Conservation Genetics (2005) 6:775–788
DOI 10.1007/s10592-005-9036-2


# Global-scale genetic identification of hammerhead sharks: Application to assessment of the international fin trade and law enforcement

Debra L. Abercrombie[1], Shelley C. Clarke[2], & Mahmood S. Shivji[1,*]
[1]*Guy Harvey Research Institute and Oceanographic Center, Nova Southeastern University, 8000 North Ocean Drive, Dania Beach, Florida, 33004, USA;* [2]*National Research Institute of Far Seas Fisheries, 5-7-1 Shimizu-Orido, Shizuoka, 424-8633, Japan (*Corresponding author: Phone: +1-954-262-3653; Fax: +1-954-262-4098; E-mail: mahmood@nova.edu)*

Received 12 October 2004; accepted 12 December 2004

*Key words:* fin DNA, fin trade, hammerhead sharks, shark conservation, *Sphyrna,*

**Abstract**

The future status of sharks is an issue of widespread conservation concern due to declines in many species in the face of high levels of exploitation to satisfy market demands for products, especially fins. Substantial declines in the large-bodied hammerhead sharks, *Sphyrna lewini*, *S. mokarran* and *S. zygaena*, even in regions where some management occurs, indicate that informed conservation measures are warranted for these circumglobally distributed species. Despite the importance of assessing shark catch and trade on a species-specific basis to detect potential overexploitation of individual species, achieving this goal for hammerheads has proven elusive due to difficulties in identification of their products. Here, we present the development and application of a diagnostic, streamlined, five-primer multiplex polymerase chain reaction assay utilizing species-specific primers based on nuclear ribosomal ITS2 for the three hammerhead species throughout their global distribution. Application of this assay to investigations of the fin market confirmed the presence of hammerhead fins in the international trade. A study of the world's largest fin market in Hong Kong revealed a high concordance between specific Chinese-name trade categories and fins from these three species (''Bai Chun'' with *S. lewini*, ''Gui Chun'' with *S. zygaena* and ''Gu Pian'' with *S. mokarran*), and clear species preferences. This concordance information allows the use of market records for monitoring species-specific trends in trade and exploitation rates. The assay is also proving useful for identification of shark body parts in U.S. fisheries law-enforcement activities. Screening of morphologically identified '' *S. lewini*'' from globally distributed areas using this assay with subsequent whole ITS2 sequencing suggests a cryptic species closely related to *S. lewini* occurs off the SE USA coast.

## Introduction

Since the mid 1980's, shark fisheries have experienced rapid growth worldwide due to increased demand for shark products (fins, meat, skin, cartilage, etc.) especially in Asian markets. Shark fins are now among the most expensive seafood items in the world, and depending on species, may fetch retail prices of over US $700 per kg in the world's largest fin market in Hong Kong (Clarke 2004). Trade in shark fins in the Chinese dried seafood market grew at 6% per year between 1991 and 2000, and the growing demand for shark fin is placing increasing pressure on shark populations (Clarke 2004). The combination of increasing exploitation and well-documented susceptibility of shark populations to collapse in response to overfishing has made conservation and management of sharks an issue of urgent and international concern (Bonfil 1994; Weber and Fordham 1997;

FAO 2000; Musick et al. 2000; NMFS 2001; Baum et al. 2003).

Because shark meat has historically been afforded relatively low economic value and due to the difficulties involved in identifying many commonly fished sharks, most fisheries do not report shark landings by species, lumping them instead in either the generic category "sharks", or into species or family groups (e.g., hammerheads, threshers, makos, etc.) at best. Additionally, the large number of sharks caught incidentally and commonly finned with the carcass discarded in high seas fisheries is often not reported in catch statistics. Thus, there is little useful information on extraction rates by which to monitor the magnitude of fisheries expansion and bycatch mortality, and accurately assess the impacts of these activities on individual shark species on a worldwide or even, with few exceptions, regional basis. In light of limited catch statistics, analysis of trade data for shark products has been proposed as a means of tracking relative extraction rates and warning of potential declines not documented by catch data (Clarke 2004).

Recent studies have shown that for the few sharks for which long-term catch data are available on a regional level, several species appear to be in severe decline ($>50\%$, Baum et al. 2003). Such large declines even in areas where some management is practiced (e.g., the northwest Atlantic) have led to concerns that the same or even greater declines have occurred in regions where catch goes largely unrecorded and management is minimal or non-existent (Bonfil 1997; Castro et al. 1999; Baum et al. 2003). Catch rate declines for "hammerheads", a group consisting primarily of three, large-bodied species: *Sphyrna lewini* (scalloped hammerhead), *S. mokarran* (great hammerhead) and *S. zygaena* (smooth hammerhead) have been estimated as high as 89% since 1986 in the northwest Atlantic (Baum et al. 2003), and these species are part of a large coastal shark complex that is considered overfished and managed under a quota system by the United States (NMFS 2001). These three species are caught incidentally in large numbers worldwide by multi-species fisheries and harvested locally in many regions for their meat (Rose 1996; Castro et al. 1999).

Accompanying the recognition that many sharks are especially sensitive to exploitation due to their life history characteristics (slow growth, late maturity, low fecundity), and that different species have varying natural capacities to respond to fishing pressure (Smith et al. 1998; Musick et al. 2000; Cortés 2002), is the realization that conservation and management measures are needed on a species-specific rather than group-specific basis to prevent the unrecognized overexploitation of any single species (Walker 1998; FAO 2000; NMFS 2001). Within the hammerheads, for example, the schooling nature of *S. lewini* and *S. zygaena* makes them vulnerable to fisheries because they concentrate in often predictable locations and are thus easily caught in large numbers. *S. mokarran* tends to be more solitary, but has a lower reproductive potential because it reproduces biennially as opposed to annually as do *S. lewini* and *S. zygaena* (Castro et al. 1999). Management of these three species as a combined group, therefore, does not adequately take into account important differences in their life histories, exploitation susceptibility, and population vulnerability.

A major impediment to implementing species-specific conservation, management and trade monitoring programs for sharks in general is the difficulty in accurately identifying many morphologically similar sharks commonly exploited in multi-species fisheries. Although hammerhead sharks as a group (family Sphyrnidae, eight described species; Compagno 1999) are easily distinguished as whole animals from other sharks due to their characteristic head (i.e., wide and flattened "hammer" or shovel-shaped head), catches are rarely recorded to species. This is partly due to difficulties in easily distinguishing the three commonly fished species (*S. lewini*, *S. mokarran*, *S. zygaena*), even as whole animals, especially when they are young (Castro 1983; Rose 1996). This identification problem is exacerbated when these animals are landed as headless and finless carcasses as is typical in commercial fisheries, and becomes severe to impossible for detached fins and other products such as meat and cartilage found in trade (Castro 1993; FAO 2000; Smith and Benson 2001; Shivji et al. 2002).

The problem of species identification of wildlife body parts in international trade is widespread, prompting the development and employment of genetic approaches to achieve accurate identification (DeSalle and Birstein 1996; Malik et al. 1997; Dizon et al. 2000; Roman and Bowen 2000). Several studies have also attempted to address the problem of shark species identification using

molecular approaches (Martin 1993; Shivji et al. 1996; Heist and Gold 1999; Hoelzel 2001; Smith and Benson 2001; Chan et al. 2003; Chapman et al. 2003). The most streamlined and economical genetic species identification method published thus far for sharks is that presented by Pank et al. (2001) and Shivji et al. (2002). This approach utilizes multiple species-specific primers in a single-reaction tube to produce species-diagnostic amplicons, incorporates an internal positive control, requires only PCR without downstream processing of the PCR amplicons by restriction enzyme digestion or DNA sequencing, and distinguishes among multiple species simultaneously. In addition to reducing assay time and costs, this species-specific primer approach also offers the advantages of being more easily adaptable to field use (e.g., on a research or enforcement vessel), and is likely to be more practical than large-volume sequencing in the context of developing country applications where financial resources available for biological management and conservation might be limited.

Planning informed conservation measures for hammerhead sharks will require assessment of their exploitation rates and the contribution that individual hammerhead species make to the fin market. Here, we expand upon the method of Pank et al. (2001) and Shivji et al. (2002) and report on the development and extensive testing of a suite of species-specific PCR primers which efficiently identify the three species of hammerhead sharks, *S. lewini*, *S. mokarran* and *S. zygaena* on a global scale. We apply this method for identifying fins in the international fin trade and law enforcement activities, and to determine the relationship between Chinese name trade categories and species in the Hong Kong fin market, allowing use of trade data for inferring exploitation rates by species.

## Methods

### *Shark tissue samples*

All reference hammerhead species samples used for DNA sequencing and species-specific primer design were collected during fishery-independent shark population abundance and tagging surveys conducted by the U.S. National Marine Fisheries Service (NMFS) or by experienced shark researchers. Shark test samples (target and non-target species) used for testing the diagnostic utility of the species-specific primers were of global distribution where possible, and collected by experienced shark researchers. Shark species, sample sizes, and geographic origins (ocean basins) are shown in Table 1.

To ensure the adequacy of the proposed species-specific primers for identifying various types of shark products, we conducted diagnostic testing on several tissue types, including wet and dried fins, white muscle, liver, heart and vertebrae with small bits of soft-tissue still attached. All wet fin and non-fin tissue samples were stored in 95% ethanol at room temperature for short-term storage or at 4 °C for long-term storage. Dried shark fins were obtained from Hong Kong fin traders as part of our complementary studies on characterization of the species composition of the Hong Kong fin market. Additional unidentified fins (wet and dry) confiscated from fishers and traders as part of law enforcement activities were obtained from the US National Oceanic and Atmospheric Administration (NOAA) Office of Law Enforcement and South African Department of Environmental Affairs and Tourism.

### *DNA Extraction, PCR amplification and DNA sequencing*

Genomic DNA was extracted from approximately 25 mg of tissue using the DNeasy Tissue Kit (QIAGEN Inc., Valencia, California) and stored at −20 °C until used. A DNA fragment (hereafter referred to as the positive control amplicon) containing the entire nuclear ribosomal DNA internal transcribed spacer (ITS2) region plus short portions of the flanking 5.8S and 28S ribosomal RNA genes was amplified from six hammerhead species (*S. mokarran, S. lewini, S. zygaena, S. tiburo, S. tudes, Eusphyra blochii*) by standard PCR, employing the shark universal primers FISH5.8S-F (forward primer 5′-TTAGCGGTGGATCACTCGGCTCGT-3′) and FISH28S-R (reverse primer 5′-TCCTCCGCTTAGTAATATGCTTAAATTCAGC-3′) and amplification conditions reported in Pank et al. (2001). Following amplification, both strands of the positive control amplicon were sequenced using standard protocols on an ABI 3100 automated sequencer. Reference sequences for the complete ITS2 locus from the three subject hammerhead species (one Atlantic and one Pacific representative of each) are

*Table 1*. Target and non-target shark species tested with the *Sphyrna lewini* ScHH401F, S. mokarran GtHH123F and *S. zygaena* SmHH630F primers in triplex and pentaplex PCR format

| Species | Ocean Basin (n) |
|---|---|
| ORDER CARCHARHINIFORMES | |
| ***Sphyrna lewini* (scalloped hammerhead)** | Atlantic (91)[a] |
| | Pacific (49) |
| ***Sphyrna mokarran* (great hammerhead)** | Atlantic (40) |
| | Pacific (5) |
| ***Sphyrna zygaena* (smooth hammerhead)** | Atlantic (7) |
| | Pacific (34) |
| *Sphyrna tiburo* (bonnethead) | Atlantic (20) |
| *Sphyrna tudes* (golden hammerhead) | Atlantic (1) |
| *Eusphyra blochii* (winghead) | Pacific (5) |
| *Rhizoprionodon acutus* (milk) | Pacific (2) |
| *Rhizoprionodon oligolinx* (grey sharpnose) | Atlantic (1) |
| *Rhizoprionodon porosus* (Caribbean sharpnose) | Atlantic (7) |
| *Rhizoprionodon taylori* (Australian sharpnose) | Pacific (1) |
| *Rhizoprionodon terranovae* (Atlantic sharpnose) | Atlantic (15) |
| *Prionace glauca* (blue shark) | Atlantic (7) |
| | Pacific (8) |
| *Carcharhinus acronotus* (blacknose) | Atlantic (15) |
| *Carcharhinus altimus* (bignose) | Atlantic (6) |
| *Carcharhinus amblyrhynchos* (gray reef) | Pacific (15) |
| *Carcharhinus amboinensis* (pigeye) | Pacific (2) |
| *Carcharhinus brachyurus* (bronze whaler) | Pacific (7) |
| *Carcharhinus brevipinna* (spinner) | Atlantic (10) |
| | Pacific (5) |
| *Carcharhinus dussumieri* (whitecheek) | Indian (4) |
| *Carcharhinus falciformis* (silky) | Atlantic (8) |
| *Carcharhinus galapagensis* (Galapagos) | Pacific (15) |
| *Carcharhinus hemiodon* (pondicherry) | Pacific (2) |
| *Carcharhinus isodon* (finetooth) | Atlantic (15) |
| *Carcharhinus leucas* (bull) | Atlantic (8) |
| | Pacific (7) |
| *Carcharhinus limbatus* (blacktip) | Atlantic (15) |
| *Carcharhinus longimanus* (oceanic whitetip) | Atlantic (3) |
| | Pacific (11) |
| *Carcharhinus melanopterus* (blacktip reef) | Pacific (5) |
| *Carcharhinus obscurus* (dusky) | Atlantic (19) |
| | Pacific (11) |
| *Carcharhinus perezi* (Caribbean reef) | Atlantic (16) |
| *Carcharhinus plumbeus* (sandbar) | Atlantic (8) |
| | Pacific (7) |
| *Carcharhinus porosus* (smalltail) | Atlantic (1) |
| *Carcharhinus signatus* (night) | Atlantic (16) |
| *Carcharhinus sorrah* (spot-tail) | Pacific (4) |
| *Carcharhinus tilstoni* (Australian blacktip) | Pacific (2) |
| *Negaprion acutidens* (sicklefin lemon) | Pacific (2) |
| *Negaprion brevirostris* (lemon) | Atlantic (15) |
| *Loxodon macrorhinus* (sliteye) | Atlantic (1) |
| *Galeocerdo cuvier* (tiger) | Atlantic (9) |
| | Pacific (6) |
| *Galeorhinus galeus* (school) | Atlantic (2) |
| | Pacific (6) |
| *Triaenodon obesus* (whitetip reef) | Pacific (1) |
| *Mustelus canis* (smooth dogfish) | Atlantic (5) |
| *Mustelus henlei* (brown smoothhound) | Atlantic (2) |
| *Mustelus norrisi* (smoothhound) | Atlantic (2) |
| *Triakis semifasciata* (leopard) | Pacific (2) |
| *Scyliorhinus retifer* (chain catshark) | Unknown Loc. (1) |
| *Apristurus profundorum* (smalleye catshark) | Atlantic (2) |
| *Cephaloscyllium ventriosum* (swell) | Pacific (2) |
| ORDER LAMNIFORMES | |
| *Carcharodon carcharias* (white shark) | Atlantic (5) |
| | Pacific (5) |
| *Isurus oxyrinchus* (shortfin mako) | Atlantic (5) |
| | Pacific (5) |
| *Isurus paucus* (longfin mako) | Atlantic (7) |
| | Pacific (3) |
| *Lamna ditropis* (salmon shark) | Pacific (10) |
| *Lamna nasus* (porbeagle) | Atlantic (10) |
| *Alopias pelagicus* (pelagic thresher) | Pacific (10) |
| *Alopias superciliosus* (bigeye thresher) | Atlantic (5) |
| | Pacific (5) |
| *Alopias vulpinus* (thresher) | Atlantic (5) |
| | Pacific (5) |
| *Carcharias taurus* (sandtiger) | Atlantic (9) |
| | Pacific (1) |

*Table 1*. (Continued)

| Species | Ocean Basin (n) |
|---|---|
| *Odontaspis ferox* (smalltooth sandtiger) | Pacific (1) |
| *Cetorhinus maximus* (basking shark) | Atlantic (4)<br>Pacific (1)<br>Unknown Loc. (5) |
| ORDER ORECTOLOBIFORMES | |
| *Ginglymostoma cirratum* (nurse) | Atlantic (10) |
| *Nebrius ferrugineus* (tawny nurse) | Pacific (1) |
| ORDER SQUALIFORMES | |
| *Squalus acanthias* (spiny dogfish) | Atlantic (5) |
| *Squalus cubensis* (Cuban dogfish) | Atlantic (5) |
| *Deania calceus* (birdbeak dogfish) | Atlantic (2) |
| *Dalatias licha* (kitefin) | Atlantic (1) |
| *Isistius brasiliensis* (cookiecutter shark) | Atlantic (1) |
| *Etmopterus pusilius* (smooth lantern) | Atlantic (2) |
| *Etmopterus spinax* (velvet belly) | Atlantic (1) |
| *Centrophorus granulosus* (gulper) | Atlantic (1) |
| *Centrophorus squamosus* (leafscale gulper) | Atlantic (1) |
| ORDER HETERODONIFORMES | |
| *Heterodontus francisci* (horn) | Pacific (2) |
| ORDER SQUATINIFORMES | |
| *Squatina californica* (Pacific angel) | Pacific (2) |
| ORDER HEXANCHIFORMES | |
| *Hexanchus griseus* (sixgill) | Pacific (4) |
| *Hexanchus vitulatus* (bigeye sixgill) | Atlantic (1) |
| *Heptranchias perlo* (sharpnose sevengill) | Pacific (1) |
| ORDER PRISTIOPHORIFORMES | |
| *Pristiophorus japonicus* (Japanese sawshark) | Unknown Loc. (1) |
| *Pristiophorus nudipinnis* (shortnose sawshark) | Unknown Loc. (1) |

Ocean basin origins of the shark test species are shown, with (n) representing the number of individuals of each species tested from each basin. Bolded taxa are the subject species of this study.

[a]The three "*S. lewini*" samples that likely represent a new undescribed cryptic species are not included (see text).

available from GenBank under the following accession numbers: *S. lewini* (Atlantic: AY858052; Pacific: AY858051), *S. mokarran* (Atlantic: AY860837; Pacific: AY860838) and *S. zygaena* (Atlantic: AY860840; Pacific: AY860839). We were unable to obtain tissue samples from the two remaining described hammerhead species *S. media* and *S. corona* despite long standing collection attempts.

To confirm that our final species-specific primers were correctly amplifying their target species only and not amplifying the two missing (untested) hammerhead species (*S. media* and *S. corona*), we sequenced the ITS2 locus from 31 (out of a total 120) randomly selected, Hong Kong market-derived dried fins identified by the primers as originating from either *S. lewini* (n = 10), *S. mokarran* (n = 10) or *S. zygaena* (n = 11). The complete ITS2 locus was sequenced for all the fins except four *S. lewini* fins from which approximately 530 bp (~ 80% of the ITS2) was determined.

### *Species-specific primer design and multiplex testing assay*

We aligned ITS2 sequences from all six hammerhead species with the alignment program ClustalX (Thompson et al. 1997), and manually refined the alignment using the sequence editing program GeneDoc (Nicholas and Nicholas 1997). Multiple (3–7) PCR primers putatively specific for each of the three hammerhead subject species (*S. lewini, S. mokarran and S. zygaena*) were designed on the basis of nucleotide differences between the target sequence and sequences from other non-target hammerhead shark taxa (Appendix).

To determine its diagnostic utility, each putatively species-specific primer was assayed for its amplification performance (i.e., species-specificity and ease of amplicon detection) following the multiplex PCR strategy detailed previously (Shivji et al. 2002). Briefly, the assay consisted of testing each primer in a 3-primer assay (triplex PCR; Figure 1) composed of the two shark universal primers and one of the putatively species-specific primers (see Discussion about the rationale for including both universal primers in the PCR). This triplex PCR assay was tested against each target species (41–140 individuals each), and against 75 non-target species (1–61 individuals each), including very closely related (other hammerhead), relatively closely related (other carcharhiniform) and distantly related (other orders) shark taxa (Table 1). Because the fin market is supplied from worldwide sources (Clarke 2004) and to ensure that potential intraspecific sequence variation in the ITS2 would not diminish the diagnostic utility of the primers, we assessed the global scale applicability of each putatively specific primer by testing its performance against a worldwide set (Atlantic and Pacific) of target samples as much as possible

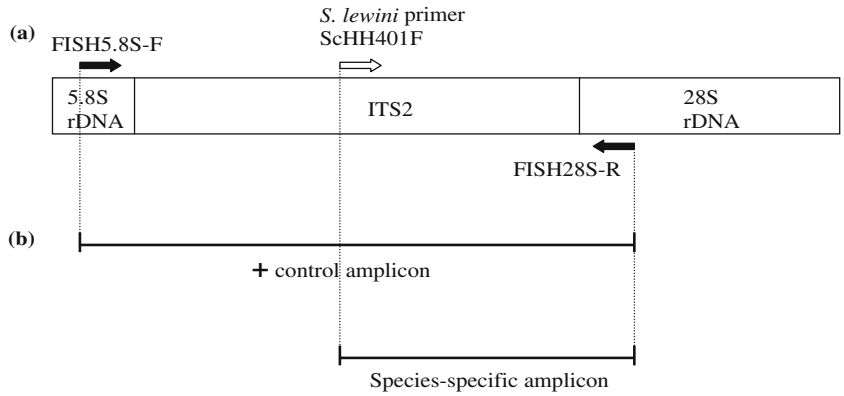


*Figure 1.* (a) Schematic representation of the shark nuclear 5.8S and 28S ribosomal RNA genes and ITS2 locus showing relative annealing sites and orientation of primers used in triplex PCR assays. Solid arrows: shark universal primers (FISH5.8S-F and FISH28S-R). Open arrow: example of a species-specific primer (*S. lewini* ScHH401F) used in this study. (b) Spatial coverage of the two amplicons expected to be produced using this combination of three primers when tested against target (*S. lewini*) DNA. In contrast, only the positive control amplicon would be produced in the *absence* of the target species.

(Table 1). The non-target taxa tested included most of the major species known or suspected to occur in global shark fisheries and trade.

All triplex PCR amplifications were performed using a Mastercycler Gradient (Eppendorf Inc.) or MJ Research PTC-100 (MJ Research Inc.) thermal cycler. Total reaction volumes were 50 $\mu$l and contained approximately 10–25 ng of the extracted DNA, 12.5 pmol of each primer, 1× PCR buffer (QIAGEN Inc.; 1.5 mM $MgCl_2$), 40 $\mu$M dNTP's and 1 unit of HotStar Taq DNA polymerase (QIAGEN Inc.). The PCR thermal cycling profile for the putatively-specific primer tests was 94 °C initial heating for 15 min to activate the hot start DNA polymerase, followed by 35 cycles of 94 °C for 1 min, 65 °C for 1 min, 72 °C for 2 min, and a 5 min extension step at 72 °C. The PCR products were checked by electrophoresis on 1.2% agarose gels. From these initial tests, one optimal primer with the following properties was selected for each of the three subject hammerhead species: the primer demonstrated unambiguous species-specificity at a high-stringency (65 °C) annealing temperature, and produced a diagnostic-sized amplicon when used in a larger multiplex (pentaplex) PCR format (see next section).

To further streamline the PCR assay and allow simultaneous testing for all three target hammerhead species, the three optimal species-specific primers were combined in a 5-primer (pentaplex) reaction and the assay tested for its diagnostic robustness. This pentaplex PCR assay included the two shark universal primers (FISH5.8S-F and FISH28S-R) and the species-specific primers for *S. lewini* (ScHH401F), *S. mokarran* (GtHH123F), and *S. zygaena* (SmHH630F) (Figure 2). With the exception of the additional primers, all pentaplex PCR conditions were identical to those used for the triplex PCR. All market and law enforcement- derived fin samples were identified using the pentaplex PCR assay.

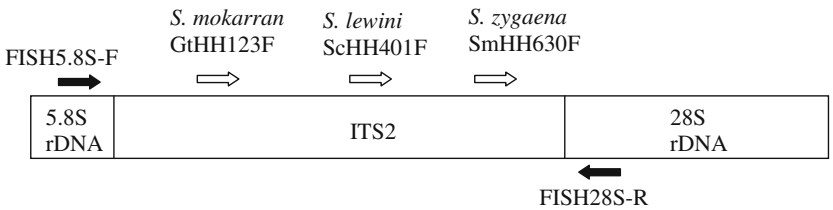


*Figure 2.* Relative annealing sites and orientation of primers used in the pentaplex PCR assay. Solid arrows: shark universal primers (FISH5.8S-F and FISH28S-R). Open arrows: the three optimal hammerhead species-specific primers.

## Results

### *Testing each species-specific primer in the triplex PCR assay*

The ITS2 locus in the six hammerhead species ranges from 667 to 672 bp, and is the smallest ITS2 for any shark lineage (M. Shivji lab, unpubl. data). The size of the entire amplicon generated by the shark universal ITS2 primers, including 5.8S and 28S rDNA flanking regions is approximately 860 bp, and is a diagnostic character for hammerheads as group compared to other shark lineages. The ITS2 sequence divergence (uncorrected) between the six hammerhead species ranged from 1.6–8.2%. The sequence of each optimal, species-specific primer (hereafter referred to as SSP) and the size of the diagnostic amplicon produced for each species are shown in Table 2.

Each SSP amplified its target species from different ocean basins, producing an easily recognizable, diagnostic-sized amplicon (Figure 3a–c). The co-amplification of the positive control occurred in most cases, albeit with reduced efficiency compared to the species-specific amplicon. Each SSP also demonstrated complete species-specificity in triplex PCR tests against the 75 non-target taxa (including non-target hammerhead species), producing only a single positive control amplicon ranging from 860–1500 bp, depending on species (Figures 3 and 4). In all cases of amplifiable sample DNA ($>97\%$ of samples), there were no cases of false negative or false positive results in our triplex PCR trials.

### *Testing the pentaplex PCR assay*

Combining the three species-specific primers (GtHH123F, ScHH401F and SmHH630F) with the two shark universal primers in a single tube, pentaplex PCR assay at 65 ° C annealing temperature produced an unambiguous, diagnostic-sized amplicon for each target species (41–140 individuals per target species tested; Table 1), regardless of global geographic origin of each sample. This assay allowed *S. lewini*, *S. mokarran* and *S. zygaena* samples to be easily distinguished from one another visually on a standard, 1.2% ethidium bromide stained agarose gel (Figure 4). Co-amplification of the positive control amplicon from target species by the two shark universal primers was mostly present at lower intensity, with relatively few reactions ($<5\%$) where it was not visible on the gel. The pentaplex assay performed robustly and as well as the triplex assay on the 75 non-target species (1–61 individuals each), producing only the positive control ITS2 in every case (Figure 4). There were no false positive or negative results (although see next for "*S. lewini*"), and the pentaplex PCR assay proved 100% accurate in discriminating the three hammerhead species from each other and non-target taxa.

An unexpected and interesting primer test result deserves comment. The *S. lewini* SSP (ScHH401F) was tested (triplex and pentaplex formats) on samples from 143 target sharks identified as "*S. lewini*" on morphological grounds and originating from globally widespread locations (NW Atlantic, Indian Ocean, NorthCentral Pacific, NE Pacific, NW Pacific). These tests produced unexpected results from 3 of the 143 animals during PCR screening, failing to amplify a diagnostic species-specific amplicon and producing only the positive control amplicon instead. The *S. mokarran* and *S. zygaena* SSPs did not amplify these three samples either. All three "anomalous" individuals were caught off the east coast of Florida (SE USA) between 1996 and 2001. To further investigate these three cases of apparent false negative results and determine the reason why the ScHH401F primer amplified the majority of *S. lewini* tested (140 out of 143 globally distributed animals) but not these three animals, we sequenced the complete ITS2 locus from the latter. Their ITS2 sequences were identical, but differed from the reference Pacific and Atlantic *S. lewini* sequences by 12 single base substitutions and 2 indels (2 bp and 8 bp; 1.26% uncorrected

*Table 2.* Species-specific primer sequences and size of the diagnostic species-specific amplicon produced (base pairs)

| Species-specific primer | Primer sequence | Amplicon size |
|---|---|---|
| *Sphyrna mokarran* (GtHH123F) | 5′-AGCAAAGAGCGTGGCTGGGGTTTCGA-3′ | 782 bp |
| *Sphyrna lewini* (ScHH401F) | 5′-GGTAAAGGATCCGCTTTGCTGGA-3′ | 445 bp |
| *Sphyrna zygaena* (SmHH630F) | 5′-TGAGTGCTGTGAGGGCACGTGGCCT-3′ | 249 bp |

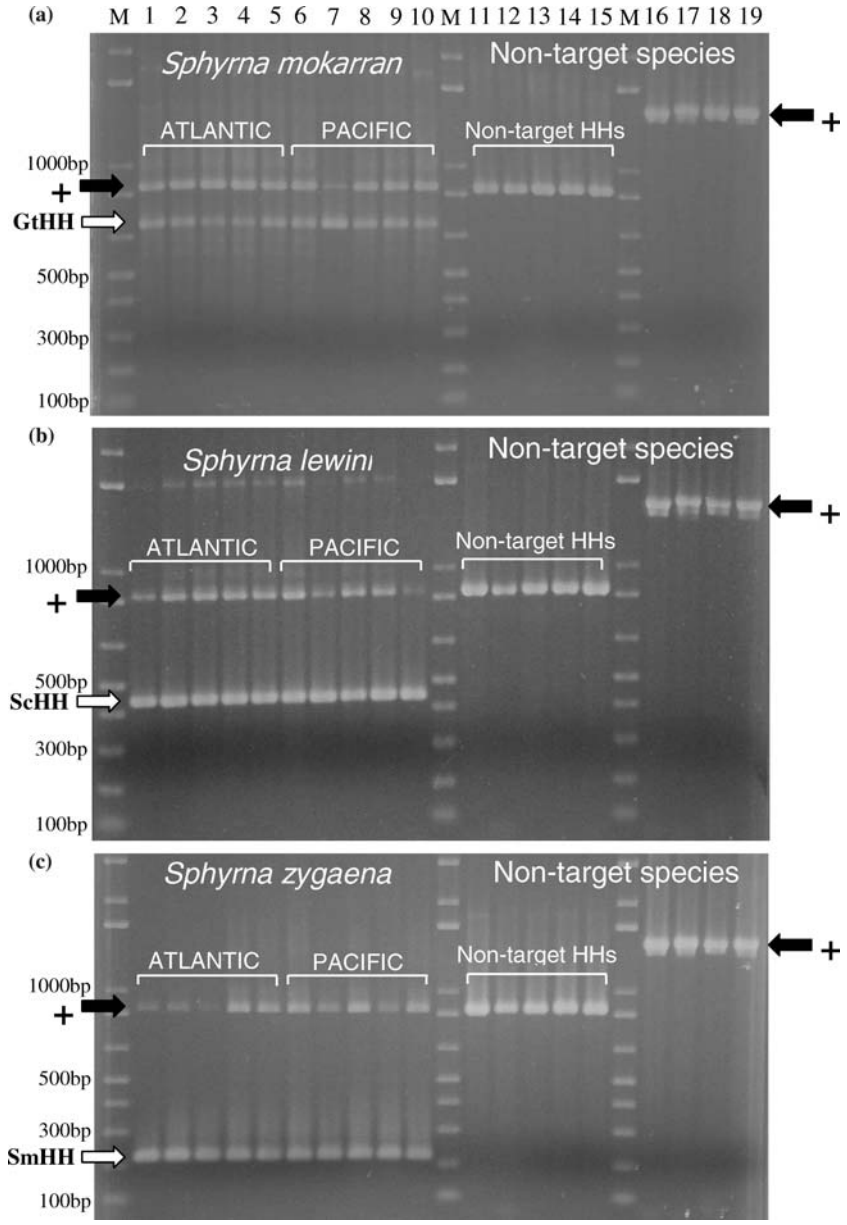


*Figure 3*. Amplification results obtained using each hammerhead species-specific primer in triplex PCR format against target and non-target shark species. Open arrows indicate the species-diagnostic amplicon (GtHH = *S. mokarran*, ScHH = *S. lewini*, SmHH = *S. zygaena*). Solid arrows indicate the positive control amplicon (+) from target and non-target species. HHs = hammerheads. Lanes labeled M contain the molecular size-standard. (a) Results from the *Sphyrna mokarran* species-specific primer (GtHH123F). Lanes 1–10: globally distributed *S. mokarran* as target. Lanes 11–15: non-target hammerhead species: 11, *S. lewini*; 12, *S. zygaena*; 13, *S. tiburo*; 14, *S. tudes*; 15, *Eusphyra blochii*. Lanes 16–19: non-target species in the closely related family Carcharhinidae: 16, *Prionace glauca*; 17, *Carcharhinus plumbeus*; 18, *C. limbatus*; 19, *C. falciformis*. (b) Results from the *Sphyrna lewini* species-specific primer (ScHH401F). Lanes 1–10: globally distributed *S. lewini* as target. Lane 11, *S. mokarran*; 12–19, same as in (a). (c) Results from the *Sphyrna zygaena* species-specific primer (SmHH630F). Lanes 1–10: globally distributed *S. zygaena* as target. Lane 11, *S. mokarran*; 12, *S. lewini*; 13–19, same as in (a).

sequence divergence; GenBank accession number AY864857). Two of the nucleotide substitutions were located in critical positions in the orthologous ScHH401F primer annealing site (Table 3), preventing primer annealing to DNA from these three "anomalous" animals.

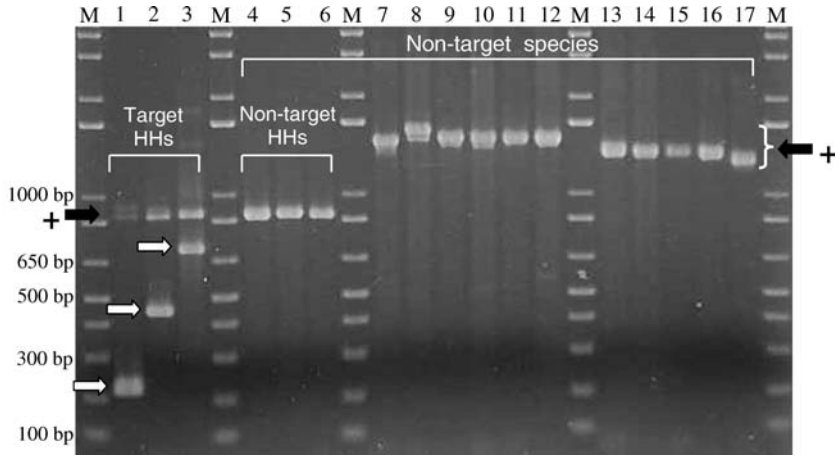


*Figure 4*. Amplification results obtained using the three hammerhead species-specific primers in pentaplex PCR format against target and non-target shark species. Open arrows indicate the species-diagnostic amplicons. Solid arrows indicate the positive control amplicons (+) from target and non-target species. HHs = hammerheads. Lanes labeled M contain the molecular size-standard. Lanes 1–3: target hammerheads: 1, *S. zygaena*; 2, *S. lewini*; 3, *S. mokarran*. Lanes 4–6: non-target hammerheads: 4, *S. tiburo*; 5, *S. tudes*; 6, *E. blochii* Lanes 7–17: non-target species in the orders Carcharhiniformes (7–12) and Lamniformes (13–17): 7, *Mustelus norrisi*; 8, *Rhizoprionodon terranovae*; 9, *Carcharhinus plumbeus*; 10, *C. falciformis*; 11, *C. obscurus* (Pacific); 12, *C. obscurus* (Atlantic); 13, *Isurus oxyrinchus*; 14, *Lamna nasus*; 15, *Carcharodon carcharias*; 16, *Alopias vulpinus*; 17, *A. superciliosus*.

*Identifying market and law enforcement-derived fins*

One of us (SCC) examined the Hong Kong fin trader auction records for 10,669 fin lots between October 1999 and March 2001 (Clarke 2003), and found 11.6% of the lots labeled as the Chinese trade category ''Chun Chi''. To determine the identity of these ''Chun Chi'' fins which trader interviews suggested were derived from hammerheads (species unspecified), we genetically analyzed 94 fins from this category sampled across 13 traders. Some traders further categorized ''Chun Chi'' fins into two sub-categories: ''Bai Chun'' and ''Gui Chun''. Genetic analysis of the subset of 35 ''Bai Chun''-labeled and 25 ''Gui Chun''-labeled fins revealed nearly perfect concordance between the ''Bai Chun'' category and *S. lewini*, and ''Gui Chun'' and *S. zygaena*. Results of these surveys are summarized in Table 4. According to traders, a fourth market category (''Gu Pian'') also contained hammerhead fins. Genetic typing of 35 ''Gu Pian'' fins with our primers revealed 30 derived from the great hammerhead, *S. mokarran*, and four fins from *S. lewini*, the ''Chun Chi'' fins most similar in color to ''Gu Pian'' (S. Clarke, J. Magnussen, D. Abercrombie, M. McAllister and M. Shivji, in press). Of the 10,669 auction lots we examined, 2.2% were labeled ''Gu Pian''.

The ITS2 sequences of the 31 randomly selected, Hong Kong market dried fins identified as either *S. lewini*, *S. mokarran* or *S. zygaena* by their respective species-specific primers were nearly identical to their respective reference species sequences (alignments of fin with reference animal sequences are available from the corresponding author upon request), providing further confirmation that each of the three species-specific primers was accurate in its diagnostic ability.

*Table 3*. Comparison of *S. lewini* ITS2 species-specific primer ScHH401F sequence (bold) with orthologous site sequences from globally distributed and ''anomalous'' (cryptic species) hammerhead individuals

| | 5′ | **G** | **G** | **T** | **A** | **A** | **A** | **G** | **G** | **A** | **T** | **C** | **C** | **G** | **C** | **T** | **T** | **T** | **G** | **C** | **T** | **G** | **G** | **A** | 3′ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *S. lewini* (W Pacific) | 5′ | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | 3′ |
| *S. lewini* (E Gulf of Mexico) | 5′ | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | 3′ |
| *S. lewini* (Caribbean) | 5′ | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | 3′ |
| *S. lewini* (SE USA Atlantic/Cryptic) | 5′ | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | T | . | . | . | C | 3′ |

Dots indicate sequence identity. The two base difference near the 3′ end prevents annealing of the species-specific primer.

*Table 4*. Summary of the concordance between Hong Kong market Chinese trade categories and taxa (n = number of fins)

| Fin trade category | Genetically identified species |
|---|---|
| Chun Chi (n = 94)[a] | *S. lewini* (n = 56) |
| | *S. zygaena* (n = 33) |
| | Non-hammerhead taxa (n = 3) |
| Bai Chun (n = 35) | *S. lewini* (n = 33) |
| | *S. mokarran* (n = 1) |
| | *Alopias pelagicus*[b] (n = 1) |
| Gui Chun (n = 25) | *S. zygaena* (n = 24) |
| | *S. lewini* (n = 1) |
| Gu Pian (n = 35)[c] | *S. mokarran* (n = 30) |
| | *S. lewini* (n = 4) |
| | Non-hammerhead taxon (n = 1) |

[a]DNA from 2 fins was unamplifiable by PCR.
[b]*A. pelagicus* was identified using a species-specific primer developed for this species as part of a separate study.
[c]Gu Pian results from Clarke et al. (in press).

Of eight shark identification cases (fins confiscated from separate fishing vessels) we investigated using this assay for the NOAA Office of Law Enforcement in 2003, three cases contained fins from *S. lewini*, with one fishing vessel containing fins from at least 10 animals. Of 104 trader-derived, dried fins confiscated by the South African Department of Environmental Affairs and Tourism, three fins originated from *S. zygaena* and two from *S. lewini*.

## Discussion

Concerns about shark population sustainability in the face of growing exploitation for fins and other products, and the consequences of large-scale apex predator removal on marine ecosystems have prompted calls for worldwide implementation of management and conservation measures for sharks (FAO 1998, 2000; Musick et al. 2000; Stevens et al. 2000). An important requirement for such measures to be effective is the availability of reliable information on shark catch and trade on a species-specific basis, data that has been largely missing for most shark species. Toward this end, we have been developing streamlined assays for identification of the major shark species believed to occur in trade or protected by national legislation (e.g., Pank et al. 2001; Shivji et al. 2002; Chapman et al. 2003). Here, we demonstrate the efficacy of a simple to use, multiplex PCR strategy using species-specific primers to simultaneously, rapidly and reliably discriminate the three, widely distributed hammerhead species, *S. lewini*, *S. mokarran* and *S. zygaena* from each other and 75 other closely and distantly related shark species that occur in global fisheries. The streamlined assay we have developed will aid in collection of catch and trade data and enforcement of potential regulations aimed at protecting these three species from overexploitation.

The assay reliably and efficiently identifies a variety of tissues, including dried fins, derived from the three species that likely make up the bulk of worldwide catch and trade for hammerheads as a group. The ITS2 locus appears particularly well-suited for the design of hammerhead species-specific primers because of its high degree of sequence conservation on a global scale, as demonstrated by each SSP amplifying all target species regardless of the sample's oceanic basin of origin. This high level of sequence conservation is further confirmed by the identical ITS2 sequences obtained from individual reference animals of each species collected from as far apart as the west Atlantic and west and south Pacific. Despite its non-coding nature, the very high degree of ITS2 sequence conservation within shark species appears to be a general characteristic of this locus, facilitating the development of SSPs applicable on a worldwide basis (Pank et al. 2001; Shivji et al. 2002; Chapman et al. 2003; M. Shivji lab, unpubl. data). The global scale diagnostic ability of each hammerhead SSP is particularly useful also because it provides the means for determining the species of origin of shark fins in the international fin trade, which is supplied by at least 86 countries worldwide (Clarke 2004).

Species-specificity of the three hammerhead primers is aided by the apparent evolutionary dynamics of the ITS2 locus in sharks. ITS2 sequence divergences between more distantly related species (i.e., outside family level) are large enough to make meaningful sequence alignments difficult (Shivji et al. 2002; Chapman et al. 2003; M. Shivji lab, unpubl. data), leaving only the most closely related species (mainly congeners) as potential candidates for SSP cross-species amplification (false positives), especially at the high stringency primer annealing temperatures used here. Indeed, we found no incidences of false positive amplifications in our trials using the three hammerhead SSPs against 75 non-target species.

We were able to confirm species specificity of each hammerhead SSP against non-target, confamilial members by direct PCR testing, and by examination of orthologous primer annealing site sequences, which were different in each hammerhead species (Appendix). Although we were unable to directly test the primers on two of the eight described hammerhead species (*S. media* and *S. corona*) due to lack of sample availability, three mitigating factors make it extremely unlikely that significant errors in identification of body parts from *S. lewini*, *S. mokarran* and *S. zygaena* will occur with the use of their SSPs. First, the nearly identical ITS2 sequence of each of the 31 randomly selected Hong Kong market-derived dried fins to its respective reference hammerhead sequence supports the fact that the SSPs are indeed amplifying the right target species, and are not cross-amplifying the two untested hammerhead species. Second, *S. media* and *S. corona* are small hammerheads (with less demand for their smaller fins) with greatly restricted distributions. *S. media* inhabits inshore continental shelves in the Western Atlantic from Panama to Brazil and in the Eastern Pacific from the Gulf of California to Ecuador and possibly Peru, while *S. corona* is only known to occur inshore on the continental shelf of the Eastern Pacific, possibly from the Gulf of California and Southern Mexico to Peru (Compagno 1984). Their limited distributions and small size suggest it is unlikely that these species are common in global fin trade. Third, the fact that each of the *S. lewini*, *S. mokarran* and *S. zygaena* SSPs does not amplify any of five other hammerhead species tested suggests that the probability of them amplifying the two untested hammerheads is low.

The typically lower yield and occasionally absent co-amplification of the positive control amplicon from target species has no impact on the functional utility of this assay (the reason for this is explained in detail in Pank et al. 2001; Shivji et al. 2002 and Chapman et al. 2003). Briefly, both shark universal primers are included in the multiplex assays only for preventing false negatives, i.e., the complete absence of any amplification (e.g., due to unamplifiable DNA, inhibitory substances, reaction set-up errors) from being interpreted as the absence of the target species. Incorporating both shark universal primers in the PCR provides an internal positive control for the diagnostic assay by requiring amplification of *only* the positive control amplicon from all non-target species. In this context, the positive control worked extremely well, amplifying robustly in the presence of all non-target species (Figures 3 and 4), including non-target hammerheads.

*A new hammerhead evolutionary lineage*

Failure of the ScHH401F primer to amplify the three "anomalous" sharks from the SE USA identified as " *S. lewini*" on morphological grounds, and the ITS2 sequence divergence observed in these animals compared to the reference *S. lewini*, initially suggested we had encountered intraspecific polymorphisms in the species-specific primer annealing site. However, the explanation that these observations represent a population-level polymorphism is inconsistent with the absence of these polymorphisms in the ITS2 of S. *lewini* from globally distributed areas, including 45 sympatric animals obtained from the SE USA. An alternative and likely explanation for the divergent ITS2 sequences is that there is an undescribed cryptic species of hammerhead in coastal waters off the US east coast that is morphologically very similar to *S. lewini*. Further investigation of these and additional "*S. lewini*" individuals by sequencing the mitochondrial control region and nuclear *LDHA6* loci supports the presence of a highly divergent evolutionary lineage within "*S. lewini*" in this region (J. Quattro, University of South Carolina, pers. comm., manuscript in review). Thus, the ScHH401F species-specific primer is also able to distinguish *S. lewini* from what may likely be a cryptic hammerhead species.

*Conservation and management applications*

Our analysis of fins derived from the Hong Kong and South African market and NOAA law enforcement activities confirms that fins from *S. lewini*, *S. mokarran* and and S. zygaena are present in contemporary international trade. In light of documented declines in these species (NMFS 2001; Baum et al. 2003), the high market value of their fins (see below), and their largely unregulated exploitation worldwide, informed conservation and management of these large apex predators is warranted. Application of the diagnostic assay reported here has already yielded data of conservation and management relevance, and

we anticipate its streamlined nature and ease of use will find additional application in these contexts.

For example, a retrospective assessment of the Hong Kong shark fin market has revealed that three Chinese market categories contain fins from two hammerhead species: ''Bai Chun'' (*S. lewini*), ''Gui Chun'' (*S. zygaena*), and the general category ''Chun Chi'' containing both species in an approximately 2:1 ratio, respectively. In our interviews, traders stated that hammerhead fins were one of the most valuable fin types in the market. Compilation of market prices from auction records (Clarke 2003) indicated an average, wholesale, unprocessed fin market value of US $135/kg for ''Gu Pian'', $103/kg for ''Bai Chun'' and $88/kg for ''Gui Chun'', indicating a species preference in the trade. Furthermore, a preference (reflected by higher prices) for the lower caudal fins of these species was also apparent. It is not clear whether the observed *S. lewini*:*S. zygaena* ratio in the combination ''Chun Chi'' category reflects the relatively higher price obtained for *S. lewini* fins or a generally higher abundance of *S. lewini* in the global catch; the latter seems a reasonable supposition based on the apparent greater abundance of *S. lewini* compared to the other two hammerhead species, and its availability in both inshore artisanal and offshore fisheries (Compagno 1984; Castro et al. 1999). Discovery of the high concordance between four Chinese market categories (i.e., three ''Chun'' categories and ''Gu Pian'') and the three hammerhead species enables for the first time, market records (available only in Chinese categories) to be used to assess the contribution of each of these hammerhead species to the overall trade, and the monitoring of relative extraction rates over time (Shivji et al. 2002; Clarke 2004; S. Clarke, J. Magnussen, D. Abercrombie, M. McAllister and M. Shivji, in press). Such trade-derived estimates provide a potentially important monitoring system for detecting declines in shark species that might not otherwise be noticed in the current absence of direct fisheries catch data.

A second, ongoing practical application is the use of the hammerhead SSPs to aid in law enforcement in the US shark fishery. Hammerheads are not believed to be targeted in the US Atlantic commercial fishery due the low price of their flesh, but are caught incidentally. Although shark finning (i.e., removal of the fins and discarding the corresponding carcass) is illegal in US waters, it is suspected that some fishers may be finning incidentally caught hammerheads and keeping just their fins for their high value, while retaining carcasses from different shark species with higher value flesh but lower value fins (Special Agent Paul Raymond, NOAA Office of Law Enforcement, pers. comm.). However, the difficulty of identifying detached fins and the headless and finless shark carcasses that are kept has made enforcing the finning prohibition difficult. We have used the hammerhead SSPs in several case studies conducted for NOAA's Office of Law Enforcement to identify fins as derived from hammerheads in suspect shark product seizures, and we anticipate an expanded role for these SSPs in detecting illegal finning of hammerhead sharks.

As an additional application, we suggest that the availability of globally diagnostic SSPs used in the efficient assay reported here allows a practical means of recording hammerhead shark landings on a species-specific basis in any managed fishery in the world. This capability provides a direct means of detecting overfishing of any single species and permits implementation of enforceable fishing quotas to allow recovery of individual species as necessary. Finally, in light of documented hammerhead declines, we suggest that the SSPs reported here might also be useful for bolstering future international agreements (e.g., CITES) that may be implemented to monitor or regulate trade in these species.

**Acknowledgements**

This research was funded by the Florida Sea Grant College Program with support from the NOAA Office of Sea Grant, Grant No. R/LR-B-54, the Wildlife Conservation Society, the Pew Institute for Ocean Science, the Eppley Foundation and the Hai Stiftung Foundation. S. Clarke is supported by the Japan Society for the Promotion of Science through the National Research Institute of Far Seas Fisheries, Shimizu, Japan. The assistance of M. Myers, H. Oosthuizen, and the NOAA Office of Law Enforcement in obtaining fin samples, and D. Chapman, M. Henning, J. Magnussen, and J. Nielsen for laboratory assistance is especially acknowledged. We are grateful to L. Beerkircher, K. Duncan, E. Heist, M. Grace, D. Grubbs, C. Lowe, R.A. Martin, L. Natanson, J. Quattro, B. Snelson, J. Tyminski, B. Wetherbee and the NOAA-NMFS-SEFSC Pelagic Observer Program for providing shark tissues.

## Appendix

Local alignment of target species-specific primer sequence (bold) with orthologous sequences from the non-target hammerheads. Dots indicate sequence identity to target sequence

| | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ***S. mokarran*** | **A** | **G** | **C** | **A** | **A** | **A** | **G** | **A** | **G** | **C** | **G** | **T** | **G** | **G** | **C** | **T** | **G** | **G** | **G** | **G** | **T** | **T** | **T** | **C** | **G** | **A** |
| *S. lewini* | G | . | . | . | . | . | . | . | . | . | . | C | . | . | . | . | . | . | . | . | . | G | . | . | . | C |
| *S. zygaena* | G | . | . | . | . | . | . | . | . | . | . | C | . | . | . | . | . | . | . | . | C | G | . | . | . | C |
| *S. tudes* | G | . | . | . | . | . | . | . | . | . | . | C | . | . | . | . | . | . | . | . | . | G | . | . | . | C |
| *S. tiburo* | G | . | . | . | . | . | . | . | . | . | . | C | . | . | . | . | . | . | . | . | . | G | . | . | . | C |
| *E. blochii* | . | . | . | . | . | . | . | . | . | . | . | C | . | . | . | . | . | . | . | . | . | G | . | . | . | C |
| ***S. lewini*** | **G** | **G** | **T** | **A** | **A** | **A** | **–** | **G** | **G** | **A** | **T** | **C** | **C** | **G** | **C** | **T** | **T** | **T** | **G** | **C** | **T** | **G** | **G** | **A** | | |
| *S. mokarran* | C | . | . | . | . | . | – | . | . | . | . | . | T | . | . | . | . | . | . | T | . | . | . | C | | |
| *S. zygaena* | C | . | . | . | . | . | A | . | . | . | . | . | . | . | . | . | . | . | . | T | . | . | . | C | | |
| *S. tudes* | C | . | . | . | . | . | – | . | . | . | . | . | . | . | . | . | . | . | . | T | . | . | . | C | | |
| *S. tiburo* | C | . | . | . | . | . | – | . | . | . | . | . | . | . | . | . | . | . | . | T | . | . | . | C | | |
| *E. blochii* | C | . | . | . | . | . | A | . | . | . | . | . | . | . | . | . | . | . | . | T | . | . | . | C | | |
| ***S. zygaena*** | **T** | **G** | **A** | **G** | **T** | **G** | **C** | **T** | **G** | **T** | **G** | **A** | **G** | **G** | **G** | **C** | **A** | **C** | **G** | **T** | **G** | **G** | **C** | **C** | **T** | |
| *S. lewini* | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | A | C | . | . | . | A | G | |
| *S. mokarran* | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | A | . | . | . | . | A | G | |
| *S. tudes* | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | A | . | . | . | . | A | G | |
| *S. tiburo* | G | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | A | . | . | . | . | A | G | |
| *E. blochii* | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | A | . | . | . | . | A | G | |

## References


Baum JK, Myers RA, Kehler DG, Worm B, Harley SJ, Doherty PA (2003) Collapse and conservation of shark populations in the northwest Atlantic. *Science*, **299**, 389–392.

Bonfil R (1994) *Overview of World Elasmobranch Fisheries*. FAO Fisheries Technical Paper No. 341. Rome.

Bonfil R (1997) Status of shark resources in the southern Gulf of Mexico and Caribbean: Implications for management *Fish. Res.*, **29**, 101–117.

Castro JI (1983) *The Sharks of North American Waters*, Texas A&M University Press, College Station, Texas.

Castro JI (1993) *A field guide to sharks commonly caught in commercial fisheries of the southeastern United States*. NOAA Technical Memorandum NMFS-SEFSC-338.

Castro JI, Woodley CM, Brudeck RL (1999) *A Preliminary Evaluation of the Status of Shark Species*. FAO Fisheries Technical Paper No.380. Rome.

Chan RWK, Dixon PI, Pepperell JG, Reid DD (2003) Application of DNA-based techniques for the identification of whaler sharks (*Carcharhinus* spp.) caught in protective beach meshing and by recreational fisheries off the coast of New South Wales. *Fish. Bull.*, **101**, 910–914.

Chapman DD, Abercrombie DL, Douady CJ, Pikitch EK, Stanhope MJ, Shivji MS (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*. *Conserv. Genet.*, **4**, 415–425.

Clarke SC (2003) *Quantification of the Trade in Shark Fins*. PhD Thesis, Imperial College London, UK.

Clarke SC (2004) Understanding pressures on fishery resources through trade statistics: A pilot study of four products in the Chinese dried seafood market *Fish Fish.*, **5**, 53–74.

Clarke SC, Magnussen JE, Abercrombie D, Mc Allister M, Shivji M (2005) Identification of shark species composition and proportion in the Hong Kong shark fin market based on molecular genetics trade records. *Conserv. Biol.*, in press .

Compagno LJV (1984) FAO Species Catalogue Vol. 4, Sharks of the World. An Annotated and Illustrated Catalogue of Shark Species Known to Date. Parts 1 and 2. *FAO Fish. Synopsis*, **125(4)** 1–655.

Compagno LJV (1999) Checklist of Living Elasmobranchs In: Sharks, Skates, and Rays: The Biology of Elasmobranch Fishes. (eds. Hamlett WC), pp. 471–498. The Johns Hopkins University Press, Baltimore, USA.

Cortés E (2002) Incorporating uncertainty into demographic modeling: Application to shark populations and their conservation *Conserv. Biol.*, **16(4)** 1048–1062.

DeSalle R, Birstein VJ (1996) PCR identification of black caviar. *Nature*, **381**, 197–198.

Dizon A, Baker CS, Cipriano F, Lento G, Palsboll P, Reeves R, eds. (2000) *Molecular genetic identification of whales, dolphins, and porpoises: Proceedings of a workshop on the forensic use of molecular techniques to identify wildlife products in the marketplace*. US Departmen, NOAA Technical Memorandum, NOAA-TM-NMFS-SWFSC-286.

FAO (1998) *International Plan of Action for the conservation and management of sharks*. Document FI: CSS/98/3, Oct. 1998. Food and Agriculture Organization, Rome.

FAO (2000) *Conservation and Management of Sharks*. FAO Technical Guidelines for Responsible Fisheries. No. 4, Suppl. 1. Rome, 37 pp.

Heist EJ, Gold JR (1999) Genetic Identification of sharks in the US Atlantic large coastal shark fishery. *Fish. Bull.*, **97**, 53–61.

Hoelzel AR (2001) Shark fishing in fin soup *Conserv. Genet.*, **2**, 69–72.

Malik S., Wilson P.J., Smith R.J., Lavigne D.M., White B.N. (1997) Pinneped penises in trade: A molecular-genetic investigation. *Conserv. Biol.*, **11(6)** 1365–1374.

Martin AP (1993) Application of mitochondrial DNA sequence analysis to the problem of species identification of sharks. In: *Conservation Biology of Elasmobranchs* (ed. Branstetter S), pp. 53–59. US Department of Commerce, National Oceanic and Atmospheric Administration Technical Report NMFS-115.

Musick JA, Burgess G, Caillet G, Camhi M, Fordham S (2000) Management of sharks and their relatives (Elasmobranchii).. *Fisheries*, **25(3)** 9–13.

Nicholas KB, Nicholas HB Jr (1997) GenDoc: Analysis and Visualization of Genetic Variation. http://www.cris.com/~Ketchup/gendoc.shtml.

NMFS (2001) *Final United States National Plan of Action for the Conservation and Management of Sharks*. US Department of Commerce, National Oceanic and Atmospheric Administration, National Marine Fisheries Service Report.

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. *Mar. Biotech.*, **3**, 231–240.

Roman J, Bowen BW (2000) The mock turtle syndrome: Genetic identification of turtle meat purchased in the southeastern United States of America. *Anim. Conserv.*, **3**, 61–65.

Rose D (1996) *An overview of world trade in sharks and other cartilaginous fishes*, TRAFFIC international, Cambridge, UK106.

Shivji MS, Tagliaro C, Natanson L, Kohler N, Rogers S, Stanhope M (1996) Utility of ribosomal DNA ITS2 for deriving shark species-diagnostic identification markers. In: Proceedings of the International Congress on the Biology of Fishes (eds. Donaldson EM, Mackinlay DD), pp. 87–93. San Francisco, USA.

Shivji M, Clarke S, Pank M, Natanson L, Kohler N, Stanhope M (2002) Simultaneous molecular genetic identification of body-parts from six pelagic shark species for conservation, management and trade monitoring. *Conserv. Biol.*, **16(4)** 1036–1047.

Smith SE, Au DW, Show C (1998) Intrinsic rebound potentials of 26 Pacific sharks. *Mar. Freshwat. Res.*, **49(7)** 663–678.

Smith PJ, Benson PG (2001) Biochemical identification of shark fins and fillets from the coastal fisheries in New Zealand. *Fish. Bull.*, **99**, 351–355.

Stevens JD, Bonfil R, Dulvy NK, Walker PA (2000) The effects of fishing on sharks, rays, and chimeras (chondrichthyans), and the implications for marine ecosystems. *ICES J. Mar. Sci.*, **57**, 476–494.

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997) The ClustalX windows interface: Flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acid. Res.*, **24**, 4876–4882.

Walker TI (1998) Can shark resources be harvested sustainably? A question revisited with a review of shark fisheries *Mar. Freshwat. Res.*, **49(7)** 553–572.

Weber ML, Fordham SV (1997) *Managing shark Fisheries: Opportunities for International Conservation*. TRAFFIC International and Center for Marine Conservation Report.