Historic divergence with contemporary connectivity in a catadromous fish, the estuary perch (*Macquaria colonorum*)

Kim Shaddick, Dean M. Gilligan,Christopher P. Burridge, Dean R. Jerry, Kiet Truong, and Luciano B. Beheregaray

Abstract: The estuary perch (*Macquaria colonorum*) represents an important model for assessing how historical changes in coastal geomorphology and current oceanographic and estuarine conditions may have impacted connectivity in a catadromous fish. A fragment of the mitochondrial control region and six microsatellite DNA markers were used to clarify connectivity in 17 populations (n = 354) of estuary perch from the southeast and southern coasts of Australia. The mtDNA data showed a latitudinal disjunction in haplotype frequencies that divided populations into two groups (Φ_{ST} = 0.419), in a pattern suggestive of isolation by geographic distance. However, no marked structure or correlation with distance was apparent within each group, a result consistent with microsatellite data that showed high contemporary population connectivity across large distances. This was contrary to expectations that the species would exhibit moderate to strong genetic structure consistent with a one-dimensional stepping stone pattern. Coalescent phylogeographic and population genetic analyses provided support for a historical divergence probably due to the emergence of the Bassian Isthmus in southern Australia. Current connectivity appears to be maintained by both large- and fine-scale oceanographic currents and processes, highlighting the important role of the marine environment for an estuarine resident species.

Résumé : La perche des estuaires (*Macquaria colonorum*) constitue un important modèle pour évaluer comment les changements historiques de géomorphologie côtière et les conditions actuelles océaniques et estuariennes affectent la connectivité chez un poisson catadrome. Un fragment de la région de contrôle mitochondriale et six marqueurs microsatellites d'ADN nous ont servi à mettre en évidence la connectivité chez 17 populations (n = 354) de perches des estuaires sur les côtes sud-est et sud de l'Australie. Les données d'ADNmt montrent une disjonction latitudinale dans les fréquences des haplotypes qui sépare les populations en deux groupes (Φ_{ST} = 0,419) en un patron qui semble relié à l'isolement par la distance géographique. Cependant, dans chacun des deux groupes, il n'y a pas de structure marquée, ni de corrélation avec la distance, un résultat qui est en accord avec les données des microsatellites qui montrent une forte connectivité actuelle entre les populations sur de grandes distances. Cela est contraire à nos attentes qui étaient que les espèces posséderaient une structure génétique moyenne à forte compatible avec un patron unidimensionnel par étapes. Des analyses génétiques phylogéographiques et démographiques de coalescence appuient l'existence d'une divergence historique probablement due à l'émergence de l'isthme de Bass dans le sud de l'Australie. La connectivité actuelle semble être maintenue par des courants et processus océaniques à la fois à grande échelle et à échelle fine, ce qui souligne le rôle de l'environnement marin chez une espèce résidante des estuaires.

[Traduit par la Rédaction]

Introduction

Estuarine ecosystems are important transitional zones between freshwater and marine environments. Accordingly, species relying on estuaries are generally predicted to exhibit levels of connectivity intermediate to those for marine and freshwater species (Farrington et al. 2000; Watts and Johnson 2004; Doukakis et al. 2005). However, the magnitude and direction of connectivity between estuarine populations depends on a number of factors, including historical

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and current biogeographic barriers to dispersal (Chenoweth et al. 1998*b*; Fauvelot et al. 2003; Dawson 2005), oceanographic processes (Mills et al. 2008), life history characteristics (Chenoweth et al. 1998*a*, 1998*b*; Jerry and Baverstock 1998), selection gradients, and local divergent selection (Beheregaray and Sunnucks 2001; Beheregaray et al. 2002).

Estuarine environments are distinct, highly variable, and characteristically ephemeral over geological and evolutionary timescales (Hodgkin 1994; Bilton et al. 2002; Durand et al. 2005). Historical events such as sea-level changes during Pleistocene glacial cycles had a substantial impact on estuarine habitats, limiting broad-scale connectivity and promoting genetic divergence among aquatic species (Chenoweth et al. 1998*b*; Beheregaray and Sunnucks 2001; Durand et al. 2005). On a more contemporary scale, estuarine ecosystems exist in a state of constant alteration with seasonal and tidal variations in salinity, turbidity, and temperature (Bilton et al. 2002). Fluctuating ecological conditions across different time scales may affect dispersal potential and hence, the population genetic differentiation of estuarine dwelling organisms (Bilton et al. 2002).

In Australia, sea level changes during glacial cycles had a dramatic impact on southeast coastal estuaries, particularly in the area of Bass Strait. In this region low sea levels periodically exposed the continental shelf creating a land bridge connecting northern Tasmania to southeast Australia (Lambeck and Chappell 2001). Tasmania was most recently connected to the mainland during the last glacial maximum around 18–15 thousand years ago (kya) (Kench 1999). By around 14 kya, sea levels rose rapidly, finally inundating the Bassian landbridge approximately 10–12 kya. Sea levels stabilized to reach near-present levels (±5 m) approximately 6 kya, creating the majority of estuarine systems along the southeast coast of Australia (Chappell 1983). Over shorter and more contemporary time periods, Australia's southeast coastal rivers and estuaries have been exposed to varying ecological conditions. The present day rivers of coastal New South Wales (NSW) are characterized by a lack of great sediment accumulations (Nanson and Erskine 1988) and very low denudation rates (Young 1983). This indicates that geomorphic changes occurring in these systems are often due to infrequent catastrophic flooding events causing the release of sediment and erosion of floodplains (Nanson and Erskine 1988). The effects of such flooding on river systems are further compounded by periods of alternating high and low flood activity (Erskine and Warner 1988).

While this spatial and temporal environmental instability has consequences for the population genetic structuring of estuarine organisms, levels of contemporary gene flow will also reflect dispersal potential and the degree of physical connectivity between estuaries. Contemporary gene flow in estuarine species is likely to conform to a pattern of isolation-by-distance (IBD), whereby genetic exchange increases with geographic distance along the coastline (Keenan 1994; Bilton et al. 2002; Durand et al. 2005). The eastern seaboard of Australia provides ample opportunity for IBD in estuarine dependant species. The coastline is fairly linear, running north–south with few major impediments to dispersal. However, there are some coastal and oceanographic features that could affect the magnitude and temporal duration of gene flow including topographic features such as Sugarloaf Point and Cape Howe, prevailing ocean currents (Hoskin 2000), eddies, floodwater plumes (Chenoweth and Hughes 1997; Jerry and Baverstock 1998), upwelling events, and temperature gradients (Dawson 2005). The southerly flowing Eastern Australia Current (EAC) and the southeasterly flowing Zeehan Current (ZC) are the two most influential current systems in the region (Fig. 1). A number of studies have examined gene flow in marine and estuarine species along the southeast and southern coasts of Australia (Jerry and Baverstock 1998; Ward and Elliot 2001; Mills et al. 2008). Many of these studies observed an east–west discontinuity across the Bass Strait region on the southern coast (reviewed in Waters 2008), but little to no genetic differentiation along the southeast coast (reviewed in Dawson 2005). In general, the lack of population differentiation detected along the southeast Australian coastline was attributed to high dispersal potential, influential current systems, and an absence of major barriers to gene flow.

In addition to physical features, life-history strategy may also affect the formation of spatial genetic structure in aquatic species. Catadromous fish, for instance, undergo specialized migrations from freshwater growth areas to spawning grounds in seawater (McDowall 1988). The population structure of catadromous fish varies amongst different species and is often associated with particular life history attributes such as spawning location and larval dispersal (Jerry and Baverstock 1998). The subject of this study is the catadromous estuary perch (*Macquaria colonorum*), a highly fecund perciform residing in coastal rivers and estuaries throughout southeastern Australia. The species' range extends from the Richmond River in northern NSW, southwest to the mouth of the Murray River in South Australia, and is also found in the Arthur and Ansons Rivers in Tasmania (Harris and Rowland 1996). Estuary perch are a popular angling fish, prized for both their aggression on the line and their excellent eating quality. Despite its broad distribution and popularity as a game fish, surprisingly little is known about the demography and biology of this species (Harris 1986; Allen 1989; McDowall 1996). Successful recruitment in estuary perch is episodic, and likely dependent on environmental factors including, water temperature, salinity, influx of flood waters, and availability of suitable spawning habitat (McCarraher and McKenzie 1986; Kirwin 2000). Estuary perch are seasonal spawners, migrating to the mouths of estuaries during the winter months to breed in salinities of 10–24‰ and temperatures between 14–19 °C (McCarraher and McKenzie 1986). Spawning occurs in male-dominant aggregations occurring near rocky estuarine shores (Llewellyn and MacDonald 1980), over submerged reefs, or areas of aquatic vegetation (McCarraher 1986). Eggs are planktonic in seawater, and the larvae, which hatch after about 2–4 days, are around 2.2 mm long (Harris and Rowland 1996). Male fish reach sexual maturity at 2 years, whilst females are not sexually mature until 3-years-old (McCarraher 1986).

Although estuary perch are thought to remain in the same estuary for the entirety of their lifecycle (McCarraher and McKenzie 1986; Kirwin 2000), adult fish have been reported in coastal waters (McCarraher and McKenzie 1986) and spawning estuary perch have been collected off the continental shelf after large floods (McCarraher and McKenzie

1986). Further, McCarraher and McKenzie (1986) suggest that monthly variations in estuary perch numbers in larger river systems may arise from transient stocks migrating along the coast and entering open estuaries. While estuary perch larvae have not been reported in marine ichthyoplankton surveys (Dempster et al. 1997; Smith and Suthers 1999), they have been caught in incoming tidal waters and can tolerate marine salinities in culture (Trnski et al. 2005). Given the presence of both adults and larvae in continental shelf waters, both life history stages may contribute to dispersal in the species (Harris and Rowland 1996; Trnski et al. 2005).

There are no published studies on the population genetic structure of estuary perch. Nonetheless, Chenoweth and Hughes (1997) suggest that levels of genetic differentiation in the species may be comparable with barramundi, which exhibits substantial structuring in northern Australia. Both barramundi and estuary perch spawn in similar salinities, close to the tidal waters of river mouths, and may have similar dispersal capabilities. Alternatively, the population genetic structuring of estuary perch may be comparable with its catadromous sister species, the Australian bass (*M. novemaculeata*), which exhibits a weak but significant population structure conforming to a pattern of IBD (Chenoweth and Hughes 1997; Jerry 1997; Jerry and Baverstock 1998). Estuary perch and Australian bass are very closely related (Jerry et al. 2001), parapatrically distributed, and exhibit similarities in morphology, habitat use, and feeding behavior; they also hybridize in southern NSW and in Victoria (Jerry et al. 1999).

Here we use variation in the mitochondrial DNA (mtDNA) genome and at nuclear microsatellite DNA markers to investigate patterns of population genetic structure and connectivity in estuary perch throughout the majority of its distributional range. This species represents an important model for assessing how historical changes in coastal geomorphology and current estuarine conditions have shaped connectivity and population structure along the southeast coast of Australia. We predict that population structure in estuary perch should correspond to a one-dimensional stepping stone model of IBD (Kimura and Weiss 1964), whereby proximate populations exhibit more genetic similarity and genetic connectivity decreases with geographic distance. It is also expected that a historical phylogeographic break (sensu Waters 2008) should be identified around the site of the now inundated Bassian Isthmus.

Materials and methods

Sampling and DNA extraction

Estuary perch, *M. colonorum*, were sampled from catchments throughout their distribution from the Richmond River in Northern NSW to the Glenelg River in Victoria, including the Arthur River in Tasmania. In total, 354 estuary perch individuals were obtained from 17 localities (Fig. 1). Whenever possible, caudal fin clips were acquired nonlethally from each individual and stored in 100% ethanol at –20 °C. Samples were collected by the authors or supplied by individuals and angling groups including Chris Walsh (NSW Department of Primary Industries (DPI)), Glen Searle, NEWTAG, and the Victorian Department of Primary Industries (Vic-DPI). Total genomic DNA was isolated from fin clips by means of a standard proteinase K digestion and salt extraction method modified from Sambrook et al. (1989).

Amplification and sequencing of mtDNA

A ~430 bp region of the mtDNA control region was amplified by polymerase chain reaction (PCR) using the oligonucleotide primers A (5′-TTCCACCTCT AACTCCCA-AA GCTAG) and E (5′-CCTGAAGTAG GAACCAGATG). These primers were first developed for use in teleost fish (Lee et al. 1995). Single-stranded conformation polymorphism (SSCP) was used to screen variation in populations prior to sequencing according to Sunnucks et al. (2000). SSCP allows the sequencing of only a subset of samples to detect most of the variation both within and between populations (Sunnucks et al. 2000). All PCR reactions were performed in a volume of 10 μL containing 1.2 μL of 10× PCR buffer (Promega), 0.4 mmol·L^{-1} of combined dNTPs, 12 pmol of each primer, 2 mmol·L^{-1} of $MgCl_2$, 0.5 U *Taq* DNA polymerase (Promega), 0.1 μL [α_{33}P]-dATP at 1000 Ci·mmol^{-1} (1 Ci = 37 Gbq), and 1 μL of template DNA (50–100 ng). The reaction mix was overlaid with one drop of mineral oil. Amplifications were performed in a MJ Research thermocycler using a "step up" PCR program (Beheregaray and Sunnucks 2001): 94 °C for 3 min, then 5 cycles at 94 °C for 30 s, 45 °C for 1 min, 72 °C for 1 min, followed by 34 cycles at 94 °C for 30 s, 58 °C for 1 min, 72 °C for 1 min, and a final extension of 72 °C for 5 min. PCR products were loaded onto a nondenaturing polyacrylamide gel and visualized by autoradiography. After visualization, unique haplotypes were selected for sequencing. A Geneclean III kit (Qbiogene, Inc., Carlsbad, California) was used to purify PCR products and sequencing was carried on an ABI 3730xl following manufacturers directions.

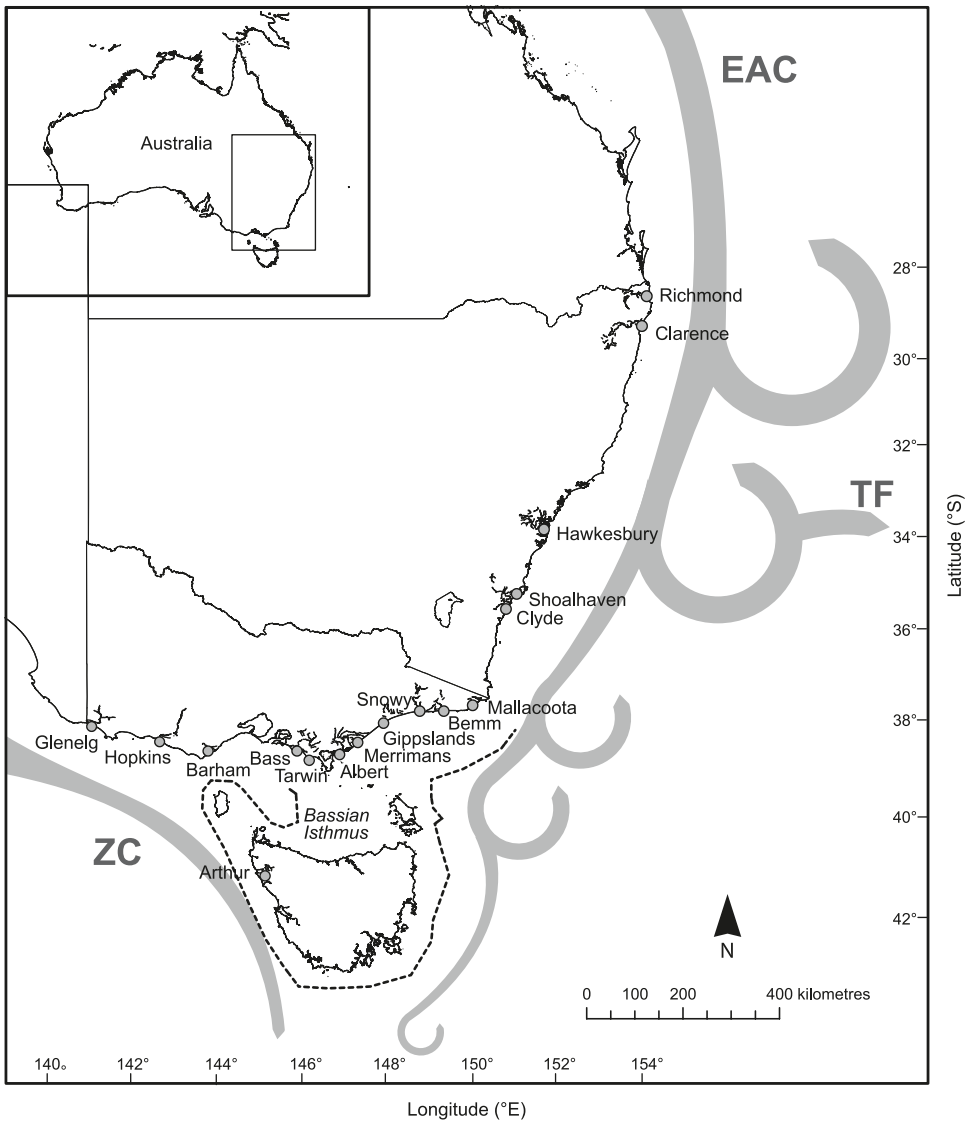
Amplification and screening of microsatellites

All estuary perch samples were genotyped at six microsatellite loci (*AB001*, *AB006*, *AB009*, *AB097*, *AB107*, and *AB114*) according to conditions described in Schwartz et al. (2005). The reactions were overlaid with mineral oil prior to amplification in a MJ Research thermocycler using settings designed by Beheregaray and Sunnucks (2000). The PCR products were loaded onto a denaturing 6% polyacrylamide gel and separated by electrophoresis. Bands were visualized by autoradiography.

Mitochondrial DNA data analysis

Mitochondrial DNA control region (mtDNA CR) sequences were edited and aligned in Sequencher version 4.1 (Gene Codes Corporation, Ann Arbor, Michigan). Genetic diversity was estimated at both the haplotype (h) and nucleotide (π) level. Nucleotide diversity was calculated from the number of substitutions that occur between sequences using Kimura's (1980) genetic distance parameter (K2P), with a gamma distribution of $\alpha = 0.5$ in PAUP* (Swofford 2001). Genetic differentiation between populations was determined using Φ_{ST} (Weir and Cockeram 1984). An analysis of molecular variance (AMOVA) (Excoffier et al. 1992) was conducted to investigate the partitioning of mtDNA variance at three hierarchical levels: (*i*) all populations grouped together into a single group, (*ii*) populations divided into two groups, one containing northern populations (Richmond to

Fig. 1. Map of 17 estuaries sampled for mtDNA and microsatellite variation in *M. colonorum* along the southeast and southern coasts of Australia. The broken line indicates a historical barrier to migration in the form of the now inundated, Bassian isthmus. Schematic representations of the main current systems are shown. EAC, Eastern Australia Current; TF, Tasman Front; ZC, Zeehan Current.



Shoalhaven), and one containing southern populations (south of Shoalhaven; see Results for justification of northern/southern division), and (*iii*) populations divided into two groups west and east of the now inundated Bassian isthmus. A permutation procedure with 1000 randomizations was used to test the significance of each comparison. Computation of all analyses was achieved using the software package Arlequin version 3.01 (Schneider et al. 2000).

Genealogical relationships between haplotypes were inferred from a haplotype network (Templeton et al. 1992). Networks were created with the computer program TCS version 1.21 (Clement et al. 2000), which uses statistical parsimony to connect haplotypes with a 95% confidence interval. Sequences with the smallest number of differences are joined first, permitting the resolution of genealogical relationships between recently diverged lineages. TCS also permits identification of the ancestral haplotype through approximation of haplotype outgroup probabilities.

We examined mismatch distributions to test for signals of demographic expansion in estuary perch populations. Mismatch analysis compares the distribution of pairwise differences between sequences to those expected under Rogers and Harpending's (1992) model of demographic expansion. Populations undergoing expansion exhibit unimodal distributions whereas populations at equilibrium tend to exhibit multimodal distributions. Three analyses of mismatch distributions were conducted: all populations grouped together, all northern populations, and all southern populations. We used Arlequin 3.01 to generate two test statistics for the model of expansion: the raggedness index and sum of squared deviations (SSD) tests. When statistical support for expansion was detected, the timing of these events ($\tau = 2\mu t$, where μ is the mutation rate per time, and t is the estimated time of expansion) was estimated using a mtDNA CR mutation rate of 3.6% per million years (Donaldson and Wilson 1999), and an estuary perch generation time of 6 years.

The divergence time between the northern and southern groups of estuary perch populations and estimates of migration rates were calculated using the Isolation with Migration model implemented in the program IM (Hey and Nielsen 2004). IM estimates the time since divergence of the two populations and indicates the directionality of gene exchange after the split. To ensure consistency of results and adequate performance of Markov chain Monte Carlo analysis, the program was run multiple times until all parameters reached an effective sample size estimate (ESS) of >100. We calculated the generational mutation rate to be $\mu = 5.47 \times 10^{-5}$, based on Donaldson and Wilson's (1999) estimate of the mtDNA CR mutation rate and a generation time of 6 years. We used the HKY model of nucleotide substitution with the priors for analysis parameters as follows: q max. = 10, m max. = 15, t max. = 0.5.

Microsatellite data analysis

Micro-Checker version 2.2.3 was used to analyse microsatellite data for genotyping errors such as null alleles, large allele drop out and stuttering (van Oosterhout et al. 2004). Fisher's exact test, carried out in Genepop version 3.3 (Raymond and Rousset 1995*a*, 1995*b*), was used to test for deviation from Hardy–Weinberg equilibrium and for linkage disequilibrium. GenAlEx version 6 (Smouse and Peakall 2006) was used to estimate the mean number of alleles per locus, allele frequency, allelic richness, and expected and observed heterozygosities in each population. Genetic differentiation among sampling localities was estimated by calculating Weir and Cockerham's fixation index, $F_{ST,}$ in Arlequin 3.01 (Schneider et al. 2000). All F_{ST} calculations were tested for significance with 10 000 permutations. The sequential Bonferroni procedure (Rice 1989) was used to adjust significance levels when performing multiple simultaneous comparisons.

We used a Bayesian method (BayesAss version 1.3; Wilson and Rannala 2003) to estimate contemporary migration rates between the northern and southern groups of estuary perch populations identified in the mtDNA analysis. The program was run five times, each with 3×10^6 Markov chain Monte Carlo iterations and a burn in of 1 000 000.

Levels of contemporary population structure and the presence of immigrants were tested with the assignment program Structure version 2.2.2 (Pritchard et al. 2000). Structure permits the assignment of individuals to any number of groups with or without any previous knowledge of population membership. We used the admixture model after initial runs detected no difference between the admixture and no-admixture models. The posterior probabilities of the data were calculated with 1 000 000 iterations after a burn-in length of 100 000 using all possible numbers of populations (K) from 1–18, and no prior population data.

The program BOTTLENECK version 1.2.02 (Cornuet and Luikart 1996; Luikart et al. 1998) was used to test for the presence of founder effects in estuary perch populations that may have arisen from an historical reduction in effective population size. BOTTLENECK, under the assumption of mutation–drift equilibrium, computes the expected distribution of heterozygosity from the observed number of alleles at each population and locus. As allelic diversity decreases more rapidly than heterozygosity, populations that have undergone a recent bottleneck will exhibit an excess of observed heterozygotes when compared with the expected values (Cornuet and Luikart 1996). The significance of any observed excess was tested using a Wilcoxon's sign-rank test based on the two-phase mutation (TPM) and the stepwise mutation (SMM) models (Di Rienzo et al. 1994). The program was run for 1000 iterations.

Isolation by distance

A Mantel Test (Mantel 1967) was used to assess whether inferred patterns of genetic differentiation in estuary perch corresponded to a model of IBD. Three Mantel tests were used to compare genetic variation (both mtDNA and microsatellites) with the geographical distance between sites (measured in kilometres along the coastline): all populations, the northern populations (between Richmond and Shoalhaven), and all populations south of the Shoalhaven. Mantel tests were conducted in Arlequin 3.01 (Schneider et al. 2000), where the significance of the correlation between two distance matrices was tested using a permutation procedure. The Arthur River in Tasmania was excluded from Mantel analyses as we were testing for a signal of IBD along the southeast coastline of the Australian mainland.

Results

Identification of hybrids between estuary perch and Australian bass

Several individuals sampled as estuary perch (n = 24) contained previously identified Australian bass alleles (Schwartz and Beheregaray 2008). These individuals were identified as hybrids between estuary perch and Australian bass using the analytical procedure developed by Schwartz and Beheregaray (2008), which combined molecular genetic data based on the same markers used in the current study, Bayesian analyses, and simulated genotypes. This procedure allows not only the identification of individuals of hybrid origin but also has a very high success rate (93%) of distinguishing introgressed individuals up to three generations later from the Australian bass (Schwartz and Beheregaray 2008). All 24 identified hybrid fish were removed from further analyses of both mtDNA and microsatellite datasets.

Mitochondrial DNA variation and genealogical relationships among haplotypes

Mitochondrial DNA data was obtained for 307 of the estuary perch samples. Samples representing different and identical SSCP phenotypes were selected for sequencing, yielding a 396 bp mtDNA CR fragment of unambiguous sequence. Analyses of aligned sequences revealed very little variation, with only three variable sites (all transitions) and no insertions or deletions, corresponding to four mtDNA haplotypes (GenBank accession numbers: EU886372–EU886375). Sequence divergence was very low (0.25%) and sequence differences were not phylogenetically informative.

The two most abundant haplotypes (haplotypes A and C) were found in over 96% of the fish sampled and were distributed across the range of the species. However, their frequencies showed a markedly distinct latitudinal disjunction. Haplotype C was more frequent south of the Clyde River,

whereas haplotype A was more common in the northern part of the species distribution (Fig. 2). Haplotype C was found in all rivers with the exception of the Richmond River (this should be interpreted with caution as only five individuals were sampled from this river). Haplotype B was found in only nine individuals from the Shoalhaven, Bemm, and Glenelg rivers, and a single fish from the Bass River possessed a unique haplotype, haplotype D. Haplotype diversity varied from 0.0 to 0.6 and was highest in the Bemm River. Most rivers had two haplotypes, with the exception of the Snowy, Barham, Hopkins, and Arthur rivers (only haplotype C), and the Richmond River (only haplotype A) (Table 1). The overall nucleotide diversity was very low (0.12%). Nucleotide diversity did not differ within populations, ranging from 0.0% to 0.18% in the Bemm River (Table 1). The genealogical analysis shown may indicate a recent evolutionary history of expansion (Fig. 2). No missing intermediate haplotypes were suggested, and all haplotypes were separated by a single mutation. The most abundant haplotype (A), showed the highest probability of being the ancestral lineage.

Mitochondrial DNA differentiation

The latitudinal disjunction in frequencies of haplotypes A and C resulted in highly significant structure for the species (Φ_{ST} = 0.419, $P < 0.01$). Populations of estuary perch were pooled into two groups that reflected this disjunction for further analysis (Fig. 2). The northern group included all samples from the Richmond River in northern NSW to the Shoalhaven River on the south coast of NSW. The southern group incorporated samples from the Clyde River on the south coast of NSW to the Glenelg River in southern Victoria (and included the Arthur River in Tasmania). The vast majority of the significant pairwise Φ_{ST} comparisons were between populations from different groups (Table 2). AMOVA results indicated that most of the variation was explained by differences between groups (46%) and not among populations within each group, although some level of variation (6%) appeared, owing to differences between populations of the southern group (Table 3). Although significant differences were also found when separating populations in groups east and west of the Bassian isthmus, the percentage of variation explained (26%) was much lower than that between southern and northern groups (46%). Further, there was no variation among populations directly east and west of the isthmus within the southern group, supporting the view that a simple east–west criterion to group populations does not efficiently explain the structure found in the mtDNA data.

Demographic history

The demographic history of estuary perch was inferred from mismatch analysis. When all samples were pooled together, a model of population expansion was rejected ($P < 0.01$ for SSD and r values). However, when samples were separated into the northern and southern groups, we were unable to reject the null hypothesis of historical expansion within each group. This was supported by both the raggedness index and SSD tests (Northern: $P = 0.46$ (r); $P = 0.05$ (SSD); Southern: $P = 0.61$ (r); $P = 0.36$ (SSD). Our estimates of time since expansion derived from the mismatch distributions suggested very recent demographic events. The time of population expansion in the northern group was estimated to have occurred around 10 kya (upper bound of 25 kya), and for the southern group around 18.6 kya (upper bound of 71 kya).

Microsatellite variability

We obtained clearly scorable PCR products for all six microsatellite loci across the 354 estuary perch sampled. There was no evidence of null alleles, large allele dropout, or scoring errors, which is consistent with previous studies that used these markers in estuary perch (Schwartz et al. 2005; Schwartz and Beheregaray 2008). Initial investigations revealed moderate levels of microsatellite variability (Appendix A, Table A1). After removal of the Australian bass alleles, owing to hybridization (as above), three of the loci became monomorphic. No significant departures from linkage disequilibrium or deviations from Hardy–Weinberg equilibrium (HWE) were observed. Populations exhibited low levels of microsatellite variability, with an overall mean number of alleles per locus of 1.8 and mean heterozygosity of 0.0212 (Table 1).

Lack of differentiation in the microsatellite data

In contrast to the overall pattern depicted by mtDNA analyses, the microsatellite data exhibited no evidence for population genetic structure (Table 2). This was reflected in an overall F_{ST} value of 0.015 ($P = 0.11$). Similarly, AMOVA revealed no evidence for genetic subdivision among populations or groups of populations, and the program STRUCTURE pooled all samples into a single population [$K = 1$; $\ln P(D) = -216.3 \pm 3.4$]. No significant heterozygote excess that could provide evidence of recent population bottlenecks was detected (P = 0.24–0.52 based on TPM and 0.14–0.48 based on SMM). However, these results were based on only three variable loci and should, therefore, be interpreted with caution (Cornuet and Luikart 1996).

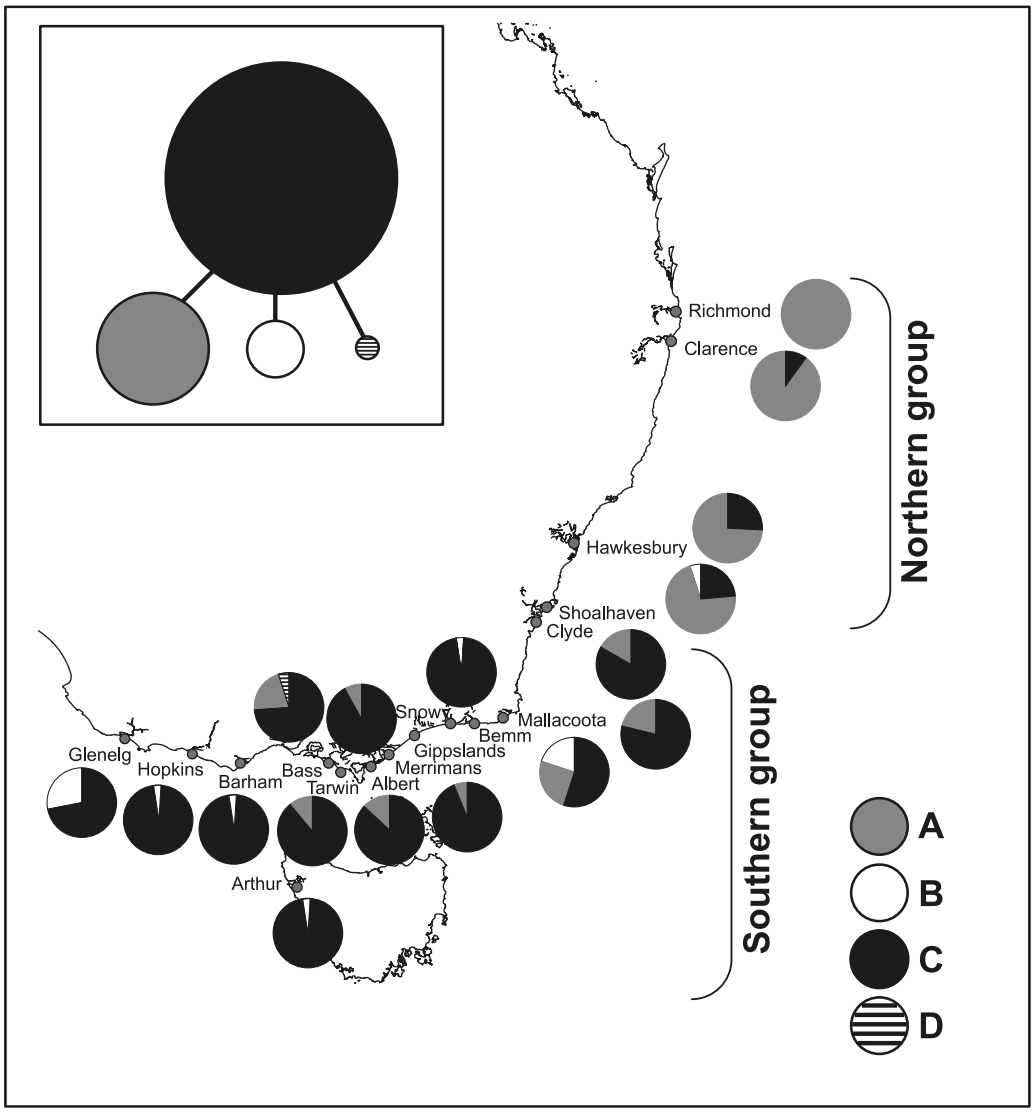
Estimates of divergence times and migration rates

The Isolation-with-Migration model based on the mtDNA data estimated that the northern and southern groups diverged from each other around 63 kya. All replicates of IM analyses indicated that following divergence, there was some southerly migration from the northern to the southern group ($M_1 = 9.27$). While this could suggest that estuary perch are using the EAC to disperse, there were still significant amounts of historical northerly migration against the EAC ($M_2 = 2.78$). The microsatellite data suggested that there was slightly more recent migration from the southern groups to the northern group (21%) than in the other direction (17%), with all five of the BAYESASS replicate runs converging on the same solution (Table 4).

Isolation by distance

Mantel tests suggest that mtDNA genetic differentiation was positively correlated with geographical distance across all samples ($P < 0.0001$). However, when populations were divided into two groups there was no correlation between genetic and geographic distances ($P = 0.23$ and 0.46 for northern and southern groups, respectively) (Fig. 3), sug-

Fig. 2. Pie charts represent the frequency distributions of the four mtDNA control region haplotypes detected in *M. colonorum* from Richmond River in northern New South Wales to the Glenelg River in South Australia. The majority of individuals are composed of either haplotype A or C. The distribution of the haplotypes into two groups is also shown (northern group: Richmond to Shoalhaven; southern group: Clyde to Arthur). Genealogical relationships of *M. colonorum* mtDNA control region haplotypes are shown (inset). Letters A–D represent individual haplotypes and single lines indicate one mutation between haplotypes. The size of each shape correlates with haplotype frequency in the same sample, and no missing haplotypes can be found in the data set. Haplotype C was suggested as the ancestral haplotype with the highest outgroup probability.



gesting moderate levels of gene flow within each group. In contrast to the mtDNA data, the microsatellite dataset revealed no signal of IBD (Fig. 3) for any population groupings.

Discussion

This study investigated population genetic structure and connectivity of the catadromous estuary perch along the southeast and southern coasts of Australia. Analyses of mtDNA sequences revealed a genetic disjunction between a northern and a southern group of populations, located in the vicinity of the Clyde and Shoalhaven rivers. Within each group there was negligible genetic differentiation and low haplotypic diversity. Moreover, there was no difference in populations on either side of the now inundated Bassian isthmus or between Tasmanian and southern mainland populations. Nuclear variability was very low, and we were unable to distinguish estuarine populations based on variation across the microsatellite markers employed. Although Mantel tests based on mtDNA data suggested an IBD relationship across all populations, no such pattern was apparent within each group. The reduced variation and population structure in the mtDNA and microsatellite datasets were surprising given predictions that estuary perch would exhibit moderate structuring, owing to apparent site fidelity, reliance on estuarine habitats, and catadromous life history. The general absence of genetic structure in estuary perch across its distribution, particularly over recent time scales, suggests the species is highly vagile occupying a region lacking obvious barriers to dispersal. These findings are dis-

Table 1. Summary of genetic variability in 17 populations of *Macquaria colonorum* based on mtDNA control region and three microsatellite loci.

	Mitochondrial DNA				Microsatellites			
	n	N_H	Haplotype diversity (*h*)	Nucleotide diversity	*n*	N_A/min.–max.	H_O	H_E
Richmond R	5	1	0.0	0.0	4	1.33/1–2	0.056	0.051
Clarence R	10	2	0.2±0.1541	0.0005±0.0008	10	1.17/1–2	0.050	0.043
Hawkesbury R	27	2	0.4±0.0838	0.0010±0.0011	30	1.17/1–2	0.011	0.021
Shoalhaven R	31	3	0.3±0.0958	0.0010±0.0011	26	1.33/1–2	0.020	0.032
Clyde R	22	2	0.2±0.1075	0.0006±0.0008	22	1.17/1–2	0.014	0.014
Mallacoota I.	9	2	0.4±0.1644	0.0010±0.0011	9	1.17/1–2	0.021	0.020
Bemm R	31	2	0.6±0.0608	0.0018±0.0016	30	1.17/1–2	0.028	0.034
Snowy R	24	1	0.0	0.0	21	1.33/1–2	0.015	0.023
Gippslands	13	2	0.3±0.1417	0.0007±0.0009	17	1.17/1–2	0.049	0.044
Merrimans Ck	21	2	0.2±0.1044	0.0005±0.0007	27	1.17/1–2	0.006	0.006
Albert R	27	2	0.3±0.0972	0.0007±0.0008	27	1.17/1–2	0.018	0.017
Tarwin R	19	2	0.2±0.1121	0.0005±0.0007	26	1.00	0	0
Bass R	22	3	0.4±0.1045	0.0012±0.0012	30	1.17/1–2	0.018	0.018
Barham R	6	1	0.0	0.0	5	1.00	0	0
Hopkins R	25	1	0.0	0.0	29	1.33/1–2	0.028	0.025
Glenelg R	6	2	0.3±0.2152	0.0008±0.0011	8	1.17/1–2	0	0.033
Arthur R.	9	1	0.0	0.0	9	1.00	0	0

Note: N_H, number of haplotypes; N_A, mean number of alleles per locus; max.–min., maximum and minimum number of alleles per locus; H_E, mean expected heterozygosity; H_O, mean observed heterozygosity; R., River; I., Inlet.

cussed within the context of species biology, coastal oceanography, and biogeographic history of the extensive region occupied by this estuarine fish.

Reduced genetic variability in estuary perch

The reduced mtDNA haplotypic and nuclear microsatellite variability detected in estuary perch may have resulted from episodic spawning (Shields and Gust 1995), high levels of gene flow, or a recent colonization from a single source population (Slatkin 1987). Spawning and successful recruitment in estuary perch is intricately tied to flooding events (McCarraher and McKenzie 1986). Coastal rivers in southeast Australia vary cyclically, owing to drought- and flood-dominated regimes, and episodic flooding events are common (Erskine and Warner 1988). The influx of floodwater into estuaries, affecting salinity, can promote, delay, or prevent spawning in estuary perch depending on the timing and magnitude of the flood (McCarraher and McKenzie 1986). In some systems the whole fishery is derived from a few successful spawning years (McCarraher and McKenzie 1986). Such episodic spawning may lead to the propagation of a small number of mtDNA haplotypes (Shields and Gust 1995) and decreased female effective population size, resulting in a reduction of genetic variability (Jerry and Baverstock 1998).

When combined with the lack of isolation-by-distance relationships, the wide-scale pattern of low genetic structure in estuary perch could also indicate recent founding events from genetically homogeneous source populations (Slatkin 1987). Ice-age variations occurring as recently as the Holocene had a dramatic impact on Australia's southeast and southern coasts, particularly in the area of Bass Strait, and the majority of the present day estuaries were only formed in the last 6000 years (Kench 1999; Lambeck and Chappell 2001). The recent events of demographic expansion detected here, especially that of the northern group (dated $\sim$10 kya) could reflect a scenario of historical colonization from a source population around Bass Strait (Burridge and Versace 2006; Crow and Aoki 1984; Chenoweth et al. 1998*b*). Finally, the low levels of haplotypic diversity and the genetic homogeneity in estuary perch may have been maintained by high levels of genetic connectivity between estuaries (Slatkin 1987).

High connectivity between estuaries

The absence of structure in the microsatellite dataset suggests substantial contemporary gene flow between estuary perch populations. Although this conclusion could be considered tentative, owing to the small number of nuclear markers used, it is consistent with the near ubiquity of mtDNA haplotype C along the species' range, with biological characteristics of the species, and with coastal oceanography. In a review of the population genetic structure in several southeastern Australian marine species, Ward and Elliot (2001) revealed that structure was limited or nonexistent in approximately half of the species studied. Dispersal was attributed to larval longevity, favourable ocean currents, and a lack of barriers to dispersal. Other studies of coastal marine taxa in the area have revealed similarly reduced genetic differentiation (reviewed in Dawson 2005, but see Chenoweth and Hughes 1997; Mills et al. 2008). However, some taxa such as Australian bass (*Macquaria novemaculeata*; Chenoweth and Hughes 1997; Jerry 1997; Jerry and Baverstock 1998) and black bream (*Acanthopagrus butcheri*; Burridge et al. 2004; Burridge and Versace 2006) display IBD relationships, while others exhibit fine-scale genetic structure due to local recruitment (sea urchins; Banks et al. 2007; abalone; Piggott et al. 2008).

Although IBD was indicated in the mtDNA data, the significance of this relationship was due to a latitudinal dis-

Table 2. Pairwise fixation indices between 17 populations of *Macquaria colonorum* based on mtDNA control region haplotypes (above diagonal) and on three microsatellite loci (below diagonal).

	RIC	*CLA*	*HAW*	*SHO*	*CLY*	*MAL*	*BEM*	*SNO*	*GIP*	*MER*	*ALB*	*TAR*	*BAS*	*BAR*	*HOP*	*GLE*	*ART*
RIC	—	0	0.11	0.03	**0.79**	**0.82**	**0.47**	**1**	**0.79**	**0.85**	**0.77**	**0.84**	**0.61**	**1**	**1**	**0.87**	**1**
CLA	0	—	0	0	**0.71**	**0.74**	**0.41**	**0.93**	**0.68**	**0.77**	**0.69**	**0.76**	**0.53**	**0.86**	**0.94**	**0.76**	**0.88**
HAW	0	0.01	—	0	**0.52**	**0.59**	**0.31**	**0.72**	**0.47**	**0.57**	**0.51**	**0.55**	**0.37**	**0.61**	**0.72**	**0.58**	**0.63**
SHO	0	0.02	0	—	**0.57**	**0.63**	**0.36**	**0.75**	**0.53**	**0.62**	**0.56**	**0.6**	**0.44**	**0.65**	**0.75**	**0.62**	**0.67**
CLY	0.01	0.04	0	0	—	**0.14**	0.06	0.1	0	0	0	0	0	0	**0.11**	0.07	0.03
MAL	0	0	0	0	0	—	0.03	0.29	0.12	0.14	0.15	0.13	**0.14**	0.06	0.3	0	**0.13**
BEM	0	0	0	0	0.01	0	—	**0.18**	0.03	**0.08**	0.06	0.07	0.03	0.07	**0.18**	0	0.1
SNO	0	0.03	0	0	0	0	0.02	—	0.16	0.06	0.11	0.07	**0.17**	0	0	0.27	0
GIP	0	0	0	0	0.02	0	0.02	0	—	0	0	0	0	0	0.16	0.05	0.04
MER	0.07	0.12	0.01	0	0	0	0.01	0.05	0.08	—	0	0	0.01	0	0.06	0.06	0
ALB	0	0.02	0	0	0	0	0	0.01	0.01	0	—	0	0	0	0.11	0.07	0.03
TAR	0.16	0.22	0.05	0.04	0.03	0.09	0.03	0.09	0.14	0	0.04	—	0	0	0.08	0.06	0
BAS	0	0.01	0	0	0	0	0	0	0	0.01	0	0.05	—	0	0.17	0.08	0.08
BAR	0	0.05	0	0	0	0	0	0.02	0.02	0	0	0	0	—	0	0	0
HOP	0.02	**0.13**	0.05	0.05	0.04	0.03	0	**0.09**	**0.1**	0.04	0.05	0.05	0.05	0	—	0.28	0
GLE	0	0.08	0.07	0.07	0.07	0.04	0	0.08	0.08	0.11	0.07	0.17	0.07	0	0	—	0.07
ART	0.04	0.1	0.01	0	0	0.01	0	0.05	0.06	0	0	0	0.01	0	0.01	0.06	—

Note: Numbers in bold are statistically significant, $p < 0.05$.

junction in haplotype frequencies. Estuary perch lacks a signal of contemporary IBD across all spatial scales for microsatellites. Similarly, Mills et al. (2008) found no evidence of IBD in two species of estuarine glassfish (*Ambassis marianus* and *A. jacksoniensis*) along the east coast of Australia. Genetic homogeneity in both species of glassfish was likely due to high levels of contemporary gene flow. This may be the case in estuary perch, with a high level of marine connectivity homogenizing haplotypic frequencies and counteracting any IBD effect (Mills et al. 2008).

The occurrence of both larval and adult estuary perch in continental-shelf waters suggests the movement of both life history stages could contribute to gene flow (Harris and Rowland 1996; Allen et al. 2002; Trnski et al. 2005). Estuary perch spawn in winter months, when flooding events are common, and larvae and juveniles may be swept out to sea by floodwaters. Floodwater plumes that form at several river mouths along the coast can coalesce creating a corridor of brackish water that could facilitate the transport of propagules between river drainages (Wolanski and Jones 1981; Grimes and Kingsford 1996). Coalescing floodwater plumes may also assist the dispersal of adult estuary perch, as is suggested for two other Australian species, black bream (Burridge et al. 2004; Burridge and Versace 2006) and Australian Bass (Chenoweth and Hughes 1997; Jerry and Baverstock 1998). However, under a scenario of dispersal via floodwater plumes, only proximate drainages would be connected, confining gene flow to nearby populations and resulting in a higher level of structuring consistent with a stepping stone model of IBD (Kimura and Weiss 1964; Chenoweth and Hughes 1997; Jerry and Baverstock 1998). Consequently, it is unlikely that long distance dispersal and recruitment of larvae and adult fish into other estuaries is solely mediated by floodwater plumes.

Other hydrographic processes, such as tidal action and ocean currents, may also facilitate gene flow in estuary perch. The two main currents systems with the potential to influence dispersal in the species are the Eastern Australia Current (EAC) and the Zeehan Current (ZC) (Fig. 1). The EAC is a highly variable western boundary current system characterized by extensive meandering and the formation of numerous eddies. The majority of the EAC departs from the coast around Sugarloaf Point (32°S) where it recirculates to the north or travels eastwards as the Tasman Front (TF) (Tilburg et al. 2001). The remainder of the current continues southward along the coast shedding anticyclonic eddies (Marchesiello and Middleton 2000). The EAC diverges from the coast at several other points including Cape Byron (28°S), Smokey Cape (30°S), and Jervis Bay (35°S) (Marchesiello and Middleton 2000). The ZC is formed from the warm waters of the Leeuwin Current that runs along the continental shelf and across the Great Australian Bight before flowing southwards along the Western coast of Tasmania (Baines et al. 1983).

While migration analyses indicate significant amounts of bi-directional migration in the species, substantial southerly dispersal was detected, consistent with movement along the EAC. Bi-directional dispersal of estuary perch in the EAC is possible as the EAC is weakest in winter months when estuary perch are spawning, and as ocean eddies of the EAC flow anticlockwise allowing some northerly movement (Til-

Table 3. Analysis of molecular variance (AMOVA) among mtDNA control region haplotypes of *Macquaria colonorum*.

Hierarchical level	Variation among populations (%)	*P*	Variation within populations (%)	*P*	Variation between groups of populations (%)	*P*
All sampling locales	42.41	<0.01	57.63	<0.01		
Group 1	0		100	= 0.4		
Group 2	6.42	<0.01	93.58	<0.01		
Group 1 vs. Group 2	43.81	<0.01	9.72	<0.01	46.57	<0.01
East vs. West	55.72	<0.01	18.4	<0.01	25.88	<0.01

Table 4. Estimates of contemporary migration rates (posterior probabilities) between the two groups of *Macquaria colonorum* populations identified using BAYESASS.

	Into			
From	Group 1 (Richmond to Shoalhaven)	df	Group 2 (Clyde to Arthur)	df
Group 1 (Richmond to Shoalhaven)	0.7806	0.6732, 0.9818	0.1723	0.0098, 0.3292
Group 2 (Clyde to Arthur)	0.2194	0.0181, 0.3268	0.8277	0.6708, 0.9902
No. times runs converged to this solution	5 of 5		5 of 5	

Note: From, source populations; Into, receiving populations.

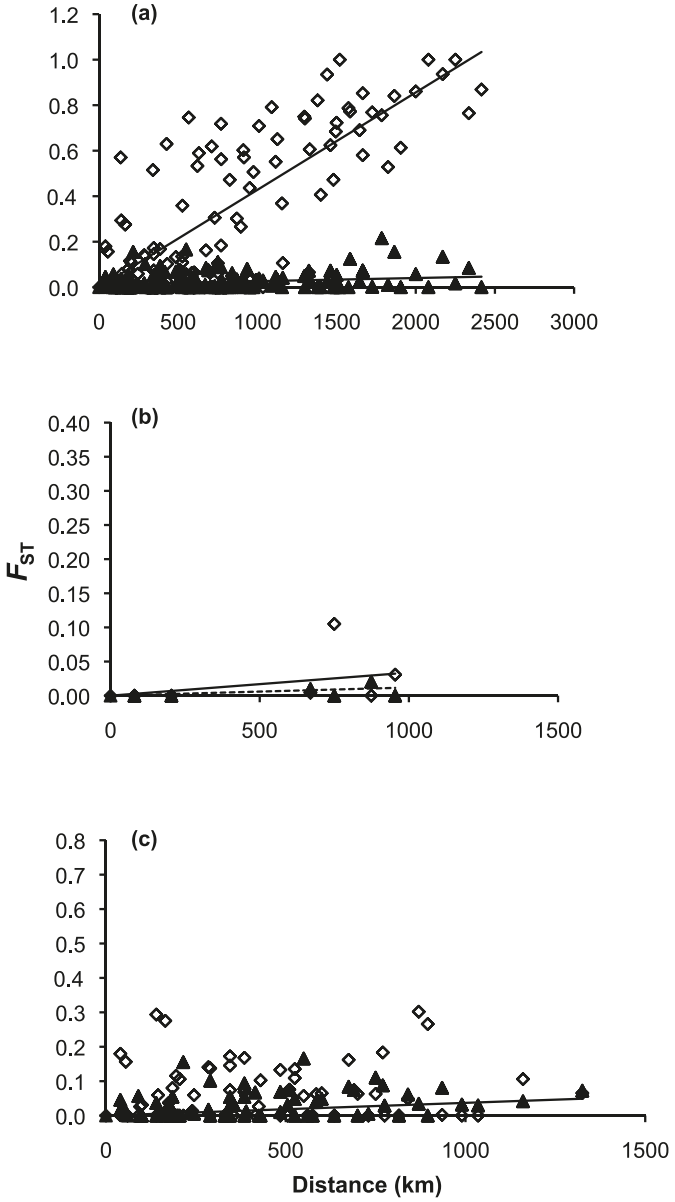
burg et al. 2001). Dispersal in the ZC is also indicated given the absence of haplotype A in the Arthur River in Tasmania and in the Glenelg, Hopkins and Barham populations in southern Victoria. Finally, the low magnitude of population genetic structure in estuary perch is more indicative of a highly dispersive, free swimming marine species (Ward et al. 1994; Bilton et al. 2002; Watts and Johnson 2004). Indeed, adult estuary perch are a large fish, fully capable of tolerating marine salinities (and thus not dependent on brackish water from flood plumes) and able to actively traverse long distances along the coastline (Trnski et al. 2005).

As tagging data suggests that estuary perch remain in the one estuary for the entirety of their life cycle (Chris Walsh, NSW Department of Primary Industries, Cronulla Fisheries Centre, Cronulla NSW 2230, Australia, personal communication 2008), the long distance dispersal of larvae may be the most likely mechanism for connectivity in the species. The passive dispersal of larvae by hydrographic processes such as flood plumes, tidal action, and ocean currents is the most common mechanism of long distance dispersal and recruitment in marine fish (Hoskin 2000; Bruce et al. 2001; Condie et al. 2005). However, the presence of transient stocks of estuary perch that migrate along the coast, occasionally entering open estuaries (McCarraher and McKenzie 1986), suggests that adult fish are also contributing towards gene flow. Consequently, dispersal in estuary perch is possible by both adult and larval life stages and may be augmented by both flood plumes and ocean currents, which when combined with the lack of any disruptive barriers in the region, could prevent the formation of strong genetic structure in the species.

Latitudinal disjunction and recent population history

The two most abundant mtDNA haplotypes (A and C) were distributed across the range of the species, though their frequencies showed distinct latitudinal disjunctions. Haplotype A was more frequent south of the Clyde River in NSW, whereas haplotype C was more common in the northern part of the distribution of the species. This pattern resulted in the significant population structure detected (Φ_{ST} = 0.443) and the division of populations into two groups. Genetic breaks dividing marine organisms with high dispersal potential usually result from the presence of physical or oceanographic barriers to dispersal (Grant and Bowen 1998) or from historical vicariant forces (Avise et al. 1987; Avise 1994; Dawson 2001). The disjunction in estuary perch haplotype frequencies did not correlate with any known temperature, geographic, or ecological barrier. However, the reduced number of highly related haplotypes, and the results from mismatch analysis and IM might suggest that the history of estuary perch populations has been predominantly influenced by rapid expansion events from homogeneous source populations, likely in the area of Bass Strait (Nei et al. 1975; Bernatchez and Wilson 1998; Cruzan and Templeton 2000). In Australia, Pleistocene variations in sea level had a dramatic impact on the Bass Strait area where low sea levels periodically created a land bridge between northern Tasmania and mainland Australia (Wells and Okada 1996; Lambeck and Chappell 2001). The episodic exposure of the Bassian Isthmus had a biogeographic effect on a number of southeastern Australian marine species. Several studies reveal an east–west phylogeographic break concordant with a hypothesis of allopatric speciation in the area of Bass Strait (e.g., *Catostylus* jellyfish, Dawson 2005; *Nerita* gastropods, Waters 2008; *Catomerus* barnacles, York et al. 2008). Similarly, the exposure of the Bassian Isthmus during the last glacial maximum may have created the historical divergence evident in estuary perch. Although the genetic discontinuity is no longer evident in the area of Bass Strait, it is possible that the geographic location of the break may have shifted in such highly dispersive organisms. Alternatively, we propose that estuary perch underwent two population expansions correlated with rising sea levels during interglacial periods. Firstly, our analyses suggest an initial postglacial eastward expansion from a putatively small refugial population in the vicinity of Bass Strait at the end of the Pleistocene. During this period favourable environmental conditions would have allowed colonization of the southeast

Fig. 3. The correlation between genetic distance (pairwise F_{ST}) and geographic distance (km) using both mtDNA (open diamonds, ◊) and microsatellites (filled triangles, ▲) is shown. Mantel tests were conducted between (*a*) all populations (*b*) populations in the northern group, Richmond–Shoalhaven, and (*c*) populations in the southern group, Clyde–Glenelg. There was no indication of isolation by distance (IBD) when populations were separated into the northern (mtDNA $P = 0.23$; microsatellites $P = 0.21$) and southern groups (mtDNA $P = 0.46$; microsatellites $P = 0.28$); however, mtDNA data revealed significant structuring in the form of IBD when both groups were analysed together as a single population (mtDNA $P < 0.0001$; microsatellites $P = 0.19$).



coast estuaries as far north as the Clyde–Shoalhaven area. A second event occurred during the early Holocene when the species expanded to colonize estuaries north to the Richmond River. This expansion may coincide with the formation of the majority of the southeast estuarine systems around 6 kya (Chappell 1983). The timing of these events may have been very recent, with IM placing the divergence time between the two groups at 63 kya. Similarly, despite having a large interval estimate, mismatch analysis suggests that the northern group expanded approximately 10 kya and the southern group expanded approximately 18.6 kya. In summary, the demography of estuary perch populations appears to be shaped by a short-term vicariant event isolating the currently characterized northern and southern groups during the late Pleistocene. This was followed by demographic expansions in both groups as a result of rising sea levels at the end of the Pleistocene and early Holocene. High levels of contemporary gene flow following recent demographic expansion events would together account for the reduced genetic structure and genetic variability in the species.

Implications for conservation and management

Estuary perch numbers are reported to be declining, and the species is of conservation concern (Hodgkin 1994). In addition to a ban on commercial fishing, conservative bag and size limits apply to recreational anglers. The species has rarely been commercially stocked and is sensitive to handling, possibly limiting their value to aquaculture (Harris 1986; Allen 1989; McDowall 1996). The overall lack of mtDNA and nuclear variation in estuary perch means that the fishery could be particularly vulnerable to stochastic events. Although genetic data indicates high gene flow in the species, only a small number of migrants are needed to homogenize gene frequencies. Consequently it is important to avoid the over-exploitation of local stocks, as the influx of immigrants may not be fast enough to replenish stocks (Waples 1987; Burridge et al. 2004; Carvalho and Hauser 1995). The mtDNA variation observed in this study suggests that the estuary perch fishery should be treated as two separate stocks. One northern stock that includes all rivers between the Richmond River and the Shoalhaven River, and one southern stock comprising all rivers between the Clyde River and the mouth of the Murray in South Australia. Although the mtDNA and microsatellite data sets were not able to differentiate between the estuaries sampled in this study, the lack of genetic divergence among populations is not necessarily indicative of complete genetic homogeneity in the species. Indeed, as tagging data suggests that adult fish are not moving between estuaries, management of the fishery needs to take into account the existence of local adaptations to variable environmental conditions within each estuary (Bilton et al. 2002; Burridge and Versace 2006). In the Australian bass, morphological variation was significantly heterogeneous throughout their distribution and was likely associated with environmental differences (Jerry and Cairns 1998). Consequently, the existence of any adaptive genetic variation in estuary perch needs to be determined in order to preserve the genetic integrity of the species.

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Appendix A

Table A1 appears on the following page.

Table A1. Allele frequency of six microsatellite loci genotyped from 17 populations of *Macquaria colonorum* along the south east coast of Australia.

Locus	Allele	*RIC*	*CLA*	*HAW*	*SHO*	*CLY*	*MAL*	*SNO*	*BEM*	*GIP*	*MER*	*ALB*	*BAS*	*HOP*	*TAR*	*BAR*	*GLE*	*ART*
AB001	224	0.86	1	1	1	1	0.79	1	0.98	0.81	1	1	1	0.98	1	1	1	1
	226	0	0	0	0	0	0	0	0	0	0	0	0	*0.02*	0	0	0	0
	232	**0.14**	**0**	**0**	**0**	**0**	**0.21**	**0**	**0.02**	**0.09**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	234	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0.06**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	238	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0.04**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
AB006	180	0.93	1	1	1	1	0.79	1	0.98	0.78	1	1	1	1	1	1	1	1
	186	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0.02**	**0**	**0**						
	188	**0**	**0**	**0**	**0**	**0**	**0.04**	**0**	**0**	**0.07**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	190	**0**	**0**	**0**	**0**	**0**	**0.11**	**0**	**0.02**	**0.09**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	192	**0.07**	**0**	**0**	**0**	**0**	**0.07**	**0**	**0**	**0.04**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
AB009	281	0	0	0	0.04	0	0	0	0	0	0	0	0	0.04	0	0	0	0
	283	0.79	1	1	0.96	1	0.79	0.96	0.97	0.67	1	1	1	0.96	0.96	0.92	0.89	1
	285	0.14	0	0	0	0	0.14	0.04	0.03	0.33	0	0	0	0	0.02	0	0.11	0
	295	**0.07**	**0**	**0**	**0**	**0**	**0.07**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0.02**	**0.08**	**0**	**0**
AB97	104	0.93	1	1	1	1	0.96	1	1	0.96	1	1	1	1	1	1	1	1
	112	**0.07**	**0**	**0**	**0**	**0**	**0.04**	**0**	**0**	**0.04**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
AB107	**290**	**0.14**	**0**	**0.02**	**0**	**0**	**0.21**	**0**	**0.02**	**0.19**	**0**	**0.02**	**0**	**0.02**	**0**	**0**	**0**	**0**
	300	0.86	1	0.98	1	1	0.79	1	098	0.81	1	0.98	1	0.98	1	1	1	1
AB114	**117**	**0.07**	**0**	**0**	**0**	**0**	**0.03**	**0**	**0.02**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	131	0.14	0.15	0.06	0.06	0.04	0.22	0.04	0.13	0.18	0.02	0.05	0.07	0	0	0	0	0
	133	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0.06**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	141	0.79	0.85	0.94	0.94	0.96	0.75	0.96	0.85	0.76	0.98	0.95	0.93	1	1	1	1	1

Note: Alleles in bold are known Australian bass alleles indicating the presence of interspecific hybrids.