Looking through glassfish: marine genetic structure in an estuarine species

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Abstract. Through the use of mitochondrial DNA (ATP8 gene), the prediction of
10   intermediate genetic structuring was investigated in two species of estuarine glassfish
(*Ambassis marianus* (Günther 1880) and *Ambassis jacksoniensis* (Macleay 1881))
(Perciformes: Ambassidae) to determine the possibility of a generalised ‘estuarine’
genetic structure. Individuals were collected from estuaries in eastern Australia
between Tin Can Bay (Queensland) in the north and Kempsey (New South Wales) in
15   the south. Analysis of the haplotype frequencies found in this region suggested
panmictic populations with star-like phylogenies with extremely high levels of genetic
diversity, but with no correlation between geographic distance and genetic distance.
Non-significant F_{ST} and Φ_{ST} suggested extensive dispersal among estuaries. However,
Tajima’s D and Fu’s F_S values suggest ‘mutation – genetic drift equilibrium’ has not
20   been reached, and that population expansions occurring 262 000 (*A. marianus*) and
300 000 (*A. jacksoniensis*) years ago may obscure any phylogeographic structuring or
isolation by distance. The finding of panmixia was contrary to the prediction of
genetic structuring intermediate between that of marine fish (shallowly structured)
and freshwater fish (highly structured), suggesting high dispersal capabilities in these
25   species.

Extra Keywords: Estuaries, phylogeography, ambassid.

INTRODUCTION

When considering the differences between freshwater and marine populations, generalized patterns of genetic structure can often be observed (Feral 2002). Numerous studies have investigated phylogeographic structuring of mitochondrial DNA (mtDNA) in freshwater and marine systems to describe the relationships between geographic and genetic distances and their concordances and discordances (Waters et al. 2000; Baker et al. 2004; Ovenden and Street 2003; Page et al. 2004; Huey et al. 2006). In general, marine populations tend to be shallowly structured and freshwater systems strongly structured (Bilton et al. 2002; Wong et al. 2004). Studies of freshwater species such as brook charr (Castric et al. 2001), hardyheads (McGlashan and Hughes, 2000), perch (Nesbo et al. 1998) and pygmy perch (Cook et al. 2007) are among many to have exemplified strong patterns of structure with high F_{ST} values. In contrast, a study by Ward and Elliot (2001) of 20 marine species in south eastern Australia detected structuring in only four of these species.

As physical connectivity can heavily influence population structure, marine populations are characterized by shallow structure, with tidal action and currents facilitating the homogenization of gene frequencies (Avisé 1998; Watts and Johnson 2004; Sheaves et al. 2007). Particularly in marine populations where ‘mutation - genetic drift equilibrium’ has been reached, any observed structure often shows a pattern of ‘isolation by distance’ (IBD), where there is a positive relationship between genetic distance between sites and geographic distance between them (Wright 1943; Slatkin and Maddison 1990). Additionally, dispersal in adults, juveniles and larvae is

relatively unimpeded by physical barriers, allowing for the free transfer of genes from population to population (Ward and Elliot 2001). In contrast, genetic structure in freshwater organisms tends to be dominated by gene frequencies that can often be quite distinct from neighboring populations (Cook et al. 2006; Ponniah and Hughes 55 2006). Hydrological connectivity in Australian freshwater systems in particular is often highly variable, with periods of low flow often disconnecting pools and reducing opportunities for gene flow (Huey et al. 2006). As a result, freshwater populations isolated from gene flow develop distinct haplotype frequencies through the process of genetic drift and environment-specific selection (Hurwood and Hughes 60 2001).

The east coast of Australia is characterized by small coastal streams, open estuaries and intermittently closed and open lakes and lagoons (ICOLLs) (Hadwen et al. 2007). Most of these estuarine systems are geomorphically young and were formed in the last 65 6 000 years (Chappell 1982). As constantly changing geomorphic features and highly productive ecosystems, estuaries are significant in the life cycles of numerous aquatic species, especially in providing nursery habitat (Vrijenhoek 1998; Edgar et al. 2000; Able 2005).

70 As estuaries represent the ecotone between marine and freshwater systems, it has been suggested that estuarine fish may be expected to have levels of gene flow that are intermediate to those observed in marine and freshwater environments (DeWoody and Avise, 2000). Organisms in estuarine environments are typically adapted to highly variable conditions, such as fluctuating salinity and temperature (Bilton et al. 2002; 75 Cook et al. 2006). Furthermore, estuarine fish utilize a variety of dispersal

mechanisms which can strongly influence patterns of gene flow (Slatkin 1987; Doherty et al. 1994; Doherty et al. 1995). Many aquatic species have several life stages that can have a distinct role in the flow of genes in and between populations (Grantham et al. 2003; Nathan 2005). Passive dispersal in the larval stage is often the
80 crucial phase for gene flow in fish (Panov et al. 2004). This is particularly true for many estuarine and marine species where larvae are carried by tidal action and currents (often over many hundreds of kilometers) to recruit into new populations (Doherty et al. 1995; Riginos and Nachman 2001; Hamer and Jenkins 2004). However, the effectiveness of this mechanism for dispersal is dependent upon the
85 duration of the larval phase, where the longer the larval phase, the greater the potential dispersal distance (Uthicke and Benzie 2003).

Many studies of marine systems exemplify IBD, particularly where the species distribution is expansive (Emerson et al. 2001). IBD is expected to characterise
90 population structure where 1) the sampling range is smaller than the distribution of the population, and 2) the sampled population is in equilibrium between mutation, gene flow and drift (Slatkin and Maddison 1990; Slatkin 1993). Given that the distribution of many marine species can cover large areas of coast and ocean, it is not surprising that populations of turbot (Feral 2002), greenback flounder (Ward and Elliot 2001)
95 and Australian bass (Chenoweth and Hughes (1997) have all shown structure following models of IBD. Due to the large, linear distribution of *Ambassis marianus* (*A. marianus*) and *Ambassis jacksoniensis* (*A. jacksoniensis*) it seems likely that these populations will be characterised by IBD.

100 *Ambassis marianus* and *Ambassis jacksoniensis* (Ambassidae) are two species of
estuarine glassfish endemic to the east coast of Australia (Allen and Burgess 1990;
Hurst et al. 2005). Both species are abundant schooling fish, and can be found in both
clear and turbid small coastal streams, permanently open large estuarine rivers and
ICOLLs (tolerating salinities of up to 35) (Hurst et al. 2005). The distribution of these
105 ambassids is reported to extend from Sydney (New South Wales (NSW), Australia) to
Hervey Bay (Queensland (QLD), Australia) (Allen et al. 2002). *A. marianus* and *A.*
jacksoniensis are relatively small fish, commonly growing to about 60mm in length
(Allen et al. 2002). They are very similar morphologically; however a few key
features distinguish them from one another. As typical of ambassids, the bodies of
110 both species are transparent and have a vivid, reflective stripe running along the
lateral line (Pusey et al. 2004). Fins are generally pale and transparent, although some
A. marianus specimens collected in this study exhibited pale yellow colouring. *A.*
marianus is easily identifiable by the dorsal fin, as the first spine is black, whereas
dorsal spines in *A. jacksoniensis* are generally uncoloured (Allen and Burgess 1990).
115 *A. jacksoniensis* is also more slender in body shape than *A. marianus*, as the latter has
more breadth longitudinally, and also has a greater spine to body size ratio (Allen and
Burgess 1990). Very little is known of either of these species in regards to their life
history.

120 This study aimed to examine the genetic partitioning found in *A. marianus* and *A.*
jacksoniensis, and investigate any correlations with geographic influences. It was
hypothesised that the populations of two study species would exhibit structuring
characterised by IBD (Wright 1943). Furthermore, this study explored the possibility
of ‘intermediate gene flow’ (i.e. levels of gene flow between that of marine and

freshwater fish), which has been suggested for estuarine systems (Farrington et al. 2000; Dawson et al. 2002; Cabral et al. 2003; Watts and Johnson 2004; Doukakis et al. 2005).

MATERIALS AND METHODS

Field Collection

Individuals were collected from 23 estuarine systems along the east coast of Australia, from Crab Creek (Tin Can Bay, QLD) in the north, to the Macleay River (Kempsey, NSW) in the south, during field trips in 2006 and early 2007 (Figure 1). Sites in QLD represented most of the estuarine habitats along the coast, whereas sites in NSW were restricted only to estuarine locations specified under the issued NSW Scientific Research Permit. Three types of estuarine systems were sampled – 1) small coastal creeks, 2) permanently open large estuarine rivers, and 3) ICOLLs. Abundance and occurrence of species fluctuated markedly from site to site, resulting in lower (or no) samples for some sites (Tables 3 and 4). Individuals ranged in size from 10mm to 80mm, and thus included juveniles and adults. Collection was carried out using a seine net (15m long and 2m high (with a 2m long bag), with a 9-mm mesh size) operated by two people. Non-target species were immediately returned to the point of capture, as were surplus individuals of the target species (if any). Individuals required for analysis were euthanised in an ice slurry and stored in liquid nitrogen, then at -80°C until required for processing.

150   **Laboratory Methods**

Species identification was made through the use of keys and sequence differences, especially in the case of juveniles (Allen and Burgess 1990). Total genomic DNA was extracted from each individual using a variation of the CTAB (cetyl trimethyl ammonium bromide) phenol-chloroform extraction procedure (Doyle and Doyle 155 1987). The polymerase chain reaction (PCR) was used to amplify the ATP8 mtDNA region to produce a fragment of 414 base pairs using the ATP8.2 and COIII.2 primers (ATP8.2: 5'-AAA GCR TYR GCC TTT TAA GC-3'; COIII.2: 5'-GTT AGT GGT CAK GGG CTT GGR TC-3') (S. McCafferty, unpublished data). Amplification occurred in 12.5µL reactions of primers ATP8.2 and COIII.2 (0.5µL of each), double- 160 distilled water (ddH₂O; 7.95µL), extracted DNA (1.0µL) and Fisher Biotec's 10 x reaction buffer (1.25µL) (www.fisherbiotec.com), MgCl₂ [25mM] (1.0µL), deoxynucleotide triphosphate (dNTPs [10mM] (0.25µL)), and *Taq* [5.5 units/µL] (0.05µL). Samples were subjected to the following conditions: 94°C for five minutes; 40 cycles of: 94°C for 30 seconds, 50°C for 30 seconds, and 72°C for one minute; 165 then 72°C for five minutes. Samples were then stored at 4°C until required for further analysis. The resulting PCR product was purified with *exo-sap* (Fermentas exonuclease (20u/µL) (www.fermentas.com)) and shrimp alkaline phosphatase (1u/µL)) where 5.0µL of PCR product was added to 0.5µL *exo* and 2.0µL *sap*, then samples underwent a thermal program (37°C for 35 minutes, 80°C for 20 minutes) in 170 a GeneAmp PCR System 2700 (Applied Biosystems www.appliedbiosystems.com). Samples were held at 4°C until required for further analysis.

Purified mtDNA samples were sequenced using the ATP8.2 primer (0.32µL) in a 10-µL reaction also containing 5X sequencing reaction buffer (2.0µL), 3.1 Big Dye

175 Terminator mix (2.0µL) (Applied Biosystems www.appliedbiosystems.com), exo-sap
product (0.5µL) and ddH₂O (5.18µL). Again, this mixture was placed in a GeneAmp
PCR System 2700 under the following thermal program: 96°C for one minute; then
30 cycles of: 96°C for 10 seconds, 50°C for 5 seconds, 60°C for four minutes.
Samples were stored at 4°C. Final reactions were cleaned then sequenced using the
180 Applied Biosystems 3130xl Genetic Analyser (www.appliedbiosystems.com).

Data Analysis

Individual sequences were aligned and edited using Sequencher 4.1 (Gene Codes
2000) for sequence comparison. A final fragment of 414bp was selected for analysis,
185 and each species was analysed as a separate entity at all times. The TCSv1.21
program (Clement et al. 2000) was used to construct parsimonious networks showing
relationships between haplotypes for each species, using the 95% most plausible
connections. Arlequin v3.1 (Schneider et al. 2000) was used for calculations of overall
 F_{ST} , a measure of genetic differentiation (Weir and Cockerham 1984) and Φ_{ST} , a test
190 of correlation between genetic divergence and genetic distance (Excoffier et al. 1992)
among populations (Excoffier et al. 2005). Mantel tests in Arlequin v3.1 (Excoffier et
al. 2005) were also used to perform correlations between genetic and geographic
distance as an indicator of IBD. This procedure used Slatkin's Linearised F_{ST} ($F_{ST} =$
 $1/(1-F_{ST})$ (Slatkin 1995) as genetic distance, and geographic distance was determined
195 as being the linear distance between pairs of estuaries, via the ocean.

To ascertain whether the study populations were in mutation – genetic drift
equilibrium, tests of neutrality were performed using the software package DnaSP
4.10.9 (Rozas and Rozas 1999). DnaSP 4.10.9 was also used to calculate the numbers

200 of synonymous and non-synonymous base changes in sequences (Rozas and Rozas
1999). As star-like haplotype networks may be indicative of a recent population
expansion, two tests of neutrality (Tajima's D and Fu's F_S) were used to analyse the
two species for the expected frequencies of mutations in the populations (Chenoweth
and Hughes 2003). Tajima's D values and Fu's F_S values were generated to determine
205 whether observed mutations reflected that of expanding populations, or populations
under selection (Rozas and Rozas 1999). Mismatch distributions were calculated from
pairwise differences - differences between sequences (Harpending et al. 1993).
Raggedness indices (r) (which range between 1 and 0) were also computed in DnaSP
to test whether observed mutational distributions were generated by stable or
210 expanding populations (Harpending et al. 1993). From this, Tau was used in
estimating the time since population expansion, using the formula: $\text{Tau} = 2\mu t$, where t
is time in generations (one/year) and μ is the mutation rate, per sequence, per
generation (Harpending et al. 1993). Sequence divergence in the fish ATP region has
been estimated to be 1.3% per million years, therefore, the mutation rate is half this at
215 0.65% per million years (Bermingham et al. 1997).

RESULTS

Patterns of Variation

220 Of the mtDNA ATP8 gene, 414 bp were analysed from 98 *A. marianus* individuals
and 149 *A. jacksoniensis* individuals. This revealed 1 non-synonymous and 18
synonymous changes for *A. marianus*, and 2 non-synonymous and 40 synonymous
changes for *A. jacksoniensis*. Analysis of the 414 bp revealed 21 haplotypes in *A.*
marianus (lodged under GenBank Accession Numbers: EU257288-EU257308), and

225 49 haplotypes in *A. jacksoniensis* (lodged under GenBank Accession Numbers: EU257313-EU257361) (Figs 2, 3 and Tables 3 and 4). Of these haplotypes, 8 of *A. marianus* and 10 of *A. jacksoniensis* were shared, and 13 of *A. marianus* and 39 of *A. jacksoniensis* were singletons (Tables 3 and 4). In both species, the arrangements of these haplotypes represented that of star-like phylogenies, radiating outwards from
230 several common internal haplotypes (Figs 2 and 3). Unresolved loops in the networks were dissolved following the guidelines of Posada and Crandall (2001), given the improbabilities of homoplasy and recombination in mitochondrial DNA (Sanderson and Donoghue 1989). Overall values of F_{ST} (0.019; $\alpha=0.05$ $P=0.205$ for *A. marianus*, 0.017; $\alpha=0.05$ $P=0.124$ for *A. jacksoniensis*) and Φ_{ST} (0.013; $\alpha=0.05$ $P=0.300$ for *A. marianus*, 0.020; $\alpha=0.05$ $P=0.106$ for *A. jacksoniensis*) were non-significant (Table 1).
235 High levels of gene flow were indicated as the scale of F_{ST} and Φ_{ST} ranges from 1 (complete population isolation) to 0 (panmixia). Mantel tests of the relationship between geographic distance and genetic distance were also non-significant (*A. marianus* 0.081; $\alpha=0.05$ $P=0.238$ and *A. jacksoniensis* 0.107; $\alpha=0.05$ $P=0.106$) (Table
240 1).

Tests of Neutrality

Both tests of neutrality yielded highly significant results (Table 2). Of particular interest were values produced for Fu's F_S , as this test is particularly sensitive to the
245 signature of population expansion (Fu 1997). Fu's F_S values of -12.779 ($\alpha=0.02$ $P=0.000$) (*A. marianus*) and -72.737 ($\alpha=0.02$ $P=0.000$) (*A. jacksoniensis*) indicated excess numbers of mutations in these populations. Tajima's D values (*A. marianus*, -1.429 ($\alpha=0.05$ $P=0.046$); *A. jacksoniensis*, -2.364 ($\alpha=0.05$ $P=0.000$)) also supported this finding.

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Mismatch Distributions and Expansion Dates

Mismatch distributions of pairwise differences in the populations closely followed models based on expanding populations (Table 2). Raggedness indices for *A. marianus* (0.020; $\alpha=0.05$ $P=0.083$) and *A. jacksoniensis* (0.069, $\alpha=0.05$ $P=0.933$)
255 showed no significant difference from the expected frequencies of mutations in expanding populations. Estimations of the time elapsed since these expansions were calculated with Tau, where values were 1.410 and 1.617 for *A. marianus* and *A. jacksoniensis* respectively. These values resulted in very similar estimates of time since expansion for *A. marianus* (~262kya) and *A. jacksoniensis* (~300kya).

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DISCUSSION

High genetic diversity – no structure

Both *A. marianus* and *A. jacksoniensis* were found to have high levels of haplotype
265 diversity (Table 3 and 4), which is typical of marine fish, for example mangrove jack (Ovenden and Street 2003), mackerel, chub mackerel (Zardoya et al. 2004) and damselfish (Domingues et al. 2005). However, this diversity was not partitioned according to the geographic scale of the study, indicating panmixia (that is the genetic diversity contained within the population is homogenous on the geographic scale)
270 (Figs 2 and 3). This implies high dispersal capabilities in these species, with no apparent barriers to gene flow. Although previous studies suggest that ‘intermediate’ structuring is often observed in estuarine organisms, the results of this study suggest estuarine ambassids are structured in a manner commonly found in marine fish (Chaplin et al. 1998; Beheregaray and Sunnucks 2001; Watts and Johnson 2004).

The high levels of diversity and the star-shaped phylogeny in both species are consistent with a relatively recent population expansion, where 1) sufficient generations have occurred to allow for divergence from ancestral haplotypes, and 2) genetic drift and selection have not yet purged the population of singletons and other less common haplotypes (Posada and Crandall 2001). The high levels of diversity in both species indicate that most of the variation detected in the ATP8 region is neutral. In coding genes, most mutations are synonymous and do not affect protein function and the amino acid produced (Altukhov and Salmenkova 2002). This is observed in the genetic diversity of both *A. marianus* and *A. jacksoniensis*, where high levels of mutations are persisting in both populations which suggests selection is not operating against lethal mutations.

Star-like networks are considered to be a signature of a recent population expansion, usually following a bottleneck or founder event (Chenoweth and Hughes 2003). Coalescent theory suggests interior haplotypes are ancestral in the population and that tip haplotypes are more recent mutations and are therefore unlikely to be as widespread in the population (Figure 2 and 3) (Posada and Crandall 2001). The population may have also been small and stable historically, but changes in some factor (such as changes in temperature, sea level or geographic composition) may have led to the population increase (Hewitt 2000). However, while these populations show no evidence of genetic structuring, the occurrence of a population expansion may act to obscure structure in the population, particularly recent expansions. Secondly, as recently expanded populations, it is unlikely these study species have been able to reach equilibrium between the effects of gene flow and genetic drift

(Crow and Aoki 1984). Consequently, under these conditions, the absence of genetic structure may be due to excess mutations in the population giving the appearance of high gene flow. We acknowledge that this study contains low sample sizes for some sites, but we believe that increasing the sample size would have little effect on the overall conclusion. For each species, there is a single common haplotype shared across all sites in similar frequencies. The rare singleton haplotypes have little effect on the overall F_{ST} values.

Effects of life cycle on gene flow

Whether dispersal and gene flow in *A. marianus* and *A. jacksoniensis* is facilitated by adults, juveniles or larvae (or all) is unknown (Allen 1991). Hadwen and Arthington (2007) provided evidence of adult *A. marianus* recruitment into an ICOLL in northern NSW which suggests that adults can be vectors of gene flow. However, as a small estuarine-dwelling fish, with perhaps limited capability for traversing long sections of open ocean as adults, it seems likely that major dispersal events occur with larval fish being passively transported and consequently recruited into new estuaries by currents and tidal cycles (Riginos and Nachman 2001). The differing effects of larval dispersal duration have been highlighted in a study of two marine fish by Purcell et al. (2006). They revealed that fish with a shorter larval pelagic time showed population structuring, whereas, longer larval durations corresponded with panmixia. Doherty et al. (1995) found the same relationship in a study of seven species, where shorter larval durations correlated with increased genetic structure. Whilst duration of the larval phase in *A. marianus* and *A. jacksoniensis* is not known, it is plausible that these species have a substantial larval duration that facilitates passage over considerable dispersal distances. Therefore, while local movements by adult fish may aid in

325 homogenising alleles, it seems likely that long distance dispersal occurring in the
larval stage may be the primary influence on allele frequencies in these species
(Uthicke and Benzie 2003).

While larval dispersal is the most likely explanation for the observed haplotype
330 distributions in these species, there are other factors may result in panmixia. For
example, Farrington et al. (2000) found similar patterns of panmixia in populations of
black bream (*Acanthopagrus butcheri*); and that it was the adult individuals dispersing
and facilitating gene flow, rather than larvae.

335 ***Isolation by distance***

Neither species of ambassid showed evidence of IBD structuring (Table 1), and fitted
Category IV of Avise et al. (1987) with haplotypes widely distributed and showing no
relationship with geography (Figs 2 and 3). Given the connectivity and high levels of
dispersal found in these populations of ambassids, and the abundance of the species, it
340 is not surprising that equilibrium in these populations has not yet been reached.
Because these populations were apparently panmictic and genetically homogenised,
this suggests that gene flow is exceeding the effect of genetic divergence, masking
any IBD effect (Purcell et al. 2006). As an endemic and abundant species occurring in
a relatively small, continuous distribution, it is likely that long distance dispersal can
345 counteract the effects of IBD (Doherty et al. 1995). This pattern is particularly
common in coral reef fish, where adult individuals have a small dispersal range, but
larvae disperse longer distances and homogenise gene frequencies (Bay et al. 2004;
Klanten et al. 2007).

350   ***Population expansions***

Many population studies have shown population expansions coinciding with late Quaternary glacial retreats. In a study of tasselfish (*Polynemus sheridani*), Chenoweth and Hughes (2003) found recent post-glacial expansions across northern Australia associated with rising sea levels. Similarly, two species of West African estuarine fish
355   (*Sarotherodon melanotheron* and *Ethmalosa fimbriata*) underwent population expansions after periods of mainland aridity and low sea levels during a glacial cycle (Durand et al. 2005). In general, there is a common pattern of population expansions in many marine and estuarine fish species occurring toward the end of the more recent glacial cycles (~10 000 to 80 000 years ago), which correspond with rising sea levels
360   (Uthicke and Benzie 2003; Domingues et al. 2005; O'Reilly et al. 2004; Bernatchez and Wilson 1998). However, expansion times of *A. marianus* and *A. jacksoniensis* greatly predate the majority of reported fish population expansions.

It is possible that an ice age depleted populations of *A. marianus* and *A. jacksoniensis*,
365   and that modern populations are the result of a population expansion after this era, when ice caps receded and seas levels rose (Hewitt 2000). The haplotype networks (Figs 2 and 3) suggest that the common haplotypes (J1 and M1) are representative of refugial populations that persisted through these periods of environmental change, from which contemporary populations have colonised new areas and diverged from
370   parent haplotypes (Posada and Crandall 2001). However, since more recent ice ages and climate changes (occurring after the calculated time since population expansion) appear to have had little impact on the genetic diversity of these populations, this explanation may be questionable. An alternative explanation is that alternating sea levels generated bottlenecks within these populations, which have resulted in genetic

375 structure that overrides any effects of more recent climatic alterations. Therefore,
possible population expansions succeeding the initial event were insignificant in
comparison, and not detected by the haplotypic resolution generated by this study.
While plausible, it is difficult to identify specific geological events accounting for
these population bottlenecks and expansions, particularly with limited geological data
380 specific to the study region. Finally, some recent studies of mutation rates in fish
mitochondrial DNA, based on known geological changes in New Zealand, suggest
that mutation rates may be much higher than currently thought (Burridge and
Smolenski 2003; Burridge et al. 2006; Waters et al. 2006). Therefore we cannot
totally discount the possibility that the expansions are much more recent than
385 calculated from our rate based on 0.65% per million years (Bermingham et al. 1997).

Population bottlenecks can be caused by a variety of factors other than glacial climate
change. Changes in food web ecology where predator-prey interactions and inter-
specific competition alter can often devastate populations (Williamson 1993;
390 Maltagliata 2002). Disease can also bottleneck populations where a substantial
proportion of the population perishes (Spear et al. 2006). Volcanic-induced
environmental destruction and climatic alterations have also been attributed to
reductions in population size (Hurwood and Hughes 1998; Hewitt 2000). In addition,
founder events are also catalysts for population expansion where colonising
395 populations rapidly expand into new regions (Lacy 1987). Population expansions
following founder events have been observed in many taxa, including freshwater
shrimp (*Paratya australiensis*) (Cook et al. 2006), black bream (*Acanthopagrus
butcheri*) (Chaplin et al. 1998) and tasselfish (*Polynemus sheridani*) (Chenoweth and
Hughes 2003). While one or a combination of these factors (including glacial-related

400 climate change) may have had severe repercussions for populations of *A. marianus*
and *A. jacksoniensis*, isolating the historical events responsible for population
bottlenecks, founder events and expansions is difficult.

Assessment of the genetic structure of *A. marianus* and *A. jacksoniensis* revealed high
405 levels of genetic diversity in a panmictic distribution, indicative of high dispersal
capabilities in these species. However, the conclusion of panmixia was accepted with
caution as there is evidence to suggest that these populations are not in ‘mutation –
genetic drift equilibrium’, having undergone population expansions ~262kya (*A.*
marianus) and ~300kya (*A. jacksoniensis*). To strengthen the conclusions reached
410 through mtDNA studies of ambassids, it would be beneficial to supplement the data
set with an additional marker. This would confirm patterns of dispersal and gene flow
found in these populations and verify ATP8 neutrality (Fu 1997). Furthermore,
mitochondrial DNA only reflects dispersal and gene flow in females, so a nuclear
marker such as microsatellites would determine whether dispersal is gender neutral
415 (Johnson 2000; Nathan et al. 2003).

Ambassis marianus and *Ambassis jacksoniensis* were used to investigate the
prediction of intermediate population structure in estuarine fish. The genetic structure
and distribution of these species suggested that these estuarine fish had population
420 structures more like those expected for marine fish. This lack of population structure
suggests these species have high dispersal capabilities, although recent population
expansions may also have contributed to this impression of panmixia.

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435 **REFERENCES**

- Able, K. W. (2005). A re-examination of fish estuarine dependence: evidence for connectivity between estuarine and ocean habitats. *Estuarine, Coastal and Shelf Science* **64**, 5-17.
- 440
- Allen, G. R. and Burgess, W.E. (1990). A review of the glassfishes (Chandidae) of Australia and New Guinea. *Records of the Western Australian Museum* **34**, 139-206.
- 445 Allen, G. R. (1991). 'Field Guide To The Freshwater Fishes of New Guinea'. Madang, Papua New Guinea, Christensen Research Institute.
- Allen, G. R., Midgley, H. and Allen, M. (2002). 'Freshwater Fishes of Australia'. Perth, Western Australian Museum and CSIRO Publishing.

450

Altukhov, Y. P. and Salmenkova, E.A. (2002). DNA polymorphism in population genetics. *Russian Journal of Genetics* **38**, 1173-1195.

455   Avisé, J. C., Arnold, J., Ball, R.M., Bermingham, E., Lamb, T., Neigel, J.E., Reeb, C.A. and Saunders, N.C. (1987). Intraspecific phylogeography: the mitochondrial DNA bridge between population genetics and systematics. *Annual Review of Ecology and Systematics* **18**, 489-522.

460   Avisé, J. C. (1998). The history and purview of phylogeography: a personal reflection. *Molecular Ecology* **7**, 371-379.

465   Baker, A. M., Hughes, J.M., Dean, J.C. and Bunn, S.E. (2004). Mitochondrial DNA reveals phylogenetic structuring and cryptic diversity in Australian freshwater macroinvertebrate assemblages. *Marine and Freshwater Research* **55**, 629-640.

470   Bay, L.K., Choat, J.H., van Herwerden, L. and Robertson, D.R. (2004). High genetic diversities and complex genetic structure in an Indo-Pacific tropical reef fish (*Chlorurus sordidus*): evidence of an unstable evolutionary past? *Marine Biology* **144**, 757-767.

470   Beheregaray, L. B. and Sunnucks, P. (2001). Fine-scale genetic structure, estuarine colonization and incipient speciation in the marine silverside fish *Odontesthes argentinensis*. *Molecular Ecology* **10**, 2849-2866.

- Bermingham, E., McCafferty, S. and Martin, A.P. (1997). 'Fish biogeography and
475 molecular clocks: perspectives from the Panamanian Isthmus'. In 'Molecular
Systematics of Fishes'. (Eds. T. Kocher and C. Stepien). pp. 113-126. Academic
Press, San Diego.
- Bernatchez, L. and Wilson, C.C. (1998). Comparative phylogeography of Nearctic
480 and Palearctic fishes. *Molecular Ecology* **7**, 431-452.
- Bilton, D. T., Paula, J. and Bishop, J.D.D. (2002). Dispersal, genetic differentiation
and speciation in estuarine organisms. *Estuarine, Coastal and Shelf Science* **55**,
937-952.
- 485
- Burridge, C.P. and Smolenski, A.J. (2003). Lack of genetic divergence found with
microsatellite DNA markers in the tarakihi *Nemadactylus macropterus*. *New
Zealand Journal of Marine and Freshwater Research* **37**, 223-230.
- 490 Burridge, C.P., Craw, D. and Waters, J.M. (2006). River capture, range expansion and
cladogenesis: the genetic signature of freshwater vicariance *Evolution* **60**, 1038-
1049.
- Cabral, H. N., Castro, F., Linhares, D. and Alexandrino, P. (2003). Genetic
495 differentiation of *Solea solea* (Linnaeus, 1758) and *Solea senegalensis* (Kaup,
1858), (Pisces : Pleuronectiformes) from several estuarine systems of the
Portuguese coast. *Scientia Marina* **67**, 43-52.

- Castric, V., Bonney, F. and Bernatchez, L. (2001). Landscape structure and
500 hierarchical genetic diversity in the brook charr, *Salvelinus fontinalis*. *Evolution*
55, 1016-1028.
- Chaplin, J. A., Baudains, G.A., Gill, H.S., McCulloch, R. and Potter, I.C. (1998). Are
assemblages of black bream (*Acanthopagrus butcheri*) in different estuaries
505 genetically distinct? *International Journal of Salt Lake Research* **6**, 303-321.
- Chappell, J. (1982). Evidence for smoothly falling sea levels relative to north
Queensland, Australia, during the past 6000 years. *Nature* **302**, 406-408.
- 510 Chenoweth, S. F. and Hughes, J.M. (1997). Genetic population structure of the
catadromous Perciform: *Macquaria novemaculeata* (Percichthyidae). *Journal of*
Fish Biology **50**, 721-733.
- Chenoweth, S. F. and Hughes, J.M. (2003). Oceanic interchange and nonequilibrium
population structure in the estuarine dependent Indo-Pacific tasselfish,
515 *Polynemus sheridani*. *Molecular Ecology* **12**, 2387-2397.
- Clement, M., Posada, D. and Crandall, K.A. (2000). TCS: A computer program to
estimate gene genealogies. *Molecular Ecology* **9**, 1657-1660.
- Cook, B. D., Baker, A.M., Page, T.J., Grant, S.C., Fawcett, J.H., Hurwood, D.A. and
520 Hughes, J.M. (2006). Biogeographic history of an Australian freshwater shrimp,
Paratya australiensis (Atyidae): the role life history transition in
phylogeographic diversification. *Molecular Ecology* **15**, 1083-1093.

- Cook, B. D., Bunn, S.E. and Hughes, J.M. (2007). Molecular genetic and stable
525 isotope signatures reveal complementary patterns of population connectivity in
the regionally vulnerable southern pygmy perch (*Nannoperca australis*).
Biological Conservation **138**, 60-72.
- Crow, J. F. and Aoki, K. (1984). Group selection for a polygenic behavioral trait:
530 estimating the degree of population subdivision. *Proceedings of the National
Academy of Science* **81**, 6073-6077.
- Dawson, M. N., Louie, K.D., Barlow, M., Jacobs, D.K. and Swift, C.C. (2002).
Comparative phylogeography of sympatric sister species, *Clevelandia ios* and
535 *Eucyclogobius newberryi* (Teleostei, Gobiidae), across the California Transition
Zone. *Molecular Ecology* **11**, 1065-1075.
- DeWoody, J. A. and Avise, J.C. (2000). Microsatellite variation in marine, freshwater
and anadromous fishes compared with other animals. *Journal of Fish Biology*
540 **56**, 461-473.
- Doherty, P.J., Mather, P. and Planes, S. (1994). *Acanthochromis polyacanthus*, a fish
lacking larval dispersal, has genetically differentiated populations at local and
regional scales on the Great Barrier Reef. *Marine Biology* **121**, 11-21.
545
- Doherty, P. J., Planes, S. and Mather, P. (1995). Gene flow and larval duration in
seven species of fish from the Great Barrier Reef. *Ecology* **76**, 2373-2391.

- Domingues, V. S., Bucciarelli, G., Almada, V.C. and Bernardi, G. (2005). Historical
550 colonization and demography of the Mediterranean damselfish, *Chromis*
chromis. *Molecular Ecology* **14**, 4051-4063.
- Doukakis, P., Birstein, V.J. and De Salle, R. (2005). Intraspecific structure within
three caviar-producing sturgeons (*Acipenser gueldenstaedtii*, *A. stellatus*, and
555 *Huso huso*) based on mitochondrial DNA analysis. *Journal of Applied*
Ichthyology **21**, 457-460.
- Doyle, J. J. and Doyle, J.L. (1987). A rapid DNA isolation procedure for small
quantities of leaf tissues. *Phytochemistry Bulletin* **19**, 11-15.
- 560
- Durand, J. D., Tine, M., Panfili, J., Thiaw, O.T. and Lae, R. (2005). Impact of
glaciations and geographic distance on the genetic structure of a tropical
estuarine fish, *Ethmalosa fimbriata* (Clupeidae, S. Bowdich, 1825). *Molecular*
Phylogenetics and Evolution **36**, 277-287.
- 565
- Edgar, G. J., Barrett, N.S., Graddon, D.J. and Last, P.R. (2000). The conservation
significance of estuaries: a classification of Tasmanian estuaries using
ecological, physical and demographic attributes as a case study. *Biological*
Conservation **92**, 383-397.
- 570
- Emerson, B. C., Paradis, E. and Thebaud, C. (2001). Revealing the demographic
histories of species using DNA sequences. *Trends in Ecology and Evolution* **16**,
707-716.

- 575 Excoffier, L., Smouse, P.E. and Quattro, J.M. (1992). Analysis of molecular variance
inferred from metric distances among DNA haplotypes: application to human
mitochondrial DNA restriction data. *Genetics* **131**, 479-491.
- Excoffier, L., Laval, G. and Schneider, S. (2005). Arlequin ver. 3.0: an integrated
580 software package for population genetics data analysis. *Evolutionary
Bioinformatics Online* **1**, 47-50.
- Farrington, L. W., Austin, C.M. and Coutin, P.C (2000). Allozyme variation and stock
structure in the black bream, *Acanthopagrus butcheri* (Munro) (Sparidae) in
585 southern Australia: implications for fisheries management, aquaculture and
taxonomic relationship with *Acanthopagrus australis* (Gunther). *Fisheries
Management and Ecology* **7**, 265-279.
- Feral, J. P. (2002). How useful are the genetic markers in attempts to understand and
590 manage marine biodiversity. *Journal of Experimental Marine Biology and
Ecology* **268**, 121-145.
- Fu, Y. (1997). Statistical tests of neutrality of mutations against population growth,
hitchhiking and background selection. *Genetics* **147**, 915-925.
- 595 Gene Codes. (2000). Sequencher 4.1, Gene Codes Corporation, Ann Arbour,
Michigan.

- 600 Grantham, B. A., Eckert, G.L. and Shanks, A.L. (2003). Dispersal potential of marine
invertebrates in diverse habitats. *Ecological Applications* **13**, S108-S116.
- Hadwen, W.L. and Arthington, A.H. (2007). Food webs of two intermittently open
estuaries receiving [15]N-enriched sewage effluent. *Estuarine, Coastal and
Shelf Science* **71**, 347-358.
- 605 Hadwen, W. L., Russell, G.L. and Arthington, A.H. (2007). Gut content- and stable
isotope-derived diets of four commercially and recreationally important fish
species in two intermittently open estuaries. *Marine and Freshwater Research*
58, 363-375.
- 610 Hamer, P. A. and Jenkins, G.P. (2004). High levels of spatial and temporal
recruitment variability in the temperate sparid *Pagrus auratus*. *Marine and
Freshwater Research* **55**, 663-673.
- 615 Harpending, H. C., Sherry, S.T., Rogers, A.R. and Stoneking, M. (1993). The genetic
structure of ancient human populations. *Current Anthropology* **34**, 483-496.
- Hewitt, G. (2000). The genetic legacy of the Quaternary ice ages. *Nature* **405**, 907-
913.
- 620 Huey, J. A., Hughes, J.M. and Baker, A.M. (2006). Patterns of gene flow in two
species of eel-tailed catfish, *Neosilurus hyrtlui* and *Porochilus argenteus*
(Siluriformes: Plotosidae), in western Queensland's dryland rivers. *Biological
Journal of the Linnean Society* **87**, 457-467.

- 625 Hurst, T., Ryan, P., Brown, M. and Kay, B. (2005). Fishing for a mosquito control
agent. *Arbovirus Research In Australia* **9**, 143-147.
- Hurwood, D. A. and Hughes, J.M. (1998). Phylogeography of the freshwater fish,
Mogurnda adspersa, in streams of northeastern Queensland, Australia: evidence
630 for altered drainage patterns. *Molecular Ecology* **7**, 1507-1517.
- Hurwood, D. A. and Hughes, J.M. (2001). Historical interdrainage dispersal of eastern
rainbowfish from the Atherton Tableland, north-eastern Australia. *Journal of
Fish Biology* **58**, 1125-1136.
- 635 Johnson, M. S. (2000). Measuring and interpreting genetic structure to minimize the
genetic risks of translocation. *Aquaculture Research* **31**, 33-43.
- Klanten, O.S., Choat, J.H. and van Herwerden, L. (2007). Extreme genetic diversity
640 and temporal rather than spatial portioning in a widely distributed coral reef fish.
Marine Biology **150**, 659-670.
- Lacy, R. C. (1987). Loss of genetic diversity from managed populations: interacting
effects of drift, mutation, immigration, selection, and population subdivision.
645 *Conservation Biology* **1**, 143-158.

Maltagliata, F. (2002). Genetic monitoring of brackish-water populations: the Mediterranean toothcarp *Aphanius fasciatus* (Cyprinodontidae) as a model. *Marine Ecology Progress Series* **235**, 257-262.

650

McGlashan, D.J. and Hughes, J.M. (2000). Reconciling patterns of genetic variation with stream structure, earth history and biology in the Australian freshwater fish *Craterocephalus stercusmuscarum* (Atherinidae). *Molecular Ecology* **9**, 1737-1751.

655

Nathan, R., Perry, G., Cronin, J.T., Strand, A.E. and Cain, M.L. (2003). Methods for estimating long-distance dispersal. *Oikos* **103**, 261-273.

Nathan, R. (2005). Long-distance dispersal research: building a network of yellow brick roads. *Diversity and Distributions* **11**, 125-130.

660

Nesbo, C. L., Magnhagen, C. and Jakobsen, K.S. (1998). Genetic differentiation among stationary and anadromous perch (*Perca fluviatilis*) in the Baltic Sea. *Hereditas* **129**, 241-249.

665

O'Reilly, P. T., Canino, M.F., Bailey, K.M. and Bentzen, P. (2004). Inverse relationship between *FST* and microsatellite polymorphism in the marine fish, walleye pollock (*Theragra chalcogramma*): implications for resolving weak population structure. *Molecular Ecology* **13**, 1799-1814.

670

Ovenden, J. R. and Street, R. (2003). Genetic population structure of mangrove jack, *Lutjanus argentimaculatus* (Forsskal). *Marine and Freshwater Research* **54**, 127-137.

675 Page, T. J., Sharma, S. and Hughes, J.M. (2004). Deep phylogenetic structure has conservation implications for ornate rainbowfish (Melanotaeniidae : *Rhadinocentrus ornatus*) in Queensland, eastern Australia. *Marine and Freshwater Research* **55**, 165-172.

680 Panov, V. E., Krylov, P.I. and Riccardi, N. (2004). Role of diapause in dispersal and invasion success by aquatic invertebrates. *Journal of Limnology* **63**, 56-69.

Ponniah, M. and Hughes, J.M. (2006). The evolution of Queensland spiny mountain crayfish of the genus *Euastacus*. II. Investigating simultaneous vicariance with
685 intraspecific genetic data. *Marine and Freshwater Research* **57**, 349-362.

Posada, D. and Crandall, K.A. (2001). Intraspecific gene genealogies: trees grafting into networks. *Trends in Ecology and Evolution* **16**, 37-45.

690 Purcell, J. F. H., Cowen, R.K., Hughes, C.R. and Williams, D.A. (2006). Weak genetic structure indicates strong dispersal limits: a tale of two coral reef fish. *Proceedings of the Royal Society B*: doi: org/10.1098/rspb.2006.3470.

Pusey, B., Kennard, M. and Arthington, A. (2004). 'Freshwater Fishes of North-
695 Eastern Australia'. Collingwood (CSIRO Publishing).

- Riginos, C. and Nachman, M.W. (2001). Population subdivision in marine environments: the contributions of biogeography, geographical distance and discontinuous habitat to genetic differentiation in a blennioid fish, *Axoclinus nigricaudus*. *Molecular Ecology* **10**, 1439-1453.
- Rozas, J. and Rozas, R. (1999). DnaSP version 3: an integrated program for molecular population genetics and molecular evolution analysis. *Bioinformatics Applications Note* **15**, 174-175.
- Sanderson, M. J. and Donoghue, M.J. (1989). Patterns of variation in levels of homoplasy. *Evolution* **43**, 1781-1795.
- Schneider, S., Roessli, D. and Excoffier, L. (2000) Arlequin ver. 2.000: A software for population genetics analysis. Genetics and Biometry Laboratory, University of Geneva, Switzerland.
- Sheaves, M., Johnston, R. and Abrantes, K. (2007). Fish fauna of dry tropical and subtropical estuarine floodplain wetlands. *Marine and Freshwater Research* **58**, 931-943.
- Slatkin, M. (1987). Gene flow and the geographic structure of natural populations. *Science* **236**, 787-792.
- Slatkin, M. and Maddison, W.P. (1990). Detecting isolation by distance using phylogenies of genes. *Genetics* **126**, 249-260.

- Slatkin, M. (1993). Isolation by distance in equilibrium and non-equilibrium populations. *Evolution* **47**, 264-279.
- 725 Slatkin, M. (1995). A measure of population subdivision based on microsatellite allele frequencies. *Genetics* **139**, 457-462.
- Spear, S. F., Peterson, C.R., Matocq, M.D. and Storfer, A. (2006). Molecular evidence for historical and recent population size reductions of tiger salamanders (730 *Ambystoma tigrinum*) in Yellowstone National Park. *Conservation Genetics*. doi: 10.1007/s10592-005-9095-4
- Uthicke, S. and Benzie, J.A.H. (2003). Gene flow and population history in high dispersal marine invertebrates: mitochondrial DNA analysis of *Holothuria* (735 *nobilis* (Echinodermata: Holothuroidea) populations from the Indo-Pacific. *Molecular Ecology* **12**, 2635-2648.
- Vrijenhoek, R. C. (1998). Conservation genetics of freshwater fish. *Journal of Fish Biology* **53**, 394-412.
- 740 Ward, R. D. and Elliot, N.G. (2001). Genetic population structure of species in the South East Fishery of Australia. *Marine and Freshwater Research* **52**, 563-573.
- Waters, J. M., Dijkstra, L.H. and Wallis, G.P. (2000). Biogeography of a southern hemisphere freshwater fish: how important is marine dispersal? (745 *Molecular Ecology* **9**, 1815-1821.

Waters, J.M., Allibone, R.M. and Wallis, G.P. (2006). Geological subsidence, river capture, and cladogenesis of galaxiid fish lineages in central New Zealand. *Biological Journal of the Linnean Society* **88**, 367-376.

750

Watts, R. J. and Johnson, M.S. (2004). Estuaries, lagoons and enclosed embayments: habitats that enhance population subdivision of inshore fishes. *Marine and Freshwater Research* **55**, 641-651.

755 Weir, B. S. and Cockerham, C.C. (1984). Estimating F-statistics for the analysis of population structure. *Evolution* **38**, 1358-1370.

Williamson, C. E. (1993). Linking predation risk models with behavioral mechanisms: identifying population bottlenecks. *Ecology* **74**, 320-331.

760

Wong, B. B. M., Keogh, J.S. and McGlashan, D.J. (2004). Current and historical patterns of drainage connectivity in eastern Australia inferred from population genetic structuring in a widespread freshwater fish *Pseudomugil signifer* (Pseudomugilidae). *Molecular Ecology* **13**, 391-401.

765

Wright, S. (1943). Isolation by distance. *Genetics* **28**, 114-138.

Zardoya, R., Castilho, R., Grande, C., Favre-Krey, L., Caetano, S., Marcato, S., Krey, G. and Patarnellos, T. (2004). Differential population structuring of two closely related fish species, the mackerel (*Scomber scombrus*) and the chub mackerel

770

(*Scomber japonicus*), in the Mediterranean Sea. *Molecular Ecology* **13**, 1785-1798.

775

780

785

Figure 1. Map of east coast of Australia, showing the 23 sites sampled for *A. marianus* and *A. jacksoniensis* across Queensland and New South Wales.

Figure 2. Geographic distribution of *A. marianus* relative haplotype frequencies along the east coast of Australia, with shading indicating corresponding network haplotype. Haplotype network for *A. marianus*, with circle size corresponding to sample size. Small black dots indicate haplotypes that were not sampled, or have become extinct through genetic drift. White circles indicate singletons.

Figure 3. Geographic distribution of *A. jacksoniensis* relative haplotype frequencies along the east coast of Australia, with shading indicating corresponding network haplotype. Haplotype network for *A. jacksoniensis*, with circle size corresponding to sample size. Small black dots indicate haplotypes that were not sampled, or have become extinct through genetic drift. White circles indicate singletons.

Table 1. Diversity values for *A. marianus* and *A. jacksoniensis* in eastern Australia showing overall F_{ST} (based on haplotypes frequencies), overall Φ_{ST} (based on pairwise differences in genetic divergence) and Mantel test correlation coefficients.

Species	Overall F_{ST}		Overall Φ_{ST}		Mantel Test	
	F -statistic	P -value	Φ -statistic	P -value	Correlation Coefficient	P -value
<i>A. marianus</i>	0.019	0.205	0.013	0.300	0.081	0.238
<i>A. jacksoniensis</i>	0.017	0.124	0.020	0.106	0.107	0.106

Table 2. Neutrality and raggedness indices for *A. marianus* and *A. jacksoniensis* in eastern Australia.

Species	Tests of Neutrality				Mismatch Distributions	
	$Tajima's D$	P -value	$Fu's F_S$	P -value	Raggedness Index	P -value
<i>A. marianus</i>	-1.429	0.046	-12.779	0.000	0.020	0.083
<i>A. jacksoniensis</i>	-2.364	0.000	-72.737	0.000	0.069	0.933

Table 3. Haplotype distributions and sample sizes for *A. marianus*. Grey shading indicates sampled sites where *A. marianus* was not captured.

Haplotype																					Sample Size			
M1	2																				1	44		
M2			10																			3		
M3			1																			1		
M4				1																		4		
M5																						1		
M6																						1		
M7	1																					1		
M8			1																			14		
M9	1	2																				9		
M10																						1		
M11																						1		
M12																						1		
M13			1																			1		
M14																						1		
M15																						1		
M16																						2		
M17	1																					7		
M18																						1		
M19																						1		
M20																						2		
M21																						1		
Sample Size	5	16	1	0	0	6	5	0	4	16	11	0	11	0	4	9	0	0	0	8	0	0	2	98

Table 4. Haplotype distributions and sample sizes for *A. jacksoniensis*. Grey shading indicates sampled sites where *A. jacksoniensis* was not captured.

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