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A multilocus comparative study of dispersal in three codistributed demersal sharks from eastern Australia

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Abstract: Demersal elasmobranchs are ecologically important mesopredators but little is known about their population connectivity or dispersal patterns. Here we use a comparative approach based on mitochondrial DNA and nuclear amplified fragment length polymorphism (AFLP) markers to examine spatial genetic structure and dispersal in three closely related demersal elasmobranchs from eastern Australia: *Orectolobus halei*, *Orectolobus maculatus*, and *Orectolobus ornatus*. We found evidence of significant spatial genetic structure, possibly indicating regional philopatry in wobbegongs. The molecular data also indicate that dispersal in wobbegongs may be sex-biased. This represents the first genetic study of dispersal and population connectivity in codistributed demersal sharks. It provides insights into the ecology of dispersal behaviours with implications for conservation management of demersal species.

Résumé : Si les élasmobranches démersaux sont des mésoprédateurs importants sur le plan écologique, les connaissances sur leurs motifs de dispersion et de connectivité des populations sont limitées. Nous employons une approche comparative reposant sur l'ADN mitochondrial et des marqueurs de polymorphisme de longueur de fragments amplifiés (AFLP) nucléaires pour examiner la structure génétique spatiale et la dispersion chez trois élasmobranches démersaux étroitement reliés de l'est de l'Australie : *Orectolobus halei*, *Orectolobus maculatus* et *Orectolobus ornatus*. Nous avons relevé des preuves d'une structure génétique spatiale significative, ce qui pourrait indiquer une philopatrie régionale chez les requins-tapis. Les données moléculaires indiquent également que la dispersion chez ces requins pourrait présenter une asymétrie dépendante du sexe. Cette première étude génétique de la dispersion et de la connectivité de populations de requins démersaux codistribués jette un nouvel éclairage sur l'écologie des comportements de dispersion et leurs conséquences pour la gestion de la conservation d'espèces démersales. [Traduit par la Rédaction]

Introduction

Whether an individual disperses or remains philopatric has important implications for individual fitness, genetic and demographic connections between populations, species distributions, and the spatial design of management strategies (Dieckmann et al. 1999). Predicting dispersal characteristics is complicated, however, because they may be the product of multiple driving forces, for example, sociality (Lukas and Clutton-Brock 2011), site fidelity (Greenwood 1980), or landscape heterogeneity (Banks et al. 2007; Möller et al. 2007). Characterizing dispersal is especially problematic for wide-ranging organisms that are elusive to sample, such as actively dispersing higher vertebrates from the marine realm (Ferriere et al. 2000). Disentangling the various influences upon dispersal behaviour can be achieved by comparative analysis of ecologically similar, codistributed species, yet this approach is rarely applied, particularly in marine organisms lacking a larval dispersal stage.

Comparative studies of dispersal in elasmobranchs (sharks and rays) could provide key insights into the ecology and evolution of dispersal behaviours in directly dispersing marine organisms because they exhibit diverse life histories, habitat preferences, and dispersal behaviour (Compagno 1990; Cortés 2000). Additionally, because elasmobranchs are among the vertebrates with greatest extinction risk, such research can inform conservation management initiatives for vulnerable elasmobranch populations by revealing dispersal patterns that require special management (Dulvy et al. 2014). Published accounts of population genetic structure and dispersal in elasmobranchs describe scenarios spanning panmixia across vast geographical areas to structure on reduced spatial scales (reviewed in Dudgeon et al. 2012; Heist 2004). However, there is an apparent bias toward studying pelagic or coastal shark species with good dispersal capacity. Dispersal behaviour may be very different for demersal species, but these have been comparatively little studied (Chapman et al. 2015; Dudgeon et al. 2012; Heist 2004).

In this study, we investigate population genetic structure and dispersal patterns among three codistributed species of wobbegong: the gulf wobbegong (*Orectolobus halei*), the spotted wobbegeong (*Orectolobus maculatus*), and the ornate wobbegong (*Orectolobus ornatus*) (Last and Stevens 2009). Previous phylogenetic and phylogeographic studies have characterized the interrelationships of these species (Corrigan and Beheregaray 2009; Corrigan et al. 2008) and their recent evolutionary and demographic histories (Corrigan 2010), providing a unique opportunity to assess contemporary dispersal patterns in demersal sharks within the context of shared biogeographic history. *Orectolobus halei* and *O. maculatus* are very closely related and show overlapping distributions from southwestern to southeastern Australia. *Orectolobus ornatus* is a more divergent lineage (Corrigan and Beheregaray 2009), despite

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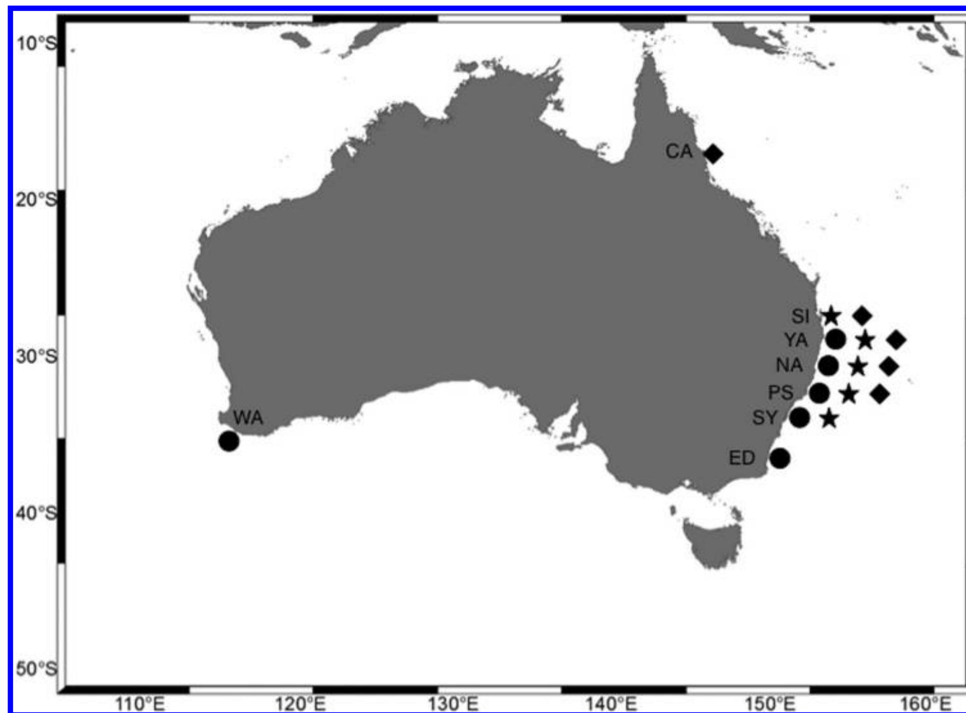
Fig. 1. Location of sampling sites for *Orectolobus halei* (circles), *Orectolobus maculatus* (stars), and *Orectolobus ornatus* (diamonds). Sampling location codes are Cairns (CA), Stradbroke Island (SI), Yamba (YA), Nambucca Heads (NA), Port Stephens (PS), Sydney (SY), Eden (ED), Augusta (WA).



possessing morphological similarities with *O. halei* (Corrigan et al. 2008; Huveneers 2006*b*). *Orectolobus ornatus* is restricted in range to the northeast coast of Australia, but overlaps with the northern part of the range of *O. maculatus* and *O. halei*, which are both also distributed through southern Australia to the west coast.

Previous studies of wobbegong habitat preference and movement using acoustic telemetry and visual survey methods described residency and site fidelity (Carraro and Gladstone 2006; Huveneers et al. 2006; Lee et al. 2014, 2015), and we hypothesize this may promote spatial genetic partitioning. We hypothesize that dispersal may be male-biased in wobbegongs given observations of sexual segregation in these species (Carraro and Gladstone 2006; Huveneers et al. 2007*b*). Although sex-biased dispersal has been demonstrated in other elasmobranch species (e.g., Daly-Engel et al. 2012; Pardini et al. 2001; Portnoy et al. 2010), it is yet to be tested in wobbegongs. We test these hypotheses using a relatively large molecular dataset collected from the mitochondrial and nuclear genomes. This work provides useful baseline information for understanding dispersal patterns and population connectivity in wobbegongs and is expected to inform the management of commercially exploited populations. More generally, it should also contribute to our understanding of dispersal patterns in demersal elasmobranchs, a topic with implications for the spatial design of management strategies for ecologically important demersal mesopredators.

Materials and methods

Sample collection

A total of 325 muscle tissue samples were collected from three wobbegong species: *O. halei* (115), *O. maculatus* (135), and *O. ornatus* (75). Samples were acquired by opportunistic sampling of commercially harvested animals at eight geographic localities around Australia: Cairns (CA), Stradbroke Island (SI), Yamba (YA), Nambucca Heads (NA), Port Stephens (PS), Sydney (SY), Eden (ED), Augusta (WA) (Fig. 1; Table 1). All individuals were captured in close proximity to the landing site at which they were sampled. Sex was determined by noting the presence of claspers in males. Tissue was preserved in either 95% ethanol or salt-saturated 20% dimethylsulfoxide.

Laboratory methods

Genomic DNA was extracted using a modified salting out protocol (Sunnucks and Hales 1996) and subsequently underwent whole-genome amplification using the REPLI-g mini kit (Qiagen Inc., Valencia, California) according to the manufacturer's instructions. The mitochondrial control region (CR) and adenosine triphosphatase subunits 6 and 8 (ATPase 6 and ATPase 8) were amplified by polymerase chain reaction (PCR), purified and bidirectionally Sanger-sequenced using BigDye Terminator chemistry on an ABI 3730*xl* genetic analyser (Applied Biosystems Life Technologies, Grand Island, USA). Reaction conditions, PCR cycling, and purification followed Corrigan and Beheregaray (2009).

Amplified fragment length polymorphism (AFLP) profiling of each individual consisted of a digestion–ligation step followed by two rounds of complexity reduction via selective amplification. Reaction conditions for the digestion and first round of selective PCR followed Zenger et al. (2006). The PCR product was diluted 1:10 for use as template in a second round of amplification. Selective amplification was carried out in 10 μL reactions containing 3 μL of diluted template, 1.5 mmol·L^{-1} $MgCl_2$, 0.2 mmol·L^{-1} each dNTPs, 1 U Taq polymerase (Qiagen), 1× Taq buffer (10 mmol·L^{-1} Tris–HCl pH 8.3, 50 mmol·L^{-1} KCl, 0.1% Triton X-100 (Qiagen), 2 pmol of fluorescently terminally labelled EcoRI selective primer carrying three selective nucleotides (5′-GAC TGC GTA CCA ATT C + ACT, AGT, ATC, or AAC-3′), and 5 pmol of unlabelled MseI selective primer carrying four selective nucleotides (5′-GAT GAG TCC TGA GTA A + CAAC, CTGC, CAGC or CTTC-3′). Selective PCR cycling involved a "touchdown" process of 30 s at 94 °C, 30 s at 65 °C for the first annealing step, and 1 min at 72 °C. At each touchdown, the annealing temperature was decreased by 0.7 °C before stabilizing at 58 °C for a further 26 cycles. Fluorescently labelled fragments were electrophoresed on an ABI 3730*xl* genetic analyser and visualized in GENEMAPPER version 4.1 and scored for presence

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Table 1. Genetic diversity at mitochondrial DNA (mtDNA) and amplified fragment length polymorphism (AFLP) markers in wobbegongs.

	mtDNA diversity					AFLP diversity				
Species	Sampling locality	n	N	h	π	Sampling locality	n	NLP	PLP	H_e
O. halei	YA	4	1	0.0000 (0.0000)	0.0000 (0.0000)	YA	4	181	100	0.2134 (0.0123)
	NA	20	2	0.2684 (0.1133)	0.0002 (0.0002)	NA	27	180	99.4	0.4528 (0.0064)
	PS	9	3	0.4167 (0.1907)	0.0004 (0.0004)	—	—	—	—	—
	SY	23	2	0.3557 (0.0995)	0.0003 (0.0003)	SY	31	180	99.4	0.4532 (0.0065)
	ED	19	3	0.4854 (0.1038)	0.0004 (0.0003)	ED	22	181	100	0.4547 (0.0062)
	WA	20	3	0.1947 (0.1145)	0.0001 (0.0002)	WA	31	178	98.3	0.4313 (0.0076)
O. maculatus	SI	19	1	0.0000 (0.0000)	0.0000 (0.0000)	SI	19	23	60.5	0.2665 (0.0287)
	YA	21	2	0.2571 (0.1104)	0.0002 (0.0002)	YA	29	18	47.4	0.2131 (0.0282)
	NA	20	3	0.5316 (0.1004)	0.0004 (0.0004)	NA	27	25	65.8	0.2528 (0.0241)
	PS	21	2	0.0952 (0.0843)	0.0001 (0.0001)	PS	30	36	94.7	0.3725 (0.0232)
	SY	20	2	0.1000 (0.0880)	0.0001 (0.0001)	SY	30	33	86.8	0.3186 (0.0254)
O. ornatus	CA	3	3	1.0000 (0.2722)	0.0034 (0.0028)	—	—	—	—	—
	SI	14	5	0.5934 (0.1438)	0.0006 (0.0006)	QLD	19	130	100	0.4335 (0.0073)
	YA	15	2	0.1333 (0.1123)	0.0001 (0.0002)	YA	14	130	100	0.4502 (0.0070)
	NA	21	1	0.0000 (0.0000)	0.0000 (0.0000)	NA	22	130	100	0.4465 (0.0076)
	PS	22	1	0.0000 (0.0000)	0.0000 (0.0000)	—	—	—	—	—

Note: n, number of individuals; N, number of haplotypes; h, haplotypic diversity; π, nucleotide diversity; NLP, number of polymorphic loci; PLP, proportion of polymorphic loci; H_e, expected heterozygosity. Sampling location codes are as in Fig. 1, except for QLD, which represents pooled CA and SI samples for *O. ornatus*.

or absence against the GeneScan 500 LIZ size standard (Applied Biosystems, Life Technologies, Grand Island, USA).

Genetic diversity

Sequences were cleaned and aligned using SEQUENCHER version 4.1 (Gene Codes Corporation, Ann Arbor, Michigan). Sequence variation was assessed through calculation of the number of observed haplotypes, as well as haplotypic and nucleotide diversities in ARLEQUIN version 3.11 (Excoffier et al. 2005). AFLP allele frequencies were estimated using a Bayesian approach (Zhivotovsky 1999) implemented in AFLP-SURV version 1.0 (Vekemans 2002). Proportion of polymorphic loci and expected heterozygosity were calculated according to Lynch and Milligan (1994) based on these frequencies, assuming Hardy–Weinberg genotypic proportions.

Population differentiation and spatial genetic structure

Mitochondrial DNA population differentiation was explored in ARLEQUIN by calculating the parameter Φ_{ST} between population pairs after applying the appropriate model of sequence evolution as specified by MODELTEST version 3.7 (Posada and Crandall 1998). Significance was tested using 100 000 permutations. Genealogical relationships among mtDNA haplotypes and their geographic distributions were depicted using a network analysis based on statistical parsimony (Templeton et al. 1992), as implemented in TCS version 1.21 (Clement et al. 2000). Pairwise genetic distances were calculated for AFLP data following the method of Huff et al. (1993) in GENALEX version 6.5 (Peakall and Smouse 2012) and used to calculate Φ_{PT}, an F_{ST} analogue for estimating population differentiation in binary data. Significance was assessed with 10 000 permutations. All significance tests were adjusted for inflated type I error by applying the Bonferroni correction (Rice 1989).

Spatial patterns of genetic structure were investigated using spatial autocorrelation. Pairwise genetic distances were calculated following Smouse and Peakall (1999). Autocorrelation coefficients (r; Smouse and Peakall 1999) were calculated across a range of distance classes (*O. halei*: within location and between 150, 500, 1050, 5000, and 6000 km; *O. maculatus*: within location and between 150, 200, 300, 450, 650, and 800 km; *O. ornatus*: within location and between 200, 400, and 2000 km). These classes were chosen to incorporate comparisons within sampling localities, among adjacent localities and more distant comparisons, while keeping sample sizes large enough to allow statistically meaningful analysis. Ninety-five percent confidence intervals (CIs) representing the null hypothesis of randomly spatially distributed genotypes were plotted, and tests for significant deviation from the null were performed using 10 000 random permutations of individual genotypes among distance classes, with 1000 bootstrap replicates (Peakall et al. 2003). The combined distance class probability metric, ω, was calculated and compared against the distribution of permuted ω values as a test of overall correlogram significance (Smouse et al. 2008).

Analyses of sex-biased dispersal

The possibility of sex-biased dispersal was initially investigated using traditional comparisons of relative structure at uniparentally (mtDNA) versus biparentally (AFLP) inherited markers (Goudet et al. 2002; Prugnolle and de Meeus 2002), under the expectation that male-biased dispersal would result in increased differentiation at maternally inherited mtDNA loci relative to the nuclear genome. This signature, however, may also occur owing to differences in the mutation rate and (or) effective population size among the markers (Chesser and Baker 1996). Analyses of sex-biased dispersal were therefore also conducted based on biparentally inherited AFLP data alone. Multilocus AFLP profiles were used to calculate pairwise relatedness (r) among individuals according to Hardy (2003) in SPAGEDI version 1.3 (Hardy and Vekemans 2002). Mean relatedness between males (MM), females (FF), and opposite-sex pairs (MF) were estimated for each species. The difference in mean r between the more philopatric and the more dispersive categories (in this case dispersal was assumed to be male-biased) was calculated as a test statistic (Goudet et al. 2002; Prugnolle and de Meeus 2002) based on the assumption that low-dispersing individuals are expected to be more closely related on average to those sampled in close proximity to themselves than to individuals selected at random from the metapopulation. The significance of this test statistic was evaluated by conducting a two-sample randomization test using POPTOOLS version 3.0.6 (Hood 2008). The probability that dispersal is not sex-biased was estimated as the proportion of times the test statistic obtained from 10 000 randomized datasets was larger or equal to the statistic obtained from the observed dataset. Each test was performed initially for the entire dataset for each species and then by population (Goudet et al. 2002).

Likelihood of local assignment was calculated for each individual as described in Paetkau et al. (1995), with modifications for dominant markers, using AFLPOP version 1.1 (Duchesne and Bernatchez 2002). Log-transformed likelihood values were corrected for population effects following Favre et al. (1997), resulting in corrected assignment indices (AI_c) that average zero per population and whereby negative values indicate lower

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than average probability of being born locally (migrants). AI_c values were compared for males and females with the expectation that the more dispersive sex would show a more negative frequency distribution (Favre et al. 1997; Mossman and Waser 1999). The difference in the means and the ratio of the variances of AI_c across sexes (Goudet et al. 2002) were used as test statistics, and significance was assessed using the same randomization procedure described above.

Multivariate spatial autocorrelation analyses (Peakall et al. 2003; Smouse and Peakall 1999) were conducted for each sex as previously described and compared following Banks and Peakall (2012). The null hypothesis of no sex-biased dispersal was accepted if there was overlap in the CIs between the sexes. Heterogeneous autocorrelation across sexes was assessed using single-distance (t^2) and multi-distance (ω) class criteria, as implemented in the nonparametric heterogeneity tests described by Smouse et al. (2008).

For all analyses of sex-biased dispersal, only those populations and samples with reliable associated sex information were used. Final sample sizes and breakdown per sampling location were 92 *O. halei* (NA: 11 males, 11 females; SY: 15 males, 14 females; ED: 8 males, 6 females; WA: 16 males, 11 females), 31 *O. ornatus* (SI: 5 males, 8 females; NA: 11 males, 7 females), and 92 *O. maculatus* (SI: 5 males, 7 females; NA: 9 males, 17 females; PS: 18 males, 11 females; SY: 9 males, 16 females).

Results

Genetic diversity

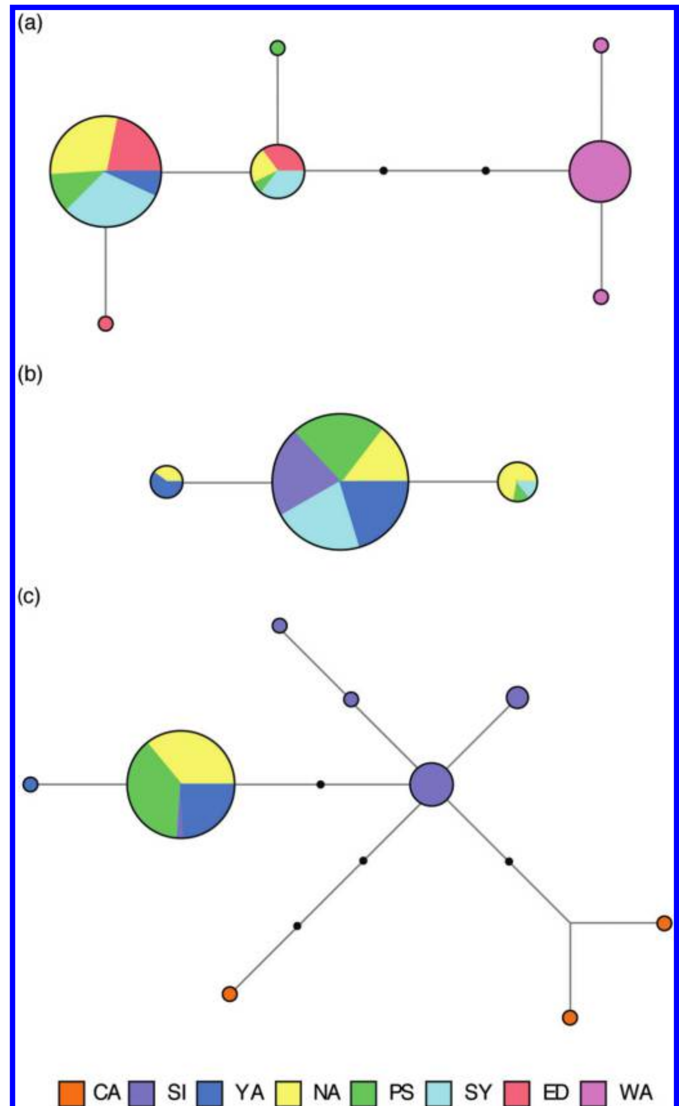
Concatenated CR-ATPase 1385 bp fragments were unambiguously resolved for 95 *O. halei*, 101 *O. maculatus*, and 75 *O. ornatus* (GenBank accession numbers: *O. halei* CR KT763479–KT763573, ATPase KT763750–KT763844; *O. maculatus* CR KT763574–KT763674, ATPase KT763845–KT763945; *O. ornatus* CR KT763675–KT763749, ATPase KT763946–KT764020). There was no haplotype sharing among species. Mitochondrial DNA diversity was low with seven, three, and nine haplotypes resolved for *O. halei*, *O. maculatus*, and *O. ornatus*, respectively. Haplotypic and nucleotide diversities were highest in *O. halei* (0.5622 ± 0.0474; 0.0011 ± 0.0008, respectively), followed by *O. ornatus* (0.3910 ± 0.0683; 0.0008 ± 0.0006, respectively), and *O. maculatus* (0.2184 ± 0.0529; 0.0002 ± 0.0002, respectively).

AFLP profiles were resolved for 115 *O. halei*, 135 *O. maculatus*, and 55 *O. ornatus* individuals. *Orectolobus halei* and *O. ornatus* sampled at PS were removed from AFLP analysis because those samples consistently produced low-quality profiles. Owing to low sample size, *O. ornatus* from CA were pooled with SI to represent a single Queensland (QLD) group for AFLP analysis. Only those loci that could be reliably scored were included in the final datasets, resulting in 181, 38, and 130 polymorphic AFLP fragments analysed for *O. halei*, *O. maculatus*, and *O. ornatus*, respectively. Genetic diversity was highest in *O. ornatus* (mean PLP = 100; mean H_e = 0.4434), followed by *O. halei* (mean PLP = 99.42; mean H_e = 0.4010) and *O. maculatus* (mean PLP = 71.04; mean H_e = 0.2847). Intraspecific population-level measures of diversity for both mtDNA and AFLP data, including sample sizes, number of haplotypes, haplotypic and nucleotide diversity, number of polymorphic loci, proportion of polymorphic loci, and expected heterozygosity, are in Table 1.

Population differentiation

There was no mtDNA haplotype sharing among species. All haplotypes within each species could be joined with 95% confidence. There was low nucleotide diversity in all species, as reflected in the very shallow mtDNA genealogies (Fig. 2). The maximum number of mutations required to link any two haplotypes was six for *O. halei* and *O. ornatus* and two for *O. maculatus* (Fig. 2). For *O. halei*, there was no haplotype sharing between the east and west coasts of Australia (Fig. 2*a*). A single abundant haplotype (62% of sampled individuals) was detected at all sampling sites, excluding WA. Singleton haplotypes were found in ED and PS. For *O. maculatus*, the network was dominated by a single abundant haplotype found at all sampling locations (88% of sampled individuals). Two additional haplotypes were detected in a very small number of individuals but at multiple sampling locations (Fig. 2*b*). The *O. ornatus* network was also dominated by a single haplotype (77% of sampled individuals) sampled at all locations (Fig. 2*c*), except CA. All haplotypes were closely related, but some structure was evident separating the northerly end of the sampling range (CA and SI) from the south (YA, NA, and PS). Four haplotypes were unique (two of which were singletons) to SI. Singleton haplotypes were also detected at YA and CA.

Fig. 2. Unrooted mitochondrial DNA haplotype networks for (*a*) *O. halei*, (*b*) *O. maculatus*, and (*c*) *O. ornatus*. The area of each haplotype is proportional to its frequency. The length of the line intersecting two haplotypes is proportional to the number of mutational differences that separate them. Unsampled or extinct haplotypes are represented by solid black circles. Sampling location codes are as in Fig. 1. [Colour online.]



Significant mtDNA population structure was found for all species (*O. halei*: Φ_{ST} = 0.8124, P < 0.0001; *O. maculatus*: Φ_{ST} = 0.0892, P = 0.0039; *O. ornatus*: Φ_{ST} = 0.7403, P < 0.0001). Levels of differentiation were low to moderate for *O. maculatus* and moderate to high for *O. halei* and *O. ornatus*. *Orectolobus halei* from WA were divergent compared with individuals sampled at all other locations. There was no significant differentiation among east coast (YA, NA, PS, SY, ED) sampling sites (Table 2*a*). For *O. maculatus*,

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Table 2. Pairwise measures of population differentiation for (*a*) *O. halei*, (*b*) *O. maculatus*, and (*c*) *O. ornatus*.

(*a*) *O. halei*.

	YA	NA	PS	SY	ED	WA
YA	—	0.045	NA	0.106*	0.103*	0.067*
NA	−0.0559	—	NA	0.034*	0.031*	0.025*
PS	−0.0435	−0.0342	—	NA	NA	NA
SY	0.0042	−0.0334	−0.0506	—	0.006	0.059*
ED	0.0118	−0.0114	−0.0538	−0.0381	—	0.077*
WA	0.9585*	0.9408*	0.9180*	0.9272*	0.9093*	—

(*b*) *O. maculatus*.

	SI	YA	NA	PS	SY
SI	—	0.048*	0.000	0.137*	0.463*
YA	0.092	—	0.008	0.138*	0.502*
NA	0.160	0.096	—	0.150*	0.497*
PS	−0.005	0.075	0.094	—	0.280*
SY	−0.003	0.073	0.088	−0.051	—

(*c*) *O. ornatus*.

	CA	SI	YA	NA
SI	0.454*	—	0.004	0.017
YA	0.826*	0.767*	—	0.000
NA	0.888*	0.823*	0.023	—
PS	0.893*	0.827*	0.000	0.027

Note: Below diagonal are Φ_{ST} values based on mitochondrial DNA. Above the diagonal are Φ_{PT} values based on amplified fragment length polymorphism (AFLP) data. Significant comparisons are marked with an asterisk. Sampling location codes are as in Fig. 1.

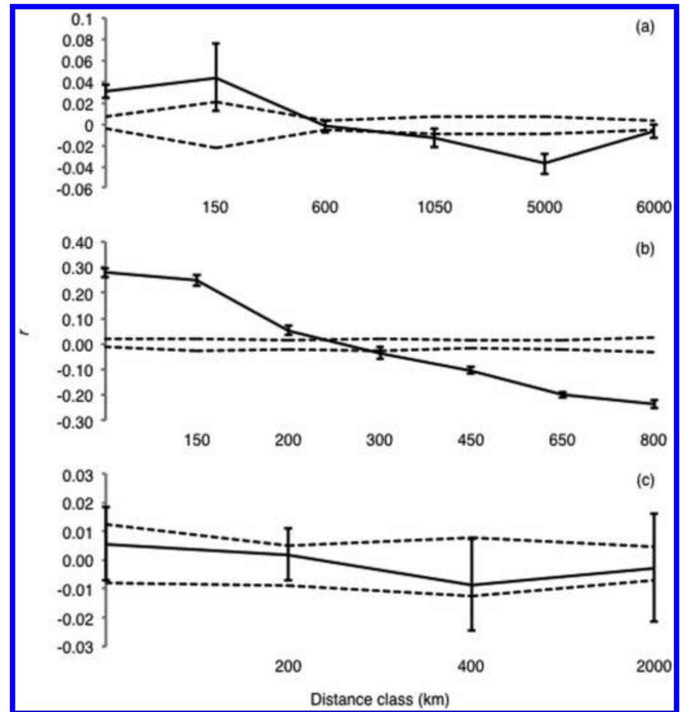
despite global Φ_{ST} significance, population pairwise estimates were all nonsignificant (Table 2*b*). The global result appears driven by two rare haplotypes detected at a subset of locations (Fig. 2; Table 1). In *O. ornatus*, CA and SI were highly differentiated from all other locations. There was no structure among New South Wales sampling sites (YA, NA, PS; Table 2*c*).

AFLP data showed weak to moderate differentiation across the sampling range for *O. halei* and *O. maculatus*. Global Φ_{PT} estimates were 0.0490 (P < 0.0001) and 0.3200 (P = 0.0010), respectively, indicating that a significant proportion of total AFLP variation could be attributed to differences among populations in these species. Most pairwise estimates of Φ_{PT} were statistically significant ($P \leq 0.01$) for *O. halei* (Table 2*a*) except between YA and NA and between SY and ED. In *O. maculatus*, the majority of pairwise comparisons were also significant; however, NA was not differentiated from either YA or SI (Table 2*b*). *Orectolobus ornatus* did not show evidence of population differentiation at AFLP loci. The global Φ_{PT} estimate was 0.0130 and approaching significance (P = 0.0660). All population pairwise estimates of Φ_{PT}, however, were relatively low and nonsignificant (Table 2*c*).

Spatial genetic structure

Orectolobus halei and *O. maculatus* showed qualitatively similar patterns of spatial genetic structure (Figs. 3*a*, 3*b*). Spatial autocorrelation analyses for both species showed significant autocorrelation, compared with the null model of random similarity among genotypes in space (ω = 73.077 and ω = 119.337 for *O. halei* and *O. maculatus*, respectively; P < 0.0001; refer to online supplementary data Tables S1*a*, S1*b*[1]) based on the overall correlogram, as well as significant positive autocorrelation for within-site comparisons and distance classes out to approximately 200 km ($P \leq 0.01$; supplementary data Table S1*a*, S1*b*[1]), suggesting localized population structure in these species. Genetic similarity among individuals decreased with increasing geographic distance class, culminating

Fig. 3. Correlogram plots of the spatial autocorrelation coefficient, *r*, as a function of geographic distance for (*a*) *O. halei*, (*b*) *O. maculatus*, and (*c*) *O. ornatus* based on amplified fragment length polymorphism (AFLP) data. Observed data are the solid black line. Dashed lines represent the upper and lower bounds for the 95% confidence interval of the null hypothesis of no spatial structure (r = 0). 95% confidence interval bootstrap error bars about r are also shown.



in negative autocorrelation among individuals sampled from the most geographically distant sites (Figs. 3*a*, 3*b*).

The comparatively small sample size in *O. ornatus* limits our ability to make reliable inferences about spatial genetic structure and dispersal patterns in this species. Results from the spatial autocorrelation analysis contrasted with *O. halei* and *O. maculatus*, as coefficients did not deviate from expectations under the null model throughout the sampling range (Fig. 3*c*; ω = 16.154, P = 0.056). This test would border on significant if using the traditional alpha of 0.05; however, Banks and Peakall (2012) recommend using 0.01 to avoid type I error. There was a trend showing positive autocorrelation at smaller distance classes in this species, similar to *O. halei* and *O. maculatus*, suggesting that *O. ornatus* may exhibit similar patterns of spatial genetic structure; however, this was not statistically supported based on the current dataset.

Tests of sex-biased dispersal

In *O. halei*, analyses of markers with different modes of inheritance did not provide evidence of sex-biased dispersal. Significant population structure was detected for both mitochondrial and nuclear markers in this species (Table 2*a*). Significant positive autocorrelation was revealed for the within-site comparisons, as well as overall correlogram significance, for both males (ω = 28.993, P = 0.004) and females (ω = 31.597, P = 0.004). This was followed by a general trend of decreasing genetic similarity with increasing geographic distance (Fig. 4*a*; supplementary data Table S2*a*[1]). Mean relatedness among females was not significantly different among males (FFr = −0.021, MMr = −0.015; P = 0.697) or among opposite sex pairs (FFr = −0.021, MFr = −0.005; P = 0.923). Female

[1]Supplementary data are available with the article through the journal Web site at http://nrcresearchpress.com/doi/suppl/10.1139/cjfas-2015-0085.

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Fig. 4. Correlogram plots of the spatial autocorrelation coefficient, *r*, as a function of geographic distance for males (solid black) and females (solid grey) of (*a*) *O. halei*, (*b*) *O. maculatus*, and (*c*) *O. ornatus* based on amplified fragment length polymorphism (AFLP) data. Observed data are represented by the solid lines. Dashed lines represent the upper and lower bounds for the 95% confidence interval of the null hypothesis of no spatial structure (*r* = 0). 95% confidence interval bootstrap error bars about *r* are also shown.

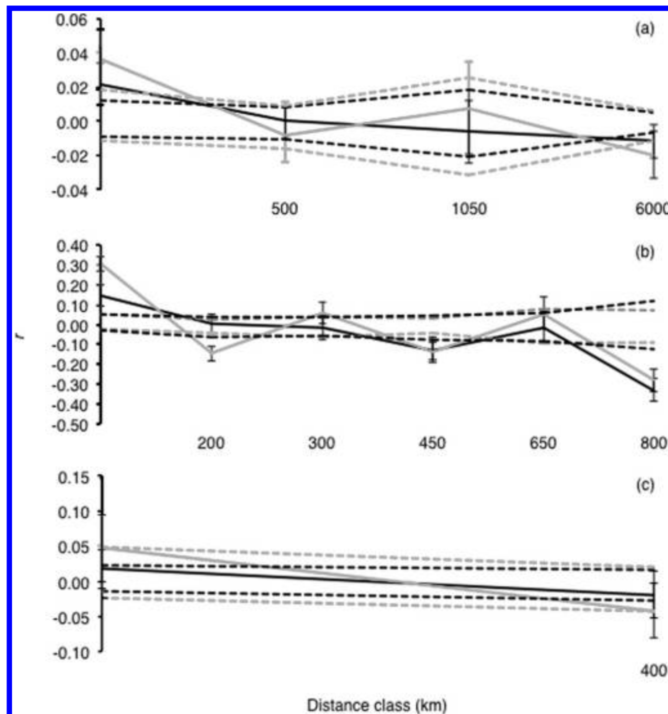
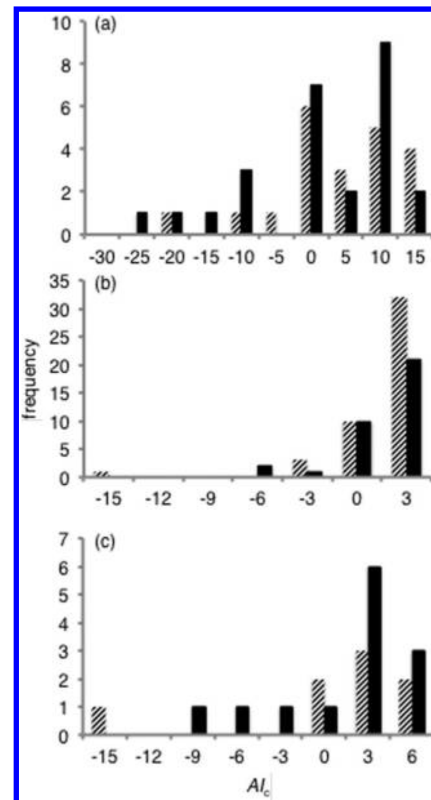


Fig. 5. Frequency distribution of corrected assignment indices (AIc) for males (solid bars) and females (hatched bars) of (*a*) *O. halei*, (*b*) *O. maculatus*, and (*c*) *O. ornatus* based on amplified fragment length polymorphism (AFLP) data.



relatedness was significantly higher than male relatedness ($P = 0.027$) in the samples from SY, but no other within-population comparisons were significant (supplementary data Table S3[1]). The mean corrected assignment index (mAIc) for *O. halei* females was often higher, and the variance lower, than for males (supplementary data Table S3[1]), suggesting that males are more likely to be migrants. These differences, however, fell within the distribution of randomized values that were independent of sex. The frequency distribution of AIc values shows slightly more males in the negative portion; however, the distributions are broadly overlapping (Fig. 5*a*).

Significant population structure was detected for nuclear AFLP markers in *O. maculatus*, but this species did not exhibit mtDNA partitioning throughout the sampling range (Table 2*b*). However, it should be noted that mitochondrial haplotypic diversity is very low in this species. Spatial autocorrelation correlograms for both sexes of *O. maculatus* showed significant positive autocorrelation within sites, a signal that decreased with increasing geographic distance (Fig. 4*b*). Overall, correlograms were significant for both males ($\omega = 89.532$, $P < 0.0001$) and females ($\omega = 53.825$, $P = 0.001$). Within-site autocorrelation was higher in females than in males, but there was no overlap in bootstrap confidence intervals about *r* between the sexes, indicating autocorrelation is significantly higher in females than in males at small distance classes. The multidistance class test of among-sex heterogeneity was significant ($\omega = 47.861$, $P < 0.0001$) as were single-class tests for the first two distance classes (supplementary data Table S2*b*[1]). Taken together, these results suggest that dispersal is male-biased in *O. maculatus*. This was not supported, however, by the tests based on relatedness or AIc. Mean FF and MM relatedness were not significantly different (FFr = –0.008, MMr = –0.011; $P = 0.458$), nor were FF and MF relatedness (FFr = –0.008, MFr = 0.001; $P = 0.660$). There was also no obvious difference in the distributions of male and female AIc values (Fig. 5*b*), and statistical tests based on the mean and variance of AIc were all nonsignificant for this species (supplementary data Table S3[1]).

Significant partitioning was revealed at mtDNA markers but was absent from nuclear AFLP data in *O. ornatus* (Table 2*c*), suggesting male-biased dispersal. The within-site autocorrelation coefficient was positive for both sexes and higher for females than for males (Fig. 4*c*). Autocorrelation decreased with increasing geographic distance for both sexes. The magnitude of the observed correlation in both sexes fell within the bounds of the null model of randomly distributed genotypes. Confidence intervals about *r* were overlapping, and single- and multidistance class tests of among sex heterogeneity ($\omega = 4.2943$, $P = 0.3676$) were all nonsignificant (supplementary data Table S2*c*[1]). FF relatedness was not significantly higher than MM (FFr = –0.022, MMr = –0.023; $P = 0.481$) and MF (FFr = –0.022, MFr = –0.013; $P = 0.627$). Although there appeared to be more males in the negative portion of the distribution of AIc values (Fig. 5*c*), tests based on the mean and variance of AIc were nonsignificant for this species (supplementary data Table S3[1]).

Discussion

Dispersal is a complex behaviour that has biological, ecological, conservation, and evolutionary implications. Studying dispersal and population connectivity over broad geographical areas in directly dispersing marine organisms, such as elasmobranchs, is complicated because they can be difficult to observe and sample. This study represents the first comparative genetic analysis of dispersal patterns and population connectivity in codistributed demersal sharks. Based on a relatively large molecular dataset, we identify spatial genetic structure in wobbegongs and provide evi-

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dence that dispersal in some of these species may be characterized by sex biases and by isolation-by-distance.

Site fidelity in wobbegongs has been documented previously (Carraro and Gladstone 2006; Huveneers et al. 2006; Lee et al. 2014, 2015). Huveneers et al. (2006) describe strong fidelity to specific resting sites in *O. halei* and long-term “residents” that could be located within the study area for up to 2 years after acoustic tagging. Similarly, most resightings of tagged individuals in a mark–recapture study of *O. ornatus* occurred within close proximity of, or often in the exact position as, the previous sighting (Carraro and Gladstone 2006). An acoustic telemetry study of *O. maculatus* also demonstrated high site fidelity with individuals returning to the same ~0.2 km^2 marine reserve annually for up to 5 years (Lee et al. 2015). Although useful for identifying behavioural tendencies, these studies are limited by the relatively small sample sizes and spatial scales that could be considered. Our genetic data confirms restricted movements by wobbegongs, as evidenced by restricted gene flow over broader spatial and temporal scales than previously assessed by telemetry studies and visual surveys. Genetic differentiation between sampling sites was detected for *O. halei*, *O. maculatus*, and for, at least, female *O. ornatus* (Table 2). Significant positive spatial autocorrelation within sampling sites and between adjacent sampling sites was also detected (Fig. 3; supplementary data Table S1[1]). Philopatric behaviour is a complex component of habitat use and population connectivity in elasmobranchs. Even some highly mobile species display a tendency to return to specific locations such as natal, nursery, mating, or feeding areas (Chapman et al. 2015; Hueter et al. 2005; Simpfendorfer and Heupel 2004; Speed et al. 2010). Reproductive philopatry can promote genetic structure throughout regions that lack physical barriers to dispersal and is thus an important behaviour to identify from a conservation planning perspective (Secor 2002). Genetic analyses of population structure have revealed regional philopatry, referring to wide-ranging individuals preferentially returning to their birth region for reproduction, but not necessarily the exact birthplace within this region (Chapman et al. 2015), in sharks, including *Carcharodon carcharias* (Blower et al. 2012; Pardini et al. 2001) *Triakis semifasciata* (Lewallen et al. 2007), *Sphyrna lewini* (Daly-Engel et al. 2012), *Mustelus henlei* (Sandoval-Castillo and Beheregaray 2015), and several species of Carcharhinidae (Karl et al. 2011; Keeney et al. 2005; Tillett et al. 2012*a*, 2012*b*). More recently, both indirect and direct evidence has revealed that *Negaprion brevirostris* (Feldheim et al. 2014) and *Carcharhinus melanopterus* (Mourier and Planes 2013) are even philopatric to their natal nursery ground (natal philopatry; Chapman et al. 2015). Thus far, this behaviour has been little documented in demersal species of elasmobranchs (although see Lewallen et al. 2007; Sandoval-Castillo and Beheregaray 2015), but is mostly known from larger, coastal species in which adults migrate to inshore habitats for parturition but are otherwise dispersed and segregated from juveniles (Springer 1967). Under such a life history model, philopatry is expected to evolve if selection favours the return to sites that have previously been favourable for offspring survival (Dudgeon et al. 2012; Portnoy 2010). In this study, spatial autocorrelation analyses of all three species of wobbegongs revealed positive genetic autocorrelation among individuals sampled at the same site (although this signal was not significant for *O. ornatus*, a result we attribute to the relatively small sample available for that species; Fig. 3*c*; supplementary data Table S1[1]), indicating that individuals sampled in close proximity to one another are more closely related than those chosen at random. This, together with observations of residency and site fidelity based on telemetry and survey studies, suggests that a scenario of local recruitment in wobbegongs is promoting population genetic structure. It is therefore possible that the spatial genetic differentiation we detected is indicative of, at least, regional philopatry in these demersal species. However, while there are no apparent major geophysical barriers to dispersal throughout the sampled region, it is also possible that observed spatial genetic differentiation represents generally restricted dispersal and a limited home range in wobbegongs, rather than habitual return to an area for breeding. Wobbegongs certainly seem to exhibit a preference for shallow water and topographical complexity (Carraro and Gladstone 2006; Huveneers et al. 2006, 2009), which may restrict dispersal over large geographic distances interspersed by areas of unsuitable habitat. That being said, juvenile sharks were not encountered during our sampling period, despite sampling a large portion of the range of these three species along the east coast of Australia (Fig. 1; Huveneers et al. 2007*a*, 2007*b*). Extensive diver surveys throughout NSW also failed to record neonate or small juvenile wobbegongs (Huveneers et al. 2009). This suggests that size segregation may occur in wobbegongs, perhaps indicating they use nursery grounds and may thus be subject to similar selective pressures proposed to promote philopatric behaviour and population structure in other coastal sharks (Dudgeon et al. 2012; Portnoy 2010). Confirming the existence and location of wobbegong nursery grounds, which are currently elusive, is essential for discriminating between restricted dispersal potential and philopatry as possible forces driving spatial genetic differentiation. Moreover, while adopting a genetic approach to identify philopatric behaviour is powerful because we can use gene flow to infer movement patterns over generational time periods, accurately discerning philopatry using genetics depends upon sampling strategy. For example, if animals are indeed philopatric, this may not be detected if individuals are sampled during their dispersed phase rather than during aggregation for breeding. While the multilocus spatial autocorrelation analyses we have used here are powerful for detecting subtle spatial genetic structure, which can help pinpoint the geographical specificity of philopatric behaviour, the analysis is sensitive to sample size with a power trade-off between scaling of distance classes and number of samples included in each class (Banks and Peakall 2012; Smouse et al. 2008). Ideally, spatial autocorrelation analysis would be conducted on a large number of individually georeferenced samples of juveniles or breeding adults collected across the spatial scale at which population connectivity is thought to occur. Unfortunately, this was not achievable here because sampling took place via interaction with the commercial fishing industry, and detailed collection information for each individual shark was not available.

If observed spatial genetic differentiation is indeed indicative of the extent of wobbegong dispersal potential, we may perhaps expect to see population genetic structure characterized by isolation-by-distance, as predicted for many directly dispersing marine taxa (Palumbi 2003) and similar to that described for another closely related orectolobiforme, *Stegostoma fasciatum* (Dudgeon et al. 2009). Based on our sampling scheme, adjacent sampling sites appeared to exchange more members. Individuals sampled at the same site were found to be positively autocorrelated, a signal also found in comparisons between individuals from adjacent sampling sites (distance classes 150–200 km) in, at least, *O. halei* and *O. maculatus*. Individuals from more geographically distant locations generally showed negative autocorrelation (Figs. 3*a*, 3*b*). This pattern suggests that gene flow may be constrained by geographic distance. Some observations are noteworthy as they are somewhat contradictory to this pattern. First, patterns of mtDNA population structure in *O. halei* and *O. maculatus* do not mirror those observed in the AFLP data. Here, the comparative analysis of three largely codistributed species is beneficial because it shows that extremely shallow, or lack of, population divergence in mtDNA is a feature common to all species (Fig. 2). Mitochondrial DNA diversity in wobbegongs is among the lowest (Fig. 2; Table 1) recorded for any shark species (Daly-Engel et al. 2010; Hoelzel et al. 2006; Schultz et al. 2008), which limits inferences about population structure using this marker dataset.

Orectolobus ornatus showed somewhat contrasting results to the other two species in that statistically, spatial genetic structure did

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not depart from the null model (Fig. 3*c*), and populations were not significantly differentiated at AFLP markers using frequency-based analyses (Table 2*c*). One possibility is that the lack of AFLP structure in *O. ornatus* is an artefact of the lower sample sizes (Table 1) used for this species. It takes only a very small amount of migration to homogenize allele frequencies and eradicate signal of differentiation in population level analyses, such as those based on *F* statistics (Spieth 1974; Waples 1998). Multilocus spatial autocorrelation analysis should be more powerful to detect subtle spatial genetic structure because it is focused at the individual level and does not require divergence in overall population allele frequencies. But, as mentioned previously, power is dependent on sample sizes and spatial scaling of distance classes (Banks and Peakall 2012; Smouse et al. 2008). Overall, patterns of spatial genetic structure in *O. ornatus* were consistent with those in *O. halei* and *O. maculatus* in that there was positive autocorrelation among individuals sampled within the same site and between adjacent sites. It is therefore possible that lack of a statistically significant result is due to limited power as a result of the smaller sample sizes available for this species. Another explanation is that *O. ornatus* disperses more than *O. halei* and *O. maculatus*. Carraro and Gladstone (2006) recorded specific cases of site fidelity in *O. ornatus* using mark–recapture methods; however, temporal changes in abundance, length distribution, and sex ratio were also reported. The authors concluded these results were demonstrative of a dynamic population of *O. ornatus* and reflected movements by individuals at a much larger spatial scale. Periods of residency were temporary and some individuals returned for short periods of time after long absences or were never sighted again (Carraro and Gladstone 2006). Residency was also described for *O. halei* but for considerably longer duration than for *O. ornatus*. Excursions away from the study site by *O. halei* were usually short, perhaps also supporting the hypothesis that *O. halei* exhibits more restricted movements than *O. ornatus* (Huveneers et al. 2006). It must be noted, however, that methodological biases complicate direct comparisons of the various datasets produced by these studies. Similarly, our genetic datasets are not directly comparable across species because of differences in numbers of samples and markers. All of these factors limit our ability to draw firm conclusions regarding the relative dispersal potentials of *O. halei*, *O. maculatus*, and *O. ornatus*. Improved fine-scale spatial sampling would allow a better assessment of whether gene flow is indeed occurring in a stepping-stone pattern as predicted by an isolation-by-distance model in these species. This certainly seems to be the case based on the spatial autocorrelation analysis of *O. maculatus*, where autocorrelation clearly decreases with increasing distance class (Fig. 3*b*), but is more ambiguous to interpret for the other two species, where confidence intervals are overlapping among distance classes.

Interpreting whether dispersal is male-biased in wobbegongs is ambiguous based on the analyses presented in this study. None of the population-level tests based on relatedness or AIc showed statistically supported evidence of male-biased dispersal (supplementary data Table S3[1]). Marker-based relatedness estimates, however, are notorious for having extreme associated variance, especially when applied to dominant markers (Lynch and Milligan 1994; Lynch and Ritland 1999), to the extent that it has been suggested that dominant markers have limited utility for relatedness estimation (Hardy 2003; Lynch and Ritland 1999). The accuracy of assignment tests may also be affected by assumptions made when estimating allele frequencies based on dominant data. Finally, the population-level randomization test based methods conducted here are most sensitive in situations of intermediate dispersal rates, intense sex-bias, and large sample size such that allele frequencies can be estimated with enough precision to distinguish "residents" from "immigrants" based on their genotypes (Goudet et al. 2002). Based on these factors, it is possible that the capacity for these tests to detect sex-biased dispersal may have been limited by the sample sizes and dominant nature of this dataset.

Individual-based multilocus spatial autocorrelation analysis is perhaps a more powerful approach to detecting sex-biased dispersal. The autocorrelation coefficient within sampling sites was indeed consistently higher in females than in males across the three species (Fig. 3), in line with the expectation under male-biased dispersal. The ability to detect statistically significant differences between the sexes using this method is also highly dependent upon sample size and requires strong spatial genetic structure in the more philopatric sex (Banks and Peakall 2012; Smouse et al. 2008). Banks and Peakall (2012) stress the importance of sampling at or below the scale to which dispersal is restricted in the more philopatric sex, rather than conducting spatially random sampling of individuals from a larger arbitrarily designated "population". The latter may be the case in the present sampling scheme since samples could not be individually georeferenced and were grouped into distance classes based on the broader geographic region within which they were sampled. Using spatial autocorrelation to detect sex-biased dispersal in wobbegongs could therefore also benefit from better fine-scale spatial information regarding sample origin.

Concluding remarks

Our study describes spatial genetic partitioning in codistributed species of wobbegongs. This contributes to our understanding of dispersal patterns in these species and informs on spatial scales that may be appropriate for managing populations of demersal mesopredator elasmobranchs. Wobbegong dispersal may be male-biased and characterized by isolation-by-distance; however, improved fine-scale spatial sampling is required to refine estimates of the spatial scale at which genetic partitioning occurs in wobbegongs. Female reproductive philopatry may also be contributing to the observed spatial genetic structure. However, the identification of nursery grounds, to allow sampling of juvenile or breeding adults, is required to confirm philopatric behaviour and to distinguish it from restricted dispersal potential. Eastern Australian wobbegong species are commercially harvested, and catch declines that possibly reflect overexploitation have been reported (DPI 2001, 2006; Scandol et al. 2008). General biological and ecological data relevant to fisheries management have been collected for these species, resulting in more aggressive management of these populations (Huveneers 2006*a*). This work now allows for species-specific information regarding population connectivity and spatial genetic structure to be incorporated into wobbegong-specific management options.

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