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Close-Kin Mark-Recapture population size estimate of *Glyphis garricki* in the Northern Territory

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EXECUTIVE SUMMARY

The Endangered Northern River Shark *Glyphis garricki* (family Carcharhinidae) is found only in northern Australia and southern Papua New Guinea. It is a euryhaline species inhabiting tidal rivers and estuaries with all life stages (neonates to adults) recorded in rivers, while adults have also been recorded in marine waters. During surveys under the National Environmental Research Program (NERP) and the National Environmental Science Program (NESP), samples were collected in the Northern Territory in the rivers of Van Diemen Gulf (VDG) and the Daly River, and in the Kimberley region of Western Australia, from Cambridge Gulf and King Sound. Previously, the species was only known from a very limited number of records (~40) and these surveys have shown its range to be more widespread than initially documented. It is also likely that the species will be recorded in other rivers and estuaries with suitable habitat as more surveys are undertaken. For the purpose of this report, the VDG encompasses the Alligator Rivers region west to the Adelaide River. Based on recent genetic population structure research, the VDG population appears demographically separate from other known populations in the western part of its range, which in turn are separate to each other.

The aim of this study was to apply Close-Kin Mark-Recapture (CKMR) to *G. garricki* to estimate population size within the Northern Territory. Samples were collected from six rivers and creeks flowing into VDG (from east to west): East Alligator River, South Alligator River, West Alligator River (collectively, these three rivers make up the 'Alligator Rivers'), Wildman River, Sampan Creek, and the Adelaide River. While sampling has been conducted across the Australian range of the species (i.e. Daly River, Cambridge Gulf, King Sound), limited sample size outside the VDG prohibited the application of a CKMR model and therefore the focus of this study is the VDG.

Within the VDG, each river shares some kin-pairs with at least one other river, clearly indicating that VDG rivers are not completely separate from each other demographically. However, they do not seem to be completely mixed either. The Wildman River in particular has very few shared pairs with other rivers, i.e. a very high proportion of 'internal' kin-pairs, so it may constitute an effectively isolated unit (i.e. one that would take a longer time than other rivers to repopulate from immigration if the current stock of adults in the Wildman River were

eliminated). The high proportion of 'internal' kin-pairs also suggests that the adult population in the Wildman River is extremely small, but more samples would be required to assess the status of the population in this river.

One hundred and thirty Half-Sibling Pairs (HSPs) were found among the VDG samples. This is ample for making a rough estimate of adult abundance (or at least a lower limit abundance estimate). However, precision is low because of limited information on age, and a limited range of cohorts sampled, which precludes population trend from being reliably estimated.

Models used to estimate adult population size included adult survival fixed at 90% or estimated, and adult abundance either constant over time or subject to a trend which was also estimated. Point estimates of adult population size in the VDG in 2015 were between 582 and 1,116. Considering the range of possible models used and spanning the lowest of the lower 90% confidence intervals and highest of the upper 90% confidence intervals, our rounded estimates are between 320 and 2,050 individuals. Point estimates for the Alligator Rivers only were between 447 and 953. Considering the range of possible models used and spanning the lowest of the lower 90% confidence intervals and highest of the upper 90% confidence intervals, our rounded estimates were between 240 and 1,750 individuals. Thus, despite juveniles being locally abundant in the VDG rivers (as indicated by the large overall sample size during NERP and NESP surveys), the adult population in this region is small.

This study has shown that it is possible to assess and monitor *G. garricki* using CKMR. There are several ways the current estimates could be improved in the future:

- The main limitation within VDG, for purposes of monitoring/predicting trends, is a lack of information on validated length-at-age. To address this would require the future collection of vertebral samples to develop a growth curve (and could be achieved with low sample size applying back-calculation ageing techniques);

- The main limitation outside VDG are small sample sizes which would require further targeted sampling. This would be feasible in the Daly River and Cambridge Gulf where recent surveys have collected more samples, but difficult in King Sound where catch-per-unit-effort is very low compared to other regions;

- Continuing to sample the VDG would give a more robust estimate of trend (this has been achieved in CKMR research on the White Shark (*Carcharodon carcharias*) by increasing sample size and the number of cohorts sampled);

- Fully understanding the demographic linkage between rivers, and whether they should be regarded as independent population units, requires analysis of mtDNA (which is in progress using current samples) and telemetry data on movements; and,

- It is not yet possible to estimate juvenile abundance and therefore total abundance of *G. garricki* (e.g. as was estimated for *C. carcharias*) as this requires estimates of age-specific juvenile survival and of age-at-maturity. The former will be possible from acoustic telemetry data in the Northern Territory, while the latter is possible with the collection of a small number of animals for vertebral ageing.

The combination of vertebral sampling for age determination, further sampling to increase sample size in the Daly River and Cambridge Gulf, analysis of mtDNA data, and analysis of existing telemetry data (to estimate juvenile survival and to assess movement between VDG rivers), would allow for more precise model estimates, and along with additional sampling in the VDG, determination of a robust population trend.

While acknowledging assumptions and limitations of the approach used here, it is possible that the entire Australian population of the species is within the Commonwealth *Environment Protection and Biodiversity Conservation Act* (EPBC) population size threshold for Endangered (<2,500 mature individuals) but is not approaching the threshold for Critically Endangered (<250 mature individuals).

1. INTRODUCTION

Euryhaline elasmobranchs represent over half of the Commonwealth *Environment Protection and Biodiversity Conservation Act* (EPBC)-listed threatened sharks and rays (elasmobranchs), with northern Australia of national importance for this threatened species community. The river sharks (family Carcharhinidae; genus *Glyphis*) along with the sawfishes (family Pristidae; genus *Pristis*) are subject to a multispecies Recovery Plan (DoE 2015). Critical information gaps remain however, limiting the implementation of Recovery Plan objectives.

This research expands on methodologies developed under the National Environmental Research Program (NERP) Marine Biodiversity Hub Project 2.4 'Supporting Management of Listed and Rare Species'. That project was a proof of concept for the use of Close-Kin Mark-Recapture (CKMR) as a tool to estimate population size in rare, poorly-known, and difficult to sample marine and aquatic species. The trial focal species for that project was the Critically Endangered Speartooth Shark (*Glyphis glyphis*) and this tool can now be applied to additional species, namely in this project, the Endangered Northern River Shark (*Glyphis garricki*).

Under the National Environmental Science Program (NESP) Marine Biodiversity Hub Project A1 'Northern Australian Hotspots for the Recovery of Threatened Euryhaline Species', targeted surveys and sampling of *G. garricki* were undertaken with the aims of: (1) documenting more accurately the species' Australian distribution; (2) examining population structure across the species' full distribution; and, (3) establishing a population size estimate by applying CKMR.

To achieve these second two aims, a stocktake of existing tissue samples (collected under NERP Project 2.4) was undertaken, and a targeted field survey was designed to collect tissue samples from known rivers of occurrence, as well as suspected rivers based on habitat suitability.

The aim of the study presented here was to apply CKMR to *G. garricki* to estimate population size within the Northern Territory. Samples were collected from six rivers and creeks flowing into Van Diemen Gulf (VDG; from east to west): East Alligator River, South Alligator River, West Alligator River (collectively, these three rivers make up the 'Alligator Rivers'), Wildman River, Sampan Creek, and the Adelaide River. While sampling has been conducted across the known Australian range of the species, a lower level of sampling effort outside the VDG prohibited the application of a CKMR model and therefore the focus of this study is the VDG.

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The lack of age and growth data as well as a limited number of cohorts sampled within VDG rivers reduces the precision in adult population size estimates (see Section 2.5).

The results presented herein can inform Recovery Plan objectives, contribute to updating the Species Profile and Threats Database (SPRAT), and to preparing a species Conservation Advice. Given a national agenda for northern Australian development (Commonwealth of Australia 2015, Infrastructure Australia 2015), this research provides data to assist in assessing referrals triggered under the EPBC Act. Importantly, it provides a baseline population size estimate and highlights that CKMR is a useful tool for monitoring the population status of *G. garricki*.

2. METHODS & RESULTS

2.1 Methods

Molecular and analytical methods followed those outlined in Bravington *et al.* (2016) and Hillary *et al.* (2017) and are similar to those already applied to *C. carcharias* (Bruce *et al.*, 2018) and Grey Nurse Shark (*Carcharias taurus*) (Bradford *et al.*, 2018).

Glyphis garricki were collected from 11 rivers, large marine embayments or estuaries (thereafter referred to as sampling locations) in 5 different regions covering the entire known geographic range (Figure 1) (Pillans *et al.*, 2010; White *et al.*, 2015; P.M. Kyne *et al.* unpublished data). Of these sampling locations, six rivers of the Van Diemen Gulf (VDG) region were the focus of this study. Each shark was measured, sexed, photographed, tagged with a passive integrated transponder (PIT) tag to individually identify animals, and sampled for genetic material before it was released at the site of capture. Total lengths (TL) of sharks ranged from 52 to 182 cm; most sharks were juveniles or sub-adults based on uncalcified claspers. Males >141 cm TL were sexually mature (possessing calcified claspers). As sexual maturity in female sharks cannot be assessed externally in sharks, female size-at-maturity was assumed to be the same as estimated for males for the purposes of this study. Size-at-maturity generally overlaps between males and females in most carcharhinid species, so this is a reasonable assumption.

Sharks from the VDG used in this study were sampled under Northern Territory Fisheries Special Permits S17/3252 and S17/3364, Kakadu National Park Research Permit RK805, and Charles Darwin University Animal Ethics Committee Approval A11041.

Figure 1. Range map of *Glyphis garricki* in northern Australia and Papua New Guinea, showing all known areas of occurrence. Cambridge Gulf includes: WC, west Cambridge Gulf; and, O, Ord River. This study used samples from Van Diemen Gulf, the core of the species' distribution (boxed area). The insert map shows the rivers sampled in Van Diemen Gulf: (A), Adelaide River; (S), Sampan Creek; (W), Wildman River; (WA), West Alligator River; (SA), South Alligator River; and, (EA), East Alligator River.
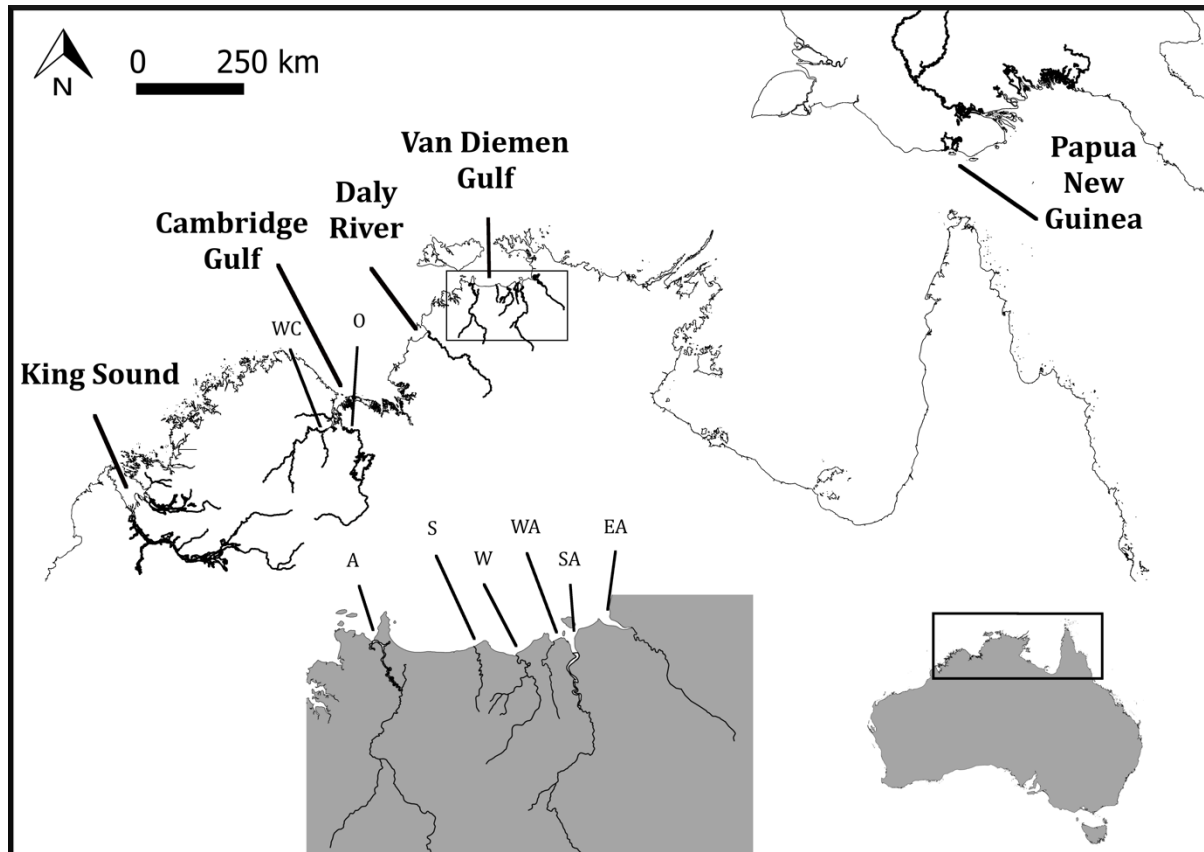
2.2 Close-Kin Mark-Recapture model

The underlying Close-Kin Mark-Recapture (CKMR) model is essentially the same that has been used for other threatened sharks: *C. carcharias* (Bruce *et al.*, 2018), *C. taurus* (Bradford *et al.*, 2018), and *G. glyphis* (Patterson *et al.*, in prep.). While each of these applications required their own set of assumptions, the common assumptions across all were:

1. Constant reproductive output for all adults (of given sex) after maturing at a fixed age;
2. Constant adult survival rate over age, time, and sex; and,
3. Steady exponential increase/decrease in adult numbers over the time period covered by birth-dates of potential Half-Sibling Pairs (HSPs) in the model (i.e. probably one decade for *G. garricki*).

In the absence of age data for *G. garricki*, we had to assume that males mature at the same age as females and that the sex ratio is equal across all ages.

The three parameters estimated are therefore:

1. Adult abundance in some reference year;
2. Adult survival rate; and,
3. Trend in adult abundance (percent change per year).

These models may be improved once sample sizes and/or age information is increased resulting in more precise results in the future.

Our analysis is based on HSPs rather than Parent-Offspring Pairs; we excluded adults from half-sibling comparisons to avoid complications from possible Grandparent-Grandchild Pairs, which are genetically indistinguishable from HSPs.

The population size was estimated for the year 2015.

2.3 Connectivity and CKMR model structure

The analysis presented within this report only addresses the VDG rivers/samples, marked in bold in Table 1 (i.e. encompassing the East Alligator River to the Adelaide River). There is clear evidence of reproductive mixing among the VDG rivers (see below, this section), and clear evidence of separation between VDG and all the non-VDG rivers based on different allele frequency distributions (see Feutry *et al.* submitted). That analysis suggested a lesser degree

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of separation between VDG and the next river sampled to the west (the Daly River) and it does not rule out *occasional* interchange (i.e. a few animals per generation) between VDG and the Daly River, but for demographic/management purposes, VDG seems to stand alone (see Feutry *et al.* submitted). Combining samples from completely different populations (e.g. with different allele frequencies) into one single CKMR model without making complex analytical adjustments, would result in misleading final estimates. While it is possible to develop more-elaborate spatially-explicit CKMR models, that would require more data than we have available for *G. garricki* at present (including the need for mitochondrial DNA data for *G. garricki*).

Table 1. Sample sizes for the final Close-Kin Mark-Recapture model. Van Diemen Gulf (VDG) rivers are in **bold**. Lower row excludes non-VDG rivers; some VDG samples were also excluded on quality control grounds (see Section 3.1).

	KS	WCG	Ord	Daly	**Adelaide**	**Sampan**	**Wildman**	**WA**	**SA**	**EA**	TOTAL
Samples collected	19	15	15	30	**49**	**30**	**47**	**41**	**213**	**70**	529
Samples analysed	.	.	.	.	**26**	**27**	**45**	**36**	**153**	**62**	349

KS, King Sound; WCG, western Cambridge Gulf; WA, West Alligator; SA, South Alligator; EA, East Alligator.

The CKMR analyses presented here therefore excluded all non-VDG samples. Allele frequencies within VDG do not seem to differ much from river to river, indicating exchange within recent generations, and the kin-finding process within VDG gave satisfactorily clear results (Section 3.2). However, the kin-patterns provide strong evidence against complete demographic mixing of all VDG 'subpopulations' (Table 2). Mixing within the Alligator Rivers system does appear to be thorough (Table 2B). Treating the Alligator Rivers as one unit (Table 2A), there is a much higher proportion of within-river HSPs than between-river which, since most HSPs will be from different cohorts and thus different breeding events, indicates reasonable 'river fidelity' on the part of male as well as female adults. In particular, the Wildman River has a sample of just 45 which leads to the remarkably large number of 30 HSPs, 28 of which are internal to the Wildman (the other 2 are shared with the Adelaide River or Sampan Creek, but not with the Alligator Rivers despite the larger sample sizes there). This high degree of 'river fidelity' led us to exclude the Wildman from the CKMR analyses. The very high number of HSPs from the Wildman River samples indicates a very small adult population so removing the Wildman from VDG CKMR estimates will not greatly influence population size estimates for the entire VDG.

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Table 2. Half-Sibling Pair (HSP) connections within the Van Diemen Gulf (VDG) of the Northern Territory. These tables show the numbers of HSPs found between and within rivers in VDG. Table A treats the Alligator Rivers as one unit, to be compared to the other three rivers sampled in VDG; Table B shows the breakdown within the Alligator Rivers system.

A	Adelaide River	Sampan Creek	Wildman River	Alligator Rivers
Adelaide River	8			
Sampan Creek	0	1		
Wildman River	1	1	28	
Alligator Rivers	3	6	0	82

B	East Alligator River	South Alligator River	West Alligator River
East Alligator River	7		
South Alligator River	4	54	
West Alligator River	3	6	8

Demographic exchange between Adelaide River/Sampan Creek and the Alligator Rivers is clearly present but incomplete (based on shared kin-pairs); i.e., some adults may breed in only one of the Alligator Rivers vs Adelaide River/Sampan Creek regions. If there was total exchange, then it should not matter whether CKMR uses samples from just a subset (e.g. from the Alligator Rivers) or from the whole (as we have done here). If there are separate 'subpopulations', then a CKMR analysis of all samples together (without using location information) can estimate the total adults across the 'population', but unless sampling happens to be in proportion to adult abundance, the estimated total will be somewhat biased. Theoretical development of CKMR and larger sample sizes collected outside the Alligator Rivers are required to fully resolve the issues around subpopulations.

In this report, we have presented two sets of analyses:

1. All VDG rivers except the Wildman River (i.e. Adelaide River, Sampan Creek, and the West, South, and East Alligator Rivers); and,
2. Alligator Rivers only.

As previously stated, sample sizes in the non-VDG rivers (Daly River and Western Australia) are currently too small to provide useful information about abundance. This becomes evident during the kin-finding phase, because allele frequency estimates are uncertain in very small samples (even though they are adequate to demonstrate qualitative differences between the rivers) and our current kin-finding code requires accurate frequencies. It is not obvious how much of that is an intrinsic limitation of the data, or something that could be improved with further methodological study, although increasing sample sizes from these locations would address this issue. Kin-finding was attempted for the best-sampled of the non-VDG rivers, the Daly River, where 28 samples survived the preliminary QC checks. While there were some kin-pairs in the Daly River samples (in which case the Daly River adult population could not be large since kin-pairs have been identified with even such a low sample size), the sample size is too small to validate those relationships, and abundance estimation was therefore not possible at this stage. Additional samples are now available from the Daly River and Cambridge Gulf (P.M. Kyne, unpublished data) and this could be re-evaluated in the future.

Note that the non-VDG samples cannot be pooled to give an abundance estimate for the western part of the species' range using CKMR, because they show clearly differing allele frequency distributions (Feutry *et al.*, Submitted).

2.4   Close-Kin Mark-Recapture results

After some experimentation to determine which factors might/might not matter, we fitted eight variants of the CKMR model (Section 2.2), corresponding to three binary choices:

- With Sampan Creek and Adelaide River, or without;
- Adult survival fixed (at 90%), or estimated; and,
- Adult abundance trend fixed (at 0), or estimated.

The results are shown in Table 3, and can be summarized as follows:

- Adult abundance estimates for the entire VDG were around 848–1,115 individuals, combined across both sexes. However, given model uncertainty as well as a coefficient of variance (CV) of 37% (Section 2.5), values as low as 400 or as high as 2,000 cannot be excluded.
- The main impact on abundance is whether the adult abundance trend is fixed *a priori* (at 0) or estimated. In the latter case, the estimates of trend were negative (i.e. declining abundance) at about -7% per year, and the absolute level of abundance is also lower.

However, the trend estimate is not significantly different from zero (i.e. constant population). The standard error is about 0.06, which is only slightly smaller than the magnitude of the point estimate; thus, even a small positive trend would still be well within two standard deviations of the point estimate and hence consistent with the data.

- Including the Adelaide River and Sampan Creek samples increases the abundance estimates by about 20%, consistent with there being some demographic separation (i.e. some adults contributing only to Adelaide River and/or Sampan Creek). This suggests that the Adelaide River and Sampan Creek samples should be included, since those rivers are not completely isolated demographically from VDG but are separated enough that adults would be missed by excluding them.

- Adult survival estimates are not shown, but the point estimates were invariably high, at 96% or more (when the Wildman River samples were included, the adult survival estimate was always 100%, which is biologically not possible). However, an adult survival rate as 'low' as 90% (but not 80%) would still be consistent with the data (i.e. inside the confidence intervals).

  o Those high point estimates of survival rate are not consistent with a downwards trend of -7%. The only way to leave the adult population is to die, so adult abundance logically cannot decline faster than adults are dying, even if no new young adults are joining it (and young *G. garricki* clearly do exist, since they make up our sample). With *C. carcharias*, we carefully formulated the CKMR model so that the contradiction could never happen (Bruce *et al.*, 2018), however, for *G. garricki* we have not yet done this. At a survival rate of 90%, a trend of -7% is still logically possible, although only marginally.

Because of the details about survival and trend, the results in Table 3 are only truly consistent when either trend is fixed at 0 or when survival is fixed at 90%. Both those cases are within the 'confidence intervals' given by the data. The point estimates of abundance for those cases range from 582 to 1,116 (including Adelaide River and Sampan Creek, as explained); this gives some measure of 'model uncertainty', but in addition there is also statistical uncertainty with a CV of ~37%, as described below in Section 2.5.

2.5 Precision

There were more than 100 HSPs in the VDG (excluding the Wildman River), so precision is potentially quite good. For example, this compares to only 20 HSPs from *C. carcharias* in Hillary *et al.* (2018) who estimated population size of this species in Australia. However, for *G.*

garricki the limited number of cohorts being compared (samples analyzed were collected over four years) means that trend and survival cannot yet be estimated precisely, and that uncertainty has a knock-on effect on the precision of absolute abundance estimates. The *reported* CV of absolute abundance (in a fixed reference year) from a single CKMR model for VDG is about 37%. This somewhat understates the true uncertainty, because it only considers certain statistically-quantifiable sources of uncertainty.

Table 3. Estimates of adult abundance and trend from different CKMR variants. Numbers in **bold** are abundance estimates for 2015, of combined male and female adults. Other numbers are estimated trends in adult abundance: -0.09 means a 9% downwards trend. Entries in italics concern adult survival rate: fixed, or free to be estimated (in which case the estimates are between 0.96 and 0.99; not shown here). 'All-VDG' (Van Diemen Gulf) includes Sampan Creek and Adelaide River samples and 'Alligator Rivers only' does not. In the lower half of the table, the trend parameter is free to be estimated. In the upper half, the trend was fixed at zero (so there is no need to report its estimate).

	No trend allowed			
	90%	*Free*		
All-VDG	**848**	**1,116**		
Alligator Rivers only	**706**	**953**		
	Trend is 'free'		Trend estimate	
	90%	*Free*	*90%*	*Free*
All-VDG	**582**	**790**	-0.09	-0.07
Alligator Rivers only	**447**	**712**	-0.10	-0.07

There are several other sources of uncertainty in relation to the application of CKMR to *G. garricki*:

1. The nature of demographic exchange within VDG. The cross-river kin-pair data suggest that we are likely dealing with several subpopulations linked by limited exchange, and our sampling is unlikely to be 'proportional' in exactly the way needed to cancel that out. An ideal CKMR model could deal with that in principle by incorporating explicit spatial factors, but this was not possible without the guidance of mtDNA data, which while not currently available for *G. garricki*, is under investigation. The only option at this time was to use a much simpler single-population model for the VDG, which will suffer some unquantifiable bias as a result. It is also worth noting that planned analysis

of acoustic telemetry data will provide some information on movement of individual sharks between rivers of the VDG.

2. The probability distribution of age-at-length is assumed to be accurate. However, there are two reasons that it may not be. The first is that the fitted growth curve will have random inaccuracy given the short timespans and modest sample sizes (of recaptures) that it was fitted to; this causes unquantifiable bias, although we would not expect that bias to be prodigiously large. The second is that estimating the variance in length-at-age from Full-Sibling Pairs (FSPs; i.e. between closely-related individuals in the same litter, by definition) may understate the true variance in length-at-age across an entire cohort. It is worth noting that this age-from-length issue is not nearly as problematic for *G. garricki* as it is for *C. taurus*; unlike *C. taurus*, almost all *G. garricki* samples were immature (i.e. the age range is restricted), and their lengths were measured accurately.

3. Male and female abundances and demographic parameters were assumed equal. This may not be the case, but this could be resolved in future with better length-at-age and maturity data as well as mtDNA data.

4. The was strong evidence of substantial clustering of HSPs (groups of individuals that were from the same litter – all HSPs of each other). This is precisely because the sampling coverage has been good relative to the small size of the adult population(s). This means that it is not valid to regard all the pairwise comparisons as mutually statistically independent, as is assumed in the CV calculations. We have encountered this with other threatened sharks, but not as dramatically. Accounting for this problem will require substantial theoretical developments in CKMR modelling.

While these factors imply that there will be some bias in our estimates of abundance, and some uncertainty about the precise definition of "population" to which they apply, we expect any effects to be minor compared to the substantial range of uncertainty in the abundance estimates. With more data, a more nuanced model could of course be developed. Furthermore, the underlying data-generation steps leading up to the CKMR model and the abundance estimates for *G. garricki* would not result in abundance estimates that could be wrong by orders-of-magnitude.

3.   ANALYTICAL STEPS & DISCUSSION

The analysis and data preparation follow broadly the same steps already used for other shark species (although in this case without attempting to extrapolate to juvenile abundance as has been done in *C. carcharias*) where genetic preparations led to finding the pairs of kin, inferring data about the age of the animals given length measurements, and combining the data into a simple Close-Kin Mark-Recapture (CKMR) population-dynamics model, to estimate abundance and other demographic parameters. The general theoretical basis for the steps is given in Bravington *et al.* (2016) and population-dynamics details in Hillary *et al.* (2018).

3.1   Data selection

A number of samples (38 out of 387) were excluded on quality control (QC) grounds, using checks we have developed while studying other species (Hillary *et al.* 2018, M. Bravington *et al.* unpublished data). We were purposefully conservative in eliminating even slightly suspect samples, since there were a high number of kin-pairs available for the final analysis.

For the CKMR model, we used only samples with measured length <140 cm TL, in order to avoid modelling complications caused by mature animals and to reduce the uncertainty associated with age-estimation from length in older animals. The final CKMR for the Van Diemen Gulf (VDG), which excluded the Wildman River, used 277 of the possible 304 samples, with 27 excluded on length grounds.

3.2   Kin-finding

Glyphis garricki appears to have rather low genetic diversity, and it was necessary to use two passes of ddRAD to find enough loci for kin-finding. We then genotyped the samples using the same ddRAD/capture-probe approach as for recent CKMR studies (Bradford *et al.*, 2018, Feutry *et al.*, submitted). To select the final set of loci for kin-finding, we used QC checks on the Alligator Rivers samples alone (where demographic exchange should certainly be adequate to ensure Hardy-Weinberg equilibrium) and ended up with 1,280 loci, which proved sufficient for accurate kin-finding (Figure 2). We then extended the checks to the other VDG rivers and identified no issues.

For CKMR purposes (i.e. for the demographic modelling to be valid), kin-finding needs to be very carefully tuned to avoid false-positives and account for likely false-negatives— something that is not possible with existing kin-finding softwares. Through various CKMR applications, we have therefore developed specific statistical algorithms for kin-finding in CKMR (M.

Bravington *et al.* unpublished data), which are aimed at large populations and/or with complete mixing (i.e. no subpopulations between which allele frequencies differ persistently). The case of limited mixing between very small populations, which may be the situation for *G. garricki*, is much more challenging statistically, and further work would be needed to get a satisfactory solution. However, for *G. garricki* in VDG, the results are adequate for at least basic CKMR modelling.

One difference between *G. garricki* and other species where we have used CKMR, is that we do not yet have mtDNA data for *G. garricki*, so we are unable to say whether connectivity is driven by male or female adult movements. The acquisition of mtDNA data for *G. garricki* is underway but was not available in time for consideration under this CKMR project.

3.2.1   Parent-Offspring Pairs

There are 4 clear Parent-Offspring Pairs (POPs), without any ambiguity. In all 4, it is obvious which is the parent: all the parents are male, and in the size range 150–160 cm TL (which is biologically plausible as these individuals were assessed as sexually mature based on the state of their claspers). In both cases, two males fathered two of the offspring. Three pairs were between the East Alligator and South Alligator Rivers, and one was where the father was sampled in the Wildman River and the offspring in the South Alligator River. With so few mature fish in the sample and only 4 POPs, we did not explicitly include the POP comparisons in the CKMR model

3.2.2   Full-Sibling Pairs

There are 34 Full-Sibling Pairs (FSPs), again without any ambiguity. In almost all cases, the two siblings were sampled in the same river, even if we separately consider East from South and South from West Alligator River. The exception had one member of the pair in Sampan Creek, and the other in the South Alligator River. In all but four pairs, the two siblings were similar in size and most likely from the same cohort (born in the same year) (Section 3.3). Overall, cross-cohort FSPs should be rare in most fish populations, because cross-cohort FSPs require one female to mate with the same male a second time (in a different year), which is unlikely if the number of potential male mates is large. Same-cohort FSPs, (individuals from the same litter, will be more common, though less common than Half-Sibling Pairs; HSPs). Therefore, most FSPs encountered will be from the same cohort. The exceptions were the Sampan Creek/South Alligator River FSP pair, plus another cluster of full-siblings from the South Alligator River, where one of the members was much bigger than its siblings and presumably born in a different cohort from a chance repeat mating. Sperm-storage is another

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possible explanation, although it can presumably only cover a small gap between cohorts. The FSP data are thus useful for comparing known-same-age-individuals, in order to study variability in length-at-age without any actual age data.

3.2.3   Half-Sibling Pairs

Half-Sibling Pairs are more weakly-related than POPs or FSPs and finding them reliably (enough for CKMR) from genetic data is challenging process requiring a large number of loci, reliable genotyping methods, and purpose-written software. Despite the small number of loci, the HSP-finding process for *G. garricki* in the VDG proceeded smoothly; note that HSP-finding did not work when pooling the VDG samples with rivers outside VDG, because of allele-frequency differences. Figure 2 shows the distribution of HSPs for the VDG. As always, there is some overlap on the left between true HSPs and either true kin that are weakly-related (or the result of some poor-quality samples inadvertently passing the QC checks, which we have noticed in other species). This is not a serious problem because the general separation of HSPs is quite clear. We deal with it by setting a tight criterion for the pseudo-log-likelihood-ratio for a pair to count as a 'definite HSP' and by adjusting the CKMR model to allow for the proportion of true HSPs that are likely to fall below that criterion (about 30%, in this case).
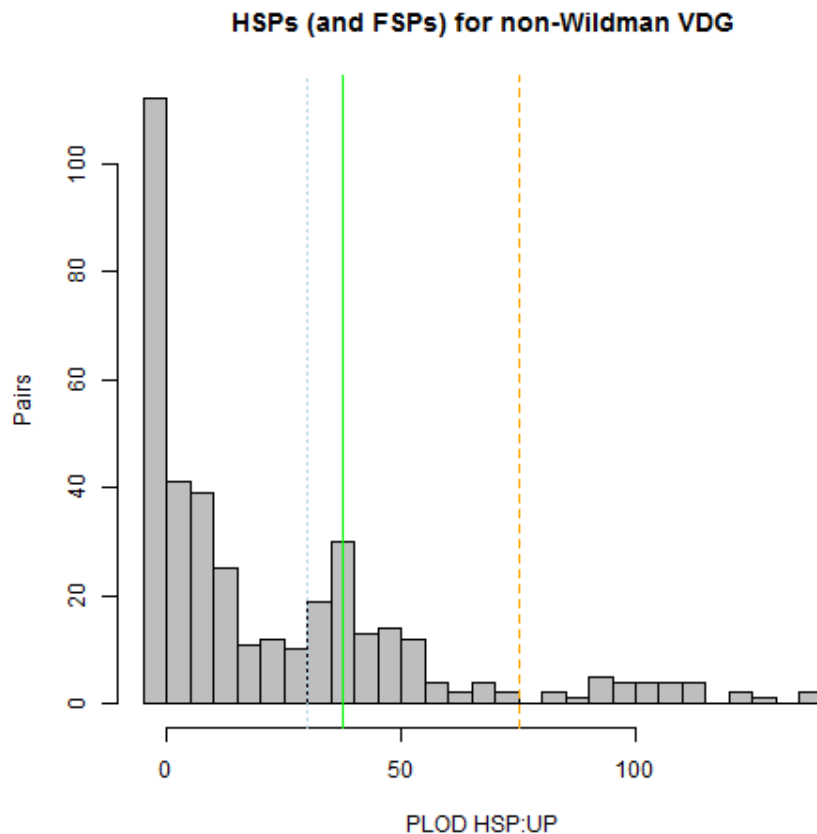
Figure 2. Half-Sibling Pair (HSP) finding in Van Diemen Gulf rivers (excluding the Wildman River). PLOD HSP:UP = pseudo-log-likelihood-ratio between HSPs and unrelated pairs. Dashed blue line is false positive cut-off for HSPs, solid green line is expected PLOD for HSPs, and dashed orange is cut-off for Full-Sibling Pairs/Parent-Offspring Pairs.

3.3   Growth and age

Close-Kin Mark-Recapture models require at least some estimate of age for all individuals, since the demographic probabilities of kinship are specific to when each animal was born; birth-date is usually determined from capture date and age. The less certain each age estimate is, the lower the information-content of the entire dataset for estimating abundance and demographic parameters. Young sharks can usually be aged to reasonable reliability based on cross-sections of vertebrae, but that requires a dead animal, whereas all our *G. garricki* samples were live-release only. We therefore needed to make some inference about age based only on length measurements. To do that, it is necessary to start with some estimate of the statistical spread of length-at-age for the ages likely to be encountered among the samples. For well-studied species, there are usually enough existing samples of known-age (dead) animals to construct those estimates directly; that is certainly the most reliable approach,

however even for well-studied carcharhinid shark species there can be a wide range of age-at-length estimates (see McAuley *et al.*, 2006). For *G. garricki*, in the absence of age-at-length data from sectioned vertebrae we used the following: (i) a growth curve based on change-in-size from individual mark-recaptures (where individual ages are not known); and, (ii) an estimate of individual variability based on the FSPs, which mostly must be belong to the same cohort as each other (see above) (Figure 3). Our estimates of age-at-length based on growth data from recaptured animals is not considered a major flaw in this process, however variability in the age-range inference will influence estimates of trend and perhaps abundance.

For *G. garricki*, the samples used were all immature and therefore the age range is restricted relative to a sample which includes neonates to adults and therefore all possible ages. An average growth-curve can be inferred for *G. garricki* using length measurements from the same individuals caught on separate occasions (these individuals were identified in the field by PIT tags inserted into all sharks at the time of capture, and also subsequently by genetics). However, the time-gap between recaptures was short (the maximum recapture interval was about 2 years). In addition, there was some apparent field measurement error, since 7 of the 29 recaptures had smaller total lengths when recaptured compared to the original capture measurements (albeit mostly by less than 2 cm).

While it would be preferable to obtain direct measurements of length-at-age from sectioned vertebrae, in the absence of these data, our estimates using mark-recapture are the best available data and enable a CKMR model to be developed. If validated age data become available, these should be incorporated into the model.

3.3.1 Treatment of uncertain age in the CKMR model

From a statistical perspective, the most efficient and reliable way to deal with uncertainty in age for CKMR is to treat each sample's age as an unknown random variable whose statistical distribution is determined by the measured length. The CKMR model is much simpler if age is (assumed) known, because then same-cohort comparisons can be excluded *a priori* and the computation of the log-likelihood (i.e. the measure of how well the data matches the working set of parameters being considered at any step of the estimation process) is much faster.

Since the estimates for *G. garricki* do not need to be very precise at present (and cannot be, given some of the issues mentioned earlier), we simplified the task by using an exact-age CKMR model; the age chosen for each individual was randomly chosen from its age-at-length probability distribution. Same-cohort comparisons were then omitted (about 10% of the total).

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We then refitted each model variant 10 times with randomly-chosen ages each time to deal with bias and uncertainty from this process.

To convert the length-at-age distribution (based on the growth curve from individual recaptures, plus the variance in length-at-age established from FSPs), some prior distribution of juvenile age is also needed. We used a uniform prior distribution, which is not strictly correct since it ignores juvenile mortality (a parameter on which we currently have no data). However, the length-at-age distribution is 'tight' enough that this source of error should not be very important.
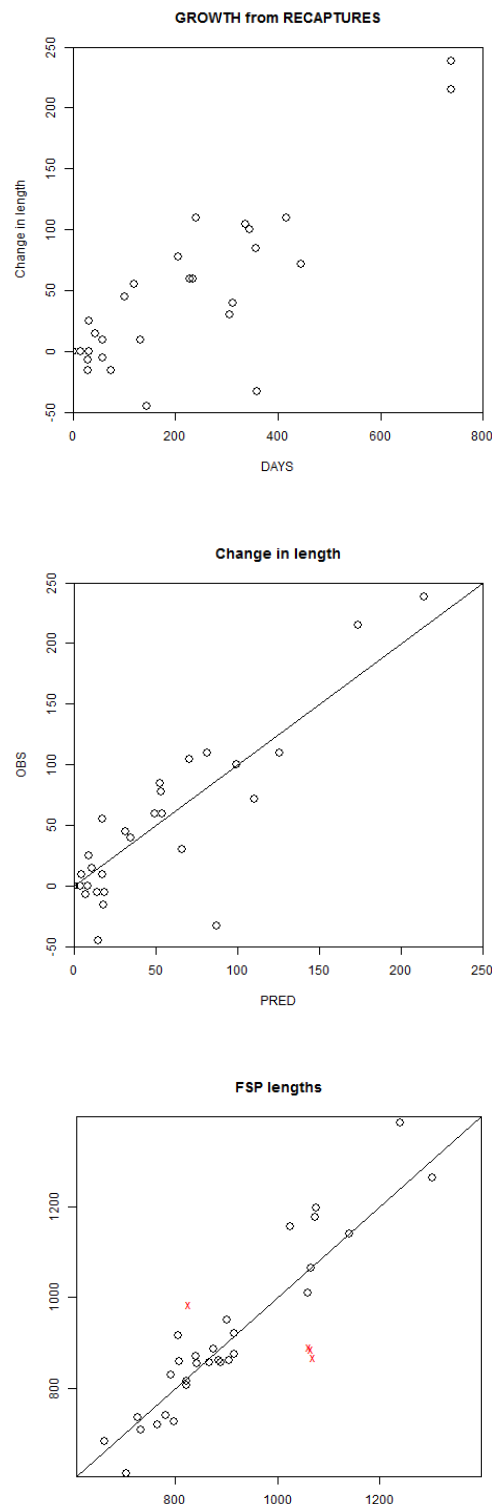
Figure 3. *Glyphis garricki* growth and age. Top and middle figures show individual-recapture data (top: raw length-change data; middle: observed and predicted. Units are millimetres. 'Shrinkage' occurs when the Y-value in the middle plot is below zero. The bottom figure compares the lengths in each Full-Sibling Pair (FSP) (which should almost all be born at the same time), after adjusting for different capture-dates using the fitted growth-curve. Four outliers are shown in red, presumably from cross-cohort FSPs as noted in the text.

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4.   CONCLUSIONS

This project has demonstrated the utility of applying Close-Kin Mark-Recapture (CKMR) to estimate population size in data-poor threatened species. Despite the simple model applied and the lack of age-at-length estimates for this species, this study was able to drastically increase our understanding of the abundance of *G. garricki*. The population size of *G. garricki* was estimated to be ~580–1,100 mature individuals in the Van Diemen Gulf (VDG) of the Northern Territory.

Within Australia, the species has also been recorded in the Daly River and the Kimberley region of Western Australia, and thus the entire Australian adult population is considered to be larger than the values above. While acknowledging assumptions and limitations of the approach used here, it is possible that the Australian population of the species is within the EPBC Act population size threshold for Endangered (<2,500 mature individuals) but is not approaching the threshold for Critically Endangered (<250 mature individuals).

Since the VDG appears to be genetically distinct from places further West (which in turn are isolated from each other), and further east in Papua New Guinea (Feutry *et al.* submitted), rather than the species existing as one wide-ranging Australian population, it is instead comprised of genetically-isolated populations that do not interbreed and should be managed as separate populations. The size of some of the isolated populations (e.g. King Sound) are likely to be smaller than the VDG population given lower catch rates in these areas.

The combination of vertebral sampling for age determination, further sampling to increase sample size in the Daly River and Cambridge Gulf, analysis of mtDNA data, and analysis of existing telemetry data (to estimate juvenile survival and to assess movement between VDG rivers), would allow for more precise model estimates, and along with additional sampling in the VDG, determination of a population trend.

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