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REPORT

Genetic connectivity and self-replenishment of inshore and offshore populations of the endemic anemonefish, *Amphiprion latezonatus*

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Abstract Globally, marine species are under increasing pressure from human activities, including ocean warming, acidification, pollution, and overfishing. Species most vulnerable to these pressures tend to be ecological specialists that have low abundance and small distribution ranges (endemics). Marine endemics often exist as metapopulations distributed among few isolated locations. Determining genetic connectivity among these locations is essential to understanding the recovery potential of endemics after local extinction events. This study examined connectivity in the endemic anemonefish, *Amphiprion latezonatus*, a habitat specialist with low abundance at most locations. Evolutionary and contemporary migration, genetic diversity, and self-replenishment among the four main locations (Sunshine Coast, North Solitary Island, Lord Howe Island, and Norfolk Island) that comprise the entire *A. latezonatus* geographic range were assessed using mtDNA and microsatellite markers. Though historical gene flow inferred from mtDNA appeared high, population genetic differentiation was evident and contemporary gene flow inferred from microsatellites was limited, alongside very high (≥89 %) self-replenishment at all locations. Together, these data suggest prolonged recovery times following severe population decline (or extirpation) and indicate a need to protect this species at all locations, particularly Norfolk Island and Sunshine Coast where marine protected areas are lacking.

Keywords *Amphiprion latezonatus* · Connectivity · Self-replenishment · Lord Howe Island

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Introduction

In the terrestrial environment, extinction rates are highest for endemic species and populations on isolated islands (Whittaker 1998). This may partly be because endemic species and isolated populations have lower genetic diversity and higher inbreeding rates than mainland populations (Frankham 1997, 1998). Endemics often possess characteristics that exacerbate extinction risk, such as occupying isolated locations, low gene flow, ecological specialization, and small population size (McKinney 1997; Frankham 1998). In marine ecosystems, recent studies indicate that endemic species and specialists are vulnerable to local and global extinction (Dulvy et al. 2003; Pratchett et al. 2008), more so if they have low abundance (Munday

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2004). However, endemic marine species and isolated populations of widespread species can have high nucleotide and haplotype diversity (Bay and Caley 2011; Hobbs et al. 2013a; Delrieu-Trottin et al. 2014), which may reduce extinction risk to some extent.

Most endemic marine fishes are not restricted to one island; instead, they exist as meta-populations distributed over several isolated islands (Kritzer and Sale 2004). Population structure may occur because of barriers to larval dispersal, with the percentage of larvae dispersing to other reefs determining the level of genetic homogeneity and the number of genetically distinct populations among scattered locations (Waples and Gaggiotti 2006; Craig et al. 2010; Selkoe et al. 2014). It was traditionally assumed that meta-populations of marine fish are "open" systems in which larvae typically disperse widely (Mora and Sale 2002). However, many reef species show high levels of *self-recruitment*—the portion of all locally settling recruits that hatched at that site (e.g., Jones et al. 1999, 2009; but see Horne et al. 2013; Nanninga et al. 2015) or *self-replenishment*—the portion of individuals having genetic loci specific to their natal site, which can be used as a proxy for self-recruitment (e.g., van der Meer et al. 2012a, 2013, 2015). Though self-recruitment is a proxy for self-replenishment, the two are measured differently. High self-recruitment and/or self-replenishment can create independent populations that rely extensively on self-persistence (Burgess et al. 2014) and reduce the ability of populations to recover after extensive declines or extirpation.

Climate change poses a major threat to marine biodiversity, due to habitat loss and ecosystem change. Coral reefs represent the most diverse marine ecosystem, and rising sea temperatures have caused bleaching events resulting in mass mortality of corals and anemones (Hoegh-Guldberg 1999; Wilkinson 2004; Hobbs et al. 2013b). Climate change-induced habitat loss has led to local extinctions of ecological specialists. For example, fishes reliant on corals or anemones for food or habitat (e.g., corallivorous butterflyfishes, coral gobies, anemonefishes) have experienced extirpation following bleaching events (Hattori 2002; Munday 2004; Pratchett et al. 2008). Determining population connectivity patterns and replenishment levels is critical to understanding the resilience of ecological specialists.

Marine protected area (MPA) networks have been proposed as a better alternative for conserving marine biodiversity than single, unconnected MPAs, or large, difficult-to-manage MPAs. To mitigate threats to meta-populations of vulnerable species, MPA networks need to be positioned to maximize connectivity between locations within the network and to replenish other, non-protected areas (Jones et al. 2007; Almany et al. 2009). MPAs need to be large enough for populations to self-persist and close enough to allow larval migration linkages (Shanks et al. 2003; Jones et al. 2005, 2007). This allows for increased population resilience within networks, as reduced or extirpated populations can be replenished by dispersal from other MPAs to facilitate recovery. Unfortunately, information on connectivity between locations is often lacking, and a "best guess" method is used to determine size, location, and spacing of MPAs (Almany et al. 2009; McCook et al. 2009). Understanding connectivity patterns allows MPAs to be positioned to maximize benefits to species by increasing inter-population resilience and connectivity (Jones et al. 2007).

Conserving biodiversity at hotspots of endemicity is considered a management priority, because these locations contain disproportionate amounts of unique biodiversity (Roberts et al. 2002). The Lord Howe Island–Norfolk Island (LHI–NI) area ranks fifth in the Indo-Pacific for endemism, with 7.2 % endemic marine fishes (Randall 1998). Although this region is comprised of remote oceanic islands and reefs, it is still vulnerable to anthropogenic effects including sedimentation, ocean acidification, and increasing temperatures (Edgar et al. 2010). These anthropogenic effects could induce changes in climate and oceanography, such as the southward movement of the Tasman Front (Bostock et al. 2006), creating more tropical conditions in the LHI–NI region, resulting in widespread coral and anemone bleaching (Harrison et al. 2011). As a result, ecological specialists that are endemic to this region, such as anemonefishes and butterflyfishes, have an elevated risk of extinction (Hobbs et al. 2011; Purcell et al. 2014).

To understand how climate change will impact marine species in this region, management agencies require an understanding of how resilient species are and how they recover from extirpations. Information on connectivity is important for understanding population recovery, while genetic diversity (the raw material on which natural selection acts) provides insights into a species' adaptability (Carvalho 1993). Collectively, information on connectivity and genetic diversity aids management agencies to develop better strategies for conserving a region's endemic biodiversity, such as designing MPAs to maximize population replenishment efficiency (Jones et al. 2007).

Although several self-recruitment, self-replenishment, and genetic connectivity studies have been performed in the LHI–NI area (see Crean et al. 2010; van der Meer et al. 2012a, 2013, 2015), none has examined gene flow between this oceanic region and coastal mainland Australia. Some restricted-range species from LHI–NI area also occur on the mainland coast, and understanding gene

flow patterns between the mainland coast and offshore locations is crucial to their management. This is particularly important because high-latitude reefs are at increased risk of climate change-induced modifications, such as warmer water temperatures that can cause altered dispersal patterns, range shifts, and habitat loss (Beger et al. 2014) and that have already impacted marine communities (Booth et al. 2007; Figueira and Booth 2010).

This study examined the genetic characteristics of the wideband anemonefish (*Amphiprion latezonatus*) throughout its range. This species is a habitat specialist that is endemic to the LHI–NI region and adjacent mainland coast. We had three principal aims: (1) to assess genetic diversity and elucidate recent population expansions and recovery potential of *A. latezonatus* after localized population declines or extirpation; (2) to determine evolutionary population structure and gene flow among locations using mtDNA to understand historic inter-population connectivity; and (3) to measure recent population structure, gene flow, and self-replenishment among and within locations using microsatellite DNA to understand contemporary connectivity and demographic isolation.

Materials and methods

Study species

Phylogenetic analyses place the wideband anemonefish, *A. latezonatus*, basal to most anemonefishes (Santini and Polacco 2006; Frédérich et al. 2013), with its closest relatives including *Premnas biaculeatus* and *A. clarkii*. *Amphiprion latezonatus* is a habitat specialist inhabiting only two anemone species (*Entacmaea quadricolor* and *Heteractis crispa*; Scott et al. 2011). Like all anemonefishes, *A. latezonatus* lays demersal eggs that hatch into pelagic larvae. *Amphiprion latezonatus* inhabits rocky and coral reefs of Lord Howe Island (LHI), Norfolk Island (NI), and scattered locations off the subtropical east Australian coast between the Sunshine Coast (SC) and South West Rocks (Fautin and Allen 1997). This study examines genetic connectivity across the four main *A. latezonatus* populations (LHI, NI, SC, and North Solitary Island, NSI), which span the entire geographic range of this species (Fig. 1). Fin clips were taken from a total of 112 *A. latezonatus* individuals that were captured using clove oil and/or hand nets at LHI ($n = 39$), NI ($n = 32$), SC ($n = 7$), and NSI ($n = 34$). Only LHI and NSI are within MPAs. *Amphiprion latezonatus* is rare at SC and has low abundance at LHI and moderate abundance at NI and NSI (Richardson 1999; Hobbs et al. 2009; Neilson et al. 2010; Scott et al. 2011; Electronic Supplementary Material, ESM).

Sequencing and genotyping

D-loop sequences from 105 *A. latezonatus* individuals were obtained using standard procedures (Bay et al. 2006; van Herwerden et al. 2009), with primers and protocols as previously reported in van der Meer et al. (2012b). Sequences were edited and aligned using ClustalW in MEGA 5.0 (Tamura et al. 2011). Missing data were minimized by trimming and manual editing of sequences. Sequences were checked for ambiguities and alignment gaps. Appropriate ambiguity codes were inserted where required.

We genotyped 22 *A. latezonatus* specific microsatellite loci as per Steinberg et al. (2015). During data compilation, 13 loci failed to amplify, or amplified poorly, in samples from the NI population (Steinberg et al. 2015), with nine or six loci having $\leq$20 or $\leq$10 % missing data at NI, respectively. DNA quality and quantity of the NI population did not differ from those of other populations and amplified with mtDNA primers, indicating that DNA quality from the NI population is not an issue, but rather that null alleles are likely to be common due to flanking region sequence differences in NI compared to other populations. All loci with $\geq$20 % missing data for NI were excluded from analyses altogether. All SC individuals were excluded due to rarity, as only seven individuals were sampled there. Limited samples combined with few loci indicated that statistically robust analyses would be impossible for this location.

Population genetic analysis: mtDNA

Haplotype number, genetic diversity [haplotype diversity (h) and nucleotide diversity ($\%\pi$)], and population pairwise F_{ST} analyses were computed in Arlequin 3.5 (Excoffier and Lischer 2010) using aligned mtDNA data. Analysis of molecular variance (AMOVA) was performed by comparing inshore (NSI and SC) to offshore (LHI and NI) populations, based on 1000 permutations using Arlequin 3.5. Isolation by distance (IBD) was evaluated using pairwise genetic and geographic distances to test for a correlation between these two distances using a Mantel test in the IBD Web Service version 3.23 (Jensen et al. 2005) with 10,000 permutations. Genetic distance and IBD were calculated using Φ_{ST} as it incorporates sequence distance information. Negative genetic distances were set to 0 (or 0.0001 for log-transformation). Geographic distances were estimated to the nearest 5 km over water distances (ESM Table S1).

Two mtDNA neutrality tests were conducted to discriminate between population stasis and spatial or temporal population expansions using Tajima's D and Fu's F_s with 1000 simulations (Tajima 1983; Fu 1997). Negative and

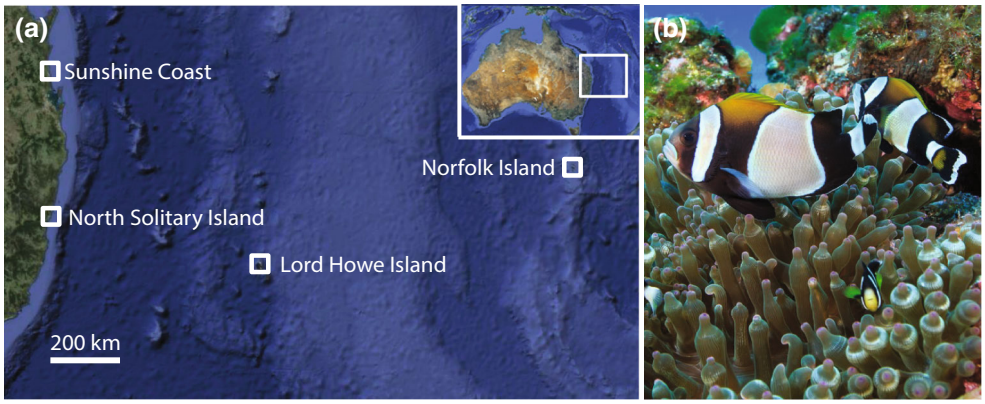

Fig. 1 Sampling locations and study species. **a** Google Earth image of Eastern Australia and Tasman Sea showing *Amphiprion latezonatus* sampling locations: *SC* Sunshine Coast, *NSI* North Solitary Islands, *LHI* Lord Howe Island, and *NI* Norfolk Island. **b** *A. latezonatus* in *Entacmaea quadricolor*, photograph by Tane Sinclair-Taylor

significant values are expected for these neutrality test parameters if a population has undergone demographic expansion. Neutrality tests are excellent statistical tests to detect population growth following a bottleneck (Ramírez-Soriano et al. 2008) and were performed using DnaSP (Librado and Rozas 2009).

Population genetic analysis: microsatellites

Microsatellite genetic diversity was estimated, and pairwise F_{ST} values were calculated in Arlequin 3.5 (Excoffier and Lischer 2010). Estimating null alleles (ENA)-corrected F_{ST} values were calculated in FreeNA (Chapuis and Estoup 2007). Spatial population partitioning between the three locations was evaluated using three tools. (1) Discriminant analysis of principal components (DAPC; Jombart et al. 2010) used allelic states to discriminate between locations, producing scatterplots of discriminant functions based on a priori spatial distributions of microsatellite genotypes. DAPC also provided posterior probabilities of population assignment for each individual. (2) A likelihood-based assignment method, implemented in GeneClass2 (Piry et al. 2004), determined significant inter-location gene flow. (3) Individuals were placed into clusters that minimize Hardy–Weinberg equilibrium (HWE) using STRUCTURE version 2.3 (Pritchard et al. 2000; Hubisz et al. 2009) which was also used to identify contemporary inter-population gene flow. An admixture model with 1,000,000 iterations and a 100,000 iteration burn-in was used, as it was found to be optimal after evaluating various models and iteration numbers. Determining the "best value" for the number of different populations (K) required two different methods. First, Pritchard et al. (2000) was followed, comparing mean log-likelihoods penalized by one half of their variance (Hubisz et al. 2009). Second, Evanno et al. (2005) was followed using STRUCTURE HARVESTER (Earl and von Holdt 2012) to determine the number of populations by calculating ΔK, the mean $\frac{|L''(K)|}{sd(L(K))}$ over ten replicate STRUCTURE runs, where $L(K)$ is the log likelihood of $K = n$, where n is any possible value for K.

Historical gene flow

Historical migration rates between NI, LHI, and NSI and effective population sizes were estimated using MIGRATE-n 3.6.4 (http://popgen.sc.fsu.edu/Migrate-n.html; Beerli and Palczewski 2010). SC was not included because of small sample size. MIGRATE-n uses a Bayesian approach to estimate migration rates and effective population size at sampled locations. We tested a combination of custom migration models (Stepping-stone, Island-n, full migration, admixture, and combining NSI and LHI as one population) and a geographic matrix, a matrix of distances between locations, all with constant mutation rate. A log marginal likelihood (Ln mL) analysis selected a full migration model with variable Θ, no geographic matrix, constant mutation rate with an F84 mutation model, and migration rate parameters [Θ and M (mutation-scaled migration rate) to a max of 0.25 and 7000, respectively]. The Bayesian analysis used a heating search strategy of one long-chain sampling every 200th of 200,000 sampled trees and applied a 100,000 iteration (50 %) burn-in. Most parameters (except effective population size for NSI and NI) converged and fell within the 90 % CI, yielding values for Θ and M per location that were consistent across three replicate analyses.

Contemporary gene flow

Self-replenishment and recent migration were estimated using a Bayesian program (BayesAss version 3; Wilson and Rannala 2003) to measure contemporary gene flow by estimating inter-population migration rates within the last two to three generations. To calculate self-replenishment BayesAss uses a Markov chain Monte Carlo (MCMC) approach with 11,000,000 steps, a 2,000,000-step burn-in, and a 100,000-step sampling interval. Migration rate priors were set to 0.3, allele frequencies to 0.4, and inbreeding coefficients to 0.5, because they generated acceptance rates within the 20–40 % range, showing MCMC convergence (Faubet et al. 2007). MCMC convergence is the point where it is reasonable to believe that samples are truly representative of the underlying stationary distribution of the Markov chain. Ten independent runs with different random seeds assessed MCMC convergence to evaluate consistency of results. BayesAss allows deviation from HWE but assumes linkage equilibrium, small migration rates, and that subpopulation allele frequencies are unaffected by recent genetic drift or migration (Wilson and Rannala 2003). If these assumptions are valid, BayesAss can produce accurate estimates of recent migration and self-replenishment, on condition that genetic differentiation is not too low ($F_{ST} \geq 0.05$). If the assumptions are violated, estimates will be accurate only if genetic differentiation is higher ($F_{ST} \geq 0.10$) and migration rates are very low ($m < 0.01$) (Faubet et al. 2007).

Results

Summary statistics: mtDNA

A 324 base pair (bp) fragment of mitochondrial DNA D-loop, of which 316 bp were usable with ≤5 % missing data, was analyzed in 105 *A. latezonatus* individuals. There were 23 polymorphic sites including 21 transitions, 2 transversions, and no indels (insertions/deletions), equating to 7.3 % variation. Thirty-two haplotypes were identified, of which 24 were shared and eight were unique to a single individual. Haplotype diversity (h) was high (>0.7) across all populations, while nucleotide diversity (%π) was highest at LHI (0.61) and lowest at NI (0.44) (Table 1).

Population genetic structure

Pairwise mtDNA F_{ST} comparisons revealed significant differentiation between the two offshore sites (LHI–NI pairwise $F_{ST} = 0.054$, $P \leq 0.001$, even after Bonferroni correction of $P \leq 0.008$) and between each offshore and each coastal site (NSI–NI pairwise $F_{ST} = 0.073$, $P \leq 0.001$; NSI–LHI pairwise $F_{ST} = 0.057$, $P \leq 0.001$; SC–NI, $F_{ST} = 0.177$, $P \leq 0.001$; SC–LHI pairwise $F_{ST} = 0.123$, $P = 0.036$). However, SC and LHI were not significantly genetically differentiated after Bonferroni correction. Further, the two coastal sites were not differentiated (NSI–SC pairwise $F_{ST} = 0.007$, $P = 0.44$). AMOVA analyses of samples structured by region (coastal vs. offshore) indicated mtDNA partitioning among regions was not significant (only 4 % of variation; $\Phi_{CT} = 0.04$, $P = 0.32$). Additionally, 91.88 % of the variation existed within locations ($\Phi_{ST} = 0.081$; $P < 0.001$) and the remaining genetic variation (4.1 %) was significant among locations within regions ($\Phi_{SC} = 0.042$; $P = 0.01$). There was significant IBD using a Mantel test of log pairwise genetic (Φ_{ST}) and log geographic (km) distance between sample locations ($z = -21.402$, $r = 0.668$, $R^2 = 0.446$, $P = 0.041$), indicating reduced gene flow between increasingly distant locations. Fu's F_s and Tajima's D tests both rejected a fixed population size hypothesis based on assuming selective neutrality in *A. latezonatus* D-loop sequences ($Fs = -36.2999$, $P < 0.001$; $D = -2.0253$, $P = 0.001$), suggesting *A. latezonatus* underwent a recent population expansion.

Nine out of 22 microsatellite markers developed for *A. latezonatus* successfully amplified for ≥80 % of NI individuals: Al1, Al3, Al5, Al7, Al9, Al10, Al15, Al16, and Al23 (see Steinberg et al. 2015). Raw microsatellite pairwise F_{ST} values indicated significant differentiation between all analyzed locations, ranging from 0.0411 to 0.1245 ($P < 0.0001$), even after Bonferroni correction ($P \leq 0.01$). ENA-corrected pairwise F_{ST} values ranged from 0.0113 to 0.0476 ($P < 0.05$) (Table 2) and P values needed no Bonferroni correction, as they were determined from confidence intervals. Three distinct populations were also characterized by discriminant function (DAPC) and likelihood (GeneClass2) analyses (Fig. 2a). Using the three locations as a priori population criteria, DAPC assigned 94–97 % of all individuals to the location from which they were sampled (Fig. 2b). The 95 % genotypic inertia ellipses did not overlap for any population, consistent with raw microsatellite and ENA-corrected pairwise F_{ST} values. Geographic structure was also obtained by likelihood (GeneClass2) analyses, as only six individuals grouped with a location from which they were not sampled (NSI = 3, LHI = 2, NI = 1). However, only two populations were recovered when HWE was minimized (STRUCTURE, $K = 2$), with NSI and NI grouped together, while LHI was distinct, suggesting sufficient contemporary gene flow between NSI and NI to maintain genetic cohesion.

Table 1 Genetic diversity estimates for *Amphiprion latezonatus* for both mitochondrial DNA (mtDNA) and microsatellites (msatDNA)

Site	n	n_h	h (mtDNA)	$\%\pi$ (mtDNA)	gd (msatDNA)
North Solitary Island	34	14	0.750 ± 0.080	0.463 ± 0.320	0.708 ± 0.377
Sunshine Coast	7	5	0.95 ± 0.103	0.512 ± 0.392	N/A
Norfolk Island	31	13	0.703 ± 0.092	0.444 ± 0.311	0.661 ± 0.372
Lord Howe Island	33	12	0.742 ± 0.073	0.612 ± 0.395	0.573 ± 0.311

Sample size (n), number of haplotypes (n_h), haplotype diversity (h), nucleotide diversity ($\%\pi$), and gene diversity (gd) are reported as mean ± SD. Gene diversity was not calculated for Sunshine Coast due to limited sample size

Table 2 Raw and ENA-corrected pairwise F_{ST} values for *Amphiprion latezonatus*

	North Solitary Island	Lord Howe Island	Norfolk Island
Raw msatDNA			
North Solitary Island	–	$P < 0.0001$	$P < 0.0001$
Lord Howe Island	0.0898	–	$P < 0.0001$
Norfolk Island	0.0411	0.1245	–
ENA-corrected			
North Solitary Island	–	0.035–0.122 ($P < 0.05$)	0.018–0.077 ($P < 0.05$)
Lord Howe Island	0.074730	–	0.062–0.180 ($P < 0.05$)
Norfolk Island	0.047591	0.112997	–

Both raw and ENA-corrected F_{ST} values were significant, indicating genetic differentiation among all locations. Values below diagonal are F_{ST}s and above diagonal are p values for raw msatDNA data, and confidence intervals for ENA-corrected data with p values in brackets

Historical gene flow

Quantifying historical gene flow inferred from mtDNA using MIGRATE-n indicated high levels of historical migration among all locations with M ranging from 765 to 4025 (Fig. 3a). Estimates of Θ, the effective population size, for NSI and NI did not converge, but all other parameters converged and were consistent among replicate analyses (Fig. 3a).

Contemporary gene flow

Microsatellite gene diversities were high at all locations, *gd* ranging from 0.573 to 0.708. This, together with pairwise F_{ST}, GeneClass2, and DAPC results, suggests that sufficient structure exists between locations to perform a BayesAss analysis (Faubet et al. 2007). Demographic independence was suggested for all location pairs except possibly NI to NSI ($m = 9$ %, Fig. 3b). Populations were considered demographically independent if migration was less than 10 % of total gene flow (following Waples and Gaggiotti 2006). BayesAss analyses inferred high self-replenishment for all locations (89–96 %) on a contemporary time scale, which suggests self-replenishment plays a greater role than migration in maintaining current *A. latezonatus* populations (Fig. 3b).

Discussion

Hotspots of endemicity are of particular conservation concern and information on genetic diversity and gene flow (or lack thereof) can elucidate the recovery capacity of populations and thus guide management actions. This study examined population structure and connectivity among *A. latezonatus* populations at a hotspot of marine endemicity (Allen 2008; Roberts et al. 2002) and found that *A. latezonatus* at NI and NSI had undergone recent population expansions. Furthermore, high genotypic diversity at both these islands (and at LHI) suggested an underlying capacity for genetic resilience of *A. latezonatus* to environmental change, notwithstanding possible limited diversity at adaptive loci under selection in contrast to the selectively neutral markers examined here (see Lamichhaney et al. 2012). *Amphiprion latezonatus* also displayed high levels of historical migration (connectivity) among the three locations, but conclusive inferences about historical population partitioning were not possible because historical population sizes could not be estimated. Contemporary migration was found to be very limited with current populations relying substantially on self-persistence, evident from very high inferred levels of self-replenishment in all populations.

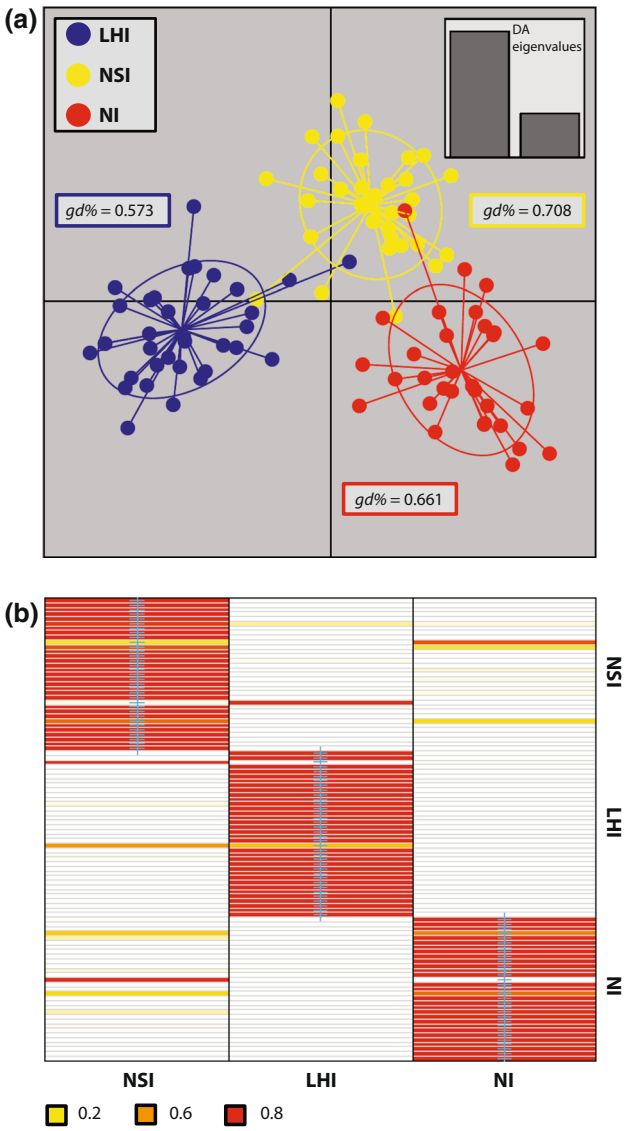

Fig. 2 *Amphiprion latezonatus* population structure based on a discriminant analysis of principal components (DAPC) analysis of microsatellite data and posterior probabilities of individual assignments to each of the sampled locations. **a** Scatterplots of the DAPC of the microsatellite data for three *A. latezonatus* locations (Lord Howe Island, LHI, *blue*; North Solitary Island, NSI, *yellow*; Norfolk Island, NI, *red*) using geographic sample site as priors for clusters. Individual genotypes appear as dots surrounded by 95 % genotypic inertia ellipses. Eigenvalues show the amount of genetic information contained in each successive principal component with *x* and *y* axes constituting the first two principal components, respectively. Boxes contain genetic diversity indices (*gd*) for each *A. latezonatus* population. **b** Posterior probability assignment of each individual genotype to each of three *A. latezonatus* populations as indicated by DAPC. Assignment populations are indicated on the *x* axis. One hundred genotypes are depicted on the *y* axis, along with the population from which they were sampled (right *y* axis). *Blue crosses* indicate sampling location of each individual. *Colored bars* below graph correspond to probabilities of assignment to a given population, ranging from 0.2 to 0.8

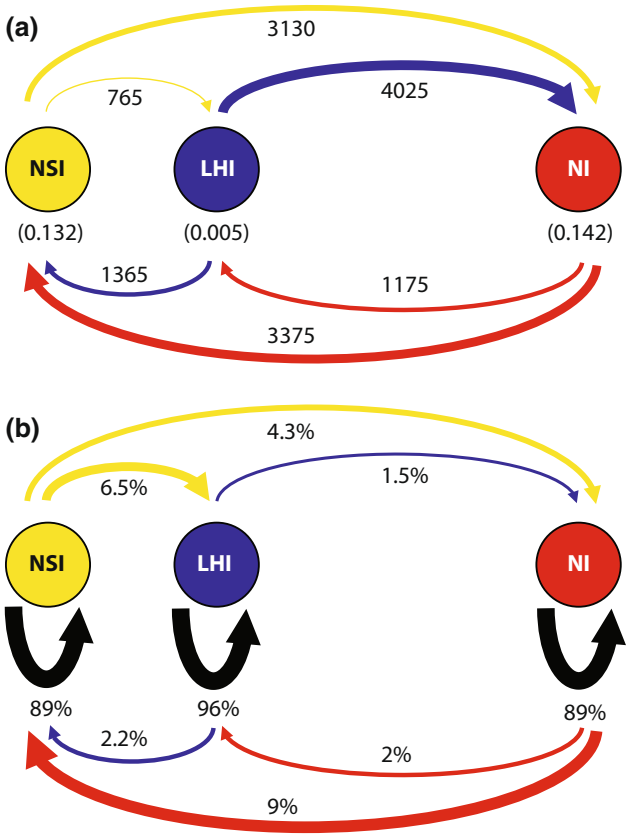

Fig. 3 Migration rates among *Amphiprion latezonatus* populations according to mtDNA and microsatellite analyses. Arrowed line thickness, direction, and color are proportional to the mutation-scaled migration rate (M) and indicate the predominant direction of gene flow. Effective population size (Θ, in parentheses) is shown for each location, where available. **a** MIGRATE-n evolutionary gene flow (mtDNA) and **b** BayesAss analysis of self-replenishment and migration rates during the last two to three generations (microsatellite), shown as a percentage

Population genetic diversity

In terrestrial ecosystems, endemics generally have low abundance, leading to low genetic diversity as a result of high inbreeding rates and genetic drift (Frankham 1996). This is not necessarily true in marine populations (e.g., van der Meer et al. 2012a, b, 2013; Hobbs et al. 2013a; Delrieu-Trottin et al. 2014), because endemics are often highly abundant (Hobbs et al. 2011). For marine fishes, *h* and $\%\pi$ values less than 0.5 indicate low genetic diversity (Grant and Bowen 1998). High haplotype and genotypic diversities were recorded for *A. latezonatus* across all locations, but nucleotide diversities differed between locations. High haplotype diversity may be attributed to a high historical abundance and the high mutation rate of mtDNA control region. Low nucleotide diversity, coupled with high

haplotype diversity, suggests a population expansion after a period of low effective population size (Grant and Bowen 1998) at NSI and NI, as supported by Tajima's D and Fu's F_s tests. Low nucleotide diversity at these two locations suggests *A. latezonatus* may have reduced resilience to change because low diversity is associated with decreased fitness and reduced adaptability (Grant and Bowen 1998; Hoelzel et al. 2002). However, these two locations have the highest population densities and this may provide some resilience.

Historical gene flow between locations

Pairwise F_{ST} values suggest differentiation among all locations except NSI and SC. The lack of population structure between NSI and SC may result from a few migrants per generation that maintain spatial genetic homogeneity (van der Meer et al. 2013) or a recent population expansion (Drew and Barber 2012), supported by Tajima's D and Fu's F_s tests. Additionally, NSI and SC are closest (geographically) of any pairwise locations and occur along the same coastline where connectivity may be enhanced by very small populations between the two locations (e.g., Moreton Bay and Cook Islands: Richardson 1996) and the south-flowing East Australian Current (Booth et al. 2007). A lack of population structure over such distances is not unusual and has been reported in several other endemic (e.g., *Chaetodon multicinctus, C. miliaris, C. fremblii, C. tricinctus, Pomacentrus maafu, Amphiprion bicinctus*; Craig et al. 2010; Drew and Barber 2012; van der Meer et al. 2013; Nanninga et al. 2014) and widespread (e.g., *Scarus frenatus*, *Chlorurus sordidus, Chrysiptera talboti, Halichoeres hortulanus*; Dudgeon et al. 2000; Drew and Barber 2012) reef fishes. High historical gene flow among endemic species may assist distant populations by increasing genetic diversity and recolonization following local extinction events (Jones et al. 2009). However, if there is limited contemporary gene flow, these benefits may be less pertinent.

High historical migration among the three locations tested was evident from mtDNA. Very high gene flow was seen from LHI to NI, NI to NSI, and NSI back to NI (Fig. 3), indicating that NI was historically highly connected to the other populations, despite the large geographic distances between them. Importantly, the population size estimates for NSI and NI did not converge and could therefore be misleading. However, LHI population size estimates did converge, suggesting that LHI historically had a very low density of *A. latezonatus*. Even though values did not converge for NSI and NI, the inferred population sizes for these two locations are 25–30 times greater than those at LHI. It is likely that historical populations at NI and NSI were considerably greater than LHI, suggesting that NSI and NI were the main reservoirs of *A. latezonatus*.

Contemporary gene flow between locations

Knowledge of contemporary patterns of gene flow among populations throughout a species geographic range is important for informing conservation management and optimizing MPA design. For *A. latezonatus*, NSI and LHI deliver limited recruitment to NI (<5 % of larvae produced), possibly due to complex currents around NI (Hamon 1965; Burrage et al. 1997; Ridgway and Dunn 2003). Moreover, high levels of self-replenishment (>89 %) predominantly maintain populations at NSI, LHI, and NI. Moderate population densities at NSI and NI (Scott et al. 2011; ESM) may reduce the risk of local extinction, but *A. latezonatus* has low abundance at LHI and SC (Richardson 1996; Hobbs et al. 2009; Neilson et al. 2010; ESM). NSI receives more than four times the contemporary gene flow from NI (9 %) compared to LHI (<2 %), which could contribute to population recovery at NSI, but not at LHI.

Limited contemporary gene flow highlights the importance of high self-recruitment for population persistence (Hastings and Botsford 2006). All locations examined here are demographically independent and therefore, largely closed on a contemporary time scale, not only for *A. latezonatus,* but also for other endemic reef fish species in this region (*A. mccullochi, Chaetodon tricinctus, Coris bulbifrons*; van der Meer et al. 2012a, 2013, 2015). This indicates that continued protection of NSI and LHI is required, while implementation of management plans for SC and NI may reduce extinction risk at these locations.

The high self-replenishment reported here for *A. latezonatus* was similar to, or slightly higher than, estimates of self-recruitment (calculated by measuring cohorts of larval recruits) reported for other anemonefishes (Almany et al. 2007; Saenz-Agudelo et al. 2011; Berumen et al. 2012) and coral reef fishes (Swearer et al. 1999; Jones et al. 1999, 2005; Planes et al. 2009). There are several reasons why self-recruitment estimates may differ somewhat from self-replenishment estimates. First, most studies estimate self-recruitment in non-endemic species after settlement, when mortality is relatively high. However, endemics recruiting to natal reefs may be better adapted (Crean et al. 2010) and more likely to survive this phase. Second, if a greater proportion of endemic recruits survive to maturity and are captured during sampling, the self-replenishment signal would be enhanced (Crean et al. 2010). Third, higher estimates of self-replenishment for *A. latezonatus* may reflect its small and isolated geographic distribution compared to widespread study species from less isolated locations. Higher and more consistent self-recruitment appears to be a feature of endemics on isolated islands

because self-recruitment is paramount to sustaining populations and ensuring the persistence of endemic species (Hobbs et al. 2011).

Patterns of contemporary genetic differentiation (inferred from microsatellite data) in *A. latezonatus* were consistent with those revealed by mtDNA. However, inferred migrant numbers based on mtDNA suggest there once was greater historical migration between locations. Such discrepancies in migrant number between evolutionary and contemporary timescales may reflect the use of different markers and analytical tools and has been increasingly documented for other coral reef fishes (e.g., Evans et al. 2010; Harrison et al. 2012), as well as in three other LHI–NI endemics, *A. mccullochi*, *Chaetodon tricinctus*, and *Coris bulbifrons* (van der Meer et al. 2012a, 2013, 2015). These differences are likely due to genetic homogeneity resulting from low levels of connectivity over evolutionary time scales (Shulman 1998), contrasting with high levels of contemporary self-recruitment (Swearer et al. 1999; Almany et al. 2007; Planes et al. 2009; Jones et al. 2005). Pertinently, only 40 % of *A. latezonatus* microsatellite loci amplified properly for the NI population (Steinberg et al. 2015), suggesting that unquantified sequence differences are scattered across the genome of this population and therefore that NI is more genetically unique than documented here. In future, a genome-wide SNP analysis may generate sufficient loci to validate this.

Management considerations for future conservation of *Amphiprion latezonatus*

Amphiprion latezonatus is vulnerable to extinction for a range of reasons. First, the effect of climate change on the East Australian Current makes this one of the most rapidly warming ocean regions (Ridgway 2007; Hobday and Pecl 2013; Robinson et al. 2015), and thus, *A. latezonatus* is expected to experience the full brunt of climate change throughout its small geographic range. Second, reproduction and growth in *A. latezonatus* are temperature sensitive (Richardson et al. 1997; Rushworth et al. 2011) and rising sea temperatures will likely affect this species because juvenile growth rates are reduced at temperatures above 23 °C (Rushworth et al. 2011). Third, ocean acidification may reduce anemonefish recruitment and survival by compromising the ability of larvae to detect settlement cues (Dixson et al. 2010; Munday et al. 2010) with direct implications of reduced survival. Fourth, extinction risk is compounded because *A. latezonatus* is a habitat specialist, inhabiting only two anemone species, both of which bleach and die during elevated temperature episodes (Richardson et al. 1997; Hattori 2002; Scott et al. 2011; Hobbs et al. 2013b).

Given limited larval export from LHI to NI and NSI, and high self-replenishment levels at all locations, the MPAs at NSI and LHI are of limited benefit to populations at NI and SC, which lack protection. Moreover, all populations are demographically independent and require protection because migration is limited. Management protection is particularly important for NI, where similar levels of low gene flow and high self-replenishment were found in two other endemic fish species, *Chaetodon tricinctus* and *Coris bulbifrons* (van der Meer et al. 2013, 2015). Given that increasing sea temperatures cause anemone bleaching and mortality (Hobbs et al. 2013b), which has resulted in local extinctions of anemonefishes elsewhere (Hattori 2002, 2005), the lack of gene flow among *A. latezonatus* populations emphasizes the need to protect all populations. The aquarium trade represents another important threat to *A. latezonatus* (Rushworth et al. 2011) and protection from collecting should be instated as at NSI (Scott et al. 2011), with a focus on meeting market demand by captive breeding (Jones et al. 2008). In short, ensuring the persistence of *A. latezonatus* requires that all populations be protected from activities that compromise their survival or recovery and that of their critical habitat (anemone hosts). Implementing protective measures such as MPAs at SC and NI will ensure that all populations have a greater chance to tolerate, acclimate, or adapt to extrinsic impacts, particularly climate change.

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