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BRIEF COMMUNICATION

Low levels of genetic structuring in King George whiting *Sillaginodes punctatus* across two geographic regions

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Running head: *SILLAGINODES PUNCTATUS* POPULATION STRUCTURE

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Postlarvae of King George whiting *Sillaginodes punctatus*, a commercially important fish species in southern Australia, were collected from multiple locations across south and south-eastern Australia. Genetic analyses using seven microsatellite markers found little evidence of genetic structure suggesting high levels of connectivity between the regions. The results found no evidence of a distinct source spawning population within the south-eastern range sampled in this study.

Key words: connectivity; fisheries management; microsatellite markers, population structure; *Sillaginodes punctatus*.

King George whiting *Sillaginodes punctatus* (Cuvier 1829) is a temperate, demersal fish species, distributed along the southern coastline of Australia from Sydney to Perth, including northern Tasmania. It forms the basis of valuable commercial and recreational fisheries, particularly in South Australia and Victoria, but the population structure and degree of connectivity between these two states is currently unknown. To determine the appropriate scale at which these fisheries should be managed, there is a need to establish whether *S. punctatus* distributed throughout South Australia and Victoria (Fig. 1) should be regarded as a single or multiple populations.

Sillaginodes punctatus has a complex life history involving a number of habitat shifts at different developmental stages; adults spawn in coastal waters around reefs in the autumn and early winter (Fowler *et al.*, 2000a) and postlarvae (approximately 20 mm standard length) settle in shallow seagrass habitat in sheltered bays a few months later (Jenkins & May, 1994; Fowler & Short, 1996). The extended larval period, which can be >160 days (Jenkins & May, 1994) means there is scope for considerable dispersal during this stage. Post-larvae regularly recruit to a number of bays in both South Australia and Victoria from August to December each year (Fowler *et al.*, 2000b; Jenkins *et al.*, 2000). As they grow they enter the deeper areas of the bays, where they reside for around four years before moving offshore when they become mature (Hyndes *et al.*, 1998; Jenkins, 2005).

Hydrodynamic modelling of probable spawning locations and larval advection pathways has suggested that post-larvae in South Australia represent numerous discrete, self-recruiting populations derived from local (40–150 km) spawning (Fowler *et al.*, 2000b), whilst it is likely that *S. punctatus* in Victoria form a single population that spawns along the

west coast of Victoria (Jenkins *et al.*, 2000). Northern Tasmania was shown to be an alternative (although less likely) spawning location for post-larvae arriving in Victoria, particularly those in Corner Inlet (Jenkins *et al.*, 2000). To date, the only known spawning grounds for this species are off the coast of South Australia (Fowler *et al.*, 1999), but it remains unclear if this spawning population is the source for post-larvae that recruit to the Victorian bays. In order to assist the management of the state-based fisheries for this species there is a need to determine whether the Victorian populations are dependent on the South Australian populations as a source of recruits, or whether they are self-recruiting (or a mix of the two), particularly because of the possible need for cross-jurisdictional fishery management.

This study builds on previous genetic work that showed little population differentiation for *S. punctatus* throughout South Australia and Victoria (Haigh & Donnellan, 1998), suggesting the existence of one large panmictic population in these waters. The study by Haigh & Donnellan (1998) did not incorporate samples from Corner Inlet in Victoria, which has the strongest indication of potentially being a discrete population and therefore a potentially important source of recruits into other Victorian populations.

Sillaginodes punctatus post-larvae were collected from three regions in South Australia (Kangaroo Island, Spencer Gulf and Gulf St Vincent) and three regions in Victoria (Port Phillip Bay, Western Port and Corner Inlet) during October and November 2011 (Fig. 1). Samples were collected using a 10 m seine net with approximately 1 mm² mesh, over shallow subtidal seagrass (*Zostera* and *Heterozostera* spp.) habitat. Thirty individuals were collected from 3 different sites within each region, with the exception of Corner Inlet which

had only 2 sites (Fig. 1). Genomic DNA was extracted from a total of 510 individuals using QIAGEN DNEasy Blood and Tissue kits (www.qiagen.com), as per the manufacturer's instructions. Levels of connectivity were assessed using seven polymorphic microsatellite markers: Sp19, Sp22, Sp32, Sp35, Sp36, Sp38 and Sp39 (Haigh & Donnellan, 2000).

Microsatellites were amplified using a PCR touchdown programme using the following thermal cycling conditions: initial hot start at 94° C for 15min; five cycles of 94° C for 45 s, 65° C for 45 s, 72° C for 45 s; five cycles of 94° C for 45 s, 60° C for 45 s, 72° C for 45 s; ten cycles of 94° C for 45 s, 57° C for 45 s, 57° C for 45 s, 72° C for 45 s; twenty cycles of 94° C for 45 s, 55° C for 45 s, 72° C for 45 s; final elongation at 72° C for 15min. PCR was conducted in 11- μ l volumes containing; 10 ng of genomic DNA; 5 μ l PCR Master Mix (QIAGEN), 4 μ l primer multiplex consisting of 0.26 μ M of each forward primer with a fluorescent dye associated tag (FAM-GCCTCCCTCGCGCCA; NED-GCCTTGCCAGCCCGC; PET-CGGAGAGCCGAGAGGTG; VIC-CAGGACCAGGCTACCGTG) and 0.13 μ M of reverse primer. PCR amplicons were electrophoresed on an ABI 3130xl Genetic Analyzer, incorporating LIZ 500 (-250) size standard (Applied Biosystems; www.appliedbiosystems.com). Alleles were scored using GeneMapper 3.7 (Applied Biosystems).

Each pairwise combination of loci was tested for linkage disequilibrium within each sample site using GENEPOP 4.2 (Rousset 2008) and conformity to Hardy Weinberg Equilibrium (HWE) was tested using Genalex 6.5 (Peakall & Smouse, 2012), with significant deviations determined using exact tests and a sequential Bonferroni correction applied for multiple tests (Rice, 1989). No significant linkage was detected between any loci and the majority of sites

were in HWE equilibrium. Significant departures from Hardy-Weinberg proportions were observed in 6 sample sites (Table I), but the levels of deviation were not large and were not consistent across loci or locations. HWE data for individual loci are provided in Supporting Information Table S1.

Estimates of population structure and patterns of connectivity were estimated as F -statistics (Weir & Cockerham, 1984) in the program FSTAT 2.9.3.2 (Goudet, 2002). Pairwise estimates of F_{ST} between all sample site comparisons were examined using Arlequin 3.5 (Excoffier & Lisher 2010) and a sequential Bonferroni correction applied for multiple tests (Rice, 1989). To ensure that underlying levels of heterozygosity in the marker system did not bias estimates of genetic differentiation Hedrick's standardised G'_{ST} (Hedrick, 2005) and Jost's D (Jost, 2008) were also calculated using the program Genalex 6.5 with upper and low 95% confidence interval estimated by bootstrapping (999 bootstraps) over loci (Peakall & Smouse, 2012).

A Bayesian analysis was performed using Structure 2.3.4 (Pritchard *et al.*, 2000), which identifies genetically similar clusters of individuals with multilocus genotypes. Estimation of the number of populations ignoring sampling location (K) was completed using ten independent runs with $K = 2-17$ at 100 000 Markov chain Monte-Carlo (MCMC) repetitions combined with a 100 000 burn-in period using an admixture model with correlated frequencies. The number of genetic clusters was determined using the Evanno (" K ") method (Evanno *et al.*, 2005) in the program STRUCTURE HARVESTER 0.6.94 (Earl & von Holdt, 2011). CLUMPAK (Kopelman *et al.*, 2015) was used to merge runs for each K and build the final plots.

Analyses showed that levels of genetic diversity did not vary significantly between sample sites or regions (Table I). The number of alleles per locus varied from 2 to 19 with a mean $\pm$ S.E. = 3.93 ± 0.33 across all loci and sample sites. This pattern was similar to that seen for expected heterozygosity (H_E), which varied from 0.29 to 0.48 with a mean $\pm$ S.E. = 0.4 ± 0.019 across all sample sites and loci. Overall levels of inbreeding were low, with mean $\pm$ S.E. $F_{IS} = 0.016 \pm 0.007$.

Overall estimates of genetic differentiation were low with a global F_{ST} estimate of 0.007 (95% C.I. 0.001 to 0.018). While the overall F_{ST} value was significantly different from zero, a comparison of pairwise estimates of F_{ST} between all sample sites found only 4 significant differences out of 136 pairwise comparisons (Table II). Three of these were for samples collect from Port Victoria suggesting that this location may be more isolated compared with others. The only other significant difference was detected between Stony Point and Corinella (both in Western Port, Victoria). Alternative estimates of genetic differentiation using Hedrick standardized G'_{ST} and Jost's D were similar to that estimated for F_{ST} (Table III). The analysis of molecular variance (AMOVA) showed that the majority of variance (98%) was due to differences within sample sites with little difference detected between sites (2%) and none of the variance attributable between regions. The Bayesian Structure analysis revealed no distinct genetic clusters with all " K values being small (Fig. 2). Visualisation of $K = 2, 4$ & 6 confirmed no structure at these levels (Fig. 2). Estimates of inbreeding (F_{IS}) were small and not significantly different from zero in any location sampled (mean $\pm$ S.E. $F_{IS} = -0.016 \pm 0.07$).

The low levels of genetic differentiation detected among the majority of sample sites suggest that there are high levels of connectivity between South Australia and Victoria. This is supported by patterns of genetic differentiation (pairwise F_{ST}), AMOVA and Structure analyses. As a result of their environment, marine species are typically characterised by high levels of dispersal and gene flow, resulting in low measures of F_{ST} between populations (Waples, 1998). Currently each state fishery is managed independently. It is crucial that the spawning sources that supply the Victorian bay and inlet fisheries are known, so that these spawning aggregations, which may be interstate, can be adequately protected. Large adults are exploited in South Australia but not Victoria and where spawning aggregations are fished, this could lead to recruitment overfishing.

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Supporting Information

Supporting information can be found in the online version of this paper.

TABLE S1. Hardy Weinberg equilibrium data for seven microsatellite loci; significant deviations were determined after adjustment of α -value applying a sequential Bonferroni correction and are displayed in bold ($***P < 0.001$).

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