Vol. 684: 103–116, 2022
https://doi.org/10.3354/meps13952

MARINE ECOLOGY PROGRESS SERIES
Mar Ecol Prog Ser

Published February 17

# Spawning sources of a coastal fishery species inferred from otolith chemistry and microstructure: implications for management

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ABSTRACT: Spawning sources of King George whiting *Sillaginodes punctatus* populations in the states of South Australia and Victoria (south-eastern Australia) were analysed using otolith chemistry and microstructure from post-larvae sampled from 3 nursery areas in each state in the spring of 2011 and 2012. Univariate and multivariate analysis of the chemistry of the core region of otoliths showed differences between states, particularly for the 2011 cohort, primarily related to higher Mg in South Australian samples, while differences in Sr and Zn also made a contribution. Even though spawning times overlapped, early larval growth rates were higher for post-larvae from South Australia than Victoria. Differences in microchemistry were most evident for elements influenced by physiological processes and were potentially influenced by the different larval growth rates. Overall, otolith chemical and microstructure analyses for post-larvae in Victoria and South Australia indicated that spawning sources for the 2 states were different, qualified by results from otolith microchemistry that were less clear for the 2012 cohort. Even though genetic analyses do not indicate genetic differentiation across the 2 states, and therefore would support cross-jurisdictional management, the results of this study give qualified support to the current arrangement wherein the *S. punctatus* fishery is managed separately by the individual jurisdictions, subject to further information on stock structure coming to light in the future.

KEY WORDS: Population connectivity · Stock structure · Otolith chemistry · Management · King George whiting · *Sillaginodes punctatus*



## 1. INTRODUCTION

Rational management of fishing depends on an understanding of the population connectivity of exploited species (Sinclair 1988). Connectivity of marine fish populations depends on metapopulation structure, which is a function of both larval dispersal and adult movement that defines the source–sink dynamics. The long duration of the pelagic larval phase of many marine fish (weeks to months) can potentially lead to dispersal over significant distances with currents (Simpson et al. 2014). Long larval duration, however, may not always lead to significant dispersal distances where currents are recirculating or larval behaviour and swimming ability can lead to modified dispersal trajectories and high rates of self-recruitment (Jones et al. 2009). For fishery species where the scale of larval dispersal is large, there is potential for the scale of the population to exceed the jurisdictional boundaries in place for fisheries management.

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Based on this understanding of metapopulation structure and connectivity, fishery management is best focussed on individual subpopulations or 'stocks' that are essentially self-reproducing (Hilborn & Walters 1992). These subpopulations can be relevant at an ecological level without necessarily showing genetic differentiation, which requires only a small exchange of individuals to maintain genetic homogeneity (Cowen & Sponaugle 2009). As such, the impacts of fishing on a stock should have negligible impact on the dynamics of other stocks.

Fisheries management is often conducted at the jurisdictional level. This approach to management may be problematic where there is potential for fish stocks to straddle jurisdictional boundaries, and more than one management regime may apply to a single stock (Fowler et al. 2017). Stocks can overlap jurisdictional management boundaries at both the national level (Strømme et al. 2016) and at the state level (Morgan et al. 2019). Ultimately, cross-jurisdictional management may need to be implemented for these fisheries (Gullestad et al. 2020).

One species to which this issue could potentially apply is the King George whiting *Sillaginodes punctatus*, which supports important commercial and recreational fisheries throughout southern Australia, particularly in the adjacent states of Victoria and South Australia (Kailola et al. 1993). In both states, post-larvae (approximately 2 cm in length) settle into shallow seagrass beds within bays and gulfs in the winter/spring (Jenkins & May 1994, Fowler & Short 1996, Jenkins et al. 1996). These juveniles then grow for 3 to 4 yr within the sheltered bays and gulfs until they move offshore at about the time of reaching sexual maturity (Fowler et al. 2000b, 2002, Hamer et al. 2004). The majority of the *S. punctatus* catch is taken in the bays and gulfs, between the times of reaching minimum legal length and the point where offshore migration occurs.

The only known spawning area for *S. punctatus* across the 2 states, based on collection of running ripe adults and eggs and young larvae, is in central South Australia (Fowler et al. 1999, 2000b). Extensive sampling of adult *S. punctatus* along the coast of Victoria did not find any evidence of significant spawning, and there was a trend for fish to be larger and older in the west of the state, indicating a possible movement of fish to the west after they leave the bays and estuaries (Hamer et al. 2004).

The duration of the larval phase of *S. punctatus* ranges from approximately 90 to 160 d (Jenkins & May 1994, Jenkins et al. 2000). Otolith microstructure of post-larvae in both states showed that *S. punctatus* spawning occurs in autumn to early winter. Computer modelling of larval dispersal confirmed that in central South Australia, spawning is likely to be relatively local (Fowler et al. 2000a). In contrast, the spawning area for post-larvae in central Victorian bays was predicted to be 100s of kilometres to the west, including western Victoria and south-eastern South Australia (Jenkins et al. 2000). The results from the modelling suggested that post-larvae in Victoria may come from spawning in South Australia, but not from as far west as the known spawning area in central South Australia (Jenkins et al. 2000).

Whether or not the current arrangement of separate jurisdictional management is suitable depends on whether the *S. punctatus* fisheries in Victoria and South Australia are dependent on fish originating from a single spawning population or separate spawning populations. Evidence from microsatellite markers suggests that *S. punctatus* fisheries in Victoria and South Australia are part of a genetically homogeneous population (Kent et al. 2018). However, populations can be separated on time and space scales that are relevant to fisheries management even though gene flow may occur that leads to genetic homogeneity (Begg & Waldman 1999).

Here we investigated the spawning sources of *S. punctatus* in Victoria and South Australia to improve our understanding of the metapopulation structure of the species across the region. We used otolith-based analyses (microstructure and chemistry) to investigate potential spawning sources for *S. punctatus*. Our aim was to determine whether there were different spawning sources for *S. punctatus* between (and within) the 2 states. In this way, we address the question of whether *S. punctatus* in Victoria and South Australia are best considered separate stocks or part of one broad stock for fisheries management purposes.

## 2. MATERIALS AND METHODS

### 2.1. Field sampling

Post-larvae of *Sillaginodes punctatus* were collected from sites within 3 nursery areas (hereafter termed 'regions') in Victoria (Port Phillip Bay, Western Port and Corner Inlet) and 3 in South Australia (Gulf St Vincent, Spencer Gulf and Kangaroo Island) (Fig. 1). Samples from Port Phillip Bay came from an annual monitoring programme of 8 sites (Jenkins & King 2006).

Samples were collected over 2 sampling years, 2011 and 2012, in the spring to early summer period

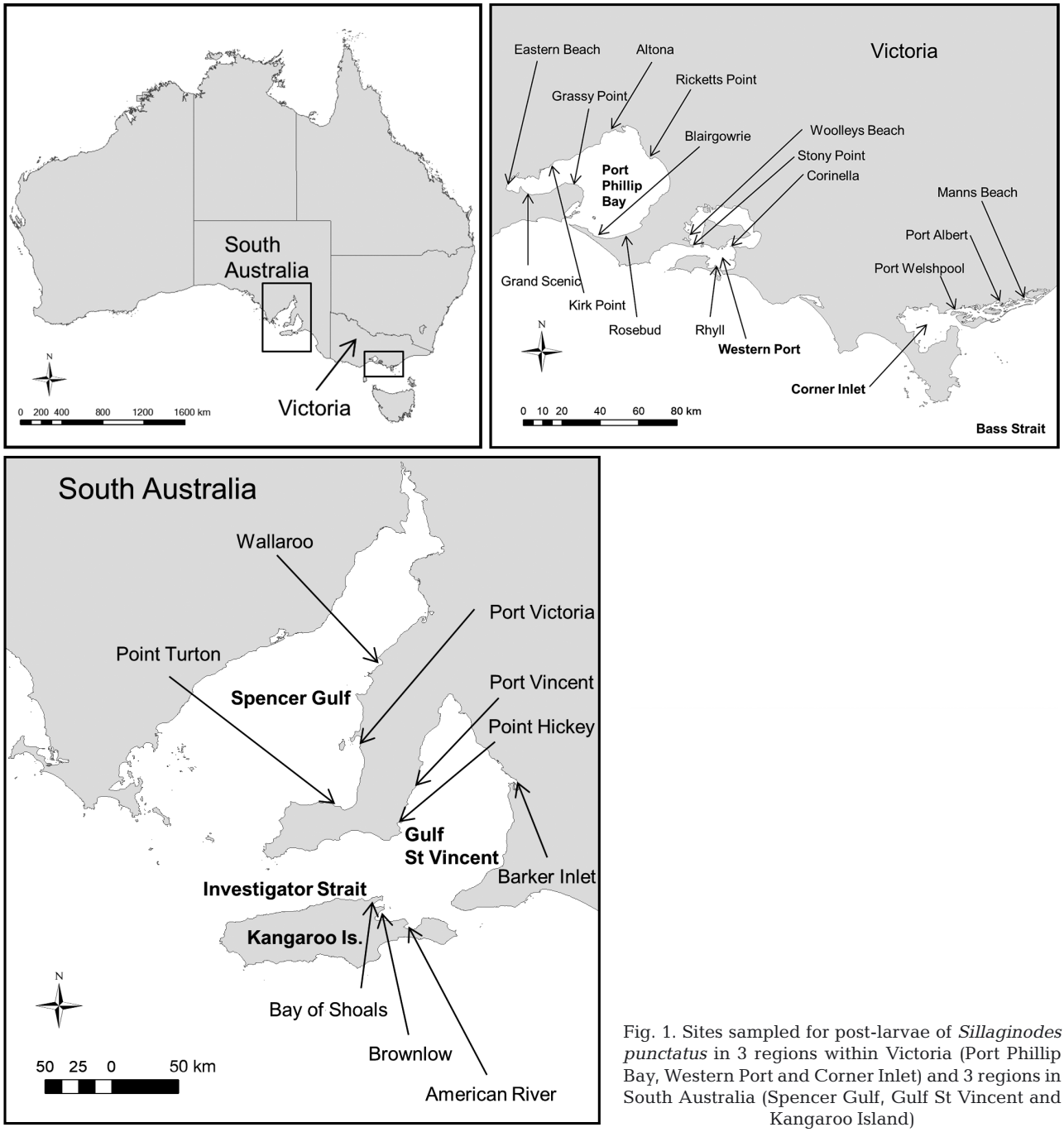


Fig. 1. Sites sampled for post-larvae of *Sillaginodes punctatus* in 3 regions within Victoria (Port Phillip Bay, Western Port and Corner Inlet) and 3 regions in South Australia (Spencer Gulf, Gulf St Vincent and Kangaroo Island)

(Table 1). One round of sampling was undertaken at sites in all regions in October, and a second round of sampling was undertaken in November in all Victorian regions and also in the Gulf St Vincent region in South Australia.

Samples were collected from intertidal to shallow–subtidal (0.5 m below Mean Low Water Spring tide) beds of the seagrasses *Zostera muelleri* and *Heterozostera nigricaulis*, with a small (10 m × 2 m), fine-mesh (1 mm) research seine. Samples were collected within 3 h of low tide. Multiple hauls of the net were taken until a minimum sample of 30 post-larvae was collected.

In 2011, individual post-larvae were placed in separate vials and frozen for later analysis. Because of some cracking of otoliths collected in 2011, a different approach was taken 2012 whereby post-larvae were preserved in absolute (95%) ethanol.

Table 1. Number of *Sillaginodes punctatus* otoliths used for chemistry and increment analysis from regions across South Australia and Victoria

| Region | 2011 | | | 2012 | | |
|---|---|---|---|---|---|---|
| | No. of sites | Chemistry | Increments | No. of sites | Chemistry | Increments |
| **South Australia** | | | | | | |
| Spencer Gulf | 3 | 54 | 32 | 2 | 54 | 58 |
| Gulf St Vincent | 3 | 108 | 41 | 3 | 108 | 26 |
| Kangaroo Island | 4 | 52 | 30 | 3 | 54 | 22 |
| **Victoria** | | | | | | |
| Port Phillip Bay | 7 | 112 | 31 | 8 | 126 | 58 |
| Western Port | 4 | 104 | 30 | 4 | 104 | 31 |
| Corner Inlet | 2 | 117 | 33 | 3 | 95 | 35 |

## 2.2. Laboratory analysis

### 2.2.1. Otolith chemistry

Post-larval *S. punctatus* were measured and their sagittal otoliths were removed under a dissecting microscope with stainless steel needles. Dissected otoliths were triple rinsed in Milli-Q water in an Eppendorf tube, and then placed in clean 0.2 ml Eppendorf PCR tubes to dry. One of the dried otoliths from each fish was embedded in a small disc of epoxy resin (Struers Epofix) set within a cut section of drinking straw. Once cured, the disks were removed from the straw and then mounted onto glass microscope slides with crystal bond, and polished in the sagittal plane to the primordium from the proximal surface down with lapping films (3M™ diamond films) lubricated with Milli-Q water. The polished sections were then moved onto a new slide for analysis, each of which had up to 24 samples fixed. The analysis slide was then sonicated in Milli-Q water for 3 min, liberally rinsed in Milli-Q water, dried and stored in a plastic container. Preparation order of individual otoliths was randomised, and the 24 samples fixed to each slide consisted of 4 randomly selected otoliths from each of the 6 regions. This meant that samples analysed within any particular block of ICP-MS time were a random assortment from all regions.

Otoliths were analysed for elemental chemistry using a laser ablation inductively coupled plasma mass spectrometer (New Wave Research UP-213 Nd: YAG ultraviolet laser microprobe coupled to a ThermoFinnigan Element 2 high-resolution ICP-MS). Each otolith section was ablated using a 40 µm diameter point sample centred on the primordium, termed the 'core'. The sample incorporated the primordium and approximately the first 15 d of larval life, which is assumed to have been insufficient time for larvae from a common spawning source to disperse into different environments.

The laser settings were 40 µm beam diameter, repetition rate of 5 Hz and fluence of 11 J $cm^{-2}$. Otolith surfaces were briefly pre-ablated (3 s with the above settings) to remove any residual surface contamination. Each sample point was ablated for approximately 30 s to obtain 30 measurements of each isotope, with an additional 20 measurements of the blank sample gases taken prior to each ablation. Data were collected by the ICP-MS for the isotopes $^{25}Mg$ (magnesium), $^{55}Mn$ (manganese), $^{65}Cu$ (copper), $^{66}Zn$ (zinc), $^{88}Sr$ (strontium) and $^{138}Ba$ (barium), along with $^{43}Ca$ (calcium) which was used as the internal standard with a concentration in otoliths of 388 000 µg $g^{-1}$ (Yoshinaga et al. 2000). The sample ablation data were blank subtracted before being integrated for the calculation of element:Ca ratios. Calibration was achieved with the National Institute of Standards (NIST) 612 glass wafer using calculation methods described elsewhere (Ludden et al. 1995, Lahaye et al. 1997, Hamer et al. 2003) and expressed as ratios to Ca (µmol $mol^{-1}$). Replicate calibration standards were analysed after every sequence of 12 otolith ablations, and each sequence consisted of a random selection of otoliths from the sampling regions. Limits of detection (LODs) were calculated for each sample based on 3 standard deviations of blank gas samples taken at the beginning and end of each analysis day and were adjusted for ablation yield of each sample (Lahaye et al. 1997). Mean LODs (ppm) were: $Mg^{25}$ = 7.17, $Mn^{55}$ = 0.44, $Cu^{65}$ = 1.03, $Sr^{88}$ = 1.72, $Ba^{138}$ = 0.17. Precision and recovery for the NIST 612 standard were: recovery %, mean/SD, Mg = 103/8, Mn = 100/4, Cu = 103/7, Zn = 100/6, Sr = 100/6, Ba = 100/6, precision RSD (%): Mg = 5, Mn = 4, Cu = 7, Zn = 6, Sr = 6, Ba = 6. The Cu:Ca ratio was not used in data analyses due to many data being below LOD, and over 20% of sample data registering negative values after blank subtractions.

### 2.2.2. Otolith microstructure and aging

Otoliths (sagittae and lapilli) were dissected from *S. punctatus* post-larvae using tungsten needles under a dissecting microscope with a polarising light source. These were different fish to those analysed

for otolith microchemistry. Sagittae were mounted on glass microscope slides in crystal bond and polished with 30 micron and 5 micron lapping film until increments from the primordium could be clearly seen. Lapilli were left to dry on slides and then placed in immersion oil. Otolith increment counts and measurements were made using a compound microscope under a magnification of 1000×.

Otoliths for increment counts were randomly selected from *S. punctatus* post-larvae collected in 2012. Sagittae were used for analysis of pre-transition/settlement increments, and lapilli were used for analysis of post-transition/settlement increments. Increments were counted from the primordium of the sagittae, where clear increments could be seen, until the transition/settlement point where increment width increased rapidly and structure became hard to interpret. Increments at the primordium of the lapilli are too narrow to be counted accurately, although post-transition increments are distinct. This method is consistent with previous studies which have looked at pelagic larval duration of *S. punctatus* post-larvae (Jenkins & May 1994, Jenkins et al. 2000). Replicate counts were made on each otolith. Increment counts were conducted for 41 and 30 *S. punctatus* otoliths from South Australia and Victoria, respectively, which included a minimum of 10 samples from each major nursery area in each state.

Sagittae were used for increment measurements. Post-larval *S. punctatus* otoliths collected from South Australian and Victorian nursery areas during 2011 and 2012 were polished until increments from the primordium could be clearly seen. Samples used for increment measurement are detailed in Table 1. Any otoliths for which the increments were unclear were discarded and not used in analyses. Otolith increment measurements were made using a compound microscope under a magnification of 1000×. A video camera was attached to the microscope, which enabled the otolith to be viewed on a computer monitor. The measurement modules for Leica Application Suite image software were used to measure increments.

Otoliths were examined blindly with respect to year of sampling and sampling location. Measurements of otolith micro-increments were made along the longest axis on either the clearest sagittal otolith (where 2 otoliths were available), or the otolith which had not been used for chemistry analysis where the sister otolith had been ablated. Jenkins & May (1994) found that results do not differ significantly between sagittae from the same fish. Increments were measured from the first increment (at approximately 10 µm from the core) to 30 µm from the core. The widths of micro-increments in the otoliths of larval fish are often used to represent somatic growth. Daily formation of micro-increments in larval *S. punctatus* has been validated by both larval rearing (B. D. Bruce and D. A. Short unpubl. data) and field evidence (Jenkins & May 1994). A strong linear relationship between otolith diameter and body length has also been recorded (B. D. Bruce and D. A. Short unpubl. data).

### 2.3. Data analysis

#### 2.3.1. Otolith chemistry

Variation in the individual element:Ca ratios was analysed by univariate ANOVA. Separate analyses were conducted for each cohort. Regions (i.e. bays/gulfs) were treated as fixed factors, with sites nested within regions and individual fish as replicates. The region term was tested against the nested terms MS and df. Tukey's HSD post hoc tests were used to determine the sources of any significant differences among regions, again using the nested terms MS and df from the main analysis.

Otolith element:Ca ratios with p-values $<0.1$ for the region main effect were retained for multivariate analysis. MANOVA (Pillai's trace) and pairwise comparisons (Hotelling's *t*-square) were used to analyse variation in the multi-element:Ca ratios among regions and states within each cohort (significant p-value $\leq 0.01$ to account for 5 tests involving an individual region). Quadratic discriminate function analysis (QDFA), with a cross validation 'leave-one-out' jackknife classification procedure, was used to assess how accurately individual fish could be assigned to their collection regions and state based on the elemental composition. Priors were chosen as equal across groups. Assessing accuracy of the classification rules for group membership needs to account for chance agreement. The kappa index is used to indicate the proportion of agreement between the actual sample group memberships and those predicted by the otolith chemistry beyond that expected by chance (Sim & Wright 2005). The kappa index ranges from 0 to 1, where 0 = no agreement between actual and predicted beyond random chance, and 1 = perfect agreement between actual and predicted beyond random chance. The kappa index is also unbiased when sample sizes differ between sample groups (Fielding & Bell 1997).

Hierarchical cluster analysis, which had no prior assumption in relation to the number of groupings

and how they related to locations, was also carried out on the otolith element:Ca ratios. The cluster analysis used Ward's linkage method and a Euclidian distance measure (Woodson et al. 2013, Miller et al. 2016). The element:Ca data were averaged for each site prior to analysis and then standardized to a value between 0 and 100 (Clarke & Gorley 2006).

For ANOVA, MANOVA, QDFA and cluster analysis, Mg:Ca, Mn:Ca, and Ba:Ca ratio data were ln($x$+1) transformed, raw Sr:Ca ratios were used, and Zn:Ca data were fourth-root transformed to meet assumptions of normality and homogeneity of variances (clear departures from assumptions were assessed using histograms, Q-Q normal probability plots, box and residual plots). QDFA was chosen over linear discriminant function analysis due to some minor inequality of covariance matrices indicated from qualitative comparisons of within-group scatterplot matrices among the 6 regional groupings (Quinn & Keough 2002). The Pillai trace statistic was used to test for significance of MANOVA as it is the most robust to any deviations from multivariate normality (Quinn & Keough 2002). $F$-to-remove statistics were used to rate the order at which elements contributed to discrimination (Wilkinson et al. 1996). Canonical discriminant function plots of the 95% confidence ellipses around the centroids for each group were used to display variation in the multi-elemental otolith chemistry among regions. For the state grouping, where only 1 canonical variate was calculated (i.e. 2 groups), data overlap/separation was displayed by plotting the canonical scores for each state as density functions.

Table 2. Results summary of ANOVA and post-hoc Tukey's pairwise tests (*p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001) for individual element:Ca ratios for the otolith core of *Sillaginodes punctatus* post-larvae sampled from 6 regions across South Australia (SA) and Victoria (VIC), and for 2 cohorts: (a) 2011 and (b) 2012. SG: Spencer Gulf; GSt: Gulf St Vincent; KI: Kangaroo Island; PPB: Port Phillip Bay; WP: Western Port Bay; CI: Corner Inlet; ns: not significant

| Element:Ca | p | Significant Tukey's pairwise comparisons between regions |
|---|---|---|
| **(a) 2011 cohort** | | |
| Ln Mg:Ca | **<0.001** | SG > GSt*KI***CI***WP***PPB***, GSt > WP***PPB***, KI > WP***PPB***, CI > PPB*, WP > PPB* |
| Ln Mn:Ca | ns | ns |
| 4th root Zn:Ca | **<0.001** | SG > KI* CI**WP***PPB***, GSt > WP**PPB***, KI > PPB* |
| Sr:Ca | ns | ns |
| Ln Ba:Ca | ns | ns |
| **(b) 2012 cohort** | | |
| Ln Mg:Ca | **<0.001** | KI > WP***CI*, SG > WP**, GSt > WP** |
| Ln Mn:Ca | ns | ns |
| 4th root Zn:Ca | ns | ns |
| Sr:Ca | **<0.001** | KI > WP**SG**PPB*, CI > SG***PPB**WP** |
| Ln Ba:Ca | **<0.01** | KI > WP**PPB*, GSt > WP* |

### 2.3.2. Otolith microstructure

The width of the first 10 daily increments in the core of post-larval *S. punctatus* was compared amongst nursery areas and sampling years using 2-factor ANOVA followed by post hoc Tukey's HSD tests to compare the significance of differences between individual nursery areas. The estimated date of first increment formation based on the pelagic larval duration of a sub-set of post-larvae was compared between states using 1-factor ANOVA. All statistical analyses were carried out using the Systat 13 version 13.2.01 software package.

## 3. RESULTS

### 3.1. Otolith chemistry

#### 3.1.1. Individual element:Ca ratios

Regional variation in element:Ca ratios was most notable for the 2011 cohort and was significant for Mg and Zn (Fig. 2a,c, Table 2a). For Mg, Spencer Gulf (SG) showed significantly higher levels than all Victorian regions, and also Gulf St Vincent (GSt) and Kangaroo Island (KI) (Fig. 2a, Table 2). Corner Inlet (CI) and Western Port (WP) were also significantly higher than Port Phillip Bay (PPB) (Table 2a), but these differences were much less than those detected between the Victorian and the South Australian locations (Fig. 2a). Samples from SG had significantly higher Zn than KI, CI, WP and PPB, and GSt was significantly higher than WP and PPB (Fig. 2c, Table 2a). Samples from KI also had significantly higher Zn than PPB (Fig. 2c, Table 2a).

For the 2012 cohort, significant regional variation was detected for Mg, Sr and Ba, but not Zn (Fig. 2a,c–e, Table 2b). Mg was much lower across all locations except PPB in the 2012 than 2011 cohort (Fig. 2a). Mg for SG and GSt was significantly higher than WP, and for

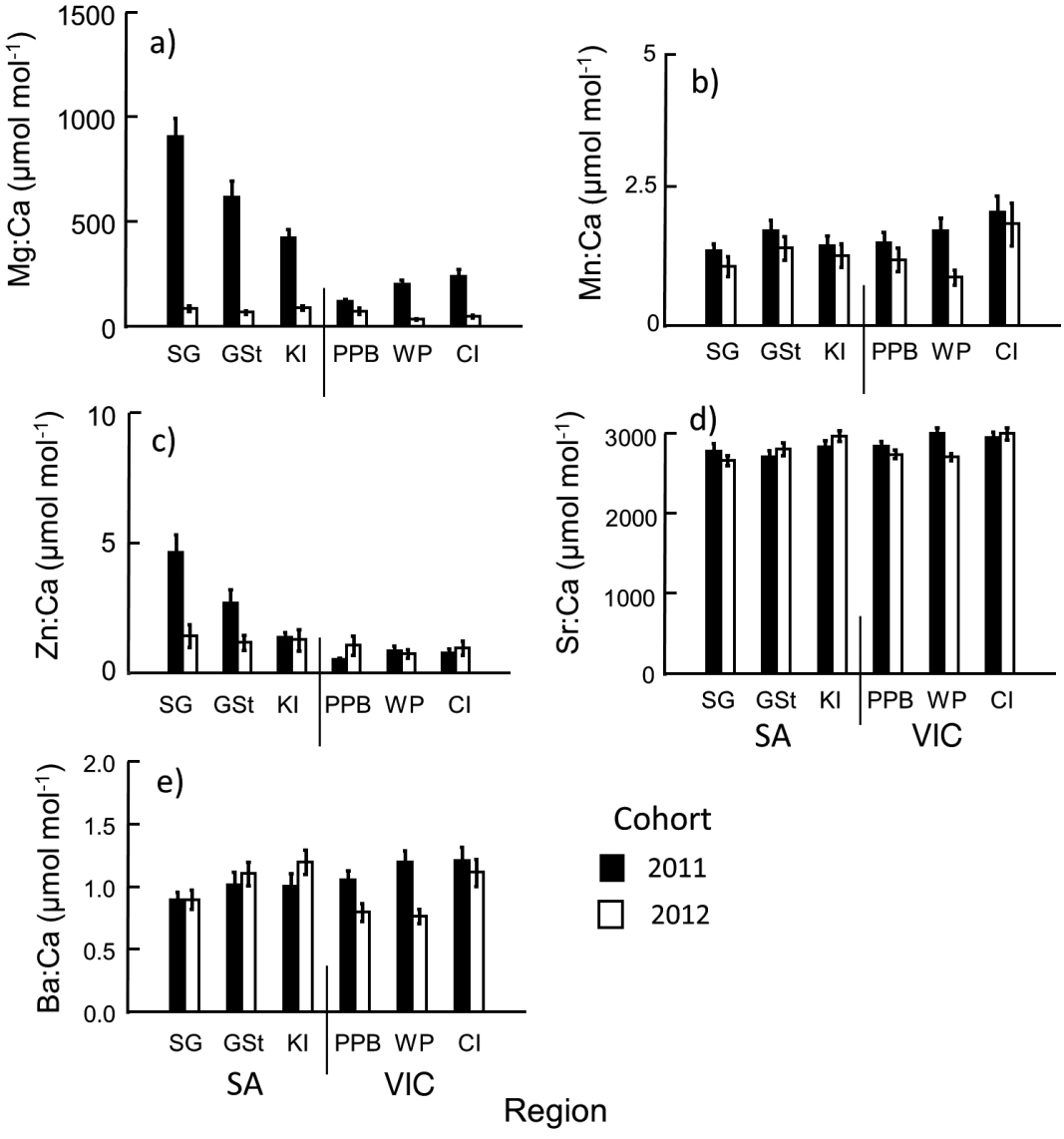


Fig. 2. Comparison of mean (±SE) otolith element:Ca ratios in otolith cores among *Sillaginodes punctatus* post-larvae sampled from regions across South Australia (SA) and Victoria (VIC), for 2 cohorts: (a) magnesium, (b) manganese, (c) zinc, (d) strontium, (e) barium. Other abbreviations as in Table 2

KI was significantly higher than WP and CI (Fig. 2a, Table 2b). Ba was significantly higher for KI than WP and PPB, and for Sr also higher than SG (Fig. 2d,e, Table 2b). CI samples were significantly higher in Sr than SG, PPB and WP (Fig. 2e, Table 2b), and GSt was significantly higher in Ba than WP (Fig. 2e, Table 2b).

### 3.1.2. Multivariate elemental chemistry

For the 2011 cohort, MANOVA was highly significant for both regional and state groupings (Pillai's trace, $p < 0.001$). Discrimination amongst the Victorian and South Australian sampling regions was high (Fig. 3a). Discrimination among regions (individual bays/gulfs) was driven mostly by variation in Mg (*F*-to-remove = 22.9) and Sr (*F*-to-remove = 10.9), with Zn adding only minor additional discrimination power (*F*-to-remove = 1.7). The pairwise regional comparisons indicated that all of the South Australian regions differed significantly from all Victorian regions (Table 3).

The jackknife cross-validation classification accuracy was high when the data were grouped at the state level (81%) but was lower at the regional level (39%) (Table 4a). Misclassifications at the regional

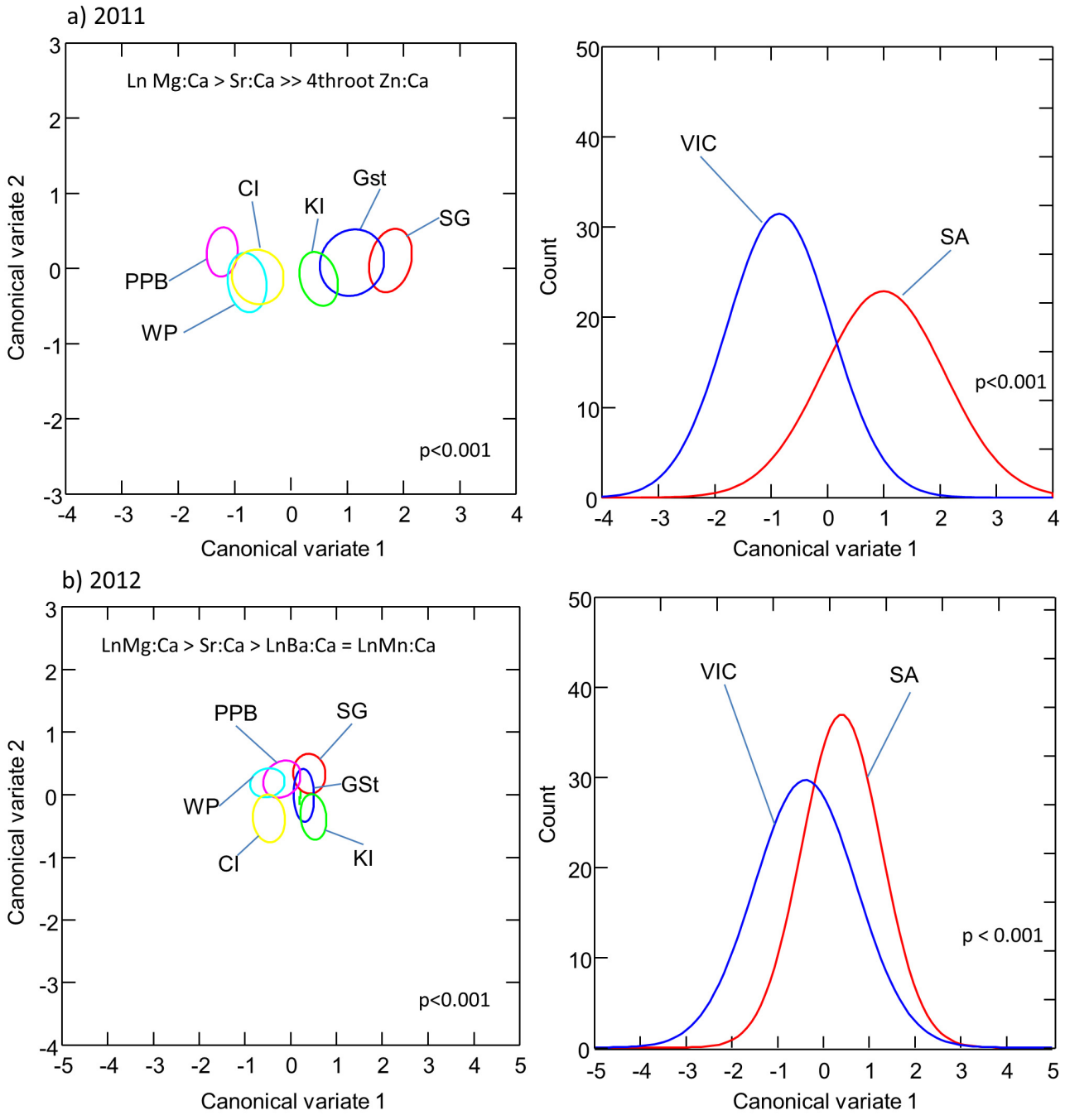


Fig. 3. Canonical variate plots from quadratic discriminant function analysis of otolith core chemistry of *Sillaginodes punctatus* post-larvae sampled from 6 regions across Victoria and South Australia for 2 cohorts: (a) 2011 and (b) 2012. Plots in the left column show data grouped according to each bay, whereas plots in the right column show data grouped according to state (because the latter included only 2 groups, only a discriminant function was possible). Abbreviations as in Table 2

level were mostly among regions within each state. Misclassification of Victorian region samples to South Australian regions were mostly to the KI region, and for South Australian regions to Victorian regions, misclassifications were mostly to PPB and CI (Table 4a). The kappa index indicated that the classification rules created from the multi-element otolith chemistry data performed better than random allocation, particularly at the state grouping (region grouping = 0.26, state grouping = 0.62) (Table 4a).

For the 2012 cohort, discrimination among Victorian and South Australian regions was less clear than for

Table 3. Results of post hoc multivariate pairwise comparisons among regions for the otolith core chemistry of *Sillaginodes punctatus* post-larvae sampled from 6 regions across South Australia and Victoria in (a) 2011 and (b) 2012. Only significant ($p \leq 0.01$) comparisons are included. Abbreviations as in Table 2

| Region | | Hotelling's *t*-square | p |
|---|---|---|---|
| **(a) 2011** | | | |
| SG | KI | 31.941 | <0.001 |
| | PPB | 195.508 | <0.001 |
| | WP | 126.690 | <0.001 |
| | CI | 101.586 | <0.001 |
| GSt | PPB | 117.046 | <0.001 |
| | WP | 70.067 | <0.001 |
| | CI | 57.388 | <0.001 |
| KI | PPB | 84.645 | <0.001 |
| | WP | 40.256 | <0.001 |
| | CI | 31.732 | <0.001 |
| **(b) 2012** | | | |
| SG | WP | 24.389 | <0.001 |
| | CI | 35.604 | <0.001 |
| GSt | WP | 21.763 | <0.01 |
| | CI | 21.183 | <0.01 |
| KI | PPB | 24.277 | <0.001 |
| | WP | 36.233 | <0.001 |
| | CI | 28.060 | <0.001 |
| PPB | CI | 14.311 | 0.010 |

Table 4. Results of jackknife cross-validation classifications at the regional and state levels of sample grouping, along with kappa values, for the otolith core chemistry of *Sillaginodes punctatus* post-larvae sampled from 6 regions across South Australia (SA) and Victoria (VIC) in (a) 2011 and (b) 2012. Abbreviations as in Table 2

| Collection | % samples classified | | | | | | Overall accuracy (%) | Kappa |
|---|---|---|---|---|---|---|---|---|
| **(a) 2011** | | | | | | | | |
| By region | SG | GSt | KI | PPB | WP | CI | 39 | 0.26 |
| SG (n = 39) | 59 | 15 | 21 | 0 | 3 | 3 | | |
| GSt (n = 36) | 39 | 8 | 33 | 14 | 0 | 6 | | |
| KI (n = 49) | 15 | 4 | 55 | 12 | 2 | 12 | | |
| PPB (n = 62) | 0 | 0 | 8 | 52 | 13 | 27 | | |
| WP (n = 47) | 0 | 2 | 13 | 28 | 19 | 38 | | |
| CI (n = 37) | 5 | 0 | 19 | 32 | 11 | 33 | | |
| By state | SA | VIC | | | | | 81 | 0.62 |
| SA (n = 120) | 80 | 20 | | | | | | |
| VIC (n = 144) | 18 | 82 | | | | | | |
| **(b) 2012** | | | | | | | | |
| By region | SG | GSt | KI | PPB | WP | CI | 29 | 0.15 |
| SG (n = 53) | 21 | 21 | 15 | 7 | 28 | 8 | | |
| GSt (n = 56) | 20 | 21 | 18 | 7 | 25 | 9 | | |
| KI (n = 54) | 17 | 18 | 41 | 4 | 15 | 5 | | |
| PPB (n = 55) | 7 | 13 | 7 | 13 | 49 | 11 | | |
| WP (n = 56) | 11 | 7 | 12 | 9 | 52 | 9 | | |
| CI (n = 57) | 5 | 17 | 4 | 5 | 44 | 25 | | |
| By state | SA | VIC | | | | | 65 | 0.30 |
| SA (n = 163) | 77 | 23 | | | | | | |
| VIC (n = 168) | 47 | 54 | | | | | | |

the 2011 cohort (Fig. 3). Similar to the 2011 cohort, Mg contributed most to discrimination (*F*-to-remove = 7.2), followed by Sr (*F*-to-remove = 3.2), with minor additional discrimination power provided by Ba and Mn (*F*-to-remove = 2.7 and 2.4, respectively). While for both the regional and state-based groupings the MANOVA was highly significant (Pillai's trace, $p < 0.001$), there was major overlap among the sample distributions from the 2 states for canonical variate 1 (Fig. 3b). Significant regional pairwise differences occurred between SG and both WP and CI, between GSt and both WP and CI, between KI and PPB, and between WP and CI. PPB also significantly differed from CI (Table 3).

Jackknife cross-validation classification accuracy was higher for the South Australian samples, with more of the Victorian samples being misclassified to South Australian regions than vice versa (Table 4b). At the regional level, most of the misclassified samples from South Australian regions to Victorian regions were to WP. PPB and CI region samples misclassified mostly to WP, but also to GSt. WP samples misclassified mostly to SG and KI (Table 4b). The kappa index for the 2012 cohort was considerably lower than for the 2011 cohort, indicating only minor improvement over random classification (region = 0.15, state = 0.30) (Table 4b).

The hierarchical cluster analysis gave results consistent with the MANOVA and QDFA analyses. For the 2011 cohort, there was a clear, high-level separation of the Victorian and South Australian sites in the dendrogram, with the exception of 1 of the Kangaroo Island sites grouping with the Victorian sites (Fig. 4a). In contrast, the dendrogram for the 2012 cohort showed no high-level separation along state jurisdictional lines, with higher-level groups containing a mixture of Victorian and South Australian sites (Fig. 4b).

### 3.2. Daily increment analysis

Two-factor ANOVA showed that the width of the inner 10 daily increments in otoliths varied significantly for post-larvae from different nursery regions ($F_{5,415} = 16.913$, $p < 0.001$; Fig. 5). There was no significant difference between settlement years ($F_{1,415} = 0.940$, $p = 0.333$; Fig. 5) and no interaction

between settlement bay and year ($F_{5,415}$ = 1.190, p = 0.313; Fig. 5). Tukey's post hoc comparisons showed that increment widths in otoliths of post-larvae in all South Australian nursery areas were higher than in all Victorian nursery areas. Within states, however, the only significant difference was that increment widths were higher in SG than GSt (Fig. 5).

The pelagic larval duration was estimated for a sub-set of post-larvae from each state collected in 2012. Estimated dates of first increment formation ranged from May to mid-July for both states, with considerable overlap (Fig. 6). There was no significant difference in the estimated date of first increment formation between the 2 states (ANOVA, $F_{1,69}$ = 0.527, p = 0.47).

## 4. DISCUSSION

### 4.1. Otolith microchemistry

The results from the otolith core chemistry for the 2011 cohort were consistent with the hypothesis that post-larval *Sillaginodes punctatus* recruiting into bay/gulf nurseries in Victoria and South Australia came from different spawning sources. South Australian samples showed clear differences to the Victorian samples, and the most notable variation was for Mg. Differences in otolith core chemistry between the South Australian and Victorian post-larvae were less clear for the 2012 cohort. This may have reflected less pronounced environmental or physiological influences on otolith chemistry in 2012. Alternatively, it is possible that the amount of overlap of the spawning areas of *S. punctata* from the 2 states can vary between years.

These results, particularly for the 2011 cohort, are consistent with reverse hydrodynamic modelling that predicted spawning areas for post-larvae from Victorian and South Australian nursery areas based on larval

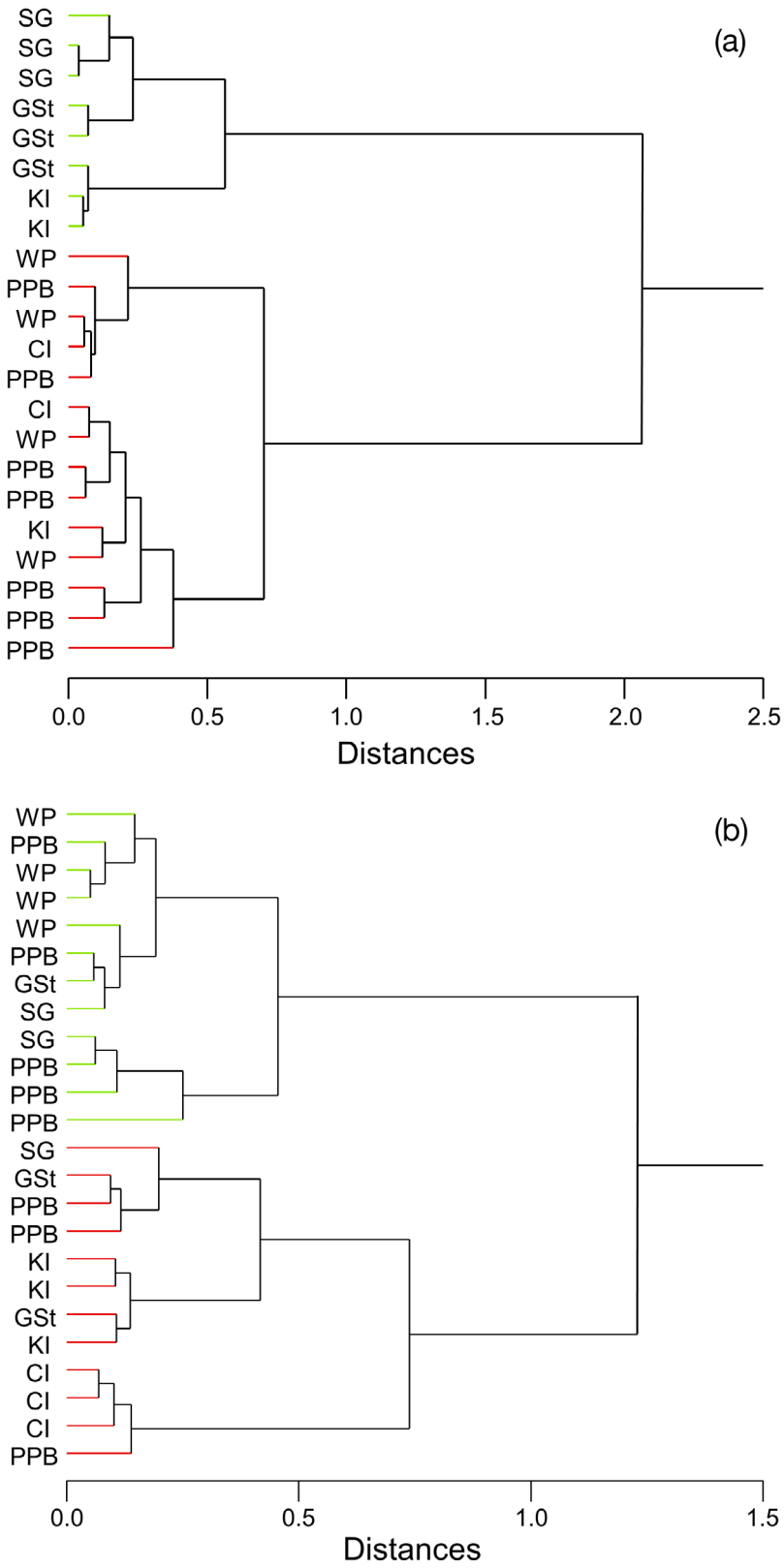


Fig. 4. Hierarchical cluster analysis results from the otolith core chemistry for the (a) 2011 and (b) 2012 cohort of *Sillaginodes punctatus* post-larvae sampled from sites within 6 regions across Victoria and South Australia. Green and red lines show sites within the 2 highest level groupings. Abbreviations as in Table 2

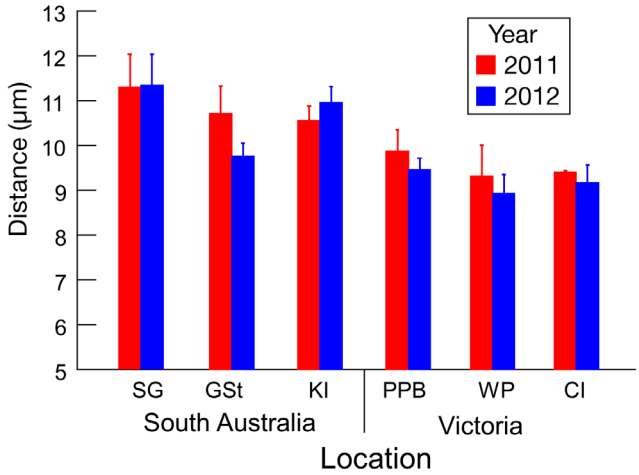


Fig. 5. Mean (±SE) distance from the core to the tenth daily increment from otoliths of *Sillaginodes punctatus* post-larvae collected from 6 regions in South Australia and Victoria. Abbreviations as in Table 2

durations and regional hydrodynamics (Fowler et al. 2000a, Jenkins et al. 2000). Post-larvae in Victorian nursery areas were predicted to have come from spawning 100s of km to the west in far west Victoria along the coast into south-eastern South Australia, but not as far west as the known South Australian spawning grounds (Jenkins et al. 2000). For post-larvae from South Australian nursery areas, spawning was predicted to be much more local, and overlapped the known spawning areas in the Investigator Strait region (Fowler et al. 2000a).

The most notable differences between post-larvae from the 2 states were for Mg in 2011. Mg is abundant in the environment and there is typically no relationship between concentrations in the water and in otoliths (Martin & Thorrold 2005, Hüssy et al. 2021). Mg concentrations are higher in blood plasma than in the endolymph (Melancon et al. 2009), indicating significant physiological regulation (Woodcock et al. 2012, Barnes & Gillanders 2013). Physiological regulation implies that Mg uptake may be affected by variables such as temperature, otolith precipitation rates and somatic growth rates (Martin & Thorrold 2005). Determining the most important mechanism is difficult given that these variables often strongly co-vary (Martin & Thorrold 2005). Results of studies have varied (Hüssy et al. 2021), with Mg showing positive (Barnes & Gillanders 2013, Stanley et al. 2015), negative (Fowler et al. 1995a,b) or no (Elsdon & Gillanders 2002, Martin & Thorrold 2005, DiMaria et al. 2010) relationship with temperature. A negative relationship has been shown between Mg and otolith precipitation and somatic growth for larval and early juvenile spot *Leiostomus xanthurus* (Martin & Thorrold 2005), a positive relationship has been shown between Mg and otolith precipitation rate for juvenile snapper *Pagrus auratus* (Hamer & Jenkins 2007), but no relationships with either of these factors were found for larval Pacific cod *Gadus microcephalus* (DiMaria et al. 2010). Hüssy et al. (2021) concluded that the pattern emerging from published studies was that otolith Mg incorporation is unrelated to somatic growth in larval fish but positively related to growth of juvenile and adult fish across a range of taxa.

Elements such as Ba and Sr are commonly found to drive discrimination in otolith chemistry studies, often in relation to variation in ambient concentrations relative to calcium (Elsdon et al. 2008). For the early oceanic larval stage of *S. punctatus*, these ele-

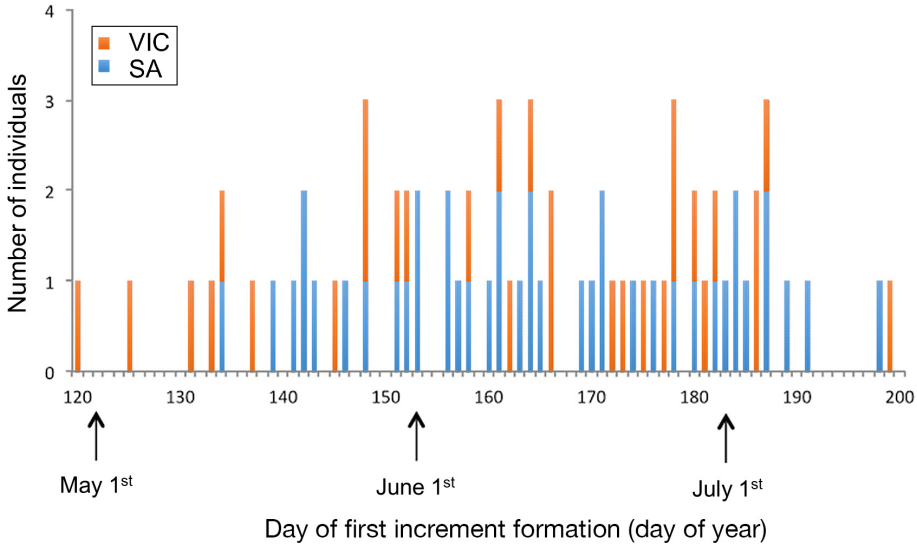


Fig. 6. Day of first increment formation for *Sillaginodes punctatus* post-larvae from Victoria (VIC; n = 30) and South Australia (SA; n = 41) collected in 2012

ments were less important for discrimination in our study. This may be due to the oceanic spawning and larval phase of the species where variation in the ambient levels of these elements in the water is likely to be low (Hamer et al. 2006). The elements that showed most regional variation during the early larval phase, i.e. Mg, Sr and Zn, are known to be influenced by physiological processes independent of water chemistry, with Sr and Zn also potentially influenced by a combination of physiological processes, food composition and ambient concentrations (Sadovy & Severin 1992, Campana 1999, Miller et al. 2006, Ranaldi & Gagnon 2008, Sturrock et al. 2015). It is likely that the variation observed in the otolith composition for these elements was related to a combination of physiological and environmental factors. The lower growth rate of the Victorian post-larvae (see Section 4.2) is clearly indicative of a physiological difference.

### 4.2. Otolith microstructure

Otolith microstructure of *S. punctatus* post-larvae from Victorian and South Australian nursery areas showed significant differences in increment widths, adding to evidence that post-larvae in each state came from different spawning areas, or at least different locations within a broad spawning area. Otolith increment widths correspond to growth rates in the larval stage of *S. punctatus* (Jenkins & King 2006). The primary determinants of larval growth rate are thought to be water temperature and food availability (Anderson 1988), although water temperature may be the more dominant variable (Meekan et al. 2003). Growth rates in the larval stage of *S. punctatus* post-larvae sampled in Port Phillip Bay were strongly influenced by inter-annual variation in water temperature in Bass Strait (Jenkins & King 2006). Our results suggest that the spawning area for post-larvae from South Australia occurs in a region with significantly higher water temperature and/or productivity than the spawning area for post-larvae sampled in Victoria.

One possible alternative explanation to this would be that post-larvae from both states were spawned in the same area, but those settling in South Australian nursery areas were spawned earlier in the spawning season when the water temperature was higher. This can be ruled out, however, as the estimated hatching dates for post-larvae from the 2 states showed significant overlap and were not significantly different for spawning in 2012.

## 5. CONCLUSIONS

The results of both the otolith chemistry for the 2011 cohort, and otolith microstructure analyses for both cohorts, suggest that post-larvae recruiting to Victorian nursery areas are not spawned in the same spawning area as *S. punctatus* in central South Australia, that is, north of Kangaroo Island and in the southern areas of Spencer Gulf and Gulf St Vincent (Fowler et al. 2000a,b). This is consistent with the modelling studies based on hydrodynamics and pelagic larval durations that predicted the spawning area for Victorian *S. punctatus* would range from west Victoria across to south-eastern coast of South Australia, but not as far west as the known spawning area in South Australia (Jenkins et al. 2000).

The results of the otolith chemistry analysis for the 2012 cohort showed much lower separation between post-larvae from the 2 states. This may indicate that there was greater overlap in the spawning sources and mixing of spawners in 2012. Alternatively, spawning sources may have been separate, but environmental differences, and corresponding otolith chemistry differences, between the 2 areas may have been less distinct in 2012.

The possible mixing of spawning individuals from the 2 states, at least in some years, is supported by genetics studies. Studies using microsatellite markers (Kent et al. 2018) and single nucleotide polymorphisms (C. D. H. Sherman et al. unpubl. data) have found no evidence for genetic differences between *S. punctatus* post-larvae sampled in Victoria and South Australia. This suggests that although the spawning locations for post-larvae in South Australia and Victoria may generally be different, there must be at least a low level of mixing of adults between these spawning areas. Evidence to date indicates that any mixing is likely to be limited in extent. Recaptures of *S. punctatus* tagged in Gulf St Vincent and near Kangaroo Island showed no evidence of movement to the south-east coast of South Australia (Fowler et al. 2002). Further, microchemical signatures in otoliths of juveniles have shown that *S. punctatus* from Victorian nursery areas are not present in the adult population in the known spawning area in central South Australia (P. A. Hamer et al. unpubl. data).

The results from otolith chemistry and increment structure suggest that *S. punctatus* from the 2 states originate from different spawning areas, qualified by the equivocal results from otolith microchemistry for the 2012 cohort, and lend support for these to be treated as separate biological stocks from a fisheries management point of view. Thus, these populations

are likely to be separated on time and space scales that are relevant to fisheries management even though there is sufficient gene flow between them for genetic homogeneity (Begg & Waldman 1999). Although genetics evidence alone would suggest that cross-jurisdictional management was appropriate, the results of this study lend qualified support for the current arrangement wherein the *S. punctatus* fishery is managed separately by the individual jurisdictions. Additional research is needed to further improve understanding of the stock structure of *S. punctatus* across Victoria and South Australia, and correspondingly to improve confidence in advice to management.

*Acknowledgements.* We thank David Hatton and Bruce Jackson for assistance with field sampling and Dr. Hannah Murphy for assistance with laboratory analyses of post-larvae. We greatly appreciate the funding provided for this project by the Fisheries Research and Development Corporation, the Victorian Recreational Fishing License Large Grants Scheme and the Victorian Government through Fisheries Victoria.

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*Editorial responsibility: Craig Radford, Warkworth, New Zealand*
*Reviewed by: 3 anonymous referees*

*Submitted: June 29, 2021*
*Accepted: November 4, 2021*
*Proofs received from author(s): February 1, 2022*