# 3 Determination of Species Composition

## 3.1 Overview and Purpose

An estimate of the number of sharks utilized by the shark fin trade each year is a fundamental component of understanding and assessing the role of this trade in the exploitation of shark resources. However, unless this estimate can be proportionally partitioned by species, it will not inform management of shark resources on a species-by-species basis. Management based on an amalgam of species is suboptimal and undesirable as it is likely to exaggerate the conservation concern for some species (e.g. those sharks with high fecundities and low age of maturity) while understating the potentially threatened nature of less prolific shark stocks (Cortés 2002a).

As presented in *Chapter 2*, this study obtained shark fin auction records which describe auction lots in terms of the Chinese trade names for various types of shark fins. Since species information is lacking in most databases describing fin production and shark catches (FISHSTAT 2002, Shotton 1999a, Hong Kong Government 2002, SEAFDEC 2001), if the trade names contained in these auction records can be mapped to taxonomic nomenclature, these records can provide the first opportunities to substantiate and quantify the species composition of shark fins in trade. Previous studies of the Hong Kong fin trade have assigned English common names or Latin names for some types of fins in trade (Parry-Jones 1996, Fong 1999, Vannuccini 1999, Yeung et al. 2000), but they have relied solely on traders' nomenclature and have had no means of independently verifying fins' species of origin.

The goal of this component of the study was to verify Chinese trade name-species name concordances for the most commonly appearing fin types in the Hong Kong auction

records. The following sections describe the methodology and results of the concordance sampling, and adjust the figures given in Chapter 2 in order to provide species-specific tallies of traded shark fin weight, shark numbers and whole landed weights. The application of these methods to future monitoring of the shark fin trade as well as to other traded wildlife products is also discussed.

## 3.2 Technique Selection

Three methods for determining the species from which dried shark fins are derived were explored for this study: visual key character methods, microscopic examination of dermal denticles, and molecular genetic techniques. Each method is discussed in the context of its applicability to the Hong Kong shark fin trade below.

### *3.2.1 Visual Key Character Methods*

Visual discrimination of fin morphology involves using shape, proportions, colour, and/or surface texture to distinguish the species of shark. Morphological shark fin keys have recently been published to aid in fin identification in Japanese fisheries (Nakano and Kitamura 1998, Fisheries Agency of Japan 1999). These keys include 11 pelagic shark species, but are based on characters observed in only one set of fins per species (i.e. n=1). Several steps in the dichotomous keys require distinctions based on fin colour which is often an impractical character when sorting fins subjected to various degrees and methods of drying. Other shark identification guides consist either of photographic catalogs of individual specimens of fins by trade name (Vannuccini 1999, Yeung et al. 2000) or do not provide sufficient information to allow identification of sharks from the fin alone (Compagno 1984, Compagno 2001, Hennemann 2001).

While traders obviously rely on visual discrimination to sort fin stocks into lots for auctioning or direct sale, the distinguishing characters for some fins are very subtle and require considerable experience to recognize. Furthermore, as described above, trader expertise extends only to trade categories and does not reliably indicate species. Nevertheless, several months of this study were devoted to observing auctions and visiting trader warehouses, in order to understand the means by which traders sort fins into categories. As a result of these efforts, it was possible to correctly identify some of the major trade categories by eye. Ultimately, due to difficulties in mastering the intricacies of visual fin classification for all the species of interest within a short timeframe, this technique was used only as a form of general corroboration for fin classifications provided by traders.

### *3.2.2 Microscopic Methods: Denticle Patterns*

Microscopic investigation of dermal denticle patterns has also been proposed as a means of species identification. An unpublished study of sharks from the Mediterranean and the Eastern Atlantic found that only some of the species assessed can be accurately identified using this technique (B. Serét, Museum National d'Histoire Naturelle, Paris, France, pers. comm.). A doctoral dissertation from the University of Port Elizabeth, South Africa has also explored this topic (Wagner 2000). Research in Japan on 13 species of mainly pelagic sharks has tentatively identified denticle patterns using electron microscopy imaging of one or more individuals of each species (Tanaka et al. 2002). This manuscript and its images were obtained and applied to 24 types of shark fin obtained from a cooperative Hong Kong trader at the beginning of this study. My investigation concluded that denticle patterns are difficult to distinguish in dried fins due to distortion during drying and abrasion during shipment and handling. Also, different patterns were observed at different points on a single fin, confirming previous research showing that denticle patterns vary within species due to position on the body and sex (Mojetta 1997).

It was concluded that although dermal denticle methods have potential for species identification, more field testing is warranted before these methods can be reliably applied, and their diagnostic utility in dried fins appears to be limited.

### *3.2.3 Molecular Genetic Methods*

The third method considered for this study involved the use of deoxyribonucleic (DNA) – based identification techniques. Molecular genetic methods are clearly capable of identifying the species origin of shark fins, but require removing tissue from fins as well as laboratory support for specialized analyses. A variety of techniques are available but must be weighed to determine advantages and disadvantages in any given sampling programme. A brief review of various molecular genetic techniques which have been applied to the identification of particular species, populations or individuals in trade is provided by way of background.

In order to draw conclusions about the origin and legality of whale meat, researchers have capitalized on extensive genetic databases and pioneered techniques for performing partial genetic analyses in the field (Baker and Palumbi 1994, Cipriano and Palumbi 1999, Dizon et al. 2000, Dalebout et al. 2002). Other studies have applied genetic methods in the laboratory to wildlife products such as caviar (Birstein et al. 1998), pinniped penises (Malik et al. 1997), turtle meat (Roman and Bowen 2000), red-meat products (Martinez and Yman 1998) and shark fins (Hoelzel 2001), with the objective of identifying the species origin of a small number of samples in various trade categories. In the main, these studies have been concerned with demonstrating the proposed technique is effective for identifying the species or origin of individual market place products, and have not attempted to characterize the species composition of the trade as a whole.

The choice of molecular markers and methods for trade monitoring will depend on the type of organism being studied and the monitoring objectives. Where existing DNA sequence databases are available to support species, population or even individual discrimination, such as for cetaceans (Baker and Palumbi 1994, Cipriano and Palumbi 1999, Baker et al. 2000, Rosenbaum et al. 2000), pinnipeds (Malik et al. 1997) or geographically confined animals (Manel et al. 2002), DNA sequencing provides a straightforward, though expensive and time consuming, means of assigning a species identification to a sample. To accommodate large scale and economical identification of samples in trade monitoring and conservation applications, alternative molecular markers and methods have been developed. These include restriction fragment length polymorphisms (Heist and Gold 1999, Gharrett et al. 2001) and species-specific PCR primers (Hoelzel 2001, Pank et al. 2001, Shivji et al. 2002), both of which screen for a small subset of nucleotides that are diagnostic for a species; and microsatellites, which sometimes can be used to identify the population of origin of individual samples (Cornuet et al. 1999, Manel et al. 2002). Microsatellite techniques are still under development for sharks and thus were not available for use in this study.

Both the species-specific primer and restriction fragment length polymorphism (RFLP) methods require considerable initial development and testing to demonstrate that the primer is indeed diagnostic of a single species. The RFLP technique is based on DNA extraction and amplification using PCR, but instead of using a species-specific primer in the PCR step, it requires one or more subsequent reactions to parse the PCR products into a number of bands that appear, after gel electrophoresis, in species-specific patterns. Like the PCR primer technique, this technique requires that a specific locus be selected within which all species of interest can be distinguished. RFLP research on sharks has identified a mitochondrial DNA locus in which species-specific differences are apparent, but problematic intra-specific differences have also been identified (Heist and Gold 1999).

While both RFLP and species-specific PCR primers have been used to identify shark products, the PCR primer technique is believed to be better suited to rapid and large-scale screening applications (Palumbi and Cipriano 1998, Hoelzel 2001, Shivji et al. 2002).

## 3.3 Methodology

### *3.3.1 Use of Polymerase Chain Reaction (PCR) Primers for Sharks*

Recent development of diagnostic DNA sequence tests using species-specific PCR primers for sharks provided an ideal opportunity to study the species composition of the shark fin trade (Pank et al. 2001, Shivji et al. 2002). Through a cooperative partnership with the innovators of the shark PCR primer techniques at the Guy Harvey Research Institute, Nova Southeastern University, this study was able to procure laboratory analysis services as part of a collaborative effort to test these methods on dried shark tissue samples from globally distributed sources.

Detailed description of the methodology of these diagnostic tests is provided in Pank et al. (2001) and Shivji et al. (2002), and summarized briefly here. This technique relies on DNA sequence differences among shark species in the nuclear ribosomal DNA ITS2 locus for the development of species-specific primers (Figure 3.1). Each primer is a short, synthetic, single stranded piece of DNA designed to recognize (anneal to) and PCR amplify DNA from a single shark species only (e.g. a primer designed for shortfin mako shark will only amplify DNA from fins of that species), producing a diagnostic sized DNA band (amplicon). Sample screening efficiency is achieved by combining multiple (up to six) species-specific primers in a single reaction (multiplex PCR), thereby testing each unknown fin for its potential origin from any one of six species at a time. At the current stage of development, this method works most efficiently when the expected

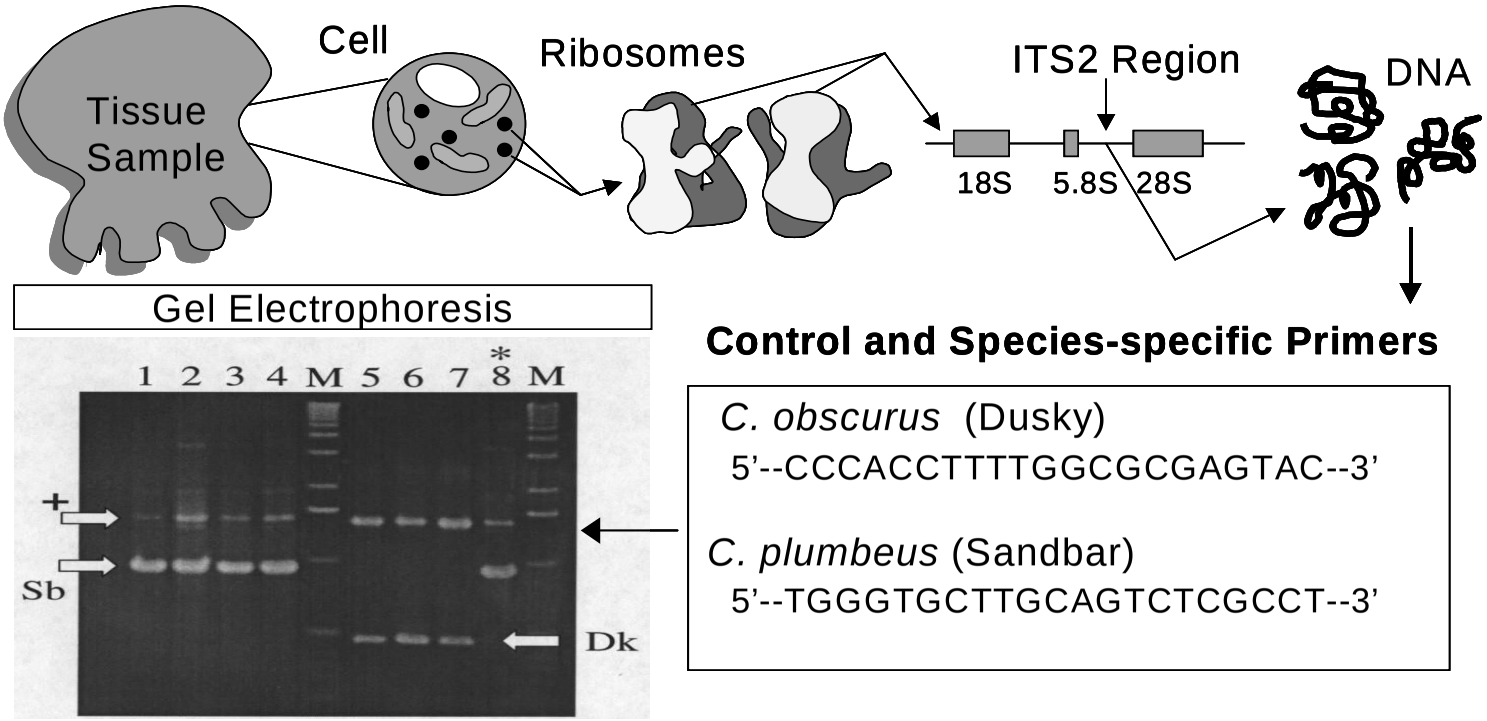


**Figure 3.1** Shark species identification using polymerase chain reaction (PCR) primers. Ribosomal DNA is isolated from the ITS2 region and exposed to control and species-species primers. If the sampled DNA matches any one of the species-specific primers, it will amplify producing multiple copies of the matching region of DNA. The primers are based on approximately 20 base pairs (bps) of amino acids (as above), but are formulated to be diagnostically different in length (e.g. 50 bps for one species versus 100 bps for another). When the products of the PCR are subjected to gel electrophoresis, the primers will migrate to diagnostically different positions along the gel lane based on their molecular weight (i.e. number of base pairs), and can be used to indicate species identity. In this example from Pank et al. (2001), samples from sharks identified in the field as sandbar (*Carcharhinus plumbeus*) and dusky (*Carcharhinus obscurus*) are exposed to the sandbar and dusky species-specific PCR primers and run in gel lanes 1-4 and 5-8, respectively. The results show that sample 8, identified in the field as tissue from a dusky shark is actually derived from a sandbar shark.

identity of the fin sample can be narrowed to a maximum of six species for which primers are available and which can be multiplexed together in a single reaction (Shivji et al. 2002). If the sample is not from one of the six initial species tested, a different suite of six primers can be tried, requiring a second round of screening. However, because a complete set of species-specific primers for all shark species possibly present in the fin market is not yet available, some fins cannot currently be identified.

Seventeen primers that are species-specific or nearly species-specific were employed to identify the shark fins in this study (Table 3.1). Detailed methodologies for the development of each species-specific primer and the multiplex PCR screening assay are given in Pank et al. (2001) and Shivji et al. (2002) for shortfin mako, longfin mako, porbeagle, silky, blue, sandbar and dusky sharks. Development of primers for common thresher, pelagic thresher, bigeye thresher, tiger, Caribbean reef, bull, bignose, smooth hammerhead, great hammerhead and scalloped hammerhead sharks will be published separately. The diagnostic reliability of each species-specific primer within the ongoing primer development process is described in Shivji et al. (2002) and in Table 3.1, with each primer ranked on a scale of 1 - 4 reflecting its diagnostic robustness (Rank of 1 being the most robust).

### *3.3.2 Selection of Sampling Sites Within the Hong Kong Shark Fin Market*

The feasibility of obtaining dried shark fin tissue samples was explored through numerous reconnaissance visits to Hong Kong's dried seafood district, Sai Yin Pun (Figure 3.2). Approximately 50 retail or wholesale establishments located there deal in shark fin products (Clarke 2002). Of this number, there are about 16 wholesalers who take turns hosting daily shark fin auctions in Hong Kong, and at least an equal number who import large quantities of fins and re-export them to Mainland China for processing without auctioning. Traders receive fins from at least 85 countries and territories (Hong

**Table 3.1** Validation status of the diagnostic reliability of shark PCR primers based on Pank et al. (2001), Shivji et al. (2002), and unpublished data. Primer reliability is ranked as follows: 1) fully validated; 2) extensively validated; 3) partially validated with high confidence; 4) partially validated but requires further testing due to some false positives with other species for which primers are not available. An asterisk indicates species whose primers amplify globally distributed samples of that species, i.e. no subpopulation variation.

| **Primer Reliability Rank** | **Primer for Shark Species** | **Primer Validation Status** |
|---|---|---|
| 1 | **Order Lamniformes**<br><br>*Family Lamnidae*<br>Shortfin mako* *Isurus oxyrinchus*)<br>Longfin mako* (*Isurus paucus*)<br><br>*Family Alopiidae*<br>Common thresher* (*Alopias vulpinus*)<br>Bigeye thresher* (*Alopias superciliosus*)<br>Pelagic thresher* (*Alopias pelagicus*) | Verified as species-specific against 68 major, non-target shark fishery species, including all major lamniform fishery sharks. |
| 1 (cont.) | **Order Carcharhiniformes**<br><br>*Family Carcharhinidae*<br>Tiger* (*Galeocerdo cuvier*) | Tiger primer verified as species-specific against 68 major, non-target shark fishery species, including 30 of 50 carcharhinid species. ITS2 sequence divergence between tiger and other carcharhinid species is large indicating tiger primer is unlikely to amplify untested species. |
| 2 | **Order Carcharhiniformes**<br><br>*Family Carcharhinidae*<br>Blue* (*Prionace glauca*)<br>Silky* (*Carcharhinus falciformis*)<br>Caribbean reef* (*Carcharhinus perezi*)<br>Bignose (*Carcharhinus altimus*)<br>Spinner* (*Carcharhinus brevipinna*) | Verified as species-specific against 68 major, non-target shark fishery species, including 21 of 30 *Carcharhinus* species. The high degree of species-specificity seen against all congeners tested thus far reduces the likelihood that these primers will show significant cross amplification against remaining untested congeners. |

**Table 3.1 (cont.)**

| | | |
|---|---|---|
| 3 | **Order Carcharhiniformes**<br><br>*Family Carcharhinidae*<br>Sandbar* (*Carcharhinus plumbeus*)<br>Bull* (*Carcharhinus leucas*)<br><br>**Order Carcharhiniformes**<br><br>*Family Sphyrnidae*<br>Smooth hammerhead* (*Sphyrna zygaena*)<br>Scalloped hammerhead* (*Sphyrna lewini*)<br>Great hammerhead* (*Sphyrna mokarran*) | Verified as species-specific against 67 major, non-target shark fishery species, including 20 of 30 *Carcharhinus* species. Sandbar primer cross amplifies bignose, and bull primer cross amplifies Caribbean reef. However, sandbar and bull shark fin identity confirmed by secondary testing with the extensively validated bignose and Caribbean reef primers.<br>Verified as species-specific against 68 major, non-target shark fishery species, including 5 of 8 sphyrnids. These 3 hammerhead species are circumglobally distributed and expected to constitute the major portion of the hammerhead fins in the market. The 3 as yet untested *Sphyrna* species are rarer and have localized distributions, and are likely to be relatively uncommon in the fin market. |
| 4 | **Order Carcharhiniformes**<br><br>*Family Carcharhinidae*<br>Dusky* (*Carcharhinus obscurus*) | Verified as species-specific against 66 major, non-target shark fishery species, including 19 of 30 *Carcharhinus* species. However, dusky primer cross-amplifies oceanic whitetip (*C. longimanus*) and Galapagos (*C. galapagensis*) sharks. The dusky primer, in combination with visual diagnostics for oceanic whitetip, permits robust identification of the latter's fins without confusing oceanic whitetip and dusky. False positive amplifications from Galapagos shark fins cannot be distinguished at present. |

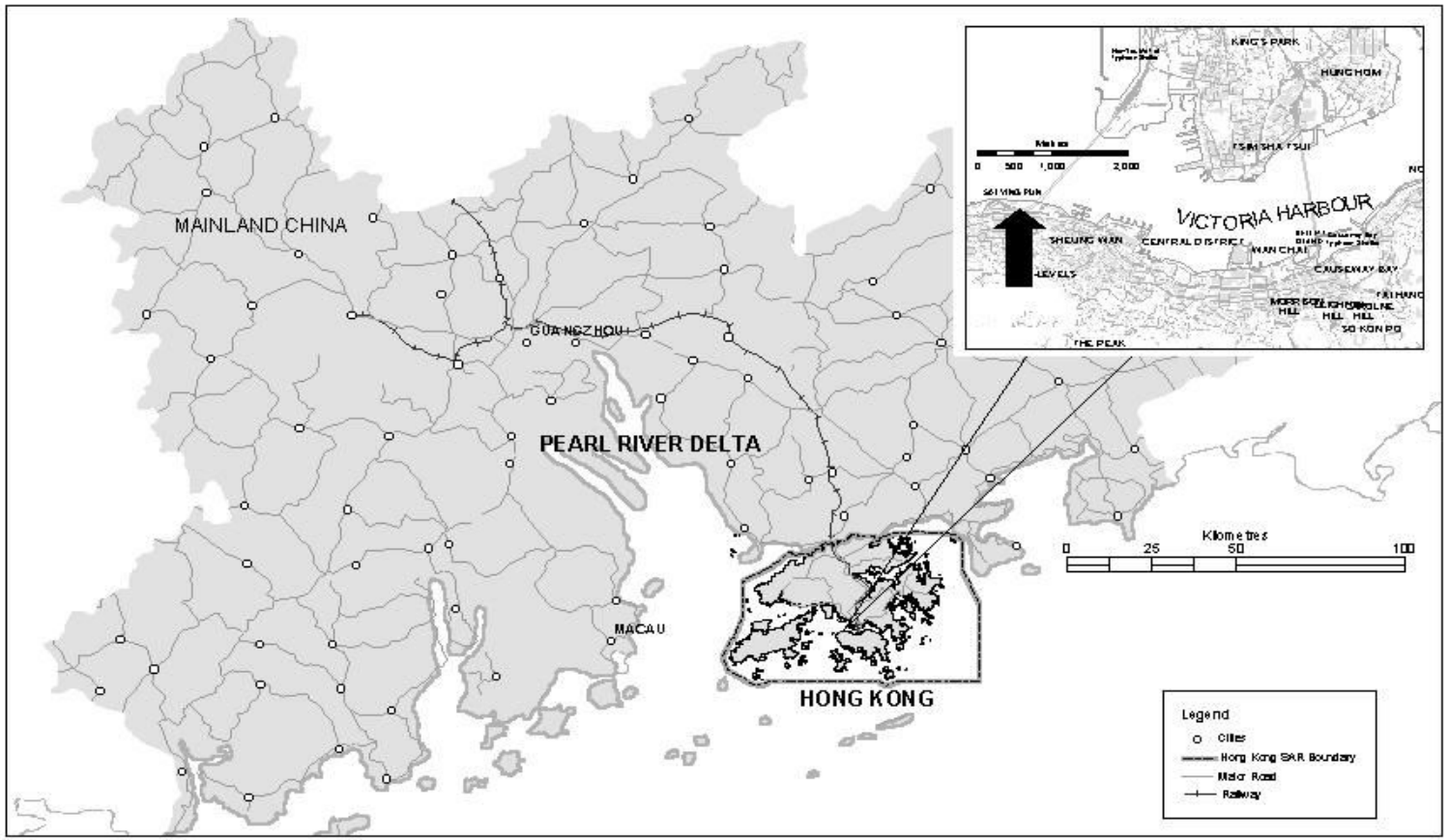


**Figure 3.2** Map of Southern China showing Hong Kong with an inset detailing the North-Western area of Hong Kong Island. The location of the dried seafood district in Sai Yin Pun is indicated by the black arrow on the inset map.

Kong Government 2002) in poorly sorted shipments and must re-sort the fins into market categories before auction or sale. However, once the fins are bagged for auctioning, and once they are sold, traders are reluctant to tamper with the fins for fear of jeopardizing the sale, thus limiting the window for sampling. Although fins are displayed during the auctions, the opportunity for sampling is too brief and most traders preferred that their participation in the study remained confidential, thus sampling could not take place at auctions. Furthermore, traders who were willing to participate in the study during fin sorting could not afford the time required to perform representative sampling of their stock. Given these restrictions, and based on the results of initial enquires at over 30 trading houses, it was decided to focus on sampling particular Chinese trade names for shark fins across as wide a range of traders and source countries as possible. The concordances between scientific names and Chinese trade names could then be linked with the information on the auction records described in Chapter 2 and used to characterize the species composition.

### *3.3.3 Statistical Design of the Sampling Program*

A total of eleven market categories were chosen for testing on the basis of being common in the trade and whether there were, or were likely to be during the course of this study, validated primers for the taxa expected to be found in each category. The expected trade name – taxonomic concordances (Table 3.2) were hypothesized based on information from traders, and comparison with published information on fins and shark morphology (Vannuccini 1999, Yeung et al. 2000, Fisheries Agency of Japan 1999, Froese & Pauly 2002). A probability, $p$, that the expected match would be found to hold for any sample of a given market category was formulated *a priori* based on trader interviews (Table 3.2).

**Table 3.2** *A priori* hypothesized matches (x→x') between traders' market categories and shark taxa for fins targeted in the sampling. Trade names are romanised using the pinyin system. Chinese characters are given to facilitate translation between Pinyin and other forms of Chinese romanisation. The probability that the expected match is correct is given by *p*. These *a priori* values of *p* were used to calculate the required number of samples (see Figure 3.1).

| **Market Category (x)** | **Expected Predominant Taxa within Market Category (x')** | **Assigned *a priori* value of *p*** | **Other Taxa Believed to be Present in the Market Category** |
|---|---|---|---|
| Ya Jian<br>牙揀 | blue<br>(*Prionace glauca*) | .95 | none |
| Qing Lian<br>青連 | shortfin mako<br>(*Isurus oxyrinchus*) | .80 | longfin mako (*I. paucus*) |
| Wu Yang<br>五羊 | silky<br>(*Carcharhinus falciformis*) | .70 | Galapagos *(C. galapagensis),* silvertip *(C. albimarginatus)* |
| Hai Hu<br>海虎 | dusky<br>(*C. obscurus*) | .90 | unknown (if any) |
| Bai Qing<br>白青 | sandbar<br>(*C. plumbeus*) | .90 | unknown (if any) |
| Ruan Sha<br>軟沙 | tiger<br>(*Galeocerdo cuvier*) | .90 | unknown (if any) |
| Chun Chi<br>春翅 | smooth and scalloped hammerheads (*Sphyrna zygaena* and *S. lewini*) | .60 | great hammerhead *(S. mokarran)* and other hammerheads (*Sphyrna* spp.) |
| Gu Pian<br>骨片 | great hammerhead (*S. mokarran*) | .85 | other hammerheads (*Sphyrna* spp.) |
| Wu Gu<br>勿骨 | threshers<br>(*Alopias* spp) | .90 | longfin mako (*I. paucus*) |
| Sha Qing<br>沙青 | bull<br>(*C. leucas*) | .80 | pigeye *(C. amboinensis)* |
| Liu Qiu<br>流球 | oceanic whitetip<br>(*C. longimanus*) | .95 | none |

As traders' profits depend on their ability to discriminate fins into market categories of varying value, it was expected that most market categories based on distinctive fins

would have high *p* values, i.e. near 1. However, for market categories based on fins that were less distinctive, and/or were believed to contain more than one species, *a priori* values of *p* were reduced in proportion to taxonomic uncertainty.

The goal of the sampling program was to estimate the probability *p* that a fin identified by a trader as market category *x* is found to be taxon *x'* with a low coefficient of variation for *p* ($CV_p$<0.10). Based on the binomial model, where *p* is the proportion correctly identified, *q* is the proportion incorrectly identified, $s_p$ is the standard deviation in *p*, and $CV_p=s_p/p$, the required number of samples *n* is calculated as:

$$n = \frac{q}{(CV_p)^2 p} \qquad \text{(Eq. 3.1)}$$

Using this formula, and the *a priori p* values in Table 3.2, the requisite number of samples for two $CV_p$ levels (0.05 and 0.10) were calculated for each market category (Figure 3.3).

Individual warehouses were sampled between November 2000 and February 2002. At each establishment, traders were shown a list of desired fin types (in Chinese characters) and asked to allow free-of-charge sampling of small pieces of tissue from the edges of dried fins. The market category to be sampled was checked using visual characters employed by traders to avoid obvious errors. In the majority of visits, not all fin types could be sampled due to limited stocks on hand. In addition, some traders were reluctant to allow sampling of high value fins, such as Hai Hu, Bai Qing and Ruan Sha, for fear of product damage. Most traders did not understand the rationale for replicate sampling within a category and this led to sample sizes of n=1 during many visits.

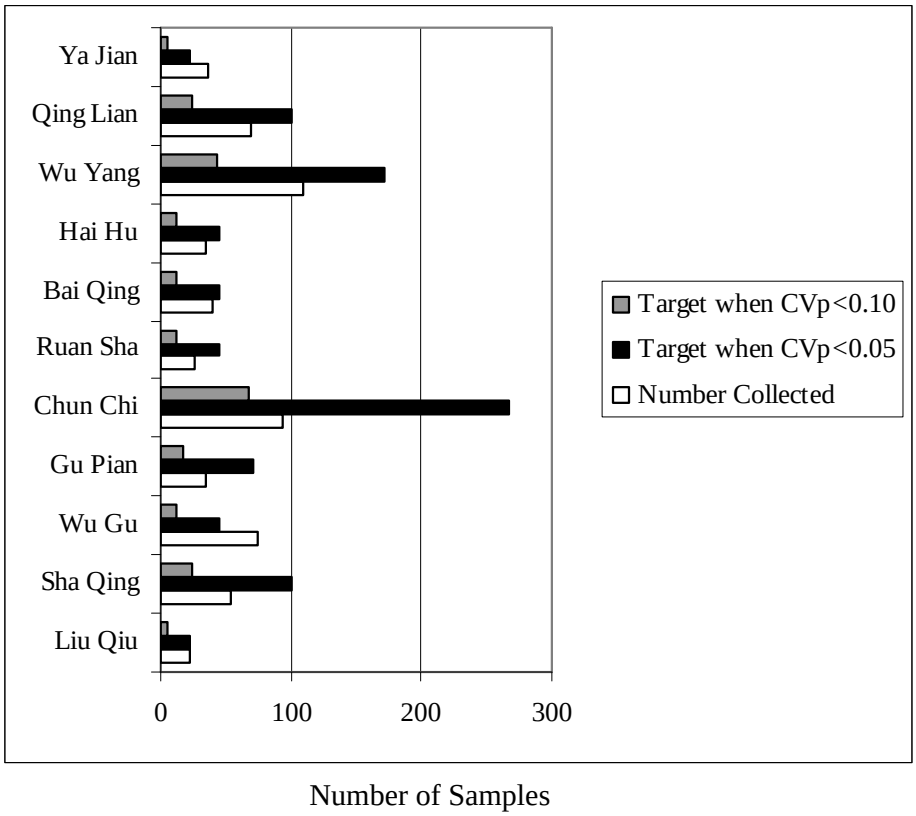


**Figure 3.3** Target number of samples and number of samples collected for coefficient of variation in $p$ ($CV_p$) set at 0.05 and 0.10 for each shark fin market category. As described in Table 3.2, *a priori* estimates of $p$ were formulated and used in conjunction with pre-selected values of $CV_p$ to calculate the required number of samples for each category.

### *3.3.4 Sampling Cutting and Handling*

In order to obtain sufficient fin tissue for analysis, samples were required to be of approximately 1 $cm^3$ but could be cut from any part of the fin, including those areas of skin or muscle tissue that are discarded during fin processing. Samples were usually cut using metal pincers or stainless steel scissors, although some traders insisted on cutting samples themselves using a jigsaw. The laboratory originally requested that the cutting tool be cleaned with 20% bleach, then water, and carefully dried before re-use. This methodology proved unnecessary for dried fins, and furthermore resulted in raised

suspicions among traders who did not understand cross-contamination issues, and/or increased impatience with delays in sample cutting. Instead, the cutting tool was carefully wiped with a cloth between samples and laboratory staff were asked to avoid using cut edges when extracting DNA in case of cross-sample contamination introduced at the point of cutting. Once cut, samples were placed into individual self-sealing plastic bags and labelled with sample number, date, trader, trade name of fin and area of origin, and a separate sample inventory was maintained in spreadsheet form.

## 3.4 Results

### *3.4.1 Representativeness and Statistical Power*

A total of 596 fin samples from the eleven target market categories was collected; opportunistic sampling of non-target fin types led to collection of an additional 239 samples that now await primer development and testing. The number of collected samples exceeded the target number for $CV_p$=0.10 for each market category, and for three categories (Ya Jian, Wu Gu and Liu Qiu), the number of collected samples also exceeds the target number for $CV_p$=0.05 (Figure 3.3; Table 3.3).

The study's ability to assess i) the fidelity of nomenclature across the trading community, and ii) traders' ability to consistently classify fins imported from different parts of the world into coherent categories, was determined by the distribution of samples among different trading houses and source ocean basins or regions. The distribution by trader (Figure 3.4) was skewed such that 372 of the 596 samples (62%) were obtained from just two traders. This situation is a reflection of the sharp decline in trade community cooperation following a shark conservation campaign conducted in Hong Kong in March 2001.

**Table 3.3** Distribution of samples within each market category by trader and source (ocean basin/region) of fins. The evenness statistic (J) (Zar 1999) represents only the equity of the distribution of samples among traders or sources that were sampled, and is not a function of the total possible number of sampled traders or sources. Higher values for J indicate that the samples were spread more evenly among the various trader or source categories being sampled. See text for further interpretation of specific J values.

| Trader's Market Category | Hypothesized Species Match | Number of Samples Collected 'n' | Number of Traders Represented (of 20 in total) | Evenness for Traders (Brillouin J) | Number of Source Ocean Basins or Regions Represented (of 8 in total, excluding unidentified) | Evenness for Sources (Brillouin J) |
|---|---|---|---|---|---|---|
| Ya Jian | *P. glauca* | 37 | 11 | 0.81 | 5 | 0.77 |
| Qing Lian | *I. oxyrinchus* | 69 | 11 | 0.68 | 6 | 0.82 |
| Wu Yang | *C. falciformis* | 110 | 13 | 0.71 | 5 | 0.58 |
| Hai Hu | *C. obscurus* | 34 | 7 | 0.52 | 4 | 0.43 |
| Bai Qing | *C. plumbeus* | 40 | 9 | 0.64 | 3 | 0.69 |
| Ruan Sha | *G. cuvier* | 26 | 6 | 0.63 | 3 | 0.48 |
| Chun Chi | *S. zygaena* or *S. lewini* | 94 | 13 | 0.68 | 7 | 0.60 |
| Gu Pian | *S. mokarran* | 35 | 8 | 0.70 | 4 | 0.29 |
| Wu Gu | *Alopias* spp. | 75 | 9 | 0.69 | 6 | 0.81 |
| Sha Qing | *C. leucas* | 53 | 10 | 0.74 | 5 | 0.64 |
| Liu Qiu | *C. longimanus* | 23 | 9 | 0.76 | 3 | 0.76 |

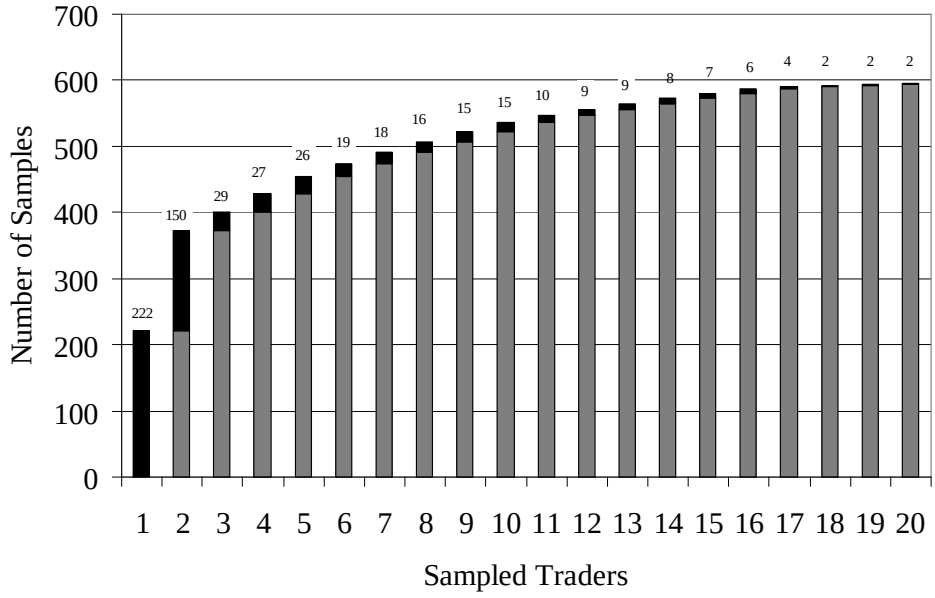


**Figure 3.4** Distribution of samples (n=596) across trading houses in Hong Kong. Samples from each sampled trader are shown in black and annotated above each column. The grey portion of each column represents the cumulative number of samples obtained from traders represented in columns to the left of each sampled trader.

The allocation of samples across geographic points of origin is shown in Figure 3.5. Since many of the traders were unable to be precise about the origin of the fins, a large proportion of fins were of unknown origin and the remaining samples were identified using general categories to represent different ocean basins or areas. The two heavily sampled traders specialized in imports from South America and this is reflected in the fact that 264 of 596 samples (44%) were said to have originated in South America. Nevertheless, one of the heavily sampled traders also received shipments of fins from other locations and these other locations contributed 50% of the samples from this trader.

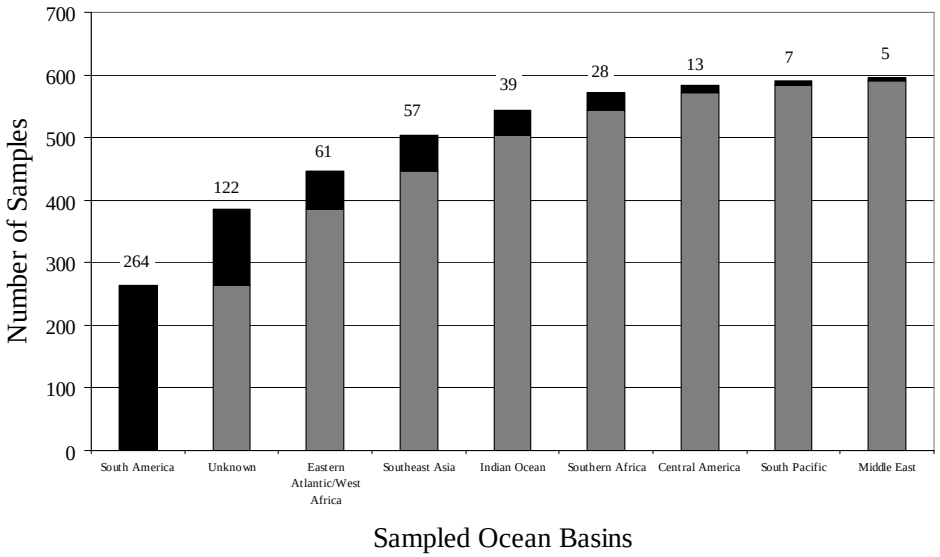


**Figure 3.5** Distribution of samples (n=596) across major ocean basins. Samples from each sampled trader are shown in black and annotated above each column. The grey portion of each column represents the cumulative number of samples obtained from traders represented in columns to the left of each sampled trader. Specific source locations cited by traders for each of the areas in the figure (left to right) are as follows: South America (Brazil, Ecuador), Eastern Atlantic / West Africa (Spain, Togo); South East Asia (Australia, Indonesia and the Philippines); Indian Ocean (India, Sri Lanka, the Maldives, Bangladesh); Southern Africa (South Africa and Angola); Central America (Gulf of Mexico, Costa Rica); South Pacific (Fiji); Middle East (including fins from unspecified African countries).

The diversity of samples in each market category by trader and source region was examined (Table 3.3) using the information-theoretic evenness measure of Brillouin for non-random samples (Zar 1999), where:

$$H = \frac{\left(\log n! - \sum \log f_i!\right)}{n} \qquad \text{(Eq. 3.2)}$$

with $n$ the total number of samples and $f_i$ the number of samples in each category. Higher values for this statistic indicate that the samples were spread more evenly among the

various trader or source categories being sampled. The lowest evenness value (0.29) by source region was calculated where 89% of the samples were collected from one source region with the remaining samples distributed among 3 other regions or unidentified (Gu Pian, n=35). By trader, the lowest evenness values (0.52) resulted when 56% of the samples were collected from one trader, with another 29% from a second trader, and the remaining samples distributed among 5 other traders (Hai Hu, n=34).

### *3.4.2 Species Identification of Samples*

All samples (n=596) were initially tested with the primer representing the expected taxonomic match (Table 3.2). If the sample failed to amplify with this primer (a *q* fin), it was consecutively tested in a multiplex PCR format against each of the other seventeen primers used in this study (Table 3.1) until a match was found (a $q_i$ sample) or until all the primers had been tried (a $q_u$ sample). Values of *p*, the proportion of samples validating the expected match, ranged from 0.64 for Sha Qing to 1.00 for Liu Qiu (Table 3.4). Calculation of *a posteriori* estimates of the coefficient of variation of *p* ($CV_p$) suggests that for the majority of market categories the sample size was sufficient to provide a robust estimate of *p* since all values of $CV_p$. are less than 0.11 (Table 3.4). The results of the testing for those samples which did not verify the expected match are given in Table 3.5.

The results for several market categories, which are easily distinguished from most other types of fins due to size, shape or colour, conformed quite closely to expectations. None of the Liu Qiu fins were from any shark other than oceanic whitetip, and the only Ya Jian that was not a blue shark fin was from a bigeye thresher shark which has similar fin dimensions. All of the fins in the Gu Pian category that were not great hammerhead were

**Table 3.4** Results of PCR primer analysis by market category. Control failures are those samples for which no valid test result could be obtained. The proportion of samples validating the expected match is *p* where *p* is equal to number of matching samples/(number of samples tested – control failures). $CV_p$ and $s_p$ are the coefficient of variation of *p* and the standard deviation of *p*, respectively.

| Trader's Market Category | Genetic Identification Necessary to Score as *p* | Number of Samples Tested (*n*) | Number of Samples with Control Failures | Number of Samples Confirmed as Matching Expected Species (*r*) | *a posteriori* Estimates | | |
|---|---|---|---|---|---|---|---|
| | | | | | *p* | $CV_p$ | $s_p$ |
| Ya Jian | *P. glauca* | 37 | 1 | 35 | 0.97 | 0.028 | 0.027 |
| Qing Lian | *I. oxyrinchus* | 69 | 2 | 57 | 0.85 | 0.051 | 0.044 |
| Wu Yang | *C. falciformis* | 110 | 2 | 86 | 0.80 | 0.049 | 0.039 |
| Hai Hu | *C. obscurus* | 34 | 0 | 29 | 0.85 | 0.071 | 0.061 |
| Bai Qing | *C. plumbeus* | 40 | 6 | 25 | 0.74 | 0.103 | 0.076 |
| Ruan Sha | *G. cuvier* | 26 | 1 | 21 | 0.84 | 0.087 | 0.073 |
| Chun Chi | *S. zygaena* or *S. lewini* | 94 | 1 | 89 | 0.96 | 0.022 | 0.021 |
| Gu Pian | *S. mokarran* | 35 | 0 | 30 | 0.86 | 0.069 | 0.059 |
| Wu Gu | *Alopias* spp. | 75 | 7 | 50 | 0.74 | 0.073 | 0.054 |
| Sha Qing | *C. leucas* | 53 | 3 | 32 | 0.64 | 0.106 | 0.068 |
| Liu Qiu | *C. longimanus* | 23 | 0 | 23 | 1.00 | 0 | 0 |
| **Totals** | | **596** | **23** | **477** | - | - | - |

**Table 3.5** Summary of samples not confirming the expected match in each market category. Samples that did not amplify with any species-specific primer ($q_u$ samples), and samples that were found to amplify with another species-specific primer ($q_i$ samples), are shown. For the latter, the true sample identity is given.

| Market Category | English Common Name of Expected Match | Number of $q_u$ Samples | Number of $q_i$ Samples | Identity of $q_i$ Samples |
|---|---|---|---|---|
| Ya Jian | blue | 0 | 1 | bigeye thresher (1) |
| Qing Lian | shortfin mako | 2 | 8 | longfin mako (6), bigeye thresher (1), scalloped hammerhead (1) |
| Wu Yang | silky | 9 | 13 | scalloped hammerhead (6), shortfin mako (4), sandbar (1), dusky or Galapagos (1), spinner (1) |
| Hai Hu | dusky | 4 | 1 | silky (1) |
| Bai Qing | sandbar | 7 | 2 | dusky or Galapagos (1), bignose (1) |
| Ruan Sha | tiger | 3 | 1 | bull (1) |
| Chun Chi | hammerhead | 1 | 3 | great hammerhead (2), pelagic thresher (1) |
| Gu Pian | great hammerhead | 1 | 4 | scalloped hammerhead (4) |
| Wu Gu | thresher | 1 | 17 | longfin mako (15), shortfin mako (1), blue (1) |
| Sha Qing | bull | 17 | 1 | tiger (1) |
| Liu Qiu | oceanic whitetip | 0 | 0 | - |
| Totals | | **45** | **51** | |

scalloped hammerhead. This was expected because both scalloped and great hammerhead fins are similarly light in colour (as opposed to smooth hammerheads which are darker). Genetic testing has confirmed that if traders further classified Chun Chi (hammerhead fins) into light and dark varieties these groups always matched the scalloped and smooth hammerhead primers respectively. Mixing of scalloped hammerhead fins in the Gu Pian category thus occurs when some traders identify any large, light coloured hammerhead fin as a Gu Pian.

Some market categories validated the hypothesized species matches at higher probabilities than expected. It was expected that the *a posteriori p* for Chun Chi fins would be relatively low due to the possible presence of other hammerhead species, for which primers have not yet been developed, in the Chun Chi category. However, except for two samples which amplified with the great hammerhead primer, and one sample which did not amplify with any primer, only scalloped and smooth hammerhead fins were present. The possibility that the scalloped and smooth hammerhead primers amplify three as yet untested *Sphyrna* congeners (*S. media*, *S. tudes*, *S. corona*) can, however, not be dismissed.

The other category which was less species-diverse than expected was Wu Yang which amplified with the silky shark primer in 80% of the samples. Other *Carcharhinus* congeners were found to be present but at much lower rates than expected. There remains the possibility, although small, that amplification of other, yet untested rarer congeners by the silky shark primer may account for the high *p* value.

Most of the remaining trade categories contained a higher diversity of species than expected. A key nomenclatural issue was discovered regarding the longfin mako which affects both the Qing Lian (shortfin mako) and Wu Gu (thresher) categories. A minority of traders will sort longfin mako fins into a separate category but most will either combine them with shortfin mako or thresher fins due to similarity of appearance and market value. Of the $q_i$ samples in Qing Lian and Wu Gu categories, 84% were longfin mako fins. All of the high value carcharhinid fins, i.e. Hai Hu, Bai Qing and Ruan Sha showed some degree of mixing with other carcharhinid species and a proportionally large number of unidentifiable fins which did not amplify with any existing primer. The market category with the lowest *a posteriori p* value was Sha Qing, which was originally expected to show a strong concordance with bull shark based on information from

traders. However, of the 50 Sha Qing samples tested, 17 could not be identified and may derive from the morphologically similar pigeye shark, *Carcharhinus amboinensis*, for which no primer is yet available.

Other unexpected results may derive from misunderstanding traders' pronunciation. For example, the presence of several shortfin makos in the Wu Yang category may arise from the fact that some of the traders call shortfin makos Wu Yang (烏羊, i.e. same romanization as silky shark, but different intonation and characters). Identification of five of the six scalloped hammerheads in the Wu Yang samples may also reflect miscommunication during sampling since on this occasion the trader used two different names for the fins during sampling and then became unco-operative.

### *3.4.3 Application of Concordance Results to Trade Records*

As described in Chapter 2, there are 16 major market names that are commonly used to describe auction lots and over 70 market categories in total. The results of the Bayesian imputation and statistical modelling exercise provide proportions, numbers and biomass of auctioned fins represented by each Chinese trade name. The *a posteriori* p-value for each of the tested trade name-species name matches generated here can be applied to these proportions to estimate adjusted proportions representing true totals by species or genus.

The WinBUGS models introduced in Chapter 2 were supplemented with code which uses the results of the genetic analysis to create a random value *p*, representing the probability that a fin labelled with one of the eleven Chinese trade names is actually derived from the expected predominant taxon associated with that trade name (see Table 3.2). This is accomplished through use of a binomial distribution of the form:

$$\frac{n!}{r!(n-r)!} p^r (1-p)^{n-r} \qquad \text{(Eq. 3.3)}$$

where $r = 0,\ 1..., n$

representing the potential number of matching samples up to *n*, the total number of samples tested (Table 3.4). The probabilities, *p*, for each of the eleven tested matches are assumed to be independent, and given a standard non-informative prior distribution conforming to a beta distribution of the form:

$$p^{a-1}(1-p)^{b-1} \frac{\Gamma(a+b)}{\Gamma(a)\Gamma(b)} \qquad \text{(Eq. 3.4)}$$

where $0 < p < 1$

and using $a = 1$ and $b = 1$ for an uninformative prior. This simple model converged within several thousand iterations; probability distributions for *p* in each trade name-species name match are shown in Figure 3.6.

Within the WinBUGS models, the random variable for *p* was used to factor the total estimate of auctioned weight of each market category of fins, for example:

$$\textit{total weight of Ya Jian dorsals} \ \ x \ \ \textit{Ya Jian}\ \text{p} = \textit{adjusted total weight of Ya Jian dorsals} \qquad \text{(Eq. 3.5)}$$

These adjusted auction weights are then directly attributable to particular species / genera and, when processed through the conversion factor portion of the model, provide taxon-specific estimates of numbers and whole landed weight (Table 3.6).

As estimated in Chapter 2 (Table 2.8) based on weight, 45.9% of all auctioned fins were described by one of the Chinese trade names tested in this genetic concordance study. The remaining 54.1% of auctioned fins were either described using a trade name that was

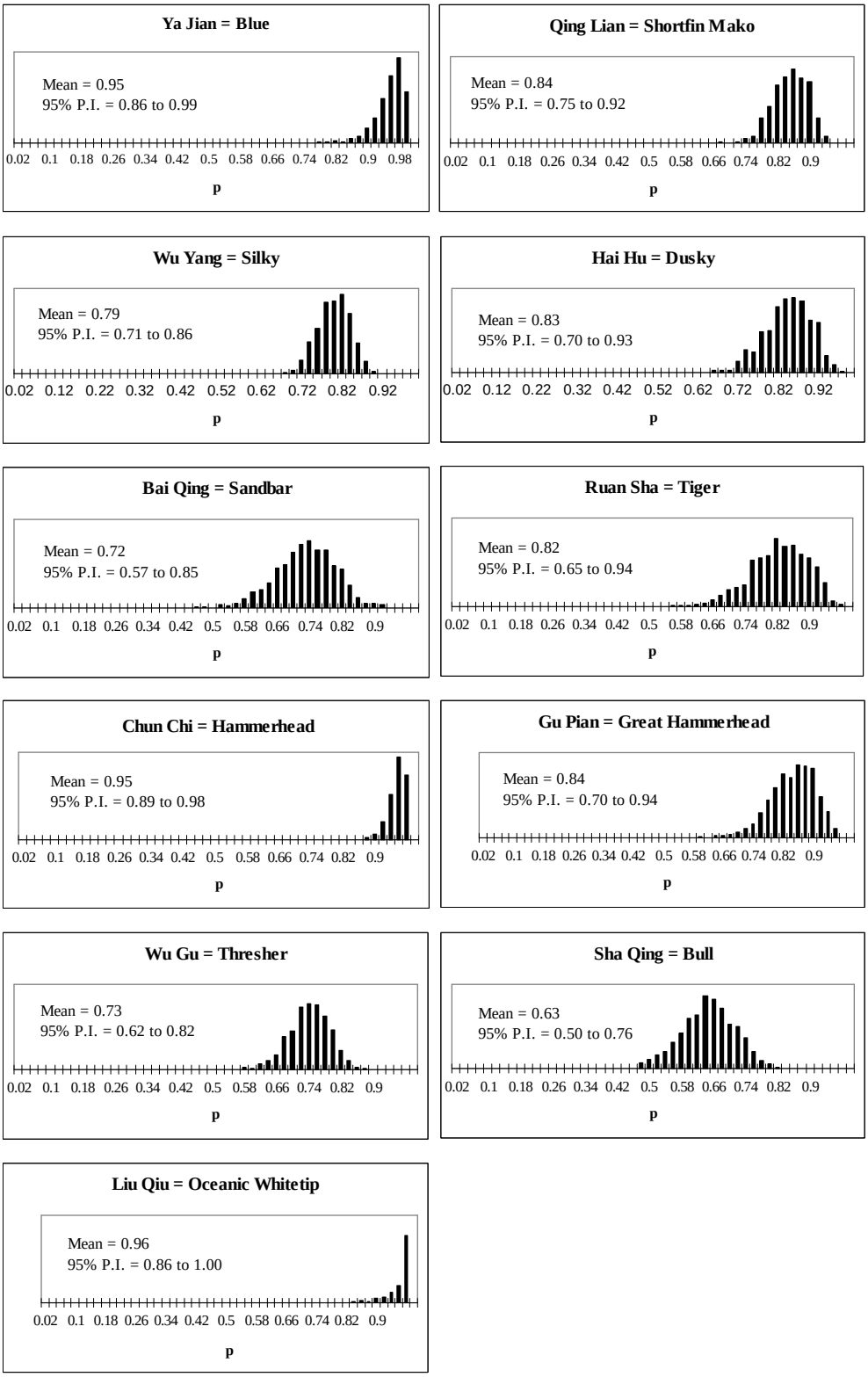


**Figure 3.6** Probability distributions for values of *p*, the proportion of fins validating the expected match between Chinese trade name and taxon. Distributions were generated using a binomial distribution based on *r*, the observed number of matches, and *n*, the sample size.

**Table 3.6** Figures for proportion in trade, number and biomass of sharks adjusted using data from genetic testing to provide results specific to individual taxa. Model output for numbers and biomass has been divided by 1.5 to express figures on an annual basis. These figures represent further Bayesian updating of results given in Chapter 2, specifically, figures on proportion in trade derive from Table 2.8, figures on the number of sharks derive from a stochastic combination of fin position results in Table 2.14, and figures on the biomass of sharks derive from a stochastic combination of fin position results in Table 2.20.

| Taxon | Median Proportion in Trade | 95% Probability Interval | Median Number of Sharks Represented (thousands) | 95% Probability Interval | Median Biomass Represented (thousand mt) | 95% Probability Interval |
|---|---|---|---|---|---|---|
| Blue (*Prionace glauca*) | 17.62 | 15.84 – 19.44 | 1,171 | 352 – 5,545 | 25,520 | 9,806 – 75,300 |
| Shortfin mako (*Isurus oxyrinchus*) | 3.23 | 2.77 – 3.72 | 64 | 27 – 177 | 4,036 | 1,503 – 11,730 |
| Silky (*Carcharhinus falciformis*) | 4.44 | 3.92 – 5.03 | 115 | 45 – 661 | 4,570 | 1,917 – 11,620 |
| Dusky (*Carcharhinus obscurus*) | 1.64 | 1.40 –1.89 | 32 | 19 – 75 | 1,520 | 738 – 3,313 |
| Sandbar (*Carcharhinus plumbeus*) | 3.21 | 2.71 –3.79 | 85 | 42 – 222 | 3,256 | 1,504 - 7,444 |
| Tiger (*Galeocerdo cuvier*) | 0.15 | 0.10 – 0.23 | 4 | 2 – 15 | 128 | 50 – 360 |
| Hammerheads (*Sphyrna* spp.) | 4.50 | 3.92 –5.12 | 213 | 74 – 1,048 | 6,093 | 2,518 – 16,090 |
| Great hammerhead (*Sphyrna mokarran*) | 1.72 | 1.42 –2.06 | 36 | 21 – 122 | 2,367 | 1,020 – 6,365 |
| Threshers (*Alopias* spp.) | 3.09 | 2.64 –3.58 | 114 | 54 - 496 | 4,566 | 1,663 – 13,300 |
| Bull (*Carcharhinus leucas*) | 3.35 | 2.86 – 3.89 | 55 | 35 – 97 | 2,909 | 1,337 – 7,030 |
| Oceanic whitetip (*Carcharhinus longimanus*) | 1.88 | 1.63 – 2.18 | 56 | 30 - 284 | 1,486 | 607 – 4,109 |

not modelled, or sold without reference to any Chinese trade name[1]. It is possible that some of these undifferentiated fins in the latter category derive from one of the eleven shark taxa genetically identified and modelled in this chapter. Traders are known to dispense with sorting small fins as they are of similarly low value, and thus lots of smaller fins are often sold as 'mixed fins' (什么翅, 'shenme chi', i.e. no trade name). For these reasons, the species-specific estimates presented above represent minimum, confirmed figures.

## 3.5 Discussion

### *3.5.1 Evaluation of the Effectiveness of the Sampling Programme*

The ability to use the results of this study to draw sound conclusions regarding the species composition of the shark fin trade is a function of three factors: i) the consistency of nomenclature within the trade community; ii) the robustness of the sampling design; and iii) the reliability and scope of the species-specific primers. Each factor is considered individually and then integrated in the context of each studied market category in the following discussion. (The uncertainties and limitations associated with the modelling of fin weights, and the conversion to numbers and biomass, as discussed in Chapter 2 also apply to the taxa-specific results presented here).

Most studies of the wildlife trade will achieve a broader characterization if trade records can be accessed and understood rather than relying on opportunistic sampling alone.

[1] The relative proportion of unnamed shark fins within the 54.1% of auctioned fins modelled as 'other' can be indicatively estimated by a comparison of simple tallies of the number of bags auctioned during the 18 month sampling period. The percentage of bags described using one of the 11 studied trade names was 49.6% (similar to the modelled percentage by weight of 45.9%). The percentage of bags described using another Chinese trade name that was not modelled was 24.3%, and the percentage of bags described without reference to any trade name was 26.2%.

However, if using trade information, potential shortcomings should be explicitly addressed in the sampling design whenever possible. For example, in this study it was acknowledged that traders' market categories are based on fin value which may have little connection with scientific taxonomy, and adherence to these categories varies by trader. This issue was addressed for the categories of interest through an *a priori* formulation of *p*, the probability of concordance between Chinese trade names and particular taxa, which was used to calculate the necessary number of samples. Subsequent post-hoc examination of $CV_p$ revealed that the sampling design achieved its objective of $CV_p<0.10$ for all categories except Bai Qing (0.103) and Sha Qing (0.106) (Table 3.4). Another example was the detection of many taxa to one market category relationships (e.g. Wu Gu category used for all species of thresher sharks) and many market categories to one taxa (e.g. the longfin mako found within several categories) nomenclatural customs. Those ambiguities which were detected early in the study were addressed through careful definition of target categories and sampling requests to traders, such as clarifying whether the fin was sometimes called by another name. The final shortcoming of the trade records, i.e. the large volume of fins (54%) traded in unstudied, and often non-specific, categories could not be overcome and was simply acknowledged by concluding that estimates based on the eleven studied categories were minimum figures.

The ability of the sampling design to produce meaningful conclusions was determined both by the underlying statistical model, and by practical aspects of obtaining the desired number and distribution of samples across traders and source regions in order to derive acceptably precise and accurate estimates of market proportions. Access constraints directed the study toward establishing concordances between market categories and shark taxa, rather than on random sampling of the trade as a whole. The lack of correspondence between the proportion of source regions sampled (Figure 3.5) and import records for Hong Kong, which indicate no South American countries amongst the major exporters of

fins to Hong Kong (Clarke 2002, Clarke and Mosqueira 2002), underscores the non-random nature of the sampling. However, the execution of the concordance testing sampling design achieved target sample sizes and distribution across a minimum of six of the 21 traders, and three of the eight source regions, for all market categories. This concordance approach provided more reasonable targets for sample collection, and in combination with auction records, allowed species composition issues to be addressed.

The final element is the reliability and scope of the primers used. PCR primer research will continue until species-specific primers for each shark utilized by the shark fin trade are confirmed through testing against a large number of globally distributed specimens. While the goal of this research is a complete set of validated species-specific primers, shark resource management requires timely information on the fin trade in order to assess questions of sustainable use, and it is therefore necessary to initiate trade studies despite gaps in the primer library. Conclusions based on confirmed primers can be considered final results, whereas use of preliminary primers can provide indicative information useful for interim decision-making. If necessary, the data produced by this study can be re-evaluated and adjusted should further primer testing lend new insights to concordances between Chinese trade names and species.

### *3.5.2 Applicability of Methods to Future Monitoring Studies*

This study has developed minimum estimates of the contributions of the taxa identified within eleven major trade categories to the overall trade. It was not able to determine all of the species represented in the shark fin market, nor the proportions derived from all of these species, although once a greater number of primers are available, similar methods could be applied to address a broader range of trade categories. Both this study and similar future studies will, however, be constrained by the large proportion (approximately 26%) of traded shark fins that are not labelled as belonging to any

particular trade category. Specifically, species composition, number and biomass estimates will be underestimates if the unlabelled fins also contain fins derived from studied species. Further studies addressing the specific issues of unsorted fins may remedy this problem, but unclassified products are likely to continue to present constraints both in studies of the shark fin trade and of traded wildlife products in general.

Another area for further study is the identification of products derived from rare species. Development of primers for those species which are listed on or have been proposed for one of the CITES appendices, i.e. basking (*Cetorhinus maximus*), whale (*Rhincodon typus*) and great white (*Carcharodon carcharias*) sharks, is currently in progress. Once primers are validated, verification of whether putative samples derive from these species can be performed quickly, easily and at low cost. A larger challenge will arise from designing a sampling program for rare species in trade. In addition to the usual problems associated with sampling coverage and statistical power, awareness of conservation concerns may lead traders to camouflage certain types of fins through cryptic labelling, as has been observed for basking shark fins. Even without deliberate subterfuge, rare fins lacking distinctive characters and market values may be inadvertently mixed within large, undifferentiated market stocks and thus become nearly impossible to detect. A more practical approach to expanding the list of species examined in this study would be to begin by identifying additional distinctive market categories used by traders and then develop primers that would be expected to resolve some of the species contained in these categories.

The market category-taxon concordances determined in this study are, at a minimum, useful for future monitoring of the Hong Kong market. Differences in nomenclature among the shark fin trading communities of Hong Kong, Singapore and Taiwan beyond what would be expected due to dialect / language alone (Vannuccini 1999) may require

similar concordance exercises in other locations. Beyond its applicability to the shark fin trade, this study has many parallels with wildlife monitoring programs operating under budgetary and sample access constraints. In its approach to integrating sampling design, statistical analysis, genetics and existing trade-derived information, it may serve as a model for similar market-based sampling efforts.

## 3.6 Conclusions

This study has produced the first verified species identifications for a number of major trade categories in the world's largest shark fin market, Hong Kong. In addition, using Bayesian statistical modelling and data-filling techniques presented in Chapter 2, the proportional contributions of key species to the trade, and taxon-specific numbers of sharks and biomass, have been estimated. These results alert fisheries managers to the types of sharks that are taken for the fin trade and the extent of utilisation. Furthermore, these taxon-specific estimates provide a step toward linking the results of this trade-based study with individual stock assessments to evaluate whether exploitation rates for particular species can be sustained. The following chapter extrapolates these results to a global total, and uses them in a simple assessment of sustainability for the global stock of blue shark.