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Population structure of Narrow Sawfish *Anoxypristis cuspidata* across northern Australia

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EXECUTIVE SUMMARY

Delineating management units is critical for species of conservation concern such as the globally Endangered Narrow Sawfish *Anoxypristis cuspidata*. In this study, we used state of the art genomic approaches to analyse the population structure of *A. cuspidata* across northern Australia. Samples were obtained from the bycatch of commercial fisheries and subject to mitogenome sequencing as well as single nucleotide polymorphism (SNP) genotyping using the DArTseq protocol. Pairwise Φ_{ST} and haplotype mitochondrial network show evidence of barriers to gene flow between all regions for which we had more than three samples, clearly demonstrating female philopatric behaviour at even finer spatial scale than previously suspected. In contrast, no evidence of population structure was detected using over 2,000 SNP nuclear markers, suggesting male-biased dispersal. Sampling of neonates in nursery areas (before they are able to disperse) would help characterise the philopatric behaviour in this species.

Keywords: connectivity, DArTseq, mitogenomics, philopatry, sex-biased dispersal, trawling

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1. INTRODUCTION

Identification of population boundaries is key to determining the spatial scale of units for appropriate conservation and management of wildlife. The development of cheap high throughput sequencing approaches, often referred to as Next Generation Sequencing (NGS), has greatly improved the resolution of genetic methods to infer population structure in non-model species. Restriction site-associated DNA (RAD) sequencing (Baird *et al.*, 2008; Miller *et al.*, 2007; Sansaloni *et al.*, 2011) in particular has changed the landscape of population genetics, making it possible to genotype thousands of single nucleotide polymorphisms (SNPs) in hundreds of individuals. Mitochondrial DNA (mtDNA), which is maternally inherited, provides a unique insight of the population historical demography and has been used to delineate management units for a long time (Moritz, 1994). It complements nuclear DNA particularly well to study species exhibiting sex-biased reproductive dispersal with philopatric females and dispersive males (Feutry *et al.*, 2017). The power of NGS can also be harnessed to gather mtDNA datasets and full mitogenomes, which are more informative than single mtDNA genes/regions (Feutry *et al.*, 2014) and are becoming more common in population genetic studies (Ovenden *et al.*, 2019).

Sharks, rays and chimaeras (chondrichthyan fishes) are a diverse group of fishes of increasing conservation concern. Rhino rays (order Rhinopristiformes) are the most threatened order of rays (IUCN, 2021). The sawfishes (family Pristidae) are a highly threatened rhino ray family with all five species assessed as either Critically Endangered or Endangered globally (IUCN, 2021). All species have undergone considerable range contractions and local extinctions across their ranges, and Australia represents the last population stronghold for the four species of sawfish occurring in the Indo-West Pacific (Yan *et al.*, 2021). Of these four species, the Narrow Sawfish *Anoxypristis cuspidata* is the smallest and most biologically productive, with a size-at-birth of 56 cm total length (TL), a maximum size of at least 350 cm TL (some other sawfish species reach 7+ m TL), female age-at-maturity of 3 years and a maximum age of 9 years (Peverell, 2009; Last *et al.*, 2016). It is predominantly a marine species with nursery areas in estuarine and coastal habitats (Last *et al.*, 2016).

Anoxypristis cuspidata is the most common sawfish in the bycatch of commercial fisheries across northern Australia (Brewer *et al.*, 2006; Field *et al.*, 2013; Fry *et al.*, 2018; A. Laird/NPF Industry unpubl. data). Bycatch levels of elasmobranchs, including *A. cuspidata*, have been reduced significantly in Australian prawn trawl fisheries through the adoption of turtle exclusion devices and bycatch reduction devices (Brewer *et al.*, 2006). However, interactions still regularly occur. Northern Australia's largest commercial fishery, both in terms of product caught and economic value, is the Northern Prawn Fishery (NPF) (Patterson *et al.*, 2020). The NPF has a large spatial management area from Cape Londonderry in Western Australia to western Cape York, Queensland (Fig. 1). Within the fishery though, effort is concentrated in productive prawn grounds so that the management area is not evenly fished (see Patterson *et al.*, 2020). *Anoxypristis cuspidata* represents the most interactions of any of the sawfish species in the fishery and there is a need to

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understand the spatial scale of management units (i.e., population boundaries) that the fishery is interacting with.
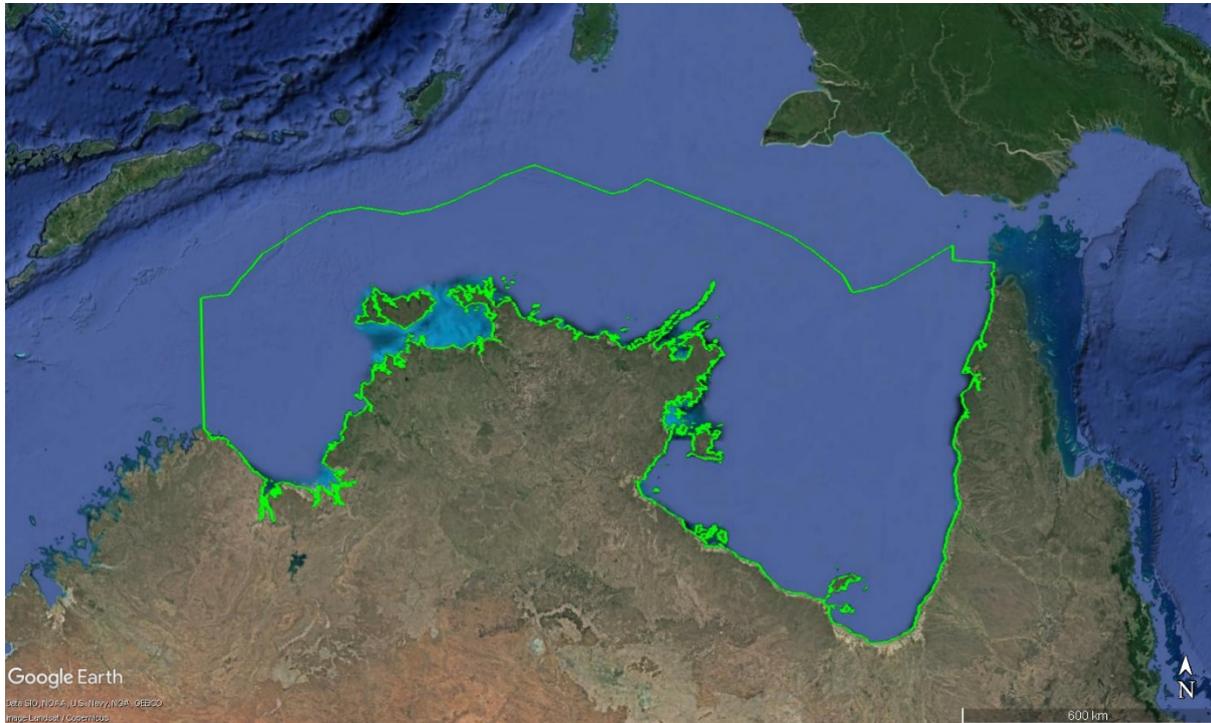
Figure 1: Management area of the Northern Prawn Fishery across northern Australia. Map source: Google Earth.

Broad-scale population structuring has been previously shown in *A. cuspidata*. Green *et al.* (2018) showed population structuring using partial mitochondrial sequences between four broad geographic regions: northwest Australia, Gulf of Carpentaria (Australia), eastern Australia, and Papua New Guinea, but there was no evidence of structuring across northern Australia with microsatellites. Such a pattern suggests female philopatry and possible male-biased dispersal (Green *et al.*, 2018). While the results of Green *et al.* (2018) are informative at the broad geographic scale, understanding structuring and barriers to gene flow at a finer-scale is needed to inform fisheries management.

Here we undertake a collaboration with the commercial fishing industry to obtain *A. cuspidata* tissue samples from across northern Australia. We used both mitogenome sequencing as well as single nucleotide polymorphism (SNP) genotyping using the DArTseq protocol. We aimed to define population boundaries of *A. cuspidata* within the spatial footprint of the Northern Prawn Fishery to better understand if this, and other commercial fisheries, are interacting with a single population or multiple populations of the species.

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2. METHODS

Tissue samples were collected through a collaboration with commercial fisheries and fishery agencies across northern Australia. Sampling in the Northern Prawn Fishery (NPF) was facilitated by the NPF Industry Pty Ltd. Tissue sampling kits were provided to vessels operating out of the main fishing ports of Darwin (Northern Territory; NT), Karumba (Queensland Gulf of Carpentaria), and Cairns (Queensland east coast). Kits consisted of sample vials containing ethanol, datasheets, sampling scissors and forceps, and an instruction sheet. Authors (AL, CLD, PMK) attended pre-fishing season crew briefings in these ports and provided information on sawfish, aims of the project, and training on tissue sampling and sample storage. Sampling was undertaken opportunistically onboard vessels during normal fishing operations by crew, crew member observers, and observers from the Australian Fisheries Management Authority (AFMA). Elasmobranchs are not permitted to be retained in the NPF and all bycatch sawfish were returned to the water after sampling. Sampling was undertaken between April 2019 and May 2020.

Additional samples were provided from NT commercial fisheries, primarily an existing collection of samples from the Offshore Net and Line Fishery, along with a smaller number of samples from the NT Barramundi Fishery and Queensland Fisheries research sampling.

Samples were collected during normal fishing operations and this did not permit an *a priori* sampling design. Samples were allocated to eight broad geographic regions reflecting the main areas fished during the course of the sampling (Fig. 2). These regions were (west to east): Eastern Kimberley (Western Australia), Western Top End (primarily Northern Territory; NT), Northern Top End (NT), Western Gulf of Carpentaria (GoC; NT), Southwest GoC (primarily NT), Southeast GoC (Queensland), Eastern GoC (Queensland), and Northeast Queensland (Fig. 2).
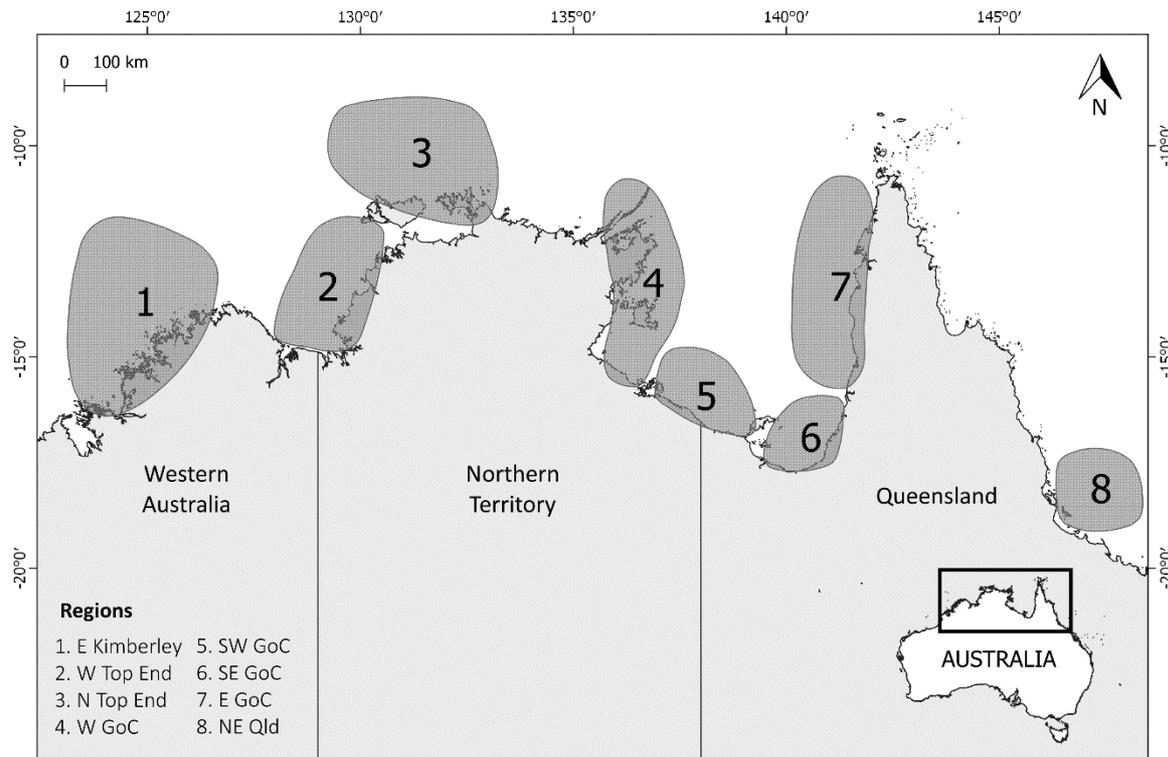
Figure 2: Sampling regions for *Anoxypristis cuspidata* across northern Australia. (1) E Kimberley (Eastern Kimberley); (2) W Top End (Western Top End); (3) N Top End (Northern Top End); (4) W GoC (Western Gulf of Carpentaria) (5) SW GoC (Southwest Gulf of Carpentaria); (6) SE GoC (Southeast Gulf of Carpentaria); (7) E GoC (Eastern Gulf of Carpentaria); (8) NE Qld (Northeast Queensland).

Mitogenome sequencing followed the approach previously described by Feutry *et al.* (2014) except for the following. Quarter reactions were used to prepare the Nextera XT libraries in order to reduce the cost of the mitogenome sequencing. We used only the forward reads for the assembly as the sequencing of the reverse reads was unsuccessful during the Miseq run. The R package pegas v. 0.12 was used to calculate the haplotype network and carry out an AMOVA to test for genetic differentiation across all sampling regions with 10,000 permutations after calculating genetic distance with apex v. 1.0.3. Pairwise Φ_{ST} were calculated for all sampling regions using haplotype v. 1.1.2, based on the same genetic distances as for the AMOVA and also with 10,000 permutations.

SNP genotyping was conducted following the DArTseq protocol as described by Grewe *et al.* (2015). The R package Radiator was used to quality control the SNP data prior to analysis. Pairwise F_{ST} values between sampling regions were calculated with 1000 bootstrap in R using StAMPP v. 1.6.1. Discriminant Analysis of Principal Component (DAPC), as implemented is adegenet 2.1.2, with sampling regions as putative groups and the alpha-score method to avoid over-fitting, was used to further investigate population structure.

3.   RESULTS

A total of 353 samples were obtained for this study, comprising 176 from the NPF, 165 from the NT Offshore Net and Line Fishery (existing samples held by NT Fisheries), 9 from the NT Barramundi Fishery, and 3 from Queensland Fisheries research. In general, size (TL, total length) and sex data were not collected by crew and so this data is limited to a small subset of samples (n=19; 18 specimens ranged 760–1110 mm TL and one was 3140 mm TL). Of the total samples collected, 182 exhibited sufficient DNA quality for mitogenome sequencing but only 168 passed the Radiator filtering and were used for mitochondrial and nuclear analyses, respectively.

The haplotype network for all samples combined shows non-random distribution of haplotypes across the sampling regions (Fig. 3), which was confirmed by the AMOVA (Global Φ_{ST} = 0.3731, p value < 0.0001) and pairwise Φ_{ST} values (Table 1). Pairwise Φ_{ST} between regions with more than three samples were all significant (p value < 0.05), ranging from 0.0160 (N Top End vs SW GoC) to 0.4446 (W Top End vs SE GoC) (Table 1; Fig. 4). Pairwise Φ_{ST} values for regions with three samples or less likely lack power to obtain statistical significance.
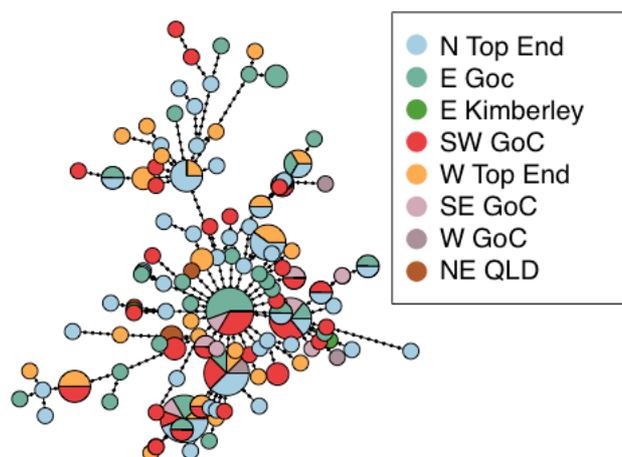
Figure 3: *Anoxypristis cuspidata* haplotype network. Size of the pie charts is proportional to the square root of the number of individuals harbouring that haplotype. Black dots on the lines connecting the pie charts indicates mutations. Abbreviations for regions are given in Figure 2.

Table 1: Pairwise Φ_{ST} values (above) and associated *p* values (below) between all sampling regions for *Anoxypristis cuspidata*. Abbreviations for regions are given in Figure 2.

	E Kimberley N=2	W Top End N=35	N Top End N=55	W GoC N=3	SW GoC N=41	SE GoC N=8	E GoC N=35	NE QLD N=3
E Kimberley	-	0.3267	0.1233	0.0635	0.0508	0.2484	0.0569	0.2370
W Top End	0.0344	-	0.4385	0.0201	0.3952	0.4446	0.3134	0.3530
N Top End	0.1199	<0.0001	-	0.4149	0.0160	0.3851	0.1063	0.0965
W GoC	0.2991	0.2587	<0.0001	-	0.3358	0.3378	0.2163	0.1696
SW GoC	0.2255	<0.0001	0.0322	0.0009	-	0.3232	0.0848	0.0402
SE GoC	0.0875	<0.0001	<0.0001	0.0049	<0.0001	-	0.2120	0.2559
E GoC	0.2585	<0.0001	<0.0001	0.0212	<0.0001	0.0009	-	0.0104
NE QLD	0.3017	0.0024	0.0895	0.1931	0.1851	0.0892	0.3806	-
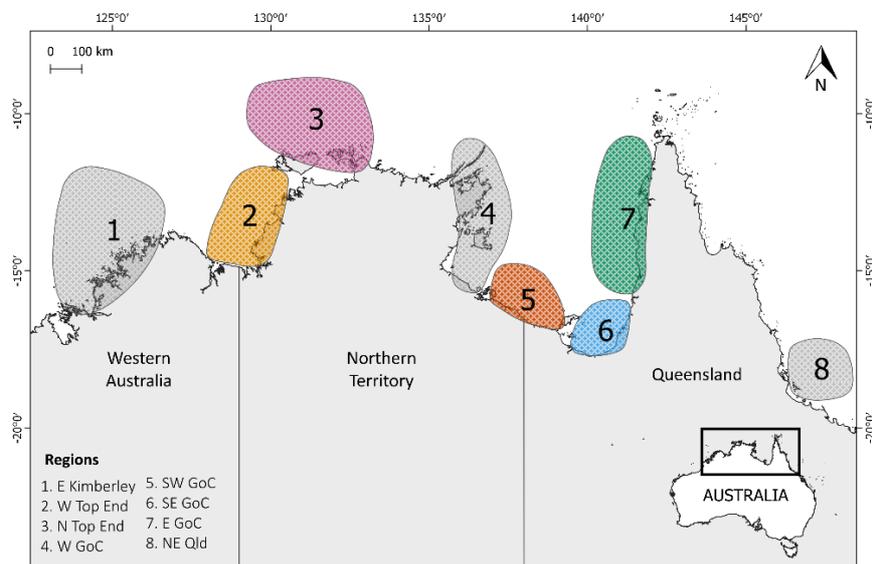
Figure 4: Population structuring of *Anoxypristis cuspidata* across northern Australia based on mitogenome sequencing. Each coloured region is significantly different from each other coloured region (pairwise Φ_{ST}). Grey regions had low sample size and likely lack power to obtain statistical significance. Abbreviations for regions are given in Figure 2.

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None of the pairwise F_{ST} differed from 0 indicating lack of population structure as reflected by the nuclear DNA (Table 2). DAPC patterns of probability membership are similar at each sampling region, each individual with a probability to belong to each sampling region roughly proportional to the sample size at that region. This is typical of an absence of population structure signal (Fig. 5).

Table 2: Pairwise F_{ST} values (above) and associated *p* values (below) between all sampling regions for *Anoxypristis cuspidata*. Abbreviations for regions are given in Figure 2.

	E Kimberley N=3	W Top End N=31	N Top End N=51	W GoC N=3	SW GoC N=36	SE GoC N=8	E GoC N=33	NE QLD N=3
E Kimberley	-	-0.0008	-0.0025	-0.0011	-0.0033	-0.0066	-0.0009	0.0055
W Top End	0.598	-	0.0002	0.0031	-0.0005	-0.0007	0.0003	0.0019
N Top End	0.820	0.364	-	0.0016	-0.0006	-0.0007	0.0001	0.0012
W GoC	0.581	0.144	0.272	-	0.0008	-0.0007	0.0027	-0.0042
SW GoC	0.891	0.849	0.963	0.385	-	-0.0026	-0.0008	0.0008
SE GoC	0.961	0.736	0.757	0.601	0.989	-	-0.0019	>0.0001
E GoC	0.614	0.273	0.467	0.185	0.952	0.963	-	0.0029
NE QLD	0.175	0.256	0.331	0.779	0.393	0.491	0.140	-

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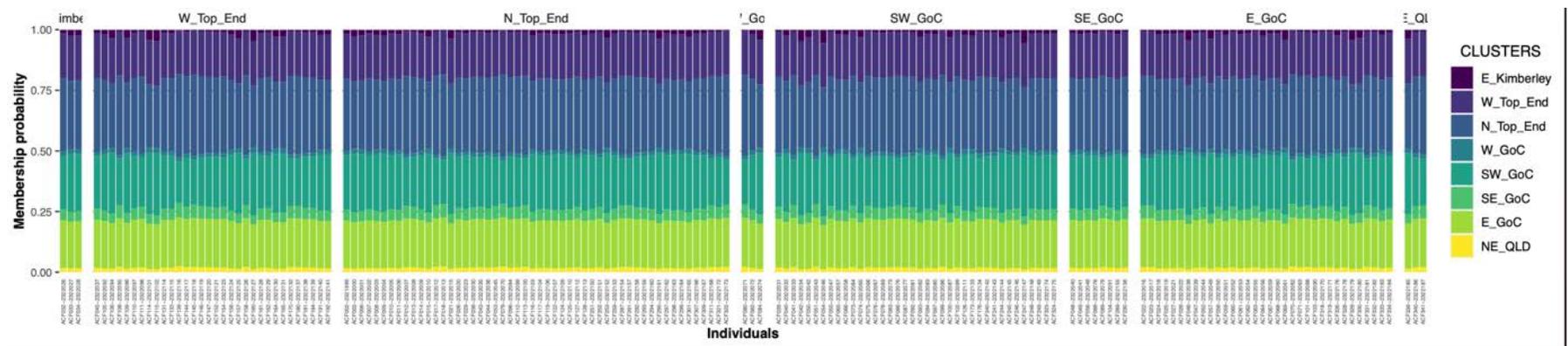
Figure 5: Discriminant Analysis of Principal Component membership probabilities for *Anoxypristis cuspidata* across northern Australia. Abbreviations for regions are given in Figure 2.

4. DISCUSSION

Isolated populations of species which have little demographic exchange represent distinct management units. We provide an analysis of population structure in a threatened sawfish, *Anoxypristis cuspidata* across its northern Australian range, primarily in the management area of northern Australia's largest commercial fishery, the Northern Prawn Fishery (NPF).

Our state-of-the-art approach including both full mitochondrial genomes and thousands of markers in the nuclear genome revealed clear signal of fine-scale female philopatry along with large-scale mixing of nuclear genes, suggesting male-biased dispersal. These results support earlier findings by Green *et al.* (2018) but provide higher spatial resolution using more advanced approaches (full mitogenomes and SNPs). Using thousands of SNPs is much more informative than a few microsatellites (as used by Green *et al.*, 2018), therefore providing more power and higher confidence in the results.

The fine-scale philopatric behaviour of females could have profound implications for the management of *A. cuspidata*. This essentially means that each region is a separate management unit given that the lack or limited amount of female dispersal mean that populations have limited ability to recover from localised depletion. This study consolidated available samples into geographic regions based on where commercial fishing was occurring during the sampling period. Sourcing samples from normal commercial fishing operations precluded an *a priori* sampling design but samples were able to be neatly categorised into distinct regions. Importantly, it is possible that female philopatry occurs at even finer-scales than demonstrated in this study. To examine this, sampling of neonates in nursery areas (before they start dispersing away from these locations) would help define the strength of female philopatry.

Nursery areas for this species occur in estuaries and adjacent coastal waters. Understanding if female philopatry occurs at the estuary-scale would refine management advice to an even finer-scale. Field sampling for sawfish can be time and resource intensive, but this study has shown that collaborating with the commercial fishing industry provides an efficient avenue to obtain high sample numbers from across a wide geographic region. NPF trawlers don't operate within the estuary environment, so to test the hypothesis that female philopatry in *A. cuspidata* occurs at the estuary-scale, a collaborative study with inshore net fisheries targeting Barramundi *Lates calcarifer* could facilitate the necessary sampling regime. Net fisheries operate in each state/territory across the Australian range of *A. cuspidata* and interact with the species as bycatch (e.g., Field *et al.*, 2013). A small number of estuaries could be selected and sampling focused on these to examine barriers to gene flow between them.

Anoxypristis cuspidata has a higher resilience to the impacts of threats relative to the other sawfish species due to it high biological productivity. In the NPF, *A. cuspidata* has a high escapement rate (73%) from turtle exclusion devices (Brewer *et al.*, 2006). Modelling of fishing mortality rates in the NPF indicated that the level of mortality was sustainable (Zhou

and Griffiths, 2008). However, it is cautioned that the cumulative impact of mortality from trawl, net, and illegal fisheries could put the species at risk (Zhou and Griffiths, 2008; Kyne *et al.*, 2021). If overall mortality exceeds productivity at the population-scale then a localised population could be easily depleted given the population structuring shown here. Field *et al.* (2013) recorded a 50% mortality rate at capture for *A. cuspidata* in the NT Barramundi Fishery, although this was based on limited observer coverage. All bycatch species are released in Australian commercial fisheries but post-release survivorship of bycatch sawfish is an important issue that has not been examined in Australian trawl and net fisheries. This is a clear future research direction. Importantly, survivorship can be increased (therefore potentially avoiding localised population depletion) through the safe handling and rapid release of bycatch sawfish.

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5.   ETHICS STATEMENT

Sampling was approved by the Charles Darwin University Animal Ethics Committee (Approval No. A19008) and undertaken through Northern Territory *Fisheries Act* Special Permit S17/3467 and Western Australian *Fish Resources Management Act* Exemption No. 3484.

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