

This project has been a collaboration between the Centre for Sustainable Tropical Fisheries and Aquaculture (CSTFA) at James Cook University, Australia, the chapter authors who have given their time freely, and WWF.



ABOUT WWF

WWF is one of the largest and most experienced independent conservation organizations, with over 5 million supporters and a global network active in more than 100 countries. WWF's mission is to stop the degradation of the planet's natural environment and to build a future in which humans live in harmony with nature, by conserving the world's biological diversity, ensuring that the use of renewable resources is sustainable, and promoting the reduction of pollution and wasteful consumption. WWF works to reverse declining shark populations through Sharks: Restoring the Balance, a global initiative.

www.panda.org sharks.panda.org



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ABOUT CSTFA

Research within the Centre for Sustainable Tropical Fisheries and Aquaculture (CSTFA) focuses not only on the aquatic and aquaculture systems that produce food, but also the industries and communities that utilise them. Multidisciplinary collaborations between our researchers provide the synergies to address substantial research problems in a way that individual research groups cannot. CSTFA provides research outputs for sustainable food production to local, state, federal and international resource managers, both in government and in the private sector. Thus, making us a key player in helping secure aquatic food production in the tropics for future generations.

www.jcu.edu.au/tropical-fisheries-and-aquaculture

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ABOUT THIS TOOLKIT

507 SPECIES OF SHARKS

545 SPECIES OF RAYS AND SKATES

The species all have different distributions and life histories, which means they are exposed to different levels of fishing pressure and have different responses to this pressure. To be able to manage a country's sharks and rays for long-term sustainability, it is crucial to accurately know which species are present within a country's waters and which species are captured in fisheries (that is, the catch composition).

SPECIES OF

CHIMAERAS

The world needs sharks and rays. These predators are an essential part of healthy marine ecosystems; and they provide food security and income to millions of people, particularly in the developing world.

But sharks and rays are in crisis and their future is in doubt. They grow slowly, take many years to mature and produce relatively few young, making them particularly vulnerable to over-exploitation. After centuries of coexistence with humans, in recent years many populations have significantly declined through overfishing, and a quarter of all species are threatened with extinction.

EXTINCTION RISK OF SHARKS AND RAYS



- Critically endangered: 25 species 2.4%
- Endangered: 43 species 4.1%Vulnerable: 113 species 10.9%
- Near threatened: 132 species 12.7%
- 2.44 : 22.20/
- Least concern: 241 species 23.2%Data deficient: 487 species 48.6%

Fig 1: The IUCN Red List status of sharks and rays. Source data: Dulvy et al. 2014.



Action is essential to restore the balance. We need to manage the fisheries that interact with sharks and rays, reduce unsustainable mortality and prevent further overfishing.

To do that, though, we need a clear picture of what's going on. Currently we lack data on almost half of all shark and ray species, and there's an absence of information on many coastal fisheries in developing countries.

That's why this Rapid Assessment
Toolkit has been created. It offers a
suite of simple tools for collecting
the sound scientific data needed for
the conservation and sustainable
management of shark and ray
populations. The kit has been designed
for use in regions with limited
capacity and resources, and it contains
practical step-by-step guidelines for
collecting data by a range of methods.
Appropriate tools can be selected
depending on the particular data gaps
relevant to local waters.

The data collected will give a baseline for studying future trends in shark and ray populations, so fisheries authorities can address areas of particular concern and determine the effectiveness of their management efforts.

PLANS OF ACTION

Concerns for the future of sharks and rays have been growing for some time. In 1999, an **§ International** Plan of Action for the Conservation and Management of Sharks (IPOA) was adopted. It aims to ensure the conservation and long-term sustainable management of sharks and rays of all species and all types of catch (target, bycatch, commercial, artisanal, recreational).

The IPOA encourages any state with fisheries that catch sharks and rays (either as target species or bycatch) to develop and implement its own tailored National Plan of Action (NPOA, or Shark Plan). This should aim to assess the state of national shark and ray populations and fisheries, and provide a strategic framework for managing them in future.

THE IPOA HAS FOUR **MAIN ELEMENTS:**

- 1. The conservation needs of some shark and ray species
- 2. The maintenance of biodiversity through viable shark and ray populations
- **3.** The need for habitat protection
- **4.** The management of sustainable shark and ray fishery resources.

THE SHARK PLAN SHOULD AIM TO ACHIEVE 10 PRINCIPLES:



1. SUSTAINABILITY



























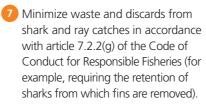












- 8 Encourage full use of dead sharks and rays.
- 9 Facilitate improved species-specific catch and landings data and monitoring of shark and ray catches.
- 10 Facilitate the identification and reporting of species-specific biological and trade data.







BIODIVERSIT









Data collection – and this Rapid Assessment Toolkit – contributes directly to principles 2, 3, 6, 9 and 10.

SHARK ASSESSMENT REPORT

To develop an effective Shark Plan with appropriate objectives, countries need to prepare a Shark Assessment Report. This collates information on species present and the fisheries that catch them, along with any data on catches, fishing effort and existing management measures. It also identifies what data is missing, helping to guide future data collection within the broader Shark Plan.

IPOA: PRACTICAL GUIDANCE

The UN FAO offers guidance on producing the Shark Plan and the Shark Assessment Report, including suggested contents.

1 Ensure that shark and ray catches from directed and non-directed fisheries are sustainable.

- 2 Assess threats to shark and ray populations, determine and protect critical habitats and implement harvesting strategies consistent with the principles of biological sustainability and rational long-term economic use.
- 3 Identify and provide special attention, in particular to vulnerable or threatened shark and ray stocks.
- 4 Improve and develop frameworks for establishing and coordinating effective consultation involving all stakeholders in research, management and educational initiatives within and between states.
- 5 Minimize the unutilized incidental catch of sharks and rays.
- 6 Contribute to the protection of biodiversity and ecosystem structure and function.

Rapid Assessment Toolkit I Sharks and Rays

DATA

Any plan to conserve and sustainably manage sharks and rays must start with good data. This data will cover these areas:

- What different shark and ray species are present in a country's waters
- Which of those species are caught by fisheries
- How many are being caught
- Whether and to what extent this has an impact on the shark and ray populations.

In order to build up this store of information, the following essential data types need to be gathered:



SPECIES IDENTIFICATION

Correct identification of the different shark and ray species present in an area is the first requirement for any data collection exercise. Species can be identified using whole animals and visual keys (taxonomy) or by laboratory testing of samples (genetics).



SPECIES PRESENT

Knowing all the species present in an area gives a measure of species

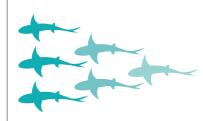
diversity, which can be compared to other areas and used as a baseline to examine future trends. A fishery may not capture all the species present – other methods such as baited underwater video surveys and citizen science may also be needed to build a full picture.



ABUNDANCE AND SIZE

Abundance is the number of individuals present from a given species – this is important baseline information to assess stocks.

Trends in abundance over time and comparisons in abundance between areas offer critical information for monitoring the health and status of sharks and rays. Size data (typically length) is used to estimate the maturity stage of specimens – this helps to reveal stock structure, and can also highlight critical information such as the presence of pupping or nursery grounds.



STOCK STRUCTURE

Shark and ray species may form separate populations. A population

is a group of individuals of the same species that live in the same area at the same time. Populations in different areas are known as separate stocks, because there tends to be little mixing between them – if one stock is threatened by overfishing, it's unlikely that individuals from another stock will move in to replenish it. For this reason, fisheries managers need to know if there are separate stocks of a species: they can then be monitored and managed separately.

The status of a stock can be more accurately assessed if its structure is also known: ie the sex, size, age and stage of maturity of the individuals comprising it.



CRITICAL HABITATS

Many species of sharks and rays have sites that they regularly use for feeding, mating and giving birth. These sites are known as 'critical habitats', as the behaviour they facilitate is essential to the continued survival of the species – and it's therefore very important for fisheries managers to be aware of where they're located. Nursery areas, where new-born or young animals are regularly found – often in shallow inshore regions – are a well-known example.



CATCH LANDINGS AND DISCARDS

Total catch – in numbers and/or weight by species – needs to be measured or estimated for every fishery, showing the removal of individuals and biomass from the ecosystem. The total catch includes all sharks and rays landed, plus those discarded at sea because they have no commercial value or aren't suitable for domestic consumption. Even when they're discarded at sea alive, some sharks and rays die due to the stress of capture: the fate of discarded catch at the time of release is valuable information. If possible, the catch should be described by species, location, numbers/weight, sex, length and maturity.



FISHERY DESCRIPTION AND FISHING EFFORT

All kinds of fisheries interact with sharks and rays, and they

need to be clearly described. They may be commercial, artisanal or recreational; and may be comprised of a fleet of similar vessels in a single area, or a complex range of boats and gears working across different areas. Various vessels and gear types catch different sizes and species of sharks and rays, so keeping up with this data is an important aspect of fisheries management.

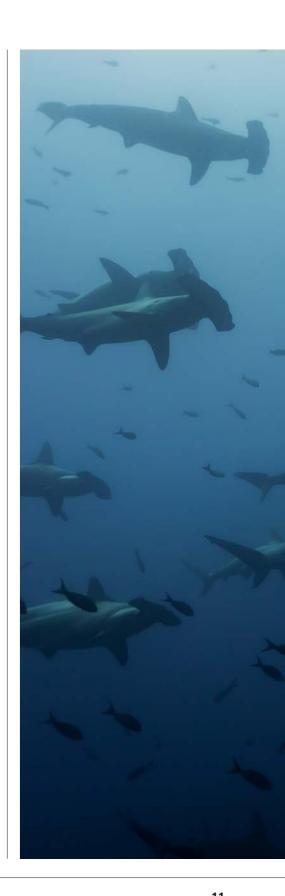
Fishing effort – the amount of time and gear used to capture the sharks and rays – also varies widely, and is another key dimension of fishery monitoring.

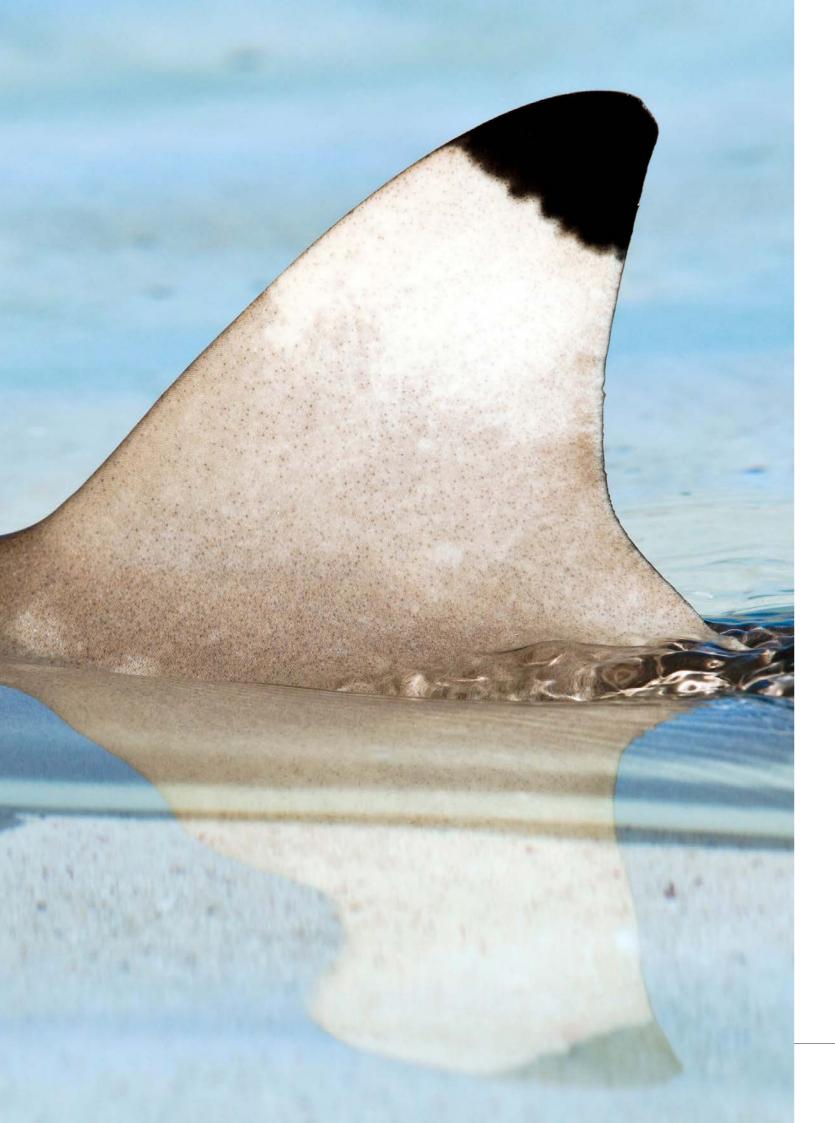




CATCH ABUNDANCE

Catch rate, or catch-per-unit effort (CPUE), is an indirect measure of stock abundance and is particularly valuable for the long-term monitoring of fishing impacts. Changes in the CPUE imply changes to stock abundance: if over time it takes longer to catch the same number of sharks and rays in a fishery, this indicates that stocks are declining. However, regular surveys of gear and operational area are also needed to confirm that CPUE changes are due to abundance rather than other changing factors. CPUE should be calculated separately for each stock, fleet and gear type.





TAXONOMY

Authors: Cassandra L. Rigby, James Cook University; William T. White, CSIRO National Research Collections Australia





9. MONITORING

DATA TYPE:

Species identification Landings and discards species identification

SHARK PLAN OBJECTIVES:

9. Improve species-specific catch and landing data, improve catch monitoring 10. Improve reporting of species-specific biological and trade data

WHY WOULD YOU USE THIS TOOL?

Taxonomy is used to identify shark and ray species accurately. A clear understanding of the species of sharks and rays present in a country's waters and captured in its fisheries provides important baseline and monitoring data for conservation and fisheries management.

WHERE WOULD YOU USE THIS TOOL?

This toolkit is aimed at coastal fisheries, so most of the sharks and rays identified are likely to be coastal species. Coastal waters are typically defined as being to a depth of up to 40m. However, in areas where the ocean floor drops steeply below 200m coastal fishers may catch deepwater species, so these may also need to be considered during identification.

RARE SPECIES

Occasionally, rare species of sharks and rays – ones that are not commonly encountered in the region in question – are identified. If this occurs, please inform the IUCN Shark Specialist Group at % iucnshark@gmail.com - they'll pass the information to the relevant scientists and projects.

WHAT IS TAXONOMY?

All living organisms are classified based on similarities in their structures and evolutionary paths. The system is arranged in levels: organisms are divided into major groups called kingdoms, which are then further subdivided into phylum, class, order, family, genus and species.

All sharks and rays belong to the class Chondrichthyes, meaning they have a flexible cartilaginous skeleton. Within this class, there are about 34 families of sharks and 26 families of rays.1

All species are given two-part scientific names through an internationally accepted system. The first is the genus name to which the species belongs, and is capitalized. The second is the specific name and is not capitalized. Both names are written in italics, and the combination must be unique for each species.







For identification purposes it's best to use scientific species names, as common names can vary by region and may change over time.

Nevertheless, when taking samples for taxonomic purposes it's also useful to record the standard English common name and the common name used in the local area, as it may be the only name with which locals are familiar.

Species are sometimes reclassified in terms of genus or family as taxonomists collect more information on them. The most accurate source for checking the currently accepted scientific name of a species is the Catalog of Fishes, available online at \$ http://researcharchive.calacademy.org/research/lichthyology/catalog/fishcatmain.asp

SUMMARY

Taxonomy is the science of classification: in this toolkit, it means identifying shark and ray species by examining their visible physical characteristics, the most important of which are shape (morphology) and colour. This is best done by comparing the whole animal – or a good quality photo (see p21) – to an identification guide or field guide (see below) that shows the different species and their main distinguishing features. Photos can also be sent to regional experts to confirm the identity of a species.

Taxonomic classification is a simple method, but it may not always provide a conclusive result: some species look similar and are hard to tell apart. In these cases, it may be necessary to take a tissue sample for genetic testing to correctly identify the species.

IDENTIFICATION GUIDES

Using the right identification guide for the area where the sharks or rays are seen or captured makes taxonomic classification quicker and more reliable.

It's best to use a regional identification guide if there's one available, as it will only include the species present in that region, considerably cutting down the total number of possibilities that need to be considered. This also reduces the chances of incorrectly identifying a shark or ray as one that does not in fact occur in the region.

As the name suggests, field guides are designed for use in the field – in ports, at markets, on boats. They're a concise version of the regional identification guides, and can be printed on waterproof paper with photos of local species.

Sharks and rays are used for their fins, gill plates, meat, cartilage, skin and liver, so sometimes the whole animal is not available for identification. Visual identification guides are available for the fins and gill plates of some sharks and rays that are used in trade or are listed as protected; although as with meat, cartilage and skin genetic tools may be required for accurate identification to species level.

Many regional shark and ray identification guides are available for free online. You can find a list of some of them at the end of this Taxonomy tool.

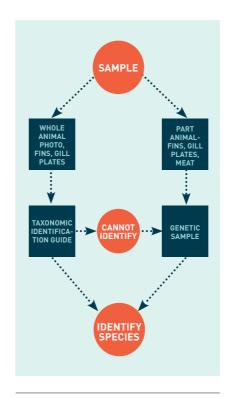


Fig 2: Steps to identify sharks and rays

HOW TO USE TAXONOMIC KEYS

Most shark and ray identification guides use taxonomic keys. These are a series of steps focused on a specimen's distinguishing features, usually with two choices: A or B. Most keys use illustrations, with arrows pointing to the features described in the A and B choices.

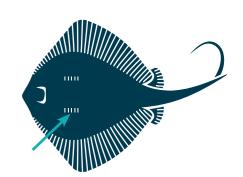
Making the correct choice at each step of the key gradually narrows down possible options and eventually leads to the correct identity of a specimen. (It's worth noting, of course, that this method only works if the species you're trying to identify is in the key in the first place.)

TAXONOMIC KEY EXAMPLE

Gill slits on side of head; body shark-like (Go to 2)



Gill slits on undersurface of head; body flattened, ray-like (Go to Rays)



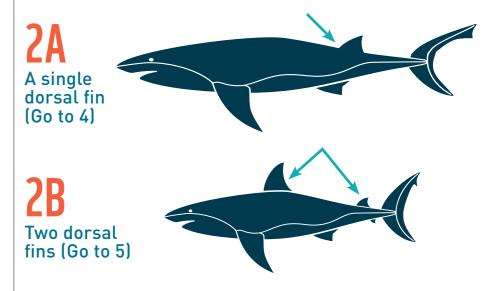


Fig 3: Example first stages in typical shark and ray taxonomic key – later stages not shown (figures from White et al. 2006)

MAIN SHARK AND RAY FEATURES

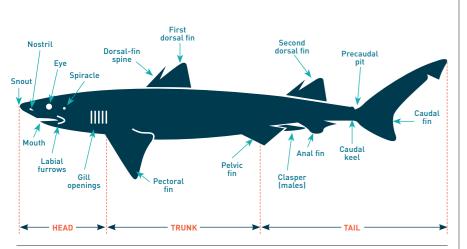


Fig 4: Main shark features. Source: Compagno 2002

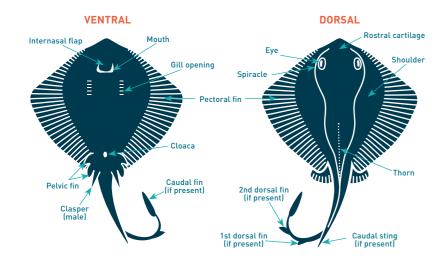


Fig 5: Ray main features. Source: White et al. 2017

METHOD: TAXONOMIC IDENTIFICATION

1 It's always good practice to record as much information as possible about the shark or ray to be identified. If it has been taken in a fishery, try to record:

- Location of catch, including place name, latitude and longitude if available
- Depth it was caught at
- Date of capture
- Gear type used
- Sex of the animal and length if measured (see § Surveys)

- Presumed species identification
- Date of identification
- Name of person who identified animal

If possible, take a photo of the animal even if it is identified, in case further verification is needed. If there are several animals to be identified, each animal should be given a unique sample identifier (USI): write this on a label and include it in the photo (see

If there's no time to identify the shark or ray in the field, take good photos of it with its USI. If possible keep them together for later laboratory identification, or if the animal can't be kept then take a labelled genetic sample (see Genetics).

For all photos, record:

- Name of photographer
- Location
- Date

3 Start with an identification guide for the area where the shark or ray was caught. If none is available, use a global guide. Follow the guide's taxonomic key, and use the key features to identify the animal (see *Figs 4 & 5*).

If only fins or gill plates are available, use a visual identification guide to fins and gill plates but ideally also take a tissue sample.

4 If visual identification guides don't help to accurately identify the species in question, take photos and genetic samples (see Senetics). Photos can be emailed to specialists for identification, and samples sent to laboratories.

HOW TO TAKE A GOOD PHOTO FOR IDENTIFICATION

A good photo can be used to identify a species, or verify an identification. Try to follow these simple steps for the best results:

- Take the photo from above looking directly down
- Use a plain, non-coloured, light background so the shape and details of head, fins and tail can be clearly seen
- Include a label in the photo to help distinguish different animals. If possible, use a unique sample identifier (see
 Surveys, labels), or place, date and name of animal.

SHARKS

A lateral (side) view is the best to photograph a shark.* Lay the shark on its side and straighten the body and all the fins. These may need to be propped up (e.g. with rocks, paper etc) so they are parallel to the camera. Ensure the head is not twisted (see *Fig 6*).

Secondary images are often also useful if the identification is not certain. These vary depending on the group, but they may include:

- Underside of the head, including pectoral fins if possible
- Close-up of each dorsal and caudal fin
- Close-up of teeth
- *Angel sharks, sawsharks and wobbegongs are the exception: use a dorsal (top) image for these. (See *Fig 7*)

RAYS

A dorsal (top) view is the best to photograph a ray. If the ray is dirty, rinse the body but be careful not to wash off any colour – this is the

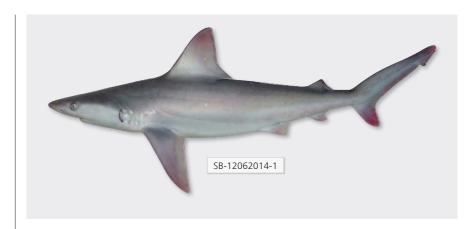


Fig 6: Sandbar shark (Carcharhinus plumbeus) (Source: C. Rigby)



Fig 7: Eastern Angelshark (Squatina albipunctata) (Source: C. Rigby)

mucous layer. Lay the ray flat with all fins unfolded. For skates, shark-like rays and electric rays, align the dorsal and caudal fins. For those with long tails (e.g. stingrays, eagle rays, cownose rays), leave the tail straight until the spine then curve the rest of the tail upwards towards the snout, as close to the disc apex as possible – there should only be



Fig 8: Brown stingray (Bathtytoshia lata) (Source: C. Rigby)

one curve in the tail, near the spine, then straight again. (See *Fig 8*)

Useful secondary images may include:

- Underside of disc
- Mouth and nostrils
- Lateral view of tail (when caudal find or skin folds are present)

SHARK FIN IDENTIFICATION GUIDES

A number of guides have been developed to help identify shark species from their fins. These guides focus on species that are most common in the international fin trade, or that are of concern because of their conservation status. They use key features of the dorsal, pectoral and caudal fins; and include photos of fresh to partially dried fins. The fin guides have been collated and are available online for free download at *www.cites.org/prog/shark/resources.php*

GILL PLATE IDENTIFICATION GUIDES

The gill plates of some mobulid rays are traded. Mobulid rays include two species of manta rays and six species of devil rays – gill plates from manta rays and devil rays can be visually distinguished. There's a free field identification guide available at *https://cites.unia.es/cites/file.php/1/files/pew-manta-ray-gill-plate-id-guide.pdf*



EQUIPMENT

Taxonomic identification doesn't require much equipment. In the field it's important to record as much data as possible, and it's worth preparing data sheets and labels in advance.



CHECKLIST

- Measuring tape
- Gloves
- Identification guide(s)
- Regional field guide(s)
- Waterproof data sheets and clipboard
- Waterproof labels
- Pencil and eraser
- Waterproof marker for labels
- Camera with batteries, charger (adaptor if needed), memory cards or mobile phone for photos



TECHNICAL LEVEL – EASY

It's useful to have some training in the use of taxonomic keys, and knowledge of the main features of sharks and rays. You should familiarize yourself with a region's identification guides before trying to use them in the field.

Training in photography for identification purposes, and recording useful field and laboratory notes, would be helpful.



TRAINING: SHARK FIN IDENTIFICATION

Training is recommended for shark fin identification, particularly for agents (e.g. customs officers) who are required to enforce fin trade regulations. Specialist NGOs and the UN FAO can offer advice and assistance on training of this kind.



COST - LOW

Visual identification using taxonomic identification guides is the simplest and lowest cost option for shark and ray species identification. Many guides can be downloaded from websites for free.

Shark fin identification costs can be higher as training is needed. However, costs can be minimized by training the trainers – regional representatives travel to a central location for training, and then train more agents in their respective regions.



REGIONAL SHARK AND RAY IDENTIFICATION GUIDES

There are numerous regional shark and ray identification guides. These websites offer free downloads:

CITES shark and ray portal:

% www.cites.org/prog/shark/resources.php
The CITES website organizes the guides by FAO geographical area, species and language.

FAO International Plan of Action for Conservation and Management of Sharks:

% www.fao.org/ipoa-sharks/tools/ipoa-sharks-documents
The FAO IPOA website organizes guides by publication date.

FAO Fishfinder Species Identification and Data Programme:

⋄ www.fao.org/fishery/fishfinder/publications

The FAO Fishfinder website includes guides to many species, and organizes them by regional guides, field guides, pocket guides and onboard guides, species synopses and fact sheets.

Shark References:

% http://shark-references.com

The Shark References site provides detailed species descriptions with links to relevant literature and identification guides.



GENETICS

Author: Sharon Appleyard, CSIRO National Research Collections Australia





SUPPOR"









GENETIC ANALYSIS PROVIDES ACCURATE **SPECIES IDENTIFICATION**



GENETIC METHODS PROVIDE UNIQUE DATA ON STOCK STRUCTURE (INCLUDING PARENTAGE), **POPULATION SIZE AND DISTRIBUTION**

DATA TYPE:

Species identification Landings and discards species identification Stock structure

SHARK PLAN OBJECTIVES:

- 2. Assess threats to shark populations
- 3. Identify and provide special attention, in particular to vulnerable or threatened
- 9. Facilitate improved species-specific catch and landings data and monitoring of shark catches
- 10. Facilitate the identification and reporting of species-specific biological and

WHY WOULD YOU USE THIS TOOL?

Genetic analysis provides accurate species identification from only a small tissue sample. It can be used when an individual animal is difficult to identify through visual examination (see § Taxonomy), or if the whole animal is no longer accessible. Genetics is also used to confirm that a visual identification is correct.

In addition, genetic analysis is the main tool used to identify species from shark and ray products including shark fins, mobula gill plates, meat, cartilage, liver oil and skin.

Genetics can be used to identify most species with certainty and is a rapid, reliable and relatively inexpensive method. The catch composition, landings and discards of fisheries can all be rigorously monitored using genetics. Numerous countries have used it to detect misidentified processed shark species in their fisheries; and it's an effective tool for monitoring and law enforcement in the domestic and international shark product trade.2

Genetic methods can provide unique data on stock structure as well as population size and geographical distribution.³ This can be useful for fisheries managers in identifying, assessing and responding to threats to stocks – but the techniques involved in these analyses are more complicated than for species identification, and they aren't described in this guide.

CITES

The Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) aims to protect species of sharks and rays threatened by international trade. Trade in products from the listed species is managed through a system of permits and certificates to ensure that the product is legally sourced, and that trade will not be detrimental to the survival of the species.

More than 20 shark and ray species are listed under the three Appendices to the Convention. They include sawfishes, hammerheads, thresher sharks, basking shark, white shark, porbeagle, silky shark, oceanic whitetip shark, mobulid rays, freshwater stingrays and whale shark. The CITES listings are regularly updated and can be checked at www.cites.org/eng/app/appendices.php.

Genetic analysis of shark and ray products can be used to separate CITES from non-CITES listed species to enforce the trade regulations.

WHAT IS GENETIC ANALYSIS?

A species' genetic code – the information that makes it develop differently to all other species – is controlled by DNA (deoxyribonucleic acid). DNA is found in every cell in an animal's body.

DNA is like a chain made of repeating units of four nucleotide bases: adenine (A), guanine (G), cytosine (C) and thymine (T). The order of these four bases is unique to each species, and it's this order that's examined to determine the species identification.

Genetic analysis involves sequencing a particular part of the DNA, usually cytochrome c oxidase subunit 1, known as COI. Sequencing is the process of determining the precise order of the four nucleotide bases within COI.

When a COI sequence has been established, it can then be matched

to international databases of COI sequences which contain known species of sharks and rays.

The most commonly used databases are the Barcode of Life Database (BOLD – **%** www.boldsystems. org) and the National Centre for Biotechnology Information (NCBI – **%** www.ncbi.nlm.nih.gov).

GENETIC SAMPLES

Genetic samples for DNA extraction can be collected from wet or dry tissue. The sampling method will depend on the country and access to the samples, preserving solutions and freezers.

Wet tissues – such as fin clips from freshly caught animals or muscle and liver tissue – are usually preserved in ethanol, high salt solutions or frozen prior to being used for DNA extractions. Dried tissues – usually a small clip taken from a shark fin – don't have to be stored in any preservatives prior to analysis.

METHOD: GENETICS

Effective tissue sampling is crucial for successful DNA analysis. It's important to note the following points:

- Sample as soon as possible after collection or access to the animals; try to keep the sampled tissue cold and dry.
- Sample tissues cleanly to avoid contamination. The knife/scalpel/ blade/scissors used should be cleaned between samples, using water or 70% ethanol.
- Only include one tissue sample (5mm cube or 150mg) from each shark or ray per sample tube/envelope.
- Place a label with a unique sample identifier (USI) into each sample tube. If possible, the data sheet that accompanies the sample should have the date of capture, latitude and longitude location, name of person that collected the sample, sex of the animal and length if measured, and the presumed species identification.
- Determine the most suitable method of preserving and storing the sample. In general, if a tissue is wet it should be stored in ethanol, DMSO, squashed onto a Whatman® FTA® Elute card, or frozen. If the tissue is dried, it doesn't require any further treatment and can be stored in a tube or small paper envelope. If it's in a tube, make sure the screwcap lid is tightened.

WHATMAN FTA ELUTE CARDS

One option for preserving and storing wet tissue samples is to use the specially developed Whatman FTA Elute sampling cards. These are designed to simplify the handling and processing of DNA, and can be used with little training – see below for more details.

WET TISSUE SAMPLING

The most common wet tissues sampled from sharks and rays include fresh fins, gill plate tissue, muscle, liver, heart, eye and tissue around the vertebrae.

- Take great care not to touch the tissue with fingers wear gloves if possible.
- 2 Cut a small piece of tissue from the animal: a 5mm cube or 150 milligrams will be enough. The tissue can come from anywhere on the shark or ray, but an easy way is to take a V-shape from the edge of the gill slit with scissors, or through the softer skin near the pelvic fin using a scalpel blade. Scissors are best for taking clips from fresh shark fins.
- 3 Place the sample in a screwcap tube.
- Cover the tissue with 70-100% ethanol (ensure the ethanol contains no methanol). Alternatively, cover the tissue with a high-salt-based solution, e.g. DMSO (see DMSO Recipe in Appendix A). The tissue should not be more than one-third of the volume of the preservative in the tube. Water itself is damaging to DNA; ethanol or high salt solutions dehydrate the tissue and help to preserve the DNA.

If there's access to a -20°C freezer, tissues can be stored frozen for up to a year – when sending them for testing, it's important to find a freight company that can transport frozen goods.

- 5 Ensure the sample is labelled with its unique sample identifier (USI). Write or print the USI on a waterproof label and place it into the tube with the sample. If ethanol is used as the preservative, only use pencil.
- 6 Make sure the screwcap lid is well tightened.

WET TISSUE SAMPLING

- WHATMAN FTA ELUTE CARDS

If available, Whatman FTA Elute cards offer a number of advantages:

- Sampling and storage of tissue in one easy step
- Suitable for use in the field and in areas with limited power, no freezer or access to preservative solutions
- Reduces labour and transport costs
- Room temperature storage, no need for freezing or buffer solutions
- Application and processing can be done in the field or laboratory
- Fast technology for analysis of DNA



Fig 1: Sampling for shark tissue – do not touch the tissue sample. (Source: Sharon Appleyard, CSIRO)



Fig 2: Place tissue into a screwcap tube. (Source: Sharon Appleyard, CSIRO)



Fig 3: Shark muscle tissue in screwcap tube containing ethanol. (Source: Sharon Appleyard, CSIRO)



Fig 4: Shark fin clip (with a USI on label) in screwcap tube containing ethanol. (Source: Sharon Appleyard, CSIRO)

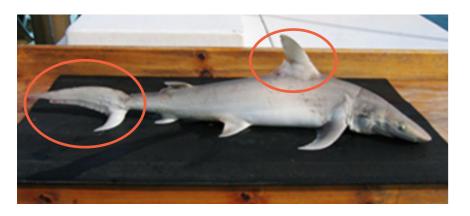


Fig 5: Caudal and dorsal fins of a shark. (Source: Sharon Appleyard, CSIRO)

HOW TO USE THE CARDS:

1 Either rub the FTA Elute card across the caudal or dorsal fin of the shark, or take a small, thin piece of tissue (e.g. fin, gills, muscle, liver, heart, skin scrapings). Fins from recently captured sharks can also be sampled in this way; while frozen shark fins or meat should be thawed and wiped dry before sampling. The cards aren't suitable for use with dried fins.

2 Use blunt end forceps to squash sampled tissue into a circle on the FTA Elute card. Clean the forceps between sampling different individuals, using water or 70% ethanol.

3 If using the whole card with four circles for one individual, take at least two samples. If cutting the card in half to use for two individuals, make sure the correct sample information is stored with each card half. It's best not to cut the card into four, as this does not leave enough room around each circle to describe the sample.

After pressing the samples on to the card, leave it open to air dry for 2-3 hours.

5 When the sample is dry, close the card and write the sample

information in the space provided:

- Presumed species identification, if there is one
- USI number
- Date of capture
- Location of capture (place name, latitude and longitude if possible)
- Name of the person that collected the sample

6 FTA Elute cards can be kept in long-term storage before the DNA is extracted, but it's important to keep them dry. Cards should be stored at room temperature in a dark, dry cupboard or drawer until they're required, preferably in a paper folder with small silica gel packs. Never store the cards in zip lock plastic bags. In tropical climates, the cards should be stored at all times in an airconditioned room.

For information on extracting DNA from the Whatman FTA Elute cards, please see Appendix B.

DRY TISSUE SAMPLING

Dry tissue samples are most commonly clips collected from dried shark fins.

1 Fin clips from dried fins should be taken using scissors.

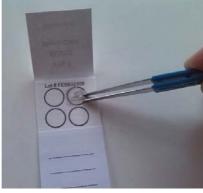


Fig 6: Squash a small piece of tissue onto the card. (Source: Sharon Appleyard, CSIRO)

2 Place the fin clip in either a screwcap tube or in a small paper envelope. Use one envelope per sample. There's no need for liquid preservative for dried fin clip samples, although they won't be damaged if they're stored in ethanol or DMSO.

3 As with all sampling, ensure that scissors are cleaned with water or ethanol between sampling of individuals and use a USI for each sample.

eDNA

A new technique for species identification using environmental DNA (eDNA) is being researched and developed for sharks and rays. It uses DNA from skin, mucus and other tissues and cells left in the water. The technique is nondestructive, meaning the animals themselves don't need to be directly sampled: they can be detected from DNA analysis of a water sample in which they have recently been present.4 The method is currently beyond the scope of this toolkit, but it may be useful in future when it has been refined and simplified.

PACKAGING SAMPLES FOR TESTING

Once genetic samples have been obtained, they can be sent for laboratory analysis.

Each sample should be placed in an appropriately labelled and packaged box. Dried fin clips and Whatman cards can be placed in an envelope.

Include as much relevant information as possible for each sample. This could include:

- USI
- Presumed species identification
- Location where the shark or ray was caught (place name, latitude and longitude if possible)
- Date of capture
- Depth it was caught at
- Gear type used
- Date of identification
- Name of person that identified the animal

- Sex of the animal and length if measured
- Name and contact details of person who collected the sample
- Preservative (if used) and dilution and Material Safety Data Sheet (MSDS)*
- * If ethanol is used as the preservative, ensure the package meets IATA Special Provision 180 for shipping non-infectious samples by air with small amounts of ethanol (UN 1170) (Special Provision in the IATA Dangerous Goods Regulations see below).

SEQUENCING COMPANIES

Prepared samples can be sent to a 'fee for service' company for DNA sequencing in a laboratory, the first step in genetic identification. Costs and procedures vary, so it's important to contact the companies first to find out what's required.

Some sequencing companies are:

- BOLD (§ www.boldsystems. org)*
- BGI (**%** www.bgi.com/global)
- Ramaciotti Centre for Genomics
 (% www.ramaciotti.unsw.
 edu.au/sequencing/sanger sequencing)
- AGRF (§ www.agrf.org.au)
- * If a sample is being sent for genetic verification of a presumed visual identification, the BOLD Systems website taxonomy tab has details of sequencing laboratories available for particular species:
- **%** www.boldsystems.org/index. php/TaxBrowser_Home.

SPECIAL PROVISION IN THE IATA DANGEROUS GOODS REGULATIONS

A180 Non-infectious specimens, such as specimens of mammals, birds, amphibians, reptiles, fish, insects and other invertebrates containing small quantities of <u>UN 1170</u>, <u>UN 1198</u>, <u>UN 1987</u>, or <u>UN 1219</u> are not subject to these Regulations provided the following packing and marking requirements are met:

- (a) specimens are:
 - 1. wrapped in paper towel and/or cheesecloth moistened with alcohol or an alcohol solution and then placed in a plastic bag that is heat-sealed. Any free liquid in the bag must not exceed 30 mL; or
 - 2. placed in vials or other rigid containers with no more than 30 mL of alcohol or an alcohol solution;
- (b) the prepared specimens are then placed in a plastic bag that is then heat-sealed;
- (c) the bagged specimens are then placed inside a another plastic bag with absorbent material then heat sealed;
- (d) the finished bag is then placed in a strong outer packaging with suitable cushioning material;
- (e) the total quantity of flammable liquid per outer packaging must not exceed 1 L; and
- (f) the completed package is marked "scientific research specimens, not restricted Special Provision A180 applies".

The words "not restricted" and the special provision number A180 must be included in the description of the substance on the Air Waybill as required by 8.2.6, when an Air Waybill is issued.

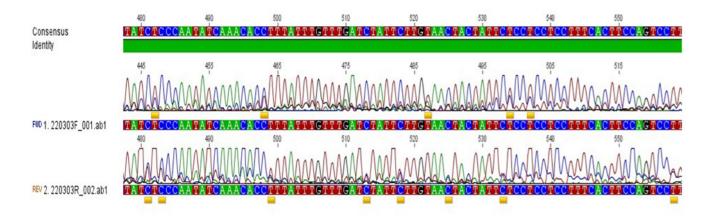


Fig 7: mtDNA CO1 sequencing in shark DNA – Forward and Reverse sequences. (Source: Sharon Appleyard, CSIRO)

DNA ANALYSIS: THE PROCESS

The identification of a species from the DNA in a tissue sample involves both laboratory and software analysis.

1 First, the DNA is sequenced in a laboratory to determine the unique order of its four nucleotide bases. This produces raw F(orward) and R(everse) sequences. Most sequencing companies provide customers with these raw F and R sequences.

These F and R sequences then need to be merged to generate a COI barcode, using sequencing software such as Geneious (§ www.geneious.com).

3 The COI barcode of the sample can then be matched to COI sequences stored in free-to-use international databases such as BOLD and NCBI. This will genetically identify the exact shark or ray species from which the sample was collected.

As a final check, verify that the USI of the genetic identification matches the sample collected.

Rapid Assessment Toolkit I Sharks and Rays

It helps if there are photos of the specimen from which the sample was taken, with the USI on a label. This final step is best taken with a person who has expertise in shark identification.

If there's no in-country expertise to generate the COI and match it

to an international database, the tissue sample or the extracted DNA can be sent to BOLD (**%** www. boldsystems.org), who will take care of the process for a fee. Generally, sequencing companies do not undertake analyses for single samples; a minimum of 48 samples is often required.

CITES

If a tissue sample is collected from a CITES-listed shark or ray species and needs to be sent out of a country for the (non-commercial) purpose of species identification, there are exemptions to the requirement for export permits. The national CITES Management Authority (MA) can provide the appropriate documentation.

The tissue samples should be sent from a scientific institution registered by the CITES MA in the country in question. A list of registered scientific institutions around the world is available at **%** *www.cites.org/common/reg/e_si.html*. If there are no institutions registered in the country from which tissues need to be sent, contact the CITES MA of the country to seek advice.

Tissue samples from CITES-listed species must also be sent to registered scientific institutions, so it's important to check the status of genetic sequencing companies.

Provisions to further simplify the export of scientific samples are currently under consideration by CITES: for up-to-date information and advice, contact the relevant MA.



EQUIPMENT

Genetic sampling requires equipment for taking the samples and storing the samples. In the field it's important to record as much data as possible, and it's worth preparing data sheets and labels in advance.



CHECKLIST:

- Knife/scalpel blade/scissors
- Cutting board
- Blunt end forceps
- 70% ethanol for cleaning implements (can use a plastic spray bottle)
- Tissues or wipes for cleaning and drying implements
- Paper envelopes and screwcap tubes; sample boxes for the tubes
- Whatman FTA Elute cards; nonindicating classic card
- 70-100% ethanol (not containing methanol) for tissue preservation
- DMSO solution
- Plastic disposable pipette for filling tubes with preservative solution
- Waterproof data sheets and clipboard
- Pencil and eraser
- Waterproof labels
- Waterproof marker for labels
- Camera with batteries, charger (adaptor if needed), memory cards or mobile phone for photos

To extract DNA from FTA Elute cards, some laboratory equipment will be needed:

- Punch tool and mat supplied with cards
- 1.7ml microfuge tubes
- Sterile water
- Heat blockCentrifuge
- Agarose gels



TECHNICAL LEVEL - MEDIUM

Tissue sampling is in itself a straightforward process, and can be undertaken after basic training on sample collection, data recording, storage and transport of samples. A key consideration is to collect the samples cleanly to avoid cross-contamination, and to keep them separated from each other at all times. It's very important that the USI for the sample accompanies the tissue, and that metadata (data on the shark species and location associated with the tissue sample) is strictly maintained.

The subsequent extraction of DNA requires a specialized molecular laboratory (see Appendix B) and trained technical staff. DNA sequencing and analysis for species identification requires specialist skills and training, specific laboratory equipment, software, computing resources that include access to the internet, and data repositories.



COST - MEDIUM

Collection of tissue samples has minimal associated costs. Dried tissue (e.g. fin clips) is the cheapest to collect, as no preservative or FTA Elute Cards are needed.

Ethanol is the best preservative for genetic tissue samples, but it can be expensive and difficult to obtain in some countries. In such cases DMSO solution is a good alternative, although long-term storage in DMSO is not advisable and transfer to ethanol is recommended. Prices of ethanol and DMSO solution vary between countries. Screwcap tubes and boxes to hold 100 vials also vary in price internationally.

Whatman FTA Elute cards can be sourced from chemical supply companies, although prices may vary. For example:

US\$115 FOR 25 CARDS US\$525 FOR 100 CARDS

The cost of obtaining a species identification from a tissue sample or DNA extract varies with the provider, the species and the quality of the tissue sample. Some closely related species may need a second marker (e.g. NADH2 in addition to COI) to confirm identification. Older or badly stored tissue samples can require repeated analysis, which can increase the cost. Most sequencing providers run the analyses on batches of samples, such as 48 or 96. Sending a large batch of samples is more cost-effective.

FURTHER INFORMATION

For more information on the Genetics tool, please contact Dr Sharon Appleyard, Australian National Fish Collection, National Research Collections Australia.

T: +61 3 6232 5458 E: sharon.appleyard@csiro.au W: www.csiro.au/en/Research/Collections/ANFC



CREEL AND MARKET SURVEYS

Author: Cassandra L. Rigby, James Cook University; William T. White, CSIRO National Research Collections Australia; Victoria Jeffers, Fran Humber, Blue Ventures Conservation





3. SUPPORT

2. Assess thre 3. Identify and threatened sh

DATA TYPE:

Species present Stock structure Catch abundance Landings and discards Fishery description and fishing effort

SHARK PLAN OBJECTIVES:

- 2. Assess threats to shark populations
- 3. Identify and provide special attention, in particular to vulnerable or threatened shark stocks

WHY WOULD YOU USE THIS TOOL?

Creel and market surveys collect information on the sharks and rays being fished in a country, and monitor catches over time to detect any changes in the stocks.

- Creel surveys (also known as fisher surveys or landing site surveys) aim to assess the status of the resource being fished and estimate fishers' catch and effort. Data collected includes area fished, fishing gear, species catch composition, length/weight of species, effort taken to catch species, costs of fishing, and income received from the sale of the catch. They're a fishery-dependent form of sampling, since the species and sizes of sharks and rays being caught will depend on the gear used and the area fished.⁵
- Market surveys aim to collect data on the catch when it is sold. Information is obtained from those selling sharks and rays direct to the public or to traders, whether for subsistence or commercial purposes. Like creel surveys, market surveys collect data on species catch composition and length or weight of the species, but the focus is on the economic value of the catch rather than the fishery description and effort.

Both methods are particularly suitable for small-scale fisheries in developing countries as they're inexpensive, require minimal equipment and can provide informative data. There are other fishery survey methods, such as on-board observers and research surveys, but these are more resource-intensive and are typically used in developed countries and large-scale commercial fisheries.

DATA USE

Data from these surveys can be used to assess the degree of threat of the fisheries to shark and ray populations by monitoring the species captured, the numbers/weights taken, and the level and area of fishing pressure. Changes in prices over time can reveal fluctuations in demand for shark products. The surveys also provide data on the stock structure of the species being captured by recording their sex, size and maturity stage.





This combined biological and fisheries data informs stock assessments. These provide scientifically robust information to fisheries managers for the regulation of fisheries to ensure shark and ray stocks are fished sustainably.

Market and creel surveys are also useful for assessing the importance of sharks and rays to communities, regions and countries. Information on how much food security and livelihoods depend on sharks and rays is essential in forming sustainable fishery management plans.

DESIGNING AN EFFECTIVE SURVEY

Creel and market surveys are focused on collecting stock and catch data in coastal fisheries to provide advice to fisheries managers, fishers and local communities about the status of sharks and rays in their area. This information can then inform action and management strategies. It's important to clearly define the purpose of a survey, along with how the information will be used and by whom.

Fisheries, markets and cultural protocols vary widely, and it's important to note that there's no such thing as a generic creel or market survey: each needs to be suited to its local context and appropriate for the interviewees. This tool describes the types of fishery and catch data that may be important to collect, along with some basic procedures for collecting it, but there are also regionally specific resources which include survey questionnaires and methods for approaching villagers, fishers and traders.



Some examples include:

White et al. 2014; Glaus et al. 2015; Kaly et al. 2016; Humber et al. 2017; SEAFDEC 2017; Johnson et al. 2018; Martins et al. 2018.

METHOD: CREEL AND MARKET SURVEYS

Define the objectives of the survey, and decide if they're best addressed by a creel or market survey, or both.

2 Design the survey(s).

Determine what information needs to be collected, and what questions will need to be answered to obtain it (see **Appendix C**).

Consider how many repeat surveys will be needed to detect trends in the fishery and catch data. It's rarely possible to survey all landing sites and markets: a well-designed survey will choose representative sites to give a realistic overview.

Identify the main landing sites of sharks and rays in the area. If there are several, select those that are most accessible, have space to work in, and where there is cooperation from local fishers and traders. Permission will also be needed from local authorities.

Research the market to ensure you're aware of, for example, the usual time of day for landings. Local contacts may be needed for this purpose.

3 Plan the logistics: who will do what, and when? As well as

conducting interviews and collecting data, consider how the data will be collated, analysed, stored, summarized and presented to fisheries managers. Identify team members, allocate responsibilities, train them if required. Develop a work plan and timetable. Is there enough budget to cover all necessary resources?

Local community members often make very good data collectors, but consider factors such as literacy, education, age, social standing and other cultural considerations when choosing a team.

Design and prepare the data sheets. Depending on the context and local infrastructure, data can either be recorded on paper or via a mobile phone app. When used appropriately, mobile monitoring can speed up data entry and produce results ready for real-time analysis, avoiding the risk of a backlog of paperwork to process.

for data components to include). At the start of every survey, clearly explain the purpose, and ensure you obtain prior informed consent from participants. Note that cultural protocols vary widely, and be aware of what's appropriate: for example, in Fiji permission to interview fishers may need to be requested from the village chief, who designates which fishers will take part.⁶ If you're likely to need to make repeated visits, consider if you can offer anything in return for continued cooperation.

6 Check and analyse the data, and report the results. This step – which of course depends on the nature and purpose of the survey – is beyond the scope of this tool. Please see the resources section for more guidance.

SURVEY DATA

GENERAL INFORMATION

The following data should always be recorded:

- Name and location of landing or market site (place name and latitude/longitude if possible)
- Date
- Name of fisher/seller (optional, to remain confidential)
- Gender of fisher/seller
- Time of survey
- Name of data collector

FISHERIES DATA

Aim to describe the fishing fleets, fishing grounds and the importance of the fishery to the community/ region/country.

Relevant information includes:

- Number of vessels
- Vessel home port and nationality
- Gear characteristics and selectivity (e.g. gillnet height and mesh size; hook type and size, distance between hooks and baits, if wire trace used; trawl net dimensions, cod-end meshsize)
- Seasonal patterns in fishing
- Fishing locality in relation to distribution of shark and ray stocks and other fleets
- Type of habitat fished (e.g. coastal, coral reef, oceanic)
- Vessel power and size
- Navigational aids to assist fishing (e.g. GPS)
- Freezer/ice capability

Background research on fishery:

 Economic and social dependence on the fishery

- Costs and benefits to the community/region/country
- Fishery access/ownership
- Fishery history and local names for fishery areas
- Identity of fishery decisionmakers
- Perceived challenges/issues faced by fisher community

Individual vessel/fisher data:

- Date(s) of fishing
- Location(s) fished
- Depth(s) fished
- Whether fishing alone or with others (if so, how many?)
- Name of main fisher (optional)
- Frequency of fishing trips
- Gear characteristics and selectivity (gillnet height and mesh size; hook type and size, distance between hooks and baits, if wire trace used; trawl net dimensions, cod-end meshsize)
- Day or night fishing
- Duration of fishing, including soak time for nets and lines or distance trawled
- If specific gear is used to catch sharks or rays
- Average number and weight of sharks and rays caught per fishing trip
- Species of sharks and rays caught
- Sex ratios of catch what proportions are male/female?
- Fate and number of discarded sharks and rays (by species if possible, including size and sex)
- Shark and ray landing location and frequency
- Costs of fishing
- Average income from shark and ray catch

CATCH DATA

The key information to record is the number of each species, location

and date. Sex, length and maturity are also important. If possible, each individual shark or ray landed should be examined, but where there are large numbers a subsample can be identified, measured and weighed. The optimum sub-sample size will depend on resources and the number of boats and animals involved

Sharks are often processed at sea and may be headed, gutted and finned at landing. The form in which they're landed needs to be noted, particularly when weights are used to record the catch: conversion factors can then be applied to estimate the biomass captured. Where possible, identify the species: if needed, photos can be taken for expert identification and tissue samples collected for genetic analysis (see & Taxonomy and & Genetics tools).

MARKET DATA

Market surveys should collect data that usually includes:

- Source of sharks and rays for sale
- Form of catch: whole/trunks/ fins etc
- Whether vendor pays for the sharks and rays – and if so, how
- Price of each shark or ray species (either whole or product)
- Costs of marketing: stall rental/ electricity/wages etc
- Whether vendor processes the sharks and rays
- How long vendor has been selling sharks and rays
- Whether vendor sells sharks and rays elsewhere – and if so, where
- What percentage of vendor's income is provided by the sale of sharks and rays

GATHERING KEY DATA ON INDIVIDUAL SHARK AND RAY SPECIMENS

SPECIES IDENTIFICATION

Wherever possible, identify shark and rays in the catch or market by species (see § Taxonomy tool). Record local names and match them to scientific names to develop a reliable list of species present.

MALE OR FEMALE? SIZE



Fig 1: Male shark with claspers. (Source: NOAA)



Fig 2: Female shark. Claspers absent. (Source: NOAA)

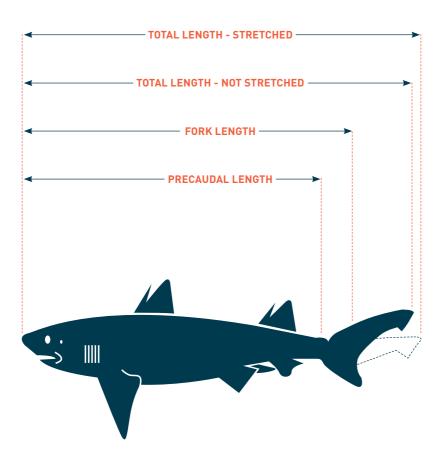


Fig 3: Length measurements of a shark.

Length – sharks

There are different ways to measure the length of a shark. Total length (stretched) is most common, where the shark's body is straightened with the top of the tail in line with the body. Fork length is used in some larger sharks with less flexible tails, while precaudal length can be used if the tail is damaged. In all cases lie the tape flat, not along the body (the latter will curve the tape and give an inaccurate measurement).

Coastal species for which total length is measured include all sharks, sawfishes (Pristidae),

wedgefishes (Rhinidae), guitarfishes (Rhinobatidae), giant guitarfishes (Glaucostegidae) and electric rays (Narcinidae, Narkinidae, Hypnidae, Torpedinidae).

Disc width – rays

Make sure the ray is lying flat, with the dorsal (top) side facing up. Coastal rays for which disc width is measured include stingrays (Dasyatidae), eagle rays (Myliobatidae), pelagic eagle rays (Aetobatidae), butterfly rays (Gymnuridae), cownose rays (Rhinopteridae), mantas and devil rays (Mobulidae).



Fig 4: Disc width measurement of a ray.

WEIGHT

For sharks and rays weighing up to 10kg, hanging scales are best – a basket can be used, with the scales adjusted for its weight. For large species, weights are estimated – ensure estimates are noted as such.

MATURITY

There are different processes for determining the maturity stage of male and female specimens.

In case of uncertainty, take photos for expert confirmation – don't forget to clearly label each specimen with a unique sample number (see § Labels).

MALE

An external examination of the claspers is the simplest way of determining male maturity. Two criteria are involved:

1 The length of the clasper in relation to the pelvic fin tip

2 The degree of development (hardness) of the clasper

These will indicate one of three male maturity stages:

1 Juvenile, immature – clasper very short, not extending past pelvic fin tip, flexible



Fig 5: 1. Juvenile immature male. (Source: W. White and L. Baje 2014)

2 Adolescent, maturing – clasper extending past pelvic fin tip, not completely hard, still flexible



Fig 6: 2. Adolescent immature male. (Source: White. W. and Baje. L 2014)

3 Adult, mature – clasper extending past pelvic fin tip, hard along entire length



Fig 7: 3. Adult mature male. (Source: C. Rigby)

FEMALE

Female maturity can't be determined externally – dissection is required in order to examine internal reproductive organs. Typically, one of five stages of maturity will be recorded:

1 Juvenile, immature – uteri very thin, ovaries small and without yolked (yellow) eggs

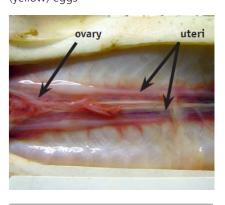


Fig 8: 1. Juvenile immature female

Adolescent, maturing – uteri slightly enlarged at one end, ovaries becoming larger and small yolked eggs visible

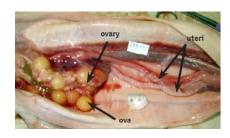


Fig 9: 2. Adolescent, maturing female

3 Adult, mature – uteri large along entire length, ovaries containing large yolked eggs



Fia 10: 3. Adult mature female

4 Pregnant – uteri containing embryos or large eggs

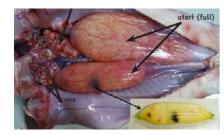


Fig 11: 4. Pregnant female

5 Post-partum – uteri very large but without embryos (birth recently occurred)



Fig 12: 5. Post-partum female

Pregnant females: if a female specimen is found to be pregnant on dissection, it's important to record data on the litter:

- Number of embryos in each uterus
- Number of male and female embryos
- Length of each embryo

BIOLOGICAL SAMPLES

Some surveys include the collection of vertebrae or dorsal fin spines for ageing in a laboratory.

To remove vertebrae:

- 1 After the internal organs are removed, the vertebrae are visible along the body cavity.
- With a sharp knife, cut either side of the vertebrae under the first dorsal fin.
- 3 Cut out (approx.) five vertebrae.
- Trim away excess flesh and place vertebrae in plastic ziplock bag with a label and USI (see **Labels**).
- 5 If possible, freeze vertebrae. If not, clean vertebrae of flesh as soon as possible depending on size 5-10 minutes in mild household bleach will speed the process.

If a genetic sample is required for the survey, see the **§ Genetics** tool.

LABELS

It's essential to label individual sharks and rays when samples are taken or whole animals are kept for later examination. Labels should also be included when photos of specimens are taken at landing sites or markets (see § Taxonomy tool – photo).

Each individual animal or sample needs a USI so that site and biological data can be linked to individual specimens in later processing.

The same numbering system should be used across the survey, and work equally for whole animals, photos, and parts such as fin clips or embryos.

EXAMPLE

Town and port name: use two initials to represent the place, e.g. AB **Date:** day, month, year, e.g. 01052018

Animal number: start at 1, and give each animal a unique sequential number

AB-01052018-1 is the USI for the first individual animal sampled at AB Port on 1 May 2018.

Write each USI on the datasheet and on waterproof labels kept with each specimen or sample. For photos, write the USI in dark pen on a label and place it next to the animal – make sure it's visible in the picture.



Fig 13: Piked spurdog (Squalus megalops) with label. (Source: C. Rigby)

MOBILE MONITORING

There are many apps and programs available for recording data on mobile phones or tablets. Some free options may have restrictions on user numbers or form submissions, so it's important to consider a survey's size and scope before choosing which tool to use.

Options include:

- Sopen Data Kit (ODK) offers a free open source suite of data collection tools to use including the ODK app, called ODK Collect. ODK offers an Excel template for form creation – a free step-by-step guide is available from Selue Ventures toolkits. The guide also includes tips on choosing and training data collectors, selecting equipment and troubleshooting in remote regions.
- Ona (§ https://ona.io) and Survey
 CTO (§ www.surveycto.com) allow forms to be built quickly and simply and then connected to an online server.
- Tails was developed for small-scale tuna fishers in Pacific countries to record fishery data, but it can also be used to record details of a large number of shark species taken as bycatch.



Take a photo of the underside of the shark's head

Take Picture
Choose Image

Fig 12: Example questions from the OKD phone-based app. These questions repeat according to the number of sharks caught per fisher. (Source: Blue Ventures)



EQUIPMENT

No specialist equipment is needed to conduct a creel or market survey.



CHECKLIST

- Identification guide(s)
- Regional field guide(s)
- Measuring tapes, plus calipers or fish measuring boards for smaller specimens
- Scales
- Maps or aerial photos of the fishing areas
- Waterproof data sheets and clipboard
- Waterproof labels
- Pencil and eraser
- Waterproof marker for labels
- Camera with batteries, charger (adaptor if needed), memory cards or mobile phone for photos

For dissections

- Sharp knife
- Plastic ziplock bags



TECHNICAL LEVEL – EASY

It's advisable to appoint a survey team leader and ensure that each team member has had some training in their role. Volunteers can conduct interviews if they follow appropriate cultural protocols.



COST - MODERATE

Many identification guides can be downloaded from websites for free. Labour costs and expenses for transfers to market and survey sites will depend on the scope of the work, but may be high in some cases. The other main cost of surveys is likely to be related to phone running and software licensing.



BAITED REMOTE UNDERWATER VIDEO SYSTEMS (BRUVS)

Author: Michelle Heupel, Australian Institute of Marine Science; Colin Simpfendorfer, James Cook University.





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DATA TYPE:

Species present Abundance and size

SHARK PLAN OBJECTIVES:

6. Contribute to the protection of biodiversity and ecosystem structure and function.

10. Facilitate the identification and reporting of species-specific biological and trade data.

WHY WOULD YOU USE THIS TOOL?

BRUVS surveys are a valuable tool for determining which species are present in an area and estimating relative abundance between areas. They're a non-destructive sampling method that's simple to repeat, reliable and cost-effective; and they're particularly useful for large-scale sampling of sharks and rays in a relatively short time.

BRUVS sample the sharks and rays in an area without humans in the water, whose presence might alter the behaviour of the animals. This unique characteristic of the sampling method sets it apart from traditional visual sampling by underwater visual census (UVC). BRUVS have minimal impact on the environment.

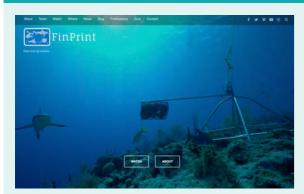
The BRUVS tool provides a permanent visual record of the species community composition. It can also be used to survey the habitat in the sampling area. Due to their ease of use in a variety of habitats, BRUVS may record the presence of

shark and ray species in areas that have not been covered by fishery surveys.

BRUVS can be used to study the effects of fishing and marine protected areas (MPAs) by comparing the diversity and abundance of sharks and rays within an MPA to those in similar nearby habitats exposed to fishing pressure. BRUVS with two cameras (stereo BRUVS) can be used to accurately measure the size of sharks and rays. Length data is useful for estimates of maturity stage, which gives an insight into the population structure of the species in the area.



S GLOBAL FINPRINT



The Global FinPrint project documents shark and ray populations in tropical coral reef ecosystems across the world. It provides important baseline data on species diversity, abundance and distribution across a large number of countries and reefs.

BRUVS data can be used to compare reefs with different features to determine the factors (e.g. habitat type, fishing pressure, prey fish densities) that influence shark and ray diversity and abundance. Global FinPrint has collected valuable information for shark and ray conservation and management, and it can be made available for local use.



- Cheap to build and use
- Collect large amounts of data
- Stereo BRUVS can measure lengths of individuals

LIMITATIONS:

- Results are biased to species attracted to bait, which may mean other species are underestimated
- Video does not work well in murky waters
- Footage analysis is labour-intensive
- Stereo BRUVS require calibration and potentially expensive software

WHAT ARE BRUVS?

BRUVS units combine bait to attract sharks and rays and an underwater video camera to record what animals come to feed on the bait. The units themselves consist of a frame with housing to hold a camera. A bait arm is attached to the frame, which includes a bait bag filled with crushed oily fish.

BRUVS are placed on the seafloor with a surface float for retrieval. Their frames are weighted to ensure they don't float or move. BRUVS are typically left on the seafloor for 60-90 minutes and then recovered. Observers can watch the video footage to determine the species and number of individuals filmed.



Fig 1: BRUVS on seafloor with bait arm with bait bag. (Source: Michelle Heupel)

Stereo BRUVS are more complicated than single camera BRUVS, with a camera mounted on each side of the bait arm. They also require camera calibration before use, so a calibration cube is needed.

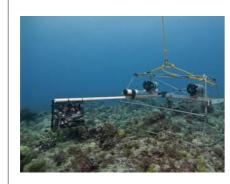


Fig 2: Stereo-BRUVS. (Source Steve Lindfield, UWA, NOAA – Image courtesy of pifsc.noaa.gov)



VIEW FOOTAGE



Fig 3: Stainless steel BRUVS frame with detachable legs cabled-tied in place. Dive weights are cable-tied on each leg. Holes have been drilled in the legs to allow the frame to be set at different heights. A current-meter is attached to the back end of the bait arm. (Source: Christian Sloater, Scubazoo)





Fig 4: Acrylic BRUVS with a camera inside each plastic drain pipe. (Source: Vanessa Jaiteh)

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DESIGNING THE PROGRAMME

Design the BRUVS sampling programme to address the objectives of the study. Consider the resources available for equipment, vessels, BRUVS deployments and analysis of the footage. This will affect the type of BRUVS used, the number of BRUVS and how many times they can be deployed.

Sampling designs vary: for example, a comparison of species diversity and abundance between areas open and closed to fishing will require a different design to an assessment of species diversity and abundance across all habitats in an area. Testing between fished and unfished areas simply requires the same number of BRUVS deployments in each location, while association of abundance with habitat requires representative numbers of deployments from all available habitat types.

For all sampling designs it's important to consider habitat type, as this can have a strong influence on the species present. Complex coral reef habitats are likely to have different types and numbers of shark and ray species to those present in open sandy areas. The habitat type must be recorded, otherwise it's difficult to determine whether any difference in diversity is due to the habitat or another factor such as the level of fishing pressure.



CONSTRUCTING BRUVS UNITS

A BRUVS unit is made of a frame, bait pole, bait bag and a camera. All units deployed in a survey should be identical in size and height off the bottom: most BRUVS are designed to stand 1m or less above the substrate, and ideally the substrate should take up half the field of view. The bait bag can either be set on the bottom, or raised in the water column.

FRAME

The BRUVS frame can be of any size and style and can be constructed from a variety of easily obtainable materials such as stainless steel, rebar, PVC pipe, acrylic, plastic or timber. Detachable leas make transport easier.

The frame needs to have weights attached to its legs to keep it steady on the seafloor. More weights are needed for lighter frames and in stronger currents – shorter frames are more stable in the latter. Points of attachment are needed for the rope and surface float.

Finally, the frame requires a housing for the camera. This should hold it steady during deployment but include a simple method for attachment and removal.

BAIT BAG, POLE AND BAIT

Bait bags can be made from plastic mesh, metal or PVC pipe with holes. In areas with a lot of larger sharks, the use of metal may prevent the sharks pulling the bait bag off the pole. Bait bags are often cable-tied to the bait pole.

The bait pole can be of any material and usually extends 1-1.5m from the camera.

Any type of oily fish makes good bait, but always use fresh bait for each deployment and ensure it's processed in the same way for all drops in a study (e.g. minced, crushed or chopped to release oil and increase attraction). It's important to plan how much bait will be needed for each field trip, where it will be sourced and how it can be stored on a vessel.

CAMERA

Any type of underwater video camera can be used in BRUVS, but GoPro cameras are particularly popular. The footage needs to be clear enough for viewers to reliably identify the sharks and rays it contains.

Filming in high definition mode of 1080p and 30 frames per second provides good resolution. Recording for 90 minutes at this setting will take up 20-25GB of storage space on a memory card.

If a number of BRUVS are to be deployed at the same time, multiple cameras, memory cards and batteries will be needed, so planning is important. Extra battery packs can allow the same camera to be used multiple times in a single day. If BRUVS are to be deployed on consecutive days, video from the memory cards will need to be downloaded and camera batteries recharged overnight. Multi-port chargers are useful to charge multiple batteries at once (*Fig 6*).

Stereo BRUVS have two cameras mounted at an angle – typically 7-8 degrees – on each side of the bait arm. Because these systems are used to make precise measurements, the cameras must be tightly fitted inside the housing



Fig 5: PVC pipe and caps with holes for bait to disperse attracts a tiger shark (Galeocerdo cuvier). (Source: Tonga-watch)



Fig 6: Multi-port charger. (Source: Samm Sherman)

so they can't move. Each stereo BRUVS has its own calibration that relies on maintaining the frame set-up and same camera on the left and right side of the bait arm for each deployment.

Camera technology continues to advance as costs go down. Fullspherical (FS) underwater videos can record a 360° field of view, so it's likely that they'll play a role in many BRUVS in future.



Fig 7: Deploying a BRUVS. (Source: Cordio East Africa-watch)



DEPLOYMENT

BRUVS are typically deployed from a boat. After deployment, it's sensible to keep the boat at least 200m away from the BRUVS to minimize the potential effects of boat noise on shark and ray behaviour.

A standard operating procedure should be followed in each study to minimize the effects of uncontrolled factors. The key factors to standardize are deployment time, amount of bait and depth range.

Deployment times are usually from 60 to 90 minutes on the seafloor. Each deployment typically uses about 1kg of bait. Depth ranges vary depending on the research question – deployments deeper than 50m require a heavy frame and a light source, and a winch may be needed to recover the unit. The length of rope from the BRUVS to the surface float should be around 1.5 times the depth of deployment. In strong currents this length may need to be increased to ensure the surface float is not pulled under.

If multiple BRUVS are deployed at the same time they need to be set far enough apart to minimize the possibility of sharks and rays swimming between them, resulting in individuals being counted more than once. The distance between deployments will depend on the study objectives – in the Global FinPrint project there was a minimum separation of 500m.



VIEWING VIDEO FOOTAGE

A standard method of recording species and counting individuals should be used when watching the video footage. Methods vary between studies, but the most widely used is to record MaxN



Depending on the camera type used, video files may need to be stitched together prior to viewing and analysis. For example, Go Pro video files are segmented during recording and stored separately on the memory card. There

are freely available and/or low cost software programs to stitch video segments into a single file for viewing, but this must be done prior to analysis.

(the maximum number of animals visible at any one time).

1 Record the habitat type the BRUVS settled in and estimate the water visibility.

2 Run the video, and pause it when a shark or ray is observed.

3 Identify the species, and sex if possible. If it's difficult to exactly identify the animal, identify it to the lowest taxonomic level possible – for example record the genus of hammerhead (*Sphyrna*) rather than the species of hammerhead (e.g. *Sphyrna lewini*).

Record MaxN when sharks or rays are present in the frame. MaxN for a species is continually checked during viewing, but it only increases if more individuals of a species become visible in a single frame. The highest MaxN for each species from each video is used for analysis. MaxN is used to assess the relative abundance of species and can be compared among deployments, habitat types and locations. This is a conservative estimate of abundance because more individuals may be present in the area but they won't be counted if they're not in the field of view at the same time.

Due to the need to measure individuals, analysis of stereo BRUVS requires purpose-built software. A commonly used option is EventMeasure (§ www.seagis. com.au).

5 Make backup copies of each BRUVS video in case something happens to the original video footage. Portable hard drives are a good option for this.



Fig 8: Spotted eagleray (Aetobatus ocellatus) feeding. (Source: FinPrint)

LABELS

Plan an effective labelling system before the start of the trip. This is likely to include initials for the trip and location, the date and consecutive numbers for each BRUVS deployment (see Surveys – labels). Film the site label at the beginning of each BRUVS drop using a slate information board (*Fig 9*).



Fig 9: Site label filmed on a slate board at beginning of BRUVS drop. (Source: Samm Sherman)

DATA SHEETS

Ensure data sheets are ready for use in the field. Standard BRUVS inclusions are:

- Date
- Location (place name and latitude/ longitude if possible)
- BRUVS label (also filmed at beginning of a BRUVS drop)
- Depth
- Time in (when BRUVS lands on seafloor)
- Time out (when BRUVS leaves seafloor)
- State of tide
- Habitat
- Bait type
- Comments



EQUIPMENT CHECKLIST:

- BRUVS frames, bait arms, bait bags, ropes and surface floats
- Equipment to assemble BRUVS (if required)
- Underwater video cameras, camera batteries, memory cards and chargers
- Camera housings
- Bait
- Weights
- GPS to record drop location
- Depth sounder (or other method to measure deployment depth)
- Waterproof data sheets and clipboard
- Waterproof labels
- Slate board
- Video file-stitching software

Stereo BRUVS - in addition

- Modified BRUVS frame
- Paired video cameras, batteries, memory cards
- Calibration cube
- Stereo BRUVS software for size analysis



TECHNICAL LEVEL -**EASY TO MEDIUM**

BRUVS surveys are usually done as a team, including someone to drive the boat, someone to deploy and recover the BRUVS, someone to record data etc. Team leaders should have some experience with BRUVS surveys – if not, then training is recommended in BRUVS sampling design, deployment and analysis of footage. Video readers require good knowledge of shark and ray species, or access to a reliable identification guide.



COST - VARIABLE

The cost of BRUVS surveys will vary considerably depending on the location and scale of the project. The major costs are likely to be in travel costs to the study site, vessel time and personnel. The cost of the BRUVS units themselves will depend on the materials used.

Good quality high resolution video can now be gained from relatively inexpensive cameras. However, analysing videos is labour-intensive and can take considerable time depending on the number of deployments and the number of sharks and rays observed on the videos. Purposebuilt software such as EventMeasure can assist with video data recording (and it's essential for stereo BRUVS calibration and measurements) but at approximately AU\$ 3,500 for video analysis software and AU\$ 2,000 for calibration software it's a significant investment.





TAGGING AND TRACKING

Author: Michelle Heupel, Australian Institute of Marine Science









DATA TYPE:

Stock structure Critical habitats

SHARK PLAN OBJECTIVES:

- 2. Assess threats to sharks and rays, determine and protect critical habitats
- 3. Identify and provide special attention, in particular to vulnerable or threatened shark and ray stocks
- 6. Contribute to protection of biodiversity and ecosystem structure and function

WHY WOULD YOU USE THIS TOOL?

Tagging and tracking provide valuable data on population size, stock structure and habitat use of sharks and rays. These are all important considerations in fisheries management, which aims to ensure fishing is sustainable.

Understanding stock structure is essential. A species population is often divided into different groups of individuals in separate areas: tagging and tracking data can identify these stocks, determining the extent of individuals' movement and whether mixing occurs. They also provide information on distribution, home range (where sharks and rays live and move on a regular basis), migration pathways and habitat use.

Sharks play an important role in the marine ecosystem, and understanding behaviours associated with their movement enables improved protection of the ecosystem. Data from tagging and tracking helps stakeholders understand and identify critical habitat use of threatened species for effective conservation planning. Habitat use can be determined by overlaying movement position data on habitat information.

Tagging and tracking data can also be used to examine the movements of shark and ray stocks in relation to the spatial distribution of a fishery. This helps to assess the level of threat a fishery poses to the stocks, and to identify threatened stocks and take steps to protect them.

FORMS OF TAGGING AND TRACKING

There are a variety of ways to study the number of sharks in an area and understand their movement patterns. Three of the main options are external tagging, acoustic tracking and satellite tracking. Each provides different types of data.

- External tagging is used to determine how far an individual travels between the point of release and point of recapture. The data informs the scale of movement of a species, and is often used to estimate population size.
- Acoustic tracking is used to examine the movement patterns of individuals within a defined area. Passive acoustic tracking of sharks and rays can provide longterm monitoring data on movement, residence and habitat use of individuals and populations. Acoustic receivers are deployed in the study area, then individuals are





TAGGING HELPS STUDY SHARK NUMBERS AND MOVEMENT PATTERNS

fitted with transmitters which the receivers record when the transmitter is within range.

With active acoustic tracking, a receiver is carried on a boat. Here an individual is followed by the boat, which records data on its location to represent the movement pattern of the tracked individual. Active tracking provides detailed information about the movement of an individual, but time constraints typically mean the data is short-term (generally 24-36 hours).

• Satellite tracking is used to study the long-range and broad-scale movements of sharks and rays. Some satellite tags report in real-time when the transmitter breaks the water surface, while others store data on temperature and light level which is only delivered when the tag releases from the animal at a pre-programmed time. Satellite tags collect data on movement over large distances, in contrast to the limited spatial coverage usually offered by acoustic tracking.

HOW DO TAGGING AND TRACKING WORK?

EXTERNAL TAGGING

Identification tags are attached externally to the shark or ray. These have a visible number, contact number or name of institute, unique to the tag that can be used to identify an individual when it's recaptured. By comparing the position and date of release to the position and date of recapture it's possible to determine the distance the tagged animal has moved in that time. When an animal is recaptured, contact the number or institute and pass on the number, date, location.

✓ ADVANTAGES:

 External identification tags are low cost and easy to apply.

XLIMITATIONS:

- Only release and recapture information is obtained, no information is gained about what the animal did between tagging and recapture.
- Recapture rates are often low, limiting the data collected.

ACOUSTIC TRACKING

Acoustic transmitters, also referred to as acoustic tags, are usually surgically implanted into sharks and rays. Each transmitter emits a unique sequence of ultrasonic pulses or pings. These unique signals can be detected when a tagged shark or ray swims within range of an acoustic receiver stationed on the seafloor. The receiver records the identity code of the tagged individual and the date and time. This data is then used to determine where individuals move and how long they spend in particular areas. The range of receiver detection depends on the transmitter and habitat conditions.

Acoustic transmitters can be equipped with additional sensors to transmit information on temperature, depth and acceleration, increasing the range of behaviours that can be studied.

✓ADVANTAGES:

- A large amount of data can be collected to define the movement patterns and habitat use of individuals within the study site.
- An individual can be tracked for up to 10 years, though transmission time depends on the battery life of the transmitter.
- Numerous individuals can be tracked simultaneously to understand population-level movements.
- Long-term data reveals the presence and residence of individuals within an area to identify habitat dependence, monitor population-

level behaviour, and to estimate mortality in some cases.

XLIMITATIONS:

- Acoustic receivers and transmitters are more expensive than external tags.
- Receivers need to be deployed and most need to be retrieved to obtain the data they store.
- No data is collected if an individual moves outside the range of the receivers.

ACTIVE ACOUSTIC TRACKING

Active acoustic tracking uses the type of transmitters described above, but the ping rate is much faster to allow an individual to be followed. A boat-mounted receiver is used rather than a deployed receiver. The signal from the transmitter guides the boat to follow the movement path of the individual fitted with it. Active tracking can also obtain data on the depth and temperature an individual is swimming through.

The labour-intensive nature of continuously following an individual through potentially changing weather conditions means most active tracking studies are short-term and not used for monitoring. Because of this, transmitters are attached externally. This decreases the amount of time an individual is handled, reducing stress, and also means the transmitter can detach after a short period since it will no longer be needed.

✓ ADVANTAGES:

- Less equipment is required than for passive acoustic tracking, reducing equipment cost.
- Fine-scale data on exact locations of individuals can be obtained.

XLIMITATIONS:

- The process is labour-intensive and may be costly based on personnel and vessel time.
- Collected data is for a single individual over a short time period.

SATELLITE TRACKING

Satellite tags are attached externally to the shark or ray. There are two main types:

- Archival tags known as pop-off satellite-linked archival tags (PSAT or PAT tags)
- Fin-mounted tags, known as smart position and temperature transmitting tags (SPOT tags)

PSAT tags are programmed to release from the animal at a predetermined date and float to the surface. Once they reach the surface, they transmit summary data to a satellite. If they're physically recovered, their full raw data record can be downloaded. PSAT tags record light, depth and temperature

data. Position data can be calculated based on recorded light levels, although this can have errors of 60-80km. PSAT tags are mainly used to study large-scale movements and temperature and depth preferences.

Fin-mounted SPOT tags track

movement by sending a signal to a satellite every time the fin/tag breaks the water surface. They measure depth and temperature data, but they don't archive data. SPOT tags provide relatively accurate position data of <250m-5km. However, they don't record or transmit data when sharks or rays are below the surface, and thus there can be gaps in the data depending on the behaviour of the animal. SPOT tags are attached via bolts through the fin which eventually corrode to allow the tag to fall off. This is a particularly good tracking system for species that regularly come to the surface and move large distances, such as tiger

sharks (Galeocerdo cuvier).

Both types of tags have an antenna that sends a signal to an ARGOS satellite used to track animals. The data transmission is relayed to a ground station where it's processed and sent to the researcher. However, using a satellite can be a considerable expense.

✓ ADVANTAGES:

- A single tag can provide data on long-range movements.
- PSAT tags store data and automatically upload summary data to a satellite.
- The tags don't have to be physically recovered to obtain their data.

XLIMITATIONS:

- Satellite tags are expensive, and satellite time produces an additional cost.
- Data resolution is sometimes poor, so these tags are best used to define migration-type movements and are less effective for local studies.

SAFE HANDLING OF SHARKS AND RAYS

Tagging procedures vary, but there are some basic points to remember in all cases to ensure the safety of the animals:⁷



- Tagging a shark or ray often involves a team and requires practice, so plan in advance.
 Designate roles for who is going to catch, tag, release, collect biological data and record.
- Even small sharks can inflict injuries, so team members with experience in handling and tagging sharks should train others before a project begins.
- Minimize handling during the tagging process, don't grip the shark across the gills, and keep the time that the shark or ray is out of the water to the minimum possible.
- Choose the right size tag for the shark, and take time to insert the tag properly. Wait until the animal settles before tagging it.

TAG DATASHEETS

Date	USI	Tag type	Species	Sex	Length (cm)	Lat/long	Depth (m)	Method capture	Name
02102018	AB-02102018-1	Fin	C. leucas	М	120	153.32-27.29	15	Line	C. Rigby

Careful record-keeping is essential. The following information should be recorded for all tagged animals:

- Unique tag number(s)
- Type of tag(s)
- Species
- Sex
- Length

- Date of tagging
- Location of capture (place name, latitude and longitude)
- Depth of capture
- Method of capture
- Name and contact details for the person or institution that tagged the animal(s)



Fig 1: Tag applicator for fin tags. (Source: Michelle Heupel)



Fig 2: Rototag male (top) and female (bottom) sections. (Source: Michelle Heupel)



Fig 3: Rototag in place on a shark fin. (Source: Michelle Heupel)

METHOD: EXTERNAL TAGGING

Two types of external tags are used on sharks and shark-like rays – fin tags and dart tags. For ray species, cinch tags are commonly used. Each tag has a unique identifier number along with contact details for whoever tagged the animal. When an externally tagged animal is recaptured, this person or institution can be contacted and provided with the tag number and location of the recapture.

A. FIN TAGS

1 Sharks or shark-like rays (e.g. wedgefish, sawfish) can be fitted with fin tags. Captured individuals can either be removed from the water (<120 cm TL) or restrained next to the boat (>120 cm TL).

2 Once an individual is secured or on board the vessel, measure and record its species identification, length and sex (see § Surveys).

When the animal is calm, punch a hole through the upper half of the dorsal fin using a leather hole punch tool.

Apply a tag to the fin using a specially designed applicator (*Fig 1*). The tag has two pieces, male and female (*Fig 2*), which clip together through the hole in the fin. For sharks or shark-like rays less than 120cm TL (*Fig 3*) use a Dalton Rototag, and for individuals greater than 100-120cm TL use a slightly larger Superflex tag.

5 Record the tag number and any additional detail on the data sheet.

B. DART TAGS

1 Dart tags are primarily used on sharks and shark-like rays and applied at the base of the dorsal fin. Captured individuals can either be removed from the water (<120 cm TL) or restrained next to the boat (>120 cm TL).

2 Once an individual is secured or on board the vessel, measure and record its species identification, length and sex (see § Surveys).

Insert the dart tag on the back of the shark at the base of the dorsal fin so that the barb of the tag catches on the fin support cartilage (dart tags inserted only into muscle tissue have low retention rates). Accessing dorsal

fin rays can be tough and dart tags are used on sharks up to 200 cm TL. Above this size, fin tags are typically more effective.

Record the tag number and any additional detail on the data sheet.

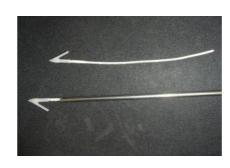


Fig 4: Plastic-headed dart tag (approximately 10cm long) and hollow tagging needle. (Source: Michelle Heupel)



Fig 5: Dart tag in place on an Atlantic sharpnose shark (Rhizoprionodon terraenovae). (Source: Michelle Heupel)



Fig 6: Dart tag with needle on tagging mount and downward-facing tag points. (Source: Cooperative Shark Tagging Program NOAA NFMS (NOAA 2017))

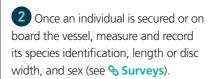
DART TAG HEADS

Dart tags can have two types of head: plastic or stainless steel. Dart tags with plastic heads are inserted using a hollow stainless steel needle that penetrates the skin and muscle (*Fig 4*). The tag remains in place when the needle is removed (*Fig 5*). If the skin is difficult to pierce with the tagging needle, a small incision can be made with a blade to allow tag insertion.

Stainless steel dart tags can be deployed in the muscle and don't necessarily need to be anchored in the dorsal fin cartilage. A stainless steel dart head fits into a slotted point in a stainless steel needle. These can be mounted on a pole, hand spear or spear gun. The tag is inserted at an angle towards the head of the shark with a strong, quick thrust.⁸ The two rear points of the dart head should face downwards into the muscle on insertion (*Fig 6*). This method is used when either the animals are too large to handle or when it's preferable to not bring the animal onto the boat.

C. CINCH TAGS

1 Cinch tags are primarily used on rays and applied through the spiracle. Rays are either removed from the water with a dip net (<70 cm DW) or restrained next to the boat (>70 cm DW). Be aware of stingray spines when handling individuals and take precautions to prevent injury (e.g. a towel can be gripped over the spine).



3 Insert cinch tag in one side of the spiracle and out the other, then close it so it forms a loop (*Figs 7*). Cinch tags are applied using hollow needle applicators similar to those used for dart tags.

4 Record the tag number and any additional detail on the data sheet.





Figs 7: Rays with cinch tags. (Source: Michelle Heupel)

With thanks to **S** VEMCO for supplying these images



Fig 8: Acoustic transmitters come in various sizes and configurations. (Source: Innova Sea Systems Inc)

METHOD: ACOUSTIC TRACKING

Most acoustic transmitters are surgically implanted internally in sharks and rays, especially for longer term tracking studies. External acoustic transmitters can still be used, but there's a risk they'll fall off or be damaged. Also, external transmitters can become fouled and cause tissue damage.

1 Sharks or rays can either be removed from the water (<120 cm TL or 70 cm DW) or restrained next to the boat (>120 cm TL or 70 cm DW).

Once an individual is secured or on board the vessel, measure and record its species identification, length or disc width, and sex (see Surveys). Record the printed identification number of the acoustic transmitter prior to insertion or attachment.

3 External acoustic transmitters can be glued to external fin tags using epoxy gel or secured to dart tags. This should be done in the laboratory, prior to taking the tags in the field. The fin or dart tag is then attached to the dorsal fin of the shark or spiracle of the ray using the procedures described above.

For internal transmitters, sharks often enter a state of tonic immobility when rolled onto their back, and this can be used to immobilize them for handling and surgical procedures. Roll the shark or ray upside down, in a tank or tub of water if on board the vessel. Ensure its gills are submerged, or use a hose to maintain flow of seawater over the gill.

5 There are a range of different types and sizes of acoustic transmitters (*Fig 8*). Select the correct size of transmitter relative to the size of the study species.

6 Internal implantation requires training on wound suturing prior to field work. To insert the acoustic transmitter, make an incision of 3-4cm in length in the abdomen with a sterile scalpel. Place the transmitter into the



Fig 9: Acoustic receiver. (Source: Innova Sea Systems Inc)

incision and push it in the direction of the head until it's completely within the body cavity. To alleviate internal damage and decrease transmitter rejection, the transmitter can be coated with a combination of paraffin and beeswax. Close the incision with surgical sutures fitted with cutting needles. Use new, sterile sutures for each individual.

7 After surgery is complete the individual can be recovered in the water bath aerated with oxygen (if required). If secured next to the boat, the animal can be rolled over to allow it to recover.

8 Sharks and rays fitted with acoustic transmitters are usually also tagged externally with dart or fin tags to assist in identification.

ACOUSTIC RECEIVERS

Sharks and rays can be actively followed in a boat after tagging. A hydrophone and receiver are used to locate the signal from the acoustic transmitter, and its position is regularly recorded. This is, however, labour-intensive – only one tagged animal at a time can be followed, and the presence of the vessel may influence how the individual moves. Often, data-logging acoustic receivers are placed within a study site

to allow passive tracking of shark and ray movements (*Fig 9*). Deployment patterns depend on the research question to be tested, ranging from grids covering an entire area to gates that monitor passage through a specific place.⁹

The data from acoustic receivers must be downloaded regularly. Some require physical recovery, while others can download data through a modem or satellite uplink – the latter options are more expensive than manual recovery.

SATELLITE TAGGING



Fig 10: Pop-off satellite tags including dart tags. (Source: Michelle Heupel)



Fig 11: Pop-off satellite tags with dart tags and needle attachment for pole. (Source: Juerg Brunnschweiler)

A. PSAT TAG

The most commonly used satellite tag – and the easiest to apply – is the PSAT or PAT tag (*Figs 10 & 11*). These tags are usually fitted with a dart and are attached near the first dorsal fin, as with external dart tags (see external tagging, above). PSAT tags can be tethered to one or two nylon-coated stainless steel leaders that are crimped to a heavy swivel and attached to a stainless steel dart head. Plastic-headed darts are also used in this same way by some research teams. For manta and devil rays (mobula species) PSAT tags can



Fig 12: Attaching PSAT tag with pole. (Source: Juerg Brunnschweiler)

be attached into the dorsal shoulder muscle using a nylon umbrella dart or nylon anchors using tagging poles.

1 Sharks or rays can either be removed from the water (<120 cm TL or 70 cm DW) or restrained next to the boat (>120 cm TL or 70 cm DW).

Once an individual is secured or on board the vessel, measure and record its species identification, length or disc width, and sex.

3 Prior to attachment, record the PSAT model, tag number,



Fig 13: Pop-off satellite tags including dart tag mounted on a tagging pole. (Source: Juerg Brunnschweiler)

programmed release time and date, external tag number and the other tagging information.

Follow the instructions for fitting an external dart tag, above.

TAGGING POLES OR SPEAR GUNS

If the animals are too large to handle or there's another reason why it's preferable not to bring them to the boat, PSAT tags can be attached using a tagging pole or a spear gun (*Figs 12 & 13*).¹⁰



Fig 14: Fin-mounted SPOT tag attached to the dorsal fin of a tiger shark (Galecerdo cuvier). (Source: \sqrt{www.himb.hawaii.edu/ReefPredator/Tools.htm.)}

B. FIN-MOUNTED SPOT TAGS

These are more difficult and timeconsuming to attach because they require the tag to be fixed with bolts, washers and nuts to go through the fin. Holes must be punched or drilled into the fin to line up with the bolt holes in the fin-mounted tag.

1 Sharks and shark-like rays can either be removed from the water (<120 cm TL) or restrained next to the boat (>120 cm TL).

2 Once an individual is secured or on board the vessel, measure and record its species identification, length and sex.

3 Punch holes through the dorsal fin of the shark using a leather hole punch or cordless drill.

4 Attach the fin-mounted satellite tag with the bolts and secure with washers and locknuts (Fig 14).

5 Record the tag number and the other tagging information.

- External tags and tagging equipment
- Acoustic receivers deployed on the

SATELLITE TRACKING

- PSAT tags or fin mounted SPOT tags
- External tags and tagging equipment
- Drill or hole punch for fin mounted
- satellite tags
- Bolts, washers and nuts for the fin mounted satellite tags

FOR ALL TAGGING ACTIVITIES

- GPS to record capture location
- Measuring board/tape
- Waterproof data sheets and clipboard
- Camera with batteries, charger, memory cards or mobile phone for photos



EQUIPMENT CHECKLISTS EXTERNAL TAGGING

- Rototag and Superflex tags
- Leather hole punch
- Tag applicator tool
- Dart tags
- Hollow stainless steel needle

ACTIVE ACOUSTIC TRACKING

- Acoustic transmitter tags mounted to an external tag
- External tags and tagging equipment
- Boat-mounted acoustic receiver
- Data sheets for position recording

PASSIVE ACOUSTIC TRACKING

- Acoustic transmitters
- Surgical tools (scalpel blade, forceps, needle holders, sterile sutures)

TECHNICAL LEVEL -SKILLED

Training is required for all aspects of tagging procedures, including the methods of shark and ray capture and handling to reduce stress to individuals and for human safety, and application of each of the tag types. Training should include time in the field and hands-on practice with people who already have experience of the method(s) employed. Internal implantation of acoustic transmitters requires training and practice in surgical procedures such as suturing.

ANIMAL ETHICS

Animal ethics permits from scientific institutions are often required for the handling of live sharks and rays. These ensure that standard operating procedures are followed, reducing adverse effects to the animals.



COST - VARIES

The cost of tagging equipment varies enormously from low-cost dart and fin tags to expensive PSAT and SPOT tags. The cost of vessel time and fishing equipment to capture sharks and rays needs to be considered, as do labour costs since tagging and tracking is usually done with a team.

Guideline costs for different tags and equipment are included below, but bear in mind that these are estimates only, and actual costs will vary considerably by country and company.

EXTERNAL TAGS

- Fin tags Dalton Rototag or Superflex – AU\$5
- Leather hole punch AU\$15
- Tag applicator AU\$0
- Dart tags
- Hallprint AU\$1+
- Tag applicator AU\$30
- Hollow stainless steel AU\$30

ACOUSTIC TAGS

- Scalpel handle and 100 blades AU\$30
- Sutures with cutting needles AU\$25/suture
- Acoustic transmitter AU\$ 350-500
- Acoustic receiver AU\$ 2,000-3,000+

SATELLITE TAGS

- PSAT tags AU\$4,000+
- Fin-mounted SPOT tags AU\$2,000-4,000+
- Both PSAT and SPOT tags also require satellite time: the cost varies depending on the number of tags deployed and the amount of data transmitted.





CITIZEN SCIENCE

Author: Andrew Chin, James Cook University, Australia



6. BIODIVERSITY



DATA TYPE:

Species present Abundance Stock structure Critical habitats Landings and discards

SHARK PLAN OBJECTIVES:

6. Contribute to the protection of biodiversity and ecosystem structure and function 10. Facilitate the identification and reporting of species-specific biological and trade data

WHY WOULD YOU USE THIS TOOL?

Depending on the scope of a project, large numbers of citizen scientists can collect shark and ray data across large areas and time scales. As well as offering significant cost savings, the approach can be particularly effective for sampling sharks and rays since they're generally highly mobile and patchily distributed, mostly in low numbers.¹¹

A network of community members collecting data across a large area can provide valuable data on uncommon shark and ray species that are otherwise difficult and costly to sample.

Another benefit of using citizen science for data collection is that it engages the public and raises awareness of shark and ray conservation. The active participation of members of the public can build trust between scientific researchers and the community that can lead to greater acceptance of management and conservation actions.¹²

HOW DOES CITIZEN SCIENCE WORK?

Citizen science is the collection of samples or data by community members for a scientific purpose. Participants are typically volunteers from the general public, and fishers often also take part.

Scientists are usually involved at some stage, most often in the design and analysis phases. There are three main types of citizen science projects:¹³

- **Contributory** designed by scientists with community members collecting data
- **Collaborative** designed by both scientists and community members with some input by community into the data collection, analyses and outputs
- Co-created scientists and community members work closely together through all project stages.

Most shark and ray citizen science projects are contributory. Fishers who record catch data can be considered citizen scientists, as they too are community members collecting data for a scientific purpose.¹⁴



CITIZEN SCIENTISTS

CAN COLLECT SHARK AND RAY DATA ACROSS LARGE AREAS AND TIME SCALES Citizen science programmes can also be linked to education programmes that include local schools, combining the monitoring aspect with education and outreach benefits.¹⁵

If they're well designed, citizen science projects can provide highquality data. This can include:

- Biodiversity the species present and records of their occurrence and distribution
- Photo identification of individual animals, particularly for iconic species such as manta rays and whale sharks
- Presence of rare and uncommon species
- Shark and ray abundance, monitoring of populations
- Catch data
- Extent and/or condition of critical habitats

WHAT ARE CITIZEN SCIENCE PROGRAMMES USED FOR?

Citizen science programmes are diverse and vary in scale from global monitoring networks to targeted national projects. As such, the methods used depend on the research question and the capabilities of local community volunteers.

Data can be collected by a variety of groups including NGOs, overseas volunteers, village communities and local fishers. While methods vary, good citizen science projects have clear objectives, appropriate data collection methods that suit the objectives, clear outputs, and transparency about all aspects of the project including the role of participants and how the data will be used.

To decide if citizen science can be a useful tool to collect information on sharks and rays, consider the species, habitats, locations and timeframe needed for sampling and

CITIZEN SCIENCE SUCCESSES





Fig 1: Sawfish species

Fig 2: Porcupine whipray (Urogymnus asperrimus)

- Citizen science data on the abundance of reef sharks in Palau has been shown to be comparable in quality to acoustic tagging data from scientists. 16
- The International Sawfish Encounter Database receives sightings of sawfish from the public to build greater knowledge of the species (www. floridamuseum.ufl.edu/fish/sawfish/ised)
- The Great Porcupine Ray Hunt used diver photographs to expand the known range of the little-studied porcupine whipray (*Urogymnus asperrimus*). ¹⁷
- In the Caribbean, REEF (www.reef.org) trained recreational divers to identify shark and ray species and survey their numbers: the data provided over 15 years has helped scientists to monitor population trends.¹⁸

how this overlaps with the interests and capabilities of potential citizen scientists in the area. For example, long-term abundance counts typically require a long-term commitment which might be best provided by dive tourist operators that repeatedly visit locations with a regular occurrence of shark or ray species.¹⁹

The most common type of citizen science programme is where people contribute photos to a project for various purposes. Citizen scientists also frequently help with censuses and habitat mapping.

PHOTOGRAPHY

Photography is used for a range of purposes in shark and ray citizen science projects:

1 To record the diversity of the sharks and rays in a country and

extend range and distribution information. A good example is Shark Search Indo-Pacific (www. sharksearch-indopacific.org): people can upload photos with location data to the website, species identifications are verified by a shark and ray taxonomist, and a living database is updated with new species records for an area. This information can contribute towards National Plans of Action.

2 For tracking abundance and migration of species such as whale sharks and manta rays. The skin patterns of these species are unique to each individual, which enables identification of particular animals. In effect, skin pattern is a non-invasive tag that can be used in long-term resighting programmes to investigate movement, abundance and habitat use.²⁰ This method is also



Fig 3: Photos from citizen scientists enabling tracking of whale sharks (Rhincodon typus) from their unique skin patterns.

useful for grey nurse sharks (*Carcharias taurus*), sevengill sharks (*Notorhynchus cepedanius*) and leopard sharks (*Stegostoma fasciatum*).²¹

The citizen scientists are mostly divers or snorkellers who submit photographs of the animals to a website – naturally, this works best for projects in areas with a tourism industry that brings people into close proximity with the animals. In some projects, such as Wildbook for Whale Sharks (% www.whaleshark.org), the system sends an email to the submitter and notifies them if the animal they photographed is seen again.

In large-scale global projects, such as for whale sharks, pattern recognition software can be used to identify animals and process large numbers of images.²² Smaller-scale projects – e.g.

Grey Nurse Shark Watch (**%** www. reefcheckaustralia.org/grey_nurse_shark_watch) – use volunteers to process photos.

In some projects citizen scientists also record size and sex ratios, which enables a better understanding of stock structure.²³

3 To document species abundance and distribution.

The Great Egg Case Hunt (**%** www. sharktrust.org/en/GEH_the_project) engages beachgoers, divers and snorkellers to photograph and collect empty shark and skate egg cases found on the beach or underwater. The project began in the United Kingdom and has expanded to receive photos from around the world: scientists use the data to document the relative abundance and distribution of egglaving sharks and skates.²⁴



Fig 4: Egg case in seaweed. (Source: ©Shark Trust)

CENSUSES

Citizen science is also commonly used to contribute to underwater censuses. Recreational divers count sharks and rays in a given area, and scientists use the information to plot population trends over time. Most of these projects are regional or local: the Great Fiji Shark Count (\$ www.fijisharkcount.com) is a good example.

eOceans (**%** www.eoceans.com) provides a global platform for a number of citizen science shark and ray census projects.

HABITATS

Citizen science can be a useful approach for mapping and monitoring shark and ray habitats. Information from recreational divers and snorkellers can inform and assist spatial management of critical habitats such as nurseries or mating areas. Citizen science programmes such as Seagrass Watch (% www. seagrasswatch.org) and Mangrove Watch (% http://mangrovewatch.org.au) have useful guidance on established protocols for mapping habitats.

FINDING VOLUNTEERS

Organizations including
Earthwatch (% http://
earthwatch.org) and Projects
Abroad (% www.projectsabroad.org) can connect
community members with
scientists running citizen
science projects that encourage
environmental sustainability.
They may also be able to provide
volunteers for in-country shark
and ray data collection projects.

METHOD: CITIZEN SCIENCE

Clearly define the research question and objectives of the project. Investigate if there are current citizen science projects with similar objectives that could provide an opportunity to collaborate. A good place to start is at registers that aim to connect scientists, volunteers and citizen science projects.

These include:

- Scistarter **%** https://scistarter.com
- Australian Citizen Science
 Association § www.
 citizenscience.org.au
- European Citizen Science
 Association Shttps://ecsa.citizen-science.net.
- Consider the types of data that are needed and the best tools for gathering it e.g. photos, surveys, BRUVS, tagging etc. Decide over what area and time period the project will run, and estimate the time and resources that will be needed. Using this information, identify potential community participants local NGOs and existing programmes may provide useful contacts. Select the best approach to the project (contributory, collaborative or co-created) and scale it to match citizen science capacity.
- 3 Establish communication and collaboration between the community members and researchers, and do due diligence on the capacity and legitimacy of any third parties (such as NGOs) who may be involved. Mutually agreed project arrangements are usually necessary, which include defined benefits to the community such as assistance with community life.
- 4 Design the sampling programme. It should have a scientific approach

and a consistent, standardized method for collection of the data.²⁵ Design also needs to consider community member needs and motivations to ensure participants will remain interested and involved. Training in data collection and management may be required – tasks that are too difficult or technical risk low levels of participation. As a general rule, community participation is maximized when activities are easy, fun and social. Assess safety risks and whether participants will need insurance to complete the activity.

- **5** Organize funding and resources.
- 6 Plan and undertake engagement with the community and other stakeholders (typically fishers and associated businesses). This could include creating information sheets or a website, but ensure that information is presented in ways that work for the community in question. It's also important to consider any intellectual property (IP) that may arise from the project. Develop and document agreement about authorship of reports and papers, access to and use and storage of data.
- 7 Design data collection quality assurance (QA) and quality control (OC) protocols – OA focuses on the people and QC focuses on the data. The greatest concern scientists and managers raise about citizen science is the quality of the data, so to allay these concerns the project must demonstrate how the data will meet the standard needed to address the project objective.²⁶ Design appropriate data systems: it may be useful to explore online systems such as iNaturalist.org or specially designed mobile apps (see 🗞 Surveys). Design, build and test databases (see § Data management).

QA may include:

- Training programmes for data collection
- Standardized sampling protocols and data sheets
- Equipment and appropriate training

QC may include:

- Expert verification of species photo identifications
- External review of sampling design and protocols
- Checks of databases for incomplete records or missing values
- Development of a data management plan

8 Collect the data and samples. The manner in which this is done will vary depending on the details of each citizen science project. Mobile apps can simplify data collection, submission and validation.

9 Enter data and perform QC on the data. Analyse the results and draw conclusions. Acknowledge participants' contributions in the report of findings as previously agreed.

Disseminate the findings to the community and stakeholders. Regular communication and feedback helps participants see their data is being used as intended: this two-way information exchange is a common feature of successful citizen science projects.²⁷

DATA MANAGEMENT

A person(s) may need to be assigned to a data management role. For example in the citizen science project on shark abundance in Palau, a dive company office staff member was made responsible for management and administration of the survey following training in data entry and data maintenance.²⁸



EQUIPMENT

Most shark and ray citizen science projects are based on photos provided by community members. In those projects, the key equipment is often a camera or mobile phone that belongs to the community member. For larger, more complex projects, equipment will vary but is likely to include standard equipment for fieldwork and data collection.



CHECKLIST:

- Regional species identification field quide
- Waterproof data sheets and clipboard
- Waterproof labels
- Pencil and eraser
- Waterproof marker for labels
- Camera with batteries, charger, memory cards or mobile phone for photos
- Snorkelling or SCUBA equipment (for in-water surveys)



TECHNICAL LEVEL - LOW

Most citizen science is undertaken by volunteers from the general public. Training is not usually required, other than in how to follow data collection protocols. Recreational divers may need training in how to identify and count shark and ray species.

More complex projects may require training in the use of standard data sheets and data entry, and specific equipment such as video cameras. Training may be required for data quality control, analyses and reporting.



COST – LOW, DEPENDING ON SCALE

Citizen science can be a low-cost approach to data collection, with the main cost being personnel time for data entry, quality control, collation, storage, analysis and dissemination of the outcomes. Projects that require large amounts of time, specialized training and/or equipment are usually more costly. It's also important to factor in the cost of time taken to engage community members and keep them informed about the project.

DATA APPENDIX A

DATA MANAGEMENT



Whichever of the tools you're working with, it's important to have a data management plan. This ensures that the integrity and usefulness of the data collected is maintained.

A data management plan should set out how data will be managed both during a project and after its completion. It can be anything from a very basic description for small projects to a documented plan for larger, more complex studies. Templates and guides for data management plans are widely available online.

Common steps in a data management plan include:

- 1 Identify data collectors, users and stakeholders, and data needs. Identify data access requirements, data security.
- 2 Design the data collection format and data sheets to suit the type of data.
- 3 Design an appropriate database structure and data management system for the required data standards. Include data quality assurance and control measures. Consider if data can be hosted in the cloud, whether an electronic reporting app is appropriate.
- Design an appropriate reporting system that presents and summarizes the data.

- 5 Assign roles for ongoing data management:
 - **a.** Entry
 - **b.** Cross-checks that the entered data matches the recorded data
 - c. Analyses and back-ups of data
 - **d.** Dissemination
 - **e.** Access, data confidentiality rules, security and protection of data
 - **f.** Long-term management.
- 6 Archive the data. The use of an open source data repository such as Dryad & https://datadryad.org provides long-term storage and allows the data to be accessed and used by others to increase the knowledge base on sharks and rays.

APPENDIX A - DMSO RECIPE

HOW TO MAKE DMSO

This popular form of DMSO – known as DESS – is useful for preserving genetic samples. It's easy to make, if you follow this procedure.

INGREDIENTS

0.25M EDTA pH 7.5 20% DMSO (Dimethyl sulfoxide) NaCl saturated

PROCESS

1 Measure out 23.27g of EDTA disodium salt (FW 372.24) for a 250ml solution (this may be different depending on the FW of your EDTA salt). Add 50ml of deionized water to the EDTA salt and stir. Make sure to use EDTA disodium salt otherwise more NaOH is needed to pH the EDTA.

Make 1M NaOH to pH the EDTA. The EDTA should be around a pH of 3 or 4 to begin with. It will take about 50ml of the 1M NaOH to pH the EDTA to 7.5. The EDTA will then begin to dissolve slowly. Be patient, heating the solution to 30°C helps.

3 Once all the EDTA salt is dissolved bring the volume up to 200ml with deionized water. Then add the 20% DMSO, which is 50ml for a 250ml solution. Return to a beaker and stir for a few minutes.

Add NaCl until it no longer dissolves – heating will help dissolve the salt. Pour the solution into a screwcap bottle, leaving most of the salt crystals in the beaker. The DMSO solution is then ready to use for sample preservation.

FREQUENTLY ASKED QUESTIONS:

1 There are crystals at the bottom of my solution: is this normal?

Yes, the solution is saturated with NaCl and salt precipitates out of solution once the liquid settles. If there is an excessive amount of crystals on the bottom of the container, transfer the solution to a new container leaving the NaCl crystals behind. Make sure the container is sealed tightly to reduce the evaporation of the water in the solution (this helps to stop the NaCl precipitation).

The crystals may also appear at the bottom of the tube in which the preserved samples are housed. Before use, samples can be rinsed in water to remove the salt and put back in DESS.

2 How do I ship samples in DESS to my colleagues in another state or country?

a) Contact your local health and safety organization for an official letter of shipment, including the chemicals' MSDS to be included in the package (i.e. MSDS for DMSO, EDTA and NACI). Include a statement from the shipper about the content of the box and general purpose of the material. b) Seal all sampling tubes/containers by tightly wrapping each one in parafilm or using cyrovials with screwcap lids to prevent leaking. Put sealed samples inside a plastic bag; place that plastic bag in another plastic bag with absorbent material and seal. Place the bag in a box with additional packing material, seal, and send. Check with local air freight companies as to requirements.

Source of DMSO Recipe and FAQ:

% www.faculty.ucr.edul~pdeleyllabl melissalDESS_protocol_f.doc.

APPENDIX B APPENDIX C

APPENDIX B – WHATMAN FTA ELUTE CARDS

DNA EXTRACTION

When working with FTA Elute cards, completing the DNA extraction incountry and sending the extracted DNA to a sequencing provider for analysis will lower the cost of the sequencing. However, this needs to be done by trained people in a laboratory with equipment such as sterile water, heat block, centrifuge and cold storage.

To extract the DNA from the FTA Elute card:

1 Use the card punch and mat supplied with the FTA cards to take 3mm punches from each circle: four 3mm punches are needed for each individual shark or ray. If two samples or circles were taken for one individual shark or ray, take two from each of the two circles to give a total of four punches. If one circle was used for one individual shark or ray, take four punches from one circle.

2 Transfer the four punches into a 1.7ml microfuge tube. One tube is needed for each individual shark or ray.

3 Before taking the next sample from a different shark or ray, the punch needs to be cleaned to avoid transferring any tissue to the new sample. To clean the punch, take a punch anywhere on the FTA Elute card that has had no sample on it. Eject the punch and then the punch tool is ready to take the next sample.



Fig 1: FTA Elute cards with shark tissues on the cards in two circles and two punches taken from each circle. (Source: Sharon Appleyard, CSIRO)

4 Add 500 microlitres (µl) of sterile water to the microfuge tube. Pulse vortex the tube five times.

5 Using a pipette tip, squeeze excess water out of the punches. Remove any remaining water with a pipette.

6 Add a further 100 µl of sterile water to the microfuge tube. Pulse vortex the tube five times. Heat the tube on a heat block at 95°C for one hour

7 Remove the tube from the heat block and pulse vortex the tube 60 times. Centrifuge the tube briefly at 13,000 rpm for two minutes. Transfer the remaining liquid to a new microfuge tube.

8 The liquid in the new microfuge tube contains the DNA. Check for quality using agarose gels and quantity with tools such as Nanodrop, Qubit and Bioanalyser. Keep at 4°C for short-term storage, -20°C for long-term storage, and preferably -80°C for archival purposes.

9 The DNA can then be sent for sequencing to determine the identification of the shark or ray.





2









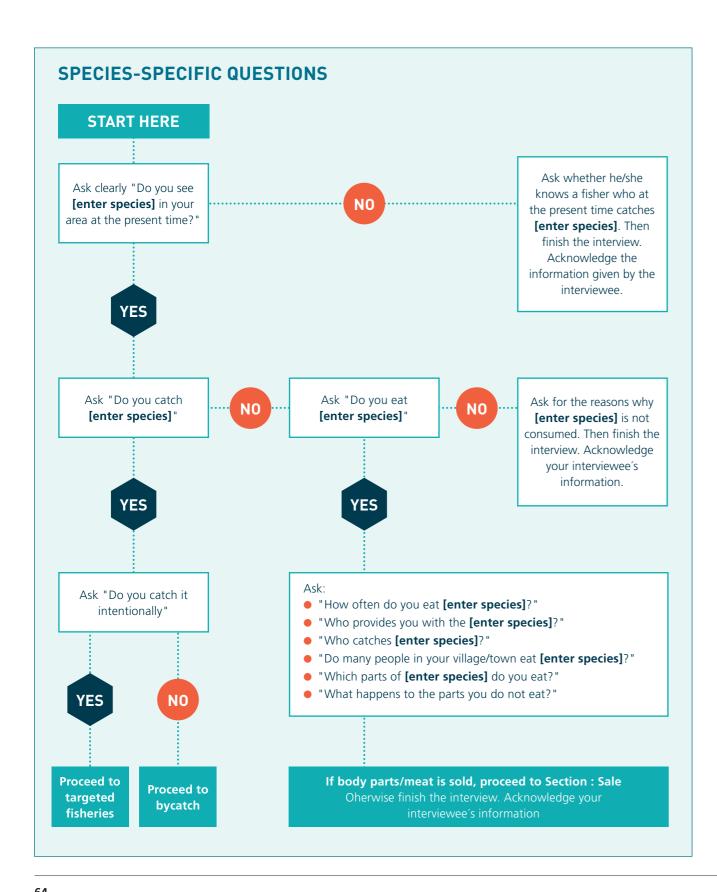
Fig 2: Whatman FTA Elute protocol

(www.gelifesciences.com/webapp/wcs/stores/servlet/catalog/en/ GELifeSciences-au/products/AlternativeProductStructure_17096/)

APPENDIX C – AN EXAMPLE CREEL SURVEY

Date
Interview No. Day
Interview No. Total
Village
Age of interviewee
Gender of interviewee
Are you a fisher?
For how long have you been a fisher?
Is fishing your main source of living?
Where do you go fishing? (e.g. reef, river, coastline, ocean)
Do you see sharks in your fishing area? If yes, where? (e.g reef, river, coastline, ocean)
What gear do you use? (e.g. speargun, gillnet, longlines, etc – select following questions depending on gear type)
Where do you put gillnets? (e.g. reef, river, coastline, ocean)?
How long do you leave your gillnet in the water before checking it?
How often do you check your gillnet? (e.g. regularly, at the end of a fishing trip)
Where do you put your longlines? (e.g. reef, river, coastline, ocean)
How long do you leave your longlines in the water?
How often do you check your longlines ? (e.g. regularly, at the end of a fishing trip)
What do you want to catch with your gillnet? (e.g. all edible seafood, crabs, fish, sharks, rays)
NATIONAL DESCRIPTION OF THE PROPERTY OF THE PR
What do you actually catch with your gillnet?
What do you want to catch with the longlines? (e.g. all edible seafood, fish, sharks, rays)
What do you actually catch with your longlines?
Do you catch sharks or rays?
Do you want to catch sharks or rays? Do you fish specifically for sharks or rays? If yes, what types of sharks do you usually catch?
Do you fish for sharks at particular times of the year?
If you catch sharks or rays, are they dead or alive?
What do you with the sharks or rays you catch (e.g. release, eat, share, sell)?
Do you take any measures to avoid catching sharks and rays? If yes, what do you do?

APPENDIX C



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The designation of geographical entities in this book, and the presentation of the material, do not imply the expression of any opinion whatsoever on the part of WWF and James Cook University concerning the legal status of any country, territory, or area, or of its authorities, or concerning the delimitation of its frontiers or boundaries.

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END NOTES

- 1. Ebert et al. 2013; Last et al. 2016
- 2. Cardeñosa et al. 2017; Feitosa et al. 2018
- 3. Dudgeon et al. 2012; Hillary et al. 2018
- 4. Goldberg et al. 2016; Simpfendorfer et al. 2016; Bakker et al. 2017
- 5. Kaly et al. 2016
- 6. Glaus et al. 2015
- 7. NOAA 2017
- 8. NOAA 2017
- 9. Heupel et al. 2006
- 10. Brunnschweiler et al. 2010
- 11. Mieras et al. 2017
- 12. WWF 2017; Chin and Pecl 2018
- 13. Chin and Pecl 2018
- 14. Chin and Pecl 2018
- 15. Saunders and Carne 2010
- 16. Vianna et al. 2014
- 17. Chin 2014
- 18. Ward-Paige et al. 2010; Ward-Paige et al. 2011
- 19. Vianna et al. 2014
- 20. Davies et al. 2012; Norman et al. 2017
- 21. Mieras et al. 2017; WWF 2017
- 22. Davies et al. 2012
- 23. Norman et al. 2017
- 24. Gordon et al. 2016
- 25. Chin and Pecl 2018
- 26. Chin and Pecl 2018
- 27. Mieras et al. 2017; Chin and Pecl 2018
- 28. Vianna et al. 2014

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