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Conservation Genetics of Basking Sharks

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Department for Environment Food and Rural Affairs

Final Project Report

for
the Department for Environment, Food and Rural Affairs
Tender CR0288

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Conservation Genetics of basking sharks

Final report for Defra
Tender CR 0288

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EXECUTIVE SUMMARY

Introduction

Populations of many large marine vertebrates are threatened by high levels of fisheries exploitation (both targeted and as bycatch). This applies particularly to sharks, skates and rays (elasmobranch fishes) that have life-history traits that make them especially vulnerable to levels of harvest mortality that are little above that of natural mortality. In particular, many elasmobranchs have a late age at maturity and low fecundity leading to low rates of reproduction. This results in little scope for the compensatory mechanisms that enable many "bony" fish species like cod or mackerel to withstand unnaturally high levels of mortality. As a consequence, elasmobranch fisheries not only exhibit rapid declines in catch rates as exploitation increases, but there is a greater potential for the population to collapse.

The basking shark is the world's second largest fish and is widely distributed in coastal waters on the continental shelves of temperate zones in both northern and southern hemispheres. Individuals take 12-20 years to reach maturity, females have long gestation intervals (1-3 years) and give birth to a few, large young. Concern over the strong possibility that populations are depleted as a result of exploitation by fisheries, and the lack of scientific knowledge of the species, has led to the basking shark being listed as **Vulnerable** worldwide and **Endangered** in the north-east Atlantic, in the 2004 IUCN Red List (IUCN, 2004). In 2002, the species was listed on Appendix II of the Convention on International Trade in Endangered Species (CITES) and, in November 2005, on Appendix I and II of the Bonn Convention on Migratory Species (CMS). Since 1998, basking sharks have also been protected in British territorial waters under Schedule 5 of the Wildlife and Countryside Act (1981).

The current approach to conservation of basking sharks relies heavily on the precautionary principle, which states that insufficient scientific knowledge about biology and stock status is no defence for a lack of action. In particular, it is not known whether there are discrete local populations of basking sharks, or whether there is a relationship between regional population abundance and global trends. There is also a need to distinguish between the effects on population status of climate change on the sharks' environments and the legacy of the impact of fisheries, and to ascertain whether stocks are depleted or recovering.

The latter concern is recognised in the UK basking shark Biodiversity Action Plan, which states that improved long-term (many decades) monitoring of the UK population is necessary to enable population trends to be identified. Though some monitoring data for this species are available, most are based on sightings of sharks feeding on plankton near the sea surface during spring and summer and no analyses done to date has provided robust information on population trends.

Aims of the study

This study set out to use modern molecular genetic methods to analyse the population structure and dynamics of basking sharks in NE Atlantic waters and those further afield. The intention was to investigate whether there are discrete populations and the degree to which mixing or inter-change occurs between populations. These results should contribute towards a scientific basis for assessment of the status of basking shark stocks and the provision of advice for decisions on conservation measures to ensure sustainability of basking shark populations. To facilitate enforcement of CITES regulations, a further aim of this study was to use the molecular genetic tools developed to provide an accurate and unambiguous method for the identification of basking sharks parts in unlabelled and processed products.

Results and conclusions

Field surveys and tissue samples

A limited number of basking shark tissue samples from SW England and NW Scotland had been collected prior to the start of the project and these were invaluable assets for developing the requisite molecular tools. However, samples collected at different times, even from the same area, might represent different populations differing substantially in their genetic diversity. Therefore, a full assessment of the population genetic structure of UK basking sharks requires representative population samples. Ideally these should consist of biopsy samples recovered from 10-20+ individuals, all at approximately the same time (or at least in the same season) and from the same area. Such population samples form a baseline against which the similarity of further samples can be compared for exclusion or inclusion to those populations.

To this end, designated ship-borne surveys were undertaken off SW England in the summers of 2004 and 2005. However, despite considerable effort (163.5 h in 2004 and 130 h in 2005), the unusually low occurrence of basking sharks (only 11 sharks observed over both years) at the sea surface off the southwest of the UK has prevented us from obtaining the necessary collection of tissue samples to obtain reliable estimates of population genetic structure. While there were many sightings of basking sharks in Scottish waters in the summers of 2004 and 2005, it was not possible to redeploy the ship-borne surveys further north at short notice because of operational constraints.

Plankton samples taken during our surveys (currently being analysed) indicate low abundance of surface zooplankton in the survey area, and it is possible that this may account for the unexpectedly low numbers of basking sharks at the surface off Plymouth during the study.

Although surveys have not provided the required population samples to conduct a detailed assessment of the population genetic structure of UK basking sharks as planned, a substantial library of 41 basking shark tissue samples from the UK (the south-west, Wales and Scotland), Italy, Portugal, South Africa, USA, Canada, Australia and New Zealand has been assembled. These have been used to develop the molecular genetic tools necessary to conduct such a study in the future.

Molecular Genetics

Development and characterization of the appropriate molecular genetic tools has been successful, providing resources that may be used to obtain reliable estimates of population genetic structure and address the proposed issues of basking shark population differentiation and ecology. We have successfully isolated many microsatellite loci specific to basking sharks using an in-house modified enrichment protocol and characterized 10 polymorphic loci; in addition to 8 polymorphic loci from non-focal species. In total, 18 microsatellite loci are now available for analysis of basking shark samples, which should provide sufficient loci to investigate population structure, relatedness of basking shark coalitions and paternity issues. Once sufficient *population* samples of basking sharks are available we are confident that the three-pronged approach for the analysis of molecular variation (SSR genotyping, and SSR flanking region and mtDNA marker sequencing) will enable high-resolution inference of intra-specific evolutionary history. These approaches will provide

complementary data that can be rigorously analyzed using modern, powerful statistical approaches.

Utility of microsatellites was proven using available samples allowing estimation of the broader levels of population structure, suggesting little gene flow between basking shark populations of the northern and southern hemispheres.

Analyses of two complete mtDNA gene regions (cytochrome b and the D-loop) have shown sufficient variation is available for population differentiation by their combined use. A simplification of this approach promises an inexpensive, rapid and simple assay of mitochondrial variation to differentiate basking shark populations and to quantify female-mediated gene flow.

We have developed a protocol to allow recovery of DNA from forensic quantities of shark tissue. This will have a significant impact on future molecular work in this and other shark species for which samples are scarce, allowing utilization of badly degraded and minute tissue samples.

Enforcement and Management

A major obstacle to obtaining data on shark catch and trade, and the implementation of conservation and management strategies on a species-specific basis, is the problem of accurately identifying exploited species. This is aggravated by the widespread practice in commercial fisheries of removing the head, tail and fin of landed sharks while at sea to minimize storage space. DNA-based methods for species identification can circumvent these problems, allowing accurate and unambiguous identification of body parts to the specific or even population level

To facilitate enforcement of CITES regulations we have successfully designed a panel of primers for two gene regions (one mitochondrial and the other nuclear) that allow accurate and unambiguous identification of basking sharks parts in processed products at extremely low concentrations (<1% and <1ng). By using more gene regions than previous approaches, this test represents a significant improvement, avoiding false negatives and providing built-in redundancy and so makes available more acceptable and robust evidence in litigation proceedings than the current existing tests.

Recommendations and further work

The unusually low occurrence of basking sharks at the sea surface off the southwest of the UK has prevented us from obtaining the necessary collection of tissue samples to obtain reliable estimates of population genetic structure and address the important issues of basking shark population differentiation and ecology in UK waters. Nonetheless, the project has provided a powerful suit of molecular genetic tools that are now available to conduct such an analysis rapidly and inexpensively and further support should be sought to conduct such a study. This will enable us to be confident that we know what part of the north-east Atlantic population the basking sharks present around Britain represent.

Conservation genetics of basking sharks

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1. Policy and scientific background

Elasmobranchs have a number of life-history traits that make them particularly vulnerable to commercial exploitation. In particular, a late age at maturity and low fecundity lead to low rates of reproduction and, therefore, potential rates of population growth are slow compared to marine teleosts (Pratt & Casey, 1990). They also have relatively little scope for the compensatory mechanisms that enable many teleosts to withstand harvest mortality well above the level of natural mortality. As a consequence, elasmobranch fisheries tend to exhibit rapid declines in catch rates at high exploitation levels, and there is a greater potential for the fishery to collapse (Holden, 1973, 1977; Casey & Myers, 1998; Dulvy *et al.*, 2000). An example is the Achill Island basking shark fishery off the west coast of Ireland, which appeared to collapse in the early 1960s after only 10 years of peak catches (Kunzlik, 1988; Sims & Reid, 2002).

The plankton-eating basking shark (*Cetorhinus maximus*) is the world's second largest fish species, attaining lengths up to 12 m. They are thought to take 12-20 years to reach maturity, have long gestation intervals (1-3 years) and produce a few, large offspring (the only recorded litter is of six 1.5-m long pups) (Kunzlik, 1988), so may be particularly prone to over-exploitation. In addition to these 'slow' life-history traits, recent satellite tagging of basking sharks in UK waters has demonstrated that, despite making long-distance movements, individuals remained in the vicinity of the continental shelf around the UK, Ireland and northern France (Sims *et al.*, 2003). Moreover, some individuals returned to specific areas visited months earlier. This indicates that populations may be relatively localised. Such philopatric behaviour, even over large scales, raises the probability that population replacement may depend more on recruitment of juveniles than immigration of adults from other parts of the population.

This inherent vulnerability, together with concern over depleted populations as a result of fisheries exploitation and the lack of scientific knowledge of the species, has led to northeast Atlantic basking sharks being listed as **Endangered** in the *2000 IUCN Red List of Threatened Species,* placed on Appendix II of CITES and is listed on Appendix I and II of the Bonn Convention on Migratory Species (CMS). Furthermore, the species is protected in British waters up to 12 miles from shore under Schedule 5 of the Wildlife and Countryside Act 1981, and in Isle of Man and Guernsey waters (UK Crown Dependencies). However, there is some doubt as to whether this protection is adequate given that basking sharks off the UK spend much of their time outside this protected zone (Sims *et al.*, 2003; 2006).

The basking shark is also a priority species under the UK Biodiversity Action Plan, with a published species action plan and identified lead partners. This plan recognises that improved long-term (many decades) monitoring of the UK population is necessary to enable population trends to be identified. Fishing for basking sharks in European waters can be regulated by the EC under the Common Fisheries Policy (CFP), and there is a zero total allowable catch in Community waters. Norway has reportedly ceased to fish for basking sharks in Norwegian waters, presumably because of poor returns.

Given the gaps in scientific knowledge about basking shark biology, the current conservation measures rely heavily on the precautionary principle. Recent satellite tagging data suggest the strong possibility that population mixing between different geographical regions may be low (Sims et al. 2003; Southall et al. 2005), but this aspect of their population biology is difficult and costly to address with tagging studies alone and, prior to the current project, no genetic studies of stock structure

had been undertaken to determine whether populations of basking sharks are discrete or not.

Clearly, there is a need to obtain more reliable data on the discreteness of populations (if any) for this species, including the rate of gene flow between the NE Atlantic region and elsewhere, in addition to information on population structure (sex ratios, relative numbers of juveniles and adults, etc.) and reproductive biology.

1.1 Scope of the Proposed Study

In accordance with the Defra requirements, the study aimed to determine, by genetic analyses, the population structure and dynamics of basking sharks in NE Atlantic waters and those further afield. Particular attention was given to investigating whether there are discrete populations, and the degree to which mixing or inter-change occurs between populations. To achieve this, modern molecular genetic techniques have been developed and adapted as appropriate to determine population structure. The results will contribute towards a scientific basis for the provision of advice for management for the recovery and sustainability of basking shark populations.

The work will also help contribute to the implementation of the UK Biodiversity Action Plan, the European Community Wildlife Trade Regulations, the Convention on International Trade in Endangered Species (CITES), the EC shark action plan and, therefore, to the implementation of the Food and Agriculture Organisation (FAO) International Plan of Action (IPOA) for Sharks.

1.2 Objectives

1. To collect tissue samples for genetic analyses (from biopsies, strandings, bycatch, archived samples) at a significant number of geographically dispersed locations within UK waters, the NE Atlantic and further afield (e.g. US), to provide sufficient samples to enable statistically robust interpretation.

2. To determine whether there are discrete populations/stocks of basking sharks, the likely boundaries between any such populations, and to what degree mixing occurs.

3. To characterise the degree and frequency of gene flow (through immigration and dispersal) within the whole population or the extent to which populations are isolated or fragmented.

4. To determine, as far as possible, whether sampled populations show any characteristics of loss of genetic diversity or related inbreeding depression that may result from small effective population size or population fragmentation.

5. To determine whether there are any sex- or age-related differences in dispersal/gene flow between populations.

6. To consider the processes, where possible, that may have resulted in the observed genetic characteristics of populations and to interpret these in light of satellite tagging results and in the light of oceanographic processes.

7. To assess the application of the genetic markers developed to the identification of the origin of parts and derivatives of basking sharks in trade.

8. To use sampling trips and liaison with fishermen, stranding co-ordinators and others to gain maximum information (e.g. morphometrics, fin photo-identification,

sex ratios/ distribution) from sharks sampled, in order to gain a better understanding of basking shark population dynamics.

2. GENERAL METHODS AND RESULTS

2.1 Tissue sampling

2.1.1 Biopsy

A limited collection of basking shark tissue samples from SW England and NW Scotland had been collected prior to the start of the project and these were valuable for developing the molecular tools required by the project. However, samples collected at different times, even from the same area, might represent different populations differing substantially in their genetic diversity and similarity. Therefore a full assessment of the population genetic structure of UK basking sharks required representative population samples. Ideally these should consist of biopsy samples recovered from 10-20+ individuals, all at approximately the same time (or at least in the same season) and in the same area. Such population samples form a baseline against which the similarity of further samples can be compared for exclusion from, or inclusion to, those populations. The original plan, as outlined in the contract tender document, was to take tissue samples by biopsy from free-ranging basking sharks using specially developed, benign techniques during annual basking shark surveys conducted by the MBA during the summers of 2004 and 2005. To sample tissue from sharks a new tagging harpoon was specifically designed for the project that secures a tissue sample as the harpoon passes into the fin and is retrieved as the shark swims away.

Designated surveys were undertaken off SW England to locate sharks for biopsy in the summers of 2004 and 2005. In 2004, ship-borne surveys to locate basking sharks in the Plymouth study area of the western English Channel (Fig. 1) were undertaken on 21 days between 14th May and 5th August, with a total search time of 162.3 hours covering a total distance of 2,004.9 km.

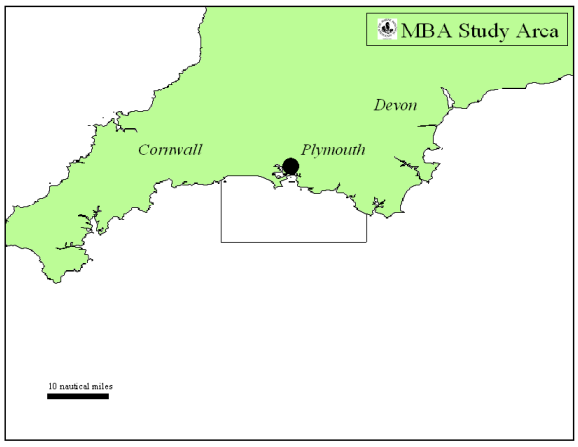


Fig. 1. The basking shark survey area in the western English Channel off Plymouth.

A total of 8 basking sharks were sighted at the surface, amounting to an overall sightings per unit effort (SPUE) for the period of 0.049 sharks h^{-1}. This SPUE for 2004 was much lower than in previous years indicating fewer sharks than normal spent time at the surface.

Because so few basking sharks were sighted during the survey period, and because of the imperative to deploy electronic tags as part of GWD contract CR0247 on the few sharks that were sighted, we were not able to take any tissue samples by biopsy

from free-swimming sharks in 2004. Had more sharks been encountered samples would have been taken. However, three tissue samples were obtained from basking sharks stranded dead on beaches in Cornwall. A full autopsy and set of samples were undertaken on a 5-m female shark that came ashore at Gerrans Bay, Cornwall on 28 May 2004. Two further sets of samples were obtained from two other 5-m female sharks examined by volunteers from the Cornwall Wildlife Trust. A fourth sample was obtained from a large 7-m long shark caught in pot ropes off the Isle of Skye in western Scotland.

In 2005 ship-borne surveys to locate basking sharks in the Plymouth study area (as above) were conducted between 9 May and 12 July 2005. A total of 17 days (130 h) were spent searching the study area where, in the past, up to 50 individual sharks have been observed each summer season. However, despite covering a total distance of 1,549 km, no basking sharks were encountered.

In response to confirmed sightings further west off north Cornwall, we spent one day searching an area between St. Agnes and St. Ives (14 July 2005). Four basking sharks were encountered during the 6-hour search but they spent very little time (10 – 15 s) at the surface, which meant approaches for biopsy samples were difficult. As a result, no samples were obtained from any of these individuals.

Because so few basking sharks were sighted during the survey period, we were again unable to take any tissue samples by biopsy from free-swimming sharks in 2005. However, a further two stranded basking sharks were reported to the Cornwall Wildlife Trust during July and samples were obtained from both individuals and sent to the molecular genetics team at Aberdeen.

The unexpectedly low occurrence of basking sharks in the Plymouth study area during the 2004 and 2005 field seasons is confirmed by data gathered by the Marine Conservation Society as part of their Basking Shark Watch programme (Table 1).

Table 1. Regional percentages of British sightings of basking sharks as recorded by the Marine Conservation Society between 1998 and 2005 (figures in brackets are the number of reports). (www.mcsuk.org, 2006)

UK REGION	**1998**	**1999**	**2000**	**2001**	**2002**	**2003**	**2004**	**2005***
Southwest	85.2 (454)	93.8 (421)	81.5 (225)	77.8 (442)	55.6 (269)	28.6 (163)	23.7 (211)	13 (90)
Scotland	9.2 (49)	0.7 (3)	9.8 (23)	16 (91)	37.4 (181)	61.2 (349)	57.8 (514)	77.7 (539)
Northeast	0	0	0	0	0.8 (4)	0.2 (1)	0.1 (1)	0.1 (1)
Northwest	0	0	0	3 (17)	0	1.1 (6)	0	0
Channel Islands	0.6 (3)	0.2 (1)	0.7 (2)	0.2 (1)	0	5.4 (31)	0.3 (3)	0.4 (3)
Isle of Man	4.9 (26)	4.5 (20)	6.2 (17)	0.2 (1)	1.7 (8)	0	11 (98)	6.3 (44)
Wales	0	0	0	0	0.8 (4)	1.4 (8)	0.8 (7)	0.1 (1)
N Ireland	0	0	0.7 (2)	0.2 (1)	2.1 (10)	0.5 (3)	0.9 (8)	0.9 (6)
Ireland	0.2 (1)	0.9 (4)	1.1 (3)	2.6 (15)	1.7 (8)	1.6 (9)	5.4 (48)	1.4 (10)
Total	100 (532)	100 (445)	100 (273)	100 (553)	100 (476)	100 (561)	100 (842)	100 (684)

* The data for 2005 are incomplete at the time of compiling this report.

While it is not clear why so few basking sharks were observed off Plymouth in the summers of 2004 and 2005, it might be that that basking shark occurrence was strongly influenced by the location of centres of zooplankton abundance. Plankton samples taken during our surveys (currently being analysed) indicate low abundance of surface zooplankton in our survey area. However, a fuller understanding of the relation between shark and plankton distributions will have to await the publication by SAHFOS (Sir Alistair Hardy Foundation for Ocean Science) of the 2005 Continuous Plankton Recorder (CPR) data.

2.1.1.i Northward movement of basking sharks?

In the summer of 2005, it was reported in the press (e.g. BBC News, 17 June) that unusually high numbers of basking sharks had been observed off north-west Scotland with relatively few sightings made off south-west England. It has been suggested by the Marine Conservation Society (Doyle et al., 2005) and some ecotourism operators that this signified basking sharks had moved further north in response to increasing sea surface temperatures as a result of climate change. However, the evidence used to support this claim is not conclusive, and the increased sightings in Scottish waters may, to a significant extent, be the result of increased observer effort (Doyle et al., 2005).

Basking sharks tracked using electronic tags have been shown to move rapidly between the two foraging areas off south-west England and north-west Scotland over time scales of a few weeks. Both south-to-north and north-to-south movements have been documented within a single foraging season, indicating long-term residence in one feeding location is unlikely for this highly mobile species (Sims et al. 2003). These distribution shifts of basking sharks have occurred in response to fluctuations in the location of centres of zooplankton abundance, which can vary greatly both within and between years (Sims & Quayle 1998; Sims & Reid 2002).

Furthermore, although surface sightings made during scientific surveys in the Plymouth study area in 2005 were the lowest for 10 years, large numbers of public sightings were made off Cornwall (Cornwall Wildlife Trust, pers. Comm.) and around the Isle of Man (Table 1) during the same summer. Because scientific surveys with bias reduction for habitat type (Sims et al. 2005) were not conducted off south-west England and north-west Scotland simultaneously, there is likely to be significant bias in the numbers reported in Scottish waters. When compared with other areas sightings are not unusually high in this region (see Table 1), but occurred within a short time frame, which gives the perception of unusually high numbers of sharks in an area.

2.1.2 Archived Samples

As part of the project we actively sought to expand our archive of basking shark tissue samples by approaching museums and individual researchers within the UK. This has been facilitated by the UK Basking Shark Research Network co-ordinated by Dr Sims that is funded through the Esmee Fairbairn Foundation. This formal grouping of six UK-based charities that routinely collect data on basking shark sightings and biology provides an unparalleled information network for collating tissue samples from around the UK.

In addition, we have contacted colleagues in the UK (Durham, R. Hoelzel and Scotland, R. Ormond), Italy (S. Clo), France (E. Stephan), Norway (IMR, Bergen), USA (Masschusetts, P. Clapham; North Carolina, F. Schwartz; Florida, D. Adams;

California; S. Van Sommeran), South Africa (M. Scholl), Australia (J. Stevens), Japan (K. Yano), Canada (Steve Campana) and New Zealand (Malcolm Francis). As a result, the project tissue library has increased to 41 samples (Table 2). These samples have been used by the team at Aberdeen University in the development of the microsatellite and mitochondrial (mt) DNA markers (below).

In conclusion, while field sampling has not been as successful as anticipated in the last two field seasons due to the very low abundance of sharks in the south west, this has not compromised development of the microsatellite and mtDNA markers (a critical and important component of the project), since only a few tissues samples are required for this. However, the inability to collect enough tissue samples (ideally 10-20+, see 2.1.1 above) from the Plymouth study area has limited the extent to which the project has been able to answer specific questions about the discreteness and structuring of basking shark populations in UK waters. This aspect is addressed more fully in the following section where we report progress and achievement with the development of the molecular genetic part of the project.

It should be noted that, while there were many sightings of basking sharks in Scottish waters in the summers of 2004 and 2005, it was not possible to redeploy the MBA's research vessel (RV SEPIA) further north as a consequence of cost and vessel availability. The project was therefore heavily dependent on appropriate numbers of basking sharks appearing off Plymouth.

2.2 Population Differentiation and Population Ecology

Reliable population differentiation estimates are crucial in conservation biology, where it is often necessary to understand the extent to which populations are genetically isolated from each other. The loss of genetic variability is thought to be detrimental to a population due to increases in homozygosity, which may reduce the ability of a population to adapt or respond to environmental change. Reduced fitness following a loss of variability may occur as a result of an increased expression of deleterious recessive alleles, inbreeding depression or the loss of heterotic effects (Hedrick and Kalinowski 2000; Keller and Waller 2002). Small populations are most vulnerable to such effects, as the rate at which genetic variability (measured as heterozygosity) is lost depends upon the effective number of breeders in a population (N_e) and time (*t*; Crow and Kimura 1970). As a consequence, small populations with low growth rates and/or low reproductive fitness are sometimes hypothesized to have undergone a historic, genetic bottleneck. However, immigration may strongly counteract the negative effects of small population size (Ingvarsson 2001). Therefore, a knowledge of population structuring, and the forces controlling it, may provide valuable guidelines for conservation strategies and management.

2.2.1 Constraints & Potential Problems

At the outset we were aware that achieving all the objectives of the project depended critically on: i) the availability of tissue samples from basking sharks, and ii) the characterization of appropriate genetic markers. Whilst the project already had access to adequate samples to develop the requisite molecular tools, and allow sufficient characterization of the mitochondrial and microsatellite markers, the lack of population samples of known provenance has constrained our initial aims. Without these suitable population samples there is no justification for pursuing the proposed rigorous population genetic analyses because it would be difficult to produce a valid biological interpretation of the results. Therefore, we have attempted to interpret the population structure at a broader level, i.e. between northern and southern hemisphere samples.

Table 2. Basking shark tissue sample library

Sample ID	Date collected	Collector	Location name	Lat. (oN)	Lon. (oW)	Status (living/dead)	Total length (m)	Sex	Tissue type	Tagged	Notes
MBA1	01/08/1994	D. Sims	Fish market, Plymouth, UK			D			muscle		
MBA2		D. Sims	Brixham Fish Quay, UK			D					
MBA3	14/12/1996	D. Sims	St. Ives Bay, Cornwall, UK			D		M	gill raker		
MBA4		D. Sims	Fish Quay, Weymouth, UK			D		F			
MBA5	01/06/2000	D. Burton	Weymouth, UK			D			skin		
MBA6	05/06/2000	D. Sims	Plymouth, UK			L	7		skin		
MBA7	24/05/2001	D. Sims	Plymouth, UK			L	5.5	F	skin	00P979	
MBA8	28/07/2001	D. Sims	Clyde Sea, Scotland, UK			L			skin	00P986	
MBA9	28/07/2001	D. Sims	Clyde Sea, Scotland, UK			L	7	F	skin		
MBA10	31/07/2001	D. Sims	Clyde Sea, Scotland, UK			L					
MBA11	31/07/2001	D. Sims	Clyde Sea, Scotland, UK			L				PLY2-6	
MBA12	26/03/2003	E. Giacomello	Malamocco, Venetian Lagoon (N. Adriatic), Italy			D	8.4	F	muscle		
MBA13	06/01/2004	D. Sims	Llyn Peninsula, N. Wales, UK			D	5	F			
MBA14	03/02/2004	N. Queiroz	Southern Portugal	36.82	8.98	D	8.2	F	skin		Mature, in post-labour state; uterus very dilated and residues of blood; captured in bottom net (291m)
MBA15	28/05/2004	D. Sims	Gerrans Bay, Cornwall, UK			D	5.03	F	muscle		Stomach contents sampled and various tissues
MBA16	09/06/2004	C. Curtis	Fal estuary, Falmouth, UK			D	2.5	F	skin		Previously stranded at M<essack Point on 28 May 2004
MBA17	14/06/2004	C. Curtis	Perranporth, Cornwall, UK			D	5	F	muscle		Conical snout also taken for analysis of gel
MBA18	02/08/2004	K. Stewart	Loch Bracadale, Isle of Skye, Scotland, UK			D	7	F	muscle		
MBA19		J. Stevens	Tasmania, Australia					M	muscle		

MBA20	06/07/2005		Culbin Sands Nat. GRIDREF (O.S) NH953617 Scotland		3	F (prob)	Skin
MBA21		M. Francis	New Zealand				
MBA22		M. Francis	New Zealand				
MBA23		M. Francis	New Zealand				
MBA24		M. Francis	New Zealand				
MBA25		M. Francis	New Zealand				
MBA26		M. Francis	New Zealand				
MBA27		M. Francis	New Zealand				
MBA28		M. Francis	New Zealand				
MBA29		S.Winter	Salt Rock, KwaZulu-Natal, E coast of South Africa, SW Indian O				
MBA30		S.Winter	Porto Santo Stefano, Grosseto province, Tuscany, Italy,				
MBA31			Bergen				
MBA32			Bergen				
MBA33			Bergen				
MBA34			Bergen				
MBA35	27/08/2005	M. Gore	Loch Maddy, Scotland	D	3.5	M	Muscle
MBA36	18/07/2005	John Morrissey	Long Island (New York), USA	D	FL = 595cm	F	Muscle
MBA37	08/09/2005	Aberdeen	St Combes, Scotland	D	385	M	Muscle
MBA38	19/07/2005	Wayne Ledwell	New Foundland, Canada	D	900		Muscle
MBA39		S. Winter	St. Helena, W. Cape, S.A.	D	391		
MBA40		S. Winter	South Africa	D	685	F	
MBA41		S. Winter	South Africa	D			

Note: The listing of basking sharks on Appendix II of CITES in 2002 imposes restrictions on the importation of basking shark material into the UK. However, there currently appears to be a low awareness of the need for CITES permits for the import of basking shark tissues unless registered as scientific institutes with CITES.

2.2.2 Molecular markers: Which ones and why?

The molecular tools for a three-pronged approach have been successfully developed and are now available to address issues of stock separation/identity. Markers developed have been derived from mitochondrial DNA (mtDNA), in addition to others found in microsatellite or simple sequence repeat (SSR) loci (genotyping of repeat length) and in DNA sequences flanking the repeat regions.

Highly variable molecular markers are essential for population genetic analyses and mitochondrial DNA has been favoured for a variety of reasons (reviewed in Avise 1994). However, mtDNA is maternally inherited and inferences of relationship apply only to female lineages, giving very limited information on population structure and migration. Differences in dispersal behaviour of mature males and females can ultimately influence the distribution of molecular variation. Hence, for a comprehensive analysis of basking shark genetics, it is essential to assess and compare the evolutionary history of both sexes by combining analyses of mitochondrial and nuclear gene markers.

Because nuclear genes in sharks evolve more slowly than mitochondrial genes (Martin 1999), standard sequence analysis of some protein-coding genes, or intronic sequence, are of insufficient resolution to reveal the requisite variation for population genetic analysis. However, microsatellites, or simple sequence repeat (SSR) loci, have been shown to harbour tremendous polymorphism due to a high rate of gain or loss of repeat motifs (Goldstein & Pollack 1997; Shimoda et al., 1999). Many recent studies have documented the immense utility of microsatellites for population genetic analysis (e.g. Goldstein et al., 1999). We believe that by isolating and characterizing microsatellites specific to basking sharks we have produced ideal markers with which to address the objectives of this investigation. Their hypervariable character provides good resolution at the population level, and allows analyses that provide for differentiation of historical and contemporary population processes, information essential for using analyses based on coalescent theory, to determine the prevailing levels of gene flow between stocks. Additionally, as PCR-based markers, they can be used to amplify loci from small tissue samples (essential for biopsy work), or partially degraded material. We proposed to utilize both mtDNA and microsatellite loci to estimate the relationships among genomes of individuals.

To-date, genetic studies of widespread marine teleosts (Grant and Bowen 1998; Graves 1998), together with analyses of the mako shark (*Isurus oxyrhinchus*) (Heist et al., 1996, 2003), suggests that most species exhibit little population genetic structure, even over vast geographic scales. This may indicate that the populations are effectively a part of a larger contiguous population, suggesting that widely separated populations are not independent and that immigration and recruitment from elsewhere in a species' range may replenish localized exploitation. Alternatively, little population genetic structure could indicate that populations simply lack sufficient genetic variation to show the underlying population differentiation. The ability to distinguish between these alternatives is essential for identifying stock boundaries for fisheries management. Ignoring this consequence could lead to the erroneous conclusion that the populations surveyed are part of the same stock, when in fact their genetic similarity is attributable to historical rather than contemporary factors (Avise, 1994; Templeton, et al., 1995). Hence, it is necessary to address the extent to which genetic similarity reflects continued gene flow between populations, or historical similarity and isolation following population fragmentation. Analyses, based on the coalescent theory (Templeton and Georgiadis 1996; Templeton 1998; Gomez-Zurita et al., 2000) of molecular data, allow inference not only of the present population structure but also of the historical population processes most likely to

have led to the observed subdivision. Such an approach will add considerably to our understanding of basking shark stock interactions.

By analysing the DNA sequences flanking the SSR repeat regions, additional variation can be uncovered that provides a clearer picture of immigration and recruitment within the species' range. Taking account of flanking region phylogenies makes it possible to identify if genetic similarity is attributable to historical or contemporary factors. Thus, for the nuclear markers, we adopted two different approaches for characterizing and analyzing molecular variation. First, we have employed methods of analysis based on estimating allelic identities from fragment sizes of amplified loci. Second, we have determined the DNA sequence of the regions immediately flanking SSRs. These regions evolve relatively quickly, and surveys of other taxa reveal an abundance of sequence variation that allows robust inference of allelic genealogies (Grimaldi & Crouau-Roy 1997; Orti et al., 1997; Fisher et al., 2000).

- Once sufficient *population* samples of basking sharks are available we are confident that the three-pronged approach for the analysis of molecular variation (SSR genotyping, and SSR flanking region and mtDNA marker sequencing) will enable high-resolution inference of intra-specific evolutionary history. These approaches will provide complementary data that can be rigorously analyzed using recently available statistical approaches.

- We have produced and tested all the molecular tools required to address the proposed questions pertaining to population genetic structure. Their development is outlined below in each section where appropriate.

2.2.3 Mitochondrial DNA variation

Partial (550 base pairs) sequences of the cytochrome b gene from samples representing the eastern and western Atlantic, the North Sea, the Mediterranean and the western South Pacific suggested the existence of only two mtDNA haplotypes, differing by 0.6% (Hoelzel 2001). With no significant frequency differences among these localities this small section of this gene is unsuitable for population genetic differentiation of basking sharks. Similarly, preliminary studies using very small (250 base pairs), partial sequences of the mtDNA D-loop (control region) from basking sharks revealed no variation (Hoelzel pers comm.). However, our recent study of great white sharks revealed most of the variation is characterized by single base substitutions, with short insertion and deletions distributed throughout the D-loop. At least 30 haplotypes were detected and pair-wise comparison of the complete D-loop sequences revealed two strongly divergent genetic lineages, which differ by 4% in sequence, with an average nucleotide diversity within oceans of between 0.12 to 1.0% (Pardini et al., 2001). Variation in hammerheads, however, appears to be clustered in the upstream portion of the D-loop sequence and limited surveys suggest, that again, two highly divergent mitochondrial genetic lineages (describing between them 22 halpotypes) were detected differing by 5.5% in sequence, and this variation exhibits significant structuring within oceans (Martin, Noble & Jones, unpublished data).

We proposed to amplify considerably more sequence from both the cytochrome b gene, and the control region D loop to assess whether these gene regions were sufficiently polymorphic to enable estimates of population differentiation. Analysis of mtDNA has followed established methods (Pardini et al., 2001), and full details are available at: http://stripe.colorado.edu/~am/WhiteSharks.html. Representative individuals from each main *region* from which samples originated were sequenced and PCR-RFLP analyses carried out for haplotype analyses.

2.2.3.i. Identification of possible region specific polymorphisms in Cytochrome b
Sequencing 859bp of Cytochrome b in *C. maximus* has identified a number of single nucleotide polymorphisms (SNPs). An SNP is a variation in the genetic code at a specific point in the DNA, for example in any given population some individuals may have a 'C' at a position where the rest of the population has a 'T'. These differences in genetic code appear slowly on a multi-generational time scale and can be inherited, making them a valuable tool for studying population interactions. Whilst individual SNPs provide less information than a microsatellite because they have far fewer alleles, variation in SNPs along a stretch of DNA can be combined to form a haplotype which vastly increases the number of alleles which can be identified, providing a very specific population/regional signature, useful for both population genetic and phylogenetic analyses. These markers differ from RFLPs (Restriction Fragment Length Polymorphisms) that reflect the size of a DNA fragment (usually recovered by PCR – hence the term PCR-RFLP) after it has been cut at a restriction site, following digestion with an appropriate restriction enzyme. The resulting fragment lengths provide the polymorphism, their length varying upon the presence or absence of the restriction site that may often be knocked out or made viable by the presence of an SNP. Hence, like SNPs, RFLPs can be used to define haplotypes. (*Note*: *In order not to compromise pending scientific publication, the base sequences for these genes have not been given here. However, this information may be made available by Defra to bona fide scientists subject to specific terms and conditions*).

We have identified 8 SNPs in Cytochrome b, of which 7 are RFLPs, more than sufficient to form an effective haplotype, and all of which can be genotyped simply and efficiently, by amplifying the PCR product and digesting it with restriction enzymes. PCR-RFLP haplotype analyses using *only* three restriction enzymes (Hae*III*, Alu*I* and Bst U*I*) from samples representing NE Atlantic (UK), the North Sea (Norway), and the east Pacific (New Zealand) suggests the existence of 4 mtDNA haplotypes, differing by 1.15% sequence divergence. Three haplotypes were found in the NE Atlantic, two of which are shared by samples from Eastern Pacific (New Zealand) and one shared with North Sea and Western Atlantic (Norway and USA). Preliminary phylogenetic analysis of the sequence data, based on small samples, clearly differentiates Pacific and Atlantic populations, though interestingly Norwegian and US east coast samples cluster, suggesting they, perhaps like the UK samples, represent a single population (see Fig. 2). However, further samples will be required to confirm this.

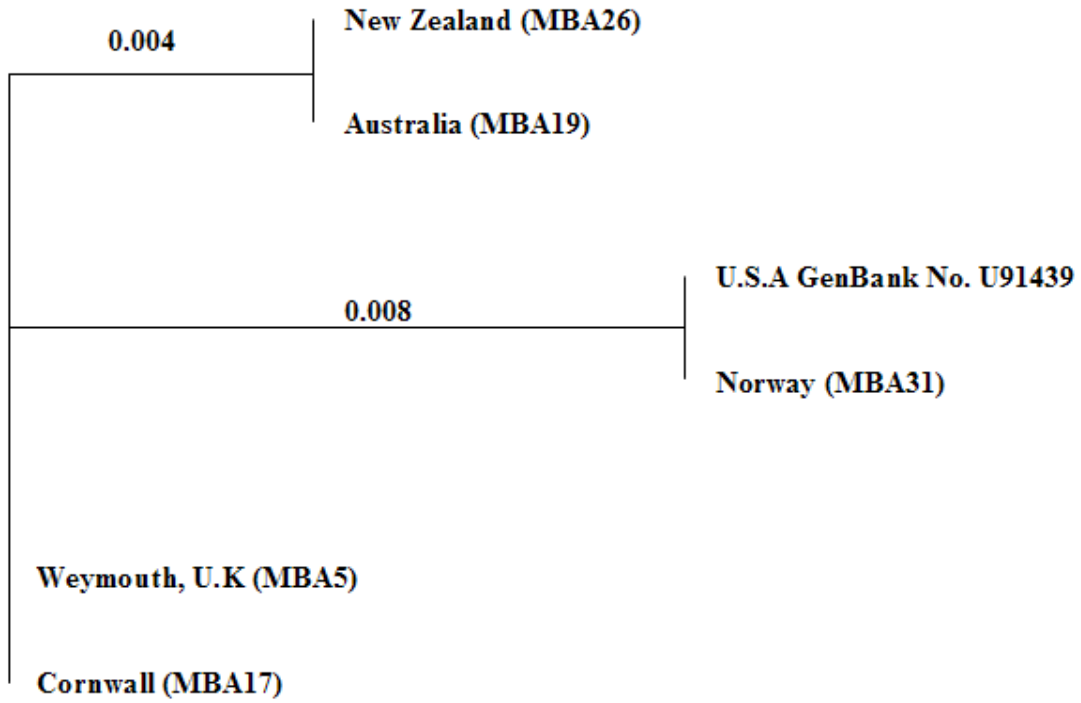


Fig. 2. Unrooted Neighbour-Joining gene tree showing relationships between representative basking shark sampling areas, based on Jukes/Cantor distance of 859 bp cytochrome b sequence.

2.2.3.ii. Identification of possible region-specific polymorphisms in mtDNA D-loop
We have amplified the *entire* non-coding D-loop using primers complementary to sequence in the tRNA genes flanking the target region (over 1000 base pairs). Focusing on sequence analysis of representative samples from *within* the western Atlantic (including waters off Wales, Cornwall and Scotland) shows there are 9 SNPs, with an average sequence divergence of 0.56% between Scotland and Welsh waters. (*Note: In order not to compromise pending scientific publication, the base sequences for the non-coding D-loop have not been given here. However, this information may be made available by Defra to bona fide scientists subject to specific terms and conditions*) This suggests, in contrast to Hoelzel (2001), that there is sufficient mitochondrial haplotype diversity to investigate the population structure of basking sharks collected *even within* a specific region such as the NE Atlantic. However, it is impossible to suggest how this diversity is distributed until larger population samples become available.

- The D-loop shows more polymorphisms than the cytochrome b gene, and is therefore, in contrast to previous workers findings which used small partial D-loop sequences, identified as a very useful marker to determine female mediated gene flow.

- Sequence analyses of two complete mtDNA gene regions (cytochrome b and the D-loop) have shown sufficient variation is available for population differentiation by their combined use. Simplified to PCR-RFLP analyses for a cheap, quick simple assay of variation, these tools promise a reliable and reproducible method of differentiating mitochondrial haplotypes of basking shark populations, permitting quantification of female mediated gene flow.

2.2.4 Microsatellites

Microsatellites have been isolated from few shark species, including lemon sharks (*Negaprion brevirostris*; Feldheim et al. 2001), sandbars (*Carcharhinus plumbeus*; Heist & Gold, 1999), nurse sharks (*Ginglymostoma cirratum*; Heist et al., 2003), and only two representative lamniforms, white sharks (*Carcharodon carcharias*; Pardini et al., 2001) and the short fin mako (*Isurus oxyrhinchus*). To date, no basking shark microsatellite markers have been published. The major drawback of microsatellites is that these markers usually have to be isolated *de novo* from each new study species. This is because microsatellites are generally located in noncoding regions with higher nucleotide substitution rates than are typical of coding regions. As a consequence the design of universal primers matching conserved flanking sequences is often problematic. However, the presence of some regions of high conservation in the flanking regions such as have been documented in cetaceans (Schlotterer et al., 1991), and fish (Rico et al., 1996), allows cross amplification from species diverged as long ago as 470 million years. Our work on white sharks has shown parts of the flanking sequences are sufficiently conserved that several loci cross amplify, and are polymorphic in several Lamniforms including the basking shark (Pardini et al., 2000); significantly, the repeat motif of one locus was preserved for 1 billion years of evolution (Martin et al., 2002). Loci recently isolated from the short fin mako have also been shown to be polymorphic in several Lamniform sharks (Schrey and Heist 2003), suggesting they too have the potential to amplify in basking sharks.

We aimed to survey variation for >10 microsatellite loci for as many individuals as possible from each major region, requiring the development of further primers *specifically* from the basking shark.

Several compelling reasons support our efforts to characterize population genetic variation for a relatively large number (> 8) of basking shark loci. First, theoretical studies indicate that, because microsatellites typically have large numbers of alleles, the variance of genetic distances between individuals or populations is large, and this compromises estimation of genetic distances (Zhivotovsky and Feldman 1995; Takezaki and Nei 1996; Goldstein et al., 1995, 1999). Therefore, reasonably large numbers of loci or individuals are recommended when inferring population level relationships and demographic history (Goldstein and Pollack 1997; Goldstein et al., 1999; Luikart and Cornuet 1999; Reich et al., 1999). Large numbers of samples are not a realistic goal for any basking shark survey, so a compromise is to screen for variation at a large number of independent loci. This allows inferences about relationships among genomes rather than genes, a particularly important point in this study, which attempts to estimate the population genetics of individuals, not single genes.

However, because the statistical power of population genetic analyses depends on the number of loci scored, together with level of polymorphism at each locus and sample size, the use of a limited number of orthologous loci might fail to provide sufficient information. Most loci are generally selected from the upper end of the repeat length distribution in the genome during the microsatellite isolation procedure, the portion known to contain the most polymorphic loci (Primmer et al., 1996). Such a bias in loci isolation may result in less polymorphism when orthologous loci are applied to non-focal species (Ellegren et al., 1995). This is an important point as loci with more alleles produce better estimates of genetic distance, regardless of the statistic used (Kalinowski 2002). Hence, observation of high polymorphism in the focal species does not guarantee similar levels will be found in related species, especially with increasing evolutionary distance (Morin et al. 1998). Additionally, there is an increased risk of null alleles occurring in non-focal species. As we are

dealing with low sample sizes for the basking sharks and only orthologous loci from related species (white sharks and mako) were available, we decided that additional loci should be isolated specifically from basking sharks.

Yields from species showing a paucity of microsatellites are generally poor with traditional isolation methods, as has been shown by our experience with white sharks (Pardini et al., 2000) and by work on other species (Heist et al., 1999; Feldheim et al. 2001). When large numbers of loci are required, such as in this study of genetic variation between populations, we propose the production of a genomic library 'enriched' for simple sequence repeat motifs (di- and tetra-nucleotide repeats).

2.2.4.i Basking shark microsatellite isolation

Two rounds of microsatellite isolation have now been completed using an in-house protocol based on the work of Hamilton et al. (1999) with some improvements taken from Koblízková et al. (1998), Gardner et al. (1999) and Glenn *et al*. (2002). The basis for this method is quite simple, equal amounts of genomic DNA, taken from geographically diverse regions, are pooled. Restriction enzymes are used to cut the DNA into appropriately sized fragments (optimum size 500-1500bp), which are then enriched for various di-, tri- and tetra- repeats (Fig. 3). The fragments are then investigated to determine if they contain repeats which are either polymorphic within or between the various *C. maximus* populations under investigation, and those which are can be developed as microsatellite markers.

In order to optimize this method for *C. maximus* a number of enzymes and enzyme combinations were tested. The most suitable enzymes identified were *Msp* I and *Taq* I combined, or *Sau3 AI* only, both alternatives were run concurrently in an attempt to increase the pool of suitably sized DNA fragments and the overall genomic coverage. The resulting fragments were tested for the di-nucleotide repeats CA and CT, the tri-nucleotide repeat AAT and the tetra-nucleotide repeats CAGA and GATA.

In the first round, 480 fragments (96 for each repeat motif) were screened using universal and repeat base primers to differentially amplify these fragments. Almost 20% of fragments tested showed some form of differential amplification. Of these, 64 were selected for sequencing, 15 CA repeats, 11 CT repeats, 13 AAT repeats, 14 CAGA repeats and 11 GATA repeats. However, many fragments sequenced contained complex (sections of sequence containing more than one type of repeat unit in close proximity) or disrupted (repeat patterns disrupted by sections of random sequence of variable length) repeat patterns. Only 25% of the fragments contained repeats of a length and complexity that suggested they could be developed into useful polymorphic markers.

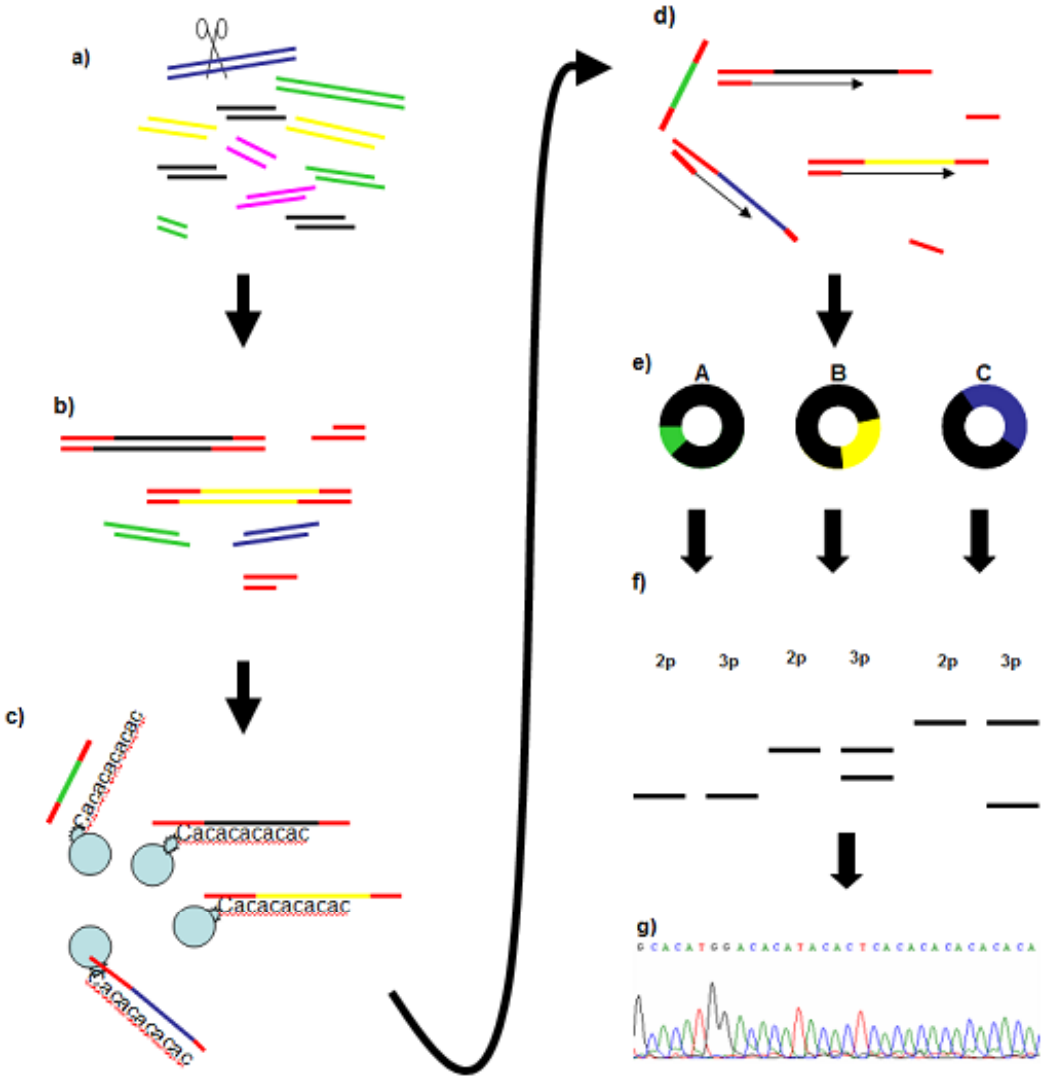


Fig. 3: Microsatellite isolation protocol for generating basking shark specific loci.
a) Equal quantities of genomic DNA from different geographical sources are pooled and digested using appropriate restriction enzymes to produce fragments of a usable size for screening (~400-1200bp). **b)** Linkers **(red)** made up of short pieces of synthetic DNA of known sequence are attached to either end of the cut DNA. **c)** The DNA fragments are screened for repeats by hybridizing (binding) the strand containing the complementary sequence of the selected repeat to magnetic beads with the synthetic repeat attached, usually one of CA, CT, AAT, CAGA or CTGT. Only those fragments containing the repeat will bind strongly, so washing the beads at different temperatures and stringencies of buffer will remove only weakly or unattached fragments, increasing the concentration of repeats within the pool. **d)** fragments are then eluted and again made double stranded by using part of the linker as primer for PCR. **e)** The resulting double stranded fragments are cloned into plasmids grown in bacteria to produce more copies, which also separates out the fragments for individual analysis. **f)** Each individual fragment is screened for repeats using a 2 and 3 primer method**.** The 2 primer (2p) reaction uses primers matching sequence within the plasmid located on either side of the inserted fragment to give the size of the fragment. The 3 primer (3p) reaction uses the primers from the 2 primer reaction and third primer made up of the repeat which is being screened for i.e. CA. The 3p reaction should produce a distinctly different pattern from the 2p reaction if a repeat is present. **g)** Any plasmids showing variation between the 2p and 3p reactions are sequenced and primers designed for sequences containing more than 10 repeats, those most likely to be polymorphic.

A number of problems were identified during the initial microsatellite isolation, such as the concatenation of sequences which, if not identified, could result in primers being designed to anneal to much more widely separated regions of the genome

than would appear from the recovered sequence, and smaller than expected fragments after the initial enzyme digest. However, most problematic was the lack of flanking sequence on many of the repeats, preventing design of flanking primers that made them unusable as microsatellite markers. As a consequence, a method was developed to recover those repeats with insufficient flanking region, by using anchored and random primers to produce additional flanking sequence for primer design, thus increasing the number of usable repeats. The first round of microsatellite isolation yielded 2 usefully polymorphic dinucleotide microsatellites, and a further 4 (3 di- and 1 tetranucleotide) were recovered from sequences with insufficient flanking regions and assessed for polymorphism.

A second round of microsatellite isolation produced far more promising results. Considering our experience of the first round, two modified methods for producing DNA fragments for microsatellite screening were employed. The first simply involved identifying an enzyme that would produce larger sized fragments reliably, in this case HaeIII, and modifying the linkers to fit. The second used a version of the PCR reaction, termed degenerate oligonucleotide-primed PCR (DOP-PCR), which employs oligonucleotides of partially degenerate sequence to randomly reproduce arbitrary sections of the genome.

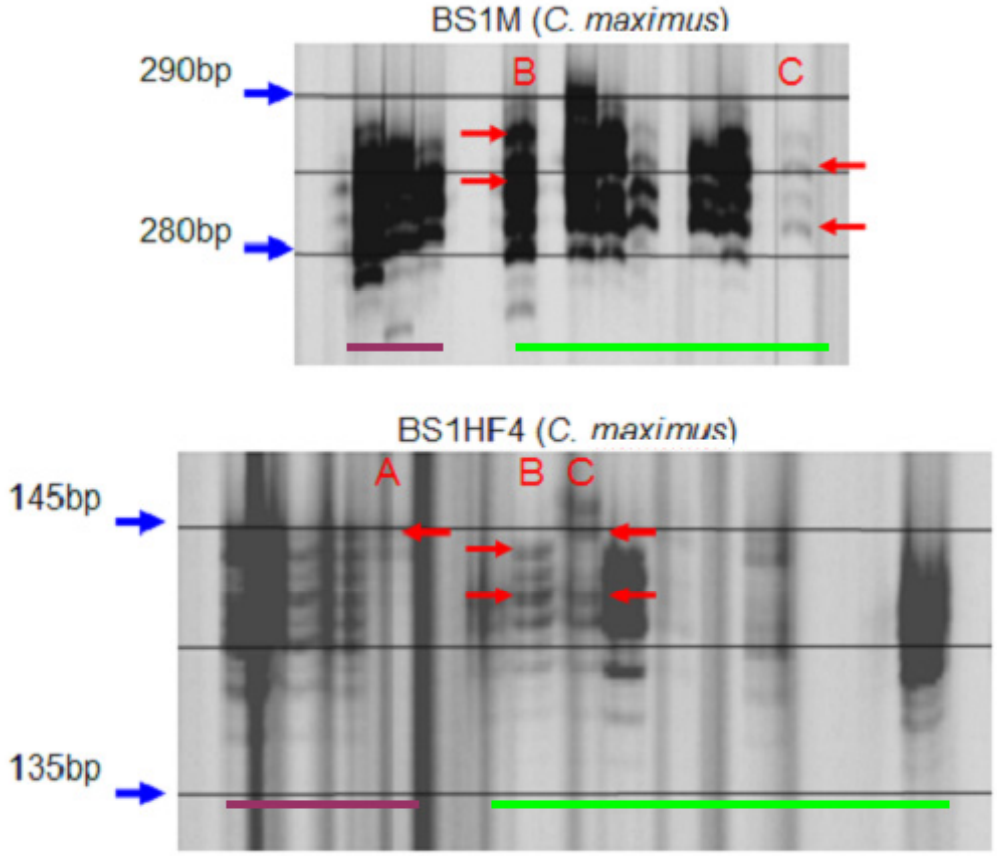


Fig. 4. The resulting microsatellite primers are assessed using basking shark DNA samples from various geographic regions. Two examples of typical dinucleotide (CA) repeats BS1M and BS1F4. Microsatellite alleles are commonly present as a series of bands of decreasing intensity called 'stutter bands' and can be used to identify the different alleles. Although stutter patterns are often specific to individual markers, as can be seen by the differences between BS1M and BS1HF4, there are common patterns. Key:- Blue arrows provide size references. Red arrows indicate alleles. A shows a typical homozygote pattern with only one allele, B shows a heterozygote in which the two alleles are close together so the stutter bands have an additive effect making the second allele appear stronger than the first, C shows a heterozygote with well separated alleles. Green lines indicate samples from the UK ; purple lines indicate samples from New Zealand.

The use of random primers, together with a PCR protocol utilizing a low initial annealing temperature, ensures priming from multiple (e.g., approximately 10^6 in human) evenly dispersed sites within a given genome (Koblizkova et al 1998; Barbaux et al. 2001), providing far better genomic coverage than the simple enzyme digest employed in the first round of microsatellite isolation. This approach has been used recently in other species for which microsatellite isolation has proven *especially* difficult, such as the house wren (Cabe and Marshall 2001) and a trematode worm (Criscione and Blouin 2005). The DNA fragments produced using this method were consistently larger (˜1.2kb) on average than those produced by the enzyme digest alone. Also, in this round of microsatellite isolation, additional samples of *C. maximus* from New Zealand, America, South Africa and Canada were included to provide a far better global coverage than was available in the first round. Some 30 dinucleotide (CA) repeat microsatellites were recovered, ten of which have been developed into usable microsatellite markers (Table 3). An additional four microsatellites are still under development. Examples of allele gels for two basking shark microsatellite loci are given in Fig. 4 (BS1M and BS1HF4), with an explanation of how these gels are interpreted.

Table 3. Repeat type, size range and number of alleles for microsatellites isolated specificially from Basking sharks. Ten reliable and polymorphic loci are now available for population genetic analyses.

ID	Repeat type	Size range (bp)	Number of alleles (number of samples tested)
BS1HF4	**CT**	141-149	5 (32)
BS1M	**CA**	278-290	7 (33)
BS1S4A	**CA**	196-204	4 (26)
BS1HA5	**CT**	221-231	6 (29)
BS1HC2	**CA**	458-466	5 (27)
BS1HA1	**CA**	342-348	3(8)
BS1HB2	**GT**	398-406	3(5)
BS1HE4	**CT**	194-202	4 (10)
BS1DE9	**CA**	425-431	3(6)
BS1HG4	**CT**	~600-650	4 (8)

Note: In order not to compromise pending scientific publication, the base sequences for these primers have not been given here. However, this information may be made available by Defra to *bona fide* scientists subject to specific terms and conditions.

Because most samples were the result of isolated biopsies or strandings, it was impossible to aggregate them into populations for the purposes of standard population genetic analyses. Using a measure of population subdivision which estimates how cohesive a unit two or more populations are (Goodman's Rst CALC) suggested, not surprisingly, there was very little gene flow between UK and Australia/New Zealand; returning an Rst value of 0.46, and Nm of 0.28, based on 10 microsatellite loci in 17 individuals. The Rst value is sufficiently high to suggest the populations are clearly different (>0.1) and the Nm low enough (<1) to suggest there is insufficient gene flow to allow the two populations to be considered part of the same evolutionary unit. However, it is difficult to place a meaningful biological interpretation on these data as few of these samples can be considered to come from the same population, and to be reliable the statistical analysis requires larger population samples.

However, 36 samples from a variety of isolated collection times and points were designated as either 'northern' or 'southern' hemisphere to allow a statistically less

demanding comparison of genetic similarity and proxy estimates of gene flow to be made at a gross level. Principal component analysis, a multivariate ordination technique which makes no *a priori* decisions based on sample classification, and produces axes the components of which describe the variance of the whole statistical sample, was used to explore the relationship of the 36 samples.

Using the microsatellite shared allele distance (SAD) based on 10 loci as a metric, the first two axes, which together describe over 46% of the sample variance, separated the samples into two fairly discrete groups, corresponding to northern and southern hemisphere (Fig. 5). This echoes the result of the Rst calculations, suggesting northern and southern hemisphere populations are genetically distinct. However, some individuals sampled in the northern hemisphere clustered with southern hemisphere populations, and *vice versa*. This may suggest there is occasional movement of individuals between hemispheres, or it may simply reflect the paucity of samples and sampling locations that cannot mitigate the large variance inherent in estimates of genetic similarity using microsatellites. Certainly our experience of other large lamniforms does suggest that individuals may undertake long transoceanic migrations, but without larger population samples to allow a full characterization of representative northern and southern hemisphere populations it is impossible to state if this is a likely explanation.

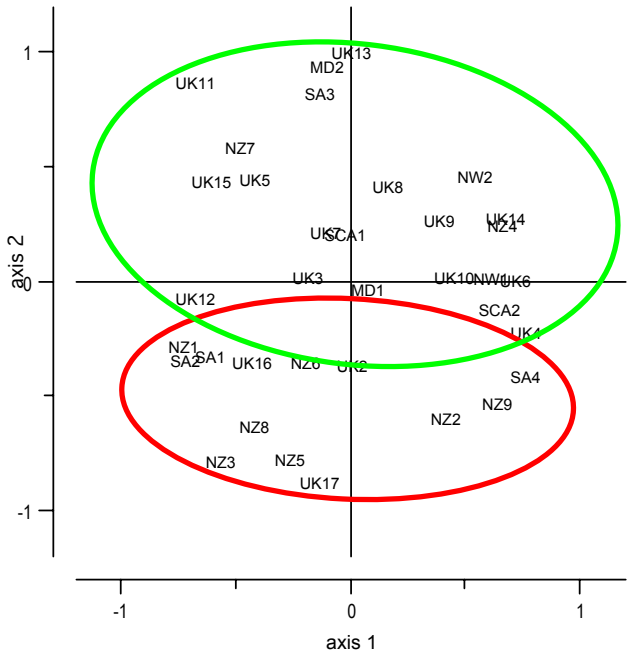


Fig. 5. Principal component analysis based on shared allele distances between Mediterranean (MD), New Zealand (NZ), North-West Atlantic US coast (NW), Norway (SCA), South Africa (SA), and British Isles (UK) samples. The first two principal components describe 46.3% of the total variance (PC1=28.8; PC2=17.5). The red ellipse delimits the *majority* of southern and green the northern hemisphere samples. While there are clear north and south groupings some individuals cluster with samples from the alternative hemisphere e.g. UK17.

- We have successfully developed basking shark specific primers for 10 polymorphic microsatellite loci, using a modified enrichment protocol used for the first time in elasmobranches.
- Although population samples were not available to us the utility of the microsatellite loci has been proven by comparison of populations on a gross

scale, suggesting little gene flow between populations in the northern and southern hemispheres.
- A panel of highly polymorphic microsatellite primers is now available for analysis of basking shark population structure.

2.2.4.ii. Survey of microsatellite variation from loci derived from non-focal shark species

Microsatellite loci developed in non-focal shark species have been tested in representative basking shark samples to assess their utility for population genetic analyses. Four dinucelotide loci from great white sharks (Pardini et al. 2000, 2001) were shown to be polymorphic in basking sharks showing between 2-3 alleles per locus (Table 4). Loci from the mako (Shrey and Heiset, 2002) appeared to be useful showing more polymorphisms with between 2–6 alleles scorable per locus (Table 4). This screening suggests an *additional* 8 polymorphic loci are available for population genetic analyses in basking sharks.

Table 4. The repeat type, size range and number of alleles in basking shark for microsatellites isolated from non-focal species of sharks including the great white (*C. carcharias)* and mako *(I. oxyrinchus)*. An additional 8 microsatellite loci are polymorphic in basking sharks and available to supplement population genetic analyses using microsatellite loci derived specifically from the focal species.

ID	Shark species Isolated in	Published	Repeat type	Size range	No. alleles (No. of samples tested)
Ccar 1	*C. carcharias*	Pardini et al (2000)	GT	168-182	3 (8)
Ccar 3.1	*C. carcharias*	Pardini et al (2000)	GT	140-146	2 (6)
Ccar 6	*C. carcharias*	Pardini et al (2001) Genbank. AF184085	CA	196-204	3 (6)
Ccar 19	*C. carcharias*	Pardini et al (2000)	GT	200-208	2 (4)
Iox-01	*I. oxyrinchus*	Shrey and Heist (2002)	GA/GT	114-128	6 (7)
Iox-12	*I. oxyrinchus*	Shrey and Heist (2002)	GT/GA	130-140	2 (3)
Iox-18	*I. oxyrinchus*	Shrey and Heist (2002)	GT	132-140	3 (8)
Iox-30	*I. oxyrinchus*	Shrey and Heist (2002)	CA	138-146	4 (6)

- An additional 8 polymorphic microsatellite loci are available to complement those developed specifically for basking sharks. Together these may provide sufficient loci to investigate relatedness of basking shark coalitions and paternity issues.

2.2.4.iii. Microsatellite flanking sequence regions for analysing population differentiation

To assess if sufficient variation was available for constructing resolved gene trees from SSR flanking regions a PCR amplified product from the *entire* internal transcribed spacer gene region 2 (ITS2), containing 2 microsatellite loci, was directly sequenced (*Note: In order not to compromise pending scientific publication, the base sequences for these microsatellite loci have not been given here. However, this information may be made available by Defra to bona fide scientists subject to specific terms and conditions*). The resulting tree from cluster analyses was used to assess variation within and between populations (regions) (Fig. 6).

Estimates of total sequence divergence (see Table 5) of four sample sources (UK, Norway, Mediterranean and New Zealand/Australia) reflected the findings of the microsatellite loci. Representing these findings by means of a Neighbour Joining tree (Fig. 8) illustrates that southern hemisphere populations are distinct from northern

hemisphere ones, with some indication of differentiation between northern hemisphere samples. There are 25 SNPs in ITS2 sequence flanking the two microsatellites loci and two variable repetitive regions (strings of Ts or single repeats such as ATC to ATCATC). In many cases more than one SNP is within a restriction enzyme recognition site, so there are only 10 RFLPs only 6 of which can be used easily. Most of the variability is between Portugal and the U.K, or Portugal / U.K and Australia.

Table 5. Total sequence divergence (upper right matrix) and number of shared sites (lower right matrix) for the ITS2 SSR flanking region. Differences between populations are evident ranging between 2-4% sequence divergence.

	Mediterranean	Scandinavian	Australia/New Zealand	UK
Mediterranean		0.0345	0.0217	0.0269
Scandinavian	0		0.0409	0.0422
Australia/New Zealand	0			0.0115
UK	1	0	0	

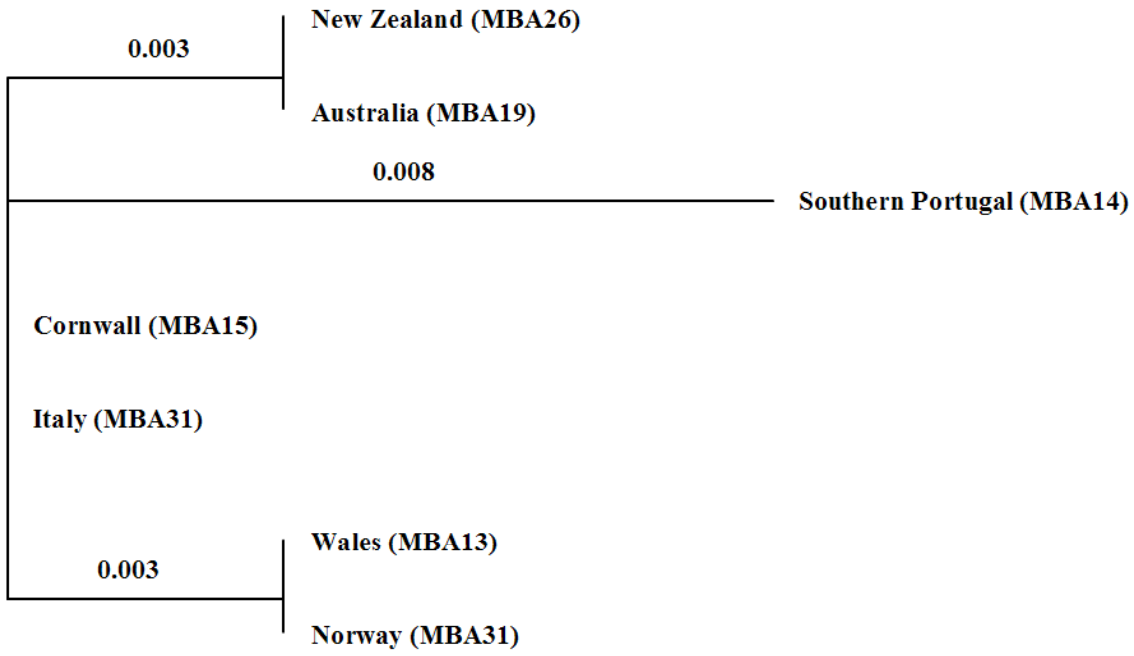


Fig. 6. Unrooted Neighbour-Joining gene tree showing relationships between representative basking shark sampling areas, based on Jukes/Cantor distance of ITS2 SSR flanking region sequences.

- The utility of three SSR flanking sequences has been demonstrated. Taking account of flanking region phylogenies makes it possible to identify if genetic similarity of populations is attributable to historical or contemporary factors, complementing data from the microsatellite repeats.

2.2.5 Analysis of tiny, degraded basking shark samples

Sample collection has been a particular problem within this project, so we have strived to find methods for optimizing the amount of DNA available to us. As many of the samples we have obtained have been either very small or from tissues with a low DNA content (e.g. cartilage and gill-rakers) and are often degraded, (e.g. with formalin preserved museum samples), we have developed a number of approaches for dealing with these problems. By using small-scale extraction protocols and modifying them to work more efficiently with formalin-preserved samples, we have been able to recover DNA from both degraded archival material and limited source material (tissue samples of less than $0.2cm^3$). In addition, we have obtained specifically designed enzymes that work at much higher efficiencies than normal PCR enzymes in degraded DNA.

However, none of these modifications resolved our main problem of DNA quantity; from many samples we could not obtain enough DNA to perform more than a few simple tests. To over come this problem we began using a genome duplication method called Degenerate Oligonucleotide-primed PCR (DOP-PCR), which employs partially degenerate primers to randomly reproduce arbitrary sections of the genome. This technique has been used with great success in humans producing approximately 10^6 evenly dispersed sites within the genome (Koblizkova et al 1998; Barbaux et al. 2001). DOP-PCR has also been used previously in this laboratory for *Lamna nasus* and *C. carcharias* with some considerable success, producing results consistent with non DOP-PCR sample for all markers tested and extending the useful life of a number of samples.

- We have developed a protocol to allow recovery of DNA from forensic quantities of shark tissue. This will have a significant impact on future molecular genetic work in this and other shark species for which samples are scarce, allowing badly degraded and minute tissue samples to be utilized.

2.2.6 Analyses to address stated population genetic objectives

- The lack of population samples has negated the use of the proposed advanced statistical *population* genetic analyses. However, with the molecular tools we have developed, all of these objectives are clearly achievable once sufficient population samples have been procured.

Modern molecular genetic techniques (e.g. microsatellite markers and mtDNA sequences, Pardini *et al.*, 2001) can now be used to investigate evidence for stock separation throughout the NE Atlantic. Connectivity between populations can be determined by assessing the variability of microsatellite loci and mtDNA haplotypes, analysed by standard population genetic protocols (such as GENEPOP, FST) to estimate population similarity and number of migrants per generation. Population isolation/fragmentation and age-related dispersal effects can be investigated by discriminant and assignment analyses of individuals/groups and parent population clusters. Differential estimates of gene flow from maternal (mtDNA) and biparental nuclear (microsatellites) markers can be used to test for sex-related differences in dispersal/gene flow. Obtaining a better understanding of basking shark population genetic structure using these approaches will indicate the relative importance of those population processes, past and present, which are likely to influence the genetic diversity and stability of stocks in the future. Additionally, depending upon the level of stock differentiation, it may be possible to use some of the population genetic markers to assign body parts recovered from commercial products to specific stocks/areas.

2.2.6.i. Genetic variation

Levels of genetic variation can be estimated from individuals sampled from all major regions (geographic scale) and a temporal scale if possible. For microsatellites, the number of alleles and heterozygosity can be measured, the latter corrected for small samples sizes. All populations can now be tested for departure from Hardy-Weinberg equilibrium at each locus and for linkage disequilibrium between pairs of loci using Fisher Exact and permutation tests (GENEPOP; Rousset and Raymond 1995); heterozygote deficiencies may imply population mixing or non-random mating. The microsatellites developed are sufficiently polymorphic to allow the detection and removal of identical genotypes in duplicate samples.

For mtDNA analyses, levels of genetic diversity within and between populations can be quantified by indices of haplotype diversity, and nucleotide divergence using DnaSP (Rozas and Rozas 1999). To visualize the relationships among the unique mtDNA haplotypes a phylogenetic tree is constructed based on uncorrected genetic distances with indels included. Distance and traditional parsimony methods perform poorly for inferring the genealogy of closely related haplotypes (Posada and Crandall 2001); therefore, the statistical parsimony methods developed by Templeton et al. (1992) using the TCS program are favoured (Clement et al., 2000). The parameter theta (2Neu and 4Neu for mitochondrial and nuclear genes, respectively) may be estimated using maximum likelihood techniques (using programs COALESCE - Kuhner et al., 1995; and MISAT -Nielsen 1997) and by calculating the average pair-wise sequence divergence (using ARLEQUIN; Schneider et al., 1997; using both the infinite allele and stepwise mutation models).

2.2.6.ii. Population structure

Population structure may now be determined by assessing variation within and between sample localities and among different sampling times (e.g. two sampling seasons), using hierarchical analysis of variance (Excoffier et al., 1992). For the mitochondrial DNA data, two levels of variation may be examined: among sea areas, and within populations within sea areas. For the nuclear marker data, a third level may be considered, that within individuals (because of diploidy). Hierarchical analysis of variance can be accomplished using ARLEQUIN (Schneider et al., 1997). Genetic divergence between all pairwise populations is assessed using classical Fst statistics (for the sequence data) and Fst and Rst (which assumes a SSM model [Slatkin 1995; Goodman 1997]) for the microsatellite genotype data. Quantitative assessment of inbreeding can be measured using Wright's (1951) inbreeding coefficient *F*is. The effective number of migrants (i.e. the average number of individuals exchanged) per generation can be estimated from private alleles (Barton and Slatkin, 1986). The analyses can be conducted for all populations with both genders considered together and separately to assess sex-biased dispersal (see later).

Classical analyses ignore the historical factors affecting the geographical distribution of allelic variation, thus, the genealogical information inherent in molecular population analyses may be explored further by employing nested cladistic analysis, developed specifically to separate historical effects from on-going processes on the distribution of variation (Templeton et al., 1992; Templeton 1998). The programs ParsProbe1.0, Chiperm1.0 and GeoDis2.0 software (Posada et al., 2000) implement these analyses for both mitochondrial and nuclear DNA data. This approach is complemetary to the use of traditional F and related statistics, but provides greater statistical rigour (Turner et al., 2000). Statistical resampling procedures for small population sizes may be employed where appropriate.

2.2.6.iii. Dispersal estimates and detection of immigrants
The individual-based population assignment tests introduced by Paetkau et al., (1995) used to quantify degrees of genetic differentiation have a variety of applications in conservation biology (Bayesian methods e.g. GENECLASS; Cornuet et al., 1999; STRUCTURE; Pritchard et al., 2001). This test can identify putative source populations of individuals of unknown origin (useful for discriminating if an animal originates from an illegal source), thus can be used to assign individuals during migration, to estimate sex-biased dispersal and gene flow, to identify potential admixture between populations, and to estimate long-term effects of population stocking. The assignment index is an indication of the frequency with which an individual's genotype occurs in a given population. A lower index indicates that the individual is less likely to have come from that population and could be an immigrant.

2.2.6.iv. Sex biased dispersal
Social organization can be an important determinant of the genetic structure of populations, and hence, of the potential for genetic differentiation and evolution of local adaptations. The predominating social organization in mammals is female philopatry and male dispersal, while the reverse is usually true among bird species (Greenwood, 1980). Such gender differences in dispersal can affect the genetic structure of populations, particularly when maternally inherited, haploid mtDNA markers and bi-parentally inherited nuclear markers are compared (Avise 1994). In the globally distributed great white shark we (Pardini et al., 2001) have shown the absence of genetic differentiation for microsatellite markers between oceans, while genetic differentiation for mtDNA markers (using Fst analyses) was significant. This was interpreted as a direct consequence of greater movement of males across oceans, consistent with females being more philopatric than males. This significant finding suggests that some species of sharks act more like some marine mammals (a similar genetic differentiation pattern was reported in the sperm whale *Physeter macrocephalu;* Lynholm et al., 1999; and humpbacked whales from the North Pacific, Palumbi and Baker, 1994) than other fish. Exploitation of a population in which immigration from surrounding stocks is low and females are philopatric could lead to a rapid decline in stock size and future sustainability.

The mtDNA variation detected in basking sharks may be enough to be useful in assessing female mediated gene flow. However, if necessary, polymorphic bi-parentally inherited markers, such as the microsatellite loci developed for basking sharks, can potentially be used to infer sex biased dispersal if the sex of the sharks has been determined (Goudet et al., 2002). Provided that dispersal occurs at the juvenile stage and samples are taken from adults, genotypes sampled from the dispersing sex should be, on average, less likely (compared to genotypes from the philopatric sex) in the population in which they were sampled. The dispersing sex should be genetically structured and present a larger heterozygote deficit. A program (http://www. Unil.ch/izea/softwares/fstat.html) is available (Goudet et al., 2002) to test this based on F-statistics (Weir and Cockerham 1984) and assignment indices (Paetkau et al., 1995; modified by Favre et al., 1997). However, Goudet et al., (2002) caution that these tests have limited power unless the bias in dispersal is extreme and large sampling efforts are undertaken.

2.2.6.v. Bottleneck detection.
There are many approaches to detecting the genetic signatures of recent bottlenecks using microsatellites (Richards and Leberg, 1996) and most of these are based upon comparisons of populations thought to have potentially experienced a bottleneck, to estimates from samples collected prior to the putative bottleneck or to populations with no known history of population reduction. Allelic richness (*A*), or allelic diversity, or the mean number of alleles per locus, is one of the commonest statistics reported.

However, because differences in sampling intensity can bias *A*, methods that standardize *A* on the basis of the size of the smallest number of samples in a comparison are required (rarefaction and repeated sub-sampling; Leberg 2002). However, it is often difficult to identify losses of variability as such, because variability prior to a population decline are often unknown, unless archived museum specimens are available. If sufficient museum specimens of basking sharks can be obtained then this approach may be feasible. A more direct method for identifying populations that have lost genetic variability during a recent bottleneck event is to examine the contemporary population for evidence of an excess of loci with an excess of heterozygosity (Spencer et al., 2000; Cornuet and Luikart 1996; BOTTLENECK) and/or calculate *M*, the mean ratio of the number of alleles to the range in allele size per locus (Garza and Williamson 2001).

2.2.6.vi. Population Ecology

The demography of populations is defined by interactions between extrinsic ecological factors such as environmental stochasticity and intrinsic ecological factors like social behaviour, reproductive strategies and migration patterns. For instance, the impact of historical fishing is suspected to have contributed to a loss of genetic diversity and inbreeding of the survivors. The current potential for inbreeding depression, an important measure of a population's 'genetic health', can now be derived for basking sharks from quantitative assessment of inbreeding measured using microsatellite data and calculated according to Wright's (1951) inbreeding coefficient *F*is, estimated using GENEPOP software.

Principally, the results from the genetic analyses of known individuals (males and females) can be interpreted in relation to satellite tagging data to examine sex- and age-related dispersal mechanisms (i.e. are females more philopatric than males?). Interpretation of the movements and distribution patterns revealed by tagging data, in terms of the probability of individual sharks belonging to spatially or temporally discrete local populations, may be evaluated against genetic evidence of population structure. If sufficient samples are available, the mixing dynamics by life stage and sex can be determined. This information provides the basis for an understanding of the impact of exploitation on basking shark populations, and can help to indicate the scale of management actions needed to ensure their sustainability.

Application of population genetic results to the conservation and management of basking shark should allow impact of issues such as sustainability, area protection and limited fishing and by-catch to be better understood. In particular, integration of genetic, telemetry and GIS data will permit development of appropriate models for use by statutory fishery and conservation bodies.

2.3 Enforcement and Management

A major obstacle to obtaining data on shark catch and trade, and the implementation of conservation and management strategies on a species-specific basis, is the problem of accurately identifying exploited species. This is aggravated by the widespread practice in commercial fisheries of removing the head, tail and fin of landed sharks while at sea to minimize storage space. DNA-based methods for species identification can circumvent these problems, allowing accurate and unambiguous identification of body parts to the specific or even population level.

An ideal test would be a single tube multiplexed reaction for identification to the specific level for identification of anonymous shark parts from commercial products. However, primers derived from single loci are undesirable where legal scrutiny is likely to be involved. Multiple loci tests are considered to enhance the accuracy of the

detection assay, providing a simultaneous, concordant diagnosis from more than one locus and reducing the likelihood of detection failure attributable to intra-specific variation at the primer annealing sites for any single primer. Microsatellites are often too variable and not sufficiently species-specific for this task, but may be suitable for recovering the origins of stock, depending upon the level and distribution of polymorphism (see genetic surveys from section 2.2.4.i). However, nuclear ribosomal Internal Transcribed Spacer 2 locus (ITS2) has low intra-specific sequence variation but sufficient inter-specific polymorphisms to yield robust diagnostic markers even between closely related shark taxa. A highly streamlined approach, based on multiplex PCR that uses species-specific primers derived from ITS2 sequences, to achieve rapid species identification of body parts has recently been developed (Pank et al., 2001; Shivji et al., 2002; Chapman et al., 2003).

These multiplexed primers can be used to identify the presence of basking shark in a commercial product prior to identification of the derivative *stock* using microsatellite markers.

2.3.1. Molecular identification of *Cetorhinus maximus*

Development of genetic methods to identify shark body parts has proceeded exceptionally well, producing a versatile and reliable test, with built in redundancy, which provides more acceptable and robust evidence in litigation proceedings than the current existing tests developed by Shivji et al. (2002) and Chapman et al. (2003). The test focuses on two genomic regions, Cytochrome b (a mitochondrial gene) and ITS2 (a nuclear sequence), and has been further developed to overcome some potential ambiguities which may arise when attempting to differentiate *C. maximus* and *some* populations of closely related species.

The use of a primer extension method to identify sequence changes has some drawbacks, most notably the fact that the primers can be prevented from working by sequence changes other than the one(s) they are intended to detect. Whilst this would not result in false positives it would produce false negatives, potentially resulting in trading of some species/populations of protected species going undetected. To compensate for this we have introduced a level of redundancy to our test by using multiple primers each targeted to a different site. In designing these we have utilised both strands of DNA, allowing multiple primers to be used without them interfering with each other. This approach allows the method to be extended to test for multiple species or identify region/population-specific haplotypes from a mixed amorphous sample (e.g. shark fin soup), whilst minimizing primer interference. Whereas Chapman et al.'s (2003) method only considers a single site per gene, our approach is an improvement not only in its versatility and level of built in redundancy, but in the amount of information it provides from each gene.

We have further improved upon previous work by making use of a far larger panel of geographically more diverse populations of closely related species than has hitherto been available to other workers in the field. It is often difficult to determine if a sequence change is truly specific to *C. maximus* or simply not present in the available panel of species. We encountered this problem when a polymorphic site thought to be *C. maximus* specific proved also to be present in some South African *Carcharodon carcharias*, demonstrating the need for more comprehensive screening of any *C. maximus* identification methods in multiple related species and populations, and the need to use multiple polymorphic sites so no single change could result in a false positive.

2.3.1.i. Cytochrome b

Although this test produced initially promising results, providing banding patterns specific to a number of species including *C. maximus*, the presence of the same sized bands for *some* populations of *C. carcharias* rendered the test ambiguous in circumstances where the target species and white sharks from these populations may have to be differentiated; a situation not encountered by other workers with access to less extensive sample resources. Additional sequencing of the Cytochrome b of the aberrant *C. carcharias* samples and a second round of primer design, taking into account these newly identified areas of sequence homology, appears to have resolved this problem. Although the availability of *C. maximus* samples is still limited, an extra step involving addition of a *C. carcharias* specific primer to the mix to discriminate between these two species has also been tested (see Fig. 7). Our large worldwide collection of *C. carcharias* samples allows us to be confident that we have produced a *C. carcharias* specific primer which will work on samples from all geographic regions. All new primers are currently being used in further large scale genotyping of *C. carcharias* and other related species in order to unambiguously confirm their specificity.

2.3.1.ii. Refinement of the mtDNA Cytochrome b molecular identification method

Further development of the mtDNA cytochrome b molecular identification method has eliminated the initial difficulties that were encountered in differentiating between *C. maximus* and *some* populations of *C. carcharias.* This has been accomplished by the expansion of the test to use a third *C. maximus* specific primer, which allows for the production of a more distinct banding pattern (Fig. 8), negating the use of the *C. carcharias* specific primer. This new three-primer method further decreases the chances of false positives compared to the previous two-primer method. Additionally, a further refinement of this test has been to redesign the universal primers to increase the range of species in which they work, thus providing a more stable platform on which to base future genetic identification methods.

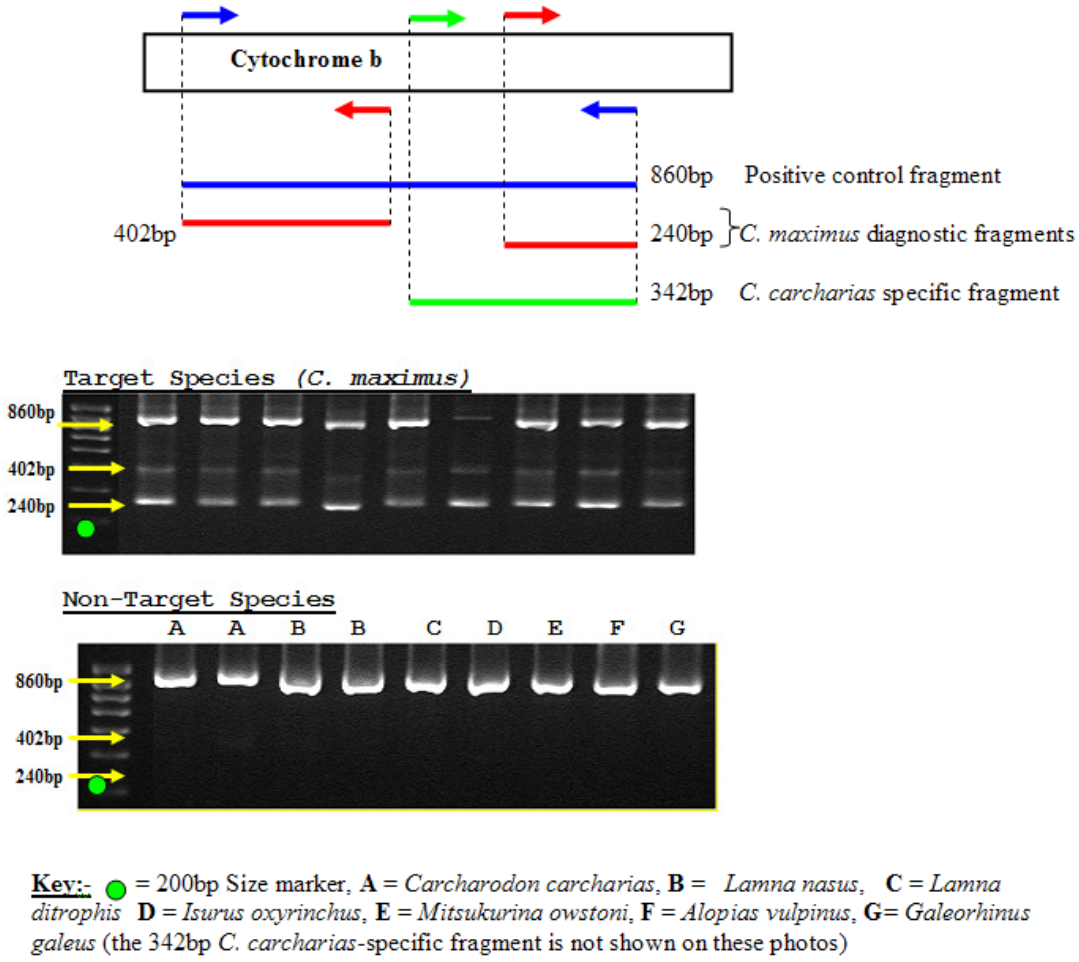


Fig. 7. Development of Basking shark molecular identification using mtDNA cytochrome b.
A set of 4 primers was designed to produce two distinct banding patterns; one pattern (3 bands) indicates the DNA was obtained from *C. maximus* and the other (a single band – or 2 if *C. carcharias*) from all others species. The positive control (blue line) fragment is produced by universal primers designed to regions of high homology (blue arrows) and is used to indicate that the PCR was successful. Two *C. maximus* specific primers (red arrows) were also designed to work in combination with one of the universal primers to produce two separate products. The production of two separate *C. maximus* specific PCR fragments (red lines) increases the confidence with which an identification of *C. maximus* can be made. An additional primer (green) specific to *C. carcharias* has been developed and is undergoing final testing to improve the genetic differentiation between the two species.

Although the availability of *C. maximus* samples is still limited, PCR amplification of cytochrome b has consistently produced all 3 *C. maximus* specific bands, plus the universal positive control band (four bands in total designated pattern A; Fig. 8) in a total of 34 samples collected from the U.K, New Zealand, Italy, Norway, South Africa, Portugal and the United States of America. In addition, the test has produced *only* the universal positive control band (one band designated pattern B; Fig. 8) in 91 *C. carcharias,* 102 *L. nasus* and a wide variety of other shark species collected from all over the world (Table 6). A low percentage (~2%) of non-*C. maximus* samples produced some non-specific banding, however, these were consistently much fainter and of different sizes than the expected bands, and thus could not mistaken for a positive result.

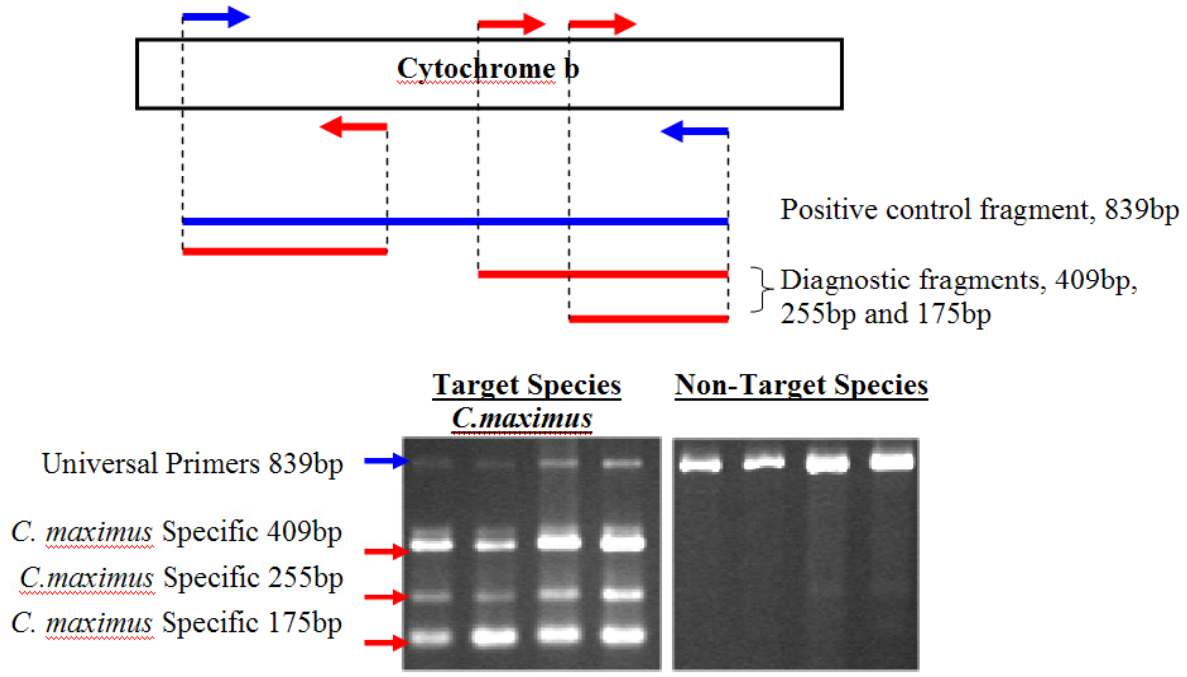


Fig. 8. Final one tube test for molecular identification of basking sharks using cytochrome b.
A set of 5 primers was designed to produce two distinct banding patterns; pattern A (4 bands) indicates the DNA was obtained from *C. maximus* and pattern B (a single band) for all others species. The positive control (depicted by the blue arrow indicating 839bp product) fragment is produced by universal primers designed to regions of high sequence homology (blue arrows) and is used to indicate that the PCR was successful. Three *C. maximus* specific primers (three red arrows indicating fragments of 409bp, 255bp and 175bp) were also designed to work in combination with one of the universal primers to produce the two separate banding patterns. Production of three separate *C. maximus* specific PCR fragments (red lines) increases the confidence with which an identification of *C. maximus* can be made.

As most shark products likely to require investigation are going to have been processed to some extent and will in all likelihood contain tissue from a number of different shark species, we undertook to test our identification method on a simulated 'shark fin soup'. This 'shark fin soup' contained equal amounts of White shark, Porbeagle, Salmon shark, Soupfin shark, Short fin Mako, Tiger shark, Smooth hammerhead, Silky shark, Bull shark, and Megamouth shark DNA. This "soup" was mixed with *C. maximus* DNA ranging from 100% to 1%, in order to test the methods ability to detect *C. maximus* products under various conditions. We were able to accurately detect the presence of *C. maximus* tissue at less than 1ng of DNA and when it accounts for less than 1% of the overall shark DNA (Fig. 9).

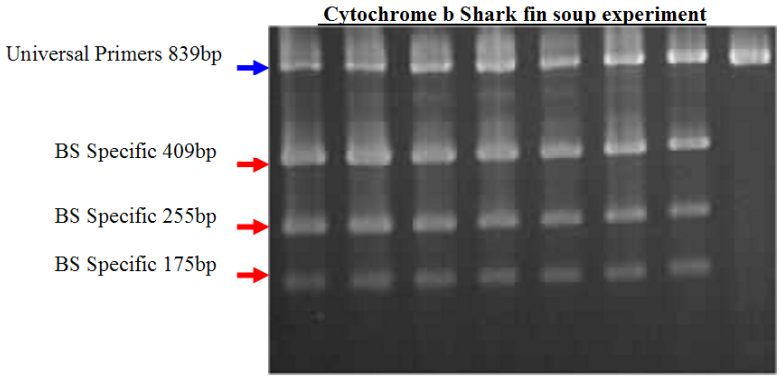
2.3.1.iii. Ribosomal Internal Transcribed Spacer 2 (ITS2)

This gene region proved more problematic than Cytochrome b to produce a primer set for, as no ITS2 sequence has been published for *C. maximus*. Initially, a set of non-species specific primers, first described in Shivji et al (2002), were used to recover and sequence the ITS2 of *C. maximus* samples from Italy, Portugal, Australia and Scotland. These sequences were combined with all available published and additional ITS2 sequences to provide a pool of 10 Carcharhiniform and 16 Lamniform species from which to design primers. A comparison of these sequences identified clear and distinct differences between the two orders spread over extended sections of ITS2, simplifying the process of identifying *C. maximus*-specific motifs.

Table 6. Global distribution of shark species tested for the mtDNA cytochrome b molecular method for identification of *C. maximus* body parts.
Key: BS+ indicates that all 3 *C. maximus* specific bands and the universal positive control are present (pattern A). **BS-** indicates that only the universal positive control band is present (pattern B). n= number of samples examined.

	Common Name	Collected	BS+ (n)	BS- (n)			Common Name	BS+ (n)	BS- (n)
C. maximus	Basking Shark	New Zealand / Australia	9			*S. Etmopterus*	Fringefin Lanternshark		1
		U.K	16			*S. rostratus*	Little Sleeper Shark		1
		Norway	2			*C. Plumbeus*	Sandbar Shark		1
		South Africa	3			*C. Leucas*	Bull Shark		1
		Italy	2			*S. Lewini*	Scalloped hammerhead		1
		Portugal	1			*I. oxyrinchus*	Short fin Mako		1
		New York	1			*G. galeus*	Soup fin Shark		3
			34			*A. Vulpinus*	Thresher Shark		1
						A. profundorum	Deepwater Catshark		1
L. nasus	Porbeagle	Falklands		14		*C. falciformis*	Silky Shark		1
		New Zealand/ Australia		18		*S. Tiburo Tiburo*	Bonnethead Shark		1
		Scandinavia		41		*A. profundorum*	Deepwater Catshark		1
		Canada		16		*C. falciformis*	Silky Shark		1
		U.K		4		*M. pelagios*	Megamouth Shark		1
		Guernsey		9		*C. taurus*	Tiger Shark		1
				102		*S. zygaema*	Smooth hammerhead		1
C.carcharias	White Shark	South Africa		73					
		New Zealand / Australia		12					
		Brazil		1					
		California		3					
		Japan		2					
				91					
L. ditrophis	Salmon Shark	Alaska		14					

A set of 4 primers was designed to produce two distinct patterns of products (Fig. 10). The positive control fragment is produced by universal primers (primers which amplify DNA in many organisms including sharks) designed within the highly conserved 5.8S and 28S ribosomal genes, and used to indicate that the PCR was successful. Two *C. maximus*-specific primers were also designed to work in combination with one of the universal primers and with each other to produce three separate products. This was done to compensate for the fact that one of the *C. maximus* specific primers produces an 1144bp product when combined with a universal primer which is difficult to distinguish from the 1248bp universal band. By combining it with the other *C. maximus* specific primer a more easily distinguishable band of 237bp is produced.



Lane 1	100% Basking Shark (~10ng) and 0% Shark fin soup (~0ng)
2	80% Basking Shark (~8ng) and 20% Shark fin soup (~2ng)
3	50% Basking Shark (~5ng) and 50% Shark fin soup (~5ng)
4	25% Basking Shark (~2,5ng) and 75% Shark fin soup (~7.5ng)
5	10% Basking Shark (~1ng) and 90% Shark fin soup (~9ng)
6	5% Basking Shark (~0.5ng) and 95% Shark fin soup (~9.5ng)
7	1% Basking Shark (~0.1ng) and 99% Shark fin soup (~9.9ng)
8	0% Basking Shark (~0ng) and 100% Shark fin soup (~10ng)

Fig. 9. Utility of the molecular identification test using mtDNA cytochome b. Simulated shark fin soup containing equal amounts of White shark, Porbeagle, Salmon shark, Soupfin shark, Short fin Mako, Tiger shark, Smooth hammerhead, Silky shark, Bull shark, and Megamouth shark mixed with known amounts of *C. maximus* DNA and in specific proportions to contain between 100% and 1% *C. maximus* DNA in order to assess the sensitivity of the test to *C. maximus* products under various conditions. It was possible to accurately detect the presence of *C. maximus* tissue at less than 1ng of DNA, and when it accounts for less than 1% of the overall DNA.

As with the mtDNA cytochrome b test, the ITS2 genetic identification method was tested on all available *C. maximus* samples and consistently produced all 3 *C. maximus* specific bands in 33 samples collected from the U.K, New Zealand, Italy, Norway, South Africa, Portugal and the United States of America. In addition, the test has produced only the universal positive control band in 92 *C. carcharias,* 102 *L. nasus* and a wide variety of other shark species collected from all over the world (Table 7), confirming the specificity of the test. A low percentage (~6%) of non- *C. maximus* samples produced some non-specific banding which was consistently much fainter and of different sizes than the expected bands and thus could not be mistaken for a positive result. The appearance of non-specific bands did increase to ~10% at lower PCR annealing temperatures, but even at very low temperatures it would be extremely difficult to mistake any of these bands for *C. maximus* specific bands, with the exception of one *G. galeus* (Soup fin shark). This specimen produced a banding pattern which at first sight could be confused with the *C. maximus* specific pattern, but when run out against a *C. maximus* sample the bands were shown to be ~100bp different in size. In addition this test allowed the presence of *C. maximus* tissue at less than 1ng of DNA, and when it accounts for less than 1% of the overall shark DNA (Fig. 11), which is consistent with the results obtained for mtDNA cytochrome b test. The development of two independent molecular identification tests from two separate gene regions (one mitochondrial and one nuclear) allows any samples giving ambiguous results from one test to be resolved using the other (Fig. 12).

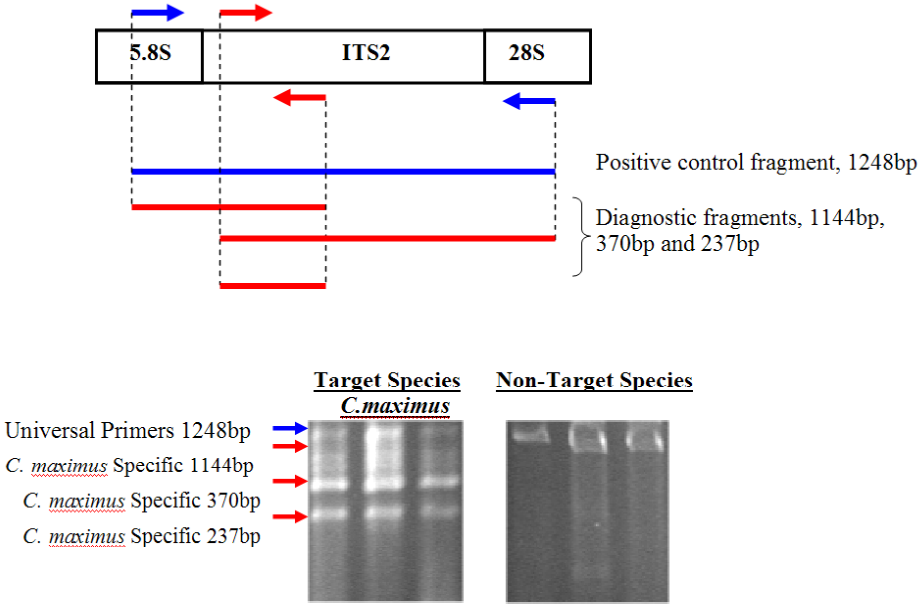
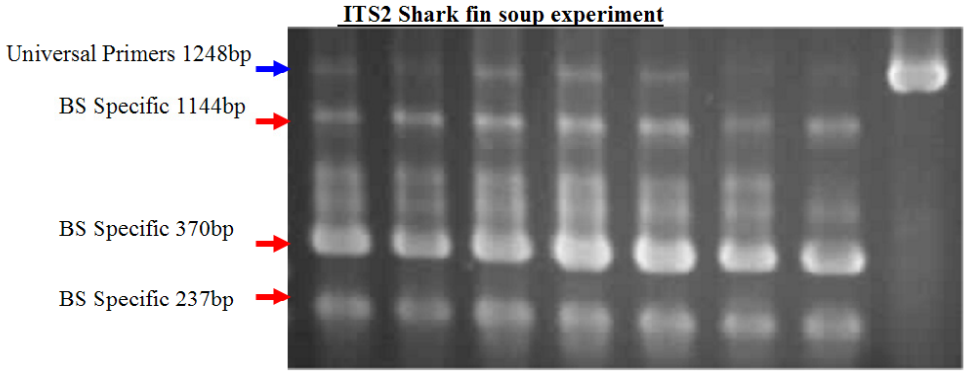


Fig. 10. Final one tube molecular identification test for basking sharks using nuclear ribosomal RNA Internal transcribed Spacer region 2 (ITS2). A set of 4 primers was designed to produce two distinct patterns of products; Non-target shark species produce a single band (1248 bp) and basking sharks (the target species) produces a pattern of 4 bands (1248bp, 1144bp, 370bp and 237bp). The positive control (blue line) fragment is produced by universal primers (blue arrows) designed within the highly conserved 5.8S and 28S ribosomal genes and is used to indicate that the PCR was successful and is the only band produced in non-target species, whereas it is one of the four bands produced in basking shark samples. Two *C. maximus*-specific primers (red arrows) were also designed to work in combination with one of the universal primers to produce two separate products. The production of three separate *C. maximus* specific PCR fragments (red lines) increases the confidence with which an identification of *C. maximus* can be made.



Lane 1	100% Basking Shark (~10ng) and 0% Shark fin soup (~0ng)
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5	10% Basking Shark (~1ng) and 90% Shark fin soup (~9ng)
6	5% Basking Shark (~0.5ng) and 95% Shark fin soup (~9.5ng)
7	1% Basking Shark (~0.1ng) and 99% Shark fin soup (~9.9ng)
8	0% Basking Shark (~0ng) and 100% Shark fin soup (~10ng)

Fig. 11. Simulated shark fin soup experiment illustrating the utility of the molecular identification test using nuclear ribosomal internal transcribed spacer gene region 2 (ITS2). Simulated shark fin soup containing equal amounts of White shark, Porbeagle, Salmon shark, Soupfin shark, Short fin Mako, Tiger shark, Smooth hammerhead, Silky shark, Bull shark, and Megamouth shark mixed with known amounts of *C. maximus* DNA and in specific proportions to contain between 100% to 1% *C. maximus* DNA in order to test the methods ability to detect *C. maximus* products under various conditions. It was possible to accurately detect the presence of *C. maximus* tissue at less than 1ng of DNA, and when it accounts for less than 1% of the overall shark DNA.

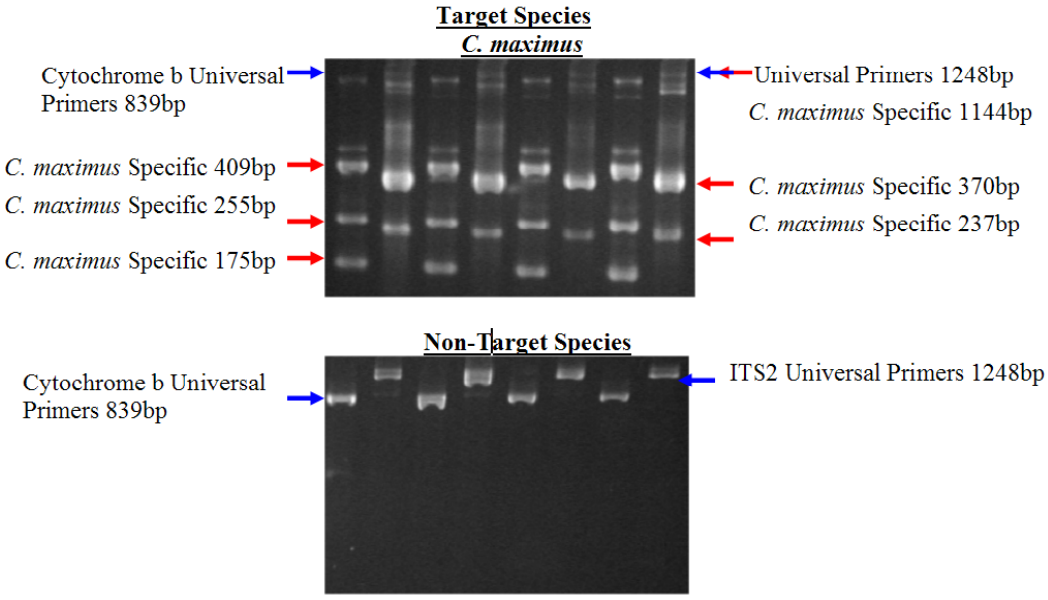

Fig. 12. Full molecular identification method (two tube test) using both gene regions ITS2 and cytochrome b, which uses a total of 5 different *C. maximus*-specific primers, and 4 universal primers. Top gel image illustrates the distinctive 4 product banding patterns produced for each gene for basking sharks, the target species; lanes 1, 3, 5 and 7 - mtDNA cytochrome b gene with product sizes bands 1248bp, 1144bp, 370bp and 237bp; lanes 2, 4, 6 and 8 ITS2 gene with product sizes 839bp 409bp, 255bp and 175bp; Basking shark samples are from Weymouth, UK, lanes 1-2, Norway, lanes 3-4, Portugal, lanes 5-6 and New Zealand, lanes 7-8. The bottom gel depicts the distinctive one band pattern produced for each gene for non-target species including white sharks lanes 1-2, mako, lanes 3-4, megamouth shark lanes 5-6 and tiger shark lanes 7-8. Lanes 1, 3, 5 and 7 show the 1248bp universal product for mt DNA cytochrome b gene and lanes 2, 4, 6 and 8 show the 839bp universal product for ITS2 gene. The use of the two gene regions significantly increases the confidence of a positive identification for basking shark parts or derivatives, especially on minute degraded samples.

Table 7. Global distribution of species tested for the ITS2 method for the identification of *C. maximus* body parts. Key:- BS+ indicates that all 3 *C. maximus* specific bands and the universal positive control are present. BS- indicates that only the universal positive control band is present. N= number of samples examined.

	Common Name	Collected	BS+ (n)	BS- (n)			Common Name	BS+ (n)	BS- (n)
C. maximus	Basking Shark	New Zealand / Australia	8			*S. Etmopterus*	Fringefin Lanternshark		1
		U.K	16			*S. rostratus*	Little Sleeper Shark		1
		Norway	2			*C. Plumbeus*	Sandbar Shark		1
		South Africa	3			*C. Leucas*	Bull Shark		1
		Italy	2			*S. Lewini*	Scalloped hammerhead		1
		Portugal	1			*I. oxyrinchus*	Short fin Mako		1
		New York	1			*G. galeus*	Soup fin Shark		3
			33			*A. Vulpinus*	Thresher Shark		1
						A. profundorum	Deepwater Catshark		1
L. nasus	Porbeagle	Falklands		14		*C. falciformis*	Silky Shark		1
		New Zealand/ Australia		18		*S. Tiburo Tiburo*	Bonnethead Shark		1
		Scandinavia		41		*A. profundorum*	Deepwater Catshark		1
		Canada		16		*C. falciformis*	Silky Shark		1
		U.K		4		*M. pelagios*	Megamouth Shark		1
		Guernsey		9		*C. taurus*	Tiger Shark		1
				102		*S. zygaema*	Smooth hammerhead		1
C.carcharias	White Shark	South Africa		74					
		New Zealand / Australia		12					
		Brazil		1					
		California		3					
		Japan		2					
				92					
L. ditrophis	Salmon Shark	Alaska		14					

- We have developed a panel of primers for two gene regions (one mitochondrial and the other nuclear) that allows the accurate and unambiguous identification of parts/derivatives of basking sharks, at extremely low concentrations (<1% and <1ng).

- The test developed with this panel is a significant improvement over existing ones as it makes use of more gene regions to produce a range of products, so avoiding false negatives and providing in built redundancy – an essential feature in litigation proceedings.

3. Conclusions

3.1 Field surveys and tissue samples

A limited collection of basking shark tissue samples from SW England and NW Scotland had been collected prior to the start of the project and these were used to develop the requisite molecular tools. However, samples collected at different times, even from the same area, might represent different populations differing substantially in their genetic diversity and similarity. Therefore a full assessment of the population genetic structure of UK basking sharks requires representative population samples. Ideally these should consist of biopsy samples recovered from 10-20+ individuals, all at approximately the same time (or at least in the same season) and from the same area, to obtain reliable estimates of population genetic structure with which to address the important issues of basking shark population differentiation and ecology in UK waters. Such population samples form a baseline against which the similarity of further samples can be compared for exclusion or inclusion to those populations.

To this end, designated ship-borne surveys were undertaken off SW England in the summers of 2004 and 2005. However, despite considerable effort (163.5 h in 2004 and 130 h in 2005) the unexpectedly low occurrence of basking sharks (only 11 sharks observed over both years) at the sea surface off the southwest of the UK has prevented us from obtaining the necessary collection of tissue samples to obtain reliable estimates of population genetic structure.

Plankton samples taken during our surveys (currently being analysed) indicate low abundance of surface zooplankton in the survey area and it is possible that this may account for the unexpectedly low numbers of basking sharks at the surface off Plymouth during the study.

Although surveys have not provided the required population samples to conduct a detailed assessment of the population genetic structure of UK basking sharks as planned, a substantial library of 41 basking shark tissue samples from the UK (the south-west, Wales and Scotland), Italy, Portugal, South Africa, USA, Canada, Australia and New Zealand has been assembled. These have been used to develop the molecular genetic tools necessary to conduct such a study in the future.

3.2 Molecular Genetics

Development and characterization of the appropriate molecular genetic tools has been successful, providing resources that may be used to obtain reliable estimates of population genetic structure and address the proposed issues of basking shark population differentiation and ecology. We have successfully isolated many microsatellite loci specific to basking sharks using an in-house modified enrichment protocol and characterized 10 polymorphic loci; in addition to 8 polymorphic loci from non-focal species. In total, 18 microsatellite loci are now available for analysis of basking shark samples, which should provide sufficient loci to investigate population structure, relatedness of basking shark coalitions and paternity issues. Once sufficient population samples of basking sharks are available we are confident that the three-pronged approach for the analysis of molecular variation (SSR genotyping, and SSR flanking region and mtDNA marker sequencing) will enable high-resolution inference of intra-specific evolutionary history. These approaches will provide complementary data that can be rigorously analyzed using modern, powerful statistical approaches.

Utility of microsatellites was proven using available samples allowing estimation of the grosser levels of population structure, suggesting little gene flow between populations of the northern and southern hemispheres.

Analyses of two complete mtDNA gene regions (cytochrome b and the D-loop) have shown sufficient variation is available for population differentiation by their combined use. A simplification of this approach promises an inexpensive, rapid and simple assay of mitochondrial variation to differentiate basking shark populations to quantify female mediated gene flow.

We have developed a protocol to allow recovery of DNA from forensic quantities of shark tissue. This will have a significant impact on future molecular work in this and other shark species for which samples are scarce, allowing utilization of badly degraded and minute tissue samples.

To facilitate enforcement of CITES regulations we designed a panel of primers for two gene regions (one mitochondrial and the other nuclear) that allow accurate and unambiguous identification of basking sharks parts at extremely low concentrations (<1% and <1ng). By using more gene regions than previous approaches this test

represents a significant improvement, avoiding false negatives and providing built in redundancy, essential features of evidence provided for litigation.

4. Recommendations and further work

The unexpectedly low occurrence of basking sharks at the sea surface off the southwest of the UK has prevented us from obtaining the necessary collection of tissue samples to obtain reliable estimates of population genetic structure and address the important issues of basking shark population differentiation and ecology in UK waters. Nonetheless, the project has provided a powerful suite of molecular genetic tools that are now available to conduct such an analysis rapidly and inexpensively and further support should be sought to conduct such a study. This will enable us to be confident that we know what part of the north-east Atlantic population the basking sharks present around Britain represent.

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6. Outputs.

The following scientific papers are in an advance state of preparation for publication in peer-review scientific journals.

SARGINSON, J., JONES C.S., SIMS, D., METCALFE, J., SHIVJI, M.S., & NOBLE, L.R. A BI-ORGANALLE SPECIES-SPECIFIC PCR IDENTIFICATION TEST FOR CONSERVATION AND TRADE MONITORING OF THE ENDANGERED BASKING SHARK *CETORHINUS MAXIMUS.* *(to be submitted to Conservation Genetics)*

Sarginson, J., Jones C.S., Sims, D., Metcalfe, J., & Noble, L.R. Polymorphic microsatellites for the endangered basking shark *Cetorhinus maximus* and cross species amplification in lamniformes. *(to be submitted to Molecular Ecology Notes).*

Sarginson, J., Gubili, C., Jones C.S., Sims, D., Metcalfe, J., & Noble, L.R. Recovering genotypes from forensic material for rare and endangered sharks. *(to be submitted to Molecular Ecology Notes).*

Appendix I

Review of the biology and ecology of the basking shark (*Cetorhinus maximus*).

Note: This is a revised and updated version of the review originally prepared in May 2002 as part of an earlier project for Defra (CR0247) on basking shark population assessment.

Review of the biology and ecology of the basking shark (*Cetorhinus maximus*)

D.W. Sims, Marine Biological Association, The Laboratory, Plymouth, U.K.

Original draft, March 2002; Updated, March 2006

1. Introduction

The majority of over 400 species of shark are macropredators and scavengers, while only three species obtain food by filtering seawater. These however, are among the largest living sharks, and among marine vertebrates only whales are larger. The basking shark (*Cetorhinus maximus*) is the second largest known fish species attaining lengths approaching 10m and a weight of approximately 4 tonnes. This species is greater in size than the rare megamouth (*Megachasma pelagios*), but smaller than the whale shark (*Rhincodon typus*) of tropical regions.

Organised fisheries for basking shark have existed in the north-east Atlantic region since at least two hundred years ago (McNally, 1976; Fairfax, 1998). Indeed, the earliest directed fisheries for pelagic shark were probably for this species (Pawson & Vince, 1999). Despite the commercial interest, little is known generally about the biology of the basking shark, although recent advances in tracking technology have allowed considerable progress to be made in identifying movements and behaviour. In this respect, a major discovery has been their whereabouts and activity patterns during winter (Sims et al. 2003b).

There have been numerous reviews of the literature available for basking shark since Kunzlik's (1988) thorough treatment, principally as part of proposals seeking to list the species on international conservation treaties (e.g. Convention on International Trade in Endangered Species (CITES), Convention on the Conservation of Migratory Species of Wild Animals (usually referred to as the Convention for Migratory Species, (CMS) or the Bonn Convention). Significant new scientific information has been added, particularly in the last five years (see Fig. 1). Therefore, the purpose of this review is to present a full description and interpretation of scientific results obtained to date with inclusion of information from "grey" literature sources where appropriate.

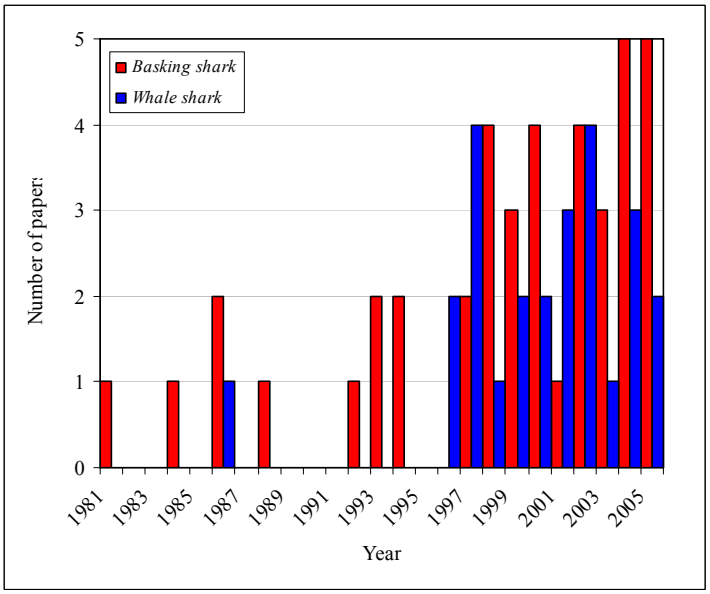

Fig. 1 *The number of peer-reviewed scientific articles by year that cite Cetorhinus maximus (basking shark) or Rhincodon typus (whale shark) in the title and abstract. Source of data: Web of Science.*

2. Description of the species

2.1 Taxonomy

The basking shark was first scientifically described and named *Squalus maximus* (literally 'largest shark') by Gunnerus in 1765. As *Squalus* was a catch-all genus for cartilaginous fish generally, Blainville in 1816 erected a new sub-genus of *Squalus* named *Cetorhinus* (literally 'whale shark'). There were many objective synonyms of *Squalus* (*Cetorhinus*) *maximus* between 1765 and 1960, including *Halsydrus pontoppidani*, *Squalus pelegrinus*, *Squalus peregrinus*, *Squalus rhinoceros* and *Cetorhinus maximus forma infanuncula* (Compagno, 2001). For example, the latter name was erected by Van Deinse and Adriani (1953) to describe a putative subspecies of basking shark which they found to lack filtering gill-rakers. This proposition was successfully refuted by Parker and Boeseman (1954) from observations that basking sharks shed gill-rakers on an apparently seasonal cycle. Despite attempts to erect subspecies, especially for individuals found between different ocean basins, it is generally considered that there is only a single species of basking shark. Springer and Gilbert (1976) rejected the concept of at least four species subdivided on the grounds of differences in body proportions between individuals in the north Atlantic/Mediterranean, south Atlantic and waters around Australia. As such differences occur naturally during growth, it was considered that separation into species merely reflected these differences and was therefore insufficient evidence for division (Springer & Gilbert, 1976; Kunzlik, 1988).

The basking shark, *Cetorhinus maximus*, is the only species placed within the family Cetorhinidae, which is considered a sister group to Lamnidae (Compagno, 1990; Martin & Naylor, 1997). These families constitute two of the seven placed within the order Lamniformes (mackerel sharks) (Compagno, 2001). Lamniformes is one of eight orders of shark within Class Chondrichthyes (subclass Elasmobranchii). The interrelationships of shark taxa including those species within Lamniformes is not without controversy. Maisey (1985) argued that the megamouth shark, *Megachasma pelagios* should be included within Cetorhinidae on account of its similarities with *C. maximus* jaw suspension and dental array, rather than forming a new monotypic family (Megachasmidae) as proposed by Taylor *et al*. (1983). However, as noted in Dulvy and Reynolds (1997), the cladistic phylogeny of the monophyletic Lamniformes (Compagno, 1990) is consistent with the molecular phylogenies of Martin *et al*. (1992) and Naylor *et al*. (1997). Furthermore, recent molecular analysis of cytochrome *b* gene sequences implies independent origins of filter-feeding within Lamniformes, and hence argues against *C. maximus* and *M. pelagios* forming sister taxa within Cetorhinidae (Martin & Naylor, 1997) (Fig. 2).

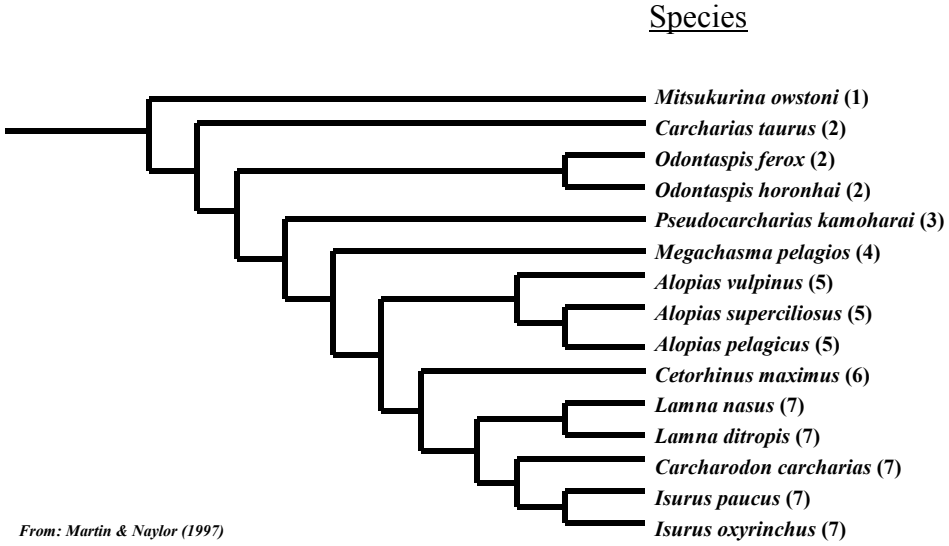

Fig. 2 *Interrelationships of species within Lamniformes derived from molecular data (from Martin & Naylor, 1997) and which is consistent with the phylogeny derived from cladistic analysis (Compagno, 1990). The same number beside species names denotes placement within the same family: 1, Mitsukurinidae; 2, Odontaspididae; 3, Pseudocarchariidae; 4, Megachasmidae; 5, Alopiidae; 6, Cetorhinidae; 7, Lamnidae.*

2.2 Morphology and structure

The basking shark is a large-bodied fish with a fusiform body shape. Detailed general descriptions of external morphology and internal anatomy are given in Matthews and Parker (1950) and which are summarised in the review by Kunzlik (1988). Nothing needs to be added to these treatments here other than to provide the reader with a brief overview of the species field marks that are of particular interest, and to describe the differences in fin dimensions between juvenile and adult individuals.

The colour of the body surface varies in descriptions, from black to dark grey through slate grey to brown (Matthews & Parker, 1950; Kunzlik, 1988). When observed in sunlight in its natural habitat, basking sharks appear grey-brown with lighter dappled or irregular longitudinal patterns along its lateral surface (Fig. 3a). When dead and out of water, basking sharks appear slate or dark grey-black (Fig. 3b). The variations in body colour reported may therefore reflect changes due to death and/or removal from water (Kunzlik, 1988).

The large body size is a feature that helps distinguish this shark from all others (Matthews & Parker, 1950; Compagno, 2001). Basking sharks have been credited with maximum total lengths between 12.2 and 15.2 m (Compagno, 2001), whilst theoretical maxima have been given as 12.76 and 13.72 m (Parker & Stott, 1965; Kunzlik, 1988). Compagno (2001) states that even if these are correct, most specimens do not exceed 9.8 m total length. However, the longest reliable measurement of a shark caught in static fishing gear in Newfoundland was found to be a 12.2-m long male (Lien & Fawcett, 1986). Consequently, the basking shark is the second largest shark species (elasmobranch, and fish-like vertebrate) in the world after the whale shark (*R. typus*). The body mass of basking sharks in relation to total length is not well known on account of the difficulties associated with weighing large specimens. Maximum body masses of 5-6 tonnes have been ascribed to adult sharks in popular accounts. However, two Californian specimens measuring 8.5 and 9.1 m total length weighed 2991 and 3909 kg respectively (Bigelow & Schroeder, 1948). The body mass of an 8.3-m total length female shark taken

off Florida was found to be 1980 kg (Springer & Gilbert, 1976), and a 6.0 m individual from Scotland weighed approximately 2000 kg (Stott, 1980). An adult female and an adult male basking shark of 6 to 7 m length taken off Plymouth weighed 1678 and 1924 kg respectively (Bone & Roberts, 1969). Kruska (1988) measured the mass of a 3.75 m long specimen to be 385 kg.

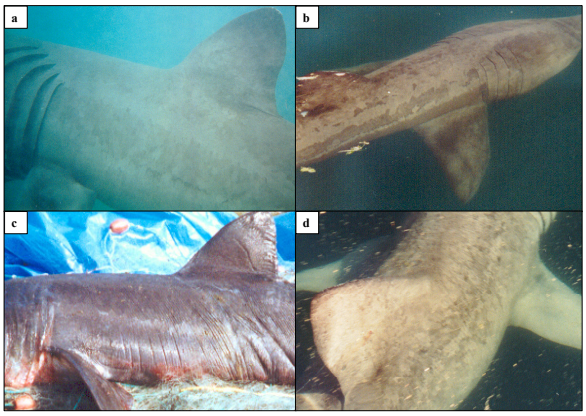

Fig. 3 *Photographs of four different basking sharks to illustrate the variation in colouration and patterning among living (a, b, d) and dead (c) specimens.*

Basking sharks have correspondingly large fins and a caudal peduncle with strong lateral keels. The first dorsal fin measured in an adult female of 8.3 m total length (L_T) was 1.1 m in height (Springer & Gilbert, 1976). The pectoral fins were similar in length (~1.3 m) to the first dorsal fin height, whereas the leading edge of the caudal fin was 1.7 m in length. In contrast, the length of the pectoral fins of a 2.6-m L_T immature female *C. maximus*, was nearly twice that of the dorsal fin height (0.22 m), whereas the leading edge of the caudal fin was nearly 0.7 m long (Izawa & Shibata, 1993). Presumably these differences in fin proportions relate to ontogenic changes in gross morphology. A table of fin measurements for 13 individuals ranging in total length from 2.6 to 8.5 m, together with a table of relative differences in fin dimensions from three of these individuals, are given in Appendix 1.

In addition to the pointed snout and huge sub-terminal mouth, there are minute hooked teeth (~5 mm in height) arranged in three to seven functional rows on the upper and lower jaws respectively (Matthews & Parker, 1950). The teeth are modified placoid dermal denticles. Small denticles of the normal type point posteriorly over the entire skin surface which is also covered with a dark-coloured mucus to the level of the summits of the denticles (Matthews & Parker, 1950). This mucus has been deposited on ropes (used to deploy plankton nets) by basking sharks as they brush past them during normal swimming (D.W.Sims, unpublished observations). Taking skin swabs of this mucus may be an effective, non-invasive method for obtaining basking shark DNA in future studies, but has yet to be achieved successfully.

Basking sharks are also typified by their enormous gill slits that virtually encircle the head (Fig. 3a). The five gill slits on each side of the pharyngeal area are openings between the gill arches upon which there are two distinct structures: the gill lamellae that enable respiration by the exchange of oxygen with seawater and, anteriorly, the gill rakers which are comb-like structures arranged in a single row along the distal portion of each gill arch. When the mouth is open, two rows of gill rakers on separate gill arches extend across each gill-slit gap and act to filter zooplankton prey from the continuous flow of seawater produced by forward swimming (so-called ram-filter feeding). The rakers are erected when

the mouth opens by contraction of the muscle on the aboral half of the foot of the raker, and are held in position against the water flow by elastic fibres in the connective tissue strip (Matthews & Parker, 1950). When the slits are closed the rakers lie flat against the surface of the arches. The gill rakers are about 0.1 m long in the centre of the gill arch and the inter-raker distance is about 0.8 mm in an adult specimen (Matthews & Parker, 1950). Early anatomical investigations of summer and winter-caught specimens suggest that the gill rakers are shed in late autumn or early winter, re-grow through the winter and erupt through the gill-arch epidermis in late winter/early spring, in time for seasonal feeding (Parker & Boeseman, 1954; Matthews, 1962). However, recent re-appraisal of these data indicate gill-raker shedding is by no means ubiquitous; basking sharks with gill-rakers present and food in their stomachs are known during winter (Sims, 1999; see section 4.3).

The liver of the basking shark is large and makes up between 15-25 % of its body weight (Kunzlik, 1988). The hydrocarbons of zooplankton pass through the basking shark alimentary canal without fractionation or structural modification, and are resorbed in the spiral valve and deposited in the liver (Blumer, 1967). Even though squalene is present only in traces in zooplankton, it is abundant in the liver of basking sharks, which contains between 11.8 and 38.0% squalene (Blumer, 1967; Kunzlik, 1988). The liver functions as both an energy store and as a hydrostatic organ for increasing static lift (Bone & Roberts, 1969; Baldridge, 1972).

The skeleton of the basking shark is cartilaginous with varying degrees of calcification throughout, but like that of other sharks, these structures are not ossified (Kunzlik, 1988). The paired sexual organs (claspers) of male sharks are located ventrally at the base of the paired pelvic fins and these become progressively calcified with maturity. Sharks have been aged by counting growth zones visualised in structures such as dorsal spines and vertebral centra (for review see Cailliet, 1990). These growth zones are comprised of opaque bands that have cells with high concentrations of calcium and phosphorus and translucent bands that are less mineralised (Yudin & Cailliet, 1990). Species such as the blue shark appear to deposit alternate dark and light concentric rings annually (Stevens, 1975), but quantifying age at length has been verified in fewer than 10 species (Cailliet, 1990). The number of rings present in the vertebra centra of basking sharks varies along an individual's body length and so ageing this species has proved problematic (Parker & Stott, 1965). Recent progress has been made, however, in ageing filter-feeding whale sharks using X-radiography of vertebral centra (Wintner, 2000).

3. Distribution and habitat

3.1 Total area

The basking shark is a coastal-pelagic shark known to inhabit the boreal to warm-temperate waters of the continental and insular shelves circumglobally. It has been recorded in the western Atlantic from Newfoundland to Florida and from southern Brazil to Argentina (Wood, 1957; Compagno, 2001; Tomas & Gomes, 1989). In the eastern Atlantic *C. maximus* is present from Iceland, Norway and as far north as the Russian White Sea (southern Barents Sea) extending south to the Mediterranean, and in the Southern Hemisphere from the western Cape province and South Africa (Konstantinov & Nizovtsev, 1980; Compagno, 2001). They are also present in the Pacific Ocean; from Japan, the Koreas, China, Australia (south of 25^{o}N) and New Zealand in the west, and from the Gulf of Alaska to Baja California, Peru and Chile in the east (Compagno, 2001). The basking shark has been recorded primarily from coastal areas, though this may not represent its entire habitat range as distribution throughout the epipelagic zone of ocean basins is possible. However, sightings data away from coastal areas are lacking, which could indicate either 'hidden' abundance at depth in oceanic regions, or a general lack of basking sharks away from productive coastal zones. Therefore, our knowledge of the total area distribution of this species may be limited at present.

3.2 Differential distribution

Although population segregation by size and sex is a general characteristic of shark species worldwide (Klimley, 1987; Sims, 2005), there is no clear evidence to indicate differential distribution in the basking shark. Juvenile (2-3 m total length, L_T) and putative sub-adult (3-5 m L_T) sharks have been frequently observed in the same areas and summer feeding aggregations as adults (Berrow & Heardman, 1994; Sims et al., 1997). However, there was some indication that juveniles and sharks <3 m L_T appeared to feed later at the surface in the summer compared to larger individuals (Sims et al., 1997), which may reflect habitat segregation. However, this may have been driven by biotic factors, such as zooplankton abundance, rather than age-segregated distribution or migration *per se*. In the years since the observations of Sims et al. (1997) were made, a shift towards smaller-sized sharks off Plymouth as the summer progresses has been less obvious (D.W. Sims, unpublished observations).

Similarly, sexual segregation of the population has not been shown unambiguously. Males and females have been observed in the same areas during summer (Matthews & Parker, 1950; Maxwell, 1952; O'Connor, 1953; Watkins, 1958; Sims et al., 2000), although more females than males have been caught in directed fisheries (Kunzlik, 1988) suggesting females may segregate from males, at least when they occur at the surface. Pregnant females are virtually unknown from these same locations so differential habitat utilisation by mature males and females at certain times in the reproductive cycle may well occur.

4. Bionomics and life history

4.1 Reproduction

Matthews (1950) gives a detailed account of reproduction in the basking shark based upon macro and microscopic anatomical investigations of dissected specimens from Scotland. To summarise the main points of interest briefly, Matthews (1950) suggests the basking shark is ovoviviparous, that is, live young are produced from eggs that hatch within the body. This mode of reproduction is common among large-bodied elasmobranchs, including the whale shark (Joung et al., 1996). In female basking sharks only the right ovary is functional, and may contain at least six million ova each about 0.5 mm in diameter (Matthews, 1950), presumably to provision oophagous foetuses for the entire gestation period. Smaller numbers of more heavily yolked ova are more commonly found in sharks (Kunzlik, 1988). Fertilisation in the basking shark, as in all other sharks is internal: the intromittent organs (claspers) are inserted via the female's cloaca into the vagina and transfer large quantities of sperm packets or spermatophores. In male basking sharks, spermatophores are up to about 3 cm in diameter, each with a core of sperm and a firm translucent cortex. The spermatophores float in a clear seminal fluid and Matthews (1950) estimates that about 18 litres of them are transferred to the female during mating. The period of gestation is not known with any certainty, but estimates as high as 3.5 years have been proposed (Parker & Stott, 1965), although a period of just over one year has been estimated from the same length-frequency data (Holden, 1974). There is only one published record of a pregnant female being captured despite organised fisheries for basking sharks in the northeast Atlantic dating back at least two hundred years. According to this single account, a female basking shark was caught in August 1936 off the mid-western coast of Norway and towed into Teigboden (Sund, 1943). Whilst being towed the shark gave birth to six pups, each about 1.5-2.0 m L_T, five of which began swimming open-mouthed at the surface, presumably feeding. The sixth pup was stillborn. Therefore, if this number of pups is representative of normal parturition rates, it seems the basking shark exhibits low fecundity even when compared to other relatively large-bodied ovoviviparous sharks (Compagno, 2001; Sims, 2005).

4.2 Growth and Maturity

Male basking sharks are thought to become sexually mature between 5–7 m, at ages between 12 and 16 years, whereas females mature at 8.1–9.8 m and possibly 16–20 years (Compagno, 2001). Maximum length is not known precisely, although 10–12 m appears to be a maximum, with individuals between 9.8 and 12.2 m having been reported (Parker & Stott 1965; Lien & Fawcett 1986). Matthews (1950) and Matthews and Parker (1950) observed mature males at lengths between 6.8 and 8.1 m. Rapid increase in male clasper length occurred between 6.0 and 7.5 m body length with little change thereafter (Francis & Duffy, 2002).

The growth rate of basking sharks is not known exactly, but has been estimated to be 0.4 m per year (Pauly 1978, 2002). Attempts to estimate age of basking shark have used two methods: (1) Length-frequency analysis has been used to derive length-at-age growth curves (Matthews, 1950; Parker & Boeseman 1954; Parker & Stott 1965), and (2) vertebral centra analysis has been used to relate observed numbers of 'age rings' to measured body length (Parker & Stott, 1965). For the length-frequency analysis, which attempts to relate successive modes in the length-frequency distribution with successive age groups, measurements of 93 fishery-caught individuals from the north-east Atlantic were used. These data resulted in suggestions that a size of not < 2 m length was typical of the first summer, a mean size of 3.09 m was attained in the following summer and that the mean size in the next winter was 3.52 m (Parker & Stott, 1965). This growth increment of 0.43 m was assumed to represent half a years growth. From these empirical data and using the assumptions that growth was asymptotic and best described by a von Bertalanffy growth function, that the length at parturition was 1.5 m L_T, and that the maximum length asymptote was 11.0 m L_T, Parker and Stott (1965) derived a growth curve for *C. maximus*. The growth curve indicates that a 5-m long shark will be about 4 years old whereas a 9 m-long individual will be at least 12.5 years old. More recent studies by Pauly (1978, 2002) however, cast some doubt on these estimates. Pauly (1978) argued that because basking sharks were thought to lose their gill rakers and could therefore not feed during colder months the annual growth was 0.43 m. The von Bertalanffy relationship based on this assumption obviously describes slower growth, with a 3.75-m long shark being at least 5 years old (Pauly,1978). Based on this derived relationship Pauly (1978) estimated longevity at about 40-50 years. Clearly new data showing basking sharks remain active in winter consistent with foraging (Sims et al., 2003) suggest this assumption about a food-limited growth increment may need to be re-assessed. Unfortunately, there are very few re-sightings of individual basking sharks to validate growth increases. An opportunistic re-sighting of a female shark by Sims *et al.* (2000) showed that this 5.0-m long shark had apparently increased in total body length by between approximately 1.4 - 2.4 m in just over 3 years. There is a need for new photo-identification studies to verify growth increments over time of individual basking sharks of different sizes.

Validation of age-at-length using growth rings in verebral centra of basking sharks has proved difficult because the number of rings apparently decreases caudally suggesting uneven laying-down of rings, and as a function of body length and with respect to time (Parker & Stott, 1965). Furthermore, there appear to be seven rings present at birth (Parker & Stott, 1965). This led Parker and Stott (1965) to suggest that basking sharks lay down two growth rings per year, a rate which was consistent with their von Bertalanffy growth curve (see above). Parker and Stott (1965) showed that vertebral centra of basking sharks between 3.5 and 5.5 m total body length contained 9–16 rings, whereas those from 7.5 to 9.0 m L_T possessed between 26 and 32 rings. The latter authors suggested for basking sharks that 2 opaque bands were deposited per year perhaps as a function of increased somatic growth during the two main periods of plankton productivity in temperate waters. Although this idea of two growth rings per year and seven at birth has been repudiated recently by Pauly (2002), the suggestions of Parker and Stott (1965) are not entirely without foundation. The Pacific angel shark (*Squatina californica*) has 6 or 7 bands present in the vertebral centra at birth and up to 42 in the largest adults (Natanson & Cailliet,

1990). It was demonstrated that these bands were not deposited annually as they are in some species (e.g. *Prionace glauca*; Stevens, 1975; Cailliet, 1990), but deposition was related to somatic growth. Nonetheless, Pauly (1978) demonstrates that von Bertalanffy growth curves derived separately from the 0.43 m growth increment, and from growth-ring data at a deposition rate of one band per year, are approximately equal. This is further supported by re-analysis using modern methods (Pauly, 2002). Despite these re-analyses and interpretation of existing data described above, there has been no contemporary work to progress age determination in basking sharks to identify growth rates.

4.3 Food and feeding

The basking shark feeds upon zooplankton prey it captures by forward swimming with an open mouth so that a passive water flow passes across the gill-raker apparatus. Unlike the megamouth and whale sharks that may rely upon suction or gulp feeding to capture swarms of zooplankton (Diamond, 1985; Clark & Nelson, 1997), the basking shark is an obligate ram filter-feeder. But exactly how the particulate prey is filtered remains unresolved. It has been assumed that the erect gill-rakers filter particulate matter of a suitable size from the passive water flow directly, that is, like a 'mechanical sieve' (dead-end filter) (Matthews & Parker, 1950; Kunzlik, 1988; for review see Gerking, 1991). Apparently, when the mouth closes the rakers collapse on the gill arches and deposit zooplankton onto mucus that is produced in vast quantities by cells at their base (Matthews & Parker, 1950). However, the gill rakers are very thin, stiff bristles so it is not easy to see how these function to retain plankton on their surfaces, because zooplankton are similarly of small diameter and unlikely to adhere to them as the rakers contain no mucus-producing cells. It seems reasonable to assume that the small gap between the rakers (the inter-raker distance), which is about 0.8 mm in adults, could prevent particulate prey from passing through. However, basking sharks only swallow plankton every 30 to 60 secs (Hallacher, 1977; D.W. Sims, unpublished observations) so it remains unclear how plankton is retained and trapped in position without loss for this length of time before swallowing. A recent study of filter-feeding in small-bodied teleost fish suggests instead that rakers function as a crossflow filter (Sanderson et al., 2001). Particles are not retained on rakers but are concentrated in the oral cavity towards the oesophagus as water exits between the rakers. Apparently the crossflow prevents particles from clogging the gaps between the rakers (Sanderson et al., 2001). Further study of the fluid dynamics in basking shark models may elucidate a similar system.

Even though the actual mechanics of filter-feeding in basking sharks remains unknown, the prey captured by them has been recorded for several specimens. Post mortem studies on basking shark stomachs show that off Scotland calanoid copepods were generally the predominant prey group (Matthews & Parker, 1950; Watkins, 1958). Matthews and Parker (1950) found *Calanus* and other copepods, in addition to fish eggs, cirripede and decapod larvae. Records of the copepods *Oithona*, *Calanus*, and *Pseudocalanus* have also been made from basking shark stomachs (Sproston, 1948 cited in Matthews & Parker, 1950). The main zooplankton species identified from shark feeding areas in the English Channel off Plymouth were *Calanus helgolandicus*, *Pseudocalanus elongatus*, *Temora longicornis*, *Centropages typicus* and *Acartia clausi* (Sims & Merrett, 1997). The density of total zooplanktonts counted from samples taken in shark feeding areas was about 2320 per cubic metre (Sims, 1999). The density of calanoid copepods ranged from 1050 to 1480 per cubic metre with *C. helgolandicus* of 2 mm mean length making up about 70% of this total by number (Sims & Merrett, 1997). Mysid larvae, decapod larvae, chaetognaths, larvaceans, polychaetes, cladocerans, fish larvae and post-larvae, and fish eggs were also recorded (Sims & Merrett, 1997). Calanoid copepods almost entirely dominated the stomach contents of a 3.3-m long female shark found tangled in nets in the English Channel (D.W. Sims, unpublished observations). However, in other regions, basking sharks utilise larger zooplankton prey. The stomach contents of an 8.1-m long basking shark off the east coast of Japan was found to contain only specimens of the pelagic shrimp *Sergestes similis*, which had been

preyed upon by the shark at a depth below 100 m at night (Mutoh & Omori, 1978). The shrimps in the shark's stomach ranged in body length from 40 to 54 mm, similar to the length-frequency distribution for shrimps sampled using trawl nets (Mutoh & Omori, 1978).

The cardiac stomach contents of a large basking shark have been found to weigh over 0.5 tonnes, of which only 30% was organic matter (Matthews & Parker, 1950). The rates of gastro-intestinal evacuation in basking sharks are unknown, however filtration rates have been estimated using measurements of swimming speed and mouth gape area. Using a swimming speed of 1.03 m s^{-1} for a 7 m shark with a mouth gape area of 0.4 m^2, a maximum filtration rate of 1484 m^3 h^{-1} was estimated (Parker & Boeseman, 1954). This estimate has perpetuated in the literature and popular accounts, however, it fails to take into account the inefficiencies associated with filter-feeding, namely buccal flow velocity was assumed to equal forward swimming velocity, and swallowing (prey handling) time was not considered. A recent study in the English Channel measured the swimming speeds of 4.0 – 6.5-m long basking sharks accurately and found that they filter feed at speeds some 24% slower than when cruise swimming with the mouth closed (Sims, 2000*a*). Basking sharks were observed filter feeding at a mean speed of 0.85 m s^{-1} (± 0.05 S.E.) and larger 9-m long sharks apparently do not swim appreciably faster (Harden-Jones, 1973). Therefore using these recent studies, a more accurate seawater filtration rate for a 7 m basking shark (mouth gape area ca. 0.4 m^2) swimming at a speed of 0.85 m s^{-1} was calculated to be 881 m^3 h^{-1}, allowing for an observed swallowing (prey handling) time of 6 s min^{-1} (Hallacher, 1977) and assuming the actual buccal flow velocity to be 80% of the forward swimming velocity (Sanderson et al., 1994). This suggests basking sharks filter seawater for food at a rate some 41% lower than previously thought.

4.4 Behaviour

4.4.1 Foraging

Basking sharks are most frequently seen around the north, west and south-west coasts of Britain, feeding at the waters' surface during summer months (Berrow & Heardman, 1994; Sims et al., 1997). In the northwest and northeast Atlantic, surface foraging occurs from around April to October, usually with a peak in sightings from May until August (Kenney et al., 1985; Berrow & Heardman, 1994). The seasonal increase in the surface sightings of basking sharks in British waters during May and early June coincides with increased zooplankton abundance at this time (Sims et al., 1997; Sims, 1999). Similarly, observations of surface-feeding basking sharks in Clayoquot Sound, British Columbia, were coincident with the season of highest plankton productivity in the region (Darling & Keogh, 1994). In contrast, basking sharks in the northeast Pacific off the central and southern California coast have been observed at the surface from October to May, with peaks in October and March (Squire, 1990). Surfacing behaviour in this particular region therefore occurred both well before and after the June peak in phytoplankton abundance (Squire, 1990). Further studies are required to establish the timing of surface behaviours with respect to seasonal trends in zooplankton abundance as relationships appear to differ between geographic regions.

Basking sharks observed at the surface feed almost continuously, and frequently occur in large aggregations. In the English Channel off Plymouth, groups of between 3 and 12 individuals were observed (Sims et al., 1997, Sims & Quayle, 1998), although fishermen have reported aggregations of up to 200 individuals off Cornwall. There does not appear to be any social organisation within these feeding groups. Basking sharks are primarily solitary, but their propensity to exhibit prolonged feeding behaviour in specific areas probably results in the formation of foraging aggregations. These have been shown to occur most often near oceanographic features (Sims & Quayle, 1998). A basking shark tracked by satellite was shown to remain close to a thermal boundary, or front, between two water masses of different temperature

(Priede, 1984). There have been similar sightings of basking sharks feeding close to frontal features (e.g. Choy & Adams, 1995).

A thermal front is a region characterised by a larger-than-average horizontal gradient in water temperature, which forms a boundary between warm, stratified and cold, mixed waters (Le Fevre, 1986). Fronts can be formed by changes in tidal current speed as a function of depth, by underwater topographical features that deflect currents to the surface, or by internal waves near shelf edges (Le Fevre, 1986; Wolanski & Hamner, 1988). Fronts have biological significance because they are often associated with enhanced primary and secondary production (plankton). This may be due to the favourable conditions presented by nutrients diffusing from cold, mixed water into warmer water that can confer higher rates of growth, or by aggregation of particulate plankton at these boundaries due to complex upwelling and downwelling currents (Le Fevre, 1986). Fronts are of significance to marine vertebrates generally (Wolanski & Hamner, 1988), and recent behavioural studies have demonstrated their role as important habitat for foraging by basking sharks.

Basking sharks were once thought to be indiscriminate planktivores that were unlikely to orientate to specific plankton-rich waters (Matthews & Parker, 1950). However, Sims and Quayle (1998) tracked basking sharks responding to zooplankton gradients and showed they were selective filter-feeders that chose the richest, most profitable plankton patches. Basking sharks foraged along thermal fronts in the English Channel and actively selected areas containing high densities of large zooplankton above a threshold density. Surface-feeding basking sharks followed convoluted swimming paths along tidal slicks associated with the front, and exhibited area-restricted searching (ARS) where zooplankton densities were measured to be high (> 1 g m^{-3}). As observed in other animals, ARS behaviour in basking sharks was characterised by increased rates of turning and decreased swimming speeds (Sims & Quayle, 1998; Sims, 1999; Sims, 2000a). Individually tracked sharks spent twice as long in areas with zooplankton densities $>$ 3 g m^{-3} compared with time spent in areas < 1 g m^{-3} (Fig. 4). Further study showed that basking sharks surface-feed in areas in which the dominant calanoid copepod prey, *Calanus helgolandicus*, was 2.5 times as numerous and 50% longer than in areas in which sharks do not feed (Sims & Merrett, 1997). In the feeding areas there were also fewer numbers of smaller zooplankton species, and therefore the biomass per cubic metre where sharks' foraged was significantly increased.

These studies emphasise the role of tidal fronts as important annual habitat utilised by large numbers of basking sharks. However, the fact that the duration of summer stratification in sea coastal areas is likely to be altered by climate warming (Wood & McDonald, 1997) raises the question of how predicted changes in the persistence of thermal fronts will affect the timing and location of foraging behaviour in this species. Between years, the changes in feeding locations of basking sharks indicated broad shifts in front-located secondary production associated with a shift in location of the seasonally persistent front as a result of local weather conditions (Sims & Quayle, 1998) (Fig. 5). Furthermore, basking sharks integrate a planktivorous fish's behaviour with zooplankton abundance directly. Therefore, it has been suggested that basking sharks may be useful detectors of the distribution, density and characteristics of zooplankton in fronts, and could provide high-trophic-level biological indication of fluxes in zooplankton assemblages that are affected by oceanographic and climatic fluctuations of the north Atlantic (Sims & Quayle, 1998). Future surveys for basking shark, where identifying large numbers of individuals becomes important (perhaps using photographic identification; Sims et al., 2000b), would benefit from efforts concentrated in these areas.

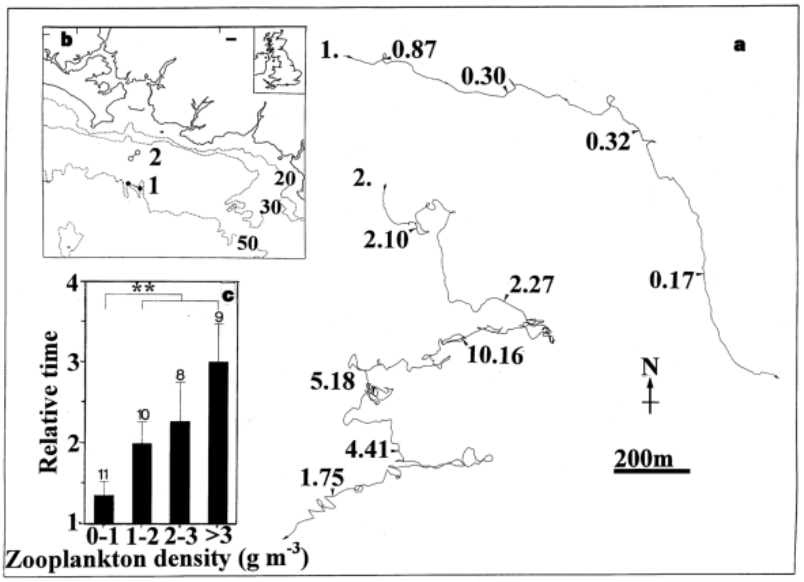

Fig. 4 *Feeding behaviour of basking sharks in relation to zooplankton density. (a) Representative fine-scale-foraging tracks of two basking sharks responding to zooplankton gradients, where track 1 denotes a non-feeding shark and track 2 a feeding shark. Numbers along each track represent zooplankton densities sampled in g m-3. (b) Positions of the tracks in relation to coastline and bathymetry off Plymouth. (c) Relationship between zooplankton density class and the time basking sharks spent within 25 m of the zooplankton-sample locations. From Sims and Quayle (1998).*

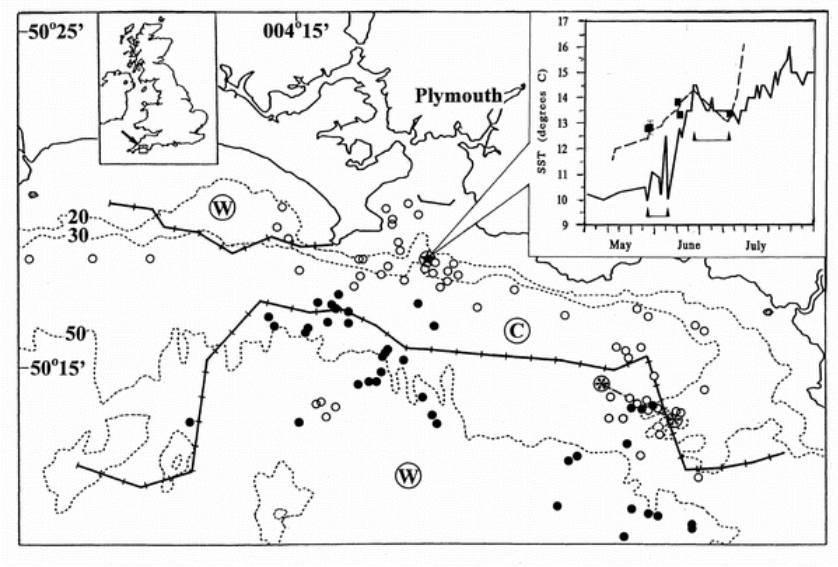

Fig. 5 *Distribution of surface-foraging basking sharks and changes in summer sea surface temperature (SST). The distribution of foraging sharks in 1996 (open circles) compared to 1997 (closed circles) and the location of the thermal front in June, 1996. The inset chart shows the change in summer SST at the sampling station S1 in 1996 and 1997, with brackets showing the periods when SST fluctuated which is consistent with decreases in frontal sharpness due to calm weather conditions. From Sims and Quayle (1998).*

There is also evidence to indicate that within feeding aggregations the amount of time individual basking sharks spend on the surface is proportional to the quantity of zooplankton present in surface waters (Sims et al. 2003a). This indicates the probability of sighting basking sharks may vary depending on habitat productivity and prey availability (see section 4.4.4), suggesting future sightings schemes for basking sharks should take into account zooplankton abundance in specific search areas. If zooplankton abundance from year-to-year is not quantified in addition to the number of sharks sighted, then it will be difficult to assess whether the number of sharks observed per unit time was due to enhanced surface zooplankton abundance in that region rather than attributable to any other factors. A recent study on minke whales (*Baleanoptera acutorostrata*) has shown that inaccurate population censuses can be obtained because the

probability of surface sightings can increase at certain times of the day, and in certain months (Stockin et al., 2001; Young, 2001).

Recent studies have also investigated the effect decreases in zooplankton density have on the foraging behaviour of basking sharks. It was found that individuals remain for up to 27 hours in rich patches that are transported by tidal currents (Sims & Quayle, 1998). In one zooplankton patch monitored, up to 23 different sharks were observed to surface feed over a period of 224 h, during which time prey density declined exponentially from between 1.47 – 8.29 g m^{-3} in the first 24 h to 0.50 – 0.80 g m^{-3} after 224 h (Sims, 1999). This indicates basking sharks have the potential to influence the density and diversity of plankton communities directly (Sims, 2000b). Furthermore, a lower threshold foraging level was also determined using empirical data from behavioural studies of individual and group-feeding sharks and theoretical calculations (Sims, 1999). This study showed that basking sharks tend to stop feeding and leave patches when prey density reaches between 0.48 and 0.70 g m^{-3}, values which were in good agreement with the theoretical threshold prey density of between 0.55 and 0.74 g m^{-3} (Sims, 1999). A previous study calculated the lower threshold to be 1.36 g m^{-3}, a relatively high value that was then used to argue that basking sharks could not derive net energy gain outside of summer months and so probably hibernate during the winter in a non-feeding state (Parker & Boeseman, 1954; Matthews, 1962). Although Parker and Boeseman's (1954) threshold estimate was only roughly double that of Sims (1999), it was found that the parameter values they used were not accurate in the light of modern data and methodology, and that in turn the prey density estimate of 1.36 g m^{-3} cannot now be considered to be correct (Sims, 1999; Weihs, 1999). The new prey threshold estimate of ~0.6 g m^{-3} is important because it questions the validity of the 'hibernation' hypothesis. The results of Sims (1999) strongly suggest that basking sharks are capable of utilising lower prey densities than 1.36 g m^{-3} for maintenance of growth rates. Because zooplankton densities between 0.60 and 1.36 g m^{-3} occur in north-east Atlantic waters outside summer months (Harvey et al., 1935; Digby, 1950), the implication of the work of Sims (1999) is that sufficient productivity to support basking shark feeding and growth may not be as spatio-temporally limited as suggested by Parker and Boeseman (1954). Therefore, basking sharks may not be limited to feeding on high densities in summer alone (Sims, 1999).

Early anatomical studies demonstrated that winter-caught basking sharks often lacked gill-raker filtration apparatus (Van Deinse & Adriani, 1953; Parker & Boeseman, 1954). This seasonal loss was used as evidence to support the idea that when zooplankton densities decrease below 1.36 g m^{-3} they shed their gill rakers and hibernate whilst re-growing their rakers during the winter months (Parker & Boeseman, 1954; Matthews, 1962). However, Sims (1999) stated that a significant proportion (~40%) of basking sharks in winter have been found with full sets of gill rakers and zooplankton prey in their stomachs (Van Deinse & Adriani, 1953; Parker & Boeseman, 1954). It appears that the chronology of autumn/winter shedding of rakers, winter re-growth and eruption of new rakers in early spring suggested by Parker and Boeseman (1954) was developed from detailed analysis of just three individual sharks. Appraisal of the entire dataset available to these workers suggests this chronology may not apply to all individuals in the population (Sims, 1999). Basking sharks may have a shorter raker development time or shedding and re-growth may be asynchronous, which would account for sharks in winter possessing rakers and having food in their stomachs (Sims, 1999).

4.4.2 Courtship

Courtship behaviour is used by animals to attract potential mates and as a prelude to mating. Comparatively little is known about courtship and mating behaviour in wild sharks as it has proved extremely difficult to study, especially in large pelagic sharks. Actual reproductive behaviour such as courtship, pairing, copulation, or post-copulatory activities have been described in only nine out of the 400 or so species of sharks, and most of these have been for captive animals (Carrier et al., 1994).

Reproduction in the basking shark has been studied only from anatomical examinations of fishery-caught individuals (Matthews, 1950). The latter study supports the hypothesis that mating occurs during summer months off the British Isles. Adult basking sharks caught off west Scotland during the summer of 1946 were in breeding condition and showed signs of having recently copulated (Matthews, 1950). Females bore recent or unhealed cloacal wounds inflicted by the claw on the clasper of the male during copulation. A female examined closely contained many spermatophores, while both males and females carried abrasions near the pelvic area possibly due to contact of the roughly denticulated skin in this region made during pairing (Matthews, 1950). On the basis of these data, Matthews (1950) concluded that the breeding season was in 'full swing' during the second half of May off west Scotland. There have been anecdotal behavioural observations of interactions between sharks before capture (Matthews & Parker, 1950), but until recently however, there have been no detailed studies of social or courtship behaviour.

Elements of courtship and putative mating behaviour among a group of 13 basking sharks at the surface over deep water (*ca*. 130 m) were recently recorded for a 5-min period off the coast of Nova Scotia, Canada (Harvey-Clark et al., 1999). In the latter study, nose-to-tail following, flank approach, close approach including rostrum-body contact, parallel and echelon swimming and possible pectoral biting were observed and interpreted to be consistent with courtship and mating behaviour. There are descriptions and observations of close-following behaviour in a number of shark species, including blacktip (*Carcharhinus melanopterus*) and whitetip (*Triaenodon obesus*) reef sharks in the wild (Johnson & Nelson, 1978), captive bonnethead (*Sphyrna tiburo*) and sandtiger (*Carcharias taurus*) sharks (Myrberg & Gruber, 1974; Gordon, 1993), and captive (Klimley, 1980) and free-ranging nurse sharks (*Ginglymostoma cirratum*) (Carrier et al., 1994). Schooling behaviour consistent with courtship interactions was observed in three large groups of basking sharks during aerial surveys in the Gulf of Maine (Wilson, 2004). Between 28 and 50 sharks were observed schooling in close echelon, cart-wheel and milling formations along a boundary between warm slope water and cold upwelled and tidally-mixed waters.

The observations of close schooling behaviour representing putative courtship and mating in basking sharks made by Harvey-Clark et al. (1999) and Wilson (2004) were opportunistic so prolonged study was not possible. Hence, the authors were unable to verify the sex of individuals exhibiting following behaviour, to characterise the behaviour over longer time-periods for quantitative comparison with those seen in other sharks species, or to determine courtship duration and its spatio-temporal occurrence. However, annual courtship-like behaviour in basking sharks from 25 separate episodes was observed and tracked during a five-year study (1995-1999) off southwest England (Sims et al., 2000) that sheds more light on the nature of courtship in basking sharks. Social behaviour was observed between paired, or three or four sharks and were consistent with courtship behaviour seen in other shark species, namely nose-to-tail following, close following, close flank approach, parallel and echelon swimming (Fig. 6). Behaviour was recorded between individuals of 5 to 8 m total body length (L_T), whereas smaller sharks (3 – 4 m L_T) did not exhibit this behaviour. In this study, lead individuals were identified as females and interactions were prolonged; the longest continuous observation of socialising was 1.8 h, although intermittent track data indicated bouts may have lasted up to 5-6 h (Sims et al., 2000).

Breaching behaviour, signified by basking sharks leaping completely clear of the water also occurred during observed social interactions in the western English Channel (Sims et al., 2000). This behaviour by basking sharks was at first thought to be improbable (Matthews & Parker 1950), but was frequently observed between May and June by shark fishermen off Scotland (Matthews & Parker, 1951).

Breaching is thought to act as social communication between predatory white sharks (*Carcharodon carcharias*) when entering their seasonal reproductive mode (Pyle et al., 1996), and between filter-feeding whales, where it may also be used as a courtship display (Whitehead, 1985). Similarly, breaching behaviour may be linked to courtship in basking sharks (Sims et al., 2000).

Courtship behaviour between basking sharks in the western English Channel over a five-year period occurred between May and July (Sims et al., 2000). These observations are consistent with the summer mating period suggested by Matthews (1950) from anatomical studies (May), and for observed breaching events (May and June) (Matthews & Parker, 1951). It also appears that basking shark courtship events are significantly associated with seasonally persistent fronts rather than mixed or stratified water (Sims et al., 2000). This spatial distribution was similar to that recorded for surface foraging locations of this species (Sims & Quayle, 1998). Close-following behaviour is only observed when large sharks were aggregated in relatively rich zooplankton patches, which indicated patch aggregation and the resultant close proximity of mature individuals was a contributing factor in whether courtship was observed (Sims et al., 2000). Therefore, courtship probably occurs as a consequence of individuals aggregating to forage in rich prey patches before initiating courtship. This suggests that locating the richest prey patches along fronts may be important for basking sharks to find mates as well as food in the pelagic ecosystem. As courtship-like behaviours occur annually off southwest England, this region may represent an important mating area for this protected species, although mating itself probably takes place at depth as it has yet to be observed at the surface (Sims et al., 2000).

Furthermore, as courtship and foraging occurs at the surface annually between May and July near fronts that are close to shore, there is the potential that these important behaviours may be at future risk of increased disturbance from anthropogenic sources, such as commercial shipping, leisure and ecotourism vessels.

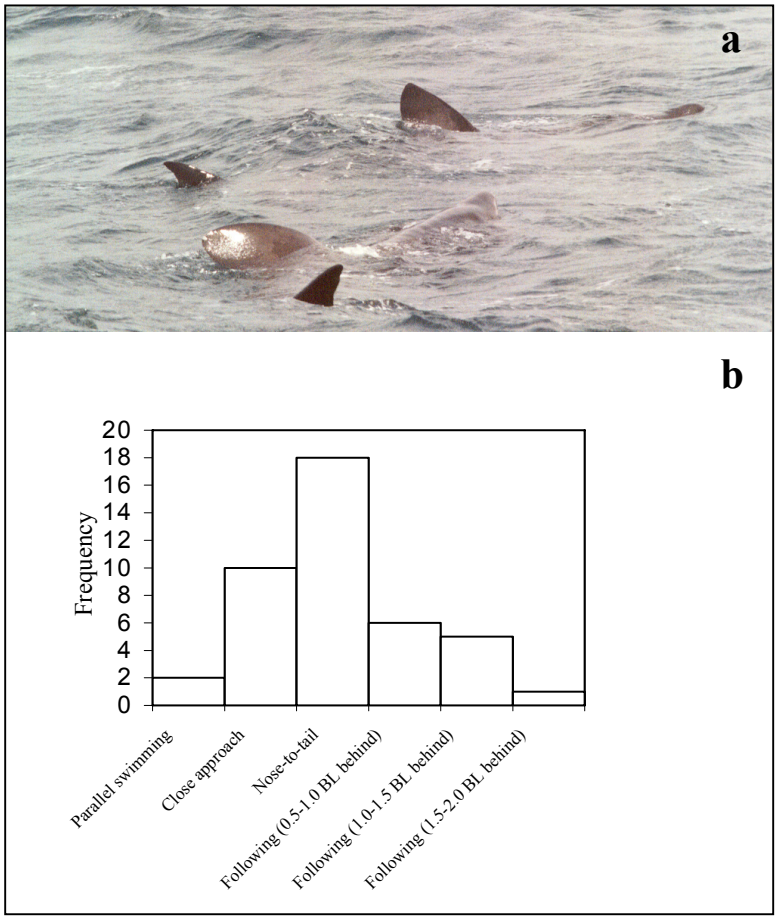

Fig. 6 *Close flank approach and following behaviour between two C. maximus off Plymouth (a), and the frequency distribution of behaviour type observed between lead and following sharks (b). From Sims et al. (2000).*

4.4.3 Local movements

Basking sharks have been tracked continuously over fine spatial scales (0.1 – 1.0 km) (Sims & Quayle, 1998), but only intermittently by visual or satellite telemetry over meso-scale (1.0 – 10 km) and broad-scale (10 to 100 km) distances (Priede, 1984; Sims & Quayle, 1998; Skomal et al., 2004). In visual tracking studies undertaken off Plymouth, three basking sharks were relocated (separately) feeding in different zooplankton patches 18 – 28 h after initial trackings and 5 – 11 km distant from the foraging areas of the previous day (Sims & Quayle, 1998). Two sharks that were originally found feeding in the same patch moved in similar directions along a zooplankton gradient from low to higher density (range: 0.47 – 1.11 g m^{-3} to 1.06 – 1.43 g m^{-3}), covering minimum distances of 9.5 km and 10.6 km in 27.6 and 23 h respectively. A basking shark tracked by a towed buoyant-satellite tansmitter spent 17 days moving in an approximately circular course and showed no signs of moving out of the Clyde Sea, Scotland (Priede, 1984). In support of this, individual sharks have been resighted in the same area after periods of up to between 14 and 45 days in studies undertaken during summer in the western English Channel and off Vancouver Island, Canada, respectively (Darling and Keogh, 1994; D.W. Sims, unpublished data). These studies indicate that basking sharks move between patches, probably in response to low prey densities encountered previously. Tracks of non-feeding sharks demonstrate that they swim on relatively straight courses and at significantly higher speeds after leaving patches where the zooplankton density has decreased below threshold levels (Sims & Quayle, 1998; Sims, 1999). It seems foraging movements may keep them within a localised area for some considerable time, but only if prey densities remain high.

4.4.4 Broad-scale Movements and Migrations

There have been long-standing debates about the movement patterns and behaviour of basking sharks over annual cycles and whether this species hibernates during winter. Studying the broad-scale movements of basking sharks over distances from 10s to 1000s of km was not possible until the advent of telemetry technology capable of long-term data recording and transmission to polar-orbiting satellites. Tracking the movements, sub-surface behaviour, and over-wintering activity of basking sharks has now been achieved (Sims et al., 2003; Skomal et al., 2004; Sims et al., 2005). To put these recent advances into context, a description of previous observations and interpretations is given prior to summarising the new work.

Basking sharks are rarely encountered at the surface outside of summer months and several theories have been forwarded to account for this apparent disappearance. One historical theory suggested that basking sharks migrate south at the end of the summer and spend the winter as a single population off the coast of Morocco, before making the return journey into northern coastal waters in spring (Kunzlik, 1988; Fairfax, 1998). However, this chronology of gradual appearance from the south in spring was disputed on the grounds that sharks were not observed first off Portugal, then Spain, France, the British Isles and Ireland, and finally off Norway as the season progressed (Stott, 1982). Subsequently, there was no southward increase in abundance at the end of the summer and during early autumn as expected in this scenario. Matthews and Parker (1950) proposed another theory based upon their own and historical observations around Britain and Ireland. They suggested that because basking sharks appeared at similar times off Ireland, southwest England and Scotland during early spring and summer, then a west to east seasonal movement pattern was more likely than a south-north migration. This idea was supported by the observation that *C. maximus* off the west coast of Ireland apparently arrive there to feed a few weeks earlier than further east (Watkins, 1958; McNally, 1976).

The high squalene content and large size of the liver in basking sharks (Blumer, 1967) was put forward as evidence that they may occupy a seasonal deep-water habit because squalene is found in large quantities only in the livers of deep-water sharks (Baldridge, 1972). The anatomical observations made by Parker and Boeseman (1954) are described in detail in section 4.3. Briefly, they showed there to be a lack of gill raker apparatus in winter-caught or stranded sharks, indicating a seasonal cessation of feeding. They coupled this observation with calculations demonstrating that winter densities of zooplankton would be too low to enable basking sharks to derive net energy gain outside summer months. Furthermore, anecdotal information from fishermen suggested that shark livers in early summer were lighter than in sharks taken later in the season (O'Connor, 1953). Taking this information together, Parker and Boeseman (1954) and Matthews (1962) hypothesised that basking sharks undergo a winter 'hibernation' by migrating into deep water away from coastal areas at the end of summer. They conjectured that by remaining inactive in deep, cold water in canyons on the continental slope they could survive this non-feeding period by subsisting entirely on the energy reserves stored in their liver for the five or more months before they emerge from this habitat to feed in spring in productive coastal areas (Parker & Boeseman, 1954; Matthews, 1962). This interpretation of basking shark seasonal movements remained largely unchanged in the subsequent scientific literature and popular accounts for almost 50 years.

Tracking of basking sharks tagged with satellite-linked archival transmitters was undertaken in the western English Channel and off western Scotland. Basking sharks were tracked over seasonal scales (1.7 to 7.5 months) and movement and activity data showed they do not hibernate during winter but instead conduct extensive horizontal (up to 3,400 km) and vertical (> 750 m depth) movements to utilise productive continental-shelf and shelf-edge habitats during summer, autumn and winter (Sims et al., 2003b). Sharks travelled long distances (390 to 460 km) to locate temporally discrete productivity 'hotspots' at shelf-break fronts, but at no time were prolonged movements into open-ocean regions away from shelf waters observed (Fig. 7). Basking sharks were tracked moving between waters off south-west England to Scotland, and *vice versa*, sometimes over periods of only a few weeks (Sims et al., 2003b). Movements between northern and southern sea areas of the UK occurred within and between seasons, suggesting a single UK population (Sims et al. 2005a). Overall, there was an absence of a synchronised migratory movement among tracked basking sharks, at least on the European shelf, indicating seasonal movements may reflect food availability rather than thermal preferences.

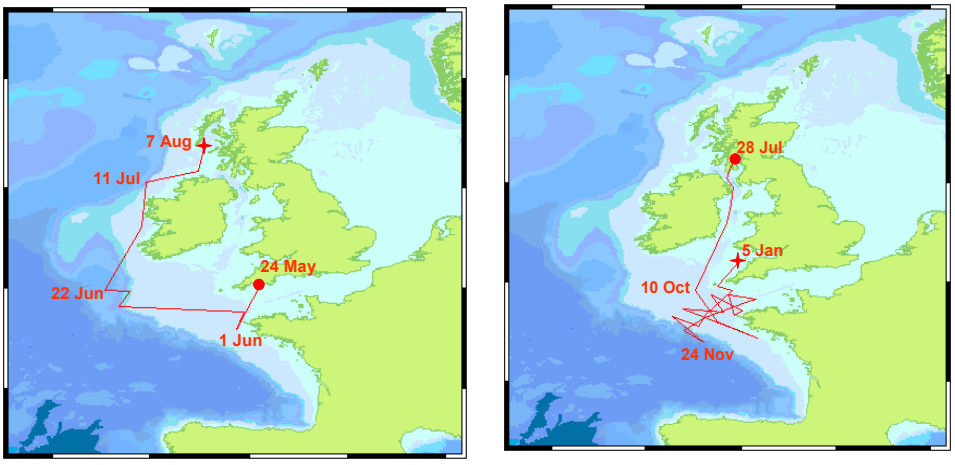

Fig. 7. *Horizontal movements of basking sharks transmitter-tagged off Plymouth (left panel) and in the Clyde Sea, Scotland (right panel). Markers denote location and time of tag attachment (red circle) and detachment (red cross).*

Although feeding behaviour was not possible to measure directly during trackings, the vertical movement patterns shown by sharks were consistent with those associated with foraging, indicating basking sharks

probably feed year-round on zooplankton (Sims et al., 2003b). The vertical pattern of movement was found to vary between different oceanographic habitat types (Sims et al. 2005b). In deep, well-stratified waters sharks exhibited normal diel vertical migration (DVM) (dusk ascent–dawn descent) by tracking migrating sound-scattering layers characterised by *Calanus* and euphausiids, whereas sharks occupying shallow, inner-shelf areas near thermal fronts conducted reverse DVM (dusk descent – dawn ascent) (Sims et al., 2005b) (Fig. 8). This difference in behaviour in fronts was due to zooplankton predator-prey interactions that resulted in reverse DVM of *Calanus*. Sharks were also tracked switching behaviour as they progressed from one habitat type to the other (Shepard et al., submitted). Hence, the probability of sighting basking sharks at the surface is dependent on habitat type and prey behaviour and may differ by several orders of magnitude (Sims et al., 2005b). These habitat-specific differences in surface occurrence has important implications for public sightings and research surveys aimed at monitoring numbers in different areas, including the UK protection zone.

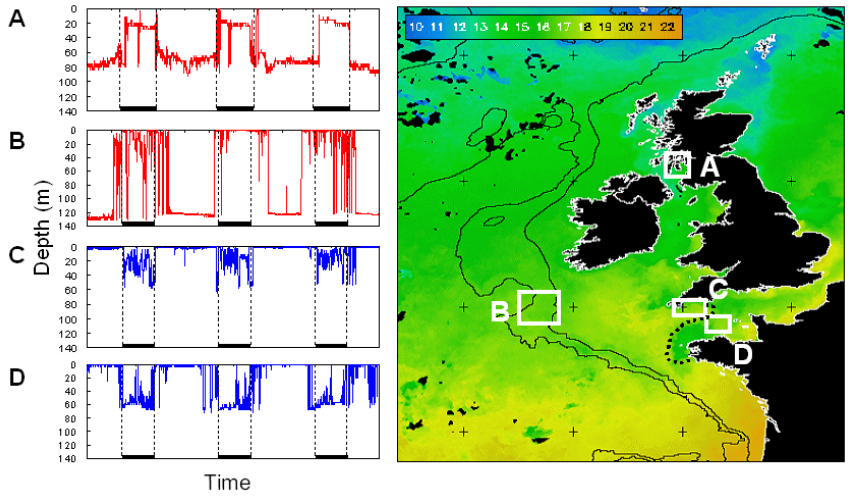

Fig. 8. *Diel vertical changes in swimming depths of four basking sharks (A-D) in relation to thermal habitat occupied on the European continental shelf. Sharks A and B followed a normal DVM in thermally stratified waters, whereas sharks C and D showed a reverse DVM pattern in frontal waters. Left panels: black bars denote nighttime and dotted lines dawn and dusk. Right panel: frontal boundary shown by black dotted line.*

5. Population

5.1 Structure

The sex ratio from fisheries data indicates there to be approximately one male for every 18 females (Watkins, 1958), which is rather less than the 30 to 40 females per male suggested by Matthews (1950). There is no reason to expect a population-level deviation from a 1:1 sex ratio, so this disparity in sex ratio may indicate pronounced spatial and seasonal segregation by sex (Compagno, 2001), or may in fact be due to fishery bias towards surface basking individuals. It is possible that females may engage in this activity more often than males and hence make up a greater percentage of the catch. In contrast, examination of 128 individual sharks caught incidentally in inshore fishing gear in Newfoundland, Canada, showed males comprised 70% of the sample (Lien & Fawcett, 1986). This suggests that sexual segregation may well occur in basking shark populations, although evidence is far from substantial.

The size composition of basking sharks from different geographic areas has not been studied in detail. The size distribution of 93 sharks caught off Scotland in the 1950s ranged from 1.7 to 9.5 m total body length (Parker & Stott, 1965). The size distribution was bimodal with peaks centred on sharks with body lengths between 3 and 4 m, and between 7.5 and 9.0 m. Incidental catches of basking sharks off

Newfoundland showed that males ranged in size from 3.0 to 12.2 m body length with a mean length of 7.5 m (± 1.87 S.D.), representing mature individuals (Lien & Fawcett, 1986). The size of females examined in the same study was slightly smaller, with a mean length of 6.9 m (± 1.82 m S.D.; range, 2.4 to 10.7 m) and consistent with immature individuals. Recent fishery-independent studies of size composition indicate that sharks sighted in the western English Channel range from 1.5 to 7.5 m body length, but that the distribution is unimodal with individuals between 4 and 5 m being most common (Sims et al., 1997; D.W. Sims, unpublished data).

Apart from some observations on sex ratio and size composition, the structure of basking shark populations has not been studied. It is not known whether basking sharks form separate populations in the north and south Atlantic, and whether these in turn are different from sharks found in the north and south Pacific Ocean. It has yet to be established whether basking sharks in the Mediterranean (Valeiras et al., 2001) are a distinct 'stock' compared to other members of the species found throughout the remaining north Atlantic Ocean. Attempts to separate basking sharks found in each of these five regions into separate species according to apparent morphological differences have been rejected (Springer & Gilbert, 1976; Kunzlik, 1988).

It has been strongly suggested that basking sharks form local populations or stocks (Parker & Stott, 1965). Local stocks were proposed for basking sharks on account of sharply declining fishery catches in certain, spatially-limited areas, e.g. Keem Bay on Achill Island, west Ireland (Parker & Stott, 1965; McNally, 1976). The rapid decline in the number of sharks caught in Keem Bay after only about 10 years of the fishery commencing was interpreted as over-exploitation of a limited stock of sharks inhabiting a locally discrete area annually. This hypothesis seems to be supported by the observations that individually-identifiable basking sharks remain in localised areas often for many days (e.g. Darling & Keogh, 1994). However, this apparent residence is probably more closely related to high zooplankton abundance than with population structure (see sections 4.4.1, 4.4.3 and 4.4.4 for discussion). Satellite-tracking data of long-range movements indicate basking sharks can remain in particular geographic regions for several months, but also can move rapidly between regions over a period of a few weeks (Sims et al., 2003b). These spatially structured movements have been shown to be driven by foraging to locate areas with the most abundant zooplankton (Sims et al. 2006). Furthermore, sharks tracked around the UK mix freely, suggesting population differentiation at a local spatial scale is unlikely (Sims et al., 2003b, 2005a). The prospect of highly philopatric stocks existing along the entire western-shelf edge of the northeast Atlantic, and which remain faithful to specific bays year after year seems doubtful. However, philopatry of basking sharks to regions at the broader scale does occur and is supported by satellite tracking data (Sims et al., 2003b). Clearly, the occurrence of regional philopatry of basking sharks has implications for interpreting past effects of fisheries on populations, and possibly predicting the likelihood of future impacts.

5.2 Abundance and density

The population abundance and density of basking sharks in any sea area of the world is not known. Fishery catches provide information on the numbers caught in particular years, but an absence of information on the variability in search times (fishing effort) prevents a systematic evaluation of relative abundance by area or year (see section Exploitation). The best available assessment of absolute basking shark abundance was provided by marine mammal aerial surveys flown between October 1978 and January 1982 (Owen, 1984; Kenney et al., 1985). Individual counts of basking sharks were made in U.S. continental shelf waters (shoreline to 9 km beyond the 1,829 m isobath) off New England, northwest Atlantic (Hudson Canyon to the Gulf of Maine) (Kenney et al., 1985). These surveys indicated an abundance there of between 6,671 and 14,295 animals. Similar aerial surveys were flown along the central and southern U.S. Californian coast between 1962 and 1985 (Squire, 1990). The

number of sharks sighted varied greatly between different 'block' areas (each block = 220 km^2). Up to 6,389 sharks were observed over the 23 year study period in the Morro Bay area, with a mean of 96.8 sharks per sighting. Lower numbers of sharks and fewer sharks per sighting occurred north of Morro Bay towards Point Sur (between 1.0 and 9.5 sharks per sighting). Whereas, in Monterey Bay, there were between 14.4 and 42.1 sharks per sighting. Further south however, the greatest number observed south of Point Conception was a mean of 6.7 individuals per sighting (Squire, 1990).

The longest running public sightings scheme for basking shark has been operated by the UK Marine Conservation Society with data collated principally over a 20-year period (Doyle et al., 2005). To date 24,013 sharks have been observed in UK inshore waters, though population-level analysis is limited by the lack of data on sightings effort. Over a smaller spatial scale, a sightings scheme was established in Ireland, mainly from fishing boats, to determine the distribution and abundance of sharks throughout Irish waters (Berrow & Heardman, 1994). The results showed that basking sharks were sighted only between April and October, with the number seen per month ranging from between 1 and 60 individuals. The total number sighted in 1993 was 425 individuals, and the abundance of sharks observed at the sea surface ranged from 1 to > 40 per 2500 km^2 area (Berrow & Heardman, 1994).

Basking shark abundance can be locally very high in productive inshore areas and will probably determine to a large degree the surface sightings of sharks (Sims et al., 1997; Darling & Keogh, 1994). Annual studies operating over relatively small spatial areas (~500 km^2) have provided information on the number of individual sharks observed per unit time (e.g. Sims et al., 1997). In the western English Channel off Plymouth, the number of sharks observed from May to August in each year between 1995 and 2001 varied from 0.01 to 0.35 per hour (D.W. Sims, unpublished data). The years 1998 and 1999 yielded uncharacteristically few sightings (0.01 and 0.02 per h), compared to 1995-1997 (0.10 to 0.35 per h) and 2000/2001 (0.30 and 0.14 per h). The abundance of basking sharks over these years have been related to prey density, with a higher number per h observed in years when the zooplankton density was high at the surface (D.W. Sims, unpublished data). As discussed in section 4.4.1 and 4.4.4, the abundance of zooplankton and its relationship to fronts must be assessed in parallel with surveys for basking sharks if the method of finding sharks based upon their surface occurrence is to be used to indicate abundance.

5.3 Recruitment

The number of female basking sharks in all sea areas of the world remains completely unknown. Similarly, because to date there has been only a single capture of a pregnant female (Sund, 1943), estimates of fecundity and hence probable recruitment rates are extremely difficult. The pregnant female captured in August off central Norway gave birth to six pups, five of which began swimming and feeding at the surface almost immediately (Sund, 1943). If this number of pups is representative of normal parturition rates, then the rate of recruitment in basking sharks must be considered to be low even compared to other shark species (Compagno, 2001; Pratt & Casey, 1990). In a preliminary demographic analysis, an effective annual female fecundity of 1.0 was determined using an assumption that each female gives birth to six pups every third year (Mollet, 2001).

It is generally agreed that total body length at parturition probably lies between 1.5 and 2.0 m (Sund, 1943; Parker & Stott, 1965). The frequency with which putative young-of-the-year basking sharks of this body length are sighted undoubtedly varies between years, but they were shown to never make up more than 2.8 % of all sightings in the western English Channel off Plymouth (Sims et al., 1997). In a study of the incidental catches of *C. maximus* in inshore fishing gear in Newfoundland, immature sharks made up only 2.6 % of captures (Lien & Fawcett, 1986). Interestingly, the frequency of sightings and capture of small-bodied basking sharks was very similar between these two studies in the

north Atlantic. In addition, it was shown in both studies that these young sharks only occurred later in the summer.

5.4 Mortality

The natural mortality rate of basking sharks is not known for any geographic region. In a preliminary demographic analysis for the basking shark, a mortality value (M) of 0.102 yr^{-1} (S = 0.903) was assumed by Mollet (2001) based on a longevity of 40 years and using the fecundity estimate of 1 per female per year. However, this author concluded that a lack of information on fecundity was not particularly problematic, rather, better mortality data and a better estimate of age-at-maturity was urgently needed.

Little is known about natural mortality from behavioural studies for obvious reasons associated with the difficulty of prolonged observation individual sharks. But because of their large body size, natural mortality of adult basking sharks through predation is probably quite low. There have been anecdotal reports from fishermen in southwest England that killer whales (*Orcinus orca*) sometimes predate on basking sharks. There is a record of a small, 2 – 3-m long basking shark being found in the stomach contents of a sperm whale (*Physeter macrocephalus*) caught off the Azores (Clark, 1956). However, such records in the literature are extremely rare.

6. Exploitation

6.1 Fishing gear and boats

The gear and boats used to hunt basking sharks have been reviewed in considerable detail by Myklevoll (1968), Kunzlik (1988) and most recently by Fairfax (1998). Briefly, most fishing operations have utilised non-explosive harpoons or harpoon guns mounted on boats to catch basking sharks, although one fishery used tethered nets within an embayment to entangle sharks (Went & Suilleabhain, 1967).

6.2 Fishing areas and seasons

Basking sharks have been exploited by organised fisheries dating back to at least the 18th Century. Several nations have prosecuted fisheries at the time when basking sharks are present in inshore areas, which in the northeast Atlantic occurs from April to September. Fisheries have operated off the U.S. Californian coast, and perhaps most importantly in the northeast Atlantic, have been undertaken annually by Norway, Ireland and Scotland (Kunzlik, 1988). The Norwegian fleet, which by 1987 numbered only seven boats, was known to hunt for basking sharks throughout the Norwegian Sea, and in areas around Scotland and Ireland outside the 12 mile belt. This was not always the case however, because Norwegian boats were frequently observed catching sharks in the Minch in Scotland during early 1950s (O'Connor, 1953).

6.3 Fishing results

Between 1946 and 1986, directed basking shark fisheries in Norway, Scotland and Ireland took a recorded 77,204 individuals (mean number per year, range, 164 – 1,495) (Kunzlik, 1988). In more recent years between 1989 and 1997, Norway landed 14,263 metric tonnes (mt) of basking shark liver (FAO, 2000). Assuming a mean liver weight of 0.5 mt per shark, this gives the number caught over this 9-year period as approximately 28,526 individuals. Taken together, the landings records in the northeast Atlantic indicate that 105,730 sharks were captured over a 51 year period. Clearly, without any knowledge of population size and inter-annual fluctuations in abundance there is no way of assessing whether capture rates were high in relation to population numbers in any one year. The geographical areas in which sharks were taken between 1946 and 1997 varied between years indicating

that broad-scale locations of aggregations may also have changed between years in the northeast Atlantic.

Less is known about the number of basking sharks caught incidentally in fishing gear where other species were the primary target. In Newfoundland between 1980 and 1983, 371 basking sharks were captured in inshore fishing gear (Lien & Fawcett, 1986). By contrast, in over forty gill-netting fishing trips in Irish waters between February 1993 and January 1994, totalling 1,167 km and 19,760 km h observed fishing effort, only one basking shark was caught incidentally (Berrow, 1994). This large difference in capture rate may reflect geographic differences in shark numbers, variations in the amount of gear deployed, and/or the fishing method employed.

6.4 Decline in numbers

An example often cited as demonstrating clear evidence for over-fishing of basking sharks (e.g. Anderson, 1990) was the fishery conducted at Achill Island, Co. Mayo, Republic of Ireland, between 1947 and 1975. After a few years of peak catches in the early 1950s the number of sharks captured at the surface (using harpoons and nets) declined sharply (Kunzlik, 1988). Between 1947 and 1975, there were 12,360 sharks taken in the fishery. The number caught over the period 1950-56 accounted for 75% of this total (mean, 1,323 sharks yr-1 ± 380 S.D.), whereas between 1957 and 1961 a mean of 345 sharks were caught per year (± 129 S.D.), and from 1962 to 1975 the mean number caught declined to 60 per year (± 29 S.D.). This downward trend was suggested as a result of a stock collapse due to over-exploitation of a localised population (Parker & Stott, 1965). A newly-published study however, related the trend in basking shark fishery catches off Achill Island to zooplankton (total copepod) abundance in four adjacent sea areas over a 27-year period (Sims & Reid, 2002). The number of basking sharks caught and copepod abundance showed similar downward trends and were positively correlated (r-value range, 0.44-0.74) (Fig. 9). A possible explanation for the downward trend in shark catches was that progressively fewer basking sharks occurred there between 1956 and 1975 because fewer copepods, their food resource, occurred near the surface off west Ireland over the same period. It was suggested by the latter authors that the decline in basking sharks may have been due to a distributional shift of sharks to more productive areas, rather than a highly philopatric, localised stock that was over-exploited (Sims & Reid, 2002). In support of this conclusion, Sims and Reid (2002) note that the catches of basking sharks in the Norwegian Sea, the main hunting ground for the Norwegian fleet (Myklevoll, 1968), remained relatively low between 1949 and 1958 when catches were highest off Achill Island. However, after 1958 the Norwegian catches increased to levels greater than those made off Achill, and remained fairly constant until 1980 (Sims & Reid, 2002). This may indicate that basking shark distribution shifted northwards in the mid-1950s, perhaps to areas with relatively higher utilisable productivity.

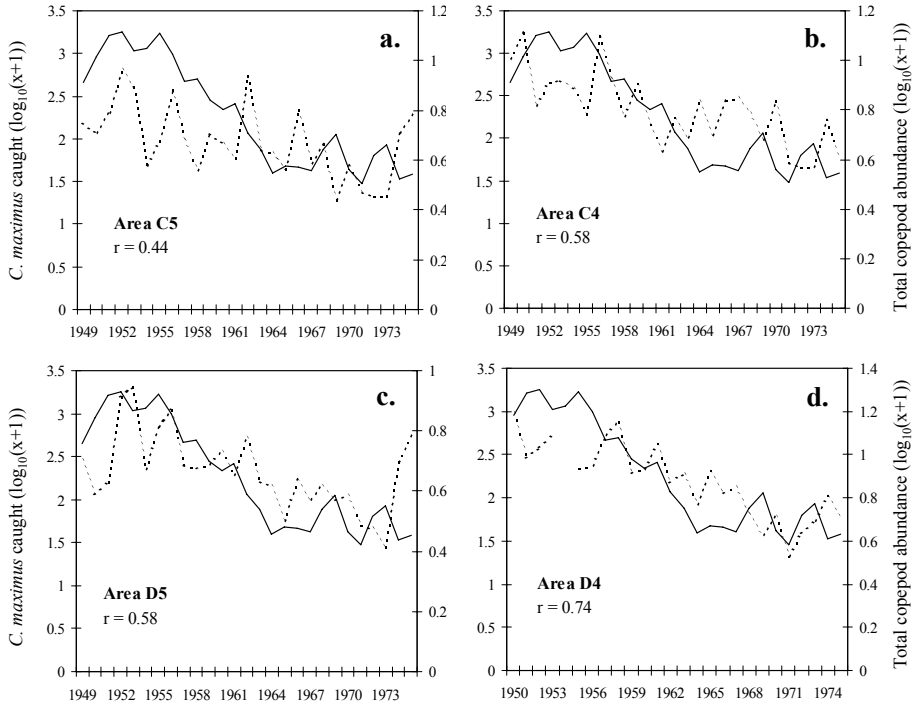

Fig. 9 *The relationships between numbers of basking sharks caught of west Achill Island (continuous line) and abundance of total copepods (dashed line) in Continuous Plankton Recorder (CPR) areas C4, C5, D4 and D5. The CPR areas were the four surrounding Achill Island. From Sims & Reid (2002).*

In other parts of the world however, there is some evidence to indicate that basking shark populations may take very many years to recover from exploitation. Basking sharks were the subject of an eradication program in Barkley Sound, Vancouver Island, Canada in the 1940s and 1950s (Darling & Keogh, 1994). The program, set up by the Canadian Department of Fisheries and Oceans, entailed sharks being rammed by a fishery vessel armed with a blade mounted on the bow below the waterline. About 100 sharks were killed in the summers of 1955 and 1956, with perhaps several hundred being killed in the area up to 1959 (Darling & Keogh, 1994). Apparently, basking sharks are still rarely observed in Barkley Sound or in other areas of Vancouver Island, although Darling and Keogh (1994) describe a small population in Clayoquot Sound. It is unclear whether the eradication program was responsible for the decline and persistent low number of sharks seasonally present off Vancouver Island in the years following the program, or whether other factors such as food availability are responsible.

7. Management and protection

7.1 Management

The only catch control on fishing for basking sharks in European waters is a total allowable catch (TAC, currently set at zero). Norway has recently issued regulations banning the taking of basking sharks, and the off-loading of any basking shark by Norwegian vessels is prohibited. For any basking shark is taken as by catch, "viable" ones must be returned to the sea immediately, and only dead or

severely injured animals can be landed. Although Norway and all other countries have now ceased to fish for basking sharks in EC waters, there is the possibility that bycatch in trawls and gillnets may be relatively high.

7.2 Protection

Sharks and rays are particularly vulnerable to exploitation on account of slow growth rates, long times to sexual maturity, long gestation periods, and relatively low fecundity (Brander, 1981; Pratt & Casey, 1990; Dulvy et al., 2000). The basking shark may take as long as 10-12 years to reach sexual maturity, probably has a gestation period of between 1 and 2 years, and has a very low fecundity rate even among elasmobranchs. Because of these basic aspects of its biology, there has been concern that past fishing activities may have affected populations.

As a result of these concerns, the basking shark is listed as Vulnerable (A1a,d, + 2d) worldwide, and Endangered (EN A1a,d) in the north-east Atlantic in the IUCN Red List (IUCN, 2004). In 2000, the species was listed in Appendix III of the Convention on International Trade in Endangered Species (CITES). In 2002, on the basis of a UK proposal, the CITES listing was upgraded to Appendix II which requires that International trade in these species is monitored through a licensing system to ensure that trade can be sustained without detriment to wild populations. Though no longer exploited there, they are also protected in British (but not Northern Irish) territorial waters under Schedule 5 of the Wildlife and Countryside Act (1981), and it is a priority species under the UK Biodiversity Action Plan (English Nature, 1999). They are also protected within the territorial waters of the Isle of Man and Guernsey (UK dependant territories), in the Mediterranean under the Bern Convention on the Conservation of European Wildlife and Natural Habitats (with EU reservation) and Barcelona Convention for the Protection of the Mediterranean Sea against Pollution (unratified). It is protected in US Federal waters (including Gulf of Mexico and the Caribbean) and is partially protected in New Zealand waters, where target fishing is banned but bycatch may be utilised. In November 2005, the basking shark was listed on Appendix I and II of the Convention of Migratory Species of Wild Animals (Bonn Convention). Listing requires that nation states which have populations of basking sharks must work with adjacent member states to introduce strict legislation to prevent capture and landing of the shark.

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