14

Shark DNA Forensics: Applications and Impacts on Genetic Diversity

Luis Fernando Rodrigues-Filho[1], Danillo Pinhal[2], Davidson Sodré[1] and Marcelo Vallinoto[1,3]
[1]*Federal University of Pará, Campus of Bragança, Institute of Coastal Studies (IECOS), Bragança, Pará*
[2]*Department of Genetics, Biosciences Institute Sao Paulo State University (UNESP), Botucatu, SP*
[3]*CIBIO/UP, Research Center in Biodiversity and Genetic Resources Campus Agrário de Vairão, University of Porto*
[1,2]*Brazil*
[3]*Portugal*

1. Introduction

Despite their worldwide distribution, an increasing conservation appeal and humans' great fascination with them, many aspects of the biology and life history of sharks remain enigmatic. Shark-focused investigations are important because these animals are prominent elements in the marine ecosystem, and the majority of species play pivotal roles as apex predators in the food web.

Over the past decades, several studies have shown the remarkable and accelerated depletion of the natural stocks of many sharks worldwide; population reductions that range from 50% to 89% have been documented (Kotas et al., 1995, Vooren, 1997; Musick et al., 2000; Baum et al., 2003; Baum & Myers, 2007; Dulvy et al., 2008). This decline has been explained to be a consequence of the extensive, unregulated exploitation of wild stocks by fisheries coupled with the restrictive biological characteristics that are intrinsic to the majority of shark species, such as slow growth, high longevity, late sexual maturity and relatively low fecundity (Branstetter, 1990). Moreover, the pressure that is experienced by shark populations is augmented by accidental capture (by-catch) during fisheries that traditionally exploit bony fish species (e.g., tuna and billfishes).

According to Dulvy et al. (2003), the major forces that drive the extinction of shark species are habitat destruction and exploitation by fisheries. Regarding the fisheries, it is well known that the majority of shark populations may not be able to recover after periods of high fishing pressure, which can lead to a permanent loss of genetic diversity in their wild populations (Stevens et al., 2000).

Currently, several coastal and oceanic sharks have reached the status of threatened species (i.e., vulnerable, endangered or critically endangered) according to data that were released by the Red List of threatened fauna of the International Union for Conservation of Nature

(IUCN, 2011). Assorted events are responsible for the current adverse condition that has been reported for diverse shark species worldwide. These events include the lack of governmental policies that regulate and control fishing activity in several countries; the inefficiency in creating priority areas for conservation; the high commercial value of shark fins, which are utilised as food and for pharmaceutical and medical purposes; and the extremely negative stereotypes, which have been acquired over the years due to relatively rare instances of attacks on swimmers, divers and surfers.

A remarkable example of overexploitation involves the daggernose shark *Isogomphodon oxyrhynchus* (Muller & Henle, 1839) (Figure 1), which is a coastal species with a low reproductive capacity (Bigelow & Schroeder, 1948) and a fragmented distribution between Venezuela and Northern Brazil (Lessa, 1999). Although it is protected by law in Brazil (MMA-IBAMA, 2004), *I. oxyrynchus* continues to be illegally caught by fisheries and is currently listed as critically endangered in the Red List of IUCN.

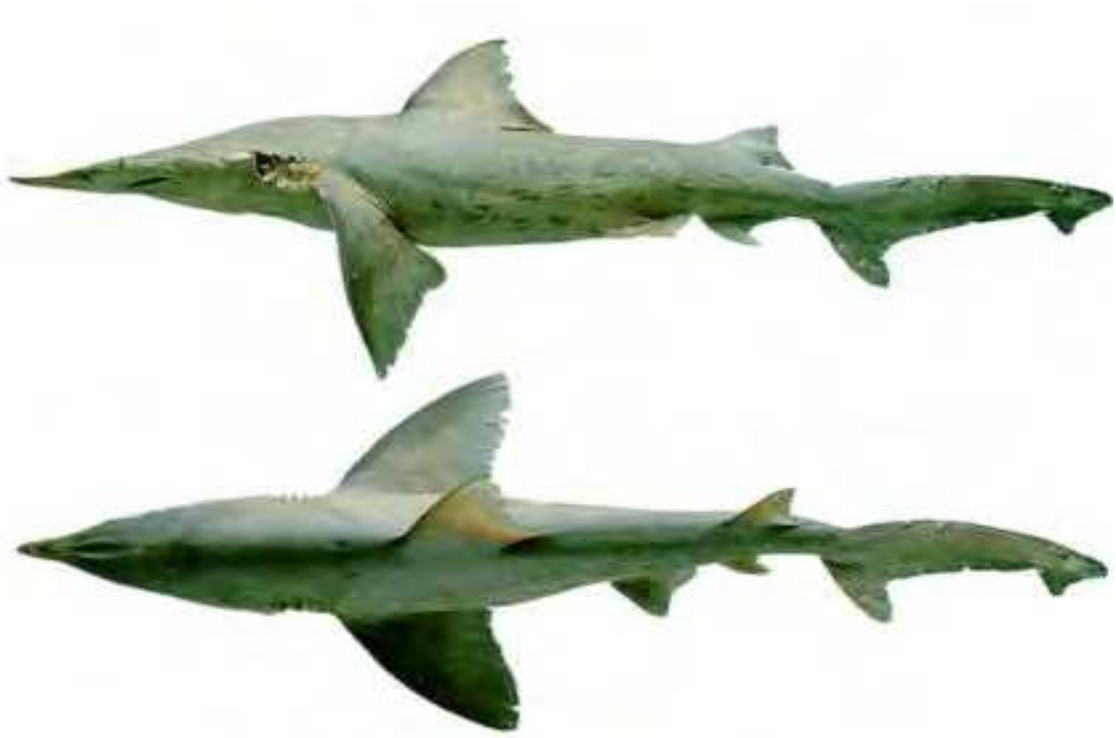


Fig. 1. A daggernose shark *Isogomphodon oxyrhynchus* specimen from the Guiana coast, Atlantic. This photo is courtesy of Dr. Keiichi Matsuura.

In response to the recognised low sustainability of shark fishing on a global scale, the Food and Agriculture Organization (FAO) developed an international plan of action, and a few countries have implemented equivalent national plans for the management and conservation of shark stocks. The primary recommendation of these plans is the collection of shark-catch and trade data on a species-specific basis because different shark species display a broad array of unique biological characteristics, which ultimately reflects their variable susceptibility to exploitation by fisheries (Heist & Gould, 1999). For this reason, precise

species identification is required to determine genetic stock structure and population subdivision (Morgan et al., 2011).

Overall, the biodiversity of sharks is high, although species-specific information that can be used to assist in sustainable resource exploitation is scarce (Ovenden et al., 2011). A major recurrent impediment that is encountered in species monitoring is the historical lack of long-term data on shark landings and commerce on a species-by-species basis; this absence virtually prevents proper inferences regarding population status and future trends (Bonfil, 1994; Castro et al., 1999). In addition, identification is prejudiced due to the illegal finning practice, in which the head and fins, which are key morphological characters for the identification of sharks, are removed at sea. Therefore, unidentifiable landed carcasses are generically recorded as "sharks", which prevents the tracking of the composition of species and detection of species that are protected by law in fisheries (Figure 2). Moreover, the difficulty of accurate species identification is exacerbated by the fact that many exploited sharks, such as those in the orders Carcharhiniformes and Lamniformes (Last & Stevens, 1994; Naylor & Marcus, 1994), are morphologically very similar. This makes the desirable taxonomic identification to the species level a challenge even for experienced taxonomists (Castro, 1993; Stevens & Wayt, 1998; Heist, 2004).

Based on this scenario, research projects that are devoted to the conservation of genetic diversity have sought simple, rapid and low-cost tools that can be immediately implemented. Diverse DNA-based techniques have been developed primarily to assist in the identification of species and the monitoring of shark fishing and trade (Lavery, 1992; Heist & Gold, 1999). These molecular approaches are a resourceful way to identify shark body parts, such as fins, carcass, meat, and processed shark subproducts, which are sold in forms such as extracts and pills (Sebastian et al., 2008; Wong et al., 2009).

Due to the emergence of forensic genetics, individuals from numerous species that were previously not easily identifiable by traditional taxonomy can now be accurately traced back to their geographical region and population of origin using diminutive amounts of DNA. Accordingly, the identification of shark species by molecular methods has contributed to control programs that are directed to fisheries management, trade monitoring and market surveillance, and it has especially facilitated the tracking of endangered and/or heavily exploited shark species (Pank et al., 2001; Clarke et al., 2006; Rodrigues-Filho et al., 2009).

In the present chapter, we review the most important molecular markers and the associated technologies that are employed to resolve species identities and their impacts on the genetic diversity of natural shark stocks.

2. Molecular markers and approaches for shark species identification

Current DNA technologies that have been developed for shark species identification are based on one- or two-step approaches that are applied to polymorphic regions of the nuclear genome, mitochondrial genome or both genomes combined. Among the most popular and well-established methodologies for the discovery and validation of informative molecular markers are polymerase chain reaction (PCR) and nucleotide sequencing.

Fig. 2. Headless and finless shark carcasses that were traded in the fish market at Bragança, Pará, Brazil.

2.1 PCR and associated methodologies

PCR (Mullis & Faloona, 1987) has been extensively used for species identification due to its simplicity, specificity and sensitivity. Among molecular markers that have been studied via PCR, the 5S rDNA has gained prominence for species identification by providing consistent results and utilising fast, low-cost strategies (Asensio et al., 2001; Imsiridou et al., 2007). The 5S rDNA is characteristically organised into multiple tandemly arrayed repeats that are distributed on one or several chromosomes in the genome. Structurally, this nuclear ribosomal region consists of highly conserved 120-base pair (bp) coding sequences that are isolated from each other by a variable nontranscribed spacer (NTS) (Long & David, 1980). Specifically, 5S rDNA is a highly informative region for studies that use the PCR technique due to the following features: (a) the 5S rRNA gene is widely conserved even in distantly related species, which makes it less laborious to isolate the whole repeat from different species by PCR; (b) the repeats do not exceed the range of PCR amplification; and (c) the sequences can be isolated from low-quality DNA because they are small in size and are tandemly organised in a large number of copies (Martins & Wasko, 2004; Pinhal et al., 2009, 2011). Although the 5S rRNA genes are conserved even among unrelated taxa, their NTSs show extensive variations in length and nucleotide composition among species; these properties make these sequences highly useful as species-specific genetic markers (Wasko et

al., 2001; Martins & Wasko, 2004; Pinhal et al., 2009). Such differences in the NTS architecture are useful for the PCR amplification of species-specific 5S rDNA profiles that can be directly discriminated with agarose gels.

Diverse studies have demonstrated the potential of 5S rDNA as a genetic marker for identifying shark species. For example, Pinhal et al. (2008) utilised 5S rDNA to discriminate eight shark species (*Carcharhinus leucas*, *C. acronotus*, *C. limbatus*, *C. obscurus*, *Galeocerdo cuvier*, *Sphyrna lewini*, *Isurus oxyrinchus* and *Alopias superciliosus*) by producing differently sized amplicons by simple PCR and analysing them in agarose gels. Later, the same genetic marker was employed to discriminate two closely related species of the genus *Rhizoprionodon* (*Rhizoprionodon porosus* and *R. lalandii*); however, in this case, the diagnostic required an extra step of enzymatic digestion in addition to PCR (Pinhal et al., 2009). A representative diagnostic 5S rDNA profile of three distinct shark species is shown below (Figure 3).

The multiplex PCR approach, which is an extension of traditional PCR, utilises a reaction in which multiple primers are simultaneously used for PCR; this permits the simultaneous amplification of various DNA fragments. This approach is used to generate amplicons that are of variable lengths between different species (i.e., unique DNA profiles), which will ultimately permit the identification of one species from another (Rasmussen & Morrissey, 2008; Carrier et al., 2010). Additional advantages of the multiplex approach include the ease of data interpretation, the feasibility of application for high-throughput screening and the overall low cost per analysed sample. Moreover, this approach does not require the sequencing of all of the samples or an extra step of enzymatic digestion to discriminate species (Magnussen et al., 2007).

Studies that have been performed on sharks have employed the multiplex PCR approach in several distinct genomic regions, such as the nuclear internal transcribed spacer 2 locus (ITS 2) and the mitochondrial genes cytochrome oxidase I (COI) and NADH dehydrogenase subunit 2 (ND2) (Pank et al., 2001; Shivji et al., 2002 ; Chapman et al., 2003; Abercrombie et al., 2005; Shivji et al., 2005; Clarke et al., 2006 ; Magnussen et al., 2007 ; Mendonça et al., 2009; Farrell et al., 2009).

The multiplex PCR technique that uses ITS 2 is the most well-established approach for the recovery of a shark's identity. For sharks, sets of species-specific primers (SSP) are used in conjunction with universal primers (positive controls) in a single reaction. Pank et al. (2001) developed SSPs for multiplex PCR using the ITS 2 region to discriminate two shark species, *Carcharhinus obscurus* and *C. plumbeus*. This work was shortly expanded by Shivji et al. (2002), who incorporated five additional species. The same approach was used by Chapman et al. (2003) and subsequently by Shivji et al. (2005) in samples with the generic name of "shark" that were obtained in Asian markets. Both of the studies proved that the meat and fins present among the samples traded were of the great white shark (*Carcharodon carcharias*), a species whose capture and trade is prohibited in many countries. In another study by the same group, Abercrombie et al. (2005) validated new SSPs to identify three large-body-sized species of hammerhead sharks (*Sphyrna lewini*, *S. zygaena* and *S. mokarran*), and their results confirmed the worldwide commerce of these species. Subsequently, Clarke et al. (2006) estimated the proportion of each taxon in the total shark fins and meat that was sold in Asian markets by using statistical approaches coupled with molecular diagnosis by multiplex PCR.

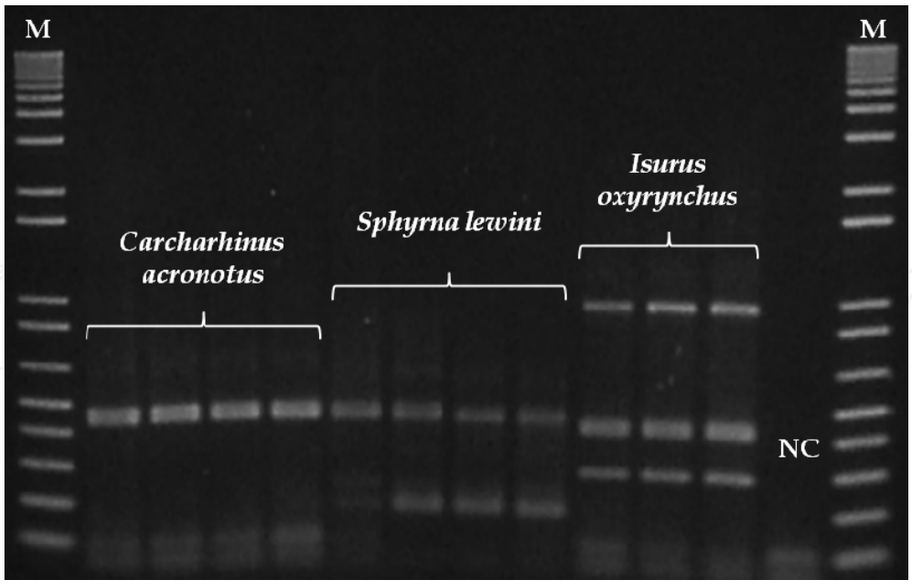


Fig. 3. The 5S rDNA profile for Carcharhiniform (*C. acronotus* and *S. lewini*) and Lamniform (*I. oxyrynchus*) species.

Although the multiplex PCR approach was originally designed to identify large pelagic and oceanic sharks, it has also has been applied to genetically profile globally distributed coastal shark species that are intensively exploited by fisheries (Pinhal et al. in preparation). In this work, the authors designed and validated seven species-specific PCR primers in a multiplex format that could simultaneously discriminate body parts from the seven known sharpnose shark species (genus *Rhizoprionodon*). Unlike the majority of shark species, *Rhizoprionodon* species exhibit life-history characteristics (rapid growth, early maturity and annual reproduction) that suggest that they could be fished in a sustainable manner if an investment in monitoring, assessment and careful management was made. Therefore, the usefulness of the developed assay is noteworthy because the acquisition of catch and trade data for species with a large quantity of landed individuals, which is the case for sharpnose sharks, will require high-throughput profiling. Currently, approximately 35 ITS2-based SSPs are available for shark species identification, and SSPs for an additional two species are currently being validated (Nachtigall et al. in preparation).

Recently a new multiplex PCR-based tool that utilises real-time PCR (RT-qPCR) and high-resolution melting (HRM) technologies to identify shark species was developed. Using a multiplex RT-HRM PCR assay, Moore et al. (2011) distinguished among three closely-related sharks (*Carcharhinus tilstoni, C. limbatus* and *C. amblyrhynchoides*) based on mutations in the mtDNA ND4 gene. Although promising, this technology still requires more sophisticated equipment, reagents and trained personnel before it can be implemented in routine species identification.

2.2 Nucleotide sequencing

DNA sequencing is a powerful and sensitive method for species identification, and it has gained prominence as a rapid, accessible methodology owing to technological advances and the decrease in the cost per sample (Wong et al., 2009).

When sequencing samples for species identification, it is important to correctly choose the genomic portion to be analysed. Distinct regions of the genome evolve at variable rates; therefore, to avoid errors in species diagnostics, the chosen portion should display high interspecific and low intraspecific variability (Bossier, 1999).

For example, Heist & Gold (1999) sequenced mitochondrial DNA (mtDNA) to discriminate among 11 species of Carcharhiniformes. Douady et al. (2003) conducted a study with a 2.4-kb fragment of the mitochondrial genome, which extended from the 12S rRNA gene to the 16S rRNA gene. They examined the phylogenetic relationships among the orders of sharks, which demonstrated that this region may be useful for resolving relationships at lower taxonomic levels. Subsequently, Greig et al. (2005) extended on this analysis by discriminating 35 shark species in the North Atlantic based on the high intraspecific and low interspecific divergence of these mitochondrial gene sequences.

Recently, Rodrigues-Filho et al. (2009) utilised the same marker to discriminate shark species that are exploited by fisheries in Northern Brazil. They revealed the occurrence of 11 species among morphologically indistinguishable landed specimens (Figure 4). Among the species that were identified, the species *Carcharhinus porosus* and sharks of the genus *Rhizoprionodon* were the most frequently caught. The former species is not listed by IUCN; however, local *C. porosus* stocks have been depleted, which has caused this species to be included in the Brazilian Red List of endangered fauna (MMA, 2004). In addition, Rodrigues-Filho et al. (2009) used genetic screening to show that the previously abundant *Isogomphodon oxyrhynchus* was not present among species that were caught by fisheries.

A different strategy that is widely used to identify species is the sequencing of the DNA barcode region (Hebert et al., 2003). In this approach, the identification of a given species is based on a profile that is generated with an approximately 650-base pair fragment of the mitochondrial COI gene. Studies have shown that this portion of COI displays low intraspecific and high interspecific variability, which is important for the discrimination of species on the basis of permanent nucleotide differences that exist among them. An advantage of this approach is the availability of a global reference databank, which is the result of international efforts that were mediated by the Consortium for the Barcode of Life (CBOL; http://www.barcoding.si.edu). The primary databank is called BOLD (Barcode of Life Data System, http://www.barcodinglife.org/views/login.php) (Ratnasingham & Hebert, 2007), which has currently assembled approximately 100,000 barcoded species. BOLD, which is the host of the FISH-BOL databank (Fish Barcode of Life campaign, http://www.fishbol.org) is dedicated to the assembly of barcode sequences from marine and freshwater species of fishes. Currently, 556 species of sharks and rays have been barcoded; this represents approximately 50% of the total number of elasmobranches that have been described (FISH-BOL, accessed in July/2011).

Some of the primordial studies that used DNA barcodes were performed by Ward et al. (2005), who sequenced the COI gene of 61 species and standardised this region for the identification of elasmobranches. Other studies that have used the DNA barcode approach have efficiently identified shark species (Ward et al., 2007; Moura et al., 2009; Ward et al., 2008; Holmes et al., 2009; Wong et al., 2009; Barbuto et al., 2010). In a wide screen, Ward et al. (2008) evaluated the variability of COI sequences from 210 species of Chondrichthyes (123 species of sharks) and discriminated 99% of the species that were screened. In addition, Holmes *et al.* (2009) identified and quantified the relative abundance of 20 shark species by

analysing dried fins that were confiscated from illegal fisheries. Similarly, the DNA barcoding approach was also useful for the detection of fraudulent mislabelling in Italian fish markets, in which 80% of the commercialised sharks did not corresponded to the referred species that were sold (Barbuto et al., 2010).

Despite of the large number of studies that have demonstrated the potential of DNA barcode techniques for species identification, its use is controversial. Many authors argue that a single, short marker is not reliable as a taxonomic tool, and traditional systematics utilises a large number of characteristics to delimit species (Dasmahapatra & Mallet, 2006). In addition, the limitations of the DNA barcode approach include its inability to detect hybridisation (which originated from gene flow between distinct species) due to the maternal inheritance of mitochondrial DNA (Moritz & Cicero, 2004). Therefore, to gauge the confidence of the DNA barcode technique for species identification, scientists have recommended the examination of a large number of individuals, especially of globally distributed species, to confirm that the genetic divergence of COI is higher between species than within species (see Shivji, 2010).

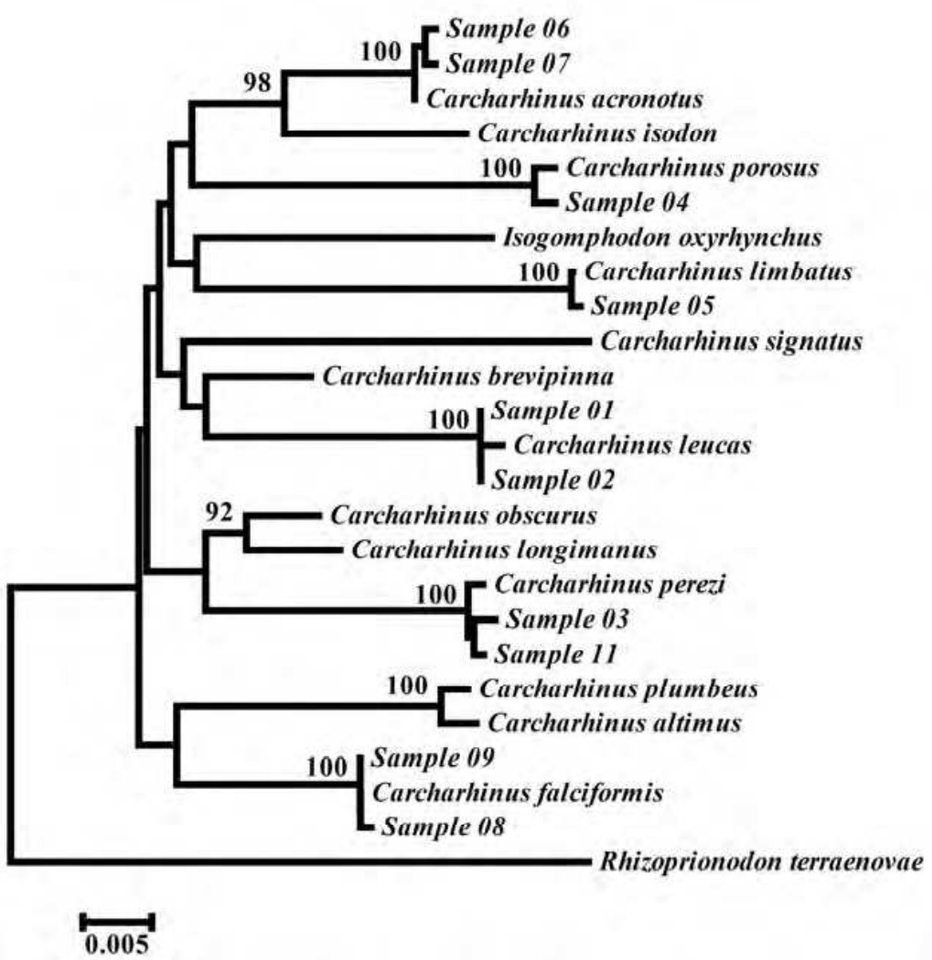


Fig. 4. Maximum likelihood tree of the mitochondrial genes 12S-16S that was used to discriminate shark samples (genus *Carcharhinus*).

2.3 Genetic diversity measured by molecular markers and implications for shark management

Among the large migratory fish species, sharks perhaps represent some of the more challenging taxa to trace demographically because they are rarely observed in the open ocean where they feed, breed and otherwise interact with conspecifics (Bradshaw et al., 2007). Despite the advances in mark–recapture and other tagging strategies that are used to estimate survival rates and long-range movements, these methods still have restrictions for deriving population estimates and exchange (Kohler & Turner, 2001), and they cannot provide a quantitative measure of the genetic diversity of the stocks.

Genetic techniques have been used with increasing frequency to inform conservation and management (Crandall et al., 2000; Allendorf et al., 2004), and they are especially useful for studying species for which ecology, demographics or behaviour limit the traditional field methods that are used to estimate the population trends.

The decreasing fish stocks and near extinction of several species will impact the economy and the whole environment. By measuring the variability of genetic stocks, it is possible to determine the real impact of fisheries on shark populations.

Because genetic diversity is correlated with population fitness (Reed & Frankham, 2003), understanding patterns of gene flow and dispersal is crucial for deriving how shark populations are distributed and if they constitute distinct genetic stocks. When this occurs populations are alleged to be strutucted. Therefore, the presence of genetic structure is indicative of low levels of migration among distinct stocks and indicates the need for the adoption of independent management measures for each stock (Schrey & Heist, 2003). This information is particularly useful for large free-living marine animals as sharks and can greatly assist for the conservation of wildlife in oceanic landscapes.

Sharks and other large marine fishes with a broad distribution frequently exhibit little population structure (Graves, 1998; Heist, 2004; Curley & Gillings, 2009). Exceptions to this pattern have been attributed to viviparous reproduction, sedentary behaviour and disjunct distributions, but they have been principally attributed to reproductive philopatry (Palumbi 1994; Pratt & Carrier, 2001; Waples et al., 2001; Keeney et al., 2003; Hueter et al., 2004; Keeney et al., 2005; Duncan et al., 2006; Karl et al., 2010). Philopatry is defined as the tendency of an individual to return to or stay in its home area, natal site, or another adopted locality; this is in opposition to nonreturning roaming behaviour or simple dispersal away from home areas (Hueter et al., 2004). Philopatric tendencies can be strong or weak for a given species, and special patterns of this behaviour can include natal philopatry (returning to the natal nursery area) and sex-specific philopatry (where one sex is more philopatric than the other, as in many male birds and female mammals) (Hueter et al., 2004). Females that exhibit philopatric behaviour return to coastal embayments that are called “nursery areas” to give birth (Pardini et al., 2001; Feldheim et al., 2002; Schrey & Heist, 2003). Nursery areas are usually located in shallow coastal waters and offer the advantages of low predation rates, prey abundance and spatial segregation from adults, which use geographically distant feeding areas, to neonates and juveniles (Morrissey & Gruber, 1993; Merson & Pratt, 2001). As a result, philopatric behaviour may clearly directly influence the levels of population structure and genetic diversity of a species, and molecular markers can be employed to detect such pattern.

Currently, a wide set of genetic markers has been utilised to infer population genetic structure in marine organisms (Baker et al., 1990; Baker et al., 1996; Amos et al., 1993; Innes et al., 1998). Due to high polymorphism and mutation rates (among other features), the molecular markers that are commonly used are those that involve the analysis of mitochondrial DNA and nuclear microsatellite loci.

In the mitochondrial genome, the non-coding control region (CR) is the molecular marker that is frequently utilised (Brown et al., 1985; Warn et al., 1986; Avise, 2000; Warn, 2000) due to its high evolutionary rates (Li, 1997; Warn, 2000). Specifically, the control region contains portions of its sequence in which the variability is even greater, which makes the region a highly informative target for population studies.

Studies that have used the CR as molecular marker for sharks are somewhat scarce in comparison to other marine species (for review, see Shivji et al., 2010). This implies incipient information regarding migration rates, dispersion movements, gene flow and the composition of genetic stocks for the majority of species. Indeed, knowledge of the genetic structure and diversity of shark populations is critical for maintaining species reproductive strength, resistance to diseases and the ability to adapt to environmental changes (Frankham et al., 2002).

Overall, most of the studies that have been performed on sharks have shown that distinct species possess lower genetic diversity when compared to other taxonomic groups (FAO). Nevertheless, despite the extensive exploitation by fisheries, some studies have reported that species still carrying high levels of genetic diversity (Pardini et al., 2001; Bowen et al., 2004; Keeney et al., 2005; Duncan et al., 2006; Martinez et al., 2006; Natoli et al., 2006) (Table 1).

Species	Pi	H	N	HAP	References
Carcharhinus limbatus (Terra Ceia Bay)	0.0106	0.720	45	8	Keeney *et al*. (2003)
Carcharhinus limbatus (Yankeetown)	0.0134	0.796	45	9	Keeney *et al*. (2003)
Carcharhinus limbatus (Belize City)	0.00077	0.680	13	4	Keeney *et al*. (2005)
Carcharhinus limbatus (Dangriga)	0.00049	0.526	19	2	Keeney *et al*. (2005)
Cetorhinus maximus	0.0013	0.720	62	6	Hoelzel *et al*. (2006)
Carcharias taurus	0.003	0.717	26	4	Stow *et al*. (2006)
Sphyrna lewini	0.013	0.8	271	24	Duncan *et al*. (2006)
Rhincodon typus	0.011	0.974	69	44	Castro *et al*. (2007)
Triakis semifasciata	0.0067	-	169	5	Lewallen *et al*. (2007)
Galeorhinus galeus	0.0071	0.92	116	38	Chabot *et al*. (2009)

Table 1. Genetic diversity of the mitochondrial control region from several shark species. **Pi** – Nucleotide diversity. **H** – Haplotype diversity. **N –** sampling. **HAP** – Number of observed haplotypes.

However, a few studies, such as the study that was published by Keeney *et al.* (2003), reported a low genetic diversity for the CR sequences from some populations of *C. limbatus* in the Atlantic coast. Other species that displayed low levels of diversity were *Cetorhinus maximus*, which is a worldwide distributed species that is highly susceptible exploitation by fisheries, and *Sphyrna tiburo*, which is heavily exploited in the Gulf of Mexico (Chapman et al., 2004; Hoelzel et al., 2006).

Within the nuclear genome, microsatellites are among the most utilised markers. These sequences can correspond to more than 20 alleles and carry heterozygosity above 95% (Heist, 2004). Other advantages of microsatellites are that they are diploid, codominant and analysable from small amounts of DNA; they also display high and constant mutational rates per generation (Jarne & Lagoda, 1996). The PCR allows access to different alleles at microsatellite loci and requires small amounts of DNA.

Recently, significant advances have been made in developing new statistical tools to analyse specific microsatellite loci (Pritchard et al., 2000). Such specificity in the analysis makes microsatellites powerful markers, and their applicability has been proven by studies in fish, such as in genetic mapping and paternity tests (Kocher et al., 1998; Keeney & Heist, 2003, Feldheim et al., 2004). Additionally, microsatellites are extensively used in studies that evaluate genetic variability among species and populations, evolutionary history, population structure and conservation and natural resource management (Bruford & Wayne, 1993; Warn, 2010). The variation in the number of repeated units in each microsatellite locus is the basis for studies that use these markers.

Currently, microsatellite markers are employed to study several shark species, and these markers can be useful for estimating genetic structure and gene flow in wild populations. In fact, DNA markers with different modes of inheritance, such as the maternally inherited mitochondrial DNA (mtDNA) and bi-paternally inherited nuclear microsatellites, are the markers that are most commonly used to infer the genetic diversity and structure of shark populations (e.g., gummy shark *Mustelus antarcticus*, Gardner & Ward 1998; great white shark *Carcharodon carcharias*, Pardini et al., 2001; blacktip sharks *Carcharhinus limbatus*, Keeney *et al*., 2003, 2005; shortfin mako *Isurus oxyrinchus*, Schrey & Heist 2003; whale shark, *Rhincodon* typus, Castro et al., 2007; bull sharks *Carcharhinus leucas*, Karl et al., 2010). Several shark studies have used a combination of both markers, which has facilitated the obtainment of improved data regarding historical and contemporary events that drive population dynamics.

3. Conclusion

Oceans are a bountiful but not unlimited source of fishes that have been extensively harvested for decades. As a consequence, several top predators, such as the majority of shark species, are now endangered or live on the edge of extinction. Given the worldwide depletion of shark populations, which has been largely documented globally, there is an urgent need to increase the knowledge of the life history traits and other fundamental aspects of shark biology to promote conservation.

Therefore, several molecular technologies and methodologies that are summarised and discussed in the present chapter are extremely relevant to monitor the exploitation and prevent the loss of genetic diversity of sharks. It is important to note that molecular

identification using distinct markers can be applied to virtually all living shark species to prevent extinction and to assist in their sustainable use as a natural resource. Furthermore, the correct monitoring of genetic stocks is extremely important to ensure the possibility of the full recovery of depleted stocks and the maintenance of genetic diversity.

4. References

Abercrombie, D.L.; Clarke, S.C. & Shivji, MS (2005) Global-scale genetic identification of hammerhead sharks: Application to assessment of the international fin trade and law enforcement. *Conservation Genetics,* 6, 775-788

Allendorf, F.W.; Leary, R.F.; Hitt, N.P.; Knudsen, K.L.; Lundquist, L.L. & Spruell, P. (2004) Intercrosses and the U.S. Endangered Species Act: should hybridized populations be included as Westslope Cutthroat Trout? *Conservation Biology*, 18, 1203–1213

Amos, B.; Schlotterer, C. & Tautz, D. (1993) Social structure of pilot whales revealed by analytical DNA profiling. *Science*, 260, 670–672

Asensio, L.; González, I.; Fernández, A.; Céspedes, A.; Rodríguez, M.; Hernández, P. & García, T. (2001) Identification of Nile perch (*Lates niloticus*), grouper (*Epinephelus guaza*), and wreck fish (*Polyprion americanus*) fillets by PCR amplification of the 5S rDNA gene. *Journal of AOAC International,* 84, 777-781

Avise, J.C. (2010) Conservation genetics enters the genomics era. *Conservation Genetetics*, 11, 665-669

Avise, J.C. (2000) Phylogeography: the history and formation of species. Cambridge, MA: Harvard University Press

Avise, J.C.; Helfman, G.S.; Saunders, N.C. & Stanton, L.H. (1986) Mitochondrial DNA differentiation in north atlantic eels: Population genetic consequences of an unusual life history pattern. *Proceedings of the National Academy of Science*, 83, 4350-4354

Baker, C.S.; Palumbi, S.R. & Lambertsen, R.H. (1990) Influence of seasonal migration on geographic distribution of mitochondrial DNA haplotypes in humpback whales. *Nature*, 344, 238–240

Baker, C.S.; Cipriano, F. & Palumbi, S.R. (1996) Molecular genetic identification of whale and dolphin products from commercial markets in Korea and Japan. *Molecular Ecology*, 5, 671–685

Barbuto, M.; Galimberti, A.; Ferri, E.; Labra, M.; Malandra, R.; Galli, P. & Casiraghi, M. (2010) DNA barcoding reveals fraudulent substitutions in shark seafood products: The Italian case of. *Food research international,* 43, 376-381

Baum, J.; Myers, R.; Kehler, D.; Worm, B.; Harley, S. & Doherty, P. (2003) Collapse and conservation of shark populations in the Northwest Atlantic. 299, 389-392

Baum, J.K. & Myers, R.A. (2004) Shifting baselines and the decline of pelagic sharks in the Gulf of Mexico. *Ecol. Lett.*, 7, 135–145

Bigelow, H.B. & Schroeder, W.C. (1948) Fishes of the western north Atlantic. *Memoirs of the Sears Foundation for Marine Research* 1, 282-292

Bonfil, R. 1994. *Overview of world elasmobranch fisheries.* FAO Fisheries Technical Paper no. 341, Rome

Bossier, P. (1999) Authentication of seafood products by DNA patterns. *Journal of food science,* 64, 189-193

Bowen, B.W.; Bass, A.L.; Chow, S.M.; Bostrom, M.; Bjorndal, K.A.; Bolten, A.B.; Okuyama, T.; Bolker, B.M.; Epperly, S.; Lacasella, E.; Shaver, D.; Dodd, M.; Hopkinsmurphy, S.R.; Musick, J.A.; Swingle, M.; Rankin-Baransky, K.; Teas, W.; Witzell, W.N. & Dutton, P.H. (2004) Natal homing in juvenile loggerhead turtles (*Caretta caretta*). *Molecular Ecology*, 13, 3797-3808

Bradshaw, C.J.A. (2007) Swimming in the deep end of the gene pool: global population structure of an oceanic giant. *Molecular Ecology*, 16, 5111-5113

Branstetter, S. (1990) Early life-history implications of selected carcharhinoid and lamnoid sharks of the northwest Atlantic. *NOAA Technical Reports NMFS,* 90, 17-28Brown WM (1985) The mitochondrial genome of animals. In: Maclntvre, R.J. (ed). Molecular Evolutionary genetics. Plenum Press, NY, p 95-130

Bruford MW, Wayne RK (1993) Microsatellites and their application to population genetic studies, *Current Opinion in Genetics and Development*, 3, 939–943

Castro, A.L.; Stewart, S.; Wilson, G.; Hueter, R.E.; Meekan, M.G.; Motta, P.J.; Bowen, B.W. & Karl, S. (2007) Population genetic structure of Earth's largest fish, the whale shark (*Rhincodon typus*). *Molecular Ecology*, 16, 5183-5192

Castro, J.I. (1993) *A field guide to the sharks commonly caught in commercial fisheries of the southeastern United States,* US Dept. of Commerce.; National Oceanic and Atmospheric Administration.; National Marine Fisheries Service.; Southeast Fisheries Science Center, Miami

Castro, J.I.; Woodley, C.M. & Brudek, R.L. (1999) *A preliminary evaluation of the status of shark species,*Fisheries technical paper 380. FAO, Rome.

Chapman, D.D.; Abercrombie, DL.; Douady, C.J.; Pikitch, E.K.; Stanhopen, M.J. & Shivji, M.S. (2003) A streamlined, bi-organelle, multiplex PCR approach to species identification: Application to global conservation and trade monitoring of the great white shark, *Carcharodon carcharias*. *Conservation Genetics,* 4, 415-425

Chapman, D.D.; Prodöhl, P.A.; Gelsleichter, J.; Manire, C.A. & Shivji, M.S. (2004) Predominance of genetic monogamy by females in a hammerhead shark, *Sphyrna tiburo*: implications for shark conservation. *Molecular Ecology,* 13, 1965-1974

Clarke, S.C.; Magnussen, J.E.; Abercrombie, D.L.; McAllister, M.K. & Shivji, M.S. (2006) Identification of shark species composition and proportion in the Hong Kong shark fin market based on molecular genetics and trade records. *Conservation Biology,* 20, 201-211

Crandall, K.A.; Bininda-Emonds, O.R.P.; Mace, G.M. & Wayne, R.K. (2000) Considering evolutionary processes in conservation biology. *Trends in Ecology and Evolution*, 15, 290–295.

Curley, B.G. & Gillings, M.R. (2009) Population connectivity in the temperate damselfish *Parma microlepis*: analyses of genetic structure across multiple scales. *Marine Biology*, 156, 381–393

Dasmahapatra, K. & Mallet, J. (2006) Taxonomy: DNA barcodes: recent successes and future prospects. *Heredity,* 97, 254-255

Douady, C.J.; Dosay, M.; Shivji, M.S. & Stanhope, M.J. (2003) Molecular phylogenetic evidence refuting the hypothesis of Batoidea (rays and skates) as derived sharks. *Molecular Phylogenetics and Evolution,* 26, 215-221

Dulvy, N.K; Sadovy, Y. & Reynolds, J.D. (2003) Extinction vulnerability in marine populations. *Fish and Fisheries*, 4, 25–64

Dulvy, N.K.; Baum, J.K.; Clarke, S.; Compagno, L.J.V.; Cortes, E.; Domingo, A.; Fordham, S.; Fowler, S.; Francis, M.P. & Gibson, C. (2008) You can swim but you can't hide: the global status and conservation of oceanic pelagic sharks and rays. *Aquatic Conservation: Marine and Freshwater Ecosystems,* 18, 459-482

Duncan, K.; Martin, A.; Bowen, B. & De Couet, H. (2006) Global phylogeography of the scalloped hammerhead shark (*Sphyrna lewini*). *Molecular Ecology*, 15, 2239- 2251

FAO (2000) Fisheries Management: 1. Conservation and Management of Sharks. FAO Technical Guidelines for Responsible Fisheries 4 (Supplement 1), pp 37

Farrell, E.D.; Clarke, M.W. & Mariani, S. (2009) A simple genetic identification method for Northeast Atlantic smoothhound sharks (*Mustelus* spp.). *ICES Journal of Marine Science: Journal du Conseil,* 66, 561

Feldheim, K.A.; Gruber, S.H. & Ashley, M.V. (2002). Breeding biology of lemon sharks at a tropical nursery lagoon. *Proceedings of the Royal Society of London B*, 269, 1655–1662

Feldheim, K.A.; Gruber, S.H. & Ashley, M.V. (2004) Reconstruction of parental microsatellite genotypes reveals female polyandry and philopatry in the lemon shark, *Negaprion brevirostris. International Journal of Organic Evolution,* 58(10), 2332-2342

Frankham, R.; Ballou, J.D. & Briscoe, D.A. (2002) *Introdução para genética da conservação*, Cambridge University Press, ISBN Cambridge

Gardner, M.G. & Ward, R.D. (1998) Population structure of the Australian gummy shark (*Mustelus antarcticus* Gunther) inferred from allozymes, mitochondrial DNA and vertebrae counts. *Marine Freshwater Research*, 49, 733-745

Graves, J.E. (1998) Molecular insights into the population structure of cosmopolitan marine fishes. *Journal of Heredity*, 89, 427-437.

Greig, T.; Moore, M.; Woodley, C. & Quattro, J. (2005) Mitochondrial gene sequences useful for species identification of western North Atlantic Ocean sharks. *Fishery bulletin-national oceanic and atmospheric administration,* 103, 516

Hebert, P.D.N.; Cywinska, A.; Ball, S.L. & DeWaard, J.R. (2003) Biological identifications through DNA barcodes. *Proceedings of the Royal Society of London. Series B: Biological Sciences,* 270, 313

Heist, E. (2004) Genetics of sharks, skates, and rays. In: Biology of Sharks and Their Relatives, Carrier, J.C.; Musick, J.A. & Heithaus, M.R., pp. 485. CRC Press, New York.

Heist, E. & Gold, J. (1999) Genetic identification of sharks in the US Atlantic large coastal shark fishery. *Fishery bulletin-national oceanic and atmospheric administration,* 97, 53-61

Hoelzel, A.; Shivji, M.; Magnussen, J. & Francis, M. (2006) Low worldwide genetic diversity in the basking shark (*Cetorhinus maximus*). *Biology Letters,* 2, 639-642

Holmes, B.H.; Steinke, D. & Ward, R.D. (2009) Identification of shark and ray fins using DNA barcoding. *Fisheries Research,* 95, 280-288

Hueter, R.E.; Heupel, M.R.; Heist, E.J. & Keeney, D.B. (2004) Evidence of philopatry in sharks and implications for the management of shark fisheries. *Journal of Northwest Atlantic Fishery Science*, 35, 7-17

Imsiridou, A.; Minos, G.; Katsares, V.; Karaiskou, N. & Tsiora, A. (2007) Genetic identification and phylogenetic inferences in different Mugilidae species using 5S rDNA markers. *Aquaculture Research,* 38, 1370-1379

Innes, B.H.; Grewe, P.M. & Ward, R.D. (1998) PCR-based genetic identification of marlin and other billfish. *Marine Freshwater Research*, 49, 383–388

IUCN (2011) 2011 IUCN Red List of Threatened Species. <www.iucnredlist.org>. Downloaded on 07 May 2011

Jarne, P. & Lagoda P.J.L. (1996) Microsatellites, from molecules to populations and back. *Trends in Ecology and Evolution*, 11, 424-429

Karl, S.A.; Castro, A.L.F.; Lopez, J.A.; Charvet, P. & Burgess, G.H. (2010) Phylogeography and conservation of the bull shark (*Carcharhinus leucas*) inferred from mitochondrial and microsatellite DNA. *Conservation Genetics*, DOI 10.1007/s10592-010-0145-1

Keeney, D.; Heupel, M.; Hueter, R. & Heist, E. (2005) Microsatellite and mitochondrial DNA analyses of the genetic structure of blacktip shark (*Carcharhinus limbatus*) nurseries in the northwestern Atlantic.; Gulf of Mexico.; and Caribbean Sea. *Molecular Ecology,* 14, 1911-1923

Keeney, D.B. & Heist, E.J. (2003) Characterization of microsatellite loci isolated from the blacktip shark and their utility in requiem and hammerhead sharks. *Molecular Ecology Notes*, 3, 501-504

Kocher, T.D., Lee, W.J., Sobolewska, H., Penman, D. & McAndrew, B. (1998) A genetic linkage map of a cichlid fish, the tilapia (*Oreochromis niloticus*). *Genetics*, 148, 1225–1232

Kohler, N.E. & Turner, P.A. (2001) Shark tagging: a review of conventional methods and studies. *Environmental Biology of Fishes*, 60, 191-223

Kotas, J.E.; Gamba, M.R.; Conoly, P.C.; Hostim-Silva, M.; Mazzoleni, R.C. & Pereira, J.P. (1995) A Pesca de emalhe direcionada aos Elasmobrânquios com desembarques em Itajaí e Navegantes/SC. Reunião Do Grupo De Trabalho E Pesquisa De Tubarões E Raias Do Brasil, VII, Rio Grande, novembro, 1995

Last, P. & Stevens, J. (1994) Sharks and Rays of Australia.; 513 pp. Australia Commonwealth Scientific and Industrial Research Organization. Melbourne

Lavery, S.; Moritz, C. & Fielder, D. (1996) Genetic patterns suggest exponential population growth in a declining species. *Molecular Biology and Evolution*, 13, 1106–1113

Lessa, R.; Santana, F.M.; Rincón, G.; Gadig, O.B. & El-Deir, A.C.A. (1999) *Biodiversidade de Elasmobrânquios no Brasil. Programa Nacional da Diversidade Biológica* (PRONABIO). 125p. (http://www.bdt.org.br/workshop/costa/elasmo/)

Li, W.H. (1997) Molecular Evolution. Sinauer Associates, Inc., Publishers, Sunderland. pp 284

Long, E.O. & David, I.B. (1980) Repeated genes in Eukaryotes. *Annual Review of Biochemestry*, 49, 727–764

Magnussen, J.; Pikitch, E.; Clarke, S.; Nicholson, C.; Hoelzel, A. & Shivji, M. (2007) Genetic tracking of basking shark products in international trade. *Animal Conservation,* 10, 199-207

Martins, C. & Wasko, A. (2004) Organization and evolution of 5S ribosomal DNA in the fish genome. *Focus on Genome Research. Nova Science Publishers, Hauppauge*, 289-319

Martinez, P.; Gonzalez, E.G.; Castilho, R. & Zardoya, R. (2006) Genetic diversity and historical demography of Atlantic bigeye tuna (*Thunnus obesus*). *Molecular Phylogenetics and Evolution*, 39, 404-416

Mendonça, F.; Hashimoto, D.; Porto Foresti, F.; Oliveira, C.; Gadig, O. & Foresti, F. (2009) Identification of the shark species *Rhizoprionodon lalandii* and *R. porosus* (Elasmobranchii, Carcharhinidae) by multiplex PCR and PCR RFLP techniques. *Molecular Ecology Resources*, 9, 771-773

Merson, R.R. & Pratt, Jr. H.L. (2001) Distribution, movements and growth of young sandbar sharks, *Carcharhinus plumbeus*, in the nursery grounds of Delaware Bay. *Environmental Biology of Fishes*, 38, 167-174

MMA – IBAMA. Estatística da Pesca 2002 Brasil: Grandes Regiões e Unidades da Federação. *IBAMA-CEPENE*, 15 agosto 2004, www.ibama.gov.br

MMA (Ministério do Meio Ambiente) (2004) Instrução Normativa n° 5, de 21 de Maio de 2004: Anexo I – LISTA NACIONAL DE ESPÉCIES DE INVERTEBRADOS AQUÁTICOS E PEIXES AMEAÇADAS DE EXTINÇÃO. Diário Oficial da União – seção 1 n°102, sexta-feira, 28 de Maio de 2004

Morgan, J.A.T.; Welch, D.J.; Harry, A.V.; Street, R.; Broderick, D. & Ovender, J.R. (2011) A mitochondrial species identification assay for Australian blacktip sharks (*Carcharhinus tilstoni*, *C. limbatus* and *C. amblyrhynchoides*) using real-time PCR and high-resolution melt analysis. *Molecular Ecology Resources*, doi: 10.1111/j.1755-0998.2011.03023.x

Moritz, C. & Cicero, C. (2004) DNA barcoding: promise and pitfalls. *PLoS Biology*, 2, e354

Morrissey, J.F. & Gruber, S.H. (1993) Home range of juvenile lemon sharks *Negaprion brevirostris*. *Copeia*, 2, 425-434

Moura, T.; Silva, M.C.; Figueiredo, I.; Neves, A.; Muñoz, P.D.; Coelho, M.M. & Gordo, L.S. (2008) Molecular barcoding of north-east Atlantic deep-water sharks: species identification and application to fisheries management and conservation. *Marine and Freshwater Research,* 59, 214-223

Musick, J.; Burgess, G.; Cailliet, G.; Camhi, M. & Fordham, S. (2000) Management of sharks and their relatives (Elasmobranchii). *Fisheries,* 25, 9-13.

Natoli, A.; Cañadas, A.; Peddemors, V.; Aguilar, A.; Vaquero, C.; Fernández-Piqueras, P. & Hoelzel, A. (2006) Phylogeography and alpha taxonomy of the common dolphin (*Delphinus* sp.). *Journal of Evolutionary Biology,* 19, 943-954

Naylor, G.J.P. & Marcus, L.F. (1994) *Identifying isolated shark teeth of the genus Carcharhinus to species: Relevance for tracking phyletic change through the fossil record*, American Museum of Natural History, New York

Ovenden, J.R.; Morgan, J.A.T.; Street, R.; Tobin, A.; Simpfendorfer, C.; Macbeth, W. & Welch, D. (2011) Negligible evidence for regional genetic population structure for two shark species *Rhizoprionodon acutus* (Ruppell, 1837) and *Sphyrna lewini* (Griffith & Smith, 1834) with contrasting biology. *Marine Biology*, 158, 1497–1509

Palumbi, S.R. (1994) Genetic divergence, reproductive isolation, and marine speciation. *Annual Review of Ecology and Systematics*, 25, 547–572

Pank, M.; Stanhope, M.; Natanson, L.; Kohler, N. & Shivji, M. (2001) Rapid and simultaneous identification of body parts from the morphologically similar sharks *Carcharhinus obscurus* and *Carcharhinus plumbeus* (Carcharhinidae) using multiplex PCR. *Marine Biotechnology,* 3, 231-240

Pardini, A.T.; Jones, C.S.; Noble, L.R.; Kreiser, B.; Malcolm, H.; Bruce, B.D.; Stevens, J.D.; Cli, G.; Scholl, M.C.; Francis, M.; Duvy, C.A.J. & Martin, A.P. (2001) Sex-biased dispersal of great white sharks. *Nature*, 412, 139–140

Pinhal, D.; Yoshimura, T.S.; Araki, C.S. & Martins, C. (2011) The 5S rDNA family evolves through concerted and birth-and-death evolution in fish genomes: an example from freshwater stingrays. *BMC Evolutionary Biology*, 11:151 doi:10.1186/1471-2148-11-151

Pinhal, D.; Araki, C.; Gadig, O. & Martins, C. (2009a) Molecular organization of 5S rDNA in sharks of the genus Rhizoprionodon: insights into the evolutionary dynamics of 5S rDNA in vertebrate genomes. *Genetics Research,* 91, 61-72

Pinhal, D.; Gadig, O.B.F. & Martins, C. (2009b) Genetic identification of the sharks *Rhizoprionodon porosus* and *R. lalandii* by PCR-RFLP and nucleotide sequence analyses of 5S rDNA. *Conservation Genetetics Research*, 1, 35-38.

Pinhal, D.; Gadig, O.; Wasko, A.; Oliveira, C.; Ron, E.; Foresti, F. & Martins, C. (2009b) Discrimination of Shark species by simple PCR of 5S rDNA repeats. *Genetics and Molecular Biology*, 31, 361-365

Pratt, H.L. & Carrier, J.C. (2001) A review of elasmobranch reproductive behavior with a case study on the nurse shark, *Ginglymostoma cirratum*. *Environmental Biology of Fishes*, 60, 157-188

Pritchard, J.K.; Stephens, M. & Donnelly, P. (2000) Inference of population structure using multilocus genotypic data. *Genetics*, 155, 945-959

Rasmussen, R. & Morrissey, M. (2008) DNA-based methods for the identification of commercial fish and seafood species. *Comprehensive Reviews in Food Science and Food Safety,* 7, 280-295

Ratnasingham, S. & Hebert, P.D.N. (2007) BOLD: The Barcode of Life Data System (http://www. barcodinglife. org). *Molecular Ecology Notes,* 7, 355-364

Rodrigues-Filho, L.; Rocha, T.; Rêgo, P.; Schneider, H.; Sampaio, I. & Vallinoto, M. (2009) Identification and phylogenetic inferences on stocks of sharks affected by the fishing industry off the Northern coast of Brazil. *Genetics and Molecular Biology*. 32(2), 405-413

Sebastian, H.; Haye, P.A. & Shivji M.S. (2008) Characterization of the pelagic shark-fin trade in north-central Chile by genetic identification and trader surveys. *Journal of Fish Biology,* 73, 2293-2304

Shivji, M.; Clarke, S.; Pank, M.; Natanson, L.; Kohler, N.; Stanhope, M. (2002) Genetic identification of pelagic shark body parts for conservation and trade monitoring. *Conservation Biology,* 16, 1036-1047

Shivji, M.S. (2010) DNA Forensic Applications in Shark Management and Conservation. Sharks and Their Relatives II: Biodiversity, Adaptive Physiology, and Conservation. CRC Press, Boca Raton, FL.

Shivji, M.S.; Chapman, D.D.; Pikitch, E.K.; Raymond, P.W. (2005) Genetic profiling reveals 33 illegal international trade in fins of the great white shark, *Carcharodon carcharias*. *Conservation Genetics,* 6, 1035-1039

Schrey, A.W. & Heist, E.J. (2003) Microsatellite analysis of population structure in shortfin mako (*Isurus oxyrinchus*). *Canadian Journal* of *Fisheries* and *Aquatic Sciences*, 60, 670-675

Stevens, J. & Wayte, S. (1998) A review of Australia's pelagic sharks resources. Fisheries Research and Development Corporation project 98/107, 64 p. *CSIRO Marine Research, GPO Box 1538, Hobart, Tasmania 7001 Australia*.

Stevens, J.D.; Bonfil, R.; Dulvy, N.K. & Walker, P.A. (2000) The effects of fishing on sharks, rays, and chimaeras (Chondrichthyans) and the implications for marine ecosystems. *ICES Journal of Marine Science*, 57, 476-494

Vooren, C.M. (1997) Elasmobrânquios demersais. *In:* Os ecossistemas costeiro e Marinho do extremo sul do Brasil. Seelinger, U., Oderbrecht, C. & Castello, J.P., pp162, Ed. Ecoscientia, Rio Grande

Waples, R.S.; Gustafson, R.G. & Weitkramp, L.A. (2001) Characterizing diversity in salmon from the Pacific Northwest. *Journal of Fish Biology*, 59, 1-41

Ward, R.; Zemlak, T.; Innes, B.; Last, P. & Hebert, P. (2005) DNA barcoding Australia's fish species. *Philosophical Transactions of the Royal Society. B. BiologicalSciences,* 360, 1847-1857

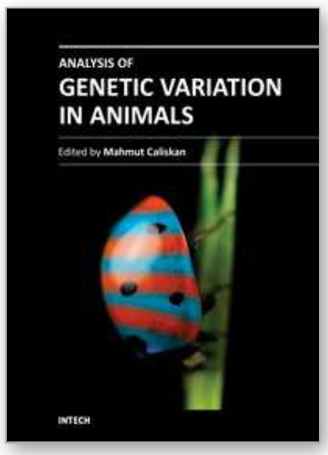
Ward, R.D.; Holmes, B.H.; White, W.T. & Last, P.R. (2008) DNA barcoding Australasian chondrichthyans: results and potential uses in conservation. *Marine and Freshwater Research,* 59, 57-71

Ward, R.D.; Holmes, B.H.; Zemlak, T.S. & Smith, P. (2007) DNA barcoding discriminates spurdogs of the genus Squalus. *Descriptions of new dogfishes of the genus Squalus (Squaloidea: Squalidae). CSIRO Marine and Atmospheric Research Paper,* 14, 117-130

Wasko, A.; Martins, C.; Wright, J. & Galetti, Jr. P. (2001) Molecular organization of 5S rDNA in fishes of the genus Brycon. *Genome,* 44, 893-902

Wong, E.H.K.; Shivji M.S. & Hanner, R.H. (2009) Identifying sharks with DNA barcodes: assessing the utility of a nucleotide diagnostic approach. *Molecular Ecology Resources,* 9, 243-256

Wynen, L.; Larson, H.; Thorburn, D.; Peverell, S.; Morgan, D.; Field, I. & Gibb, K. (2009) Mitochondrial DNA supports the identification of two endangered river sharks (*Glyphis glyphis* and *Glyphis garricki*) across northern Australia. *Marine and Freshwater Research,* 60, 554-562



Analysis of Genetic Variation in Animals
Edited by Prof. Mahmut Caliskan

ISBN 978-953-51-0093-5
Hard cover, 360 pages
Publisher InTech
Published online 29, February, 2012
Published in print edition February, 2012

Analysis of Genetic Variation in Animals includes chapters revealing the magnitude of genetic variation existing in animal populations. The genetic diversity between and within populations displayed by molecular markers receive extensive interest due to the usefulness of this information in breeding and conservation programs. In this concept molecular markers give valuable information. The increasing availability of PCR-based molecular markers allows the detailed analyses and evaluation of genetic diversity in animals and also, the detection of genes influencing economically important traits. The purpose of the book is to provide a glimpse into the dynamic process of genetic variation in animals by presenting the thoughts of scientists who are engaged in the generation of new idea and techniques employed for the assessment of genetic diversity, often from very different perspectives. The book should prove useful to students, researchers, and experts in the area of conservation biology, genetic diversity, and molecular biology.

How to reference
In order to correctly reference this scholarly work, feel free to copy and paste the following:

Luis Fernando Rodrigues-Filho, Danillo Pinhal, Davidson Sodré and Marcelo Vallinoto (2012). Shark DNA Forensics: Applications and Impacts on Genetic Diversity, Analysis of Genetic Variation in Animals, Prof. Mahmut Caliskan (Ed.), ISBN: 978-953-51-0093-5, InTech, Available from: http://www.intechopen.com/books/analysis-of-genetic-variation-in-animals/shark-dna-forensics-applications-and-impacts-on-genetic-diversity

INTECH
open science | open minds

InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

IntechOpen