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Stock structure of *Lethrinus laticaudis* (Lethrinidae) across northern Australia determined using genetics, otolith microchemistry and parasite assemblage composition

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Abstract. The grass emperor *Lethrinus laticaudis* is a conspicuous element of the commercial and recreational catch from nearshore reef systems across northern Australia. The nearshore reef systems across northern Australia are exposed to increasing levels of fishing pressure from commercial and recreational fishers. To inform ongoing management of this species, the present study examined the stock structure of *L. laticaudis* across northern Australia using a combination of complementary techniques. In all, 342 *L. laticaudis* samples were collected from 13 locations in the coastal waters of northern Australia ranging from the Pilbara region of Western Australia to Moreton Bay in south-east Queensland. Population genetic analyses using microsatellite markers demonstrated that there were at least four genetically distinct populations across northern Australia with gene flow between management jurisdictions (with significantly more separation between Western Australian and Northern Territory locations than between Northern Territory and Queensland locations). An isolation by distance effect was evident (genetic differences increasing linearly with distance). Otolith microchemistry and parasitology analyses indicated some spatial structuring of populations within broader regions. These findings of restricted connectivity at small spatial scales suggest that *L. laticaudis* is vulnerable to localised depletion in areas where fishing effort is concentrated. This conclusion is consistent with recent observations of fishery declines in heavily fished locations.

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Introduction

Knowledge on the separation of populations into biological stocks, where there is limited interbreeding between groups over a given geographical scale (Stewardson *et al.* 2016), is important for sustainable fishery management. Fish caught in different areas may come from separate biological stocks or a single biological stock may be shared across different management jurisdictions (Stewardson *et al.* 2016). A powerful method for

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elucidating the structure of biological stocks is to use multidisciplinary approaches that provide complementary information on different aspects of the life history across a range of temporal and spatial scales (Begg and Waldman 1999; Buckworth *et al.* 2007; Welch *et al.* 2015; Izzo *et al.* 2017). Population genetic analyses that look at changes in allele frequencies among populations provide information over intergenerational time scales (Cowen and Sponaugle 2009), whereas other techniques, such as parasitology, otolith structure and microchemistry and demographic characteristics, provide information on intragenerational time scales (Begg and Waldman 1999; Welch *et al.* 2015). The integrated use of multiple techniques is becoming more common for the identification of biological fish stocks to support the spatial management of fisheries (Welch *et al.* 2015; Izzo *et al.* 2017).

Lethrinus laticaudis (common name 'grass emperor') occurs in tropical waters of the western Pacific and south-eastern Indian oceans throughout southern Indonesia, Papua New Guinea, Solomon Islands, New Caledonia and Australia (Carpenter 2001). In Australia, it occurs along the northern coastline from Shark Bay in Western Australia (WA) to Bass Point in south-east New South Wales (Ayvazian *et al.* 2004; Hoese *et al.* 2006). This distribution covers four major jurisdictional fishery management areas (based on the states and territories of Australia) and at least six provincial bioregions (North-west, North-west Transition, Northern, North-east Transition, North-east, Central Eastern Transition) as determined by the Integrated Marine and Coastal Regionalisation of Australia (Pope *et al.* 2015, fig. 2). The species is a high-quality food fish and is exploited by commercial fishers and recreational anglers (Ayvazian *et al.* 2004; Coleman 2004; Knuckey *et al.* 2005; Grubert *et al.* 2010), indicating that the species could be vulnerable to overfishing. Commercial catch data from Queensland (Qld) (the only jurisdiction that has kept catch records over a period of time) show that overall catches of *L. laticaudis* increased from ~3 tonnes in 2000 to 26 tonnes in 2010 before reducing to 15 tonnes in 2015 (Queensland Government Department of Agriculture and Fisheries; https://www.daf.qld.gov.au/fisheries, accessed 4 September 2017).

The stock structure of *L. laticaudis* within Australian waters remains unknown and there is limited information upon which to base assessments of the sustainability of potentially exploited populations. A study by Ayvazian *et al.* (2004) on populations in an embayment on the mid-west coast of Australia found that *L. laticaudis* grows to nearly 600-mm total length (TL), with age at 50% maturity of 2–3 years and a maximum estimated age of 16 years. Mature *L. laticaudis* occur most commonly in depths of 5–35 m over coral reef habitats, with juveniles thought to use inshore seagrass meadows (Carpenter 2001; Travers *et al.* 2010). Based on otolith stable isotope ratio analysis, *L. laticaudis* adults are inferred to exhibit site fidelity to reef complexes unless patches of suitable habitat are close enough to allow movement between sites (Ayvazian *et al.* 2004), with 82% of tagged fish along the Qld coast recaptured within 2 km of their release site, even after 2 years (Sumpton *et al.* 2008).

The aim of the present study was to elucidate the population structure of *L. laticaudis* across northern Australia using a combination of genetics, otolith microchemistry and parasite assemblage composition. We used microsatellite markers to investigate the genetic population structure of *L. laticaudis* and to identify potential barriers to gene flow across generations. Variability in otolith multi-elemental microchemistry and parasite assemblages of *L. laticaudis* was also used to examine population structure at the temporal scale of individual life histories. The results of these analyses have the potential to support future spatial management of the species.

Materials and methods

This project was conducted under Charles Darwin University Animal Ethics Approval A13014.

Sampling

In all, 342 *L. laticaudis* (333 mm TL, range 175–547 mm; mean age 5.1 years, range 2–14 years) were collected from 13 locations across northern Australia from Locker Point in the Pilbara region of WA to Moreton Bay in south-east Qld between January 2013 and August 2015 (Fig. 1; Table 1).

L. laticaudis were collected by line fishing and fish traps by research staff of the relevant state and territory fisheries departments, Indigenous marine rangers and various recreational and commercial fishers. Fish were killed in an ice slurry, placed on ice or frozen and transported to the laboratory for processing. Fish collected from the Sunshine Coast and Moreton Bay were provided as frames by recreational fishers as part of the Queensland Fisheries Long-term Monitoring Program (https://www.daf.qld.gov.au/fisheries, accessed 4 September 2017). TL (mm) and sex were recorded for all fish.

Muscle or fin clip samples were collected for genetic analysis and placed into vials containing molecular grade 95% ethanol or 20% dimethyl sulfoxide (DMSO) solution in 5 M NaCl and frozen. The pair of sagittal otoliths was dissected from the fish, cleaned and rinsed thoroughly, dried and stored in paper envelopes before trace element analyses. The gills, pharyngeal teeth plates and internal body organs were placed in labelled bags and frozen until examined for parasites.

Microsatellites

Genomic DNA from all samples for genotyping was extracted using an ISOLATE II Genomic DNA Kit (Bioline, Sydney, NSW, Australia) according to the manufacturer's instructions. All DNA extracts were quantified using the Qubit (ver. 3, ThermoFisher Scientific, Seventeen Mile Rocks, Qld, Australia) fluorometric method. Ten species-specific microsatellite loci (*Lel011*, *Lel040*, *Lel033*, *Lel012*, *Lel013*, *Lel032*, *Lel028*, *Lel027*, *Lel044*, *Lel039*) were genotyped across all the samples, as described in Taillebois *et al.* (2016).

The potential for null alleles, large allele dropout and stuttering to interfere with scoring accuracy was checked for each microsatellite locus in each sample using Microchecker, ver. 2.2.3 (Van Oosterhout *et al.* 2004). Summary statistics for microsatellite loci, including the number of alleles, allelic richness, expected and observed heterozygocity and fixation indices were obtained for each sampling location using GenAlEx, ver. 6.5 02 (Peakall and Smouse 2006). Tests of conformance of genotypic proportions to Hardy–Weinberg proportions (HWP) and tests of genotypic equilibrium between pairs of microsatellites (linkage disequilibrium) were performed

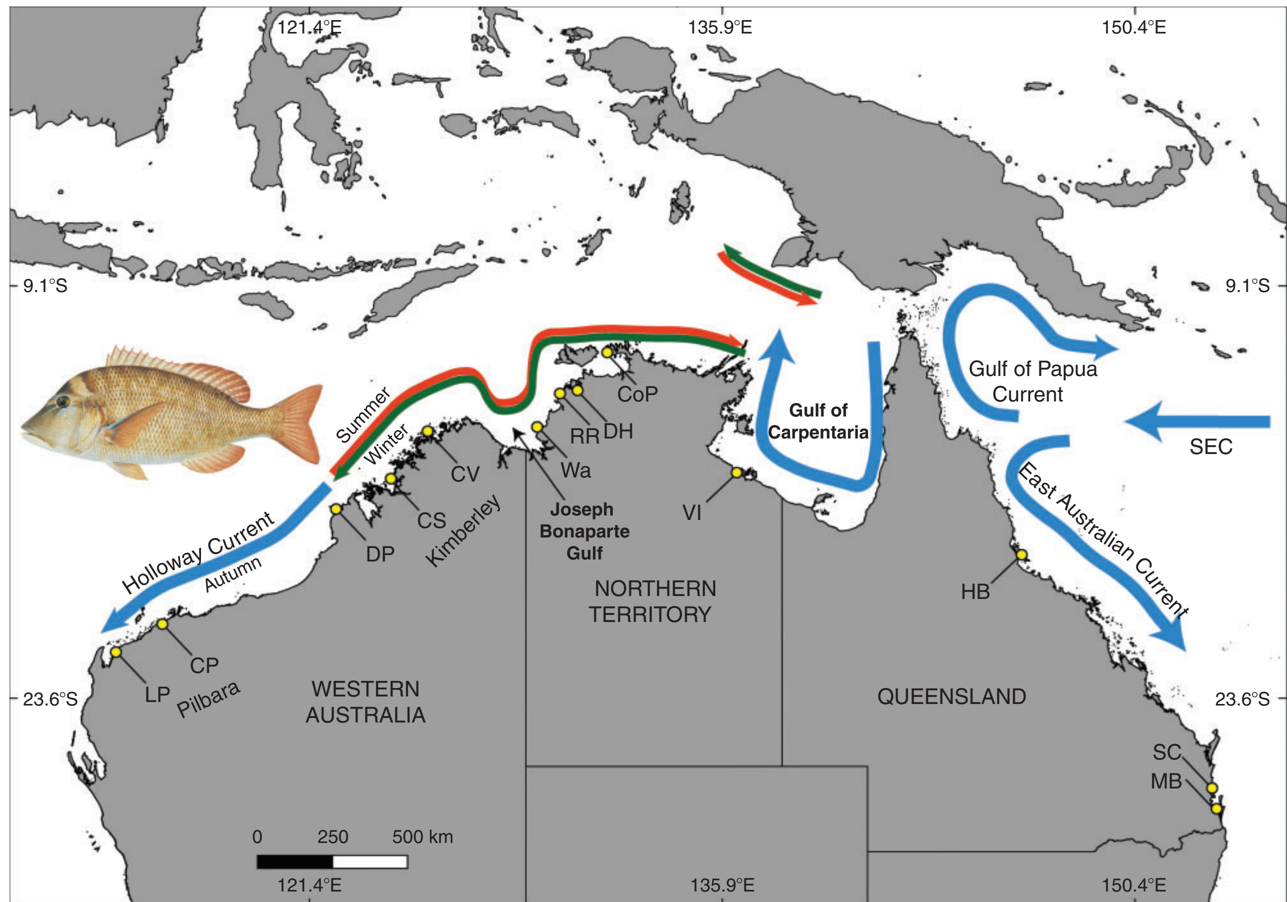

Fig. 1. Map of 13 sampling locations for *Lethrinus* laticaudis (yellow dots) across northern Australia, showing the three Government jurisdictions (Western Australia, Northern Territory and Queensland). The details for each collection locality are provided in Table 1. The dominant near-surface circulation patterns of northern Australia are illustrated (adapted from Sprintall *et al.* 2002; Domingues *et al.* 2007; D'Adamo *et al.* 2009; Schiller 2011). Illustration of *L. laticaudis*, reproduced with permission from www.anima.net.au (accessed 25 October 2017), credit R. Swainston.

for each sample location, using an exact probability test as implemented in Genepop, ver. 4.5 (Rousset 2008). The exact test was estimated using a Markov Chain that used 1000 dememorisations, 500 batches and 1000 iterations per batch. The fixation index (F_{ST}) between pairs of sample locations was estimated as implemented in Arlequin (ver. 3.5.2.2, Excoffier and Lischer 2010) to assess levels of genetic differentiation between sample locations.

In order to assess whether locations could be treated as independent genetic cohesive units and to test for the extent of admixture within the groups, two clustering methods were used. Population assignment and clustering was performed using the Bayesian model-based clustering program STRUCTURE, ver. 2.3.4 (J. K. Pritchard, W. Wen, and D. Falush, Pritchard Lab, Stanford University, CA, USA, see https://web.stanford.edu/group/pritchardlab/structure.html, accessed 1 August 2015). A dissimilar method, discriminant analysis of principal components (DAPC; Jombart *et al.* 2010), available in the *adegenet* package (Jombart 2008) for R (ver. 3.3.1, R Foundation for Statistical Computing, Vienna, Austria, see http://www.R-project.org/, accessed 4 August 2017) was also used. STRUCTURE attempts to cluster individuals based on minimising Hardy–Weinberg and gametic disequilibrium but does not test for types of genetic structure, such as isolation by distance or hierarchical population structure. The program estimates the proportion of individual genotypes that are derived from a user-defined number of clusters. The discriminant analysis of principal components does not make *a priori* assumptions on underlying population genetic models and may be more efficient than a model-based approach at identifying genetic clines and hierarchical structure (Jombart *et al.* 2010). Both methods make individual assignments and determine the posterior probability that an individual's genotype originates from its capture location. Posterior probabilities of the assignment of individuals to locations were performed through *dapc* in R and plotted using *assignplot* in R.

In STRUCTURE, varying numbers of possible clusters (K) within the dataset were tested from 2 to the number of sampling locations ($K = 2$–12; no genetic samples were available for Moreton Bay). The analysis was run 10 times for each K tested with 100 000 generations after 100 000 of burn-in. We performed the analysis using the no admixture model, correlated allele frequencies (Falush *et al.* 2003) and the sampling location as a prior to improve the ability to find clusters (Hubisz *et al.* 2009). The results were visualised in Pophelper (Francis 2017)

Table 1. Collection locations ($n = 13$) for *Lethrinus laticaudis* across northern Australia separated into state and territory government jurisdictions (see Fig. 1), with the subsample of fish (of the total number) used for each technique in the present study
WA, Western Australia; NT, Northern Territory; Qld, Queensland; TL, total length

Jurisdiction	Location	Code	Total number	Number of fish for genetic samples	Number of fish for otoliths	Number of fish for parasites	Month and year sampled	Mean (range) TL (cm)	Mean (range) age (years)
WA	Locker Point	LP	34	30	30	34	July 2014	351.7 (285–489)	5.8 (3–14)
	Cape Preston	CP	35	32	30	35	July 2014	373 (259–489)	5.8 (2–10)
	Dampier Peninsula	DP	28	24	28	28	Oct. 2013	338.9 (260–477)	4.4 (2–9)
	Camden Sound	CS	29	28	29	29	Sep.–Oct. 2013	306 (220–400)	3.6 (3–5)
	Cape Voltaire	CV	30	30	30	29	Aug. 2015	359.6 (264–427)	5.5 (3–8)
NT	Wadeye	Wa	30	27	30	30	June–July 2015	396.2 (290–440)	7.7 (3–11)
	Roche Reef	RR	29	26	25	29	Aug. 2013, June 2015	300 (250–395)	3.1 (2–4)
	Darwin Harbour	DH	24	24	24	24	July–Aug. 2015	209.6 (175–250)	2 (–)
	Coburg Peninsula	CoP	33	33	33	33	Aug. 2015	308.8 (230–370)	4 (3–8)
	Vanderlin Islands	VI	30	29	30	30	Nov. 2014	307.9 (245–433)	4.9 (2–7)
Qld	Halifax Bay	HB	14	12	14	14	May 2014	354.1 (239–405)	3 (–)
	Sunshine Coast	SC	14	9	14	14	Apr. 2013–Jan. 2014	440 (333–547)	7 (4–9)
	Moreton Bay	MB	12	0	12	12	Jan. 2013–Apr 2014	305.2 (268–356)	8.2 (4–13)
Overall			342	304	329	341		333.2 (175–547)	5.1 (2–14)

and the optimal number of clusters was estimated using the second-order rate of change between runs of different K (ΔK) as described by Evanno *et al.* (2005). However, given the large degree of uncertainty around the statistical estimation of K (Meirmans 2015), all the clustering patterns that warranted a biological interpretation were examined.

Evidence of genetic clusters was also examined in DAPC by running successive K-means clustering in the *find.clusters* function. We tested the same values of K as for STRUCTURE (i.e. $K = 2$–12), with 10 runs at each value of K. The optimal number of clusters was determined using the Bayesian information criterion (BIC; i.e. K with the lowest BIC value is ideally the optimal number of clusters). However, BIC values may continue to decrease after the true K value in the case of genetic clines and hierarchical structure (Jombart *et al.* 2010). Therefore, the rate of decrease in BIC values was examined visually to identify values of K after which BIC values decreased only subtly (Jombart *et al.* 2010). Once the number of genetic clusters was selected, the dataset was accordingly subdivided into locations or groups of spatially continuous locations (the number of groups equals the number of genetic clusters identified) by taking into account the STRUCTURE results. The *dapc* function was then executed using this grouping, retaining axes of principal components analysis (PCA) sufficient to explain at least 90% of the total variance of the data.

In order to be able to choose from the different patterns of genetic structure revealed by our different approaches, we assessed the percentage of genetic variance explained by the groupings from STRUCTURE and DAPC results using an analysis of molecular variance (AMOVA) as implemented in Arlequin. The significance of differentiation was determined by permutation of 22 000 replicates. The grouping revealed by the genetics was used to partition the data for the otolith and parasites analyses. To test whether *L. laticaudis* is constrained by a pattern of isolation by distance (IBD), we performed a Mantel test (Mantel 1967) of G ($F_{ST} \div (1 - F_{ST})$) *v.* seascape distances (D) among locations using Genodive, ver. 2.0b23 (Meirmans and Van Tienderen 2004). To distinguish between the effect of geographic distance and population structure on the observed IBD across the sampling range, we also performed a Mantel test within each of the genetic clusters identified with STRUCTURE.

Otolith microchemistry

The left sagittal otolith was selected from each individual and embedded in epoxy resin (West System 105 epoxy resin and West System 206 hardener; ATL Composites, Molendinar, Qld, Australia) with the sulcus facing downwards. A low-speed saw was used to cut ~350-µm transverse sections through the primordium of each otolith. Sections were polished using three grades of 3M diamond lapping film (30, 9 and 3 µm) (St Paul, MN, USA), mounted on glass microscope slides using resin, rinsed thoroughly with Milli-Q water and air dried.

Elemental concentrations were determined by laser ablation–inductively coupled plasma–mass spectrometry (LA-ICP-MS) using an Agilent 7700x (www.agilent.com, accessed 31 July 2017) quadrupole inductively coupled plasma mass spectrometer coupled to a custom-built RESOlution laser ablation system (https://service.asi-pl.com.au, accessed 31 July 2017) with a HelEx cell (Laurin Technic; www.agilent.com, accessed 31 July 2017). The RESOlution system is constructed around a Compex 110 ArF excimer laser (Lambda Physik; www.coherent.com, accessed 31 July 2017), which was operated using an ablation spot size of 72 µm in diameter with laser energy at 2.7 J cm^{-2} and a repetition rate of 5 Hz. Laser software (GeoStar, ver. 6.14; Resonetics, Nashua, NH, USA) was used to digitally plot three ablation locations on each otolith section: the primordium; the 'near core' located just inside the first opaque zone (representing the early juvenile life after settlement); and the ventral margin adjacent to the sulcus acousticus (referred to hereafter as the 'margin' and representing the most recent period of growth before collection).

Eleven trace elements (^{7}Li, ^{25}Mg, ^{23}Al, ^{49}Ti, ^{53}Cr, ^{55}Mn, ^{60}Ni, ^{63}Cu, ^{66}Zn, ^{88}Sr, ^{138}Ba) and the internal standard (^{43}Ca)

were analysed from the ablation zones in each otolith. The laser ablation spot sample consisted of a 20-s blank, followed by an ablation period of 50 s, of which the first 5 s and the last 1 s were excluded from data integration to allow for signal stabilisation. Data reduction and processing were completed using the trace elements data reduction scheme (Woodhead *et al.* 2005) of Iolite, ver. 3 (Paton *et al.* 2011). Subtraction of background ion counts from otolith counts was followed by the normalisation of each element to ^{43}Ca and the National Institute of Standards and Technology (NIST) 612 glass standard, which was used as the external calibration standard and was analysed after every 10 otolith samples to correct for any long-term drift in the instrument. The limits of detection (LOD) were calculated based on 3× s.d. of the blank background measurements. For all elements, the ratio of element isotope intensity to ^{43}Ca intensity was used to estimate the element : ^{43}Ca ratio (mmol mol^{-1} or μmol mol^{-1}).

Because data from otolith primordia are potentially affected by maternal effects that may influence their usefulness as natural tags (see Ruttenberg *et al.* 2005; Macdonald *et al.* 2008), we limited statistical analyses to data for the near core and margin. All multi-elemental otolith data were examined and subsequently $\log_{10}$ transformed to meet assumptions of normality and homogeneity of variance (Quinn and Keough 2002). A Pearson correlation was undertaken to test whether there was an effect of fish size (TL) with each element ratio, but no significant relationships were detected.

Parasites

After defrosting, gills were separated into individual arches, washed in water and examined. The stomach and intestinal tract were separated from the mesenteries and associated organs, slit along their length and washed for parasite examination. The supernatant of the washings was decanted and the sediment searched for parasites. The separated mesenteries were washed and examined; encysted parasites were removed from the mesenteries and released from their associated cysts before fixation. For female fish, ovaries were slit along their length and examined for parasites. Representative samples of each type of parasite from each location were placed into 70% ethanol. Parasites were identified as far as possible.

Summary statistics were compiled and included mean abundance (total number of individuals of a particular parasite per collection location divided by the total number of fish from that location examined, including uninfected fish) and prevalence (number of fish infected with a particular parasite divided by the number of fish examined, expressed as a percentage) for each of the parasite species, following the terminology of Bush *et al.* (1997). Only parasites with prevalence ≥10% in at least one of the locations were used in the analysis (Bush *et al.* 1990); additional parasites were removed from analyses if they could not be easily identified or accurately counted (MacKenzie and Abaunza 1998). Data were $\ln(x + 1)$ transformed to meet assumptions of normality and homogeneity of variance. Pearson correlation was used to explore the relationships between the TL of fish and abundance for individual parasite species within collection locations. For parasites that showed a significant correlation, abundances were adjusted to the mean host TL as described in Moore *et al.* (2003). No adjustment was made if the parasite abundance was zero.

Data analysis for otolith microchemistry and parasites

The otolith microchemistry and parasite datasets were divided into groups of collection locations based on groupings (hereafter 'regions') revealed by the genetic analyses and current jurisdictional management units for *L. laticaudis* (Table 1; Fig. 1). Spatial variation in otolith near core and margin microchemistry and parasite assemblages of *L. laticaudis* among regions and locations within regions were investigated using single-factor multivariate analysis of variance (MANOVA). Linear discriminant function analysis (LDFA) was conducted to examine variation in the multi-elemental signatures and parasite assemblages across all locations and among locations within each region. Classification success for the LDFA was calculated by jack-knife cross-validation matrices. All the above analyses were conducted using R (ver. 3.3.1, R Foundation for Statistical Computing). Classification success rates and an associated proportional chance criterion (the expected proportion of correct classification by chance alone; Poulin and Kamiya 2015) was calculated for comparison with calculated reclassification success.

To test the effect of distance between locations on *L. laticaudis* otolith elemental concentrations, a Mantel test of the mean differences in concentrations of each element between each pair of locations *v.* geographic distances along the coast among locations was performed using Matlab (ver. 2013a, The Mathworks Inc., MA, USA, see https://au.mathworks.com/products/matlab.html, accessed 28 October 2016), separately for near core and margin results. Similarity in parasite communities between locations was analysed using the Jaccard index, which was calculated as:

$$c \div (a + b - c)$$

where *a* and *b* are the species richness in two communities being compared and *c* is the number of parasite species they have in common (Poulin and Morand 1999). Jaccard indices were then compared against distance between locations by Pearson correlation (see Poulin and Morand 1999).

Results

Microsatellites

Genotypes from 10 microsatellite loci were obtained for 304 individuals of *L. laticaudis* from 12 locations (Table 1) with 2.56% missing data that was kept and identified as missing data in subsequent analyses (the percentage of missing data was calculated as the number of pairs of alleles that could not be scored over the total number of pairs of alleles present in the ideal dataset, 10 pairs of alleles × 304 fish). The number of alleles per locus ranged from 14 (*Lel013* and *Lel044*) to 24 (*Lel012, Lel033* and *Lel028*; see Table S1, available as Supplementary material to this paper). Locus *Lel012* was removed from the dataset due to the lack of heterozygotes at 6 (Locker Point, Camden Sound, Dampier Peninsula, Cape Voltaire, Roche Reef and Vanderlin Islands) of the 12 locations examined, which reduced the number of loci to 9 and the missing data

Table 2. Pairwise fixation index (F_{ST}) estimates based on 9 microsatellite data from 304 individuals of *Lethrinus laticaudis* between sampling locations

Lower diagonal, F_{ST} estimates; upper diagonal, P-values of the F_{ST} estimates. Comparisons that differed significantly from zero ($P < 0.05$) are bolded. Populations are abbreviated following Table 1

	LP	CP	DP	CS	CV	Wa	RR	DH	CoP	VI	HB	SC
LP		0.543	0.537	0.314	0.585	**0.001**	0.09	0.076	**0**	**0**	**0**	**0**
CP	0.001		0.897	0.235	0.22	**0.011**	0.296	0.069	0.21	**0**	**0.001**	**0**
DP	0	−0.004		0.44	0.173	0.058	0.66	0.126	0.188	**0.003**	**0.023**	**0.005**
CS	0.003	0.003	0.001		0.164	0.082	0.06	**0.02**	**0.022**	**0.001**	**0**	**0**
CV	0	0.003	0.004	0.004		**0.001**	**0.007**	0.065	**0.009**	**0**	**0.001**	**0**
Wa	**0.015**	**0.01**	0.007	0.006	**0.016**		0.84	0.428	0.077	0.099	**0.024**	**0.004**
RR	0.006	0.003	−0.002	0.007	**0.01**	−0.003		0.888	0.194	0.394	0.055	**0.045**
DH	0.006	0.006	0.005	**0.009**	0.006	0.001	−0.005		0.201	0.591	0.157	**0.005**
CoP	**0.015**	0.003	0.003	**0.009**	**0.009**	0.006	0.003	0.003		**0.009**	0.123	**0.001**
VI	**0.016**	**0.016**	**0.013**	**0.014**	**0.019**	0.005	0.001	−0.002	**0.009**		**0.011**	**0.005**
HB	**0.031**	**0.022**	**0.014**	**0.035**	**0.025**	**0.016**	0.011	0.005	0.007	**0.015**		**0.018**
SC	**0.049**	**0.033**	**0.024**	**0.037**	**0.044**	**0.027**	**0.014**	**0.022**	**0.031**	**0.021**	**0.021**	

to 1.57%. For locus *Lel012*, Microchecker confirmed that the deficit of heterozygotes was not due to scoring errors. A common explanation for a deficit of heterozygotes is the occurrence of null alleles. This was not explored further for this locus, although the rate of genotyping failures for this locus was high, which is consistent with the presence of null homozygotes. For the remaining loci, Microchecker indicated the possible occurrence of null alleles at Locker Point for loci *Lel040* and *Lel027*, at Cape Preston for locus *Lel039* and at Camden Sound for locus *Lel033* with no stuttering or scoring errors. Overall deviation from HWP (all with a deficit of heterozygotes) was low and no further data were removed. Only 1 of 45 tests for linkage disequilibrium between pairs of loci were significant (i.e. $P < 0.05$) at one collection location (*Lel011* × *Lel044* for population Camden Sound), which accounts for the experiment-wide error rate (here 0.90; $(1-(1-\alpha)^k)$ with $\alpha = 0.05$ and k the number of independent tests), which is the probability that at least one test will be significant by chance (Waples 2015). Heterozygosity was high for all locations (mean ± s.d.; 0.799 ± 0.074) and slightly above the expectation (~0.7 in marine species; DeWoody and Avise 2000; Table S1).

The overall F_{ST} value was low (0.008) and some locations comprised a panmictic population. However, other locations were significantly differentiated with significant pairwise F_{ST} ranging from 0.009 to 0.049 (Table 2). The pattern revealed that the Sunshine Coast was genetically distinct from all other locations in the study, whereas Vanderlin Islands and Halifax Bay were genetically distinct from the majority of other locations in the study. Other locations could be grouped into a Locker Point to Cape Voltaire group (no difference between any locations) and a Wadeye to Coburg Peninsula group (minor differences).

The Bayesian model-based approach STRUCTURE illustrated a pronounced genetic break between Cape Voltaire in the Kimberley and Wadeye in the Northern Territory (NT), supporting the F_{ST} results. Although the optimum number of clusters returned by ΔK was 7, the range of K tested (K = 2–12) in STRUCTURE consistently observed a clear separation between Cape Voltaire and Wadeye locations into a western and an eastern cluster (Figs 2*a*, S1). The two clusters were independently reanalysed in STRUCTURE, both with an optimum number of ΔK as 3. The western cluster did not show a pattern of genetic structure (Fig. 2*b*), whereas the two extreme locations of Halifax Bay and Sunshine Coast differed from all other locations within the eastern cluster, possibly suggesting a hierarchical clustering in this region (Fig. 2*b*). The groups revealed by STRUCTURE were consistent with the groups revealed by the F_{ST} after the iterative pooling.

The DAPC was run over the whole dataset using collection locations as priors and a plot of the probability of assignment of each individual to the different collection locations showing that individuals from one location have a high probability of being assigned to the location of collection (Fig. 3). A DAPC was also run for both the western and eastern clusters using the collection locations as priors. In the western group, DAPC revealed overlapping distributions of all locations on the ordination plot, indicating a low degree of genetic differentiation between all locations (Fig. 2*c*). In the eastern group, DAPC separated Halifax Bay and Sunshine Coast from all other locations along the first principal component axis (eigenvalue = 29.22). Along the second component axis (eigenvalue = 19.18), Halifax Bay was also plotted distant from Sunshine Coast (Fig. 2*c*).

An AMOVA was used to evaluate the scenarios supported by STRUCTURE and DAPC. The four-group scenario supported by STRUCTURE and DAPC explained 1.17% ($P = 0$) of the total genetic variation. To evaluate the spatial processes that drive population structure and to test whether there was an isolation by distance effect, we performed Mantel tests in Genodive. The Mantel test revealed that when considering all locations across the sampling range, geographic distance was significantly correlated with genetic differentiation ($r = 0.844$, $P = 0.001$). Within the western cluster only, geographic distance was not significantly correlated with genetic differentiation ($r = -0.120$, $P = 0.404$), but it was when considering samples from the eastern cluster only ($r = 0.787$, $P = 0.01$; Fig. 4).

Otolith microchemistry

In all, 329 otoliths were examined for elemental concentrations (Table 1). Of the 11 trace elements examined, concentrations of

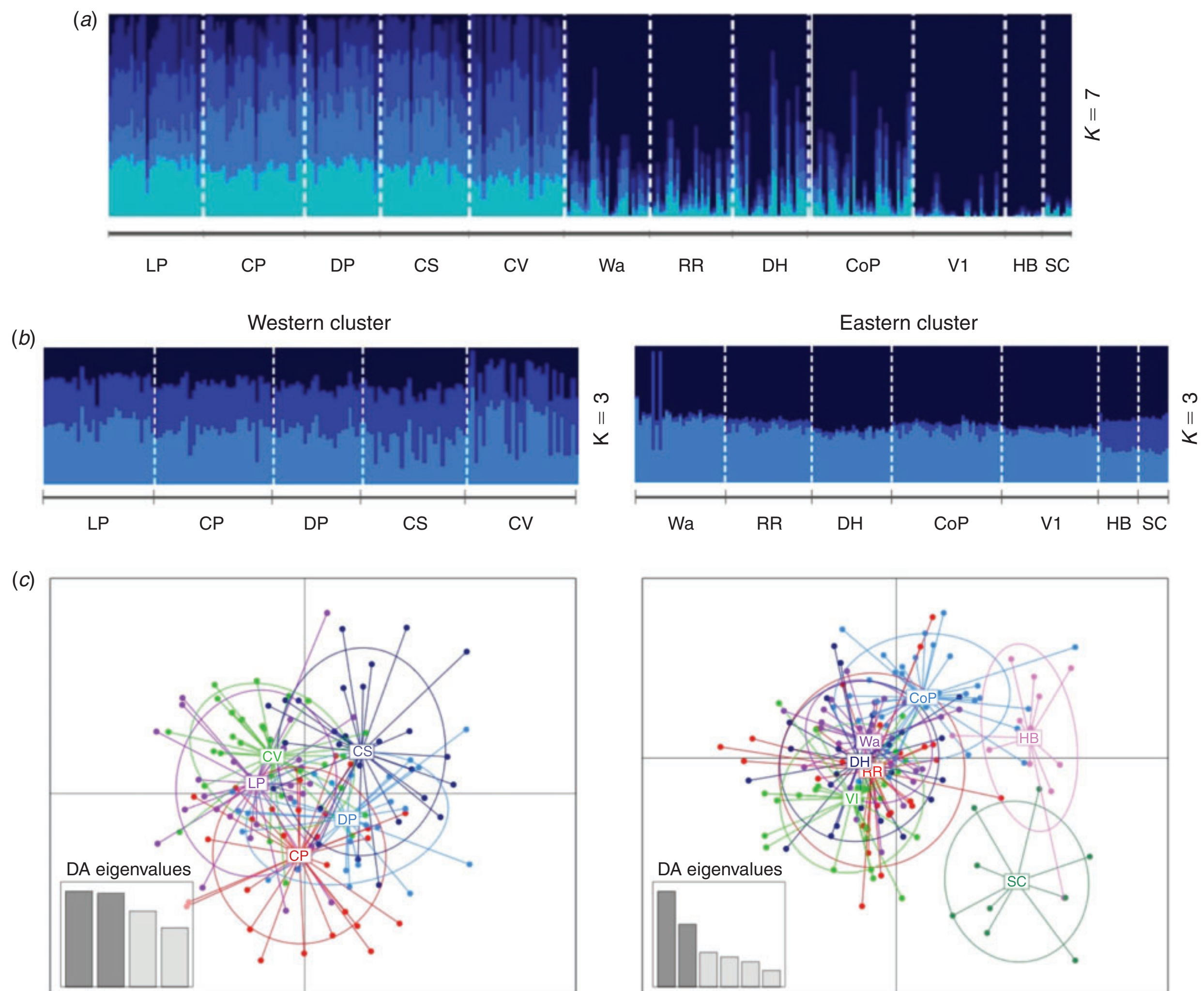

Fig. 2. Results from the Bayesian model-based clustering and discriminant analysis of principal component (DAPC) of microsatellite data from *Lethrinus laticaudis* using the software STRUCTURE and R. (*a*) Plot from STRUCTURE for $K = 7$ genetic clusters. Each vertical line represents an individual and the posterior probability proportions of its genotype assigned to the different genetic clusters. Individuals are plotted from west to east along the sampling gradient and populations are abbreviated following Table 1. Population information was used as a prior in the analysis. (*b*) Plots from STRUCTURE for each of the two geographic clusters: western and eastern; the optimal number of clusters returned by ΔK was $K = 3$ for both analyses. (*c*) Scatterplots of the DAPC for the western and eastern clusters using geographic collection location as priors for genetic clusters. Individual genotypes appear as dots and locations are depicted by colours and 95% inertia ellipses. Eigenvalues show the amount of genetic information contained in each successive principal component with the *x*- and *y*-axis constituting the first two principle components respectively.

6 elements were above the LOD at the near core (^{7}Li, ^{25}Mg, ^{63}Cu, ^{88}Sr, ^{138}Ba and ^{55}Mn) and 5 were above the LOD at the margin (^{7}Li, ^{25}Mg, ^{63}Cu, ^{88}Sr and ^{138}Ba). Results of the analysis comparing elemental signatures from the near core and margin indicated that the individual elemental ratios varied among the 13 locations (Table S2). Multi-elemental signatures differed significantly overall (i.e. across all locations) and among locations within the three regions (WA, NT and Qld) for both the near core and margin (MANOVA, all tests $P < 0.001$; Table S3).

Overall average classification success in the LDFA was 29% in the near core and 39% in the margin (Table 3). Classification success was greater when the data for each region were analysed separately with 41% (WA), 33% (NT) and 63% (Qld) based on near core microchemistry, and 48% (WA), 58% (NT) and 75% (Qld) based on margin microchemistry (Tables 3, S4; Fig. 5). The otolith margin elemental concentrations were not significantly correlated with geographic distance.

Parasites

In all, 341 *L. laticaudis* were examined for the parasitology component of the present study (Table 1). All 341 *L. laticaudis* examined were infected with at least one parasite individual. Mean abundance was 45.8 (range 1–274) parasite individuals per host, with the mean parasite species richness 5.1 (range 1–13). A total of 39 different parasites was identified. Of these, 15 were excluded from the analyses based on prevalence or due to issues with accurate taxonomic identification or counts. Removal of these parasites slightly reduced overall prevalence

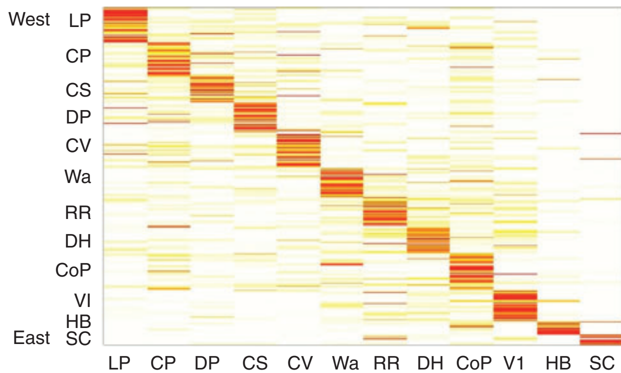

Fig. 3. Plot of the probability of assignment of each individual of *Lethrinus laticaudis* to the 12 locations calculated by discriminant analysis of principal component (DAPC). Populations are abbreviated following Table 1. Each individual is represented by a horizontal line and individuals are ordered according to their collection location (indicated on the *y*-axis) from west to east. The corresponding locations are indicated on the *y*-axis. Assigned locations are indicated on the *x*-axis from west to east. Heat colours represent membership probabilities: red = 1; white = 0. Collection locations were provided as a prior for DAPC.

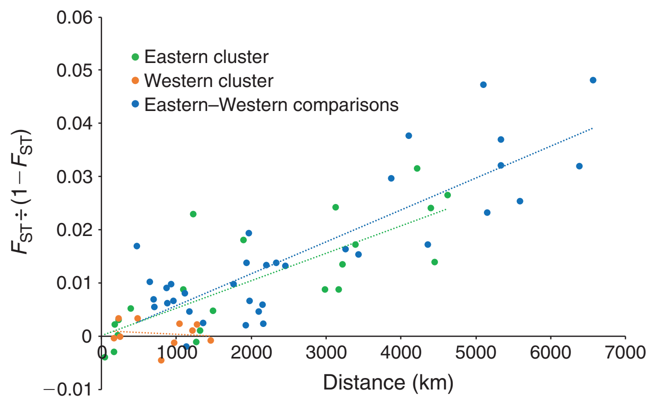

Fig. 4. Isolation by distance for all *Lethrinus laticaudis* samples illustrating the relationship between geographic distance and $F_{ST} \div (1 - F_{ST})$, where F_{ST} is the fixation index. The dashed lines indicate the best linear fit.

of infection (95.9%), mean abundance (mean 32.5; range 0–250) and species richness (mean 3.6; range 0–11). The remaining parasites used in subsequent analyses are presented in Table S5 of the Supplementary material. The parasite assemblages differed significantly overall and among the three broad-scale regions (MANOVA, $P < 0.001$; Table S3).

LDFA of the overall parasite assemblage data across the entire sample range successfully reclassified 56% of fish back to their collection location (Table 3). As for the otolith chemistry analyses, partitioning the data into regions returned improved classification success with 65% (WA), 78% (NT) and 85% (Qld) correctly reclassified (Table 3). Reclassification success ranged from 46% (Cape Preston) to 93% (Camden Sound) in WA, from 67% (Wadeye) to 92% (Darwin Harbour) in the NT and from 79% (Halifax Bay and Sunshine Coast) to 100% (Moreton Bay) in Qld (Tables 3,S6; Fig. 5). The correlation of Jaccard index

Table 3. Jack-knife reclassification success of the linear discriminant function analysis for the overall otolith near core and margin chemistry and parasite assemblage of *Lethrinus laticaudis* sampled from all locations, five locations in Western Australia (WA), five locations in the Northern Territory (NT) and three locations in Queensland (Qld)
Poulin and Kamiya's (2015) proportional chance criterion is shown in parentheses after the total classification success within each region

	Percentage correct		
	Near core	Margin	Parasites
Among regions			
All locations	29 (8)	39 (8)	56 (8)
Within WA			
Locker Point	63	47	68
Cape Preston	17	27	46
Dampier Peninsula	46	61	64
Peninsula			
Camden Sound	59	24	93
Cape Voltaire	53	50	55
Total	41 (20)	48 (20)	65 (20)
Within the NT			
Wadeye	57	20	67
Roche Reef	52	4	79
Darwin Harbour	79	17	92
Coburg Peninsula	58	58	82
Vanderlin Islands	50	57	74
Total	33 (20)	58 (20)	78 (20)
Within Qld			
Halifax Bay	86	86	79
Sunshine Coast	79	57	79
Moreton Bay	58	42	100
Total	63 (34)	75 (34)	85 (34)

against geographical distance showed a decline in similarity in parasite communities with increasing distance (r = 0.29, $P < 0.01$).

Discussion

The present study used an integrated methodological approach to analyse stock discrimination for an inshore reef fish species across the coastline of northern Australia that spans three separate management jurisdictions across six provincial marine bioregions and a plethora of mesoscale bioregions. Emphasis was placed on comparing the results from three discriminatory techniques (microsatellite genetics, otolith microchemistry and parasite assemblage composition) to determine levels of population connectivity to inform spatial management of the species.

The genetic analyses suggest the existence of a substantial restriction to gene flow along the coastline between Cape Voltaire (Kimberley region, WA) and Wadeye (on the western coast of the NT), separating stocks of *L. laticaudis* into a western and eastern cluster (Figs 1, 2). Similar separation of fish stocks into western and eastern populations, with a boundary in the same region of north-western WA, has also been reported in grey mackerel *Scomberomorus semifasciatus* (Welch *et al.* 2015) and the black croaker *Protonibea diacanthus* (Taillebois *et al.* 2017). Pope *et al.* (2015) analysed genetic diversity data from multiple species in order to identify regions of high or low

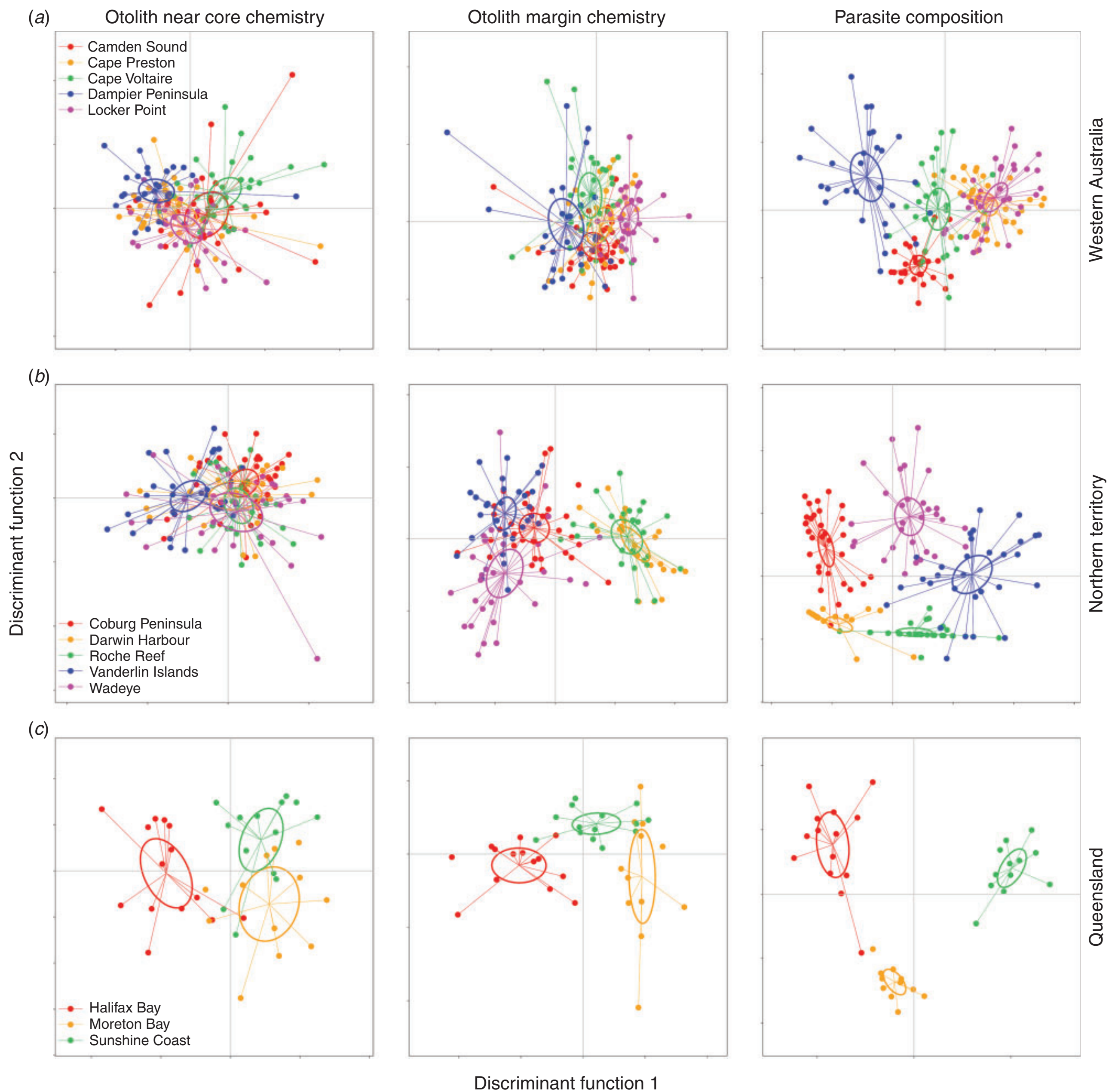

Fig. 5. Plot of the first two discriminant function scores showing spatial variation in the otolith near core and margin microchemistry and the parasite assemblages of *Lethrinus laticaudis* within (*a*) Western Australian, (*b*) Northern Territory and (*c*) Queensland fish. Ellipses are 95% confidence intervals around the group centroid.

genetic diversity and reported that the area of the Joseph Bonaparte Gulf (in which Wadeye is located) and the north-western coast possessed high levels of genetic diversity, potentially representing admixture, where distinct evolutionary units overlap. This may suggest that this region represents a substantial biogeographic feature that affects gene flow in several fish species. In addition to this pronounced genetic discontinuity between the Kimberley region and the west coast of the NT, there was a contrasting pattern of IBD between the two genetic clusters. A strong IBD effect was evident among the eastern cluster samples, but not in the western cluster. This difference of pattern between the two clusters suggests that the IBD pattern observed across the whole sampling range is driven by the clear genetic break between clusters, as well as an effect of geographic distance. Indeed, locations that are far apart geographically are more likely to be separated by multiple barriers and less connected by prevailing currents. As a consequence, sampling locations were genetically most similar to their closest neighbours and least similar to distant locations. The changing slope of IBD within the different genetic clusters, as outlined above, is a

consequence of distance, with collection locations spreading across a linear distance of ~4600 km within the eastern cluster and only ~1450 km within the western cluster.

Moreover, differences in the hydrodynamics and geomorphology of the coastline in this area may help explain this genetic pattern. A point of distinction between the ecological systems of the Kimberley region and the Joseph Bonaparte Gulf is thought to occur at Cape Londonderry (North-west Marine Bioregional Planning Section, Marine and Biodiversity Division, Department of the Environment, Water, Heritage and the Arts 2007) and this region could effectively be acting as an impediment for genetic dispersal. On the western side of Cape Londonderry, there is a diversity of substrates, dominated by algal reefs (North-west Marine Bioregional Planning Section, Marine and Biodiversity Division, Department of the Environment, Water, Heritage and the Arts 2007; Wilson 2013). The Joseph Bonaparte Gulf to the immediate east of Cape Londonderry is a large, shallow water body characterised by weak circulation patterns, muddy substrate, high bottom stress and a lack of reef development (North-west Marine Bioregional Planning Section, Marine and Biodiversity Division, Department of the Environment, Water, Heritage and the Arts 2007; Condie 2011). Isolation of coastal populations in the eastern Joseph Bonaparte Gulf from those to the west appears to be related to the restricted movement of water at the base of the Gulf compared with stronger more directional water movement offshore where currents flow north-east during summer (Condie 2011; Schiller 2011), at a time when this species is known to spawn in WA (Ayvazian *et al.* 2004). Thus, it may be relevant that the pairwise F_{ST} estimates were greater between Cape Voltaire and Wadeye than between Cape Voltaire and Roche Reef, despite the distance between the latter two locations being greater than the former (Fig. 1). Travers *et al.* (2006) showed that *L. laticaudis* were abundant in shallow-water (10–20 m) reefs along the northern WA coast, with their early life spent in seagrass beds before moving into reef habitats (Carpenter 2001). The absence of substantial seagrass habitats in the Joseph Bonaparte Gulf region (North-west Marine Bioregional Planning Section, Marine and Biodiversity Division, Department of the Environment, Water, Heritage and the Arts 2007; Seagrass-Watch HQ, see http://www.seagrasswatch.org, accessed 6 September 2016) may thus restrict settlement and subsequent dispersal of post-settlement stages. The apparent paucity of reef systems in the Joseph Bonaparte Gulf (North-west Marine Bioregional Planning Section, Marine and Biodiversity Division, Department of the Environment, Water, Heritage and the Arts 2007) combined with the pattern of water flows may also present a sufficient boundary to adult dispersal. In future research, examination of populations of *L. laticaudis* from locations closer to this apparent boundary may help determine its significance to gene flow for *L. laticaudis* in this region, particularly with regard to potential effects on larval and adult dispersal.

Further analysis of the genetics data using F_{ST}, STRUCTURE and DAPC provided insights within the western and eastern populations of *L. laticaudis*. The western cluster did not present any hierarchical structure: pairwise comparisons were not significant between locations in the western cluster, STRUCTURE did not reveal any evident pattern of genetic variation and DAPC showed largely overlapping locations within the western cluster (Table 2; Fig. 2*a–c*). Unlike for *P. diacanthus* (Taillebois *et al.* 2017), another inshore fish species, there was no apparent genetic break between populations located around the Dampier Peninsula (Cape Preston and Locker Point to the south; Camden Sound and Cape Voltaire to the north). Taillebois *et al.* (2017) suggested that underlying differences in coastal geomorphology and tidal currents accounted for these differences in *P. diacanthus*, a species known to inhabit river mouth areas (Bray 2017). In addition, DiBattista *et al.* (2017) found that the inshore reef-associated *Lutjanus carponotatus* exhibited genetic differences either side of a genetic 'transition zone' of admixture spanning a distance of 180 km at the border of the Kimberley and Canning bioregions, including the Buccaneer Archipelago and adjacent waters, which collectively experiences the largest tropical tidal range and some of the fastest tidal currents in the world. It would appear that *L. laticaudis*, although an inshore species, has larvae or juveniles with the potential to overcome the restrictions to movement imposed by local hydrodynamics, such as strong tidal currents. In the eastern cluster, the F_{ST} pairwise comparisons revealed three distinct locations (Vanderlin Islands, Halifax Bay and Sunshine Coast) on the eastern end of the sampling range, whereas all other locations were grouped into a northern cluster (Wadeye–Roche Reef–Darwin Harbour–Coburg Peninsula; Table 2). Taillebois *et al.* (2017) also demonstrated that for *P. diacanthus* the Vanderlin Islands were genetically distinct from a 'northern' grouping of locations, incorporating many of the same areas as in the present study. Taillebois *et al.* (2017) suggested that the location of Vanderlin Islands, in the western base of the Gulf of Carpentaria, was isolated from the northern coastline due to patterns of water circulation and seasonal winds. Again, further sampling of locations along the coastline and into the top of the Gulf of Carpentaria may provide more information on apparent population breaks.

The Bayesian analyses and DAPC performed on the eastern cluster showed that Halifax Bay and Sunshine Coast were different from all the others in the cluster (Fig. 2*b*, *c*). It is possible that the restriction of water at Torres Strait acts as a barrier to gene flow between populations of *L. laticaudis* on the east coast of Australia with the more western locations, as reported by van Herwerden *et al.* (2009) for the congeneric *L . miniatus* and for nearshore species by Taillebois *et al.* (2013; for the Sicydiinae (amphidromous gobies)) and Horne *et al.* (2011, 2012, 2013; for threadfin species of the Polynemidae). Unfortunately, sample sizes for these east coast locations were small and samples for genetic analyses were not available for the Moreton Bay population, the easternmost sample location in the present study. Thus, further research is required to determine the extent and strength of this apparent genetic boundary.

Analysis of otolith microchemistry and parasite assemblages showed population structuring at finer spatial scales than the regions identified in the genetic analyses, as was found in Taillebois *et al.* (2017) for *P. diacanthus*. Only Locker Point and Cape Preston in WA had substantial overlap in the multivariate analysis of parasite assemblages, with high percentages of fish collected from both locations reclassifying back to the other location. The otolith chemistry data exhibited some spatial differentiations, but there was substantial overlap in otolith chemistry

signatures among some locations, particularly in the near core analyses. Lower classification success in the near core than the margin was expected in the present study *a priori* because the fish analysed were 2–14 years of age and the near core material therefore represented growth laid down over a wide range of years (i.e. multiple year classes were present in the near core samples). Previous studies have demonstrated strong interannual variation in otolith chemistry signatures in marine systems (e.g. Gillanders 2002; Hamer *et al.* 2003). Temporal variation has the capacity to reduce discrimination among locations using near core samples from fish of different ages (Gillanders 2002), indicating that temporal variation needs to be considered in the integrated interpretation of data with regard to discerning spatial dynamics. In contrast, the otolith margin represents recent growth and less temporal variation in chemical signatures would be expected because our samples were collected over a much more discrete period (2013–15). Although analysing fish from the same age class is an optimal approach, it was not logistically possible to collect sufficient samples of fish from the same age class from all locations in the present study. Nonetheless, the highly significant differences among sampling locations in the MANOVA and LDFA provide strong evidence of some fine-scale population structuring across the life history for *L. laticaudis*. However, the proportion of misclassified fish in the LDFA that can be attributed to interannual variation in near core otolith chemical signatures *v.* other potential causes (e.g. immigration from other locations) cannot be resolved from our analyses.

In addition to the likelihood of interannual variation in otolith signatures within locations, it is also possible that the lower classification success of the near core samples reflects movement by some individual *L. laticaudis*, although such an explanation is not supported by the parasite analyses. This also potentially indicates that parasite loading is likely to be occurring at a different time scale from the deposition of otolith carbonate in the life history dynamics of some species and needs to be considered when integrating different lines of evidence. Although there are some uncertainties in the interpretation of the otolith chemistry analyses, there was a highly significant effect of sampling location in the MANOVA and much higher classification success in the LDFA than would be expected from random for both the near core and margin otolith chemistry data. The limited classification success rates from the otolith chemistry data indicate that although some individuals remain within a defined sampling location, others exhibit evidence of having occupied additional environmental regimes.

The otolith chemistry results of the present study differ from those of Ayvazian *et al.* (2004), who demonstrated high site fidelity for *L. laticaudis* in the Shark Bay area by the use of whole otolith carbon and oxygen stable isotopes. The analysis of carbon and oxygen stable isotopes in whole and sectioned otoliths has been used in many studies across the northern and western Australian region (e.g. Newman *et al.* 2000, 2009, 2010*a*, 2010*b*, 2011; Fairclough *et al.* 2011) and has revealed population subdivision and limited longshore movements at a range of spatial scales for demersal, nearshore and pelagic species. The otolith chemistry findings from the present study reveal that *L. laticaudis* differs from other nearshore species that exhibit far greater fine-scale spatial subdivision indicative of much more restricted movements (Newman *et al.* 2010*b*, 2011; Taillebois *et al.* 2017). Furthermore, these results are in contrast with the parasite analyses that provide evidence of population structuring (i.e. location fidelity) at fine spatial scales in *L. laticaudis* across its range.

Poulin and Kamiya (2015) suggested that small spatial scales (<100 km) would decrease the usefulness of parasites as biological tags for fish stocks. Our results were mixed in this regard, with higher levels of reclassification for locations adjacent to each other (e.g. Locker Point *v.* Cape Preston (175 km), Roche Reef *v.* Darwin Harbour, Darwin Harbour *v.* Coburg Peninsula (180 km)), but not always (Sunshine Coast *v.* Moreton Bay (80 km)). Similarly, pairwise F_{ST} between locations identified through genetic analysis was higher between non-adjacent groups than between adjacent groups, indicating that differentiation increased with distance. This was confirmed by the Mantel test showing an overall positive correlation between G ($F_{ST} \div (1 - F_{ST})$ and D (seascape distances), indicating isolation by distance. In addition, the similarity in parasite communities, as indicated by the Jaccard index, did decrease with distance, despite some parasite species occurring across the entire range of collection. Similar to Grutter (1994), who sampled fish species from Lizard and Heron islands on the Great Barrier Reef, which are separated by over 1000 km, differences in the parasite community between locations was due to differences in the abundance of only one or two species. Thus, the differences in parasite communities detected in the present study show that interpretation of parasite loads can be heavily affected by just a few species. Ideally, the spatial dynamics of such parasites would assist interpretation of the spatial dynamics of their hosts.

A diverse range of parasites was found to infect *L. laticaudis* across the geographical range of this study (see Table S5). Not all parasites could be identified to the lowest taxonomic unit possible; however, we are confident in the differentiation of the parasite groupings. Although groupings of closely related parasites (or the inability to correctly separate them) may reduce the overall significance of the results (see Bray and Cribb 2015), it should not inflate them. If anything, Bray and Cribb (2015) suggest that finer-scale differentiation of parasite species will only increase the significance of the results.

Small distances, combined with the current circulation patterns along the western coastline (see Condie 2011), could assist in the intermixing of sediments and parasite larval stages to account for the higher reclassification scores between locations for both parasites and otoliths for the collection locations between Wadeye and Coburg Peninsula. However, the Vanderlin Islands were always distinct from the other NT locations, even for the otolith near core results. The Qld locations were distinct from each other for both parasites and otoliths, with only slight overlap between Moreton Bay and Sunshine Coast, separated by less than 100 km, for the otolith near core results. This could suggest that the populations in south-east Qld may represent an admixture of larval stages, with adult populations remaining separate; further genetic analysis of populations from Moreton Bay are required to confirm this. However, it would appear, through all three techniques used, that there is the possibility of disjunct populations of *L. laticaudis* in Far North Queensland (Halifax Bay) and along the south-eastern Qld coast. Similar to the situation with the WA–NT barrier, the lack of collections along a finer scale prevents a more precise estimate of the location of this boundary.

Table 4. End-user summary table of the results from all for *Lethrinus laticaudis*, based on sampling locations in the present study

Within each technique, statistically similar samples are grouped into 'regions'. Genetic population analysis was not undertaken for Moreton Bay. WA, Western Australia; NT, Northern Territory; Qld, Queensland

	Otoliths (margin)	Parasites	Genetics
WA	Locker Point	Central WA	North WA
	North WA	Dampier Peninsula	
		Camden Sound	
		Cape Voltaire	
NT	Wadeye	Wadeye	NT
	Darwin Area	Roche Reef	
		Darwin Harbour	
	Coburg Peninsula	Coburg Peninsula	
	Vanderlin Islands	Vanderlin Islands	
Qld	Halifax Bay	Halifax Bay	Halifax Bay
	South-east Queensland	Sunshine Coast	Sunshine Coast
		Moreton Bay	

To summarise the study's findings in the context of potential spatial management of *L. laticaudis*, we have presented an integration of the results of the otolith microchemistry, parasite and genetic analyses (Table 4). Calculation of a stock differentiation index (*sensu* Izzo *et al.* 2017) would assist in a more rigorous quantification of the results presented, but this was not possible in the present study due to the small number of techniques used (3 *v.* 14 in Izzo *et al.* 2017). Overall, the genetic analyses suggest at least four genetically distinct groups of *L. laticaudis* across northern Australia. However, the parasite analyses suggest much finer levels of spatial structuring within the temporal scales of individual life histories. Similar results have been reported previously, where an apparently homogeneous genetic population is spatially structured over fine temporal scales (see Sturrock *et al.* 2012; Welch *et al.* 2015; Taillebois *et al.* 2017). Thorrold and Swearer (2009) suggested that many shallow-water fish, like *L. laticaudis*, have a bipartite life cycle in which demersal adults spawn clutches of benthic or pelagic eggs that hatch into pelagic larvae and that the spatial scales of dispersal of these pelagic larvae are not well understood. Subsequently, this can lead to a decoupling of local reproduction and recruitment so local populations will be at least partially demographically open, which is similar to the results found here.

The otolith and parasite results were generally consistent with each other in suggesting fine spatial scale structuring of adult populations, even within genetically distinct populations among NT sampling locations only. The otolith and parasite results were inconsistent among the WA sampling locations. Although the genetic results indicated populations across large areas are likely to be linked by larval connectivity, given the likely sedentary nature of adults, as indicated by parasite results and, to a lessor degree, otolith chemistry, the spatial scale of separation between most locations sampled is an important consideration for fishery management (Table 4). These results overall suggest that *L. laticaudis* populations have an amount of connectivity over the scale of locations in the present study (probably by larval dispersal), but with a pattern of declining relatedness with distance (isolation by distance). However, once recruited it appears that post-larval fish tend to be fairly site attached at the scale of locations sampled in the present study and may thus be vulnerable to localised depletion if overfished. As such, location-specific stock assessments and location-specific management arrangements may need to be considered by fisheries managers or, alternatively, location-specific management areas or reef complexes need to be considered within assessment, monitoring and management frameworks. Moreover, if individual management areas or reef complexes are overfished, they are unlikely to receive sufficient recruitment in time frames that balance exploitation, mixing and life history dynamics to sustain ongoing fisheries.

The use of a range of stock identification techniques on the same samples, which cover multiple aspects of the life history of that fish species, provides a stronger capacity to infer potential stock structure (Begg and Waldman 1999; Izzo *et al.* 2017). The use of this integrative approach for identifying fish stocks has been applied several times in northern Australia on threadfins, Spanish mackerel, grey mackerel, school mackerel and black croaker (e.g. Begg *et al.* 1997; Buckworth *et al.* 2007; Welch *et al.* 2009, 2010; Taillebois *et al.* 2017) and on southern Australian stocks of sardines (Izzo *et al.* 2017). Because this approach uses a combination of techniques, it can provide the necessary information to define populations at time scales that are relevant to management. Population genetic analysis, as performed in the present study (i.e. variation of allele frequencies between populations) provides information on population structure across generations, whereas techniques like otolith microchemistry and parasitology are intragenerational, providing information on early life history (otolith near core microchemistry) and recent adult biology (otolith margin microchemistry and parasite assemblage; Begg and Waldman 1999; Welch *et al.* 2015; Izzo *et al.* 2017). In combination, the three marker systems provide insights that a single technique could not have provided. For example, results here suggest that dispersal between populations occurs during the presettlement stages, whereas post-settlement stages are more sedentary. The parasite and otolith data allow a robust test of hypotheses that are often posed by genetic studies of stock structure. Similarly, in studies of population structure defined by otolith chemistry and parasite composition, it is challenging to test hypotheses of connectivity in life history stages before the acquisition of parasites or the development of otoliths. Studies like this, which produce contrasting results between inter- (genetics) and intragenerational (otoliths and parasites) methods, allow the outcome of fine and broader spatial scale management arrangements to be predicted. However, fine-scale management is needed to provide the greatest certainty that local populations will be protected. In areas like the NT, where overfishing of *L. laticaudis* is suspected, location-specific (fine-scale) management arrangements may need to be considered by fisheries managers in order to preserve spawning stocks.

Conflicts of interest

The authors declare that they have no conflicts of interest.

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