Molecular insights into the invasion dynamics of *Carcinus* crabs in South Africa

Clova A. Mabin · Tamara B. Robinson · John R. U. Wilson · Heidi Hirsch · Maria L. Castillo · Michelle Jooste · Johannes J. Le Roux

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**Abstract** Knowledge of the introduction history and spread dynamics of invasive species can provide important insights for management (Ens et al. in Environ Rev (in press), 2022), however such information is often unavailable for accidental introductions. Here we infer how the European shore crab, *Carcinus maenas*, and its congener, the Mediterranean shore crab, *C. aestuarii*, were introduced to and spread within South Africa. We do this using nuclear microsatellite data and Bayesian assignment tests and Approximate Bayesian Computation (ABC) modelling that included samples from the native and other invasive ranges of these two species. We also compared the genetic diversity and structure of one of the South African populations during and after intensive management, with that of another, unmanaged, population. South African populations had higher genetic diversity than invasive *Carcinus* populations from elsewhere in the world. Moreover, the ABC analyses suggest that South African populations originated from an admixture event between individuals of *C. maenas* from a population in the native range and an invasive population from Canada. We also identified instances of hybridisation between *Carcinus maenas* and *C. aestuarii* in South Africa. South African populations showed no genetic structure, suggesting either extensive migration between them or that populations arose from the same initial introduction. Management of *Carcinus* did not affect genetic diversity or structure, and we suspect that the management duration was insufficient to target a full generation of crabs. Together these results suggest multiple introductions and/or high propagule pressure to South Africa, crab (larval or adult) movement between existing populations, and some hybridisation. For eradication from South Africa to be achieved, management would need to concurrently target all known invasive populations and clearly establish that new introductions could be prevented.

**Keywords** Admixture · Eradication · Hybridisation · Invasion history · Marine crab · Propagule pressure
Introduction

Understanding invasion histories and post-introduction demographic dynamics of invasive species are important considerations for the development of effective management approaches (Johnston et al. 2009; Le Roux and Wieczorek 2009; Wilson et al. 2017). For taxa that have been deliberately introduced, these aspects can sometimes be quantified through historical introduction records (Richardson 1998). However, such information is rarely available for accidental introductions, as is the case for most marine invasions (Hewitt and Campbell 2007; Mead et al. 2011). In addition, many marine species have pelagic life stages in highly connected ecosystems, potentially diluting pathway signatures (Johnston et al. 2009). Molecular approaches can, however, provide insights into historical and contemporary population-level processes (Le Roux and Wieczorek 2009; Chown et al. 2015; Sherpa and Després 2021). Estimates such as the number of founding individuals, introduction frequency, the occurrence of admixture, and hybridisation, can all be reliably inferred from molecular data (Rius et al. 2012; Rius and Darling 2014; Le Roux 2021).

Shore crabs in the genus *Carcinus* are some of the world’s most widespread marine invaders. For instance, the European shore crab, *Carcinus maenas*, has established invasive populations in four continents: Africa, Australia, North America and South America (Carlton and Cohen 2003). Its congener, the Mediterranean shore crab, *C. aestuarii*, is also invasive in Japan (Sakai 1986). Such widespread invasion success suggests that these crabs are easily transported over long distances, perhaps because of their pelagic larval life stages (Queiroga 1996). Yet, except for invasions in Japan and South Africa, these two species rarely co-invade (Geller et al. 1997). *Carcinus* crabs are considered high-impact invaders across many of their invasive ranges (for reviews on *C. maenas* see Behrens Yamada 2001; Walton et al. 2002; Chen et al. 2004; Ens et al. 2022). In regions such as Canada, multiple introductions of *C. maenas* have led to genetically diverse invasive populations (Roman 2006) while, in others, such as on the Pacific coast of North America, secondary introductions founded populations with limited genetic diversity (Darling et al. 2008). The latter population, covering approximately 1,000 km, stemmed from a single introduction event from the Atlantic coast (Darling et al. 2008; Tepolt et al. 2009).

*Carcinus maenas* was first detected in South Africa in 1983 (Joska and Branch 1986). Previous genetic studies identified both *C. maenas* and *C. aestuarii* in South Africa (Geller et al. 1997; Darling et al. 2008). Hybridisation between the two species has also been detected in Japan (Darling 2011). It has been suggested that the Japanese population likely resulted from a single introduction event from a hybrid zone in the native range (Darling 2011). Genetic studies on South African *Carcinus* populations suggest a complex introduction history characterised by multiple introductions (Darling et al. 2008). Subsequent morphometric analyses of thousands of individuals, however, found no evidence of *C. aestuarii* in the country (Robinson et al. 2005). In South Africa, *Carcinus* is largely restricted to two harbours (Table Bay and Hout Bay) (Mabin et al. 2017). Table Bay harbour is Cape Town’s largest commercial harbour with substantial international traffic. Hout Bay harbour, located approximately 30 km to the south of Table Bay harbour, is relatively small and used by commercial fishing boats and recreational watercraft. A 2013 study estimated that these two harbours contained approximately 164,000 and 7,000 *Carcinus* crabs, respectively (Jooste 2013).

In recognition of the negative impacts of *Carcinus* crabs elsewhere in the world (e.g. Grosholz et al. 2000, Matheson et al. 2016, Ens et al. 2022), and in support of authorities charged with managing biological invasions in South Africa, a management trial was implemented during 2014 and 2015 in Hout Bay harbour (Mabin et al. 2020). The primary aim of that project was to assess the feasibility of eradicating *Carcinus* crabs from South Africa. However, approximately five times more crabs (36,244 individuals) were caught than the previously estimated total population size and extirpation was not achieved (Mabin et al. 2020). This management trial also highlighted the need to understand the movement of individuals within and between populations that are targeted for management. In the context of the South African *Carcinus* invasion, three important questions arose that would need to be addressed to assess the feasibility of a nationwide eradication attempt. First, is there significant movement of crabs between Table Bay and Hout Bay harbours? If so, eradication attempts need to be strictly concurrent between harbours. Second,
to what degree did the management trial in Hout Bay Harbour reduce the genetic diversity of the population? And third, if both crab populations could be extirpated, is reintroduction likely to occur from elsewhere in the world?

Using a population genetic approach, this study aimed to provide insights to these management questions by establishing: (1) whether the two South African populations of *Carcinus* have similar levels of genetic diversity and structure and how these attributes compare to other invasive and native populations, (2) the most likely scenarios that explain the colonisation history of *Carcinus* in South Africa, (3) whether there is any genetic evidence for *C. maenas* and *C. aestuarii* co-invading South Africa and, if so, whether they hybridise, and (4) whether management efforts have affected the genetic diversity and structure of the *Carcinus* population in Hout Bay harbour.

We hypothesised that the two South African populations would have similar genetic diversity and no structure, in line with the attributes observed in invasive *Carcinus* populations globally. Support for this hypothesis will indicate frequent movement between the two harbours and multiple introductions, implying a high likelihood of reintroduction if both populations were successfully extirpated. We also hypothesised that no *C. aestuarii* individuals or *Carcinus* hybrids would be present in South Africa (Robinson et al. 2005). Lastly, we hypothesised that a *Carcinus* trial management programme would reduce the genetic diversity and structure of the Hout Bay harbour population, compared with the unmanaged Table Bay population (Hampton et al. 2004; Abdelkrim et al. 2007).

### Materials and methods

#### Sample collection and microsatellite genotyping

Samples were collected from native and invasive populations of *Carcinus maenas* and *C. aestuarii* around the world in 2015 (Table 1). South African samples were collected at Hout Bay and Table Bay harbours in 2014, 2015 and 2016, i.e. before, during and after the management trial in Hout Bay, with Table Bay as a control (Mabin et al. 2020).

In South Africa, *Carcinus* individuals were collected using baited traps containing 200 g of crushed sardines in a muslin bag with a soak time of two hours. Captured crabs were euthanised by freezing at −20 °C and frozen gill tissue was removed for DNA extractions. International samples were provided as gill tissue, except for samples from the USA, which consisted of chelae tissue. All tissue samples were stored in 90% ethanol until DNA extraction. DNA was extracted from all samples using a QIAGEN DNeasy Blood and Tissue Kit (QIAGEN, California, USA, supplied by Whitehead Scientific, Cape Town, South Africa) according to the manufacturer’s protocol, with the following modifications. After the...

| Species       | Sampled population          | Range     | No. of samples |
|---------------|-----------------------------|-----------|---------------|
| *C. maenas*   | Plymouth, England           | Native    | 49            |
| *C. maenas*   | Newtownards, Northern Ireland | Native    | 49            |
| *C. maenas*   | Aveiro, Portugal            | Native    | 48            |
| *C. maenas*   | Port Gawler, Australia      | Invasive  | 30            |
| *C. maenas*   | Stephenville, Canada        | Invasive  | 50            |
| *C. maenas*   | Bodega Bay, United States of America | Invasive | 49            |
| *C. aestuarii*| Chioggia, Italy             | Native    | 43            |
| *C. aestuarii*| Tokyo Bay, Japan            | Invasive  | 30            |
| *Carcinus* spp.* | Hout Bay, South Africa 2014 | Invasive  | 60            |
|               | Hout Bay, South Africa 2015 | Invasive  | 65            |
|               | Hout Bay, South Africa 2016 | Invasive  | 76            |
|               | Table Bay, South Africa 2014| Invasive  | 26            |
|               | Table Bay, South Africa 2015| Invasive  | 72            |
|               | Table Bay, South Africa 2016| Invasive  | 71            |
addition of Buffer AW2, the solution was centrifuged for 5 min at 13,300 rpm. The DNeasy mini spin column was then removed from the collection tube and centrifuged for a further 3 min at 13,300 rpm in a clean collection tube to remove excess ethanol. The final elution step was modified using 50 µL Buffer AE to increase final DNA concentrations. DNA quality and concentrations were determined using the Nanodrop spectrophotometer (Infinite 200 PRO NanoQuant, Tecan Group Ltd, Männedorf, Switzerland) and all samples were diluted to a final concentration of 20 ng/µl.

Polymerase chain reaction (PCR) was used to test the amplification of 23 microsatellite loci using primers previously developed for *C. maenas* and *C. aestuarii* (Tepolt et al. 2006; Marino et al. 2008). After PCR optimisation, successful amplification was consistently achieved for eight loci (Table S1). PCR amplifications for these loci were performed in two multiplex reactions using the QIAGEN Multiplex PCR kit (multiplex 1) and the KAPA2G Fast Multiplex PCR kit (multiplex 2). PCR reactions were performed in 96 well plates (with nine technical replicate samples and two water samples per plate as positive and negative controls, respectively). For multiplex 1, PCR reactions contained 1.5 µL x 0.2 µM primer mix (primers mixed in equal concentrations), 7.5 µL QIAGEN multiplex mix, 1.5 µL Q solution, 3 µL water and 1.6 µL DNA template. For multiplex 2, PCR reactions contained 4.5 µL water, 7.5 µL 2x Kapa2G Fast Multiplex mix, 1.5 µL x 2 µM primer mix, and 1.6 µL template DNA. PCR cycling conditions for multiplex 1 consisted of 15 min initial denaturation at 95 °C, followed by 30 cycles of denaturation at 94 °C for 30 s, annealing at 52.7 °C for 90 s and extension at 72 °C for 60 s, before a final extension for 30 min at 60 °C. PCR cycling conditions for multiplex 2 consisted of denaturation at 95 °C for 3 min, followed by 30 cycles of denaturation at 95 °C for 15 s, annealing at 54.4 °C for 30 s and extension at 72 °C for 25 s, and a final extension for 1 min at 72 °C. Purified PCR fragments were separated on an ABI Prism 3100 Genetic Analyzer (Applied Biosystems, Foster City, California, USA), using GENESCAN TM-500 (-250) as an internal size standard (Applied Biosystems). Allele sizes were visualised and scored using GENE-MARKER version 1.95; SoftGenetics LLC, State College, Pennsylvania, USA). Samples that failed to amplify at more than four loci were excluded from the final dataset, resulting in a total of 718 usable genotypes.

**Genetic diversity**

The number of alleles per locus were calculated using the ‘NB’ package in the R statistical environment (Hui 2014; R core team, 2016). Observed heterozygosity (*H*<sub>O</sub>), expected heterozygosity (*H*<sub>E</sub>), allelic richness (*A*<sub>R</sub>), and the inbreeding coefficient (*F*<sub>IS</sub>), and significant departures of allele frequencies from Hardy Weinberg equilibrium (HWE) expectations were calculated in the ‘diveRsity’ package (Keenan et al. 2013).

Population genetic diversity metrics (*A*<sub>R</sub>, *H*<sub>O</sub>, *H*<sub>E</sub> and *F*<sub>IS</sub>) were compared between invasive- and native-range *C. maenas* populations using student t-tests. We only included data from 2016 for South African samples (highest sample size) in these analyses to avoid pseudo replication.

**Global population genetic structure**

The final global dataset (including South Africa) was assessed for the presence of null alleles using the FreeNA software (Chapuis and Estoup 2007). Pairwise fixation indices (*F*<sub>ST</sub>) were calculated in FreeNA, with and without null allele correction (Chapuis and Estoup 2007). To compute 95% confidence intervals for *F*<sub>ST</sub> values, 10,000 simulations were performed. *F*<sub>ST</sub> values for each population were then used to test whether the presence of null alleles significantly influenced the results using a Kruskal-Wallis test.

The genetic structure of all sampled crab populations was estimated using Bayesian assignment tests implemented in the STRUCTURE version 2.3.4 software (Pritchard et al. 2000). STRUCTURE models were run on all populations of *Carcinus* (*n* = 14). Including both *Carcinus* species in our models allowed us to infer instances of hybridisation between them. The number of genetic clusters (i.e. *K*-values) used for each analysis was between one and the number of populations included in each analysis. Twenty model iterations were run for each value of *K*. Each run consisted of 500,000 generations of which the first 100,000 were discarded as burnin. The admixture model was applied with correlated allele frequencies as hybrids between *C. maenas* and *C. aestuarii* were suspected to be present in the dataset (Darling
et al. 2008). Optimal \( K \) values were determined using the method described by Evanno et al. (2005) and as implemented in STRUCTURE Harvester (Earl and von Holdt 2012). The programme CLUMPP (version 1.1.2; Jakobsson and Rosenberg 2007) was used to merge the results from STRUCTURE, utilising the greedy option method with 10,000 repeats. The resultant output file was visualised in DISTRUCT (version 1.1; Rosenberg 2004).

Inferring putative the source(s) of Carcinus introductions to South Africa

The potential source(s) of Carcinus populations in South Africa was inferred using an Approximate Bayesian Computation (ABC) approach and the DIYABC software (version 2.1.0; Cornuet et al. 2008, Cornuet et al. 2014). This approach allows the simulation of a large number of genetic datasets for different potential introduction scenarios that are compared with the observed dataset to identify the most likely scenario (Beaumont et al. 2002). A hierarchical approach was adopted as this reduced the number of scenarios and thus computational power required and has been used successfully in previous studies (Boissin et al. 2012; Stone et al. 2017; Wauters et al. 2018). In total, 78 DIYABC analyses were conducted, with several variations to the biological and historical parameters (results not shown). These variations included the size, source and founding date of all populations considered, the presence and strength of potential bottlenecks, rate of admixture, mutation models and rates. The three best-performing analyses are detailed below.

**Analysis 1** involved simulations that considered all potential sources of Carcinus. The probabilities of three invasion scenarios were estimated, including a direct introduction from the ranges of \( C. maenas \) or \( C. aestuarii \) and an admixture event between these two ranges. These ranges included three native and three invasive \( C. maenas \) populations and one native and one invasive \( C. aestuarii \) population (Fig. 1).

**Fig. 1** Sequential ABC analyses conducted to assess the likely origin of South African Carcinus populations. Analysis 1: Scenario (1.1) A direct introduction from the native or invasive range of \( C. maenas \) to South Africa; Scenario (1.2) A direct introduction from the native or invasive range of \( C. aestuarii \); Scenario (1.3) An admixture event between \( C. maenas \) and \( C. aestuarii \) leads to the founding of the South African population. DIYABC output: Posterior probability comparison using a logistic regression approach identifies Scenario 1.1 as the most parsimonious. Analysis 2: Scenario (2.1) Direct introduction from the native range of \( C. maenas \) to South Africa; Scenario (2.2) Direct introduction from the invasive range of \( C. maenas \) to South Africa; Scenario (2.3) The South African population formed as a result of admixture between native and globally invasive \( C. maenas \); Scenario (2.4) A ghost population in the \( C. maenas \) native range founds the South African population; and Scenario (2.5) A ghost population in the \( C. maenas \) invasive range founds the South African population. DIYABC output: A comparison of the posterior probabilities inferred that Scenario 2.3 is the most likely. Analysis 3: The South African populations is the result of admixture between populations in the \( C. maenas \) native range and invasive populations from Scenario (3.1) Canada; Scenario (3.2) the USA; or Scenario (3.3) Australia. DIYABC output: Posterior probability comparison indicated that Scenario 3.1 is the most likely.
with ghost populations (i.e. unsampled populations) and strong population genetic bottlenecks. However, these were not included in the final analyses due to low support (see Results). The complete global dataset was used for this analysis, excluding the 2014 and 2015 South African samples to avoid pseudo replication while maximising sample sizes.

Analysis 2 was conducted at higher resolution, with different invasion scenarios developed using the inferred results from Analysis 1. This led to more detailed modelling to infer the contribution of native and invasive C. maenas populations to South African invasions. Scenario 2.1 involved a direct introduction from the native range of C. maenas while Scenario 2.2 represented a direct introduction from the invasive range of C. maenas to South Africa. Scenario 2.3 modelled an admixture event between a native and invasive C. maenas population. Scenario 2.4 and 2.5 simulated an introduction to South Africa from a ghost population in the C. maenas native and invasive range respectively.

Analysis 3 was based on the results from analysis 2 and aimed to identify the most likely invasive range source of invasive populations in South Africa: Canada (Scenario 3.1), the USA (Scenario 3.2), and Australia (Scenario 3.3), noting in each case that the invasive population in South Africa appears to have been the result of admixture of other invasive populations with populations from the native range.

All ABC models were parameterised by drawing on the biology of Carcinus and presence data in invaded areas. It was assumed that the two congeners shared demographic parameters where data were unavailable for C. aestuarii (the least studied of the two species). The timing of events was measured in terms of generations, which was one year in this case, based on the life history characteristics of C. maenas (Behrens Yamada 2001). The values estimated for the temporal priors were assumed from historical shipping and detection records (Joska and Branch 1986; Carlton and Cohen 2003; Mead et al. 2011). The prior parameters (estimated with uniform distributions) for temporal events, population sizes and bottleneck reductions are detailed in Table S2. Loci were estimated to have an allelic range between 40 and 72. The minimum value of 40 represented the DIYABC default value, which is greater than recorded in this study, but allowed for the inclusion of unsampled alleles. The final model utilised a single-step mutation model due to improved performance over the default generalised stepwise mutation model. Microsatellite mutation rates have not been determined for crustaceans, however for mammals, rates are between $1 \times 10^{-3}$ and $1 \times 10^{-5}$ (Dallas 1992; Ellegren 1995) while in arthropods (e.g. Drosophila melanogaster), mutation rates of $6 \times 10^{-6}$ have been observed (Schlötterer et al. 1998). Accordingly, rates in this study ranged from $1 \times 10^{-2}$ to $1 \times 10^{-6}$. The DIYABC default rates of admixture were used, i.e., 0.001–0.999. The summary statistics used to differentiate between the different scenarios modelled included the mean number of alleles and the mean genic diversity (Nei 1987; as measures of the diversity among the sampled populations), in addition to the fixation index ($F_{ST}$) (Weir and Cockerham 1984) and genetic distance ($d\mu^2$; Goldstein et al. 1995) to distinguish between populations. Datasets were simulated $1 \times 10^6$ times for each scenario. The pre-evaluation analysis allowed a visual comparison between the simulated and observed datasets of each scenario in a principal coordinate analysis (PCA) as well as a ranked comparison between the summary statistics of each dataset. The posterior probabilities were then compared in a Linear Discriminant Analysis using a logistic regression approach to select the 1% closest simulated dataset to that observed, with non-overlapping 95% confidence intervals. The accuracy of the posterior distributions of the model parameters were evaluated using the relative bias. In order to evaluate the confidence in scenario choice, various scenario-parameter combinations were analysed in the prior and posterior distributions. Type I and II errors were calculated using 100 pseudo-observed datasets (pods) for each scenario. Then for each analysis, type I errors were estimated by determining the number of times that the correct analysis was not selected, over all 100 pods. Type II errors were calculated based on the proportion of times the correct scenario was selected when it did not have the highest posterior probability.
Genetic insights into the invasion dynamics of Carcinus crabs in South Africa

Allelic richness ($A_R$) of the three sampling periods in Table Bay and Hout Bay was calculated using the ‘diveRsity’ (Keenan et al. 2013) package in R with a rarefaction approach to account for unequal sample sizes. $A_R$ was analysed with respect to harbour (2 levels: Table Bay and Hout Bay) and year (3 levels: 2014, 2015 and 2016), using a two-factor generalised linear model (GLM) with a quassipoisson error distribution. The Genalex (version 6.5; Peakall and Smouse 2012) programme was used to calculate the number of private alleles ($P_A$) and these were analysed with respect to harbour and year using a Pearson’s Chi-Squared test.

Temporal changes in the genetic structure of South African populations were evaluated using Bayesian assignment tests in STRUCTURE (version 2.3.4; Pritchard et al. 2000). This methodology was identical to that of the global population structure analysis described above, except that $K$ values between 1 and 6 were tested for the South African-only analysis. Assignment values for the South African populations were analysed with respect to harbour (2 levels: Table Bay and Hout Bay) and year (3 levels: 2014, 2015 and 2016) and the interactions between the factors, using a two-factor generalised linear model (GLM) with a quassipoisson error distribution.

Mitochondrial DNA analyses

Fourteen South African samples (collected in 2014 and 2015) were identified as potential hybrids in our STRUCTURE analysis (i.e. being admixed), using an approach adopted in previous studies (Le Roux et al. 2015, Castillo et al. 2021). To confirm the maternal identities of these genotypes, a portion of the $COI$ mtDNA gene was sequenced. PCR amplification was done using the primers COIF-PR115: TCW ACN AAY CAY TTA ATY G 3’ and the reverse primer jgHCO2198 (5’ TAI ACY TCI GGR TGI CCR AAR AAY CA 3’) (Geller et al. 2013). Each PCR reaction contained 3 µL template DNA, 1.25 µL of each primer (5 µM), 2.5 µL 10 x Buffer, 2.5 µL MgCl$_{2}$, 0.1 µL Super Isotherm Taq, and 13.4 µL distilled water. PCR cycling conditions were: initial denaturation at 94 °C for 2 min, 30 denaturation cycles at 94 °C for 1 min, annealing at 50 °C for 60 s, and a final elongation step at 72 °C for 15 min.

DNA sequences were aligned and edited in BIOEDIT (version 7.0.5, Hall 1999) along with Carcinus sequences from Genbank (http://www.ncbi.nlm.nih.gov, FJ159008 to FJ159102). Haplotype networks were constructed using sequence data in TCS (version 1.21; Clement et al. 2000) and visualised using the Population Analysis with Reticulate Trees (PopART, version 3; Leigh and Bryant 2015) software.

Results

Global genetic diversity

Null alleles were detected in our dataset but at a low frequency, with a mean of 0.036 (SD: 0.056) across all loci. Seven of the microsatellite loci were identified as polymorphic in all populations included, with a maximum of 36 alleles detected per locus. One locus was monomorphic in six of the 14 populations but had a total of five alleles across all populations. Thirty-one (28%) loci by population combinations showed significant departures ($p < 0.05$) from HWE in the 14 populations.

Native C. maenas populations had significantly higher high levels of allelic richness ($A_R$ of 10.8 to 11.3, Table S3) compared to most invasive populations ($A_R$ between 4.5 and 7.3; t = -2.448, df=6, $p < 0.05$). It is noteworthy, however, that South African populations had high levels of $A_R$. 

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Across all populations, observed heterozygosity ($H_0$) was lower than the expected heterozygosity $H_E$ (Table S3). Levels of $H_E$ were similar between native and invasive populations ($t = -2.167$, df = 6, $p > 0.05$). $H_0$ was significantly higher in native populations ranging between 0.65 and 0.67, while in invasive populations ranging between 0.58 and 0.64 ($t = -3.796$, df = 6, $p = 0.009$). The number of private alleles ($P_a$) in native range populations varied from four to seven; in invasive populations there were fewer than three per population), with the exception of South Africa (mean = 4). Inbreeding ($F_{IS}$) estimates were higher in invasive (mean = 0.067) than in native populations (mean = 0.05) (Table S3), however, this difference was non-significant ($t = 1.219$, df = 6, $p > 0.05$).

Global population genetic structure

There was no significant difference between pairwise $F_{ST}$ values when using data that were uncorrected or corrected for the presence of null alleles (Table S4 and S5 respectively, $H = 0.394$, $p = 0.530$), therefore subsequent analyses were conducted using the uncorrected dataset. Among global Carcinus populations, excluding South African populations, pairwise $F_{ST}$ distances ranged from zero between Portugal and Ireland, to 0.2473 between the USA and Japan (Table S4). Unsurprisingly, the highest differentiation was observed between C. maenas and C. aestuarii populations. STRUCTURE analyses identified four genetic clusters ($K = 4$) within the global range of Carcinus (Fig. 2a, Fig. S1). These consisted of a

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**Fig. 2 a** STRUCTURE barplots where vertical axes illustrate the proportional assignment of individual genomes to the inferred genetic clusters where $K = 4$ for global Carcinus populations sampled, including both native and invasive regions. This analysis included South African samples collected in 2014, 2015 and 2016. **b** Haplotype network based on the mtDNA sequences analysed using POPART (including samples from 2013). The size of circles indicates the number of samples with those haplotypes. Lengths of connections are not to scale. $C_{m\_native} = C. maenas$ native range populations; $C_{a\_native} = C. aestuarii$ native range populations.
distinct *C. aestuarii* cluster (including native Italian and invasive Japanese populations), a North American *C. maenas* cluster (comprised of the USA and Canadian populations), a *C. maenas* cluster (including native European populations) and a less distinct *C. maenas* cluster, that corresponded mostly to populations from Australia.

Putative source(s) of *Carcinus* in South Africa

The first DIYABC analysis (which assessed the likelihood of a direct introduction from the *C. maenas* or *C. aestuarii* native and invasive ranges as well as an admixture event between these two ranges) indicated that a *C. maenas* population most likely founded the South African population, i.e. Scenario 1.1 (Fig. 1, Table S6). This scenario had the highest posterior probability with 95% confidence intervals that did not overlap with the other tested scenarios (P = 0.6800, 95%CI = 0.6690–0.6910). The type I error associated with the selection of this scenario was 0.07 using the direct approach and 0.08 for the logistic approach. The mean type II errors calculated under the direct and logistic approach were 0.12 (ranging from 0 to 0.24) and 0.06 (with a range of 0 to 0.11) respectively. The next most likely scenario inferred was that the South African population was an admixture between *C. maenas* and *C. aestuarii*, i.e. Scenario 1.3 (P = 0.3143, 95%CI = 0.3034–0.3253). The last scenario (which tested that the founding population in South Africa came from a *C. aestuarii* population) was not well supported by the model (P = 0.0057, 95%CI = 0.0008–0.0105).

The second analysis (which involved scenarios focusing on *C. maenas* populations only, grouped as native or invasive) strongly supported the scenario of South African populations originating from an admixture event between populations in the native and invasive range (i.e. Scenario 2.3) (P = 0.6886, 95%CI = 0.6791–0.6982) (Fig. 1, Table S6). Type I errors associated with the selection of Scenario 2.3 were 0.4 using the direct approach and 0.2 for the logistic approach. The mean type II errors associated with the false acceptance of Scenario 2.3 under the direct and logistic approach were 0.05 (ranging from 0.03 to 0.06) and 0.19 (with a range from 0 to 0.4) respectively. The next most probable scenario for the genetic patterns observed in South Africa was that the colonists originated from a native-range population of *C. maenas* (P = 0.135, 95%CI = 0.129–0.142).

The third analysis (which addressed the *C. maenas* invasive populations at a country level, in combination with populations from the native range) indicated that Canada [instead of invasive populations from the USA (P = 0.197, 95%CI = 0.190–0.204) or Australia (P = 0.287, 95%CI = 0.279–0.295)] was the likely source of South African populations that subsequently admixed with a native range population (P = 0.516, 95%CI = 0.508–0.525) (Fig. 1, Table S6). Type I errors associated with this most likely scenario were 0.24 and 0.25 (for the direct and logistic approaches respectively). The mean type II errors associated with the false acceptance of this scenario under the direct approach were 0.15 (ranging from 0.13 to 0.17) and 0.22 (with a range from 0.16 to 0.27) for the logistic approach.

The effective population size (i.e. the number of individuals that are reproductive in a population) of the South African *Carcinus* populations according to the posterior distributions of the Bayesian model parameters in analysis 1 was 3,410 (95% CI: 720–8,840) while analysis 2 estimated the populations to comprise 6,570 individuals (95% CI: 2,520–9,780) and analysis 3 estimated this population to contain 7,870 (95% CI: 4,210–9,890) (Table S6). The most-supported invasion scenario in analysis 1 estimated that *Carcinus* arrived in South Africa in 1895 (95% CI: 1683–1979). Analysis 2 estimated that the crab was introduced in 1864 (95% CI: 1660 and 1962), while Analysis 3 supported an introduction occurring in 1843 (95% CI 1488 and 1995).

Demographic impacts of trial management interventions against *Carcinus* in South Africa

Within South African populations, there was no main effect of harbour or year (or an interaction between them) on *A_R* (p > 0.05) (Fig. 3a). The *A_R* of the Hout Bay population did not change through time because of management or stochastic loss via genetic drift. The Hout Bay population had higher *P_A* (18 alleles) compared with Table Bay (6 alleles) (χ² = 14, df = 5, p = 0.016) but this did not change over time [χ² = 6, df = 6, p = 0.423, (Fig. 3b)].

Among South African populations, genetic differentiation was extremely low, ranging from 0 to 0.0076. STRUCTURE analyses of these populations
assigned individuals to three genetic clusters ($K=3$), but with all individuals assigned with equal probabilities to all three clusters (Fig. S2). Such complete and equal admixture indicates the true value of $K$ is more likely to be one, i.e., a panmictic population (Le Roux et al. 2008).

There was no main effect of year or site on the Bayesian assignment values for South African populations (q1-4) (Table S7, Fig. 4) when considering data from the full global model ($p>0.05$). None of the interactions between the two factors were significant, except for q2, where there was a significant interaction between site and year ($F=5.559$, df=2, $p=0.004$).

Mitochondrial DNA diversity of Carcinus in South Africa

Of the 62 samples sequenced, only one $C. aestuarii$ individual was identified. This specimen was collected during a minor sampling event in 2013, rather than the more extensive sampling of 2014 and 2015. The haplotype network (Fig. 2b) showed considerable genetic distance between the congeners, with 33 mutational steps separating $C. maenas$ and $C. aestuarii$ haplotypes. The network clearly identified one South African individual that has a haplotype that closely resembles $C. aestuarii$, in contrast to the majority of samples collected in South Africa that were identified as $C. maenas$.

Discussion

Reintroductions and movement of individuals between invasive populations of an alien species that is the focus of management action can undermine control efforts. As such, understanding these demographic aspects can help to identify incursions that can be effectively and efficiently managed. Following a trial to manage invasive Carcinus crabs in South Africa, this study used a genetic approach to consider introductions and within-region movement in relation to two invasive populations to inform future management. It was found that the two harbour populations of Carcinus in South Africa had high and very similar
genetic diversity and no structure. This most likely resulted from multiple independent introductions to both sites and/or ongoing gene flow between the two harbours. Extensive sampling during this study confirmed that *C. maenas* is the predominant species in South Africa and there is limited evidence of hybridisation with *C. aestuarii*. Management action in Hout Bay harbour did not affect the genetic diversity of that population. If both harbour populations were successfully extirpated in future, these findings suggest that reintroduction is likely to occur from elsewhere in the world.

Except for South African populations, invasive *Carcinus* populations harbour significantly lower genetic diversity than native-range populations, indicative of lower propagule pressure and possible founder effects in these regions. These findings fit the general observation that introduced populations often exhibit reduced genetic diversity compared to their native range source populations (Dlugosch and Parker 2008; Le Roux 2021). By contrast invasive South African populations had remarkably high levels of genetic diversity, comparable to that found in native range populations. *Carcinus* populations in Hout Bay and Table Bay harbours represent a panmictic population suggesting that these they likely stemmed from the same introduction and/or experience frequent gene flow. Our approximate Bayesian computation estimated that *Carcinus* was introduced to South Africa earlier than previously thought (Joska and Branch 1986), in 1843. One of the earliest recorded sightings of *C. maenas* outside its native range...
range was in the Red Sea in approximately 1817, before the Suez Canal opened in the 1860s (Carlton and Cohen 2003). Therefore, this introduction may have occurred via a shipping route from the native range, around the tip of Africa (Carlton and Cohen 2003). In North America, lag phases of between two and four years have been documented for *C. maenas* (Medof and Dickie 1955; Cohen et al. 1995) but other marine crustaceans have been known to have experienced lag phases of up to 50 years (Witte et al. 2010). However, there is a wide confidence interval around the introduction dates estimated in this study and considering that the crab is highly conspicuous, it is unlikely to have gone undetected for so long in such a well-studied region (Awad et al. 2002). The discharge of ballast water in South African waters began in the late 1800s and completely replaced the use of dry ballast by the 1950s (Griffiths et al. 2009). However, the first detection of an alien species that was thought to have arrived in South Africa via ballast water occurred in the 1940s (Griffiths et al. 2009). Therefore, it is plausible that the upper range of introduction dates estimated by our ABC analyses are closer to the true arrival date of the crab.

In addition to multiple introductions from global *Carcinus* populations, the similarities in the genetic diversity and structure of the *Carcinus* populations in South Africa indicates frequent secondary dispersal within the country. This might have occurred through the natural or human-mediated transfer of propagules. It is possible that coastal advective currents flowing South (Nelson and Polito 1987), enabled larval dispersal from Table Bay to Hout Bay. Oceanographic processes were identified as a possible factor contributing to the invasion of *C. maenas* on the Pacific coast of Canada (Brasseale et al. 2019). However, an experimental study in South Africa found that adult *C. maenas* are unable to effectively grip rocky substrata under high wave action (Hampton and Griffiths 2007) that typifies much of the intertidal coastline between Hout Bay and Table Bay. As such, environmental conditions might limit the establishment of adults outside protected harbour areas (Mabin et al. 2017). Alternatively, predatory driven biotic resistance might restrict the spread of the crabs (Mabin 2018). In terms of human-mediated spread, the most plausible vectors between the two harbours are pelagic fishing vessels and recreational yachts. Such transfer by coastal vessels has been documented for *C. maenas* in Canada (Blakeslee et al. 2010). However, in the absence of clear evidence, it is not possible to determine the vector(s) responsible for the initial or secondary introductions and maintenance of genetic diversity found in South African populations.

In agreement with previous reports (Geller et al. 1997; Darling et al. 2008), our STRUCTURE analyses identified 13 admixed genotypes (based on microsatellites) between the two *Carcinus* species. Yet, almost all of these admixed genotypes had mtDNA haplotypes corresponding to *C. maenas*, suggesting that *C. maenas* is the predominant invader in South Africa and that *C. aestuarii* is likely no longer present in the country. The latter is supported given the absence of ‘pure’ *C. aestuarii* multi-locus microsatellite genotypes in South African populations. The most parsimonious introduction scenario inferred through the ABC approach also suggests that current South African *Carcinus* populations are the result of at least one admixture event between native and non-native North American *Carcinus* populations. This seems more likely when considering that most marine taxa introduced to South Africa came from Europe and North America (Faulkner et al. 2017). However, to date, no evidence exists for the presence of *C. aestuarii* or *Carcinus* hybrids in Canada (Darling et al. 2008). Therefore, the presence of admixed genotypes in South Africa may have arisen under various circumstances. First, individuals of both species could have been directly introduced to South Africa from their native range where they co-occur and hybridise. The assignment analysis found evidence for *C. aestuarii* genotypes in the native range of *C. maenas*. An oceanographic mixing zone between Atlantic and Mediterranean waters, off the coast of Spain in the Gibraltar Strait (Tintore et al. 1988) has been proposed as an important phylogeographic break for several marine organisms such as the Mediterranean mussel, *Mytilus galloprovincialis* (Quesada et al. 1995) and the European flat oyster, *Ostrea edulis* (Saaavreda et al. 1995) and possibly the genus *Carcinus* (Geller et al. 1997). This region might therefore have acted as the source of South African *Carcinus* populations. The port of Gibraltar is an important contributor to contemporary and historical global species flow within this zone as a result of its position at the entrance to the Mediterranean Sea (Xu et al. 2014). Alternatively, it is possible that larvae of both species were collected in ballast water in multiple
ports by international vessels, prior to discharge in South Africa. The third possibility is that there were multiple introductions and that the two species were introduced on different occasions in South Africa, *C. maenas* in high numbers and *C. aestuarii* in low numbers. The absence of *C. aestuarii* individuals from the extensive sampling of 2014 and 2015 suggests the Mediterranean shore crab has not established in South Africa.

Tracking temporal changes in genetic diversity can be useful in assessing the demographic responses of invasive species to disturbances (Mairal et al. 2021) and management interventions (Abdelkrim et al. 2007; Vardien et al. 2013). For example, three years after a ten-year-long management programme against *Spartina alterniflora* in California, significant changes in the genetic diversity of the species were observed (Ort and Thornton 2016). In the current study, similar and high levels of genetic diversity in *Carcinus* populations were maintained over three years in both Table Bay (unmanaged) and Hout Bay (managed) harbours. There are three possible explanations for the apparent stability of the Hout Bay population through time. First, the diversity and structure trends simply reflect the presence of juveniles in the population who were below the minimum catchable size during the period of management (Mabin et al. 2020) that have since grown to a sufficient size to be retained in the traps. These juveniles would carry most, if not all, standing genetic variation from the previous generation. Second, it could be that continued natural or human-mediated spread from Table Bay during management maintained the genetic diversity of the Hout Bay population. Lastly, and most likely, our management duration may have been insufficient to lower the Hout Bay harbour population size to levels that would affect its genetic diversity.

Considering the limited resources that are available to address threats to biodiversity (Echols et al. 2019), it is vital that management of invasive species is effective and efficient (Loureiro et al. 2020). Key to supporting such an outcome is scientific feasibility. While this can relate to the susceptibility of the target taxa to control techniques (Panetta 2015) or the biology of the species (Dana et al. 2019), reintroductions together with movement of individuals from within the invaded range into management areas, can also moderate management success (Williams and Grosholz 2008). While tracking such movement can be challenging (especially in aquatic systems), this study used genetic approaches to investigate the role it may have played in a management trial that was unsuccessful at removing a population of *Carcinus maenas*. It seems likely that there have been multiple introductions from multiple ranges into South Africa, possibly earlier than previously reported. Thus, we would predict a high likelihood of re-colonisation even if removal was feasible. As such, eradication is unlikely to be achieved unless the vectors of this crab are correctly identified and managed. In addition, there seems to be extensive gene flow between the harbours. While it is not clear how frequently dispersal occurs, this suggests that any attempts at eradication would need to be concurrent between all invasive populations and that invasive populations are likely to establish in other harbours along the South African coast. In conclusion, understanding inter- and intra-regional dispersal of target alien species can offer invaluable insights into the feasibility of management actions.

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Data Availability  (all data will be deposited into the Dryad online repository upon acceptance of the manuscript)

Code Availability  Not applicable.

Declarations

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