGAGTCTTC	HEX	Shinohara et al. 1997	57	2.0	59	2.0
D18	5'-CCCAAAACCGACAGACAGAC 5'-GATCTGGGGATGCAGG	HEX	Shinohara et al. 1997	56	2.0	64	1.5
D22	5'-GGAAATGCTCTGAGAAGGTC 5'-CCAGAGCACCTATGTGGAC	FAM	Shinohara et al. 1997	54	2.0	54	1.5
MK8	5'-TCCTGGAGCATCTTATAGTGGC 5'-CTCTTTGACATGCCCTCACC	NED	Krützen et al. 2001	60	1.5	62	1.5
$TtruAAT_{44}$	5'-CCTGCTCTTCATCCCTCACTAA 5'-CGAAGCACCAAACAAGTCATAGA	FAM	Caldwell et al. 2002	55	1.5	52	1.5

at 72°C for 1 minute. This was followed by 24 cycles of denaturation at 92°C for 30 seconds, annealing temperature (see Table 4.2) for 30 seconds, extension at 72°C for 1 minute with a final extension step of 10 minutes at 72°C. Finally for TtruAAT$_{44}$ cycle conditions were: denaturation at 92°C for 5 seconds, 30 cycles of denaturation at 92°C for 5 seconds, annealing temperature (see Table 4.2) for 30 seconds, extension at 72°C for 10 seconds, with a final extension step of 40 minutes at 72°C.

For the bone and tooth samples PCR amplification was carried out in 20 μL reactions using 2 μL of DNA extract. Reaction conditions were 15 mM Tris-HCl pH 8.0, 50 mM KCl, 0.2 mM each dNTP, $MgCl_2$ at the concentration specified in Table 4.2, 26 ng/μL of each primer 0.08 μg/μL of bovine serum albumina, and 0.5 units of Taq (Bioline™). Cycle conditions were: denaturation at 95°C for 8 minutes, 46 cycles of denaturation at 94°C for 45seconds, annealing temperature (see Table 4.2) for 1 minute 30 seconds, extension at 72°C for 1 minute 30 seconds with a final extension step of 10 minutes at 72°C. Alongside amplification of bone and tooth samples, PCR amplification was also attempted on all extraction controls to check for contamination that may have occurred during the extraction. A negative PCR control, using 2 μL of water instead of a DNA extract, was also included to test for contamination that took place during the set-up of the PCR reaction. Finally, a positive control, using 1.5 μL of water and 0.5 μL of a 100-200 ng/μL solution of skin sample of bottlenose dolphin DNA, was included to ensure the PCR reaction was working. Loci KWM1b and KWM9b were not successfully amplified from the bone and tooth samples.

4.2.2.3. Interpretation of results.

Microsatellite PCR products were run, without further purification, on 6% polyacrylamide gels. DBS Genomics (University of Durham) ran them on a 377 ABI

polyacrylamide slab gel automated sequencers. As mentioned in the earlier section on PCR, each product had been labelled by the use of a flourescently labelled primer, allowing the product to be detected by the sequencer. ABI Prism™ fluorescent labels of FAM, HEX, and NED were used. The PCR products were then added in specific amounts (0.2 μL for FAM dyed PCR products, 0.3 μL for the HEX dyed products and 0.4 μL for the NED dyed products) to a 1.625 μL mixture of ABI loading buffer. Sets of loci were assembled taking care not to overlap allele sizes on the same given dye before run together on the 377 ABI sequencer. Therefore, two sets were assembled: 1) FAM: GT011, 417/418 and TAA031; HEX: EV104 and EV94; and NED: GT015 and 415/416; and 2) FAM: IGF-I, HEX: GT101 and EV96; and NED: GT136 and GATA 053. Running of a ROX labelled DNA size ladder in each lane allowed sizing of the detected PCR products. Visualization of PCR product sizes to a resolution of 1 bp was possible on a chromatogram produced by analysis of the output of the automated sequencer using ABI Genescan™ and Genotyper™ software.

Microsatellite alleles were considered reliable and used in the analysis if the peaks met certain criteria. First, the highest amplitude peak, used as the allele size, was only considered valid if it had an amplitude higher than 50 on the chromatogram. Most alleles, especially in skin samples, were well above this amplitude, and any peaks below 100 were duplicated before use in the analysis. Second, alleles deemed reliable had to show the expected signature structure. Each locus showed a pattern in the shape and prominence of the stutter peaks associated with an allele, and any peaks not showing this pattern were considered to be background 'noise' in the chromatogram or unspecific amplification.

4.2.3. Statistical analyses.

4.2.3.1. Morphometric characters.

Statistical analyses for morphological characters to detect population structure were already described in Chapter 2.

4.2.3.2. Microsatellite loci analysis.

Statistical analyses for microsatellite loci to detect genotyping errors, allelic richness, number of alleles, deviation from Hardy Weinberg expectations, degree of random mating and population structure were already described in Chapter 2.

4.3. Results.

4.3.1. Cranial measurements.

No significant differences were found between sexes or between age classes within populations. Therefore all data was pooled within populations. The results from the MANOVA that tested for differences in the skull morphology among the populations was significant for all characters at $p<0.001$ (Bonferroni correction applied). Table 4.3 shows basic statistics for each trait and for each population. The discriminant analysis (DA) results showed that there were significant differences in the Wilks' λ test for both discriminant functions among the population centroids (both $p<0.001$). This suggests that the populations were distinguishable based on skull morphology (table 4.4).

The percentages of successful classification for each group are presented in table 4.5. For each population the percentage of correct classification was much higher than the 33% expected by chance.

Table 4.6 shows the structure matrix of the DA. This matrix shows the relationship between each trait and the discriminant functions. The higher the correlation index for

Table 4.3. Means and standard deviations of standardised measurements for each population. All traits are relative ratios in respect to the condylobasal length (CBL). CBL is reported in cm. Upper (UDG) and lower (LDG) dental growth are reported in number of teeth.

	WNAC		WNAP		GOC
	Juveniles	Adults	Juveniles	Adults	All
CBL	39.6 ± 4.9	44.6 ± 1.5	42.2 ± 4.2	45.8 ± 2.5	48.7 ± 3.1
WON	0.063 ± 0.02	0.047 ± 0.01	0.063 ± 0.01	0.058 ± 0.01	0.054 ± 0.01
AOT	0.440 ± 0.01	0.443 ± 0.01	0.437 ± 0.01	0.447 ± 0.01	0.439 ± 0.01
FTL	0.236 ± 0.01	0.245 ± 0.01	0.234 ± 0.01	0.237 ± 0.01	0.236 ± 0.01
OL	0.141 ± 0.01	0.138 ± 0.01	0.132 ± 0.01	0.134 ± 0.01	0.135 ± 0.01
COD	0.147 ± 0.03	0.136 ± 0.01	0.142 ± 0.02	0.132 ± 0.01	0.147 ± 0.01
MW	0.154 ± 0.01	0.160 ± 0.01	0.155 ± 0.01	0.162 ± 0.01	0.169 ± 0.01
LOP	0.148 ± 0.01	0.156 ± 0.01	0.145 ± 0.01	0.151 ± 0.01	0.136 ± 0.01
LAP	0.093 ± 0.01	0.099 ± 0.01	0.093 ± 0.01	0.099 ± 0.01	0.110 ± 0.01
WRAB	0.135 ± 0.03	0.114 ± 0.02	0.129 ± 0.02	0.112 ± 0.02	0.124 ± 0.01
LOR	0.856 ± 0.02	0.879 ± 0.014	0.856 ± 0.02	0.869 ± 0.02	0.867 ± 0.01
HOR	0.188 ± 0.01	0.193 ± 0.01	0.188 ± 0.01	0.194 ± 0.01	0.187 ± 0.01
LMF	0.290 ± 0.01	0.295 ± 0.01	0.290 ± 0.01	0.298 ± 0.01	0.271 ± 0.01
UDG	23.5 ± 1.1	24.09 ± 0.9	23.5 ± 1.4	23.7 ± 1.1	21.6 ± 2.1
LDG	23.4 ± 1.1	23.9 ± 1.1	23.6 ± 1.1	23.3 ± 1.3	20.5 ± 1.3

Table 4.4. Results of the Wilks' λ test.

Discriminant Function	Wilks' λ	χ^2	df	Significance
1	0.234	117.003	11	***
2	0.723	26.083	11	***

*** $p<0.001$

a skull character in respect of a discriminant function, the more that trait contributed to the separation between populations. The discriminant function 1 explained 63% of the variance, therefore the skull characters that correlated highest with discriminant function 1 are the most important skull characters for distinguishing among the three putative populations.

Table 4.5. Adequacy of classification results from the discriminant analysis. Left column indicates the original group while the top row indicates the predicted group. Values are as percentage. Correct classifications are italicised.

	GOC	WNAC	WNAP
GOC	*85.2*	6.3	8.5
WNAC	0	*91*	9
WNAP	0	3.3	*96.7*

The population centroid of the Gulf of California population showed a value of 4.50 for the discriminant function 1, this value represented the largest degree of separation among the groups (table 4.7, figure 4.3).

Discriminant function 1 discriminated the Gulf of California population from the two WNA populations, thus the traits that correlated higher with this discriminant function are the most important traits to distinguish the GOC population from the WNA populations. Likewise, discriminant function 2 (and the traits that correlated higher with it) contributed more to the separation of the two WNA populations from each other (table 4.7, figure 4.3).

4.3.2. Microsatellite analysis.

4.3.2.1. Genotyping errors, test for Hardy Weinberg equilibrium and genetic diversity.

The test for a non-homogenous distribution of excess in the number of homozygotes across all loci for each population to assess genotyping errors was not significant. However, deviations from the Hardy Weinberg (HW) equilibrium expectations were found for loci D08 and D22 for the Gulf of California population, TexVet 7 for the WNAC population, and loci KWM1b, KWM2a, and KWM2b for the WNAP population.

Table 4.6. Pooled within-groups correlations between discriminating variables and standardised canonical discriminant functions. Variables ordered hierarchically by absolute size of correlation within function. The largest absolute correlations with either discriminant function are shown in bold and italicised.

	Discriminant Function	
	1	2
LAP	***0.618***	0.162
COD	***0.467***	0.019
AOT	***0.384***	0.312
HOR	***0.383***	0.244
MW	***0.381***	0.341
OL	***0.284***	0.019
WRAB	***0.232***	0.162
LOR	0.311	***0.363***
LMF	0.192	***0.361***
FTL	0.290	***0.329***
WON	0.091	***0.304***
LOP	0.096	***0.109***

Omission of the loci that depart from HW equilibrium expectations did not significantly change the pattern of differentiation of the populations so they were retained for the analyses. However, for the Bayesian analysis implemented in Structure 2.0 all loci that departed from HW equilibrium were omitted, meaning the analysis was conducted in a total of 7 loci. No significant heterozygote excess was observed at any locus in any population. Each pair of loci was tested for linkage disequilibrium and genotypic independence was confirmed. A summary of the statistics computed for the genotyping of microsatellites is shown in table 4.8.

The number of alleles in the three populations had a combined range of 2 to 25. Average allelic richness (table 4.9) was lowest in the WNAC population (5.4) and highest for the GOC (6.9). Allelic richness was not significantly different among populations (χ^2=4.53, df=2). Private alleles were found in every population. The degree of random mating (F_{IS}) was based on observed and expected heterozygosity

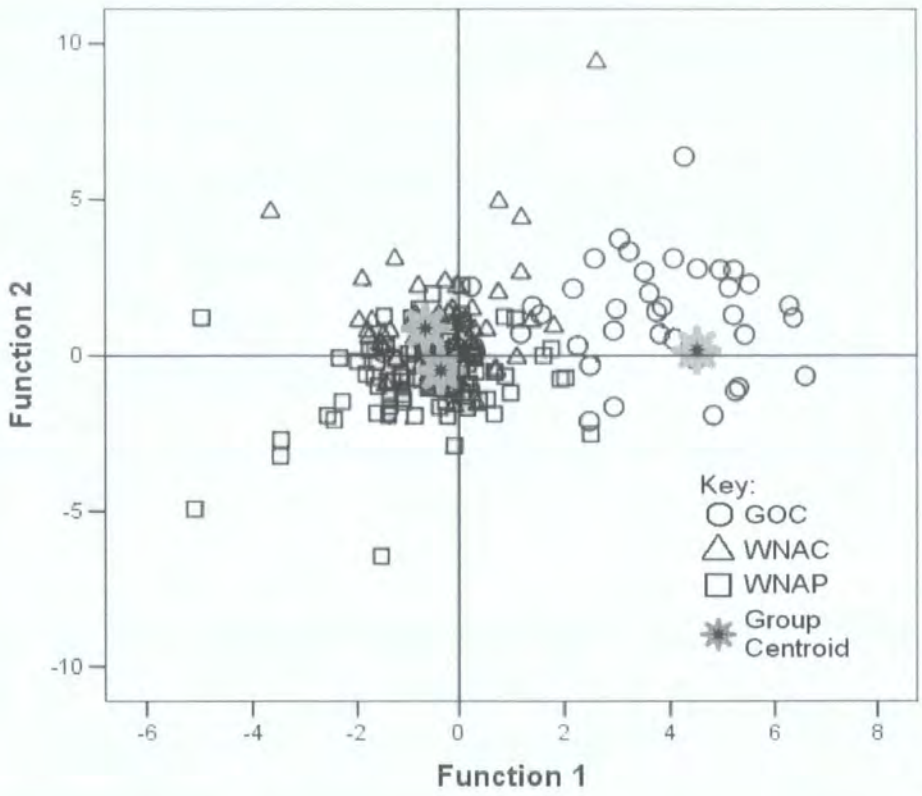

Figure 4.3. Plot of the discriminant function scores for bottlenose dolphin from the Gulf of California and the western North Atlantic based on skull morphology.

Table 4.7. Discriminant functions at population centroids.

	Function	
	1	2
GOC	4.50	0.18
WNAC	-0.64	0.88
WNAP	-0.35	-0.47

values for each locus within the three populations. Large positive values of F_{IS} at particular loci over the populations can be an indicator of homozygosity excess at that locus and is an indicator of non random mating. The WNAP population showed the highest degree (0.131) of F_{IS}.

Table 4.8. Pairwise t-tests in the morphometric traits among the populations studied.

	WNAC vs. WNAP			GOC vs. WNAC			GOC vs. WNAP		
	t	df	Sig.	t	df	Sig.	t	df	Sig.
WON	-2.45	171	**	3.24	102	**	0.41	157	n.s.
AOT	-1.31	143	n.s.	1.13	134	n.s.	5.68	135	***
FTL	-2.95	143	***	5.32	83	***	3.17	132	**
OL	-1.72	164	n.s.	-10.52	77	***	1.51	146	n.s.
COD	-0.87	139	n.s.	4.37	90	***	5.17	141	***
MW	-3.83	166	***	10.88	96	***	7.69	148	***
LOP	-1.28	139	n.s.	1.41	61	n.s.	0.68	104	n.s.
LAP	-2.14	154	*	11.20	89	***	9.59	143	***
WRAB	0.78	172	n.s.	5.96	105	***	1.60	159	n.s
UDG	0.99	152	n.s.	-6.38	83	***	-6.39	127	***
LDG	0.66	145	n.s.	-10.01	71	***	-9.46	112	***
LOR	6.55	143	***	2.98	73	***.	5.22	114	***
HOR	-2.62	144	**	2.73	71	***	3.36	107	***
LMF	-3.59	144	***	3.14	71	***	2.00	148	*

Significance= * $p<0.05$; ** $p<0.01$; *** $p<0.001$, Bonferroni correction applied.

4.3.2.2. Population structure.

Figure 4.4 shows a bar plot of the results of the likelihood of each individual to belong to a sub-population. The conditions of consistency among different runs for the estimation of $P(X|K)$ and the prior α were met. This indicates that the burn-in length and the lengths of the runs were appropriate. K=3 had the highest likelihood for $P(K|X)$ considering both the independent and the correlated allele frequency models.

Table 4.9. Number of alleles (A), private alleles, allelic richness, allele size range, expected (He) and observed (Ho) heterozygosity and F_{IS} for each population at each microsatellite locus.

		GOC *n*=46	WNAC *n*=31	WNAP *n*=56
KWM1b	A	-	2	6
	Private A	-	-	4
	Allelic Richness	-	1.9	3.7
	Size range	-	190-192	170-192
	H_o	-	0.400	0.214*
	H_e	-	0.367	0.560
	F_{IS}	-	-0.226	0.609
KWM2a	A	11	5	10
	Private A	2	-	1
	Allelic Richness	7.2	3.8	6.3
	Size range	141-167	145-155	143-161
	H_o	0.886	0.629	0.630*
	H_e	0.903	0.653	0.868
	F_{IS}	0.035	0.035	0.269
KWM2b	A	9	9	9
	Private A	-	1	1
	Allelic Richness	3.5	3.7	4.3
	Size range	170-186	160-182	166-186
	H_o	0.839	0.269	0.340*
	H_e	0.863	0.466	0.553
	F_{IS}	0.03	0.388	0.387
KWM9b	A	-	5	5
	Private A	-	3	2
	Allelic Richness	-	4.1	4.4
	Size range	-	171-185	173-181
	H_o	-	0.478	0.736
	H_e	-	0.719	0.782
	F_{IS}	-	0.321	0.047
KWM12a	A	13	7	14
	Private A	3	-	3
	Allelic Richness	6.4	4.9	6.2
	Size range	151-183	167-179	159-187
	H_o	0.903	0.724	0.760
	H_e	0.914	0.772	0.846
	F_{IS}	-0.016	0.049	0.095
EV37	A	23	16	18
	Private A	6	3	1
	Allelic Richness	11.7	7.0	7.9
	Size range	192-260	200-248	198-244
	H_o	0.914	0.758	0.872
	H_e	0.946	0.859	0.906
	F_{IS}	0.013	0.118	0.038
TexVet 5	A	13	6	8
	Private A	5	-	2
	Allelic Richness	6.0	4.4	5.2
	Size range	196-220	204-216	202-216
	H_o	0.878	0.640	0.642
	H_e	0.923	0.737	0.783
	F_{IS}	0.051	0.109	0.179

TexVet 7	A	9	6	9
	Private A	-	-	-
	Allelic Richness	8.4	4.3	5.6
	Size range	154-170	156-168	154-170
	H_o	0.836	0.645*	0.750
	H_e	0.804	0.716	0.836
	F_{IS}	-0.022	0.1	0.089
D08	A	15	8	11
	Private A	4	-	-
	Allelic Richness	5.8	5.1	5.3
	Size range	99-127	95-123	95-121
	H_o	0.647*	0.689	0.750
	H_e	0.897	0.742	0.794
	F_{IS}	0.27	0.064	0.046
D18	A	10	6	9
	Private A	2	-	3
	Allelic Richness	5.3	6.0	5.4
	Size range	80-98	80-94	80-106
	H_o	0.818	0.833	0.875
	H_e	0.730	0.909	0.804
	F_{IS}	-0.105	0.074	-0.09
D22	A	9	8	10
	Private A	-	-	1
	Allelic Richness	8.2	7.4	7.0
	Size range	120-136	120-136	118-136
	H_o	0.673*	0.714	0.794
	H_e	0.882	0.912	0.898
	F_{IS}	0.225	0.221	0.113
MK8	A	14	8	11
	Private A	3	-	1
	Allelic Richness	8.8	7.4	6.5
	Size range	87-113	93-111	95-115
	H_o	0.833	0.857	0.636
	H_e	0.923	0.923	0.850
	F_{IS}	0.113	0.065	0.243
TtruAAT$_{44}$	A	4	3	8
	Private A	-	-	4
	Allelic Richness	3.9	4.3	4.2
	Size range	84-94	84-92	82-96
	H_o	0.945	0.666	0.794
	H_e	0.631	0.651	0.704
	F_{IS}	-0.502	-0.25	-0.129
Total	Mean no. A ± SD	11.1 ± 5.3	7.5 ± 3.6	9.9 ± 3.3
	Mean Allelic Rich.	6.9 ± 2.5	5.4 ± 156	5.8 ± 1.2
	Mean He ± SD	0.856 ± 0.097	0.725 ± 0.166	0.783 ± 0.114
	Mean F_{IS}	0.025	0.095	0.131

Asterisks indicate those loci with a *P*-value <0.001 (Bonferroni correction applied) when tested for heterozygote deficiency.

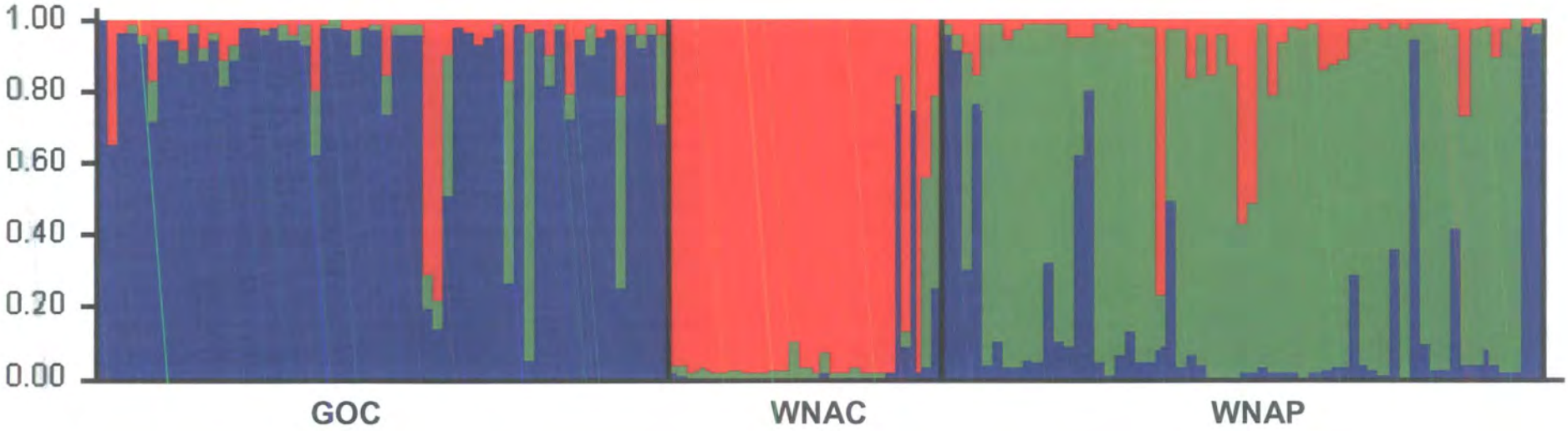

Figure 4.4. Graphic representation of the proportion of each individual to belong to a sub-population based on the coefficient of admixture. Each bar represents and individual. Blue=Gulf of California, red=coastal, western North Atlantic, green=pelagic, western North Atlantic.

Table 4.10. Estimated posterior probabilities of *K*.

K	*P*(*K*|*X*)	lnPr(*X*|*K*)
1	~0	-5624
2	~0	-5336
3	0.999	-5177
4	~0	-5184
5	~0	-5205
6	~0	-5287

Table 4.10 shows the estimated posterior probabilities for each value of *K* and the natural logarithm of Pr(*X*|*K*). This indicated the presence of three distinct subpopulations: GOC, WNAP and WNAC. Table 4.11 shows the proportion of each individual from a predefined population to belong to an inferred cluster.

Genetic differentiation among pairwise populations using F_{ST} and Rho_{ST} values are displayed in table 4.12. Significant differences were found in all the pairwise comparisons. These results are consistent with the results of the morphometric and the Bayesian analyses of Structure 2.0. For F_{ST} and Rho_{ST} the pairwise comparison between WNAC and WNAP was the largest (0.192 and 0.308 respectively). A comparison between F_{ST} and Rho_{ST} to assess the role of allele size in population differentiation (after Hardy et al. 2003) indicated no significant role for allele size.

4.4. Discussion.

4.4.1. Distinction between the two populations of the WNA.

The skull characters that distinguish the two WNA populations were found along the discriminant function 2 (fig 4.4). The correlation index of the first three skull characters showed similar magnitudes (table 4.6). They not only showed the higher correlations with the discriminant function 2, but also were involved in the same

Table 4.11. Proportion of individuals from the pre-defined populations allocated to the inferred clusters.

Predefined Population	Inferred Clusters		
	GOC	WNAC	WNAP
GOC	0.847	0.074	0.079
WNAC	0.139	0.782	0.079
WNAP	0.146	0.076	0.779

Table 4.12. Genetic differentiation among pairwise populations.

	GOC	WNAC	WNAP
GOC		0.212***	0.138***
WNAC	0.124***		0.308***
WNAP	0.077**	0.192***	

Rho_{ST} values are reported above the diagonal, F_{ST} values below the diagonal (p<0.01, ***p<0.001, Bonferroni correction applied) .**

functional process. These characters included two mandibular traits, length of the maxilla (LOR), and length of the mandibular fossa (LMF). The third one is the length of the temporal fossa (FTL). The temporal fossa is mainly occupied by the temporalis muscle; this muscle is involved in the mastication process by elevating the mandible, thus closing the jaws; and its posterior fibres retract the mandible after protrusion (Getty 1975). Since the coastal population had proportionally larger mandibles (0.84 in juveniles and 0.86 in adults) than the pelagic population (0.80 in juveniles and 0.84 in adults, table 4.3), and confirmed by the result of the pairwise t-test comparison (t=6.55, df=143, p<0.001 Bonferroni correction applied, table 4.8) this finding suggests that coastal bottlenose dolphins have larger beaks than the pelagic animals

The findings in this study support the theory suggested by Heyning and Perrin (1994) that in delphinids moderate to strong morphological variation over short geographical distances are common.

The results from the microsatellite study showed that these two populations, although living in parapatry, were also well differentiated. The Bayesian analysis of the allelic frequencies implemented in Structure 2.0 (Pritchard et al. 2000) identified a clear boundary between these two populations. Measures of population differentiation found in this study, F_{ST} and Rho_{ST}, also suggested the presence of two distinct putative populations. The pairwise comparison for both measures showed the highest differentiation in the comparison between the two WNA. However Rho_{ST} (0.308) was higher than the F_{ST} value (0.192).

Although both, F_{ST} and Rho_{ST}, are measures of population differentiation, they are based in different mutation models. F_{ST} (Wright 1951) is based in the infinite allele model. This model stipulates an infinite number of populations, with no selection, and the amount of gene flow is not affected by the geographic distance between the two populations (Neigel 2002). Rho_{ST} (Goodman 1997) is based on R_{ST} an analogous measure to F_{ST}. Among its main differences is that R_{ST} (Slatkin 1995) is based on the stepwise mutation model that is better suited for microsatellite loci. This model involves the addition or subtraction of a small number of repeat units according to a stepwise mutation model where the size of new mutant alleles depends on its progenitor (Weber and Wong 1993). The above may explain why Rho_{ST} showed a higher value since F_{ST} usually underestimates the true genetic differentiation (Slatkin 1995).

Nevertheless, these results are in agreement with a previous study done by Hoelzel et al. (1998a) that included microsatellite loci and mitochondrial DNA. They also

found a clear distinction between the two populations. They reported an R_{ST} value of 0.373 on average for the five microsatellite loci studied. For the mtDNA they found that the average number of nucleotide differences between populations was D_{xy}=0.039. For Φ_{ST}, 60.4% of the variation was explained by differences between the two populations.

In the eastern North Pacific two different ecotypes of another delphinid species, the common dolphin *Delphinus delphis*, are found in parapatry (Rosel et al. 1994). The long beaked form usually inhabits coastal regions whereas the short beaked form is normally seen in off-shore waters (Heyning and Perrin 1994). The taxonomy of this genus is contentious. One study in particular (Heyning and Perrin 1994) used morphological characters and colouration patterns to distinguish between these two ecotypes. The results suggested that the level of differentiation found in the analysis were equal to or greater than those for some other full species of dolphins. They proposed the revision of the genus and named the coastal long-beaked form as *Delphinus capenisis*, whereas the pelagic short-beaked form retained the previous name *Delphinus delphis*.

In a parallel study, the same two forms (long vs. short-beaked) of common dolphin for the same area were studied genetically by Rosel et al. (1994). They used mtDNA sequences from the control region and the cytochrome b gene to determine whether these two forms of common dolphins were genetically distinct. They found that there were no shared mtDNA haplotypes between the two forms, and both gene regions exhibited frequency and fixed nucleotide substitutions between the two morphotypes. This genetic differentiation, supported the findings of the morphological study of Heyning and Perrin (1994) that the morphotypes although parapatric, are reproductively isolated from one another and may represent separate species.

Genetic differentiation between sympatric populations could have been evolved by several mechanisms. Hoelzel (1998) has suggested three: First, differentiation could be due to a drift in allopatry followed by a posterior rejoining and the subsequent resumption of gene flow between the two populations. However, because both populations showed private alleles this explanation is unlikely. Second, the two populations may represent two different species with restricted gene flow due to physiological or behavioural barriers. However in spite of the amount of evidence gathered in later years to support this theory (see introduction); Natoli et al. (2004, 2005), and the results found in this study have found that comparisons between populations more geographically distant show similar values of population differentiation than the two WNA populations reported in this study.

The third possible explanation is that local differentiation could be driven by behavioural isolation, mainly based on different foraging strategies. Hoelzel et al. (1998b) considered this the most likely possibility. However, how this affects gene flow is not well understood. Species that have shown phenotypic plasticity for a given characteristic that is environmentally induced, and become specialists in respect to a feeding pattern are most likely to show differences in feeding behaviour (Adams et al. 2003). Hoelzel (1991, 1993, 1994) has suggested that the difference in exploitation of resources may lead to the specialisation of different feeding strategists that later could become differentiated for reproductive strategy. Further reproductive isolation could limit the gene flow between them (Hoelzel et al. 1998b).

The majority of the examples of reproductive isolation based on behavioural differences in the literature come from research done in fish species, mainly because among this taxon trophic polymorphism appears to be common (Skulason and Smith 1995). Probably the best known example is the cichlid *Perissidus microlepis* that shows a lateralisation of the morphology of the mandible. Morphotypes of fish that

show left-handed mandibles prey on the right side of its host, and *vice versa* (Hori 1993). Since behaviour is considered more flexible than morphology, differences in behaviour are most likely to precede differences in morphology and life history characters. Differences in resource exploitation are likely to be the result of ecological pressures in sympatric conspecific populations, therefore intraspecific competition and the availability of niches are essential for the differentiation within a species (Skulason and Smith 1995).

In bottlenose dolphins several studies have shown a relationship between habitat type and the behaviour shown by the dolphins to exploit the specific resources found in those particular habitats. Barros and Wells (1998) analysed the potential factors that lead to patterns in habitat use of bottlenose dolphins in Sarasota Bay, Florida. They found that coastal populations of bottlenose dolphins are found all year round preying on fish that inhabit estuaries and seagrass habitats. A similar study (Gannon and Waples 2004) reported that coastal bottlenose dolphins of the eastern north Atlantic showed variation in diet according to habitat type and a dependence of the strategy of foraging according to the habitat and resources found.

4.4.2. Comparison between the two populations of the WNA and the GOC.

The skull characters that explained more of the discrimination of the GOC population from the two WNA populations were along the discriminant function 1. The characteristics that correlated to this function were more diverse than seen for discriminant function 2. However, there is some consistency among the traits. First the longitude of the antorbital process of the lacrimal (LAP), the length of the zygomatic arch (orbit length, OL), and the distance from the antorbital notch to the hindmost external margin of the raised suture of the posttemporal fossa (AOT) are involved in vision. The function of the LAP is to protect the eye (Perrin 1975) and the function of the orbit is to contain the eye itself. The population of dolphins from the

Gulf of California had relatively larger LAP (0.112) than those from the WNA (range from 0.090 to 0.099) and was significantly different (GOC vs. WNAC: t=11.2, df=89, and GOC vs. WNAP: t=9.6 df=143, all p<0.001, Bonferroni correction applied). However OL values are smaller in the GOC when compared with WNAC (0.135 vs. 0.141 for juveniles and 0.138 for adults of the WNAC), and also significantly different (t=-10.5, df=77, p<0.001, Bonferroni correction applied). AOT also scored a higher (0.384) correlation with discriminant function 1, and was significantly different when compared with the WNAP population (t=5.86, df=135, p<0.001, Bonferroni correction applied).

The second consistent result has to do with the measurements that dealt with the breadth of the skull: the width of the maxilla (MW) and the width of the rostrum at its base (WRAB). MW is relatively larger in the skulls of the dolphins from the Gulf of California (0.169 vs. 0.148-0.162) and is significantly different in the pairwise comparisons with the WNA populations (GOC vs. WNAC: t=10.9, df=96, and GOC vs. WNAP: t=7.69, df=148, all p<0.001, Bonferroni correction applied). In fact this trait also scored high (0.341) along discriminant function 2 that separated the two WNA populations, and was significantly different between the WNA populations (t=-3.8, df=166, p<0.001, Bonferroni correction applied). For WRAB, significant differences were found in the comparison between GOC and WNAC (t=5.96, df=105, p<0.001, Bonferroni correction applied). However this trait scored the lowest correlation (0.204) with discriminant function 1.

Both tooth counts (UDG and LDG) are significantly different between the GOC and the WNA populations (GOC vs. WNAC: t=-10.01, df=71, and GOC vs. WNAP: t=-9.46, df=112, all p<0.001, Bonferroni correction applied). Average counts showed that the GOC had fewer teeth than the WNA populations (~21 for GOC vs. ~24 for WNA). This result may be related to the high correlation (0.467) showed by the

height of the mandible (HOR) and the length of the mandible (LOR). HOR was significantly different in the pairwise comparisons between GOC and WNA (GOC vs. WNAC: t=2.73, df=71, and GOC vs. WNAP: t=3.36, df=107, all p<0.001, Bonferroni correction applied). Although LOR correlated higher (0.363) with discriminant function 2, it showed a similar correlation score (0.311) with discriminant function 1. Significant differences were found in the pairwise comparisons (GOC vs. WNAC: t=2.98, df=73, and GOC vs. WNAP: t=5.22, df=114, all p<0.001, Bonferroni correction applied). This result may suggest the presence of different type of prey inhabiting the two ocean basins.

Finally, COD also scored high along discriminant function 1 (0.467), it is also significantly different (GOC vs. WNAC: t=4.37, df=90, and GOC vs. WNAP: t=5.17, df=141, all p<0.001, Bonferroni correction applied). The occipital condyles in the bottlenose dolphin are robust and fit into the fossa of the atlas (Rommel 1990). The difference in size of COD may be because the skull size in the GOC was larger and therefore a stronger joint is required to move also larger muscle masses. Although a direct comparison between age class groups between each population was not possible because of the lack of information for the GOC populations, on average the GOC populations had the larger skulls (table 4.3). This finding is in accordance with the theory that animals in tropical waters have larger body sizes (Wells and Scott 2002).

The results from the Bayesian analysis implemented in Structure 2.0 showed that the boundary between the clusters represented by the animals that inhabit the Gulf of California from the western North Atlantic populations is strong. It is probably mainly due to the closing of the Panama Isthmus three million years ago (Emiliani et al. 1972). Therefore, gene flow is no current expected between these two populations. The Isthmus has been open artificially in the form of the Panama Canal early in the

20th century, however it is unknown if currently there is gene flow between these three populations.

Nevertheless, the results of the population differentiation indices (F_{ST} and Rho_{ST}) were significant for the pairwise comparisons between the GOC population and the two WNA populations. Natoli et al. (2004) reported values of F_{ST} for pairwise comparisons between bottlenose dolphins from the eastern north Pacific and the WNA populations of 0.270 for WNAC and 0.219 for WNAP ($p<0.05$). For Rho_{ST} the values reported were 0.511 for WNAC and 0.272 for WNAP ($p<0.0001$). However in this study the highest population differentiation for both indexes was between the two geographically close populations, WNAC and WNAP (0.192 for F_{ST} and 0.308 for Rho_{ST}).

The results presented in this study support the idea that habitat use and resource exploitation may be important in the differentiation of sympatric populations of bottlenose dolphin (Hoelzel 1998, Natoli et al. 2004 and Natoli et al. 2005). It is acknowledged (Hoelzel 1991, 1998, Natoli et al. 2004, and Natoli et al. 2005) that in odontocetes, and especially delphinids, geographic distance and genetic differentiation are not always correlated. Local variations in habitat, and marine coastal environments are different from the pelagic and could lead to niche specialisation (Hoelzel 1998). Adaptation to these habitats could then lead to reproductive isolation and as a consequence of this, social facilitation of feeding strategies may be important in defining phylopatry in both females and males and in establishing social groups that prey together and transfer the knowledge over generations, leading to fine-scale structure at the intra-specific level that could lead to relatively frequent speciation within the genus (see Natoli et al. 2004).

Chapter 5: The relationship between developmental stability, genomic diversity and environmental stress in the Bottlenose Dolphin (Tursiops truncatus).

5.1. Introduction

Developmental stability is a term used to describe controlled developmental processes that buffer against external forces in the form of stress that could disrupt the development of an organism during ontogeny (see chapter 1 for review). Developmental stability reflects the ability of individuals to produce a pre-determined phenotype under given genetic and environmental conditions (Møller and Swaddle 1997). The appropriate control of development (stability) gives an advantage to individuals by providing them with an optimal phenotype (Shykoff and Møller 1999). However developmental buffering is not always precise, and therefore, could account for inaccuracies during development (Leary et al. 1983) and the mechanisms are not well understood (see chapter 3).

Developmental stability is an indirect measure of fitness (Clarke 1995). One effect of genetic and/or environmental stress during the development of an organism could be reflected in the symmetry of bilateral characters in the form of fluctuating asymmetry (FA) -random asymmetric characters in right or left counterparts of paired structures-. FA is the only asymmetry present in nature that is useful for determining developmental stability (Palmer and Strobeck 1986). Two other types of asymmetry present in nature have a functional component, directional asymmetry and antisymmetry (Palmer 1994). Directional asymmetry is present in the skull of odontocetes as a physiological adaptation to live in an aquatic environment. The skull has been reshaped to allocate the organs that are used for echolocating and sound production (Mead 1975, Yurick and Gaskin 1988, see chapter 1 for further explanation).

The bottlenose dolphin *Tursiops truncatus* is an odontocete that has a cosmopolitan distribution. In the western North Atlantic, it mainly occurs in two different ecotypes: coastal and pelagic; which have been differentiated from one another by different studies over the last years by using haematology, morphology, genetic variability, diet, stable isotopes, parasite and contaminant load (Duffield et al. 1983, Hoelzel et al. 1998, Mead and Potter 1995, Walker 1981, Walker et al. 1999 chapter 4 of this study).

Abundance estimates for the coastal population have considered this population as depleted in respect to the optimum sustainable population (OSP) and it is listed as depleted by the United States Marine Mammal Protection Act (NOAA 2001). Hoelzel et al. (1998a) and Natoli et al. (2004) have considered the coastal population as a founder population that may have originated from the pelagic population. Abundance for the pelagic population is unknown, and it is considered neither as threatened nor as endangered by the Endangered Species Act of the USA (Waring et al. 1998).

In the Gulf of California a previous study based in a genetic analysis of mtDNA (Segura-Garcia et al. 2004) found that the population of bottlenose dolphins in the Gulf of California is structured into coastal and pelagic ecotypes. The abundance for this population is unknown; however the bottlenose dolphin is listed as a species subject to special protection under the Mexican Official Norm, here are included those species that are considered as threatened.

Along its distribution the bottlenose dolphin encounters threats to the welfare of the species. Chemical pollution has been documented in this species in extremely high concentrations of polychlorinated hydrocarbons, especially DDT residues in the eastern tropical Pacific (Hansen 2004, O'Shea 1999). The level of pollutants found

in the tissues of bottlenose dolphins are of concern especially in the context of reproductive and immune system health (Wells and Scott 2002). In the western North Atlantic the population suffered an epizootic of morbillivirus in the late 1980's that caused a mass mortality (Lipscomb et al. 1994). Large numbers of dolphins have been removed by incidental catches, especially in the purse-seine fisheries of tuna, sardine and anchovetas (Wells and Scott 1999). In Mexico the species is exploited in different ways, e. g. to be presented in aquariums (Ortega-Ortiz 1996), or to be used as aid in therapy for some medical conditions such as autism (Simmonds 1991 cited in Perez-Cao 1996). Interactions with fisheries include, but are not limited to, use as shark bait and fishermen killing them since they consider the dolphins as a competitor for some target species (Delgado-Estrella 1991, Gallo 1986, López-Hernández 1997).

The US and Mexican government have shown concern for the status of the species, the threats mentioned above may put the species under genetic and environmental stress; possibly resulting in fluctuating asymmetry (FA). In this study the level of developmental stability was determined to give an idea of the impact of anthropogenic activities on populations of bottlenose dolphins.

The level of genetic diversity is another factor that is also considered important to know. Hoelzel et al. (1998a) and Natoli et al. (2004) have already reported low levels of genetic diversity in the coastal population of the western North Atlantic (WNAC). Since human activities in the form of environmental stress are placing pressure on the welfare of this species it is expected that fitness has been compromised in these populations. If there is regional variability in levels of environmental stress, then this may be reflected in regional variation in levels of FA and genetic diversity.

5.2. Material and Methods.

5.2.1 Determination of developmental stability.

5.2.1.1 Morphometric measurements and determination of asymmetry.

5.2.1.1.1 Collection of skulls.

The information of the two museums sampled, number of skulls measured for each population, and the fifteen traits measured have already been provided in Chapter 4.

5.2.1.1.2. Indices of fluctuating asymmetry used and statistical analyses.

The indices of asymmetry and statistical analyses used in this study were explained in Chapter 3. Since two meristic traits were included, the tooth count of the maxilla and the mandible, two different composite indices were used, one for the metric traits and another for the meristic.

5.2.2. Genetic analyses and determination of genomic diversity.

5.2.2.1. Samples obtained and previously published data used.

Details of the samples obtained and the techniques used to extract and isolate DNA, and information about the thirteen microsatellites used, the amplification and interpretation of the microsatellite data has already been provided in Chapter 4.

5.2.2.2. Genomic diversity indices used.

The genomic diversity indices used, mean d^2, scaled mean d^2, multilocus individual heterozygosity, standardised heterozygosity and internal relatedness were explained in Chapter 3.

5.2.2.3. Evidence for historical bottleneck in the different populations sampled.

The methods used to investigate if the study populations had experienced a recent reduction in population size have been explained in chapter 3.

5.2.3 Environmental stress

Published results of environmental stress that bottlenose dolphins are facing in the areas studied included chemical pollutant levels (PCB's and DDT's). In the WNA the study of Hansen et al. (2004), King (1987), and Kuehl et al. (1991) analysed PCB and DDT congeners from bottlenose dolphins collected in the WNA. The data used to describe the chemical pollutant burden of the Gulf of California dolphins came from a study done by Schafer et al. (1984) in the south western coast of the USA. This was the closest data set that could be found, but can only be used as a reference. Details of the results found are listed in table 5.7. For by-catch rate, the study of Waring et al. (1998) was used for the WNAP population, NOAA (2001) for the WNAC, and Perez-Cortez and Rojas-Bracho (2002) for the GOC; details of the results found are listed in table 5.8.

5.3. Results.

5.3.1. Determination of developmental stability.

5.3.1.1. Detecting traits that depart from ideal FA.

The between sides variation (FA) was significantly greater in all cases ($p<0.001$) than expected due to measurement error when tested with the two-way ANOVA (sides by individuals, Palmer 1994). For all traits the mean of (R-L) is more than five times the standard deviation of the repeated measurements. Measurement error did not appear to differ among sub-populations or among traits, since no statistical significance was found when tested using a two-way ANOVA.

When tested for departures from normality, no trait showed the effects of antisymmetry as not a single scatter plot of traits revealed a bimodal curve; neither was platykurtosis present in the traits. Also no trait showed statistically significant departures from normality when tested with the Kolomogorov-Smirnov test (table 5.1).

Table 5.1. Statistical analyses for each trait in relation to ME, departures from normality, DA and size dependence. ***$p<0.001$, Bonferroni correction applied.

Effect tested	Statistic used	CBL n=190	WON n=218	AOT n=185	FTL n=182	OL n=205	COD n=188	MW n=208	LOP n=155
Measurement Error	two- way ANOVA	***	***	***	***	***	***	***	***
Departures from normality	Kolomogorov-Smirnov	0.10	0.07	0.06	0.07	0.08	0.03	0.12	0.16
	Sig.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Directional asymmetry	Mean	-0.01	0.04	-0.14	0.20	-0.16	0.01	0.02	0.01
	t-test t	-0.14	6.29	-12.2	13.8	31.2	0.71	0.62	1.60
	Sig.	n.s.	***	***	***	***	n.s.	n. s.	n.s.
Size dependence of FA	Spearman ρ	n. s.	n. s.	n. s.	n. s.	n. s.	n. s.	n. s.	n. s.

Effect tested	Statistic used	LAP n=196	WRAB n=221	UDG n=184	LDG n=167	LOR n=168	HOR n=164	LMF n=163
Measurement Error	two- way ANOVA	***	***	***	***	***	***	***
Departures from normality	Kolomogorov-Smirnov	0.15	0.15	0.05	0.10	0.12	0.16	0.18
	Sig.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Directional asymmetry	Mean	-0.27	0.16	0.01	0.02	0.15	0.07	0.06
	t-test t	-38.8	22.5	-0.14	0.25	11.5	16.9	7.47
	Sig.	***	***	n. s.	n. s.	***	***	***
Size dependence of FA	Spearman ρ	n. s.	n. s.	n. s.	n. s.	n. s.	n. s.	n. s.

Table 5.2. Basic statistics of FA between the sub-populations. Metric traits are given in cm.

	SUB-POPULATIONS								
	COASTAL WESTERN NORTH ATLANTIC			PELAGIC WESTERN NORTH ATLANTIC			GULF OF CALIFORNIA		
Trait	Mean ± S.D.	Min - max	n	Mean ± S.D.	Min - max	n	Mean ± S.D.	Min - max	n
CBL	0.06 ± 0.10	0.00 - 0.40	51	0.05 ± 0.06	0.00 - 0.20	100	0.05 ± 0.09	0.00 - 0.50	39
COD	0.10 ± 0.09	0.00 - 0.33	37	0.10 ± 0.09	0.00 - 0.39	72	0.08 ± 0.05	0.00 - 0.19	46
MW	0.12 ± 0.10	0.00 - 0.43	57	0.11 ± 0.07	0.00 - 0.31	110	0.10 ± 0.06	0.01 - 0.26	39
LOP	0.15 ± 0.14	0.01 - 0.78	48	0.13 ± 0.11	0.00 - 0.56	92	0.10 ± 0.07	0.03 - 0.21	14
UDG	0.44 ± 0.54	0.00 - 2.0	54	0.32 ± 0.53	0.00 - 2.0	99	0.31 ± 0.47	0.00 - 1.0	29
LDG	0.53 ± 0.57	0.00 - 2.0	53	0.50 ± 0.54	0.00 - 2.0	94	0.35 ± 0.49	0.00 - 1.0	20
CFA-MET	0.35 ± 0.23	0.00 - 1.08	60	0.33 ± 0.17	0.02 - 0.81	112	0.24 ± 0.16	0.01 - 0.67	46
CFA-MER	0.87 ± 0.81	0.00 - 4.0	60	0.74 ± 0.70	0.00 - 3.0	107	0.48 ± 0.57	0.00 - 2.0	33

Met-Metric
Mer-Meristic

Table 5.3. Levene's test for homogeneity of variances to test for differences in FA among sub-populations.

Trait	Levene Statistic	Significance
CBL	4.93	**
COD	4.62	**
MW	6.99	***
LOP	2.88	n. s.
UDG	1.51	n. s.
LDG	1.51	n. s.
CFA-MET	4.69	**
CFA-MER	0.59	n. s.

Significance: **$p<0.01$, ***$p<0.001$, Bonferroni correction applied.

From the results of the two way ANOVA, and from the two-tailed one sample t-test against a mean of zero performed to test for directional asymmetry (DA), DA was found in WON, AOT, FTL, OL, LAP, WRAB, LOR, HOR, and LMF (table 5.1). Finally no trait showed size dependence for FA when tested using the Spearman bivariate rank correlation (table 5.1). The traits that showed FA were CBL, COD, MW, LOP, UDG, and LDG. No further statistics were attempted for the remaining traits. No correlation was found across FA traits using the Spearman coefficient therefore FA traits are independent from each other.

5.3.1.2. Differences in FA between sexes and age groups.

The results of *F*-tests with respect to variance between sex and age groups found no statistical significance. Therefore these were pooled for subsequent analyses.

Table 5.4. Basic statistics of several genomic diversity indices in the sub-populations.

	n	Mean d^2	Scaled Mean d^2	Heterozygosity	Standardised Heterozygosity	Internal Relatedness
WNAC	28	22.3 ± 19.2	0.010 ± 0.009	0.58 ± 0.15	0.935 ± 0.24	0.21 ± 0.22
WNAP	52	40.8 ± 32.2	0.014 ± 0.011	0.67 ± 0.13	0.996 ± 0.13	0.13 ± 0.16
GOC	44	38.9 ± 23.4	0.032 ± 0.040	0.80 ± 0.11	1.054 ± 0.20	0.07 ± 0.13

Table 5.5. ANOVA to test for differences of several genomic diversity indices among the sub-populations.

	Mean d^2	Scaled Mean d^2	Heterozygosity	Standardised Heterozygosity	Internal Relatedness
$F_{(2,124)}$	4.80	7.87	26.32	3.47	6.30
Significance	**	***	***	*	**

Significance: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, Bonferroni correction applied.

Table 5.6. Correlation between indices of genomic diversity (Pearson coefficient).

	Mean d^2	Scaled Mean d^2	Heterozy-gosity	Standardised Heterozygosity
Scaled Mean d^2	0.33***			
Heterozygosity	0.21n.s.	0.26***		
Standardised Heterozygosity	0.16n.s.	0.07n.s.	0.79***	
Internal Relatedness	-0.13n.s.	-0.14n.s.	-0.89***	-0.88***

Significance: n.s.=not significant, *** $p < 0.001$, Bonferroni correction applied; n=124.

5.3.1.3. Differences in FA among the sub-populations of bottlenose dolphin studied.

Basic statistics for each of the populations are shown in table 5.2. The results of the Levene's test for testing differences in the magnitude of FA among the populations was significant for CBL, and COD at $p<0.01$ (Bonferroni correction applied), while MW was significant at the $p<0.001$ level (Bonferroni correction applied, table 5.3). The mean FA of the six traits was highest in the WNAC population and lowest values were found in the GOC population.

5.3.2. Genomic diversity.

Table 5.4 summarise the results of the different indices of genomic diversity used in the subpopulations studied. The WNAC population showed the lowest level of genomic diversity for all the indices studied.

The five indices of genomic diversity are significantly different among the populations studied. However when the Bonferroni correction was applied

Table 5.7. Value of *M* for the microsatellite data for each population.

	M
WNAC	0.580
WNAP	0.782
GOC	0.729

standardised heterozygosity was no longer significant (table 5.5). Table 5.6 shows the linear correlations of the five indices of genomic diversity. High correlation across the indices indicates that these are not independent from each other, similar results where already explained on section 3.3.2.

5.3.3. Relationship between developmental stability and genomic diversity.

No single population had significant relationships between FA and the indices of genomic diversity for single traits and for the composite indices of asymmetry.

5.3.4. Detection of a recent bottleneck in the study populations.

None of the performed bottleneck tests, -mode shift and heterozygosity excess analyses- implemented in the software Bottleneck 1.2 detected a recent reduction in population size for any of the three populations. However by using *M* one population was detected to have suffered from recent reduction in size. The value showed by the WNAC is the lowest among the three populations (0.580). Garza and Williamson (2001) established the threshold of 0.68, and any population with seven loci or more below this threshold is considered as reduced. Table 5.7 shows the value of *M* for each study population.

Table 5.8. Geographical comparison of chemical pollutants.

Pollutant	Region	Mean	Min-max	Sex	Age	n	Source
ΣPCB	WNAC	45.3[a]	8.3 - 110.1	Both	Juvenile	31	Hansen et al. (2004)
		92.8[a]	10.2 - 110.1	M	Adult	18	Hansen et al. (2004)
		18.3[a]	2.0 - 29.1	F	Adult	13	Hansen et al. (2004)
	WNA	138.4	5.7 - 31.8	M	-	3	Kuehl et al. (1991)
		62.3	17.4 - 157	F	-	9	Kuehl et al. (1991)
		97.7	5.7 - 195	M	-	8	King (1987)
		22.4	2.2 - 41.2	F	-	7	King (1987)
	California	100.4	13.8 - 182.8	M	-	3	Schafer et al. (1984)
		38.7	13.9 - 65.5	F	-	3	Schafer et al. (1984)
tDDT's	WNAC	46.6[a]	7.86 - 87.2	Both	Juvenile	31	Hansen et al. (2004)
		97.6[a]	8.29 - 87.2	M	Adult	18	Hansen et al. (2004)
		12.7[a]	1.15 - 22.1	F	Adult	13	Hansen et al. (2004)
	WNA	38.5[b]	3.7[b] - 80[b]	M	-	3	Kuehl et al. (1991)
		7.4[b]	0.52[b] - 21.2[b]	F	-	9	Kuehl et al. (1991)
		31.8	4.3 - 63.9	M	-	8	King (1987)
		4.6	0.6 - 8.0	F	-	7	King (1987)
	California	1297.6	328 - 2745	M	-	3	Schafer et al. (1984)
		1356.6	180 - 2957	F	-	3	Schafer et al. (1984)

Concentrations are in mg Kg^{-1} lipid in blubber.
[a]Geometric mean.
[b]DDE

5.3.5. Environmental stress.

Published information for the two different kinds of environmental stress studied for the bottlenose dolphin is shown in tables 5.8 for the chemical pollutants and 5.9 for the by-catch rate. Note that table 5.8 shows the results of the concentration of chemical pollutants in the California area of the eastern North Pacific, and not the Gulf of California proper.

Table 5.9. Geographical comparison of yearly estimates of bottlenose dolphin catches.

Region	Fishery	By-catch rate (per year)	CV	Year	Reference
WNAC	All	9	0.67	1994-1998	NOAA (2001)
WNAP	Drift Gillnet	13.6	0.06	1992-1996	Waring et al. (1998)
	Pair trawl	44.8	0.28	1992-1995	Waring et al. (1998)
GOC	All	0[a]		2000	Perez-Cortez and Rojas-Bracho (2002)

[a] 15 dolphins captured alive.

5.3.5.1. Chemical pollutants.

The concentration of chemical pollutants in the different regions is very similar for ΣPCB's in adult males. The concentration of DDT's in the blubber of bottlenose dolphin is higher (1297.6 for males, 1356.6 for females) in the California population, although the sample size is small (only three animals, table 5.8).

5.3.5.2. By-catch rate.

The by-catch rate in the western North Atlantic is low, involving only a small number of animals. In the Gulf of California, there has been no report of a direct catch in fisheries, although the dolphins are captured alive so they can be used in aquariums around the world (table 5.9).

5.4. Discussion.

No difference in FA between sex and age class was found within each population. Sexual dimorphism has not been found in the skull of the species (Tolley et al. 1995). Stability during development is a stochastic process therefore no difference in age classes or sex should be expected.

No single population showed a significant correlation between FA and the indices of genomic diversity. One reason could be sample size. In the study of harbour porpoise presented in chapter three, the sample size of the populations that had significant relationships (after Bonferroni correction) between FA and genetic diversity were around 130 individuals, whereas in the present study the largest sample size was for the pelagic population of the western North Atlantic, which comprised 52 individuals. The smallest population (Norway, n=47) in the harbour porpoise study also failed to show a significant correlation.

An inverse relationship between FA and genomic diversity was found at the population level. The WNAC population showed the least genetic diversity for the five indices, and also contained the most asymmetrical individuals; while the opposite was present in the GOC. This finding suggests that the most genetically stressed population is also the population with the highest level of developmental instability. However, since only three independent populations were assessed it is difficult to establish a general pattern on this trend. More independent populations should be assessed so a general idea could be established.

High levels of genetic diversity are seen as an evolutionary advantage to resist stress (Amos and Harwood 1998). Acevedo-Whitehouse et al. (2003) found that more inbred California sea lions *Zalophus californianus* were more susceptible to diseases and the treatment to cure diseases took longer than less inbred animals. Valsecchi et al. (2004) found that striped dolphins *Stenella coeruleoalba* with high levels of inbreeding died early during a morbillivirus outbreak and they also strand more easily than those with higher levels of genetic diversity.

The results found in this study, that less genetically diverse organisms are more asymmetrical, support the theory of genetic stress as an important factor to

produce disturbances during development (Leary et al. 1983, Clarke 1995). This finding is in accordance with the results showed in chapter 3 of this study and with previous studies (e.g. Hoelzel et al. 2002a, Leary et al. 1983, Lens et al. 2000a, b). Nevertheless, recently some studies have failed in established a direct relationship between measures of genomic diversity and inbreeding depression. Overall et al. (2005) did not find any relationship between two measures of fitness, birth weight and neonatal survival, and several measures of genomic diversity including mean d^2, standardised heterozygosity and internal relatedness. They explained the need of better information on pedigrees to estimate inbreeding depression and the use of large numbers of markers. Balloux et al. (2004) also found that approximately 200 markers are needed to have a good estimation of the role of heterozygosity on fitness.

Recent events suggests that the WNAC population may be facing inbreeding depression as a consequence of depletion in the population size, especially during the 1987-88 mass mortality event which was estimated to decimate the population by as much as 53% (Scott el al. 1988, cited in NOAA 2001). Another fact that may have an impact in population fragmentation of the WNAC and that may have been going on for several decades is that the interaction of the species with the fishery industry may be more serious than the data available and presented in this study in the form of by-catch rate. The National Oceanic and Atmospheric Administration of the US (NOAA) concluded that this population exceeded the potential biological removal (PBR, an index to calculate the sustainability of the stock) in the mid and late 1990's (NOAA 2001) based on the abundance of strandings that showed fisheries related mortality.

The results from testing for reduction in size with M (Garza and Williamson 2001) showed that the WNAC population could have experienced a recent reduction in

size, therefore another explanation for the loss of genetic diversity in the WNAC population may be that this population could represent a founder event originating from the WNAP (Hoelzel et al. 1998a, Natoli et al. 2004). New coastal areas may become available after the retreat of ice at the end of the ice age (Hoelzel 1998), but because the founder genotypes are rare in the source population, may be they were either unsampled or are currently extinct in the WNAP (Holezel et al. 1998a).

Inbreeding reduces genetic variation in a population and also increases homozygosity as the number of unique alleles at loci is reduced. This could lead to a reduction in fitness. If there are specific dominant genes that influence developmental stability (Falconer 1989), the expression of recessive alleles may produce a decrease in stability in inbred populations (Leary et al. 1983, Møller and Swaddle 1997). Coltman et al. (1999) reported that inbred Soay sheep (*Ovis aries*) were more susceptible to parasitic infections and were also less likely to survive. In a laboratory based study a comparison on 20 generations of the bank vole (*Clethrionomys glareolus*), Zakharov and Sikorski (1997) found that more asymmetrical characteristics in the skull of the voles were found in animals that were experimentally inbred over time compared with the founder population.

The magnitude of the association between FA and inbreeding may itself depend on how much stress is experienced during development (Palmer 1996, Lens 2000a). In this study environmental pressures that study populations are facing were difficult to asses mainly because there was limited availability of data. However, this could be a factor to consider in explaining the differences in FA and genetic diversity among the populations studied. The evolutionary potential of a trait can be hypothesised to increase under increasing stress because more of the underlying genetic variability is being revealed, thus strong association

between inbreeding and FA under severe habitat disturbance, but not under more relaxed conditions could be expected (Lens et al. 2000a). Inbreeding depression increases under relatively stressful conditions (see review in Armbruster and Reed 2005).

If an organism is exposed to suboptimal environmental conditions, the efficiency in which this organism manages its energy will be reduced, thus the stability of developmental pathways will be affected, as energy is used to maintain stability this will lead to a greater phenotypic variance and to a tendency of the organisms to produce bilaterally asymmetric traits. There may be an interaction between environment and genotype due to genetic factors that influence the susceptibility of individuals and populations to the effects of the environment (Møller & Swaddle 1997).

It has been established that the relationship between FA and environmental stress are typically weak and difficult to detect (Hoffman and Woods 2003). However, it has been proven that relatively high levels of chemical pollutants have an influence on other measures of fitness such as reproduction (e.g. Hendrickx et al. 2003), and susceptibility to infectious diseases and related mortality (e.g. Jepson et al. 1999, 2005).

The use of developmental stability as an indirect measure of fitness of individuals in the bottlenose dolphin populations could be used to identify populations subject to stress before these populations are impacted in other fitness components, or before a population is irreversibly affected (Clarke 1995). The coastal population of the western North Atlantic population of bottlenose dolphin has been identified in this study as a population that is subject to genetic stress, for that reason remedial action should be taken to avoid inbreeding depression.

This study corroborates the importance in maintaining genetic diversity as an advantage for natural populations to subsist.

Chapter 6: Summary of results and recommendations for further research.

In this study the relationship between developmental stability and genetic diversity in harbour porpoises of the eastern North Atlantic and in bottlenose dolphins of the western North Atlantic and the Gulf of California has been established. Three populations of harbour porpoises and three of bottlenose dolphin dolphins showed a direct relationship between levels of fluctuating asymmetry and levels of genetic diversity. However, three problems were also identified: 1) composite indices of asymmetry showed that two traits were correlated, therefore non-independent on the estimation of FA. 2) The five measurements of genomic diversity are somehow correlated across each other. This also means that these measurements are not independent and that they may be measuring the same thing; 3) finally, only three populations on each study were assessed. This may make difficult the interpretation of the results obtained since only two degrees of freedom were involved. The relationship between developmental stability and environmental stress was not significant and it was also very difficult to assess.

In addition population structuring for both species was determined for the geographic locations studied using morphological skull characters and microsatellite loci.

6.1. Population structure in the eastern North Atlantic population of the harbour porpoise.

In the eastern North Atlantic both morphometric and genetic analyses classified harbour porpoises into three distinct stocks: Norwegian, British and Danish. The Norwegian stock showed the greatest level of morphological differentiation. Relatively smaller maxillae and mandibles suggested an adaptation to the

environment and type of prey found off the coast of Norway, since porpoises prey mainly on pelagic and mesopelagic fish, in contrast with benthic species that are more often predated by porpoises in the British and Danish population (Aarefjord et al. 1995). The Norwegian population also showed the highest level of genetic differentiation (F_{ST}=0.178 and Rho_{ST}=0.237). This is in agreement with previous studies based on microsatellite loci (Andersen et al. 2001) and mtDNA (Tolley et al. 2001, Wang and Berggren 1997).

The Danish sub-population was also found to be a single stock. This result does not support the theory of sub-structuring into Danish North Sea-Skagerrak and Inner Danish Waters sub-populations. F_{ST} values are similar to those reported by Andersen et al. (2001) but they are not significant. Rho_{ST} values are also small but not significant. The results of percentages of correct classification for the discriminant function analysis for morphological traits and for the Bayesian analysis for the genetic data were also consistent with this observation.

Finally, the results from the morphological and genetic analyses showed that the British sub-population was found to be a single unit and not sub-structured into two sub-populations as proposed by several studies (e.g. Andersen et al. 2001, Gaskin 1984, IWC 1996, Walton 1997). Percentages of correct classification for morphological and Bayesian analyses for the genetics did not show a separate unit. F_{ST} and Rho_{ST} values were small and non significant when porpoises from the British North Sea and the Irish-Welsh subpopulations were compared. However, these values were similar to those reported by Andersen et al. (2001). They found significant differences to support the sub-structuring of the British population.

6.1.1. Recommendation for future research.

The use of other genetic markers such as mtDNA could offer the opportunity to study female philopatry. In marine mammal species, population structure is generally more evident from mtDNA than microsatellite loci (Natoli et al. in press). This could be explained by the difference in effective population size represented by the two genomes, and also because of the more frequent dispersal of males (Hoelzel et al. 2002c). The development of Y-chromosome genetic markers would allow for an assessment whether genetic variation between males from different populations exists in addition to quantifying levels of male relatedness between populations (Engelhaupt 2004). Larger sample sizes will also allow a better understanding by increasing statistical power. The use of other ecological characteristics like contaminant analysis, parasite load, stable isotopes, feeding ecology, movement patterns and acoustic profiles, additional to morphologic and genetic studies may be useful to detect population boundaries.

6.2. The relationship between developmental stability, genomic diversity and environmental stress in the eastern North Atlantic population of harbour porpoise.

Different levels of FA and genomic diversity were found in the eastern North Atlantic population of harbour porpoise. Two approaches were incorporated to analyse the relationship between developmental stability with genomic diversity and environmental stress. The first took into account the three putative populations found in chapter 2. The second approach taken was to subdivide the three putative populations into five management units as proposed by several authors (e.g. Andersen et al. 2001, Gaskin 1984, IWC 1996, Walton 1997). The results found on both approaches indicated that the Norwegian population showed the highest level of FA and the lowest level of genomic diversity. Significant relationships between FA and the indexes of genomic diversity were found for the British North Sea

management unit, and for the British sub-population. In particular the composite index of individual asymmetry had more power to detect the relationship with the indexes of genomic diversity than single traits. Populations that had larger sample sizes recorded significant correlations. The relationship between FA and environmental was difficult to assess since only three populations were involved in the analysis, and the availability of data was limited. Reasons to explain this included sample size, and the possibility that selection occurs in organisms with high level of FA under stressful conditions (Hendrickx et al. 2003).

6.2.1. Recommendation for future research.

Further research is needed to establish a relationship between FA and environmental stress. This could give a better picture of the different levels of FA and genetic diversity found in this study. More populations should be used to give a better picture of what is happening in the eastern North Atlantic population of harbour porpoise. Environmental stress should be assessed taking into account more variables that should be measured together. The inclusion of other pollutants, such as the concentration of polychlorinated and polybrominated organic pollutants, e.g. toxaphene and bromide diphenyl ethers, would provide a better understanding of the role of chemical pollution as a threat for this species. Another possibility would include the increase in sample size of the porpoises in respect to environmental data.

6.3. Morphological and genetic comparison between two populations of bottlenose dolphin (*Tursiops truncatus*) from the western North Atlantic and one from the Gulf of California.

This chapter focused in a morphological and genetic comparison of two bottlenose dolphin populations in the western North Atlantic (WNA), and used an out-group, the population of the Gulf of California (GOC), in order to compare the magnitude of

differentiation between the coastal and the pelagic population of the WNA. DFA found that the morphological traits that discriminate between the two populations were related with the size of the beak. Pelagic bottlenose dolphins had relatively shorter mandibular traits than the coastal population. This finding has also been reported for the common dolphin (Heynning and Perrin 1994). The population from the GOC showed relatively larger skulls. This result also supports the theory that bottlenose dolphin living in tropical areas have larger body sizes (Wells and Scott 2002). The main morphological differences in the skull of the GOC from those of the WNA had to do with traits related with vision.

The magnitude of genetic differentiation between the two WNA populations was the highest among the pairwise comparisons (F_{ST} 0.192 and Rho_{ST} 0.308). This result showed that in cetaceans geographic distance is not always correlated with genetic distance (Hoelzel 1998, Natoli et al. 2004). The results presented in this chapter also supported the idea that habitat use and resource exploitation may be important in reproductive isolation, therefore genetic and morphological differences in sympatric populations are evident (Natoli et al. 2004, 2005).

6.3.1. Recommendation for future research.

The two populations of the WNA have been studied intensively in respect to several ecological (e.g. Mead and Potter 1995, Walker et al. 1999), physiological (Hersh and Duffield 1990) morphological (Mead and Potter 1995, chapter 4) and genetic (Hoelzel et al. 1998a, Natoli et al. 2004, chapter 4 of this study) characteristics. The amount of evidence gathered in the last years has made clear the need for a revision of the genus. Differentiation between populations for post-cranial skeleton and other body structures would provide valuable information to consider changes at alpha-level taxonomy.

6.4. The relationship between developmental stability, genomic diversity and environmental stress in the bottlenose dolphin.

An inverse relationship between developmental stability and genomic diversity was found among the three populations of bottlenose dolphins studied. The WNAC population showed the highest level of FA and the least for genomic diversity. A recent bottleneck was also detected using *M* (Garza and Williamson 2001). One reason to explain the low level of genetic diversity found in this population is that it has been considered for some authors (e.g. Hoelzel et al. 1998a) as a founder population diverged possibly from the pelagic population. This study also supports the hypothesis of the importance in maintaining genetic diversity as a condition for natural population to subsist (Hoelzel et al. 2002a). No significant relationships between developmental stability and genomic diversity were found. An explanation to this was relatively smaller sample sizes than the study of harbour porpoise. The relationship between developmental stability and environmental stress was difficult to assess because the data available was not abundant for the WNA and non-existent in the case of the GOC.

6.4.1. Recommendation for future research.

An increase in sample size, in the number of traits that show FA, in the number of study populations and adding other measures of environmental stress may provide a better understanding of the relationship between developmental stability and stress. Comparisons between the WNAC and other coastal populations of bottlenose dolphins around the world for FA and measures of genomic diversity would provide an idea of the level of developmental instability experienced in the WNAC. A comparison of the samples of the GOC used in this study with other samples from the same area would clarify to which of the populations present in the GOC belong the samples analysed in this study.

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Appendix 1: Records held in the museums for the animals sampled in this study.

A.1. Harbour porpoise.

A.1.1. Zoology Museum, University of Oslo, Oslo, Norway.

Consecutive	ZM lab number	ID	Sex	Date	Location	Source	Length (cm)	Mass (Kg)
1	31	2000-7	M	21-Mar	LEPSØY	GILLNET	136.5	35.5
2	32	99-11	F	wk 5 april	Vadsø	Gillnet	132	37
3	33	99-05	M	wk 5 april	Karmøy	Gillnet	129.5	37
4	34	2000-8	F	21-Mar	LEPSØY	GILLNET	138.5	48.5
5	35	99-15	M	wk 5 april	Vadsø	Gillnet	129	38
6	36	99-06	M	wk 5 april	Karmøy	Gillnet	128	37
7	37	2000-9	M	17-Mar	KARMØY	GILLNET	131	36.8
8	38	99-26	M	mid may	Kirkenes	gillnet	118	30
9								
10								
11	41	98-01	F	29-Jun-98	Sognefjord	Stranding	71.5	7.4
12	42	2000-36	F	04-Apr	SKILLEFJORD I ALTAFJORD	GILLNET	104.5	37
13	43	2000-54				GILLNET		
14	44	2000-50	M	21-Mar	60 47N 5 15E, Masfjord	GILLNET	125	35.5
15	45	2000-51	M	03-Apr	70 04N 29 06E	GILLNET	148	49.5
16	46	2000-30	F	21-Mar	59 18 03N 5 06 75E	GILLNET	140	39
17	48	2000-4	F	30-Mar	MANDAL	GILLNET	139	39
18								
19	49	99-32	F	28-Apr-99	Lurøy (Norland)	Gillnet	133.5	49
20	50	99-21	M	wk 12 april	Egersund	Gillnet	147	45
21	51	99-12	M	wk 5 april	Vadsø	Gillnet	139	47
22	52	2000-25	F	18-Feb	HARDANGERFJORD	GILLNET	130	40.5
23	53	99-27	M	mid may	Kirkenes	gillnet	131	43

24	54	99-23	M	mid apr 99	Kjøllefjord	Gillnet	124	35
25	55	99-24	M	24-Apr	9791 Gamvik	gillnet	135.5	43.5
26	56	2000-22	M	07-Mar	TRÆNA	GILLNET	121	36
27	57	99-22	M	19-Apr-99	Mandal	Gillnet	116.5	30
28	58	99-14	F	wk 5 april	Vadsø	Gillnet	153	59
29	59	2000-31	M	14-Mar	Karmøy, 59.25N	GILLNET		35
30	60	2000-44	M	04-Apr	65 13.02N 11 53.43E	GILLNET	136	38.5
31	61	99-13	F	wk 5 april	Vadsø	Gillnet	150	62.5
32	62	2000-37	M	02-May	69 54.5N 20 48 E, Rotsund	GILLNET	120	32
33	63	2000-39	F	05-Apr	70 25N 22 55E, KVALFJORD	GILLNET	130	42.5
34	64	99-07	M	wk 5 april	Karmøy	Gillnet	123	35
35	65	2000-5	M	27-Mar	LEPSØY	GILLNET	133	43.5
36	66	2000-6	F	24-Mar-00	LEPSØY	Gillnet	133	34.5
37	67	2000-48-F	M		FEDJE		59	3.014
38	68	2000-29	M	23-Mar	LEPSØY	GILLNET	130	31
39	69	99-08	F	wk 5 april	Karmøy	Gillnet	133	41
40	70	VF-06-99	F	04-Jun-99	Jarfjord	Kilenot	164	63
41	71	99-10	F	wk 5 april	Vadsø	Gillnet	149	69.5
42	72	99-03	M	29-Mar-99	Kjøllefjord	Gillnet	151	53.5
43	73	99-09	F	wk 5 april	Vadsø	Gillnet	156.5	74
44	74	2000-48	F	06-Apr	FEDJE	GILLNET	144.5	49.5
45	75	2000-46	M	27-Mar	65 13.15N 11 53.21E	GILLNET	123	33
46	76	99-28	M	mid may	Kirkenes	gillnet	145	55
47	77	99-04	F	29-Mar-99	Kjøllefjord	Gillnet	158	73
48	78	99-30	M	28-Apr-99	Lurøy (Norland)	Gillnet	117	28.5
49	79	VF-04-99	M	04-Jun-99	Jarfjord	Kilenot	138	51
50	80	VF-03-99	M	04-Jun-99	Jarfjord	Kilenot	131	42

A.1.2. Zoology Museum, University of Copenhagen, Copenhagen, Denmark.

Consecutive	Year	Date	ID	Length	SEX	METHOD	ICES AREA
1	1986	11.22	CN346	155	F	IC	IIIc
2	1986	10.01	CN353	134	F	IC	IIIc
3	1987	04.08	CN361	111	F	IC	IIIas
4	1987	04.07	CN365	126	M	IC	IIIc
5	1987	04.24	CN370	140	M	IC	IIIas
6	1987	04.24	CN371	122	M	IC	IIIas
7	1987	04.24	CN372	129	F	IC	IIIas
8	1987	04.26	CN373	100	M	IC	IIIas
9	1987	04.26	CN374	113	F	IC	IIIas
10	1987	04.27	CN375	108	F	IC	IIIas
11	1987	04.27	CN376	118	M	IC	IIIas
12	1987	04.14	CN377	123	F	IC	IIIc
13	1987	05.02	CN378	173	F	IC	IIIas
14	1987	07.20	CN404	138	M	IC	IIIc
15	1987	08.22	CN405	95	M	IC	IIIc
16	1987	03.27	CN408	101	F	IC	IIIas
17	1987	06.05	CN410	153	F	ST	IIIas
18	1987	04.02	CN414	106	F	IC	IIIas
19	1987	10.25	CN422	99	F	IC	IIIc
20	1988	01.28	CN428	148	F	ST	IIIas
21	1988	02.22	CN435	117	M	IC	IIIas
22	1988	03.09	CN436	119.5	F	IC	IIIc
23	1988	03.09	CN437	121	F	IC	IIIc
24	1988	03.19	CN438	131	M	IC	IIIas
25	1988	03.19	CN439	124	M	IC	IIIas
26	1988	03.21	CN440	110	M	IC	IIIas

27	1988	03.24	CN441	120	F	IC	IIIc
28	1988	03.24	CN442	114	M	IC	IIIc
29	1988	03.29	CN443	113	M	IC	IIIas
30	1988	04.05	CN444	124	M	IC	IIIas
31	1988	04.05	CN445	117	F	IC	IIIas
32	1988	04.05	CN446	112	M	IC	IIIas
33	1988	04.05	CN447	114	F	IC	IIIas
34	1988	04.05	CN448	113	M	IC	IIIas
35	1988	04.05	CN449	99	M	IC	IIIas
36	1988	04.05	CN450	132	M	IC	IIIas
37	1988	04.05	CN451	120	M	IC	IIIas
38	1988	04.06	CN452	101	F	IC	IIIc
39	1988	04.06	CN453	120	M	IC	IIIc
40	1988	04.07	CN455	123	F	IC	IIIas
41	1988	04.12	CN457	157	F	IC	IIIc
42	1988	04.10	CN458	118	F	IC	IIIas
43	1988	04.12	CN459	48	M	NN	IIIc
44	1988	04.16	CN478	129	M	IC	IIIas
45	1988	04.16	CN479	134	M	IC	IIIas
46	1988	06.30	CN503	119	F	IC	IIIc
47	1988	00.00	CN536	140	M	ST	IIIas
48	1988	08.29	CN537	137	M	ST	IIIc
49	1988	09.15	CN538	137	M	ST	IIIc
50	1988	08.16	CN576	148	F	ST	IIIc
51	1988	09.19	CN577	89	M	ST	IIIc
52	1988	09.21	CN578	126	F	ST	IIIc
53	1988	10.13	CN579	112	M	ST	IIIc
54	1988	10.28	CN580	103	F	ST	IIIc
55	1989	03.05	CN584	112	M	IC	IIIas
56	1989	03.10	CN585	126	F	IC	IIIc

57	1989	05.08	CN591	110	M	IC	IIIc
58	1989	05.10	CN592	144	F	IC	IIIc
59	1989	05.09	CN593	122	F	IC	IIIas
60	1989	05.24	CN594	120	M	ST	IIIas
61	1989	07.15	CN613	123	F	ST	IIIan
62	1990	01.04	CN677	147	M	ST	IVb
63	1989	12.19	CN679	119	F	ST	IIIc
64	1990	03.21	CN680	151	F	IC	IVb
65	1990	04.17	CN681	139	M	IC	IVb
66	1990	04.25	CN684	131	M	IC	IIId
67	1990	05.25	CN687	173.5	F	ST	IVb
68	1990	05.25	CN688	89	M	NN	IVb
69	1990	06.11	CN689	121	F	IC	IIIc
70	1990	06.13	CN690	163	F	ST	IIIc
71	1990	06.21	CN692	132.5	M	IC	IIIas
72	1990	06.21	CN693	124	M	IC	IIIc
73	1990	08.28	CN711	151	M	IC	IIIc
74	1990	05.25	CN713	117	F	ST	IIIc
75	1990	08.27	CN714	148	M	IC	IIIc
76	1990	10.07	CN715	94	M	ST	IIIc
77	1990	11.09	CN716	170	F	IC	IIIas
78	1990	11.09	CN717	111	F	IC	IIIas
79	1991	03.24	CN749	0	F	NN	IIIas
80	1991	04.05	CN750	125.5	F	ST	IIIas
81	1991	09.23	CN826	149	F	IC	IIIas
82	1991	07.29	CN827	155	M	ST	IIIas
83	1991	07.31	CN828	95	M		IVb
84	1991	08.01	CN829	155	M		IVb
85	1991	09.04	CN830	148	M		IIIan
86	1991	09.04	CN831	108	M		IIIc

87	1991	09.04	CN832	130	M		IIIc
88	1991	09.16	CN839	123	M		IVb
89	1991	09.16	CN840	138	F		IVb
90	1991	09.23	CN844	101	M		IV
91	1991	09.23	CN845	139	M		IV
92	1991	04.05	CN889	110.5	M	IC	
93	1992	03.23	CN890	167	F	IC	IIIc

IC incidental catch
ST stranding
IIIan Skagerrak
IIIas Kattegat
IIIc Belt Sea
IIIb Sound
IIId Baltic proper
IV b cental North Sea

A.1.3. Natural History Museum, London, UK.

Consecutive	ID	Date	Sex	Length	Location
1	1867.4.12.204				
2	1897.7.30.1				
3	1951.12.14.1		male	5' 2"	
4	1953.3.3.2				Burnham on Crouch, Essex.
5	1954.9.18.1	No history.			
6	1958.11.21.1				Oldany Harbour, Lairg, Sutherland.
7	1961.5.29.1				20 miles East of Muckle Flogga, Shetlanda.
8	1965.3.30.1.	30 January, 1960	male	4' 3"	Mablethorpe, Lincolnshire, England.
9	1965.3.30.2		male		St. Leonards, Sussex.
10	1968.1.12.1.	Found on or ca. 4 Aug.1967			Found in Strand-line ca. 5M.

11	1981.910	1 September, 1977			Bay of Creekland, Hoy, Orkney Isles, Scotland.
12	1992.263	13 May 1912	male	3' 8"	
13	1992.265		male		Cullercoats, Scotland.
14	1992.266	18 October 1923			Hernsley Beach.
15	1992.86	No history.			
16	365b	registered in 1846 (= 1846.12.15.6)			British Isles, English Coast.
17	365g	No history.			British Isles, English Coast.
18	365a	registered in 1841 (= 1841.1.12.14)			British Isles.
19	365e	registered in 1865 (= 1865.10.9.23)			Margate, Kent, England.
20	365h				
21	SW 1916/18	24 August, 1916		5' 0"	Skinningrove, Yorkshire.
22	SW 1926/29	20 August, 1926	female	4' 7"	South Shields, Durham.
23	SW 1928/45	16 October, 1928		ca. 4' 0"	Grimsby, Lincolnshire.
24	SW 1928/49	03 November, 1928	male	5' 5.5"	Greencastle. W. Belfast, Antrim, Northern Ireland
25	SW 1929/13	14 May, 1929	female	5' 0"	Lowestoft, Suffolk.
26	SW 1933/15	02 June, 1933		5' 9"	Aberystwyth. Cardigan, Wales
27	SW 1933/24	13 August, 1933		4' 6"	Faversham, Kent.
28	SW 1933/50	30 December, 1933	male	4' 10.5"	Lyme Regis, Dorset.
29	SW 1934/14	01 March, 1934	female	5' 0"	Hope Cove, Devon.
30	SW 1934/32	08 June, 1934	male	4' 1.5"	Ballycastle, Antrim, Northern Ireland.
31	SW 1934/43	21 August, 1934	female	5' 3"	Heysham, Lancashire.
32	SW 1934/45	18 September, 1934	male	3' 8.5"	Haverigg, Cumberland.
33	SW 1934/48	25 September, 1934	male	5' 4"	Pembrey. Carmarthen.
34	SW 1934/51	03 October, 1934	female	5' 3"	New Quay, Aberystwyth, Cardiganshire.
35	SW 1936/29	14 September, 1936		ca. 6' 6"	Pembrey, Carmarthenshire.
36	SW 1937/14	14 July 1937	female	5' 0"	Maryport, Cumberland.
37	SW 1937/24	16 August, 1937	female	5' 0"	Porthcawl. Glamorgan.
38	SW 1937/36	24 December, 1937	female	5' 4.5"	Porteynon, Glamorgan.
39	SW 1938/11	06 May, 1938		4' 8"	Cromer, Norfolk.
40	SW 1939/15	12 June 1939	male	4' 7"	Winchelsea, Sussex.

41	SW 1950/21	01 August, 1950		59' 4"	Maryport. Cumberland.
42	SW 1950/28	19 October, 1950	female	4' 6.1/4"	Gorleston. Suffolk.
43	SW 1954/15	06 September, 1954		5' 8"	Seaham. Durham.
44	SW 1956/11	03 April, 1956	male		Kingsgate Bay, Broadstairs, Kent.
45	SW 1965/36	05 December, 1965		4' 10"	Gibraltar Point. Lincolnshire.

A.1.4. National Museum of Scotland, Edinburgh, UK.

Consecutive	NSM ID	Sex	Length (cm)	Location	Date
1	1991.145.25	F	152	England, Essex, Clacton, TM1715	16.9.1991
2	1995.224.1	F	141	England, Cornwall, Porth Cothan, SW860720	15.3.1995
3	1995.224.2	M	123	England, Cleveland, Seaton Carew, NZ531295	20.4.1995
4	1997.112.7	F	171	Wales, Dyfed, Ynys-las, SN609947	24.6.1996
5	1997.116.47	M	153	Scotland, Aberdeen beach, NJ963128	18.12.1995
6	1997.116.48	M	152	Scotland, Orkney, South Walls, Myre Bay, ND327910	17.11.1995
7	1997.116.49	F	131	Scotland, Sutherland, Brora, Dalchalm beach, NC915054	6.12.1995
8	1997.116.52	M	152	Scotland, Aberdeen beach, NJ954076	4.9.1995
9	1997.116.58	F	132	Scotland, Banffshire, Buckie, Portessie, NJ445667	3.7.1995
10	1997.116.66	F	126	Scotland, Morayshire, Lossiemouth, NJ215710	31.3.1996
11	1997.116.70	F	118	Scotland, Morayshire, Portgordon, NJ388643	7.11.1996
12	1997.116.74	M	119	Scotland, Morayshire, Covesea, NJ190712	2.5.1996
13	1997.116.75	M	145	Scotland, Morayshire, Hopeman, NJ143698	3.5.1996
14	1997.116.76	M	123	Scotland, Morayshire, Lossiemouth, NJ214713	7.5.1996
15	1997.116.77	F	106	Scotland, Argyllshire, Arrochar, NN297044	13.6.1996
16	1997.116.80	M	122	Scotland, Aberdeen, Balmedie, NJ978178	21.5.1996
17	1997.116.82	M	148	Scotland, Banffshire, Portsoy, Sandend Bay, NJ560662	13.9.1996

18	1997.116.84	F	137	Scotland, Banffshire, Scotstown, NJ677647	23.6.1996
19	1997.116.92	M	132	Scotland, Morayshire, Findhorn, NJ065647	17.11.1996
20	1997.116.95	F	152	Scotland, Shetland, 25 miles NW Foula	18.3.1997
21	1997.116.97	M	144	Scotland, Fife, North Queensferry, NT134803	19.12.1996
22	1997.116.107	F	126	Scotland, Banffshire, Gardenstown, NJ793645	9.5.1997
23	1997.116.109	M	154	Scotland, Banffshire, Portsoy, NJ585665	31.5.1997
24	1997.116.113	M	152	Scotland, Sound of Harris (bycatch)	25.6.1997
25	1997.116.115	M	150	Scotland, Fife, Pittenweem (bycatch)	22.7.1997
26	1997.116.116	F	161	Scotland, Western Isles (bycatch)	24.7.1997
27	1997.116.117	M	121	Scotland, Western Isles, Minches (bycatch)	21.8.1997
28	1997.116.118	F	151	Scotland, Caithness, Crosskirk Bay, ND029701	13.9.1997
29	1997.116.119	M	119	Scotland, Banffshire, Gardenstown, Crovie, NJ807656	13.9.1997
30	1997.116.120	F	143	Scotland, Western Isles, Minches (bycatch)	20.8.1997
31	1997.118.32	F	129	Wales, Dyfed, Pendine, SN283068	10.8.1996
32	1997.118.34	M	124	Wales, Dyfed, Borth, SN607893	30.11.1996
33	1997.118.36	F	c.150	England, Northumberland, Berwick upon Tweed, Spittal Point, NU005522	22.12.1996
34	1997.118.37	M	132	England, Norfolk, Great Yarmouth, TG531038	6.1.1997
35	1997.118.38	M	107	England, Northumberland, Whitley Bay, NZ350753	8.1.1997
36	1997.118.39	F	131	England, Norfolk, Hemsby, TG506180	12.1.1997
37	1997.118.40	F	110	England, Yorkshire, near Scarborough, off Cromer Point, TA037930	18.4.1997
38	1997.118.42	F	123	North Sea, 26 miles east of Runswick Bay, Yorkshire, England	14.5.1997
39	1997.118.45	F	154	England, Yorkshire, Bridlington Bay, TA2165	29.1.1997
40	1997.118.47	F	119	England, Isle of Man, north of Ramsey, SC455988	22.1.1997
41	1997.118.53	F	160	Wales, Dyfed, Aberystwyth, SN579812	13.6.1997
42	1997.118.59	M	144	England, Cumbria, Nethertown beach, NX990075	3.8.1997
43	1997.118.60	M	151	England, Lancashire, Overton, Bazil Point, SD430560	3.8.1997
44	1997.118.63	M	143	North Sea, 7.75 miles northeast of Sandend, Yorkshire, England	10.9.1997
45	1998.73.3	M	129	Wales, Dyfed, Tenby, SS125995	8.2.1998
46	1998.159.16	F	163	Scotland, Fife, Largo Bay, NO4203	25.6.1998
47	1998.159.23	M	116	Scotland, Fife, Caiplie, NO312472	6.12.1997

48	1998.159.24	F	120	Scotland, Shetland, Mainland, Sand, HU350474	6.1.1998
49	1998.159.27	M	136	Scotland, Ayrshire, Troon, NS323315	1.3.1998
50	1998.159.28	M	143	Scotland, Bute, Scalpsie Bay, NS057583	25.2.1998
51	1998.159.34	F	164	Scotland, Banffshire, Gardenstown, Crovie, NJ807657	26.5.1998
52	1998.159.37	F	140	Scotland, Banffshire, Findochty, NJ467684	10.6.1998
53	1998.159.41	M	143	Scotland, Ross and Cromarty, Portmahomack, NH904842	1.8.1998
54	1999.44.2	F	147	England, Norfolk, Gorleston, TG532024	26.11.1997
55	1999.44.8	M	141	England, Norfolk, Hunstanton beach, TF670408	1.5.1998
56	1999.44.18	F	110	Wales, Dyfed, Tenby, SS127994	7.9.1998
57	1999.44.23	F	141	England, Devon, Noss Mayo, SX548476	23.10.1998
58	1999.44.6	F	114	England, North Yorkshire, 1 mile east of Cromer Point, TA047933	20.4.1998
59	1999.45.4	M	147	Wales, Dyfed, Newport, SN041400	28.9.1998
60	1999.47.8	M	122	Scotland, Morayshire, Burghead, NJ111673	14.9.1998
61	1999.47.10	M	145	Scotland, Ayrshire, Girvan, NX167946	14.1.1999
62	1999.47.11	F	160	Scotland, Ayrshire, Troon, NS324300	18.1.1999
63	1999.122.2	F	123	Wales, Dyfed, Ynyslas, SN602940	27.11.1998
64	1999.122.3	M	117	England, Northumberland, Seaton Sluice, NZ332777	16.12.1998
65	1999.122.4	F	149	England, West Sussex, Worthing, TQ147023	18.12.1998
66	1999.122.5	M	120	Wales, Dyfed, Pwllgwaelod	13.1.1999
67	1999.122.6	F	148	England, Northumberland, Druridge Bay	17.1.1999
68	1999.122.7	F	101	England, Yorkshire, Bridlington, South Bay	28.1.1999
69	1999.122.8	M	146	England, Cornwall, Bude, Crooklets Beach	16.2.1999
70	1999.122.9	F	167	England, Lincolnshire, Gibraltar Point	22.2.1999
71	1999.122.10	F	104	England, Norfolk, Waxham Beach	3.3.1999
72	1999.122.13	F	115	England, Suffolk, 2 miles east of Sizewell Power Station	12.3.1999
73	1999.250.26	M	143	Scotland, Ross and Cromarty, Hilton of Cadboll, NH877765	7.5.1999
74	1999.250.27	M	145	Scotland, Aberdeen, River Don Mouth, NJ947093	21.5.1999
75	1999.250.30	M	126	Scotland, Bute, Glecknabae, NS005682	22.6.1999
76	1999.250.33	F	159	Scotland, Aberdeen, Footdee, NJ958058	10.8.1999
77	1999.250.36	M	143	Scotland, Aberdeen, south of Don Estuary, NJ954090	12.3.1999

78	1999.250.37	F	148	Scotland, Ross and Cromarty, Ullapool, Rhue, NH094976	23.6.1995
79	1999.264.3	F	155	Scotland, Skye, Waternish, NG246587	29.10.1999
80	1999.264.4	M	133	Scotland, Angus, Montrose, NO720569	29.7.1999
81	1999.264.5	M	149	Scotland, Ayrshire, Barassie beach, NS324321	2.10.1999
82	1999.264.6	M	135	Scotland, Morayshire, Burghead, Cummingstown, NJ133693	8.10.1999
83	2000.375.5	F	122	Scotland, Aberdeen, Footdee, NJ957058	3.4.2000
84	2000.375.7	F	111	Scotland, Morayshire, Portgordon, NJ387643	27.4.2000
85	2000.375.9	F	106	Scotland, Kincardineshire, St Cyrus, NO757647	23.4.2000
86	2000.375.12	M	104	Scotland, Banffshire, Findochty, NJ462679	15.3.2000
87	2000.375.14	F	118	Scotland, Bute, Ettrick Bay, NS040653	18.12.1999
88	2000.382.18	M	124	England, Cornwall, Looe, Hannafaore	13.2.1999
89	2000.382.19	M	114	Wales, Dyfed, Ynyslas	23.3.1999
90	2000.382.22	F	165	Wales, Dyfed, Wallog	12.6.1999
91	2000.382.26	M	134	Wales, West Glamorgan, Porthcawl, Newton Beach	17.8.1999
92	2000.382.29	M	125	Wales, Dyfed, Pembrey Country Park	25.9.1999
93	2000.382.31	F	125	Wales, West Glamorgan, Gower, Langland Bay	26.12.1999
94	2000.382.32	M	125	Wales, West Glamorgan, Gower, Horton Beach	7.2.2000
95	2000.382.34	M	134	England, Devon, River Avon near Bantham	22.2.2000
96	2000.382.46	M	140	North Sea, 18 miles off Bridlington, Yorkshire, England	9.8.2000
97	2000.382.47	M	136	North Sea, off Bridlington, Yorkshire, England	25.8.2000
98	2000.382.49	M	139	North Sea, off Bridlington, Yorkshire, England	29.8.2000
99	2000.382.50	M	141	England, Yorkshire, 700m off north side of Filey Brigg	30.8.2000
100	2000.386.18	M	118	Scotland, Aberdeen beach, MJ954087	26.6.2000
101	2000.386.20	F	148	Scotland, Shetland, Mainland, West Voe of Skellister, HU470553	19.7.2000
102	2000.386.22	M	140	Scotland, Angus, Montrose, NO738617	28.8.2000
103	M36/01	F	104	Scotland, Skye, Broadford, NG647236	10.4.2001
104	M38/01	M	124	Scotland, Montrose, Lunan Bay, NO693507	9.4.2001
105	M55/01	M	103	Scotland, Dornoch, NH817896	4.5.2001
106	M83/01	F	110	Scotland, Strathclyde, Arrochar, NN298047	22.6.2001
107	M100/01	F	164	Scotland, Moray, Lossiemouth, NJ245697	18.7.2001

108	M105/01	M	157	Scotland, Skye, Lower Breakish, NG672236	23.7.2001
109	M111/01	F	164	Scotland, Dumfries & Galloway, Creetown, NX467583	29.7.2001
110	M120/01	F	119	Scotland, Banffshire, Buckie, NJ419657	17.8.2001
111	M121/01	F	140	Scotland, Angus, Lunan Bay, NO693495	19.8.2001
112	M136/01	F	137	Scotland, Moray, Burghead, NJ113684	15.9.2001
113	M147/01	M	154	Scotland, Sutherland, Embo, NH819924	1.11.2001
114	M2/02	F	106	Scotland, Banffshire, Findochty, NJ459681	6.1.2002
115		F	171	Crow Point Devon	24/01/01
116		F	105	Swansea Beach, Swansea	07/03/01
117		F	122	Angle Bay Pembrokeshire	21/03/01
118		F	122	Aberarth, Ceredigion	21/04/01
119		F	112	Winterton-on-Sea, Norfolk	27/04/01
120		M	113	Tresaith, Ceredigion	16/09/01
121		M	146	Pembrey, Carmanthenshire	06/10/01
122		M	98	Newport, Pembrokeshire	09/10/01
123		F	108	Aberafon, Near Port Talbot	09/11/01
124		F	113	Aberarth, Cerdigion	28/12/01
125	1991.123.11	M	125	England, Durham, Sunderland	1990
126	1991.145.4	M	112	England, Kent, Isle of Sheppey	29.11.1990
127	1991.145.5	F	178	England, Cornwall, Sennen Cove	12.12.1990
128	1991.145.7	F	189	England, Cornwall, Penzance, Wherry Beach, SW4730	1.1991
129	1991.145.8	M	141	England, Kent, Broadstairs, Joss Bay, TR390670	19.1.1991
130	1991.145.9	F	137	England, Isle of Wight, Shanklin, Hope beach, SZ580810	5.2.1991
131	1991.145.11	F	132	England, Suffolk, Southwold, TM500760	5-7.2.1991
132	1991.145.12	M	152	England, Durham, South Shields, Marsden Bay, NZ400650	14.2.1991
133	1991.145.13	M	143	England, East Sussex, Bexhill-on-Sea, Little Galley Hill, TQ763077	16.2.1991
134	1991.145.14	F	132	England, Yorkshire, Hull, TA0929	7.3.1991
135	1991.145.15	F	131	England, Cornwall, St Ives, Porthmeor, SW4337	12.2.1991
136	1991.145.17	M	100	England, Durham, South Shields, NZ3967	27.3.1991
137	1991.145.18	F	151.5	England, Isle of Wight, Victoria Fort, SZ600830	2.4.1991

138	1991.145.21	F	148	England, Yorkshire, Filey Brigg, TA030880	20.4.1991
139	1991.145.23	F	128	England, Essex, Foulness Point, TG240410	13.5.1991
140	1991.145.27	F	82	England, Norfolk, Great Yarmouth, Gorleston beach, TG520030	7.6.1991
141	1991.145.28	M	142	England, Yorkshire, Hornsea, TA2047	29.8.1991
142	1991.145.30	M	88	England, Norfolk, Hunstanton, TF670410	26.6.1991
143	1991.145.31	M	78	Wales, Pembroke, Tenby, Amroth beach, SN1607	29.6.1991
144	1991.145.33	F	134	England, Yorkshire, Robin Hood's Bay, Boggle Hole, NZ9505	5.9.1991
145	1991.145.34	F	114	England, Yorkshire, Reighton Sands, TA1476	8.9.1991
146	1991.145.35	F	117	Wales, Dyfed, Pembrey Country Park, SN425013	23.9.1991
147	1992.24.10	F	111	England, Durham, Sunderland, NZ3957	30.11.1991
148	1992.24.13	M	144	Wales, Dyfed, Cardigan Island, SN1650	22.10.1991
149	1992.150.22	M	136	Wales, Dyfed, Tresaith, SN278518	23.9.1992
150	1992.150.27	M	148	Wales, Dyfed, Towyn, SN087429	19.7.1992
151	1992.150.29	M	78	England, Kent, Isle of Sheppey, Leysdown-on-sea, TR030706	24.6.1992
152	1992.150.30	M	89	England, Essex, Sizewell, TM481614	2.7.1992
153	1992.150.31	F	158	England, Yorkshire, Spurn Point, TA3614	13.4.1992
154	1992.150.33	M	80	England, Yorkshire, Hedon Creek, TA180280	14.7.1991
155	1992.150.48	F	135	England, Lincolnshire, Sutton-on-Sea, TF529817	15.1.1992
156	1992.150.49	M	168	England, Cornwall, Portscatho, SW881342	30.1.1992
157	1993.114.2	M	132	England, Lancashire, Formby Point, SD268066	7.3.1993
158	1993.114.4	F	130	Wales, Clwyd, Point of Ayr, SJ128858	17.5.1993
159	1993.114.20	M	152	Wales, Dyfed, Ynyslas, SN601921	23.11.1993
160	1993.114.21	F	143	England, Isle of Man, Port St Mary, SC211681	19.9.1993
161	1993.114.22	F	161	Wales, Dyfed, Aberaeron, SN450630	23.8.1993
162	1993.114.23	F	147	Wales, Dyfed, Aberaeron, SN426620	30.8.1993
163	1993.53.10	M	143	England, Devon, Torbay, Godrington beach, SX895581	15.2.1993
164	1993.53.11	M	142	England, Suffolk, Dunwich, Minsmere, TM4868	4.3.1993
165	1993.73.2	F	76	England, Lincolnshire, Skegness	12.6.1991
166	1993.73.3	M	72	England, Norfolk, Titchwell, TF76444644	17.6.1991
167	1993.73.7	M	136	England, Yorkshire, Hornsea, TA2047	6.10.1991

168	1993.73.8	M	87	England, Suffolk, Lowestoft, TM5493	1.8.1991
169	1993.73.11	M	c.102	England, Northumberland, Blyth Harbour, NZ3181	22.9.1991
170	1993.73.14	M	94	England, Northumberland, Amble, HU2604	9.3.1991
171	1993.73.15	M	130	England, Yorkshire, Saltburn, NZ6024	3.10.1991
172	1993.73.16	M	127	Wales, Gwynedd, Llandwg beach, SH562284	10.6.1991
173	1993.73.17	M	111	England, Northumberland, Seahouses, NU203335	13.3.1991
174	1993.73.18	M	103	England, Yorkshire, Bridlington Bay, TA190640	18.2.1991
175	1994.12.10	M	119	England, Durham, Sunderland, NZ393578	5.3.1993
176	1994.12.66	M	122	England, Isles of Scilly, Bryher, SV874152	11.6.1993
177	1994.12.67	M	98	England, Yorkshire, Redcar, NZ606248	12.8.1993
178	1994.12.70	M	109	England, Suffolk, Lowestoft, Pegfield Beach, TM539898	28.10.1993
179	1994.12.71	M	105	Wales, Dyfed, Borth, SN602895	12.11.1993
180	1994.12.72	M	151	England, Yorkshire, Spurn Point, TA3910	23.1.1994
181	1994.13.18	F	85	Scotland, South Uist, Milton, NF726264	14.11.1993
182	1994.54.15	M	117	England, Yorkshire, Bridlington, TA166632	28.4.1994
183	1994.54.16	F	167	Wales, Gwynedd, Morfa Dyffryn, SH5624	10.4.1994
184	1994.54.17	M	127	England, Devon, Westward Ho!, SS419294	4.3.1994
185	1994.54.18	M	105	England, Devon, Westward Ho!, SS435302	3.2.1994
186	1994.54.21	F	148	Wales, Dyfed, Borth, SN606912	16.3.1994
187	1994.54.22	F	136	Wales, Dyfed, Saundersfoot, SN155065	25.5.1994
188	1994.54.23	M	149	Wales, Gwynedd, Barmouth, SH605163	4.8.1994
189	1994.54.24	M	152	Wales, Gwynedd, Criccieth, SH508381	10.7.1994
190	1994.54.25	M	120	Wales, Gwynedd, Tywyn, SH564028	2.6.1994
191	1994.54.26	M	122	Wales, West Glamorgan, Port Talbot, SS734900	6.5.1994
192	1994.54.27	M	145	Wales, Dyfed, Ynyslas, SN606916	9.7.1994
193	1994.54.28	M	99	England, Lancashire, Liverpool, Crosby, SJ304989	14.6.1994
194	1994.54.29	M	139	Wales, Dyfed, Borth, SN606916	31.3.1994
195	1994.54.30	M	78	Wales, Cardigan Bay	28.6.1994
196	1994.54.31	M	90	Wales, Dyfed, Tresaith, SN278514	4.9.1993
197	1994.54.32	F	85	Wales, Gwynedd, Fairbourne, SH611124	1.7.1994

198	1994.54.47	F	160	England, Devon, Salcombe, SX737402	20.2.1994
199	1994.54.48	M	123	England, Gloucestershire, Framilode, SO749104	1.3.1994
200	1994.54.49	M	136	England, Devon, Salcombe, SX737385	4.2.1994
201	1994.54.50	M	144	England, Cornwall, 60 miles off Newlyn	11.3.1994
202	1994.55.8	F	101	Scotland, Aberdeenshire, Fraserburgh, NK054635	23.2.1994
203	1994.55.9	F	129	Scotland, Morayshire, Burghead, NJ115692	2.4.1994
204	1994.55.23	M	138	Scotland, West Lothian, Blackness, NT045801	25.2.1994
205	*1993.114.5	M	135	Wales, Dyfed, Ynyslas, SN600920	26.6.1993
206	1995.39.8	F	110	Scotland, Barra, Tangasdale, NF647006	28.10.1994
207	1995.39.10	M	142	Scotland, Kincardineshire, Gourdon, NO834712	28.10.1994
208	1995.39.12	F	104	Scotland, Ross and Cromarty, Nigg, NH802687	21.9.1994
209	1995.40.2	F	138	England, Cornwall, Porthluney Cove, SW973412	23.10.1994
210	1995.40.7	M	77	England, Cornwall, Penzance, SW472297	11.5.1994
211	1995.40.8	F	178	England, Cornwall, Marazion, SW500310	5.12.1994
212	1995.40.9	F	176	England, Cornwall, Marazion, SW505312	3.12.1994
213	1995.93.7	F	100	Scotland, East Lothian, Longniddry, NT440773	23.1.1995
214	1995.113.5	F	121	Scotland, Fife, St Andrews, NO503175	1.12.1994
215	1995.224.3	F	172	Wales, Anglesey, Treandour Bay	7.7.1995
216	1995.224.4	M	138	Wales, Dyfed, Aberystwyth	14.5.1995
217	1995.224.5	M	111	England, Yorkshire, Filey Brigg	2.7.1995
218	1995.224.6	F	154	Wales, Dyfed, Penbryn	9.7.1995
219	1995.224.7	M	144	Wales, Dyfed, Tresarth	11.6.1995
220	1995.224.8	F	79	Wales, Dyfed, Abermawr	9.6.1995
221	1995.224.9	M	67	Wales, Dyfed, Pendine	25.6.1995
222	1995.224.10	F	86	England, Norfolk, Gorleston	5.7.1995
223	1995.224.11	F	90	Engand, Lincolnshire, Gibraltar Point	11.8.1995
224	1997.112.3	M	110	Wales, Anglesey, Borthwen, SH297877	8.9.1995
225	1997.112.4	F	102	Wales, Gwynedd, Fairbourne, SH610119	6.10.1995
226	1997.112.5	F	131	Isle of Man, Port Erin, SC454978	28.1.1996
227	1997.112.6	M	159	Wales, Gwynedd, Porthmadog, SH528372	31.10.1996

228	1997.112.8	F	191	Wales, Dyfed, Fishguard, SM959373	23.10.1996
229	1997.112.10	M	164	England, Lancashire, Crosby Sands, SJ307985	31.7.1996
230	1997.112.11	F	166	Wales, Dyfed, Rhosselli Beach, SS410912	26.5.1996
231	1997.112.12	F	172	Wales, Dyfed, Pembrey, SN365030	19.6.1997
232	1997.112.13	F	70	Wales, Dyfed, Pendine, SN260070	24.6.1997
233	1997.112.14	F	78	Wales, Dyfed, Pembrey, SN370030	13.7.1997
234	1997.112.15	M	160	Wales, Dyfed, Fishguard, SM946380	17.7.1997
235	1997.112.16	F	85	Wales, Dyfed, New Quay, SN400597	17.7.1997
236	1997.112.17	F	172	Wales, Clwyd, Prestatyn, SJ065844	18.7.1997
237	1997.112.18	F	129	Wales, Dyfed, Borth, SN607901	30.7.1997
238	1997.116.53	M	104	Scotland, Banffshire, Portknockie, NJ387687	24.8.1995
239	1997.116.54	M	109	Scotland, Aberdeen beach, NJ954086	28.1.1996
240	1998.159.19	F	90	Scotland, Orkney, Burray, Glimps Holm, ND4798	7.8.1998
241	1998.159.21	M	137	North Sea, east of Scotland, 57.37N1.23W	23.10.1997
242	1998.159.22	M	103	Scotland, Argyllshire, Appin, NM925499	28.11.1997
243	1998.159.25	M	152	Scotland, Aberdeenshire, Rosehearty, Quarryhead, NJ906660	26.1.1998
244	1998.159.26	F	161	Scotland, Ayrshire, Troon, NS325295	28.2.1998
245	1998.159.29	F	152	Scotland, Banffshire, Buckie, Portessie, NJ442667	8.3.1998
246	1998.159.30	F	110	Scotland, Caithness, Dunnet Bay, ND218705	17.3.1998
247	1998.159.31	M	133	North Sea, off Shetland, 60.56N1.40W	23.3.1998
248	1998.159.32	M	147	North Sea, off Fraserburgh, Aberdeenshire, Scotland	26.3.1998
249	1998.159.39	F	102	Scotland, Banffshire, Findochty, NJ467684	2.7.1998
250	1998.159.42	M	86	Scotland, Banffshire, Whitehills, NJ655658	13.8.1998
251	1999.44.1	M	137	England, Devon, Sidmouth, SY125871	24.11.1997
252	1999.44.3	F	156	England, Northumberland, Whitley Bay, NZ353736	5.12.1997
253	1999.44.4	F	131	England, Devon, Westward Ho!, SS432300	13.3.1998
254	1999.44.5	M	138	England, Yorkshire, Scarborough, TA051883	9.4.1998
255	1999.44.7	M	141	England, Yorkshire, Scarborough, east of Cromer Point, TA457932	22.4.1998
256	1999.44.9	F	133	Wales, Dyfed, Caldy Island, SS137969	21.5.1998
257	1999.44.10	M	146	England, Lincolnshire, Mablethorpe, TF511851	28.6.1998

258	1999.44.11	M	77	Wales, Dyfed, Borth, SN605923	28.6.1998
259	1999.44.12	M	82	England, Suffolk, Corton beach, TM540970	4.7.1998
260	1999.44.13	F	154	England, Norfolk, Sea Palling, TG418286	7.7.1998
261	1999.44.15	M	84	England, Norfolk, Snettisham, TF648340	23.7.1998
262	1999.44.17	M	142	Wales, Dyfed, Llanon, SN507670	25.8.1998
263	1999.44.19	F	113	England, Hampshire, near Lymington, off Hurst, SZ310890	9.9.1998
264	1999.44.20	M	119	Wales, Dyfed, Fishguard, SM960373	11.10.1998
265	1999.44.24	F	173	England, Norfolk, Salthouse, TG078446	3.11.1998
266	1999.44.22		76	England, Devon, Airey Point, SS443347	17.9.1998
267	1999.45.1	F	74	Wales, Dyfed, Ynyslas, SN606925	25.6.1998
268	1999.45.2	M	76	Wales, Dyfed, Borth, SN608905	18.7.1998
269	1999.45.3	M	143	Wales, West Glamorgan, Swansea, SS650922	17.9.1998
270	1999.44.22		76	England, Devon, Airey Point, SS443347	17.9.1998
271	1999.44.4	F	131	England, Devon, Westward Ho!, SS432300	13.3.1998
272	SW 2001/198				
273				No history	
274				No history	

A.1.2. Bottlenose dolphin.

A.1.2.1. National Collection of Mammals, Institute of Biology, University of Mexico, Mexico City, Mexico.

Consecutive	ID	Sex	Location		Date
1	35175		BC	Punta Estrella, 0.5 Km S, mpio. Mexicali	1992
2	4028		BC	Isla San Esteban S	14 feb 1985
3	37900		SONORA	3 Km NW de la Choya, mpio. Puerto Peñasco	22 may 1993
4	32930		SONORA	2 Km S del Estero Morúa, mpio. Puerto Peñasco	24 nov 1990
5	32932		SONORA	3 Km S del Estero Morúa, mpio. Puerto Peñasco	29 nov 1990
6	16570		SONORA	Bahía Kino	8 dic 1976
7	32923	M	SONORA	Punta Borrascosa, N Bahía Adais	Abril 1991

8	32935	F	SONORA	Punta N de la Boca del Estero Morúa, mpio. Puerto Peñasco	Abril 1991
9	32934	M	SONORA	Punta N de la Boca del Estero Morúa, mpio. Puerto Peñasco	Abril 1991
10	36580		SONORA	2 Km S del Estero Morúa, mpio. Puerto Peñasco	Mayo 1993
11	35173		SONORA	300m E del Estero Morúa, mpio. Puerto Peñasco	27 nov 1992
12	36747	M	SONORA	2 Km SE del Golfo de Sta. Clara, mpio. Puerto Peñasco	23 may 1993
13	34640	M	SONORA	1.5 Km W Golfo de Sta. Clara	12 may 1992
14	36750		SONORA	44 Km SE Golfo de Sta. Clara, mpio. San Luis Río Colorado	28 may 1993
15	4162		SONORA	Isla Tiburón, Bahía de Aguadulce, Pta. Norte	18 nov 1985
16	34639		SONORA	44 Km SE Golfo de Sta. Clara, mpio. San Luis Río Colorado	10 may 1992
17	36582	M	SONORA	42 Km SE Golfo de Sta. Clara, mpio. San Luis Río Colorado	28 may 1993
18	34638		SONORA	44 Km SE Golfo de Sta. Clara, mpio. San Luis Río Colorado	10 may 1992
19	35174		SONORA	37 Km SE Golfo de Sta. Clara, mpio. San Luis Río Colorado	9 dic 1992
20	32929	M	SONORA	41 Km SE Golfo de Sta. Clara, mpio. San Luis Río Colorado	20 oct 1991
21	36751		SONORA	5 Km E del Estero Morúa, mpio. Puerto Peñasco	21 may 1993
22	32936	M	SONORA	2.5 Km E del Estero Morúa, mpio. Puerto Peñasco	14 oct 1991
23	36581		SONORA	5 Km E del Estero Morúa, mpio. Puerto Peñasco	21 may 1993
24	37899		SONORA	2 Km E del Estero Morúa, mpio. Puerto Peñasco	21 may 1993
25	34634		SONORA	Lado E Boca del Estero Morúa, mpio. Puerto Peñasco	30 abr 1992
26	34636		SONORA	Roca del Toro, Puerto Peñasco	30 abr 1992
27	34635		SONORA	Lado E Boca del Estero Morúa, mpio. Puerto Peñasco	30 abr 1992
28	36749	F	SONORA	33 Km SE Golfo de Sta. Clara, mpio. San Luis Río Colorado	28 may 1993
29	32924		SONORA	30 Km SE Golfo de Sta. Clara, mpio. San Luis Río Colorado	6 dic 1990
30	30981	F	SONORA	2 Km S Golfo de Sta. Clara, mpio. San Luis Río Colorado	24 abr 1991
31	32933		SONORA	2 Km S Puerto Peñasco	29 nov 1990
32	32926		SONORA	20 Km S Golfo de Sta. Clara, Campo el Tornillal, mpio. San Luis Río Colorado	
33	32925		SONORA	SE Golfo de Sta. Clara, mpio. San Luis Río Colorado	6 dic 1990
34	4031		SONORA	W Bahía de Agua Dulce, Isla Tiburón	14 feb 1985
35	4020		SONORA	Isla Tiburón, Bahía de Agua Dulce, Punta Norte	18 nov 1985
36	4021		SONORA	Isla Tiburón, Bahía de Agua Dulce, Punta Norte	18 nov 1985
37	4022		SONORA	Isla Tiburón, SE Ensenada de Perros	11 feb 1986

38	4019		SONORA	Isla Tiburón, Punta SE	13 vov 1985
39	4026		SONORA	Isla Tiburón, E Ensenada de Perros	11 feb 1986
40	4025		SONORA	Isla Tiburón, SE Ensenada de Perros	11 feb 1986
41	4023		SONORA	Isla Tiburón, SE Ensenada de Perros	11 feb 1986
42	4024		SONORA	Isla Tiburón, SE Ensenada de Perros	11 feb 1986
43	4030		SONORA	Isla Tiburón, SE Ensenada de Perros	11 feb 1986
45	4027		SONORA	Isla Tiburón, SE Ensenada de Perros	11 feb 1986
46	3871		SONORA	Isla Tiburón, Playa SE	14 may 1985
47	32928	M	SONORA	21 Km SE Golfo de Sta. Clara, mpio. San Luis Río Colorado	20 oct 1992
48	32927	M	SONORA	5 Km NW Golfo de Sta. Clara, mpio. San Luis Río Colorado	18 oct 1991

A.1.2.2. National Museum of Natural History, Smithsonian Institution, Washington, D. C., USA.

Consecutive	ID	Date	State	County	Nature	Sex	Weight	Total Length
1	504348	19760429	NORTH CAROLINA	DARE	STRANDING	F	0172KG*	0247CM
2	504501	19761021	VIRGINIA	NORTHAMPTON	STRANDING	F	0230KG	0256CM
3	571085	19870731	VIRGINIA	VIRGINIA BEACH	STRANDING	F		0270CM
4	571096	19870808	VIRGINIA	VIRGINIA BEACH	STRANDING	F		0188CM
5	571097	19870807	VIRGINIA	VIRGINIA BEACH	STRANDING	F		0159CM
6	571101	19870808	VIRGINIA	VIRGINIA BEACH	STRANDING	F		0242CM
7	571104	19870809	VIRGINIA	VIRGINIA BEACH	STRANDING	M		0148CM
8	571905	19950630	VIRGINIA	NORFOLK	STRANDING	M		0284CM
9	571907	19950801	VIRGINIA	VIRGINIA BEACH	STRANDING?	F		0213CM
10	572359	19981126	NORTH CAROLINA	CURRITUCK	STRANDING	M	0211KG	0264CM
11	572366	19980930	NORTH CAROLINA	DARE	STRANDING	M	0230KG	0208CM
12	572367	19981207	NORTH CAROLINA	CARTERET	STRANDING	F		0240CM
13	572368	19981007	NORTH CAROLINA	DARE	INCIDENTAL CATCH	M		0285CM
14	572558	20000720	VIRGINIA	NORTHAMPTON	INCIDENTAL CATCH	F		0181CM
15	291424	192802--	NORTH CAROLINA	DARE	CAPTURE	?		?
16	291425	192802--	NORTH CAROLINA	DARE	CAPTURE	?		?

17	291426	192802--	NORTH CAROLINA	DARE	CAPTURE	?		?
18	291431	192802--	NORTH CAROLINA	DARE	CAPTURE	?		?
19	291434	192802--	NORTH CAROLINA	DARE	CAPTURE	?		?
20	291443	192802--	NORTH CAROLINA	DARE	CAPTURE	?		?
21	291446	192802--	NORTH CAROLINA	DARE	CAPTURE	?		?
22	291454	192802--	NORTH CAROLINA	DARE	CAPTURE	?		?
23	291458	192802--	NORTH CAROLINA	DARE	CAPTURE	?		?
24	291463	192802--	NORTH CAROLINA	DARE	CAPTURE	?		?
25	291466	192802--	NORTH CAROLINA	DARE	CAPTURE	?		?
26	291472	192802--	NORTH CAROLINA	DARE	CAPTURE	?		?
27	291474	192802--	NORTH CAROLINA	DARE	CAPTURE	?		?
28	291489	192802--	NORTH CAROLINA	DARE	CAPTURE	?		?
29	291493	192802--	NORTH CAROLINA	DARE	CAPTURE	?		?
30	291494	192802--	NORTH CAROLINA	DARE	CAPTURE	?		?
31	291495	192802--	NORTH CAROLINA	DARE	CAPTURE	?		?
32	305767	--------	VIRGINIA	MATHEWS	STRANDING	?		?
33	307545	195805--	VIRGINIA	ACCOMACK	STRANDING	F		?
34	307546	195805--	VIRGINIA	ACCOMACK	STRANDING	?		?
35	395179	19681104	VIRGINIA	ACCOMACK	STRANDING	F		0217CM
36	395670	19691010	MARYLAND	WORCESTER	STRANDING	M		0267CM
37	500857	19740112	SOUTH CAROLINA	BEAUFORT	STRANDING	M		0189CM
38	504095	19740622	MARYLAND	ANNE ARUNDEL	STRANDING	F		0235CMEST
39	504273	19750614	NORTH CAROLINA	DARE	STRANDING	F		0275CM
40	504325	19760116	NORTH CAROLINA	DARE	STRANDING	F		0241CM
41	504326	19760114	NORTH CAROLINA	DARE	STRANDING	F		0203CM
42	504501	19761021	VIRGINIA	NORTHAMPTON	STRANDING	F	0230KG	0256CM
43	504550	19770227	NORTH CAROLINA	DARE	STRANDING	F	0103KG	0201CM
44	504551	19770211	NORTH CAROLINA	DARE	INCIDENTAL C	M	0145KG	0216CM
45	504561	19770305	NORTH CAROLINA	DARE	STRANDING	M	0118KG	0206CM
46	504562	19770305	NORTH CAROLINA	DARE	STRANDING	F	0026KG	0132CM

47	504739	19780208	NORTH CAROLINA	DARE	STRANDING	M	0157KG	0250CM
48	504755	19780325	NORTH CAROLINA	DARE	STRANDING	F	0270KG	0268CM
49	571135	19870829	VIRGINIA	VIRGINIA BEACH	STRANDING	M		0151CM
50	571141	19870901	VIRGINIA	VIRGINIA BEACH	STRANDING	M		0201CM
51	571146	19870904	VIRGINIA	VIRGINIA BEACH	STRANDING	F		0190CM
52	571166	19870918	VIRGINIA	VIRGINIA BEACH	STRANDING	M		0158CM
53	571179	19870929	VIRGINIA	VIRGINIA BEACH	STRANDING	F		0142CM
54	571193	19871005	VIRGINIA	VIRGINIA BEACH	CAPTURE	F		0249CM
55	571195	19871006	VIRGINIA	VIRGINIA BEACH	CAPTURE	F		0226CM
56	571196	19871007	VIRGINIA	VIRGINIA BEACH	CAPTURE	M		0259CM
57	571197	19870718	VIRGINIA	MATHEWS	STRANDING	M		0222CM
58	571199	19871008	VIRGINIA	VIRGINIA BEACH	CAPTURE	F		0258CM
59	571241	19871124	GEORGIA	CHATHAM	STRANDING	M		0181CM
60	571242	19871123	SOUTH CAROLINA	CHARLESTON	STRANDING	M		0210CM
61	571254	19880831	MARYLAND	WORCESTER	STRANDING	M	0191KG	0242CM
62	571266	19881203	NORTH CAROLINA	DARE	INCIDENTAL C	F		0165CM
63	571317	19890120	NORTH CAROLINA	CARTERET	STRANDING	M		0218CM
64	571356	19890520	NORTH CAROLINA	DARE	STRANDING	F		0298CM
65	571381	19890826			INCIDENTAL C	F	0114KG	0209CM
66	571382	19890103			INCIDENTAL C	F	0054KG	0158CM
67	571383	19890103			INCIDENTAL C	M	0059KG	0158CM
68	571385	19890105			INCIDENTAL C	M	0056KG	0159CM
69	571393	19900317	NORTH CAROLINA	DARE	STRANDING	F		0261CM
70	571426	19900817			INCIDENTAL C	M		0293CM
71	571427	19900918			INCIDENTAL C	M		0178CM
72	571428	19900918			INCIDENTAL C	F		0175CM
73	571439	19901109			INCIDENTAL C	M		0174CM
74	571465	19910702	NEW JERSEY	ATLANTIC	STRANDING	F		0251CM*
75	571466	19910702	NEW JERSEY	CAPE MAY	STRANDING	F		0197CM*
76	571494	--------	NEW YORK		STRANDING	M		0298CM

77	571496	19920229	NEW JERSEY	ATLANTIC	STRANDING	M		0202CM
78	571548	19920603	VIRGINIA	ACCOMACK	INCIDENTAL C	M		0203CM+
79	571553	19920719	VIRGINIA	ACCOMACK	STRANDING	F		0270CM
80	571615	19921213	MASSACHUSETTS	BARNSTABLE	STRANDING	M		0230CM
81	571617	19921213	MASSACHUSETTS		STRANDING	M	0250KG*	0263CM
82	571687	19930213	NEW JERSEY	OCEAN	STRANDING	M	0312KG	0301CM
83	571781	19940521	VIRGINIA	VIRGINIA BEACH	INCIDENTAL C	F	0093KGES	T* 0199CM
84	571785	19940603	VIRGINIA	VIRGINIA BEACH	INCIDENTAL C	ATCH M	0107KG*	0196CM
85	571794	19940510	VIRGINIA	ACCOMACK	INCIDENTAL C	ATCH M	0149KG*	0209CM
86	571795	19940801	VIRGINIA	ACCOMACK	INCIDENTAL C	ATCH M	0288KG+*	0265CMEST
87	571797	19940805	VIRGINIA	ACCOMACK	STRANDING	F	0096KG*	0194CM
88	571798	19940831	VIRGINIA	NORTHAMPTON	INCIDENTAL C	ATCH F	0100KG*	0206CM*
89	571799	19940907	VIRGINIA	VIRGINIA BEACH	INCIDENTAL C	ATCH M	0133KG*	0223CM
90	571904	19950613	VIRGINIA	NORFOLK	STRANDING	M		0256CM
91	571912	19940502	VIRGINIA	NORTHAMPTON	STRANDING	M		0227CM
92	571957	19950401	NORTH CAROLINA	DARE	INCIDENTAL C	ATCH? M	?	0233CM
93	571978	19931026	VIRGINIA	NORFOLK	STRANDING	F		0184CM
94	571980	19941113	VIRGINIA	ACCOMACK	INCIDENTAL C	ATCH M	0263KG*	0265CM
95	572029	19960627	VIRGINIA	VIRGINIA BEACH	STRANDING	F		0196CM
96	572048	19970106	NORTH CAROLINA	DARE	INCIDENTAL C	ATCH F		0187CM
97	572049	19970106	NORTH CAROLINA	DARE	INCIDENTAL C	ATCH F		0257CM
98	572075	19950430	NORTH CAROLINA	CARTERET	INCIDENTAL C	ATCH? F	?	0175CM
99	572086	19950803	VIRGINIA	VIRGINIA BEACH	STRANDING?	F		0270CMEST
100	572093	19970531	VIRGINIA	VIRGINIA BEACH	STRANDING	M	0018KG	0101CM
101	572097	19970802	VIRGINIA	VIRGINIA BEACH	STRANDING	F		0265CM
102	572102	19970609	VIRGINIA	VIRGINIA BEACH	STRANDING	F	0231KG	0246CM
103	572103	19970605	VIRGINIA	NORFOLK	STRANDING?	M	0172KG	0234CM*
104	572104	19970528	VIRGINIA	NORTHAMPTON	STRANDING	M	0168KG	0250CM
105	572106	19970429	VIRGINIA	VIRGINIA BEACH	STRANDING	F	0043KG	0160CM
106	572109	19970526	NORTH CAROLINA	CARTERET	STRANDING	M		?

107	572113	19971008	NORTH CAROLINA	PENDER	STRANDING	M		0260CM
108	572114	19971009	NORTH CAROLINA	BRUNSWICK	STRANDING	F		0207CM
109	572116	19970507	NORTH CAROLINA	DARE	STRANDING	?		0108CM*
110	572117	19970520	NORTH CAROLINA	DARE	INCIDENTAL C	ATCH F		0180CMEST
111	572118	19970527	NORTH CAROLINA	HYDE	STRANDING	M		0270CM
112	572120	19970606	NORTH CAROLINA	DARE	STRANDING	F		0248CM
113	572121	19970623	NORTH CAROLINA	HYDE	INCIDENTAL C	ATCH M		0190CMEST
114	572125	19971030	NORTH CAROLINA	HYDE	STRANDING	F		0190CM
115	572131	19970414	NORTH CAROLINA	CARTERET	INCIDENTAL C	ATCH M		0216CMEST
116	572134	19970601	NORTH CAROLINA	CARTERET	STRANDING	M		0264CM
117	572136	19970613	NORTH CAROLINA	DARE	STRANDING	M		0223CM
118	572137	19970812	NORTH CAROLINA	DARE	VESSEL COLLI	SION M		0192CM
119	572139	19970916	NORTH CAROLINA	DARE	INCIDENTAL C	ATCH M		0215CM
120	572140	19970917	NORTH CAROLINA	DARE	STRANDING	M		0134CM
121	572141	19970929	NORTH CAROLINA	DARE	STRANDING	M		0195CM
122	572144	19971028	NORTH CAROLINA	CARTERET	STRANDING?	F		0235CM
123	572146	19971204	NORTH CAROLINA	DARE	INCIDENTAL C	ATCH F		0269CM
124	572147	19980330	DELAWARE	SUSSEX	INCIDENTAL C	ATCH M	0250KGES	T* 0284CM*
125	572157	19980129	NORTH CAROLINA	CARTERET	STRANDING	?		0230CM
126	572158	19980129	NORTH CAROLINA		STRANDING	F		0157CMEST
127	572162	19960805	NORTH CAROLINA	DARE	STRANDING	M		0280CM
128	572163	19941026	NORTH CAROLINA	CARTERET	INCIDENTAL C	ATCH M		?
129	572164	19941103	NORTH CAROLINA	CARTERET	INCIDENTAL C	ATCH F		?
130	572167	19971004	NORTH CAROLINA	CARTERET	STRANDING	M		0203CM
131	572217	19970730	VIRGINIA	NORFOLK	STRANDING	M	0035KG	0150CM
132	572224	19980712	VIRGINIA	VIRGINIA BEACH	STRANDING	M		0121CM
133	572227	19980722	VIRGINIA	VIRGINIA BEACH	STRANDING	F		0223CM
134	572229	19980506	VIRGINIA	VIRGINIA BEACH	STRANDING	M		0247CM
135	572230	19980704	VIRGINIA	VIRGINIA BEACH	STRANDING	M	0032KG	0138CM
136	572232	19980707	VIRGINIA	ACCOMACK	STRANDING	M		0240CMEST

137	572235	19980614	VIRGINIA	NORTHAMPTON	STRANDING	M		0241CM
138	572238	19980804	VIRGINIA	VIRGINIA BEACH	STRANDING	?		0283CM
139	572239	19980805	VIRGINIA	NORFOLK	STRANDING	?		0294CM
140	572240	19980806	VIRGINIA	VIRGINIA BEACH	STRANDING	?		0241CM
141	572242	19980810	VIRGINIA	VIRGINIA BEACH	STRANDING	M	0109KG	0210CM
142	572243	19980813	VIRGINIA	VIRGINIA BEACH	STRANDING	F	0188KG	0250CM
143	572244	19980812	VIRGINIA	VIRGINIA BEACH	STRANDING	?		0216CM
144	572245	19980812	VIRGINIA	VIRGINIA BEACH	STRANDING	M		0248CM
145	572246	19980816	VIRGINIA	HAMPTON	STRANDING	M	0073KG	0194CM
146	572247	19980818	VIRGINIA	VIRGINIA BEACH	STRANDING	F	0036KG	0130CM
147	572248	19980820	VIRGINIA	VIRGINIA BEACH	STRANDING	?		0184CM
148	572250	19980911	VIRGINIA	HAMPTON	STRANDING	F		0220CM
149	572253	19960119	VIRGINIA	HAMPTON	STRANDING	F		0188CM
150	572255	19960529	VIRGINIA	VIRGINIA BEACH	STRANDING	F		0215CM
151	572263	19970523	NEW JERSEY	CAPE MAY	STRANDING	F	0182KGES	T* 0262CM*
152	572271	19980419	NORTH CAROLINA	CARTERET	INCIDENTAL C	ATCH M		0155CM
153	572276	19980331	NORTH CAROLINA	CARTERET	STRANDING	M		0277CM
154	572281	19980329	NORTH CAROLINA	DARE	STRANDING	F		0246CM
155	572307	19980518	NORTH CAROLINA	DARE	STRANDING	F		0110CM
156	572310	19931012	VIRGINIA	NORFOLK	STRANDING	M	0035KG	0140CM
157	572345	19981025	VIRGINIA	VIRGINIA BEACH	STRANDING	M	0225KG	0270CM
158	572347	19981101	VIRGINIA	GLOUCESTER	STRANDING	F	0076KG	0255CM
159	572348	19981102	VIRGINIA	NORTHAMPTON	STRANDING	F		0287CM
160	572349	19981109	VIRGINIA	ACCOMACK	STRANDING	M		0283CM
161	572350	19981110	VIRGINIA	ACCOMACK	STRANDING	F		0264CM
162	572351	19981117	VIRGINIA	NORTHAMPTON	STRANDING	M		0198CM
163	572374	19970817	VIRGINIA	VIRGINIA BEACH	STRANDING	M	0039KG	0138CM
164	572418	19981221	VIRGINIA	VIRGINIA BEACH	STRANDING?	F	0125KG	0218CM
165	572419	19990101	NORTH CAROLINA	CURRITUCK	STRANDING?	F		0270CM
166	572554	20000809	VIRGINIA	VIRGINIA BEACH	INCIDENTAL C	ATCH M	0099KG	0202CM

167	572557	19991119	VIRGINIA	VIRGINIA BEACH	INCIDENTAL C	ATCH F	0147KG	0215CM
168	572565	20000522	VIRGINIA	VIRGINIA BEACH	INCIDENTAL C	ATCH M		0235CM
169	572566	20000506	VIRGINIA	ACCOMACK	STRANDING	F		0225CM
170	572781	19950901	VIRGINIA	VIRGINIA BEACH	STRANDING	F	0126KG	0260CMEST*
171	572782	19960610	NEW JERSEY	CUMBERLAND	STRANDING	M	0227KGES	T* 0286CM*
172	MME06292	19910306	NORTH CAROLINA	HYDE	STRANDING	F	?	0153CM
173	MME08449	19930112	NORTH CAROLINA	CARTERET	STRANDING?	F		0200CM
174	MME10243	19931030	NORTH CAROLINA	CARTERET	INCIDENTAL CATCH?	F		0215CM
175	MME10601	19941028	NORTH CAROLINA	CARTERET	STRANDING	F		?

Appendix 2: Allele frequencies for each locus and location.

A.2.1. Harbour porpoise.

A.2.1. Divided in three sub-populations.

	Norwegian	Danish	Bitish
Locus: GT101			
93	0.011	NA	0.000
99	0.021	NA	0.032
101	0.000	NA	0.183
103	0.106	NA	0.392
105	0.319	NA	0.183
107	0.362	NA	0.091
109	0.064	NA	0.027
111	0.043	NA	0.081
113	0.074	NA	0.011
Locus: EV104			
136	0.000	0.014	0.043
142	0.000	0.005	0.000
144	0.000	0.017	0.005
146	0.000	0.005	0.000
148	0.106	0.012	0.016
150	0.181	0.094	0.054
152	0.160	0.130	0.113
154	0.138	0.156	0.151
156	0.202	0.139	0.038
158	0.138	0.196	0.220
160	0.064	0.170	0.188
162	0.000	0.050	0.140
164	0.011	0.014	0.032
Locus: 415/416			
201	0.000	0.000	0.005
207	0.000	0.000	0.005
209	0.000	0.026	0.038
211	0.000	0.002	0.000
213	0.037	0.059	0.065
215	0.000	0.671	0.597
217	0.610	0.197	0.199
219	0.280	0.040	0.048
221	0.073	0.005	0.043
Locus: GT011			
101	0.000	0.002	0.000
103	0.000	0.016	0.005
105	0.000	0.305	0.000
107	0.074	0.250	0.000

109	0.053	0.016	0.323
111	0.330	0.002	0.392
113	0.170	0.032	0.005
115	0.000	0.043	0.022
117	0.000	0.052	0.011
119	0.043	0.100	0.016
121	0.043	0.136	0.022
123	0.085	0.039	0.075
125	0.053	0.005	0.102
127	0.106	0.000	0.027
129	0.032	0.002	0.000
131	0.011	0.000	0.000

Locus: 417/418

161	0.000	0.014	0.000
165	0.056	0.000	0.000
167	0.022	0.000	0.000
169	0.167	0.000	0.000
173	0.044	0.000	0.000
175	0.322	0.603	0.505
177	0.211	0.239	0.269
179	0.000	0.021	0.043
181	0.022	0.064	0.134
183	0.078	0.037	0.038
185	0.078	0.007	0.011
187	0.000	0.016	0.000

Locus: EV94

196	0.191	0.000	0.000
198	0.000	0.241	0.290
200	0.160	0.011	0.016
202	0.223	0.282	0.156
204	0.128	0.191	0.306
206	0.181	0.169	0.156
208	0.117	0.068	0.022
210	0.000	0.038	0.048
224	0.000	0.000	0.005

Locus: GT136

83	0.064	0.000	0.000
85	0.011	0.000	0.000
87	0.021	0.018	0.038
89	0.011	0.011	0.000
91	0.043	0.000	0.000
93	0.245	0.009	0.032
95	0.202	0.173	0.204
97	0.191	0.225	0.129
99	0.043	0.250	0.328

101	0.053	0.141	0.081
103	0.011	0.052	0.054
105	0.021	0.048	0.032
107	0.032	0.039	0.043
109	0.032	0.030	0.059
111	0.021	0.005	0.000
Locus: GT015			
118	0.000	0.000	0.011
120	0.000	0.007	0.000
122	0.021	0.000	0.000
126	0.000	0.002	0.000
128	0.011	0.000	0.011
130	0.011	0.002	0.022
132	0.011	0.000	0.048
134	0.064	0.034	0.065
136	0.170	0.110	0.108
138	0.128	0.092	0.038
140	0.074	0.062	0.011
142	0.074	0.028	0.038
144	0.106	0.025	0.027
146	0.032	0.028	0.016
148	0.000	0.046	0.016
150	0.053	0.060	0.048
152	0.021	0.037	0.081
154	0.032	0.030	0.065
156	0.032	0.039	0.038
158	0.000	0.037	0.054
160	0.064	0.037	0.070
162	0.021	0.053	0.059
164	0.011	0.087	0.086
166	0.000	0.064	0.022
168	0.000	0.050	0.011
170	0.021	0.025	0.005
172	0.021	0.018	0.016
174	0.021	0.014	0.011
176	0.000	0.014	0.027
Locus: GATA053			
201	0.202	0.007	0.000
205	0.043	0.034	0.011
209	0.277	0.874	0.952
213	0.479	0.085	0.038
Locus: IGF-1			
132	0.000	0.005	0.000
134	0.032	0.002	0.000
136	0.021	0.000	0.011

138	0.000	0.023	0.005
140	0.074	0.030	0.005
142	0.021	0.018	0.022
144	0.138	0.041	0.065
146	0.160	0.157	0.167
148	0.160	0.236	0.188
150	0.032	0.180	0.177
152	0.096	0.095	0.118
154	0.000	0.070	0.102
156	0.064	0.048	0.086
158	0.032	0.055	0.048
160	0.011	0.020	0.000
162	0.011	0.007	0.005
164	0.149	0.014	0.000

Locus: EV96

185	0.050	NA	0.672
187	0.000	NA	0.011
189	0.050	NA	0.306
191	0.025	NA	0.011
193	0.300	NA	0.000
199	0.025	NA	0.000
201	0.175	NA	0.000
205	0.025	NA	0.000
207	0.100	NA	0.000
209	0.225	NA	0.000
213	0.025	NA	0.000

Locus: TAA031

214	0.000	0.009	0.000
217	0.043	0.048	0.032
220	0.117	0.086	0.038
223	0.404	0.475	0.575
226	0.053	0.039	0.038
229	0.117	0.084	0.043
232	0.096	0.068	0.054
235	0.170	0.134	0.204
238	0.000	0.034	0.011
241	0.000	0.018	0.005
244	0.000	0.005	0.000

A.2.1. Divided in five management units.

	BNS	IRL-W	DKNS	IDW	NOR
Locus: GT101					
93	NA	NA	0.000	0.000	0.011
99	NA	NA	0.038	0.025	0.021
101	NA	NA	0.179	0.188	0.000
103	NA	NA	0.368	0.425	0.106
105	NA	NA	0.217	0.138	0.319
107	NA	NA	0.113	0.063	0.362
109	NA	NA	0.019	0.038	0.064
111	NA	NA	0.066	0.100	0.043
113	NA	NA	0.000	0.025	0.074
Locus: EV104					
136	0.014	0.015	0.047	0.038	0.000
142	0.000	0.010	0.000	0.000	0.000
144	0.009	0.025	0.009	0.000	0.000
146	0.005	0.005	0.000	0.000	0.000
148	0.009	0.015	0.019	0.013	0.106
150	0.100	0.091	0.047	0.063	0.181
152	0.145	0.111	0.151	0.063	0.160
154	0.132	0.177	0.123	0.188	0.138
156	0.100	0.172	0.019	0.063	0.202
158	0.227	0.167	0.198	0.250	0.138
160	0.168	0.177	0.189	0.188	0.064
162	0.068	0.030	0.160	0.113	0.000
164	0.023	0.005	0.038	0.025	0.011
Locus: 415/416					
201	0.000	0.000	0.000	0.013	0.000
207	0.000	0.000	0.009	0.000	0.000
209	0.023	0.030	0.019	0.063	0.000
211	0.000	0.005	0.000	0.000	0.000
213	0.068	0.050	0.066	0.063	0.038
215	0.668	0.675	0.575	0.625	0.000
217	0.182	0.210	0.226	0.163	0.628
219	0.050	0.030	0.047	0.050	0.256
221	0.009	0.000	0.057	0.025	0.077
Locus: GT011					
101	0.005	0.000	0.000	0.000	0.000
103	0.018	0.014	0.009	0.000	0.000
105	0.282	0.327	0.000	0.000	0.000
107	0.259	0.238	0.000	0.000	0.074
109	0.014	0.019	0.311	0.338	0.053
111	0.000	0.005	0.406	0.375	0.330
113	0.023	0.042	0.009	0.000	0.170

115	0.050	0.037	0.019	0.025	0.000
117	0.068	0.037	0.000	0.025	0.000
119	0.109	0.089	0.019	0.013	0.043
121	0.127	0.145	0.019	0.025	0.043
123	0.036	0.042	0.066	0.087	0.085
125	0.005	0.005	0.094	0.113	0.053
127	0.000	0.000	0.047	0.000	0.106
129	0.005	0.000	0.000	0.000	0.032
131	0.000	0.000	0.000	0.000	0.011
Locus: 417/418					
161	0.018	0.005	0.000	0.000	0.000
165	0.000	0.000	0.000	0.000	0.057
167	0.000	0.000	0.000	0.000	0.023
169	0.000	0.000	0.000	0.000	0.182
173	0.000	0.000	0.000	0.000	0.023
175	0.586	0.625	0.509	0.500	0.352
177	0.248	0.231	0.302	0.225	0.216
179	0.027	0.014	0.038	0.050	0.000
181	0.063	0.063	0.113	0.163	0.023
183	0.041	0.034	0.028	0.050	0.068
185	0.009	0.005	0.009	0.013	0.057
187	0.009	0.024	0.000	0.000	0.000
Locus: EV94					
196	0.000	0.000	0.000	0.000	0.191
198	0.208	0.274	0.255	0.338	0.000
200	0.013	0.009	0.019	0.013	0.160
202	0.270	0.297	0.179	0.125	0.223
204	0.204	0.184	0.283	0.338	0.128
206	0.199	0.137	0.198	0.100	0.181
208	0.071	0.061	0.009	0.038	0.117
210	0.035	0.038	0.057	0.038	0.000
224	0.000	0.000	0.000	0.013	0.000
Locus: GT136					
83	0.000	0.000	0.000	0.000	0.064
85	0.000	0.000	0.000	0.000	0.011
87	0.027	0.010	0.047	0.025	0.021
89	0.009	0.014	0.000	0.000	0.011
91	0.000	0.000	0.000	0.000	0.043
93	0.004	0.014	0.047	0.013	0.245
95	0.156	0.190	0.151	0.275	0.202
97	0.192	0.252	0.123	0.138	0.191
99	0.259	0.243	0.396	0.238	0.043
101	0.143	0.143	0.047	0.125	0.053
103	0.058	0.043	0.047	0.063	0.011
105	0.067	0.029	0.047	0.013	0.021

107	0.049	0.029	0.057	0.025	0.032
109	0.031	0.029	0.038	0.087	0.032
111	0.004	0.005	0.000	0.000	0.021
Locus: GT015					
118	0.000	0.000	0.019	0.000	0.000
120	0.004	0.010	0.000	0.000	0.000
122	0.000	0.000	0.000	0.000	0.021
126	0.000	0.005	0.000	0.000	0.000
128	0.000	0.000	0.009	0.013	0.021
130	0.004	0.000	0.019	0.025	0.043
132	0.000	0.000	0.066	0.025	0.000
134	0.036	0.029	0.085	0.038	0.064
136	0.089	0.136	0.094	0.125	0.181
138	0.080	0.107	0.019	0.063	0.128
140	0.071	0.049	0.009	0.013	0.064
142	0.040	0.015	0.038	0.038	0.064
144	0.018	0.034	0.047	0.000	0.106
146	0.045	0.010	0.009	0.025	0.032
148	0.049	0.039	0.000	0.038	0.000
150	0.058	0.063	0.028	0.075	0.064
152	0.018	0.058	0.085	0.075	0.032
154	0.031	0.029	0.085	0.038	0.032
156	0.049	0.029	0.047	0.025	0.011
158	0.027	0.049	0.075	0.025	0.000
160	0.040	0.024	0.066	0.075	0.043
162	0.045	0.058	0.038	0.087	0.021
164	0.076	0.102	0.094	0.075	0.011
166	0.076	0.053	0.009	0.038	0.000
168	0.058	0.044	0.009	0.013	0.000
170	0.013	0.039	0.000	0.013	0.021
172	0.022	0.015	0.028	0.000	0.021
174	0.022	0.005	0.009	0.013	0.021
176	0.027	0.000	0.009	0.050	0.000
Locus: GATA053					
201	0.009	0.005	0.000	0.000	0.202
205	0.013	0.051	0.000	0.025	0.043
209	0.920	0.827	0.981	0.913	0.277
213	0.058	0.117	0.019	0.063	0.479
Locus: IGF-1					
132	0.005	0.005	0.000	0.000	0.000
134	0.005	0.000	0.000	0.000	0.021
136	0.000	0.000	0.009	0.013	0.021
138	0.023	0.024	0.000	0.013	0.000
140	0.036	0.024	0.000	0.013	0.074
142	0.018	0.019	0.019	0.025	0.021

144	0.054	0.028	0.047	0.087	0.138
146	0.153	0.165	0.160	0.175	0.160
148	0.203	0.274	0.189	0.188	0.191
150	0.194	0.160	0.189	0.163	0.021
152	0.099	0.090	0.123	0.113	0.074
154	0.081	0.052	0.142	0.050	0.000
156	0.050	0.047	0.066	0.113	0.064
158	0.045	0.066	0.057	0.038	0.021
160	0.023	0.019	0.000	0.000	0.011
162	0.009	0.005	0.000	0.013	0.011
164	0.005	0.024	0.000	0.000	0.170
Locus: EV96					
185	NA	NA	0.689	0.650	0.050
187	NA	NA	0.019	0.000	0.000
189	NA	NA	0.283	0.338	0.050
191	NA	NA	0.009	0.013	0.025
193	NA	NA	0.000	0.000	0.300
199	NA	NA	0.000	0.000	0.025
201	NA	NA	0.000	0.000	0.175
205	NA	NA	0.000	0.000	0.025
207	NA	NA	0.000	0.000	0.100
209	NA	NA	0.000	0.000	0.225
213	NA	NA	0.000	0.000	0.025
Locus: TAA031					
214	0.004	0.014	0.000	0.000	0.000
217	0.076	0.019	0.028	0.038	0.043
220	0.063	0.114	0.038	0.038	0.117
223	0.491	0.457	0.528	0.637	0.404
226	0.036	0.043	0.047	0.025	0.053
229	0.067	0.095	0.047	0.038	0.117
232	0.076	0.062	0.047	0.063	0.096
235	0.147	0.119	0.264	0.125	0.170
238	0.027	0.043	0.000	0.025	0.000
241	0.009	0.029	0.000	0.013	0.000
244	0.004	0.005	0.000	0.000	0.000

A.2.2. Bottlenose dolphin.

	GOC	WNAC	WNAP
Locus: KWM1b			
170	NA	0.000	0.036
176	NA	0.000	0.048
186	NA	0.000	0.036
188	NA	0.000	0.155
190	NA	0.200	0.655
192	NA	0.800	0.071
Locus: KWM2a			
141	0.059	0.000	0.000
143	0.088	0.000	0.033
145	0.118	0.074	0.098
147	0.103	0.056	0.120
149	0.044	0.278	0.239
151	0.147	0.074	0.022
153	0.118	0.000	0.098
155	0.088	0.519	0.196
157	0.132	0.000	0.120
159	0.074	0.000	0.065
161	0.000	0.000	0.011
167	0.029	0.000	0.000
Locus: KWM2b			
160	0.000	0.019	0.000
162	0.000	0.019	0.000
166	0.000	0.000	0.034
168	0.000	0.038	0.034
170	0.068	0.019	0.000
172	0.205	0.058	0.068
174	0.159	0.058	0.068
176	0.182	0.750	0.659
178	0.102	0.000	0.091
180	0.125	0.019	0.023
182	0.045	0.019	0.000
184	0.057	0.000	0.011
186	0.057	0.000	0.011
Locus: KWM12a			
151	0.040	0.000	0.000
155	0.100	0.000	0.000
157	0.100	0.000	0.010
161	0.000	0.000	0.010
163	0.000	0.000	0.010
165	0.060	0.000	0.010
167	0.060	0.017	0.050
169	0.140	0.103	0.070

171	0.020	0.034	0.090
173	0.080	0.034	0.280
175	0.140	0.310	0.240
177	0.100	0.362	0.100
179	0.100	0.138	0.080
181	0.040	0.000	0.020
183	0.020	0.000	0.000
185	0.000	0.000	0.020
187	0.000	0.000	0.010

Locus: EV37

192	0.014	0.000	0.000
194	0.081	0.000	0.000
196	0.041	0.000	0.000
198	0.041	0.000	0.053
200	0.108	0.017	0.053
202	0.000	0.034	0.181
204	0.095	0.017	0.085
206	0.068	0.000	0.202
208	0.095	0.328	0.043
210	0.000	0.052	0.032
212	0.041	0.034	0.064
214	0.014	0.103	0.032
216	0.027	0.190	0.032
218	0.000	0.034	0.032
220	0.000	0.052	0.053
222	0.014	0.000	0.011
224	0.014	0.017	0.021
226	0.014	0.000	0.032
228	0.014	0.034	0.000
230	0.014	0.000	0.043
232	0.027	0.000	0.000
234	0.000	0.017	0.021
236	0.014	0.000	0.000
238	0.014	0.034	0.000
240	0.000	0.017	0.000
244	0.000	0.000	0.011
248	0.054	0.017	0.000
250	0.041	0.000	0.000
252	0.149	0.000	0.000
260	0.014	0.000	0.000

Locus: TexVet5

196	0.014	0.000	0.000
198	0.071	0.000	0.000
200	0.086	0.000	0.000
202	0.057	0.000	0.024
204	0.114	0.020	0.048
206	0.114	0.000	0.036

208	0.057	0.220	0.143
210	0.114	0.040	0.381
212	0.071	0.480	0.214
214	0.114	0.160	0.095
216	0.057	0.080	0.060
218	0.043	0.000	0.000
220	0.086	0.000	0.000

Locus: TexVet7

154	0.012	0.000	0.063
156	0.047	0.016	0.219
158	0.209	0.048	0.177
160	0.256	0.468	0.292
162	0.326	0.194	0.083
164	0.058	0.242	0.083
166	0.023	0.000	0.021
168	0.058	0.032	0.042
170	0.012	0.000	0.021

Locus: D08

95	0.000	0.086	0.025
99	0.024	0.000	0.000
101	0.061	0.017	0.025
103	0.110	0.052	0.100
105	0.134	0.500	0.350
107	0.159	0.207	0.275
109	0.098	0.052	0.075
111	0.195	0.052	0.087
113	0.024	0.000	0.013
115	0.024	0.000	0.025
117	0.024	0.000	0.013
119	0.024	0.000	0.000
121	0.049	0.000	0.013
123	0.037	0.034	0.000
125	0.024	0.000	0.000
127	0.012	0.000	0.000

Locus: D18

80	0.102	0.167	0.109
82	0.500	0.250	0.344
84	0.023	0.083	0.000
86	0.091	0.167	0.219
88	0.034	0.167	0.047
90	0.114	0.000	0.031
92	0.068	0.000	0.000
94	0.034	0.167	0.000
96	0.023	0.000	0.000
98	0.011	0.000	0.156

102	0.000	0.000	0.031
104	0.000	0.000	0.031
106	0.000	0.000	0.031

Locus: D22

118	0.000	0.000	0.029
120	0.071	0.286	0.147
122	0.119	0.071	0.074
124	0.202	0.143	0.118
126	0.095	0.071	0.132
128	0.048	0.000	0.147
130	0.167	0.071	0.088
132	0.083	0.143	0.147
134	0.060	0.071	0.088
136	0.155	0.143	0.029

Locus: MK8

87	0.026	0.000	0.000
89	0.066	0.000	0.000
91	0.158	0.000	0.000
93	0.013	0.071	0.000
95	0.066	0.071	0.121
97	0.079	0.000	0.030
99	0.066	0.000	0.091
101	0.039	0.143	0.000
103	0.079	0.214	0.121
105	0.066	0.214	0.333
107	0.105	0.143	0.015
109	0.026	0.071	0.061
111	0.132	0.071	0.060
113	0.079	0.000	0.136
115	0.000	0.000	0.030

Locus: TtruAAT44

82	0.000	0.000	0.029
84	0.261	0.167	0.015
86	0.000	0.000	0.074
88	0.534	0.667	0.412
90	0.170	0.000	0.029
92	0.000	0.167	0.353
94	0.034	0.000	0.029
96	0.000	0.000	0.059

Locus: KWM9b

171	NA	0.043	0.000
173	NA	0.000	0.342
175	NA	0.000	0.263

177	NA	0.000	0.211
179	NA	0.152	0.105
181	NA	0.304	0.079
183	NA	0.478	0.000
185	NA	0.022	0.000