# Manual for data collection and species identification of sharks from longline vessels in Papua New Guinea

- observer training guide



March 2014

### Shark manual - contents

Gear checklist	2
Data collection protocol	3
Basic shark data required	4
Shark anatomy	5
abelling	6
Photography	7
Male maturity data	. 11
Female reproductive data	. 12
Collection of vertebrae	. 15
Additional information	. 17
Guide to sharks and rays likely to occur on longlines	. 18
ist of the species of sharks and rays	. 31

Prepared by W. White (CSIRO) for the ACIAR-funded project: Sustainable management of the shark resources of Papua New Guinea: socioeconomic and biological characteristics of the fishery

### Gear checklist

### List of gear needed for each trip

- SPC and new (modified LL-4 form) data sheets for entire trip
- camera with batteries, charger (adaptor if needed) and memory sticks
- rolls of pre-printed waterproof labels (with appropriate observer number)
- plain water proof labels
- this manual and SPC species manual
- pencils and eraser (for data entry)
- small, medium and large ziplock bags (for vertebrae)
- knife and sharpener (for vertebrae removal)
- clippers/secatuers (for vertebrae removal)
- scissors (for genetic samples)

### Data collection protocol

### Prioritising data collection

It will not always be possible to collect all of the data in this manual for every shark landed during fishing trips.

It is important to collect the most important information first:

The critical data which must be collected are:

- 1. Date and duration of each longline set
- 2. The location of each longline set (images with GPS on)
- 3. The species, total length and sex of each shark landed

The data which is important to collect

- 4. Maturity information from key species (see below)
- 5. Vertebrae from key species (see below)
- 6. Sex and length of embryos from key species (see below)

The data which can be collected if time permits (i.e. all above data has been collected)

- 7. Maturity information from other species
- 8. Vertebrae from other species
- 9. Sex and length of embryos from other species
- 10. Genetic samples from mother and embryos of pregnant sharks

#### **Key species**

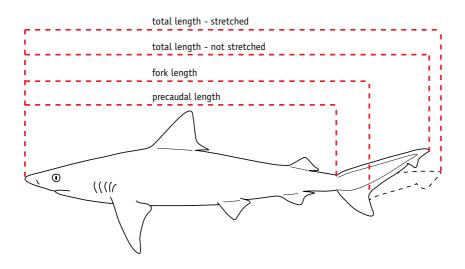
The key species which should be focused on for obtaining more detailed data are:

a.	Silky Shark	Carcharhinus falciformis	FAL
b.	Oceanic Whitetip	Carcharhinus longimanus	<b>0CS</b>
с.	Scalloped Hammerhead	Sphyrna lewini	SPL
d.	Grey Reef Shark	Carcharhinus amblyrhynchos	AML
e.	Silvertip Shark	Carcharhinus albimarginatus	ALS
f.	Shortfin Mako	Isurus oxyrinchus	SMA

### Basic shark data required

### Length

There are many different methods of measuring length of a shark:



Total length (stretched) is the preferred length measurement to take.

When measuring the total length of a shark on the deck of the boat:

- Straighten the body and tail

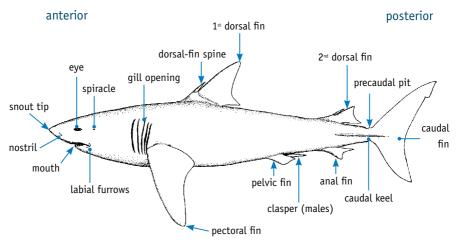
- Run tape measure over the body from snout to tail tip but try and keep the tape as straight as possible (don't curve over head and body too much)

Note: if any rays are encountered, the standard measurement should be disc width (DW) which is taken across the body from wing tip-to-wing tip

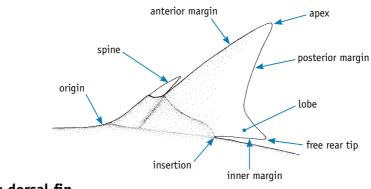
#### Weight

It is difficult to obtain weight for large sharks while at sea. If it is possible to obtain a weight, this will be useful. But it is not necessary to weigh every single shark recorded.

### Shark anatomy



Structural features of sharks



Shark dorsal fin

# Labelling

### Importance of tracking samples

When collecting samples, one of the most critical steps is correct labelling of the samples and a good system to track what specimen they came from.

It is important to have a unique number for a sample which is linked to the specimen it came from.

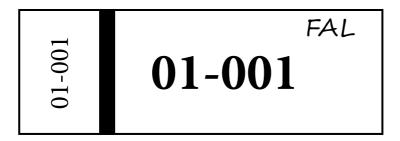
### Labelling

Waterproof paper is essential. Rolls of labels provided consist of unique numbers pre-printed onto waterproof paper.

Numbering system: 01-001

- first 2 numbers identify an observer
- last 5 numbers identify a shark and increase incrementally

On an observer trip to sea, each shark will receive a number and this number will be the link between images and samples kept, etc.



#### Use of labels:

- 1. Each individual shark landed will have an image of the whole shark which includes the sequential label with the species code written in pencil above the number
- 2. Image of claspers or female reproductive tract (see later) will also include the label
- 3. After processing each shark, the vertebral section retained will be placed into a ziplock plastic bag with the label
- 4. If any additional samples are kept (e.g. tissue samples), the small part of the label containing the same number can be used

The digital cameras will assist with verification of samples collected (including GPS location) and have the potential to save a large amount of processing time while at sea.

### Cameras

Canon D20 cameras are:

- waterproof (to 10 m)
- shockproof
- GPS capable

Supplied with:

- 2 x 8 GB memory cards
- 2 x batteries
- 1 x battery charger

#### Settings:

The automatic setting will be the best all round setting to use. The 'Func. Set' button allows the following settings to be changed (from top to bottom)

- GPS function: turns on or off GPS capability
- Timer (no need to use)
- Image aspect ratio (leave on 4:3)
- Image size: L, M1, M2, S (leave on M1)
- Video size: leave on 1920 (use only if necessary)

#### Images

The following images are required:

- Yourself at the beginning of the trip
- Start and end of each longline set (GPS enabled)
- Each shark recorded (with label)
- Reproductive stage of females (with label)
- Clasper development of males (with label)
- Fishing gear, hauling long lines, landing sharks (if time permits)

#### Yourself at beginning of trip

This is so we can double check the identity of the observer on each trip (important if images get mixed up between observers).

Start and end of each longline set

1. Enable GPS function:

- press 'Func. set' when camera on >> press up button (above func. set) to GPS settings >> press right button to enter GPS settings >> turn on (up) or off (down) GPS function >> press 'Func. set'

- 2. With camera on, hold camera so it's top is facing towards the sky (not inside cabins) until the GPS logo stops flashing (see below)
- 3. Take image over water where line has been set
- 4. Take similar image just as the line is being retrieved with GPS on
- 5. Turn off GPS function (see step 1)

FF GPS function is not on (see Step 1 above)



GPS function is on and is looking for a signal

#### (flashing)



GPS function is on and signal received



GPS function is on but there is no GPS signal

The GPS function may drain battery quickly so only turn on at the start and end of each longline set and then turn back off.

If a signal cannot be obtained within 2-3 minutes while on the deck then a problem receiving GPS signal exists. In this case do not worry about these images.

Each shark landed

Since there will be collection of vertebral samples from specimens, it is important to obtain images of each shark landed.

Lateral images of sharks (see guide at back of manual) are the best images to take, although they do not need to be perfectly aligned as in the guide.

- Place one of the supplied waterproof labels on the side of shark and take a roughly lateral image



- If shark identity is very certain (e.g. blue sharks, mako's etc) then 1 lateral image with a label is  $\mathsf{OK}$ 

- If shark identity a bit uncertain, some additional images would be ideal e.g.:



ventral head



interdorsal ridge



upper teeth



1<sup>st</sup> dorsal fin

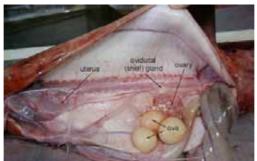
#### Female maturity stage

The condition of the reproductive tract of females can be difficult to record without prior experience (see maturity stage section later). Females which are pregnant are easy to determine but it is also important to know whether a female is mature (capable of breeding) or maturing (not yet capable of breeding).

The maturity staging section of this manual explains how the reproductive tracts are staged, but the use of a good image can bypass the need to record this as long as the image shows the necessary details.

When cutting into the belly of the shark:

- 1. Remove liver
- 2. Remove stomach and intestine
- 3. Reproductive tract will be what remains; an image showing the main elements (uteri, ovary, ova, etc) should allow staging of maturity by using the image alone



For pregnant females, take an image of the entire litter (include label).

#### Male maturity stage

The level of development (calcification) of the claspers is a very useful method for assessing the maturity of males. If the claspers are assessed, the internal male organs do not need to be examined.

An image of the claspers can be useful for verifying maturity status.



### Male maturity data

#### <u>Claspers</u>

Sharks can easily be sexed externally by the presence or absence of the male sex organs, the claspers. Even embryos inside pregnant females will have small claspers visible long before birth.

The maturity status of males is recorded using 2 criteria:

1. The length of the clasper (outer length)

Multiple ways to measure the clasper.

The outer length (see right) is the simplest measurement to take on large sharks



2. The level of development (hardness) of the clasper

Three categories

- a. NC non-calcified
- b. PC partially calcified
- c. FC fully calcified
- a. <u>Non-calcified</u>: clasper very short, not extending past pelvic fin tip
- <u>Partially calcified</u>: claspers longer, extending past pelvic fin tip; not entirely hard, still flexible
- c. <u>Fully calcified</u>: claspers long; hard along almost entire length





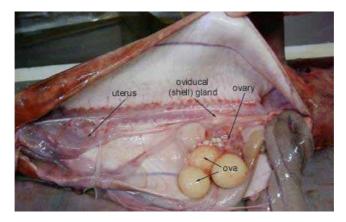


### Female reproductive data

### **Maturity Stage**

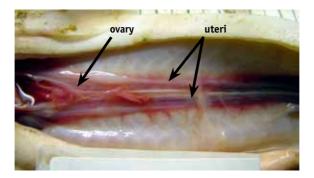
The female reproductive tract of sharks vary greatly, but in general consist of:

- 2 uteri
- 2 ovaries (only one or both may be functional)
- ova (eggs) inside the ovaries
- nidamental or oviducal gland (at front end of uteri)



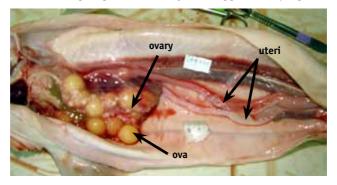
Maturity status of females is recorded using a 1-5 system:

1. <u>Immature</u> - uteri very thin; ovaries small and without yolked (yellow) eggs

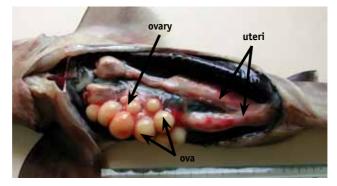


# Female reproductive data

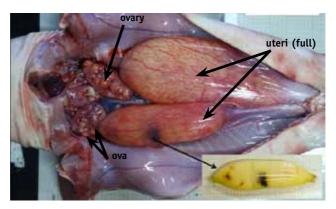
2. <u>Maturing</u> - uteri slightly enlarged at one end; ovary(ies) becoming larger and small yolked eggs developing



3. <u>Mature</u> - uteri large along entire length; ovary(ies) containing some large yolked eggs

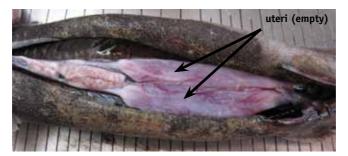


4. <u>Pregnant</u> - uteri containing embryos or large eggs



### Female reproductive data

5. <u>Post-partum</u> - uteri very large but without embryos (birth recently occurred)



#### **Pregnant females**

The litters from pregnant females typically consist of similar sized individuals. Data to be recorded are:

- 1. How many embryos in each uterus
- 2. How many male and female embryos
- 3. Minimum and maximum total lengths (TL) of embryos



### **Collection of vertebrae**

#### Shark vertebrae

The vertebrae of sharks are extremely useful for determining the age of each individual as they contain growth rings which can be counted like rings on a tree trunk.

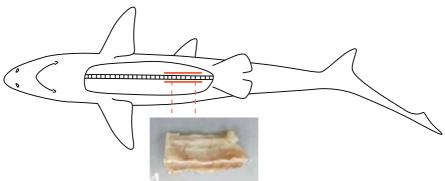
Vertebrae are about their widest (not longest) in the gut cavity, roughly below the 1<sup>st</sup> dorsal fin in most species. This is the best and easiest place to remove vertebrae.



#### Removal of shark vertebrae

After the liver, gut and reproductive organs have been removed from the gut cavity, the vertebrae can usually be seen running along the middle of the cavity.

- 1. With a shark knife, make a deep cut along one edge of the vertebrae with the knife angled inwards
- 2. Make a similar cut along the other edge of the vertebrae with the knife angled inwards
- 3. Use the clippers to cut through the vertebrae at each end (aim to collect 4 or 5 whole vertebrae per shark) of the section to be kept
- 4. Section should be able to be pulled away by hand
- 5. Roughly trim the excess flesh from the vertebrae and then place in a small plastic ziplock bag with the label for the shark



### **Collection of vertebrae**

#### Vertebrae per species

For age and growth work, it is critical that a good size range of individuals is collected for each species as well as good numbers of both females and males.

- try to collect vertebrae from a wide size range of individuals
- collect from females and males of each species
- don't focus on only large sharks, smaller individuals are also important

Focus on the key species (see page 3), but if time permits, it will be good to have vertebrae from other species caught as well.

In any one month, aim to collect up to 30 vertebrae per key species. If this goal is reached, then shift focus onto other shark species.

#### Freezer storage

Each shark processed should have an associated small ziplock bag containing a section of vertebrae. The vertebrae from one day should be placed into a larger ziplock bag and a waterproof paper label placed inside with the date.

Bags containing daily samples will reduce chances of vertebrae becoming mixed up in case there is a problem with the numbering system.

At the end of a trip, all of the daily bags should be placed into a larger bag(s) or box(es) with the date of the trip and observer name included on a label.

Vertebrae need to be kept frozen at all times, both onboard and back at port in Rabaul or Port Moresby.

#### Why kept frozen?

Genetic samples are also required from the sharks processed during the observer trips.

These are very time consuming to take and require washing of scalpels and forceps between each sample which is difficult while at sea.

If vertebrae are kept frozen, genetic samples can be taken from the vertebrae at a later date and not at sea.

### Additional information

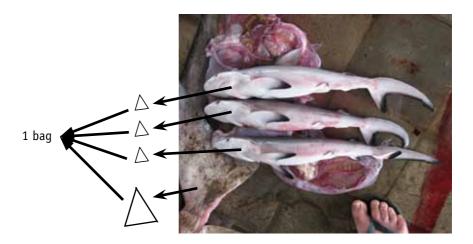
#### Genetic samples from pregnant females

Very useful information an be gained from determining whether the litter of embryos in a pregnant female was the result of mating with 1 male or with multiple males. To determine this, genetic techniques are used and tissue samples are required.

Note: this should only be done if time permits and if all other required data has been collected.

- 1. Using scissors, take a small, v-shaped piece of skin from the gills of the pregnant female
- 2. In the same way, take a smaller-sized piece of skin from the gills or fins of **each** embryo
- 3. Place all clips together in a small ziplock bag
- 4. Add a waterproof label with Species Code, Unique fish number (from main label) and date
- 5. Freeze together with the vertebrae collected for that day

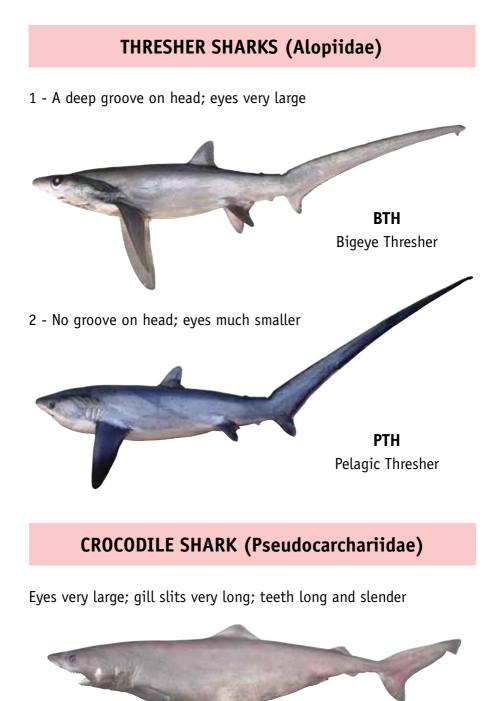
Note: make sure the sample from the mother is much larger than the embryo samples



# GUIDE TO SHARKS AND RAYS LIKELY TO OCCUR ON LONGLINES

### Key to the families of sharks

1a	Tail extremely long, about as long as body
	a. Body heavily spottedStegostomatidae (p. 28)
	b. Body not spottedAlopiidae (p. 19)
1b	Tail not as long as body 2
2a	Head hammer-shaped Sphyrnidae (p. 27)
2b	Head not hammer-shaped 3
3a	No anal fin present Deepwater sharks (p. 29)
3b	Anal fin present 4
4a	Upper and lower lobes of caudal fin similar in length
4a	
4a 4b	Upper and lower lobes of caudal fin similar in length Lamnidae (p. 20) Upper lobe much longer than lower lobe 5
	Lamnidae (p. 20)
4b	Upper lobe much longer than lower lobe 5
4b 5a	Lamnidae (p. 20) Upper lobe much longer than lower lobe



**PSK** Crocodile Shark

### MAKO SHARKS (Lamnidae)

Upper and lower caudal-fin lobes equal in length; strong keels on side of caudal peduncle; snout very pointed

1 - Pectoral fins shorter than head length; underneath of snout white

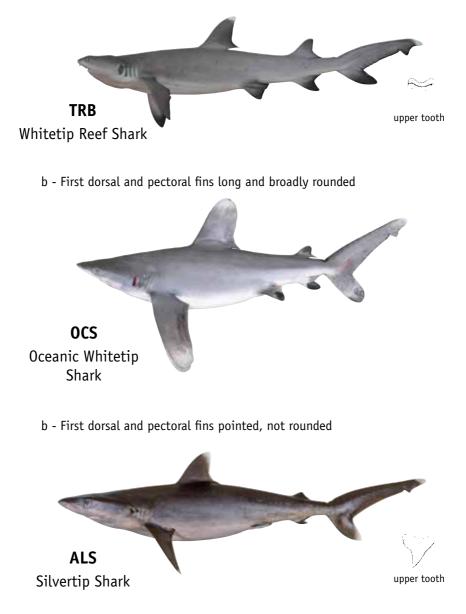


2 - Pectoral fins as long as head; underneath of snout darker



LMA Longfin Mako

- 1 First dorsal fin with a distinct white tip or margin
  - a Second dorsal fin almost as high as first; small species



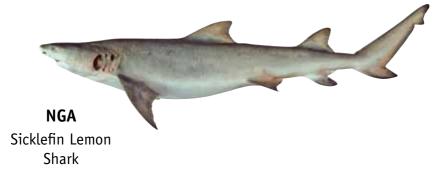
2 - Body bright blue; 1<sup>st</sup> dorsal fin slightly closer to pelvic fins than to pectoral fins; snout long

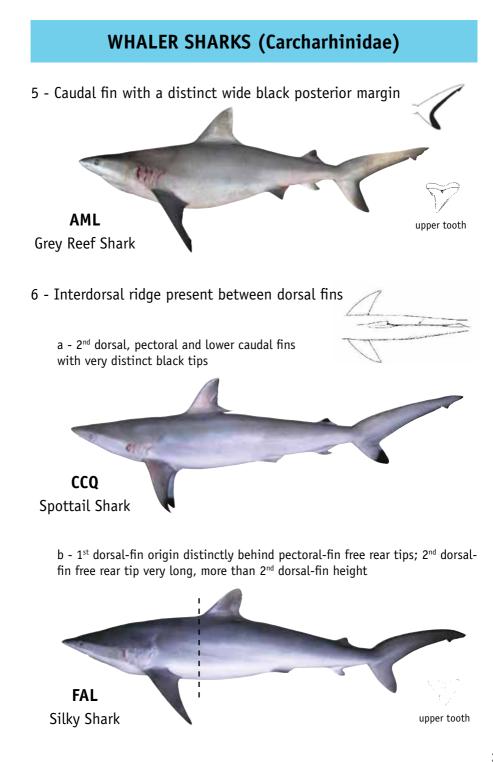


3 - Body with vertical bars on side; teeth very distinctive

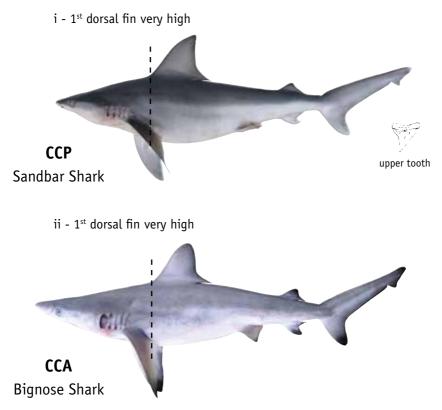


4 - Dorsal fins similar in height; body pale yellow brown

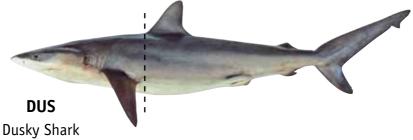




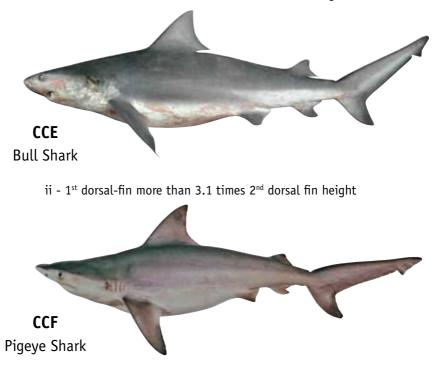
c -  $1^{\mbox{\scriptsize st}}$  dorsal-fin origin well forward, closer to pectoral fin insertions than to their free tips



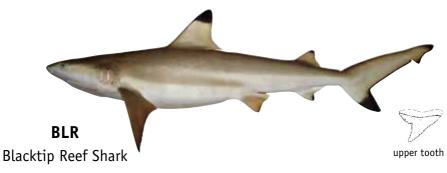
d -  $1^{\mbox{\scriptsize st}}$  dorsal-fin origin further back, closer to pectoral fin free tips than the insertions



- 7 No interdorsal ridge between dorsal fins
  - a Upper teeth broad, triangular and serrated
    - i  $1^{st}$  dorsal-fin less than 3.1 times  $2^{nd}$  dorsal fin height

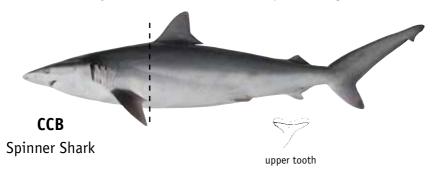


b - 1st dorsal fin with a very distinct black tip; upper teeth narrower

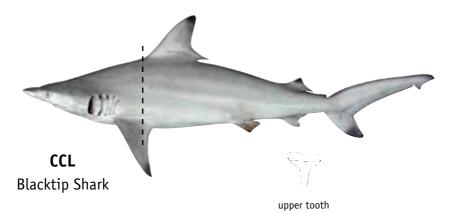


c - Upper teeth narrow and not serrated

i - Teeth short; 1<sup>st</sup> dorsal-fin origin over pectoral-fin free tips; 1<sup>st</sup> dorsal fin relatively low; fins with distinct black tips when large

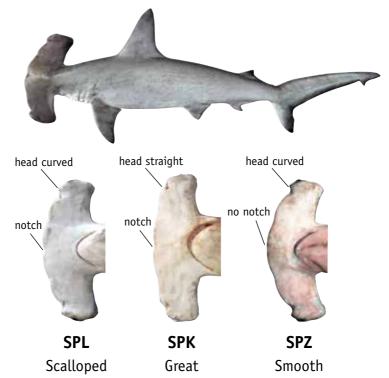


ii - Teeth longer; 1<sup>st</sup> dorsal-fin origin over pectoral-fin insertions; 1<sup>st</sup> dorsal fin high; fins with distinct black tips young (plain in adults)



# HAMMERHEAD SHARKS (Sphyrnidae)

1 - Head width much less than half body length



2 - Head about half of body length



# ZEBRA SHARK (Stegostomatidae)

Caudal fin very long; body heavily spotted



### NURSE SHARKS (Ginglymostomatidae)

Caudal fin moderately long; body plain; dorsal fins behind pelvic fins



**ORZ** Tawny Nurse Shark

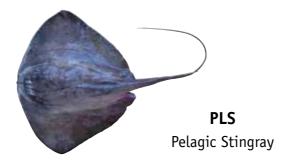
### DEEPWATER SHARKS (rare) - no anal fin

1 - One dorsal fin; 6 gills on each side of head

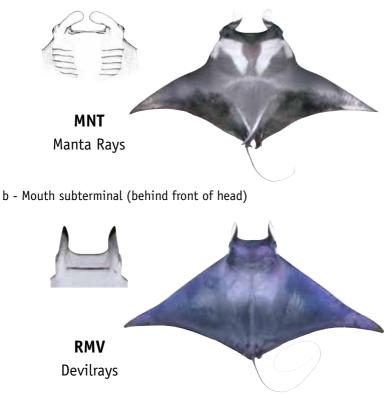


### PELAGIC RAYS

1 - Disc rounded; blackish on above and below



- 2 Disc diamond-shaped; 2 lobes extending forward of head
  - a Mouth terminal (at front of head)



# List of the species of sharks and rays

Family	Common Name	Scientific name	Code	Page
Hexanchio	dae			
	Bluntnose Sixgill Shark	Hexanchus griseus	SBL	29
Dalatiidae				
	Kitefin Shark	Dalatias licha	SCK	29
	Cookiecutter Shark	Isistius brasiliensis	ISB	29
Somniosio	lae			
	Velvet Dogfish	Zameus squamulosus	SSQ	29
Ginalymo	stomatidae	·		
Gingtymot	Tawny Nurse Shark	Nebrius ferrugineus	ORZ	28
Stegotom	5			
Jiegotom	Zebra Shark	Stegostoma fasciatum	0SF	28
Lamnidae		Stegostoma Jusciatam	051	20
Lammuae	Shortfin Mako	Isurus oxyrinchus	SMA	20
	Longfin Mako	Isurus paucus	LMA	20
	Longini Mako	isulus pudeus	LINA	20
Alopiidae	Delerie Threeher	Alemine nelezioue	וודם	19
	Pelagic Thresher Bigeye Thresher	Alopias pelagicus Alopias superciliosus	PTH BTH	19 19
	5.5	Alopius superciliosus	ып	19
Pseudoca			DCI	
	Crocodile Shark	Pseudocarcharias kamoharai	PSK	19
Carcharhi				
	Silvertip Shark	Carcharhinus albimarginatus	ALS	21
	Bignose Shark	Carcharhinus altimus	CCA	24
	Grey Reef Shark	Carcharhinus amblyrhynchos	AML	23
	Pigeye Shark	Carcharhinus amboinensis	CCF	25
	Spinner Shark	Carcharhinus brevipinna	CCB FAL	26 23
	<b>Silky Shark</b> Bull Shark	<b>Carcharhinus falciformis</b> Carcharhinus leucas	CCE	23 25
	Blacktip Shark	Carcharhinus limbatus	CCL	25
	Oceanic Whitetip Shark	Carcharhinus longimanus	0CS	20
	Blacktip Reef Shark	Carcharhinus melanopterus	BLR	25
	Dusky/Galapagos Shark	Carcharhinus obscurus/galapag	CCG	24
	Sandbar Shark	Carcharhinus plumbeus	ССР	24
	Spottail Shark	, Carcharhinus sorrah	CCQ	23
	Tiger Shark	Galeocerdo cuvier	TIG	22
	Sicklefin Lemon Shark	Negaprion acutidens	NGA	22
	Blue Shark	Prionace glauca	BSH	22
	Whitetip Reef Shark	Triaenodon obesus	TRB	21
Sphyrnida	e			
	Winghead Shark	Eusphyra blochii	EUB	27
	Scalloped Hammerhead	Sphyrna lewini	SPL	27
	Great Hammerhead	Sphyrna mokarran	SPK	27
	Smooth Hammerhead	Sphyrna zygaena	SPZ	27
Dasyatida	e			
-	Pelagic Stingray	Pteroplatytrygon violacea	PLS	30
Mobulidae				
	Giant Manta	Manta birostris	MNT	30
	Devilrays	Mobula spp.	RMV	30
	-			

Manual for data collection and species identification of sharks and rays from prawn trawl bycatch in Papua New Guinea

observer training guide



March 2014

### Shark and ray trawl bycatch manual - contents

Gear checklist2
Data collection protocol3
Basic shark and ray data required4
Shark anatomy6
Ray anatomy7
Labelling8
Photography9
Genetic samples12
Guide to sharks and rays likely to occur in trawl bycatch
List of the species of sharks and rays

### Gear checklist

### List of gear needed for each trip

- data sheets for entire trip
- camera with batteries, charger (adaptor if needed) and memory sticks
- rolls of pre-printed waterproof labels (with appropriate observer number)
- plain waterproof labels
- this manual
- pencils and eraser (for data entry)
- small and large plastic bags
- scissors (for genetic samples)

#### Data collection protocol

#### Data collection

The aim is to collect data on all of the sharks and rays landed by the trawl fishery.

The most difficult component will be identification. To make this simpler, we ask that smaller specimens are retained and that images of specimens not retained are taken (with appropriate label).

The critical data which must be collected are:

- 1. Date and duration of each trawl
- 2. The location (images with GPS on) and depth of each trawl
- 3. The species, size and sex of each shark or ray landed that is not retained whole

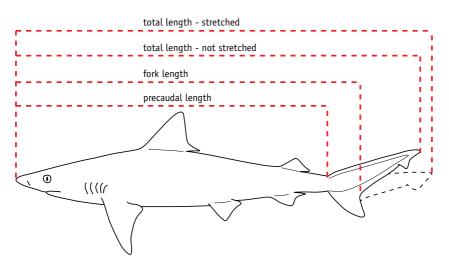
For smaller specimens, place into a large plastic bag with the next sequential label. On the datasheet for that trawl, write down the label number and write 'mixed frozen samples'. This way we can trace which trawl those samples belong to. It would be beneficial to take a rough photograph of the specimens retained with the label number included in the image.

Specimens kept need to be kept frozen until back at port.

#### Basic shark and ray data required

#### Length

There are many different methods of measuring length of a shark:



Total length (stretched) is the preferred length measurement to take (TL).

When measuring the total length of a shark on the deck of the boat:

- Straighten the body and tail

- Run tape measure over the body from snout to tail tip but try and keep the tape as straight as possible (don't curve over head and body too much)

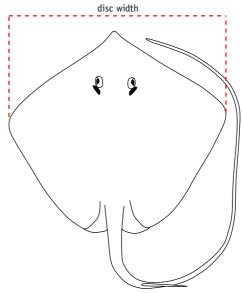
Sharks and rays for which TL is used:

- all sharks
- shark rays (Rhinidae)
- wedgefishes (Rhynchobatidae)
- guitarfishes (Rhinobatidae)
- numbfishes (Narcinidae)

#### Basic shark and ray data required

#### Disc width (DW)

The width of the disc (pectoral fin span) is the standard measurement used for rays which have a long, slender tail. Total length is not as accurate since it can easily be damaged.



When measuring the disc width of a ray, make sure the ray is laying flat on the floor facing upwards (ventral side down).

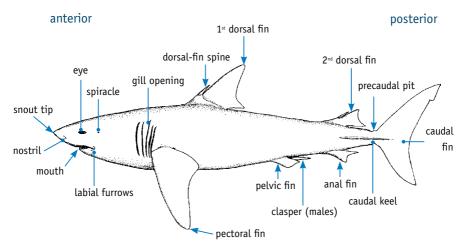
Rays for which DW is used:

- stingrays and whiprays
- eagle rays
- butterfly rays
- cownose rays

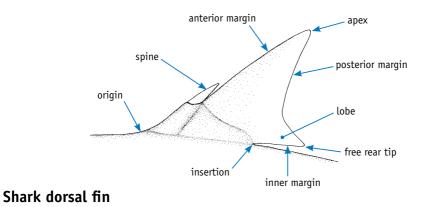
#### Weight

It is difficult to obtain weight for large sharks while at sea. If it is possible to obtain a weight, this will be useful. But it is not necessary to weigh every single shark or ray recorded.

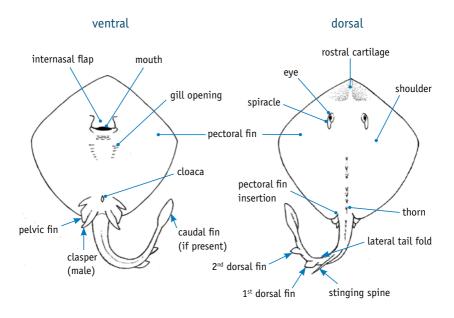
#### Shark anatomy



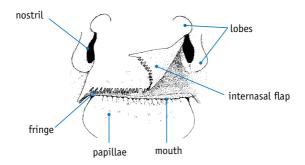
Structural features of sharks



#### Ray anatomy



#### Structural features of rays



#### Ray nostrils and mouth

## Labelling

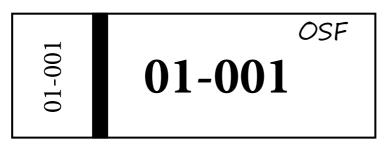
#### Labelling

Waterproof paper is essential. Rolls of labels provided consist of unique numbers pre-printed onto waterproof paper.

Numbering system: 01-001

- first 2 numbers identify an observer
- last 5 numbers identify a shark and increase incrementally

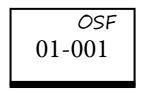
On an observer trip to sea, each shark or ray not retained will receive a number and this number will be the link between images and any samples kept, etc.



#### Use of labels:

After the catch from one trawl is landed:

- 1. Keep any small to medium-sized sharks and rays whole. Place in a plastic bag with the next sequential label
- 2. Any shark or ray not kept (e.g. large animals) will need an image of the whole animal with the next sequential label (with species code written in pencil above the number)
- 3. If any genetic sample are taken from animals not kept, place the small part of the tag in with the sample in a small plastic bag (see below)



#### Photography

The digital cameras will assist with verification of samples collected (including GPS location) and have the potential to save a large amount of time while at sea.

#### Cameras

Canon D20 cameras are:

- waterproof (to 10 m)
- shockproof
- GPS capable

Supplied with:

- 2 x 8 GB memory cards
- 2 x batteries
- 1 x battery charger

#### Settings:

The automatic setting will be the best all round setting to use. The 'Func. Set' button allows the following settings to be changed (from top to bottom)

- GPS function: turns on or off GPS capability
- Timer (no need to use)
- Image aspect ratio (leave on 4:3)
- Image size: L, M1, M2, S (leave on M1)
- Video size: leave on 1920 (use only if necessary)

#### Images

The following images are required:

- Yourself at the beginning of the trip
- Start and end of each trawl (GPS enabled)
- Each shark or ray landed which was not kept (with label)
- Sharks and rays retained with label (all kept in one photo)
- Fishing gear, hauling long lines, landing sharks (if time permits)

## Photography

#### Yourself at beginning of trip

This is so we can double check the identity of the observer on each trip (important if images get mixed up between observers).

#### Start and end of each trawl

1. Enable GPS function:

- press 'Func. set' when camera on >> press up button (above func. set) to GPS settings >> press right button to enter GPS settings >> turn on (up) or off (down) GPS function >> press 'Func. set'

- 2. With camera on, hold camera so it's top is facing towards the sky (not inside cabins) until the GPS logo stops flashing (see below)
- 3. Take image over water where line has been set
- 4. Take similar image just as the line is being retrieved with GPS on
- 5. Turn off GPS function (see step 1)

FF GPS function is not on (see Step 1 above)



GPS function is on and is looking for a signal

#### (flashing)



GPS function is on and signal received



GPS function is on but there is no GPS signal

The GPS function may drain battery quickly so only turn on at the start and end of each trawl and then turn back off.

If a signal cannot be obtained within 5 minutes while on the deck then a problem receiving GPS signal exists. In this case do not worry about these images.

#### Photography

Each shark or ray landed

Lateral images of sharks and dorsal (top) images of rays and flattened sharks (see guide at back of manual) are the best images to take, although they do not need to be perfectly aligned as in the guide.

- Place the next sequential waterproof label on the side of the animal and take a roughly lateral or dorsal image



- If it's identity is very certain (e.g. zebra sharks, etc) then 1 image with a label is  $\mathsf{O}\mathsf{K}$ 

- If shark identity a bit uncertain, some additional images would be ideal e.g.:



ventral head (sharks)



interdorsal ridge (sharks)



nostrils and mouth (rays)



1st dorsal fin

#### Sharks and rays retained

Take an image of the sharks and rays kept from each trawl. Include all specimens and the label in the one photograph.

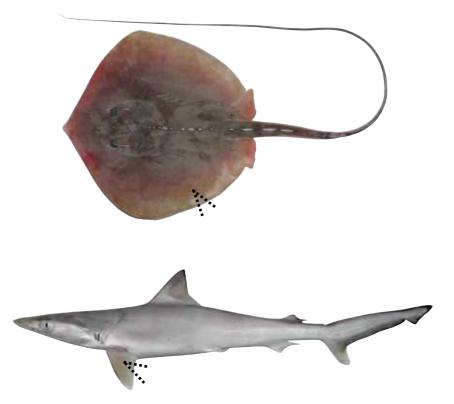
#### **Genetic samples**

Genetic tissue samples

If possible, please take a small piece of tissue from any shark or ray not kept.

Using the small scissors, take a small v-shaped piece of flesh (< 1 cm) from the fins or edge of disc and place in a small ziplock bag with the small part of the sequential label used for that specimen (with species code written in corner).

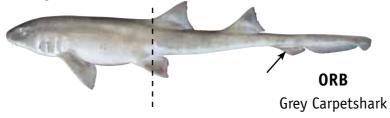
Examples of where to take tissue sample from:



## GUIDE TO SHARKS AND RAYS LIKELY TO OCCUR IN TRAWL BYCATCH

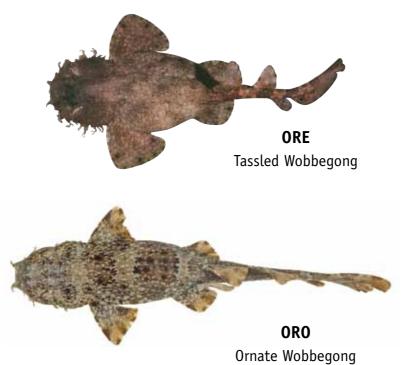
## LONGTAIL CARPETSHARKS (Hemiscylliidae)

Anal fin touching lower caudal-fin lobe;  $1^{\mbox{\scriptsize st}}$  dorsal-fin origin behind pelvic-fin origin



#### **WOBBEGONGS** (Orectolobidae)

Head and body flattened, with bright colour patterns; flaps of skin present around mouth and sides of head



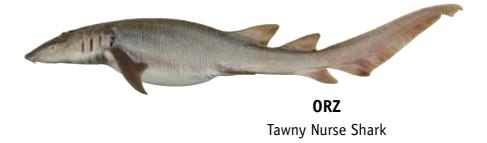
## ZEBRA SHARK (Stegostomatidae)

Caudal fin very long; body heavily spotted



#### NURSE SHARKS (Ginglymostomatidae)

Caudal fin moderately long; body plain; dorsal fins behind pelvic fins



## CATSHARKS (Scyliorhinidae)

Anal fin between pelvic fins and caudal fin (not touching lower caudal lobe); 1<sup>st</sup> dorsal-fin origin behind pelvic-fin origin



Eastern Banded Catshark

#### WEASEL SHARKS (Hemigaleidae)

Small spiracle present behind eyes;  $2^{\mbox{\scriptsize nd}}$  dorsal fin about half height of  $1^{\mbox{\scriptsize st}}$ 

a - Teeth noticeably protruding from mouth when closed



b - Teeth not protruding from mouth when closed



Australian Weasel Shark

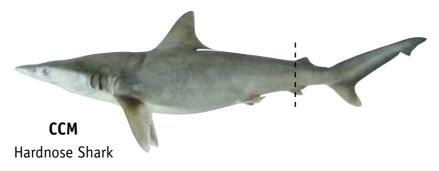
1 - Body with vertical bars on side; teeth very distinctive



2 - First dorsal fin with a distinct white tip;  $2^{nd}$  dorsal fin almost as high as  $1^{st}$ 

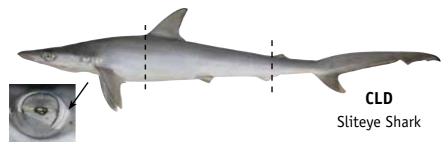


3 - Snout long and hard; 2<sup>nd</sup> dorsal and anal fins similar in size and shape; 2<sup>nd</sup> dorsal-fin origin about level with midbase of anal fin



4 - Snout long;  $2^{\rm nd}$  dorsal much smaller than anal fin;  $2^{\rm nd}$  dorsal-fin origin about level with anal-fin insertion

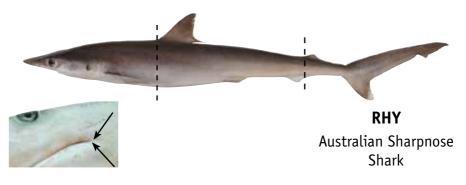
a - Eye with a small notch at back; 1st dorsal-fin origin behind pectoral fins



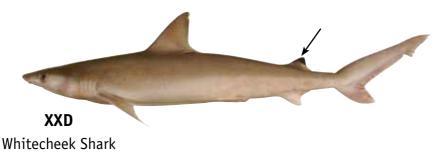
b - Eye without a notch;  $1^{\mbox{\tiny st}}$  dorsal-fin origin in line with pectoral-fin tips

i - Grooves at corner of mouth long

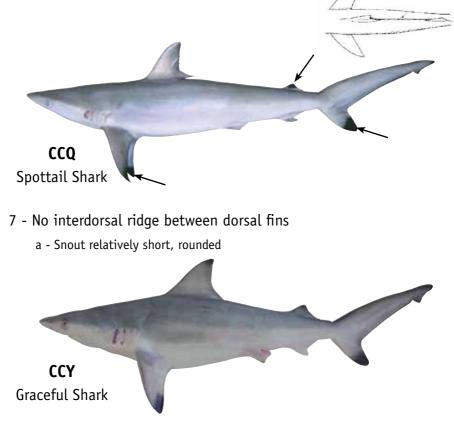
ii - Grooves at corner of mouth short, confined to mouth corners



5 - Large black spot on 2nd dorsal fin, all other fins plain

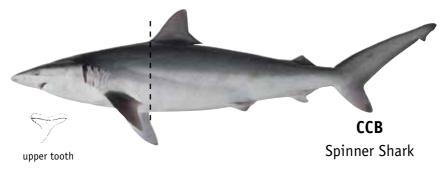


6 - Interdorsal ridge present between dorsal fins;  $2^{nd}$  dorsal, pectoral and lower caudal fins with very distinct black tips

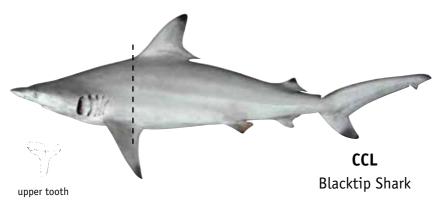


b - Snout longer, more pointed

i - Teeth short; 1<sup>st</sup> dorsal-fin origin over pectoral-fin free tips; 1<sup>st</sup> dorsal fin relatively low; fins with distinct black tips when large

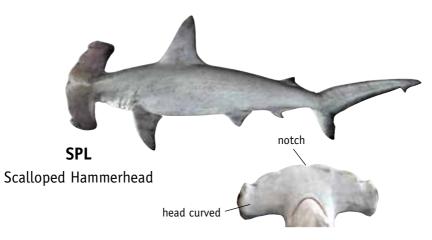


ii - Teeth longer; 1<sup>st</sup> dorsal-fin origin over pectoral-fin insertions; 1<sup>st</sup> dorsal fin high; fins with distinct black tips young (plain in adults)

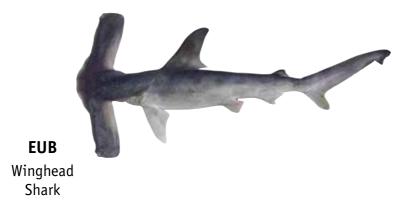


## HAMMERHEAD SHARKS (Sphyrnidae)

1 - Head width much less than half body length



2 - Head about half of body length



## SAWFISHES (Pristidae)

1 - Lower lobe of caudal fin very distinct; teeth on saw absent from basal quarter



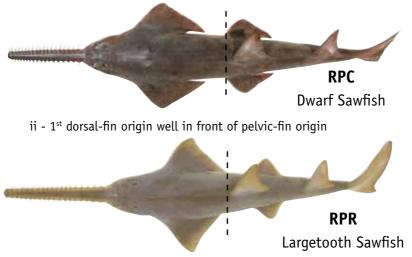
2 - Lower lobe of caudal fin not distinct; teeth on saw present along entire length

a - Teeth on saw more widely space near base than at tip; 1<sup>st</sup> dorsal-fin origin well behind pelvic-fin origin



b - Teeth on saw equally spaced along length;  $1^{\mbox{\scriptsize st}}$  dorsal-fin origin over or in front of pelvic-fin origin

i - 1st dorsal-fin origin above pelvic-fin origin



## SHARK RAY (Rhinidae)



## WEDGEFISHES (Rhynchobatidae)

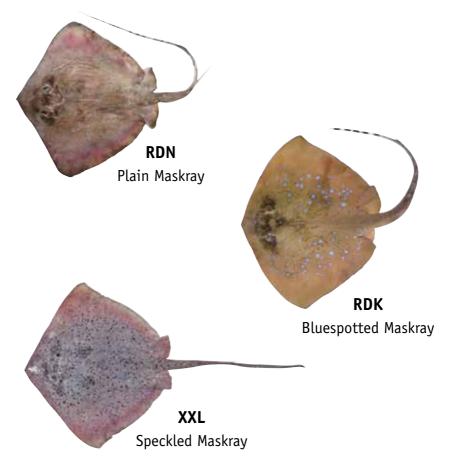


## **GUITARFISHES (Rhinobatidae)**



# STINGRAYS (Dasyatidae) 1 - Body covered in thorns; no stinging spine

- Porcupine Ray
- 2 Dark mask-like band across eyes; tail banded behind sting



- 3 Tail base circular in cross-section; no skin folds along top or bottom of tail (= whiprays)
  - a Disc mostly oval in shape; tail usually white behind sting
    - i Disc with small white spots



DHR Mangrove Whipray

ii - Disc plain (can reach a very large size)



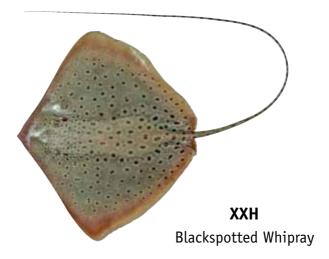


Note: samples of this species are urgently required

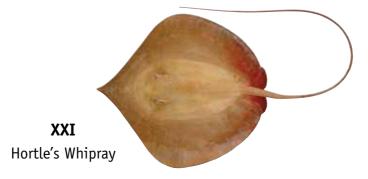
- b Disc quadrangular; tail tip not entirely white beyond sting
  - i Tail banded
    - Disc plain or with faint white spots



- Disc with distinct black spots



- ii Tail not banded, disc plain
  - Snout long, disc yellow above and below



- Row of enlarged thorns along midline of body and tail



- No enlarged thorns along midline of body or tail



**DHF** Pink Whipray

- iii Tail not banded, disc brightly coloured
  - Pattern of leopard-like spots in adults

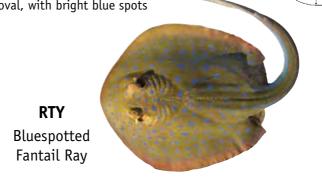


- Pattern of fine spots or reticulations

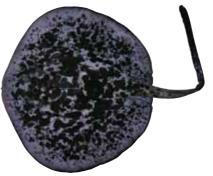


3 - Tail base slightly flattened in cross-section; low or deep skin folds along top and/or bottom of tail

a - Disc oval, with bright blue spots



b - Disc circular, with black and white mottling

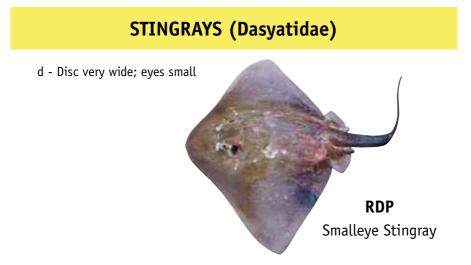


RTE Blotched Fantail Ray

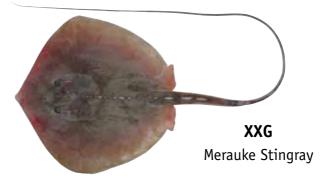
c - Lower skin-fold very deep; sting located a long way behind tail base



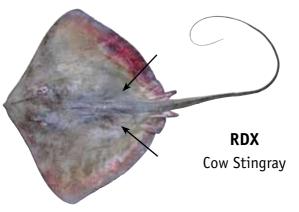
XXM **Cowtail Stingray** 



e - Tail very long; small species; row of enlarged thorns along midline



f - Disc usually with large irregular thorns; large species; row of fine white spots on either side of midline near back of disc



#### **NUMBFISHES (Narcinidae)**

Two dorsal fins; body soft; colour pattern of spots; capable of small electric shocks



XXF Ornate Numbfishes

**BUTTERFLY RAYS (Gymnuridae)** 

Disc very broad; tail very short



#### EAGLE RAYS (Myliobatidae)

1 - Spine present near base of tail; flap in front of mouth with a deep v-shaped notch



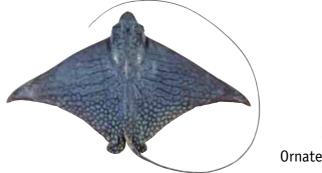
- 2 No spine on tail; flap in front of mouth without a notch
  - a Disc with a series of blue bands





**RYH** Banded Eagle Ray

b - Disc with a complex pattern of spots and reticulations



**RYE** Ornate Eagle Ray

## **COWNOSE RAYS (Rhinopteridae)**

1 - Tail very long; head relatively narrow



2 - Tail shorter; head relatively broad



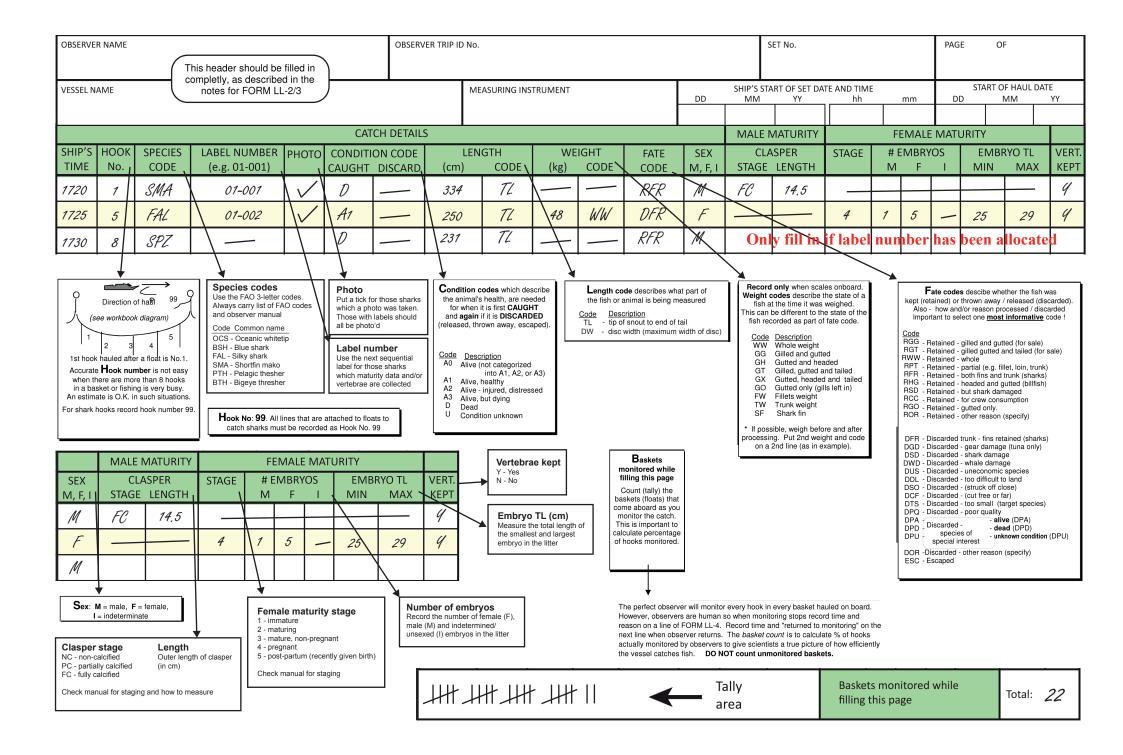
## List of the species of sharks and rays

Scientific Name	Common Name	FAO code	Page			
HEMISCYLLIIDAE			-			
Chiloscyllium punctatum	Grey Carpetshark	ORB				
ORECTOLOBIDAE						
Eucrossorhinus dasypogon	Tassled Wobbegong	ORE				
Orectolobus ornatus	Ornate Wobbegong	ORO				
	offiate wobbeyong	UNU				
STEGOSTOMATIDAE	7	005				
Stegostoma fasciatum	Zebra Shark	OSF				
GINGLYMOSTOMATIDAE						
Nebrius ferrugineus	Tawny Nurse Shark	ORZ				
SCYLIORHINIDAE						
Atelomycterus marnkalha	Eastern Banded Catshark	XXB				
HEMIGALEIDAE						
Hemigaleus australiensis	Australian Weasel Shark	XXC				
Hemipristis elongata	Snaggletooth Shark	HEE				
CARCHARHINIDAE						
Carcharhinus amblyrhynchoides	Graceful Shark	CCY				
Carcharhinus brevipinna	Spinner Shark	CCB				
Carcharhinus oreviprima Carcharhinus coatesi	Whitecheek Shark	XXD				
Carcharhinus limbatus	Blacktip Shark	CCL				
Carcharhinus macloti	Hardnose Shark	CCM				
Carcharhinus sorrah	Spottail Shark	CCQ				
Galeocerdo cuvier	Tiger Shark	TIG				
Loxodon macrorhinus	Sliteye Shark	CLD				
Rhizoprionodon acutus	Milk Shark	RHA				
Rhizoprionodon taylori	Australian Sharphose Shark	RHY				
Triaenodon obesus	Whitetip Reef Shark	TRB				
SPHYRNIDAE						
Eusphyra blochii	Winghead Shark	EUB				
Sphyrna lewini	Scalloped Hammerhead	SPL				
, ,	Seattoped Hammernedd	SIL				
PRISTIDAE	Name Carifich	DDA				
Anoxypristis cuspidata	Narrow Sawfish	RPA				
Pristis clavata Pristis pristis	Dwarf Sawfish Largetooth Sawfish	RPC RPR				
	Green Sawfish	RPZ				
Pristis zijsron	Green Sawiish	KFZ				
RHINIDAE						
Rhina ancylostoma	Shark Ray	RRY				
RHYNCHOBATIDAE						
Rhynchobatus spp	Wedgefishes	XXE				
RHINOBATIDAE						
Glaucostegus typus	Giant Guitarfish	RBQ				
DASYATIDAE						
Dasyatis longicauda	Merauke Stingray	XXG				
Dasyatis microps	Smalleye Stingray	RDP				
Dasyatis interops Dasyatis ushiei	Cow Stingray	RDX				
Himantura astra	Blackspotted Whipray	XXH				
Himantura fai	Pink Whipray	DHF				
Himantura granulata	Mangrove Whipray	DHR				
	5 r 5	-				

## List of the species of sharks and rays

Scientific Name	Common Name	FAO code	Page				
DASYATIDAE (cont.)							
Himantura hortlei	Hortle's Whipray	XXI					
Himantura jenkinsii	Jenkins' Whipray	DHJ					
Himantura leoparda	Leopard Whipray	XXJ					
<i>Himantura</i> sp	'a whipray'	XXK					
Himantura toshi	Brown Whipray	DHT					
Himantura uarnak	Reticulate Whipray	DHV					
Neotrygon annotata	Plain Maskray	RDN					
Neotrygon kuhlii	Bluespotted Maskray	RDK					
Neotrygon picta	Speckled Maskray	XXL					
Pastinachus atrus	Cowtail Stingray	XXM					
Taeniura lymma	Bluespotted Fantail Ray	RTY					
Taeniurops meyeni	Blotched Fantail Ray	RTE					
Urogymnus asperrimus	Porcupine Ray	RUA					
NARCINIDAE							
Narcine ornata	Ornate Numbfish	XXF					
GYMNURIDAE							
Gymnura australis	Australian Butterfly Ray	RGU					
MYLIOBATIDAE							
Aetobatus ocellatus	Whitespotted Eagle Ray	XXN					
Aetomylaeus nichofii	Banded Eagle Ray	RYH					
Aetomylaeus vespertilio	Ornate Eagle Ray	RYE					
RHINOPTERIDAE							
Rhinoptera javanica	Javanese Cownose Ray	MRJ					
Rhinoptera neglecta	Australian Cownose Ray	MRN					
Kimopteru neglectu	Australian cownose Ray	PILL					

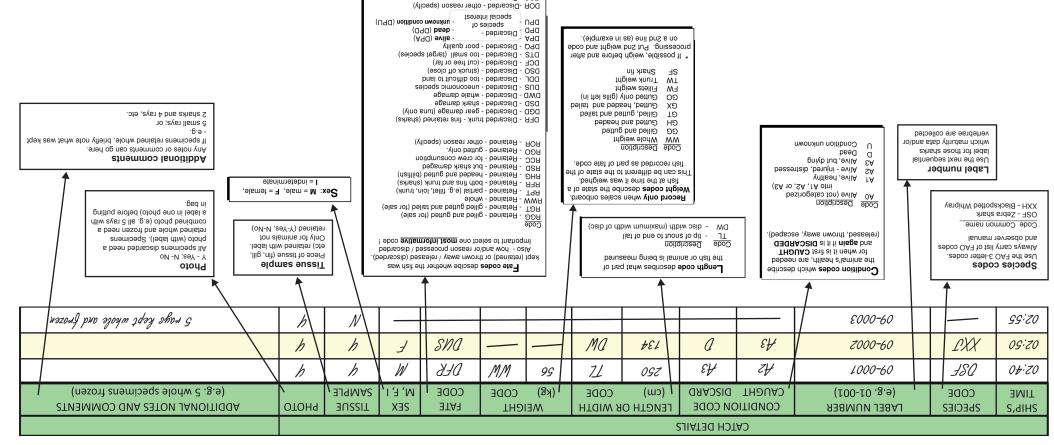
PNG LONGLINE OBSERVER (MODIFIED) LL-4 FORM SHARK MONITORING MODIFIED MAR. 2014																						
OBSERVE		14				OBSERV	ER TRIP ID No						S	ET No.				PAG	E O	F		
VESSEL NAME MEASURING INSTRUM					TRUMENT	RUMENT SHIP'S START OF SET DATE AND DD MM YY						TE AND TIME START				OF HAUL DATE MM YY						
CATCH DETAILS																						
SHIP'S	HOOK	SPECIES	LABEL NUMBER	рното				NGTH	WE	IGHT	FATE	SEX					MALE MATURITY			VERT.		
TIME	No.	CODE	(e.g. 01-001)	THOTO		DISCARD	(cm)	CODE	(kg)	CODE	CODE	M, F, I		LENGTH			F		MIN	MAX	КЕРТ	
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MODIFIED N OBSERVER N			OBSERVER	TRIP ID No.	SHOT	No.	START OF SHO	DT - DATE & TIN	1E			LOCA			DEPTH		
			C DOLLAR			-	DD	MM		hh	mm	Lati	tude	Longitude			
VESSEL NAM	IE		MEASURIN	NG INSTRUMEN	Т		END OF SHOT DD	- DATE & TIME MM	YY	hh	mm	LOCATI	ON itude	Longitude	DEPTH		
				CA	TCH DETAIL	s	I			I							
SHIP'S	SPECIES	LABEL NUM		-			H OR WIDT	н м/	EIGHT	FATE	SEX	TISSUE	рното		AL NOTES AN		
TIME	CODE	(e.g. 01-0			DISCARD	(cm)			CODE	CODE	M, F, I	SAMPLE			vhole specim		
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	DEPTH	əbutignol	LocAtioN	աա	ЧЧ	, TIME YY	8 JTAD - TOH MM	S TART OF S DD	.oN TOHS	OBSERVER TRIP ID No.	OBSERVER NAME

#### The data below is based on an example shot which contained: 5 small rays, 1 large shark and 1 large ray



ESC - Escaped

#### **SECTION 1: BUSINESS DETAILS**

1. Name:	
2. Position in the business:	

## **3. What activities (including non-shark fin activities) does the business currently undertake?** (Please tick all relevant activities; if an activity is not listed please tick 'Other' and specify details)

(	
Operating boats	
Processing and packaging	
Marketing	
Local transportation/freight	

I / /	/
Sales to local businesses	
Sales to domestic consumers	
Exporting overseas	
Other: please specify	

#### 4. What products generated the business revenue in 2014?

(Please tick all relevant products; if a product is not listed please tick 'Other' and specify details).

Shark fin	
Shark meat	
Other shark products	
Tuna	
Beche De Mer	
Prawn	

Lobster	
Agricultural products	
Non-agricultural food products	
Other: please specify	
Other: please specify	
Other: please specify	

## 5. Rate the importance of shark fin for the business's *profitability* relative to other products indicated in question 4. (Please tick only one)

Most important	
Very important	
Important	

Slightly important Not important

#### 6. What has been the usual number of staff by gender in the business in 2014?

Female:

Male:	

#### SECTION 2: SHARK FIN SOURCES

#### 7. Who has the business sourced shark fin from in 2014? (Tick all relevant boxes, if 'Other' specify)

Boats owned by the business		
External fishers		
Fisher and/or village co-operatives		

Shark fin processors			
Other shark fin buyers and middlemen			
Other: please specify			

## 8. Please list in order the top 4 locations (villages, towns or cities) from where the business has sourced shark fin in 2014

1 <sup>st</sup>		3 <sup>rd</sup>	
2 <sup>nd</sup>		4 <sup>th</sup>	

#### 9. Please provide the average price the business paid for shark products purchased in 2014.

Note: The average price of fins depends on the quantities of fin bought across all categories (fin type and size) throughout 2014 and their associated prices. If average prices are difficult to estimate, please use the optional section to provide more detailed price information by fin type and size. (Prices should be Kina per kilogram. If a product was not bought, please leave row blank).

	Average
Shark fin – Dried	K/kg
Shark fin – Undried	K/kg
Whole shark carcass with fins attached	K/kg

#### **SECTION 3: SHARK FIN DESTINATIONS**

#### 10. Who has the business sold shark fin to in 2014?

(Please tick all relevant buyer types. If not listed, please tick 'Other' and provide details)

Local businesses that export	
Local businesses that sell locally	

Local businesses that further process

Overseas businesses

Local consumers	
Local restaurants	
Other: please specify	

### 11. Please list in order the top 4 locations (villages, towns or cities) to which the business has sold its

shark fin in 2014. (If exported, it is only necessary to specify relevant countries)

1 <sup>st</sup>	
2 <sup>nd</sup>	

3 <sup>rd</sup>	
4 <sup>th</sup>	

#### 12. Please provide the average price the business received for shark product sales in 2014.

Note: The average price of fins depends on the quantities of fin sold across all categories (fin type and size) throughout 2014 and their associated prices. If average prices are difficult to estimate, please use the optional section to provide more detailed price information by fin type and size. (Prices should be Kina per kilogram. If a product was not bought, please leave row blank).

	Average
Shark fin – Dried	K/kg
Shark fin – Undried	K/kg
Other shark products: please specify	K/kg
Other shark products: please specify	K/kg

#### THANK YOU

Please feel free to use the space below to provide additional comments or concerns you have about the industry.

100%

#### **OPTIONAL SECTION**

#### PLEASE ONLY FILL THIS OUT IF YOU WISH TO PROVIDE MORE DETAILED PRICE INFORMATION FOR QUESTIONS 9 AND 12

#### (a) What proportion of shark fin bought in 2014 was dried? (please tick appropriate box)

19% or less		40% - 59%		90% - 100%	
20% - 39%		60% - 89%			

(b) What proportion of shark fin sold in 2014 was dried? (please tick appropriate box)

19% or less		40% - 59%		90% -
20% - 39%		60% - 89%		

(c) For each fin type (pectoral, dorsal, caudal, anal) please use the space provided to indicate fin size category, average buying prices and selling prices in 2014. If readily available, please also provide the quantity (in kilograms) of each fin bought in 2014.

#### Please tick relevant box:

Dried fin weight prices have been provided below	
Wet fin weight prices have been provided below	

#### **PECTORAL FINS**

Size category (eg '6–8 inches' or '20-40 cm' or 'mixed')	Average buying price	Average selling price	Quantity bought
	K/kg	K/kg	kg

#### **DORSAL FINS**

Size category	Average buying	Average selling	Quantity
(eg '6-8 inches' or '20-40 cm' or 'mixed')	price	price	bought
	K/kg	K/kg	kg

#### **CAUDAL FINS**

Size category (eg '6-8 inches' or '20-40 cm' or 'mixed')	Average buying price	Average selling price	Quantity bought
	K/kg	K/kg	kg

#### **ANAL FINS**

Size category (eg '6–8 inches' or '20-40 cm' or 'mixed')	Average buying price	Average selling price	Quantity bought
	K/kg	K/kg	kg

Interviewer:

#### Location and date:

#### **RESOURCE BENEFITS**

In parts of PNG, shark fishing has increased substantially and now makes a large contribution to community income. Part of this project's aim is to visit communities in PNG to better understand this contribution. Can I ask you some questions about your shark fishing in VILLAGE NAME?

Respondent name: Position:			
1. For how many years have you been fishing?			
2. For how many years have you <u>landed</u> shark?			
3.	3. For how many years have you <u>targeted</u> shark?		

#### 4. How do you rate the importance of shark to your boat's profitability? (Please tick one)

Most important	
Very important	
Important	

Low importance	
No importance	

## 5. In PNG, shark fishing is sometimes viewed as an activity that is important for the community's culture, traditions and beliefs. Do you think this is the case here? YES\_\_\_\_ NO\_\_\_\_ UNSURE\_

If yes or unsure, why?

#### 6. How important are the following factors in explaining why you land shark? (read out factors first)

	Very	Important	Slightly	Not
	important		important	important
Income				
The value of shark fin				
The value of non-fin products including meat				
Food source for personal or family consumption				
Opportunistic – it's caught with other target species				
Spiritual beliefs				
Tradition (e.g. parents did it)				
Debt or contractual commitments				

7. If shark fin had no value, would you still land shark? Yes\_\_\_\_ No\_\_\_\_ Unsure\_\_\_\_

#### **FISHING CHARACTERISTICS**

Another aim of the project is to better understand the characteristics of vessels that catch shark and the value of these catches. Can I ask you some questions about your vessel and activities?

#### 8. Which months of the year do you typically fish and which months do you typically catch shark?

All fishing:	
Shark:	
9. What is the average fishing trip length?	
10. What is the average time between fishing trips?	
11. Estimated number of trips per year:	

Location and date:

12. What fishing gears and bait do you use?

13. Can you provide a description of a typical fishing trip? (Start to finish, activities, times, duration)

14. Can you please provide a description of where you fish?

15. What species (shark or non-shark) did your vessel target in 2014?

16. What other non-targeted species groups (shark or non-shark) were landed as bycatch in 2014?

17. Please list in order of <u>quantity landed</u> (high to low) the <u>shark products and/or species landed in</u> 2014, the average <u>quantity landed per trip</u> and its <u>average price</u> in 2014.

Shark product	Kg/trip	K/kg
1.		
2.		
3.		
4.		

Shark product	Kg/trip	K/kg
5.		
6.		
7.		
8.		

18. Please list in order of <u>quantity landed</u> (high to low) the <u>non-shark products</u> caught in 2014, the average quantity landed per trip and its average price in 2014.

Non-shark product	Kg/trip	K/kg
1.		
2.		
3.		
4.		

Non-shark product	Kg/trip	K/kg
5.		
6.		
7.		
8.		

19. On average, what is total income (before deducting costs) from landings per trip?

**20. When fishing, what are the most important factors that allow shark to be targeted?** E.g. location, depth, time of day/year, moon, gear, bait etc.

**21.** Describe how many owners, skippers and crew operate the vessel and describe how boat income is shared. (If on a share of income basis, request percentages. If wage basis, request Kina amounts).

Role	Number	Details of income earned
Owner:		
Skipper:		
Crew:		
Notes:		

#### 22. Please describe any onshore activities the crew and skipper do in relation to the boat.

(e.g. boat/gear repairs, selling of fish, purchasing of supplies. If possible, get estimate of time spent)

CAPITAL			
23. Vessel type and material?			
24. What is the vessel's length?			
25. How old is the vessel?			
26. What is the vessel's typical expected life?			

27.	Is the vessel engine powered? Yes	No	
	(a) If yes, what size is the engine (horsepor	wer)?	
	(b) If no, how is it powered? (e.g. sail, row	ing)	

#### 28. How did you acquire the fishing vessel? (Please tick)

- a) Purchased and/or paid someone to build \_\_\_\_\_ ......Complete questions (a1) and (a2)
- b) Personally built \_\_\_\_\_ .....Complete questions (b1) and (b2)

#### (a1) In what year did you purchase the vessel?

(a2) How much did you pay for the vessel?

(b1) In what year did you build the vessel?

(b2) Describe the materials, the costs and time spent building the vessel:

29. Is the vessel associated with debt? (please tick) Yes\_\_\_\_ No \_\_\_\_ Unsure\_\_\_\_ If yes, outline the <u>current debt</u> and <u>repayments</u> (amounts, frequency, debt life).

Current debt:

Debt repayment details:

#### **OPERATING COSTS**

#### **30.** Fuel costs (if boat doesn't have an engine OR electricity generator skip this question)

(a) What was your average fuel use per trip?	K/trip
(b) What was the average fuel price paid in 2014?	K/L
Calculated fuel cost per trip (use as a check)	K/trip
(c) From where do you source your fuel?	
Notes:	

#### **31.** Purchased bait costs (if no bait is purchased skip this question)

32. (a) What quantity of purchased bait is used per trip?	
(b) What was the average bait price in 2014?	
(c) Where do you source your purchased bait from?	
Notes:	

#### 33. Do you incur costs related to freight, marketing or packaging of catch? Yes: \_\_\_\_ No: \_\_\_\_

If yes, provide details including total cost or unit cost (e.g. cost per box, per kilogram etc.)

#### 34. Describe any fishing gear replaced in 2014? (Lines, hooks, nets, ropes, floats. Not boat or engine)

35. Total cost of above gear replacements?	К
36. Where is fishing gear sourced from?	

## 37. Please outline any repairs and maintenance (painting, engine work, hull work, etc.) undertaken on the vessel in 2014 including cost, the supplier and location of the supplier.

Repair activity	Supplier Location of supplier		Cost

#### 38. Please provide details of any additional fishing costs which have not yet been discussed?

(List below. Eg. ice, administration, insurance, licences/permits, community co-operative fees)

Cost item	Expense in Kina
	К
	К
	К
	К
	К

Location and date:

#### **VIEWS, FUTURE PROSPECTS AND ALTERNATIVES**

**39.** How does current fishing compare to the last 5 years? (prices, catches, profitability)

For shark:

For other species:

#### 40. In the time that you've been catching shark, have you noticed changes in shark <u>numbers</u> or <u>sizes</u>? If so, please provide <u>details</u> about the change and <u>why</u> you believe these changes have occurred.

41. Have you ever adjusted anything about the way you fish to improve shark catch rates or profitability? Please <u>describe</u> any changes and the <u>reasons</u> for making them.

(Allow respondent to respond to the best of their ability, then prompt with: fishing location, distance travelled, fishing gear or techniques, new technology, new species).

**42.** How do you expect the following characteristics will change in the next 5 years? (if retiring, assume will continue)

Boat numbers	Increase	Decrease	No change	Variable	Unsure
Your shark catch	Increase	Decrease	No change	Variable	Unsure
Shark size	Increase	Decrease	No change	Variable	Unsure
Your non-shark catch	Increase	Decrease	No change	Variable	Unsure
Distance to fishing grounds	Increase	Decrease	No change	Variable	Unsure
Your profitability	Increase	Decrease	No change	Variable	Unsure

#### 43. Would you encourage younger generations to fish? Yes \_\_\_\_ No \_\_\_\_ Unsure \_\_\_\_

-			
Why/why not?			

44. If shark prices or catches decreased and shark generated insufficient income, how would you change your fishing activity? (Different methods? Species? Locations?).

45. If fishing in general became insufficient for providing you with a reliable income, what other income earning activities would you consider pursuing?

46. What is your age?	
47. Number of sons/daughters?	S: D:
<b>48.</b> Total number of people in household?	
<b>49.</b> Is your fishing income the household's main income?	
<b>50.</b> Are fish caught also consumed by the household?	Yes No

## **51. What other activities provide food or income for the household? (**Specify whether food or income, the person generating, and its relative importance)

#### 52. What options do you believe exist to improve the income generated from fishing?

#### 53. List the main management rules that you have to follow when fishing and who enforces them?

#### 54. Are there rules that you think should be changed? Why?

#### 55. Do you believe that fishery management rules are generally well followed by other vessels? Yes \_\_\_\_ No \_\_\_\_ Unsure \_\_\_\_

Why or why not?

#### 56. Are you part of a local fishing association, co-operative or fishery community group? Describe.

Sharks and Rays of Papua New Guinea

Papua New Guinea has an amazing diversity of sharks and rays and many of these are poorly known to science. Divers can help scientists to document the fauna and provide new insights into these species.

We are undertaking a 4-year project to gain a better understanding of PNG's shark and ray resources to ensure long-term sustainable use of these apex predators. The project is a collaborative project between the National Fisheries Authority in PNG and CSIRO and James Cook University in Australia and funded by the Australian Centre for International Agricultural Research (ACIAR).

The project will be collecting detailed data from all the fisheries catching sharks and rays as well as investigating the biodiversity of sharks and rays in PNG. This is where we need your help.

How you can help!!!

Divers and snorkelers exploring Papua New Guinea's fascinating underwater habitats can provide valuable information about sharks and rays! If you take photographs of any sharks or rays, simply record these four basic details:

- Location e.g. 'reef off Kokopo Beach, New Britain' (GPS location would be great)
- Habitat type and depth e.g. 'coral reef edge, 5 m depth'
- **Number** of individuals seen and any **behavioural** notes e.g. 2, resting on bottom
- Estimated size of individuals e.g. <1 m

Send these details with your photos to: william.white@csiro.au

or post image and information on our facebook page: <u>https://www.facebook.com/sharksPNG</u>

### Prizes

A prize for the best photo/most notable record will be given out in each October and March in 2014–2017











# Guide to the sharks and rays of Papua New Guinea:

Species possibly encountered by divers

### MAKO SHARKS (Lamnidae)

Upper and lower caudal-fin lobes equal in length; strong keels on side of caudal peduncle; snout very pointed





Photo: M. Conlin

### **THRESHER SHARKS (Alopiidae)**

Upper caudal lobe extremely long, about same length as body

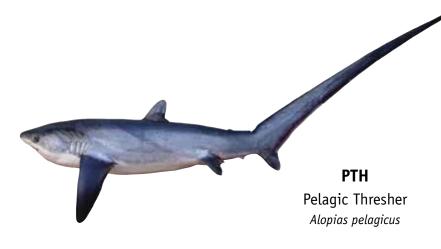




Photo: K. Stiefel

### LONGTAIL CARPETSHARKS (Hemiscylliidae)

Plain in colour in adults; brown bands in juveniles





Photo: J. Randall

### LONGTAIL CARPETSHARKS (Hemiscylliidae) - cont...

Brightly patterned - walking sharks



**ORK** Papuan Epaulette Shark *Hemiscyllium hallstromi* 



Photo: G. Allen

XXX Leopard Epaulette Shark Hemiscyllium michaeli



Photo: reef-fishes.com

### WOBBEGONGS (Orectolobidae)

ORQ

Hooded Epaulette Shark Hemiscyllium strahani

Head and body flattened, with bright colour patterns; flaps of skin present around mouth and sides of head

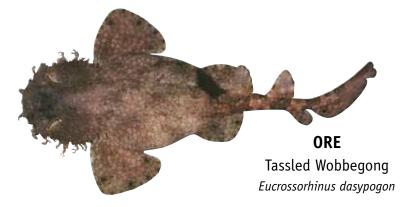




Photo: L. Low

Narrower head and body; smaller number of skin flaps around head





Photo: A. Green

### ZEBRA SHARK (Stegostomatidae)

Caudal fin very long; body heavily spotted



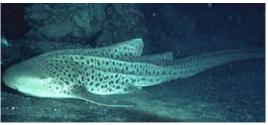
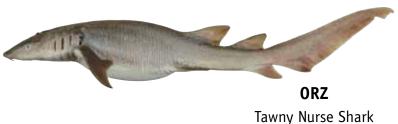


Photo: J. Randall

### NURSE SHARKS (Ginglymostomatidae)

Caudal fin moderately long; body plain; dorsal fins behind pelvic fins



lawny Nurse Shark Nebrius ferrugineus



Photo: V. Taylor

WHALE SHARK (Rhincodontidae)



KHN Whale Shark Rhincodon typus



Photo: W. White

### **CATSHARKS** (Scyliorhinidae)



ATY Coral Catshark Atelomycterus marmoratus



Photo: J. Randall

### WHALER SHARKS (Carcharhinidae)

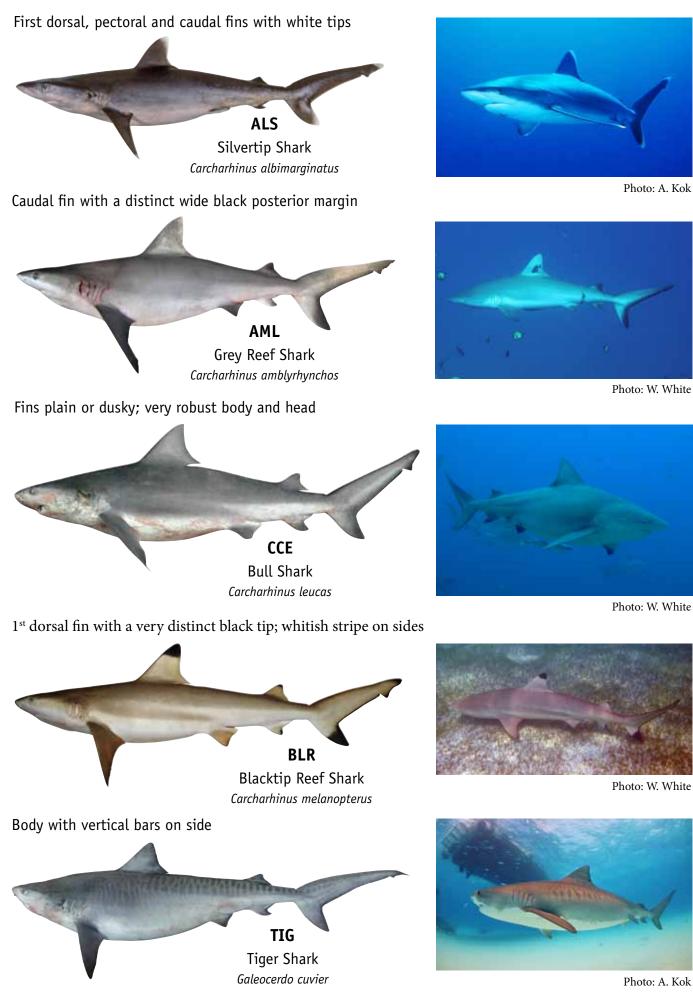


Photo: A. Kok

Photo: A. Kok

### WHALER SHARKS (Carcharhinidae)

Dorsal fins similar in height; body pale yellow brown



icklefin Lemon Shark Negaprion acutidens



Photo: J. Steinitz

First dorsal fin with a distinct white tip;  $2^{nd}$  dorsal fin almost as high as  $1^{st}$ 

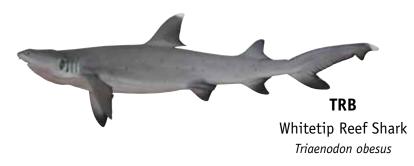
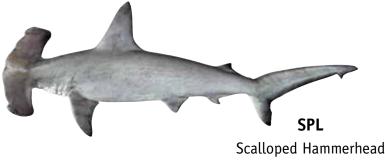




Photo: W. White

### HAMMERHEAD SHARKS (Sphyrnidae)

First dorsal fin not very tall; anterior profile of head broadly curved



Sphyrna lewini



Photo: J. Randall

First dorsal fin very tall; anterior profile of head usually straight

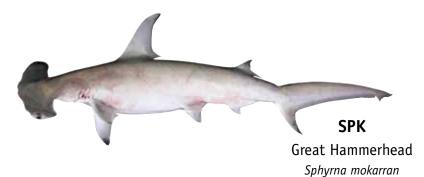




Photo: V. Taylor

### SHARK RAY (Rhinidae)





Photo: Georgia Aquarium

### WEDGEFISHES (Rhynchobatidae)





Photo: S. Gingins

### **GUITARFISHES (Rhinobatidae)**

Glaucostegus typus





Disc very wide; eyes small; tail base very broad

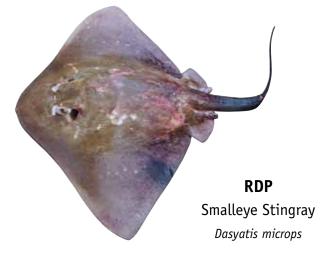
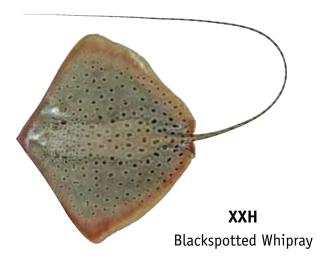




Photo: A.D. Marshall

Disc with distinct black spots; tail banded after sting



No enlarged thorns along midline of body or tail; pale pinkish to brown in colour





Photo: J. Randall

**DHF** Pink Whipray *Himantura fai* 

### STINGRAYS (Dasyatidae)

Disc mostly oval in shape; tail usually white behind sting; small white spots on disc





Photo: www.scuba-equipment-usa.com

Row of enlarged thorns along midline of body and tail; body yellow-brown in colour

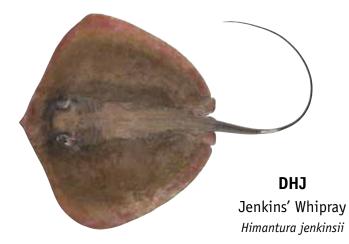




Photo: W. White

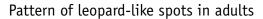






Photo: A. Murch

### **STINGRAYS (Dasyatidae)**

Pattern of fine spots or reticulations





Photo: W. White

Dark mask-like band across eyes; tail banded behind sting

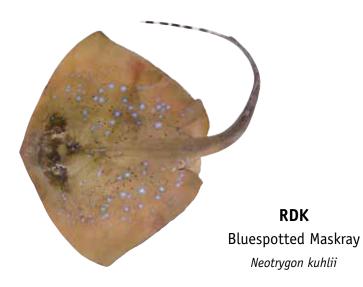




Photo: W. White

c - Lower skin-fold very deep; sting located a long way behind tail base





Photo: W. White

### STINGRAYS (Dasyatidae)

Disc oval, with bright blue spots





Photo: W. White

Disc circular, with black and white mottling

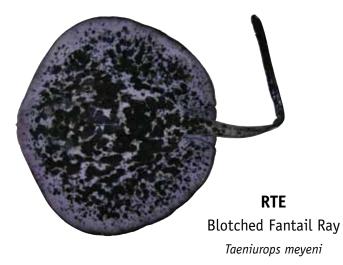
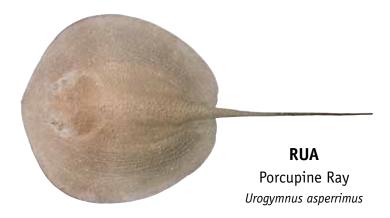




Photo: W. White

Body covered in thorns; no stinging spine



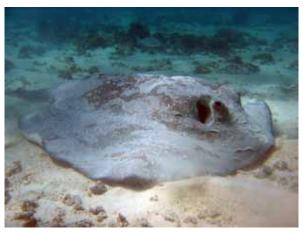
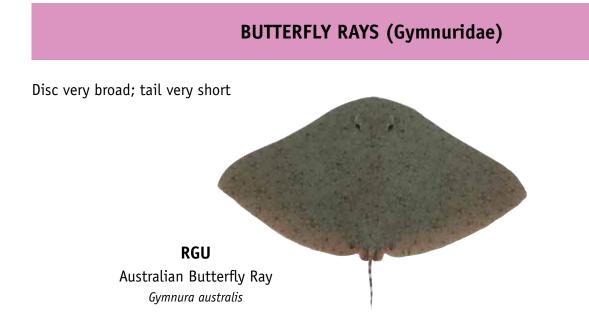


Photo: W. White



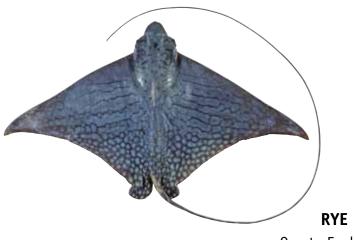
### EAGLE RAYS (Myliobatidae)

White spots on dorsal surface; spine present near base of tail





Photo: R. Field

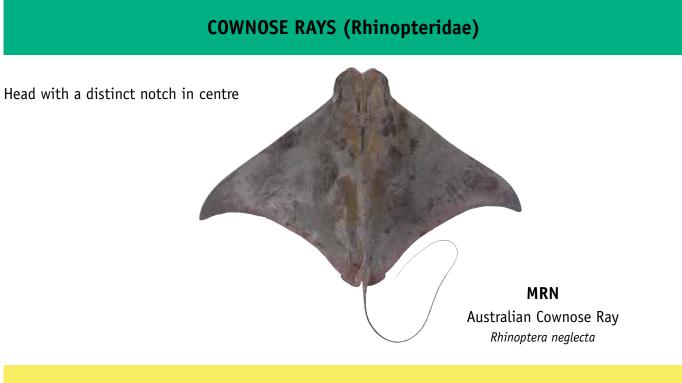


Ornate Eagle Ray Aetobatus vespertilio



Photo: W. White

Disc with a complex pattern of spots and reticulations



### MANTA & DEVILRAYS (Mobulidae)

Disc diamond-shaped; 2 lobes extending forward of head; head very wide; mouth at front of head





Disc diamond-shaped; 2 lobes extending forward of head; head narrower; mouth behind front of head





Photo: J. Randall

## Sampling for Shark Genetics – FTA Elute Cards

Sharon Appleyard and Will White

## Whatman<sup>™</sup> FTA Elute<sup>™</sup> cards for tissue preservation

FTA Elute<sup>™</sup> cards use patented Whatman FTA technology

(http://www.gelifesciences.com/webapp/wcs/stores/servlet/ catalog/en/GELifeSciences-

au/products/AlternativeProductStructure 17096/?gclid=CPDE uMWdvdACFZaTvQodOxYAzA). The cards are designed to store tissues that are needed for DNA extractions and to simplify the handling and processing of DNA. The FTA Elute<sup>™</sup> matrix in the card is chemically treated with proprietary reagents that preserve the tissue on contact with the card. DNA is then recovered from the FTA card through a simple elution process using water and heat. The cards are optimised for biosafety through the use of anti-microbial agents. Samples can be collected and shipped at room temperature with no need for ethanol preservation. FTA cards are routinely used in human, plant and animal research.

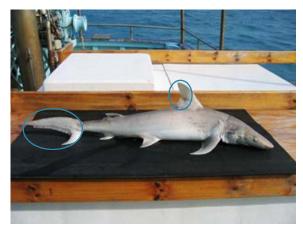
Here we use FTA Elute cards for tissue storage and for DNA extraction. The DNA is used to determine the species of the shark that has been caught (as part of Shark Assessment Reports, we need to determine the species that are being impacted, the number of individuals (i.e. abundance) and where the animals are found).

#### Advantages

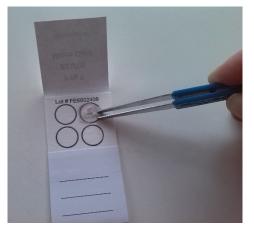
- sampling and storage of tissue in one easy step
- suitable for collection in the field
- each card can be divided in half for sampling of two individuals (2 circles per individual)
- reduced costs for labour and transport
- application and processing in the field or lab
- fast technology for analysis of DNA
- room temperature storage, no need for freezing or buffers

#### Method for tissue sampling

 either rub the FTA Elute card across the caudal/dorsal fin of the individual, or alternatively take a small, thin piece of tissue from the individual (e.g. muscle, liver, heart, skin scrapings) and using blunt end forceps, squash the sample into the circles on the FTA Elute card



Caudal (left) and dorsal (right) fins of a shark



Squash a small piece of tissue onto the card

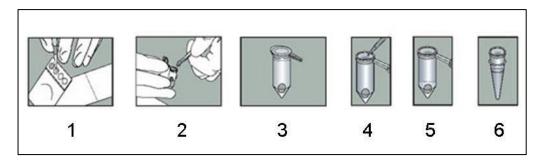
- once the sample has been pressed onto the card, leave the FTA Elute card open to air dry (2-3hrs)
- once dry, close the card, write the sample information (e.g. date of catch, length, species) on the space provided and return the card (and any additional sampling information (e.g. sex of the shark, catch location) in an envelope to the relevant NFA provincial officers or NFA's head offices in Port Moresby.
- as it is important to keep the FTA Elute cards dry during long term storage, cards should be stored at room temperature in a dark, dry cupboard/drawer (preferably in a paper folder with small silica gel packs; do not store in zip lock plastic bags) until DNA extraction is undertaken

#### **Method for DNA extraction**

- 1. using the supplied card punch and mat, 4 × 3mm punches are taken from each sample card
- 2. punches are transferred into a 1.7ml microfuge tube. Between each sample, take a cleaning punch (using a piece of card), eject the punch & start on the new sample

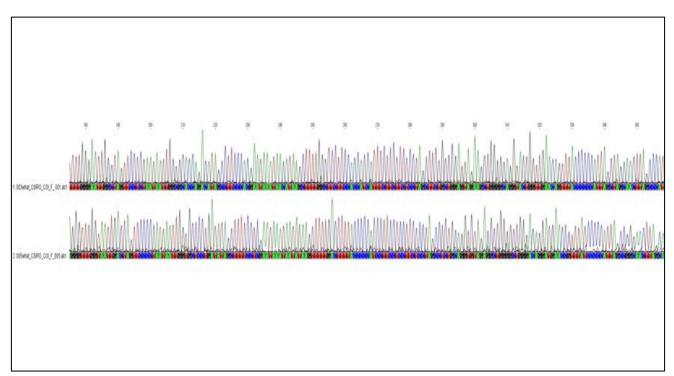


FTA Elute cards with shark tissues pressed onto the cards



Whatman FTA Elute<sup>™</sup> protocol (<u>http://www.gelifesciences.com/webapp/wcs/stores/servlet/catalog/en/GELifeSciences-au/products/AlternativeProductStructure\_17096/</u>)

- 3. 500ul of sterile water is added to the tube; the tube is pulse vortexed 5 times
- 4. excess water is squeezed out of the punches; remaining water is removed with a pipette
- 5. a further 100ul of sterile water is added to the tube containing the punches and vortexed for 5 seconds; the tube is heated on a heat block at 95°C for 1 hour
- 6. after 1 hour, the tube is removed from the heat block, pulse vortexed 60 times and briefly centrifuged (13 000 rpm for 2 minutes); remaining liquid is transferred to a new microfuge tube
- 7. the liquid in the tube contains the DNA and is now checked for quality and the DNA is stored at  $4^{\circ}$ C (short term) or  $-20^{\circ}$ C (freezer) for archival purposes
- 8. the DNA is used in sequencing to determine the shark species



mtDNA cytochrome c oxidase subunit I (COI) sequencing in shark DNA

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AT CSIRO, WE DO THE EXTRAORDINARY EVERY DAY

We innovate for tomorrow and help improve today – for our customers, all Australians and the world.

We imagine. We collaborate. We innovate.

FOR FURTHER INFORMATION

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**w** www.csiro.au/ National Collections and Marine Infrastructure



### OPEN ACCESS

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## Effects of Including Misidentified Sharks in Life History Analyses: A Case Study on the Grey Reef Shark *Carcharhinus amblyrhynchos* from Papua New Guinea

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### Abstract

Fisheries observer programs are used around the world to collect crucial information and samples that inform fisheries management. However, observer error may misidentify similar-looking shark species. This raises questions about the level of error that species misidentifications could introduce to estimates of species' life history parameters. This study addressed these questions using the Grey Reef Shark Carcharhinus amblyrhynchos as a case study. Observer misidentification rates were quantified by validating species identifications using diagnostic photographs taken on board supplemented with DNA barcoding. Length-at-age and maturity ogive analyses were then estimated and compared with and without the misidentified individuals. Vertebrae were retained from a total of 155 sharks identified by observers as C. amblyrhynchos. However, 22 (14%) of these were sharks were misidentified by the observers and were subsequently re-identified based on photographs and/or DNA barcoding. Of the 22 individuals misidentified as C. amblyrhynchos, 16 (73%) were detected using photographs and a further 6 via genetic validation. If misidentified individuals had been included, substantial error would have been introduced to both the length-at-age and the maturity estimates. Thus validating the species identification, increased the accuracy of estimated life history parameters for C. amblyrhynchos. From the corrected sample a multi-model inference approach was used to estimate growth for C. amblyrhynchos using three candidate models. The model averaged length-at-age parameters for C. amblyrhynchos with the sexes combined were  $\bar{L}_{\infty}$  = 159 cm TL and  $\bar{L}_{0}$  = 72 cm TL. Females mature at a greater length ( $I_{50}$  = 136 cm TL) and older age ( $A_{50}$  = 9.1 years) than males ( $I_{50}$  = 123 cm TL;  $A_{50}$  = 5.9 years). The inclusion of techniques to reduce misidentification in observer programs will improve the results of life history studies and ultimately improve management through the use of more accurate data for assessments.



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#### Introduction

Life history information such as growth and maturity are fundamental prerequisites for many demographic and population dynamics models [1]. Without life history estimates, demographic assessments can be produced using life history theory, although the estimates will contain higher levels of uncertainty [2]. Producing accurate life history information is therefore crucial to inform fisheries management and conservation. However, in instances where available life history information has been inaccurate, population declines have occurred through incidental overfishing [3]. The production of accurate life history estimates or a quantifiable uncertainty around them is therefore imperative for sustainable fishing and effective population management.

The Grey Reef Shark *Carcharhinus amblyrhynchos* is a medium bodied whaler shark (Family Carcharhinidae) which is reef associated and has a Indo–West and Central Pacific distribution [4]. *Carcharhinus amblyrhynchos* are caught in tropical fisheries throughout their range [5, 6] and are often landed as incidental catch in some commercial fisheries [7, 8]. In Papua New Guinea (PNG) a dedicated shark long-line fishery existed until July 2014 which developed from the tuna fishery in the 1990s [9]. *Carcharhinus amblyrhynchos* was a common species caught in this fishery, where they comprised ~11% of the total catch [9]. Despite being susceptible to fisheries across much of its range, life history information for *C. amblyrhynchos* is only available from Australia [10, 11], with some limited data available from Hawaii [12, 13] and Indonesia [5]. However, as *C. amblyrhynchos* is caught in larger numbers in PNG, life history information is needed from the local population to form the basis of effective fisheries management and conservation.

Many elasmobranch life history studies have used observer programs as an effective source for collecting life history samples [14, 15, 16]. However, many tropical fisheries do not have operational observer programs and as a result many reef associated shark species are still data deficient with regards to life history information. Recent studies have started to fill these gaps by providing life history information for reef elasmobranchs through fishery independent sampling—where researchers conducted field work to collect the samples [10, 17, 18]. While these studies are valuable for species that cannot be sampled by other means, they add mortality to the population and are logistically disadvantaged as they cannot match the level of fishing effort that observer programs can sample. Observer programs therefore have several benefits for collecting life history samples including larger sample sizes, shorter sampling time frames, greater spread of samples across size ranges, and greater geographic coverage. The opportunistic use of observer programs to source life history samples can therefore have considerable benefits for species that have previously been difficult to sample.

While observer programs provide several benefits in collecting biological data, an important factor to consider is the accuracy of species identification. When collecting life history samples for sharks, many observer programs require observers to record basic biological information (species, length and sex), record the maturity status of an individual when possible, and remove a section of vertebrae for ageing. While this allows a great amount of information to be collected quickly without the need for storing large volumes of biological samples, only the observer witnesses the whole specimen. Therefore, an important assumption of observer data is that species identification is accurate. However, realistically some level of error is inherent in observer species identifications and only recently has this been quantified [19]. Genetic validation has shown that observer error can be substantial for carcharhinid sharks caught in multispecies fisheries in northern Australia [19]. In the northern Australian study, species misidentification occurred at different rates depending on a combination of factors such as species, sex and size [19]. The highest misidentification rates (~20%) occurred for *C. limbatus* and

*C. tilstoni*; two species that are morphologically similar and known to hybridise [<u>19</u>, <u>20</u>]. When using observer sourced samples, these findings raise questions about how often misidentified sharks are unintentionally included in life history analyses and the level of error this introduces into estimates.

Species validation is becoming increasingly feasible due to recent technological advances. Identifying species in the field can be complicated as closely examining morphological features such as dentition or fin morphology can be difficult in field conditions, and for cryptic or "look-alike" species. However, preserving entire specimens is often not possible for fisheries observers as sharks are typically processed at sea. Recent advances in digital camera technology are beginning to overcome this issue as many "all weather" rugged camera models are now available that survive exposure at sea and can store large numbers of images. This technology facilitates the post-cruise validation of species identifications using photographs taken by fisheries observers at sea. While digital cameras have great potential for species validation in situ, genetic analyses in the laboratory are increasingly being used for species identifications. DNA barcoding of the cytochrome c oxidase I (COI) mitochondrial (mtDNA) gene has become an important tool that can rapidly and accurately assist in species identification and can overcome issues such as unknown or poorly defined morphological characteristics that complicate accurate identification of individuals at sea [21]. Due to these advantages, the use of DNA barcoding is becoming increasingly common in fisheries science [21] and has already been used to validate species identifications for fisheries observer programs [19]. Both DNA barcoding and the post-fishing trip inspection of specimen photos provide an opportunity to determine what effects species misidentification might have on life history estimates and ultimately minimise them.

In order to determine the effects of species misidentification in life history analyses, a case study is presented using *C. amblyrhynchos* sampled from the PNG longline fishery. Two types of species validation techniques were used to identify the misidentification rate: 1) diagnostic photographs of the specimens taken on-board by the fisheries observers; and 2) DNA barcoding using the COI gene. This integrated approach of combining genetic and life history analyses allowed the effects of including misidentified individuals in life history studies to be explored.

#### Methods

#### **Ethics Statement**

Vertebrae from *Carcharhinus amblyrhynchos* were collected from commercial longline operations operating in Papua New Guinea by an observer placed on the vessels by the National Fisheries Authority (NFA), the governing fisheries authority in Papua New Guinea. No specific permits or approvals were required to collect samples from the sharks caught by the longliners. All sharks from which vertebrae were taken were to be retained by the fishing vessels as part of their quota.

#### Sample collection

Samples were collected in May and June 2014 by observers on board longline vessels operating in the Bismarck and Solomon Seas. The vessels targeted shark species by setting their gear close to the surface while using a maximum of 1200 hooks per set for an average soak time of 8–10 hours [9]. Biological information was recorded for each landed individual including the total length (TL), sex and maturity stage. The TL of each individual was measured to the nearest 1 mm following [22]. A section of vertebrae consisting of about 4–6 centra were removed from the vertebral column below the first dorsal fin and stored frozen. Frozen vertebral sections were sorted at the NFA provincial office in Rabaul, East New Britain, and then sent to the

laboratories at James Cook University (JCU) in Townsville. Tissue samples (approximately 150 mg) for DNA barcoding were later excised from the remaining muscle around the vertebrae or from the vertebral chord and preserved in 100% analytical-grade ethanol.

While on board the vessels, the NFA observers photographed each individual before processing. These images usually consisted of a roughly lateral view of the shark (Fig 1a), but sometimes also included secondary images of other key diagnostic features (e.g. ventral view of the head, upper dentition, close-ups of fins). These images were later examined by WTW to verify on-board species identifications. Most *C. amblyrhynchos* identifications were easily confirmed from images of the caudal fin as this species has a distinctive black margin on the anterior edge of the fin (Fig 1b). In some instances, the image did not include the key diagnostic feature, i.e. the caudal fin, and thus accurate confirmation could not be made from the image.

#### DNA barcoding of tissue samples

DNA from vertebral chord or muscle samples was extracted using the Wizard<sup>®</sup> SV Genomic DNA Purification system (Promega, Australia) with starting material of approximately 0.25 g. Tissue extractions were undertaken using SV minicolumns following manufacturer's instructions (including an overnight digestion at 55°C on an Eppendorf Thermomixer Comfort (Eppendorf, Australia) and the modifications of 400  $\mu$ g Proteinase K and DNA precipitated in 160  $\mu$ l nuclease free water. Each DNA sample was quantified on a Nanodrop 8000 UV-Vis Spectrophotometer (Thermo Scientific, USA).

Genetic species identification through barcoding of the COI mtDNA gene was undertaken using the universal Fish-BCL (5' –TCAACYAATCAYAAAGATATYGGCAC–3') and Fish-BCH (5' –ACTTCYGGGTGRCCRAARAATCA–3') primers [23]. PCRs were undertaken in 25  $\mu$ l using GoTaq<sup>®</sup> Green Master Mix (Promega, USA), Bovine Serum Albumin (Promega, USA), 10  $\mu$ M primers and DNA quantities of between 8 and 20 ng. PCRs were performed in an Applied Biosystems GeneAmp<sup>®</sup> PCR System 9700 (Life Technologies, Thermo Fisher Scientific, USA) with cycling conditions of 94°C × 3 min; 35 cycles of 94°C × 1 min, 50°C × 1 min 30sec, 72°C × 1 min; and a final extension of 72°C × 10 min. PCR products were visualised on 2.5% TAE agarose gels and fragments cleaned using an Agencourt AMPure XP PCR purification kit (Beckman Coulter, Australia) according to the manufacturer's instructions.

PCR products were sequenced bi-directionally using the same primers as in the original PCR, BigDye<sup>®</sup> Terminator v3.1 Cycle sequencing kit (Life Technologies) and an annealing stage of 50°C × 5 sec across 25 cycles. Cycle sequenced products were cleaned using the CleanSEQ kit (Beckman Coulter) according to the manufacturer's instructions and run on an ABI 3130XL AutoDNA sequencer (Life Technologies).

Forward and reverse sequences (per gene fragment) were assembled into consensus sequences in Geneious<sup>®</sup> R8.1.4 (Biomatters Ltd Auckland, New Zealand; <u>http://www.geneious.com</u>) using the de novo assembly tool. Consensus sequences were aligned within Geneious using the MUSCLE algorithm and sequence identity was confirmed by using the BLAST module in Geneious (<u>http://blast.ncbi.nlm.nih.gov/Blast.cgi;Megablast</u>) against GenBank (<u>http://www.ncbi.nlm.nih.gov/genbank/</u>). COI sequences were additionally compared to sequences publicly available in the Barcode of Life database (BOLD, <u>http://www.boldsystems.org/index.php/IDS\_OpenIdEngine</u>).

#### Vertebrae sectioning

Vertebrae processing and sectioning followed [24]. Vertebrae were defrosted and the remaining muscle tissue was removed using a scalpel while also separating individual centra and removing the haemal arches. Individual centra were then soaked in a 4% sodium hypochlorite



Fig 1. Diagnostic photographs of *C. amblyrhynchos* taken by the NFA observers on board long line vessels. These photographs include (a) a ventral view of the whole specimen and (b) a view of the caudal fin. *Carcharhinus amblyrhynchos* have a very distinctive, broad black posterior margin on the caudal fin.

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solution for 30 min and rinsed under tap water to remove any remaining connective tissue. They were then placed in a drying oven at 60°C for 24 hours. A single centrum from each individual was sectioned using a low-speed circular saw with two diamond-tipped blades (Beuhler, Illinois, USA). These sections were made through the centrum focus at a thickness of 400  $\mu$ m. After sectioning, each centrum was mounted onto a microscope slide using Crystal Bond adhesive (SPI supplies, Pennsylvania, USA).

#### Age determination

Individual ages were estimated by counting translucent and opaque bands in the *corpus calcar-eum* of the centra under transmitted light [24]. Annual growth deposition could not be validated in this study as the short sample collection period precluded validation techniques such as marginal increment analysis. However, age validation was previously attempted for *C. amblyrhynchos* from northern Australia using oxytetracycline mark recapture methods [10]. While these attempts were unsuccessful, individuals that were at liberty for 10 months displayed growth consistent with annual growth band deposition [10]. Based on this evidence and a strong body of literature which has validated the ages of several carcharhinid species [17, 25, 26] annual growth band deposition was assumed in this study.

Growth bands were counted by two independent readers to reduce growth read bias [24]. When counts differed between readers the samples were re-examined until a consensus age was reached. If no consensus age was reached, that centrum was removed from analysis. In order to simulate the scenario where misidentified individuals were incidentally included in growth analysis; individuals that were mistakenly identified as *C. amblyrhynchos* were also included in the samples. Neither reader had any knowledge of which individuals had been misidentified nor how many were included.

Inter-reader precision was conducted on the original counts of both readers for verified *C. amblyrhynchos* (i.e. misidentified individuals were not included). Percent agreement  $\pm$  1 year (*PA*  $\pm$  1 year) was calculated between growth band reads [24]. Bowker's test of symmetry [27, 28], average percent error (*APE*) and Changs coefficient of variation (*CV*) [29] were used to test precision and whether the inter-reader variability was systematically biased. These statistics were calculated using the FSA package [30] in the 'R' program environment [31].

#### Growth modelling

A contemporary framework using multi-model inference (*MMI*) was used to estimate growth following [32]. This approach incorporated *a priori* a set of three candidate models: the von Bertalanffy, Gompertz and logistic growth models (<u>Table 1</u>) and used Akaike's information criterion (*AIC*) to evaluate model performance and produce a set of weighted model average length-at-age estimates [32]. This approach provides more robust growth estimates than the *a priori* use of the von Bertalanffy growth function (VBGF) [33, 34]. All three models were

Table 1. Model equations of the three a priori gr	rowth functions used to estimate length-at-age.
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Growth function	Equation	Reference
von Bertalanffy growth function (VBGF)	$L_t = L_0 + (L_\infty - L_0)(1 - \exp(-kt))$	[35]
Gompertz function	$L_t = L_0 \exp\left(\ln\left(\frac{L_\infty}{L_0} ight)(1 - \exp(-gt)) ight)$	[36]
logistic function	$L_t = rac{L_\infty L_0(\exp(gt))}{L_\infty + L_0(\exp(gt) - 1)}$	[37]

where  $L_t$  is length-at-age t,  $L_0$  is length-at-age 0,  $L_\infty$  is asymptotic length, k and g are the different growth coefficients of the respective models (which are incomparable).

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parameterised to include a length-at-birth parameter ( $L_0$ ) and an asymptotic length parameter ( $L_\infty$ ) as both of these can be compared directly between growth functions (<u>Table 1</u>).

The best fit parameter estimates of all three growth models were estimated using the 'nls' function in the 'R' program environment [<u>31</u>]. The *AIC* values were also calculated in the 'R' program environment [<u>31</u>] and incorporated an additional bias correction algorithm (*AICc*) as the number of samples was less than 200 [<u>38</u>]. The *AICc* was calculated as:

$$AIC_{c} = AIC + \frac{2k(k+1)}{n-k-1}$$

where  $AIC = nlog(\sigma^2) + 2k$ , *k* is the total number of parameters +1 for variance ( $\sigma^2$ ) and *n* is the sample size. The model with the lowest *AICc* value (*AIC<sub>min</sub>*) was the most appropriate. The remaining models were ranked using the *AIC* difference ( $\Delta$ ) which was calculated for each model (*i* = 1–3) as:

$$\Delta = AIC_{c} - AIC_{min}$$

Models with  $\Delta$  of 0–2 had the highest support while models with  $\Delta$  of 2–10 had considerably less support and models with  $\Delta$  of >10 had little or no support [39]. *AIC* weights (*w*) represent the probability of choosing the correct model from the set of candidates and were calculated for each model (*i* = 1–3) as:

$$w_i = \frac{\exp\left(-\frac{\Delta_i}{2}\right)}{\sum_{j=1}^{3} \exp\left(-\frac{\Delta_j}{2}\right)}$$

As  $L_{\infty}$  was comparable between the three growth functions, a model averaged value was calculated for both parameters as:

$$\bar{L}_{\infty} = \sum_{i=1}^{3} w_i * L_{\infty,i}$$

where  $\bar{L}_{\infty}$  was the model averaged asymptotic length [33, 40]. The unconditional standard error of  $\bar{L}_{\infty}$  was estimated as:

$$SE(\bar{L}_{\infty}) = \sum_{i=1}^{3} w_i * (var(L_{\infty,i}|g_i) + (L_{\infty,i} - \bar{L}_{\infty})^2)^{1/2}$$

where  $var(L_{\infty,i}|g_i)$  is the variance of parameter  $L_{\infty}$  of model  $g_i$  [34]. As  $L_0$  is also comparable between model candidates, a model averaged value and unconditional standard error were also calculated for it using the same methods. The three growth completion parameters (k,  $g_{logistic}$ and  $g_{Gompertz}$ ) are incomparable between candidate models and therefore cannot be averaged between them [32].

A likelihood ratio test [41] was used to determine if growth should be estimated for separate or combined sexes. This test was only conducted on the verified *C. amblyrhynchos* individuals using the method outlined by [42] in Microsoft Excel. An assumption of likelihood ratios tests is that the age ranges of the data are equivalent. Therefore, as females younger than 3 years old were missing from the sample, the age range of the males was truncated to be equivalent for this analysis. Likelihood ratio tests cannot be conducted on model averages. Therefore, this analysis was conducted for all three candidate models to ensure that sexual dimorphism of growth was not model dependent and avoid a type II error.

Growth analyses were carried out on two data sets: 1) with all the individuals identified as *C. amblyrhynchos* in the field and 2) with individuals misidentified as *C. amblyrhynchos* removed. A likelihood ratio test [41] was used to statistically test for coincident curves between the two data sets.

# Maturity estimation

The maturity of each individual was staged on board using an index modified from [43] (Table 2). Male maturity stages were based on clasper condition (C = 1–3) and female maturity stages were based on uteri condition (U = 1–5) (Table 2). Maturity stage data was converted to a binary maturity category (immature = 0 and mature = 1) for statistical analysis. Estimates of length-at-maturity were produced for males and females using a logistic regression model [43]:

$$P(l) = P_{max} \left( 1 + e^{-\ln(19)(rac{l-l_{50}}{l_{95} - l_{50}}} 
ight)^{-1}$$

where P(l) is the proportion of the population mature at TL, l and  $P_{max}$  is the maximum proportion of mature individuals. The lengths that 50% and 95% of the population were mature  $(l_{50} \text{ and } l_{95})$  were estimated using a generalised linear model (*GLM*) with a binomial error structure and a logit-link function in the 'R' program environment [31]. Estimates of age-at-maturity ( $A_{50}$  and  $A_{95}$ ) were estimated using the same methods.  $l_{50}$  and  $A_{50}$  were used as metrics to describe the approximate length and age at maturity for the population.

Maturity estimates were also estimated twice: 1) with all the individuals identified as *C*. *amblyrhynchos* in the field and 2) with individuals misidentified as *C*. *amblyrhynchos* removed. A statistical difference between two sets of population maturity estimates was tested for using a likelihood ratio test with a  $\chi^2$  distribution using the 'drop1' function in the 'R' program environment [31].

## Results

## Effects of species misidentification on life history estimates

A total of 155 sharks were originally identified as *C. amblyrhynchos* by the on-board fisheries observers. However, 22 of these individuals (14.2%) were subsequently found to be misidentified and were not *C. amblyrhynchos*. Sixteen of these identification errors (72.2%) were originally detected by examining the photographs taken by the observers. DNA barcoding corroborated these corrections and also detected an additional six misidentified individuals (<u>Table 3</u>). Three of the misidentified individuals were larger than the typical length range for *C. amblyrhynchos* (*c*.190cm TL) [<u>11</u>]; these larger individuals were detected from the observer

	Index	Description	Binary maturity condition
Female Uterus	U = 1	Uniformly thin tubular structure. Ovaries small and without yolked ova	Immature
	U = 2	Thin, tubular structure which is partly enlarged posteriorly. Small yolked ova developing	Immature
	U = 3	Uniformly enlarged tubular structure. Yolked ova developed	Mature
	U = 4	In utero eggs or embryos macroscopically visible	Mature
	U = 5	Post-partum—enlarged tubular structure distended	Mature
Male Clasper	C = 1	Not calcified; pliable with no calcification	Immature
	C = 2	Partly calcified	Immature
	C = 3	Rigid and fully calcified	Mature

Table 2. Indices for staging maturity condition. Adapted from [43]Organ.

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Corrected species ID	Total Length (cm)	Age (Vertebral growth band count)	Detected via photograph	Detected via DNA barcoding
Carcharhinus lecuas	284	21	Yes	Yes
Carcharhinus limbatus	145	7	Yes	Yes
Carcharhinus falciformis	90	1	No	Yes
Carcharhinus falciformis	92	1	Yes	Yes
Carcharhinus falciformis	95	1	Yes	Yes
Carcharhinus falciformis	95	2	No	Yes
Carcharhinus falciformis	108	5	Yes	Yes
Carcharhinus falciformis	112	5	No	Yes
Carcharhinus falciformis	112	4	Yes	Yes
Carcharhinus falciformis	121	6	Yes	Yes
Carcharhinus falciformis	123	4	No	Yes
Carcharhinus falciformis	124	6	Yes	Yes
Carcharhinus falciformis	127	7	Yes	Yes
Carcharhinus falciformis	127	8	Yes	Yes
Carcharhinus falciformis	137	9	Yes	Yes
Carcharhinus falciformis	146	9	Yes	Yes
Carcharhinus falciformis	149	7	Yes	Yes
Carcharhinus falciformis	150	11	Yes	Yes
Carcharhinus falciformis	170	8	No	Yes
Carcharhinus falciformis	174	5	No	Yes
Carcharhinus falciformis	192	13	Yes	Yes
Carcharhinus falciformis	230	13	Yes	Yes

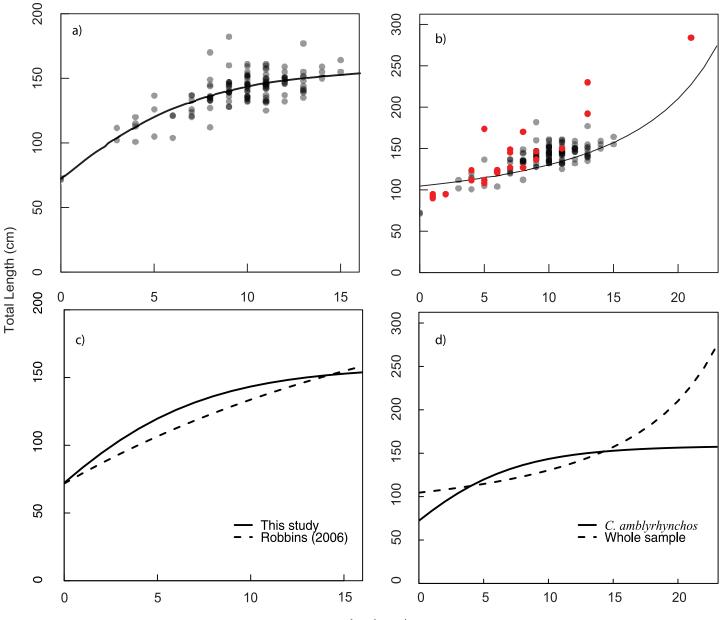
#### Table 3. Individuals misidentified as C. amblyrhynchos by on-board observers.

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photographs (<u>Table 3</u>). The species that had been incorrectly identified as *C. amblyrhynchos* were the bull shark (*C. leucas*), common blacktip shark (*C. limbatus*) and silky shark (*C. falciformis*).

Likelihood ratio tests determined that the misidentified individuals produced a significantly different growth curve to C. amblyrhynchos when they were not removed (VBGF [ $df = 3, \chi^2 =$ 20.19,  $p = \langle 0.0001 \rangle$ ; logistic function [ $df = 3, \chi^2 = 28.92, p = \langle 0.0001 \rangle$ ; Gompertz function [ $df = 3, \chi^2 = 27.80, p = < 0.0001$ ]). The  $L_0$  and  $L_\infty$  parameter estimates did not resemble empirical length-at-birth or maximum length values and were extremely inflated (Fig 2b). The inclusion of misidentified individuals produced an  $\bar{L}_0$  estimate of 105 cm TL which is well outside of the length-at-birth range of C. amblyrhynchos (63-72 cm TL) [11]. However, the greatest amount of error was introduced to the older age ranges of the growth curve (Fig 2b and 2d). The  $\bar{L}_{\infty}$  estimate with the misidentified individuals included was 5640000 cm TL; a nonsensical value which demonstrated the inability of the model to include anomalous data produced by misidentification. This value was produced as the data was best fit by models that indicated growth increased continuously and therefore did not asymptote (Fig 2b and 2d). Subsequently all of the growth completion parameters (k,  $g_{logistic}$  and  $g_{Gompertz}$ ) were extremely low (<u>Table 4</u>). This growth trajectory occurred due to the inclusion of two individuals (230 and 284 cm TL) that were far larger than any of the verified C. amblyrhynchos individuals included in this study (Table 3).

The maturity estimates were less affected than the growth estimates when misidentified individuals were included (Fig 3). Likelihood ratio tests determined that failing to remove misidentified individuals altered the maturity ogives for males (Length [ $df = 1, \chi^2 = 7.66, p = 0.005$ ] and age [ $df = 1, \chi^2 = 4.03, p = 0.045$ ]) but not for females (Length [ $df = 1, \chi^2 = 0.26, \chi^2 = 0.26$ ])



Age (years)

Fig 2. Length-at-age curves for: a) *C. amblyrhynchos*, b) *C. amblyrhynchos* (grey points) with misidentified individuals (red points) included, c) a comparison between *C. amblyrhynchos* from PNG (solid line) and northern Australia [10] (dashed line), and d) comparison of curves for *C. amblyrhynchos* (solid line) and *C. amblyrhynchos* with misidentified individuals included (dashed line). The species of the misidentifications are given in Table 3. All curves were fitted using the model averages of the *MMI* results except for the results from [10] which are the respective VBGF length-at-age estimates.

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p = 0.61]; age  $[df = 1, \chi^2 = 0.03, p = 0.85]$ ). However, the  $l_{50}$  and  $A_{50}$  estimates for males with misidentified individuals included were 123.3cm TL (SE = 3.12) and 5.5 years (SE = 0.85) respectively which were only marginally different to confirmed *C. amblyrhynchos*. The  $l_{50}$  and  $A_{50}$  estimates for females when misidentified individuals were included were 138.6 cm TL (SE = 2.96) and 9.5 years (SE = 0.52) respectively. Despite there being no significant difference



Table 4. Summary of model parameters and AIC<sub>c</sub> results for the observed length-at-age for C. amblyrhynchos and C. amblyrhynchos with misidentified individuals still included.

Model	n	AICc	Δ	w (%)	$L_{\infty}$ (± SE)	<i>L</i> <sub>0</sub> (± <i>SE</i> )	k (± SE)	g <sub>Gompertz</sub> (± SE)	g <sub>logistic</sub> (± SE)	RSE
Carcharhinus an	Carcharhinus amblyrhynchos and misidentified individuals									
VBGF	155	1288.55	5.02	0.07	1.04e+4 (± 4.87e+5)	104 (± 5.69)	5.32e+4 (± 4.87e+5)	-	-	15.2
Logistic	155	1283.53	0.00	0.93	6.10e+6 (± 1.29e+11)	105 (± 4.37)	-	-	0.04 (± 0.02)	14.95
Gompertz	155	1545.85	262.33	0.00	1.27e+5 (± 9.41e+6)	105 (± 10.97)	-	5.93e+3 (± 0.06)	-	34.85
Model average	155	-	-	-	5.64e+6 (± 1.2e+11)	105 (± 4.45)	-	-	-	-
Carcharhinus amblyrhynchos										
VBGF	133	1000.52	0.32	0.30	163 (± 6.27)	71 (± 6.46)	0.15 (± 0.03)	-	-	9.92
Logistic	133	1000.20	0.00	0.35	156 (± 3.77)	73 (± 5.81)		-	0.26 (± 0.04)	9.91
Gompertz	133	1000.22	0.02	0.35	158 (± 4.65)	72 (± 6.14)		0.21 (± 0.03)	-	9.91
Model average	133	-	-	-	159 (± 5.62)	72 (± 6.20)		-	-	-

n is the sample size,  $AIC_C$  is the small-sample bias adjusted form of Akaike's Information Criteria,  $\Delta$  is the difference in  $AIC_C$  values between models, w (%) are the  $AIC_C$  weights,  $L_{\infty}$  is asymptotic length parameter in cm,  $L_0$  is the length-at-birth parameter in cm, k is the growth completion parameter in yr-1 for the VBGF, g is the growth parameter for Logistic and Gompertz functions (but is incomparable between the two), *SE* is the standard error of the adjacent parameter and *RSE* is the residual standard error of the model.

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between maturity ogives for females when misidentified individuals were included, the  $l_{50}$  and  $A_{50}$  estimates were more disparate than the males.

## Life history of C. amblyrhynchos

The confirmed number of *C. amblyrhynchos* used in the analyses was 133. This sample consisted of 90 males (71–182 cm TL) and 43 females (102–177 cm TL). The age ranges for males and females were 0–13 and 3–15 years, respectively. The *PA* ± 1 year was 46% with no systematic bias detected by Bowker's test of symmetry ( $df = 39, \chi^2 = 43.15, p = 0.30$ ). Precision was greatest at younger age classes (< 5 years) (Fig 4). The *APE* and *CV* were 9.46% and 13.38% respectively which are typical for long lived species that have a greater number of growth bands to read [44].

Likelihood ratio tests determined that there was no significant difference between male and female growth curves for any candidate model (VBGF [ $df = 3, \chi^2 = 1.92, p = 0.58$ ]; logistic function [ $df = 3, \chi^2 = 2.10, p = 0.55$ ]; Gompertz function [ $df = 3, \chi^2 = 2.05, p = 0.56$ ]). Therefore, length-at-age estimates were produced with the sexes combined (Fig 2a). All three candidate models produced similar length-at-age estimates that were biologically reasonable; with estimate ranges being  $L_0 = 71-73$  cm TL and  $L_{\infty} = 156-163$  cm TL (Table 4). Subsequently, the residual standard error (*RSE*) was similar between all three candidate models and *AICc* determined that they provided equal support for the data (Table 4). Therefore, *MMI* was used to produce model averaged length-at-age estimates (Table 5). The model averaged  $\bar{L}_0$  and  $\bar{L}_{\infty}$  were 72 cm TL and 159 cm TL respectively (Table 4). Length-at-age estimates for *C. amblyr-hynchos* from this study (PNG) were similar to estimates from northern Australia [10] (Fig 2c).

Male and female *C. amblyrhynchos* mature at different lengths and ages. The maximum likelihood estimates of  $l_{50}$  and  $A_{50}$  predicted for males were 123 cm TL (SE = 2.9) and 5.9 years (SE = 2.03) respectively (Fig 3a and 3b). Female estimates of  $l_{50}$  and  $A_{50}$  were predicted as 136 cm TL (SE = 0.64) and 9.1 years (SE = 0.65), respectively, demonstrating that females mature at greater lengths and older ages than males (Fig 3c and 3d).

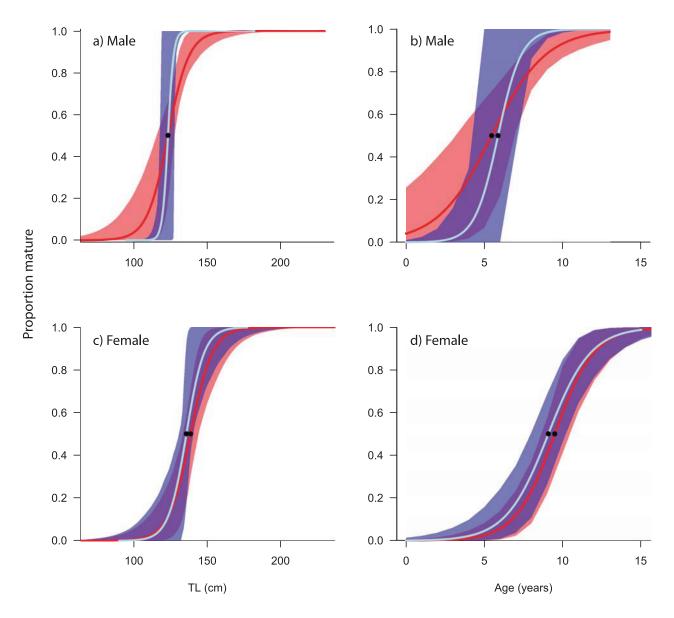


Fig 3. Length- and age-at maturity ogives for: (a, b) male and (c, d) female *C. amblyrhynchos* (light blue line) with 95% confidence intervals (blue area). The maturity ogives for *C. amblyrhynchos* when misidentified individuals were included with 95% confidence intervals are shown by the red line and red area respectively for comparison.

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## Discussion

The misidentification of sharks by observers can have significant effects on the results of life history studies. The inclusion of individuals of species other than *C. amblyrhynchos* added substantial error to the life history analyses from growth models. The greatest error was introduced to the growth analysis which produced inaccurate length-at-age and parameter estimates. In contrast, the amount of error introduced to the maturity ogive analysis was marginal relative to the growth analysis, demonstrating that error can be variable between life history parameters.

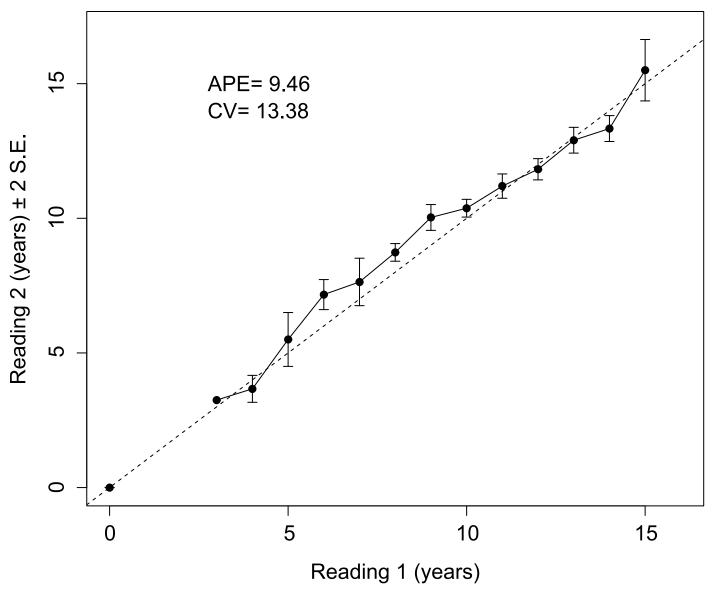


Fig 4. Age-bias plot for C. amblyrhynchos incorporating the age-specific agreements between Readers 1 and 2. Mean age-specific agreements ± 2 standard errors are plotted along a 1:1 equivalence line.

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The maturity estimates ( $l_{50}$  and  $A_{50}$ ) produced for both sexes when misidentified individuals were not removed were similar to those of *C. amblyrhynchos*. However, despite producing biologically realistic  $l_{50}$  and  $A_{50}$  estimates, including misidentified individuals produced male maturity ogives that were significantly different from those of *C. amblyrhynchos*. These maturity ogives along with the length-at-age estimates would have introduced substantial error to future demographic analyses had species identifications not been verified. Consequently, failing to use accurately identified individuals would have precluded this life history information from being usable due to the obvious magnitude of its error.

Regional variability in growth can occur for carcharhinid species [45]. *Carcharhinus amblyrhynchos* from PNG grows slightly faster than the northern Australian population, although the length-at-birth and the lengths at older ages are similar between the two

Age	Model averaged TL estimate (cm)
0	72
1	84
	94
2 3	104
4	112
5	120
6	126
7	132
	136
8 9	140
10	143
11	146
12	148
13	150
14	152
15	153
16	154

Table 5. Model averaged total length-at-age estimates for *C. amblyrhynchos* over the age range included in this study.

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populations [10]. However, no sexual dimorphism in growth curves occurred for *C. amblyr-hynchos* in this study nor from northern Australia [10]. Additionally, females matured at greater lengths and older ages than males for both populations, a trait typical of many carchar-hinid species [17, 46]. Validation techniques such as marginal increment analysis and mark and recapture were precluded for this study. However, annual growth band deposition is likely based on partial results from validation attempts in northern Australia [10]. In the PNG population, *C. amblyrhynchos* were aged to a maximum of 15 years which was younger than in northern Australia (19 years) [10]. This is likely an artefact of the length-dependent mortality of the PNG population by the dome-shaped selectivity of longline fishing. As increased adult mortality prevents individuals from reaching maximum age, these individuals are often rarer in fished populations and are under-represented in stock assessments [47].

This study has shown that substantial error may be introduced when misidentified individuals are unknowingly included in life history analyses. The misidentification rate detected in this study for C. amblyrhynchos is similar to the largest misidentification rate quantified in the northern Australia observer program [19]. Therefore, this study likely demonstrates the full impact of species misidentification on subsequent life history analyses. The severity of this impact was magnified by the inclusion of misidentified individuals that were far larger and older than verified C. amblyrhynchos individuals. As growth curves are fitted by minimising the sum of squared residuals, they are strongly influenced by the oldest and youngest data points in the sample [42]. Therefore, the inclusion of two misidentified individuals that had disparate length-at-ages to C. ambly hynchos inflated the  $L_{\infty}$  estimate of the candidate growth models. As growth parameters co-vary with one another [48] an inflated  $L_{\infty}$  estimate also caused an overestimated  $L_0$  parameter. The maturity analyses were not influenced as strongly by these misidentifications as sex-specific ogives meant fewer misidentifications were included in each sample. Further as the two largest misidentified individuals were both males, the female maturity ogive was therefore unaffected. Despite minimal error added to the maturity parameters for males, the shape of the ogive was still inaccurate with these misidentifications included.

Therefore, the greatest amount of error will be added to life history estimates when misidentified individuals that have length-at-ages which are substantially larger than the true population are incidentally included.

When life history data include outliers, an argument could be made for removing potentially spurious data points. However, removing these individuals from the data without verifying their identity is poor practice. In this study, a *C. leucas* individual was identified as *C. amblyr-hynchos* with a length of 284 cm TL; a value far larger than any other individual in the sample. However, there are confirmed records of *C. amblyrhynchos* that were larger than 250 cm TL [49] despite individuals rarely exceeding 190 cm TL [4]. Therefore, removing this large *C. leucas* individual from the sample could have potentially removed an individual from an underrepresented demographic of the population. In reality *C. amblyrhynchos* individuals that reach this maximum size would likely be older than a comparably sized *C. leucas* individual. Therefore, a growth curve produced with *c.*250 cm TL *C. amblyrhynchos* individuals in this study. This situation demonstrates that removing supposedly spurious data points should not be a valid option without a reasonable justification.

The recent advancements in genetic techniques means that they are now an important tool in fisheries science [21]. DNA barcoding detected all of the species misidentifications in this study; avoiding the estimation of inaccurate life history parameters. However, the diagnostic images taken by the observers were also an important resource. While they did not detect all of the species misidentifications, the post cruise inspection of images detected the majority of them; including the two outliers that introduced the majority of the error to the growth curve. In a number of instances, some observers took multiple diagnostic images for individuals whose identities were uncertain in order to maximise their identification accuracy. Therefore, providing the observers with cameras not only allowed misidentifications to be detected (in a cost efficient way) but also meant that observers were more vigilant for potential misidentifications. The presence of misidentifications in observer datasets also highlights the need for improved regional species identification guides in many instances, particularly in developing nations.

Genetic analyses are the only option for determining species identifications when poorly resolved images or only parts of an animal (e.g. fin clips or fillets) are available. However, the cost of such an approach means that the incorporation of DNA barcoding into any life history analyses which emanate from observer programs can be cost prohibitive and not always a realistic tool. In contrast, images are a cost effective means for species identifications (particularly from field observations) as long as the image resolution is suitable and the correct lateral view of the animal (with diagnostic features) are taken. Providing observers with cameras so that they can take diagnostic photographs of each specimen (or at least those to be used in subsequent life history analyses) should be considered a feasible addition to observer program sampling methodologies. Such an approach would be especially beneficial for studies that focus on species that are morphologically similar to others and which are likely to be misidentified; genetic validation however still provides the greatest species resolution [19]. By verifying species identifications, accurate data is available to form the basis of life history information and demographic estimates on which informed fishery and population management can be based.

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## **Author Contributions**

Conceived and designed the experiments: JS AC CS SA WW. Performed the experiments: JS AC LB MG SA. Analyzed the data: JS MG SA. Wrote the paper: JS AC SA AT CS WW.

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