

## Levels of connectivity between longnose skate (*Dipturus oxyrinchus*) in the Mediterranean Sea and the north-eastern Atlantic Ocean

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**Abstract** Sequencing of a partial region of the mitochondrial control region has revealed no shared haplotypes between longnose skate (*Dipturus oxyrinchus* L.) sampled in the north-eastern Atlantic (Norway and Rockall) and those sampled in the Mediterranean (Mallorca). Bayesian estimation of the migration rate suggests little, if any, gene flow occurs between the regions and that the populations separated 20,000 years ago. These conclusions provide a genetic basis for long-standing observations, based on egg capsule and adult size, that longnose skate in the Mediterranean may be genetically isolated from other stocks. This result has important conservation implications for the threatened longnose skate.

**Keywords** Elasmobranch · Marine fishes · Population · Management · Genetic differences · Conservation

### Introduction

There is mounting concern regarding the conservation status of large oceanic predators, including large species of skates (Dulvy et al. 2000). Their life histories that incorporate large size, slow growth rate, late maturity and low fecundity, renders them particularly vulnerable to overfishing (Dulvy and Reynolds 2002). Historically, the longnose skate (*Dipturus oxyrinchus* L.) had wide distribution occurring on benthic habitats of the north-east Atlantic continental shelf and slope from Norway, southwards to Senegal in Africa, and the Mediterranean. It is a demersal fish occurring at depths between 90 and 950 m, although mostly deeper than 200 m, and growing to a total length of 1.5 m (Wheeler 1978). Whilst many aspects of its biology remain unknown, it appears to have become locally extinct in the Irish Sea and is now virtually absent from the North Sea (Dulvy and Reynolds 2002).

Until recently, fishery catches of many skate species, including longnose skates, have usually been recorded under a single, aggregated landing name. The lack of species-specific data means that a decline in one species can effectively be masked by stable trends in others (Dulvy et al. 2000). A recent morphological study of skate landed at French ports found misidentification was frequent and of 4110 skate landed under the marketing names of *Dipturus batis* and *D. oxyrinchus* only a single individual was confirmed as *D. oxyrinchus* (Iglésias et al. 2010). The near disappearance of longnose skates in landings led the latter

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authors to suggest the immediate upgrading of the species from ‘Near Threatened’ to ‘Critically Endangered’ on the IUCN Red List of threatened species ([www.icunredlist.org](http://www.icunredlist.org)).

Given the absence of pelagic eggs or larvae, elasmobranch dispersal potential is dependent on sub-adult and adult movement. The failure of longnose skate to re-colonise areas from which they have become extirpated, combined with tagging studies of the closely related common skate (*Dipturus batis*) showing remarkable site fidelity (Wearmouth and Sims 2009), suggests that there may be limited connectivity between the remaining populations of longnose skates. Indeed, Clark (1922) suggested that the Mediterranean and north-eastern longnose skates may be distinct stocks, based on differences in the size of adults and of egg capsules. The analysis of molecular markers in other species of coastal sharks and skates (Chevolot et al. 2006; Ovenden et al. 2009) has revealed significant genetic structure within oceans, and occasionally along contiguous coastline. This has often been associated with sedentary behaviour, disjunct distributions and/or reproductive philopatry (Palumbi 1994). Information gathered from molecular markers can therefore be useful in determining levels of gene flow between the populations and provide valuable data on stock structure that can help form the basis for better informed management and conservation plans. Accordingly, the aim of this study was to investigate using molecular markers whether populations of longnose skate sampled from regions of the Mediterranean and north-eastern Atlantic are genetically segregated and estimate the timescales of their divergence.

## Materials and methods

Two individuals of longnose skates were caught on Rockall Bank and 12 along the coast of Norway. The 14 specimens from the Mediterranean all originated from the waters surrounding Mallorca and Menorca (Table 1). Between June 2007 and July 2009, samples were obtained opportunistically during research cruises, by catch assessments, and via quantitative surveys. Tissue was immediately preserved in absolute ethanol prior to storage at  $-20^{\circ}\text{C}$ . Extraction of genomic DNA was undertaken using the Promega (Madison, Wisconsin, USA) Wizard extraction kit.

An approximately 1,000 base pair section of the mitochondrial DNA control region was amplified using primers RayDloopFor (5'-CATTAATCGACTRTCAACTATTTCA TT-3') and Ray12s (5'-TACTGAGGCTAGGACCAAAC-3'), following Griffiths et al. (2010). PCR products were cleaned and sequenced by Macrogen, Seoul, Korea using the forward primer RayDloopFor. The resulting sequences were checked in BioEdit 7.0.9 (Hall 1999), and aligned using Clustal W (Thompson et al. 1994).

The control region was chosen for phylogeographic analysis due to the high levels of nucleotide polymorphism it has demonstrated in skate species (Valsecchi et al. 2005). To reconstruct the genealogical relationships amongst haplotypes, a maximum parsimony haplotype network was constructed using TCS 1.2.1 (Clement et al. 2000), with the connection limits set at 95%. Control region diversity indices (haplotype and nucleotide diversity) were calculated in DnaSP v5 (Librado and Rozas 2009). Arlequin 3.0 (Excoffier et al. 2005) was used to estimate levels of genetic divergence between skates in the Atlantic and Mediterranean. Pairwise estimation of  $F_{ST}$  was completed using haplotype frequencies and  $\Phi_{ST}$  was estimated using percentage sequence divergence, based on the Tamura-Nei model (Tamura and Nei 1993), as selected by ModelTest (Posada and Crandall 1998). The significance of these measures was determined at the 95% level (16,000 permutations).

Mdiv (employing a Bayesian Markov chain Monte Carlo method, Nielsen and Wakeley 2001), was used for the joint estimation of migration rate and isolation time between the samples originating from the Mediterranean and north-east Atlantic, by estimating the following parameters  $\theta = 4N_e\mu$ ,  $M = 2N_em$  and  $T = t/2N_e$  ( $N_e$  = effective population size,  $\mu$  = mutation rate in the region sequenced,  $m$  = migration rate and  $t$  = time since divergence), and their 95% credibility intervals. The mutation rate was calculated by applying a 0.65% divergence between lineages per million years [estimated from control region sequences in Griffiths et al. (2010)] using a divergence time of 31 Mya years between Rajini and Amblyrajini (Valsecchi et al. 2005), and a generation time of 20 years. This is based on longnose skates attaining sexual maturity at a relatively large size of 1.2 m (Wheeler 1978) and estimates of generation times between 8 and 14 years that have been made in four smaller species of north-western Atlantic skate (McPhie and Campana 2009). The HKY model of sequence evolution was selected and 5,000,000 cycles were employed in the Markov chain, incorporating a 10% burn-in. Multiple chains were run to check the consistency of estimation, and after excluding very large values, migration and population divergence times were estimated for values from 0.02 to 10, in increments of 0.02.

## Results

Analysis of partial control region sequences produced a 733 base pair alignment with five polymorphic sites, of which three were parsimony informative. Global nucleotide diversity was 0.00223 (which falls within the range of  $\pi = 0.000$ – $0.010$  described for a shorter sequence of the

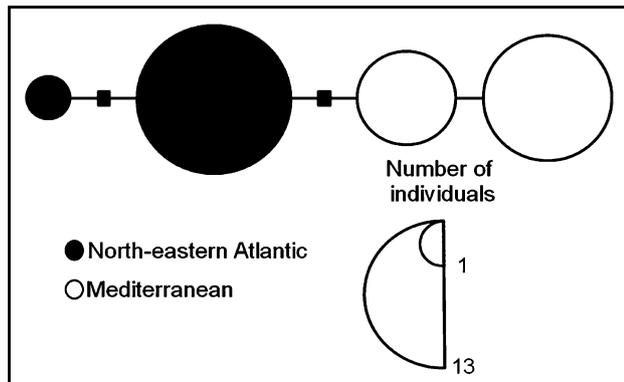
**Table 1** Longnose skate (*Dipturus oxyrinchus*) capture locations, sample details and control region sequence accession numbers

Sample	Location	Length (mm)	Disc width (mm)	Weight (g)	Sex	Date	Latitude	Longitude	Haplotype	GenBank accession no.
ARV-001	Norway	939	–	–	Male	28/06/07	63.73	9.95	1	GU595172
ARV-016	Norway	900	580	2780	Female	17/05/09	64.34	11.24	1	GU595172
ARV-023	Norway	1040	720	6050	Female	23/06/08	63.62	9.77	1	GU595172
ARV-027	Norway	–	–	6200	Female	28/07/08	62.57	7.69	1	GU595172
190609_I	Norway	–	–	–	–	19/06/09	63.62	9.75	1	GU595172
190609_II	Norway	–	–	–	–	19/06/09	63.62	9.75	1	GU595172
ARV-025	Norway	1310	880	12000	Female	04/07/08	63.62	9.75	1	GU595172
ARV-047	Norway	1150	760	7300	Male	18/07/09	63.62	9.75	1	GU595172
ARV-048	Norway	1220	790	10000	Female	18/07/09	63.62	9.75	1	GU595172
ARV-038	Norway	1100	–	5600	Female	15/12/08	64.33	11.23	1	GU595172
ZMUB19782	Norway	1311	892	–	Female	30/05/08	67.67	14.25	1	GU595172
ZMUB19783	Norway	1135	774	–	Male	30/05/08	67.67	14.25	1	GU595172
R-8	Rockall	1330	–	–	Male	21/04/08	56.40	–15.87	1	GU595172
R-10	Rockall	700	–	–	Female	21/04/08	56.40	–15.87	2	GU595173
33.17	Mallorca	811	520	2104	Male	13/05/09	~39.81	~3.57	3	GU595174
33.18	Mallorca	229	151	32	Female	13/05/09	~39.81	~3.57	4	GU595175
33.19	Mallorca	310	201	88	Male	13/05/09	~39.81	~3.57	3	GU595174
33.20	Mallorca	375	245	157	Female	13/05/09	~39.81	~3.57	3	GU595174
33.21	Mallorca	399	255	196	Male	13/05/09	~39.81	~3.57	3	GU595174
33.23	Mallorca	315	204	80	Male	16/05/09	~39.81	~3.57	4	GU595175
33.24	Mallorca	319	206	86	Female	16/05/09	~39.81	~3.57	4	GU595175
33.25	Mallorca	554	361	628	Female	16/05/09	~39.81	~3.57	4	GU595175
33.26	Mallorca	315	491	1556	Male	16/05/09	~39.81	~3.57	3	GU595174
33.27	Mallorca	1040	689	5044	Male	16/05/09	~39.81	~3.57	3	GU595174
33.28	Mallorca	673	440	1068	Female	16/05/09	~39.81	~3.57	4	GU595175
33.29	Mallorca	165	115	94	Male	16/05/09	~39.81	~3.57	3	GU595174
33.30	Mallorca	1170	756	6410	Female	16/05/09	~39.81	~3.57	3	GU595174
33.32	Mallorca	765	516	1877	Female	16/05/09	~39.81	~3.57	3	GU595174

**Table 2** Control region diversity indices

Parameter	North-east Atlantic	Mediterranean	Pooled
N	14	14	28
S	2	2	4
V	2	1	5
h	0.143 (0.119)	0.495 (0.088)	0.672 (0.052)
$\pi$	0.00039 (0.00032)	0.00670 (0.00012)	0.00223 (0.00021)

N number of individuals, S number of haplotypes (excluding insertions/deletions), V number of variable sites, h haplotype diversity,  $\pi$  nucleotide diversity (the standard error for each diversity measure is given within the brackets)



**Fig. 1** Network of relationships among mitochondrial control region haplotypes. Small black boxes represent steps (or unsampled sequences) required to join the haplotypes in the network

CR analysed in Mediterranean skate species, Valsecchi et al. 2005), and global haplotype diversity was 0.672. These diversity indices were also lower in skate collected from the north-east Atlantic when compared to the Mediterranean (Table 2). Skate sampled from the north-eastern Atlantic and Mediterranean shared no common haplotypes (Fig. 1). Pairwise  $F_{ST} = 0.681$  and  $\Phi_{ST} = 0.860$ , were both high, and highly significant ( $P < 0.001$ ).

The Bayesian estimation of migration rate and isolation time between the samples originating from the Mediterranean (Mallorca) and north-east Atlantic (Rockall and Norway) with Mdiv revealed that levels of migration were not statistically distinguishable from zero ( $M = 0.02$ ,  $CI = 0.00–0.04$ ). However, divergence time was estimated at 19,980 years ago ( $T = 3.92$ ,  $CI = 3.72–4.14$ , 18,960–21,100 years). Varying the generation time to 25 or 15 years, altered the estimation of divergence time between 15,984 and 26,640 years. Using a range of estimates of sequence divergence calculated for other elasmobranch species to alter the mutation rate [1.62% divergence between lineages per million years in white sharks, C. Gubili personal communication; 0.4% in blacktip sharks, Keeney and Heist (2006)], produced divergence times of 8,016 and 32,466 years.

## Discussion

The analysis of mitochondrial DNA sequences from longnose skates originating from the north-east Atlantic (Norway and Rockall) and Mediterranean (Mallorca), has revealed evidence of significant population structure in this threatened species. This is consistent with observations of differences in egg case and adult size between the north-east Atlantic and the Mediterranean populations (Clark 1922). Whilst the level of sequence divergence between these groups is not of the order demonstrated by different species (Griffiths et al. 2010), or the recently proposed standard for designating operational species (Hart and Sunday 2007), it provides a genetic basis for treating these groups as distinct management units. It is also consistent with results showing significant genetic differences between the Mediterranean and north-east Atlantic population types of many marine species (e.g. Chow and Takeyama 2000), although this conclusion is by no means universal (Patarnello et al. 2007). Further investigation into the morphology and ecology of these population types may also lead to their recognition as evolutionary significant units, provided reproductive isolation has led to the development of adaptive differences (Palsbøll et al. 2007).

Simultaneous estimation of migration rate and divergence time of the population demonstrated that there is little, if any, gene flow between longnose skate sampled in the north-east Atlantic and Balearic Sea. The estimated split between these populations, approximately 20,000 years ago, broadly corresponds to the last glacial maximum (Yokoyama et al. 2000). During this period, populations of many species were separated in glacial refugia, which eventually resulted in strong genetic heterogeneity between the isolated groups (Hewitt 1996). A similar pattern of isolation may have caused the divergence in longnose skates. Alternatively, the wide confidence intervals connected with the divergence time means that a more recent split is also possible, associated with the colonisation of very remote northern areas once the ice sheets retreated. This pattern of divergence between northern and southern populations within Europe bears some

resemblance to the phylogeographic patterns demonstrated by thornback rays (*Raja clavata*) within Europe (Chevolot et al. 2006). That the two populations of longnose skate shared no haplotypes is suggestive of a high degree of isolation between them, although it is also possible that overfishing may have acted to remove any common haplotypes they once shared.

This study represents an initial step in determining the scales of connectivity between longnose skate populations. A number of other marine organisms have demonstrated population structuring at much narrower scales than that examined here, both within the Mediterranean Sea (Natoli et al. 2008), or north-eastern Atlantic (Reiss et al. 2009). Increasing evidence for philopatry in elasmobranchs (Hueter et al. 2005), combined with the lack of pelagic larval dispersal, would argue that finer scale structure between longnose skate populations could occur, although opposing conclusions have been drawn in other European skate species (Chevolot et al. 2006; 2007). The challenge now is to determine patterns of connectivity across the whole range of the species. Particular focus is now required on additional nuclear markers that would allow investigation of ontogenetic or sex-linked differences in habitat use and philopatry, which could have a bearing on the effective management of this declining species. However, the data presented in this study clearly demonstrate that the longnose skate does not comprise of a single homogeneous unit for management. In particular, the results suggest that if further local extirpations occur then re-colonisation from neighbouring areas may not be straightforward (Dulvy and Reynolds 2002). Low fecundity, low dispersal potential and significant population structure may be characteristic of this species, and could therefore present an important barrier to the recovery of longnose skate populations. These factors, combined with the scarcity of longnose skate during sample collection undertaken for the study, strengthen previous calls for the conservation status of the longnose skate to be upgraded, according to the IUCN Red List criteria (Iglésias et al. 2009).

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