# Pelagic early life stages promote connectivity in the demersal labrid *Choerodon rubescens*

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## Abstract

Population connectivity has profound ecological and evolutionary implications. In marine species with complex life cycles, the nature of these implications depends on both the amount of dispersal and the life-cycle stage(s) through which dispersal occurs. For demersal fishes with such life cycles, the pelagic early life stages (ELS) are generally considered the main dispersive phase, though this assumption has rarely been tested. This research investigates genetic connectivity in the reef-dwelling labrid *Choerodon rubescens*, which is a prized eating fish endemic to ca 1200 km of the west coast of Australia. This species has demersal juveniles and adults, and pelagic ELS that are predicted to last about 25–30 days. The aim of the study was to use patterns of variation at 12 microsatellite loci to test the hypothesis that *C. rubescens* is genetically homogeneous across its main distribution. The genetic analyses were based on samples of 26–40 individuals from six locations, which collectively span all of the areas where *C. rubescens* is common. The values of global $F_{ST}$ (0.0019) and $D_{EST}$ (0.0010) were not significantly different from zero and Bayesian clustering indicated that all individuals belonged to a single genetic group. In addition, Mantel tests showed no evidence of isolation by distance. These

results support the view that *C. rubescens* is genetically homogeneous over all or most of its geographic range. Since published otolith microchemistry evidence indicates that the juveniles and adults of *C. rubescens*are relatively sedentary, these results also imply that the pelagic ELS are the main avenue of dispersal in this species. This study highlights the value of combining the results of complementary methods for assessing the relative importance of the different life-cycle stages in dispersing a fish species. The results also have implications for the management of *C. rubescens*, which has shown signs of localised overfishing. Specifically, the dispersal of the ELS provides a mechanism for the recruitment of *C. rubescens* to non-natal reefs. Such connectivity could help this species to recover from localised depletions in abundance and ameliorate the potential evolutionary consequences of any localised overfishing, such as reductions in genetic diversity or selective responses to harvesting.

**Keywords**: Baldchin Groper; Microsatellite; Larval dispersal; Early life stages; Population genetics; Reef fish

## 1. Introduction

Connectivity refers to the exchange of individuals between assemblages of a species in different locations (Cowen and Sponaugle, 2009). The degree of connectivity influences the ecology and evolution of the species, including population growth, resilience to disturbance and rates of divergence and adaptation (Berry et al., 2012a; Botsford et al., 2009; Cowen and Sponaugle, 2009; D'Aloia et al., 2013), and is therefore of fundamental practical and theoretical importance (Curley et al., 2013; Selkoe and Toonen, 2011; Treml et al., 2008). In marine invertebrates and fishes, it is especially important to understand the life-cycle stage(s) at which connectivity occurs (Berry et al., 2012b; Frisk et al., 2013; Tobin et al., 2010) because the demographic implications will vary greatly according to whether dispersal occurs during the egg/larval, juvenile and/or adult stages (Botsford et al., 2009).

Most demersal and benthic invertebrates and fishes in coastal marine environments possess pelagic early life stages (ELS) (Weersing and Toonen, 2009). These pelagic stages, which usually include the eggs, larvae and sometimes also young juveniles (Govoni, 2005), are often assumed to be the main dispersive phase in the life cycles of such species (Cowen and Sponaugle, 2009). This assumption has, however, rarely been tested, partly because it is very difficult to disentangle the relative contributions of the different life-cycle stages to dispersal (Selkoe and Toonen, 2011). Some recent studies have, however, used data from complementary methods to assess these contributions (e.g., Berry et al., 2012a, 2012b; Longmore et al., 2014; Schmidt et al., 2011), and have provided support for the above assumption. For example, the combined results of genetic, otolith microchemistry and particle modelling analyses indicate that the pelagic ELS of the West Australian Dhufish *Glaucosoma hebraicum* disperse relatively widely, while the adults and juveniles are fairly sedentary (Berry et al., 2012a; Fairclough et al., 2013). In contrast, the juveniles and/or the adults were found to be the main dispersive phases in some other species (Frisk et al., 2013; Sagarese and Frisk, 2011; Wirgin et al., 2014).

The Baldchin Groper *Choerodon rubescens* (Günther, 1862) is a long-lived, reef-dwelling labrid that is endemic to about 1200 km of the west coast of Australia between Coral Bay (ca 24°S) and Geographe Bay (ca 34°S) (Allen, 2009). It is particularly abundant at the Houtman Abrolhos Islands, which are located at approximately the centre of its distribution. This species contributes to commercial and recreational fisheries and evidence suggests that overfishing has occurred at some locations (Fairclough et al., 2011, 2014; Ryan et al., 2013; Wise et al., 2007).

An understanding of connectivity in *C. rubescens* is required to ensure that the management of this species is occurring at appropriate spatial scales (see Fogarty and Botsford, 2007; Toonen et al., 2011). The results of an otolith microchemistry study indicate that the movements of the adults and juveniles of this demersal species are likely to be limited (Fairclough et al., 2011), probably to within 10s of km. The extent of dispersal of the pelagic ELS, however, remains uncertain. Information on genetic connectivity in *C. rubescens*, when integrated with the otolith microchemistry results, can be

used to address this uncertainty. This is because genetic connectivity will reflect the amount of gene flow over all stages of the life cycle, including the ELS (Johnson et al., 1986).

The duration of the pelagic ELS of *C. rubescens* is not known, but is predicted to be in the order of 25–30 days. This prediction is based on the fact that pelagic larval durations (PLD) in congeneric labrids in similar geographic locations tend to be similar (Victor, 1986) and the PLD of the Blackspot Tuskfish *Choerodon schoenleinii*, which co-occurs with *C. rubescens*, is about 25–30 days (Yamada et al., 2009). Preliminary analyses of the number of growth rings between the nuclei and settlement marks in otoliths of *C. rubescens* support this prediction (K. Cure, pers. comm.). Furthermore, *C. rubescens* is a broadcast spawner with buoyant eggs (Fairclough, 2005), which probably hatch in one to two days, like those of *C. schoenleinii* (see Yamada et al., 2009). The relatively protracted duration of the pelagic ELS provides considerable potential for dispersal and thus gene flow (see Shanks, 2009), possibly across the entire distribution of the species. The extent to which this dispersal potential is realised will, however, depend strongly on the hydrology of the environment (Cowen and Sponaugle, 2009; Shanks, 2009).

The hydrology of the west coast of Australia within the range of *C. rubescens* is dominated by northward-flowing, wind-driven shelf currents, as well as intrusions onto the continental shelf from the Leeuwin Current (which mainly flows southwards along the shelf break at these latitudes) (Cresswell et al., 1989). The latter dominates in late-autumn and winter, while the former are predominant in summer, although the strength and peak flow of each vary from year to year (Cresswell et al., 1989; Feng et al., 2010). Both of these water flows probably influence the dispersal of the ELS in *C. rubescens*, which spawns mainly in spring and early summer (Fairclough et al., 2011). The results of hydrodynamic modelling of passive particles released in water around the Houtman Abrolhos Islands, and further south off Perth, suggest that the prevailing water movements could transport 28-day pelagic ELS in multiple directions, sometimes over hundreds of kilometres, particularly during the peak spawning period of *C. rubescens* (see Feng et al., 2010; Penton and Pattiaratchi, 2013). Some areas, including one south of the Houtman Abrolhos Islands, may, however, have higher retention rates than others (Feng et al., 2010). Overall the data imply that the hydrology

of the broader environment may facilitate widespread mixing of the pelagic ELS of *C. rubescens* (also see Kennington et al., 2013). Nevertheless, it is difficult to predict the extent and pattern of the dispersal of pelagic ELS, particularly in species where these stages are relatively protracted (Selkoe and Toonen, 2011). Reasons for this include the complexities of marine hydrodynamics and that larval dispersal is not necessarily entirely passive (Stanley et al., 2012; Weersing and Toonen, 2009).

The aim of this study was to use the patterns of variation at 12 microsatellite DNA loci to test the hypothesis that *C. rubescens* is genetically homogeneous across its main distribution. Such a finding would imply the presence of widespread gene flow brought about via the dispersal of the pelagic ELS, since the demersal juveniles and adults have already been shown to be relatively sedentary.

## 2. Methods

### 2.1. Sample collection

Samples of *C. rubescens* were obtained from six localities throughout the main part of the distribution of this species (Fig. 1; Table 1). These represent a subset of the samples used by Fairclough et al. (2011) for assessing connectivity in *C. rubescens* via otolith microchemistry. Samples were collected from all the fishery management districts in which this species occurs, except for the South-West Area (see Fig. 1), where this species is typically rare (see Hutchins, 2001). A total of 193 fish, with between 26 and 40 per site, were caught by line fishing between May 2008 and April 2010 (Table 1). The ages of 153 of these fish were determined and ranged from ca 2 to 19 years. Approximately 5 g of white muscle tissue was dissected from each fish at the time of capture and stored in 100% ethanol for subsequent genetic analyses.

### 2.2. Molecular analyses

Total genomic DNA was extracted from approximately 5 mg of ethanol-preserved muscle tissue using a MasterPure™ DNA Purification Kit (Epicentre Technologies, Sydney). This process essentially

followed the manufacturer's recommended methodology for soft tissue. Each DNA extract was air dried and then suspended in 50 μL of TE buffer at pH 8.0 and stored at − 20 °C.

Polymerase chain reaction (PCR) was used to amplify 8 tetranucleotide and 4 dinucleotide microsatellite loci from the DNA extracts. The PCR protocols, primer sequences and locus characteristics are as described by Gardner et al. (2011).

PCR products were screened using capillary electrophoresis and laser detection on a 3730 DNA Analyzer (Applied Biosystems). The PCR products from two loci, with either different dye labels (VIC or FAM) or non-overlapping allele size ranges, were typically multiplexed. Each assay contained 0.5 μL of each PCR product combined with 15 μL of Hi-Di™ Formamide and 0.17 μL of GeneScan™ LIZ 600 size standard (Applied Biosystems).

Genotypes were scored manually, using GeneMarker demo v 1.92 (SoftGenetics Inc.), providing that the relative fluorescent unit for each allele peak was ≥ 1000. Another researcher blind scored approximately 10% of the samples (see Morin et al., 2010). On any occasion where a discrepancy in calls occurred, the chromatogram was re-examined and then either a consensus was reached or, if that was not possible, the assay was repeated. If a consensus on the scoring of the repeat assay could not be reached, the data were discarded.

A positive control (i.e., a PCR assay using a DNA sample that had previously yielded product) and a negative control (i.e., a PCR assay with no added DNA) were incorporated into each PCR run and plate.

### 2.3. Data analyses

The total (A) and standardised ($A_S$) number of alleles, and the observed ($H_o$) and expected ($H_e$) heterozygosities were calculated for each locus in each sample of *C. rubescens* and for all samples pooled. Calculations of $A_S$ for a locus were based on the smallest number of individuals assayed for that locus in any of the six samples, using 1000 iterations in the standardisation procedure in PopTools v 3.2.5 (Hood, 2010).

Friedman rank tests (Friedman, 1937), with loci as the unit of replication, were applied to test the null hypothesis that the values of $A_S$ and $H_e$ did not differ between samples.

Tests for departures from Hardy–Weinberg Equilibrium (HWE) conditions at each locus in each sample, and for each locus across all samples, were conducted using exact probability tests and the Fisher exact test, respectively. These tests were conducted with GENEPOP v 4.0.10 (Raymond and Rousett, 1995; Rousset, 2008), which used Markov chain resampling to estimate the exact probability of a type I error with 1000 batches, 10,000 iterations per batch and a dememorisation number of 10,000. A sequential Bonferroni correction (Rice, 1989) was applied to these and all subsequent analyses involving multiple tests, to control for Type I errors. Micro-Checker v 2.2.3 (van Oosterhout et al., 2004) was used to test for evidence of null alleles, large allele drop out and other genotyping errors.

Tests for departures from non-random associations of genotypes between pairs of loci in each sample, and between pairs of loci across all samples, were conducted using exact probability tests and the Fisher exact test, respectively, via GENEPOP. These tests used the batch, iteration and dememorisation numbers described above for this software. The tests found no evidence of non-random associations of genotypes, following Bonferroni correction (data not shown), and the patterns of variation at the different loci were therefore assumed to be independent of each other.

Simulations in POWSIM v 4.0 (Ryman and Palm, 2006) were used to evaluate the power of the microsatellite data to detect genetic differentiation at five levels of divergence. Each simulation mimicked the subdivision of a base population into six subpopulations (corresponding to the six samples) that then drifted to a specified level of divergence. The divergence level was specified by a combination of the effective size of the subpopulations ($N_e$) and the number of generations of divergence (t). For all simulations, $N_e$ was set at 10,000 and t was varied according to the level of divergence required (see Appendix 10 in the POWSIM manual). After drift, POWSIM mimicked the sampling of alleles from n diploid individuals in each subpopulation, where the values of n corresponded to the maximum number of individuals assayed in the six samples of *C. rubescens*. These data were then used to estimate the value of $F_{ST}$ among the six subpopulations and to assess

whether the $F_{ST}$value was significantly different from zero, using Fisher's method. For a particular simulation/level of divergence, this process was replicated 1000 times with replacement. The proportion of 1000 replicates in which the $F_{ST}$ values were statistically significant reflects the power of the microsatellite data to detect genetic differentiation at that divergence level. Each simulation was based on the allele frequencies at the 12 microsatellite loci for all samples pooled and run using the default number of batches, iterations per batch and dememorisations (see Ryman and Palm, 2006).

The genotypic frequencies of each pair of samples were compared using the log-likelihood ratio G tests in GENEPOP, using the batch, iteration and dememorisation numbers described above for this software. The results of the G tests are presented as the exact probability of incorrectly rejecting the null hypothesis, which states that the genotype frequencies at the sampling locations under consideration are identical.

Pairwise and global $F_{ST}$ values were estimated using the method of Weir and Cockerham (1984), as implemented in GENEPOP. $F_{ST}$ measures the proportion of the total variance in allele frequencies that is due to differences among subpopulations. Values range from zero (subpopulations are identical) to a maximum ≤ one, depending on the magnitude of heterozygosity. The statistical significance of the values of pairwise and global $F_{ST}$ was calculated via 10,000 permutations in FSTAT v 2.9.3.2 (Goudet, 2002) and Arlequin v 3.5.1.3 (Excoffier and Lischer, 2010), respectively. Values of pairwise and global Jost's (2008)$D_{EST}$ were calculated, using 1000 bootstrap replicates in SMOGD (Crawford, 2010). The statistical significance of pairwise and global $D_{EST}$ values was determined using 10,000 permutations in GenoDive v 2.0b22 (Meirmans and Van Tienderen, 2004).

The relationships among individuals, based on their multi-locus genotypes, were investigated using factorial correspondence analysis (FCA) via the software GENETIX v 4.05.2 (Belkhir et al., 1996–2004). The analysis used a multiple contingency table of n individuals (rows) x p alleles (columns). The data for an individual for a particular allele were recorded following She et al. (1987), such that scores of 2 and 1 were used to indicate, respectively, a homozygote and heterozygote, while a score of zero meant that the allele was absent. These tabulated data represent a cloud of n points in p-dimensional space. The analysis searched for the factor axes (combinations of alleles) that maximised

the (weighted chi square) distance between individuals in this space and thereby explained the greatest proportion of the overall inertia (overall chi-square value). Individuals were plotted on the two factor axes that explained the most inertia, with different symbols used to distinguish individuals from different sampling locations. Bayesian clustering, via STRUCTURE v 2.3.3 (Pritchard et al., 2000), was used to detect the number of genetically differentiated clusters (K) within the sampled individuals. The log probability of the data, Pr(X/K), was determined for 1 to 8 K values. This analysis applied the LOCPRIOR setting and invoked the assumptions that the allele frequencies were correlated between populations and that the populations were admixed (see Hubisz et al., 2009). Runs were carried out using 10 replicates, with a burn-in period of 100,000 steps and 100,000 Markov chain Monte Carlo replications. Data were collated and presented using STRUCTURE HARVESTER (Earl and vonHoldt, 2012).

The relationship between pairwise population genetic differentiation and geographical distances was analysed in order to test for the presence of isolation by distance. The geographic distances between sampling localities were estimated, as the shortest route across water, via Google Earth v 6.0.1.2032. Mantel tests, implemented via IBDWS v 3.23 (Jensen et al., 2005), were used to compare the regression matrix of the pairwise geographical distances (following a one-dimensional model, see Rousset, 1997) vs (i) $F_{ST} / (1 - F_{ST})$ (Weir and Cockerham, 1984; Rousset, 1997) and (ii) exact values of $D_{EST}$.

## 3. Results

### 3.1. Microsatellite characteristics

The number of individuals genotyped per locus in each sample was usually ≥ 26 (Table 2). The exception was the Kalbarri sample, for which sample sizes were lower due mainly to poor quality DNA.

For all samples pooled, the mean number of alleles per locus was 11.25 (SE ± 1.78), and the number per locus ranged from 2 (*CruC120-4*) to 24 (*CruA115*) (Table 2). The mean value of $H_e$ was 0.70 (± 0.05), and the value per locus ranged from 0.21 (*CruC120-4*) to 0.90 (*CruA115*) (Table 2).

For all of the individual samples, the mean values of both $A_S$ and $H_e$ were very similar, ranging from, respectively, 6.86 to 7.17 and 0.68 to 0.71, and did not show an obvious latitudinal trend or other spatial pattern (see Table 2). Furthermore, Freidman's test did not detect any significant differences in $A_S$ ($\chi^2_r = 4.66$; $P > 0.05$) or $H_e$($\chi^2_r = 5.33$; $P > 0.05$) between the samples.

The observed genotype frequencies for each locus-sample combination were typically consistent with those expected under HWE (Table 2). In the case of *CruC111*, however, the values of $H_o$were less than those of $H_e$ in all samples, and were significantly different to those expected under HWE for the Monkey Rock sample and overall, even after Bonferroni correction. Tests using Micro-Checker v 2.2.3 indicated that these differences were due to the presence of null alleles because homozygotes were in excess over most allele classes. Regardless, the results of all analyses were very similar, irrespective of whether they were carried out with (data shown) or without (data not shown) *CruC111*.

Power analysis, via the simulations in POWSIM, indicated that the microsatellite data had sufficient power to detect a $F_{ST}$ of ≥ 0.005 with ≥ 99% confidence (Fig. 2), i.e., could potentially detect even subtle levels of genetic differentiation in *C. rubescens*, if present.

### 3.2. Genetic connectivity

None of the following analyses provided clear evidence of differences in the genetic composition of *C. rubescens* among the six sampling localities.

The values of global $F_{ST}$ and $D_{EST}$ were low and did not differ significantly from zero ($F_{ST} = 0.0019$, $P = 1.000$; $D_{EST} = 0.0010$, $P = 0.094$). Likewise, the genetic compositions of each pair of samples, whether based on $F_{ST}$, $D_{EST}$ or genotype frequencies, were similar, and almost invariably not significantly different (Tables 3 and 4). The only exception was the comparison between Monkey Rock and Abrolhos Island South, which was significantly different when comparing genotype frequencies using the G test and close to significance with the other two tests (Tables 3 and 4).

The points for the multi-locus genotypes of individuals from each sampling locality were interspersed in the FCA plot, apart from a few outliers from three sampling localities, particularly Monkey Rock (Fig. 3). The outliers reflect the presence of rare alleles. Regardless, the total amount of inertia (variation) in the data set explained by each axis was ≤ 2.54%, emphasising that the differences among individuals were limited.

The log-likelihood value from the Bayesian clustering was the highest for one group and then tended to decline exponentially as the number of groups increased (Appendix A, Supplementary material).

The was no correlation between genetic and geographic distances for the samples from the six localities (Fig. 4), irrespective of whether genetic distance was estimated as either $F_{ST} / (1 - F_{ST})$ ($r = -0.110$; $P = 0.663$) or Jost's $D_{EST}$ ($r = 0.079$; $P = 0.613$).

## 4. Discussion

### 4.1. Genetic connectivity

Analyses of the variation at 12 microsatellite loci support the hypothesis that *C. rubescens* is genetically homogeneous across its main distribution. As otolith microchemistry data indicate that the movements of demersal juveniles and adults of this species are limited (Fairclough et al., 2011), probably to within 10s of kilometres, these genetic data imply the presence of widespread dispersal via the pelagic ELS. *Choerodon rubescens* thus adds to a growing list of demersal species for which there is evidence that the pelagic ELS are critical for their dispersal over moderate to large spatial scales (e.g., Berry et al., 2012a; Damerau et al., 2012; Pusack et al., 2014; but see Ackiss et al., 2013). This study highlights the value of employing complementary methods, such as genetic and otolith microchemistry analyses, to investigate connectivity in marine species, and, in particular, for assessing the likely extent of dispersal during different phases of the life cycle (e.g., see Berry et al., 2012a; Collins et al., 2013; Pujolar et al., 2013).

The results of this study are based on samples of *C. rubescens*from six localities, between ca 26 and 31°S, which collectively span the vast majority of the geographical distribution of this species. Only the margins of the distribution, where this species is typically rare (Hutchins, 2001), were not represented in the sampling. The results therefore suggest that *C. rubescens* is genetically homogeneous across all, or at least most, of its distribution.

The genetic analyses were based on data from 12 microsatellite loci in samples of (usually) 26–40 individuals. Although the minimum numbers of loci and individuals required to provide a robust test for genetic differentiation in marine species with pelagic ELS have been the subject of much debate (Hellberg, 2009; Paetkau et al., 2004), the number of loci used in the present study was comparable with those employed in many similar studies (e.g., Ball et al., 2007; Bekkevold et al., 2005; Buonaccorsi et al., 2002; Damerau et al., 2012; Pujolar et al., 2013). Furthermore, comparisons of simulated and empirical data sets, by Hale et al. (2012), indicate that the use of sample sizes greater than ca 25–30 individuals does not have a significant impact on estimates of genetic differentiation. The view that the numbers of loci and individuals were appropriate for achieving the aim of the present study is also supported by the results of the power analysis, which demonstrated that the data set contained sufficient information to detect an $F_{ST}$ of $\geq 0.005$ with $\geq 99\%$ confidence.

The genetic results show that the amount of gene flow is, or has been, sufficient to preclude genetic differentiation in *C. rubescens*at neutral markers across all or most of its geographic distribution. The pelagic ELS provide an obvious mechanism for dispersal, and thus for gene flow, given their relatively protracted duration and the hydrodynamics of the environment (see Introduction). Conversely, the otolith microchemistry data indicate that the movements of adults and juveniles of *C. rubescens* are limited (Fairclough et al., 2011) and thus these phases are unlikely to be contributing to gene flow in any significant way. It is, however, recognised that such a contribution cannot be discounted entirely because: i) there are circumstances such that otolith microchemistry data do not accurately reflect the movements of individuals (reviewed by Elsdon et al., 2008; Chang and Geffen, 2013); and ii) gene flow via the exchange of a very small number of individuals is sufficient to maintain genetic homogeneity (Waples, 1998).

Given the potential for widespread dispersal via the ELS, the genetic homogeneity of *C. rubescens* is considered to reflect contemporary rather than historical gene flow. When a species is genetically homogeneous, it is difficult to quantify the amount of gene flow because even a small amount of gene flow is sufficient to maintain homogeneity (Waples, 1998), particularly in a species with a large effective population size ($N_e$) (see Hutchison and Templeton, 1999; Marko and Hart, 2011; Waples, 1998). The possible basis for the genetic homogeneity of *C. rubescens*ranges from complete panmixia (e.g., Moore and Chaplin, 2013), through consistent but low levels of gene flow, to episodic pulses of gene flow (e.g., Zeigler and Fagan, 2014). Although it is not possible to determine which of these scenario(s) is applicable, a study of the population dynamics of *C. rubescens* at the southern margin of its distribution has provided evidence of interannual variation in recruitment strength in this area (Cure et al., 2015), which is consistent with the concept that gene flow is episodic. It is also worth noting that many long-lived demersal species undergo considerable interannual variation in recruitment strength, including the labrid *Achoerodus gouldii* (e.g., Coulson et al., 2009; Hesp et al., 2002).

Although marine species with pelagic ELS were once almost invariably assumed to be genetically homogeneous over large spatial scales (Cowen et al., 2000; Ward et al., 1994), recent studies have demonstrated that such homogeneity is far less common than previously envisaged (e.g., Horne et al., 2011). Nevertheless, there are numerous reports of species that are genetically homogeneous over large areas (e.g., Kennington et al., 2013; Moore and Chaplin, 2013; Reece et al., 2011). The underlying reasons for such homogeneity vary. For example, in some species, extensive movements occur at all stages of the life cycle (Moore and Chaplin, 2013), whereas in others, like *C. rubescens*, mixing is likely to mainly occur during the early phases of the life cycle. Also, given only a small amount of gene flow is required to maintain genetic homogeneity (see above), the extent of connectivity may be vastly different in these species.

### 4.2. Management implications

The conclusion that the pelagic ELS of *C. rubescens* provide an avenue for dispersing this labrid over moderate to large spatial scales has important management implications. In particular, it implies that

there is a mechanism for the recruitment of *C. rubescens* to non-natal reefs, which could be vital for replenishing any local assemblages of this species that become depleted. It is, however, important to recognise that the recovery of biomass via recruitment of eggs and larvae would take time (see Ottersen et al., 2006). Furthermore, the rate and timing of any recovery process will be intricately linked with the extent of ELS dispersal, as well as any temporal variability (see Cure et al., 2015; Hellberg, 2009; Waples, 1998).

ELS dispersal would reduce the potential evolutionary consequences of any localised overfishing, such as changes to life history, behavioural or morphological traits (Pandolfi, 2009), as well as the risk of localised reductions in genetic diversity (Strathmann et al., 2002). While it was not possible to test for reductions in genetic diversity directly, due to a lack of historical samples, the levels of microsatellite diversity in *C. rubescens* did not vary spatially. There is thus no evidence of an appreciable loss of genetic diversity in *C. rubescens* at any location, even the Abrolhos Islands, where there have been indications of overfishing (Fairclough et al., 2014; Wise et al., 2007). This study provides baseline data on genetic diversity in *C. rubescens*, whichare likely to be useful in the future for determining whether exploitation is having deleterious genetic effects (see Schwartz et al., 2007).

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Table 1. The collection date(s), numbers of *Choerodon rubescens* collected and approximate coordinates of the six sampling locations.

| Sampling location | Coordinates | Number of individuals | Date of collection |
|---|---|---|---|
| Monkey Rock | 26°10′S, 113°06′E | 40 | May-08 |
| Kalbarri | * | 14 | Feb-09 |
| | * | 14 | Feb-10 |
| Abrolhos Island North | 28°10′S, 113°40′E | 31 | Nov-08 |
| Abrolhos Island South | 28°50′S, 113°45–55′E | 35 | Mar-09 |
| Jurien Bay | * | 30 | Apr-10 |
| Two Rocks | 31°25′S, 115°15′E | 1 | Nov-08 |
| | 31°25′S, 115°15–20′E | 5 | Dec-08 |
| | 31°20–25′S, 115°15–20′E | 4 | Jan-09 |
| | 31°35′S, 115°20–30′E | 12 | Apr-09 |
| | 31°20′S, 115°15–20′E | 4 | May-09 |
| | 31°45′S, 115°25′E | 3 | Jun-09 |

*Exact coordinates not known.

Table 2. Numbers of individuals scored (N), numbers of alleles (A), numbers of alleles standardised to the smallest sample size ($A_S$), expected heterozygosities, assuming Hardy–Weinberg Equilibrium ($H_e$) and observed heterozygosities ($H_o$) for 12 microsatellite loci for six samples of *Choerodon rubescens* and for all samples pooled. *P* is the probability of incorrectly rejecting the null hypothesis, which states that the observed genotype numbers are the same as those expected under Hardy–Weinberg equilibrium. *P* values that are statistically significant following a sequential Bonferroni correction are indicated in bold**.**

| | | **Samples pooled** | **Monkey Rock** | **Kalbarri** | **Abrolhos Island North** | **Abrolhos Island South** | **Jurien Bay** | **Two Rocks** |
|---|---|---|---|---|---|---|---|---|
| *CruA1* | N | 178 | 35 | 26 | 30 | 28 | 30 | 29 |
| | A | 6 | 5 | 5 | 4 | 4 | 4 | 4 |
| | $A_S$ | – | 4.69 | 5 | 3.86 | 3.94 | 3.99 | 3.89 |
| | $H_e$ | 0.64 | 0.61 | 0.68 | 0.6 | 0.64 | 0.65 | 0.62 |
| | $H_o$ | 0.68 | 0.74 | 0.61 | 0.73 | 0.82 | 0.6 | 0.55 |
| | *P* | 0.6649 | 0.586 | 0.0645 | 0.7766 | 0.1343 | 0.1691 | 0.1945 |
| *CruD118* | N | 165 | 30 | 14 | 31 | 31 | 30 | 29 |
| | A | 7 | 6 | 6 | 6 | 5 | 7 | 5 |
| | $A_S$ | – | 4.96 | 6 | 5.05 | 4.44 | 5.51 | 3.44 |
| | $H_e$ | 0.6 | 0.56 | 0.61 | 0.61 | 0.6 | 0.67 | 0.47 |
| | $H_o$ | 0.59 | 0.53 | 0.5 | 0.64 | 0.58 | 0.77 | 0.48 |
| | *P* | 0.3249 | 0.6527 | 0.1928 | 0.291 | 0.799 | 0.2476 | 0.7272 |
| *CruD7-2* | N | 171 | 30 | 22 | 31 | 31 | 28 | 29 |
| | A | 11 | 6 | 8 | 8 | 9 | 6 | 7 |
| | $A_S$ | – | 5.94 | 8 | 7.52 | 8.08 | 5.55 | 6.5 |
| | $H_e$ | 0.69 | 0.68 | 0.62 | 0.69 | 0.73 | 0.66 | 0.72 |
| | $H_o$ | 0.67 | 0.6 | 0.73 | 0.68 | 0.71 | 0.57 | 0.72 |
| | *P* | 0.0762 | 0.7325 | 0.1434 | 0.1009 | 0.4104 | 0.2588 | 0.2284 |
| *CruC111* | N | 154 | 30 | 12 | 28 | 30 | 28 | 26 |
| | A | 5 | 4 | 3 | 5 | 4 | 4 | 3 |
| | $A_S$ | – | 3.64 | 3 | 4.41 | 3.32 | 3.89 | 2.73 |
| | $H_e$ | 0.6 | 0.57 | 0.62 | 0.69 | 0.49 | 0.63 | 0.5 |
| | $H_o$ | 0.42 | 0.3 | 0.42 | 0.5 | 0.43 | 0.43 | 0.42 |
| | *P* | **0.0001** | **0.0007** | 0.116 | 0.1123 | 0.3295 | 0.0415 | 0.477 |
| *CruC10* | N | 176 | 30 | 21 | 31 | 35 | 30 | 29 |
| | A | 8 | 5 | 6 | 7 | 7 | 8 | 7 |
| | $A_S$ | – | 4.97 | 6 | 6.32 | 5.68 | 7.28 | 6.36 |
| | $H_e$ | 0.67 | 0.66 | 0.68 | 0.62 | 0.62 | 0.73 | 0.66 |
| | $H_o$ | 0.68 | 0.63 | 0.67 | 0.68 | 0.63 | 0.8 | 0.66 |
| | *P* | 0.3802 | 0.0116 | 0.3036 | 0.6709 | 0.9553 | 0.6016 | 0.5533 |
| *CruA115* | N | 169 | 30 | 14 | 31 | 35 | 30 | 29 |
| | A | 24 | 15 | 14 | 19 | 18 | 16 | 19 |
| | $A_S$ | – | 12.27 | 14 | 11.76 | 11.88 | 12.16 | 12.81 |
| | $H_e$ | 0.9 | 0.91 | 0.87 | 0.85 | 0.88 | 0.87 | 0.89 |
| | $H_o$ | 0.9 | 0.9 | 0.86 | 0.93 | 0.86 | 0.93 | 0.93 |
| | *P* | 0.2071 | 0.4203 | 0.1915 | 0.9135 | 0.6887 | 0.4105 | 0.7089 |
| *CruC120-4* | N | 178 | 30 | 23 | 31 | 35 | 30 | 29 |
| | A | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| | $A_S$ | – | 2 | 2 | 1.98 | 2 | 2 | 2 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | $H_e$ | 0.21 | 0.28 | 0.19 | 0.09 | 0.16 | 0.23 | 0.29 |
| | $H_o$ | 0.22 | 0.27 | 0.22 | 0.1 | 0.17 | 0.27 | 0.28 |
| | *P* | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| *CruA2* | N | 165 | 27 | 14 | 30 | 35 | 30 | 29 |
| | A | 17 | 13 | 7 | 10 | 8 | 11 | 10 |
| | $A_S$ | – | 9.04 | 7 | 7.28 | 6.51 | 8.12 | 7.84 |
| | $H_e$ | 0.73 | 0.79 | 0.72 | 0.67 | 0.73 | 0.72 | 0.68 |
| | $H_o$ | 0.73 | 0.85 | 0.57 | 0.63 | 0.77 | 0.8 | 0.65 |
| | *P* | 0.1095 | 0.697 | 0.0518 | 0.2635 | 0.0034 | 0.5368 | 0.6628 |
| *CruD1* | N | 166 | 30 | 12 | 30 | 35 | 30 | 29 |
| | A | 13 | 10 | 6 | 9 | 10 | 9 | 9 |
| | $A_S$ | – | 7.45 | 6 | 7.63 | 7.85 | 6.99 | 6.89 |
| | $H_e$ | 0.83 | 0.82 | 0.79 | 0.84 | 0.83 | 0.83 | 0.8 |
| | $H_o$ | 0.86 | 0.73 | 0.92 | 0.9 | 0.89 | 0.87 | 0.9 |
| | *P* | 0.0327 | 0.012 | 0.9101 | 0.548 | 0.3868 | 0.8797 | 0.553 |
| *CruD124* | N | 173 | 39 | 12 | 30 | 33 | 30 | 29 |
| | A | 15 | 13 | 9 | 10 | 10 | 13 | 11 |
| | $A_S$ | – | 10.02 | 9 | 8.16 | 8.08 | 9.89 | 9.12 |
| | $H_e$ | 0.88 | 0.87 | 0.86 | 0.84 | 0.83 | 0.88 | 0.88 |
| | $H_o$ | 0.89 | 0.82 | 1 | 0.8 | 0.94 | 0.93 | 0.93 |
| | *P* | 0.1245 | 0.2019 | 0.9741 | 0.1034 | 0.1954 | 0.0782 | 0.5465 |
| *CruD112* | N | 181 | 40 | 18 | 30 | 35 | 29 | 29 |
| | A | 11 | 9 | 8 | 8 | 10 | 11 | 9 |
| | $A_S$ | – | 7.85 | 8 | 7.53 | 8.52 | 9.69 | 8.07 |
| | $H_e$ | 0.82 | 0.8 | 0.8 | 0.81 | 0.8 | 0.85 | 0.82 |
| | $H_o$ | 0.82 | 0.75 | 0.83 | 0.93 | 0.83 | 0.79 | 0.83 |
| | *P* | 0.2431 | 0.2116 | 0.7519 | 0.4032 | 0.1537 | 0.1105 | 0.0465 |
| *CruD2* | N | 180 | 37 | 19 | 30 | 35 | 30 | 29 |
| | A | 16 | 12 | 12 | 12 | 10 | 13 | 13 |
| | $A_S$ | – | 10.71 | 12 | 10.83 | 8.49 | 11.37 | 11.55 |
| | $H_e$ | 0.84 | 0.85 | 0.79 | 0.86 | 0.79 | 0.84 | 0.84 |
| | $H_o$ | 0.83 | 0.92 | 0.89 | 0.87 | 0.74 | 0.73 | 0.86 |
| | *P* | 0.7356 | 0.4814 | 0.6043 | 0.8669 | 0.0828 | 0.2883 | 0.7702 |

Table 3. The extent of genetic differentiation, as measured by $F_{ST}$ (above the diagonal) and G tests (below the diagonal), between pairs of samples of *Choerodon rubescens* from six locations.
The *P* value (in parentheses) for a measure of $F_{ST}$ is the probability of obtaining, by chance, a value as large or larger than the observed value from 10,000 permutations. The results of the G tests are presented as the exact probability of a type I error. *P* values that are statistically significant following a sequential Bonferroni correction are indicated in bold.

| | **Monkey Rock** | **Kalbarri** | **Abrolhos Island North** | **Abrolhos Island South** | **Jurien Bay** | **Two Rocks** |
|---|---|---|---|---|---|---|
| Monkey Rock | – | − 0.0006 (0.533) | 0.0048 (0.197) | 0.0062 (0.003) | − 0.0008 (0.060) | 0.0008 (0.150) |
| Kalbarri | 0.302 | – | 0.0040 (0.313) | 0.0060 (0.113) | − 0.0012 (0.233) | 0.0051 (0.040) |
| Abrolhos Island North | 0.234 | 0.315 | – | 0.0015 (0.067) | − 0.0004 (0.667) | 0.0031 (0.623) |
| Abrolhos Island South | **0.002** | 0.064 | 0.098 | – | 0.0019 (0.080) | 0.0011 (0.093) |
| Jurien Bay | 0.214 | 0.237 | 0.575 | 0.175 | – | − 0.0011 (0.500) |
| Two Rocks | 0.432 | 0.005 | 0.216 | 0.105 | 0.652 | – |

Table 4. The extent of genetic differentiation, as measured by $D_{EST}$ (above the diagonal), and associated *P* values (below the diagonal), between pairs of samples of *Choerodon rubescens* from six locations. $D_{EST}$ measures the proportion of total allelic diversity that occurs in subpopulations, and is unaffected by the magnitude of heterozygosity (see Jost, 2008). Values range from zero (subpopulations are identical) to one. NB negative values are a function of a correction for sampling bias in the calculation. The *P* value is the probability of obtaining, by chance, a value as large or larger than the observed value from 10,000 permutations of the data. No *P* values were statistically significant.

| | **Monkey Rock** | **Kalbarri** | **Abrolhos Island North** | **Abrolhos Island South** | **Jurien Bay** | **Two Rocks** |
|---|---|---|---|---|---|---|
| Monkey Rock | – | 0.0012 | 0.0009 | 0.0039 | 0.0003 | 0.0010 |
| Kalbarri | 0.487 | – | 0.0015 | 0.0032 | 0.0000 | 0.0037 |
| Abrolhos Island North | 0.097 | 0.194 | – | 0.0001 | − 0.0001 | 0.0004 |
| Abrolhos Island South | 0.066 | 0.059 | 0.285 | – | 0.0001 | 0.0000 |
| Jurien Bay | 0.554 | 0.452 | 0.535 | 0.279 | – | 0.0000 |
| Two Rocks | 0.368 | 0.094 | 0.181 | 0.348 | 0.574 | – |

Fig. 1. The approximate locations of the six sampling sites for *Choerodon rubescens* and associated fisheries management zones. The distribution of this species covers two fisheries management bioregions, the Gascoyne Coast Bioregion (GCB) and the West Coast Bioregion (WCB), which is further subdivided into four inshore areas (Kalbarri, Mid-West, Metropolitan and South-West) and an Offshore Area.

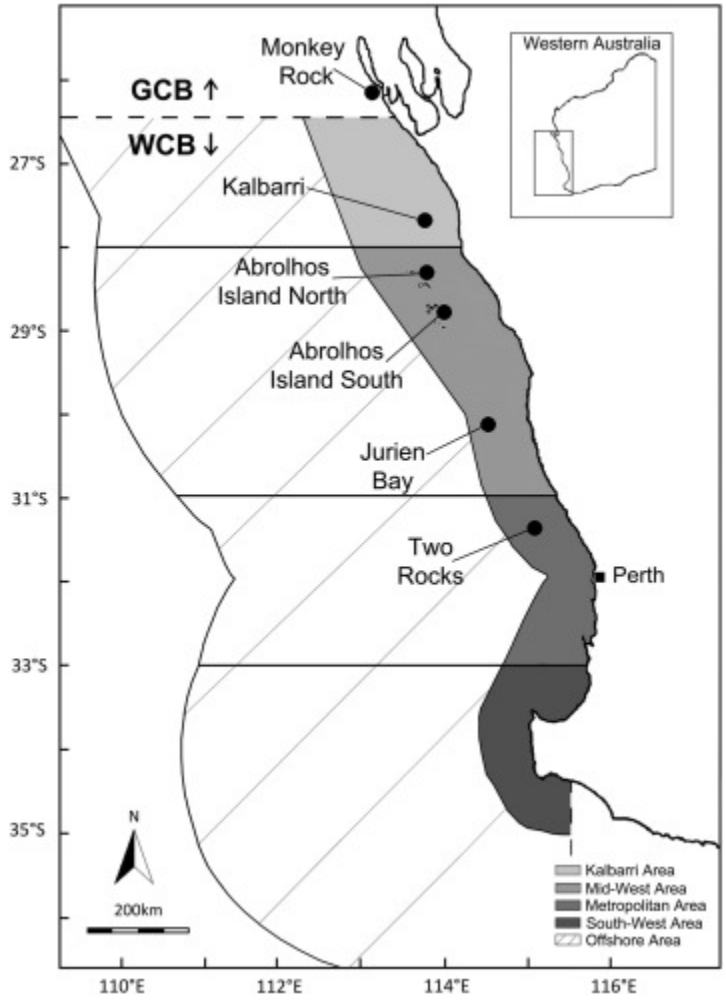


Fig. 2. The results of a POWSIM analysis of the power of the microsatellite data to detect genetic differentiation in *Choerodon rubescens* at five levels of divergence, corresponding to $F_{ST}$ values of 0.001, 0.0025, 0.005, 0.0075 and 0.01. The analysis simulated the subdivision of a population into a series of subpopulations that then drifted to a specified level of divergence. For each level of divergence, the results show the mean value of $F_{ST}$ for the 1000 replicates, and the proportion of 1000 replicates for which the $F_{ST}$ values were significantly different from zero, based on Fisher's method.

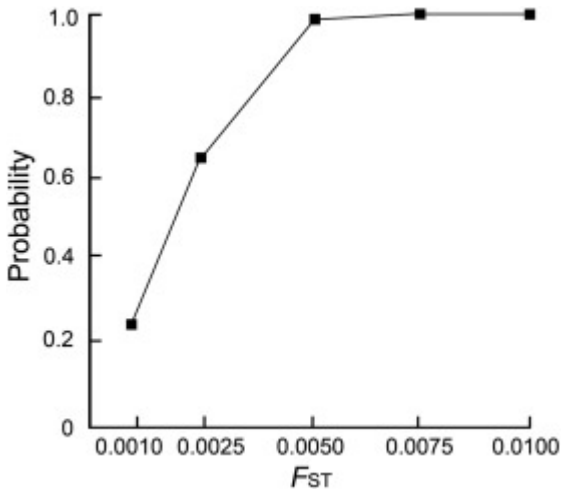


Fig. 3. A factor plot, produced via a factorial correspondence analysis, illustrating the relationships among individuals of *Choerodon rubescens*based on their multi-locus genotypes. The two factor axes represent combinations of alleles that maximised the (chi square) distance between individuals and thereby explained the greatest proportion of the overall inertia (variation). The proportion of the total inertia explained by each axis is given as a percentage.

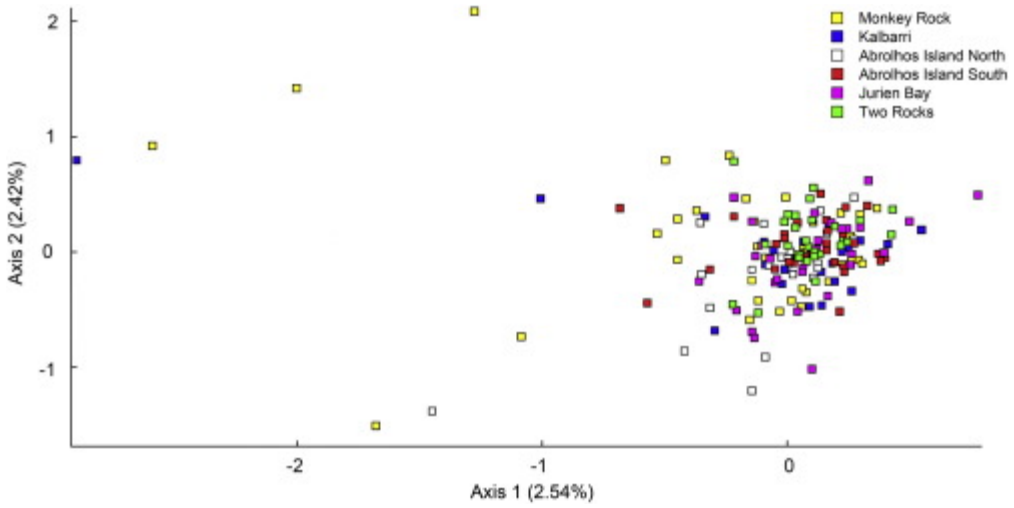
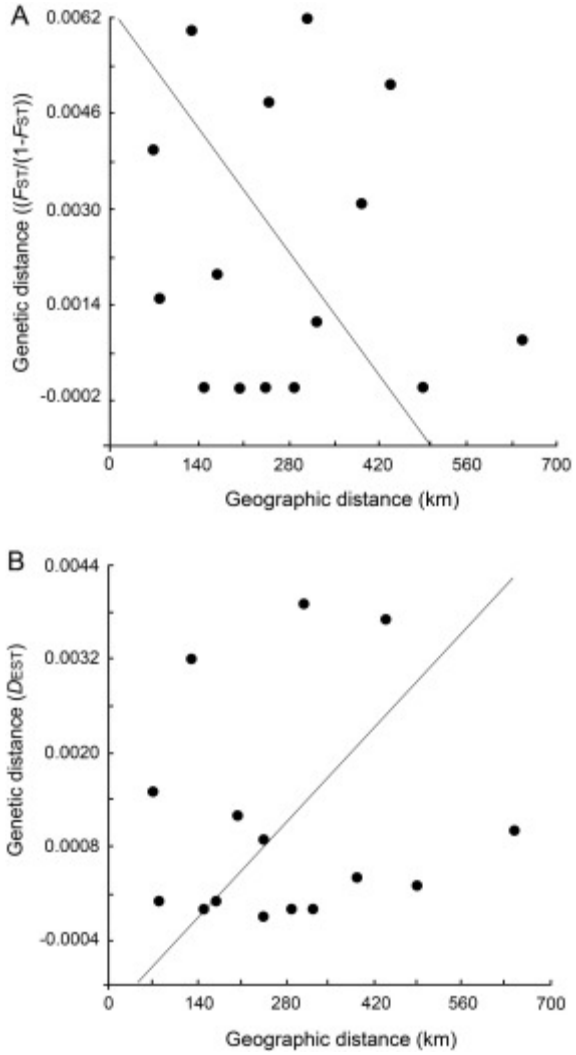


Fig. 4. Isolation by distance analysis for data from 12 microsatellite loci in samples of *Choerodon rubescens* from Monkey Rock, Kalbarri, Abrolhos Island North, Abrolhos Island South, Jurien Bay and Two Rocks. The analysis compares genetic versus geographic distances for all pairwise comparisons of samples. Genetic distance was measured as (A) Rousset's $F_{ST}$ / (1 − $F_{ST}$) and (B) Jost's $D_{EST}$. The relationships were tested using a Mantel test with 10,000 permutations.



Supplementary figure. Estimates of population genetic structure detected in *Choerodon rubescens* using the STRUCTURE program. Bayesian estimates are based upon the genotypes of a total of 193 individuals at 12 microsatellite loci from Monkey Rock, Kalbarri, Abrolhos Island North, Abrolhos Island South, Jurien Bay and Two Rocks. Estimates were calculated via the admixture model with LOCPRIOR option and show the mean of the natural log of the probability of K clusters (ranging from 1 - 8) averaged over 10 iterations. The errors bars indicate the SD for each K value.