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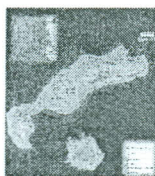
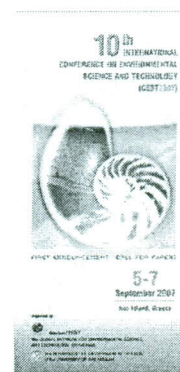
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**Genetic polymorphism and population structure of the striped
dolphin, *Stenella coeruleoalba*, and the common dolphin, *Delphinus
delphis*, within the ACCOBAMS area.**

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Abstract

The aim of the present work is to evaluate the genetic polymorphism of various populations of the common dolphin (*Delphinus delphis*) and the striped dolphin (*Stenella coeruleoalba*) in the Mediterranean Sea and north eastern Atlantic. The effort to estimate genetic diversity was achieved with the use of microsatellite DNA as a molecular genetic marker. A total of 156 were screened representing 3 Mediterranean populations (western Med, eastern Med, Korinthiakos Gulf) and one from the north east Atlantic Ocean. Five polymorphic cross-species microsatellites were tested (15-25 alleles). H_{exp} was higher than the H_{obs} in all populations at all loci. Unbiased estimates of Hardy-Weinberg exact P -values, using the Markov chain method, indicated an heterozygosity deficit ($P < 0.01$). The proportion of randomisations gave a larger F_{IS} than the observed ($P < 0.01$) and the values of F_{ST} were quite low (0.018 ± 0.014) within the Mediterranean Sea. Relatively quite high levels of gene flow values (mean $N_m = 2.45$) were estimated among populations, despite the significant heterogeneity in allele frequencies. These N_m values were sufficiently high to imply near-panmixia between populations, indicating the

possibility of a probable movement of migrants within the Mediterranean Sea and this is consistent with Nei's minimum genetic distance; D didn't show any clear spatial separation between western and eastern Mediterranean Sea. The most distant population was that from the north eastern Atlantic.

Introduction

The Agreement on the Conservation of Cetaceans of the Black Sea, Mediterranean Sea and Contiguous Atlantic Area (ACCOBAMS), which came into effect in 2001, has proposed that the status of common dolphins in the Mediterranean should be evaluated in a comprehensive manner, with the goals of estimating distribution and abundance throughout the basin, identifying critical habitats and characterizing threats. Such an evaluation would entail a series of localized surveys to locate any concentrations of animals that might remain, with a priority in the eastern Mediterranean (ACCOBAMS, 2002).

Studies of population biology are scarce in Mediterranean striped dolphins (*Stenella coeruleoalba*) mostly because of the lack of samples (Calzada & Aguilar, 1996, Notarbartolo di Sciara 2002). According to Bourret *et al.* (2007) a significant departure from Hardy-Weinberg equilibrium, suggest that the western Mediterranean population might possibly be further subdivided and significant genetic differentiation was detected between the Mediterranean and Pacific populations, and between the Mediterranean and Atlantic populations. Valsecchi *et al.* (2004) showed that, on average, all stranded animals showed elevated levels of inbreeding, suggesting that animals dying from disease may venture towards the shore more than those dying of old age. The striped dolphins have significantly increased their stranding rate and are restricted to warmer waters (MacLeod *et al.*, 2004). García-Martínez *et al.* (1995) did not reveal any population subdivision in the Mediterranean Sea by using mtDNA; according to Garcia-Martinez *et al.* (1999) no haplotype was shared between Mediterranean and Atlantic macroscale and all the analyses indicated the existence of two different populations with a very limited gene flow across the Strait of Gibraltar. Remarkably, previous studies on cetaceans have suggested that nuclear gene flow may occur whereas studies on mtDNA reveal a strong structuring among populations (Valsecchi *et al.* 1997; Bérubé *et al.* 1998).

Like most other cetaceans *Delphinus delphis* is not panmictic and occurs as a series of geographically separate populations (Heyning & Perrin, 1994; Perrin & Brownell, 1994; Jefferson & Van Waerebeek, 2002, Bearzi et al. 2003). The unresolved taxonomy and the wide distribution range of species of the genus *Delphinus* make populations difficult to identify and mating strategies challenging to study (Heyning & Perrin 1994; Rosel et al. 1994; LeDuc et al. 1999). More recently, a phylogeographic study of the genus *Delphinus* based on control region sequences and microsatellites revealed some differentiation between populations from different Oceans and different sides of the same ocean, but little or no differentiation among populations from the same side of an ocean basin (Natoli et al. 2006). Morphological diversity had led to more than 20 different species being described in the past, although they were all subsequently considered local variations of a single species *Delphinus delphis* (Hershkovitz, 1966). The present classification within this genus is still uncertain, although two different species are generally accepted: a long-beaked form (*Delphinus capensis*) and a short-beaked form (*Delphinus delphis*); low genetic differentiation has been observed among such short-beaked populations across a large geographical scale (Natoli et al. 2006).

The aim of this research was to apply genetic methods to a better understanding of the evolutionary processes and construct a population genetic model for the striped and common dolphin inhabiting within their geographical range in the Mediterranean Sea, within the ACCOBAMS protected area, and the eastern north Atlantic Ocean. The effort to estimate genetic diversity and the assessed level of genetic differentiation between the Mediterranean and eastern north Atlantic populations was achieved with the use of microsatellite DNA as a molecular genetic marker.

Materials and Methods

Samples from striped and common dolphins ($n=156$), were collected from the Mediterranean Sea (Eastern Mediterranean, Western Mediterranean and Korinthiakos Gulf) and from the North Eastern Atlantic Ocean. Seven samples from the Eastern Mediterranean and all from the North East Atlantic Ocean ($n=101$) were obtained from stranded animals while the rest of the samples were collected from free-ranging animals with the use of biopsy dart method. For the genetic analysis 5 highly

polymorphic microsatellites primers were used (Table 1). The DNA extraction method followed that of Sambrook's & Russel (2001), with some amendments. A total of 10 µl PCR volume reaction was used with the following cycles: 95°C for 3 min, followed by 30 cycles of 1 min at 95°C, 50 sec at T °C annealing of the primer set, and 30 sec at 72°C, with a final step of 15 min at 72°C. The PCR amplification protocol and the PCR products for all specimens were screened on an ABI PRISM 3700 DNA Sequencer. For the genetic analysis Genepop 3.4, FSTAT 2.9.3, POPULATIONS 1.2.28 and TreeView 1.6.6 genetic software were used.

Table1. T °C annealing and MgCl₂ concentration for the microsatellite primers

Genebank name	Code name	References	T-annealing (°C)	MgCl ₂ (%)
KWM2b	D1	Hoelzel et al. 1998b	52	1.5
KWM9b	D2	Hoelzel et al. 1998b	58	1.5
KWM12a	D3	Hoelzel et al. 1998b	58	1.5
D08	D4	Shinohara et al. 1997	58	3
TexVet7	D5	Rooney et al. 1999	49	1.5

Results

Five highly polymorphic cross-species microsatellites were tested (15-25 alleles). Values of F_{ST} , F_{IS} and heterozygosity values were similar for both species. H_{exp} was higher than the H_{obs} in all populations at all loci. Significant values were observed, by the Markov chain method, for deficiency of heterozygotes using the inbreeding index F_{IS} across all loci and all populations. Unbiased estimates of Hardy-Weinberg exact P -values, by the Markov chain method, showed a heterozygote deficit ($P < 0.01$). Mean genetic heterogeneity value (F_{ST}), under the sequential Jack-knifing method, was quite low (0.018 ± 0.014) within the Mediterranean samples but quite high between populations from the Mediterranean Sea and the eastern north Atlantic Ocean (0.164 ± 0.010). Gene flow values were quite high ($N_m = 2.45$) estimated when using private alleles (Barton & Slatkin, 1986). Fig. 1 represents a UPGMA genetic tree under Nei's minimum genetic distances.

Table 2. The mean values of F_{IS} , H_{exp} and H_{obs} and the mean number of alleles per locus.

Populations	F_{IS}	H_{exp}	H_{obs}	Mean number of alleles/locus per population
Western Med.	0.426	0.847 (± 0.04)	0.507 (± 0.06)	11
Eastern Med.	0.332	0.816 (± 0.06)	0.608 (± 0.16)	7.8
Korinthiakos Gulf	0.255	0.834 (± 0.05)	0.640 (± 0.23)	10.8
North eastern Atlantic	0.567	0.825 (± 0.05)	0.623 (± 0.05)	8.5

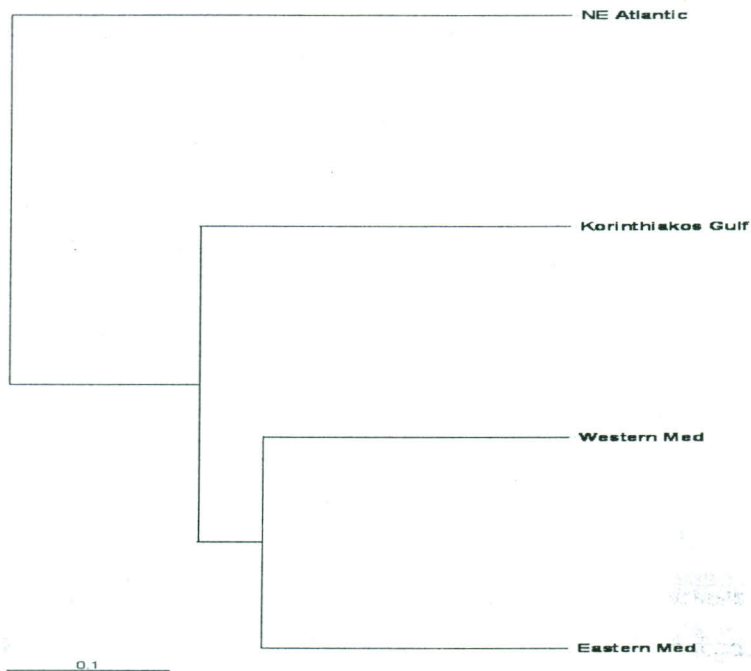


Figure 1. The Nei's minimum genetic distance UPGMA genetic tree.

Discussion

Little is known about the striped and common dolphin's (*Stenella coeruleoalba* and *Delphinus delphis*) social and genetic structure in the Mediterranean Sea; only a few studies on population genetic structure of the species in question are available in the bibliography. Contrasting aspects of their known ecology and life history suggest that a comparative approach would facilitate a better understanding of dolphins' social behaviour in general.

These observed low values of heterozygosity could be a result of genetic divergence or small sample sizes. The F_{ST} values indicate evidence of genetic differentiation among the two large geographic areas (Mediterranean Sea, Atlantic

Ocean). The N_m values were sufficiently high to imply near-panmixia between the Mediterranean populations, indicating the possibility of a probable movement of migrants in the area. Nei's minimum genetic distance, using the UPGMA method did not reveal any clear-cut separation within and among Mediterranean populations but significant differences were detected between the Mediterranean and Atlantic ones. A hierarchical pattern of structure was clearly influenced by social group structure and their dispersal behaviour, though any differences revealed between the populations, were probably related to differences in social structure and/or habitat use.

Further data on population distribution coming from ongoing visual and acoustic surveys, may provide the scientific background for a better understanding of the species' population dynamics and contribute to the design of a conservation strategy. In the determination of population structure in wild species, the genetic information about population subdivision has practical applications to establish short- and long-term strategies for animal management, conservation and protection. (Lande, 1991; Dizon *et al.*, 1992; Avise, 1994; Moritz, 1994; Hoelzel, 1998).

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