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Genetic structure of *Carcinus maenas* in southeast Australia

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ABSTRACT: The European shore crab *Carcinus maenas* is a highly successful marine invader, and has displayed rapid range expansion following its introduction to many parts of the world. In Australia, it was first reported in Port Phillip Bay, Victoria in the late 1800s. Despite predictions that it would expand its range northward, its distribution has remained limited to the southeast coast of the mainland and to Tasmania. Using microsatellite loci and mitochondrial DNA, we assessed whether low connectivity among southeastern Australian estuaries might be contributing to the limited distribution of this invasive species. Under this hypothesis, we expected that sampling of *C. maenas* from 6 estuaries, roughly evenly spaced along the southeast coast, would reveal: (1) greater genetic variability among than within estuaries; (2) increasing genetic dissimilarity with distance from Port Phillip Bay; and (3) in the absence of human-mediated dispersal, declining genetic variation with distance from Port Phillip Bay. Contrary to these predictions, we found that genetic variability was no greater among than within mainland southeast Australian estuaries — indicating significant gene flow. Some slight genetic differentiation was, however, evident between Tasmania and the mainland. Multiple introductions appear to have contributed to the Australian population. The high connectivity of populations among southeast Australian estuaries suggest that management strategies focused on eradication of *C. maenas* from individual localities will not be effective. Factors other than limited connectivity appear to be responsible for the slow range expansion up the east Australian coast.

KEY WORDS: *Carcinus maenas* · Establishment · Estuary · Genetic variation · Invasive species · Microsatellite marker · Mitochondrial DNA · Population genetics

INTRODUCTION

Introductions of non-indigenous species are considered a leading cause of biodiversity loss (Bax et al. 2003, Sala & Knowlton 2006). Although not all non-indigenous species have negative impacts (e.g. Williamson 1996), some disrupt native biota and communities through competition for resources, predation, disease and parasitism (Vitousek et al. 1996), often resulting in the loss of individuals or species, or loss of ecosystem function (Bax et al. 2003, Molnar et al. 2008). Once established, an invader may be nearly impossible to eradicate, highlighting the need for rapid identification of vectors (Vitousek et al. 1996). A key task for ecologists is therefore to understand the factors involved in the spread and successful establishment of invasive species in order to inform conservation management, ameliorate impacts, and formulate plans for the prevention of future invasion events and establishment (Ruiz & Carlton 2003).

Genetics is an increasingly used tool for tracing invasion pathways, the number of establishment

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events, and the level of connectivity across the distribution of successfully established invasive species (Grosholz 2002, Ficetola et al. 2008). Invasion pathways can be reconstructed by characterising genetic variability at molecular markers such as microsatellites and sequences of mitochondrial DNA (mtDNA; Estoup & Guillemaud 2010, Gauthier 2010). Ultimately, the application of genetic techniques to marine bioinvasions allows rapid risk assessment of spread and establishment, subsequently providing vital information to conservation biologists and resource managers (Holland 2000).

The European shore crab *Carcinus maenas* is listed among the world's top 100 worst invaders by the IUCN's Invasive Species Specialist Group (GISD 2009). It inhabits a wide range of coastal habitats, including rocky intertidal and shallow subtidal sediments (Moksnes 2002, Amaral et al. 2009); it is capable of withstanding air exposure for up to 10 d (Crothers 1968), temperatures ranging from 0°C to 30°C (Roman & Palumbi 2004, Audet et al. 2008), and salinities of 4 to 54 psu (Anger et al. 1998). In addition, it has a long planktonic larval stage (up to 50 d; Williams 1968, Dawirs 1985) and high fecundity (Broekhuysen 1936). These traits have enabled *C. maenas* to establish on the east and west coasts of North America, in Australia, South Africa, Japan, and Argentina (Grosholz & Ruiz 1996, Carlton & Cohen 2003, Thresher et al. 2003, Darling et al. 2008, Blakeslee et al. 2010). In many parts of its invasive range, it has caused significant ecological and economic impacts, including the collapse of shellfisheries (Yamada & Hauck 2001, Walton et al. 2002) and shifts in native food web structures (Grosholz et al. 2000).

Carcinus maenas has been present in Australia since at least the late 1800s. It is thought to have initially been introduced to Port Phillip Bay, Victoria, in discarded ship ballast from Europe (Carlton & Cohen 2003, Thresher et al. 2003, Ahyong 2005). Subsequently, the crab has expanded its range to include the coasts of southeast Victoria, South Australia, Tasmania and southern New South Wales (NSW; Thresher et al. 2003). *C. maenas* has been reported as far north as Botany Bay, Sydney, which is separated from Port Phillip Bay by around 1200 km of coastline (Ahyong 2005), yet has been persistently observed through time in no more than 10 out of 130 estuaries in NSW — all of which are located on the state's far south coast, less than 900 km from Port Phillip Bay (Thresher et al. 2003, Ahyong 2005). This is despite predictions that *C. maenas* should have reached southern Queensland (around 1200 km further north of its present established distribution) over the last 100 yr (Cohen et al. 1995, Carlton & Cohen 2003, Compton et al. 2010).

The coastline of southeastern Australia is dominated by intermittently closed and open lakes and lagoons (ICOLLs), estuaries characterised by periodic isolation from the ocean by a sandbar (Haines et al. 2006). Conditions in ICOLLs are influenced by the rate of freshwater inflow, evaporation and tidal exchange. When closed, they may range in salinity from fresh to hypersaline, and may reach temperatures that far exceed air temperature (Lill et al. 2011). Consequently, they are highly variable environments. In previous reviews, persistent observations of *Carcinus maenas* have been notably absent from Australian ICOLLs (Thresher et al. 2003). The dynamic entrance morphology of ICOLLs may lead to environmental conditions that challenge even euryhaline and eurythermal species, and limit the dispersal of pelagic larvae. *C. maenas* zoeae are typically released during ebb tides (Zeng & Naylor 1996) which are not present in closed ICOLLs. Closed estuary entrances also present a physical barrier to new recruits from the ocean, with estuaries disconnected from the ocean for extended periods of time notably lacking *C. maenas* (C. Garisde unpubl. data). Hence, juxtaposed against the significant dispersal capabilities of *C. maenas*, the dynamics of ICOLLs may act to limit connectivity of *Carcinus* in southeastern Australia.

While the genetic structure of *Carcinus maenas* elsewhere in the world has been examined (Roman & Palumbi 2004, Brian et al. 2006, Darling et al. 2008, Pascoal et al. 2009, Tepolt et al. 2009, Blakeslee et al. 2010, Silva et al. 2010), no such study has been conducted for the populations in Australia. Here, we use both microsatellite loci and a region of mtDNA (cytochrome oxidase subunit I; COI) to assess (1) whether *C. maenas* displays greater genetic variability among rather than within estuaries — a symptom of low connectivity, and (2) whether the current distribution of *C. maenas* in Australia can be explained by a single or multiple introduction events. In the event that all Australian populations of *C. maenas* originated from a single Port Phillip Bay introduction, we would expect to find increasing genetic dissimilarity and decreasing allelic richness with geographic distance from Port Phillip Bay. If ICOLLs are limiting connectivity, we would expect individuals within an estuary to have greater genotypic similarity with each other than to individuals sampled in other estuaries.

MATERIALS AND METHODS

Sample collection

Between 28 and 30 *Carcinus maenas* were collected from each of 6 southeast Australian estuaries within the introduced range of *C. maenas* between January and July 2011 (Fig.1, Table 1). Crabs were collected from shallow waters (0 to 5 m deep) using a combination of large crab traps lined with 50 mm mesh, small folding crab traps lined with 13 mm mesh, bait traps lined with 5 mm mesh, and hand collection from rocky substrate. To adequately represent populations, collections were done from multiple locations in each estuary. Specimens were then frozen at −20°C or preserved in 70 to 95% ethanol until DNA extraction.

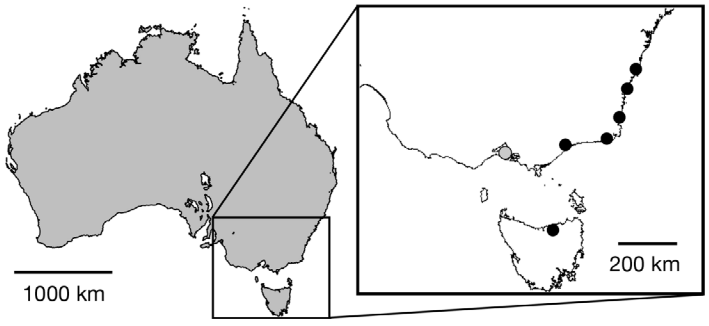

Fig. 1. Location of study sites in southeast Australia (●, see Table 1), and of Port Phillip Bay (◎), the site of the first Australian record of *Carcinus maenas*

Microsatellite DNA amplification and genotyping

DNA was extracted from leg muscle tissue of *Carcinus maenas* using the 'salting out' protocol of Sunnucks & Hales (1996). Each individual was genotyped at up to 8 microsatellite loci (n = 28 to 30 ind. per estuary) using primers previously described by Tepolt et al. (2006). For 7 of the loci (Cma01EPA, Cma03EPA, Cma04EPA, Cma05EPA, Cma08EPA, Cma09EPA and Cma14EPA), the PCR conditions were 0.5 U *Taq* DNA Polymerase (Promega), 0.5 µM each of forward and reverse primer, 0.8 µM dNTPs, 1.5 mM $MgCl_2$, and 2 µl 5× Go Taq Flexibuffer (Promega). Cma02EPA had identical PCR conditions as all other loci with the exception of primer concentrations — 1.0 µM each of forward and reverse primer. PCR amplifications were carried out using an MJ Research PTC 100 thermocycler with an initial denaturation at 94°C for 3 min, followed by 6 'touchdown' cycles of 30 s denaturation at 94°C, annealing temperatures (65°C, 63°C, 61°C, 59°C, 57°C, 55°C) for 30 s, and an extension step of 72°C for 45 s. On completion of the last touchdown cycle, another 35 cycles were carried out at 55°C annealing temperature, followed by a final extension step for 10 min at 72°C. PCR products were electrophoresed using a 3130xl Genetic Analyzer (Applied Biosystems), and allele sizes scored using Peak Scanner software v.1.0 (Applied Biosystems), GeneMapper® software v.4.0 (Applied Biosystems) and checked by eye. In order to ensure consistency in amplification and genotype scoring, an additional 10% of samples were rerun and genotyped.

Table 1. Estuary sampling locations and summary haplotype data collected in 2011. NSW: New South Wales; VIC: Victoria; TAS: Tasmania; N: no. individuals sampled; h (n): no. haplotypes (no. sampled ind.); *H*: mean (±1 SD) haplotype diversity; π: mean (±1 SD) nucleotide diversity; Hap. Freq.: observed frequencies of individual haplotypes within estuaries

Location	GPS	N	Date	h (n)	*H*	π	Hap. Freq. (h1/h2/h3/h4/h5/h6)
Candlagan Creek, NSW	35°84′S, 150°18′E	28	July	4 (8)	0.694 ± 0.147	0.0025 ± 0.0007	0.250/0.500/0.125/0.000/0.125/0.000
Corunna Lake, NSW	36°29′S, 150°13′E	30	March	4 (10)	0.778 ± 0.091	0.0032 ± 0.0007	0.100/0.200/0.000/0.000/0.400/0.300
Fisheries Creek, NSW	37°12′S, 149°93′E	30	Jan	5 (10)	0.756 ± 0.130	0.0033 ± 0.0006	0.100/0.500/0.100/0.100/0.200/0.000
Mallacoota Inlet, VIC	37°56′S, 149°76′E	30	May	5 (9)	0.806 ± 0.120	0.0035 ± 0.0007	0.111/0.111/0.222/0.000/0.444/0.111
Lake Tyers, VIC	37°85′S, 148°08′E	30	May	5 (10)	0.667 ± 0.163	0.0024 ± 0.0008	0.600/0.100/0.100/0.000/0.100/0.100
Tamar River, TAS	41°10′S, 146°82′E	30	March	3 (10)	0.511 ± 0.164	0.0017 ± 0.0006	0.200/0.100/0.700/0.000/0.000/0.000

Mitochondrial DNA amplification and sequencing

Species-specific primers designed by Roman & Palumbi (2004) were used to amplify a 403 bp segment of the mitochondrial COI gene for 10 randomly selected crabs per estuary. We selected COI to contribute towards the database of sequences already available for this species. Given the reported levels of variability at this region for *Carcinus maenas,* we anticipated that sequences from a total of 60 individuals would provide a reliable and cost effective estimate of Australian haplotype frequencies. The total volume of PCRs was 50 µl, including 3 U *Taq* DNA Polymerase (Promega), 0.5 µM each of forward and reverse primer, 0.8 µM dNTPs, 1.5 mM $MgCl_2$, and 10 µl 5× Go Taq Flexibuffer (Promega). PCR amplifications were carried out using an MJ Research PTC 100 thermocycler with an initial denaturation at 94°C for 3 min, followed by 6 'touchdown' cycles of 30 s denaturation at 94°C, annealing temperatures (60°C, 58°C, 56°C, 54°C, 52°C, 50°C) for 30 s, and an extension step of 72°C for 45 s. On completion of the last touchdown cycle, another 35 cycles were carried out at 50°C annealing temperature, followed by a final extension step for 10 min at 72°C. PCR product was visualised on a 2% agarose gel by electrophoresis prior to purification and sequencing by Macrogen (Korea).

Data analyses

The total number of alleles, observed heterozygosity (H_o) and expected heterozygosity (H_e) were calculated for each locus using GENALEX v.6.41 (Peakall & Smouse 2006). Significant deviations from Hardy-Weinberg equilibrium and linkage disequilibrium for pairs of loci were tested using the software GENEPOP v.4.0.10 (Rousset 2008) and FSTAT v.2.9.3.2 (Goudet 1995), respectively. Scoring errors and possible null alleles were identified using MICRO-CHECKER v.2.2.3 (Van Oosterhout et al. 2004). Mitochondrial sequence data were aligned and edited by eye in MEGA v.5.05 (Tamura et al. 2011). The number of haplotypes, number of polymorphic sites, haplotype diversity (H) and nucleotide diversity (π) were calculated for mitochondrial data using DnaSP v.5.10.01 (Librado & Rozas 2009). A haplotype network was created, and the putative ancestral haplotype was determined via parsimony using TCS 1.21 (Clement et al. 2000)

Genetic partitioning among estuaries was assessed by calculating pairwise F_{ST} values for microsatellite data, and both F_{ST} and Φ_{ST} for mitochondrial data using Arlequin v.3.5.1.3 (Excoffier & Lischer 2010). The 95% confidence intervals around F_{ST} and Φ_{ST} values were generated by permutation and used to evaluate whether estimates were different from zero (0 = no structure; 1 = maximum difference). Nonetheless, gene flow may be high enough to offset the effects of genetic drift (and thus differentiation as measured via F_{ST}). Consequently, we also carried out analyses based on genotypic arrays, which, being shuffled at each generation, provides a more sensitive measure of genetic differentiation (Stow & Magnusson 2012). The spatial distribution of genotypic similarity can be used to infer patterns of dispersal and gene flow. Low dispersal is predicted to result in high genotypic similarity among individuals in close proximity, with similarity decreasing with geographic distance (Stow et al. 2006).

Spatial autocorrelation of genotypic similarity (s) was evaluated using GENALEX v.6.41, in order to test (1) whether there is higher genotypic similarity within estuaries than among estuaries, and (2) whether genetic similarity would decrease with geographic distance. The s values obtained range from +1 to −1 with a mean of zero (no autocorrelation). Positive s indicates greater genotypic similarity than expected by chance for the given data set, and negative values indicate less similarity. For each distance class, the significance of any deviation from zero was assessed by 999 permutations (Peakall & Smouse 2006). The 95% confidence intervals around s were obtained via bootstrapping 999 times. Pairwise relatedness (Queller & Goodnight 1989) between individuals sharing an estuary was estimated with reference to allele frequencies generated from all sampled individuals. Because relatedness estimates are based on allele frequencies generated from the whole sample, isolation is expected to result in elevated within-estuary relatedness compared to the overall level of relatedness in more connected estuaries, and variation in the level of within-estuary relatedness would indicate variation among estuaries in the degree to which they are connected. The 95% confidence intervals around relatedness were obtained via bootstrapping 999 times. Migrant flow between estuaries was further examined using a Bayesian clustering analysis performed using BayesAss 3.0.3 (Wilson & Rannala 2003).

RESULTS

DNA for analysis using microsatellite markers was successfully extracted from 178 crabs from 6 estuar-

Table 2. Summary statistics for microsatellite loci for *Carcinus maenas* in southeast Australian estuaries. N_A: number of alleles; N: number of samples; bp: base pairs; H_o: observed heterozygosity; H_e: expected heterozygosity; sig: statistical significance; ns: not significant at $\alpha = 0.05$; *p < 0.00625

Locus	N_A	N	Size range (bp)	H_o	H_e	Sig
Cma01EPA	2	148	191–195	0.453	0.450	ns
Cma02EPA[a]	7	148	196–256	0.503	0.740	*
Cma03EPA	8	148	152–196	0.689	0.705	ns
Cma04EPA	13	148	118–296	0.811	0.836	ns
Cma05EPA	2	148	92–104	0.412	0.441	ns
Cma08EPA	14	148	118–154	0.777	0.839	ns
Cma09EPA	8	148	165–197	0.757	0.828	ns
Cma14EPA	4	148	229–255	0.230	0.279	ns

[a]Locus excluded due to possible null alleles

ies. One sample from the Tamar River failed to amplify at more than 3 microsatellite loci, and was consequently removed from analyses. Early data exploration suggested some genetic structuring between mainland estuaries and the Tamar River estuary in Tasmania. Therefore, to test for any non-Mendelian inheritance, summary statistics were generated for mainland estuaries only (Table 2). No pairs of loci showed any evidence of linkage disequilibrium. A homozygote excess and significant deviation from Hardy-Weinberg equilibrium was evident for Cma02EPA. Analyses performed in MICRO-CHECKER (Van Oosterhout et al. 2004) supported the possibility of null alleles at this locus, and therefore the Cma02EPA locus was excluded from all subsequent analyses.

A total of 58 mitochondrial DNA sequences were used in the analyses. Following alignment, a 403 bp COI amplicon contained 5 polymorphic sites, 4 of which were parsimony-informative across the 6 identified haplotypes (GenBank accession numbers; h1: KF709201; h2: KF709202; h3: KF709203; h4: KF709204; h5: KF709 205; h6: KF709206). Four haplotypes were separated from the putative ancestral haplotype (h1) by a single mutational difference, while the last haplotype was separated by 2 mutational differences (Fig. 2). Five haplotypes were shared between 3 or more estuaries, whereas haplotype h4 was sampled in only one estuary (Fisheries Creek, NSW). The most abundant haplotype on the mainland was h2, whereas the most abundant haplotype in Tasmania was h3 (Table 1). Four or more haplotypes were found in each mainland estuary, while Tamar River, Tasmania exhibited only 3 haplotypes. Overall haplotypic diversity was relatively high (H = 0.803 ± 0.017 [SD]), and consistent with nucleotide diversity (π = 0.0033 ± 0.0002 [SD]; Table 1).

Our data revealed higher levels of gene flow among estuaries located on the mainland compared with levels of gene flow between the Tasmanian Tamar River estuary and mainland estuaries. Overall, microsatellite data revealed weak but significant differentiation between mainland estuaries and Tasmania, with the F_{ST} value for this contrast significantly differing from zero (mean F_{ST} = 0.047, 95% CI = 0.016 to 0.076). Whereas each of the pairwise F_{ST} values between mainland estuaries and Tasmania were significantly different from zero, 90% of comparisons between mainland estuaries were non-significant (Table 3). This pattern of greater gene flow between estuaries of the mainland and Tasmania than among mainland estuaries was also supported by Bayesian clustering. A lower average level of migrants per generation was evident for pairwise comparisons between the mainland estuaries and the Tasmanian estuary (mean ± SD = 0.034 ± 0.027) than for comparisons between mainland estuaries (0.058 ± 0.076). Overall, mitochondrial data indicated significant genetic structure among estuaries (Φ_{ST} = 0.22, p < 0.000001). This result appeared to be driven by the single Tasmanian population, as pairwise F_{ST} values calculated using mitochondrial data indicated little genetic structure among mainland estuaries (70% of comparisons non-significant), but all pairwise F_{ST} values involving Tasmania were significantly different from zero (Table 3).

The average within-estuary relatedness of *Carcinus maenas* was similar for each of the mainland estuaries (range: −0.009 to 0.050; all bootstrapped 95% CI overlap), suggesting little variation in the

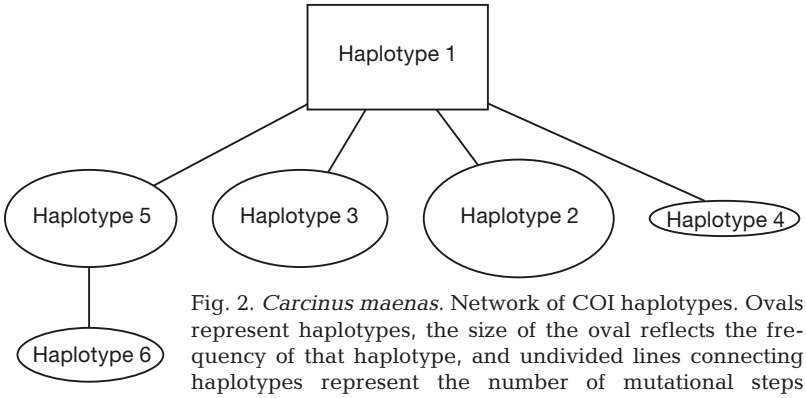

Fig. 2. *Carcinus maenas.* Network of COI haplotypes. Ovals represent haplotypes, the size of the oval reflects the frequency of that haplotype, and undivided lines connecting haplotypes represent the number of mutational steps separating them

Table 3. Genetic differentiation between populations of *Carcinus maenas* in southeast Australian estuaries. Pairwise F_{ST} values for microsatellite data are given above the diagonal; pairwise F_{ST} values for mtDNA (COI) are given below. Negative values are adjusted to zero. * denotes a significant result

	Candlagan Creek	Corunna Lake	Fisheries Creek	Mallacoota Inlet	Lake Tyers	Tamar River
Candlagan Creek		0.003	0.005	0.006	0.000	0.058*
Corunna Lake	0.319*		0.007	0.017*	0.006	0.076*
Fisheries Creek	0.000	0.226*		0.000	0.000	0.040*
Mallacoota Inlet	0.214*	0.000	0.127		0.009	0.032*
Lake Tyers	0.103	0.150	0.018	0.000		0.046*
Tamar River	0.394*	0.535*	0.337*	0.331*	0.296*	

degree to which these locations are connected. *C. maenas* within the Tamar River estuary, Tasmania, by contrast, displayed significantly higher within-estuary relatedness than mainland estuaries (r = 0.158). This reflects the significant partitioning in mainland Tasmanian allele frequencies — put another way, Tasmanian individuals are more related to each other than they are to individuals sampled on the mainland, implying less connectivity between the Tasmanian and mainland locations. Across mainland estuaries, spatial autocorrelation analysis revealed that there was no significant relationship between geographic distance and genotypic similarity, and no evidence that genetic similarity was higher within mainland estuaries than genotypic similarity between individuals occupying different estuaries (s = 0.006 and –0.001, respectively; both values overlapping with bootstrapped 95% CI).

DISCUSSION

Contrary to the hypothesis that there would be limited connectivity among *Carcinus maenas* populations of southeast Australia, we found that genetic variation was no greater among than within mainland estuaries. A lack of genetic partitioning among mainland estuaries was evident over both relatively long (pairwise Φ_{ST} and F_{ST}) and short time scales (via comparisons of within and between estuary genotypic similarity), and also supported by migration estimates using Bayesian clustering analyses. This lack of genetic structure implies high levels of gene flow among mainland estuaries.

High genetic connectivity has been found in other introduced populations of *Carcinus maenas*, including the west and mid-Atlantic east coasts of North America (Roman 2006, Tepolt et al. 2009). Despite the high genetic connectivity of *C. maenas* among mainland estuaries, slight but significant genetic partitioning was evident between mainland estuaries and Tasmania. This was demonstrated by measures of genetic distance (F_{ST} and Φ_{ST} results) and comparatively low genetic diversity in the Tamar River.

The East Australian Current (EAC) likely plays a major role in setting patterns of connectivity for *Carcinus maenas* along the east Australian coast. The EAC, which moves warm water down the east coast of Australia from its source in the Coral Sea to the east coast of Tasmania, is the continent's strongest boundary current, reaching speeds of up to 3.6 m s^{-1} (Mata et al. 2007). The current generates a characteristic cyclonic and anticyclonic eddy field in summer and autumn (Mata et al. 2007) which, combined with the weakening of the current in winter (Ridgway & Godfrey 1997), can result in considerable counter-current flow. Previous studies have found a lack of clear spatial genetic structuring along the east coast of Australia in a range of dispersing organisms (e.g. sea anemone: Hunt & Ayre 1989; sea urchin: Banks et al. 2007; damselfish: Curley & Gillings 2009; kelp: Coleman et al. 2011). It has been suggested that in contrast to weak currents, which result in 'stepping stone' patterns of dispersal that leads to patterns of isolation by distance, stronger currents such as the EAC result in 'mosaic' patterns of genetic structure (Coleman et al. 2011). Genetic connectivity between Tasmania and the mainland may, however, be limited because the 240 km wide Bass Strait lacks suitable habitat for the benthic phases of *C. maenas*.

Given the high level of genetic connectivity found in this study, it seems unlikely that the dynamics of ICOLLs are significantly impacting genetic connectivity of *Carcinus maenas* among estuaries in southeast Australia. In this study, however, only 3 of the 6 estuaries examined (Corunna, Fisheries Creek and Mallacoota Inlet) exhibit periodic entrance closure, and each was open at the time of this study. A more extensive survey that samples ICOLLs during times when they are both open and closed would provide a more extensive test of this question.

Invasion patterns in Australia

Levels of genetic diversity can reflect the size of founding populations, the number of introductions and their provenance. For example, multiple inva-

sions from genetically distinct sources merge genetic diversity, potentially resulting in rapid increases in genetic diversity over short time periods (Roman 2006). In the event that Australian populations of *Carcinus maenas* resulted from a single small establishment event, we would expect them to be of lower genetic diversity than native populations, as a consequence of founder effects (Brandt et al. 2009, Lye et al. 2011, Rollins et al. 2011).

We found similar genetic diversity of *Carcinus maenas* in southeast Australia, compared to previously sampled populations elsewhere in the world, of both native and introduced origin. The levels of microsatellite heterozygosity, number of alleles, and mtDNA haplotype and nucleotide diversities of *C. maenas* sampled during this study did not differ from previously reported values for native European *C. maenas* (Darling et al. 2008, Blakeslee et al. 2010) or introduced *C. maenas* on the east and west coasts of North America and in South Africa (Roman 2006, Tepolt et al. 2006, Darling et al. 2008, Tepolt et al. 2009). Furthermore, in a study including 3 Australian sites, Darling et al. (2008) similarly concluded that Australian *C. maenas* display similar genetic diversity to other invasive populations that are known to have resulted from multiple introductions (Roman 2006, Blakeslee et al. 2010). The similar genetic diversity of *C. maenas* in Australia to that within its native distribution, suggests either multiple introductions of *C. maenas* to southeast Australia or a single, large founding event. We tentatively suggest that multiple introductions are more likely. This hypothesis is consistent with the large number of international shipping ports along the southeast coast of Australia (see Bishop & Hutchings 2011), which are regularly visited by ships from Asia, Europe and North America, and have resulted in this coastline being among the most invaded in the world (Hewitt et al. 2004, Bishop & Hutchings 2011).

Implications

In summary, this study has demonstrated high connectivity of *Carcinus maenas* among mainland estuaries of southeastern Australia. This suggests that ICOLL entrance dynamics are unlikely to be limiting connectivity, and hence range expansion of *C. maenas*. Instead, the southward flow of the EAC or biotic interactions may be more plausible explanations for the failure of the crab to remain established above southern New South Wales. The blue swimmer crab *Portunus pelagica* is an abundant epibenthic predator in shallow habitats of east Australian estuaries, as far south as the Victorian border (just south of Fisheries Creek). The southern limit of its distribution overlaps with the northernmost distribution of *C. maenas*. It is possible that competitive interactions between *P. pelagicus* and *C. maenas* limit the invader's northward range expansion on the east Australian coast. On the east coast of North America, another portunid crab, *Callinectes sapidus,* limits the distribution and abundance of *C. maenas* through aggressive interactions and predation (deRivera et al. 2005). An ongoing trapping program in southern NSW estuaries has detected a negative relationship between the abundance of *P. pelagicus* and *C. maenas* (C. Garside unpubl. data).

The high connectivity of *Carcinus maenas* along the east Australian coast has important implications for management. It suggests that interventions aimed at eradicating the crab from a single location are unlikely to be effective, and more holistic approaches to management may be needed. Given the high dispersal capabilities of *C. maenas,* it will be important to understand the factors that are presently limiting its northward range expansion so that further spread of this invader, which can cause costly damage to shellfish industries (e.g. Walton et al. 2002), can be avoided.

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