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Social and population structure of striped and Risso's dolphins in the Mediterranean Sea

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Social and Population Structure of Striped and

Risso's Dolphins in the Mediterranean Sea

by

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Doctor of Philosophy

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We do not really see the Sea from the bow of our small boat, we feel it.

I wish to dedicate this work to the animals that have offered me a

unique opportunity to be at sea and share with them some

unforgettable moments.

DECLARATION

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

Abstract

The aim of this research was to apply genetic methods to a better understanding of the evolution of population genetic structure in two dolphin species inhabiting a shared geographic range in the Mediterranean Sea and eastern North Atlantic (ENA). In support of this I analysed the pattern of population subdivision, genetic variability and group kin structure of striped (*Stenella coeruleoalba*) and Risso's dolphins (*Grampus griseus*) in the Mediterranean Sea, and compare theassessed the level of genetic differentiation between Mediterranean and ENA populations. The specific objectives were to: 1- study their distribution in the core study area (the Ligurian Sea) and group size variation as a possible behavioural responses to environmental features; 2- assess patterns of kinship and association within and between social groups (based on estimates of R); and 3- investigate patterns of genetic subdivision among the putative striped and Risso's dolphin populations within the Mediterranean Sea, and in comparison with the ENA.

Risso's dolphins showed a clear preference for the steeper continental slope, while striped dolphins did not show this distribution preference.

Samples from 165 striped dolphins were collected from the Mediterranean Sea (Adriatic, Tyrrhenian, and Spain) and ENA, and were analysed at 8 microsatellite DNA loci. The comparison between the Mediterranean and the ENA populations showed a clear separation (F_{ST}=0.055). All loci were polymorphic (4-22 alleles), and mean Ho ranged from 0.68 in the Tyrrhenian to 0.8 in the ENA. All comparisons between putative populations showed significant differentiation, including the populations of the eastern (Adriatic) and western (Tyrrhenian) seas of Italy. Analysis of the Risso's dolphin samples (n=50) collected in the Mediterranean and the ENA showed that the two populations were significantly differentiated (F_{ST}=0.0296). All loci were polymorphic (up to17 alleles), and mean Ho was 0.467 in the Mediterranean, 0.548 in the ENA. Analysis of 619 bp of sequence of the mtDNA control region revealed 28 variable sites defining 16 unique haplotypes among the two populations. The degree of differentiation was highly significant for both F_{ST} (0.260) and ϕ_{ST} (0.542).

Individual relatedness within and between social groups of striped dolphins was investigated in the Ligurian population. On average the values were higher for intra- than inter-group comparisons, and females showed significantly higher relatedness within than between groups. The kin structure of the Risso's dolphins in the Ligurian population showed that females had a considerably higher relatedness within groups than among groups. For both species, the data suggest a relatively fluid model of kin structure with a trend for female philopatry, and male dispersal.

My results indicate fine-scale population structure in both species, and significant differentiation between the Mediterranean and North Atlantic populations. A hierarchical pattern of kin structure is clearly influenced by social group structure and the dispersal behaviour of males and females, though there are some differences between the species, probably related to differences in social structure and habitat use.

ACKNOWLEDGEMENTS

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CHAPTER ONE

INTRODUCTION

INTRODUCTION

Aims

The principal aim of this study was to provide insights into the evolution of social and population structure in two species of dolphins, the Risso's and the striped dolphin in the Mediterranean Sea. In general these two species have received relatively little attention, especially the Risso's dolphin. Little is known about their social and genetic structure in the Mediterranean Sea, but contrasting aspects of their known ecology and life history suggested that a comparative approach would facilitate a better understanding of dolphin social behaviour in general.

The social systems of mammals are a result of the complex interactions of both internal constraints and external forces (van Schaik & van Hoof 1983). Biological factors and the physical characteristics of the habitat are important sources of ecological pressure acting on sociality. Ecological factors are generally recognised as the most fundamental determinants of social systems. The social system exhibited by a particular species, or population, is best considered as the outcome of strategies that individuals employ to meet their basic requirements, such as obtaining food, avoiding predators and finding mates (van Schaik & van Hoof 1983). Distinguishing the key factors which shape a particular system is difficult, the ecological factors themselves are often little understood, and the relationship between them may be complex and compounding (Gosling & Petrie 1981). A promising approach would be to manipulate the environment and observe the changes in the animals' social behaviour, but such opportunities, however, are rare especially in large free ranging mammals. The comparative approach between two different species subjected to a similar environmental pressure may provide great potential. By comparing the social system of animals of related species in the same environment one may be able to identify how certain social systems have evolved.

The dolphins' distribution along the Ligurian continental zone were considered in order to see whether different habitat characteristics had an influence on their group size and composition. Social behaviour was studied through the analysis of association behaviour and group size.

I have also aimed to examine the mechanisms involved in the evolution of social structure through the application of molecular markers, both nuclear and mitochondrial, in order to investigate the phylogeographic patterns of variation across different areas of the North Mediterranean Sea, and to determine the genetic distance between geographically structured sample sets. Measures of kinship within and between social groups were assessed from samples collected non-invasively from free ranging dolphins in the Tyrrhenian Sea.

Thesis outline

My thesis is organised into six chapters in which the different objectives outlined in the aims are addressed.

A general introduction on the existing knowledge available in the literature about sociality in general, and in particular on cetaceans, as well as a description of the two dolphin species and the study area is presented in this Chapter.

Chapter 2   presents the distribution and group sizes of both species within the tudy area in relation to the possible influences of season and environmental features.

Chapter 3   investigates patterns of genetic subdivision among putative striped dolphin populations within the Mediterranean Sea, and also compares Mediterranean with North Atlantic populations. Kinship was also assessed within and between social groups, based on microsatellite DNA markers. The determination of gender allowed an assessment of the possible role of same-sex coalitions and sex biased dispersal.

Chapter 4   assesses information on Risso's dolphin associations based on
             photo-identification of individuals and groups. The duration and
             stability of individual bonds are interpreted as indications of the
             nature of social structure in this species.

Chapter 5   describes the genetic structure of Risso's dolphins in the
             Mediterranean sea and compares it with a population in the North
             Atlantic, based on both microsatellites and mitochondrial DNA
             markers. In this chapter I also investigated individual relatedness
             within versus between groups. The determination of gender
             allowed an assessment of the possible role of same-sex coalitions
             and sex biased dispersal.

To conclude, Chapter 6 provides an overview of the results achieved, together
with a comparison between the two species.

Historical background

        The idea that ecological adaptation played an important role in the
formation of animal societies was rooted in Darwinian thought. The British
philosopher and sociologist Herbert Spencer, who was one of the principal
proponents of evolutionary theory in the 19th century, explored the notion that
animal societies are highly structured relationships among individuals of a
species. He discovered that such structures varied independently from the
taxonomic relationships of species, and he therefore interpreted them as an
expression of direct adaptation to ecology rather than of phylogenetic descent.
        The school of Konrad Lorenz (1932-1966) and Niko Tinbergen (1932-
1972) dominated the European study of social behaviour in animals from the mid
1930s until the publication of important critiques by Lehrmann (1953) and Hinde
(1969-1966). Social behaviour was interpreted in terms of interactions between

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individual organisms in which the fixed action patterns of one individual elicited reciprocal behaviour in another, as in the courtship movements of the stickleback, for example. The role of social behaviour within groups or within populations was only rarely considered and never a focus of serious attention. In 1962, Wynne Edwards drew attention to the possibility that many behaviours determining social organization might often be the result of group rather than individual selection. He argued that group selection was responsible for a very wide range of social attributes including flocking and mating systems. Most people nowadays reject this interpretation.

Through the work of Hamilton, Trivers, Williams, Dawkins, Wilson and many other evolutionary theorists, socioecology became far more explicitly evolutionary, and behaviour was seen as a direct consequence of natural selection operating through the differential reproductive success of individuals. Hamilton was one of the greatest evolutionary theorists. Trivers (2001) states that Hamilton's first work (1964), the theory of inclusive fitness, was his most important work, since it was a true advance since Darwin, in our understanding of natural selection. The work of Hamilton (1964) provided the basis for a neo-Darwinian account of biological altruism that had ramification for the animal kingdom as a whole. Trivers (2001) further suggests that Hamilton's work is an extension of Darwinian logic. In Darwin's system, natural selection refers to individual differences in reproductive success (RS) in nature, and RS is the number of surviving offspring produced. Hamilton extends the concept to include RS effects on other relatives: that is, not just fitness or reproductive success but inclusive fitness, defined as an individual RS plus effects on the RS of relatives. Hamilton's key realisation (1964), was that genetic inheritance is a matter of involving all relatives and not only parents and children. Any action by an adult that increases the fitness of an indirect relative such as a brother's child will improve the probability that genes shared in common between them will be passed to the next generation. This probability increases with the degree of relationship. It is possible to calculate the degree of relatedness (the coefficient of relatedness, r) and hence to predict relationships within which assistance from one relative to another will be beneficial in terms of 'inclusive' fitness to the donor.

That is to say, an individual fitness depends not only on its own survival and reproductive success but also on that of its kin to which it may contribute by fitness enhancing acts of "altruism", less the costs to its personal fitness. This is the principle of kin selection.

Trivers (1971) theorises a model for reciprocal altruism which aimed to show evidence of cross-species altruistic relationships, and non kin-based exchanges in terms of helping another at some cost to themselves. This idea of the "cost/benefit" value of any act that will help another has an underlying drive concerned with promoting the overall success of the individual's genes. Trivers realised that reciprocal altruism, independent of kin selection, could arise in circumstances where individuals live in close groups with ample opportunity for mutual recognition, and exchange benefits. Such behaviour, however, would be accompanied by high risk of the beneficiary cheating by failing to provide a return benefit. On the other end, Dawkins explains that acts of altruism don't really exist as such, since they are the product of a purely selfish mechanism, in which an individual is primarily thinking about his own welfare above the welfare of anybody else. In this sense, then, the notion of "reciprocal altruism" in itself is an antagonism.

In 1951, Wright developed the classical models of population structure, where individuals mate randomly within sub-populations. Under this model, Wright's fixation indices are used to interpret the breeding structure of a population. However, in many species, random mating within sub-populations does not occur. Instead, individuals associate in social groups, within which behaviours determine the mating system. Hence, while under the classical model, maintenance of genetic variation relies upon differentiation among sub-populations and rates of inbreeding, social structuring provides an additional hierarchical level at which genetic variation may be maintained, and within which behaviours may minimize inbreeding (Sugg *et al* 1997).

Theoretical background

Group living is only adaptive when the benefits of sharing resources with others counter-balance the costs, such as competition for food, the increased risk of detection by predators and the transmissions of diseases (Alexander 1974, (Wrangham & Rubenstein 1986). A simple model of group formation is seen when animals passively aggregate where food is concentrated, or in taking refuge from a predator. This is often referred to as a non- mutualistic grouping behaviour where there are no benefits due to the presence of other individuals, and the aggregation formed is thus referred to as non-social group (Alexander 1974, Connor 2000). When individuals actively seek companions to gain benefits from each other, this promotes social group formation, which depends on relations between individuals, as in mating, resource defence and predator defence.

Many mammals live in social groups and this facilitates many important aspects of their life such as feeding, reproduction, communication, learning, and defence (Hamilton 1964, Alexander 1974, Axelrod & Hamilton 1981, Norris & Schilt 1988). They have evolved a wide variety of social systems. The evolution of this behaviour has been attributed to a variety of factors. These include: the distribution and abundance of food resources (Jarman 1974, Macdonald 1983); the behaviour of their prey (Würsig *et al* 1991); the level of competition for food (Lott 1984, Foster 1985); the distribution of mates (Macdonald 1983); levels of predation (Seghers 1974, Cresswell 1994); population density (Emlen & Oring 1977; Albon *et al* 1992); information transfer requirements (Ward & Zahavi 1973); habitat type (Macdonald 1983, Würsig *et al* 1989) and requirements for rearing young (Wells 1991). The identification of the relative importance of these factors is necessary for the understanding of the mechanisms involved in the evolution of a particular social system.

One of the fundamental questions in evolutionary biology is "why should animals form groups, and even more interestingly, cooperate or exchange altruistic acts-when benefit may be unequal among group members? Over the past decade, four models have been developed to explain the evolution and the maintenance of cooperative and altruistic behaviour on an individual level: by-

products mutualism, reciprocity, pseudo-reciprocity and kin selection (Hamilton 1964, Trivers 1974, West-Eberhard 1975, Connor 1986). The first three models do not assume that cooperating individuals are related, but genetic relatedness among individuals is a key assumption of the kin selection model. Ever since the seminal work of Hamilton (1964) on kin selection, genetic relatedness has been considered as one of the crucial variables in the understanding of any social phenomenon. The occurrence in many species of groups of close kin raises additional questions about whether individuals can somehow assess their genetic relatedness to one another, and perhaps adjust competitive, cooperative, altruistic, or other behaviours accordingly (Waldman 1988). The knowledge of the social structure of a population is important for a range of fundamental and applied purposes (Whitehead 1997). Social structure defines an important class of ecological relationships. The identification of the relative importance of these factors is necessary for the understanding of the mechanisms involved in the evolution of a particular social system. Thus, social structure is often an important element of the population biology of a species, influencing gene flow, spatial pattern and scale, and the effect of predation and/or exploitation by humans (Wilson 1975).

Analysis of the social structure of a population requires detailed information on the interactions between individual members of that population collected over a considerable period of time. This has been possible for some primates (e.g. Goodall 1986). To understand the association pattern within a species, it is not sufficient to consider only ecological parameters, without taking social organisation into account (Wrangham & Rubenstein 1986).

Molecular studies

Molecular-based studies have already addressed many questions relating to the evolution and structure of populations of different species of whales and dolphins. The data obtained from mitochondrial and nuclear markers are qualitatively different, and maximum information is achieved by parallel analyses of these genomes. Mitochondrial DNA (mtDNA) can tell us about matrilineal

population structure, and nuclear markers can tease out close family relationships and provide information about gene flow between subpopulations (Amos 1997). The analysis of animal mitochondrial DNA polymorphism represents the most commonly used means for revealing phylogenetic relationships among closely related species, and among populations of the same species (Avise & Lansam, 1983, Wilson *et al* 1985, Avise 1986, Moritz *et al* 1987, Harrison 1989). MtDNA is strictly maternally inherited in mammals and devoid of recombination (Dawid & Blackler 1972, Hutchison *et al* 1974, Giles *et al* 1980). The lack of recombination makes it possible to detect past evolutionary events such as migration, bottlenecks and population division by the patterns of coalescence of the different maternal lineages. Nuclear markers are inherited in a Mendelian fashion which allows for identification and delineation of panmictic units, assessment of levels of genetic variability and the assessment of the degree of relatedness between individuals. A combined use of genetic and behavioural data provides the best insight into the dynamics of social group structure and the evolution of dolphin societies.

Cetacean sociality

Many animals, whose social structures appear complex and interesting, however, live in situations that make it difficult to collect detailed data on interactions between individuals. Those include many species of cetaceans (Tyack 1986). The social structure and evolution of cetaceans have recently been reviewed in considerable detail, and compared and contrasted with those of terrestrial mammals (Connor *et al* 1998, Mann *et al* 2000). Many dolphin species are highly social, and a number of factors have been proposed as important in the evolution of this behaviour in these species. One factor may be cooperative foraging and feeding behaviour.

In the long-finned pilot whale (*Globicephala melas*), social groups typically consist of 50-200 animals whose strong herding instincts have been exploited by native peoples to drive entire pods into shallow bays for mass

slaughter. Preliminary DNA-fingerprint analyses of tissue samples from several such Faroe Island harvests revealed that adult males are not closely related to adult females within a pod, and furthermore 90% of foetuses could not have been fathered by a resident male (Amos *at al* 1991). From these and other behavioural observations, the authors conclude that the social groups in the pilot whale are built around matrilineal kinship, with considerable interpod genetic exchange mediated by males. Matrilineal relationships within other cetacean species have been examined more directly by mtDNA analyses, for example, among pods of killer whales (*Orcinus orca*) near Vancouver Island, British Columbia. In this area detailed field studies based on photo-identification of killer whales began in the 1970s, and since then much was revealed about the life of killer whales. Maybe the most striking feature of these odontocetes is their dispersal pattern, or rather the lack of dispersal pattern of the *resident* killer whales. Two distinct mtDNA types have been observed that appear to correspond to a long recognised behavioural distinction between sympatric groups with fish-hunting (resident) versus mammals-hunting (transient) social traditions (Hoelzel & Dover 1990, Hoelzel 1991, Hoelzel 1998, Hoelzel *et al* 2002). On the other end, in similar mtDNA analyses of groups of spinner dolphins (*Stenella longirostris*), no clustering of matrilines within specific schools or neighbouring morphotypes was detected, suggesting significant and recent genetic interchange (Dizon *et al* 1991).

THE RISSO'S DOLPHIN

Taxonomy

Grampus is a monotypic genus of the family Delphinidae. Taxonomist have long puzzled over the classification of Risso's dolphins, basic questions about the relationship between *Grampus* and other delphinids abound. They are thought by some to share important morphological similarities with *Tursiops* (Fraser & Purves 1960), while others think that Risso's is distinctive enough to be put in its own subfamily (Mead 1975), and yet others believe that *Grampus* are most closely allied with *Globicephala* (Caldwell *et al* 1968, Paul 1968).

Classification

The French scientist G. Cuvier first described this species under the name of *Delphinus griseus* after an amateur Italian M. Risso found a specimen on a beach near Nice in 1811. Griseus is Latin for grey. In 1828 the British naturalist Gray created a new genus, for this species, called *Grampus* from the Latin *grandis* which means big and *pisces* which means fish. It was True in 1889 who finally classified the species as *Grampus griseus*. *Grampus griseus* belongs to the species of *delphinidae*, and the latest systematic revision of Delphinidae based on cytochrome b analysis (LeDuc 1999), assigns *Grampus griseus* to the family of *Globicephalinae*, but its classification is still a matter of debate.

Morphology

Risso's are relatively large, the body is torpedo-shaped measuring 3m-4m in length, there is no documented sexual dimorphism in the genus though. They weigh between 350-500 kg, having a stocky, robust body that becomes slender behind the dorsal fin to the tail fluke. Size at birth is about 1.5 m, adults attain a maximum length of about 4 m, and large animals weight about 500 kg. The animal's most distinctive morphological trait, the head, is blunt with no beak. A V shaped groove in the forehead, creates a distinctive cleft down the animal's face. Risso's dolphins have reduced dentition specialised for taking cephalopod prey. Typically there are no teeth in the upper jaw and two to four pairs in the lower jaw.

Figure 1.1. Adult Risso's dolphins head seen from above, where is visible the V shaped groove in the forehead.

The abundance of white marks on their bodies make them easy to identify individually. The dorsal fin is tall, erect and falcate and their pectoral fins are long and sickle-shaped. At sea the best identification characteristic is the coloration and scarring. Adults range from uniform grey to nearly white as the animals age, typically covered with white scratches, spots and blotches. They are uniformly grey at birth, changing to dark brown and with age turn to light grey as scars accumulate. The scarring is believed to be caused by the teeth of other Risso's dolphins and from interactions with the cephalopod prey. There is a large anchor-shaped light grey patch on the dark ventrum similar to that found in pilot whales.

Diet

The main prey for Risso's dolphins are cephalopods. Cockcroft *et al* (1993) examined the stomach content of Risso's dolphins stranded along the east coast of South Africa over a 22 years period (1969-1991). Prey consisted exclusively of cephalopods from which 17 species were identified. The dominant prey was *Loligo vulgaris reynaudu*, a shallow water, semi-pelagic subspecies which constituted 81% by mass of the total prey and 48.1% and 29.2% by number and frequency of occurrence, respectively. Other important species were *Lycoteuthis diadema, Argonauta nodosa, Octopus magnificus* and *Ancistrocherius lesueuri*. These data suggest a partitioning of food resources between subgroups. Wuertz *et al* (1992) report that the cephalopod remains from the stomachs of Risso's dolphin entangled in a fishing net off the Ligurian coast include squids *Ancistroteuthis lichtensteini, Histioteuthis bonnelli, H reversa* and *Todarodes sagittatus* and the sepiolid *Heteroteuthis dispar*. All these cephalopods live in oceanic water over the steep continental slope. Clarke & Pascoe (1985) found that cephalopod remains from the stomach of a Risso's dolphin stranded in South Devon included an octopus *Eledone cirrhosa*, a cuttlefish *Sepia officinalis* and four squid *Loligo forbensi, Todaropsis eblanae, Todarodes sagittatus* and an oceanic species. Santos *et al* (1995) on stomach analysis of three Risso's dolphins stranded in Scotland found the main prey species to be the octopus *Eledone cirrhosa*. Five Risso's dolphins stranded in NW Spain had mainly cephalopod remains in their stomachs mainly *Octopus vulgaris,* and *Eledone cirrhosa.*

Reproduction

Risso's dolphins become sexually mature at about 3 m length and at about 9 years of age (Leatherwood *et al* 1982). Kruse (1987) observed the smallest calves during the month of November in Monterey Bay, California. Gill & Atkinson (1996) observed nursery groups up to 6 mothers and neonates of similar

ages during the months of August and September in the coastal waters of the Isle of Lewis, Scotland. In 1998 I observed a mother with a newborn calf in the Ligurian Sea that still showed the foetal grooves in September. It may be that the breeding season is extended and variable and may differ with geographical localities. Information from 23 Risso's dolphins stranded in Japan, suggest that gestation time may be 13-14 months (Kasuya 1985).

Seasonality

In some areas, Risso's dolphins are reported to be migratory (Australia: Troughton 1931, Walker *et al* 1968). Summer reproductive migrations and winter feeding migrations have been observed off Japan (Mizue & Yoshida 1962). Others suggest that Risso's dolphins are equally common throughout the year, showing no evidence of seasonal migration (Great Britain: Fraser 1953, Cowan & Guinet 1956). In Monterey Bay, California, there appeared to be a seasonal influx into the bay probably related to temperature (Kruse 1989). Leatherwood *et al* (1980) reports that resightings of identifiable individuals indicate that shifts in abundance did not reflect a cohesive seasonal population movement along the coast. Individuals sighted between seasons indicate that animals may not follow a strict seasonal or migratory pattern of residency but may visit the bay at any time. Dohl *et al* (1978) describes a correlation between population size, distributional expansion/contraction and water temperature in the southern Carolina Bight area, USA. They found that as the water cooled, Risso's dolphins appear to leave the Bight, moving offshore and to the south. Thus it appears that abundance patterns fluctuate with sea surface temperatures independent of the season (Dohl *et al* 1983). Kruse (1989) reports that the dolphins were more abundant when the temperatures were stable than when large temperature fluctuations were recorded, suggesting that they prefer warmer well-mixed surface waters. Dohl *et al* (1983) reported that between 1980-1983, Risso's were moderately abundant all year on the north and central California coast during which time records showed only minor seasonal fluctuations.

THE STRIPED DOLPHIN

General Characters

The general body plan of *Stenella coeruleoalba* is similar to that of most small oceanic delphinids: a largely fusiform body with a long beak, well demarcated from the melon, falcate dorsal fin, and long slim flippers. It is a relatively robust dolphin; the longest recorded specimen reaching 2.56 m (from the western Pacific) and the heaviest specimen weighing 156 kg (from the southwest Indian Ocean). On average, males measure 11-15 cm longer than females in the western Pacific and 2 cm longer in the Mediterranean (Kasuya 1972, 1976, Miyazaki 1983, Calzada & Aguilar 1995). South western Mediterranean striped dolphins are 5-8 cm shorter than their eastern Atlantic conspecifics (Calzada & Aguilar 1995). Mean maximum body length has been estimated to be 236 cm for males and 220 cm for females. Maximum estimated age for both males and females is 57.5 years (Kasuya 1972, Miyazaki 1984, Kasuya 1985).

The species name *coeruleoalba*, refers to the bold blue and white colour pattern of this species, which may serve to break up body outlines or be used for interspecific cohesion (Wursig *et al* 1990). The dorsal cape is often a muted blue or bluish-grey, while the eye-to-anus and flipper stripes are of a darker blue coloration. The lateral and ventral fields and the spinal blaze can range from white to grey.

Figure 1.2. Anterior end of a striped dolphin where the head and lateral coloration are visible.

Reproduction

Much is known about the growth and reproduction of this species as a result of a long series of studies on specimens caught in the Japanese drive and hand-harpoon fisheries. Mating occurs in winter and early summer. Gestation lasts from 12 to 13 months, during which time the average foetal growth rate is 0.29 cm per day. In the western Mediterranean, gestation is approximately 13 months, similar to that in the west Pacific. Length at birth is approximately 92.5 cm, at an average weight of 11.3 kg. There is a single mating and calving period in autumn, which allows the mother to take advantage of the relatively high seasonal regional oceanic productivity (Frontier & Viale 1985; Aguilar 1991). Body length at birth has been estimated to be 100 cm. Lactation lasts approximately 1.5 years. After birth, there is a rapid increase in size for both sexes for the first two years, with length reaching 166 cm in the first year and 188 cm in the second year. Sexual dimorphism begins at two or three years of age,

with males exceeding females by approximately 4 cm (Kasuya 1976, Miyazaki 1984). Males enter sexual maturity at between 7 and 15 years of age at an average body length of 220 cm, with social maturity being reached at the age of 17. Females become sexually mature between 5 and 13 years old. The mean length at sexual maturity for females is 212 cm. Females go through a 0.2 to 0.5 year resting period, which makes for an average reproductive cycle of 3 years. Females are estimated to ovulate once every two years, with a decline in fecundity at about 30 years of age.

Diet

Fish in the family Myctophidae are one of the important documented prey items in stomachs of striped dolphin found off the coast of Japan and South Africa, comprising 63% and 82.5% respectively of all fish prey recorded (Miyazaki *et al* 1973, Ross 1984). In the Northeast Atlantic, cod is taken more frequently, accounting for 63% of the total fish found in 25 stomachs (Fraser 1953, Desportes 1985). Several studies have found from 50% to 100% of the stomachs in Mediterranean striped dolphins to contain only cephalopods, with the majority of the remaining samples containing a combination of cephalopods and fish (Duguy *et al* 1978, Raduan & Raga 1982, Wurtz & Marrale 1991). The most frequent prey taken were the squid families Ommastrephidae and Histioteuthidae (Desportes 1985, Pulcini *et al* 1992,Wurtz & Marrale 1991), see Perrin *et al* (1994) for a comprehensive list of prey families.

The known range records of recorded prey indicate that striped dolphins often feed in the pelagic or benthopelagic zone along the continental slope or just outside in oceanic waters (Desportes 1985; Ross 1984; Santos *et al* 1993; Wurtz & Marrale 1991). The majority of the prey (74%-80%) have luminescent organs, suggesting that the dolphins may be feeding at great depth, possibly diving from 200 to 700 m, to reach potential prey. They may also feed at night in order to take advantage of the diurnal vertical migrations made by many of their prey species (Miyazaki *et al* 1973; Ross 1984,Wurtz & Marrale 1991).

THE MEDITERRANEAN SEA

The Mediterranean Sea is located between 30°N and 45°N, and 6°W and 36°E. It is an almost totally land-locked sea, joined to the Atlantic, through a single, relatively narrow entrance at the Strait of Gibraltar. In 1869, the Suez Canal was opened to connect the Mediterranean to the Indian Ocean and, in its far north-eastern corner the Mediterranean is also linked to the land-locked Black Sea though the Sea of Marmara and the Bosphorus. The Mediterranean Sea extends over 3000 km in longitude and over 1500 km in latitude and has a surface area of more than one million square kilometres. Its floor is made up of a complex system of ridges, troughs and deep basins. In comparison with those of larger oceans, the Mediterranean Sea continental shelf regions are extremely narrow and deeply incised by submarine canyon systems. The Mediterranean is divided into two distinct eastern and western basins by a narrow and relatively shallow (400 m) constriction at the Strait of Sicily. The Western Basin is characterised by broad, generally smooth abyssal plains. In contrast, the Eastern Basin is dominated by the Mediterranean ridge system and has a much lower proportion of smooth abyssal plain. The Mediterranean Sea is noted for its unique water circulation. The surface circulation in the Mediterranean generally flows eastwards transporting low salinity, Atlantic surface waters across the basin. At the Strait of Sicily, approximately one third of the water is diverted and enters the Tyrrhenian Sea, flowing northward along the Italian coast. The remaining two-thirds flows through the Straits into the Ionian Sea, forming a weak but persistent eastward current. Along the western coasts of Sardinia and Corsica, the surface water generally flows north towards the Ligurian Sea forming the western Corsican current. This water continues to flow westward along the French coast, as the Ligurian current, and eventually west along the Spanish coast (Millot 1987). This permanent cyclonic circulation in the north-western basin leads to divergences and fronts. The most important ones are the Ligurian divergence, centred approximately at 42°N/8°E, and the Algerian-Provençal divergence, centred at 41°N/5°E. These divergences have a seasonal character and bring to

the surface a mass of intermediate water, hence supplying nutrients to the euphotic layer.
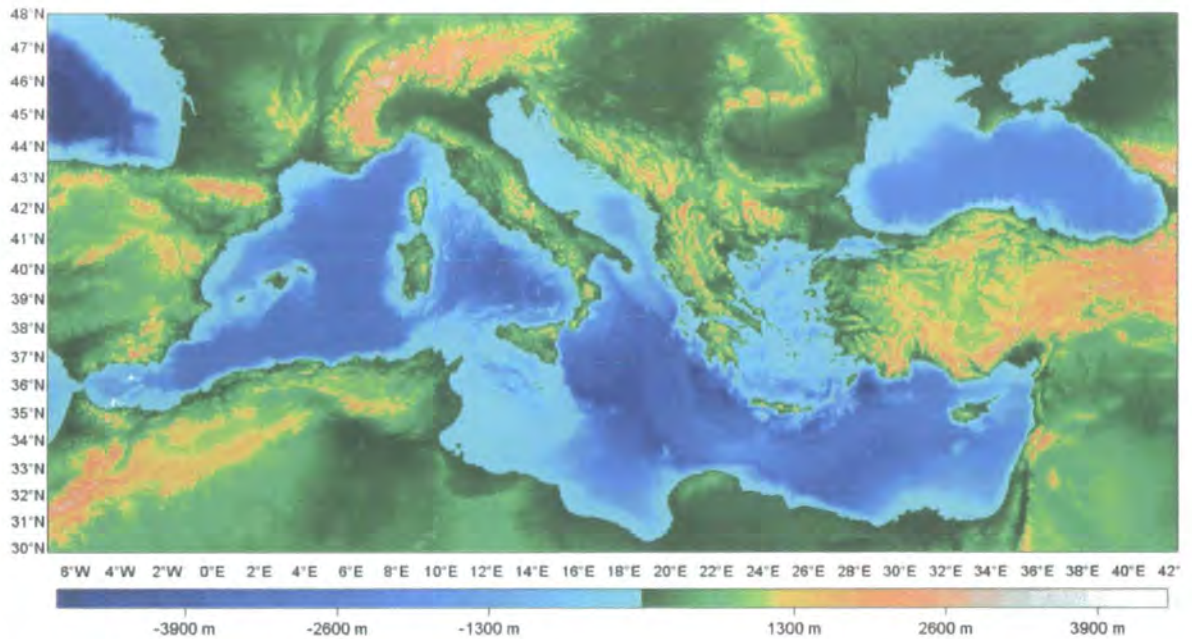
Figure 1.3. Map of the Mediterranean Sea showing longitude and latitudes, sea depth are also indicated.

The Mediterranean Sea is described as oligotrophic (meaning 'with low levels of primary production'). Some sites of increased productivity do, however, occur locally and seasonally. Primary production is particularly heterogeneous in the Western basin (Jacques & Treguer 1986). The Alboran Sea and Gulf of Lion are recognised as being permanently mesotrophic, with superficial biomass ranging from 0.5 to 1mg Chla. The Ligurian Sea appears to be relatively rich in summer, with surface biomass ranging between 0.3 and 0.5 mg Chla. The Tyrrhenian Sea is characterised by a marked oligotrophy throughout the year. In the eastern Mediterranean, the completion of the Aswan Dam in 1970 drastically reduced the Nile River outflow, which was possibly the largest source of nutrients in the region. This has resulted in a particularly low productivity in this region.

Mediterranean cetacean populations are not endemic, and it is known that they have originated from the adjacent Atlantic populations, but there is little information about the degree of exchange between the populations inhabiting the Atlantic and the Mediterranean basin. Moreover, the connection with the Atlantic ocean that governs the water circulation of the basin, combined with the topography of the bottom (max depth 5000 m) and the shape of the coasts, determine the bio-oceanographic feature of the Mediterranean Sea, which is characterized by high salinity (36-38%), strong seasonal fluctuation of the water temperature, scarce upwelling and the non homogenous distribution of nutrients. The ecology of cetaceans in the Mediterranean Sea is likely to have been influenced by these factors.

CHAPTER TWO

DISTRIBUTION AND GROUP SIZE OF RISSO'S AND

STRIPED DOLPHINS IN THE NORTH WEST

MEDITERRANEAN SEA

INTRODUCTION

The geographical distribution of delphinids has been related to a great variety of environmental and biological factors. Theses include: prey distribution (Würsig & Würsig 1980, Irvine *et al* 1981, Heimlich-Boran 1996, Würsig *et al* 1991), water depth (Wells 1996; Ballance 1990), tidal variation (Fellman *et al* 1991), submarine topography (Evans 1971, Hui 1979, 1985, Heimlich-Boran 1988; Würsig *et al* 1991), and surface temperature (Perrin 1984, Kasuya *et al* 1988, Mate 1989). Several factors are known to influence the spatial and temporal distribution of cetaceans. When considering the environmental context, we generally include physiochemical, climatological and geomorphological characteristics, as well as biotic (i.e. prey distribution and predation), and anthropogenic factors. All these factors play an important role in structuring cetacean communities (Borcard *et al* 1992, Jaquet 1996). However, often it is difficult to quantify the contribution of each of these factors, or to specifically attribute them to a particular event (Jaquet 1996). Cetaceans are an elusive study species due to their diving behaviour. This characteristic, and the difficulty of making a continuous record of all factors possibly involved with their presence or absence over certain areas complicate the study of cetacean movements and habitat preferences. In particular, in the Mediterranean Sea, studies on the factors controlling their distribution have been rather inconclusive. It is generally assumed that the forces driving the patterns of movements include the need to perform activities in specific habitats, at certain times, and the need to optimize the use of resources in that habitat to maximize fitness (Stevick *et al* 2002). Information on cetacean movements has often been infered from simpleobservations of animals in certain places at certain times.

Environmental variables such as predation and prey resources may have an important influence on group sizes and distribution, but also broad features of the environment, such as the water depth, have been related to group size. Gygax (2002) published a quantitative comparative analysis of different Delphinoidea

species, in relation to environmental characteristics, including water depth. He found some indication that open habitat leads to bigger group sizes, which would support the hypothesis of groups as an anti-predator strategy. A study on humpback whale (*Megaptera novaeangliae*) habitat preference (Ernst & Robembaum 2003) showed that their distribution by depth could not be considered as a function of group size but could be described as a function of social organisation, with mother-calf pairs showing a strong preference for shallower water compared to all other group types.

I investigate group sizes and distribution of both species in order to understand whether group size could be related to specific areas and/or specific seasons, and to understand whether group formation may be in response to specific environmental factors. Most studies on the evolution of group sizes focus on costs and benefits of behavioural strategies in the context of cooperative foraging, and defence of resources against predators (Lee 1994, Rodman 1988, van Schaik & van Hoof 1983, Terborgh & Jason 1986, Wrangham 1980). However, other factors should be considered when trying to understand a certain distribution pattern and group size formation. In fact, factors such as bottom geomorphology, may play an important ecological role in determining the distribution of different species and may also influence social organization.

Risso's dolphin world-wide distribution

Risso's dolphins are abundant world-wide and are widely distributed throughout tropical and temperate seas (Letherwood *et al* 1980). They show a preference for deep offshore waters but will inhabit coastal areas around oceanic islands and where there are narrow continental shelves. In the western Atlantic, Risso's dolphins have been reported as far north as Greenland (60° N) (Vibe 1950) and as far south as Cape Horn (53° S) (Leatherwood *et al* 1980) with sighting records at Cape Cod (True 1989, Gunter 1954, Paul 1968, Fahlgren-Torres 1988) the Virgin Islands (Erdman 1970) the Caribbean (Caldwell *et al* 1971), Brazil (Geise 1987) and Argentina (Mitchell 1975). In the eastern Atlantic

sightings range from the Shetland Islands, Scotland (60° N) (Fraser 1953) and as far south as the Cape of Good Hope (34° S) (Bernhard 1954) with sighting records from Norway (Oen 1987), Sweden (Schultz 1970, Lepiksaar 1974), the Outer and Inner Hebrides and the Mediterranean and Adriatic seas (Trois 1883, Tamino 1953, Pilleri 1967, Pilleri 1970, Duguy *et al* 1978, Raga 1986, Notarbartolo di Sciara 1990). They occur throughout the Indian Ocean (Ross 1984, Alling 1987) and the Indo-Australian Archipelago (Medway 1977, Hembree 1980). In the western Pacific they have been recorded as far north as Kuril Island (50° N), east China sea and Japan (Nishiwaki 1967) and as far south as New Zealand (45° S) (Oliver 1922, Baker 1974, 1983) with sightings from Australia (Troughton 1931) and the Soloman Islands (Dawbin 1966).

In the Eastern Pacific they occur as far north as the Aleutian Islands (50° N) (Clark 1945) and as far south as the Chilenean Coast (Aguayo 1975) with sightings from the Gulf of Alaska (Clark 1945, Braham 1983), Vancouver Island (Guinet & Pike 1965, Pike & MacAskie 1969, Hatler 1971, Reimchen 1980), Washington (Stround 1968, Oregon (Wick 1969), California (Orr 1966, Ficus & Niggol 1965, Dohl *et al* 1978, 1983), Baja California (Hubbs 1960, Leatherwood *et al* 1979) and throughout the eastern tropical Pacific (Au & Perryman 1985).

The Risso's dolphin in the Mediterranean

Risso's dolphin is a pelagic species which is most commonly encountered seaward of 1,000 fathoms (Leatherwood *et al* 1982, Dohl *et al* 1983). Only in areas where the edge of the continental shelf is close to shore, are these animals likely to be observed in coastal waters. In the Mediterranean basin, this species is found from Gibraltar to the Aegean Sea, and it is mostly encountered in deep pelagic waters, in particular over steep shelf slopes and submarine canyons. Although Risso's dolphins are regularly sighted in the western Mediterranean, no population estimate exists for this species. The few published population estimates do not come from recognised estimation methods. However, a figure of 3,000 animals in the entire western Mediterranean is published in the report of the

International Workshop on Mortality of Cetaceans (Perrin *et al* 1990). Di Natale (1983), from a study on the distribution of the seven most common cetacean species among different regions in the seas surrounding Italy from 1978 to 1982, concluded that *G. griseus* is consistently encountered in the central Mediterranean Sea.

Outside the Mediterranean Sea, only a few distribution studies on Risso's dolphins have been undertaken to date. From October 1985 to November 1987, Kruse conducted a study on photo-identification of *Grampus griseus* along the west coast of California (Kruse 1989). A photo ID study on the species around the Azores was started in 1987 from Song of the Whale, with 58 individuals catalogued. Since 1992, the Alnitak, which is a non-profit NGO founded in 1990 with the aim of carrying out marine environmental investigations in the Mediterranean Sea, has been conducting a research programme on cetaceans along the southern coast of Spain, and has identified 300 individuals (Cañadas & Sagarminaga 1995). After four years of research on photo identification and distribution (1992-95), Cañadas concluded that the highest concentration of *G. griseus* seems to occur mainly in the Ligurian sea, where they are seen all year. Notarbartolo di Sciara from the Tethys Research Institute conducted a series of cruises in the central Mediterranean sea (1986-1989), studying the distribution and relative abundance of six species of cetaceans. He too concluded that the highest sighting frequencies of Risso's dolphins occurred in the Ligurian sea. It is therefore likely that a resident population of Risso's dolphins inhabit the Ligurian sea. In order to establish this, in 1997, the Tethys Research Institute started a photo identification study on *Grampus griseus* in the Ligurian sea, as part of a wider, long-term project. Another similar study, based on individual photo identification, was started in 1987 by P. Evans in the coastal waters of the Isle of Lewis in Scotland (Gill & Atkinson 1996). This particular study revealed that a population of Risso's dolphins may be resident in that area for at least six months of the year, and possibly all year. They also suggested that prey availability and distribution plays an important role in the distribution of Risso's dolphins off Scotland, especially during the winter months.

The striped dolphin in the Mediterranean

The striped dolphin is the most frequently occurring dolphin in the Mediterranean Sea (Aguilar 1991), well documented from the coasts of Spain, France, Italy, and Greece. Records from countries bordering the southern Mediterranean are not as common, perhaps as a result of lower sighting effort. In the western Mediterranean, the species was considered rare in the 1960s, until an apparent population explosion occurred in the mid 1970s, causing it to become the most abundant species in the region (Viale 1985). Later, from 1990 to 1992, several thousand were estimated to have died in an epizootic caused by morbillovirus infection (Aguillar & Raga 1993). Post-epizootic abundance estimates were on the order of 118,000-225,000 individuals (Forcada *et al* 1992, 1994). As no pre-epizootic abundance estimates exist, the total impact of this event on the population remains uncertain. There is no overall population estimate in the Mediterranean Sea. However, the striped dolphin is now considered the commonest cetacean encountered in the Mediterranean basin. Line-transect surveys in 1991 and 1992 yielded population sizes respectively, of 117,880 individuals over a large portion of the western Mediterranean, and about 25,600 individuals in the Ligurian-Corsican-Provençial (LCP) basin (Notarbartolo di Sciara *et al* 1993). Key areas of distribution include the deep offshore waters of the central and western Mediterranean Sea, particularly the LCP basin. Striped dolphins are also frequent in the Ionian Sea and open waters of the southern Adriatic Sea. Their abundance appear to decrease towards the eastern portion of the Mediterranean basin.

Description of the study area

The study area was situated in the Ligurian-Corso-Provençial (LCP) basin which is one of the few areas in the Mediterranean where the

continental shelf is close to the coast, and this has favoured the study of pelagic species such as Risso's dolphins. The core study area is approximately encompassed by the longitude of Cap Ferrat (7.33 °E), close to the French/Italian border, to the longitude/latitude of Imperia (8.02 °E; 43.8 °N) and the 50-m and 2000/2500-m isobaths (Fig 2.1). All cetaceans regularly observed in the Mediterranean can be found in this region and include the fin whale (*Balaenoptera physalus*), sperm whale (*Physeter macrocephalus*), Cuvier's beaked whale (*Ziphius cavirostris*), long-finned pilot whale (*Globicephala melas*), bottlenose dolphin (*Tursiops truncatus*), Risso's dolphin (*Grampus griseus*), striped dolphin (*Stenella coeruleoalba*), and common dolphin (*Delphinus delphis*).

Figure 2.1. The Sanctuary for the protection of marine mammals in the Northwest Mediterranean. The core study area is the dark blue area (Mar Ligure).

The LCP basin was declared a Marine Sanctuary for the protection of marine mammals in 1999. This protected area extends over 90,000 km^2

of the Ligurian Sea, the sea of Provençe and the Corsican- Sardinian basin.
The Sanctuary covers the inland seas, the territorial waters of the three
countries (Italy, France, and Montecarlo), and the open sea. A series of
ecological conditions make this basin a very productive pelagic zone with a
rich variety of life, and a strong cetacean presence, both in terms of quantity
and diversity of species. This is a complex area from different points of
view, due to the presence of several islands, and its proximity to the Atlantic
water exchange area. It is characterised by a very high level of primary
productivity, in contrast to the generalised oligotrophy of the Mediterranean
sea (Barale & Zin 2000). The large volume of water, the cyclonic
movement of the currents, the deep water formation and the consequent
upwelling of water rich in nutrients lead to the development of complex
food chains. The Ligurian sea in particular supports an abundant biomass of
Mediterranean krill, the euphausid *Meganyctiphanes norvegica,* that
constitutes a nodal prey in many marine trophic chains.

METHODS

From 1990 to 2000 (June to the end October), sighting cruises from Imperia (Italy) to Nizza (France) were carried out on board different sized sailing boats, ranging from 9 m to 20 m. From 1996 to 2000 an additional source of sightings data was available: a whale watching boat operating in the area of Imperia.
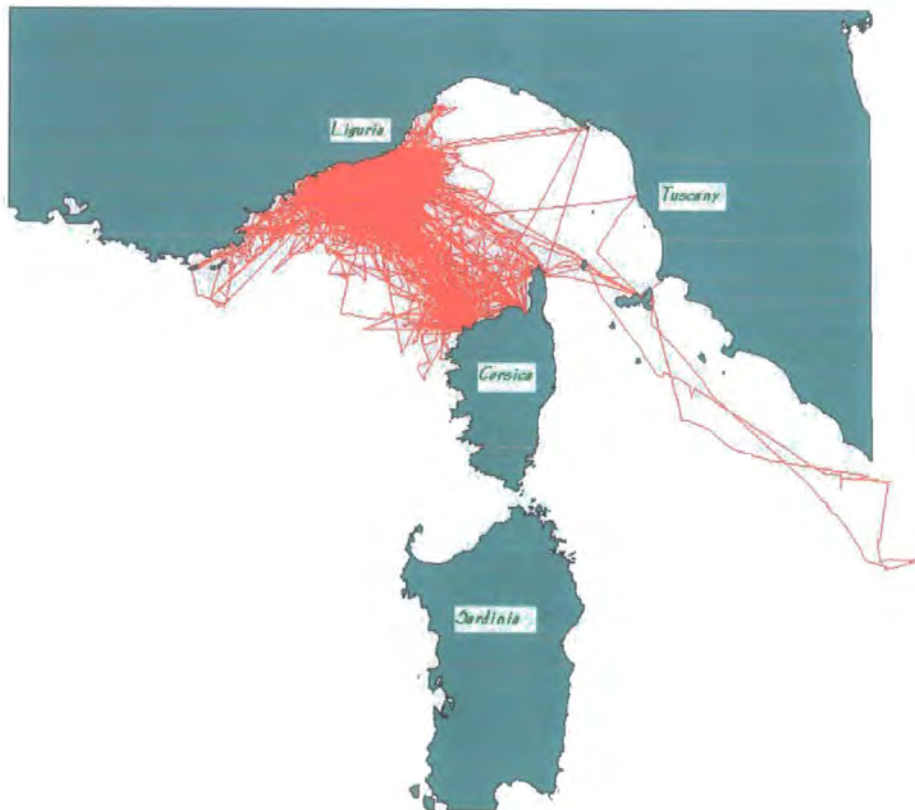
Figure 2.2. The map of the northwest Mediterranean sea shows the total track lines during the study period.

During surveys, at least two observers were constantly monitoring the area, both by naked eyes and with binoculars. Ship locations were collected at regular time intervals using a GPS data-logging system (IFAW-LOGGER Data Logging Software), to track the effort line of the vessel and, once the animals were located, to track their movements during the sighting . The position of the boat was recorded every ten minutes when *off effort* (i.e. not searching or in the presence of the dolphins), and every three minutes when *on effort* (i.e. when searching or in the presence of dolphins) and outputs the surveys tracks were produced. Trips at sea were conducted whenever weather conditions permitted. During the sighting events, the searching was interrupted for periods ranging from a few hours to the whole day in order to collect records on the encounters. Logsheets were used to maintain an *ad libitum* record of events (Altman 1974). Information recorded (1990-2000) includes start and end times of the vessel trips, course information such as heading, start and end times of each dolphin group observation, group size, presence, and general notes on their behaviour. Dolphins were distinguished in three age categories: adults, young and calves. Risso's dolphins were distinguished depending on size and coloration, details are given in the methods of chapter 4 (Figure 4.1a-c). Also striped dolphins were distinguishable from size and coloration.

A dolphin group was defined as all animals within 200 m of each other exhibiting similar behavioural characteristics, such as direction and activity. To investigate the distribution data, the study area was divided into cells by using a grid of 3 nautical miles. A G.I.S. software (MapInfo) was used to create the grid and to prepare the dataset for the following statistical analysis, and the cell unit was chosen as the basic unit for the analysis. The cell encounter rate (i.e. number of sightings/km surveyed) was calculated by dividing the number of encounters by the effort (i.e. length of track lines comprised within a cell). The cell encounter rate was calculated on an annual and monthly basis. All cells surveyed were grouped by year or by month and treated as populations to compare the central tendency of the annual or the monthly encounter rates. Statistical comparisons of inter-annual, inter-seasonal and group size differences for non-normal data were determined by applying the non-parametric Kruskal-Wallis test

to the mean rank of the cell encounter rate. Normality was tested using the Shapiro-Wilk test when the sample size was lower than 30; the Kolmogorov-Smirnov test was applied with the Lilliefors correction when the sample size was higher than 30). For normally distributed data, an ANOVA was used. Statistical analysis was performed with STATISTICA 5.0 software.

RESULTS

Surveys covered approximately an average of 10,000 km, and a total of 1080 hours of surveys effort per season on board of different vessels. During the 10 years of surveys including all platforms, a total of 1494 striped dolphin sightings and 110 Risso's dolphin sightings were recorded.
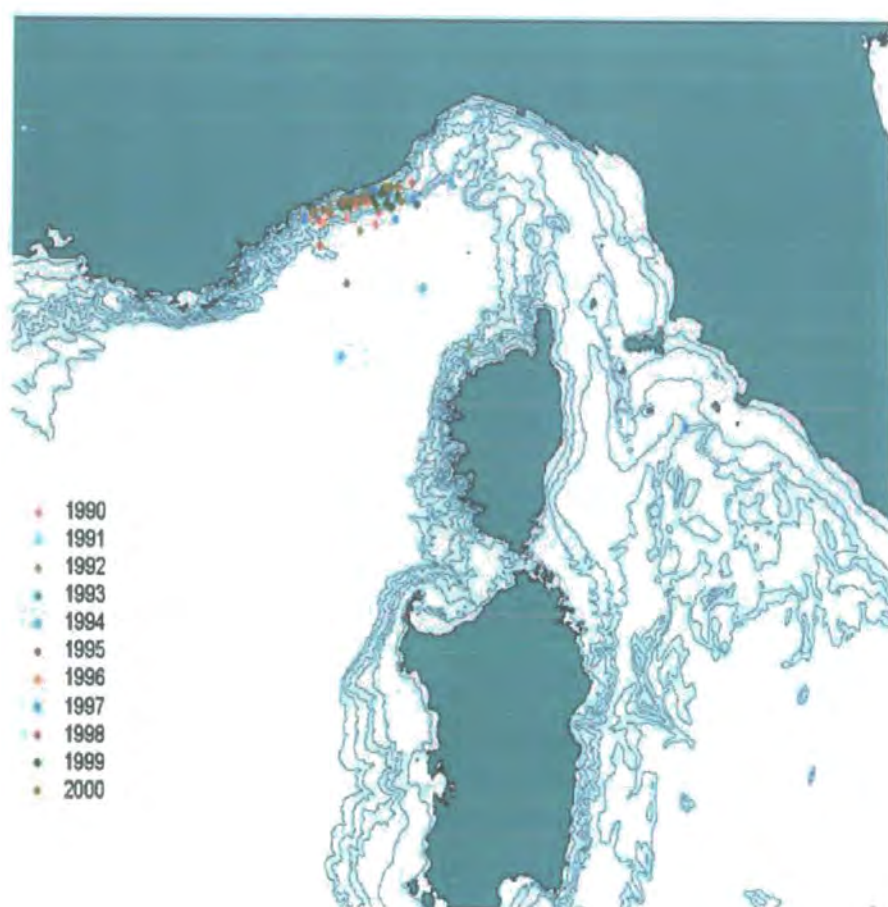
Figure 2.3a. Map of the Northwest Mediterranean with the Risso's dolphins sightings per year.

Figure 2.3b. Zoomed in map of the core study area showing the Risso's dolphins sightings per year. The different colours correspond to the different year. The bathymetry is also indicated.
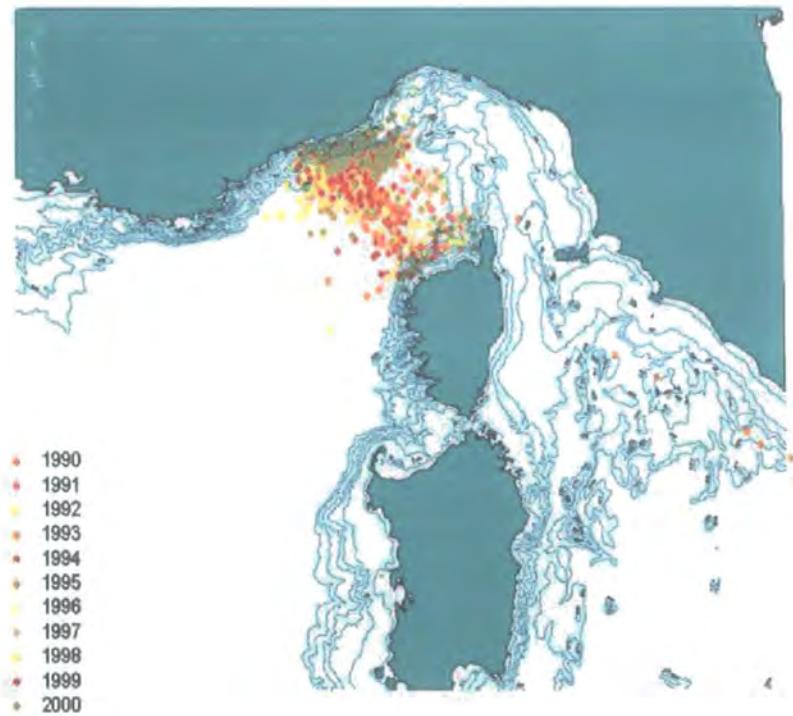
Figure 2.4. Zoomed in map of the core study area showing the striped dolphin sightings per year. The different colours correspond to the different years. The bathymetry is also indicated.

Table 2.1. Records of the number of sightings for each species during the study period 1990-2000.

Year	Sc	Gg
1990	97	5
1991	125	7
1992	129	6
1993	163	9
1994	77	4
1995	113	7
1996	89	6
1997	129	19
1998	207	18
1999	200	14
2000	165	15
Total	1494	110

The whale watching boat collected a total of 814 sightings, including all the species occurring in the area: *Stenella coeruleoalba* was the most abundant cetacean sighted in the area (69.3 %), and *Grampus griseus* represented 6.3 % of the sightings.

RISSO'S DOLPHINS

Distribution

During ten years of study, Risso's dolphins were sighted with a mean frequency of 11 sightings per season. The sighting frequency per unit effort is reported in Figure 2.5. Data on track-lines and effort are not available for the first year of survey (1990).
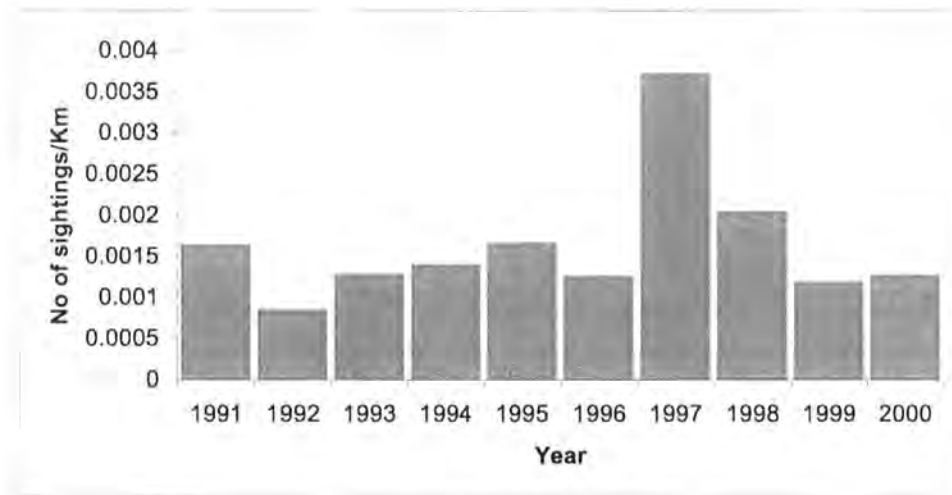
Figure 2.5. Sighting frequency per unit effort (Km) of *Grampus griseus* from 1991 to 2000.

The number of encounters increased during 1997 when a specific study on odontocetes along the continental slope began and data were collected by three

sighting platforms. No significant difference was found in the encounter rate between different years (Kruskal-Wallis H: 0.484, p > 0.90), or between different months (Kruskal-Wallis H: 3.38, p > 0.40).

In relation to water depth, Risso's dolphins were not homogeneously distributed across the study area, fifty one percent were encountered between 500 m and 1000 m depth, where the continental slope was deeper and steeper.

Group size

During the ten years of study, single individuals were sighted four times (3.63%), and group size ranged from 2 to 70 individuals, although sightings of group of 50-70 individuals were rare. Figure 2.6 shows that 84 % of the encounters consisted of groups ranging from 2 to 20 individuals, and that larger groups were not as common.
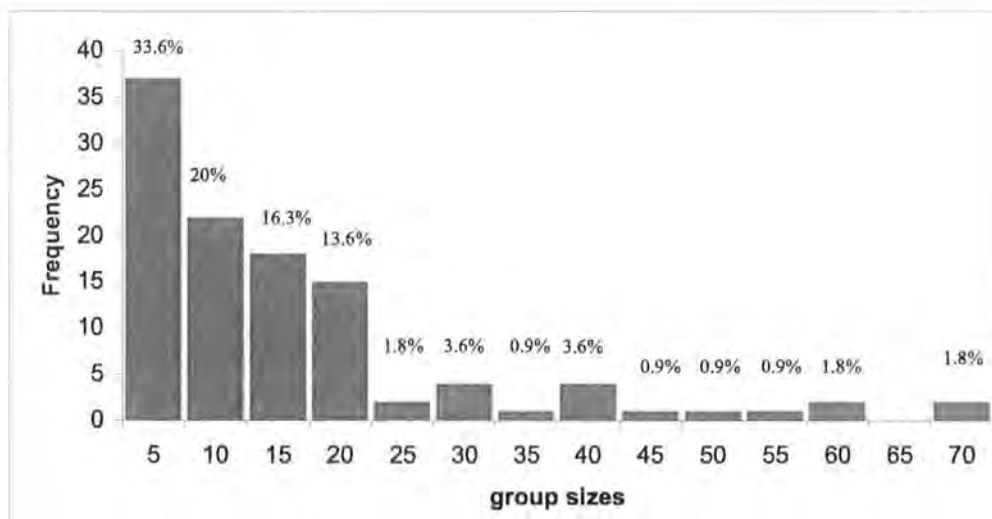
Figure 2.6. Frequency distribution of Risso's dolphin group size, values above the bar are the frequency values in percentage.

Average group size was 14.5, including those encounters where group size was larger than 50, (mode = 10; N = 117; SE 1.23). Detailed values for each single year are given in Table 2.2.

Table 2.2. The Table reports the general statistics of Risso's dolphin group sizes including all sightings for each year, from 1990 to 2000.

Year	Mean group size	Mode	SD	SE	N
1990	10.4	5	9.449	4.226	5
1991	7.428	6	4.894	1.849	7
1992	8.833	7.5	6.524	2.663	6
1993	6.777	5	5.494	1.831	9
1994	6	5	2	1	4
1995	10.142	6	7.470	2.823	7
1996	9	10	5.176	2.113	6
1997	12.894	10	13.295	3.050	19
1998	24.481	20	16.282	3.133	27
1999	15.214	12	12.873	3.440	14
2000	16.333	10	15.112	3.90	15

Risso's dolphins were most frequently encountered in groups of four and five individuals, and the highest frequency group size was five. During the study period the average group size varied significantly between years (Kruskal-Wallis H: 16.63, p = 0.01, N = 105), but not significantly between months (Kruskal-Wallis H: 4.69, p > 0.335, N = 105).

Risso's dolphins were identified by age category (details on their identification is given in the methods of chapter 4, Table 4), and their seasonal variation of group composition was investigated (Figure 2.7.).
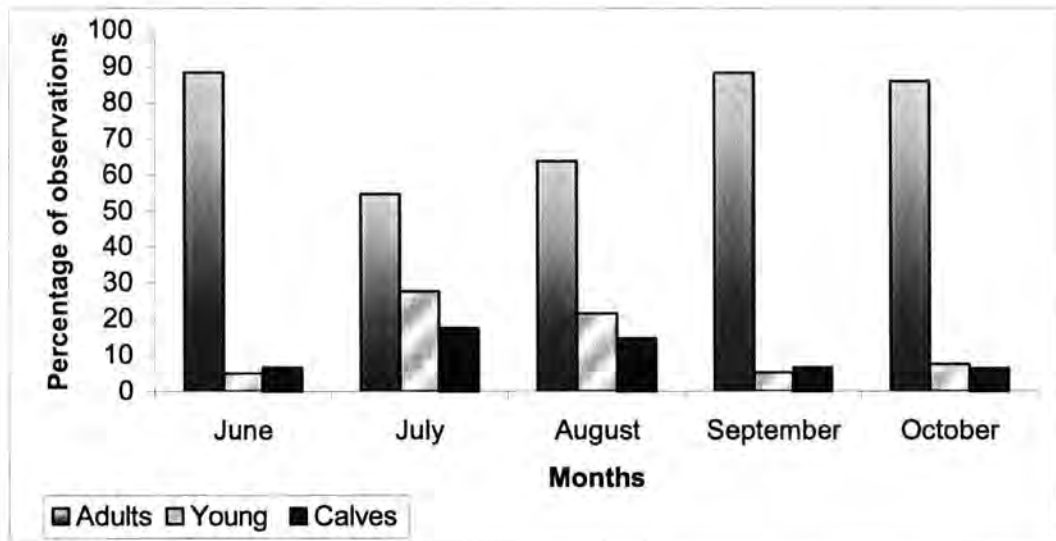
Figure 2.7. Seasonal variation in age group composition of Risso's dolphins. Individuals of different ages are indicated with different colours.

The proportion of adults and young within groups did not vary significantly during the different months of observation. However, a significant seasonal difference in number of calves within groups was found (Kruskal-Wallis H: 13.03, p = 0.01, N = 38); July was the month with the highest proportion of calves.
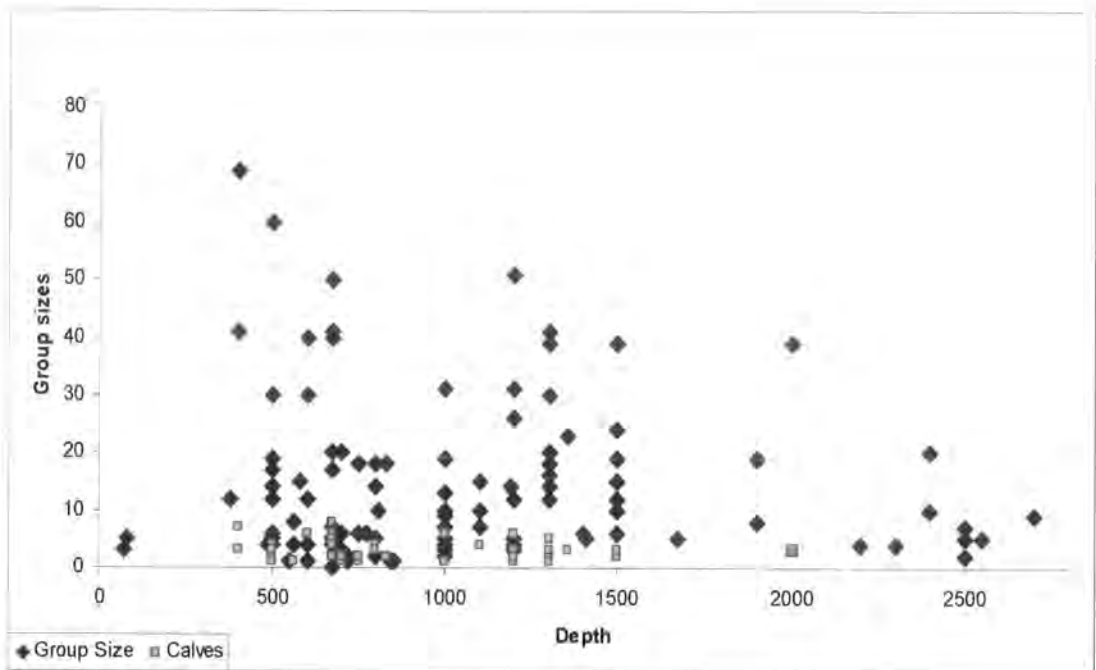
Figure 2.8. Group size distribution in relation to water depth. Group size is shown in blue and distribution of calves is represented in green. There is a single sighting of a group with 3 calves at 2000 m depth represented in red.

Risso's dolphins group size was not significantly correlated with water depth. However, groups with calves were mainly observed between 400- 1500 m depth, with a single sighting with three calves at 2000 m (Figure 2.8). Calf presence showed a significant and negative correlation with water depth ($r = -0.194$; $p < 0.05$) (Figure 2.9).
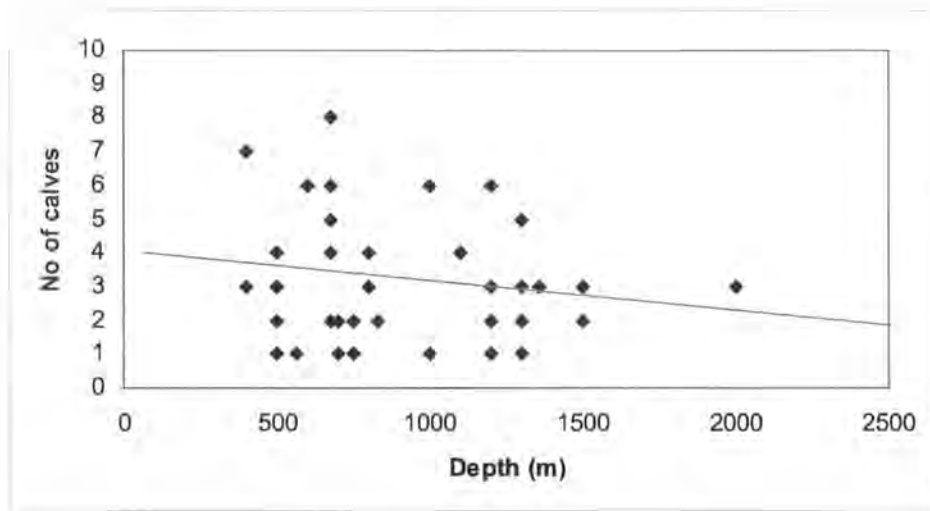
Figure 2.9. Negative correlation between calf presence and water depth.

STRIPED DOLPHINS

Distribution

During the study period, striped dolphins were sighted with a mean frequency of 150 sightings per year. The sighting frequency per unit effort are reported in Figure 2.10. Data on track-lines and effort are not available for the first year of survey (1990), as for Risso's dolphins. Significant differences were found in the encounter rate between different years (Two-way ANOVA test for the cell encounter rate vs year vs month F_{year}: 19.32, p = 0.000) and between different months (F_{month}: 3.911, p = 0.012).
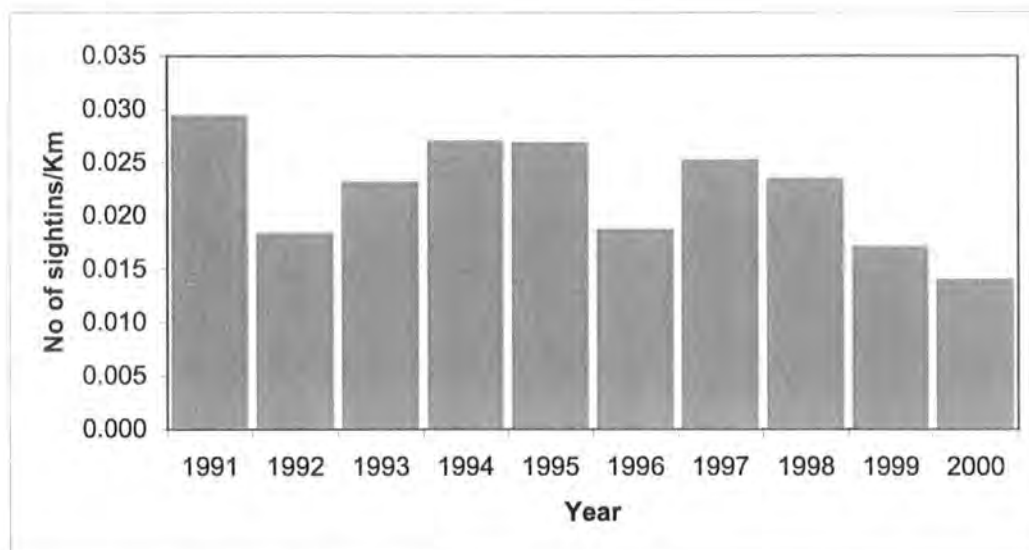
Figure 2.10. Sighting frequency per unit effort (Km) of *Stenella coeruleoalba* from 1991 to 2000. The year 1990 is not reported because unit effort for that first year was not available.

In relation to water depth, striped dolphins were widespread and homogeneously distributed in the study area.

Group size

Group size ranged from 2 to 200 (Figure 2.11), single individuals represented 3.7 % of total sightings. Group size varied greatly, but fifty percent of the encounters were groups of between 2 and 20 individuals. Groups larger than 50 individuals were rare, and there was only one sighting of 200 individuals. The average group size was 17 and the mode 11. Table 2.3 provides details for each year.
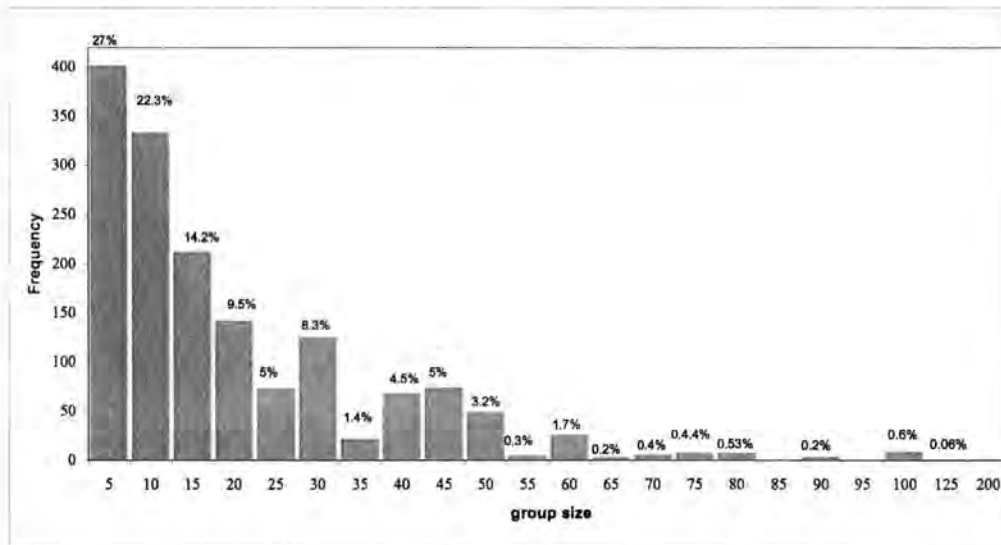
Figure 2.11. Frequency distribution of striped dolphin group size, with percentage value indicated above the chart bar.

Table 2.3. Striped dolphin general statistics of group sizes including all sightings for each year, from 1990 to 2000.

Year	Mean Group Size	Mode	SD	SE	N
1990	12.886	7	12.827	1.302	97
1991	17.48	11	21.375	1.911	125
1992	16.96	11	18.756	1.651	129
1993	14.889	8	19.561	1.532	163
1994	15.259	10	14.243	1.623	77
1995	16.663	10	17.417	1.638	113
1996	18.955	15	17.109	1.813	89
1997	18.492	12	16.003	1.414	128
1998	18.922	10	22.951	1.595	207
1999	22.753	15	20.481	1.451	199
2000	17.733	15	15.017	1.169	165

During the study period, the average group size varied significantly between years (Kruskal-Wallis H: 23.49, $p < 0.005$, N= 1327), (Figure 2.11); it also varied significantly between months (Kruskal-Wallis H: 18.55, $p = 0.000$, N = 1327) (see Table 2.4).
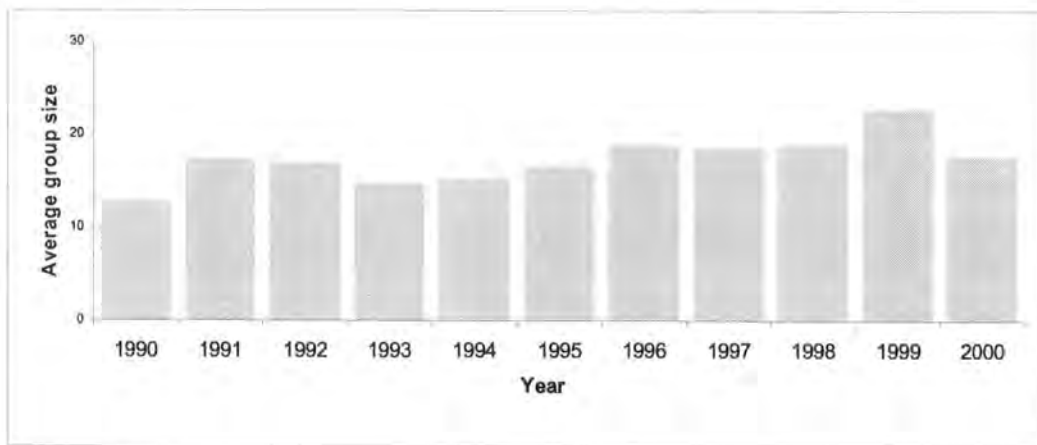
Figure 2.12. Striped dolphin yearly average group size variation.

Table 2.4. Striped dolphins seasonal group size variation during different months from 1990 to 2000.

Month	Group size	N
June	19.427	219
July	15.982	482
August	21.165	530
September	13.838	235
October	20.899	21

Variation of group composition was investigated for the different years (Figure 2.13), and no significant difference in group composition was found between months. October was excluded from this analysis, since the effort during this month was very irregular and scarce.

Striped dolphins were encountered frequently beyond 2500 m depth. However, overall, they were widespread and homogenously distributed. There was not a zone were they were predominantly found. Adults and calves were encountered in both costal and pelagic waters.
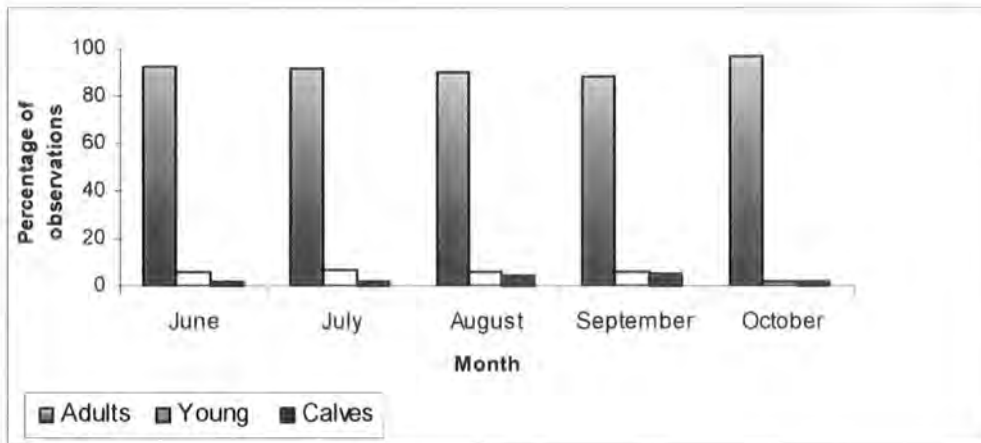
Figure 2.13. Seasonal variation in age group composition of striped dolphins. Individuals of different ages are indicated with different colours.
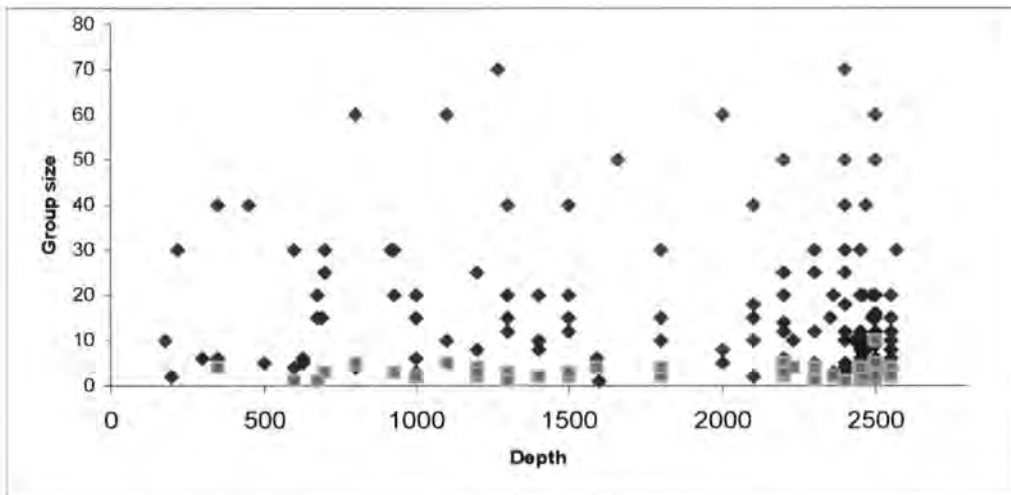
Figure 2.14. Striped dolphin group size distribution in relation to water depth. Blue square represent adults and young, while green square, represent calves.

Neither group size nor calf presence was related to water depth. From Figure 2.14, it can be seen that unlike Risso's dolphins (Figure 2.8), calves are present throughtout the depth range, and they are also numerous in pelagic waters and within small groups.

DISCUSSION

During the last decade, research has revealed that cetaceans are common in many areas of the Mediterranean Sea (Cagnolaro & Notarbartolo di Sciara 1992; Bearzi *et al* 1992; Marini *et al* 1993; Notarbartolo di Sciara 1993, Forcada *et al* 1995), with a total of nine cetacean species (Notarbartolo di Sciara & Demma 1994) regularly seen. General cetacean abundance in the Mediterranean is supposedly higher west of the Italian Peninsula than in the eastern basin (Notarbartolo di Sciara 1993). However, quantitative comparative surveys have never been conducted. Striped dolphins, were the most frequently encountered species in the study area. During the ten years of surveys, striped dolphins were sighted at a frequency about 14 times higher than Risso's dolphins. The difference in sighting frequency between the two species, may possibly be attributed to an effective difference in the abundance of the two species in the study area, but it could also reflect a different use of the habitat. In fact, although the two species extensively overlap, unlike striped dolphins, Risso's dolphin encounter rate apparently suggests a "transient" use of the habitat within the study area, based on a Monte Carlo randomization test which was applied to the occurrence matrix of Risso's presence/absence data during the study period. (Azzellino *et al* 2004). Furthermore, I suggest that the differences in habitat use also reflect a difference in resource exploitation. Risso's dolphin are known to prey on cephalopods, and analysis of stomach contents of dolphins in the study area confirm this (Clarke & Pascoe 1985, Desportes 1985, Podestà & Meotti 1991). Therefore, they may use the environment in a vertical manner performing deep dives to forage, whereas striped dolphins are more flexible in choice of food resources, and are considered to be opportunistic feeders, and may therefore use the same area but in a horizontal way, hunting fish in the upper part of the water column. Although striped dolphins are capable of deep dives and were reported to feed also on cephalopods, in the study area they may primarily feed on fish in order not to overlap with other teuthophagic species present in the area. Azzellino

et al (2004) suggest that, in the Ligurian Sea, competition for food between different species of cetaceans, such as Cuvier's beaked whales, sperm whales, and Risso's dolphins, may be high, and these species have adopted different feeding strategies. It is therefore conceivable that an opportunistic species such as the striped dolphin may have opted to feed on prey that do not coincide with those of the deep diving species, such as Risso's dolphins. During behavioural observation of Risso's dolphins, they were observed spending much time in dive bouts of about eight to nine minutes, suggesting at best foraging at depth. While striped dolphins were never observed diving for long periods; the maximum dive that we have recorded was three minutes, but more typically were between one and two minutes.

Notarbartolo di Sciara & Gordon (1996), classified the species regularly found in the Mediterranean in three categories based on their habitat preferences: pelagic (fin whale, pilot whale, Cuvier's beaked whale, and striped dolphin); slope (sperm whale and Risso's dolphin), and costal (bottlenose dolphin and common dolphin). My results regarding striped dolphins, are not in agreement with the previous classification, and are also supported by results of Azzellino *et al* (2004). In fact, striped dolphins were encountered across all the three habitats classified by Notarbartolo & Gordon (1996), rather than preferentially in one (pelagic). Unlike Risso's dolphins, striped dolphins were homogenously distributed in costal, pelagic waters, and on the continental slope (Azzellino *et al* 2004). Moreover, striped dolphins' opportunistic feeding habits, may contribute to their widespread distribution. In a specific study on habitat preference of cetaceans on the continental slope by Azzellino *et al* (2004), fin whales and striped dolphins were the only species encountered in the LCP, that showed no apparent preferences for physical habitat features. On the other hand, Risso's dolphins were found associated with a definite depth and slope gradient, suggesting a feeding specialization. Oceanographic mechanisms may concentrate prey along the steep section of the continental slope, and this may be what attracted Risso's dolphins. Relationships between the distribution of cetaceans and physiographic features have been demonstrated for several species, including common dolphins (*Delphinus delphis*) (Evans 1975, Hui 1979, 1985, Selzer & Payne 1998), short

finned pilot whales (*Globicephala macrorhyncus*) (Hui 1985), Atlantic white-sided dolphins (*Lagenorhynchus acutus*) (Selzer & Payne 1998), and humpback whales (*Megaptera novaeanglie*) (Sutcliffe & Brodie 1977, Payne *et al* 1986). These relationships suggest that complex seafloor relief can concentrate prey species through oceanographic mechanisms such as topographically induced upwelling of nutrients, increased primary productivity, and aggregation of zooplankton due to the enhanced secondary production, or convergence of surface waters. Information on Risso's dolphin dietary habits has come only from stomach contents of dead animals. Detailed analysis of Risso's stomach contents are reported by Clarke and Pascoe (1985), Desportes (1985), Podestà & Meotti (1991). Risso's dolphins prey on cephalopods, and may dive to considerable depths to catch their prey. They feed primarily on mesopelagic cephalopods which live in waters deeper than 300 m. In the western Ligurian Sea, where Risso's dolphins seem to occur most frequently, this species can find a suitable feeding areas very close to the coast. Similar results on the habitat preference of Risso's dolphins were reported by Baumgartner (1997), for a study on the distribution of Risso's dolphin with respect to physiography, conducted in the Gulf of Mexico. He, too, found that the species was non uniformly distributed, and that they utilize the steep sections of the continental slope, and concluded that their distribution was not solely related to water depth, but also to depth gradient.

Risso's and striped dolphins are gregarious animals, with group sizes varying from a few up to about one hundred individuals. Risso's dolphin group size varied significantly during the ten years of monitoring, being considerably higher in 1997. These years coincide with " El Niño". It is possible that an increase in water temperature recorded during " El Niño" event may have had consequences on prey abundance and distribution, and in turn on the number of group members.

Very often when Risso's dolphins were sighted in small groups of two to five individuals, they would perform long dives of about eight to nine minutes, remaining in a relatively restricted area. Once these activities, presumably feeding, were finished, the animals would gather in larger groups and stop the

dives, and engage in social behaviour. Twice, during these bigger group gatherings, I have observed sexual display behaviours. These larger groups usually formed during the afternoon. Group size seemed to be dependent both on animal activities and on the time of the encounter. Risso's dolphin prey may not require large group hunting, as animals herding fishes may do, this possibly suggests why Risso's dive preferentially in pairs or in small groups.

In dolphin species, it is generally assumed that larger groups are associated with pelagic environments (Wells *et al* 1980); it was suggested that as the environment becomes more uniform, the sizes of groups increase. A uniform habitat may present less refuges for prey. Thus, in uniform open water habitats, prey species for the dolphins are distributed in patches, which once found, provide an abundant source of food. Locating these patches is difficult and dolphins can take advantage of the searching capacity of conspecifics to locate prey (Norris & Dohl 1980). In more complex, topographically variable, environments there are more refuges for prey and so they no longer form such large groups; concordantly, the advantages of grouping for the dolphins similarly decrease as competition for less patchy food supplies increases. Although this may be true for some species and for some areas, I found that in both species there was not a tendency for larger groups to be found in pelagic waters. Both striped and Risso's dolphin group size range occurred throughout the study depth range. For Risso's dolphins, larger groups were encountered between 500 and 1500 m depth and not in deeper water. This may be due to the fact that Risso's dolphins are mainly concentrated within this area, but it is more likely that this is due to the presence of calves in the larger groups. In fact, I have found a significant negative correlation between calf presence and water depth. Larger groups may function as an anti-predator system for protecting calves, and it is conceivable that they could support alloparental behaviour, as has been documented for other species that forage at depth, such as sperm whales (Whitehead 1996), bottlenose whales (Gowans 1999), and bottlenose dolphins (Mann & Smuts 1998). In striped dolphin groups, calves were present both in smaller and larger groups with no correlation with water depth.

CHAPTER THREE

PATTERNS OF POPULATION STRUCTURING OF STRIPED DOLPHINS (*Stenella coeruleoalba*)

INTRODUCTION

The striped dolphin has a world-wide distribution in tropical and temperate waters. Distribution records have been summarized in Perrin *et al* (1994). Across its range it is typically pelagic, preferentially inhabiting the deep waters of the continental shelf where it feeds on mesopelagic fish, cephalopods and plaktonic crustaceans.

In this study I investigate patterns of genetic subdivision of striped dolphins within the Mediterranean Sea, and also compare Mediterranean populations to the North Atlantic populations. I also assess kinship within and between social groups.

Studies on the genetic variation of striped dolphins, based on mtDNA (Archer 1996, Garcia-Martinez *et al* 1995, Garcia-Martinez *et al* 1999), and morphological characteristics (Archer 1996), have found differentiation between samples from the Mediterranean and North Atlantic. Archer (1996) considered five populations of striped dolphins: Eastern and western Pacific, eastern and western Atlantic, and the western Mediterranean. He demonstrated geographical variation based on external and skeletal morphology, reproductive parameters, and food habits among 368 specimens. The extent of sexual dimorphism in cranial characters was also examined to determine if it could influence the analysis of morphological variation among populations, and dimorphism was detected for some populations (most pronounced in the Pacific), with males typically larger than females. In the two Pacific populations, males were also found to have relatively shorter, wider, and more robust rostra. For most cranial measurements, dolphins from the western Pacific were largest, and those from the Mediterranean were smallest. The difference in skull size found between the eastern Atlantic and the Mediterranean samples supported the findings of previous studies on variation in external body size between these populations (Sylvester 1985, Calzada & Aguilar 1995).

A study conducted by Calzada & Aguilar (1995) investigated variation in adult body size in the Mediterranean, and found that animals in southern regions were significantly larger. Garcia-Martinez *et al* (1995) analyzed mtDNA RFLP (restriction fragment length polymorphism) variation of 44 stranded striped dolphins in Spanish Mediterranean waters, and found no population subdivision, or any variation among sampled years (samples collected between 1983 and 1990). In a more recent study, Garcia-Martinez *et al* (1999) extended their analyses to a broader European distribution of the species, examining genetic variability based on mtDNA RFLP analysis for 98 dolphins stranded along the Mediterranean and European Atlantic coast. Their results based on Φ_{ST} and G_{ST} statistics suggest differentiation between the Atlantic Ocean and Mediterranean Sea populations, with very low gene flow between them. However, due to the maternal mode of inheritance of the mtDNA, it has limited value in assessing gene flow when species have a complex social behaviour (O' Corry *et al* 1997).

As for most delphinid species, the striped dolphin is typically found in social groups. These can vary in size from less than 20 to more than 200 animals. I compare individuals within and among social groups of varying size, and over a small-scale geographic range to assess the hierarchical pattern of genetic structure in this species. These data may help to understand patterns of dispersal and the role of long-term kin associations. Genetic studies of highly mobile terrestrial species have shown that population differentiation is not necessarily correlated with distance when geographical ranges are relatively continuous (Lehman & Wayne 1991, Forbes & Boyd 1997). This could be expected to be even more relevant in the marine environment, where few geographical boundaries exist, and various studies have found genetic structure among cetacean populations, not correlated with geographic structure or distance (see review in Hoelzel 1998). Often intra-specific differences in foraging behaviour and the differential use of habitat appear to be important in structuring patterns of gene flow in cetacean species (see Hoelzel *et al* 2002).

The "resource-competition hypothesis" (Greenwood 1980) is based on the benefits brought by philopatry through the exploitation of local resources. In polygynous species, the benefits of resource competition are more valued by

females, as females are mainly responsible for rearing their young, and are therefore under stronger selective pressure than males to remain philopatric (Favre 1997). Male dispersal could also be favoured by local mate competition (e.g. Dobson 1982). Several studies on cetacean species have suggested examples of male-biased dispersal, such as: harbour porpoise (*Phocoena phocoena*) (Rosel *et al* 1999), Dall's porpoise (*Phocoena dalli*) (Escorza *et al* 2000), and long-finned pilot whales (*Globicephala melas*) (Amos 1993), though little is known about delphinid breeding systems.

Breeding tactics and social behaviour can significantly affect the distribution of genetic variation within populations (Chesser 1991). Studies on mammalian carnivores generally support the idea that dispersal functions to minimize inbreeding and reduce competition for resources (Greenwood 1980; Cockburn *et al* 1985; Clutton-Brock 1989; Wolff 1993, 1994). The main focus of this study is on fine-scale patterns of genetic and social structure of putative sub-populations in the Mediterranean Sea.

METHODS

Sample collection

Genetic analysis was carried out on a total of 165 samples of adult striped dolphins collected from different parts of Mediterranean Sea and eastern North Atlantic (Figure 3.1 and Table 3.1). Samples were collected from both free-ranging and stranded animals.

Figure 3.1. Map of origin of Striped dolphin samples showing sample locations.

Boat surveys in the Ligurian sea were conducted from June to October, from 1990 to 2000.

Table 3.1. Number and origin of samples used for the analysis.

Location	Free-ranging	Stranded
Eastern Mediterranean		
Greece	9	
Puglia		12
Croatia		1
North-West Mediterranean		
Tuscany		28
Ligurian Sea	84	
Spain		15
Eastern North Atlantic		
Scotland		16
		Tot 165

Behavioural observations of the Ligurian population, and data on group sizes refer to the period from 1996 to 2000. Kinship analysis was based on the 84 free-ranging adult dolphins in the Ligurian Sea. The samples were collected in different areas, sea depths and from different social groups.

Groups were identified as assembled individuals behaving in a coherent manner within approximately 200 meters of each other on a given sighting. Associations of dolphins at the time of sampling may or may not have included the same individuals on different occasions, but for our sample, no genetic matches were found between groups. Twenty-two of the 84 Ligurian samples represented the only individual sampled from a given group. Samples from most of the free-ranging dolphins were collected non-invasively using a scrubber attached on a long pole which caused very little or no reaction from the sampled animals. Figure 3.2 shows the pole with the scrubber, and a researcher attempting to scrub a striped dolphin. All animals sampled were of adult size.

Figure 3.2a. The photograph shows a researcher attempting to sample a dolphin with the pole. The figure gives an idea of how we used this sampling method when the animals were close to the boat.

Figure 3.2b. The photograph shows the pole with the scrubber attached to it while sampling an individual just underneath the water surface. The scrubber was used to get skin samples from the back of the dolphins, we tried to hit the area just behind the dorsal fin, in order to minimize disturbance. It was easier to sample them while still underwater rather then out of water. This also allowed us to better aim the body part to be sampled, since the animal was fast but steady.

DNA Extraction and PCR amplification

Skin was preferred among available tissues because of its lower degradation rate, but when not available, other tissues were also used, including liver, muscles, and heart. DNA was extracted and purified from tissue samples preserved in 20% DMSO NaCl 5M, by standard phenol/chloroform extraction method (after Hoelzel 1992). A total of 30 published universal cetacean primer sets were tested and the amplification conditions optimized (see Table 3.2). Eight polymorphic loci were chosen for this study. Primers KWM1b, KWM2a, KWM2b, KWM12a KWM5c were derived from *Orcinus orca* (Hoelzel *et al* 1998), EV37Mn from *Megaptera novaeangliae* (Valsecchi & Amos 1996), D08 and TexVet7 from *Tursiops truncatus* (Rooney *et al* 1999, Shinohara *et al* 1997). Amplified DNA from all specimens was analysed for length variation on 6% polyacrilamide denaturing gels using fluorescent imagining on an automated ABI PRISM 377 DNA sequencer, after incorporation of 1/10 fluorescent labelled primer (PCR reaction conditions: 200μM dNTPs, 0.75-1.5 mM MgCl$_2$, 10 mM Tris-HCl pH 8.4, 50 mM KCl, 250pM of each primer, 0.004 U/μL Taq polymerase. PCR cycling profile: 5 min at 95°C; then 35 cycles: 40 sec at 94°C, 1 min at the T° ann, 1 min at 72°C; 10 min at 72°C). The gel images obtained were analysed by Genescan and Genotyper programmes. Gender was usually recorded in the field for samples obtained from stranded animals but not for samples taken from biopsied and scrubbed animals. For those samples gender was determined genetically. Differential amplification of the zinc finger gene regions present in the X and Y chromosomes (ZFX and ZFY, respectively) was performed using three primers described by Bérubé & Palsbøll (1996). PCR products were separated by electrophoresis on 2 % gels, and gender was determined from resulting banding patterns.

Diversity analyses

The level of polymorphism was estimated as the number of alleles per locus and as the observed heterozygosity (Ho). Homogeneity of allele

distributions for all pairs of populations was tested using exact tests (Raymond & Rousset 1995). Since the number of alleles in a sample is dependent on sample size, allelic richness per locus and per sample was estimated. Evaluations of possible deviations from the expected HW genotypic frequencies and linkage disequilibrium were performed using Fisher exact tests and the Markov chain method. To test against sampling bias from the inclusion of close kin, those same calculations were repeated excluding one individual from pairs with high R-values ($R \geq 0.5$, $P < 0.01$ after 5000 simulations).

Putative inshore and offshore populations were tested for the free-ranging Ligurian samples, using a nested method. Individuals were classified as either "inshore" or "offshore" depending on where they were sampled (see Figure 3.3)

Figure 3.3. Map of the Ligurian Sea. The map shows the main contours and the distance in nautical miles from the coast to the bathymetry of 2,500 m (about 10 nautical miles). Core area of sampling in the Ligurian Sea is encircled.

The possibility that the striped dolphin population was differentiated between "inshore" and "offshore" was then assessed, varying the assignation of different depths to putative groups, in order to look for a point of division between "inshore" and "offshore". In the first instance, "inshore" animals were classified as all individuals sampled within 2000 m of depth, and "offshore" as all individuals sampled beyond 2000 m depth, then the animals were separated in three groups: within 600 m depth, between 600 m and 2000 m, and beyond 2000 m (see Figure 3.3). Subsequently the animals that were sampled between 600 m and 2000 m were excluded, and the comparison between animals found in the two non-contiguous segments, within 600 m and, beyond 2000 m was tested.

Two extreme models of mutations have been considered for microsatellite loci (Deka *et al* 1991): the infinite mutation model (IAM, Kimura & Crow 1964) and the stepwise mutation model (SMM, Kimura & Ohta 1978). The SMM describes mutation of microsatellite alleles by the loss or gain of single tandem repeat, and hence alleles may possibly mutate towards allele states already present in the population. In contrast, under the IAM, a mutation involves any number of tandem repeats and always results in an allele state not previously encountered in the population. The level of differentiation among populations was estimated using F_{ST} (Weir & Cockerhem 1984). The statistic is based on the variance in allele frequencies and assumes an infinite allele model. It describes the proportion of variation in subpopulations relative to total variance. This value relies on the assumption that all populations have descended from a common ancestor, that they are maintained under the same conditions, and that gene frequencies are at equilibrium (Balloux & Lugon-Moulin 2002). *Rho$_{ST}$* value represents the fraction of the total variance of allele sizes that is due to genetic differences between population and is based on the step-wise mutation model. RSTCALC calculates the statistical significance of *Rho$_{ST}$* by permutation tests, and uses bootstrapping to provide 95% confidence intervals. The number of interactions was set at 1000. Both F_{ST} and *Rho$_{ST}$* estimators range from 0, which signifies no differentiation, to 1, which indicates complete differentiation between populations.

Genetic distance between populations was estimated using Nei's unbiased genetic distance, D_A (Nei 1983), and $(\delta\mu)^2$ (Goldstein *et al* 1995). D_A was calculated with the program GeneDist (www.biology.ualberta.ca/jbrzusto/GeneDist.html): The distance $(\delta\mu)^2$ was created for microsatellite loci and incorporates features of the stepwise mutation model. Calculations were performed using GENEPOP 3.3 (Raymond & Rousset 2001), Fstat 2.9.3 (Goudet 2001), GENETIX 4.02 (Belkhir 2001), and RST Calc (Goodman 1997) Packages. Distance matrices were used to reconstruct a Neighbor Joining tree as implemented in PHYLIP version 3.56 (Felsenstein 1993)

Kinship Assessment

Levels of kinship were assessed by a comparison of allele frequencies at microsatellite loci, and comparative estimates based on allele sharing between individuals of the same and different groups. The analyses were carried out using the computer packages RELATEDNESS 5.8 and KINSHIP 1.3 (Queller & Goodnight 1989). For these analyses, the Ligurian population was divided into the twelve groups that were classified as such in the field. Each group was represented by at least three individuals sampled within the same group. Pairwise relatedness values were calculated for all possible pairs in the population, and the level of relatedness among the two sexes and within versus between the groups defined in the field, was compared using a Mann-Witney test, with Monte Carlo method (20000 resamplings). "RELATEDNESS" and KINSHIP are programs for testing genetic relatedness among and between groups of individuals. The programs estimate Hamilton's relatedness coefficient (R) between two individuals, which measures the extent to which they have alleles that are identical by descent, using allele frequencies in the population and each individual genotype. The index weights each allele by its frequency in the population, so rare alleles are given relatively higher weight. The index of Relatedness (R) varies between -1 and 1. A positive R value indicates that two individuals share more alleles that are identical by descent than expected by chance. When either of the two individuals possesses uncommon alleles, a negative R value is expected for a

pair (De Ruiter & Geffen 1998). In a sample representing a population in Hardy-Weinberg equilibrium, the relatedness coefficients should average 0.5 for parents and offspring or full-siblings (first-degree relatives), 0.25 for half-siblings and zero for randomly related individuals (Queller & Goodnight 1989). "KINSHIP" uses the R-values, the population allele frequencies, and the genotype of the two individuals under consideration to calculate the likelihood that this genotype combination could have been produced by the specified relationship.

Sex-biased dispersal

To test sex-biased dispersal, I have used an approach described by Goudet (2002), who compares several methods for detecting sex-biased dispersal using bi-parental inherited genetic markers. If gene flow is restricted among populations, then the genotype of an individual tells something about its origin. Provided that dispersal occurs at a juvenile stage before reproduction and that sampling is carried out on adults, genotypes sampled from the dispersing sex should on average be less common (compared to genotypes from the philopatric sex) in the population in which they were sampled. The dispersing sex should be less genetically structured and should present a larger heterozygote deficit (Goudet 2002). The test assumes that there are no overlapping generations, and that individuals are sampled after dispersal. Under these conditions F statistics and the Assignment Index are used to indicate sex-biased dispersal. F_{IS} is a statistic describing how well the genotype frequencies within populations fit with Hardy-Weinberg expectations (Wright 1978). If only males disperse, the males sampled from a single patch will be a mixture of two populations, resident and immigrants; due to the Wahlund's effect, the samples should therefore display a heterozygote deficit and a positive F_{IS} (Weir & Cockerhem 1984). In general, members of the dispersing sex should therefore display a higher F_{IS} than the more philopatric sex. F_{ST} is a statistic expressing the proportion of the total genetic variance that resides among populations (Hartl & Clark 1997). Allelic frequencies for the individual of the more dispersing sex should be more similar

than those for individuals of the more philopatric sex. Therefore F_{ST} is expected to be higher in the more philopatric sex.

The test also performs an Assignment Index (AI) (Paetkau *et al* 1995, Favre *et al* 1997). The distribution of AI is centred around 0. A positive value indicates a genotype more likely than average to occur where it was sampled, implying philopatry, while a negative value suggests a possible immigrant. Because members of the dispersing sex will include both residents and immigrants, variance AI (vAI) for the dispersing sex should be larger. Therefore, biased dispersal provides us with the possibility of using an indirect method to infer sex-specific dispersal from the distribution of alleles. Tests for sex-biased dispersal were performed using Fstat 2.9.3 (Goudet *et al* 2001, 2002).

RESULTS

Thirty universal primers were tested for optimal conditions of annealing temperature and magnesium ($MgCl_2$) concentration for amplification, see Table 3.2 for details. I have chosen eight that were the more polymorphic and gave better results.

Phylogeographic patterns of variation

Striped dolphin samples were compared among putative populations in Croatia, Puglia, Greece, Tuscany, Liguria, Spain, and the eastern North Atlantic (see Figure 3.1). After testing genetic differentiation between each of these areas, samples could be pooled into four main populations: Adriatic (Croatia, Puglia, Greece: $F_{ST} = 0.0302$; $p = 0.226$), Tyrrhenian (Tuscany and Liguria $F_{ST} = 0.0045$; $p = 0.44$), Spain, and the eastern North Atlantic (ENA). All loci were polymorphic, having between four and 22 alleles per locus. The mean observed heterozygosity ranged from 0.68 ± 0.05 in the Tyrrhenian to 0.8 ± 0.05 in the ENA. Allelic richness and the observed (Ho) and expected (He) heterozygosity values are reported in Table 3.3. Hardy-Weinberg equilibrium was tested for each population at each locus using the Fisher exact test, significant deviations are indicated by p-values ($p < 0.001$ – Bonferroni correction applied). Each locus was tested for linkage disequilibrium, and genotype independence was confirmed.

Table 3.2. Optimized conditions obtained for 30 universal primers, those shown in bold were those used in this study to amplify striped dolphin microsatellites.

Primer	Allele size	MgCl$_2$	Best annealing Temperature ^{0}C
EV1	100-170	2%	50
EV5	100-170	2%	60
EV14	120-160	1,5%	56
EV21	110-170	1,5%	56
EV37	170-210	1,5%	56
EV76	220-260	1,5%	48
EV92	190-260	1,5%	62
EV94	150-200	1,5%	62
KWM1b	170-210	1,5%	48
KWM 2a	130-170	1,5%	48
KWM 2b	150-190	1,5%	52
KWM 5c	140-155	1,5%	50
KWM 9a	100-170	1,5%	57
KWM 9b	160-190	1,0%	60
KWM 9c	100-170	1,5%	57
KWM12a	150-190	1,5%	54
WM415/416	200-240	1,5%	45
WM 417/418	150-180	1,5%	47
WM 468/469	100-170	1,5%	47
WM199/200	130-160	1,5%	46
WM 409/470	100-170	1,5%	46
WM 464/465	140-160	1,5%	46
D08	80-130	1,5%	58
D14	120-150	1,5%	52
D18	80-120	1,5%	54
D22	130-170	1,5%	50
D28	130-150	1,5%	48
Tex Vet 3	200-160	1,5%	52
Tex Vet 5	190-260	1,5%	52
Tex Vet 7	150-170	1,5%	52

Table 3.3. Number of alleles (private alleles are shown in parentheses, and alleles richness is shown in square brackets), observed (Ho) and expected (He) heterozigosities for each population at each locus. Deviation from the Hardy-Weinberg equilibrium was tested and loci showing a significant deviation after Bonferroni correction are indicated with an asterisk.

	Adriatic (22)			Tyrrhenian (106)			Spain (15)			ENA (16)		
Locus	A	Ho	He	A	Ho	He	A	Ho	He	A	Ho	He
KWM1b	5 [4.77]	0.571	0.719	8(2) [5.64]	0.718	0.755	4 [3.73]	0.800	0.660	5 [4.65]	0.875	0.693
KWM2a	12 [9.56]	0.952	0.868	16 (2) [8.67]	0.845	0.858	9 [8.10]	1.000	0.820	14 (1) [11.52]	0.937	0.918
KWM2b	10 (2) [7.52]	0.800	0.803	6 [4.73]	0.652	0.725	5 [4.98]	0.846	0.727	9 (1) [7.56]	0.812	0.750
KWM5c	6 (1) [5.52]	0.538	0.538	8 [4.73]	0.350*	0.413	6 [5.33]	0.600	0.588	7 (1) [6.37]	0.466	0.731
KWM12a	8 [7.01]	0.762	0.744	14 (3) [7.35]	0.672	0.740	8 (1) [7.05]	0.666	0.746	9 [8.12]	0.866	0.842
EV37Mn	9 [7.31]	0.750	0.725	22 (3) [9.60]	0.753	0.804	10 [8.66]	0.71*	0.775	14 [12.86]	1.000	0.908
D08	8 [6.77]	0.70*	0.780	22 (5) [11.12]	0.864	0.896	12 [10.5]	0.600	0.880	12 (1) [12.00]	0.727	0.871
TexVet7	6 [5.53]	0.857	0.667	10 (2) [6.62]	0.634	0.683	6 [8.1]	0.714	0.591	9 (2) [8.10]	0.714	0.783
Mean	8 [6.75]	0.741	0.731	13.25 [7.32]	0.686	0.734	7.5 [6.73]	0.742	0.723	9.875 [8.99]	0.8	0.812
SE	0.823 [0.531]	0.048	0.034	2.234 [0.831]	0.056	0.052	0.96 [0.795]	0.047	0.037	1.140 [1.032]	0.058	0.029

Genetic differentiation among pairwise populations was estimated as F_{ST}, Rho_{ST} and $(\delta\mu)^2$ (Table 3.4). Overall genetic differentiation among the four populations was Fst = 0.027 (p = 0.014) Rho_{ST} = 0.0457 (p = 0.000). The Mediterranean population (considered as the Adriatic, Tyrrhenian, and Spanish populations pooled together) was first compared with the ENA population and the F_{ST} value was 0.055, significantly different from zero (p = 0.001). Subsequently, the Mediterranean population was subdivided in three putative populations: the Adriatic, Tyrrhenian, and Spanish population.

Table 3.4. Genetic differentiation among pairwise populations, estimated by F_{ST} values reported in the lower matrix, $(\delta\mu)^2$ values shown parenthetically under the F_{ST} values, Rho_{ST} values are reported in the upper matrix. Level of significance is indicated in parenthesis.

	Adriatic	Tyrrhenian	Spain	ENA
Adriatic (22)		0.0151 (p<0.05)	0.0523 (p<0.05)	0.0811 (p<0.01)
Tyrrhenian (106)	0.0047 (p<0.01) (0.0607)		0.0237 (p<0.05)	0.0966 (p<0.01)
Spain (15)	0.0154 (p<0.01) (0.1562)	0.0161 (p<0.05) (0.0805)		0.1067 (p<0.01)
ENA (16)	0.0632 (p<0.01) (0.2579)	0.0595 (p<0.01) (0.284)	0.0281 (p<0.01) (0.3207)	

All comparisons between putative populations showed significant differentiation, including the comparison between the eastern (Adriatic) and western (Tyrrhenian) sides of Italy. The ENA population showed the highest differentiation compared with all the other populations. A phylogenetic reconstruction based on a Da distance matrix and using *Grampus griseus* as an out-group is shown in Figure 3.4. This analysis illustrates the hierarchical genetic structure with respect to geography, where the Mediterranean populations cluster together and the ENA population clusters with the Spanish samples.
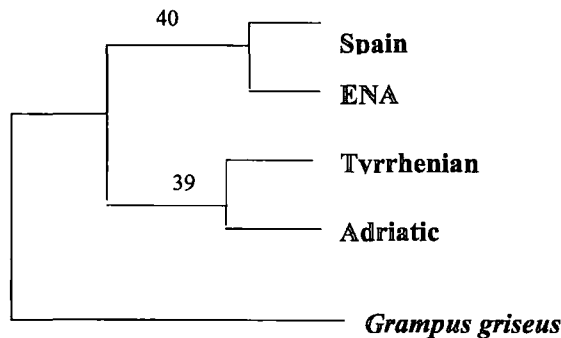
Figure 3.4. Neighbour-joining consensus tree based on Nei's (1978) genetic Distance. The number above the branches represent percentage bootstrap values. *Grampus griseus* was used as out-group.

The Adriatic population includes two sample sets, one from Puglia and one from Ionian Greece, while Tyrrhenian includes Ligurian and Tuscany sample sets. As indicated above, the two sample sets within each population are not significantly differentiated. But when a sample includes distinct demes, structuring within samples may lead to an underestimation of between sample structuring (Balloux & Lugon-Mulin 2002). One way to test this is to estimate the inbreeding coefficient F_{IS}. I have calculated the F_{IS} values for each possible subpopulation within the Adriatic and Tyrrhenian regions. The F_{IS} values for the two sub-samples within the Adriatic (Croatia-Puglia and Greece) were respectively 0.055 and −0.016. The F_{IS} values for the two sub-samples within the Tyrrhenian (Tuscany and Liguria) were respectively: −0.049 and 0.103.

The Tyrrhenian Sea population had the largest sample size (n = 106) among putative population samples, and most of these samples were collected from free ranging individuals in the Ligurian Sea. When compared with the other populations, the Tyrrhenian showed the lowest heterozygosity. Various reasons may account for low heterozygosity in the Tyrrhenian population, including kin structure and population structuring. I have calculated the heterozygosity of the Tyrrhenian population excluding one individual from pairs of related individuals (those that had an R value ≥ 0.3) in an attempt to exclude possible bias in the

samples. After the exclusion of close kin, the heterozygosity became higher (mean increased from 0.686 to 0.734), very similar to the rest of the Mediterranean populations (see Table 3.3), and consistent with Hardy-Weinberg expectations after Bonferroni correction.

The possibility that the Ligurian population was structured as an inshore and offshore population was also tested. To test the hypothesis of possible inshore and offshore populations, the sample set was divided with a nesting criteria into inshore and offshore individuals (see methods). The only point of separation between "inshore" and "offshore" comparisons that showed significant differentiation was a comparison of the extremes: up to 600 m depth (inshore n = 22) vs beyond 2000 m depth (offshore n = 27). No contiguous comparisons showed significant differentiation (see methods). The F_{ST} value comparing these two groups was 0.0175, p = 0.04. F_{IS} values were also considerably different between the two groups: inshore = 0.206, offshore = 0.008. The inshore group also showed significant deviation from Hardy-Weinberg expectations. Considering that kin-bias may be an important confounding factor, I repeated the comparison excluding close kin. The inshore (n = 16) and offshore (n = 17) populations remained differentiated (F_{ST} = 0.0199, p = 0.04), and F_{IS} values were respectively 0.151 and –0.041. The inshore group still showed significant deviation from Hardy-Weinberg expectations, but less so (p = 0.03).

Individual relatedness

Individual relatedness was investigated in the Ligurian population (n = 84) where all samples were collected from free ranging individuals. Individual relationships were estimated based on genetic similarity among individuals within and among identified groups. All individuals were assigned to their group of origin in the field (with letters from A to N, n = 12 groups (see Table 3.5). Based on these group formations, I compared relatedness within and between groups. From an analysis of all pairwise combinations including dolphins of both sexes, the estimated average

relatedness within groups was larger (0.1184 ± 0.023) than the estimated average relatedness between groups (0.0742 ± 0.004), but this difference was not significant at the α = 0.05 level after applying a Mann-Whitney-U test (Z = 1.546, p = 0.122).

Table 3.5. Average R pairwise comparisons within groups identified in the field. Sample and group size, and standard error are given for each group.

Group	Sample Size	Group Size	Mean R	SE
F	4	20	0.369703	0.135927
I	3	20	0.11992	0.20866
M	6	25	0.225424	0.086345
N	6	25	0.136431	0.071159
B	3	30	0.089884	0.222068
D	5	50	0.358313	0.085127
E	5	50	0.120039	0.067449
G	3	60	0.133783	0.272313
L	6	60	0.021765	0.070537
A	5	80	-0.09046	0.093832
H	6	100	0.204056	0.057733
C	10	200	0.034513	0.036762

There was no significant linear correlation between group size and R-value (data not shown), but when dolphin groups were divided into two categories of group size: one from 20 to 50 animals and a second from 60 to 200 animals, smaller groups showed a significantly higher kinship than larger groups (Table 3.5). Average R values were respectively 0.1436, and 0.0607 (Z = 2.98, p = 0.003).

Sex-specific tests

In the Ligurian samples, there were 43 females, and 41 males. Pairwise R-values were used to examine the within sex relatedness, and a

significant difference was found comparing all females and all males (Z = 5.375, p = 0.000). Females had an average R = 0.107 ± 0.009 and, males had an average R = 0.035 ± 0.008. When comparisons within vs between groups were restricted to one sex, females showed significantly higher relatedness within than between groups (Z = 2.464, p = 0.013); mean R within groups = 0.228 ± 0.044 (n = 43), mean R between groups = 0.112 ± 0.009 (n = 855). For males, mean relatedness within groups was not statistically different from mean relatedness between groups, although the trend was clearly the same as for females: R within groups = 0.116 ± 0.040 (n = 49), R between groups = 0.031 ± 0.008 (n = 766), (Z = 1.88, p = 0.06). I also tested whether, within the same group, striped dolphins tended to be more closely related to individuals of the same sex or to individuals of the opposite sex.

Table 3.6. Comparison of R values within the same groups for both sexes. Female kin associations with other females and with males of the same group were compared, as well as, male kin associations with other males and females of the same group. The Table reports the nature of association, N is the number of comparisons.

	N	Mean R	Standard Error
Female/Female	35	0.248	0.049
Female/Male	61	0.037	0.033
Male/Male	51	0.113	0.039

Comparisons of kinship for females with other females was significantly higher than for females with males (Z = 3.375; p = 0.0007), while comparison of kin association for males with other males was higher than with females, but not statistically different (Z = -1.265; p = 0.126) (see Table 3.6).

To test for evidence of sex-specific dispersal, I used the method of Goudet *et al* (2002). Several measures were used to assess sex -biased

dispersal: F_{IS}; F_{ST}; mean of Assignment Index (AI); and variance of AI (vAI).

Table 3.7. Comparison of values between females and males after a randomisation test for sex-biased dispersal (5000 reiterations), and statistical significance.

	F (n = 43)	M (n = 41)	P- values
F_{IS}	0.0447	0.0589	0.7244
F_{ST}	0.0228	-0.0118	0.0914
mean AI	0.1497	-0.1519	0.6344
v AI	10.9514	16.1062	0.1960

The results are shown in Table 3.7. While none of the comparisons were significant, the direction of the magnitude of the values is in each case consistent with female philopatry. The non significance of the values may be due to low power, given the sample size.

DISCUSSION

Phylogeographic patterns

Throughout the geographic regions included in this study, there is evidence for genetic structure among putative populations. Differentiation between Mediterranean and North Atlantic samples based on microsatellite DNA analyses is in accordance with previous findings based on mitochondrial DNA (Archer 1996, Garcia-Martinez *et al* 1995, Garcia-Martinez *et al* 1999). My results based on microsatellite DNA data also revealed small but significant differences over relatively small geographic scales within the Mediterranean Sea.

The Mediterranean can be subdivided into three main regions: the western basin (including the Algerian-Provençial basin and the Tyrrhenian Sea), the eastern basin (including the Adriatic-Ionian and the Levantino basins), and the Black Sea basin. The Italian Peninsula, which links Europe and Africa, divides the western basin from the eastern basin, and is surrounded by seas that show very diverse ecological characteristics. The Ligurian Sea is deep and productive, while the Tyrrhenian Sea, which lies between the Italian Peninsula, Corsica, Sardinia and Sicily, is deep only in its southern part; on the other hand, the Adriatic Sea is relatively shallow and eutrotrophic. Striped dolphins inhabiting the eastern side of the North Mediterranean are significantly differentiated from the western populations and, within the western part of the Mediterranean, populations sampled in different seas also seem to be distinct (comparing samples from Spain with the samples from west of Italy). This may suggest that a combination of physical and the consequent ecological characteristics contribute to the pattern of gene flow that has led to population genetic structure. Although the bathymetric lines along the Ligurian continental slope are very close (Figure 3.3), there was a small effect of differentiation between nearshore and offshore samples in the Ligurian Sea, but only when most of the samples were omitted from the comparison (i.e. samples collected within 600 m n = 16, and 2000 m n = 17 were

excluded). Much of the effect was likely due to the sampling of kin, especially in the nearshore sample.

All populations showed a high level of genetic variation, although heterozygote deficiency was found at a couple of loci, especially in the Tyrrhenian population. This could reflect further population subdivision (Wahlund effect), or a biased sampling of close kin. Removing close kin accounted for most of the observed heterozygote deficiency. Allelic dropout is a less likely explanation since the same loci showed no heterozygote deficiency in other populations.

Kin structure in the Ligurian Sea

Most analyses of the relationship between group size and food intake of social carnivores have shown a discrepancy between the group size that maximises energy intake and that which is most frequently observed. In fact, the group sizes of social hunters are often larger than the predicted optima (Smith & Warburton 1992, Clark & Mangel 1996). This is likely due to the benefit of social factors such as an increased vigilance and protection against predators, and the co-operative protection of young. If larger groups form as a response to their habitat, it seems likely that some measure of fine-grained social organisation is maintained in these large groups, and that kin-associations could be adaptive in this context. Subgroups of two to six striped dolphins were often observed to be in more close contact, and my results confirm that dolphins in smaller groups tend to be more closely related to each other, though there was no strict correlation between group size and kinship.

The kin structure of the Ligurian population showed a significantly greater association among female than among male kin, and while females were significantly more related within than between groups, males were not. The sex-biased dispersal test was not statistically significant; however, the direction of the magnitude of the values was in each case consistent with

female philopatry. However, a larger sample size may provide the necessary power for significant results in a future study. The results indicate that females disperse less than males; this is also supported by the relatedness values, which all indicate that females are more philopatric than males in this species. Within groups, there was a trend for males to associate with male, but not with female kin, suggesting that males disperse from the maternal group early, and may form associations in groups with male kin as adults. These results are expected if females could gain through association with female kin (perhaps through allomaternal care), while males disperse to avoid inbreeding, and sometimes join groups with male kin to maximise inclusive fitness. This analysis of kin-associations within and among groups was, however, most consistent with a relatively fluid model of social structure. There was significantly greater kinship among females within groups, but the distributions overlap extensively.

CHAPTER FOUR

ASSOCIATION PATTERNS OF RISSO'S DOLPHINS

(*Grampus griseus*)

INTRODUCTION

One of the fundamental issues in any study of social species, is the size and stability of social groupings. Grouping behaviour entails a variety of costs and benefits to individuals (reviewed extensively elsewhere, e.g. Krebs & Davis 1987, Lee 1994, Gygax 2002). Some of these costs and benefits such as, predators and food-related factors, relate to all forms of groups; others, such as access to helpers for rearing young, relate primarily to membership of long term groups. The size and stability of social groups may vary with the types of interactions and strengths of relationships between individuals. Information on the nature and duration of bonds between individuals can aid in the assessment of the value of sociality for a species (Myers 1983). Among mammals, female grouping behaviour is thought to be primarily related to resource acquisition and predation avoidance (Bradbury & Vehrencamp 1977, Emlen & Oring 1977, Wrangham & Rubenstain 1986), therefore the pattern of social groups can provide insight into the ecology of a species. Female grouping behaviour may be a strong determinant of male social behaviour (Emlen & Oring 1977; Wrangham & Rubenstein 1986), and therefore strongly constrain mating strategies and systems.

The quantification of associations within a social group is an important aspect of the study of social behaviour, and all members of the same group can be regarded as being associated. However, quantifying social behaviour between individuals poses many problems. In numerous studies, where quantification of direct social interaction is difficult, the presence of two individuals within the same social grouping has been seen as a measurable expression of social linkage (Sailer & Gaulin 1984, Cairns & Schweger 1987). Here I use membership in the same group as a measure of association, as it is likely that most interactions take place within groups (Bräger 1999). The proportion of co-occurrence of any two individuals can be measured using association indices. This method was originally developed by ecologists to measure the co-occurrence of plant species in a given habitat (Dice 1945). Association indices, subsequently adapted from

ecological studies of plant community assemblage, have been used in a wide range of studies to describe the various aspects of animal societies (Schaller 1972, Clutton-Brock *et al* 1982, Lott & Minta 1983, Wells *et al* 1987, Smolker *et al* 1992, Slooten *et al* 1993), and to calculate coefficients of associations (CoA) between individuals in populations (Ginsberg & Young 1992). The underlying assumptions are that physical co-occurrence signifies social affiliation, and the amount of time together correlates with the strength of affiliation (Bejder *et al* 1998). Indices of association among pairs of animals in a social population naturally vary (Whitehead 1999). An important question for the study of social structure is whether this variation can be accounted for by purely random associations, or whether the data give evidence for preferred associations (Whitehead 1997, Bejder *et al* 1998, Whitehead & Dufault 1999). The purpose of this study is to describe quantitatively, for the first time in this species, the social organisation of Risso's dolphins (*Grampus griseus*) by the analysis of association patterns, to determine whether the observed associations are significantly different from random, and to assess the possible existence of particular social groups.

Little is known of the social structure and behaviour of Risso's dolphins as this species has received little attention. Kruse (1989) conducted a photo–identification study on Risso's dolphins in the waters surrounding Monterey Bay, California between 1985-1987. Monterey Bay is a deep water embayment on the central California coast, with the Monterey subcanyon bisecting the bay creating a productive marine environment. Deep water is reached and the continental shelf edge is within 1.6 km of the shore in Carmel Bay. Kruse identified 800 individuals from 59 encounters of schools of dolphins; however, only 294 animals were identified by photographs taken of both sides of the fin, with 298 only identified by their left side and 208 by their right sides. The average school size was 63 (sd 88) ranging from 3-500 individuals. However, only 26% of the dolphins were resighted and she suggested that like other pelagic dolphin species, the Risso's dolphins proved to be highly fluid aggregations of all age and sex classes. She saw no evidence for resident schools of Risso's dolphins in the Monterey Bay suggesting that some resightings occur when the dolphins repeatedly visit the bay, instead of residing there for long periods.

Kruse reports that distinctive groups of large, calfless animals, juveniles, and females with calves (nursery groups) were clearly obvious and that the most cohesive units were composed of large, similarly-sized, calfless individuals. She observed mother/calf pairs in small groups of other calfless individuals but also in large nursery groups. These subgroups may be associations between same-sex and same-age animals, animals in the same reproductive condition, genetically related individuals (Wells *et al* 1987), or animals that are just associates. Kruse reports that female/calf pairs formed extremely close social bonds which lasted at least 11 months. Mothers with slightly larger offspring would clump together with their offspring with calves interacting more with one another, exhibiting social and play behaviour. Large calf groups were often seen within the school and these were extremely mobile and could be seen charging around within the area of the school.

Outside of this, Risso's dolphin society was extremely labile. Fluctuation of school size suggested that Risso's dolphin schools were dynamic aggregations of groups and subgroups. However, several subgroups maintained their membership over a period of 10-15 months. Limited data on Risso's dolphin strandings suggest that some of these cohesive subgroups may be same-age, same-sex animals (Leatherwood *et al* 1979, Baker 1983, Blizzard 1989). Evans (1987) has noted from photo-identification studies of Risso's dolphins in the coastal waters of the Isle of Lewis Scotland that the dolphins appear to live in groups of stable composition. His studies indicate that groups comprise one adult male, typically 4-6 females and young of both sexes, although, the sex of each individual cannot be determined with certainty. Recognisable individuals were observed within the same group over an extended period of several weeks and from one year to the next. The same area is apparently occupied by the group seasonally over at least 15 years. Cohesive subgroups within a fluid school structure have been observed in spinner dolphins (Norris & Dohl 1980), dusky dolphins (Würsig & Würsig 1980) and bottlenose dolphins (Irvine & Wells 1972, Würsig & Würsig 1977, 1979, Wells 1986).

METHODS

Fieldwork and Photo-Identification

Between 1990 and 2000, individual Risso's dolphins were photographically identified in the Ligurian Sea (Figure 3.3), this is an area included in the recently established International Marine Sanctuary. From June to the end of October, trips at sea were conducted whenever weather conditions permitted. Surveys were carried out on board different sized sailing boats, ranging from 9 m to 20 m. At least two observers were constantly monitoring the area, both by eye and with binoculars. Once Risso's dolphins were located, the animals were counted and group sizes defined. Group size was estimated in the field and was later compared with photo-ID data. A group was defined as an aggregation of dolphins that moved in a coordinated fashion within approximately 200 meters of each other on a given sighting. All members of a group were assumed associated at the time of the sighting.

After the dolphins were located, the vessel slowly approached them, and whenever possible they were photographed for later identification. Risso's dolphins were identified on the basis of natural marks and scars on the dorsal fin and back. The aim was to photograph as many individuals as possible, ideally from both sides. Since the two sides of the animals are different, it was therefore difficult to match the left and the right side of the same individual, unless there were identifiable nicks on the dorsal fin which were recognisable from both sides. Risso's dolphins' skin coloration varied with age, thus colour variations and body size allowed the identification of age categories. Calves are born blue-grey above and creamy-white below. They have a white, anchor-shaped patch between the pectoral fin which resembles the chest chevron of pilot whales. As calves grow, they turn silver-grey, then darken to a gunmetal grey. As the animal ages, its colour lightens, and this in conjunction with characteristic body scars which Risso's dolphins accumulate throughout life, makes some animals appear almost pure white. It is not clear whether the scars are the result of struggles with prey or

come from the teeth of their own kind. According to their coloration and size (Table 4.1), Risso's dolphins were divided into five categories: new-borns, calves, juveniles, young adults, and adults. Young adults were considered those animals that were grown full size but had few marks on their body (Figure 4.1c).

Table 4.1. Risso's dolphins characteristics for age determination.

	Coloration	Scars	Size
Newborn	Uniformly grey	No	~1,5 m
Calves	Uniformly grey-brownish	No	~ 2 m
Juveniles	Dark grey	No / Few	~ 3 m
Young Adults	Grey	20%- 40% body cover	Full ~ 4 m
Adults	Grey-White	40%- 90% body cover	Full ~ 4m

Figure 4.1a. Risso's dolphin mother with a new born calf. On the new-born dolphin are visible the foetal grooves, and the typical grey homogeneous colour, with no scars.

Figure 4.1b. Full size adult Risso's dolphin. The number of scars that cover the animal body classify this individual as an adult.
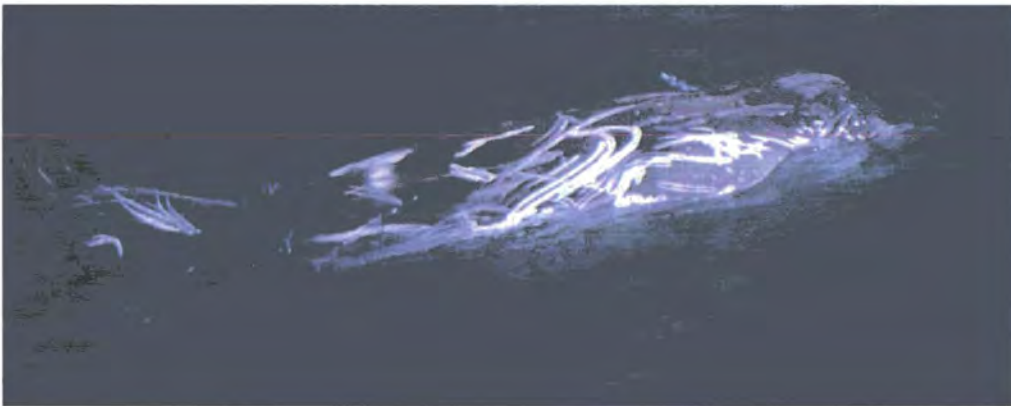
Figure 4.1c. Risso's dolphin of adult size but the number of scars that cover the dolphin body classify it as a young adult.

Colour slides were taken with a Canon EOS 5 equipped with an autofocus 70-200 mm zoom lens. All slides were examined visually for identifiable dolphins at the end of the fieldwork season. Both sides of individuals' dorsal fins were drawn for the Photo ID catalogue. Any dolphin that was constantly accompanied by a small calf was assumed to be a mother and therefore female. Photographs of calves or sub-adults with no recognisable marks were not included in the catalogue since they would not provide any guarantee of repeat identification.

From a total of 77 encounters, 54 sightings were documented photographically, and only those documented photographically were considered in the analysis. For this analysis of association patterns, only the individuals photo-identified a minimum of three times (n = 58) were included. Mother-calfmpairs were photo identified and catalogued but not included in the association analysis. Individuals were given an ID name on the basis of: year of first identification (1990 = A, 1991 = B, etc.), followed by a number corresponding to the order of first identification. If they were sighted at least 30% of the time with a calf, they were considered females and, the letter F was added to the ID name (e.g. I21F was identified the first time in 1998 and was a female).

Data analysis

There are three primary indices that are currently in use; these are summarised in Table 4.2.

Table 4.2. Formulae of association indices. Where X is the number of times that both animals a and b were seen in the same group, Ya is the number of times that a was seen but not b, Yb is the number of times that b was seen but not a and Yab is the number of times that the two animals were seen but in different groups.

Half-Weight	$\dfrac{X}{X + Yab + \frac{1}{2}(Ya + Yb)}$
Twice-Weight	$\dfrac{X}{X + 2Yab + Ya + Yb}$
Simple Ratio	$\dfrac{X}{X + Y_{ab} + Y_{a} + Yb}$

Ginsburg and Young (1992) compared these three measures of association and considered the Simple Ratio Association Index to be the least biased. The Half Weight Index tends to overestimate levels of association since it averages the count of Ya and Yb, thus reducing the denominator. On the other hand, the Twice Weight Index tends to underestimate association since it doubles the counts in samples in which members of the pair are located separately (Y_{ab}). The Simple Ratio Index quantifies associations as a simple proportion of the number of times a pair was seen together compared to the total number of samples in which either member of a pair was sighted. For reasons of minimising bias and to enhance compatibility within studies, I use the Simple Ratio Index (SRI) (Cairns & Schwager 1987). I calculated the SRI for all dyads in which both individuals were photo identified at least three times during the study period. The index ranges between zero (individuals never seen together) and one (individuals always seen together). This association index measures the frequency of association between two individuals only for those encounters during which both individuals where identified.

A test of random association was performed using a Monte Carlo method, in which testing is carried out using simulated data sets implemented by SOCPROG, a series of programs developed in MATLAB 5.2 by Hal Whitehead,

University of Dalhousie; program available at http://is.dal.ca/whitelab/index.html. The data sets are randomly generated in such a way as to retain important features of original data. This general approach was used by a number of authors in the analysis of association (Lott & Minta 1983, Myers 1983; Wilkinson 1985; Mitani *et al* 1991; Smolker *et al* 1992; Slooten *et al* 1993; Whitehead 1999). The Monte Carlo randomisation test was applied to determine which of the observed associations were significantly different from random. In this test, association data were randomly permuted, and statistics of the original data were compared with those of the random data. In order to define higher order of level associations, such as long term social grouping, association values were calculated for each individual identified during the period of study paired against each other. These calculations produced matrices of SRI values.

Cluster analysis was used to infer the existence of particular social groups within the considered population. The result of this clustering is a tree-like hierarchy showing the structure of the clustering from all individuals as separate clusters to all individuals as members of one large cluster (Digby & Kempton 1987). For the analysis of associations, I used SOCPROG.

RESULTS

Mean group size values ranged from 11 to 47, the overall mean was 28, and the mode was 28, SD = 9 (Table 4.3). The group size range was wide for all identified individuals, in fact standard deviation of group size values were also high. Females (those individuals indicated with the letter F on the right side of the ID name) were always sighted in the biggest groups.

Table 4.3. Details of the groups for the 58 photo-identified individuals. The table indicates the number of sightings in which each individual was identified; the size range of the different groups in which each individual was identified; mean group size of the groups of the identified individuals and the standard deviation.

Individual ID	Number of Sightings	Group size range	Mean Group size	Group size sd	Individual ID	Number of Sightings	Group size range	Mean Group size	Group size sd
A9	5	4-30	18	9	F22	8	6-70	32	19
A13	6	3-70	28	25	F25	3	5-34	19	15
A14	3	3-30	16	14	F27	8	6-70	32	19
B1	4	5-20	12	8	F29	3	18-41	30	12
B3F	7	5-70	27	22	F30	6	18-70	37	19
B4	4	17-41	32	11	G1	8	10-70	28	21
B8F	6	17-70	33	20	G5	4	5-30	16	11
B10F	6	17-70	33	19	G6	5	10-41	24	13
B11	6	17-41	28	9	G9	4	10-41	28	13
B13	7	5-70	27	23	H1	3	20-70	43	25
B14	5	4-41	28	14	H2	6	14-41	28	10
B15	4	6-30	18	10	H5	4	2-20	11	9
B16	3	30-40	33	6	H10	6	18-70	29	21
B17	3	6-30	18	12	H27	3	30-70	47	21
B19	7	6-77	33	20	H21	3	18-70	36	30
D2	6	3-70	30	23	H26	3	18-41	30	12
D3	4	3-70	30	29	H30	3	18-30	22	7
F2	4	18-40	24	11	H31	5	18-70	34	21
F4	8	10-70	25	20	I1	3	6-19	14	7
F6	3	13-20	17	4	I2	4	6-41	28	15
F7	12	13-70	30	16	I3	4	6-41	28	15
F9	4	13-19	16	3	I4	4	6-41	27	19
F10	3	19-70	43	26	I8F	6	18-70	35	19
F13	3	14-30	21	8	I11	4	14-70	34	25
F15	5	19-70	38	19	I15	3	30-41	32	3
F16	3	14-30	21	8	I17	3	30-41	32	3
F17	2	10-19	16	5	I19	3	31-70	45	22
F18F	6	18-70	33	20	I21F	4	20-70	39	22
F20	4	18-41	31	10	I41F	3	20-70	40	26

Association patterns

There were a total of 1624 possible pairwise combinations. Association indices (SR ≥ 0.5) showed that about 4.3% of all possible pairwise interactions between dolphins were observed. The associations in this population of Risso's dolphins were dominated by Coefficient of Association of zero (44%), and non zero values were highly skewed (Figure 4.2).

Freq.	1420	804	558	182	97	72	25	34	2	0	8
	0	0,1	0,2	0,3	0,4	0,5	0,6	0,7	0,8	0,9	1
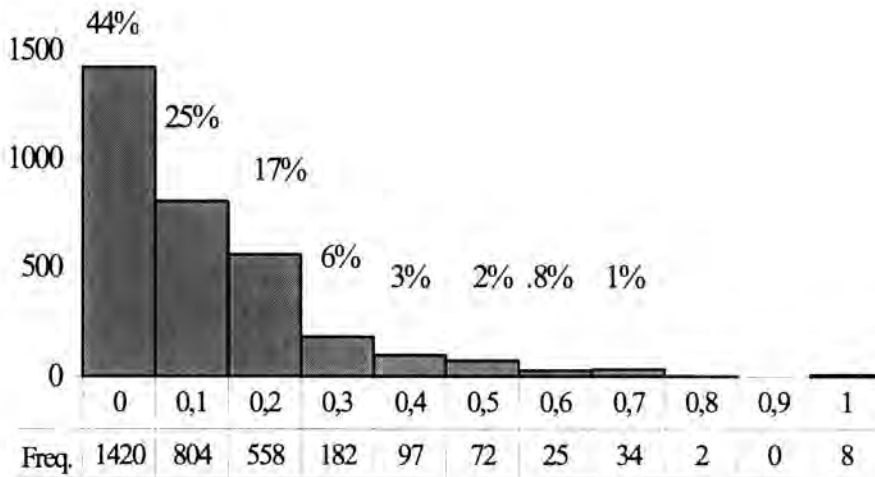
Figure 4.2. Frequency distribution of Simple Ratio Index (SRI) values with frequency value reported under the Association index values in the x-axis.

Randomisation test

A test of random associations was performed using a Monte Carlo method performing 30,000 unrestricted permutations. I started with a low number of randomisations (100) and increased the number until the number of significant dyads stabilised at 30,000. After 30,000 randomisations, the population

contained 59 dyads that had a degree of association significantly stronger than expected assuming random associations (p < 0.01) (Table 4.4). Eighty-eight percent of the individual associations of dolphin pairs tested were found to be significantly different (p < 0.01) from random distributions.

Table 4.4 Significant dyads that had SRI values ranging from 1 to 0.5.

Dyads	F16	F27	I17	I3	D2	D3	D3	B17	I1	I15	F30	I17	F30	H31	I4	I15	
	F13	F22	I15	I2	A13	B13	D2	B15	B15	F20	F22	F20	F27	H30	I2	I2	
CoAs	1.00	1.00	1.00	1.00	0.86	0.83	0.83	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	
Dyads	I17	I3	I4	I17	I41	I21F	D3	I41	D3	D2	I8	B13	B10F	H21	H30	I15	I17
	I2	I15	I3	I3	I11	I19	D2	I21F	A13	B13	F18F	A13	B8F	A9	A9	B14	B14
CoAs	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.71	0.71	0.71	0.63	0.63	0.60	0.60	0.60	0.60
Dyads	F10	H1	F15	I3	H21	I21F	I21F	F15	B10F	F15	F30	I8	F10	F22	F27	F15	
	F2	F2	F10	F20	H10	I11	B10F	D2	B3F	A13	B8F	B8F	D2	F20	F20	F7	
CoAs	0.60	0.60	0.60	0.60	0.60	0.60	0.57	0.57	0.56	0.50	0.50	0.50	0.50	0.50	0.50	0.50	

The permutation test also revealed that some individuals preferentially associate with other individuals (p < 0.01) (Table 4.4). Four pairs (eight individuals) had a SRI of 1; these pairs were always identified within the same group. Two dyads (F13–F16 and F22-F27) were sighted repeatedly in three different years, and the other two (I1-I3 and I15-I17) were observed repeatedly within the same year (Table 4.5). With the exception of the pair F13-F16, the other three pairs appeared to be member of larger groups.

Table 4.5. Dyads that have a coefficient of association of 1. The Table shows in which sighting the pairs were seen together and their mean group size and mean values of association. All individuals were adults.

Mean Assoc	Max Assoc	Mean Gr.size	Individual ID	Date of Sighting						
0.1	1	21	F13	1\7\95	1\9\97	6\7\98				
0.1	1	21	F16	1\7\95	1\9\97	6\7\98				
0.2	1	32	F22	24\9\95	14\7\97	31\7\98	2\8\98	13\7\98	27\8\98	1\9\98
0.2	1	32	F27	24\9\95	14\7\97	31\7\98	2\8\98	13\7\98	27\8\98	1\9\98
0.16	1	20	I2	25\4\98	13\7\98	31\7\98	2\8\98			
0.16	1	20	I3	25\4\98	13\7\98	31\7\98	2\8\98			
0.17	1	32	I15	13\7\98	31\7\98	2\8\98				
0.17	1	32	I17	13\7\98	31\7\98	2\8\98				

Cluster analysis

In order to define higher order levels of association, a symmetrical matrix of association indices between all possible pairs of combinations was analyzed for natural occurring clusters. Cluster analysis identified four groups indicated as Y1; Y2;Y3; Y4 (Figure 4.3).

Figure 4.3. Dendrogram of associations between individual Risso's dolphins. Average linkage cluster analysis of association matrix of the 58 photo-identified individuals.

Cluster Y1 contains four individuals that have a SRI of 1 (I2-I3 and I5-I17); as shown in Table 4.5, those pairs were always sighted together. Cluster Y1, as well as Y3, was observed only in 1998. In cluster Y2 one dyad has a SRI of 1. The group shows high individual fidelity, and was sighted in three different years. Cluster Y4 was first seen in 1995 and was sighted in three different years. The four Y groups were always sighted within bigger groups (Table 4.5).

Table 4.6. Details of the four groups resulting from the cluster analysis. For each group the mean and maximum association values, the mean group size and the number of individuals in each cluster are given.

Group	N	Individual ID	Mean Assoc.	Max Assoc.	Mean Group size
Y1	*6*		*0.16*	*0.92*	*26*
		F20	0.17	0.75	31
		I2	0.16	1	20
		I3	0.16	1	20
		I4	0.14	0.75	20
		I15	0.17	1	32
		I17	0.17	1	32
Y2	*3*		*0.2*	*0.92*	*27*
		F22	0.2	1	32
		F27	0.2	1	32
		F30	0.2	0.75	27
Y3	*4*		*0.15*	*0.75*	*40*
		I11	0.13	0.75	34
		I19	0.17	0.75	45
		I21F	0.18	0.75	39
		I41F	0.13	0.75	40
Y4	*4*		*0.15*	*0.85*	*29*
		A13	0.15	0.86	28
		B13	0.13	0.83	27
		D2	0.16	0.86	30
		D3	0.15	0.83	30

In Table 4.6 are reported all individuals included in the Cluster Analysis groups. For each individual is given the mean and the max SRI, in relation to the rest of the dolphins in the population. For example, the group Y1 contains two pairs that have an SRI of 1, their mean Association index value SRI in relation to

the rest of the individuals is 0.16 (I2 and I3) and 0.17 (I15 and I17). This indicate that they show a strong preference for specific individuals, and to associate with them and not with other members.

DISCUSSION

In odontocetes, group stability varies from the stable family of *resident* killer whales (*Orcinus orca*), where pods typically encompass 1-3 related matrilineal units, each consisting of roughly two to nine individuals (Bigg *et al* 1990, Ford *et al* 2000), to the fluid fission-fusion society of spinner dolphins (*Stenella longirostris*). In fission-fusion society, individuals associate in small groups in which composition changes very dynamically several times per day (White 1992). This is a fluid system where all member of a group are never seen all together at one time. This is not to say that stable relationships are not formed in such a society; often there are long-term associations between particular individuals.

In this study, Risso's dolphins appear to be another example of fission-fusion society. During the study period, the dolphins were encountered in groups of variable sizes suggesting that social groups were fluid. Variation of group sizes may be an indication of group instability, and individuals of any group may be interchangeable. In fact, in Table 4.3 we see that for each individual, group size varied greatly, a dolphin was observed to be a member of different group composition, indicating that members moved between groups. Also those individuals that were found to have strong associations with some conspecifics, varied their groups, the pairs of Risso's dolphins that had a coefficient of association of one were always encountered together, but they were seen in larger groups with different membership composition. Kruse (1989), in her study on the ecology of *Grampus,* based on behavioural observations, in Monterey Bay (USA), also found that school composition of Risso's dolphins was highly variable. This group behaviour may be similar to that suggested for bottlenose dolphins. More than 12 studies of different bottlenose dolphin populations, spanning from tropical to temperate waters, have shown that the species typically lives in societies in which relationships among individuals are predominantly fluid (Lusseau *et al* 2003). Among the few well studied marine delphinid species, group stability appears to decrease with body size: killer whale > pilot whale > bottlenose

dolphin > spinner dolphin > Hector dolphin (Bräger 1999). Even though body size itself is unlikely to be a factor that determines group stability, it seems to be related to longevity and the duration of nursing in odontocetes (Perrin & Reilly 1994). On this basis, we would expect Risso's dolphin social structure to be comparable to that of bottlenose dolphins. Although most associations within the Ligurian Sea population of Risso's dolphins were weak, consistent relationships between individuals were found over periods of months and, in some cases years. Pairs or small cliques may have such stable bonds that they form sub-units in larger changeable groups. Limited data on *Grampus griseus* subgroups from stranding, suggest that some of these cohesive subgroups may be same-age, same-sex animals. Risso's dolphins were frequently observed to gather in big after-feeding aggregations, and engage in social activities. The few encounters that counted over 50 individuals within a group were of this kind. Often small groups, typically four to eight individuals, appeared to be engaged in, presumably, feeding activity and then aggregate with different members to form much bigger groups to rest and socialise. A very similar behaviour was observed in Argentine dusky dolphins (Würsig 1980). Social groups in which members know each other, allow for a choice of partners and for long-term fidelity. Even extremely stable social systems such as resident killer whales sometimes gather in bigger social groups. These gatherings may be intrinsic to delphinid social systems in general, and may be important in getting all individuals of a population together periodically, so that animals that cooperate together get to know each other well (Würsig *et al* 1991), or for mating purpose.

CHAPTER FIVE

PATTERNS OF POPULATION STRUCTURE IN

RISSO'S DOLPHIN (*Grampus griseus*)

INTRODUCTION

Although the Risso's dolphin has a wide distribution, it is not particularly well known anywhere. Most of the studies undertaken on the species were done with stranded specimens, and those on free- ranging individuals mainly report behavioural and photo-identification observations. Kruse (1989), studied aspects of the ecology and behaviour of Risso's dolphins in which she conducted a photo identification project between 1985 and 1987 in Monterey Bay, California, identifying 800 individuals from 59 schools. Gill & Atkinson (1998) conducted a study in the Isle of Lewis, Scotland, and highlighted the feasibility of using photo-identification to recognise individual Risso's dolphins; they have also catalogued a number of individuals that with time will enable them to understand aspects of the species' social structure and life history. To date however, results are limited.

Studies on the genetic structure of cetacean population in the Mediterranean have only recently started. Berubè *et al* (1998) conducted a study on fin whales (*Balaenoptera physalus*) to determine whether the Mediterranean population was isolated from the Atlantic population, and found that the two populations were markedly genetically differentiated. Other Mediterranean species that have been investigated with respect to their genetic structure include striped dolphins (Garcia –Martinez *et al* 1999, Gaspari 2001); bottlenose and common dolphins (Natoli 2004) and Risso's dolphins (Gaspari this chapter).

Social organisation can be an important determinant of the genetic structure of populations and, hence the evolution of local adaptations. Among mammals, the predominant social organisation is female philopatry and male dispersal (Greenwood 1980). Such gender differences in dispersal can also profoundly influence the genetic structure of populations, particularly when the haploid and maternally inherited mtDNA is compared with the biparentally inherited nuclear genome (Avise 1994). For example, pronounced and significant differentiation in mtDNA but not in nuclear markers has been found in populations of macaques (Melnick & Hoelzer 1992) and humpback whales in the

North Pacific (Palumbi & Baker 1994). In both cases, it was suggested that the observed patterns of variation might be due to the limited dispersal of females and extensive dispersal of males. Baker (1990) suggested that this female-based social structure provides a mechanism for cultural transmission of migratory destinations and local home ranges, and thus the formation of population subdivision. Backer *et al* (1990) illustrated the point with examples from humpback whales, killer whales, bottlenose dolphins, and Hector dolphins.

In this chapter I describe the application of nuclear and mtDNA loci, and assess the possible differentiation between populations of Risso's dolphins from the North Atlantic and the Mediterranean Sea. I also investigate individual relatedness within versus between groups of Risso's dolphins identified in the field. Moreover, the molecular genetic determination of gender allows an assessment of the possible role of same-sex coalitions. It is generally assumed that close genetic relationships among individuals facilitate cooperative behaviour and, further, that this cooperation may evolve through the indirect effect of kin selection. The assessment of kinship between pairs of individuals is considered within a single geographic population, in the northwest Mediterranean Sea. This is all the more relevant as Kruse (1989), in her study near Monterey Bay, California, suggested that, like other pelagic dolphins, Risso's dolphins' social groups are highly fluid aggregations of all age and sex classes. However, limited data on Risso's dolphin strandings have suggested the opposite, that some of these cohesive subgroups may be same-age, same-sex animals (Baker 1983, Evans 1987).

METHODS

Sample collection

Genetic analyses were carried out on a total of 50 Risso's dolphins using tissue collected from stranded animals in different parts of the Mediterranean Sea and the eastern North Atlantic, and from free-ranging animals in the Ligurian Sea (Table 5.1 and Figure 5.1.).

Figure 5.1. Map of origin of Risso's dolphin samples showing sample locations.

The analysis of kinship was carried out on 30 individual free-ranging dolphins collected in the Ligurian Sea. These samples from free-ranging individuals were

collected during the summer months from 1997 to 2000, employing three different methods: (i) biopsy darting, (ii) a pole, (iii) a scrubber on a pole. Biopsy darting (i) is the most commonly used method around the world for collecting tissue samples of cetaceans. It involves shooting at the animals with either a crossbow or shotgun that fires a dart with a modified tip shown in Figure 5.2 that removes a piece of skin tissue from an individual. The pole (ii) consists of a long pole that has attached at its extremity the same dart as used in biopsy darting, and allows sample collection from the animals that come close the boat. Tissue collection (iii) with the scrubber is described in chapter 3 and illustrated in Figure 3.2a and 3.2b. Although this method may be difficult to employ on species that do not usually bow ride, such as Risso's dolphins, it has advantages for this species, since it seems to be very sensitive to darting disturbance.

Figure 5.2. Biopsy dart, showing tissue inside the tips. (Photo Cristophe Guinet).

When I used biopsy darting and the pole, animals always displayed a strong reaction, regardless of the outcome of the shooting, e.g., even when they were not touched by the pole or the dart. On the other hand though they did not respond so violently to scrubbing, they would still try to avoid it, by diving deeper. But usually they would not disappear from view. During boat surveys conducted from 1997 to 2000, tissue samples and also behavioural observations and other data on groups of dolphins were collected; details of the tissue samples are reported in Table 5.2.

Table 5.1. Number and origin of samples collected in the North West Mediterranean and used for assessing kinship.

Location	Free-Ranging	Stranded	Total
Ligurian Sea	24	3	27
Tuscany		2	2
Sicily		1	1
			30

Groups of dolphins were identified as assembled individuals behaving in a coherent manner within approximately 200 meters of each other on a given sighting.

Kinship analysis was based on 30 adults of the northwest Mediterranean, reported in Table 5.1. From the kinship analysis I consider a total of 30 animals sampled along the Italian coast; of these, six were identified in the field. I have excluded samples from Greece and Spain since they were only represented by single individuals.

DNA extraction and PCR amplification

For the genetic analyses, skin was preferred among other tissues because of its lower degradation rate, but other tissues were also used, including muscles, liver, brain, and heart. DNA was extracted and purified from tissue samples preserved in 20% DMSO NaCl 5M, by a standard phenol/chloroform extraction method (after Hoelzel 1992). A total of 29 published universal cetacean primer sets were tested and the amplification conditions optimised (see Table 5.3 for details). The most polymorphic loci were chosen for this study. Primers KWM1b, KWM2a, KWM12a and KWM5c were derived from *Orcinus orca* (Hoelzel *et al* 1998), EV37Mn from *Megaptera novaeangliae* (Valsecchi & Amos 1996), and D08 and TexVet7 from *Tursiops truncatus* (Rooney *et al* 1999, Shinohara *et al* 1997). Details on DNA amplification, sequencing, PCR reaction conditions, and sex determination are given in the methods section of Chapter 3.

Microsatellite diversity analyses

The level of polymorphism was estimated as the number of alleles per locus and the observed heterozygosity (Ho) using GENETIX 4.02 (Belkhir 2001). Homogeneity of allele distributions for all pairs of populations was tested using exact tests (Raymond & Rousset 1995). Evaluation of possible deviations from the expected HW genotypic frequencies and linkage disequilibrium were performed using Fisher exact tests that employ a Markov chain approach, as performed by GENEPOP 3.3 (Raymond & Rousset 2001). Since the observed number of alleles in a sample is dependent on sample size, allelic richness per locus and per population (R_S) was estimated as implemented in the program Fstat 2.9.3 (Goudet 2001).

The level of differentiation among populations was estimated as F_{ST} (Weir & Cockerham 1984), and Rho_{ST} (Goodman 1997) using Fstat 2.9.3 (Goudet 2001) and GENETIX 4.02 (Belkhir 2001). Calculations were performed using GENEPOP 3.3 (Raymond & Rousset 2001). Further details regarding the analyses are given in the Methods section of chapter three.

MtDNA analyses

The mitochondrial DNA control region was amplified with universal primers MTCRf (5' - TTC CCC GGT GTA AAC C) and MTCRr (5' - ATT TTC AGT GTC TTG CTT T) after Hoelzel (1998). The PCR reaction conditions were as follows: 0.2 μM dNTPs, 1.5 mM MgCl$_2$, 10mM Tris-HCl pH.4, 50mM KCl, 200 - 250pM of each primer, 0.02 U/μl Taq polymerase and 3 - 6ng/μl of DNA. The amplification involved the steps: 4 minutes of denaturation at 94°C and 35 cycles of 90 sec at 50°C (annealing temperature), 90 sec at 72°C, and 45 sec at 90°C. PCR products were then purified with QIAgen PCR purification columns, and sequenced directly using the dye-terminator method for the ABI system. The DNA sequencing reactions were performed using standard conditions: 25 cycles of 10 sec at 96°C, 7 sec at 50°C and 4 min at 60°C.

The PCR products were run on a 6% denaturing polyacrilamide DNA sequencing gel for fluorescent imaging on an automated ABI PRISM 377 DNA sequencer. The sequence files were then aligned using the program Clustal X (Thomson *et al* 1997) (www.igbmc.u-strasbg.fr/BioInfo/).

The first 619 bps at the 5' end of the mtDNA control region were sequenced in a total of 41 samples: 24 samples from the Mediterranean Sea (12 males, 11 females, and 1 unknown sex); and 17 from the eastern North Atlantic (4 males, 10 females, and 3 unknown sex).

The level of polymorphism (π) was measured as nucleotide diversity (Nei 1987), assuming the Tamura-Nei (1993) model of sequence evolution and that heterogeneity in the substitution rates across nucleotide sites followed a gamma distribution with a value of α = 0.47 (as estimated for the 5' hypervariable segment of the human control region by Wakeley 1993). The Tamura-Nei correction assumes a higher rate of transitional substitutions than of transversional substitutions and also allows for different rates between purine (A and G) transitions and pyrimidine (T and C) transitions. Nei's (1987) measure of gene diversity (*h*) was also calculated. It is comparable to the expected heterozygosity

for diploid data and it is defined as the probability that two randomly chosen haplotypes are different in the population. The calculations were run in the program ARLEQUIN 2.1 (Schneider *et al* 2001).

Allelic richness was also calculated for mtDNA sequences. Allelic richness, denoted as *r(g)* and originally defined for biallelic systems, is the number of different alleles found when *g* genes –the specified sample size– are sampled. This index is corrected for sample size by the rarefaction method. This method allows comparison of the number of haplotypes found in two regions when the sampling effort differed. The method uses the data from the larger sample to determine how many haplotypes would have been found in a smaller sample. Thus, if a total of N ($N > g$) individuals are analysed in the larger sample, the expected number of different haplotypes in a sample of g individuals can be obtained by the formula:

$$\hat{r}(g) = \sum_{i} \left[1 - \frac{\binom{N - N_i}{g}}{\binom{N}{g}} \right]$$

where N_i represents the number of occurrences of the *i*-th allele among the N sampled genes. No underlying distribution of allelic frequencies is assumed to derive $\hat{r}(g)$; the formula is purely based on the observed relative abundance of the alleles in the sample of N genes. The rarefaction method was proposed by Sanders (1968) and corrected by Hurlbert (1971) and Simberloff (1972). The corrected version was calculated with the software RAREFACTOR calculator (Brzustowski 2001) The programme also gives the standard deviation of the index.

A mismatch distribution test (Rogers & Harpending 1992) was run for each population with the program DnaSP (DNA Sequence Polymorphism) version 3.53 (http://www.ub.es/dnasp/). The test can provide an indirect assessment of demographic history. It analyses the observed distribution of the nucleotide site differences between pairs of haplotypes and compares it with the expected

distribution. This is usually multimodal in samples drawn from populations at demographic equilibrium and unimodal in populations that have passed through a recent demographic expansion. The program computes the raggedness index (r) of the observed distribution, which takes larger values for multimodal distributions commonly found in stationary populations than for unimodal and smoother distributions typical of expanding populations.

Another test for assessing demographic history is provided by tests of neutrality. Each population was analysed with Tajima's test of selective neutrality, which is based on the infinite-site model without recombination (Tajima 1989). In a population of constant size, variation at a neutrally evolving locus is expected to a have a D value of approximately zero. Following a reduction in population size, rare frequency mutations are lost more readily than are common mutations and transient positive D values are expected. On the contrary, following an increase in population size there is a temporary excess of new mutations segregating at rare frequencies, and negative D values are expected (Fay & Wu 1999).Values of Tajima's D were tested for the hypothesis of selective neutrality and population equilibrium using DNASP 3.5.3 (Rozas & Rozas 1997). Fu's test (Fu 1997) of selective neutrality was also run on each sample in the same program. As for the Tajima's test, it is based on the infinite-site model without recombination. It evaluates the probability of observing a random neutral sample with a number of alleles similar or smaller than the observed value. Genetic differentiation between populations was also quantified and tested for the mtDNA data using both F_{ST} and Φ_{ST}, calculated with ARLEQUIN 2.1. Estimates of ϕ_{ST} used the Tamura-Nei genetic distance model (Tamura & Nei 1993) with a gamma correction of $\alpha = 0.47$. F_{ST} evaluates the difference in overall haplotype frequency, while Φ_{ST} takes into account the relationships between the haplotypes based on both haplotype frequency and molecular distance (Excoffier et al 1992). The statistical significance of F_{ST} and Φ_{ST} values were tested by 10 000 permutations of the data with ARLEQUIN 2.1 (Schneider 2001).

Genetic distances between populations were calculated using the program DNASP 3.5.3. Uncorrected nucleotide divergence values (D_{xy} and D_a) were calculated by the program following Nei (1987; equations 10.20 and 10.21). D_{xy}

represents the average number of nucleotide substitutions per site between populations, and D_a the number of net nucleotide substitutions per site between populations (i.e. gross divergence minus within-population diversity). The total number of shared mutations and the number of fixed differences between populations (i.e. nucleotide sites at which all of the sequences in one population are different from all of the sequences in the second population) were also calculated with the same program.

Phylogenetic relationships among the control region sequences were analysed following different approaches. First, a maximum parsimony (MP) analysis was run with PAUP 4.0b10 (Swofford 1998), using a killer whale sequence as outgroup. A majority-rule consensus tree was constructed from 1000 bootstrap replications and a 50% criterion for the retention of nodes was applied. With the same program, a neighbour-joining (NJ) tree was also constructed from 1000 bootstrap replications; the distance matrix was based on the Tamura-Nei model and a gamma distribution with $\alpha = 0.47$, and the transition/transversion ratio was set at a level of 4.5, based on observed values

A median-joining network was generated to infer phylogenetic relationships among the ENA and Mediterranean mtDNA haplotypes, by use of the program Network 2.0 (www.fluxus-engineering.com, Bandelt *et al* 1999).

Analysis of genetic relatedness

Individual relatedness was investigated in the Mediterranean population, considering a total of 30 animals sampled along the Italian coast.

Levels of kinship were assessed by a comparison of allele frequencies at microsatellite loci and comparative estimates based on allele sharing between individuals of the same groups and from different groups. The analysis was carried out using the computer packages RELATEDNESS 5.8 and KINSHIP 1.3 (Queller & Goodnight 1989, Goodnight & Queller 1999); details of the analyses are given in the methods section of Chapter 3. Pairwise relatedness values were calculated for all possible pairs in the population

RESULTS

General information about all samples collected and used for the analyses are given in Table 5.2.

Table 5.2. General information about the samples used for the analyses: gender, identification code for each individual, the haplotypes identified for the 41 samples sequenced, the precise location of collection, how samples were obtained and what tissue was obtained, the group size at the moment of sampling when available. Asterisks indicate those individuals that were photo identified in the field. The haplotype numbers correspond to those given in Figure 5.2. Those individuals that are enclosed in a bold square were sampled within the same group.

Sex	ID Code	Haplo	Location	Method	Tissue	group size
?	G1	2	Tuscany	Stranded	Brain	
M	G2	4	Tuscany	Stranded	Heart	
M	G3		Genoa- Ligurian	Stranded	Spleen	
M	G4	2	Genoa- Ligurian	Stranded	Skin	
F	G5	4	Sicily	Stranded	Skin	
M	G6	5	Genoa- Ligurian	Stranded	Skin	
M	G7*	1	Ligurian	Pole	Skin	60
M	G8*	4	Ligurian	Biopsy	Skin	15
F	G9*		Ligurian	Scrubber	Skin	15
F	G10	6	Ligurian	Scrubber	Skin	15
F	G11*		Ligurian	Scrubber	Skin	?
M	G12	6	Ligurian	Scrubber	Skin	?
M	G13		Ligurian	Scrubber	Skin	40
M	G14	7	Ligurian	Scrubber	Skin	40
F	G15		Ligurian	Scrubber	Skin	40
M	G16		Ligurian	Scrubber	Skin	40
F	G17		Ligurian	Scrubber	Skin	40
F	G18	12	Ligurian	Biopsy	Skin	30
F	G19		Ligurian	Scrubber	Skin	35
M	G20	12	Ligurian	Biopsy	Skin	30
M	G21		Ligurian	Scrubber	Skin	100
M	G22	3	Ligurian	Scrubber	Skin	100
F	G23	6	Saronika gulf-Greece	Stranded	Skin	
F	G24	9	Alboran Sea-Spain	Stranded	Muscle	
M	G25	8	Ligurian	Scrubber	Skin	15
F	G26	3	Ligurian	Scrubber	Skin	15
F	G27	10	Ligurian	Biopsy	Skin	50

F	G28	3	Ligurian	Scrubber	Skin	50
M	G30*	11	Ligurian	Scrubber	Skin	50
M	G31*	6	Ligurian	Scrubber	Skin	4
F	G33	1	Ligurian	Scrubber	Skin	25
F	G34	1	Ligurian	Scrubber	Skin	25
F	G53	13	Israel	Stranded	Skin	
F	G35	14	Western Isles-Scotland	Stranded	Skin	
F	G36	15	Western Isles-Scotland	Stranded	Skin	
F	G37	15	Western Isles-Scotland	Stranded	Skin	
F	G38	16	Western Isles-Scotland	Stranded	Skin	
M	G39	16	Sutherland-Scotland	Stranded	Skin	
M	G40	16	Western Isles-Scotland	Stranded	Skin	
?	G41	15	Western Isles-Scotland	Stranded	Skin	
?	G42	16	Orkney-Scotland	Stranded	Skin	
?	G43	16	Western Isles-Scotland	Stranded	Skin	
F	G44	16	Western Isles-Scotland	Stranded	Skin	
?	G45	15	Strathclyde-Scotland	Stranded	Skin	
F	G46	16	Highland-Scotland	Stranded	Skin	
M	G47	16	Western Isles-Scotland	Stranded	Skin	
F	G48	16	Western Isles-Scotland	Stranded	Skin	
M	G49	15	Western Isles-Scotland	Stranded	Skin	
F	G50	16	Dyfed	Stranded	Skin	
F	G51	16	Cornwall-South England	Stranded	Skin	
F	G52	16	Cornwall-South England	Stranded	Skin	

Twenty-nine heterologous primer sets for microsatellite DNA loci were tested for optimal conditions of annealing temperature and magnesium ($MgCl_2$) concentration for amplification. Eight of the tested primers were chosen for analysis of Risso's dolphins (Table 5.3).

Table 5.3. Optimised conditions obtained for 29 universal primers; those indicated in bold were used to amplify Risso's dolphin microsatellites.

Primer	Allele size range	$MgCl_2$	Best annealing Temperature (^0C)
EV1	100-200	2 mM	55
EV5	150-155	2 mM	59
EV14	120-160	1.5 mM	62
EV21	110-170	1.5 mM	54
EV37	170-260	1.5 mM	54
EV76	120-170	1.5 mM	49
EV92	230-250	1.5 mM	50
EV94	200-260	1.5 mM	58

KWM 1b	180-210	1.5 mM	49
KWM 2a	135-170	1.5 mM	44
KWM 2b	100-120	1.5 mM	52
KWM 5c	140-142	1.5 mM	52
KWM 9a	180-200	1.5 mM	53
KWM 9b	180-200	1 mM	55
KWM 9c	180-220	1.5 mM	57
KWM 12a	150-210	1.5 mM	52
WM 415/416	200-230	1.5 mM	48
WM 417/418	155-170	1.5 mM	45
WM199/200	100-120	1.5 mM	48
WM 409/470	200-230	1.5 mM	48
WM 464/465	130-140	1.5 mM	48
D08	80-140	1.5 mM	48
D14	100-120	1.5 mM	48
D18	80-100	1.5 mM	48
D22	100-140	1.5 mM	52
D28	100-150	1.5 mM	48
Tex Vet 3	200-270	1.5 mM	58
Tex Vet 5	180-260	1.5 mM	52
Tex Vet 7	140-160	1.5 mM	53

Phylogeographic patterns of variation

Microsatellite results

Risso's dolphin samples were collected from several areas in the Mediterranean and Eastern North Atlantic (ENA), as shown in Figure 5.1. For the two populations, all loci were polymorphic, having between two and 17 alleles per locus. Each pair of loci in each population was tested for linkage disequilibrium, and genotype independence was confirmed for all loci, except for Kwm12a-D08, and Kwm12a-EV1 in the Mediterranean population. The mean observed heterozygosity was 0.467 ± 0.07 in the Mediterranean population and 0.548 ± 0.09 in the ENA. The observed (Ho) and expected (He) heterozygosity, number of private alleles and allelic richness are reported in Table 5.4. Hardy-Weinberg equilibrium was tested for each population at each locus, and neither of the populations was at equilibrium. A significant heterozygote deficiency was found for five loci (Kwm2a, Kwm12a, D08, TexVet7, EV1, EV37) in the Mediterranean population, and for one locus (Kwm12a) in the ENA population.

Table 5.4. Number of different alleles (with the number of private alleles shown in parentheses, and allele richness value in squared brackets), observed (Ho) and expected (He) heterozygosities for each population at each locus. Deviation from Hardy-Weinberg equilibrium was tested, and loci showing a significant deviation are indicated with an asterisk (P < 0.05).

| Locus | Mediterranean (n = 32) | | | East North Atlantic (n = 18) | | |
	Alleles	Ho	He	Alleles	Ho	He
KWM1b	3 (1) [2.46]	0.392	0.322	4 (2) [3.86]	0.800	0.557
KWM2a	10 (6) [7.98]	0.533*	0.818	6 (2) [5.85]	0.500	0.586
KWM5c	3 (1) [2.05]	0.031	0.090	2 [1.72]	0.055	0.054
KWM12a	15 (10) [10.7]	0.645*	0.880	8 (3) [7.5]	0.611	0.762
D08	11 (7) [8.46]	0.428*	0.825	6 (2) [6]	0.615	0.742
TexVet7	6 (2) [4.74]	0.500	0.445	4 [3.70]	0.555	0.442
EV1	17 (8) [12.66]	0.607*	0.905	10 (1) [9.71]	0.687*	0.873
EV37Mn	10 (5) [7.71]	0.600*	0.824	5 [4.81]	0.562	0.722
Mean	9.37 (5) [7.09]	0.467	0.638	5.6 (1.25)[5.39]	0.548	0.592
SE	1.821(1.195) [1.332]	0.069	0.109	0.885 (0.411)[0.873]	0.077	0.090

The Mediterranean population showed a higher level of variability than the ENA population, as indicated by the mean allelic richness values, 7.09 for the Mediterranean and 5.39 for the ENA (table 5.4). Allelic richness is considered to be more sensitive to the effects of bottlenecks than is heterozygosity. Although both the Mediterranean and the Eastern North Atlantic populations showed private alleles, the Mediterranean population had a larger number.

The two populations were found to be marginally but significantly differentiated F_{ST} = 0.0296 (p < 0.05); Rho$_{ST}$ = 0.018 (p < 0.05).

MtDNA sequence analysis

The analysis of 619 bp of sequence data of the mtDNA control region revealed 28 variable sites defining 16 unique haplotypes among two populations; shared haplotypes were not observed. All substitutions were transitions. In the Mediterranean population there were 13 haplotypes among 24 individuals, and 26

polymorphic sites, while in the ENA population there were three haplotypes, and
two polymorphic sites among 17 individuals (Figure 5.5).

```
             Polymorphic sites                    Frequency

             1112222333 3333334444 44445555
             2684788002 3335693334 49993346
             6072125267 0175460128 91242434

MED G1       TCGCCCAGCA TTGGGTCCCT ACTGCCCT3    Med    ENA
MED G2       .T..T..... .......... ........      2
MED G3       .T..T.GATG ...A.C..TC G......C      3
MED G4       .T....G... C..A...... ........      3
MED G5       .T.TT..... .C.AA...T. ........      1
MED G6       .T..T.G... C..A...... ........      4
MED G7       .T..T.GAT. ...A.....C .T.A.T..      1
MED G8       .T..T.GAT. ...A.....C .T.A....      1
MED G9       .T..T.GAT. ...A....T. ..C...T.      1
MED G10      CT.TTTGAT. ..AA..T.T. ......TC      1
MED G11      .T..T.G... C..A...T.. ........      1
MED G12      .T........ .........C ........      2
MED G13      .T.TT.G... C..A...... ......T.      1

ENA G14      .TATT.G.T. .C.A....T. ..C.T...             1
ENA G15      .TATT.G... .C.A....T. ..C.....             5
ENA G16      .TATT.G.T. .C.A....T. ..C.....             11
```

Figure 5.5. Polymorphic sites among 16 haplotypes are shown (left). The
numbers indicate variable sites along the sequence. Haplotypes were identified by
an abbreviation of their geographic region followed by the individual ID number
which is reported in Table 5.1. Dots indicate identity with the reference sequence.
Haplotype abundance (right) is reported for each haplotype in each population.

Gene (h) and nucleotide (π) diversities were estimated for the two populations,
and varied greatly (Table 5.6).

Table 5.5. Genetic variability based on mtDNA analysis (619 bp). For each
population, number of individuals (n), number of polymorphic sites (Poly sites),
number of haplotypes (Haplo), nucleotide diversity (π), and gene diversity ($\hat{H}$)
are shown, SE are in parentheses.

Population	n	poly sites	Haplo	π	H
Mediterranean	24	26	13	0.010 (0.005)	0.930 (0.027)
ENA	17	2	3	0.0009 (0.0008)	0.522 (0.100)

The ENA mtDNA diversity was lower than that of the Mediterranean. However, the observed number of mtDNA haplotypes in a sample is highly dependant on sample size. In order to compensate for this problem, haplotype richness was calculated to standardize for sample size. The Mediterranean population had a haplotypic richness (h) of 10.75 (instead of 13) which is still considerably higher than the value observed for the ENA population (H = 3). Hence, the difference in haplotypic diversity was not due to differences in sample size.

Both Tajima's D and Fu's neutrality tests were negative but not significant for either population (Mediterranean: Tajima's D = -0.185, Fs = -1.334; ENA: Tajima's D = -0.138, Fs = -0.126). The mismatch distribution was carried out on both populations, Mediterranean and ENA. The ENA population had too few haplotypes (3) to allow robust interpretation of the results, therefore I only show the Mediterranean mismatch distribution in Figure 5.7. The multimodal distribution indicates the population is at demographic equilibrium.
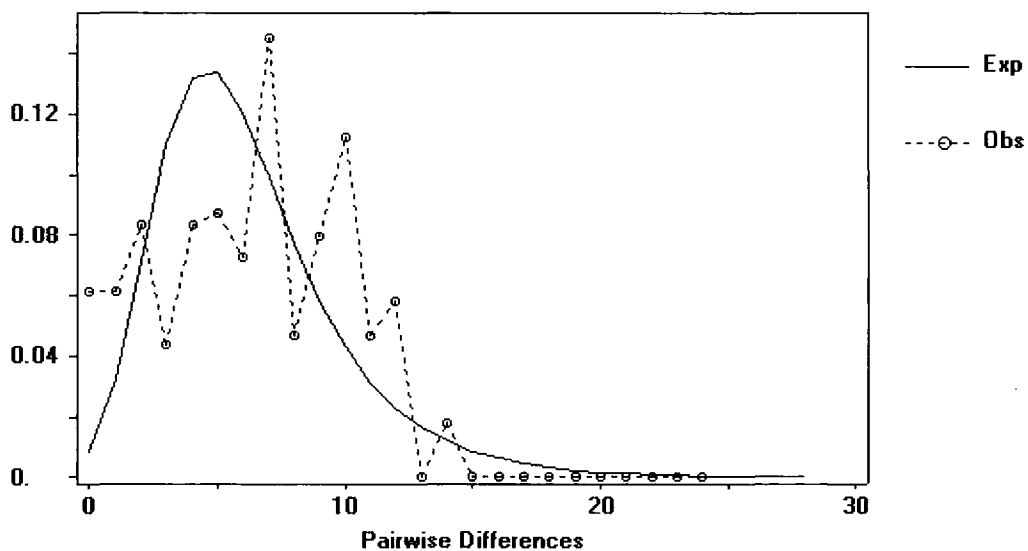
Figure 5.7. Mismatch distribution for the control region of the Mediterranean population.

The degree of differentiation in the mtDNA data was estimated by F_{ST} and ϕ_{ST}, which indicated a highly significant difference between the two populations (respectively 0.260, $p < 0.001$ and 0.542, $p < 0.001$).

Genetic distance was determined by nucleotide divergence and *Da* and *Dxy* were 0.008 and 0.0135 respectively.

Rooted (*Orcinus orca*) neighbour-joining and maximum parsimony trees were reconstructed using 16 haplotypes. The trees confirmed the separation between the Mediterranean and the ENA populations (Figure 5.8).

A minimum-spanning network (MSN) is reported in Figure 5.9. Both the NJ tree (Figure 5.8) and MSN (Figure 5.9) show that the two populations are well separated. Individuals from the ENA population are grouped together, and although the Mediterranean population is separated, it does not appear to be sub-structured. There is no predominant haplotype among the Mediterranean samples, as there is in the ENA population (haplotype G16).
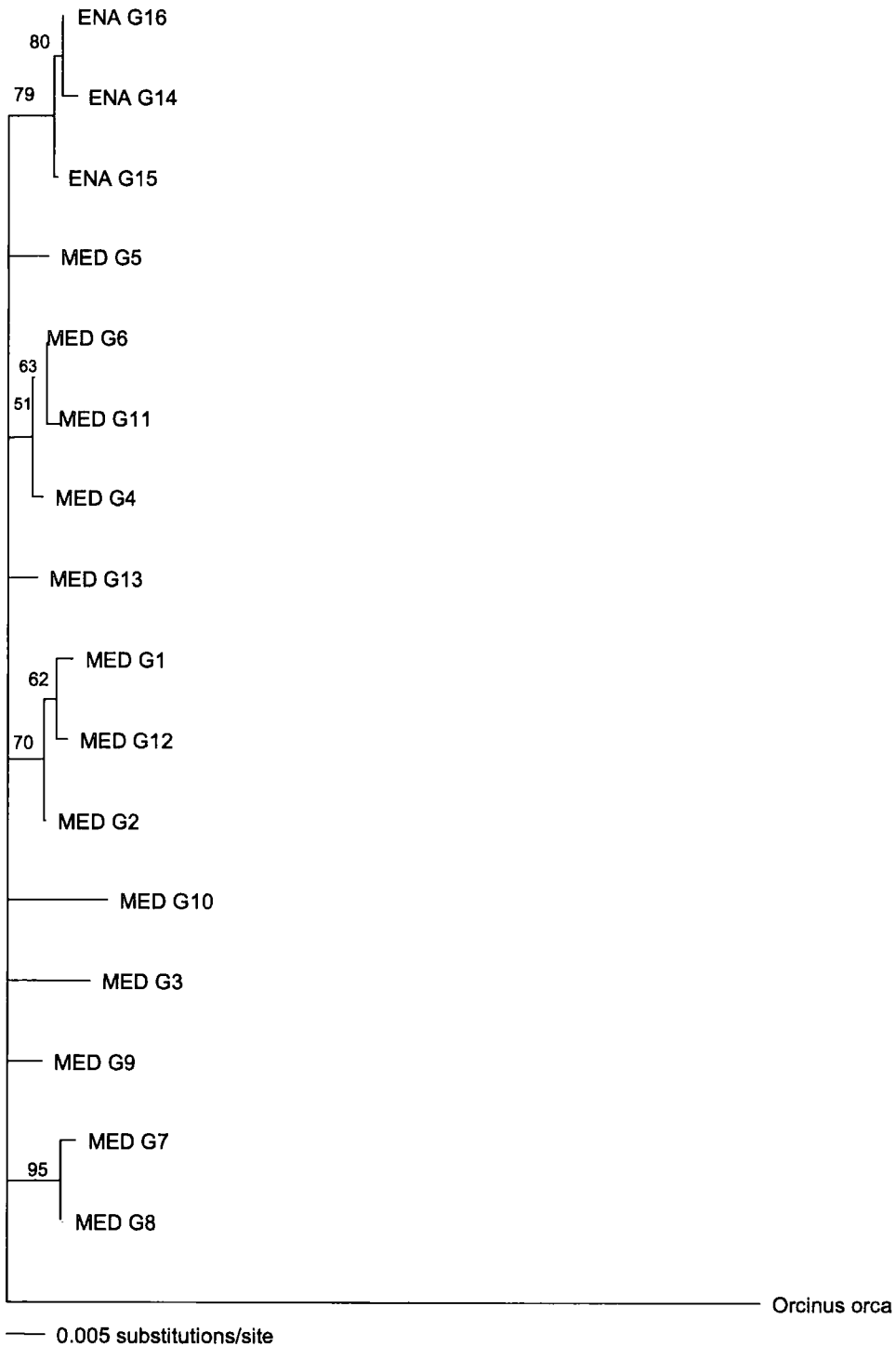
Figure 5.8 Phylogenetic relationship of Risso's dolphins based on mtDNA control region haplotypes. Neighbour Joining rooted tree based on Tamura & Nei (1993) distance measure. The out-group is represented by a Killer whale. Geographic origins are indicated in capital letters, and the numbers correspond to the haplotypes reported in Table 5.2.Bootstrap value are indicated.
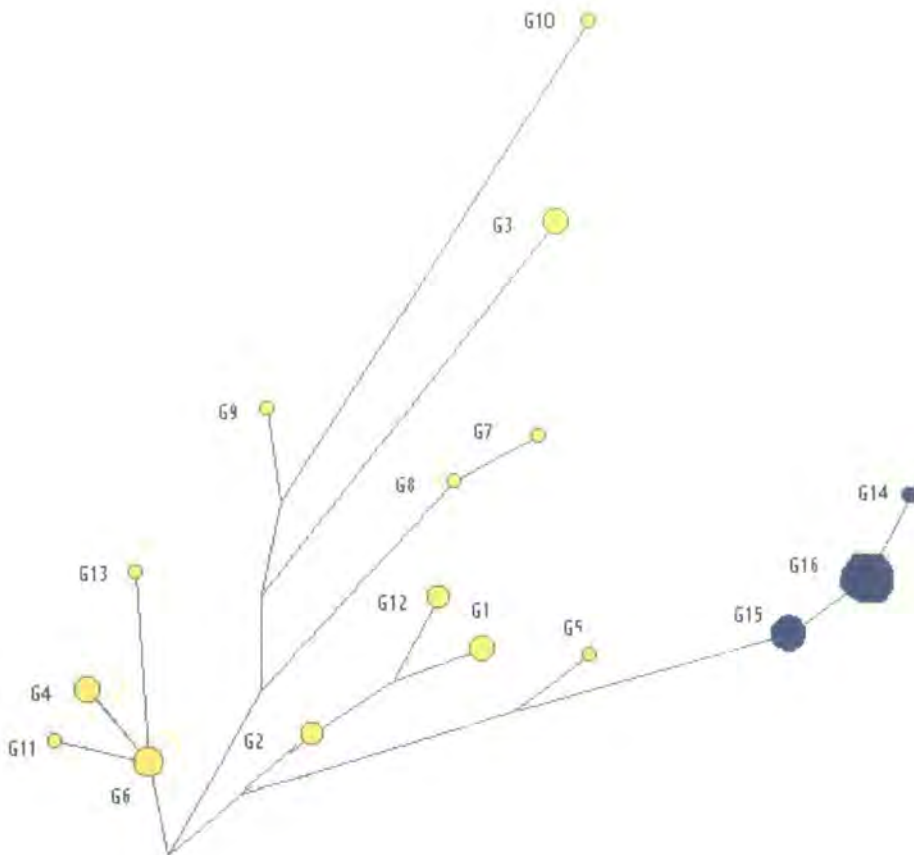
Figure 5.9. Minimum-spanning network (MSN) of 16 haplotypes of Risso's dolphins. The size of the circles represents the frequency of haplotypes in the sample. G stands for *Grampus* while the numbers are the haplotype identification reported in Table 5.2. Haplotypes in blue represent the ENA population. In the network graphical display, each node represents either an observed haplotype or a hypothetical intermediate haplotype; the area of the circle representing each haplotype is proportional to the number of individuals with that haplotype; the length of the links is proportional to the number of mutations.

Individual relatedness

Individual relatedness was estimated based on genetic similarity among individuals within and among groups (n = 30). All individuals were assigned to their group of origin in the field (with letters from A to G, n = 7) (Table 5.7).

Based on group membership, I compared relatedness within versus between groups. From an analysis of all pairwise combinations including dolphins of both sexes, the estimated average relatedness within groups was -0.046 ± 0.059 and the estimated average relatedness between groups was 0.008 ± 0.012. Both within and between group values were low; in particular, within groups, it was lower than expected by chance. Relatedness values of between groups versus within were not significantly different. Except for one pair of females (G33 and G34 that had an R = 0.876), the higher R values were all found between individuals that were not sampled within the same group.

Table 5.6. Average pairwise comparison within groups identified in the field.

Group	Sample size	Mean R	SE
A	3	0.0553	0.0476
B	2	0.2219	-
C	5	-0.1344	0.1777
D	2	-0.1354	-
E	2	-0.0358	-
F	3	-0.1803	0.2002
G	2	0.8765	-

Relatedness values ranged from a minimum of -0.4 and a maximum of 0.8 within groups, and a minimum of -1 to a maximum of 1 between groups.

Relatedness between sexes

In the Northwest Mediterranean samples, there were 13 females and 16 males. Pairwise R-values were used to examine the relatedness among sexes, and a significant difference based on the Mann-Whitney U test was

found comparing all females and all males (Z = 3.418, p = 0.000). Females had an average R of -0.053 ± 0.038, values ranged from a minimum of -1 to a maximum of 1. Males had an average R of 0.048 ± 0.021, values ranged from a minimum of -0.344 to a maximum of 1. When comparisons within versus between groups were restricted to one sex, females showed a considerably higher relatedness within than between groups; mean R within groups = 0.208 ± 0.229 (n = 4), R values ranged from a minimum of -0.163 to a maximum of 0.876; mean R between groups = -0.064 ± 0.036 (n = 72), R values ranged from a minimum of -1 to a maximum of 1. The difference was not statistically different possibly due to the small sample size of females within groups (Z = -1.495 p = 0.067). Also for males, mean relatedness within groups was not statistically different from mean relatedness between groups. However, R values within groups showed an opposite trend, mean R within groups = -0.118 ± 0.061 (n = 4), R between groups = 0.054 ± 0.021 (n = 115), (Z = 1.828 p = 0.067).

DISCUSSION

Phylogeographic patterns

This study on *Grampus griseus* genetic variation revealed that Risso's dolphins inhabiting the Mediterranean Sea are genetically distinct from those in the North Atlantic. This result was supported by both microsatellite and mitochondrial DNA analyses. One possible reason for this differentiation could be the geophysical characteristics of the Mediterranean Sea, which has oceanographic and ecological characteristics that greatly differ from the Atlantic Ocean. The nature of a semi-enclosed sea such as the Mediterranean may have contributed to isolation between the Atlantic and the Mediterranean populations. The Mediterranean Sea is relatively young in terms of geological time, about 4.5 – 5.5 Mya (late Miocene), and underwent cycles of dessiccation as a result of the closing of the Strait of Gibraltar. A relatively recent separation between Mediterranean and Atlantic Risso's dolphin populations is supported by the values of Da and Dx genetic distances. Moreover, the NJ Tree (Fig 5.8) and the MSN (Fig 5.9) showed a clear separation between the two populations, but no lineage sorting. The ENA population falls in a separate lineage and it is depauperate in variation. The Mediterranean mismatch distribution suggests a stable population at demographic equilibrium.

In a study on the genetic differentiation of striped dolphins in the Mediterranean and Atlantic (Garcia-Martinez *et al* 1999), it was suggested that there was a higher nucleotide diversity in the North Atlantic than in the Mediterranean. This was explained by the more ancient origin of the Atlantic population with respect to the Mediterranean population, or attributed to a broader distribution of the Atlantic samples. My Risso's dolphin results show the opposite pattern. Both microsatellites and mtDNA show that the Mediterranean population was more variable than the East North Atlantic population. The Mediterranean population is presumably a more recent population in relation to

the ENA, and may have retained a higher variability. The North Atlantic is mainly represented by samples from Scotland, and this region represents the extreme range limit for this species; it is therefore conceivable that adaptation to this particularly extreme environment in some way led to the isolation of this population. Another study on bottlenose (*Tursiops sp*) and Common (*Delphinus sp*) dolphins, comparing Mediterranean and North Atlantic populations, also suggested the possible genetic isolation of Scotland (Natoli 2004). According to the microsatellite results, the two populations showed high genetic variability, but the Mediterranean population was not at equilibrium for most loci considered. Samples obtained in the Mediterranean Sea, were mostly collected in one area, the Ligurian Sea (Table 5.1), during the summer months. During that time of year, the Ligurian Sea is particularly rich in food, and cetaceans may migrate to the area for feeding, as is known for fin whales. The deviation from HW equilibrium may therefore indicate an internal population structuring (Wahlund effect), but to test this hypothesis a greater number of samples are needed.

Previous studies on cetaceans have suggested that nuclear gene flow, which is a consequence of genes transmitted through both sexes, may be high whereas mtDNA variation shows a strong structuring among populations (Valsecchi *et al* 1997, Bérubé *et al* 1998), presumably due to limited female dispersal since it is only transmitted by females. Thus, the analysis of nuclear loci could potentially paint a different picture than that from the mtDNA. When we consider the ENA population, microsatellites show genetic variability, while mtDNA diversity was very low. It is often true that microsatellites show a difference in diversity level, while mtDNA does not. This is due to the smaller effective population size represented by the mtDNA genome (1/4 that of the nuclear genome). MtDNA is more liable to demographic fluctuations and the effects of drift. Populations may naturally pass trough successive bottlenecks and founder events that are due to past range contractions, followed by (re)colonization by a small number of individuals. Alternatively, low genetic variability may reflect intense selection. The low variability in mtDNA haplotypes in the ENA population may also imply low female gene flow and possible founder events.

Kin structure in the Ligurian Sea

Social structure may have significant influences on the genetic properties of populations (Matthews & Porte 1993, Sugg *et al* 1996), and differences between sexes in breeding and dispersal patterns have major consequences for genetic subdivision and the kin structure of populations (Pope 1998). As a result, investigation of genetic structure within and between social groupings can be highly informative regarding social structure (Amos *et al* 1993).

The sample size on which kin assessment was based was small, but what evidence there is suggests that Risso's dolphins have a fluid social structure. This was also indicated by association data. When individual relatedness of both sexes together was compared, the within groups versus among groups r was not significantly different; dolphins did not appear to associate on the basis of genetic relatedness. When Risso's samples were separated by sex, a significant difference was found between male and female within-group relatedness. The results suggest that males do not form kin groups in general; the relatedness of all males in the population was low R = 0.05. But it is interesting to note that when we consider the relatedness of males within groups, R value dropped drastically to - 0.118. This may indicate that males avoid clustering in kin groups, possibly to avoid inbreeding. The opposite was true for females. In fact, females within groups appeared to associate with other kin-related females. Although we never found groups which were formed only by females, the relatedness values within groups was considerably higher that that between groups. However, sample size is a limiting factor for conclusive interpretation of the results. I therefore only speculate on possible scenarios that are suggested by the results obtained.

Grampus griseus is considered to be a deep diving dolphin species, and consequently during the mother's feeding bouts, the young may be left unattended. It is, therefore, conceivable that females form kin groups possibly to cooperate in rearing the young, practising some form of alloparental behaviour. Allomaternal behaviour was also suggested for other species such as spinner

dolphins (Johnson & Norris 1994), bottlenose whales (Gowans 1999), killer whales (Haenel 1986), and sperm whales (Whitehead 1996). The evolution of female sociality has been a topic of considerable debate and research in recent years, particularly among primatologists. A great deal of discussion was stimulated by Wrangham's (1980) "female bonding" model, with stable cooperative relationships among females evolving as a response to strong between-group competition for patchy resources, yet it has been shown to have little empirical support (van Schaik & van Hoof 1983, Dunbar 1988, Janson 1992). Gregariousness leads to resource competition, both within and between groups. The form of resource competition is determined by the distribution of food resources relative to group size, and it is this that determines the nature of female social relationships (van Schaik & van Noordwijk 1988). Where resources are not defensible, leading to "scramble" competition, variations in individual power cannot be translated in access to food, so social phenomena such as dominance and alliance formation are of no benefit to individuals; relationships between individuals are undifferentiated, and individuals may frequently move between groups (van Schaik 1989). By contrast, if the resource is monopolisable, "contest" competition results, and differences in power lead to differential access to resources. In such a society, dominance and alliances are favoured, since coalitionary aggression is necessary for individuals to gain access to resources (Mitchell *et al* 1991). Thus contest competition leads to the formation of stable female groups, and consequently female philopatry and matrilineal group structure (van Schaik 1989). If we consider social organisation as adaptive to environmental constrains such as food distribution, then we would consider Risso's dolphins food resources as patchily distributed and not monopolisable. In such a context, a fluid social organisation would be adaptive and female kin associations may have a significance other than for access to food.

CHAPTER SIX

GENERAL DISCUSSION

DISCUSSION

The aim of this thesis was to study the hierarchical genetic structure of Risso's and striped dolphin in the Mediterranean Sea and North Atlantic towards a better understanding of the evolution of population genetic structure in dolphin species. In support of this, I investigated the population and social structure of these two species using a combination of molecular techniques, distributional data and photo-identification. This comparative assessment of the two species has provided some insight into the evolutionary processes that underlie the differences between them, and this is discussed in the broader context of our current knowledge on dolphin population and social structure.

Distribution

Social behaviour has important implications for processes such as spatial distribution, reproductive success, and gene flow (Whitehead 1997), and can therefore be an important variable when analysing habitat selection and use (Reed & Dobson 1993, Dobson & Poole 1998). Habitat preference is driven by complex interactions among behavioural patterns, biological requirements, and environmental conditions. Furthermore, for many gregarious animals, the type of social organisation is inextricably linked with the nature of the environment (McDonald 1983). Social organization and patterns of habitat use are critical to understanding the distribution and behaviour of species in the wild. In general, organisms occupy regions that contain resources that meet their daily requirements (Burt 1943), and spatial partitioning and social organization are often affected by the distribution and defensibility of these resources (Crook & Gartlan 1966, Crook 1970). However, criteria for habitat selection can be quite different for each organism, and distribution and differential patterns of habitat use may be based on seasonal fluctuations in availability of resources such as water (Kgathi & Kalikawe 1993), availability of shelter and nesting sites (Kroon

de Graaf & Liley 2000), foraging strategies (Funston *et al* 1998) or a complex interaction of multiple factors. Moreover, for some species, habitat selection and temporal distribution are driven more by access to conspecifics than by levels of predation or the availability of resources, such as food.

In the present study, I found some evidence for the environment having a significant impact on dolphin distribution, despite the relatively small geographic area investigated. In particular, Risso's dolphins showed a marked habitat preference for the steeper continental slope, where presumably most of their prey were concentrated.

Risso's dolphins are known to frequent subsurface seamounts and escarpments where they are thought to feed on vertical migrant and mesopelagic prey (Norris & Dohl 1980, Clarke & Pascoe 1985). Local submarine topography associated with the continental slope may concentrate prey by creating upwelling, convergence and divergence zones, and current ridges that may serve to increase local productivity. However, little is known about the pelagic distribution of squid and its relation to bottom topography in the study area. Theoretical models of squid distribution suggest that animals aggregate along thermal fronts prior to spawning (Thompson & Frey 1975). These fronts are characteristic of high relief sea floor topography (Braker & Broenkow 1989), areas where Risso's dolphins are most abundant. It is plausible that Risso's dolphins are attracted to these depths by squid aggregating along these thermal fronts, and that would explain their distribution preference. Other species such as bottlenose whales have also been found to show a discrete distribution within the canyon associated with water depth (500 to 1500 m) and relatively steep topography (Gowans *et al* 2001). The results of a specific study (with which I collaborated) that considers the distribution of cetaceans in the study area (Azzellino *et al* 2004) support the hypothesis that physiography plays an important role in partitioning cetacean distribution. They showed that the cetacean distribution in the Western Ligurian Sea could effectively be explained by depth and slope. Out of the eight species examined in the study, bottlenose dolphin, Risso's dolphins and Cuvier's beaked whales, showed definite non-overlapping depth and slope preferences respectively for the shelf-edge, the upper and the lower slope. Similar results have been found

for the same species elsewhere, suggesting that the observed habitat preferences are the outcome of the different feeding habits and of the species' response to changes in the environmental conditions. (Baumgartner *et al* 2001, Gulf of Mexico: Davis *et al* 1998, North East US shelf-break: Waring *et al* 2001, Mediterranean waters off Southern Spain: Cañadas *et al* 2002). In general, species with a diet known to be mostly teuthophagic, such as Risso's dolphin (Clark & Pascoe 1985, Wurtz *et al* 1992, Kenney *at al* 1995), have been shown to be most closely linked to the physiography of the upper and lower slope.

I found that the distribution of the two study species was very different. Unlike Risso's dolphins, there was little evidence for a correlation between environmental characteristics and striped dolphin distribution, which showed no particular habitat preference. This may suggest that striped dolphins are more opportunistic in their prey choice, and therefore less directly linked to physiographic changes, or more influenced by prey movements and seasonal variations.

The differences found in the encounter rate of the two species also indicated a possible difference in the temporal use of the habitat. Azzellino *et al* (2004) suggested that the transient use of the habitat by Risso's dolphins may be the result of interspecific competition for prey resource (e.g. with sperm whales) or temporal changes in the habitat due to oceanographic processes. Information about the dietary habits of the cetaceans living in Mediterranean waters is scarce; however, the few available data on stomach contents suggest that Risso's dolphin (Wurtz *et al* 1992), Cuvier's beaked whale (Blanco & Raga 2000) and sperm whale probabily feed on the same species. The potential for competition among these three species may explain some observed spatial and temporal habitat partitioning (Azzellino *et al* 2004). In the Ligurian sea, both Risso's dolphin and sperm whales were found to use the same habitat within definite temporal intervals. However, only Risso's dolphin encounters were associated with a clear temporal pattern.

The EUROMARGE Program (Macquart-Moulin & Patriti 1996) showed the geomorphology of the Western Ligurian Sea (i.e. the continental slope and the submarine canyons at the boundary between neritic and oceanic domains) in

combination with the changeable wind and the current regimes off the coast, which create the conditions for the accumulation of migratory micronektonic species in the slope waters. These pelagic species, such as euphausiids, hyperiids and mysids are transported by surface currents which are generated by winds blowing landwards, during their upward migration and their nocturnal accumulation at the surface; afterwards, they are trapped in slope waters during their downward morning migrations. The Liguro-Provençal coast is frequently exposed to strong gusts of seawards wind that generate a strong offshore current in the superficial layers.

In general, a coastal upwelling is associated with this offshore current carrying the neritic bentho-pelagic species that stratify at the surface during the night out off the shelf. This periodic pattern of concentration of pelagic zooplankton near the bottom above the slope (Macquart-Moulin & Patriti 1996) may provide an abundant food source for the organisms living in the slope area. Risso's dolphin's transient use of the habitat may therefore follow those currents that concentrate food along the slope. Risso's dolphins may move up and down along the upper slope as a foraging strategy to find the mesopelagic squid, which in turn may be attracted by the wind-driven accumulation of zooplankton. Conversely, striped dolphins may be less linked to the sea bottom biocenosis and feed mainly on the organisms inhabiting the first 500 meters below the surface.

Group size

In social species, optimal foraging theory predicts an optimal group size as a result of relationships between hunting success, calorific intake and group size. However, various factors can increase the group size beyond the optimal number. Although resource exploitation and predation avoidance have been suggested as the main determinants of group size in dolphin species, factors such as social facilitation may also produce groups larger than the optimum for foraging. Females, for example, may prefer groups larger than optimal foraging groups to favour alloparental care of their young (Norris & Dohl 1980). Males may join

groups of already optimal size in search of oestrous females and thus occupy larger groups. Undoubtedly, the determinants of group size are complex and possibly conflicting; in fact, individuals may have different requirements, for example in terms of nutrition or reproduction, and therefore each individual may prefer groups of different sizes, depending on the individual requirement. This individual variability may somehow account for the wide variety of group sizes, both between different species and within the same species depending on the individual's activities. Risso's and striped dolphins showed a wide group size range. In general, striped dolphin groups were larger than those of Risso's dolphins, which varied considerably. I suggest that Risso's dolphin group size variability was determined by individual activities. The difference found in group size variation between the two species suggests that the group size was possibly influenced by prey distribution in striped dolphins, and by individual activities in Risso's dolphins.

Phylogeographic patterns of variation

The phylogeographic analyses of striped and Risso's dolphins indicated differentiation between the Mediterranean and the ENA populations. Genetic differentiation between the Mediterranean and the Atlantic has also been demonstrated for other species including bottlenose dolphins, common dolphins (Natoli 2004) and fin whales (Berubé *et al* 1998). The differentiation between the Mediterranean and the ENA for Risso's dolphins was established for the first time in this thesis, using both nuclear and mitochondrial DNA markers.

Striped dolphins also showed greater variability in the ENA than in the Mediterranean. This was previously reported in another study based on mtDNA (Garcia-Martinez *et al* 1999). Garcia-Martinez *et al* (1999) suggested that more ancient origins may account for the higher variability in the ENA population. Unlike striped dolphins, Risso's dolphins showed a greater variability within the Mediterranean population by comparison with an ENA population off Scotland. Results similar to those found for Risso's dolphins were obtained by Natoli (2004)

for bottlenose and common dolphins. However, ENA samples of Risso's, bottlenose and common dolphins were mainly collected from regional populations around Scotland. For those species, this area represents the extreme range limit, and therefore dolphins may have adapted to this specific environment in isolation from other conspecific populations, thereby reducing their genetic variability. However, it is also possible that Risso's dolphins from the ENA population underwent a bottleneck or a founder event that reduced their genetic variability. A comparison between the Mediterranean population and a wider sample-set from the ENA may help address this question.

For striped dolphins within the Mediterranean Sea, I further demonstrated a pattern of differentiation between the eastern and the western regions that is consistent with habitat differentiation and with the population genetic patterns seen in other dolphin species inhabiting these waters (Natoli 2004). The eastern and the western basin of the Mediterranean are ecologically and geologically very different. The eastern and the western part of the Mediterranean formed in two distinct geological processes, with the western side of the Mediterranean Sea being more recent (Oligocene; Rosenbaum et al 2002). The implication is a reduction in gene flow, perhaps related to these environmental differences. On a finer scale, I also found genetic differentiation between the local seas east (Adriatic) and west (Tyrrhenian) of Italy. Furthermore, within the Tyrrhenian Sea, which is the youngest basin in the western Mediterranean, I found a small effect of differentiation between dolphins sampled inshore and offshore in the Ligurian Sea. Although the interpretation of this pattern is limited by the small sample size, and emphasizes the need for further research, it also underlines the potential role of a combination of physical and ecological characteristics on gene flow in this species, even on a relatively small geographic scale. Other dolphin species show a differential habitat use with respect to the utilization of inshore and offshore habitat, and often inshore and offshore animals show different morphological characteristics. For example spotted dolphins (*Stenella attenuata*) differ in tooth and jaw structure (Douglas et al 1984) and common dolphins (*Delphinus delphis*) differ in beak length (Rosel 1994). For striped dolphins there

are no data available on the morphological differentiation between the two possible morphs.

Individual relatedness

My analysis of kin-associations within and among groups for striped and Risso's dolphins was most consistent with a relatively fluid model of social structure, where females tend to be philopatric and males disperse. However, my data on inter-and intragroup relatedness suggest important differences between the two species that may underlie different social strategies, although the data are stronger for striped than for Risso's dolphins.

Kinship is a central issue in the evolution of sociality, and the elucidation of relatedness between interacting individuals is a key variable in understanding social organisation and its evolution (Hamilton 1964, Pamilo *et al* 1997). While human have achieved a complexity of social organisation far greater than any other species, non-humans mammals exhibit levels of social complexity greater than other vertebrates (Wilson 1975). Elephants, primates and cetaceans are considered to have independently evolved peaks in brain size, and concomitantly peaks of social complexity (Wilson 1975, Connor *et al* 1998,). Hamilton (1964) endorses the idea that cooperation among related individuals may be favoured by kin selection, because individuals can increase their inclusive fitness by assisting relatives' reproduction and by helping in raising their offspring, even if the direct benefits of cooperation in terms in increased reproductive success accrue only to one or a few of the cooperating individuals. Nevertheless, group living has disadvantages in terms of competition and the spread of socially transmitted diseases. However, three major factors are generally recognised as beneficial in group living, especially in birds and mammals. First, high predator pressure can favour the formation of large groups. For example, birds and ungulates often decrease the risk of predation by clumping together (Emlen & Oring 1977). Second, resource distribution can favour group living if resources are defendable and the costs of sharing are low. Third, intraspecific competition often interacts

with resource distribution and can be important to develop long-term bonds. Nonetheless, group formation, which promotes social bonds arises only if the benefits of being with others outweigh the disadvantages.

Although, Hamilton's inclusive fitness theory represents one of the most important development in evolutionary biology and has been very successful in explaining a wide range of phenomena, especially cases of supposed altruism, recent works have emphasized how the importance of kin selection can be overestimated. Moreover, it has recently been suggested that the structure of relatedness within many groups may be a consequence of the direct benefit of natal philopatry (Lambin *et al* 2001, Clutton-Brock 2002). An estimate of high relatedness between interacting individuals is not considered sufficient evidence for kin selection to be responsible for promoting altruism. Furthermore, new theoretical models (Queller 1992, 1994, Taylor & Frank 1996) suggest that competition between relatives can counteract kin selection (West 2001). Therefore, today the debate about the function of kin groups remains open.

Risso's dolphins were characterised by a low background level of relatedness, and kin biased affiliations were found only within groups of females. Low average relatedness values recorded in other social mammals, including bats (*Rhinolophus ferrumequinum*, Rossiter *et al* 2002), savannah baboon (*Papio cynocephalus*, Altmann *et al* 1996), bonobo (*Pan paniscus*, Gerloff *et al* 1999), and hairy-nosed wombat (*Lasiorhincus krefftii*, Taylor *et al* 1997) have often led to the conclusion that sociality in such cases cannot be attributed to kinship, but is more likely to represent mutualism or reciprocity. Risso's dolphins showed a clear and marked difference in kin association between the two sexes. While females within groups tended to associate with kin females, and females outside the groups were not related to each other, a similar pattern was not detected for males.

The larger odontocetes have a low adult mortality but substantial infant mortality (Jefferson *et al* 1991) and in the open marine habitat, the ability to protect infants is important in determining female reproductive success. The possible role of alloparental care has not been investigated in Risso's dolphins. However, as for sperm whales, Risso's dolphins pursue prey in deep water

(primarily squid). Best (1979) and Whitehead & Weilgart (2000) proposed two principal functions for sociality in female sperm whales: cooperative foraging, and communal care of calves. To forage, sperm whale mothers may need to dive to depths that could be beyond the capabilities of young calves. This foraging strategy may have led to the evolution of stable female groups, where the calf can be left without its mother among other adults. This could also be true at some level for other cetacean species that pursue prey in deep water. For female mammals that invest heavily in their offspring, ecological and social benefits accruing to philopatric females may be strong (Wrangham 1980). Therefore, it is not surprising that many gregarious mammals have groups with matrilineal social structure, and in fact among social carnivores, female natal philopatry appears to be fairly widespread (Greenwood 1980, Waser & Jones 1983).

Female striped dolphins as well as Risso's dolphins, were more related within than between groups, although the difference was far more noticeable for Risso's dolphins. Female striped dolphins may form kin groups for reasons other than alloparental care. Females that raise their offspring within groups of kin females may increase the probability of successfully raising their calves. Furthermore, females may also obtain other benefits from bonds with other females, such as cooperating against harassing males (Connor *et al* 1992, Richards 1996).

The evident difference between the two species was especially seen in the male pattern of kin association. In striped dolphins, males showed a similar pattern to females. They were more closely related to males within groups than between groups, although the pattern was weaker than that for females. On the contrary, male Risso's dolphins were negatively related to each other within groups (less related than expected by chance). This is consistent with solitary male dispersal and dispersion among groups, while striped dolphin males may continue associations with male kin (as suggested for bottlenose dolphins) (Lusseau *et al* 2003). As for other social mammals, male dolphins may disperse to avoid inbreeding and reduce competition among relatives, and sometimes join groups with kin males to maximise their inclusive fitness.

Individual associations of Risso's dolphins

Although most associations within the Ligurian Sea population of Risso's dolphins were weak, consistent relationships between individuals were found over periods of months and, in some cases, years.

Risso's dolphin individual associations seemed to be consistent with a fluid social structure system, with few strong individual bonds, typical of a fission-fusion society. Fission-fusion grouping patterns, in which individuals associate in small groups that change composition, often on a daily or hourly basis are also characteristics of all populations of *Tursiops* (Würsig & Würsig 1977, Wells *et al* 1987, Smolker *et al* 1992). The strongest and longest lasting associations were observed between individuals that were not classified as females, and were possibly males (never seen in close association with calves). In the bottlenose dolphins of Shark Bay and Sarasota it was found that the strong bonds occurred between males (Connor *et al* 2000). My molecular results on male relationships suggested that male Risso's dolphins may not associate on the basis of their kinship, though the sample size was small. Sperm whale association studies (Christal 1998) also showed no correlation between patterns of association and genetic relatedness with a unit, suggesting that closely related males do not affiliate preferentially.

Conclusions

In this study I compared two species that overlap in geographic range, but differ in aspects of their behaviour. The hierarchical genetic structure in these two species was similar in some respects (especially in the correlation between environmental and genetic structure, as seen for other dolphin species over this geographic range), but differed in ways that may relate to their differential use of local habitats.

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