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Genetic bottlenecks in *Pristis* sawfishes in northern Australian waters

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ABSTRACT: Northern Australia has been identified as the last stronghold for the dwarf sawfish *Pristis clavata*, green sawfish *P. zijsron*, and largetooth sawfish *P. pristis*, making these populations key in global conservation efforts for each species. This research assesses the levels of genetic diversity in these 3 sawfishes in Australian waters, testing for the presence of population bottlenecks using data at microsatellite loci. Levels of observed heterozygosity in each species from the west coast of Australia and the north-eastern Gulf of Carpentaria were generally high. *M* ratio tests suggest that assemblages of *P. zijsron* and *P. pristis* on the west coast and *P. clavata* and *P. zijsron* in the Gulf of Carpentaria may have experienced population bottlenecks. The bottlenecks are especially pronounced in *P. zijsron* populations and in *P. clavata* from the Gulf of Carpentaria. Demographic analyses, based on mtDNA data, indicate relatively recent (evolutionarily) range expansions in *Pristis* sawfishes in northern Australian waters, which could account for the population bottlenecks. A more recent range expansion in each of *P. clavata* and *P. zijsron*, as evidenced by more recent population divergence and more recent/higher rates of historic maternal gene flow, could account for the more pronounced bottlenecks in these species when compared to *P. pristis*. Given that *Pristis* sawfishes in Australian waters have experienced population bottlenecks, whether they be historic, contemporary or both, the preservation of remaining genetic diversity should be a high conservation priority.

KEY WORDS: Founder effect · *Pristis clavata* · *Pristis zijsron* · *Pristis pristis*

INTRODUCTION

Over the last century, sawfishes have faced vast declines in both range and abundance, resulting in an increased threat of their global extinction (Dulvy et al. 2016). These declines are attributed to a combination of anthropogenic activities, such as bycatch in fisheries and direct human use (Seitz & Poulakis 2006, Dulvy et al. 2016), as well as habitat degradation (Kyne et al. 2013). In the midst of these large global declines, Australia is regarded as the last stronghold for each of 3 *Pristis* sawfishes: the dwarf sawfish *P. clavata*, green sawfish *P. zijsron*, and the largetooth (or freshwater) sawfish *P. pristis* (Pogonoski et al. 2002, Phillips et al. 2011). Nevertheless, there is evidence of recent population declines in *Pristis* sawfishes in Australian waters. For example, the historic distribution of *P. zijsron* extended from Coral Bay on the west coast, across the north coast and along the east coast as far south as Sydney, New South Wales (Last & Stevens 2009). *P. zijsron* has suffered from a 30% range contraction in these waters and has not been recorded off New South Wales since the 1970s, the decline largely being attributed to anthropogenic activities occurring over the last century (White & Kyne 2010, Dulvy et al. 2014, 2016). *P. zijsron* is now considered extirpated from New South Wales waters and rare on the east coast of Queensland (Pogonoski et al. 2002, Dulvy et al. 2014, 2016). Sawfish are especially vulnerable to

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such declines because they are long-lived, mature late in life and have low fecundity, making population recovery a slow process (Simpfendorfer 2000, Peverell 2005, Thorburn et al. 2007).

While it is generally agreed that *Pristis* sawfishes have suffered from declines in range and abundance in Australian waters, the severity of these declines and the impact on the health of extant populations is largely unknown. This is because the evidence for the declines is largely anecdotal, with limited reliable estimates of abundance or population trend data (Kyne et al. 2013). Populations that have suffered from severe declines or population bottlenecks are at risk of having substantially reduced levels of genetic diversity (Frankham 2005). The maintenance of genetic diversity in sawfish populations is important in their conservation because long-term recovery is dependent upon populations being genetically diverse and large enough to adapt to changes in the environment, resist disease, and avoid inbreeding (Peery et al. 2012). Assemblages of each of *P. clavata*, *P. zijsron*, and *P. pristis* on the west coast and Gulf of Carpentaria in Australia have low to moderate levels of genetic diversity, based on data from the mitochondrial (mtDNA) control region (Phillips et al. 2011). However, levels of genetic diversity in each of *P. clavata* and *P. zijsron* in the Gulf of Carpentaria are reduced when compared to those for the west coast (Phillips et al. 2011). Whether or not the levels of genetic diversity in *Pristis* sawfishes are a consequence of population declines (whether contemporary or historic) remains unresolved (Phillips et al. 2011). The current research, therefore, aims to assess genetic diversity in *Pristis* sawfishes in Australian waters using nuclear DNA (nDNA) markers (microsatellite loci), and to test whether these populations have undergone population bottlenecks, thus providing valuable information on the genetic 'health' of these sawfish populations.

MATERIALS AND METHODS

Sampling

Genetic data were generated from tissue biopsies (preserved in 100% ethanol or 20% dimethyl sulfoxide saturated with NaCl) or skin taken from contemporary dry rostra from the dwarf sawfish *Pristis clavata*, green sawfish *P. zijsron*, and largetooth sawfish *P. pristis* from sites across northern Australia, as described by Phillips et al. (2011, 2017). Tissue samples were collected from sites on the west coast of Australia for *P. clavata* (N = 40), *P. zijsron* (N = 26), and *P. pristis* (N = 51) (Phillips et al. 2011, 2017). Samples were also collected from the Gulf of Carpentaria for *P. clavata* (N = 25), *P. zijsron* (N = 18), and *P. pristis* (N = 88) (Phillips et al. 2011, 2017). Since the number of samples collected per site was generally small, samples from a single geographic region were pooled for analysis (Phillips et al. 2011, 2017). Microsatellite data were not necessarily generated for all samples, because statistical power was not substantially increased by larger sample sizes once data were generated for 30 to 40 samples from a single geographic region (e.g. *P. pristis* from the Gulf of Carpentaria).

Genetic methods

A Masterpure™ DNA extraction kit (Epicentre Technologies) was used to extract total genomic DNA from approximately 5 mg of tissue, according to the manufacturer's protocol for preserved tissue and following the protocol of Phillips et al. (2009) for the rostral tissue. PCR was used to amplify alleles at 6 microsatellite loci in *P. pristis* and 8 loci in each of *P. clavata* and *P. zijsron* (Feldheim et al. 2010, Fields et al. 2015, Phillips et al. 2017) (see Tables 1, 2 & 3). The primers, PCR cycling conditions, and allele scoring were performed according to the methods of Phillips et al. (2017). Genetic data were generated for a 351–, 352–, and 351–353 base pair (bp) portion of the mitochondrial control region in each of *P. clavata*, *P. zijsron* and *P. pristis*, respectively, using the primers, PCR cycling conditions and DNA sequencing methods of Phillips et al. (2011).

Data analysis

Linkage disequilibrium and deviation from Hardy-Weinberg equilibrium (HWE) were assessed for each of *P. clavata*, *P. zijsron*, and *P. pristis* in GENEPOP version 1.2, with a dememorization number of 10 000, 1000 batches, and 10 000 iterations per batch (see Raymond & Rousset 1995) and a Bonferroni correction for multiple tests (Rice 1989). Micro-Checker v.2.2.3 was also used to check for genotyping errors and null alleles for all loci (van Oosterhout et al. 2004).

Genetic diversity

Levels of genetic diversity at microsatellite loci were assessed via expected and observed hetero-

zygosity and allelic richness using rarefaction to account for uneven sample sizes, as calculated in FSTAT (www2.unil.ch/popgen/softwares/fstat.htm).

Demographic history

Heterozygosity excess, mode-shift, and *M* ratio tests were used to test for genetic bottlenecks in each of *P. clavata*, *P. zijsron*, and *P. pristis*. The heterozygosity excess test uses the expectation that a declining population will experience a greater reduction in the number of alleles (via loss of rare alleles) than in heterozygosity, resulting in an excess of heterozygosity in bottlenecked populations (Nei et al. 1975). The mode-shift test evaluates the allele frequency distribution at a locus and determines whether there has been a shift of the mode from the expected (L-shaped) distribution (Luikart et al. 1998). Heterozygosity excess and mode-shift tests were conducted in BOTTLENECK version 1.2.02 (Cornuet & Luikart 1996) using the stepwise mutation model (SMM) and the 2-phase model (TPM) with 10 000 simulations per locus. The TPM consisted of 90% single-step mutations and 10% multi-step mutations with the variance for mutation size set to 12, as suggested by Piry et al. (1999). The *M* ratio is the ratio of the number of alleles observed in a population to the range in allele size at each locus (Garza & Williamson 2001). In bottlenecked populations, *M* is predicted to decrease because the number of alleles declines faster than the range in allele sizes (Garza & Williamson 2001). *M* ratios were estimated at each locus and over all loci for each population and species in M_P_Val and the critical *M* values (M_C) were determined in Critical_M (Garza & Williamson 2001). The mean percent of single-step mutations was set to 0.88 and the mean size of larger mutations was set to 2.8 (Garza & Williamson 2001). Theta was estimated in IMa2 (Hey & Nielsen 2007) using the methods described below, although lower and higher theta values were also used to assess the impact, if any, on values of *M*.

To explore the hypothesis that a population bottleneck is the result of a founder effect during historic range expansions (e.g. 1000s of years ago; see Phillips et al. 2011), historic gene flow was investigated in each species. Migration-scaled divergence time (*t*) was estimated for the west coast and Gulf of Carpentaria assemblages for each species in IMa2 using mtDNA data for all samples collected (Hey & Nielsen 2007). When divergence of populations was supported, mutation-scaled migration (*m*) and timing of migration events were estimated. Posterior probability distributions of *t* were produced using the Hasegawa-Kishino-Yano (HKY) model (Hasegawa et al. 1985) and final runs had a minimum of 3 000 000 steps in the Markov chain Monte Carlo (MCMC) chain with the first 1 000 000 steps discarded as burn-in. Preliminary runs used a prior of 10 for *m*, and final runs used the estimates from these preliminary runs as priors. These priors produced consistent results between 5 final runs with different seed numbers. Estimates were left in mutation-scaled units due to the lack of reliable mutation rates for sawfishes and the potential for considerable error with the use of an incorrect mutation rate (i.e. Henn et al. 2009).

RESULTS

At each locus, the numbers of homozygotes and heterozygotes in each of the dwarf sawfish *Pristis clavata*, green sawfish *P. zijsron*, and largetooth sawfish *P. pristis* were similar to those expected under HWE after a Bonferroni correction (Tables 1, 2 & 3). There was no evidence of null alleles or errors in genotyping for any locus and there was no evidence of linkage disequilibrium after a Bonferroni correction.

Genetic diversity

Levels of observed heterozygosity in each of *P. clavata*, *P. zijsron*, and *P. pristis* from the west coast and Gulf of Carpentaria were generally high (Tables 1, 2 & 3). Allelic richness was similar in *P. clavata* (10.316–11.576) and *P. zijsron* (10.161–10.235) and slightly higher in *P. pristis* (14.073–14.399), likely due to the large sample sizes of the latter (Tables 1, 2 & 33). There was no apparent pattern when comparing heterozygosity and allelic richness in assemblages from the west coast and Gulf of Carpentaria for any species.

Demographic history

The heterozygosity excess and mode shift tests did not provide evidence of a population bottleneck in any species (Table 4). The *M* ratio tests suggest that the west coast assemblages of *P. zijsron* and *P. pristis* and the Gulf of Carpentaria assemblages of *P. clavata* and *P. zijsron* are bottlenecked (Table 4). This is evidenced by *M* ratio values less than the *M* critical val-

Table 1. Summary statistics for 8 microsatellite loci in *Pristis clavata* from the west coast (WC, N = 34) and the Gulf of Carpentaria (GoC, N = 25) in Australia. N: number of individuals, *k*: number of alleles, *A*: allelic richness, H_e: expected heterozygosity, H_o: observed heterozygosity, p: outcome of tests for Hardy-Weinberg equilibrium. No values were statistically significant after a Bonferroni correction (p < 0.003)

Region		*Ppe4*	*Ppe5*	*Ppe69*	*Ppe122*	*Ppe152*	*Ppe165*	*Ppe179*	*Ppe186*	Average
WC	N	34	34	34	34	29	26	31	31	
	k	14	19	10	9	16	16	13	11	13.50
	A	12.443	14.643	7.600	8.126	13.888	14.285	11.474	10.150	11.576
	H_e	0.915	0.925	0.795	0.793	0.928	0.933	0.879	0.880	
	H_o	1.000	0.912	0.853	0.824	0.897	1.000	0.871	0.871	0.904
	p	0.983	0.873	0.658	0.423	0.112	0.439	0.504	0.969	
GoC	N	22	23	23	25	18	18	21	23	
	k	11	16	5	8	12	10	10	14	10.75
	A	10.750	15.062	4.941	7.365	12.000	10.000	9.660	12.730	10.316
	H_e	0.910	0.935	0.566	0.834	0.910	0.887	0.856	0.879	
	H_o	1.000	0.957	0.696	0.720	0.889	0.667	0.783	0.739	0.806
	p	0.417	0.005	0.699	0.106	0.144	0.003	0.057	0.019	

Table 2. Summary statistics for 8 microsatellite loci in *Pristis zijsron* from the west coast (WC, N = 24) and the Gulf of Carpentaria (GoC, N = 18) in Australia. N: number of individuals, *k*: number of alleles, *A*: allelic richness, H_e: expected heterozygosity, H_o: observed heterozygosity, p: outcome of tests for Hardy-Weinberg equilibrium. No values were statistically significant after a Bonferroni correction (p < 0.003)

Region		*Ppe4*	*Ppe88*	*Ppe152*	*Ppe165*	*Ppe172*	*Ppe179*	*Ppe180*	*Ppe186*	Average
WC	N	23	23	23	24	23	24	24	23	
	k	13	22	4	17	10	12	12	7	12.13
	A	11.067	17.482	3.211	13.564	9.180	10.405	9.880	6.497	10.161
	H_e	0.870	0.962	0.274	0.930	0.879	0.902	0.865	0.752	
	H_o	0.783	1.000	0.304	1.000	0.826	0.958	0.917	0.870	0.832
	p	0.371	0.812	1.000	0.344	0.581	0.599	0.151	0.216	
GoC	N	14	15	18	15	15	15	16	16	
	k	8	17	4	13	12	10	12	9	10.63
	A	8.000	16.326	3.725	12.660	11.533	9.798	11.225	8.611	10.235
	H_e	0.831	0.949	0.303	0.924	0.885	0.892	0.879	0.853	
	H_o	0.929	0.933	0.333	0.867	0.867	1.000	0.813	0.875	0.827
	p	0.903	0.341	1.000	0.126	0.535	0.510	0.225	0.629	

ues or *M* values that were ≤0.70, the suggested threshold for bottleneck detection; although non-bottlenecked populations are expected to have *M* > 0.820 (Garza & Williamson 2001). The assemblage of *P. pristis* from the Gulf of Carpentaria had an *M* ratio value less than the *M* critical value; however, the *M* ratio was well above 0.70 (Table 4). The low values of *M* do not appear to be driven by a single locus for any species (see Table A1 in the Appendix).

The results of the analysis of population divergence, based on mtDNA data, indicate that assemblages of each of *P. clavata*, *P. zijsron*, and *P. pristis* from the west coast and Gulf of Carpentaria diverged in the relatively

Table 3. Summary statistics for 6 microsatellite loci in *Pristis pristis* from the west coast (WC, N = 36) and the Gulf of Carpentaria (GoC, N = 68) in Australia. N: number of individuals, *k*: number of alleles, *A*: allelic richness, H_e: expected heterozygosity, H_o: observed heterozygosity, p: outcome of tests for Hardy-Weinberg equilibrium. No values were statistically significant after a Bonferroni correction (p < 0.004)

Region		*Ppe4*	*Ppe5*	*Ppe122*	*Ppe172*	*Ppe180*	*Ppe186*	Average
WC	N	35	33	36	29	29	32	
	k	12	23	7	18	13	17	15.00
	A	11.622	21.107	6.792	18.000	13.000	15.875	14.399
	H_e	0.890	0.952	0.677	0.940	0.767	0.938	
	H_o	0.914	0.879	0.556	0.897	0.655	0.906	0.801
	p	0.219	0.152	0.156	0.055	0.116	0.308	
GoC	N	67	65	68	66	63	63	
	k	13	31	7	21	18	19	18.17
	A	11.396	20.197	5.815	17.505	13.823	15.700	14.073
	H_e	0.864	0.957	0.706	0.926	0.842	0.918	
	H_o	0.836	0.969	0.765	0.908	0.794	0.905	0.863
	p	0.581	0.298	0.272	0.062	0.032	0.108	

recent (evolutionary) past (Fig. 1). The time of divergence of these assemblages for *P. clavata* and *P. zijsron* probably occurred more recently than that for *P. pristis* (Fig. 1). The analysis of mutation-scaled migration rates suggests that there was maternal gene flow between populations and that the direction of gene flow in each species was asymmetrical, with migration occurring in an east to west direction in each of *P. clavata* and *P. zijsron*, and in a west to east direction in *P. pristis* (Fig. 2). In all species, this maternal gene flow is historic, rather than contemporary, because the distributions of migration events over time have a low probability at time zero (Fig. 3). It appears as though this historic gene flow was higher and slightly more recent in each of *P. clavata* and *P. zijsron* than in *P. pristis* (Figs. 2 & 3).

Table 4. Bottleneck tests for each of *Pristis clavata*, *P. zijsron*, and *P. pristis* from the west coast (WC) and the Gulf of Carpentaria (GoC) in Australia. Expected heterozygosity (H_e) excess is presented as p-values from the Wilcoxon/sign rank tests using the stepwise mutation model (SMM) and 2-phase model (TPM). M_c are the critical M values

Species	Region	H_e excess		Mode shift	M ratio	M_C
		SMM	TPM			
P. clavata	WC	0.770/0.445	0.320/0.424	Normal	0.885	0.841
	GoC	0.473/0.576	0.320/0.307	Normal	0.744	0.821
P. zijsron	WC	0.902/0.185	0.629/0.427	Normal	0.659	0.818
	GoC	0.980/0.053	0.844/0.169	Normal	0.634	0.799
P. pristis	WC	0.578/0.527	0.500/0.532	Normal	0.711	0.828
	GoC	0.344/0.519	0.281/0.518	Normal	0.842	0.858

DISCUSSION

The present study provides evidence that each of the *Pristis* sawfishes in northern Australian waters has gone through a population bottleneck, although the geographic region affected and the severity of the bottleneck differs between species. The number of loci and sample sizes of this study were moderate. Therefore, there is a note of caution in that the data underlying the interpretation are limited and more extensive sampling

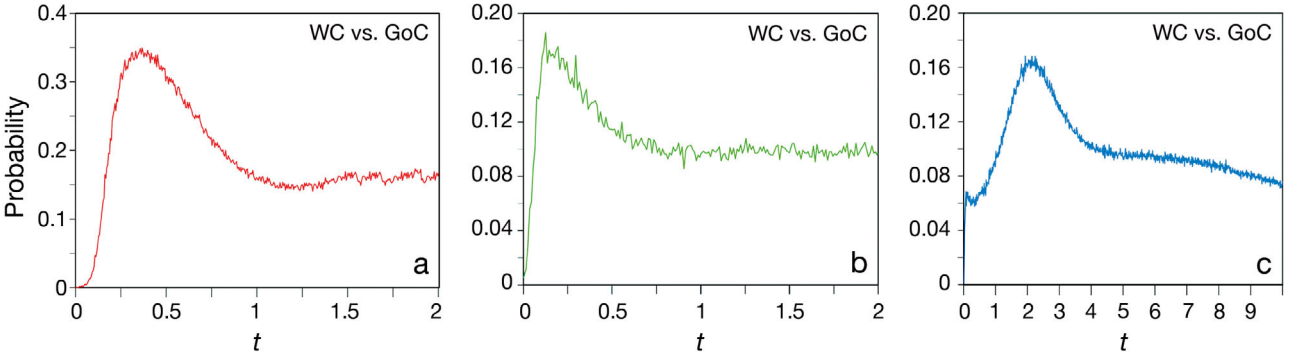

Fig. 1. Posterior probability distributions of mutation-scaled time since divergence (t), based on nucleotide sequence data from the mtDNA control region, of assemblages from the west coast (WC) and the Gulf of Carpentaria (GoC) in (a) *Pristis clavata*, (b) *P. zijsron*, and (c) *P. pristis*

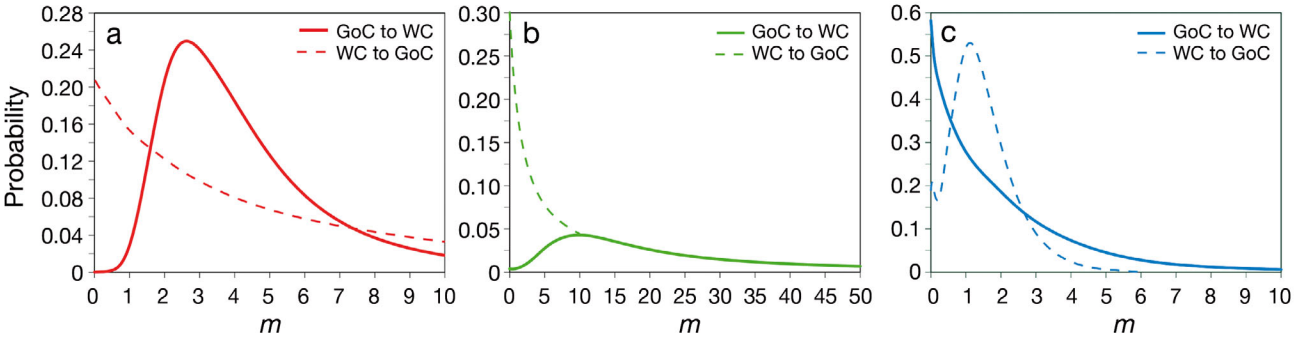

Fig. 2. Posterior probability distributions of estimates of mutation-scaled migration rates (m) of 'genes', based on nucleotide sequence data from the mtDNA control region, between assemblages from the west coast (WC) and the Gulf of Carpentaria (GoC) in (a) *Pristis clavata*, (b) *P. zijsron*, and (c) *P. pristis*

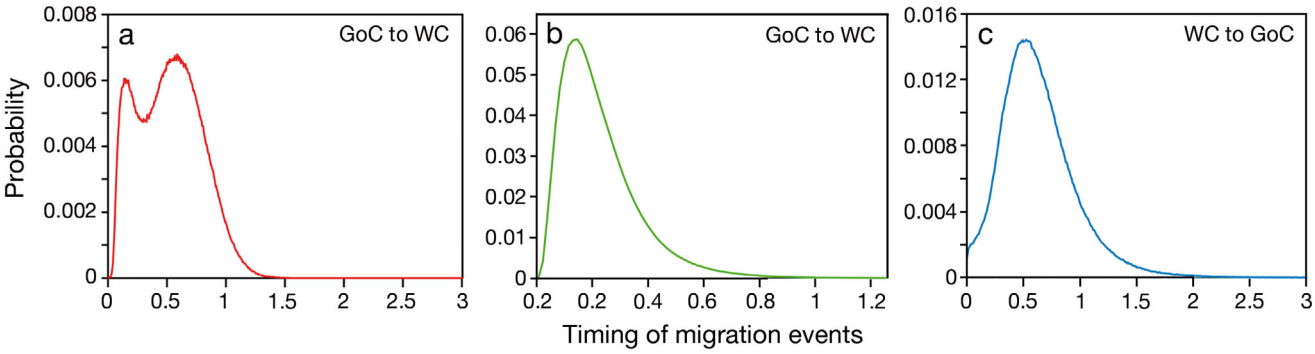

Fig. 3. Posterior probability distributions of estimates of the mutation-scaled timing of migration events, based on nucleotide sequence data from the mtDNA control region, between assemblages from the west coast (WC) and the Gulf of Carpentaria (GoC) in (a) *Pristis clavata*, (b) *P. zijsron*, and (c) *P. pristis*

and additional genetic markers are needed to confirm the proposed demographic histories of *Pristis* sawfishes in Australian waters. It is possible, for example, that the use of a microsatellite library developed for another species (the smalltooth sawfish *P. pectinata*) has contributed to lower values of *M*, although it seems unlikely to be the sole explanation, because the levels of polymorphism and genetic diversity in each of the dwarf sawfish *P. clavata*, green sawfish *P. zijsron*, and largetooth sawfish *P. pristis* were similar to those for *P. pectinata* (see Chapman et al. 2011). It is also possible that the low values of *M* are due to a violation of the SMM mutation model, although there is no evidence to support this (see Guinand & Scribner 2003, Williamson-Natesan 2005). There are no reliable historic catch records available for any of these species in Australian waters to assess changes in population sizes (Peverell 2005), so despite the limitations of the present study, it provides novel information obtainable only via genetic approaches.

Genetic diversity

Assemblages of each of *P. clavata*, *P. zijsron*, and *P. pristis* on the west coast and the Gulf of Carpentaria appear to have moderate to high levels of genetic diversity at microsatellite loci that are within the ranges reported for other elasmobranchs, including sawfish (Chapman et al. 2011, Daly-Engel et al. 2012, O'Leary et al. 2015), with no apparent spatial pattern in genetic diversity. These results are in contrast to the moderate to low levels of genetic diversity found in the mtDNA control region for each species (Phillips et al. 2011). Furthermore, both *P. clavata* and *P. zijsron* had reduced levels of mtDNA diversity in the Gulf of Carpentaria assemblage when compared to values for the west coast, with *P. clavata* being genetically depauperate in the Gulf of Carpentaria (Phillips et al. 2011), a pattern not observed in the microsatellite loci.

The pattern of lower levels of genetic diversity in mtDNA markers compared to microsatellite loci (nDNA) has been observed in other elasmobranchs such as the bull shark *Carcharhinus leucas* and lemon shark *Negaprion brevirostris* (Schultz et al. 2008, Karl et al. 2011). This discordance could be due to the characteristics of mtDNA versus nDNA markers (microsatellite loci), changes in population size, sex-biased dispersal and/or selection. Selection alone is not thought to be driving the differences in levels of genetic diversity at mtDNA and nDNA markers given that similar selective forces would have to be operating across *Pristis* sawfishes as well as other species of sharks. It is difficult to hypothesize a scenario where similar selective forces would be operating across such different species in different geographic regions (Schultz et al. 2008, Karl et al. 2011). Although the discordance in levels of genetic diversity in mtDNA and nDNA markers in *P. pristis* could be explained by sex-biased dispersal with female philopatry to parturition sites, there is currently no evidence to support sex-biased dispersal in either *P. clavata* or *P. zijsron* (Phillips et al. 2011, 2017, Feutry et al. 2015).

The lower levels of genetic diversity in mtDNA markers, when compared to those in nDNA markers, are most likely due to the characteristics of the markers combined with changes in population size. Levels of genetic diversity in nDNA markers should, theoretically, be higher than those in mtDNA markers simply due to differences in their modes of inheritance and effective population sizes (Ballard & Whitlock 2004). Being maternally inherited, the mtDNA has a 4-fold smaller effective population size when

compared to bi-parentally inherited nDNA (Ballard & Whitlock 2004). These characteristics of the markers, when combined with the evidence of changes in population size, could explain the lower levels of genetic diversity in the mtDNA control. The smaller effective population size of the mtDNA marker makes it highly susceptible to long-term genetic erosion from bottlenecks (Grant & Leslie 1993, Canino et al. 2010) and given the slow rate of mutation in the mtDNA of elasmobranchs (Martin et al. 1992, Martin 1995), the recovery of genetic diversity at these markers would be an (evolutionarily) slow process.

Demographic history

The discrepancies in the results of the *M* ratio tests and the heterozygosity excess and mode shift tests have been found in a number of other studies (e.g. Hundertmark & Van Daele 2010, Nance et al. 2011, O'Leary et al. 2015). *M* ratio tests are thought to be more powerful at detecting bottlenecks in general, compared to the heterozygosity excess or mode shift tests (Peery et al. 2012). Typically, *M* ratio tests are better at detecting slightly older bottlenecks (up to 50 generations) than very recent events (1 to 5 generations), which could account for the inability to detect bottlenecks in some species with contemporary declines (Peery et al. 2012). Heterozygosity excess methods are less powerful in detecting bottlenecks after approximately 10 generations have passed because mutation-drift equilibrium can be reached quickly after a period of sudden growth (see Nei et al. 1975, Hundertmark & Van Daele 2010, Peery et al. 2012). *M* ratios, by contrast, take longer to recover post-bottleneck because new alleles arise slowly and do not necessarily increase values of *M* (Peery et al. 2012).

Evidence from *M* ratio tests suggests that assemblages of *P. zijsron* and *P. pristis* on the west coast and *P. clavata* and *P. zijsron* in the Gulf of Carpentaria may have experienced population bottlenecks. Detection of genetic bottlenecks in endangered species is often hindered by the combination of life history parameters (long-lived, overlapping generations) and low statistical power due to concomitant small sample sizes and an insufficient number of genetic markers (Peery et al. 2012). Mounting evidence suggests that in order to detect bottlenecks in such species, the bottleneck must be severe and of sufficient duration, and the population should be sampled many generations after the event (Peery et al. 2012). Given the difficulty in detecting bottlenecks in endangered species, the bottleneck that occurred in *Pristis* sawfishes in Australian waters must have been severe and have occurred some time ago (see Peery et al. 2012). In comparison, genetic bottlenecks were not detected in the remaining population of *P. pectinata* in southwest Florida, despite this species experiencing a 95 to 99% decline in US waters (Simpfendorfer 2000, Chapman et al. 2011). The inability to detect a bottleneck in *P. pectinata* was attributed to the longevity of the species, which slows the process of genetic drift, or the southwest Florida population never reaching severely low numbers for a sufficient duration to produce a bottleneck (Chapman et al. 2011). Despite the apparent difficulty in detecting bottlenecks in long-lived species such as elasmobranchs, bottlenecks have also been detected using *M* ratios in the scalloped hammerhead *Sphyrna lewini* in the eastern Pacific Ocean (Nance et al. 2011) and the white shark *Carcharodon carcharias* in the northwest Atlantic (O'Leary et al. 2015). The values of *M* for *S. lewini* and *C. carcharias* were comparable to those for *Pristis* sawfishes in Australian waters (Nance et al. 2011, O'Leary et al. 2015). The bottleneck in *S. lewini* was attributed to historic processes following the Last Glacial Maximum (~18 000 to 20 000 yr ago, Nance et al. 2011), while that for *C. carcharias* was suggested to be the result of population declines during the mid to late 20th century (O'Leary et al. 2015).

Population bottlenecks can result from contemporary (i.e. 100 to 200 yr) declines in abundance or from historical processes, such as founder effects during range expansions over time scales of 1000s to 10 000s of years (Hauser et al. 2002, Stow et al. 2006). mtDNA control region data collectively suggest there has been a relatively recent (evolutionarily) range expansion in each of the *Pristis* sawfishes in Australian waters (Phillips et al. 2011). This is evidenced by signals of population growth in the mtDNA marker (Phillips et al. 2011), the presence of historic (i.e. not contemporary) maternal gene flow in each species between the west coast and the Gulf of Carpentaria, and the signature of population bottlenecks. The population bottlenecks could indicate founder effects occurring during the colonization of new habitats during range expansions. A more evolutionarily recent range expansion in each of *P. clavata* and *P. zijsron*, as evidenced by more recent population divergence and more recent/higher rates of historic maternal gene flow, could account for the more pronounced bottlenecks in these species. Range expansions in coastal marine species are usually attributed to changes in coastline morphology associated with

fluctuating sea levels during the glacial cycling of the Pleistocene (Lukoshek et al. 2007, Swatdipong et al. 2009). In northeastern Australia, the Pleistocene was characterized by the repeated emergence of the Torres Strait land bridge (in the area that is currently the Gulf of Carpentaria) at low sea levels (Voris 2000). The assemblages of *P. clavata* and *P. zijsron* in these waters may have been subject to cycles of range expansion and contraction as habitat availability changed during this time period in the Gulf of Carpentaria (Phillips et al. 2011), contributing to the more pronounced population bottlenecks in these species. The range expansion in *P. pristis*, on the other hand, occurred much earlier, perhaps allowing more time for populations to recover from a founder effect during range expansion. *P. pristis* may not have been as adversely affected by the emergence of the Torres Strait land bridge because they utilize freshwater rivers as juveniles, and expansive rivers remained in the Gulf of Carpentaria even at low sea levels (Voris 2000, Phillips et al. 2011).

Demographic history provides a plausible explanation for the observed levels of genetic diversity in *Pristis* sawfishes in Australian waters. However, this explanation does not exclude the possibility that contemporary bottlenecks have also occurred as a result of anthropogenic activities (Cavanagh et al. 2003). In the absence of historic records for *Pristis* sawfishes in Australian waters, it is difficult to disentangle the relative influence of contemporary versus historic factors on contemporary patterns of genetic diversity (see Costello et al. 2003, Johansson et al. 2006). The most direct way of resolving this uncertainty is to compare levels of genetic diversity in historic and contemporary populations, for example, of sawfishes from approximately 100 yr ago and today, to determine if levels of genetic diversity have been fairly stable for the past 100 yr (e.g. Ludwig et al. 2000, Hoelzel et al. 2002, Larson et al. 2002, Leonard 2008). While this is not a viable possibility for many species of conservation concern, for sawfishes, historic dried rostra readily available in public and private collections provide a means to generate genetic data for historic sawfish populations (see Leonard 2008, Phillips et al. 2009). Given that *Pristis* sawfishes in Australian waters have experienced population bottlenecks, whether they be historic, contemporary or both, the preservation of remaining genetic diversity should be a high conservation priority. This is especially true for the maternal lineages of each species, as levels of mtDNA diversity were moderate to severely low, and recovery of genetic diversity is a slow, evolutionary process (Phillips et al. 2011).

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Appendix

Table A1. *M* ratio tests at each microsatellite locus in each of *Pristis clavata*, *P. zijsron*, and *P. pristis* from the west coast (WC) and the Gulf of Carpentaria (GoC) in Australia

Species	Locus	WC	GoC
P. clavata	*Ppe4*	0.933	0.846
	Ppe5	0.826	0.667
	Ppe69	1.11	0.625
	Ppe122	1.000	0.889
	Ppe152	0.889	0.800
	Ppe165	0.533	0.714
	Ppe179	0.867	0.714
	Ppe186	0.917	0.700
P. zijsron	*Ppe4*	1.00	0.800
	Ppe88	0.667	0.515
	Ppe152	0.500	0.571
	Ppe165	0.586	0.684
	Ppe172	0.500	0.400
	Ppe179	0.800	0.770
	Ppe180	0.522	0.429
	Ppe186	0.700	0.900
P. pristis	*Ppe4*	0.923	1.000
	Ppe5	0.920	1.148
	Ppe122	0.700	0.700
	Ppe172	0.692	0.741
	Ppe180	0.325	0.462
	Ppe186	0.708	1.00

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