

Image: David Harasti

## Sizing up Australia's eastern Grey Nurse Shark population

*A new estimate of adult population size for Australia's eastern Grey Nurse Shark drew on widespread genetic sampling and forensic exploration of family trees.*

Grey Nurse Sharks are found across tropical and temperate regions of the North and South Atlantic, Indian and western Pacific oceans, to depths of at least 230 metres. Internationally, they are listed as *Vulnerable* on the International Union for Conservation of Nature Red List of Threatened Species.

Australia has distinct eastern and western populations, each inhabiting an approximate 2700-kilometre stretch of coastal waters. The eastern population ranges from central Queensland to at least the New South Wales/Victoria border and the western population ranges from Western Australia's North West Shelf to at least Cocklebidy in the Great Australian Bight. The eastern population is listed as *Critically Endangered* under the Australian *Environment Protection and Biodiversity Conservation Act 1999*.

Objective 1 of the 2014 national recovery plan for the Grey Nurse Shark is to: *Develop and apply quantitative monitoring of the population status (distribution and abundance) and potential recovery of the Grey Nurse Shark in Australian waters.*

Previous population estimates for Grey Nurse Shark in 2009 and 2010 relied on photo identification, but this technique can have challenges relating to covering the full geographic range of the population, and the accuracy of matching the sharks' spot markings.

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This CSIRO-led project, funded by the National Environmental Science Program Marine Biodiversity Hub, built on data collections and analytical techniques developed in a suite of related projects completed under this partnership.

It collated existing tissue samples and collected new samples from Grey Nurse Sharks at aggregation sites and from shark control programs in Queensland and New South Wales (in partnership with the University of Queensland and the NSW Department of Primary Industries).

Adult population size and trend for the eastern population was estimated using close-kin mark-recapture (CKMR): an approach that combines advanced genetics and statistical modelling. Two maturity scenarios were used, due to uncertainties relating to the age of Grey Nurse Sharks at maturity.

**Scenario 1** estimated the number of adult sharks for the eastern population at 2167 (range: 1257–3078), with an adult survival rate of 97 percent a year and a 3.4% (range 1.2–5.7%) annual rate of increase. **Scenario 2** estimated the number of adult sharks for the eastern population at 1686 (range: 956–2417), with an adult survival rate at 95 percent a year and a 4.5% (range 2.5–4.7%) annual rate of increase.

A total population estimate could not be produced, due to a lack of available information on juvenile rates of survival.

Based on modelled estimates of a positive annual rate of increase, the eastern Grey Nurse Shark population is believed to have made some overall recovery since protection was implemented. This presumably is as a result of a range of protection measures including the voluntary ban on capture by game-fishers in 1979 followed by NSW Government protection in 1984, and the implementation of critical habitat areas throughout their eastern distribution. (Associated management measures included no-fishing zones, on-the-spot fines, a code of conduct for divers, site specific fishing gear and bait restrictions and guidelines for fishing methods and safe-release.)

	<i>Scenario 1</i> female maturity: 10, male maturity: 7	<i>Scenario 2</i> female maturity: 14, male maturity: 11
total adult population	<b>2167 (range 1257–3078)</b>	<b>1686 (range 956–2417)</b>
adult survival	0.97 per year	0.95 per year
annual rate of increase	3.4% (range 1.2–5.7%)	4.5% (range 2.5–4.7%)

### Close-kin mark-recapture (CKMR)

The close-kin mark-recapture technique used for the eastern Grey Nurse Shark population estimate was developed by CSIRO to estimate Southern Bluefin Tuna populations and refined in studies supported by the Marine Biodiversity Hub for northern river sharks and white sharks.

Advanced genotyping of tissue samples is used to identify family relationships, specifically parent-offspring pairs, grandparent-grandchild pairs, and half-sibling pairs. Given adequate sampling, the number of kin-pairs found, and their spread in time, can be used in a demographic population model to estimate adult abundance, and potentially whether numbers are going up or down.

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The modelling works on the premise that the number of close-kin pairs in a population is related to the size of the population being estimated. For example, a small adult population typically would have a high number of close-kin pairs such as half-siblings, while a large adult population would have a small number of close-kin pairs.

The benefit of this approach is that it allows the use of samples from dead animals, and circumvents problems such as tag loss and tag reporting rates from live sharks. The approach also ensures a broad geographic spread of sampling effort, as was the case in this study, which found close-kin matches covering the whole range of the species on the east coast.

### Sampling and analysis

Sampling of live Grey Nurse Sharks for this study was done by SCUBA. The method involves a diver carefully approaching a shark and obtaining a tissue sample through the use of a biopsy needle attached to a tagging pole. The biopsy needle is darted into the dorsal surface close to the dorsal fin.

A total of 514 tissue samples were sourced for this project: 381 collected from live sharks, six from sharks killed in the Queensland shark control program, one from a shark caught and killed in Victorian waters, and 126 from the NSW DPI archive (including live and dead animals). Mitochondrial DNA from 374 of these sharks was of sufficient quality for analysis, and was used to identify:

- 108 half-sibling pairs/grandparent-grandchild pairs;
- 26 parent-offspring pairs; and
- 11 full-sibling pairs.

Gene sequencing conducted for this project provided an opportunity to identify a genetic sex marker present only in male Grey Nurse Sharks. This marker can be used on past and future samples to verify the sex of individual samples where sex was ambiguous.

### Strategies to guide future monitoring and research

The project made the following recommendations to guide future monitoring and research on Grey Nurse Shark populations in Australia.

#### Improved understanding of age structure: two key strategies

Knowledge of the length of sharks at certain ages is important to CKMR models because it tells the time between the two samples and whether they are related as parent-offspring, or sibling pairs. Because these estimates are not available for Australia, however, growth curves (derived from age-at-length) used in this study were based on United States data.

Accurate Australian estimates would be invaluable, and as a priority, they should be developed using vertebrae sourced, for example, through shark control programs. Vertebrae can be used to derive an age for an animal based on the number of rings, typically formed annually, in much the same way as growth rings are used to age trees.

An emerging epigenetic technique that is being investigated for a number of marine species aims to estimate age based on the methylation of DNA, and may at some point be feasible to apply to Grey Nurse Shark. This technique can provide an estimate of age to within  $\pm 3$  years or better for some mammal species. If developed for the Grey Nurse Shark it would reduce the uncertainty in age estimates and could, retrospectively, be applied to the existing samples to improve estimates of population size.

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### Tissue sampling

Tissue sampling of live animals should be continued on a regular basis. Coupled with the sampling, a paired laser system or hand-held stereo video would ideally be routinely used to provide better length estimates. Because growth rates are highest in young animals, accurate length measurements from this demographic are likely to be most informative.

### Expanded surveys of southern waters on the east coast

Although Grey Nurse Sharks have been recorded from all Australian waters except Tasmania, they have not been reported from the southern coast for many years. Recent sightings, however, suggest the sharks may be reappearing in southern waters on the east coast. Surveys including the New South Wales/Victorian border to Wilsons Promontory are recommended to confirm this potential reoccurrence, initially using aircraft and baited remote underwater video systems.

### Expanded sampling of the western population

A lack of known western population aggregation sites for Grey Nurse Shark has meant there are few tissue samples available, but three potential sites have recently been found: one at Exmouth, WA, and two further south. It is recommended that these sites be surveyed (using BRUV). Substantially more samples from the western population are necessary before the CKMR strategy to estimate abundance could be applied.

### Continue photo identification and citizen science

Photo identification is invaluable for establishing a baseline index of abundance, and possibly, given sufficient time, population trend. Tissue sampling could be added to improve accuracy. Existing photo-ID programs off eastern Australia should be continued (Grey Nurse Shark Research & Community Engagement Program: [https://www.reefcheckaustralia.org/grey\\_nurse\\_shark\\_watch](https://www.reefcheckaustralia.org/grey_nurse_shark_watch)).

### Assemble the Grey Nurse Shark genome

A genome assembly for Grey Nurse Shark would help to overcome difficulties encountered with identifying half-sibling pairs by providing information about the physical linkages between genetic markers.

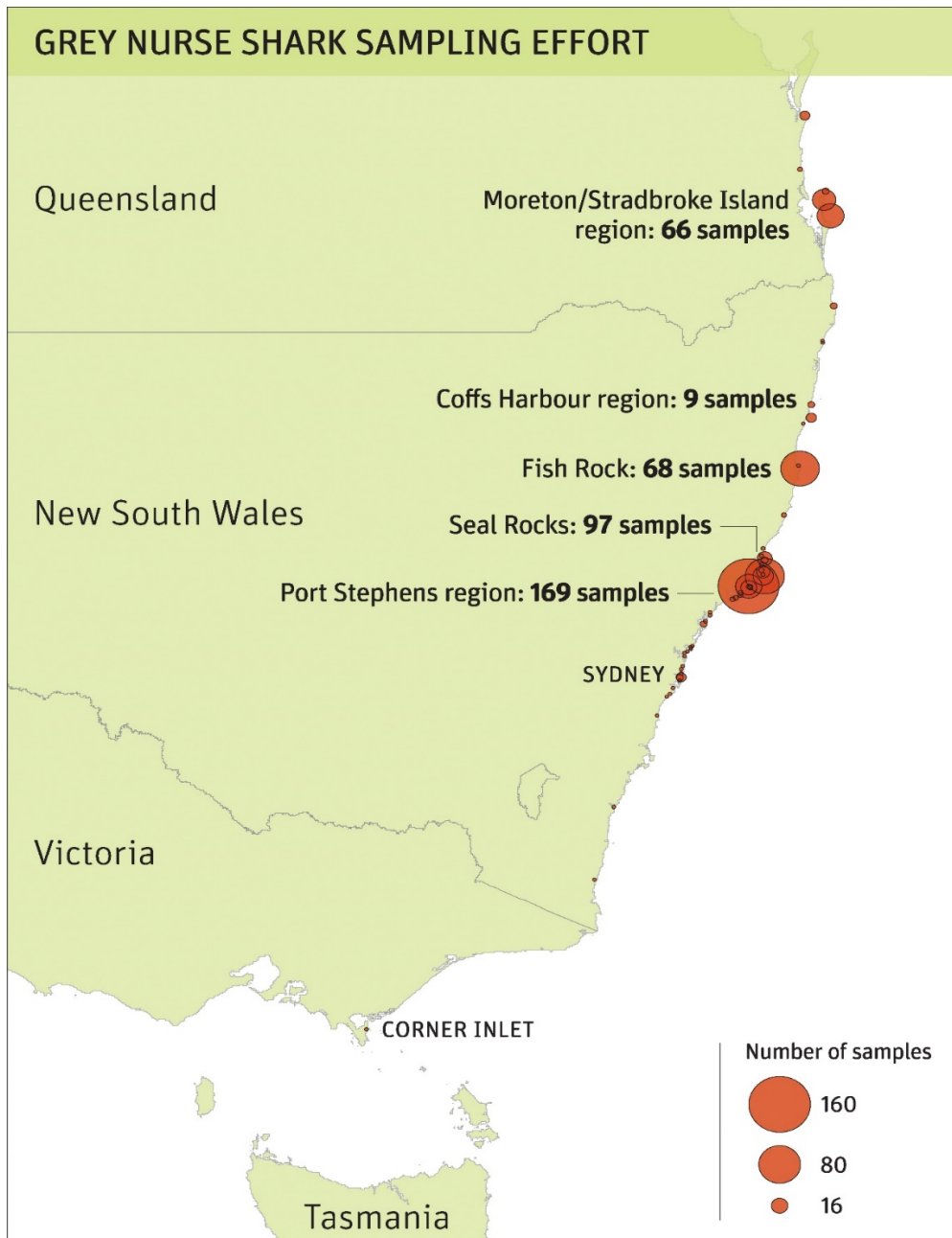
## Archiving the data

Since 2014, CSIRO has partnered with the University of Queensland, New South Wales Department of Primary Industries (NSW DPI), and the Office of the Threatened Species Commissioner (through the Queensland Department of Natural Resource Management – now Queensland Department of Environment and Science) to collect tissue samples and extract DNA from live eastern Grey Nurse Sharks. NSW DPI provided additional tissue samples collected as part of the NSW Shark Control Program and targeted field sampling.

Samples collected for this project (not including those from the NSW DPI archive) have been deposited with the Australian Museum's Centre for Wildlife Genomics to create a lasting archive for future research collaborations.

Field data, including sample locations, have been deposited in the Atlas of Living Australia through MERIT (<https://fieldcapture.ala.org.au>).

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The NESP Marine Biodiversity Hub is funded by the Australian Government's National Environmental Science Programme. Our goal is to assist decision-makers to understand, manage and conserve Australia's environment by funding world-class biodiversity science.

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