2. SAMPLING METHODS FOR MONITORING WHALE SHARKS IN AUSTRALIAN WATERS

The most common techniques used for monitoring whale shark populations are variants of capture-mark-recapture/resight (CMR/S) approaches, where animals are individually recognisable so that they can be followed through time for the calculation of demographic rates (Lebreton et al. 1992). Individual recognition can be achieved by applying an artificial mark to an animal or by using an animal's natural markings (Neumann et al. 2002). The former technique is pervasive in ecological studies, ranging from the purely theoretical (Booth 2004) to the highly applied (Kohler & Turner 2001) and has been used in both marine and terrestrial environments on taxa ranging from insects (Auckland et al. 2004) through to whales (Watkins et al. 1993).

Though successful in many situations, the physical marking or tagging of animals is not without drawbacks. For example, the application of artificial marks to wildlife can alter natural behaviour and reduce individual performance (Gauthier-Clerc et al. 2004). The marking process itself may also be disruptive (Bateson 1977) due to the necessity of handling and restraining for mark application (Ogutu et al. 2006) and the loss of marks over time (Bradshaw et al. 2000), and the non-reporting of retrieved marks (Schwarz & Seber 1999) may cause severe bias in parameter estimates (Stevick et al. 2001). Additionally, there are often a host of ethical and welfare issues that can arise from the application of permanent or semi-permanent marks (McMahon et al. 2006, Wilson & McMahon 2006). The artificial marking of individuals may also be costly and impractical when dealing with large populations (Kelly 2001).

Due to the vulnerable status of whale sharks, it is essential that sampling techniques are as benign as possible to ensure minimal impact on the remaining individuals. The size of whale sharks also limits sampling techniques to those that do not require the physical restraint of the animals for mark application. The aim of this chapter is to describe the techniques trialled for individual identification of whale sharks at Ningaloo Reef.

2.1 STUDY SITE AND SAMPLING

Whale sharks frequent Ningaloo Reef, WA (22º 50' S, 113º 40' E) between March and July each year (Figure 2.1). Due to their cryptic nature, light planes are used to locate animals. Once an animal is spotted at the surface, the light plane circles above while the research vessel (Figure 2.2.) is directed towards vicinity of the shark. When alongside an animal, researchers are able to swim to the shark from the boat to collect data (Figure 2.3).

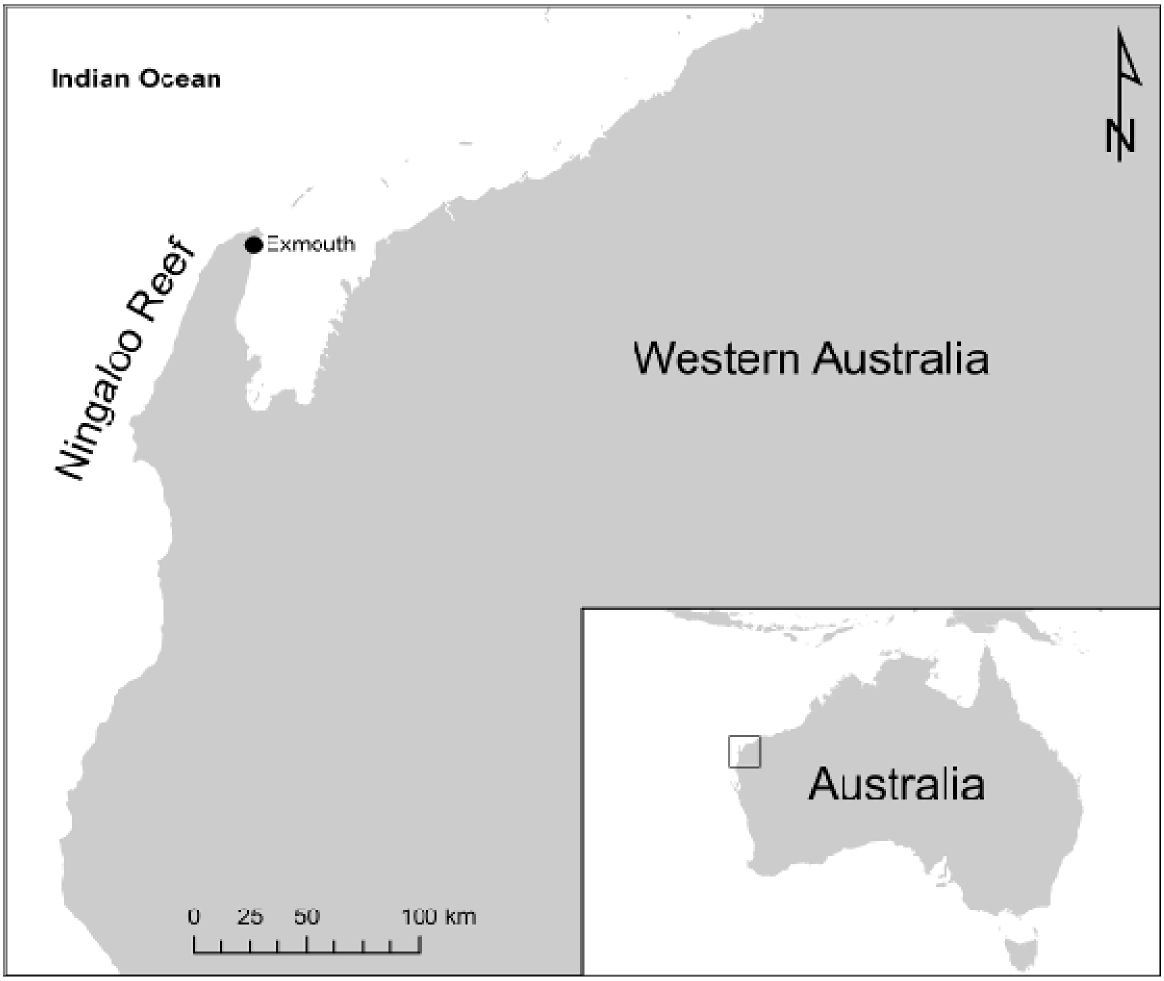

Figure 2.1. Map of study site, Ningaloo Reef, WA.

Figure 2.2. Research vessel used for whale shark work during 2005-7. (Photo – © C. Speed).

Figure 2.3. A) Researchers exiting vessel in pursuit of whale shark. B) Researcher positioning in relation to whale shark for photo-id (far) and satellite tag attachment (close) (Photos – © F. Baronie).

2.2 CONVENTIONAL TAGS

Prior to the 1990s there was relatively little reliable information on the abundance and distribution of whale sharks. At Ningaloo, the only available information was based on direct counts of sharks from boat and aerial surveys (Taylor 1996). Taylor (pers. comm.) attempted to tag whale sharks using conventional fish tags, which were numbered plastic spaghetti-shaped tags inserted by a speargun below the dorsal fin (Fig. 2.4).

Figure 2.4. The left flank of a whale shark tagged using a conventional fish tag (yellow dart below dorsal fin). (Photo – © G. Taylor).

These tags allowed researchers to recognise immediately whether a shark was a new individual or a resight, and also acted as a form of double tagging when used in conjunction with identification photographs (Geoff Taylor, pers. comm.). The physical tagging of whale sharks using standard tags has been discontinued at Ningaloo Reef due to tag loss and advances in photo-identification and satellite tagging technology; however, similar tags are still being used to assist with individual identification of whale sharks in other aggregations such as in the Seychelles (Taylor 1996, Rowat 1997).

2.3 PHOTO-IDENTIFICATION

Photo-identification is one of the most effective and popular methods of recording natural markings of an animal. It permits individual identification, which can then allow the study of animal movement patterns, site fidelity, population size and other parameters (Karlsson et al. 2005), with the only field requirement being a suitable camera. In addition to the other benefits of non-intrusive 'marking' of individuals, this method allows storage of photos in a library for cross-matching and generation of capture-history matrices (Fujiwara & Caswell 2001, Meekan et al. 2006, Bradshaw et al. 2007). There have been an increasing number of photo-identification studies of long-lived animals that rely on natural markings, including predatory cats (Kelly 2001, Karanth & Nichols 1998, Maffei et al. 2004, Ogutu et al. 2006), cetaceans (Hammond et al. 1990), and elasmobranchs (Arzoumanian et al. 2005, Domeier & Nasby-Lucas 2006, Meekan et al. 2006, Van Tienhoven et al. 2007b).

Image capture techniques vary among studies largely due to the accessibility and ease of observation of study animals. In recent photo-identification studies, most images have been captured using digital or video cameras. Images may be captured directly on land (Kelly 2001), remotely by camera trap (Karanth, 1998 #270; Maffei, 2004 #293}), by aerial photography (Hiby & Lovell 2001), on the surface of the ocean (Hiby & Lovell 2001, Langtimm et al. 2004, Parra et al. 2006), as well as underwater (Corcoran & Gruber 1999, Arzoumanian et al. 2005, Castro & Rosa 2005, Domeier & Nasby-Lucas 2006, Meekan et al. 2006, Van Tienhoven et al. 2007b). Underwater photography has a host of problems that are not associated with standard photographic techniques (Meekan et al. 2006) in terrestrial environments, such as light refraction and backscatter from particulate matter in the water. These issues, as well as the complicating factor of maintaining the line of sight of animals being photographed, can make collection of underwater images for photo-identification particularly challenging.

The standard method of photographing whale sharks for photo-identification captures images while swimming along the flank of the animal. The area on the flank of sharks directly behind the fifth gill slit is typically chosen for use for identification of whale sharks (Figure 2.5) for a variety of reasons, including consistency with past studies, the lack of contortion of this part of the animal during swimming and also because of the ease with which photographers can focus on this area (Arzoumanian et al. 2005, Meekan et al. 2006). In addition to the host of problems associated with underwater photography, whale sharks swim at a speed of approximately 2 knots, which makes taking clear photographs exceedingly difficult, especially in rough weather (Meekan et al. 2006).

Photo-identification studies of whale sharks are relatively new in comparison to photo-identification of marine mammals. Nevertheless, similar problems associated with the manual identification and matching of individuals by eye have emerged. Image libraries can be examined manually (by eye) to build a history of individual matches (Meekan et al. 2006); however, as the number of photos in a library increases beyond a person's capacity to process the potential candidate matches reliably, the development of faster, automated techniques to compare new photographs to those previously obtained is required (Mizroch et al. 1990, Arzoumanian et al. 2005). Several automated matching algorithms have been trialled with some success (e.g.,Mizroch et al. 1990, Wilkin et al. 1998, Evans 2003, Hillman et al. 2003, Arzoumanian et al. 2005, Lapolla 2005, Urian 2005), but these are often highly technical, species/morphological feature specific, or unavailable for public use.

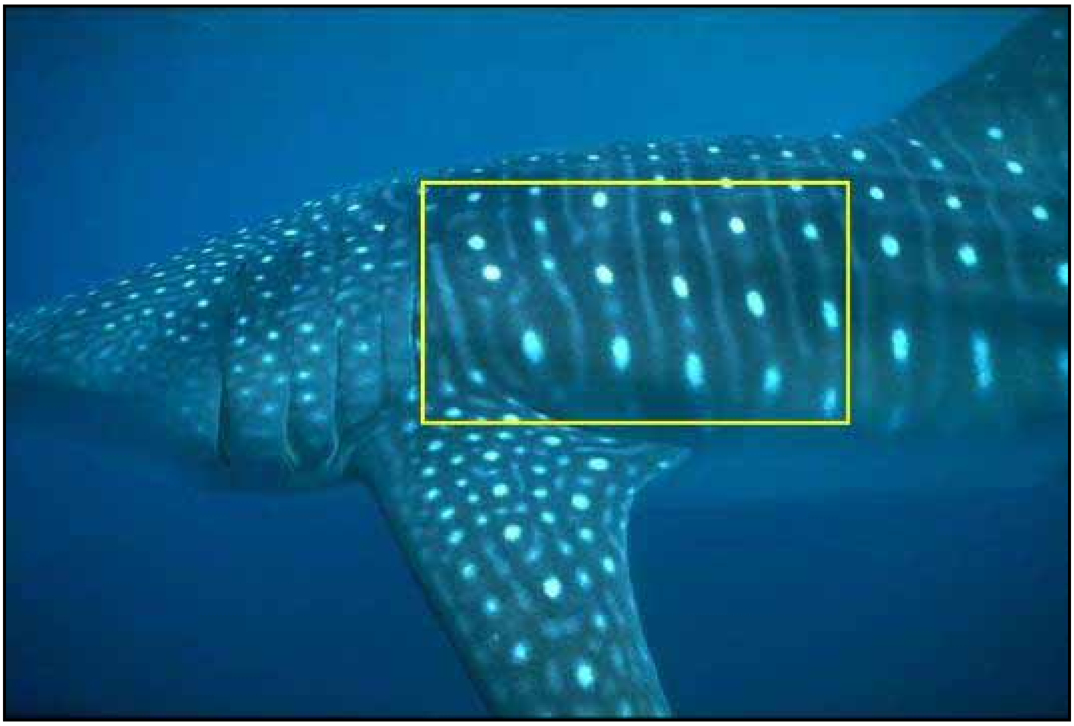

Figure 2.5. Standard reference area use for photo-identification of whale sharks (Photo – © G. Taylor).

The methods of computer-assisted image matching currently used for photo-identification of whale sharks are: 1) a method adopted from a stellar-pattern recognition software by NASA (Arzoumanian et al. 2005), and 2) a program developed for matching the spot-patterns of grey nurse (*Carcharius taurus*) sharks called Interactive Individual Identification System (I³S) (Van Tienhoven et al. 2007b)Van Tienhoven et al. 2007a. The first method is currently being employed to match images in the online Ecocean database (repository), where the public can submit photos taken while swimming with whale sharks (Arzoumanian et al. 2005). The matching algorithm incorporated into the software is insensitive to image magnification, rotation, and inversion via the use of triangulated triplets of coordinates, which can then be used to match similar patterns from the database (Arzoumanian et al. 2005). This method is almost completely automated, but like other such methods, the final validation process involves a manual by-eye component. This software has been used successfully to identify individual whale sharks; however, the program is of little use to researchers as the software is not open-access so its limitations and biases cannot be investigated. The inaccessibility of the software also means that matching cannot be done by individual researchers. In contrast, the I³S program can be freely downloaded from the internet (see www.reijns.com/i3s) and can successfully identify and match individual sharks (Speed et al. 2007). Furthermore, a validation technique using information theory has been developed to aid this program, providing the user with a relatively non-subjective means of confirming individual matches (Speed et al. 2007). The limitations, protocol and use of this matching software is discussed in Chapter 4.

Until recently, data collection for photo-identification studies of the whale shark population at Ningaloo Reef had been done largely in an *ad hoc* manner. Photographs were taken by various researchers, tourists and ecotourism operators, and libraries were held separately. Recently, a new initiative to encourage collaboration and standardise effort has been implemented by the Western Australia Department of Environment and Heritage (DEC) where all photographs and measurements taken by researchers and tour operators are submitted to DEC at the end of each whale shark season. This has vastly increased the size of the library of photographs available for researchers.

2.4 TISSUE SAMPLING – COLLECTION TECHNIQUES

2.4.1 Faecal material

As an alternative to traditional tagging methods and photo-identification, individual animals can be also be identified using genetic information from nuclear microsatellite markers (Palsboll et al. 1997). Within the field of ecology, a number of methods have been trialled for obtaining tissue samples such as biopsies, sloughed skin, shed hair and faecal material collection (Palsboll et al. 1997). The collection of scat/faecal samples from animals for individual identification and mark-recapture purposes has been used in a number of circumstances (see review by Lukacs & Burnham 2005). Indeed, one such sample was collected from a whale shark during the 2007 research trip (Figure 2.6). This collection technique has been used successfully to identify prey species of whale sharks (Jarman & Wilson 2004); however it is unlikely that this would be viable for individual identification of sharks due to the relatively rare observation of the deposition of faecal matter, as well as the rapid dispersion of faeces by currents.

Figure 2.6. Whale shark faecal sample collected at Ningaloo Reef (photo – © F. Baronie)

2.4.2 Biopsy Spear

During April and May of 2005-2007 genetic samples were collected using a Hawaiian-sling pole that had a biopsy tip fastened to the end of the spear (Figure 2.7A). The tip was made by Ceta-Dart (Virum, Denmark – Finn Larsen fl@difres.dk), constructed of stainless steel and was 40 mm in length and 27 mm in diameter (Figure 2.7B). Samples were taken from the sub-dermal layer of either the upper-left or the upper-right flank of the sharks. Samples were then placed in vials of 10 % salt-saturated dimethylsulfoxide (DMSO) for genetic analysis (Figure 2.7C). The biopsy tip was cleansed with bleach after each use to avoid cross-contamination of samples.

This method of obtaining tissue samples from whale sharks proved to be successful, once the researcher had mastered the spearing technique. One major disadvantage of the technique was that once speared, sharks rarely remained in the immediate vicinity for further observations. For this reason, genetic samples were taken after length and sex information was collected and identification images had been captured.

2.4.3 Microplanes

Microplanes were trialled as an alternative and possibly less intrusive sampling technique than the biopsy probe. Two types of microplanes were trialled that had differing gauges of blade:

1. Coarse Grater – stainless steel, 12.4 × 5 cm grating area, 27.5 cm length and 6.88 cm width (Figure 2.8A).
2. Medium Ribbon grater – stainless steel, 12.4 × 5 cm grating area, 27.5 cm length and 6.88 cm width (Figure 2.8B).

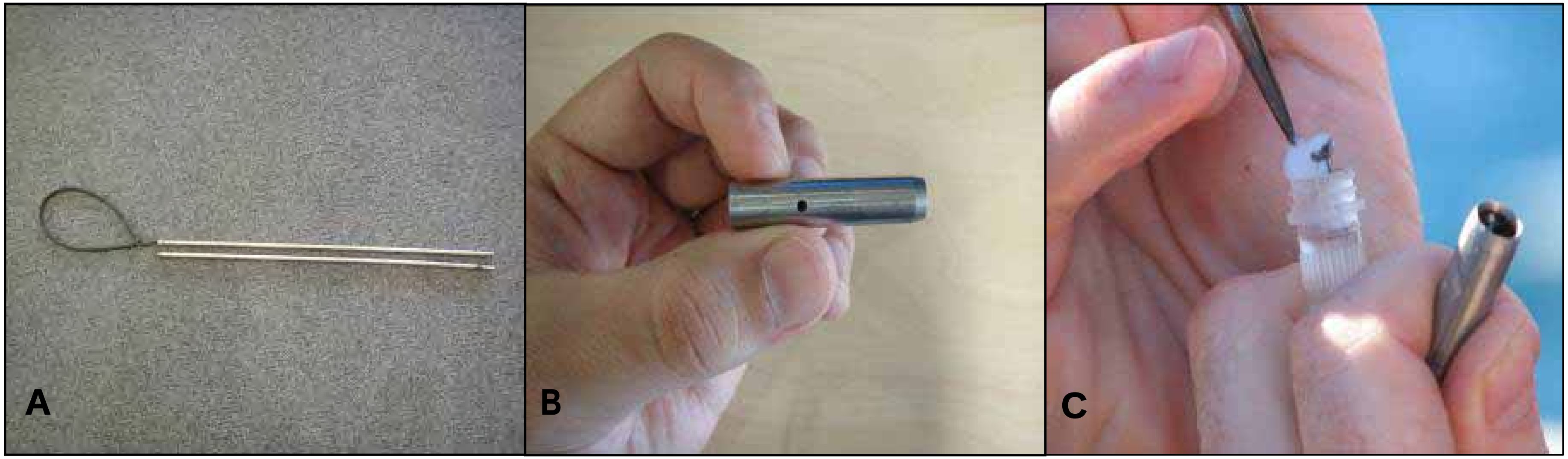

Figure 2.7. A) Hawaiian-Sling pole spear with biopsy tip attached, B) .Biopsy tip for tissue sampling of whale sharks, C) Biopsy tip and whale shark tissue sample being placed in vial of DMSO (Photos A & B – © C. Speed & photo C – © F. Baronie).

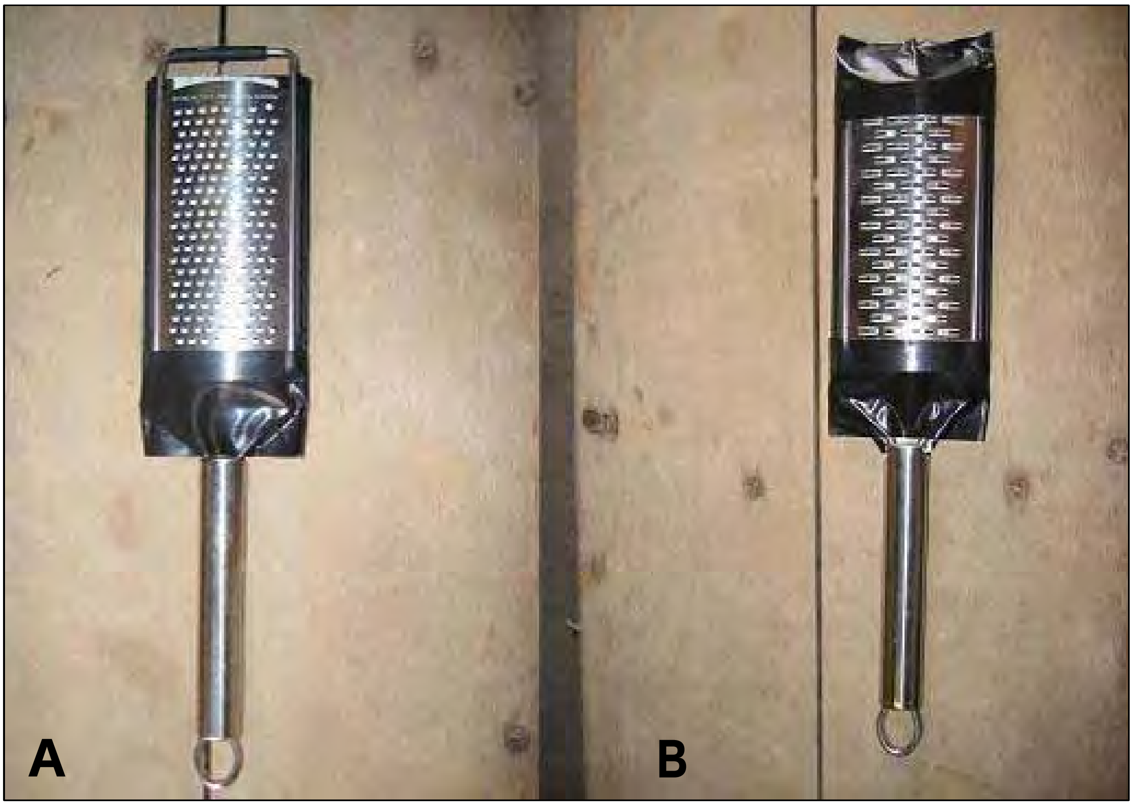

Figure 2.8. Microplanes trialled for taking skin samples from whale sharks. A) Coarse grade, B) medium ribbon grade. (Photos – © C. Bradshaw).

Electrical tape was used to seal the underside of the microplanes, so that skin samples would be retained. While swimming alongside the animals, microplanes were used to remove skin from the flank by scraping in a forward motion (the opposite direction to which the denticles face). A number of potential issues arose with this technique: 1) inadequate amounts of skin were removed by the micro plane due to the toughness of whale shark skin; 2) the convoluted nature of the microplanes meant that sterilization between sampling occasions was laborious; 3) samples may be contaminated by oxidisation due to prolonged contact with sea water, over multiple sampling occasions; and 4) whale sharks appeared to respond negatively to the scraping sensation, often more noticeably than their reaction to the biopsy spear.

To surmise, the ineffectiveness of the microplanes to collect skin samples, coupled with other logistical problems, meant that this technique was not suitable for collecting genetic samples for individual identification of whale sharks. The toughness of whale shark skin limits the use of scraping devises for skin collection purposes. For this reason, biopsy spears provided the most appropriate means to collect tissue samples for genetic studies.

2.5 SATELLITE TRACKING OF WHALE SHARKS

Satellite tracking to monitor large- and small-scale movement patterns of animals commenced during the mid- to late 1980s (Fancy et al. 1988). It was not until the early to mid-1990s however that this technology was used successfully to monitor the movement patterns of whale sharks (Eckert & Stewart 2001). The success of this study subsequently lead to numerous other tracking studies of the horizontal and vertical movements of whale sharks (see Eckert et al. 2002, Rowat & Gore 2006, Wilson et al. 2006). While this method of monitoring is more expensive than using conventional tags or photo-identification, satellite tags allow researchers to observe horizontal and vertical movement patterns over shorter time scales and at higher spatial resolution. A summary of the number and type of satellite tags that have been deployed on whale sharks by our study is given in Appendix 2.

2.5.1 Satellite tags

The satellite tags used to track movement patterns of whale sharks at Ningaloo Reef were made by Wildlife Computers (Redmond, Washington, USA) with custom tag housings and an applicator developed by the Marine Technology Group at the Australian Commonwealth Scientific and Industrial Research Organisation (CSIRO) Marine and Atmospheric Research (Figure 2.9).

The satellite transmitters were contained in a torpedo-shaped float that was attached to the shark's dorsal fin via a one-metre tether and fin clasp. Fin clasps were covered with neoprene to reduce the risk of infection caused by friction of the clasp rubbing on the fin. Tags transmitted location, depth and water temperature information to polar-orbiting satellites fitted with Argos receivers (Myers et al. 2006).

Figure 2.9. A) Applicator & bolt, B) Applicator, fin clasp and satellite tag and C) Satellite tag ready for deployment (Photos A & C © – C. Bradshaw, B © – C. Speed).

Each tag was coated with an antifouling agent prior to attachment to minimise algal growth that could increase drag. The tags were attached to the base of the leading edge of the first dorsal fin by the custom-designed applicator that fired a stainless steel bolt through the fin clasp and fin (Figure 2.9 & Figure 2.10). A snorkeler attached tags while swimming alongside the shark.

A total of 6, 5 and 4 towed Splash satellite tags were attached to sharks during the April/May field trips to Ningaloo Reef in 2005, 2006 and 2007 respectively. Tags were retained for up to 4 months in 2005, however in 2006 and 2007 towed tags were removed by the animals after only a few weeks (2006) or months (2007). The application of satellite tags was relatively successful; however, the trigger mechanism of the applicator misfired on a few occasions. The lower retention time of the towed tags in 2006 and 2007 were most likely due to a combination of sharks actively trying to dislodge the tags on the reef and weak attachment to the dorsal fin due to problems with the applicator.

Figure 2.10. A double-tagged whale shark showing fin clasp on leading edge of 1st dorsal fin and satellite tag (above left pectoral fin) and 2 PSATs below dorsal fin on left and right flanks (Photo – © Department of Environment and Conservation). * Note: The orange material below the satellite tag is biofouling, most likely a species of brown algae.

2.5.2 Fin tags

As with the satellite tags, fin tags were attached with the same applicator and to the same position on the shark (i.e., the base of the leading edge of the first dorsal fin). Unlike the satellite tags, fin tags did not have a tether that connected the clasp and the transmitter. Rather, fin tags housed the attachment device and transmitter in one unit (Figure 2.11). As with the fin clasps used to attach satellite tags, fin tags also had a neoprene covering to minimise friction.

Figure 2.11. Fin tag without neoprene cover (Photos – © C. Speed).

The aim in placing the satellite tag in the fin clasp was to avoid issues of fouling of the towed tag by weed and flotsam (see Fig. 2.10) and to attempt to prevent the shark catching the tether on the reef and pulling the tag off. In both 2006 and 2007 all fin tags deployed on sharks failed to report position data to satellites and this approach has now been discarded. These tags were adapted from an approach in common and very successful use in other species such as great white and salmon sharks. Likely reasons for failure in whale sharks was due to the dorsal fin not clearing the water surface for periods of time sufficient for contact with a satellite to be made by the transmitter.

2.5.3 Pop-up Archival Satellite Tags (PSAT)

Both Wildlife Computers and model PTT-100, Microwave Telemetry, Inc., Columbia, MD, USA PSAT tags were deployed on whale sharks at Ningaloo Reef. (Figure 2.12). These tags measure and store light, depth and temperature at pre-determined intervals, and then later transmit raw data (Microwave tags) or data summaries (Wildlife Computer tags) to Argos satellites when tags have detached and floated to the surface (Wilson et al. 2006). In the case of Wildlife

Computer tags, if the tag is retrieved after pop-off the entire archive can then be downloaded.

Figure 2.12. PTT-100, Microwave Telemetry Pop-up archival tag showing titanium dart and tether. (Photo - © S. Wilson)

Prior to deployment, each tag was coated with an antifouling agent to help minimise the settlement of algae and other micro-organisms. PSATs were connected to a titanium dart by a tether of either monofilament or nylon-coated stainless steel (Wilson et al. 2006). PSATs were deployed using a Hawaiian-sling polespear (Figure 2.13), with each dart being embedded into either the left or right flank of sharks below the first dorsal fin. Darts were implanted several centimetres into the sub-dermal tissue on the dorsal surface of the animal near the first dorsal fin (Wilson et al. 2006) (Figure 2.14).

A total of 15, 9, 5 and 1 PSATs were deployed during the 2004, 2005, 2006 and 2007 field trips at Ningaloo respectively. The lower number of deployments in 2007 reflects the fact that very few whale sharks were seen in this year, probably as a result of El Nino effects on oceanographic phenomena in the Ningaloo Reef region (see Chapter 6 for a detailed analysis of whale shark abundance patterns in relation to climatic events). PSATS were also deployed at Ningaloo in 2002 and 2003. For analysis and discussion of the track and diving information obtained from these earlier deployments, see Wilson et al. 2006.

Figure 2.13. Application of Pop-up Archival Tag (PSAT). (Photo - © C. McLean)

Figure 2.14. PSAT tag embedded in sub-dermal layer of whale shark flank/dorsal fin. (Photo- ©C. McLean)