Mark-recapture population estimate and movements of Grey Nurse Sharks

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EXECUTIVE SUMMARY

- The Grey Nurse Shark is an inshore, coastal-dwelling species that favours boulder or sand-filled gutters, cave and gutters in rocky reefs in waters 15 – 50 m deep (Otway *et al*. 2003). The shark's life history comprises late onset of sexual maturity (i.e. at 6 – 8 years of age), longevity of at least 25 years, and extremely low fecundity (i.e. 2 pups born biennially). These attributes, combined with its inshore distribution and specific habitat requirements, make the shark extremely vulnerable to human-induced mortality.

- Research on the endangered Grey Nurse Shark in NSW commenced in 1998 and focused on documenting the: (1) distribution and abundance; (2) population demography; (3) movements; (4) critical habitat sites; (5) human-induced threats; and (6) population genetics.

- It was not possible to identify what proportion of the total Grey Nurse Shark population was observed during the 10 coastwide surveys. To estimate the proportion of sharks observed requires that the size of the total population is known. Given the movements exhibited by Grey Nurse Sharks, the size of the total population is best estimated using a standard mark-recapture technique (or mark/resighting technique in the case of these protected animals).

- The current project was established to: (1) estimate the total population of Grey Nurse Sharks using "mark-recapture" techniques; and (2) document possible large-scale, migratory movements of Grey Nurse Sharks in coastal waters of SE Australia.

- Examples of shark tagging studies spanning the period 1945 - 2002 were reviewed to identify the most appropriate techniques used to catch and tag large, sharks. In reviewing these studies, emphasis was placed on identifying: (1) the techniques used to capture the sharks; (2) how and where the animal was tagged; (3) the type of tag used and where it was attached to the shark; (4) how many sharks were tagged and subsequently recaptured; and (5) the length of the recapture period and recapture rate.

- The chosen tagging technique involved a scuba diver feeding a Grey Nurse Shark a baited, barbless hook attached to 8 mm polypropylene rope fed down from the dive boat. Once hooked, the sharks were slowly pulled to the surface and into a stretcher that was suspended in the water alongside the boat (i.e. the shark remained submerged). Once in the stretcher the animal was restrained at the head and tail. The person doing the tagging used an air-powered drill to make a 5 mm diameter hole in the first and second dorsal fins for attaching the tags. The drills were fitted with hollow, stainless steel coring tubes that retained the tissue for subsequent DNA analysis. Numbered, cattle-ear tags were then attached using purpose-built pliers. Once tagged, the total length of the shark was measured to the nearest 1 cm. The hook was then removed from the jaw and the shark released.

- To enhance the awareness and reporting of tagged Grey Nurse Sharks amongst scuba divers, spearfishers and the wider community, 8000 posters providing details of: (1) the key identification features of Grey Nurse Sharks; (2) the tagging program; (3) details of the information to record if a tagged shark was sighted, and (4) the 24-hour hotline phone number to report a tagged shark, were distributed to scuba diving shops, and diving and spearfishing clubs, commercial aquaria, primary and high schools in the coastal zone, council offices, NSW Fisheries and NSW National Parks and Wildlife Service offices, environmental groups, commercial fishing cooperatives, recreational fishing clubs, and bait and tackle shops.

- The locations and dates of re-sighted, tagged Grey Nurse Sharks were used to construct a data set of large-scale movements among the sites in the coastal waters of SE Australia. Data used to compile the large-scale movements were constrained to the period commencing with the initial tagging and ending in July 2003.

- Tagging was done at four sites: Tollgate Islands (Batemans Bay); Little Broughton Island (Port Stephens), Fish Rock (South West Rocks); South Solitary Island (Coffs Harbour) in New South Wales coastal waters and at one site in Queensland coastal waters, Flat Rock (North Stradbroke Island). Twenty-four sharks were tagged ranging in size from 1.00 m to 2.61 m. Of these, 20 individuals (83.3%) have been resighted on at least one occasion after tagging.

- The tagging of Grey Nurse Sharks has enabled the preliminary documentation of large-scale, possibly migratory, movements. The unidirectional distances travelled ranged from 25 to 681 km and included journeys from sites in Queensland to sites in central NSW and vice-versa.

- Abundances of Grey Nurse Sharks were quantified in a 2-week long mark-recapture (resighting) survey in June 2003 using underwater visual counts of sharks over a 15 minute period at sites along the NSW and southern Qld coastlines. At each site, divers recorded the number of sharks, their size and sex and if the shark was tagged or not. If a tagged shark was observed, they also recorded the tag number.

- Of the 44 sites sampled the June 2003 survey, 20 (45.5%) had no Grey Nurse Sharks present. The proportion of Grey Nurse Sharks present or absent did not differ significantly among the winter surveys in 1999, 2000 and 2003. The proportions of sites sampled with Grey Nurse Sharks present or absent did not differ between the northern and southern coastal sections over the 3 winter surveys. Finally, the proportion of sites sampled with Grey Nurse Sharks present or absent did not differ significantly among the 3 winter surveys within the northern and southern coastal sections.

- A total of 313 Grey Nurse Sharks was counted by divers and spearfishers in the coastal waters of SE Australia in June 2003. The total number observed was greater than in the previous winter surveys in 1999 and 2000 (i.e. 207 and 292, respectively). Of the sharks observed, 224 (71.6%) occurred in the northern coastal section compared to 89 (28.4%) in the southern coastal section.

- Seven of the 24 tagged Grey Nurse Sharks (i.e. 29.2%) showed evidence of having been hooked within a year of tagging. Six of these were first observed in critical habitats at Fish Rock (off South West Rocks), The Pinnacle (off Forster), and Big Seal Rock (off Seal Rocks).

- The mark-recapture (re-sighting) estimates using probability distribution-based formulae (i.e. non-Bayesian) showed that the total population of Grey Nurse Sharks in the coastal waters of SE Australia was between 410 and 461 individuals with upper 95% confidence values ranging between 541 and 766 individuals. This is a refinement of previous estimates from the coastwide surveys.

- The mark-recapture (re-sighting) estimates suggest that 74 - 89% (mean = 81.5%) of reproductively mature individuals and 68 - 79% (mean = 73.5%) of all individuals (i.e. irrespective of size or sexual maturity) are observed during diver surveys in the coastal waters of SE Australia.

1. GENERAL INTRODUCTION

1.1. Background

The Grey Nurse Shark is an inshore, coastal-dwelling species that favours boulder or sand-filled gutters, cave and gutters in rocky reefs in waters 15 – 50 m deep (Otway *et al*. 2003). The shark's life history comprises late onset of sexual maturity (i.e. at 6 – 8 years of age), longevity of at least 25 years, and extremely low fecundity (i.e. 2 pups born biennially). These attributes, combined with its inshore distribution and specific habitat requirements, make the sharks' population numbers extremely vulnerable to human-induced mortality. Research on the endangered Grey Nurse Shark commenced in 1998 and focused on documenting the: (1) distribution and abundance; (2) population demography; (3) movements; (4) critical habitat sites; (5) human-induced threats; and (6) population genetics.

The 10 previous underwater surveys over a 2.5 year period and at an average of 57 sites scattered along the entire NSW coast and into southern Queensland showed that the maximum number of Grey Nurse Sharks observed at any one time was 292. The sharks were most numerous at 14 sites (almost 90% of the observed population) and totally absent from 63% of the sites sampled. Importantly, during the 1960s, substantial numbers of Grey Nurse Sharks could be found at all of the sites where they are now no longer present. These surveys (Otway *et al*. 2003) also showed that the Grey Nurse Shark population exhibited sexual and size segregation. Proportionally more juvenile and adult male sharks were found off Forster and sites to the north and proportionally more juvenile and adult females sharks were found off Seal Rocks and sites to the south. Furthermore, sexually mature females and juveniles were generally found at sites in shallower (inshore) waters whereas the males tended to spend some time in deeper waters. These segregated patterns of abundance make sexually mature females and juveniles more prone to human-induced threats such as accidental hooking because much of the commercial and recreational fishing effort is located in inshore waters.

While the results of these previous coastwide surveys showed clear and consistent patterns, it was not possible to identify what proportion of the total Grey Nurse Shark population was observed during the surveys. To estimate the proportion observed requires that the size of the total population is known. Given the movements exhibited by Grey Nurse Sharks, the size of the total population is best estimated using a standard mark-recapture technique. Consequently, much of the research documented in this report focuses on obtaining an estimate of the total population of Grey Nurse Sharks in the coastal waters of SE Australia.

1.2. Overall objectives of the research

1) To estimate the total population of Grey Nurse Sharks using "mark-recapture" techniques.

2) To document possible large-scale, migratory movements of Grey Nurse Sharks in coastal waters of SE Australia.

1.3. Fulfilment of objectives

1.3.1. "Mark-recapture" population estimate

Grey Nurse Sharks were tagged at various sites in SE Australian waters. Chapter 2 describes the tagging process and the associated data arising from this research. Given that Grey Nurse Sharks are a threatened species and that it is illegal to capture them, a mark-recapture estimate could not be based on recaptures but required underwater re-sightings of tagged individuals. A mark-recapture (re-sighting) survey was done in June 2003 and the results are described in Chapter 3. Direct and indirect sampling schemes were used and 6 different formulae were used to obtain estimates. Estimates of the total population of Grey Nurse Sharks (Chapter 4) were obtained for (a) all individuals (i.e. irrespective of sexual maturity) and (b) for adult individuals. The latter estimates were then converted to total population estimates using the ratio of juveniles to adults established from the mark-recapture (re-sighting) survey in June 2003.

1.3.2. Large-scale, migratory movements

The movements of Grey Nurse Sharks in the coastal waters of SE Australia are described in Chapter 2. The data include the distances travelled and time since tagging.

2. TAGGING AND MOVEMENTS OF GREY NURSE SHARKS

2.1. Introduction

Tagging studies all share the same basic format. Individuals in a population are captured, tagged and then released. The properties of this readily identifiable "sample" of the population are then used to estimate the same properties of the entire population. The properties include: (1) movement; (2) growth rates; (3) age-specific rates of fecundity; (4) age-specific rates of mortality; (5) total population abundance; (6) rates of birth/immigration; (7) rates of death/emigration; (8) rate of harvesting; and (9) rate of increase of the population.

The estimation of these properties relies on a number of assumptions (see Section 4.2.1), the fulfilment of which can be assessed in a number of ways. However, with populations of sharks it is often very difficult to test some of these assumptions because of the risk of injury to the shark or the researcher.

Examples of shark tagging studies published in internationally recognised marine ecological journals spanning the period 1945 - 2002 were reviewed to identify the most appropriate techniques used to catch and tag large, potentially dangerous sharks. In reviewing these studies, emphasis was placed on identifying: (1) the techniques used to capture the sharks; (2) how and where the animal was tagged; (3) the type of tag used and where it was attached to the shark; (4) how many sharks were tagged and subsequently recaptured; and (5) the length of the recapture period and recapture rate. It is also important to note that tagging studies focussing on large species of sharks have to use techniques that minimise injury to the animal whilst simultaneously avoiding serious injury to the researchers involved.

The results from published studies that have reported tag recaptures for 10 species of shark have been summarised in Table 2.1. The table summarises the information listed above. Three techniques: (1) gillnets; (2) longlines with barbed hooks; or (3) handlines (or rod & reel) with barbed hooks, have been used predominantly to catch sharks for tagging. Once caught, the sharks are either: (1) tagged with M-type Dart tag whilst in the water at the surface and adjacent to a boat, or (2) tagged on deck (i.e. out of water) with a dorsal fin tag (e.g. Petersen disk or rototag/cattle ear tag). With the more recent advances in digital and computer technology, sharks in more recent studies have been tagged with computerised archival tags and satellite tags (e.g. West & Stevens 2001). The number of sharks tagged varied greatly among the studies (Table 2.1). This most likely reflects the resources available and duration of the study. The recapture rates also differed markedly among species and studies. School sharks had the highest and lowest recapture rates (30.0% - 2.1%, Olsen 1953, West & Stevens 2001). Seven (50%) of the studies listed in Table 2.1 had recapture rates less that 10%, five studies (35.7%) had recapture rates between 10 - 20%, and only two studies (14.3%) had recapture rates of 20 – 30%. The recapture periods also varied greatly among studies (Table 2.1). Adjusting the recapture rates by the recapture period gives an annual recapture rate, which can then be compared across studies. The mean annual recapture rates varied from a minimum of 0.15 sharks per annum for sandbar sharks (Casey *et al.* 1985, Kohler *et al.* 1998) to a maximum of 15 sharks per annum for school sharks (West & Stevens 2001).

Tagging-related mortality has rarely been assessed with sharks owing to the numerous problems associated with research on these animals, and none of the studies reviewed address this issue. With the advent of computerised archival tags, it will now be possible to quantify tagging-related mortality of large, potentially dangerous sharks. Such studies will, however, be expensive owing to the cost of the computerised tags.

There are 2 notable, long-term studies that have involved the tagging of Grey Nurse Sharks. First, the National Marine Fisheries Service Cooperative Shark Tagging Program (Kohler *et al*. 1998) has tagged sharks on the east coast of the USA for over 30 years. Over the period 1962 – 1993 (i.e. 31 years), 562 Grey Nurse Sharks were tagged. Of these, 257 were male, 242 were female and 63 of unknown sex. A total of 31 individuals was recaptured over the 31 year period with a maximum time at liberty of 3.2 years. These data give a mean annual recapture rate of 0.18% per annum. Finally, the maximum distance travelled was 1172 km. No mark-recapture estimates were calculated using these data.

Second, the Oceanographic Research Institute based in Durban, South Africa, has also tagged sharks for over 30 years with tagging commencing in 1964 (Davies & Joubert 1966). Over this time (also 31 years), 1637 Grey Nurse Sharks were tagged with 79 individuals (4.8%) being recaptured. This gives a mean annual recapture rate of 0.15% per annum. The maximum distance travelled by Grey Nurse Sharks along the east – coast of South Africa was 1416 km.

The large-scale, migratory movements of Grey Nurse Sharks off the east coasts of the USA and South Africa have been linked to water temperatures and the sharks' reproductive cycle (see Otway *et al.* 2003 for details). As with most animals, there is an extensive literature on shark tagging and its role in estimating many of the above parameters. More recent reviews (e.g. Kohler & Turner 2001, Voegeli 2001) have summarised the state of knowledge and examined the benefits of newer electronic tagging technology in documenting many of the above parameters. However, as the present study was aimed primarily at estimating the total population of Grey Nurse Sharks in SE Australian waters, an older, conventional tagging method was used. Given that Grey Nurse Sharks are a threatened species, it is illegal to capture the animal so a mark-recapture estimate could not be based on recaptures but required underwater re-sightings of tagged individuals. This necessitated using a tag that could be easily seen underwater, readily applied, and was inexpensive. Cattle ear tags (rototags – Table 2.1) met these requirements and a large, numbered tag could be read with ease up to 5 m away from the animal given good water clarity (visibility). Moreover, recent studies (e.g. Heupel & Bennett 1997, Heupel *et al*. 1998) have shown that such conventional tagging techniques (i.e. dart tags and cattle ear tags) have minimal effects on the surrounding tissues (i.e. muscle and dorsal fin) of tagged sharks.

Consequently, large, numbered cattle ear tags were used in this study.

The aims of this section of the research were to: (1) identify the best technique for tagging Grey Nurse Sharks; (2) tag Grey Nurse Sharks at various sites along the SE coast of Australia; and (3) document the large-scale movements of these tagged sharks.

Table 2.1. Examples of shark tagging studies over the period 1945-2002 and their associated recapture results, arranged in chronological order.

Shark Species	Tagging Technique	Tag Type	Number Tagged	Number Recaptured	Recapture Rate (%)	Recapture Period (years)	Mean annual recapture rate (%/yr)	Reference
Galeorhinus galeus (school shark)	Caught using barbed hook on longline. Shark tagged aboard boat.	Petersen Disk	3,580	75	2.1	6	0.35	Olsen (1953)
		Internal tag	105	3	2.9	6	0.48	Olsen (1953)
Carcharhinus plumbeus (sandbar shark)	Caught using barbed hook on longline. Tagged in the water and aboard boat.	Dart (M-type) or Rototag in 1st dorsal	15,617	727	4.7	31	0.15	Casey *et al.* (1985) Kohler *et al.* (1998)
Ginglymostoma cirratum (nurse shark)	Caught using barbed hook on longline or handline. Sharks tagged aboard boat.	Rototag in 1st dorsal	152	44	28.9	10	2.89	Carrier & Luer (1990)
Triakis semifasciata (leopard shark)	Caught using barbed hook on longline. Shark tagged aboard boat.	Rototag in 1st dorsal	948	101	10.7	10	1.07	Smith & Abramson (1990)
Negaprion brevirostris (lemon shark)	Caught using gillnets or on barbed hook on longline and handline. Shark anesthetised and tagged aboard boat.	PIT below 1st dorsal	76	15	19.7	3	6.58	Manire & Gruber (1991)
		and Dart (M-type)	563	57	10.1	6	1.69	Manire & Gruber (1991)
Isurus oxyrinchus (shortfin mako)	Caught with barbed hook on longline or handline. Wire trace cut and then hook and trace left in buccal cavity.	Dart (M-type)	2,459	231	9.4	20	0.47	Casey & Kohler (1992)

Table 2.1 continued

Shark Species	Tagging Technique	Tag Type	Number Tagged	Number Recaptured	Recapture Rate (%)	Recapture Period (years)	Mean annual recapture rate (%/yr)	Reference
Carcharias taurus (grey nurse/sand tiger shark)	Caught using barbed hook on longline. Tagged in water and aboard boat.	Jumbo rototag and Dart (M-type).	562	31	5.5	31	0.18	Kohler *et al.* (1998)
	Caught using gillnets. Sharks tagged in water and aboard boat.	Rototag and Dart (M-type) tag	1637	79	4.8	31	0.15	Davies & Joubert (1966)
Carcharhinus tilstoni (Australian blacktip shark)	Caught using gillnets and on barbed hooks on handlines and longlines. Sharks tagged aboard boat.	Jumbo Rototag	4,846	402	8.3	15	0.55	Stevens *et al.* (2000)
Carcharhinus sorrah (spot-tail shark)	As above	Jumbo Rototag	2,919	83	2.8	15	0.19	Stevens *et al.* (2000)
Galeorhinus galeus (school shark)	Caught using gillnets or barbed hook on longlines.	Rototag in 1st dorsal with dummy Archival	243	47	19.3	5	3.90	West & Stevens (2001)
		Rototag in 1st dorsal with Archival tag	30	9	30.0	2	15.00	West & Stevens (2001)
Carcharhinus obscurus (dusky shark)	Caught using gillnets or barbed hook on longlines.	Rototags in 1st dorsal	2,199	374	17.0	5	3.40	Simpendorfer (2000) Simpendorfer *et al.* (2002)

2.2. Materials and methods

2.2.1. Tagging procedure

2.2.1.1. In New South Wales

Small amounts of burley (fish frames and discarded tissues) were placed in crevices in and around the gutters occupied by the Grey Nurse Sharks. The presence of the burley attracted the sharks and provided the necessary stimulus for feeding. The Grey Nurse Sharks were then caught by divers feeding the shark a baited, barbless hook attached to 8 mm polypropylene rope which was fed down from the dive boat. Once the shark had taken the bait, the diver then tugged on the rope to set the hook into the tissue of the lower jaw (occasionally the upper jaw). Once hooked, the sharks were slowly pulled to the surface and into a stretcher that was suspended in the water alongside the boat (Fig. 2.1). The use of this stretcher ensured that the shark remained in the water (i.e. was submerged) for the duration of the tagging procedure. Once the shark was in the stretcher, two people held the animal around the head and tail. Once held in this position, the shark stopped struggling and could be readily tagged by a third person. The person doing the tagging used an air-powered drill to make a 5 mm diameter hole for attaching the tags (Fig. 2.2). The drilling of the fins took only 5 - 10 seconds per fin. The drills were fitted with hollow, stainless steel coring tubes that retained the tissue for subsequent DNA analysis. The numbered (3 digits), white, cattle-ear tags were attached to the first and second dorsal fins using purpose-built pliers (Fig. 2.3). Once tagged, the total length (TL) of the shark was measured to the nearest 1 cm with a waterproof tape measure (Fig. 2.4). The hook was then removed from the jaw (Fig. 2.5) and the shark released. Figure 2.6 shows a tagged shark swimming at Fish Rock about 3 months after being tagged at this same site. The immediate and subsequent (i.e. weeks later) underwater observations of tagged Grey Nurse Sharks indicated that behaviour was similar to untagged individuals and this suggested that the tagging procedure caused minimal harm and/or distress to the animals.

2.2.1.2. In Queensland

Tagging in Queensland coastal waters was done in cooperation with Sea World and the Queensland Parks and Wildlife Service (QPWS) aboard Sea World's research vessel. Several changes to the overall tagging protocol were required because of the large size of the Sea World vessel. Grey Nurse Sharks were caught underwater with a lasso and then placed in a perspex housing (Fig. 2.7). This housing was then used to carry the animal to the surface. The shark was then hoisted aboard the vessel, placed on a stretcher and submerged in a large, rectangular, water-filled tank on the quarterdeck (Fig. 2.8). The same procedure as used in NSW waters was then used to tag the shark. The tags used in Queensland waters were blue in colour to enable a quick, visual discrimination from sharks tagged in NSW (Fig. 2.9). After the total length had been measured the stretcher (with shark) was lifted out of the tank and lowered into the sea astern of the vessel. The stretcher was then opened and the shark released.

Figure 2.1. Hooked shark being pulled into the submerged stretcher.

Figure 2.2. First dorsal fin being drilled using hollow, stainless steel coring tubes.

Figure 2.3. Cattle tag being applied to the first dorsal fin.

Figure 2.4. Tagged shark being measured before release.

Figure 2.5. Barbless hook being removed from shark.

Figure 2.6. Tagged shark 220 at Fish Rock in December 2002 three months after it was tagged at this site. Note a large hook is visible in the right side of the jaw.

Figure 2.7. A Grey Nurse Shark brought to the surface in the perspex housing.

Figure 2.8. The stretcher in the water filled tank aboard the Sea World vessel.

Figure 2.9. Grey Nurse Shark tagged with blue tags and waiting release.

2.2.2. Reporting of tagged sharks

Several approaches were adopted to enhance the awareness and reporting of tagged Grey Nurse Sharks amongst divers (i.e. scuba divers and spearfishers) and the wider community. First, 8000 posters providing details of: (1) the key identification features of Grey Nurse Sharks; (2) the tagging program; (3) details of the information to record if a tagged shark was sighted, and (4) the 24-hour hotline phone number (02 4916 3888) to report a tagged shark, were distributed to scuba diving shops, diving and spearfishing clubs, commercial aquaria, primary and high schools in coastal areas, council offices, NSW Fisheries and NSW National Parks and Wildlife Service offices, environmental groups, commercial fishing cooperatives, recreational fishing clubs, and bait and tackle shops.

Divers were also encouraged to report sightings to the authors by phone, fax, or email. Regular phone contact was maintained with dive shops along the coast throughout the project to further enhance reporting of tagged sharks. The awareness of the tagging program was also enhanced by television and radio interviews and in numerous articles in national, state and local print media.

2.2.3. Movements of tagged sharks

The locations and dates of re-sighted, tagged Grey Nurse Sharks were used to construct patterns of large-scale movement among the sites in the coastal waters of SE Australia. Data used to compile the large-scale movements were constrained to the period commencing with the initial tagging and ending in July 2003. Maps of the large-scale movements were developed using ArcView 8.3. GPS positions for Grey Nurse Shark sites were overlayed on a base map of NSW and southern Queensland. Start and end points of each shark's movements were located on the map and the approximate path of travel was drawn. The distance travelled (kms) along the coast was estimated using the measuring tool.

2.3. Results

2.3.1. Tagging

Tagging was done at four sites: Tollgate Islands (Batemans Bay); Little Broughton Island (Port Stephens), Fish Rock (South West Rocks); South Solitary Island (Coffs Harbour) in New South Wales coastal waters and at one site, Flat Rock (North Stradbroke Island), in Queensland coastal waters starting in March 2002. Twenty-four sharks have been tagged; 16 in NSW and 8 in Queensland (including the 3 individuals tagged by Sea World in 1999 on behalf of NSW Fisheries). The tagged sharks ranged in size from 1.00 m to 2.61 m TL and 20 individuals (83.3%) had been re-sighted on at least one occasion by June 2003 (Table 2). The re-sighting rates of the sharks did not appear to differ between the capture methods used in New South Wales and Queensland.

By February 2003, it was apparent from underwater observations and photographs of tagged individuals that the tags on some of the sharks were becoming fouled by algae and barnacles. An examination of photographs showed that the tags on 5 individuals had been fouled by barnacles with a similar number of sharks exhibiting algal growth on the tags. The fouled tags appeared to be causing slight abrasions to some Grey Nurse Sharks. The tagging program was halted at this point and a review of the tagging procedures was undertaken by the Department of Environment and Heritage (DEH).

2.3.2. Movements of tagged sharks

Descriptions of the large-scale movements of tagged Grey Nurse Sharks were compiled for 4 of the 5 sites where tagging occurred. It was not possible to document the movements of the shark tagged at Little Broughton Island because there has been no confirmed re-sighting of this individual. The movements of 3 Grey Nurse Sharks tagged and released at Flat Rock by Sea World (on behalf of NSW Fisheries) in August 1999 are also included. Finally, the unidirectional distances travelled minimum and maximum times at liberty and the most distant site (from site of tagging) where a shark was re-sighted are summarised in Table 2.2 for all the sharks tagged. The period in which re-sightings were made was constrained to July 31st 2003 to permit calculation and presentation in this report. The minimum time at liberty was defined (and calculated) as the number of days from when the shark was tagged to the date of the last re-sighting. The maximum time at liberty was simply defined (and calculated) as the number of days from when the shark was first tagged until July 31st 2003. However, this latter estimate assumes that the shark was alive at July 31st 2003.

2.3.2.1. Sharks tagged at Flat Rock, Queensland

The 3 sharks tagged by Sea World (shark # 140, 141 & 142) all moved south. Shark 140 was re-sighted at South Solitary Island (off Coffs Harbour) in August 2002 after 1112 days at liberty (Table 2.2, Fig. 2.10). Shark numbers 141 and 142 were re-sighted at Fish Rock on June 2002 and December 2002, respectively having been at liberty for at least 1121 and 1274 days (Table 2.2, Fig. 2.10).

Of the sharks tagged in July 2003 (Table 2.2), 2 individuals moved north and the remaining 3 moved south. The shark tagged with blue tag number 216 (hereafter # B216) was caught and released alive by a commercial fisher in September 2002 having travelled 681 km in 45 days (Table 2.2, Fig 2.10). This shark has not been seen since its capture and release. While it is presumed to be alive, it is possible that the shark died as a result of its capture. The shark that moved north (# B218) was re-sighted at Flat Rock off North Stradbroke Island (Qld) in August 2002 and then at Cherubs Cave off Moreton Island (Qld) in mid August, 2002 having been at liberty for 51 days (Table 2.2, Fig. 2.10). The shark disappeared from the site and then reappeared in July 2003 having been at liberty for at least 359 days (Table 2.2).

Two of the sharks tagged in July 2002 (# B210 & B215) were re-sighted at Julian Rocks off Byron Bay (NSW) in June 2003 having travelled a minimum of 263 km in the 300+ days at liberty (Table 2.2, Fig. 2.10). One of the sharks (# B210) was then observed back at Flat Rock in July 2003 having travelled a minimum of 283 km (Table 2.2). The last individual (# B217) also travelled south and was re-sighted at the Pinnacle off Forster (NSW) in March 2003 after at least 239 days at liberty (Table 2.2, Fig 2.10).

Table 2.2. Locations of tagging and re-sighting, unidirectional distances moved and times at liberty (see text for details) for Grey Nurse Sharks tagged in SE Australian waters. NCR denotes no confirmed re-sighting, * denotes distance unknown as shark disappeared and then was re-sighted at the same site many months later.

Tagging Site	Date	Tag No.	Most Distant Site where Re-sighted	Unidirectional Distance (km)	Time at Liberty (Days)	
					Min	Max
Flat Rock, QLD						
	20.8.99	140	South Solitary Is. (Coffs Harbour, NSW)	300	1112	1441
	20.8.99	141	Fish Rock (South West Rocks, NSW)	408	1121	1441
	20.8.99	142	Fish Rock (South West Rocks, NSW)	408	1274	1441
	23.7.02	B210	Julian Rocks (Byron Bay, NSW)	283	355	374
	23.7.02	B215	Julian Rocks (Byron Bay, NSW)	263	327	374
	23.7.02	B216	Yeppoon (QLD)	681	333	374
	23.7.02	B217	The Pinnacle (Forster, NSW)	552	239	374
	25.7.02	B218	Cherubs Cave (Moreton Is. QLD)	25	359	372
South Solitary Is., NSW						
	8.8.02	116	NCR	-	-	358
	8.8.02	125	South Solitary Is. (Coffs Harbour, NSW)	0 *	351	358
	9.8.02	117	South Solitary Is. (Coffs Harbour, NSW)	0 *	3	357
	9.8.02	118	NCR	-	-	357
	9.8.02	119	NCR	-	-	357
Fish Rock, NSW						
	25.6.02	215	Julian Rocks (Byron Bay, NSW)	263	334	402
	25.6.02	216	Fish Rock (South West Rocks, NSW)	0 *	22	402
	25.6.02	217	South Solitary Is. (Coffs Harbour, NSW)	101	363	402
	26.6.02	213	South Solitary Is. (Coffs Harbour, NSW)	101	47	401

Table 2.2. continued

Tagging Site Date	Tag No.	Most Distant Site where Re-sighted	Unidirectional Distance (km)	Time at Liberty (Days) Min	Max
Fish Rock continued					
26.6.02	214	South Solitary Is. (Coffs Harbour, NSW)	101	7	401
26.6.02	218	Flat Rock (North Stradbroke Is., QLD)	408	394	401
26.6.02	219	South Solitary Is. (Coffs Harbour, NSW)	101	348	401
24.9.02	220	The Pinnacle (Forster, NSW)	159	288	311
25.9.02	226	Little Seal Rock (Seal Rocks, NSW)	183	307	311
Little Broughton Is., NSW					
19.3.02	610	NCR	-	-	500
Tollgate Is., NSW					
16.5.02	820	Green Is.(South West Rocks, NSW)	622	436	442

2.3.2.2. Sharks tagged at South Solitary Island, NSW

Of the 5 sharks tagged at South Solitary Island in August 2002, only 2 have been re-sighted. Both sharks (# 117 & 125) were re-sighted at South Solitary Island after at least 3 and 351 days at liberty, respectively (Table 2.2 & Fig. 2.11).

2.3.2.3. Sharks tagged at Fish Rock, NSW

Nine Grey Nurse Sharks were tagged at Fish Rock and all have been re-sighted after various times at liberty (Table 2.2). One shark was sighted at Fish Rock, 2 were observed to the south and the remaining 6 were re-sighted at 3 sites to the north of Fish Rock (Table 2.2). The 2 Grey Nurse Sharks (# 220 & 226) that travelled south were re-sighted at the Pinnacle off Forster (NSW) in May 2003 and Seal Rocks (NSW) in February, March and April 2003 having travelled 159 and 183 km, respectively. The sharks had been at liberty for at least 288 and 307 days. Shark # 220 was regularly observed at Fish Rock after being tagged in September 2002. This shark remained at Fish Rock for almost 6 months prior to leaving and travelling south. Four sharks (# 217, 213, 214, & 219) all swam north to South Solitary Island covering 101 km (Fig. 2.12). Their minimum times at liberty varied between 7 and 363 days (Table 2.2).

A further 2 sharks (# 215 & 218) travelled even further north to Julian Rocks (NSW) and Flat Rock (Qld) covering 263 and 408 km, respectively (Table 2.2, Fig. 2.12). These sharks had been at liberty for at least 334 and 394 days, respectively when they were re-sighted (Table 2.2). The final shark (shark # 216) was re-sighted at Fish Rock 22 days after being tagged. The shark left then left Fish Rock and has not been re-sighted as at 31 July 2003.

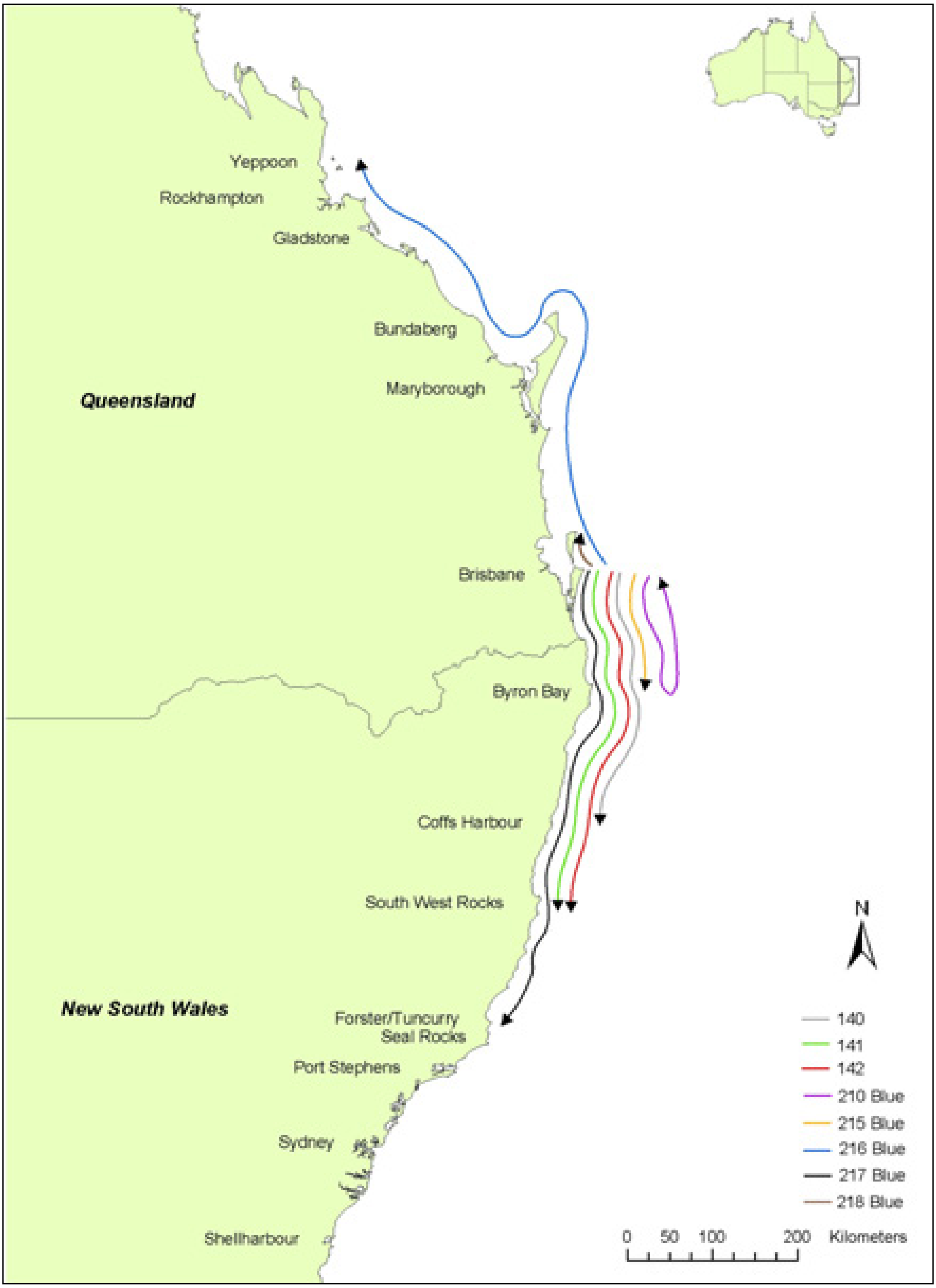

Figure 2.10. Map showing the movements of Grey Nurse Sharks tagged at Flat Rock (off North Stradbroke Island, QLD) in 1999 and 2002.

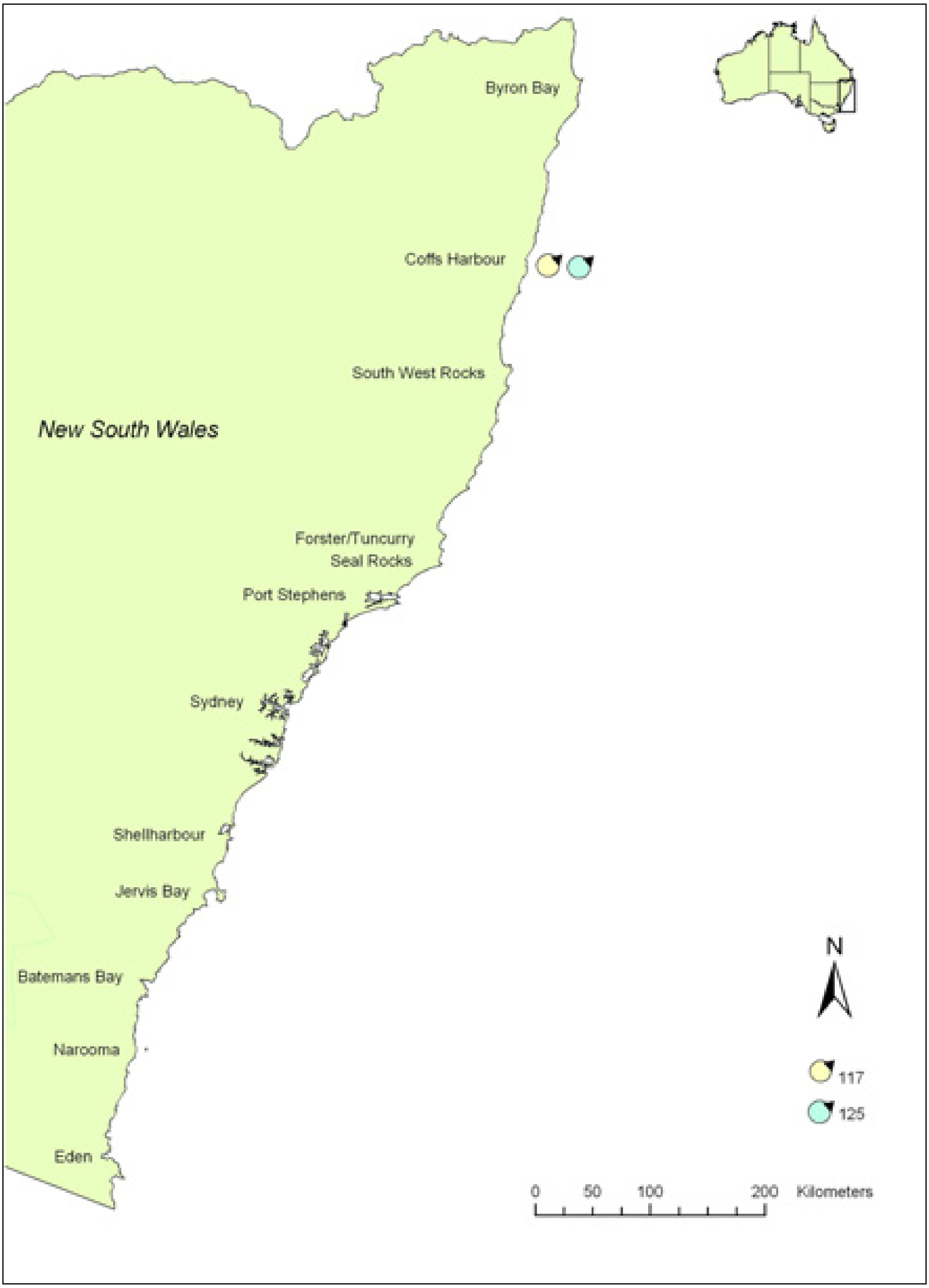

Figure 2.11. Map showing movements of Grey Nurse Sharks tagged and released at South Solitary Island (off Coffs Harbour) in 2002.

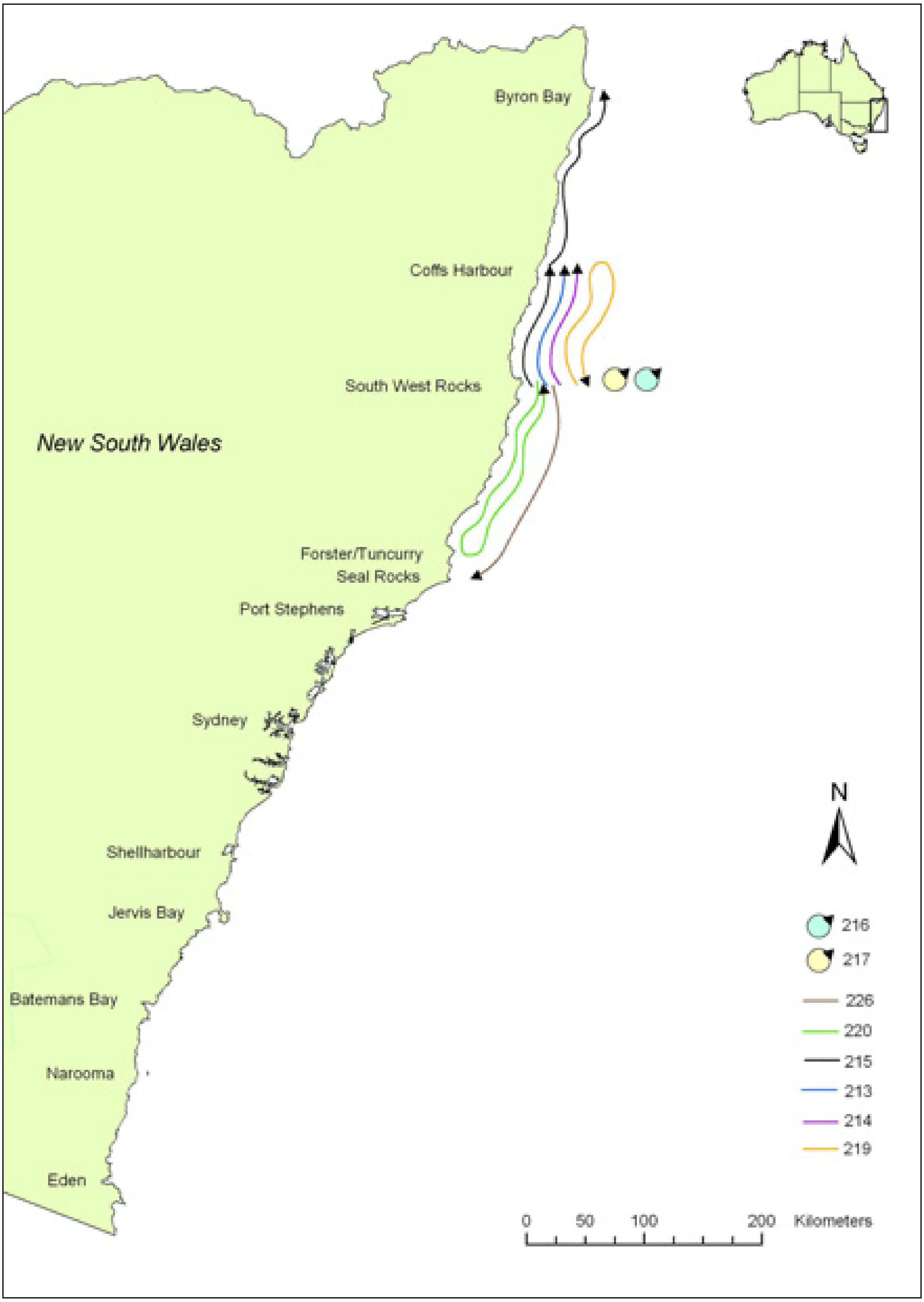

Figure 2.12. Map showing the movements of Grey Nurse Sharks tagged and released at the Fish Rock (off South West Rocks).

Figure 2.13. Map showing movements of a Grey Nurse Shark tagged and released at the Tollgate Islands (off Batemans Bay) in 2002. The small track and ? denotes a presumed movement north similar to the northerly track in 2003 (see text for details).

2.3.2.4. Sharks tagged at the Tollgate Islands, NSW

One Grey Nurse Shark (shark # 820) was tagged at the Tollgate Islands in May 2002. The shark left before June 2002 and was re-sighted at this site in March 2003 (Fig. 2.13). The shark again left the Tollgate islands in May 2003 and travelled north to Fish Rock and Green Island (off SW Rocks, NSW) having been at liberty for at least 436 days (Table 2.2, Fig. 2.13). The shark was re-sighted at Fish Rock in July 2003, having moved 620 km in approximately 2 months (Table 2.2).

2.4. Discussion

2.4.1. Tagging

The tagging techniques used enabled 24 sharks to be tagged across 5 sites in the coastal waters of SE Australia; 3 in 1999 and 21 in 2002. By 31 July 2003, 20 of the tagged sharks (83.3%) had been re-sighted. This re-sighting rate far exceeds the mean annual recapture rates reported in previous studies (Table 2.1). For example, the highest annual recapture of 15% per annum was achieved by West & Stevens (2001) whilst working with the school shark *Galeorhinus galeus*. Furthermore, 85.7% of the annual recapture rates were less than 5% per annum (Table 2.1). However, all the other studies relied on recaptures not re-sightings and thus a direct comparison may not be entirely appropriate. Nevertheless, the high re-sighting rates recorded in this study, indicated that the sharks were not greatly affected by the capture technique used. On the other hand, such re-sighting rates should be cause for significant concern because re-sighting rates of this magnitude would be very unlikely unless the total Grey Nurse Shark population was very small. This issue will be addressed in more detail in subsequent chapters.

Tagging using cattle ear tags also enabled the collection of additional morphometric data and tissue samples for DNA analysis. Preliminary results of the genetic work (Hill 2003, Harcourt *et al*. 2003) have shown that the Grey Nurse Shark populations on the east and west coasts of Australia are genetically distinct. However, the research was constrained by the small number of samples. The sample size for the genetic analyses will need to be increased to enable the examination of genetic ‘bottlenecks’ and the estimation of minimum viable population sizes.

The fouling of the tags by barnacles on 5 of the sharks was unexpected. Reviews of shark tagging (e.g. Davies & Joubert 1966, Kohler *et al.* 1998, Kohler & Turner 2001) have not documented the incidence of barnacles fouling external tags used on Grey Nurse Sharks. Observations of tagged individuals suggested that the tagging technique (because of unexpected fouling) was associated with some skin abrasions on a few sharks. Future tagging research will seek alternative ways of marking the animals individually and/or attaching acoustic tags to minimise such problems.

2.4.2. Movements

The tagging of Grey Nurse Sharks at various sites in the coastal waters of SE Australia has enabled the preliminary documentation of large-scale, possibly migratory movements. The unidirectional distances travelled ranged from 25 to 681 km and included journeys from sites in Queensland to sites in central NSW and vice-versa. One individual also travelled from the Tollgate Islands (southern NSW) to Fish Rock off South West Rocks (northern NSW). These preliminary movements are in general agreement with the hypothetical movements proposed by Otway & Parker (2000). Substantially more observations over longer periods of time will be needed to enable a robust test of the proposed migratory movements. Such movements would be better addressed using acoustic and pop-up archival tagging technologies. This information will obviously come at greater initial cost, but this will be offset by the greater quantity of more detailed data. Nevertheless, information on the localised and migratory movements will be crucial for future management of the recovery of the species.

3. MARK – RESIGHTING SURVEY

3.1. Introduction

Estimating the total number of individuals in an animal population is fraught with difficulties and is exacerbated when the species migrates over large distances (Caughley 1977, Strong *et al.* 2003). It is also important to consider whether the species segregates by size and or sex and exhibits biased sex ratios (Seber 1982), all of which may contribute to bias in the population estimate. Consequently, it is important to eliminate, where possible, as many potential sources of bias/error when making such estimates. Previous surveys of Grey Nurse Sharks (Otway & Parker 2000, Otway *et al*. 2003) have shown that, in winter, the SE coastal population of Grey Nurse Sharks occurs northward from Sydney to southern Queensland, and exhibits a 1:1 sex ratio along this stretch of coast. With this in mind, the aims of this section are to: (1) document the abundances, size-structure and sex ratios of Grey Nurse Sharks from a mark-recapture survey carried out in winter, and (2) compare these results with those from the winter surveys in 1999 and 2000 to identify any potential sources of bias when the data are used to calculate estimates of the total population in Chapter 4.

3.2. Materials and methods

3.2.1. Sampling sites and protocol

The sites sampled in this study were similar to those documented in Otway and Parker 2000 and Otway *et al.* 2003. The sites were chosen by reviewing the scientific and “grey” literature (i.e. popular books, diving magazines, newspaper articles, etc.) to document where Grey Nurse Sharks had been observed over the past 50 years. A total of 44 sites were sampled from southern Queensland to the New South Wales and Victorian border (hereafter SE Australian waters).

Divers from commercial scuba diving charter operators, universities, dive clubs, underwater research groups and spearfishing clubs assisted with the survey. Previous surveys were done over a 4-week period (Otway & Parker 2000, Otway *et al.* 2003). However, preliminary results of the tagged sharks showed that individuals can move from one site to another over 2 weeks. Therefore, the 4-week survey period was shortened to 2 weeks to reduce the possibility of double counting. At each site, the divers were asked to swim for a 15 minute period in and around habitats known to be occupied by Grey Nurse Sharks (e.g. gutters, caves and over-hangs – Pollard *et al*. 1996; Otway & Parker 2000, Otway *et al*. 2003). The divers and spearfishers recorded the total number of sharks present, estimated total lengths of the sharks in 3 size classes: 1 – 2 m, 2 – 3 m and > 3 m, the sex of each shark, and these same details for any tagged sharks present and their tag number. When sex could not be determined, the shark was recorded as sex “unknown”. In addition, the divers and spearfishers recorded the presence of mating scars, fishing gear (hooks, wire trace, line etc.) and noted the bottom depth and water temperature to the nearest 1˚ C.

3.2.2. Population size-structure and segregation by size and sex

Previous survey results (Otway & Parker 2000, and Otway *et al.* 2003) showed that males and females segregated by size and sex along 2 sections of coast from (1) Forster and sites north (hereafter called northern coastal section), and (2) Seal Rocks and sites south (hereafter called southern coastal section). Consequently length-frequency data were re-partitioned in the same manner. The size-structures of the Grey Nurse Shark populations along the entire coast and for the northern and southern coastal sections were plotted for the 3 size classes. Possible biases in the sex-ratios were examined using χ^2 analyses with Type I error-rates of $\alpha = 0.05$.

3.3. Results

3.3.1. Sampling effort

Inclement sea conditions (i.e. rough seas and moderate swell) prevented sampling in the latter part of the second week and the last weekend of the survey. Nevertheless, 44 sites from southern Queensland to southern New South Wales were sampled by NSW Fisheries and volunteer divers and at a similar intensity to the coastwide surveys of Otway *et al.* 2003. All the sites identified in Otway *et al.* 2003 as key aggregation sites (critical habitats) were sampled over the survey. Of the 44 sites sampled, 20 (45.5%) had no Grey Nurse Sharks present. However, the proportion of Grey Nurse Sharks present or absent did not differ significantly among the winter surveys in 1999, 2000 and 2003 (Table 3.1; $\chi^2 = 0.26$, $P > 0.85$). Moreover, the proportions of sites sampled with Grey Nurse Sharks present or absent did not differ between the northern and southern coastal sections over the 3 winter surveys (Table 3.1; $\chi^2 = 0.05$ and 4.50, respectively, $P > 0.10$). Finally, the proportion of sites sampled with Grey Nurse Sharks present or absent did not differ significantly among the 3 winter surveys within the northern and southern coastal sections (Table 3.1; $\chi^2 = 1.78$ and 0.65, respectively, $P > 0.25$).

Table 3.1. The number of sites sampled and sites with Grey Nurse Sharks present or absent in the winter surveys in 1999, 2000 and 2003.

Year	**Coastal Section**	**Number of Sites Sampled**	**Number of Sites Sampled with**	
			GNS Present	**GNS Absent**
1999	Entire	**50**	**25**	**25**
	North	22	15	7
	South	28	10	18
2000	Entire	**62**	**31**	**31**
	North	36	19	17
	South	26	12	14
2003	Entire	**44**	**24**	**20**
	North	23	14	7
	South	21	10	13

3.3.2. *Patterns of Abundance*

A total of 313 Grey Nurse Sharks was counted by divers and spearfishers in the coastal waters of SE Australia (Fig. 3.1a). This was greater than the previous winter surveys in 1999 and 2000 (i.e. 207 and 292, respectively). Of the sharks observed, 224 (71.6%) occurred in the northern coastal section compared to 89 (28.4%) in the southern coastal section (Fig. 3.1a). Incorporating the sampling effort distributed along the coast (Fig. 3.1b) showed the same patterns of relative abundance of Grey Nurse Sharks along the entire coast and in the northern and southern coastal sections.

The numbers of male, female and sharks of unknown sex (Fig. 3.2a) were similar for the entire coast. For individuals of known sex, the ratio of males to females did not differ significantly from a 1:1 sex ratio (Table 3.2). Comparing the sex ratios from the previous 2 winter surveys (i.e. in 1999 and 2000) showed the same patterns with 1:1 sex ratios evident along the entire coast (Table 3.2).

Partitioning the coast into the 2 coastal sections (Fig. 3.2a & c) showed a very different pattern with males and females distributed differently along the coast. Males comprised 58% of the population in the northern section. This also resulted in the sex ratio significantly differing from a 1:1 ratio with a bias towards males (Table 3.2). Comparisons of the sex ratios from the previous 2 winter surveys with those of winter 2003 showed identical patterns. In contrast to the northern coastal section, the Grey Nurse Sharks of known sex (Fig. 3.2c) on the southern coastal section were dominated by females and comprised almost 80% of the population. The dominance of females also resulted in a significantly biased sex ratio in favour of females (Table 3.2). The pattern of bias towards females in the southern coastal section was identical to the previous two winter surveys (Table 3.2).

Grey Nurse Sharks of unknown sex comprised 31% of the population observed along the entire coast (Fig. 3.2a). In comparison to the previous winter surveys, the proportions of individuals of known and unknown sex differed significantly (Table 3.2; $\chi^2 = 14.53$, $P < 0.01$). Proportionally more individuals were classified as “unknown sex” and proportionally fewer individuals allocated a sex in the winter 2003 survey compared to the previous surveys in 1999 and 2000. The sex of almost 23% of the sharks in the northern coastal section could not be determined (Fig. 3.2b) compared to 51% in the southern section (Fig 3.2c).

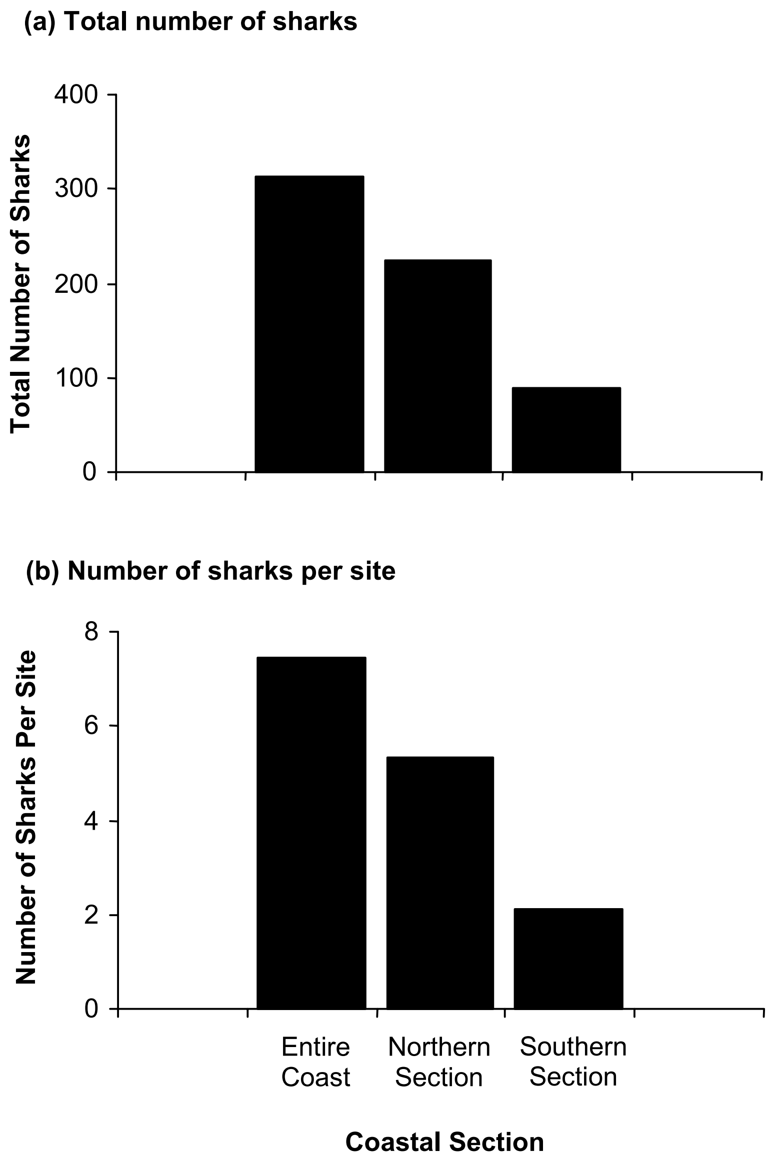

Figure 3.1. (a) Total number of Grey Nurse Sharks along the entire coast and along the 2 sections of coast: Forster and sites north, and Seal Rocks and sites south, and (b) the number of Grey Nurse Sharks per site along the entire coast and along the 2 sections of coast: Forster and sites north (23 sites), and Seal Rocks and sites south (21 sites).

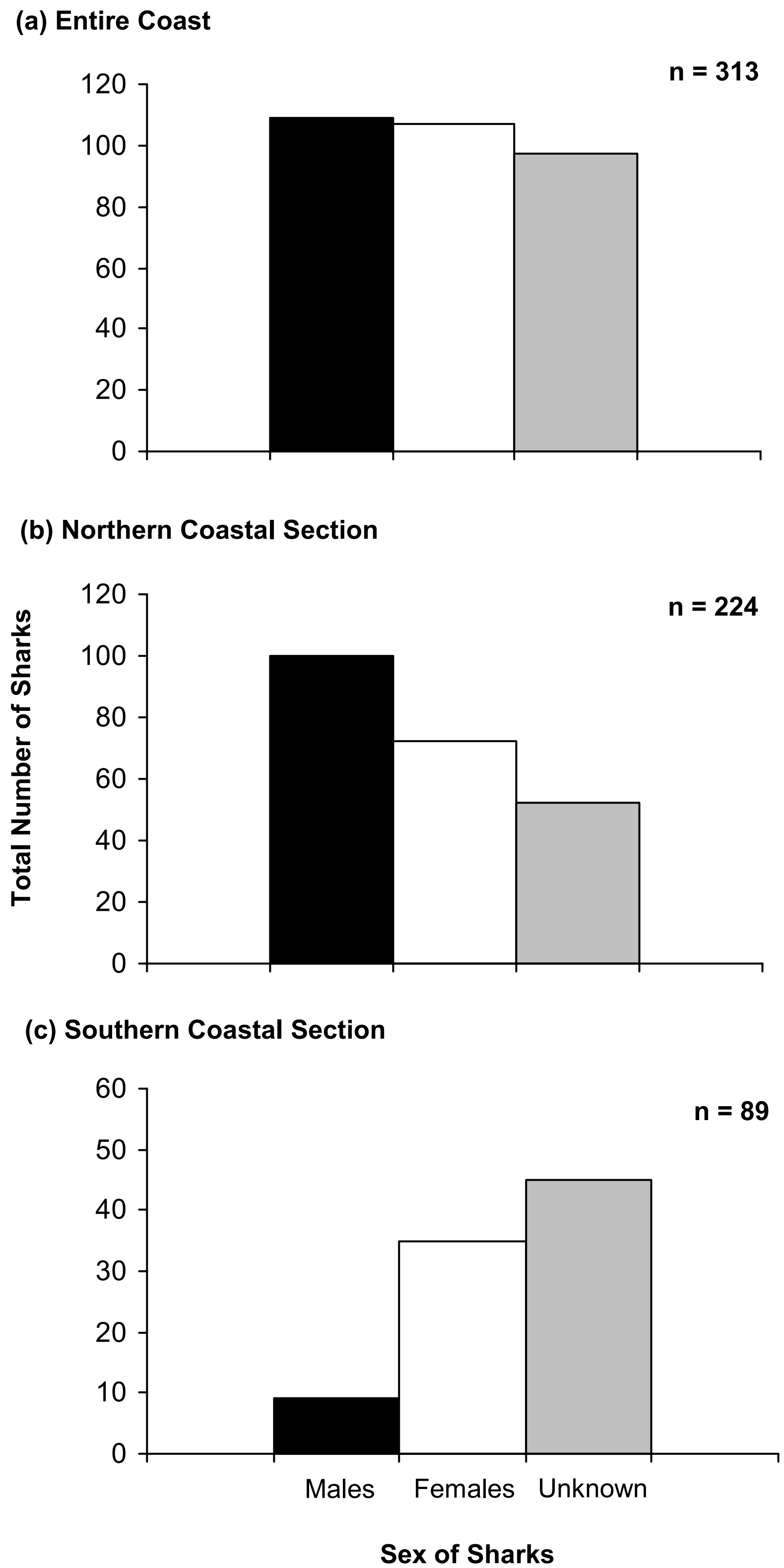

Figure 3.2. Total number of male (■), female (□) and individuals of unknown sex (■) Grey Nurse Sharks (a) along the entire coast, (b) along the northern coastal section (Forster and sites north), and (c) along the southern coastal section (Seal Rocks and sites south) in winter 2003.

3.3.3. Population size–structure

3.3.3.1. General observations

Individuals in the < 2 and 2 – 3 m TL size classes dominated the length frequency distribution of Grey Nurse Sharks along the entire coast (Fig. 3.3a). Despite this, 42.5% of the sharks observed were of a reproductively mature size (i.e. 2 – 3 m or > 3 m TL). For individuals of known sex, 26.2% of the males and 20.0% of the females were of a reproductively mature size. On partitioning the coast into the northern and southern sections, it was apparent that the vast majority (97%) of the reproductively mature males were observed in the northern section (Fig 3.3b & c). Similarly, the majority (73%) of females of a reproductively mature size were observed in the northern coastal section (Fig. 3.3b & c).

The length frequency distributions (Fig. 3.3) suggested that the Grey Nurse Shark population was segregated by size and sex as has been the case in the past (see Otway & Parker 2000, Otway *et al.* 2003 for details). To examine the statistical significance of these observations, the data for males and females were partitioned into 2 size classes: (1) < 2 m TL (i.e. juvenile) and (2) ≥ 2 m TL (i.e. adult). These were further subdivided into the 2 coastal sections: (1) the northern section – from Forster and sites north and (2) the southern section – from Seal Rocks and sites south. Finally, the data obtained in the mark-recapture survey (i.e. winter 2003) were compared with those obtained in the winters of 1999 and 2000. This comparison was done to highlight the similarities and differences in the spatial patterns of size and sexual segregation exhibited by Grey Nurse Sharks over winter. Similarities in the patterns would clearly suggest that the mark-recapture population estimate would be representative and not biased because of anomalous, uncharacteristic events affecting the Grey Nurse Shark Nurse population when sampled in 2003.

3.3.3.2. Comparisons between coastal sections

The proportions of male to female Grey Nurse Sharks observed across the sites in the 2 coastal sections (i.e. Forster and sites to the north versus Seal Rocks and sites to the south) differed significantly in the winter 2003 survey (Table 3.2 and χ^2 = 19.90, P < 0.001). There were proportionally more males and fewer females in the northern coastal section than would be expected by chance alone (Table 3.2). In contrast, there were proportionally fewer males and more females in the southern coastal section (Table 3.2). Comparing the proportions of male to female Grey Nurse Sharks observed in the northern coastal section in winter 1999, 2000 and 2003 showed that that there were no significant differences among the 3 winter surveys and that males were consistently more numerous (Table 3.2 and χ^2 = 1.67, P > 0.40). Similarly, the proportions of male to female Grey Nurse Sharks in the southern coastal section did not differ significantly among the 3 winter surveys (Table 3.2 and χ^2 = 1.67, P > 0.40), but females were consistently more abundant than males.

The proportions of male to female juvenile Grey Nurse Sharks differed significantly between the northern and southern coastal sections in winter 2003 (Table 3.2 and χ^2 = 5.07, P > 0.05). Proportionally fewer juvenile males and more females occurred in the southern coastal section in winter 2003 (Table 3.2). The proportions of male to female adult Grey Nurse Sharks also differed significantly between the 2 coastal sections (Table 3.2 and χ^2 = 14.67, P < 0.001). There were proportionally more adult males and fewer females in the northern coastal section and proportionally fewer adult males and more females in the southern coastal section (Table 3.2). Comparing the proportions of male to female juvenile Grey Nurse Sharks observed in the northern and southern sections in winter 2003 with those documented in both coastal sections in winter 1999 and 2000 showed that proportions of male to female Grey Nurse Sharks in the northern and southern coastal section did not differ significantly among the 3 winter surveys (Table 3.2 and χ^2 =

0.03 and 0.13 respectively, $P > 0.90$). Similarly, proportions of male to female adult Grey Nurse Sharks in the northern and southern coastal section did not differ significantly among the 3 winter surveys (Table 3.2 and $\chi^2 = 3.55$ and 0.13 respectively, $P > 0.10$ and 0.70, respectively).

The number of juvenile males to females in the northern coastal section did not differ significantly from a 1:1 sex ratio in winter 2003 (Table 3.2). In contrast, the number of juvenile males to females in the southern coastal section differed significantly from a 1:1 sex ratio in winter 2003 with a strong bias towards females (Table 3.2). Comparing the sex ratios of the juvenile Grey Nurse Sharks observed in winter 2003 results with those documented in the northern coastal section in winter 1999 and 2000 (Table 3.2) showed that a 1:1 sex ratio occurred in all 3 years. A comparison among the 3 winters for the southern section showed an identical pattern with the sex ratios biased towards females in all 3 years.

Table 3.2. The numbers of male and female Grey Nurse Sharks in 2 size-classes (i.e. < 2 m TL & ≥ 2 m TL) observed along the entire coast and in the 2 coastal sections in the winter surveys in 1999, 2000 and 2003.

Year	Coastal Section	Size Class	All Individuals	Males	Females	Unknown	M:F	χ^2	P
1999	North coast	Total	**134**	**73**	**38**	**23**	1 : 0.52	11.40	**
		< 2	31	13	18	-	1 : 1.38	0.81	ns
		≥ 2	80	60	20	-	1 : 0.33	20.00	**
	South coast	Total	**70**	**8**	**41**	**21**	1 : 5.13	22.22	**
		< 2	24	5	19	-	1 : 3.80	8.17	**
		≥ 2	25	3	22	-	1 : 7.33	14.44	**
2000	North coast	Total	**134**	**98**	**64**	**24**	1 : 0.65	7.14	**
		< 2	22	9	13	-	1 : 1.45	0.73	ns
		≥ 2	140	89	51	-	1 : 0.57	10.31	**
	South coast	Total	**70**	**15**	**62**	**29**	1 : 4.13	28.69	**
		< 2	27	6	21	-	1 : 3.50	8.33	**
		≥ 2	50	9	41	-	1 : 4.56	20.48	**
2003	North coast	Total	**224**	**100**	**72**	**52**	1 : 0.72	4.56	*
		< 2	68	34	34	-	1 : 1.00	-	ns
		≥ 2	104	66	38	-	1 : 0.58	7.54	**
	South coast	Total	**89**	**9**	**35**	**45**	1 : 3.89	26.00	**
		< 2	28	7	21	-	1 : 3.00	7.00	**
		≥ 2	16	2	14	-	1 : 7.00	9.00	**

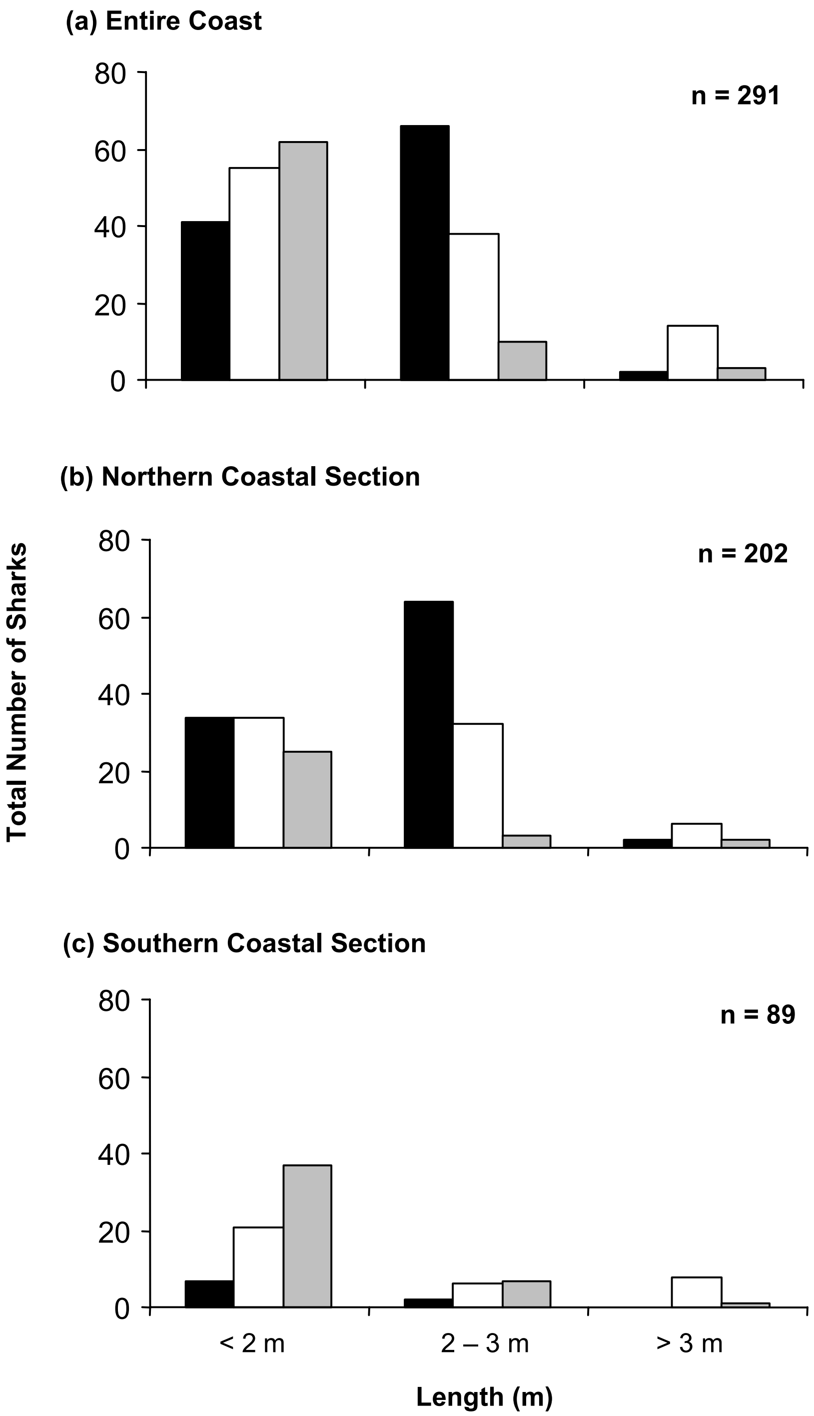

Figure 3.3. Total number of male (■), female (□) and individuals of unknown sex (■) Grey Nurse Sharks (a) along the entire coast (b) along the northern coastal section (Forster and sites north), and (c) along the southern coastal section (Seal Rocks and sites south). Total lengths estimated visually and placed into size-classes: < 2 m, 2 – 3 m and > 3 m in winter 2003.

The number of adult males to females in the northern coastal section differed significantly from a 1:1 sex ratio in winter 2003 with a strong bias towards males (Table 3.2). Similarly, the number of adult males to females in the southern coastal section differed significantly from a 1:1 sex ratio in winter 2003, but with a pronounced bias towards females (Table 3.2). Comparing the sex ratios of adult Grey Nurse Sharks observed in winter 2003 results with those documented along the northern and southern coastal sections in winter 1999 and 2000 (Table 3.2) showed identical patterns were evident. The sex ratios of adult Grey Nurse Sharks in the northern section were biased towards males in all 3 winters, whereas the sex ratios of adult Grey Nurse Sharks in the southern section were consistently biased towards females.

3.3.3.3. Comparisons within each coastal sections

(1) Northern coastal section

The proportions of male to female Grey Nurse Sharks did not differ significantly between juveniles and adults in winter 2003 (Table 3.2 and $\chi^2 = 3.06$, $P > 0.05$). Comparing the proportions of male to female juvenile Grey Nurse Sharks observed in winter 2003 with those documented in the winter surveys in 1999 and 2000 showed that proportions of male to female juvenile Grey Nurse Sharks did not differ significantly among the 3 winter surveys (Table 3.2 and $\chi^2 = 3.50$, $P > 0.05$). Similarly, the proportions of male to female adult Grey Nurse Sharks did not differ significantly among the 3 winter surveys (Table 3.2 and $\chi^2 = 3.55$, $P > 0.05$).

(2) Southern coastal section

The proportions of male to female Grey Nurse Sharks did not differ significantly between juveniles and adults in winter 2003 (Table 3.2 and $\chi^2 = 0.98$, $P > 0.30$). Comparing the proportions of male to female juvenile Grey Nurse Sharks observed in winter 2003 with those documented in the winter surveys in 1999 and 2000 showed that proportions of male to female juvenile Grey Nurse Sharks did not differ significantly among the 3 winter surveys (Table 3.2 and $\chi^2 = 0.13$, $P > 0.90$). Similarly, the proportions of male to female adult Grey Nurse Sharks did not differ significantly among the 3 winter surveys (Table 3.2 and $\chi^2 = 0.59$, $P > 0.70$).

3.3.3.4. Usage of aggregation sites

Of the 313 sharks observed during the winter 2003 survey, 201 (64.2%) were seen at 8 of the 14 key aggregation sites identified by Otway *et al.* (2003). A further 53 Grey Nurse Sharks (i.e. 16.9% of the observed population) were seen at Mermaid Reef. This reef has numerous gutters utilised by Grey Nurse Sharks and is located approximately 20 kms south-east of the Cod Grounds (off Laurieton – see Otway & Parker 2000 for detail).

3.3.4. Incidence of hooking

Untagged Grey Nurse Sharks with hooks embedded in their jaws and gills were seen at various sites from Julian Rocks (off Byron Bay) to Big Seal Rock (off Seal Rocks). By 31 July 2003, 7 of the 24 tagged Grey Nurse Sharks (i.e. 29.2%) had been hooked since tagging. Of these, 6 were observed in critical habitats at Fish Rock (off South West Rocks), The Pinnacle (off Forster), and Big Seal Rock (off Seal Rocks). Another tagged individual (# B216) was caught by a commercial fisher in 70 m of water NE of Yeppoon in September 2002. The shark was caught on a nylon dropline with two hooks and brought to the surface. The line was then cut leaving the hooks in place and the shark swam away slowly upon its release. This individual has not been re-sighted since its capture.

3.4. Discussion

3.4.1. Patterns of abundance

The number of Grey Nurse Sharks (i.e. 313 individuals), whilst greater than the 292 individuals counted in winter 2000, is still small given that the species has been protected since 1984. The dispersion of the population between southern Queensland and Sydney was consistent with the previous winter surveys in 1999 and 2000 (Otway & Parker 2000, Otway *et al.* 2003). Moreover, the patterns of segregation by size and sex observed in the winter 2003 survey were almost identical to those documented in the winter surveys in 1999 and 2000. The degree of consistency among years in these attributes of the Grey Nurse population in SE Australian waters indicates that the mark-recapture (re-sighting) estimate (see Chapter 4) should be representative. Therefore, it is unlikely that any unforeseen events or random perturbations have affected the population in such a way that would unknowingly bias the mark-recapture estimates.

3.4.2. Population size-structure

The usage by Grey Nurse Sharks of the key aggregation sites identified by Otway *et al.* (2003) was also consistent among the winter surveys in 1999, 2000 and 2003. This further reinforces the importance of these sites as critical habitats (see NSW Fisheries 2002). Aggregations of Grey Nurse Sharks at Mermaid Reef are also consistent with previous observations (Otway & Parker 2000, Otway *et al.* 2003). The relative importance of Mermaid Reef was not to be assessed by Otway *et al.* (2003) because of the infrequent sampling at this site. However, the aggregation of Grey Nurse Sharks at Mermaid Reef represented almost 17% of the sampled population in winter 2003. This indicates that the site is likely to be important during the northward movement of the sharks over the winter months. Presumably, the sharks would also use this site when travelling south in spring. Further sampling over time will enable a better understanding of the site. In the meantime, and given the low numbers of Grey Nurse Sharks in SE Australian waters, consideration should be given to affording Mermaid Reef some protection, at least as a strictly precautionary measure. A review of such measures could be done when further data become available.

3.4.3. Incidence of hooking

The hooking of 7 of the 24 tagged individuals within 1 year of tagging (i.e. 29.2% per annum) provides an independent estimate of the rate of hooking of Grey Nurse Sharks. Otway *et al.* (2003) showed that the incidence of hooking had significantly increased over the decade to 2002 with 12% of the Grey Nurse population displaying hooks and fishing tackle of various forms. Autopsies of accidentally caught and killed Grey Nurse Sharks have shown that 6 of 8 individuals (75%) were hooked (Otway unpub. data). More importantly, these 6 sharks were hooked internally and thus the hooks were not evident on external examination. This statistic indicates that the incidence of hooking, based on external observations, is an underestimate. The hooking of 29.2% of the tagged sharks shows that hooking is occurring at a relatively high rate. Furthermore, as 6 of the tagged sharks were first observed at critical habitat sites, the efficacy of the current fishing regulations at these sites should be examined and revised if required.

4. MARK - RESIGHTING POPULATION ESTIMATE

4.1. Introduction

The idea of using marked individuals to estimate the total size of a population of animals was first proposed by Petersen (1896), but not actually used in the field until Dahl (1919) used the method to estimate the size of a trout population in Norway. The Petersen estimate is the simplest and most commonly used technique to estimate the total size of a population. The method also forms the basis of many of the other mark-recapture techniques for estimating population numbers (Caughley, 1977; Seber, 1982).

The aim of this section of the research was to use various sampling protocols and associated Petersen estimates to obtain estimates of the total population of Grey Nurse Sharks in SE Australian waters.

4.2. Mark-recapture estimation of total population size

4.2.1. Petersen estimate

A Petersen mark-recapture experiment involves the capture of a randomly chosen group of animals. These animals are marked using individually identifiable tags (numbered in this study) and then released. A period of time is allowed to occur to permit the tagged animals to mix with the remaining untagged population. A second sample is then taken and the numbers of tagged and untagged animals are recorded.

This approach yields: $N = t\,n / R$ (1)

Where:

- N = the estimated number of individuals in the total population,
- t = the number of individuals caught, tagged and then released,
- n = the total number of individuals (tagged & untagged) observed in the second (recapture/re-sighting) sample, and
- R = the number of tagged individuals recaptured/re-sighted in the second (recapture/re-sighting) sample.

The accuracy of the Petersen mark-recapture estimate is, however, dependent on meeting several key assumptions. These are listed below:

(1) the tagged individuals are unaffected by the tagging process and behave in the same manner as untagged animals (i.e. the tagging does not affect the recapture/re-sighting of the animal);
(2) the tagged individuals must disperse throughout the untagged population;
(3) all animals have the same probability of being tagged initially:
(4) the tags are not lost in the time between the two samples;
(5) the second sample is a random sample (i.e. each of the possible samples has an equal probability of being chosen;
(6) the animal population is closed (i.e. the effects of emigration, immigration, mortality and recruitment are negligible); and
(7) all tagged animals seen in the second sample are reported.

These assumptions are not mutually exclusive (Seber, 1982). For example, the validity of assumption 4 is dependent on the validity of assumptions 3 and 5.

A Petersen estimate will often lead to a positively biased estimate (i.e. an over-estimate) of the total population (Caughley, 1977; Seber, 1982) because of an inability to satisfy the underlying assumptions. For example, if the some of the tags are lost then the number of animals recaptured (re-sighted) would be reduced and this would result in an overestimated population. It is also important, when designing a Petersen mark-recapture experiment, to ensure that the sampling results in 7 or more recaptures/re-sightings (i.e. $R \geq 7$ and $\mu = t\,n / N > 4$) because this will ensure, with 95% confidence, that the bias of the total population estimate (i.e. N*) will be negligible (Robson & Regier, 1964). The use of Equation 1 is only valid when the number of tagged individuals recaptured/re-sighted in the second sample is large (ideally $R \geq 20$ - Seber, 1982). This formula has, however, been modified for small "R" and the modifications (see below) result in less biased estimates.

The estimation of the total number of individuals in a population via a Petersen mark-recapture experiment is based on the hypergeometric distribution (Chapman, 1951; Seber, 1982). A more detailed mathematical description of this distribution together with approximations by the binomial, poisson and normal distributions can be found in Johnson and Kotz (1969). The use of the binomial, poisson and normal distributions, in combination with modifications permitting the use of small sample sizes, have enabled the development of a range alternative formulae for the calculation of a Petersen estimate.

In this study, six separate Petersen mark-recapture (re-sighting) estimates were calculated using various sampling protocols (i.e. direct & inverse) and modifications to the basic formula for the Petersen estimate (see Equation 1). These estimates are based on several probability distributions and the calculation of these is described in more detail below.

4.2.1.1. Direct Sampling

Ideally, the number of tagged individuals to be recaptured/re-sighted should be determined prior to sampling. Once underway, the sampling should continue until this number is reached. However, given the practical constraints and difficulties associated with sampling in the real world (particularly at sea), it is often not possible to determine, *a priori*, the number of tagged individuals to be recaptured/re-sighted (i.e. R) nor how many individuals (i.e. n - tagged & untagged) in total should be sampled in the second sample (Caughley, 1977; Seber, 1982). When this occurs, R and n change from "fixed" to "random" parameters, but this has negligible effects on the estimates of N or N* and their respective variances V and V* (Seber, 1982). Moreover, any effects that do occur will result in over-estimates of the total population.

Sampling to determine a Petersen estimate without the *a priori* determination of R or n is referred to as a "direct" sampling procedure. Two direct, Petersen estimates were calculated using modifications of Equation 1 derived by Chapman (1951, 1952) and Bailey (1951, 1952).

Method 1.

Chapman (1951) developed a Petersen estimate using the hypergeometric distribution (see Equation 1). However, when the number of recaptures (re-sightings) is small the bias is often large. With this in mind, Chapman (1951) modified the equation to enable an estimate with small "R."

This estimate is calculated as: $N^* = [(t + 1)(n + 1) / (R + 1)] - 1$ (2)

Where: N^* = the estimated number of individuals in the total population,
t = the number of individuals caught, tagged and then released,
n = the total number of individuals (tagged & untagged) observed in the second (recapture/re-sighting) sample, and
R = the number of tagged individuals recaptured/re-sighted in the second (recapture/re-sighting) sample.

This estimate has a poisson approximation of the variance (V^*) of:

$$V^* = N^2 (\mu^{-1} + 2\mu^{-2} + 6\mu^{-3}) \qquad (3)$$

where: $\mu = t\,n / N$

An alternative and approximately unbiased estimate of the variance (V^*) attributable to Seber (1970) and Wittes (1972) is given by:

$$V^* = [(t + 1)(n + 1)(t - R)(n - R)] / [(R + 1)^2 (R + 2)] \qquad (4)$$

This latter equation is used in this report. When $(t + n) \geq N$ the modified estimate (N^*) is exactly unbiased, but if $(t + n) < N$ the modified estimate (N^*) is a reasonable approximation with minimal bias and especially if the conditions of Robson and Regier (1964) are met.

Method 2.

Bailey (1951,1952) developed a Petersen estimate using a binomial approximation to the hypergeometric distribution and allowed modifications for small "R" (i.e. $R < 20$) – the number of tagged individuals recaptured/re-sighted in the second sample.

The estimate is calculated as: $N = [t\,(n + 1) / (R + 1)]$ (5)

Where: N = the estimated number of individuals in the total population,
t = the number of individuals caught, tagged and then released,
n = the total number of individuals (tagged & untagged) observed in the second (recapture/re-sighting) sample, and
R = the number of tagged individuals recaptured/re-sighted in the second recapture/re-sighting) sample.

This estimate has a variance (V) of: $V = [t^2 (n + 1)(n - R)] / [(R + 1)^2 (R + 2)]$ (6)

This estimate will be exact with random sampling with replacement (Seber, 1982).

4.2.1.2. Inverse Sampling

Inverse sampling requires the *a priori* determination of the number of tagged individuals to be recaptured/re-sighted (i.e. R) or the total number of individuals (i.e. n - tagged & untagged) to be obtained in the second sample (Caughley, 1977; Seber, 1982). When inverse sampling occurs, R and n are treated as "fixed" parameters and the estimates of N or N* and their respective variances V and V* are more accurate and less biased (Seber, 1982). Inverse sampling is also demonstrably more efficient than the direct Petersen estimate and will, for a given coefficient of variation, yield a smaller estimate of the total population size than direct sampling (Chapman, 1952; Seber, 1982).

Method 1

Bailey (1951) developed a Petersen estimate using inverse sampling with R (i.e. the number of tagged individuals to be recaptured/re-sighted), the negative hypergeometric distribution and sampling without replacement. Modifications for small "R" (i.e. $R < 20$) – the number of tagged individuals recaptured/re-sighted in the second sample were also incorporated.

The estimate is calculated as: $N = [n\,(t + 1) / R\,] - 1$ (7)

Where:
- N = the estimated number of individuals in the total population,
- t = the number of individuals caught, tagged and then released,
- n = the total number of individuals (tagged & untagged) observed in the second (recapture/re-sighting) sample, and
- R = the number of tagged individuals recaptured/re-sighted in the second (recapture/re-sighting) sample and specified *a priori*.

This estimate has a variance (V) of: $V = [\{t - (R + 1)\}(N + 1)(N - t)] / [(R(t + 2)]$ (8)

Note that the estimate of the variance will be exact with "random sampling with replacement" (Seber, 1982).

Method 2

Chapman (1952) developed a Petersen estimate using inverse sampling with u (i.e. the number of untagged individuals to be obtained), the negative hypergeometric distribution and sampling without replacement. Modifications for a small number of tagged individuals recaptured/re-sighted in the second sample (i.e. $R < 20$) were also incorporated.

The estimate is calculated as: $N = [n\,(t + 1) / (R + 1)\,] - 1$ (9)

Where:
- N = the estimated number of individuals in the total population,
- t = the number of individuals caught, tagged and then released,
- n = the total number of individuals obtained in the second (recapture/re-sighting) sample (i.e. untagged & tagged individuals, where $n = u + R$),
- u = the number of untagged individuals to be obtained in the second (recapture/re-sighting) sample determined *a priori*, and
- R = the number of tagged individuals recaptured/re-sighted in the second (recapture/re-sighting) sample.

This estimate has a variance (V) of: $V = [\{t - (R + 1)\}(N + 1)(N - t)] / [(R(t + 2)]$ (10)

Method 3

When a Petersen estimate is obtained using sampling with replacement the underlying probability density function is then the negative binomial distribution (Chapman, 1952; Seber, 1982). This distribution can also be used when the number of tagged individuals recaptured/re-sighted in the second sample (determined *a priori*) is small (i.e. $R < 20$).

The estimate is calculated as: $N = t\,n / R$ (11)

Where:
- N = the estimated number of individuals in the total population,
- t = the number of individuals caught, tagged and then released,

n = the total number of individuals (tagged & untagged) observed in the second (recapture/re-sighting) sample, and
R = the number of tagged individuals recaptured/re-sighted in the second (recapture/re-sighting) sample.

This estimate has a variance (V) of: $V = [t^2 n(n - R)] / R^2(R + 1)$ (12)

4.2.1.3. Bayesian Petersen estimate

Gaskell and George (1972) developed a Bayesian variation of the Petersen estimate by incorporating a prior, independent estimate of the population. The number of tagged individuals to be recaptured/re-sighted in the second sample is also determined *a priori*.

The estimate is calculated as: $N = (t\,n + 2N^1) / (R + 2)$ (13)

Where:
N = the estimated number of individuals in the total population,
N^1 = the prior, independent estimate of the number of individuals in the total population,
t = the number of individuals caught, tagged and then released,
n = the total number of individuals (tagged & untagged) observed in the second (recapture/re-sighting) sample, and
R = the number of tagged individuals recaptured/re-sighted in the second (recapture/re-sighting) sample and determined *a priori*.

This estimate has a variance (V) of: $V = [t^2 n(n - R)] / R^2(R + 1)$ (14)

4.2.1.4. Confidence Limits

As the Hypogeometric distribution can be approximated by the Binomial, Poisson and Normal distributions (Chapman, 1948; Lieberman & Owen, 1961; Johnson and Kotz, 1969), 95% confidence limits can also be calculated using these distributions. Seber (1982) has provided a guide to their use and 95% confidence limits based on the Poisson distribution are summarised below.

The 95% confidence limits are given by: $N^* \pm 1.96 \sqrt{V^*}$ (15)

Where:
N* = is the estimated number of individuals in the total population, and
V* = is the estimated variance.

However, Ricker (1958) has shown that 1 / N* is more normally distributed than N*. Hence, 95% confidence limits are more appropriately based around "R". Chapman (1948) provided a table for up to R = 50. However, an abridged version of this table can also be found in Seber (1982) and the latter was used for the calculation of 95% confidence limits in our study. The table provides the shortest 95% confidence limits for N / λ.

With: $\lambda = t\,n$

And
t = the number of individuals caught, tagged and then released, and
n = the total number of individuals (tagged & untagged) observed in the second (recapture/re-sighting) sample.

4.2.1.5. Coefficient of Variation

The coefficient of variation (CV) for N or N* is given by: $CV = 1 / \sqrt{\mu}$ (16)

where: $\mu = t\,n / N \approx R$

Hence: $CV \approx 1 / \sqrt{R}$

4.2.2. *A priori* choice of 'R' and 'u' for inverse sampling

Inverse sampling (as described in Section 4.2.1.2) requires that 'R' and 'u' (depending on the method) are chosen prior to doing the mark-recapture (re-sighting) survey. However, it is possible that the values of 'R' and 'u' chosen *a priori* might not be obtained during the survey. To allow for this possibility, a second, mutually exclusive set of values, with 'R' and 'u' of smaller magnitudes, was determined. The 2 sets of numbers with higher and lower magnitudes were used as first and second options, respectively.

The choice of values for 'R' and 'u' was assisted by utilising the number of tagged Grey Nurse Sharks re-sighted to the end of May 2003 together with the results of the previous abundance surveys (Otway & Parker 2000, Otway *et al.* 2003). By May 2003, 15 of the 24 sharks had been re-sighted at various sites along the SE Australian coast (see Chapter 3). Hence, R = 15 and R = 10 were chosen as the first and second options. These values were used for inverse sampling methods 1 and 3.

Inverse sampling method 2 requires that the number of untagged individuals to be sampled is determined *a priori*. With this in mind, the previous abundance surveys yielded 207 and 292 (mean = 249.5) individuals in winter 1999 and 2000, respectively. These population estimates suggested that the mean value and a value close to the smallest abundance of Grey Nurse Sharks would likely provide realistic *a priori* values. Consequently, u = 250 and u = 200 were chosen as the first and second options, respectively.

4.2.3. *Size-based approach to data analysis*

As described in Chapter 3, the mark-recapture (re-sighting) survey in June 2003 recorded 16 tagged individuals in a total of 313 Grey Nurse Sharks. Consequently, the mark-recapture (re-sighting) estimates utilising inverse sampling were based on the first set of *a priori* values (i.e. 'R' = 15 and 'u' = 250). Moreover, given that 22 of the tagged Grey Nurse Sharks (i.e. 91.7% of all tagged individuals) were at least 2 m TL, two separate approaches were adopted when estimating the size of the total Grey Nurse Shark population. The population estimates arising from these two approaches are given below.

4.2.3.1. All individuals

This approach used data arising from all tagged and untagged Grey Nurse Sharks, irrespective of their total lengths and/or their reproductive status, to estimate the size of the total population. The formulae for the 2 direct and 3 inverse sampling schemes, together with the single Bayesian sampling scheme were used to calculate the total population estimates utilising the inputs documented in Table 4.1.

4.2.3.2. Individuals ≥ 2 m TL

This approach was confined to tagged and untagged Grey Nurse Sharks with total lengths of 2 m or greater (i.e. adults). This necessitated modifying the values of t, n, and, u. There were 22 Grey Nurse Sharks ≥ 2 m TL tagged and thus t = 22. The numbers of tagged and untagged Grey Nurse Sharks ≥ 2 m TL were obtained from the data on population size-structure (see Chapter 3). The 22 individuals of unknown size were allocated to the 2 size classes based on the ratio of Grey Nurse Sharks (of known size) in the 2 size classes. The values of 'n' for the 6 estimates are documented in Table 4.3. The number of untagged individuals to be sampled via the inverse sampling method 2 was determined using data for the population size-structure of the 250 individuals used in the estimates based on all individuals. This value was determined as u = 105.

The total population of Grey Nurse Sharks was also estimated using the results for the individuals ≥ 2 m TL.

4.2.4. Assessment of bias in the Petersen estimate

An assessment of the bias in the Petersen estimate was done by examining the degree of agreement with the conditions specified by Robson & Regier (1964) (see Section 4.2.1 for details).

4.2.5. Validity of the assumptions

An assessment of each of the assumptions underlying the Petersen estimate was done to: (1) identify any potential shortcomings (bias), and (2) highlight the degree of confidence in the population estimates.

4.3. Results

4.3.1. Total Population Estimates

The vast majority (91.7%) of tagged Grey Nurse Sharks had a total length of at least 2 metres. Consequently, the mark-recapture (re-sighting) estimates of the total population were calculated using the 2 separate approaches below.

4.3.1.1. All individuals

The first approach used the data for all individuals (tagged and untagged), irrespective of their total length, in the calculations. Each of the formulae for the 2 direct, 3 inverse and the single Bayesian sampling schemes were used to calculate population estimates. The values used in these formulae differ (as described in Section 4.2.1) and are provided in Table 4.1.

The two direct estimation techniques (Table 4.1) had the same inputs, but gave estimates of total population size of 461 and 443 for the Hypergeometric and Binomial probability distributions, respectively. The maximal upper 95% confidence value was 766 and was associated with a Poisson approximation to the Binomial distribution. The inverse sampling scheme with sampling without replacement, based on the negative Hypergeometric distribution (Table 4.1), gave total population estimates of 426 and 413 individuals, respectively. Again the upper 95% confidence values differed, with a maximal value of 682 individuals associated with a Poisson approximation to the negative Hypergeometric distribution. The inverse sampling scheme with sampling with replacement, based on the negative Binomial distribution (Table 4.1), gave a total population estimate of 410 individuals with an upper 95% confidence value of 672 individuals. Finally, the

Bayesian technique gave an estimated total population of 396 individuals, but 95% confidence limits could not be calculated.

Overall, the traditional (i.e. non-Bayesian) estimates were similar in magnitude and ranged from 410 to 461 individuals with a maximal upper 95% confidence value of 766 individuals (Table 4.1).

Table 4.1. Calculation inputs and Petersen estimates of the total population of Grey Nurse Sharks irrespective of size and/or sexual maturity, in SE Australian waters. Calculations based on direct and indirect sampling – see Section 4.2.1 for more detail.

Estimation Technique *Sampling scheme* Method	**Probability Distribution**	**Reference**	**Calculation Inputs**					**Population Estimate**			
			t	**n**	**R**	**u**	**N^1**	**N**	**Variance**	**95% CL**	**CV (%)**
Direct											
1	Hypergeometric	Chapman (1951)	24	313	16	-	-	461	3,585	$369 \le N \le 647$	24.8
2	Binomial	Bailey (1951, 1952)	24	313	16	-	-	443	10,326	$263 \le N \le 766$	24.3
Inverse											
Sampling without replacement											
1	Negative hypergeometric	Bailey (1951)	24	256	**15**	-	-	426	3,521	$224 \le N \le 682$	26.3
2	Negative hypergeometric	Chapman (1952)	24	265	15	**250**	-	413	1,315	$291 \le N \le 541$	25.5
Sampling with replacement											
3	Negative binomial	Chapman (1952)	24	256	**15**	-	-	410	9,871	$148 \le N \le 672$	25.8
Bayesian											
1	-	Gaskell & George (1972)	24	256	**15**	-	**292**	396	-	-	25.4

Table 4.2. Calculation inputs and estimates of 'μ' for assessing the bias of the Petersen estimate for the total population of Grey Nurse Sharks, irrespective of size and/or sexual maturity, in SE Australian waters. Note Petersen estimate is unbiased with 95% confidence if $\mu > 4$ and $R \geq 7$. For further details see Section 4.2.1 and Robson & Regier (1964).

Estimation Technique *Sampling scheme* Method	**Probability Distribution**	**R**	**Calculation Inputs for μ**			**Consistency with Robson & Regier (1964) Conditions**		
			t	**n**	**N**	**μ**	**μ > 4**	**R ≥ 7**
Direct								
1	Hypergeometric	16	24	313	461	16.30	Yes	Yes
2	Binomial	16	24	313	443	16.96	Yes	Yes
Inverse								
Sampling without replacement								
1	Negative hypergeometric	15	24	256	426	14.42	Yes	Yes
2	Negative hypergeometric	15	24	265	413	15.40	Yes	Yes
Sampling with replacement								
3	Negative binomial	15	24	256	410	14.99	Yes	Yes
Bayesian								
1	-	15	24	256	396	15.52	Yes	Yes

There were no biases in the total population estimates (i.e. all individuals irrespective of their size or sexual maturity) with 95% confidence limits (Table 4.2) in accordance with the criteria of Robson and Regier (1964).

4.3.1.2. Individuals ≥ 2 m TL

The second approach used only the data for individuals (tagged and untagged) with total lengths of 2 m or greater were used in the calculations. Each of the formulae for the 2 direct, 3 inverse and the single Bayesian sampling schemes were then used to calculate population estimates for the portion of the population that had a total length of 2 m or greater (i.e. the reproductive individuals – see Otway *et al*. 2003 for further details). The values used in these formulae differ (as described in Section 4.2.1) and are provided in Table 4.3

Table 4.3. Calculation inputs and Petersen estimates of the total population of adult Grey Nurse Sharks (i.e. sharks $\geq$ 2 m TL) in SE Australian waters. Calculations based on direct and indirect sampling – see Section 4.2.1 for more details.

Estimation Technique — *Sampling scheme* / Method	Probability Distribution	Reference	Calculation Inputs for sharks ≥ 2m TL					Population Estimate for sharks ≥ 2m TL			
			t	**n**	**R**	**u**	**N[1]**	**N**	**Variance**	**95% CL**	**CV (%)**
Direct											
1	Hypergeometric	Chapman (1951)	22	143	16	-	-	194	485	$176 \leq N \leq 232$	24.8
2	Binomial	Bailey (1951, 1952)	22	143	16	-	-	186	1,702	$110 \leq N \leq 321$	24.3
Inverse											
Sampling without replacement											
1	Negative hypergeometric	Bailey (1951)	22	110	**15**	-	-	169	417	$85 \leq N \leq 247$	26.4
2	Negative hypergeometric	Chapman (1952)	22	120	15	**105**	-	183	346	$139 \leq N \leq 241$	26.3
Sampling with replacement											
3	Negative binomial	Chapman (1952)	22	110	**15**	-	-	161	1,405	$58 \leq N \leq 264$	25.8
Bayesian											
1	-	Gaskell & George (1972)	22	110	**15**	-	**215**	168	-	-	26.3

Table 4.4. Calculation inputs and estimates of 'μ' for assessing the bias of the Petersen estimate for the total population of adult Grey Nurse Sharks (i.e. sharks ≥ 2 m TL) in SE Australian waters. Note Petersen estimate is unbiased with 95% confidence if $\mu > 4$ and $R \geq 7$. For further details see Section 4.2.1 and Robson & Regier (1964).

Estimation Technique *Sampling scheme* Method	**Probability Distribution**	**R**	**Calculation Inputs for μ**			**Consistency with Robson & Regier (1964) Conditions**		
			t	**n**	**N**	**μ**	**μ > 4**	**R ≥ 7**
Direct								
1	Hypergeometric	16	22	143	194	16.22	Yes	Yes
2	Binomial	16	22	143	186	16.91	Yes	Yes
Inverse								
Sampling without replacement								
1	Negative hypergeometric	15	22	110	169	14.32	Yes	Yes
2	Negative hypergeometric	15	22	120	183	14.43	Yes	Yes
Sampling with replacement								
3	Negative binomial	15	22	110	161	15.03	Yes	Yes
Bayesian								
1	-	15	22	110	168	14.40	Yes	Yes

The two direct estimation techniques (Table 4.3) had the same inputs, but gave estimates of total population size of adults of 194 and 186 for the Hypergeometric and Binomial probability distributions, respectively. The maximal upper 95% confidence value was 321 and was associated with a Poisson approximation to the Binomial distribution. The inverse sampling scheme with sampling without replacement, based on the negative Hypergeometric distribution (Table 4.3), gave population estimates of 169 and 183 adults, respectively. Again the upper 95% confidence values differed, with a maximal value of 247 adults associated with a Poisson approximation to the negative Hypergeometric distribution. The inverse sampling scheme with sampling with replacement, based on the negative Binomial distribution (Table 4.3), gave an estimate of 161 adults with an upper 95% confidence value of 264 individuals. Finally, the Bayesian technique gave an estimated population of 168 adults, but 95% confidence limits could not be calculated.

In summary, the traditional (i.e. non-Bayesian) estimates were similar in magnitude and ranged from 161 to 194 adults with a maximal upper 95% confidence value of 264 individuals (Table 4.3).

The mark-recapture survey (see Chapter 3) showed that the ratio of juvenile (i.e. sharks with total lengths < 2 m) to adult Grey Nurse Sharks in the winter 2003 survey was 1.189. Consequently, the estimate of the juvenile population was obtained by multiplying the estimate of the total number of reproductive individuals by 1.189 (i.e. the ratio of juveniles to adults) to obtain the number of juveniles. The numbers of adults and juveniles were then summed to give an estimate of the total population (i.e. all individuals irrespective of reproductive status). This process was done for each of the 6 population estimates obtained for the adults. The resulting number of juveniles ranged from 191 to 231 individuals. On summing the respective adult populations, the estimates of the total population ranged from 352 to 425 individuals. While generally smaller, the estimated population sizes are similar in magnitude to those estimated via all tagged individuals (i.e. irrespective of their reproductive status).

There were no biases in the total population estimates of with 95% confidence limits (Table 4.4) in accordance with the criteria of Robson and Regier (1964).

Finally, the mark-recapture (re-sighting) estimates indicated that dependent on the mark-recapture/re-sighting formula used, as much as 68 – 79% of the Grey Nurse Shark population is observed and counted during a diver survey in SE Australian waters.

4.3.2. Assessment of the Validity of the Assumptions

(1) *Tagged individuals are unaffected by the tagging process and behave in the same manner as untagged animals*

Twenty of the 24 tagged sharks were re-sighted on numerous occasions within 1 year of tagging. Video footage of Grey Nurse Sharks showed that tagged and untagged displayed similar behaviours. Moreover, observations of untagged (but recognisable individuals) and tagged sharks moving between the same sites also indicated that tagging had had little effect on the animals. These lines of evidence are qualitative, but suggest that the sharks are unaffected by the tagging process and thus this assumption appears valid.

(2) *Tagged individuals disperse throughout the untagged population*

The tagged individuals moved over a range of sites within the known range of the SE Australian population of Grey Nurse Sharks, and were re-sighted at many sites distant from the site where the sharks were originally tagged. These observations suggest that the tagged individuals have dispersed throughout the untagged population and thus this assumption also appears valid.

(3) *All animals have the same probability of being tagged initially*

Sharks of a particular size or sex were not targeted when the tagging was done at a range of sites. The particular animals tagged comprised a random sample of the sharks present at any given site. Given this approach, it is highly likely that this assumption is valid.

(4) *Tags are not lost in the time between the two samples*

All the sharks were double tagged and none of the 20 sharks re-sighted over the period of the study had lost either of their tags. Given this result, it is unlikely that tag shedding prevented the remaining 4 sharks from being re-sighted. Hence, for the purpose of this study we have assumed that this assumption is valid.

(5) *The second sample is a random sample*

The mark-recapture (re-sighting) survey was done following established techniques (Otway *et al.* 2003). The sharks counted were observed at 44 sites over a two-week period. The observation of tagged and untagged sharks in this survey comprised a random sample of the population at sites within the sharks' known range. Thus, it appears that this assumption is valid.

(6) *The effects of emigration, immigration, mortality and recruitment are negligible*

The sampling covered almost the entire range of the population (see Otway & Parker 2000, Otway *et al.* 2003) and thus emigration and immigration would likely not be evident and hence not affect the population estimate. The mark-recapture (re-sighting) survey was done in excess of a year after the first Grey Nurse Shark was tagged. This was done to enable sufficient time for the tagged animals to disperse amongst the untagged population by participating in the migratory movements along the SE Australian coast. A single period of recruitment would have occurred in late winter – early spring, 2002. However, tagging did not finish until after the period of recruitment. Any effect of recruitment, if present, would lead to an over-estimation of the true population size (Seber, 1982).

(7) *All tagged animals seen in the second sample are reported*

Sixteen individual, tagged sharks were reported during the mark recapture survey in June 2003. Numerous, independent reports of the same shark were made by divers on the same dive. Divers also reported seeing tagged sharks with the tag numbers obscured by algae. These data suggest that all tagged sharks were seen and were also reported. It is important to note that under-reporting of tagged animals will give a total population estimate greater than the true population. This is not likely to be a problem in this study.

4.4. Discussion

The mark-recapture (re-sighting) technique using probability distribution-based formulae (i.e. non-Bayesian) gave an estimate of the total population of Grey Nurse Sharks in the coastal waters of SE Australia of between 410 and 461 individuals with upper 95% confidence values ranging between 541 and 766 individuals. Furthermore, the same methods gave an estimate for the total number of adult Grey Nurse Sharks (i.e. sharks $\geq$ 2 m TL) of between 161 and 194 individuals with upper 95% confidence intervals ranging between 232 and 321 individuals.

The mark-recapture (re-sighting) estimates suggest that 74 - 89% (mean = 81.5%) of reproductively mature individuals and 68 - 79% (mean = 73.5%) of all individuals (i.e. irrespective of size or sexual maturity) are observed during the diver surveys that have been done in the coastal waters of SE Australia.

It is extremely important to consider whether the mark-recapture (re-sighting) estimates are representative and unbiased. There are 5 lines of evidence that strongly suggest that the mark-recapture (re-sighting) estimates are accurate, representative and unbiased. First, the re-sighting of 20 of the 24 tagged sharks (83.3%) by 31 July 2003 is a simple and compelling statistic. Moreover, the tagged sharks have been re-sighted at various locations along the coast with individuals travelling in excess of 600km. Second, the patterns of abundance, size, and sexual segregation exhibited by the Grey Nurse Shark population were consistent and almost identical between the mark-recapture (re-sighting) survey in 2003 and the 2 previous winter surveys in 1999 and 2000.

Third, the total population estimated using only the reproductively mature (tagged and untagged) Grey Nurse Sharks and the ratio of adults to juveniles in winter 2003 was very similar, but slightly lower in magnitude, to the population estimate based on all tagged and untagged individuals irrespective of their size and/or sexual maturity. Fourth, the conditions ensuring that bias of the total population estimate would be negligible with 95% confidence recommended by Robson and Regier (1964) were met for all the population estimates for adults and all individuals irrespective of their size and/or sexual maturity. Fifth, the assumptions underlying the use of the Petersen estimate were met.

The estimates of the total number of reproductively mature individuals and individuals of all sizes (i.e. irrespective of size or sexual maturity) clearly support the declaration of the Grey Nurse Shark population in SE Australian waters as critically endangered under Commonwealth legislation (EPBC 1999), by the IUCN shark specialist group (Cavanaugh *et al.* 2003), and as endangered: the highest level available under NSW legislation (FMA 1994). Given the reproductive biology and low fecundity of this species (i.e. 2 pups born biennially after intrauterine cannibalistic and oviphagous phases – for more details see Otway *et al*. 2003) combined with established rates of fishing-related mortality, the species may face extinction in SE Australian waters in the not too distant future if actions are not taken soon to increase the survival rates of the species and especially juvenile females (Otway *et al*. 2004).

5. SUMMARY AND CONCLUSIONS

The current project quantified the distribution and abundance of Grey Nurse Sharks along the SE coast of Australia. The maximum of 313 sharks, observed in the winter 2003 mark-recapture/re-sighting survey, supports the declaration of the Grey Nurse Shark as an endangered species under *NSW Fisheries Management Act 1994 & Amendments* (FMA 1994). Similarly, the data also support the declaration of the species as critically endangered under Commonwealth's *Environment Protection and Biodiversity Conservation Act 1999* (EPBC, 1999). The numbers of Grey Nurse Sharks exhibited substantial spatial variation along the SE Australian coast and occupied sites from Sydney northwards. Despite this, there were obvious patterns that were consistent with the previous winter surveys in 1999 and 2000. These included the segregation by size and sex along the northern and southern sections of the coast, and a 1:1 sex ratio along the entire coastline occupied.

The tagging showed that Grey Nurse Sharks moved over several hundreds of kilometres, covering these distances in less than 2 months in some instances. The directions of movement appeared to follow the movements hypothesised by Otway and Parker (2000). Males and females tended to move north over autumn and winter. Females then moved south over spring and summer.

The mark-recapture/re-sighting estimates suggest that the total population of Grey Nurse Sharks (i.e. all individuals irrespective of size and sexual maturity) in the coastal waters of SE Australia is between 410 and 461 individuals with a maximum 95% confidence value of 766 individuals. However, the total number of sexually mature (adult) Grey Nurse Sharks is between 161 and 194 individuals with a maximum 95% confidence value of 321 individuals. Similarities in the abundances and population size-structure of Grey Nurse sharks between the mark- recapture/re-sighting survey in winter 2003 and previous winter surveys in 1999 and 2000, combined with analyses showing that the Petersen mark-recapture/re-sighting estimates were not biased, indicate that the total population estimates provide a realistic indication of the total number of Grey Nurse Sharks in SE Australian waters. These results also show that almost 75% of all individuals (irrespective of size and sexual maturity) and 81% of adult sharks are likely to have been observed in the diver surveys.

Given what is currently known about Grey Nurse Sharks in SE Australia, if management actions do not increase the shark's survival, this population may well be extinct before the end of the 21st century (Otway *et al.* 2004). The results of the tagging study have provided a more precise estimate of the total population than was previously available, and have confirmed that the SE Australian population is in a very precarious position. To assist in the recovery of this population, management actions need to be devised to increase the survival of Grey Nurse Sharks of all ages and particularly juvenile females. Determining which management alternatives will be the most efficacious will require additional research to: (1) further quantify the survival/mortality rates and other demographic parameters; (2) size/age-specific rates of fishing-related mortality; (3) document the reproductive biology and size/age-specific fertility rates; and (4) localised and migratory movements and the factors affecting them.

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