Elemental fingerprints of southern calamary (*Sepioteuthis australis*) reveal local recruitment sources and allow assessment of the importance of closed areas

Gretta T. Pecl, Sean R. Tracey, Leonid Danyushevsky, Simon Wotherspoon, and Natalie A. Moltschaniwskyj

Abstract: Movement of individuals over a range of temporal and spatial scales is a critical process in determining the structure and size of populations. For most marine species, a substantial amount of movement that is responsible for connecting subpopulations occurs when individuals are too small and numerous to be tagged using conventional methods. Using the elemental fingerprints of the statoliths of the squid *Sepioteuthis australis* and a robust machine learning classification technique, this study determined that newly hatched squid had elemental signatures that exhibited sufficient spatial variation to act as natural tags for natal origin and that elemental signatures can be used to allocate adult squid back to their natal site. Between 55% and 84% of the adult squid caught throughout the east and southeast of Tasmania, Australia, were classified back to an area that is closed to commercial fishing over much of the peak spawning period, and this was the only location with substantive evidence of natal recruitment. Although many studies have demonstrated the potential of this approach to discern connectivity between population units, few studies have successfully done so by then examining the trace element profiles of adults in addition to those of hatchlings as we have demonstrated with *S. australis*.

Résumé : Les déplacements des individus sur une gamme d'échelles temporelles et spatiales sont des éléments essentiels dans la détermination de la structure et de la taille des populations. Pour la plupart des espèces marines, une partie majeurs des déplacements qui sont responsables des liens entre les sous-populations se produit lorsque les individus sont trop petits et nombreux pour être marqués à l'aide des méthodes conventionnelles. En utilisant les empreintes en éléments des statolithes du calmar *Sepioteuthis australis* et une technique robuste de classification par apprentissage automatique, notre étude démontre que les calmars nouvellement éclos possèdent une signature d'éléments qui montre suffisamment de variation spatiale pour servir d'étiquette naturelle afin de déterminer leur origine à la naissance; les signatures d'éléments peuvent aussi servir à associer les calmars adultes à leur site de naissance. Entre 55 % et 84 % des calmars adultes capturés sur l'ensemble de l'est et du sud-est de la Tasmanie, Australie, ont été classifiés comme provenant d'une région fermée à la pêche commerciale durant une grande partie de la période maximale de fraie; c'est la seule région qui présente des preuves solides de recrutement natal. Bien que plusieurs études aient démontré le potentiel de cette approche pour discerner la connectivité entre les unités démographiques, peu de travaux ont réussi à le faire en examinant les profils des éléments en trace des adultes en plus de ceux des nouveau-nés comme nous l'avons fait chez *S. australis*.

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Introduction

Quantifying many of the processes that structure marine metapopulations is an enigmatic problem in ecology, although it is widely acknowledged that populations of marine species are connected on temporal and spatial scales larger than their local habitat through dispersal of juveniles and (or) the migration of adults. Movement of individuals among habitat patches is an essential component of both the maintenance and establishment of populations. The capacity of individuals to recolonize areas following localized extinction events, because of natural or human-mediated change, is highly dependent on the strength of connectivity among subpopulations. Given that migration affects persistence, coloni-

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G.T. Pecl and S.R. Tracey. Institute for Marine and Antarctic Studies, University of Tasmania, Private Bag 49, Tasmania, Australia 7001.
L. Danyushevsky. Centre for Ore Deposit Research, University of Tasmania, Private Bag 79, Tasmania, Australia 7001.
S. Wotherspoon. School of Mathematics and Physics, University of Tasmania, Private Bag 37, Tasmania, Australia 7001.
N.A. Moltschaniwskyj. National Centre for Marine Conservation and Resource Sustainability, University of Tasmania, Locked Bag 1370, Tasmania, Australia 7250.

Corresponding author: Gretta T. Pecl (e-mail: Gretta.Pecl@utas.edu.au).

zation, and extinction, we need to explicitly measure and quantify migration rates among spawning populations. The source of new individuals, either via recruitment or migration, is usually unknown, and although the sum of the influx is measureable, quantifying the relative contribution of new individuals from a number of different sources is extremely problematic. In particular, tracking or marking large quantities of larvae or juveniles is often challenging, and yet this is frequently a life history stage where large-scale movements occur (Thorrold et al. 2007).

The successful use of spatial and temporal control measures to manage exploitation of wild populations requires knowledge of the likely sources of recruitment to local populations (i.e., is the population reproductively self-sustaining or dependent on immigration for the replacement of individuals; Holmes et al. 2003). This is necessary for sustainable management, including the placement and expected function of marine reserves (Cowen and Sponaugle 2009) or correct placement and timing of areas closed to commercial fishing to protect spawning individuals (Pecl et al. 2006; Fogarty and Botsford 2007). Without knowledge of the real population structure, the assumption in fisheries management that population units are homogeneous risks overfishing of less productive stocks (Begg et al. 1999), potentially affecting the long-term stability and sustainability of the entire stock (Hilborn et al. 2003). The level of genetic exchange arising from immigration between populations will also influence their long-term stability given naturally occurring levels of stochasticity in survival and growth (Havenhand 1995).

The integration of naturally occurring trace element signatures into the perennial biomineralized hard structures of fish and invertebrates provide ideal natural tags because of metabolic inertness of the structures and the chronological incorporation of elements that are influenced by environmental variables (Campana 1999; Gillanders 2005; Doubleday et al. 2008). Natural tags are suited to answering ecological questions regarding life histories, as all individuals regardless of size or life history stage are essentially tagged (Elsdon and Gillanders 2005), all individuals potentially represent recaptures, and it is possible to detect small-scale movement (Gillanders and Kingsford 2000). Trace element tags in the hard structures of a wide range of taxa have been used to distinguish between groups, including gastropods (Zacherl et al. 2003), squid (Ikeda et al. 2002; Warner et al. 2009), octopus (Doubleday et al. 2008), mussels (Becker et al. 2007), and fish (Gillanders et al. 2001). More rarely, studies have used trace element profiles of structures formed during the embryo phase to identify the geographic locations where the hard part was formed, thereby allowing the reconstruction of the natal source of adults from across a range of locations (Campana 1999; Becker et al. 2007). The overwhelming advantage of trace element analysis over genetic approaches is that it allows direct determination of natal origins of a large number of adults and can provide a direct measure of exchange over a discrete and defined period (Becker et al. 2007).

Squid are important components of many marine ecosystems from the poles to the equator, as their mid-level position in the food web means that they have important roles as both predators and prey. Worldwide, catches of cephalopods have increased rapidly over recent decades (Hunsicker et al. 2010), and unique aspects of their growth and reproduction mean that they are likely to play an increasingly important role in the oceans that are changing because of climate change (Pecl and Jackson 2008). Functionally, cephalopods are ecological equivalents of teleost fish except that they grow faster and have shorter life spans, and therefore they achieve much faster turnover of generations. Given the annual life span of many squid species, new "recruits" generally constitute the entire population for any one year and are solely responsible for producing the future population. The occurrence of spatially and temporally predictable spawning events of many inshore squids means they are vulnerable to targeted fishing of the spawning aggregations, and the single generation structure of populations can result in susceptibility to overfishing.

Sepioteuthis australis is a relatively large (~1–4 kg) but short-lived (<1 year), multiple-spawning inshore squid (Pecl 2001). A low level of spawning for this species occurs all year round; however, in the Australian spring and summer adults aggregate over seagrass beds in large numbers to spawn in shallow waters (<15 m), laying benthic strands attached to the substrate with 5–7 large eggs (6–8 mm) inside each strand, with multiple strands forming discrete egg masses (Moltschaniwskyj et al. 2003). Following escalating catch and effort in Tasmania, Australia, the fishery has been managed via short-term spatial and temporal closures of the major spawning areas. These closures have been put in place in the east coast of Tasmania (Great Oyster Bay and Mercury Passage), as most of the spawning activity is concentrated within these regions (Moltschaniwskyj and Pecl 2003). However, survival of southern calamary hatchlings is size selective (Steer et al. 2003*a*), indicating that not all spawning activity contributes equally to the next generation. Until now, we have not been able to show a direct link between putative "most important" spawning areas and adult fished recruits.

In this study we use trace element profiles to explore the extent of self-seeding, the degree of connectivity among local populations, and the extent of dispersal of hatchlings in southern calamary (*S. australis*). The key objectives of this study were to determine whether (*i*) hatchling elemental signatures exhibit sufficient spatial variation over regional scales to act as tags for natal origin and (*ii*) if the elemental signatures of the hatchlings from one year can be used to allocate adults caught the following year to a natal location. As the life span of southern calamary is less than 1 year, there is no overlap of generations, minimizing any confounding temporal effects. Additionally, as hatchlings are taken straight from very late-stage embryos laid directly attached to seagrass, the exact natal location is known for all hatchling individuals. This is in contrast with most studies examining natal origins in fish where only the broad region of origin is estimated for free-swimming larvae, reducing the potential to determine small-scale differences.

Materials and methods

Sample collection

A total of 218 southern calamary hatchlings were collected across six regions on Tasmania's east and southeast coast: Great Oyster Bay (GOB), Mercury Passage (MP), Frederick Henry Bay (FHB), Bruny Island (BI), Tasman Peninsula (TP), and D'Entrecasteaux Channel (DEC) in December 2004 and January 2005 (Table 1; Fig. 1). Hatchlings were collected in

Table 1. Location and date of collection of hatchling and adult *Sepioteuthis australis* used for statolith element analysis.

					Mantle length (mm)	
Region	Date	Sex	Collected *n*	Analysed *n*	Mean	Range
Hatchlings						
BI	7 December 2004	—	30	25	—	—
FHB	9 December 2004	—	20	11	—	—
MP	14 December 2004	—	20	10	—	—
TP	9 December 2004		20	19		
DEC	17 December 2004	—	30	20	—	—
GOB	22 December 2004	—	60	54	—	—
GOB	18 January 2005	—	100	79	—	—
Adults						
GOB	August 2005	M	95	31	343	225–493
		F	76	33	282	224–340
DEC	September 2005	F	2	2	281	280–282
MP	August 2005	M	78	20	360	214–457
		F	54	17	278	196–346
	September 2005	M	88	26	395	269–492
		F	10	5	296	270–318
TP	August 2005	F	11	6	264	190–300
	September 2005	M	16	5	326	258–490
		F	20	7	290	240–325

Note: Great Oyster Bay (GOB), Mercury Passage (MP), Bruny Island (BI), Tasman Peninsula (TP), Frederick Henry Bay (FHB), and D'Entreacasteaux (DEC).

situ from very late stage egg masses (Moltschaniwskyj et al. 2003) located on shallow (<10 m) seagrass beds by SCUBA divers. Hatchlings were collected by placing a plastic bag over the egg mass and gently agitating the mass to stimulate the late stage embryos (stage 28–29; Steer et al. 2003*b*) to hatch. Hatchlings were examined and those that had completely absorbed their yolk sac were retained and stored in 95% ethanol until statolith extraction. Owing to the small size of hatchling statoliths (~160–375 μm), fine-gauge sterile hypodermic needles were used to remove each statolith from the cartilaginous case. Hatchling statoliths were prepared for mounting directly after extraction.

Male and female adult southern calamary were collected during August and September 2005 from four geographic regions on Tasmania's east and southeast coast: GOB, MP, TP, and DEC (Table 1; Fig. 1). The animals were caught over or in close proximity to seagrass beds using squid jigs on rod and reel. Adults were frozen soon after capture and thawed prior to dissection and statolith extraction. Statoliths from adult calamary were extracted using ceramic-tipped forceps to reduce the possibility of contaminating the samples. Once extracted, the statoliths were stored in sealed polycarbonate multiwell trays.

Statolith preparation

One statolith from each animal was randomly selected for analysis. Up to 30 hatchling statoliths were placed longitudinally (to expose as much surface area to grinding as possible) in a circular mould, and epoxy resin was poured over the statoliths to produce a 1 cm deep mount. Adult statoliths were ground individually from the lateral dome to expose the hatch check in the lateral plain. The plane of grinding was precisely controlled using the edge of a glass slide as described by Lipinski and Durholtz (1994). Adult statoliths were then mounted in epoxy resin blocks (10–30 statoliths per block) with the ground surface exposed for polishing.

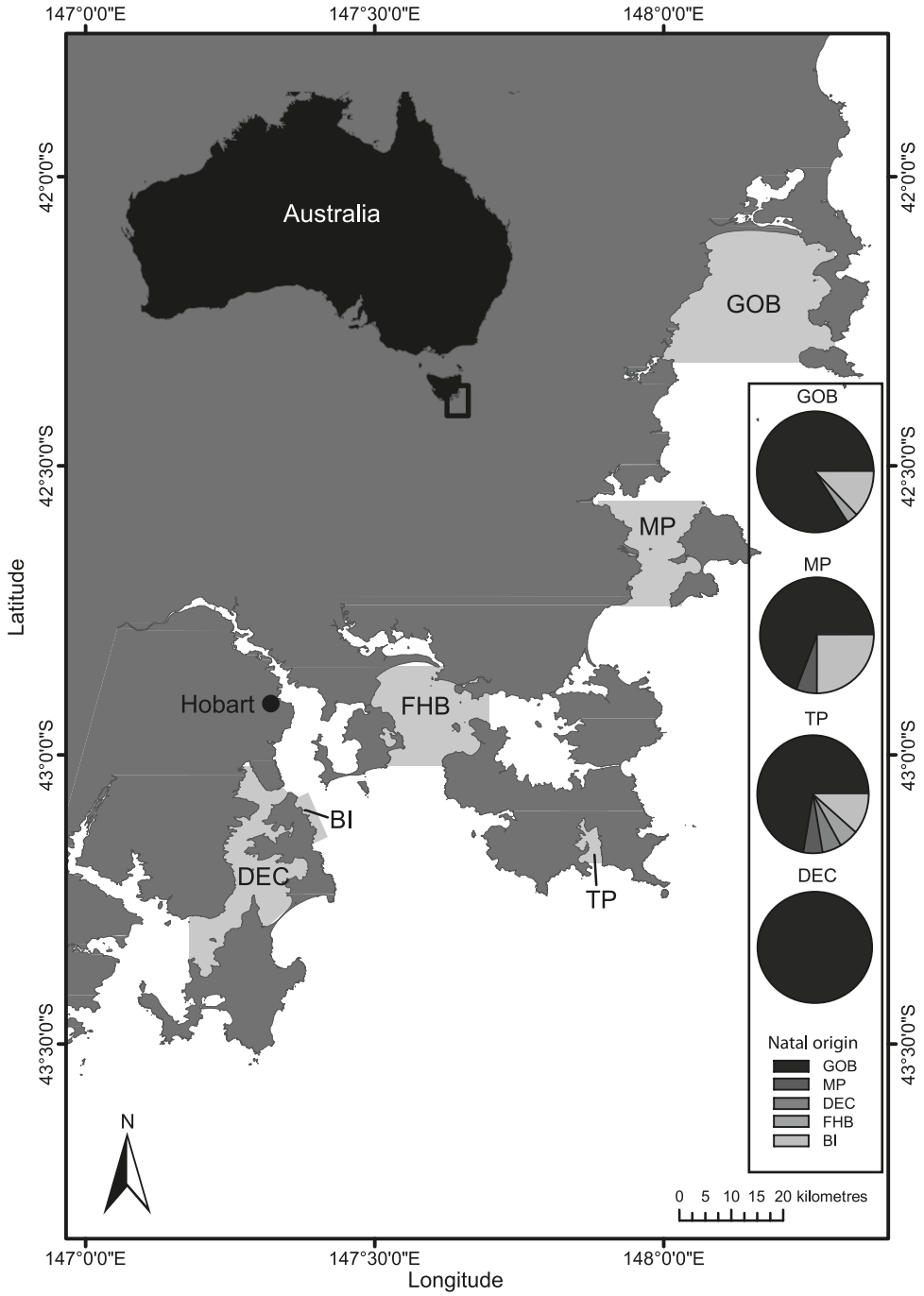
Once the epoxy blocks holding the statoliths were cured in an oven at approximately 40 °C, the statoliths in the blocks were ground using 1500 grit carborundum paper with 18.2 MΩ·cm^{-1} Milli-Q water then polished using 0.3 μm alumina powder on a suede polishing disc to reveal the inner ring structure in the dome of the statolith. Following polishing, the mounts were ultrasonically cleaned for 5 min in Milli-Q water and then rinsed further using Milli-Q water to remove surface contaminants before being allowed to air dry in a laminar flow fume hood.

Trace element analysis

Trace element analysis was conducted by laser ablation inductively coupled plasma – mass spectrometry (LA-ICP-MS) using a New Wave UP-213 Nd:YAG Q-switched laser ablation system controlled by the MEOLaser 213 software package, coupled with an Agilent HP4500 Quadrapole ICP-MS. The following suite of elements was recorded for each statolith: ^{7}Li, ^{24}Mg, ^{45}Sc, ^{47}Ti, ^{53}Cr, ^{55}Mn, ^{57}Fe, ^{60}Ni, ^{65}Cu, ^{66}Zn, ^{75}As, ^{85}Rb, ^{88}Sr, ^{137}Ba, and ^{43}Ca, with the latter used as an internal standard.

Ablations were conducted in "spot mode", with the statolith stationary under the pulsating laser beam, resulting in a continuous deepening in the cylindrical hole during ablation. Ablations were carried out in an atmosphere of ultra-high-purity helium using a custom-designed small-volume (~3 cm^3) sample cell. This cell ensured rapid transport of the ablated material to the mass spectrometer, resulting in accurate documentation of chemical variations with depth. For statolith analysis, the beam diameter was set nominally at

Fig. 1. Southern calamary hatchlings were collected from six known spawning regions from southeast Tasmania: GOB, Great Oyster Bay; MP, Mercury Passage; FHB, Fredrick Henry Bay; TP, Tasman Peninsula; BI, Bruny Island; and DEC, D'Entrecasteaux Channel. Adult calamary were caught from GOB, MP, TP, and DEC. The figure inset shows the proportion of adults that were classified as originating from each spawning region.



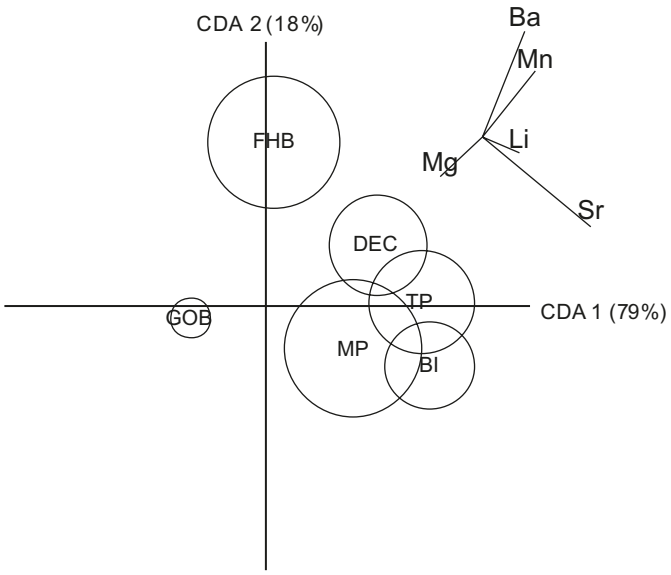
55 μm with a pulse rate of 5 Hz. To avoid error introduced by surface contamination, each statolith was pre-ablated at 2 Hz for 2 s. The average laser energy output was ~13 $J \cdot cm^{-2}$. Prior to each ablation, background levels were collected for 30 s, and the average of these measurements was subtracted from the following sample measurement to correct for background levels. The acquisition time for each sample ranged between 40 and 60 s.

Elemental concentrations were calibrated against the international standard NIST612 following the procedure of Longerich et al. (1996). To adjust for instrument drift over the day, the standard was ablated approximately each hour or when a new mount was inserted into the ablation chamber and drift corrected using linear interpolation. The concentration of calcium in the statoliths was assumed to be constant at 40.04 percent weight CaO, based on $CaCO_3$ stoichiometry. Detection limits for each element were estimated from three standard errors of the background signal. Analytical precision is dependent on the concentration level and varies from ~2% (1 standard error) at high concentrations to ~30%–50% at

concentrations near the detection limits. Accuracy is difficult to estimate because of the lack of international secondary standards, but is believed to be >50% based on our experience with analysis of other materials.

Hatchling statoliths were ablated in the centre of the dome region, which is the widest region of the statolith. No data was collected for 11% of the hatchling statoliths generally because the statoliths were too small for the laser beam width or they cracked during the ablation process. A much greater percentage (65%) of the data from the adult statoliths was rejected, as it was difficult to get a sufficient amount of ablation sample from the hatchling portion of the statolith, particularly if the statolith sections were too thin to allow sufficient time for data acquisition (Table 1). Adult statoliths were ablated in the middle of the hatch check, which is the dark region within the ring structure. An increase in the concentration of ^{55}Mn at the time of ablation was used as a diagnostic confirmation of successfully ablating within the prehatching zone (Brophy et al. 2004; Ruttenberg et al. 2005). Using ^{55}Mn as a diagnostic of the correct position of the ablation was useful if the statolith was not ground sufficiently and part of the posthatch statolith material was being ablated. Only the portion of the ablation signal where increased ^{55}Mn concentrations occurred was used for data analysis. There was greater than an order of magnitude difference between the concentration of ^{55}Mn in the statolith cores and the statolith material deposited after hatching.

Fig. 2. Canonical discriminate plot showing the variation in microchemistry of the hatchling statoliths from six regions along the east coast of Tasmania: Great Oyster Bay (GOB), Mercury Passage (MP), Bruny Island (BI), Tasman Peninsula (TP), D'Entreacasteaux Channel (DEC), Fredrick Henry Bay (FHB). Trace elements used in the analysis were Li, Mn, Mg, Sr, and Ba. Circles represent the 95% confidence ellipses around the centroid mean for each region.



Data analysis

Of the 15 elements recorded, only seven (Li, Mn, Mg, Fe, Sr, Ba, and the internal standard Ca) occurred consistently in the statoliths at concentrations greater than the detection limits. Concentrations of Fe were confounded between the analysis of the hatchling and the adult statoliths because of calibration issues. The remaining elements were used in the data analysis. All variables, within each regional group of hatchlings in the analyses, were checked for normality using quantile–quantile plots. Minor departures from normality were corrected by log transformation, and three outliers were removed. Spatial differences in the trace elements from the statoliths of hatching squid from the six regions were explored with a one-way multivariate analysis of variance (MANOVA), with a canonical discriminant analysis (CDA) then used to determine which regions differed and which of the trace elements could explain the pattern of differences. However, it should be noted that the allocation of hatchlings and adults to source regions was conducted with random forests (below). CDA and random forests operate via very different mechanisms. Insight gained from the discriminant procedures of CDA may not be consistent with the basis of sample allocation with the random forests analysis.

To characterize the trace element signatures of statoliths from hatchlings obtained from each of the six geographic regions, and to then classify the trace element signatures of adults caught from these locations in the following year, we used the random forests method of classification using Li, Mn, Mg, Sr, and Ba as predictor variables. Random forests (Breiman 2001) is a modern, computer intensive, ensemble classification technique that generalizes classification trees (Breiman et al. 1984). Random forests have quickly proven to be one of the most important algorithms in the machine learning literature, showing robust and improved results of classifications on standard data sets (Boinee et al. 2006). They also confer advantages in being resistant to overfitting and do not require the strict distributional assumptions required by classical discriminant methods (Breiman 2001; Izenman 2008).

Like classification trees, random forests generates a rule to classify observations into one of K predefined classes, given training data consisting of observations whose class is known. Random forests draws bootstrap samples from the training data and fits a classification tree (Breiman et al. 1984) to each bootstrap sample. New observations are classified by applying each of the fitted trees to the new observation and assigning the class selected by the majority of trees, with probabilities of class membership derived from the proportions of trees that select each class. In random forests, cross-validation or a separate test to get an unbiased estimate of the test set error are not necessary as the general classification accuracy can be judged from the "out-of-bag" (OOB) misclassification rate. Approximately two-thirds of the data in the training sample are extracted for each bootstrap sample, with the remaining one-third of the cases left as OOB data (Boinee et al. 2006). For each tree, the misclassification rate is computed for the OOB training data excluded from the bootstrap sample used to fit the tree, and these are then pooled to give an overall measure of classification accuracy. In this way the OOB data are used to get a running unbiased estimate of the classification error as trees are added to the forest. We assessed which trace elements played a greater role in partitioning the data into defined classes with the Gini importance measure, which incorporates a (weighted) mean of the individual tree's improvement in the splitting criterion produced by each variable (Friedman 2001). Every

Fig. 3. Mean concentrations (± standard error) of Li, Mn, Mg, Sr, and Ba in the statoliths of hatchlings from six sites in the southeast of Tasmania: Great Oyster Bay (GOB), Mercury Passage (MP), Bruny Island (BI), Tasman Peninsula (TP), D'Entreacasteaux Channel (DC), Fredrick Henry Bay (FH).

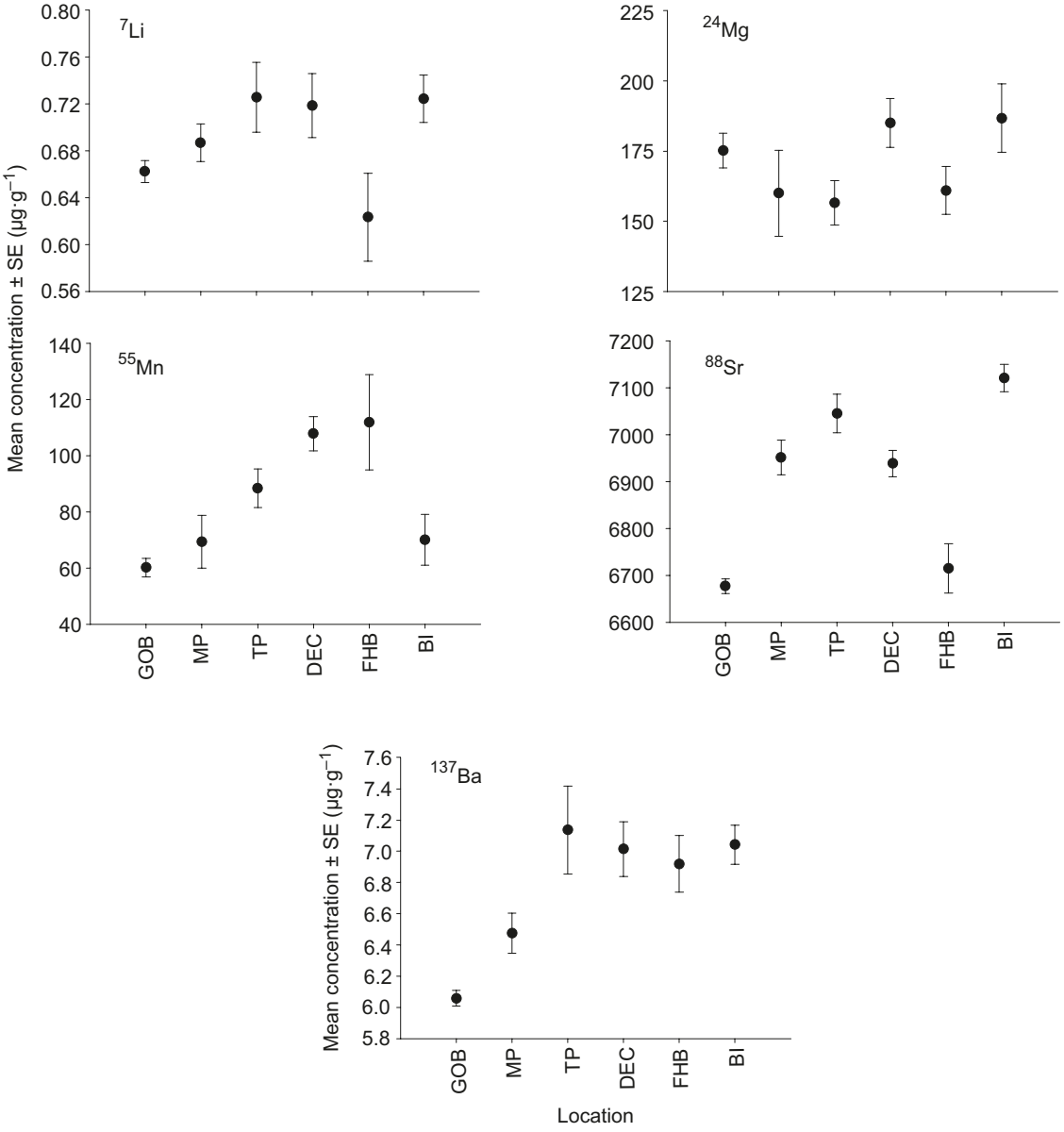
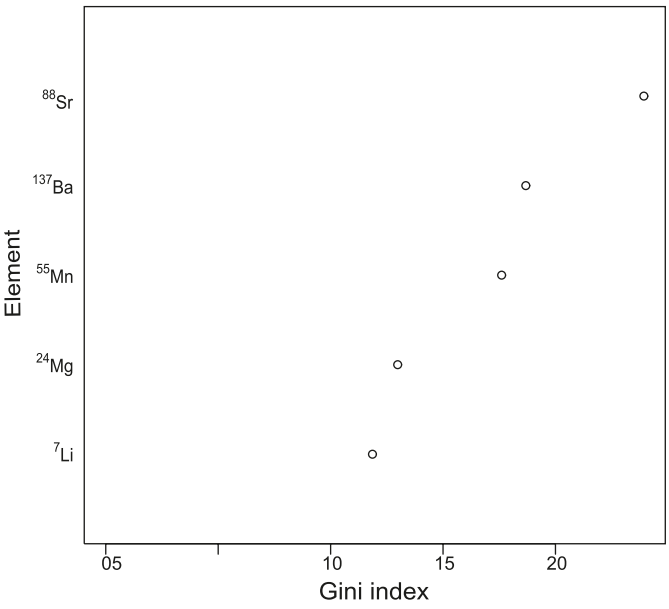


Table 2. Random forests accuracy of hatchlings allocated to each of the regions based on trace element profiles of the statolith.

			n						
Region	*N*	Accuracy (%)	GOB	MP	TP	DEC	FHB	BI	% misclassified to GOB
GOB	131	83.2	109	3	3	6	4	6	—
MP	10	20.0	1	2	1	2	0	4	10
TP	18	11.1	0	2	2	6	1	7	0
DEC	20	50.0	2	0	3	10	3	2	10
FHB	11	27.3	3	0	1	3	3	1	27
BI	25	60.0	2	2	5	1	0	15	8
Total	215	65.6							

Note: *N* is total number of hatchlings from each region; *n* is number of hatchlings classified to belong to each region.

Fig. 4. Variable importance in the random forest generation for classifying hatchlings to natal location, according to the Gini importance measure.



time a split of a node is made on a variable, the Gini impurity criterion for the two descendent nodes is less than the parent node. Adding up the Gini decreases for each individual variable over all trees in the forest gives a measure of variable importance.

Because of the nature of the sampling, samples from GOB were over-represented in the training data, and it is known that classifiers can perform poorly in such circumstances (Izenman 2008). To compensate, GOB was downsampled in the bootstrap, so that in each bootstrap sample only 20 individuals were drawn from GOB. Random forests were fitted using the random forests package (Liaw and Wiener 2002) in the R statistical computing environment (R Development Core Team 2010).

Results

Spatial variation in the elemental composition of hatchling statoliths

Significant differences were seen in the statolith elemental composition of squid hatched among the six spawning regions ($F = 8.708$; df = 25, 1045; $P < 0.001$). The statoliths of the hatchlings from GOB and FHB had distinctly different elemental compositions from all other regions (Fig. 2). GOB hatchlings had statoliths with relatively greater concentrations of Mg, but lower concentrations of Ba and Mn, while FHB hatchlings had statoliths with relatively lower concentrations of Sr and Li (Figs. 2 and 3). In contrast, hatchlings from DEC, MP, TP, and BI had statoliths with greater concentrations of Sr than hatchlings from other regions (Fig. 2).

Allocation of hatchlings and adults to regions based on statolith elemental composition

Generalized error was stabilized from 1000 trees; therefore, for high robustness of a forest and stable importance values we generated a forest of 5000 trees. The overall forest accuracy for hatchlings from the six regions, using Ba, Li, Mg, Mn, and Sr as classifiers, was 65.6% (Table 2). Hatchlings from MP, TP, and FHB had trace element profiles that resulted in particularly poor classification success. Hatchlings from DEC, BI, and GOB fared better with 50%, 60%, and 83% correct classification, respectively. Importantly, although hatchlings from one of the two closed regions (MP) had very poor classification success, hatchlings from the other closed region (GOB) were allocated with very high classification accuracy, effectively allowing the discrimination between those two key regions of interest. Additionally, very few individuals were misclassified between GOB and MP (Table 2). Ranking of the five trace elements using the Gini importance measure suggests that the most important variable for hatching site classification is Sr, followed by Ba, Mn, Mg, and finally Li (Fig. 4). The Gini variable importance curve does not drop off sharply, indicating significant discrimination ability is likely contributed by all elements.

Using the hatchling allocation rules generated with the random forests, the 152 adults caught throughout the eastern and southeastern waters of Tasmania were classified as originating from one of the six sampled natal regions. We examined and present classifications for every observation and also only those classifications where the probability of class membership exceeded 0.5 (Table 3). Based on elemental analysis, the majority of adults caught from across eastern and southeastern Tasmania were sourced from GOB, with each region indicating at least 55% contribution from GOB (Table 3). Other than the 79.7%–84.4% of the individuals caught in GOB being sourced from GOB, no other regions showed evidence of self-recruitment at a greater than 0.5 level of probability (Table 3). Both GOB and MP are considered the major spawning grounds for southern calamary, although MP appears to be largely supplied by adults from GOB. There was evidence of individuals moving substantial distances; for example, between 55% and 72% of adults caught in TP were classified as originating in GOB, and both GOB and MP showed indications of small contributions from BI. Over two-thirds of the adults were allocated to a natal region with greater than 0.5 probability (Fig. 5), suggesting that we have likely characterized trace element signatures from the major spawning regions across eastern and southeastern Tasmania.

Discussion

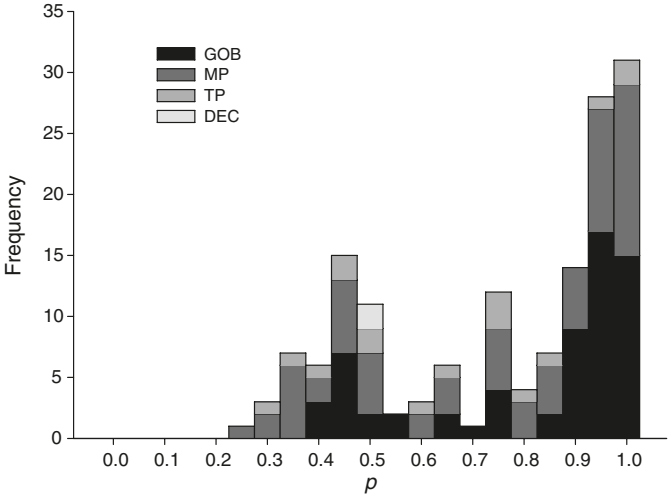
This study used trace element profiles of statoliths to explore the extent of self-seeding, the degree of connectivity among local populations, and the extent of dispersal of hatchlings in southern calamary (*S. australis*). We demonstrated that hatchlings from some regions had distinctive differences in statolith trace element profiles, which allowed us to discriminate among key spawning areas, including two regions closed to commercial fishing during the main spawning period of southern calamary (GOB and MP). By comparing hatchling trace element profiles with those of spawning adults captured at the same locations in the following year, we identified the major source of southern calamary recruits over a large proportion of the east and southeast Tasmanian coast and estimated the relative contribution of different natal areas to the fished adult population. Extensive examinations of southern calamary age and growth from within these re-

Table 3. The percentage (and number in parentheses) of adult *Sepioteuthis australis* that were allocated to one of six hatching regions, with allocations greater than 0.5 probability specified separately, from the random forest analysis.

		Classified to natal region					Classified to natal region with $P > 0.5$		
Capture location	*N*	GOB	MP	DEC	FHB	BI	GOB	FHB	BI
GOB	64	84.4% (54)	—	—	3.1% (2)	12.5% (8)	79.7% (51)	—	1.6% (1)
MP	68	69.1% (47)	5.8% (4)	—	—	25.0% (17)	61.8% (42)	—	5.9% (4)
TP	18	72.2% (13)	5.5% (1)	5.5% (1)	5.5% (1)	11% (2)	55.5% (10)	5.5% (1)	—
DEC	2	100.0% (2)	—	—	—	—	—	—	—
Total	152								

Note: Shaded cells indicate self-recruitment.

Fig. 5. Probability of adults caught from GOB, MP, TP, and DEC being allocated back to a natal region with the random forests analysis using Li, Mn, Mg, Ba, and Sr as classifiers.



gions provide strong evidence that these adults correspond to those that would have hatched at the time our hatchling samples were collected (Pecl et al. 2004). To our knowledge, this is the first study to use trace element profiles of hatchling and adult squid to successfully directly estimate the extent of population connectivity among different spawning populations. As with the majority of studies utilizing trace element analysis to examine population connectivity, we have not been able to sample all possible natal areas. However, we have 15 years of extensive fishery-independent and fishery-dependent surveys that support the selection of our sample locations as the main spawning areas for southern calamary on the east and southeast coast of Tasmania (e.g., Moltschaniwskyj and Pecl 2003, 2007).

This study revealed an important source of recruits to coastal populations of southern calamary. GOB likely contributed at least 55% and up to 84% of the fished adults caught from along the east and southeast Tasmanian coast, and this was the only region with any evidence of self-recruitment. Extensive acoustic tracking and t-bar tagging of adult calamary in GOB and MP, undertaken in the same year as the adults were obtained for the current study, allowed us to determine the phase of the life cycle that movement among regions occurs (Pecl et al. 2006). Reproductively mature adult squid spend the last 2–4 months of their life moving extensively within spawning regions (e.g., within GOB), but not moving between them (e.g., from GOB to MP or vice versa; Pecl et al. 2006). Thus, the movement of individuals originating from GOB occurs during the subadult period and not as adult migration. Southern populations appeared to be substantially augmented by very young individuals from GOB and are therefore likely dependent on the continued persistence of recruitment from this region. This has major implications for the assessment of the potential benefits and risks of given management strategies, including the designation of the location and timing of closed areas, analogous to issues associated with optimal marine reserve design.

Elemental analysis of southern calamary statoliths had sufficient resolution to allow discrimination of the natal source of hatchlings on a spatial scale relevant to management; however, there was poor spatial discrimination among some regions. Using trace elements in open marine systems or with nondiadromous species is difficult because of subtler gradients in the properties of the water compared with stronger elemental signals generated within river and estuarine systems (Thorrold et al. 2007). Despite this, over two-thirds of the adult calamary were able to be allocated to a natal region with reasonable confidence, suggesting we have been able to describe the major population linkages occurring over a broad section of the Tasmanian coast. The individuals that were unable to be allocated to one of the sampled natal areas may be from poorly discriminated hatching regions, areas or times that were not sampled, or individuals with unclear elemental profiles. Greater resolution of population linkages may be obtained by combining trace element analyses with other approaches, such as bacterial, parasites, morphology, otolith shape, and lectins used as specific gene markers to characterize frequencies of blood groups (Arrizabalaga et al. 2004), among others. Genetic analyses, like allozyme electrophoresis (Triantafillos and Adams 2001) and comparison of microsatellite allele frequencies (Shaw et al. 1999; 2004) can also produce dispersal estimates; however, these reflect average movement over evolutionary time scales (Peery et al. 2006).

Any exchange of individuals among spawning aggregations will potentially alter the genotypic composition and biological characteristics of the next generation. Furthermore, in very short-lived species with no mixing of generations, migration could very quickly change the life history characteristics of a population. Quantifying migration rates is therefore only the first step in truly understanding the role of population connectivity in modifying the structure and dynamics of populations. Several questions remain to be answered: What

biological characteristics allow individuals to successfully migrate between local groups, and are these characteristics spatially and temporally stable? Do migrants contribute to the reproductive output of the population? What influence does this have on the next generation? Effective dispersal, where individuals survive to reproduce in the new environment, is the primary determinant influencing demography and colonization on ecological time scales and local adaptation, speciation, and extinction on evolutionary time scales (Lester et al. 2007). The outcomes from this study effectively provides the groundwork in establishing evidence of self-recruitment in a short-lived marine species and as such provides a model that allows a quantitative assessment of migration and movement as processes determining population structure and dynamics.

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