Genetic structure and diversity of Australian freshwater crocodiles (*Crocodylus johnstoni*) from the Kimberley, Western Australia

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Abstract

The Australian freshwater crocodile (*Crocodylus johnstoni*) is endemic to the northern mainland tropics of Australia and is widespread across the Kimberley region in the northwest Australia. Currently, there is limited understanding of the genetic structure and diversity of these populations, which impacts on our ability to evaluate the conservation status of the species. Population genetic analyses of 173 freshwater crocodiles from the Ord River, Fitzroy River, and Lennard River basins were conducted using single-nucleotide polymorphisms (SNPs). After filtering, 1185 SNPs were retained for downstream population genetic analysis. STRUCTURE and principal component analyses identified three clusters consistent with the three river basins. Population differentiation gave an F_{ST} of 0.15 between western and eastern Kimberley and the pairwise F_{ST} range was 0.06–0.18 among the three river basins. Assignment tests identified three migration events between the adjacent Fitzroy River and Lennard River basins, which may be explained by possible overland movement across these river basins. The population structure found here indicates that delimitation of management units should be based on river basins with the proximity of adjacent river basins taken into consideration when gene flow exists. Estimates of effective population size showing a low ratio of effective population size to census size in Lake Argyle may raise the concern of future monitoring in this area. Further population genetic studies across the species' full range are required to better understand the extent of river basins acting as discrete population units, gene flow, population dynamics, and demographic history.

Keywords Crocodiles · Species distribution · ddRADseq · Genetic structure · Genetic diversity

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Introduction

The Australian freshwater crocodile (*Crocodylus johnstoni*) is widely distributed in tropical areas of northern mainland Australia (Webb et al. 1983a, b) where it inhabits freshwater environments including creeks, rivers, lagoons, and pools, and occasionally extends into saline tidal areas (Webb 1985). The ecological role of freshwater crocodiles is yet to be understood (Somaweera et al. in press), but they arguably play a crucial role as prey and predators in freshwater ecosystems. They are also important for ecotourism and in Aboriginal communities where there can be strong cultural and spiritual connections to this species (Delaney et al. 2010). While this species is currently accessed as "Least Concern" in the IUCN Red List (Isberg et al. 2017), there is a growing body of literature showing impacts from cane toads, feral ungulates, land use change, invasive weeds and agrochemicals on local populations of freshwater crocodiles (Yoshikane et al. 2006; Letnic et al. 2008; Somaweera et al. 2019).

Defining discrete population units is crucial for species conservation because it helps delimit management units to best protect genetic diversity (Kolomyjec et al. 2009; Muniz et al. 2018; Kay 2005) and could assist in investigating the relationship between population structure, dispersal and local geographical features (Moritz 1999). Currently, the understanding of the population dynamics of the freshwater crocodile is limited. Mark-recapture studies have reported strong site fidelity among freshwater crocodiles, with movement limited to a small home range (Webb et al. 1983a, b). Some male juveniles showed an extensive movement range (~ 30.3 km) compared with other individuals (~ 1 to 1.9 km) within river systems, and this could relate to mating competition (Tucker et al. 1997). In addition, freshwater crocodiles occasionally move overland in the dry season when they move between drought pools to seek refuge (Lang 1987; Webb et al. 1983a) and can aestivate in suitable terrestrial refugia when these pools dry (Kennett and Christian 1993). However, the scale or distance of movement of this is not documented, making it difficult to evaluate the potential for migration between river basins.

Population genetic and genomic studies have provided insights into gene flow, population structure and genetic diversity of crocodile species (Kay 2005; Muniz et al. 2018; Luck et al. 2012; Versfeld 2016; Russello et al. 2007) but this has been very limited in *C. johnstoni*. For example, an analysis of 532 unlinked ddRAD loci for Cuvier's dwarf caiman (*Paleosuchus palpebrosus*) in Brazil suggested very high population differentiation among four river basins, and four management units consistent with the river basins were proposed (Muniz et al. 2018). Using short tandem repeat (STR) markers, Nile crocodiles inhabiting the Kunene and Okavango Rivers showed some population differentiation, and each river basin population was suggested to be a single management unit (Versfeld 2016). For saltwater crocodiles (*C. porosus*) in the Kimberley region, Western Australia, management units were suggested at the level of river basin because of moderate genetic differentiation and a 10% first-generation migration rate (Kay 2005). Another saltwater crocodile genetic study based on 8312 SNPs genotype data analyzed the genetic structure, genetic diversity, translocation and homing ability of *C. porosus* in the Northern Territory, Australia (Fukuda et al. 2019). However, there is no genetic understanding of freshwater crocodile populations including those from the Kimberley region, a region of high ecological importance (Pepper and Keogh 2014). Freshwater crocodiles have established large populations in the Kimberley region, where they inhabit the Ord River basin, including Lake Kununurra and Lake Argyle in eastern Kimberley and the Fitzroy River and Lennard River basins in western Kimberley (McNamara and Wyre 1993). These three river basins are independent river systems without water path connections, with a minimum distance apart of about 50 km

(Department of Water 2008). Since the freshwater crocodile is characterized by limited overland movement ability, this may be driving some level of population differentiation between river basins, a hypothesis that is yet to be examined. To address this, we investigated the genetic diversity and differentiation among three river basins from the Kimberly region in northern Western Australia, using single-nucleotide polymorphisms (SNPs) generated by double-digest restriction site-associated DNA sequencing (RADseq).

Materials and methods

Sample collection and DNA extraction

Freshwater crocodile tissue samples were collected from the Fitzroy River (Geikie Gorge and water pools downstream), Lennard River (Windjana Gorge and water pools downstream), and Ord River (Lake Argyle and creeks flowing in) basins, which are known to contain large populations of freshwater crocodiles (Mawson 2004). Hatchling and sub-adult crocodiles were caught at night using hand nets from boats. Adult crocodiles were caught using nooses (Fitzroy and Ord Rivers) or drift nets swept across drying water pools (Lennard River), the latter during annual mark and recapture surveys by the Department of Biodiversity Conservation and Attraction and Bunuba Rangers. All the captured animals were restrained using methods appropriate for their size (Webb and Messel 1977; Combrink et al. 2012). Dorsal tail scutes were cut using scissors or a sharp pocketknife sterilized in 70% ethanol and dried using a clean tissue. The harvested tissue samples were then stored in 100% ethanol. The animals were released at the site of capture. In addition to the above, two samples from Duck Hole and six samples from Lake Kununurra along the Ord River (Fig. 1, Table 1) were included. In total, 174 samples from five sample sites representing three river basins were used for genomic DNA extraction. DNA was extracted using the DNeasy Blood and Tissue Extraction Kit from Qiagen (Germany) or the phenol–chloroform protocol (Green and Sambrook 2012) Qubit (Life Technologies, USA). The concentration and quality of the extracted DNA was determined using Nanodrop spectroscopy and 1% agarose gels. The samples were then sent to the Australian Genomic Research Facility for dd-RADseq, as described below.

Library preparation and SNP discovery

Library preparation was performed according to the protocols by Peterson et al. (2012). In brief, 200 ng of genomic DNA was double-digested using *ecoRI* and *NlaIII*, with the ligation-compatible barcode adapters A and P2 adapter and restriction site overhang. The fragmented DNA was
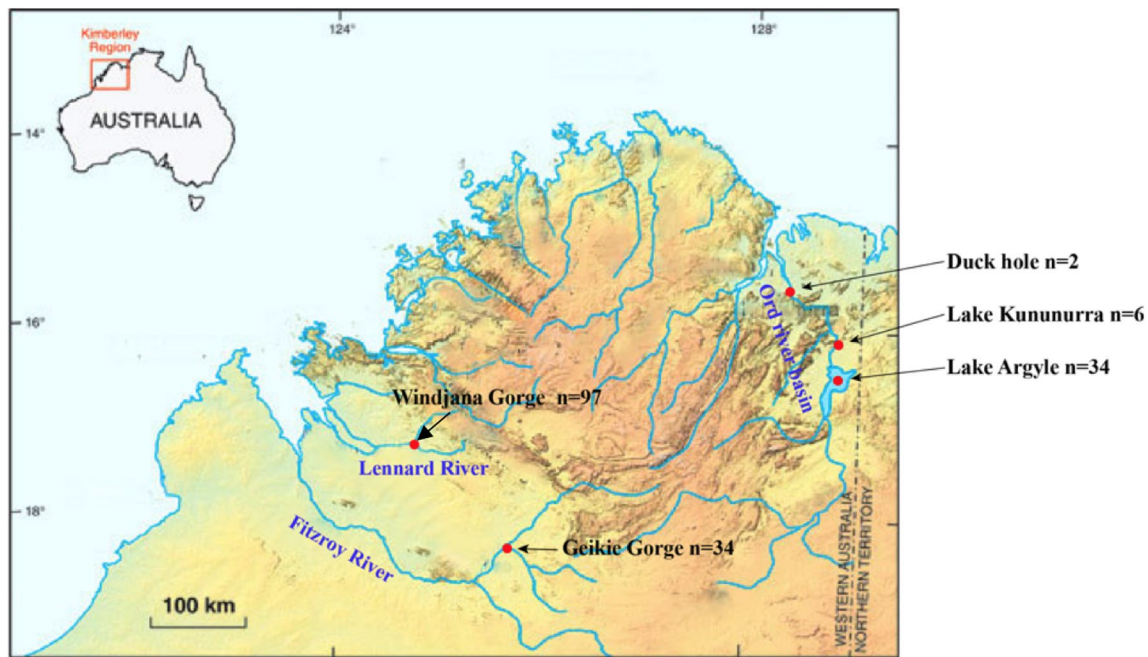
Fig. 1 The map shows the sample collecting sites and the numbers from each location. The blue lines represent the river and the dot represent the collecting site of samples, with the specific river basin names labelled in blue and sample size indicated by arrow. The red dot indicates the samples collecting site, with the number of samples collected there

Table 1 The summary of the samples from three river systems

Collecting site	River basin	Geographical area	Coordinate/location description
Lake Argyle	Ord River	Eastern Kimberley	16.1126S, 128.73967E
Duck Hole	Ord River	Eastern Kimberley	15.6985S, 128.0878E
Lake Kununurra	Ord River	Eastern Kimberley	15.90333S, 128.81944E
Windjana gorge	Lennard River	Western Kimberley	17.40503S, 124.94619E
Geikie gorge	Fitzroy River	Western Kimberley	18.092039S, 125.708293E

enriched by polymerase chain reaction, and only the tags with ligation of both P2 and A adapters being amplified. The enriched fragments were size-selected using Blue Pippin, which selected 60-bp fragments for sequencing. The prepared libraries were sequenced using the NextSeq platform (Illumina, USA) in four lanes, following the manufacturer's instructions.

Raw sequences were demultiplexed and assembled using Stacks 1.47 (Catchen et al. 2011, 2013b). Demultiplexing was performed using *process RAD-tags*, which excluded reads with uncalled bases as well as low-quality data with Phred scores < 20 and barcode and RAD-tags. The resulting reads were analyzed for SNP discovery de novo since a reference genome sequence from freshwater crocodile is not available. We employed UStack with a maximum likelihood statistical model (Catchen et al. 2011), which requires a minimum of two raw reads to create a stack. Cstack allowed a maximum of one mismatch to generate the catalogue and SStack verified individuals with a default parameter. All

generated files were used in the population component to link individuals to their respective populations and generate a data matrix stored in a VCF file. The genotype rate and locus depths can cause bias in downstream analysis (Fumagalli 2013; Chattopadhyay et al. 2014; Linck and Battey 2019). Therefore, we filtered the dataset to include only those loci that provided genotypes in at least 50% of all individuals to maximize population structure informativeness parameters (Streicher et al. 2016). The SNP filtering steps were conducted using VCFtools (Danecek et al. 2011). Finally, the filtered VCF file was converted into a compatible format with PGDSpider v. 2.0.8.2 (Lischer and Excoffier 2011) for analysis using GenoDive (Meirmans and Van Tienderen 2004), and STRUCTURE (Evanno et al. 2005).

Population structure and differentiation

The genetic relatedness of individuals was investigated to determine whether the genetic structure was consistent with

river basin location. Principal component analysis (PCA) was performed in GenoDive (Meirmans and Van Tienderen 2004) to infer population structure and identify and adjust for ancestral differences among the sampled animals. A covariance matrix was used to calculate eigenvalues of individual principal components (PCs), with 100 replications for significance testing. The two PCs with the highest eigenvalues were plotted. Another PCA analysis were performed by removing Duck Hole and Lake Kununurra samples due to their small sample size.

Population clustering was further investigated in STRUCTURE (Pritchard et al. 2000) using an admixture model and correlated variance. In order to highlight the genetic structure between western and eastern Kimberley, we further performed a STRUCTURE analysis with combination of the samples from the Lennard and Fitzroy River as the western Kimberley group and the Ord River as the eastern Kimberley group. These analyses were run with K values of 2–6, 5 replications per K, 20,000 burn-in steps, and 20,000 iterations. The K values were selected using the *delta K* method described by Earl (2012) in Structure Harvester (https://taylor0.biology.ucla.edu/structureHarvester/) and then summarized and drawn using CLUMPP (Jakobsson and Rosenberg 2007) and Distruct (Rosenberg 2004).

Pairwise FST values between the identified populations were calculated using GenoDive (Meirmans and Van Tienderen 2004). We further combined data from the Lennard and Fitzroy River to estimate genetic differentiation between the western Kimberley and the Ord River in the eastern Kimberley. All of the calculations were performed with 10,000 replications to determine significance.

Population assignment test

Assignment tests were used to calculate the probability of an individual belonging to the source population (Rannala and Mountain 1997) using the allele-frequency-based method in GenoDive (Meirmans and Van Tienderen 2004). The likelihood ratio L_{home}/L_{max} with 100 permutations (significance threshold 0.05) was also calculated (Piry et al. 2004). Misassignments with > 95% probability indicated genotypes that were likely the result of immigration rather than random combinations of the original population.

Genetic diversity and effective population size

We estimated genetic diversity based on the SNP data stored in a VCF file; thus, population statistics only included variable positions. Private alleles, nucleotide diversity (π), expected heterozygosity, observed heterozygosity and inbreeding coefficient (F_{IS}) were measured for each identified population using the POPULATIONS program in Stacks (Catchen et al. 2011, 2013b). We further employed

GenoDive to perform Hardy–Weinberg equilibrium with 10,000 permutation to test the significance of F_{IS} (Meirmans and Van Tienderen 2004). To assess the genetic diversity at the genome level, we calculated nucleotide diversity in the all position of RAD-tag in Stacks, to compare genetic diversity to other species. Effective population size was estimated in NeEstimator (Do et al. 2014) using the linkage disequilibrium approach with a minimum allele frequency cutoff of 0.02 and random mating model for the Lake Argyle, Fitzroy River and Lenard River samples (Larson et al. 2014), when Lake Kununurra and Duck Hole were dropped due to the too small sample sizes. We further compared the effective population size to the census size in Lake Argyle (WMI Pty Ltd 2010) and the Fitzroy River (Mawson 2004).

Results

Sequencing data quality and processing

Four lanes of sequencing produced approximately 361 million reads derived from 174 samples. The number of RAD-tags obtained per individual ranged from 58,000 to 210,000, with the tag depth between 2.4–3 (Supplementary Information, Table S1). After de novo assembly and SNP calling, 89,516 SNPs were merged in a data matrix. We summarized the percentage of un-genotyped SNPs across 174 samples and found very high missing rate per loci across individuals and populations (Supplementary Information, Fig S2). We filtered SNP loci by requiring that they be successfully genotyped in 50% of individuals. During data filtering, one individual was eliminated as no loci were available after SNP quality control. Finally, 173 individuals and 1185 SNP loci were retained for the downstream analysis of population genetics.

Population structure and differentiation

Three clusters were identified based on PCA of 1185 SNP loci that are consistent with the three river basins (Fig. 2). Some overlap between the two populations from western Kimberley was observed, as well as a few individuals from Fitzroy River and Duck Hole on the Ord River. The extra PCA analysis excluding Lake Kununurra and Duck Hole show three clear clusters consistent with the river basins, but again some overlap within western Kimberley (Supplementary Material S6). The STRUCTURE analysis detected three genetic clusters (K = 3, Supplementary Material S3, S4), which is consistent with the river basin origins of the samples (Fig. 3). One cluster grouped individuals from Lake Kununurra, Lake Argyle, and Duck Hole, all from the Ord River basin, although some Duck Hole samples showed overlap with the Fitzroy River. The remaining two
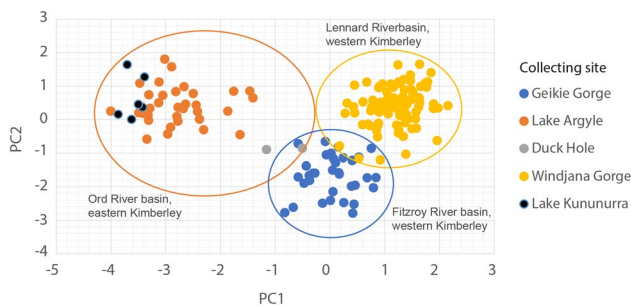
Fig. 2 Principal Component analysis plot of average scores of individuals from five collecting sites. The first and second principle components (PC1 and PC2) are shown, representing the *x* axis and *y* axis respectively. The first PCA axis explains 6.797% and the second PCA axis explains 2.319% of the total variation. The first 10 PC axes explain 21.223% of variation. The individual sourced from collecting points were represented by different colour, which indicates in the right. Three circles represent 3 river basins: Orange: Ord River basin, Yellow: Lennard River basin, Blue: Fitzroy River basin. Although some overlap between three circles, the identified three clusters generally concordant to the three river basins indicate the presence of genetic structure among river basins

clusters grouped individuals from the Lennard River or Fitzroy River, although there was some genetic admixture. The STRUCTURE analysis comparing western Kimberley and eastern Kimberley groups shows the same result, which illustrates the clear differentiation between western and eastern Kimberley (Supplementary Material S7).

We further analyzed the pairwise F_{ST} among the three rivers. The largest differentiation was between Ord River and Lennard River ($F_{ST} = 0.177$, $P < 0.0001$), followed by 0.119 between Ord River and Fitzroy River ($P < 0.0001$). Only a moderate differentiation ($F_{ST} = 0.061$, $P < 0.0001$) was identified between Lennard River and Fitzroy River. Genetic differentiation between western (combined Lennard and Fitzroy Rivers) and eastern Kimberley (Ord River) was $F_{ST} = 0.152$ ($P < 0.0001$).

Assignment of individuals

The population assignment test correctly assigned 170 of 173 samples to their source populations. Three individuals (20-I6_GCAT, 94-I6_TAGA, and WG378-I19_GGATTG GC) were identified as unlikely to belong to their sample populations and were therefore identified as immigrants. Based on the three reference populations, two samples (20-I6_GCAT and 94-I6_TAGA) were from the Fitzroy River and assigned to the Lennard River. One sample (WG378-I19_GGATTGGC) was from the Lennard River and assigned to the Fitzroy River.

Genetic diversity and effective population size

Based on the SNP data matrix of variable positions the π value for all three basins combined was 0.101, observed heterozygosity was 0.073, expected heterozygosity was 0.10, and F_{IS} was 0.394 (Table 2). The π value for each basin ranged between 0.09 and 0.101 and was the highest for the Fitzroy River (0.101) and lowest for the Lennard River

Table 2 Population genetic parameters for three populations of *Crocodylus johnstoni* based on variable SNP loci including number of private alleles, observed heterozygosity, expected heterozygosity, nucleotide diversity (π), inbreeding coefficient

Population ID	Private alleles	Obs het[a]	Exp Het	π	F_{is}[b]
Ord River basin	141	0.077****	0.097	0.099	0.188**
Fitzroy River basin	84	0.084****	0.098	0.101	0.140**
Lennard River basin	263	0.070****	0.092	0.092	0.330**

[a]Asterisks indicate significant Hardy–Weinberg test result

[b]Asterisks indicate F_{IS} significantly different from 0
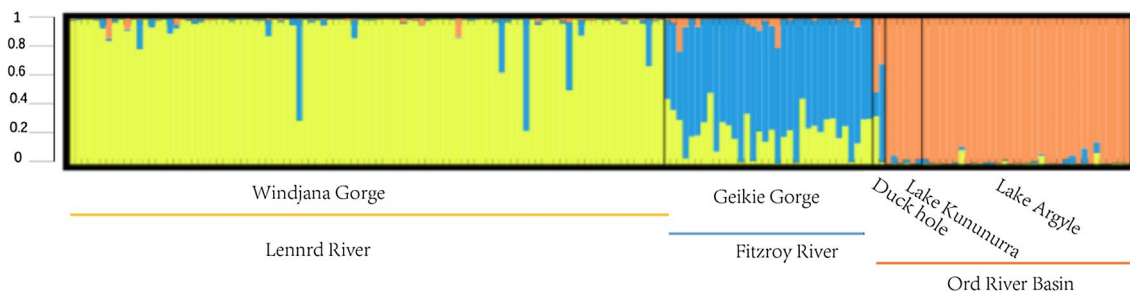
*p < 0.05, **p < 0.01, ****p < 0.001

Fig. 3 Population structure inferred by 173 individuals from five collecting sites. Each vertical represents an individual. The collecting sites belonged to the river basin placed below the figure, and three collecting sites from Ord River basin was extra labelled above the Ord River basin. The color proportion represents the probability of assignment of each individual to each cluster. K = 3 was the best fit our result based on DeltaK method (Supplement Material). The collecting site is arranged by the geographical location and place adjacent river basin together. Each River basin represent a clear cluster, but some admixture occurs between Lennard River and Fitzroy River

(0.09). The number of private alleles was higher in the Lennard River (263) than in the Ord River (141) and Fitzroy River (84). All clusters showed observed heterozygosity lower than expected heterozygosity and the Hardy–Weinberg Equilibrium test among three cluster (P < 0.001) support the significant difference between observed Heterozygosity and expected heterozygosity. The Lennard River population had the highest value (F_{IS} of 0.33), followed by the Ord River (F_{IS} of 0.18) and Fitzroy River basins (F_{IS} of 0.14). All of the F_{IS} showing statistical significance (P < 0.01). The estimated effective population size was 5678 (2599.8 to infinite) in the Lennard River, 5064 (1134.6 to infinite) in the Fitzroy River, and 2047 (831 to infinite) in Lake Argyle. All of the effective population size estimates contained a large confidence interval showing an infinite size for the upper limit. The ratio of estimated effective population size to census size Ne/N was 0.389 in the Fitzroy River and 0.082 for Lake Argyle (Table 3). Finally, we compared the nucleotide diversity from all positions of RAD-tag to other species and found that the genetic diversity observed in our study population was low (Table 4).

Discussion

This is the first study identified 1185 SNP loci in freshwater crocodiles, this was higher than the 532 loci used in a study of Cuvier's dwarf caiman (Muniz et al. 2018), and lower than 8312 SNPs loci used in a study of *C. porosus* (Fukuda et al. 2019). Some studies compared the effectiveness of microsatellites and SNPs in population genetics. Fernández et al. (2013) found 2–3 SNPs were equivalent to 1 STR marker in getting the same exclusion power. Gärke et al. (2011) found that 29 SSR loci were equivalent to 70 SNPs for effectiveness in PCA-based partitioning. Liu et al. (2005) found on average that the informativeness of microsatellite loci was four to twelve times that of SNPs for population comparisons. Considering various population genetics using microsatellites in crocodilian species used up to 20 loci (e.g. Kay 2005; Davis et al. 2002; Velo-Antón et al. 2014; Russello et al. 2007), the number of SNP loci in this study should be sufficient to inform the population structure. The sampling regime allowed comparisons of populations in the adjacent Fitzroy and Lennard River basins in the western Kimberley, to the Ord River basin in the eastern Kimberley. The pairwise estimates of genetic structure (F_{ST}) were 2–3 times greater between the Ord River basin and either the Fitzroy or Lennard River, than between the Fitzroy and Lennard River. This pattern of genetic structure was expected given the geology of the region. In the central Kimberley region, a large plateau comprising the uplands of the Prince Regent Plateau, Gibb Hills and the Karunjie Plateau separates the Ord River basin from the Fitzroy River and Lennard River basins (Pepper and Keogh 2014). Sample locations in the two regions were located a minimum of 400 km distant across extensive rocky terrain that would impede immigration. Three genetic clusters were identified based on PCA and STRUCTURE analysis, which were concordant with those found in the three river basins, which could be an indication that these streams are acting as discrete units. One of the factors that may be driving the genetic structure is the strong site fidelity observed in the species. It has been found that relocated freshwater crocodiles have been recaptured in the original water hole after a year of having been released (Webb et al. 1983c) and movement of natural populations appear to be restricted within river basins (Tucker et al. 1997).

Table 3 Estimates of effective population size Ne from three river basin and five collecting sites based on 1185 SNP loci

River basin	Collecting site	Ne	Census size	Ne/N
Lennard River	Windjana Gorge	5678 (2599.8 to infinite)	NA	NA
Fitzroy River	Geikie Gorge	5065 (1134.6 to infinite)	13,000 (Webb Pty Ltd 1989)	0.389
Ord River	Lake Argyle	2047 (813 to infinite)	30,000 (WMI Pty Ltd 2010)	0.068

The ratio of effective population size to census size (Ne/N) is also reported

Table 4 Observed nucleotide diversity of all position of RADtag SNP loci in species based on RADseq techniques

Scientific name	Common name	Locality	Nucleotide diversity	References
Gasterosteus aculeatus	Threespine stickleback	USA, Oregon	1.1 to 3.4×10^{-3}	Catchen et al. (2013a)
Anguilla japonica	Japanese eel	China	$0.5–0.8 \times 10^{-3}$	Gong et al. (2019)
Phoebastria nigripes	Black-footed Albatrosses	Japan, Hawaii	0.65×10^{-3}	Dierickx et al. (2015)
Anguilla anguilla	European eel	France, Ireland	5.2×10^{-3}	Pujolar et al. (2013)
Crocodylus johnstoni	Freshwater crocodile	Kimberley, Western Australia	0.2×10^{-3}	This study

Within the western Kimberley, the observed genetic admixture and the identification of three possible migration events between the Lennard River and Fitzroy River basins suggests limited contemporary gene flow between these basins. Although the sample locations in the two collecting site were ~ 110 km apart, overland movement of freshwater crocodiles may have occurred where distances are ~ 50 km through the King Leopold Range. Avenues for temporary connectivity between these rivers may have been facilitated by a number of small stream anabranches found throughout the area between Geikie Gorge (Fitzroy River) and Windjana Gorge (Lennard River) (Department of Water 2008). It may be surmised that during the wet season these creeks, anabranches and temporary water holes may facilitate occasionally suitable habitats and paths for freshwater crocodiles to disperse when the distance apart between river basins is at its minimum. In years of high rainfall due to tropical cyclones (Wende 1997), increased rainfall may have provided aquatic pathways as a stepping-stone for limited dispersal and gene flow between freshwater crocodiles in the Lennard River and Fitzroy River basins. Alternatively, gene flow may have occurred at the mouths of the Lennard River and Fitzroy River, as both rivers empty into King Sound, and during high flood events the entire area can be flooded (Wolanski and Spagnol 2003). However, this scenario would be more difficult since it requires migration distances between the sample's sites of ~ 500 km river distance.

Low genetic diversity and effective population size

The observed heterozygosity of 1185 SNPs (H_o range 0.077–0.084) were lower than the C. *porosus* (average $H_o = 0.203$) based on the 8,312 SNPs (Fukuda et al. 2019). We further compared nucleotide diversity at all position to our species using RADseq and also found the considerably low genetic diversity (Table 4). This evidence support that the genetic diversity of freshwater crocodile in the Kimberley region is low. At present it is not possible to establish a reason for this but some mechanism of genome evolution in crocodilians (Green et al. 2014) may provide a partial explanation. At the genome level, crocodilians show lower genome heterozygosity values and evolutionary mutation rates compared to chicken, turtle or human DNA, which could be related to the longer generation interval and generation overlap, large body size and low evolutionary rate (Green et al. 2014), which may have influenced the genetic diversity in this species.

Estimated effective population sizes for the three rivers ranged from 3480 to 5670, which were not consistent with limited data on population size. Lake Argyle contains the largest C. *johnstoni* population known and was estimated at over 30,000 non-hatchling individuals in 2010 (WMI Pty Ltd 2010). Limited surveys show that the population

at Fitzroy River basin is 13,000 non-hatching (Webb Pty Ltd 1989), which is smaller than that of Ord River basin. Therefore, the expectation of effective population in Lake Argyle would be much higher than Fitzroy River. However, the estimated effective population size of 2047 was much lower than that of the Fitzroy River (5065). An estimated average value of Ne/N in wildlife populations is 0.10–0.11 (Frankham 1995), which is lower than estimated for the Fitzroy River (0.389), but higher than estimated for Lake Argyle (0.068). The interpretation of relatively high effective population size in the Fitzroy River could be related to the genetic admixture between the adjacent Lennard River and Fitzroy River described in the current study. In contrast, the relatively low effective population size in Lake Argyle, needs to be understood for effective management and conservation. The population at the lake arguably has gone through a bottleneck when the Ord River was dammed to create the lake. Currently, the spread of invasive weeds in nesting sites pushes crocodiles to nest in suboptimal substrates causing higher embryo mortality (Somaweera et al. 2019), while selective nest predation pressures by dingoes (Somaweera et al. 2011) and direct mortality through ingestion of invasive cane toads (Somaweera and Shine 2012) continues. While the actual long-term direct and indirect impacts of these interactions are not fully understood yet, they may further influence the effective population size. Continued monitoring of the population is recommended. Future studies with additional genetic markers are required to confirm the effective population size in the Kimberley region given the estimates of Ne had a large confidence intervals. Use of whole genome sequencing, which has been used with other Australian freshwater vertebrate lineages (Martin et al 2018), along with population surveys may provide more genetic markers in shedding the demographical history in Kimberley and determine whether the freshwater crocodiles went through past genetic bottlenecks in this region.

Conclusions

This study found strong population differentiation with statistical significance between the western and eastern Kimberley, and possible limited gene flow between the adjacent Lennard River and Fitzroy River basins in the western part of this region. Although the specific factors that may have led to this gene flow are unclear, we propose a scenario to explain this, in which freshwater crocodiles may have occasionally dispersed through temporary aquatic pathways in streams, creeks, and floodplains between these two river basins. We recommend additional surveys involving mark-recapture, tracking and genetic analysis between these two river basins and the King Leopold Range to enhance our understanding. Based on the current study, the river basins

appear to act as discrete population units for freshwater crocodiles, and thus the delimitation of management units could be considered at the level of river basin. However, a comprehensive study with more sampling sites, including multiple sites within river basins, such as Duck Hole, is required to identify habitat boundaries. The continued threat from the invasion of cane toads across the Kimberley, and variable results concerning effective population size identified in this study should raise the concerns for continued population monitoring in this ecologically important region to ensure the conservation status of freshwater crocodiles remains of "least concern".

Compliance with ethical standards

Conflicts of interest The authors declare no conflicts of interest.

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