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Population genetic structure of barramundi (*Lates calcarifer*) across the natural distribution range in Australia informs fishery management and aquaculture practices

Shannon R. Loughnan[A], *Carolyn Smith-Keune*[B], *Luciano B. Beheregaray*[A], *Nicholas A. Robinson*[C,D] *and Dean R. Jerry*[B,E,F]

[A]College of Science and Engineering, Flinders University, PO Box 2100, Adelaide, SA 5001, Australia.
[B]Centre for Sustainable Tropical Fisheries and Aquaculture, College of Science and Engineering, James Cook University, Townsville, Qld 4811, Australia.
[C]Nofima, PO Box 5010, N-1432 Ås, Norway.
[D]Sustainable Aquaculture Laboratory – Temperate and Tropical (SALTT), School of BioSciences, The University of Melbourne, Vic 3010, Australia.
[E]Tropical Futures Institute, James Cook University, 149 Sims Drive, 387380, Singapore.
[F]Corresponding author. Email: dean.jerry@jcu.edu.au

Abstract. Clarifying population structure of fish stocks is important for the sustainable exploitation of fisheries, along with informing collection of founder broodstock for the genetic improvement of aquaculture programs. Using 16 microsatellite DNA markers, the most comprehensive genetic survey to date (1297 individuals from 49 sample collections) of the population structure and genetic diversity of wild Australian barramundi (*Lates calcarifer*) was undertaken. The results point to the existence of two distinct genetic stocks (east and west) with isolation by geographic distance (IBD), and a central region of admixture between the stocks, located in an area where a historic land bridge once connected northern Australia with Papua New Guinea. Global levels of population differentiation were moderate (fixation index, $F_{ST} = 0.103$, $P < 0.001$) and IBD was identified as a factor influencing population structure across the sampled region. There was also evidence of temporal stability of population genetic structure over a period of 25 years. This study provides valuable information for improving programs of translocation, restocking and captive breeding for both the wild barramundi fishery and the aquaculture industry.

Additional keywords: Asian seabass, connectivity, genetic diversity, microsatellite.

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Introduction

The population genetics of wild fish stocks helps define distinctive populations for the purposes of fisheries management, monitoring, conservation and aquaculture. Understanding population genetic structure is critical for advising translocation policy, because such knowledge informs the movement of genetically disparate fish between different stocks, either through deliberate stock enhancement efforts or by avoiding accidental aquaculture release (Cross 2000). Different genetic stocks may exhibit variation in the expression of commercially important quantitative traits for aquaculture, whereas specific stocks can also be targeted to help maintain genetic diversity and reduce inbreeding within captive populations.

Barramundi (*Lates calcarifer*), also known as Asian seabass in south-east Asia, is a euryhaline, protandrous hermaphrodite fish species that supports valuable farmed, commercial and recreational wild fisheries in tropical Australia. Barramundi is widely distributed throughout the Indo-West Pacific, ranging from India, across south-east Asia to Taiwan, to Papua New Guinea and tropical Australia (Grey 1986). Being euryhaline, barramundi inhabit both freshwater and marine environments, with juveniles generally growing to maturity in fresh water before returning to the mouths of estuaries and nearshore regions to spawn.

Initially, the Australian barramundi wild fishery was being managed as a single, or panmictic, stock across the entire geographic range of the species (Shaklee and Salini 1983), possibly due to tag and recapture studies suggesting that there was some movement between river systems (Moore 1982; Moore and Reynolds 1982; Davis 1986). However, the earliest

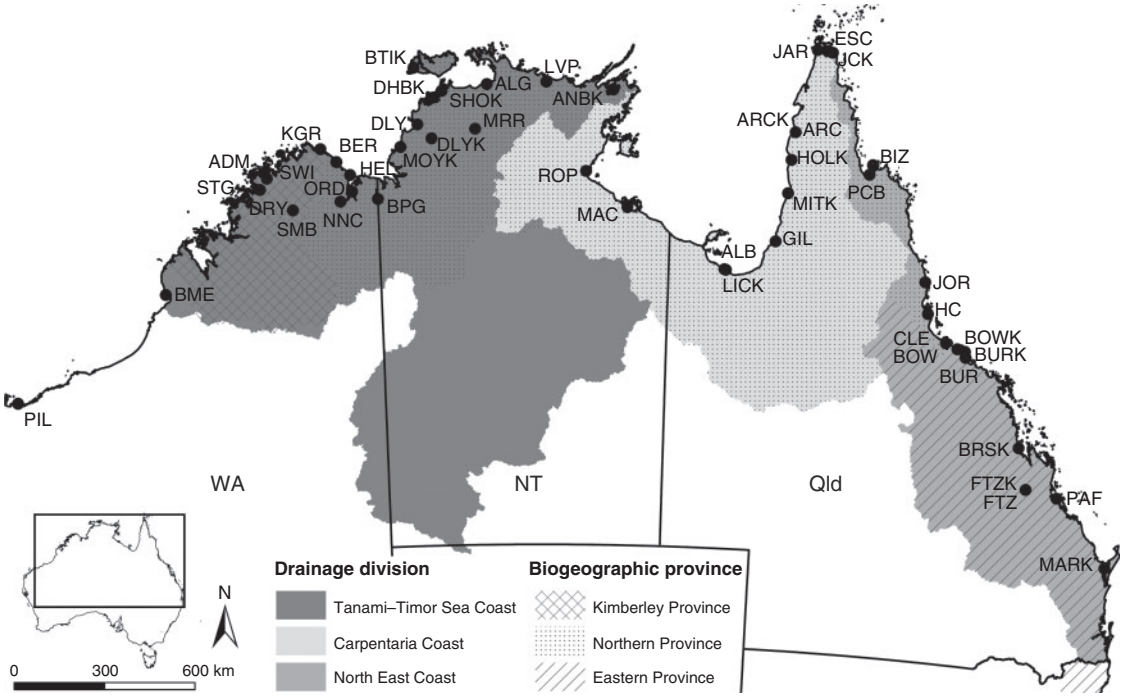

Fig. 1. Map of 49 *Lates calcarifer* sample collections in Australia where 1297 individuals were collected for the study, including topographic drainage divisions and historical biogeographic provinces. Descriptions of the labels representing each collection site are given in Table 1.

population genetic studies of wild Australian barramundi using allozyme electrophoresis initially detected two distinct stocks in Queensland and one in the Northern Territory (Shaklee and Salini 1983, 1985), with a further study recognising five stocks in Queensland and two in the Northern Territory (Salini and Shaklee 1987). Salini and Shaklee (1988) sampled eight locations and detected seven distinct stocks, including the Ord River in Western Australia and six stocks within the Northern Territory. Later, seven distinct Queensland stocks were identified (Shaklee *et al.* 1993). Based on these initial findings, Keenan (1994) went on to conduct the most comprehensive study at the time and used allozymes to compare barramundi from 50 locations, from the Ord River in Western Australia, across northern Australia to the Mary River in south-eastern Queensland. Three broad genetic stocks were identified in the study of Keenan (1994), with the species exhibiting an overall fixation index (F_{ST}) of 0.064 among populations. Several later studies using mitochondrial (mt) DNA control regions have since confirmed the presence of this broad-scale genetic structuring (mean nucleotide diversity (Φ_{ST}) = 0.328, Chenoweth *et al.* 1998*a*, 1998*b*; and mean Φ_{ST} = 0.563, Doupé *et al.* 1999). However, no single study to date has comprehensively sampled barramundi populations across the entire Australian distribution of the species. For example, the allozyme study of Keenan (1994) exhibited a north-eastern bias in sampling design, whereas the studies of Doupé *et al.* (1999) and Marshall (2005), which investigated western populations, included little representation of barramundi from the north-east. In this study we use 16 polymorphic microsatellite DNA markers to assess the contemporary genetic stock structure and diversity of wild barramundi across the full species distribution in Australia, including some previously unsurveyed populations.

Materials and methods

Sample collection

Sampled barramundi genotyped in the present study originated from two distinct temporal collections. The first collection comprised archived muscle tissue sampled from barramundi between 1988 and 1993 and were used in the allozyme studies of Shaklee *et al.* (1993) and Keenan (1994). The second collection comprised newly collected fin clip samples obtained between 2006 and 2013. These latter samples were collected by recreational and commercial fishers, plus state and territory fisheries personnel from Western Australia, Queensland and the Northern Territory. In total, 2312 samples of barramundi muscle tissue and fin clips were collected from 49 sample collections (Fig. 1; for the latitude and longitude coordinates, see Table S1, available as Supplementary material to this paper), which was an increase of six sample collections from the work initiated by Jerry and Smith-Keune (2014). The samples were either frozen in liquid nitrogen or preserved in 70% ethanol or a saturated salt solution containing 20% dimethyl sulfoxide and 0.25 M EDTA preservative. Because of the substantial intervening time between the sampling of barramundi in the two collections (18–25 years), temporal stability of barramundi population genetic structure was tested by resampling six sites common to the two collections (Table 1). These common sites were the Daly River, Leichardt and Albert rivers in the Northern Territory, which were treated as one locality due to their proximity, and the

Table 1. Measures of genetic diversity and Hardy–Weinberg equilibrium for 49 *Lates calcarifer* sample collections genotyped with 16 microsatellite DNA loci

Note: sample size (n) for the western stock, central region, eastern stock and all sample collections is the total count, whereas the remaining values are means. Years in parentheses represent the year of sample collection. A, mean number of alleles; A_r, mean allelic richness; F_{IS}, mean inbreeding coefficient; H_e, mean expected heterozygosity; H_o, mean observed heterozygosity; NT, Northern Territory; PA_r, private allelic richness; Qld, Queensland; WA, Western Australia

Sample collection number	Locality	State	Code	n	A	H_o	H_e	A_r	PA_r	F_{IS}
1	Pilbara	WA	PIL	31	3.6	0.489	0.481	2.81	0.07	−0.000
2	Broome	WA	BME	14	3.3	0.451	0.440	2.85	0.05	0.013
3	St George Basin	WA	STG	30	4.5	0.536	0.528	3.38	0.09	0.001
4	Admiralty Gulf	WA	ADM	37	4.4	0.461	0.487	3.10	0.01	0.068
5	Swift Bay	WA	SWI	17	4.6	0.515	0.552	3.64	0.01	0.098
6	Drysdale River	WA	DRY	26	4.7	0.546	0.532	3.47	0.00	−0.005
7	Salmon Bay	WA	SMB	25	4.9	0.550	0.526	3.46	0.05	−0.025
8	King George River	WA	KGR	24	4.6	0.563	0.529	3.42	0.01	−0.042
9	Berkeley River	WA	BER	24	4.8	0.537	0.536	3.57	0.01	0.018
10	Helby River	WA	HEL	24	4.4	0.523	0.508	3.40	0.00	−0.009
11	Nulla Nulla Creek	WA	NNC	21	4.3	0.478	0.480	3.17	0.04	0.032
12	Ord River	WA	ORD	63	5.4	0.515	0.522	3.42	0.00	0.022
13	Bonaparte Gulf	WA	BPG	26	4.9	0.529	0.517	3.48	0.01	−0.003
14	Moyle River[A] (1988–93)	NT	MOYK	29	4.8	0.531	0.515	3.40	0.00	−0.012
15	Daly River[A] (1990)[B]	NT	DLYK	22	4.8	0.548	0.524	3.50	0.01	−0.023
16	Daly River (2008)[B]	NT	DLY	24	4.8	0.513	0.529	3.52	0.00	0.052
17	Bathurst Island[A] (1988–93)	NT	BTIK	24	4.4	0.492	0.498	3.34	0.07	0.033
18	Darwin Harbour[A] (1988–93)	NT	DHBK	24	4.9	0.473	0.492	3.43	0.04	0.061
19	Shoal Bay[A] (1988–93)	NT	SHOK	24	4.6	0.512	0.490	3.37	0.04	−0.025
20	Mary River	NT	MRR	24	4.6	0.503	0.489	3.40	0.00	−0.007
21	Alligator River	NT	ALG	13	4.3	0.538	0.495	3.52	0.01	−0.048
	Western stock			546	4.6	0.514	0.508	3.36	0.03	0.009
22	Liverpool River	NT	LVP	32	4.1	0.498	0.515	3.15	0.05	0.049
23	Arnhem Bay[A] (1988–93)	NT	ANBK	22	4.6	0.523	0.506	3.42	0.06	−0.010
24	Roper River	NT	ROP	24	4.6	0.523	0.487	3.46	0.08	−0.055
25	McArthur River	NT	MAC	24	4.8	0.513	0.514	3.54	0.06	0.024
26	Leichhardt River[A] (1990–91)[B]	Qld	LICK	24	4.9	0.521	0.500	3.53	0.02	−0.020
27	Albert River (2011)[B]	Qld	ALB	24	5.3	0.508	0.528	3.69	0.07	0.060
28	Gilbert River	Qld	GIL	24	5.5	0.516	0.529	3.68	0.06	0.047
29	Mitchell River[A] (1988–93)	Qld	MITK	24	5.1	0.536	0.525	3.68	0.01	−0.000
30	Holroyd River[A] (1988–93)	Qld	HOLK	21	5.1	0.536	0.531	3.68	0.05	0.016
31	Archer River[A] (1993)[B]	Qld	ARCK	24	5.9	0.555	0.547	3.82	0.04	0.007
32	Archer River (2011)[B]	Qld	ARC	33	5.8	0.528	0.525	3.66	0.02	0.009
33	Jardine River	Qld	JAR	16	4.4	0.539	0.509	3.52	0.06	−0.028
34	Jackey Jackey Creek	Qld	JCK	30	4.7	0.557	0.542	3.45	0.07	−0.008
35	Escape River	Qld	ESC	24	4.8	0.549	0.548	3.56	0.03	0.019
	Central region			346	5.0	0.529	0.522	3.56	0.05	0.008
36	Princess Charlotte Bay	Qld	PCB	24	4.1	0.549	0.543	3.20	0.04	0.009
37	Bizant River	Qld	BIZ	15	3.9	0.533	0.539	3.30	0.00	0.045
38	Johnstone River	Qld	JOR	48	5.1	0.570	0.533	3.36	0.03	−0.059
39	Hinchinbrook	Qld	HC	50	5.6	0.565	0.555	3.43	0.06	−0.008
40	Cleveland Bay	Qld	CLE	23	4.6	0.584	0.569	3.46	0.02	−0.005
41	Bowling Green Bay[A] (1988)[B]	Qld	BOWK	24	4.4	0.534	0.540	3.31	0.02	0.033
42	Bowling Green Bay (2008)[B]	Qld	BOW	24	4.5	0.555	0.535	3.24	0.02	−0.016
43	Burdekin River[A] (1989–90)[B]	Qld	BURK	24	4.5	0.583	0.547	3.34	0.00	−0.045
44	Burdekin River (2008)[B]	Qld	BUR	24	4.8	0.581	0.551	3.50	0.01	−0.034
45	Broad Sound[A] (1988–93)	Qld	BRSK	15	3.6	0.532	0.519	3.27	0.01	0.010
46	Fitzroy River[A] (1988–90)[B]	Qld	FTZK	44	5.1	0.530	0.519	3.15	0.03	−0.010
47	Fitzroy River (2013)[B]	Qld	FTZ	48	4.3	0.526	0.511	3.03	0.01	−0.019
48	Port Alma	Qld	PAF	24	3.7	0.480	0.453	2.85	0.05	−0.036
49	Mary River[A] (1989–90)	Qld	MARK	18	4.4	0.552	0.539	3.39	0.02	0.004
	Eastern stock			405	4.5	0.548	0.532	3.27	0.02	−0.009
	All sample collections			1297	4.6	0.528	0.519	3.39	0.03	0.004

[A]Historical samples from Shaklee *et al.* (1993) and Keenan (1994); all other samples are contemporary and were collected between 2006 and 2013.
[B]Locations tested for temporal differences.

Archer River, Bowling Green Bay and Burdekin and Fitzroy rivers in Queensland. Replicate temporal samples were collected less than 10 km from the original collection locality.

This study received animal ethics approval from Flinders University Adelaide (project number E345).

DNA extraction and microsatellite genotyping

DNA extraction was attempted from all 2312 samples using a modified cetyltrimethylammonium bromide (CTAB) chloroform : isoamyl (24 : 1) extraction buffer (Adamkewicz and Harasewych 1996); however, due to the age and poor preservation of many of the older muscle samples, only 1297 samples passed quality control criteria for subsequent microsatellite genotyping. Extracted DNA from the samples were amplified at 17 microsatellites using two multiplex polymerase chain reactions (PCRs; Loughnan *et al.* 2013), followed by genotyping on an Applied Biosystems (Foster City, CA, USA) DNA Analysis System 3730 at Georgia Genomics Facility (Athens, GA, USA) incorporating LIZ 500 (ThermoFisher Scientific, Waltham, MA, USA) as the size standard. Fragment analysis was performed using GENEMAPPER software (ver. 4.1, Applied Biosystems). From the 17 loci genotyped, *Lca287* was difficult to score, deviated significantly from Hardy–Weinberg Equilibrium (HWE) and null alleles were identified. As a result, this microsatellite marker was removed from subsequent statistical analyses, reducing the number of loci for analyses to 16 (for the full genotypes, see Table S2, available as Supplementary material to this paper).

Data analysis

Genetic diversity statistics

MICRO-CHECKER software (ver. 2.2.3, see https://www.microchecker.hull.ac.uk/, accessed 20 November 2016; Van Oosterhout *et al.* 2004) for identifying and correcting genotyping errors in microsatellite data was used to scan for scoring errors and the presence of null alleles. The average number of alleles (A), expected (H_e) and observed (H_o) heterozygosities and tests for HWE were calculated in GENEPOP (ver. 4.5.1, see https://kimura.univ-montp2.fr/~rousset/Genepop.htm, accessed 20 November 2016; Rousset 2008), and their significance determined after correction for the false discovery rate (FDR; Benjamini and Hochberg 1995). Exact P-values under the Markov chain method were determined with a dememorisation step of 10 000 iterations, followed by 20 batches of 5000 iterations per batch. The inbreeding coefficient (F_{IS}) was also calculated in GENEPOP and significance in heterozygote excess or deficiency ($P < 0.05$) was calculated using the method of Weir and Cockerham (1984), following FDR correction to 5%. Allelic richness (A_r) and private allelic richness (PA_r) were calculated in HP-RARE (ver. June-6-2006, see https://montana.edu/kalinowski/software/hp-rare.html, accessed 15 November 2016; Kalinowski 2005), incorporating a rarefaction approach for a minimum of 14 genes per sample.

Genetic differentiation

The Bayesian method of individual clustering applied in STRUCTURE (ver. 2.3.4, see https://web.stanford.edu/group/pritchardlab/structure_software/release_versions/v2.3.4/html/structure.html, accessed 8 December 2017; Pritchard *et al.* 2000) was used to identify population genetic structure. STRUCTURE assigns the most probable individuals to k groups per threshold q-values and enables the visualisation of the grouping of individuals into genetic clusters. Enabling sample location as a prior reference ('locprior') is designed to detect weak population structure, and this was compared to the 'no locprior' model. Admixture and correlated allele frequencies were also considered in both models. Ten replicate runs at each k (1–49) were performed (Gilbert *et al.* 2012) using a burn-in length of 100 000 iterations and one million Markov chain Monte Carlo (MCMC) repetitions. STRUCTURE HARVESTER (ver. 0.6.94, see http://taylor0.biology.ucla.edu/structureHarvester/, accessed 8 December 2017; Earl and vanHoldt 2012) was used to detect the number of genetic groups (Δk) that best represented the data (Evanno *et al.* 2005). CLUMMP (ver. 1.1.2, see https://rosenberglab.stanford.edu/clumppDownload.html, accessed 8 December 2017; Jakobsson and Rosenberg 2007) was used to average the admixture proportions for the best k of each individual over the 10 replicates and bar plots were graphed in DISTRUCT (ver. 1.1, see https://rosenberglab.stanford.edu/distruct.html, accessed 8 December 2017; Rosenberg 2004).

Next, a model-free method was applied to the analysis of population genetic structure. Discriminant analysis of principal components (DAPC) is a multivariate method to identify clusters of genetically related individuals. Using the R package *adegenet* (Jombart 2008; Jombart and Ahmed 2011; see https://cran.r-project.org/bin/windows/base/, accessed 29 April 2018), groups were organised by sampling location and the optimal number of principal components (PCs) was retained following a-score calculations.

Genetic differentiation among datasets was estimated as pairwise F_{ST} with GENODIVE (ver. 2.0b27, see http://www.bentleydrummer.nl/software/software/GenoDive.html, accessed 11 February 2019; Meirmans and Van Tienderen 2004) and the significance of associated P-values was determined by FDR correction (Weir and Cockerham 1984). To test for patterns of population structure influenced by isolation by distance (IBD; Meirmans 2012), GENODIVE was also used to conduct stratified Mantel tests, investigating the relationship between population genetic distances ($F_{ST} \div (1 - F_{ST})$) and geographical distances. Three regions were classified as separate strata (i.e. western, central and eastern), with 10 000 permutations. Pairwise F_{ST} and Fisher's exact tests (calculated in GENEPOP) of genetic differentiation were used to test for temporal stability at the six common sites sampled between historical and contemporary collection periods.

To assess putative hierarchical population differentiation, an analysis of molecular variance (AMOVA) was conducted with ARLEQUIN (ver. 3.5.2.2, see http://cmpg.unibe.ch/software/arlequin35/Arl35Downloads.html, accessed 20 November 2016; Excoffier *et al.* 2005), including 1000 permutations. First, AMOVA was run with sample sites grouped into historical biogeographic regions according to Unmack (2001): Kimberley Province, Northern Province and Eastern Province. The sample collection from the Pilbara was included in the Kimberley Province. Second, sampled sites were grouped according to three topographic drainage divisions outlined by the Australian

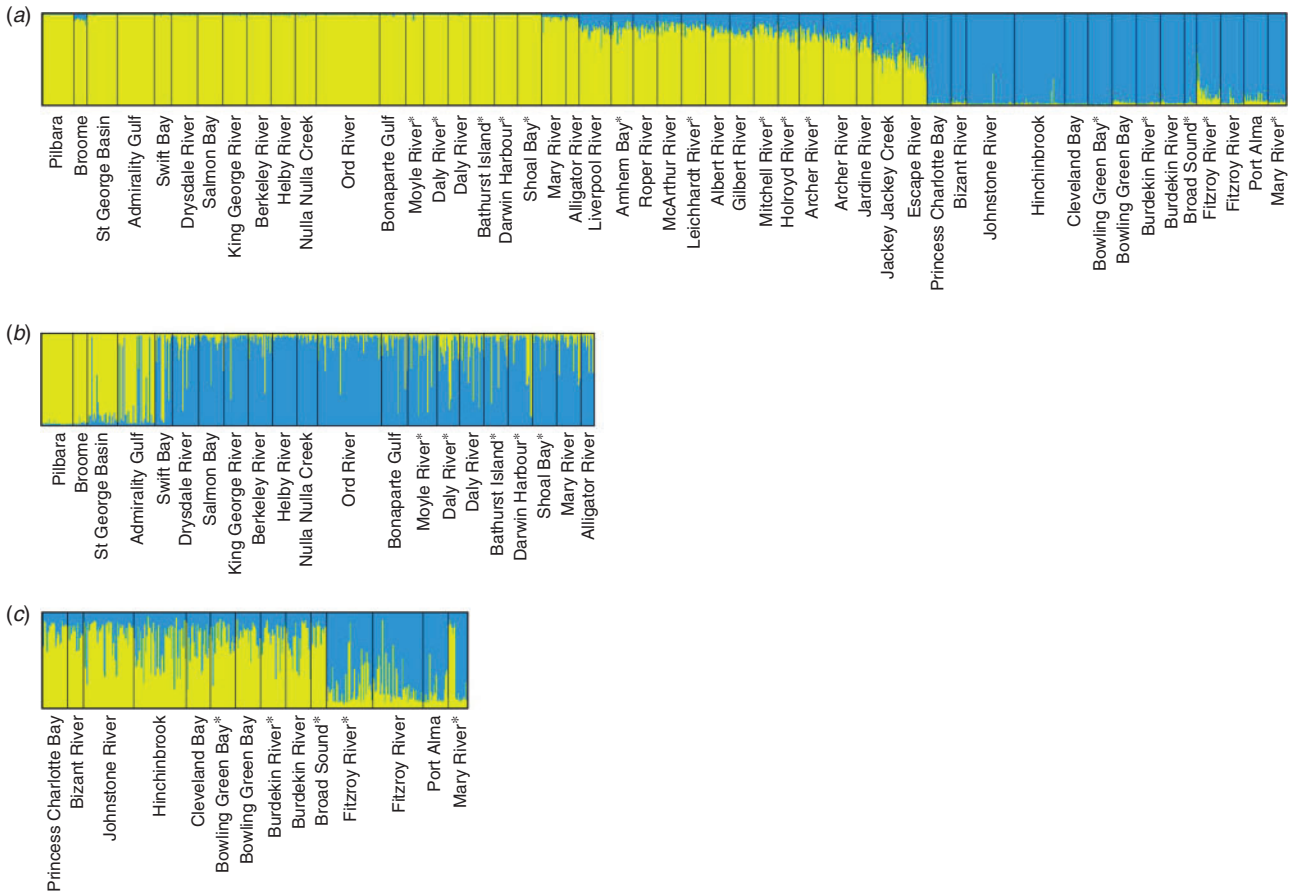

Fig. 2. STRUCTURE bar plots based on 16 microsatellite loci for *Lates calcarifer*. Horizontal lines represent individuals and black lines separate each sample collection. (*a*) Including 49 sample collections for 1297 individuals, $k = 2$. (*b*) The western stock includes 21 sample collections ($k = 2$) and (*c*) the Eastern stock includes 14 sample collections ($k = 2$). Sample collections ending with an asterisk denote historical samples from Shaklee *et al.* (1993) and Keenan (1994).

Hydrological Geospatial Fabric (Geofabric; http://bom.gov.au/water/geofabric/documents/BOM002_Map_Poster_A3_Web.pdf, accessed 3 May 2018): Tanami–Timor Sea Coast, Carpentaria Coast and North East Coast. The Pilbara sample collection was included in the Tanami–Timor Sea Coast drainage division.

Results

Measures of genetic diversity and HWE

According to MICRO-CHECKER, null alleles were detected in six sample collections for six loci: Berkeley River for locus *Lca070*, Daly River (2008) for loci *Lca020* and *Lca021*, Bathurst Island for locus *Lca178*, Darwin Harbour for locus *Lca058*, McArthur River for locus *Lca371* and the Escape River for locus *Lca058*. However, there was only one sample collection that deviated from HWE ($P < 0.05$) following FDR correction, namely that of the Ord River for locus *Lca058*. Across all datasets, mean A_r and PA_r were 3.39 and 0.03 respectively (Table 1) and the highest level of A_r was recorded for the Archer River (3.82) and was from a dataset previously collected by Shaklee *et al.* (1993). The St George Basin had the highest level of PA_r (0.09). Mean F_{IS} was positive (0.004) and was not significantly different from 0 for any of the 49 localities.

Discrimination of population structure

Bayesian STRUCTURE analysis revealed the presence of two distinct genetic populations ($k = 2$; referred to herein as stocks) and a central region of admixture for barramundi in Australia (Fig. 2*a*). The western stock was defined as comprising barramundi sampled from localities from the Pilbara in Western Australia, across northern Australia through to the Alligator River in the Northern Territory (q-membership >93% to the western stock), whereas the eastern stock was located from Princess Charlotte Bay down the east coast to the Mary River in Queensland (q-membership >85% to the eastern stock). Between the two stocks, a central region of admixture spanned the Liverpool River in the Northern Territory (q-membership 82% to the western stock and 18% to the eastern stock), through the Gulf of Carpentaria to the Escape River in Queensland (q-membership 47% to the western stock and 53% to the eastern stock), whereby barramundi were observed to possess varying levels of genome ancestry to both east and west genetic stocks. Mean A_r and PA_r values were 3.36 and 0.03 respectively for the western stock, 3.56 and 0.05 respectively for the central region of admixture and 3.27 and 0.02 respectively for the eastern stock (Table 1). Upon further discrimination within the western

(Fig. 2*b*) and eastern (Fig. 2*c*) stocks, two further genetic clusters were estimated within each stock ($k = 2$). The sample sites from the Pilbara to Admiralty Gulf were not in panmixia with other western sites (q-membership >75% to Cluster 1). Within the eastern stock, Fitzroy River and Port Alma were not in panmixia with other eastern sample sites (q-membership >76% to Cluster 2), whereas the Mary River demonstrated evidence of admixture between these two clusters (40 and 60% q-membership).

Similarly, DAPC analysis suggested a major grouping of the barramundi sampled into western and eastern stocks with a central region of admixture (Fig. 3). DAPC suggested subdifferentiation of the Pilbara and Broome sample sites from others in Western Australia, and of the St George Basin and Admiralty Gulf from others in eastern Australia. According to a-score calculations, the optimal number of retained PCs was 62.

Global levels of population differentiation were moderate ($F_{ST} = 0.103$, $P < 0.001$; Fig. 4; for pairwise F_{ST} values, see Table S3, available as Supplementary material to this paper), and significant hierarchical genetic structure in Australian barramundi was detected by AMOVA (Table 2). High but similar estimated F_{ST} values were calculated among historical biogeographic provinces ($F_{ST} = 0.134$) and among hydrological boundaries ($F_{ST} = 0.132$). These results, coupled with the existence of population structure within groups, indicates that both historical biogeographic history and currently recognised hydrological provinces have been important in generating and delineating population structure in Australian barramundi.

Stratified Mantel tests revealed a pattern of IBD for Australian barramundi that was supported by a significant correlation between population genetic distances and geographic distances (Mantel $R = 0.756$, $r^2 = 0.571$, $P < 0.001$), and ~57% of the variation observed could be attributable to IBD. The results are consistent with a pattern of two stocks with IBD in a hierarchically structured system.

Six sample locations were investigated for temporal stability over the periods from 1988 to 2013 (Table 3). Neither pairwise F_{ST} nor Fisher's exact tests returned a statistically significant result for any comparison performed, with F_{ST} values ranging from −0.001 to 0.003. This indicates that barramundi genetic population structure has remained temporally stable at the six sites tested for a period of between 18 and 25 years.

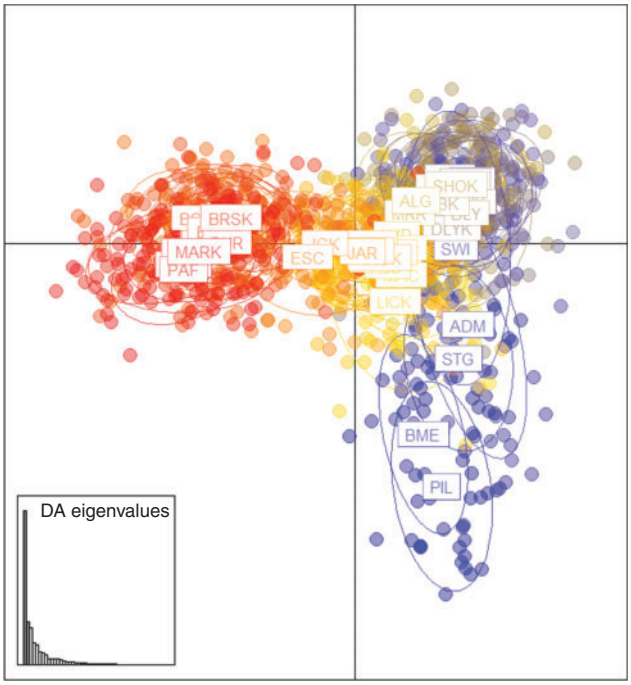

Fig. 3. Discriminant analysis of principal components (DAPC) for 49 *Lates calcarifer* sample collections (for collection code descriptions, see Table 1). Individuals are represented by dots and sample groups are within inertia ellipses. DAPC analysis suggested major grouping of the barramundi sampled into western (right quadrants) and eastern (left quadrants) stocks, with a central region of admixture. Eigenvalues display the amount of genetic information contained in the first two principal components, representing the x- and y-axes respectively. DA, discriminant analysis.

Discussion

Defining the population genetic structure of wild fish stocks can help identify distinct populations or management units for the effective management of fisheries, translocation and restocking programs, and the present study provides valuable information for the delineation of stock boundaries for Australian barramundi. Current Australian fishery regulations are based on two separate management units in Western Australia and the Northern Territory, and seven discrete populations in Queensland (Saunders *et al.* 2016).

Large captive populations of *L. calcarifer* currently exist in a growing Australian aquaculture industry (Loughnan *et al.* 2016) and understanding the genetic stock structure of wild fish populations can be informative for captive breeding programs. The results from this study can be used to highlight regions for broodstock collection that will contribute high levels of genetic diversity to a founding captive population and help minimise inbreeding throughout the life of the program. Local adaptive genetic differences, such as thermal tolerance (Edmunds *et al.* 2010; Newton *et al.* 2010), can also benefit a breeding program, and information about the genetic stock structure and signatures of selection occurring in natural populations can help identify natural sources of variation. Many farmed fish species under production have benefitted from the establishment of a foundation population sourced across a broad natural distribution (Gjedrem *et al.* 1991; Eknath *et al.* 1993); however, few captive breeding populations have been established using detailed knowledge about wild fish stock structure and genetic diversity (for a review, see Attard *et al.* 2016).

Overall population genetic differentiation

Our results confirm that barramundi populations across tropical Australia are genetically structured, with the overall level of genetic divergence reported here ($F_{ST} = 0.103$) higher than earlier allozyme studies, such as those of Keenan (1994; $F_{ST} = 0.064$), Shaklee and Salini (1985; $F_{ST} = 0.028$), Salini and Shaklee (1988; $F_{ST} = 0.087$) and Shaklee *et al.* (1993; $F_{ST} = 0.031$). Compared with Keenan (1994), the present study significantly expanded the sampling region of the distribution of naturally occurring barramundi in Australia further south-west to the Pilbara region and is the first to cover the entire natural

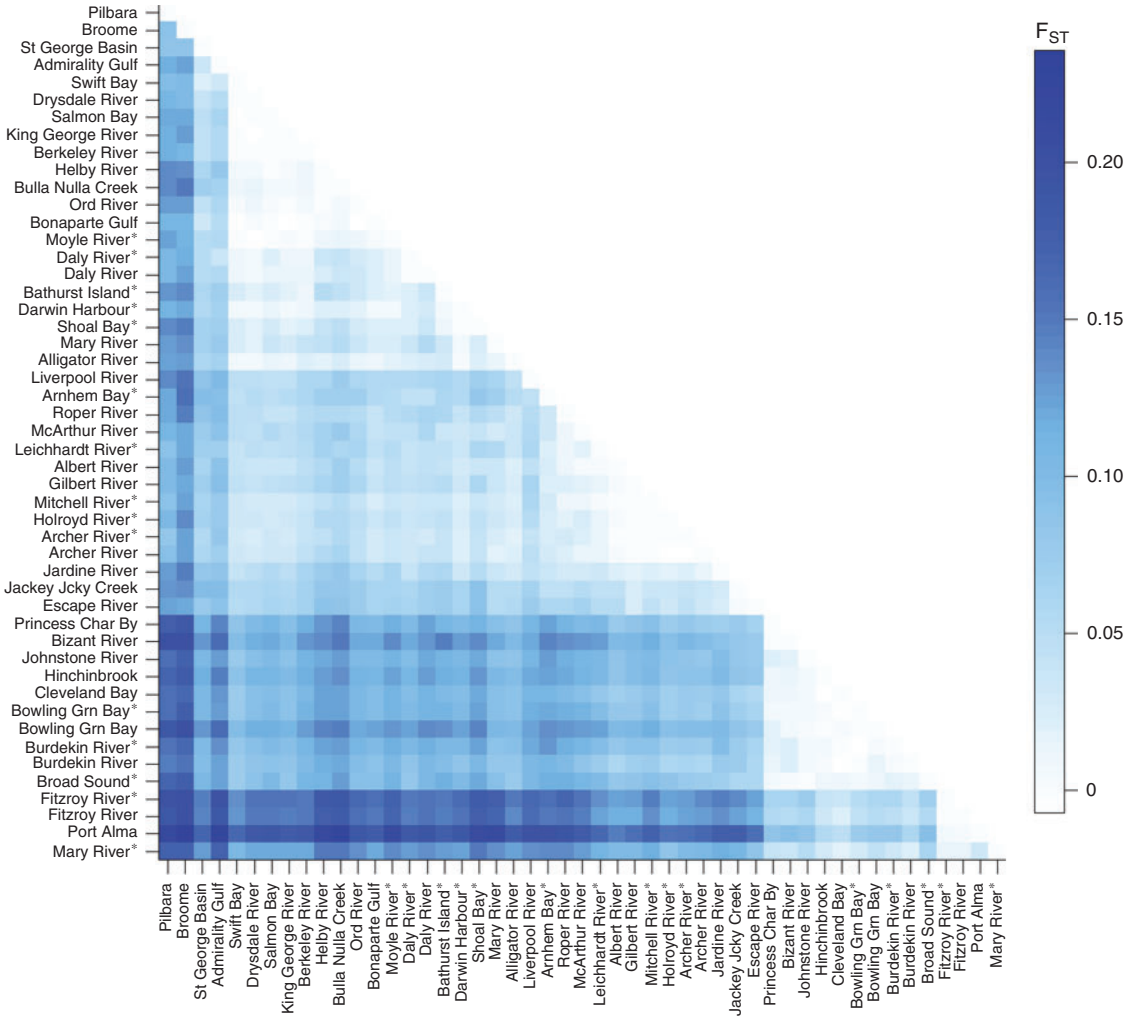

Fig. 4. Heat map of population differentiation measured as pairwise fixation index (F_{ST}) values for 49 collections of *Lates calcarifer* genotyped with 16 microsatellite loci. Sample collections ending with an asterisk denote historical samples from Shaklee *et al.* (1993) and Keenan (1994).

Table 2. Analysis of molecular variance tests based on fixation index calculations showing analysis for three groups based on either biogeographic provinces as outlined in Unmack (2001) or hydrological boundaries

F_{CT}, variance among groups relative to total variance; F_{SC}, variance among subpopulations within groups; F_{ST}, variance among subpopulations relative to total variance

Source of variation	Percentage variation	*F*-statistic	*P*-value
Analysis based on biogeographic provinces			
Among groups	8.58	$F_{ST} = 0.134$	<0.001
Among populations within groups	4.82	$F_{SC} = 0.053$	<0.001
Within populations	86.59	$F_{CT} = 0.086$	<0.001
Analysis based on hydrological boundaries			
Among groups	8.90	$F_{ST} = 0.132$	<0.001
Among populations within groups	4.30	$F_{SC} = 0.047$	<0.001
Within populations	86.80	$F_{CT} = 0.089$	<0.001

distribution of the species in one study. The difference in global F_{ST} reported here may result from the use of a different type of genetic marker (microsatellites *v.* allozymes), but may also reflect the inclusion of genetically divergent populations from areas to the west and south-west. In addition, mtDNA markers have detected differing levels of genetic structuring between

Table 3. Pairwise F_{ST} and Fisher's exact tests obtained from six temporally replicated sample collections
Note, historical samples taken from the Leichardt River were compared with the modern collection from the Albert River due to the proximity of the river mouths (<10 km apart). F_{ST}, fixation index

Dataset	Sampling periods	Pairwise F_{ST}		Fisher's exact test	
		F_{ST}	*P*-value	χ^2	*P*-value
Daly River	1990 and 2008	−0.002	0.633	26.062	0.672
Leichardt and Albert rivers	1990–91 and 2011	0.003	0.290	33.384	0.400
Archer River	1993 and 2011	−0.005	0.881	19.663	0.957
Bowling Green Bay	1988 and 2008	−0.001	0.533	37.719	0.224
Burdekin River	1989–90 and 2008	0.001	0.379	39.014	0.184
Fitzroy River	1988–90 and 2013	−0.003	0.848	23.595	0.859

barramundi populations; Chenoweth *et al.* (1998*a*, 1998*b*) and Doupé *et al.* (1999) demonstrated strong levels of genetic structuring with Φ_{ST} values of 0.328 and 0.563 respectively, although a smaller effective population size (N_e) of mtDNA combined with high levels of divergence can cause a disparity of Φ_{ST} and F_{ST} (Beheregaray *et al.* 2003). To date, the only other population study conducted on wild Australian barramundi using microsatellites incorporated five loci across six geographic sites from the western half of the Australian range (Marshall 2005).

Previous studies on the genetic structure of wild populations from south-east Asia (Yue *et al.* 2009) indicated higher levels of genetic diversity compared with Australian barramundi populations. However, this could be due to comparing farmed stocks from Australia with wild south-east Asian stocks, or that south-east Asia is the centre of the natural range and perhaps the origin of barramundi (Keenan 1994, 2000; Yue *et al.* 2009).

Larger-scale patterns of genetic stock structure suggested a strong influence of several historical biogeographical barriers to gene flow across the species distribution. The dominant structure detected in this study was that of an eastern and western stock, joined by a central admixed region (Fig. 2, 3). The presence of an east–west division in barramundi stocks was first suggested in allozyme and mtDNA studies (Salini and Shaklee 1988; Keenan 1994, 2000; Chenoweth *et al.* 1998*a*, 1998*b*), although this was later challenged by mtDNA investigations (Doupé *et al.* 1999; Marshall 2005). Long-term historical separation of eastern and western barramundi populations caused by the formation of an extensive land bridge between Australia and Papua New Guinea at times of lower sea levels is the likely driver behind this east–west split (Keenan 1994; Chenoweth *et al.* 1998*b*). Support for the effect of this historic land bridge on local marine species in the region has also been shown in sea turtles (Dethmers *et al.* 2006), sharks (Duncan *et al.* 2006) and reef fishes (Mirams *et al.* 2011). The slightly higher levels of genetic diversity detected in the central admixed region would be expected if the total species distribution consisted of two large stocks with gene flow between them.

In Queensland, the historical differences in the shape of the coastline, the direction of riverine discharge (Keenan 1994; Chenoweth *et al.* 1998*b*) and a strong historical gradient in environmental (climatic) conditions along the east coast (Unmack 2001) may have all contributed to past reductions in gene flow.

Temporal stability

Previous studies have shown temporal stability of allozyme allele frequencies at time scales ranging from several months (Shaklee and Salini 1985) up to 7 years (Keenan 1994), and there are currently no published reports of temporal stability of more rapidly evolving microsatellite loci for barramundi over the time scales sampled in the present study. No temporal shifts in allele frequency were found for any of the six locations sampled between 18 and 25 years ago from the Northern Territory, Queensland gulf and the central coast region of Queensland. Australian barramundi are abundant and cover a wide natural distribution range. As a result, temporal stability with the current dataset would be expected unless translocation, restocking and accidental aquaculture releases have substantially affected the genetics of wild populations in recent decades.

Geographical patterns of genetic structure

We detected a pattern of IBD for barramundi across the full species distribution in Australia such that spatially limited gene flow results in an increase in genetic divergence as geographic distance increases between sampling sites. This was also identified in earlier studies using allozymes (Keenan 1994) and mtDNA markers (Chenoweth *et al.* 1998*a*; Marshall 2005). Given the linear and near continuous distribution of barramundi along the northern Australian coastline, the detection of such a pattern was largely to be expected. The demonstration of IBD within the two stocks indicates inhibition of panmixia; however, within each stock there are no distinct genetic populations.

Conclusion

This study on the genetic population structure of Australian barramundi has shown clear delineation of two genetic stocks in Australia. The stock structure detected has been stable over the last quarter of a century, but short-term perturbations in genetic stock structure due to heavy restocking and widespread flooding could occur in the future. This study provides valuable information for translocation and fishery stock management, as well as identification of possible genetic sources for future captive barramundi genetic improvement programs.

Conflicts of interest

The authors declare that they have no conflicts of interest.

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