Colonization and demographic expansion of freshwater fauna across the Hawaiian archipelago


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Abstract

It is widely accepted that insular terrestrial biodiversity progresses with island age because colonization and diversification proceed over time. Here, we assessed whether this principle extends to oceanic island streams. We examined rangewide mtDNA sequence variation in four stream-dwelling species across the Hawaiian archipelago to characterize the relationship between colonization and demographic expansion, and to determine whether either factor reflects island age. We found that colonization and demographic expansion are not related and that neither corresponds to island age. The snail Neritina granosa exhibited the oldest colonization time (~2.713 mya) and time since demographic expansion (~282 kya), likely reflecting a preference for lotic habitats most prevalent on young islands. Conversely, gobiod fishes (Awaous stamineus, Eleotris sandwicensis and Sicyopterus stimpsoni) colonized the archipelago only ~0.411–0.935 mya, suggesting ecological opportunities for colonization in this group were temporally constrained. These findings indicate that stream communities form across colonization windows, underscoring the importance of ecological opportunities in shaping island freshwater diversity.

Introduction

The biodiversity of oceanic islands has served as a crucible for deriving fundamental principles in ecology and evolutionary biology, including the seminal theory of island biogeography (Wallace, 1880; MacArthur & Wilson, 1963, 1967; Paulay, 1994; Whittaker et al., 2008; Valente et al., 2014). Comparative assessments of insular terrestrial biodiversity have yielded consistent support for the idea that colonization and diversification progress with island age (MacArthur & Wilson, 1963, 1967; Carson, 1983; Baldwin & Robichaux, 1995; Tarr & Fleischer, 1995; Price & Clague, 2002; Cowie & Holland, 2008; Parent et al., 2008; Husemann et al., 2014). Although conditions in other ecosystems also adhere to this principle (e.g. Jordan et al., 2003; Goodman & O’Grady, 2013), the relationships between time since formation, colonization and diversification have not been well characterized for the biota of oceanic island streams.

Stream ecosystems on oceanic islands are a particularly interesting test case for predictions about island age because insular freshwater communities do not exhibit many of the characteristics of insular terrestrial biota. For instance, oceanic island streams can be remarkably depauperate. This is most evident in streams of the Hawaiian Islands that support a native aquatic fauna consisting of only five fishes, two crustaceans and two molluscs. Also, most, if not all, species exhibit an amphidromous life history, a form of...
stream fishes (Fitzsimons et al., 1990; Nishimoto & Kamitori, 1991, 1997; Keith, 2003). Because stream configuration and habitat availability change over the course of island ontogeny (Craig et al., 2001; Craig, 2003; Smith et al., 2003), colonization may instead be episodic with establishment contingent on species-specific ecological requirements. Alternatively, colonization may not proceed according to discrete windows of habitat availability, but instead occur along a time continuum (Cook et al., 2010).

After colonization, it is expected that species will diversify through spatial and demographic expansion as habitat availability and heterogeneity increase with island age (Parent & Crespi, 2006). Terrestrial biodiversity often conforms to the old-to-young progression rule (Funk & Wagner, 1995), which is characterized by diversity (i.e. genetic diversity, species diversity) progressively declining from older to younger islands (Slattin & Hudson, 1991; Funk & Wagner, 1995; Excoffier et al., 2009). If aquatic biota similarly conform to the old-to-young progression rule (Jordan et al., 2003; McDowall, 2003; Rubinoff, 2008), then measures of diversity and associated processes would be expected to follow island age (Price & Clague, 2002; Jordan et al., 2003; McDowall, 2003; Rubinoff, 2008). Thus, the timing of the last demographic expansion might be coupled to colonization time and island age (Cook et al., 2008, 2010). On the other hand, if colonization has occurred at some point after the emergence of extant high islands (Lindstrom et al., 2012), diversity and associated demographic measures might not conform to the progression rule because multiple islands could have been colonized simultaneously. If driven by factors other than island age, demographic expansion also might proceed well after colonization.

In this study, we evaluated relationships between island age, colonization time and demographic history in freshwater fauna across the Hawaiian Islands. Using geologically independent and comparative approaches, we assessed rangewide patterns of mitochondrial DNA (mtDNA) sequence variation to infer (1) time of colonization and demographic expansion for half of the endemic species; and (2) whether these factors and related measures of genetic diversity correspond to island endemic species; and (2) whether these factors and related measures of genetic diversity correspond to island age. We also assessed (3) whether colonization of the islands is best described by the dispersal mode, colonization window or continuous colonization hypothesis. Doing so enabled us to infer the relative contributions of geology and ecology in shaping the origin and assembly of aquatic communities across the archipelago.

Materials and methods

Study area

The Hawaiian Islands originated from a volcanic hot spot beneath the Pacific tectonic plate that drifts in a
north-west direction. The linearly arranged five main high islands progress in age from the south-eastern end of the chain to the north-west (Fig. 1), with Hawai‘i (0.43 my) being the youngest main island and Kaua‘i (5.1 my) being the oldest (Carson & Clague, 1995). The archipelago encompasses several other high islands (e.g. Lāna‘i) and the Northwestern Hawaiian Islands formation, which extends thousands of kilometres to the north of Kaua‘i, but only the five main high islands have perennial streams that harbour aquatic fauna (Clague, 1996). The entire archipelago is thought to have existed for 80–85 my, but a continuous chain of high islands has existed for only 23 mya, which has served as a hypothetical maximum time limit for colonization of the archipelago by terrestrial and semi-aquatic biota (Clague, 1996).

Aquatic habitat formation and availability shift during the course of geomorphic development. Volcanic islands initially increase in size due to deposition of new lava until a maximum area is reached, after which islands enter a phase of declining size and submergence due to erosion (Whittaker et al., 2008). Watershed and stream geomorphology are also shaped by erosion, weathering and soil formation over the progression of island evolution. For example, newly formed islands may not support systems with permanent surface flow because rainwater can percolate through porous volcanic material. As an island ages, erosion can transform fast-running, steep-slope cascading streams with terminal waterfalls on younger islands to slower flowing rivers with larger estuaries on older islands. With increases in catchment area, stream networks and specialized microhabitats also develop due to variation in volcanic material and succession of riparian vegetation (MacArthur & Wilson, 1963; Zink et al., 1996; Chubb et al., 1998; Craig et al., 2001; Donaldson & Myers, 2002; Fitzsimons et al., 2002; Craig, 2003; McDowall, 2003; Smith et al., 2003). Thus, as islands age, erosion may lead to higher topographical complexity and habitat diversity that can favour more diverse communities. However, habitat complexity may subsequently decline as erosion and submergence lead towards submergence.

Sea level changes associated with global interglacial-glacial cycling also had an effect on the configuration and availability of aquatic habitat on oceanic islands. For example, during the peak of the Last Glacial Maximum (ca. 17–19 kya), sea level was up to 130 m below present conditions, resulting in a 75% reduction of shallow coastal and estuarine habitats in Hawai‘i and up to a 92% reduction on south Pacific islands (Ludt & Rocha, 2015). The islands of Maui and Moloka‘i, and Lāna‘i, which were once a single landmass, became reunited by limestone bridges conforming with the superisland of Maui Nui during this period. The bridges were subsequently drowned by rising sea level during the Holocene Transgression (ca. 12 kya), which separated all five main high islands (Price & Elliott-Fisk, 2004).

**Study species**

The native aquatic fauna of Hawaiian Islands streams consists of only nine species including five fishes: *Awaous*...
stamineus (Eydoux and Souleyet, 1850), Lentipes concolor (Gill, 1860), Sicyopterus stimpsoni (Gill, 1860) and Stenobopus hawaiiensis Watson, 1991 from the family Gobiidae, and Eoleotris sandwicensis Vaillant and Sauvage, 1875 from the family Electrotridae (McDowall, 2003); two gastropods: Neritina granosa Sowerby, 1825 and Neritina vespertina (Sowerby, 1849); and two decapods: Macrobrachium grandimanus (Randall, 1840) and Attyida bisilicata Randall, 1840 (Short & Marquet, 1998; McDowall, 2003; Bebler & Foltz, 2004). With the exception of M. grandimanus, which also occurs in the Ryukyu, Philippine and Fiji islands (Short & Marquet, 1998), all species are endemic to the Hawaiian Islands. Phylogenetic relationships with Indo-Pacific congeners have not been well examined; thus, colonization and demographic histories remain largely unknown (McDowall, 2003; Lindstrom et al., 2012; Tailliebois et al., 2014).

Here, we examined three fish and one mollusc: A. stamineus, S. stimpsoni, E. sandwicensis and N. granosa. All exhibit either an obligate or facultative amphidromous life history (Radtke et al., 1988; McDowall, 1992; Hogan et al., 2014). The duration of marine dispersal also differs among the species, varying from 57–248 days in A. stamineus (mean = 118 days; Hogan et al., 2014), 119–151 days in S. stimpsoni (mean = 135 days; Radtke et al., 1988), approximately 60–120 days in E. sandwicensis (Maeda & Tachihara, 2005) and up to 1 year in N. granosa according to patterns observed in congeneric species (Ford, 1979; Hodges & Allendorf, 1998).

The freshwater species of Hawaiʻi have different ecological requirements reflecting physiological conditions that define the longitudinal distribution of habitat use in streams. The five native fishes, for example, differ in climbing ability due to variation in fin morphology, musculature and climbing kinematics (Blob et al., 2006), whereby waterfalls and flow velocity effectively partition habitat use by elevation and substrate type (Fitzsimons & Nishimoto, 1990; Nishimoto & Kuamoto, 1991, 1997; Keith, 2003). Additionally, the species differ in trophic ecology, where some are herbivores or predators, and others are omnivores (Kido et al., 1993; Kido, 1996; Keith, 2003). Currently, A. stamineus is the most abundant and widespread native fish; it inhabits lower- and middle-elevation reaches of streams on every island and is tolerant of in-stream degradation (Keith, 2003; Blum et al., 2014). Like A. stamineus, S. stimpsoni can climb and disperse beyond moderately high waterfalls and reach higher elevations within watersheds, but it is intolerant of stream degradation (Blum et al., 2014). As E. sandwicensis lacks fused pelvic fins, it is unable to disperse upstream of precipitous waterfalls, and thus, it exhibits a more restricted distribution, particularly on younger islands because watersheds often end in terminal waterfalls (Fitzsimons et al., 1990). The two native neritid snails also differ in habitat use: N. vespertina are largely restricted to hard substrates below the first major elevation change (i.e. in estuaries or lower reaches), whereas N. granosa utilize hard substrates across a broad longitudinal range within watersheds (Ford, 1979; Bebler & Foltz, 2004). Historically, all four of our study species were found on all five high islands, but S. stimpsoni and N. granosa are now virtually absent on Oʻahu due to anthropogenic factors (Burr, 2001; Henderson, 2003; Walter et al., 2012; Blum et al., 2014; Hawaiʻi Watershed Atlas, available at: http://hawaiiwatershedatlas.com).

### Specimen collection and DNA sequencing

Fishes were netted by hand in 41 watersheds from the islands of Kauaʻi, Oʻahu, Maui, Molokaʻi and Hawaiʻi between 2009 and 2011. Tissue samples were taken from the caudal fin and preserved immediately in 95% ethanol. Specimens were released after processing except for post-larvae, which were killed due to the difficulty of morphological species identification in the field (Tate et al., 1992; Lindstrom, 1999). We collected N. granosa by hand from 20 watersheds, taking and preserving non-lethal clips of 10–30 mg of foot tissue from each individual. Sample sizes across all species ranged from 50 to 462 per island, and from 497 to 2229 per species (Fig. 1, Table 1).

Colonization, diversification and demographic and range expansions may result in signature patterns of variation detectable at neutral genomic regions like mtDNA (Slatkin & Hudson, 1991; Excoffier et al., 2009). Accordingly, we amplified a fragment of the mitochondrial cytochrome c oxidase I gene (coI) in N. granosa using primers LCO1490 and HCO12198 (Folmer et al., 1994). For all three fish, the mitochondrial cytochrome b gene (cytb) was amplified using primers GluFish and ThrFish2 (Sevilla et al., 2007; Appendix S1). Due to the very low number of cytb haplotypes found in an initial screening of variability in S. stimpsoni, additional sequences of the coI gene were obtained, using primers L6468 and H7696 (Thacker & Hardman, 2005; Appendix S1). All analyses were performed for the S. stimpsoni coI data sets except for the molecular clock and mismatch distribution analyses, which were performed independently for each mitochondrial gene. Also, because of differences in the size of fish cytb gene fragments, genetic diversity estimates for E. sandwicensis were calculated using a shorter data set to match that of A. stamineus. Sequences of all haplotypes are available in GenBank under accession numbers KX134284–KX134661, and complete gene alignments for all species are available from the Dryad repository (http://dx.doi.org/10.5061/dryad.n21f4).

### Genetic diversity

For each species and island, DnaSP 5.10.01 (Librado & Rozas, 2009) was used to determine the number of
haplotypes \( (h) \), variable sites \( (S) \) and average number of pairwise differences between sequences \( (k) \), and to calculate haplotype \( (H_d) \) and nucleotide diversity \( (\pi) \). Because of differences in sample sizes between taxa and islands, rarefaction was used to estimate the expected number of haplotypes among 50 individuals \( (h_{n50}) \); the minimum sample size for \( N. \) granosa in Kaua‘i) using the program EstimateS 9 (Colwell, 2013). We also calculated the number of unique haplotypes per island divided by the total number of haplotypes \( (U_n50) \), number of haplotypes controlled by the minimum sample size and standard deviation; \( \Pi \), Faith’s Index.

### Genetic structure

Measures of genetic differentiation might be expected to adhere to the old-to-young progression rule if the vagility of propagules in amphidromous species is sufficient to reach every island, but rare enough to maintain integrity of each deme and allow local differentiation (Chubb et al., 1998; Cowie & Holland, 2008; Moody et al., 2015). To depict relationships among haplotypes for each species, haplotype networks were constructed using the package pegas (Paradis, 2010) in R 3.0.3. Also, neighbour-joining gene trees were constructed for unique haplotypes using the package ape 3.1-2 (Paradis et al., 2004) with node support assessed from 1000 bootstrap replicates. Genetic differentiation among islands was assessed according to pairwise \( \Phi_{ST} \) for each species with significance estimated from 10 000 random permutation tests and \( P \)-values adjusted according to sequential Bonferroni corrections for multiple tests (Holm, 1979) in Arlequin 3.5.1.3 (Excoffier & Lischer, 2010). Hierarchical structure among all islands and among watersheds within islands was evaluated using an analysis of molecular variance \( (\text{AMOVA}) \). We tested for patterns of isolation-by-distance, as would be expected from a progression arising from island age, using Mantel tests to compare pairwise geographic distances and linearized pairwise differentiation \( [\Phi_{ST}/(1-\Phi_{ST})] \) among islands. Statistical significance was estimated from 5000 random permutations in Arlequin 3.5.1.3.

### Table 1 Genetic diversity estimates for every species and island.

<table>
<thead>
<tr>
<th>Species (gene, bp)</th>
<th>Island</th>
<th>( n )</th>
<th>( S )</th>
<th>( h )</th>
<th>( H_d ) (SD)</th>
<th>( \pi ) (SD)</th>
<th>( k )</th>
<th>( h_n50 ) (SD)</th>
<th>( \Pi )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( N. ) granosa (coi, 578 bp)</td>
<td>Kaua‘i</td>
<td>50</td>
<td>26</td>
<td>23</td>
<td>0.732 (0.070)</td>
<td>0.002 (0.000)</td>
<td>1.292</td>
<td>0.111 11</td>
<td>0.478</td>
</tr>
<tr>
<td></td>
<td>O‘ahu</td>
<td>154</td>
<td>75</td>
<td>75</td>
<td>0.811 (0.034)</td>
<td>0.003 (0.000)</td>
<td>1.655</td>
<td>0.362 47</td>
<td>0.627</td>
</tr>
<tr>
<td></td>
<td>Moloka‘i</td>
<td>112</td>
<td>62</td>
<td>60</td>
<td>0.832 (0.037)</td>
<td>0.003 (0.000)</td>
<td>1.935</td>
<td>0.290 37</td>
<td>0.617</td>
</tr>
<tr>
<td></td>
<td>Hawai‘i</td>
<td>289</td>
<td>104</td>
<td>107</td>
<td>0.793 (0.026)</td>
<td>0.003 (0.000)</td>
<td>1.565</td>
<td>0.517 72</td>
<td>0.673</td>
</tr>
<tr>
<td></td>
<td>All</td>
<td>606</td>
<td>149</td>
<td>207</td>
<td>0.799 (0.018)</td>
<td>0.003 (0.000)</td>
<td>1.633</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( A. ) stamineus (cytb, 512 bp)</td>
<td>Kaua‘i</td>
<td>421</td>
<td>33</td>
<td>35</td>
<td>0.538 (0.028)</td>
<td>0.001 (0.001)</td>
<td>0.678</td>
<td>0.350 8</td>
<td>0.229</td>
</tr>
<tr>
<td></td>
<td>O‘ahu</td>
<td>657</td>
<td>41</td>
<td>51</td>
<td>0.526 (0.023)</td>
<td>0.001 (0.000)</td>
<td>0.735</td>
<td>0.310 19</td>
<td>0.373</td>
</tr>
<tr>
<td></td>
<td>Moloka‘i</td>
<td>352</td>
<td>32</td>
<td>34</td>
<td>0.519 (0.032)</td>
<td>0.001 (0.000)</td>
<td>0.701</td>
<td>0.340 9</td>
<td>0.265</td>
</tr>
<tr>
<td></td>
<td>Hawai‘i</td>
<td>482</td>
<td>32</td>
<td>40</td>
<td>0.588 (0.025)</td>
<td>0.002 (0.000)</td>
<td>0.821</td>
<td>0.400 12</td>
<td>0.300</td>
</tr>
<tr>
<td></td>
<td>All</td>
<td>337</td>
<td>33</td>
<td>38</td>
<td>0.538 (0.032)</td>
<td>0.001 (0.000)</td>
<td>0.706</td>
<td>0.380 12</td>
<td>0.316</td>
</tr>
<tr>
<td>( E. ) sandwicensis (cytb, 512 bp)</td>
<td>Kaua‘i</td>
<td>141</td>
<td>35</td>
<td>26</td>
<td>0.653 (0.046)</td>
<td>0.004 (0.001)</td>
<td>2.206</td>
<td>0.591 13</td>
<td>0.500</td>
</tr>
<tr>
<td></td>
<td>O‘ahu</td>
<td>121</td>
<td>13</td>
<td>13</td>
<td>0.413 (0.065)</td>
<td>0.002 (0.000)</td>
<td>0.913</td>
<td>0.295 4</td>
<td>0.308</td>
</tr>
<tr>
<td></td>
<td>Moloka‘i</td>
<td>80</td>
<td>13</td>
<td>13</td>
<td>0.533 (0.065)</td>
<td>0.002 (0.000)</td>
<td>0.835</td>
<td>0.295 2</td>
<td>0.154</td>
</tr>
<tr>
<td></td>
<td>Hawai‘i</td>
<td>73</td>
<td>24</td>
<td>13</td>
<td>0.370 (0.073)</td>
<td>0.002 (0.001)</td>
<td>1.161</td>
<td>0.295 1</td>
<td>0.077</td>
</tr>
<tr>
<td></td>
<td>All</td>
<td>497</td>
<td>47</td>
<td>44</td>
<td>0.508 (0.028)</td>
<td>0.003 (0.000)</td>
<td>1.521</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( S. ) stimpsoni (coi, 612 bp)</td>
<td>Kaua‘i</td>
<td>221</td>
<td>17</td>
<td>12</td>
<td>0.580 (0.019)</td>
<td>0.003 (0.000)</td>
<td>1.808</td>
<td>0.545 8</td>
<td>0.667</td>
</tr>
<tr>
<td></td>
<td>O‘ahu</td>
<td>269</td>
<td>9</td>
<td>7</td>
<td>0.592 (0.022)</td>
<td>0.003 (0.000)</td>
<td>1.802</td>
<td>0.318 3</td>
<td>0.429</td>
</tr>
<tr>
<td></td>
<td>Moloka‘i</td>
<td>170</td>
<td>13</td>
<td>8</td>
<td>0.533 (0.002)</td>
<td>0.003 (0.000)</td>
<td>1.655</td>
<td>0.364 5</td>
<td>0.625</td>
</tr>
<tr>
<td></td>
<td>Hawai‘i</td>
<td>156</td>
<td>9</td>
<td>5</td>
<td>0.526 (0.020)</td>
<td>0.003 (0.000)</td>
<td>1.637</td>
<td>0.227 2</td>
<td>0.400</td>
</tr>
<tr>
<td></td>
<td>All</td>
<td>818</td>
<td>28</td>
<td>22</td>
<td>0.564 (0.011)</td>
<td>0.003 (0.000)</td>
<td>1.747</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\( n \), number of samples; \( S \), number of polymorphic sites; \( h \), number of haplotypes; \( H_d \), haplotype diversity and standard deviation; \( \pi \), nucleotide diversity and standard deviation; \( k \), average number of sequence pairwise differences; \( h/n50 \), percentage of haplotypes; \( U_{h/n50} \), number of unique haplotypes divided by the total number of haplotypes; \( \Pi \), Faith’s Index.

\( n, S, h, H_d, \pi, k, h/n50, \Pi \) are measured from 5000 random permutations in Arlequin 3.5.1.3. The Faith’s Index \( (\Pi) \) and island age. The effect of island age on genetic diversity across all species and standard deviation; \( \Pi \), Faith’s Index.
Estimates of colonization time

Time to the most recent common ancestor (tmrca) was inferred for all haplotypes for each species as a proxy for the time of colonization, using relaxed molecular clock analyses performed in BEAST 1.8.0 (Drummond et al., 2012) via the CIPRES portal (Miller et al., 2010). Previously published sequences and fossil record data from gobioid fishes and Neritidae gastropods were gathered to reconstruct time-calibrated molecular phylogenies for each species group. The analysis of Neritidae gastropods included, in addition to the *N. granosa* haplotypes, 84 *coi* sequences from 9 genera of Neritidae, with the majority from *Neritina* and *Nerita*, and one genus of Phenacolepidaeidae (Table S1). A molecular phylogeny was constructed anchored by 10 fossil calibration points with mean ages ranging from 5 to 56 mya (Table S2). For the gobioid fishes, two data sets were built, one for each mitochondrial gene. In addition to the haplotypes obtained for the focal species, both data sets included 45 sequences from 35 genera of Gobiiformes and 2 genera of Kurtiformes (S1), which were used to calibrate the gobioid phylogeny using 4 fossil data points with mean ages ranging from 12.5 to 92 mya (Table S2).

Time to the most recent common ancestor and corresponding confidence intervals [95% highest posterior density (HPD)] were estimated for the Hawaiian haplotypes of each species, using a relaxed molecular clock with uncorrelated lognormal distribution of rates and the HKY substitution model. A birth–death speciation model was assumed as a tree prior. Two independent analyses were performed for each data set, running the MCMC for 5 × 10^7 generations, with trees sampled every 5 × 10^4 generations, and 10% of the samples discarded as burn-in. Runs were combined and checked for convergence and adequate effective sampling size (ESS) using LogCombiner 1.8.0 and Tracer 1.5 (available at: http://tree.bio.ed.ac.uk/software/tracer/). To calibrate the molecular clocks, all fossil data points were included as soft calibration points to account for uncertainties in fossil dates or conflicts between fossil and molecular data (Yang & Rannala, 2006) using lognormal prior distributions. Mean substitution rates and their 95% HPD intervals were estimated for each data set in Tracer 1.5.

Estimates of time since demographic expansions

The recent demographic history of each species across all islands and on each island was first estimated according to mismatch distribution analyses in Arlequin 3.5.1.3. Estimates for the time since the last population expansion derived from the population growth statistic τ (Rogers & Harpending, 1992) were calculated using the substitution rates obtained from the fossil-calibrated molecular clocks. Also, for comparative purposes, times since expansion were calculated using previously published mitochondrial *cytb* rates that are commonly used in phylogeographic studies (Burrage et al., 2008), and references therein. We applied a slow rate of 5.3 × 10^{-3} substitutions/site/million years (s/s/my; Dowling et al., 2002), an intermediate rate of 7.6 × 10^{-3} s/s/my (Zardoya & Doadrio, 1999) and a fast rate of 1 × 10^{-2} s/s/my (Near & Bernard, 2004).

Deviations of the observed mismatch from expectations under the sudden-expansion model were assessed from 1000 bootstrap replicates using the sum of squared deviations (SSD) and the Harpending raggedness index (*HR*; Harpending, 1994). To further test for deviations from neutrality arising from selection or demographic changes, Tajima’s *D* (Tajima, 1989) and Fu’s *Fs* (Fu, 1997) tests were used. Statistical significance was determined from 1000 coalescent simulations in Arlequin 3.5.1.3. Lastly, past population dynamics were estimated from Bayesian skyline plots (BSP) as implemented in BEAST 1.8.0, which generates a posterior distribution of the effective population size (*N_e*) through time by estimating changes in the relative genetic diversity along a genealogy using MCMC sampling. A strict molecular clock was assumed incorporating the substitution rates estimated for each species group and gene as previously described. Two independent analyses were run for 5 × 10^7 generations for each species and island and sampled every 5 × 10^4 generations, except in *A. stamineus*, for which 1 × 10^8 generations sampled every 1 × 10^7 generations were necessary to reach convergence. The analyses were carried out in the CIPRES web portal. After combining the runs and checking for convergence, the median and corresponding 95% HPD intervals of each BSP were depicted using Tracer 1.5.

We performed Spearman correlations for all islands to test for correlations between tmrca and time since the last demographic expansion. We also performed multivariate linear regressions to test whether island age explains tmrca and time since the last demographic expansion. Both analyses were performed for all islands and species, as well as for *Neritina* and fish separately, with species as a cofactor in R 3.0.3.

Results

Five mitochondrial gene alignments were constructed consisting of the following: 606 *coi* sequences (578 bp) for *N. granosa*, 2229 *cytb* sequences (512 bp) for *A. stamineus*, 497 *cytb* sequences (943 bp and 512 bp) for *E. sandwicensis* and 110 *cytb* sequences (430 bp) as well as 818 *coi* sequences (612 bp) for *S. stimpsoni*.

Genetic diversity and island age

Although this study represents the most intensive molecular genetic assessment across multiple
amphidromous species to date, we failed to capture the full haplotype diversity in most of the study species and islands due to the prevalence of rare haplotypes (Fig. S1). Not even >2200 samples of A. stamineus (mean \( n \) per island = 445.80 ± 128.57) were enough to yield saturation of the haplotype sampling curve. However, in populations with relatively low genetic diversity, such as S. stimpsoni from Moloka‘i and Hawai‘i, our sampling was representative of extant genetic variability (Fig. S1).

Rarefaction curves adjusted to the minimum sample size (\( n = 50 \)) indicated that a much higher number of haplotypes occur in N. granosa on all islands (mean \( h_{n50} = 15.21–21.77 \)) than in other species. This was accompanied by larger haplotype and nucleotide diversity values (Table 1). Across all species, N. granosa exhibited the highest number of unique haplotypes per island, with up to 67% of the total number of haplotypes being exclusive to one island. Sicyopterus stimpsoni exhibited a much lower number of haplotypes, but not haplotype or nucleotide diversity, than other species (Table 1). Among fish, S. stimpsoni showed the highest proportion of unique haplotypes per island (mean \( U_h/h = 0.530 ± 0.135 \)), whereas the lowest proportion was exhibited by A. stamineus (mean \( U_h/h = 0.296 ± 0.054 \)).

The levels of genetic diversity were not consistent across islands for different species. Fish populations on Kaua‘i consistently showed the highest levels of genetic diversity for E. sandwicensis and S. stimpsoni, including the highest proportion of unique haplotypes per island (\( U_h/h = 0.500 \) and 0.667) as well as the highest number of haplotypes (\( h_{n50} = 13.09 \) and 5.37) and Faith’s index values (\( F_t = 0.074 \) and 0.028). Conversely, Maui and Hawai‘i exhibited higher values for the same statistics in N. granosa (Table 1). This translated to a negative correlation between genetic diversity and island age in N. granosa (\( r_2 = −4.389, P = 0.048 \)), indicating that the species exhibits lower genetic diversity on older islands. When considering only fish species, we found a non-significant trend towards higher genetic diversity (\( F_t \)) on older islands (\( P_{1,8} = 4.134, P = 0.076 \)).

Genetic structure

Neighbour-joining gene trees support monophyly of all four species examined. All of the trees were characterized by shallow branches and a lack of well supported relationships among haplotypes (Fig. S2). Only E. sandwicensis exhibited two moderately supported clades (\( P \)-distance = 1.162 ± 0.320%), although these groups did not exhibit phylogeographic structure. The average genetic distance among all haplotypes within species was lowest for A. stamineus (\( P \)-distance = 0.569 ± 0.088%) and N. granosa (\( P \)-distance = 0.582 ± 0.052%), followed by S. stimpsoni (\( P \)-distance = 0.698 ± 0.158%) and E. sandwicensis (\( P \)-distance = 0.716 ± 0.106%). All haplotype networks exhibited a starlike topology, with one extremely common and central haplotype, and several-to-many low-frequency haplotypes separated by a small number of mutations, with the exception of S. stimpsoni, which exhibited two comparably frequent and closely related haplotypes without phylogeographic structure (Fig. 2).

Intraspecific genetic differentiation among islands was low for all species. Significant pairwise comparisons were observed only once in N. granosa (\( \Phi_{ST} \) Maui–Hawai‘i = 0.007), four times in E. sandwicensis (\( \Phi_{ST} = 0.008–0.025 \)) and five times in A. stamineus (\( \Phi_{ST} = 0.002–0.004 \)). No comparisons were significant for S. stimpsoni (Table S3). Among these, only the significant comparisons between O‘ahu and Hawai‘i and between Moloka‘i and Hawai‘i were shared by two fish species. Overall genetic differentiation among islands was not significant for all species. Only N. granosa and A. stamineus exhibited significant genetic structure among watersheds within islands and among sites.

![Image](https://example.com/image.png)

Fig. 2 Haplotype networks estimated for the mitochondrial haplotypes of Neritina granosa (coi), Awaous stamineus (cytb), Eleotris sandwicensis (cytb) and Sicyopterus stimpsoni (coi). Circles are proportional to haplotype frequency, and internode lengths are proportional to the number of mutational steps between haplotypes.
across the archipelago (AMOVA \( \Phi_{SC} = 0.012, P = 0.004, \Phi_{ST} = 0.007, P = 0.014 \) for \( N. \) granosa; and \( \Phi_{SC} = 0.011, P < 0.001, \Phi_{ST} = 0.011, P < 0.001 \) for \( A. \) stamineus). Mantel tests did not detect significant correlations between genetic and geographic distances in any species.

**Time of colonization**

The fossil-calibrated molecular clocks estimated mitochondrial substitution rates that fall well within the range of what has been reported for other neritid gastropods (Frey & Vermeij, 2008) and fishes (Near & Bernard, 2004; Burridge et al., 2008). We estimated a substitution rate for the Neritidae coi gene of \( 2.59 \times 10^{-3} \text{ s/s/my} \) (95% HPD: \( 2.02 \times 10^{-3} - 3.22 \times 10^{-3} \)), whereas we estimated a substitution rate of \( 1.11 \times 10^{-2} \text{ s/s/my} \) (95% HPD: \( 7.79 \times 10^{-3} - 1.54 \times 10^{-2} \)) for the gobiod fish data set and \( 1.2 \times 10^{-2} \text{ s/s/my} \) (95% HPD: \( 8.59 \times 10^{-3} - 1.49 \times 10^{-2} \)) for the gobiod coi data set.

Inferred colonization times (tmrca) clustered into two distinct time periods. One period corresponded to the tmrca of \( N. \) granosa, which was estimated at 2.713 mya (95% HPD: \( 1.737 - 4.465 \)). The second period corresponded to the mean estimated tmrca for the three gobiod fish based on cytb and coi data, which ranged between 0.411 mya and 0.935 mya (\( A. \) stamineus mean = 0.672, 95% HPD: \( 0.265 - 1.149 \); \( E. \) sandwicensis mean = 0.935, 95% HPD: \( 0.498 - 1.425 \); \( S. \) stimpsoni mean = 0.411 mya, 95% HPD: \( 0.079 - 0.916 \) for the cytb haplotypes, and 0.726 mya, 95% HPD: \( 0.388 - 1.142 \) for the coi haplotypes (Fig. 3).

Demographic history

Unimodal mismatch distributions, as expected under scenarios of demographic expansion, were observed in all but one species (Fig. 4). Unlike the other species, \( S. \) stimpsoni exhibited two modes for both the coi and the cytb data, which is indicative of stationary demographic population sizes (Hudson, 1990; Rogers & Harpending, 1992). The SSD and \( H_{ri} \) indicated that the demographic history of \( N. \) granosa on all islands does not significantly deviate from the sudden-expansion model. However, for each fish species, at least one of the statistics indicated significant deviations from the sudden expansion on two or more islands (Table 2). Both neutrality tests (Tajima’s \( D \) and Fu’s \( F \)) showed significantly negative values suggesting expansion or selection for all species except in \( S. \) stimpsoni (Table 2).

Based on the demographic parameters obtained from mismatch analyses, the estimated mean times since the last expansion was around 235 000–362 000 years ago for \( N. \) granosa and around 31 000–45 000 years for \( A. \) stamineus and \( E. \) sandwicensis (Table S4). Time for \( S. \) stimpsoni was estimated between 137 000 and 146 000 years ago, but the multimodal mismatch distribution and nonsignificant neutrality tests suggest a stationary population size, which precludes inferences about time since expansion. Finally, when times since...
Table 2  Neutrality tests and demographic parameters obtained for every species and island.

<table>
<thead>
<tr>
<th>Species</th>
<th>Island</th>
<th>SSD</th>
<th>Hri</th>
<th>Tajima’s D</th>
<th>Fu’s Fs</th>
<th>( \tau )</th>
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</thead>
<tbody>
<tr>
<td><em>N. granosa</em> (coi, 578 bp)</td>
<td>Kaua’i</td>
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<td>0.043</td>
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<td>-27.586*</td>
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<td></td>
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<td>-27.492*</td>
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<td>0.045</td>
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<td>-27.922*</td>
<td>1.564</td>
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<tr>
<td></td>
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<td>0.036</td>
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<td>-27.459*</td>
<td>1.717</td>
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<tr>
<td></td>
<td>All</td>
<td>0.000</td>
<td>0.045</td>
<td>-2.733*</td>
<td>-27.922*</td>
<td>1.564</td>
</tr>
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<td><em>A. stamineus</em> (cytb, 512 bp)</td>
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<td>0.005</td>
<td>0.106*</td>
<td>-2.412*</td>
<td>-34.028*</td>
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<td>-34.028*</td>
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<td>0.094*</td>
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<td>-34.028*</td>
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<td>0.098*</td>
<td>-2.412*</td>
<td>-34.028*</td>
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<tr>
<td><em>E. sandwicensis</em> (cytb, 512 bp)</td>
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<td>0.013*</td>
<td>0.059*</td>
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<tr>
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<td>0.053</td>
<td>2.733</td>
<td>4.217</td>
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<tr>
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<td>0.439*</td>
<td>-1.411*</td>
<td>-6.826</td>
<td>4.111</td>
</tr>
</tbody>
</table>

SSD, sum of squared deviations; Hri, Harpending raggedness index; \( \tau \), population expansion constant.

*\( P < 0.05 \).
the last expansion were calculated using universal fish mitochondrial rates, estimates were ~2.1× older for the slowest rate, ~1.46× older for the intermediate rate and ~1.1× older for the fastest rate (Table S4). Overall, effective population sizes appear to be increasing through time in all species, although the extent of change differed among species and islands (Figs 5 and S2). For example, the populations of *E. sandwicensis* on Hawai‘i and *S. stimpsoni* on Hawai‘i and Moloka‘i exhibited little evidence of population growth. In contrast, the populations of *N. granosa* for all islands, up to 773 000 years for *N. granosa* in Kaua‘i and 861 000 years for *E. sandwicensis* in Maui. In both species, tmrca estimates for populations on Moloka‘i were much more recent: 541 000 and 221 000 years, respectively. In contrast, tmrca estimates for *A. stamineus* were more recent for all islands (mean tmrca: 104 000–154 000 years). As observed from the BSP analyses, the times at which *N* eff started increasing were oldest for *N. granosa* for all islands (ca. 250 000–350 000 years ago), whereas *A. stamineus* and *E. sandwicensis* exhibited much younger and similar times of demographic expansion (20 000–40 000 and 30 000–42 000 years ago, respectively; Fig. 5). Considering that *S. stimpsoni* did not significantly deviate from expectations under demographic equilibrium, the recent increase in *N* eff (ca. 3600–6400 years ago) is probably an artefact of nonsegregating mutations accumulating at the branch tips.

Time to the most recent common ancestor and time since the last demographic expansion were not significantly correlated across islands and species (*t* 13 = 2.323, *P* = 0.264). When only fishes were considered, a significant negative correlation was observed (*t* 11 = −4.324, *P* = 0.001). The effect of species was also significant (*t* 2 = 9.802, *P* < 0.001). On the other hand, we detected a positive but nonsignificant correlation between tmrca and demographic expansion for *N. granosa* (*t* 2 = 0.206, *P* = 0.180). Additionally, island age was not related to either tmrca or demographic history (*F* 2,14 = 0.143, *P* = 0.867). Likewise, no relationship was observed when fishes and *Neritina* were analysed separately (*F* 2,10 = 0.173, *P* = 0.843; and *F* 2,1 = 5.757, *P* = 0.283, respectively), but species had a highly significant effect when only fishes were considered (*F* 2,10 = 44.370, *P* < 0.001).

![Fig. 5 Bayesian skyline plots estimated for each of the four native Hawaiian freshwater species and island. Time estimates were calculated relative to the substitution rates inferred from the molecular clock analyses for each species. For clarity, only the median effective population sizes (*N* eff) are shown. All the plots showing median and associated 95% HPD values are included in Fig. S3.](image-url)
Discusson

Examining communities on oceanic islands can enrich understanding of ecological and evolutionary mechanisms that generate biodiversity, including the tempo and mode of island colonization and subsequent demographic expansion (Cowie & Holland, 2008). Here, we show that colonization and demographic expansion of insular freshwater fauna are not positively correlated and that neither tracks island age across the Hawaiian Islands. Colonization of the archipelago by freshwater fauna appears to have occurred during at least two distinct windows of time during the Mid-Pliocene and Pleistocene, far later than the initial emergence of the archipelago and the origin of the oldest extant high island. Each window is represented by different taxonomic groups, suggesting that ecology (i.e. climatic or habitat requirements), rather than geology (i.e. island age), governs assembly of insular freshwater communities. Likewise, it appears that demographic processes are more likely governed by the variable influence of ecological and local stochastic factors on different species.

Island age and colonization

We found that tmrca estimates for stream fauna range from the Pleistocene to the Mid-Pliocene (Fig. 3). These tmrca intervals largely agree with estimates for the origin of terrestrial assemblages, waterbirds and anchialine crustacea (Fleischer & McIntosh, 2001; Craft et al., 2008; Russ et al., 2010; Santos & Weese, 2011), but differ from estimates that indicate colonization by semi-aquatic invertebrates predated the age of the extant high Hawaiian Islands (Jordan et al., 2003; Goodman & O’Grady, 2013). Our findings also indicate that like some semi-aquatic and anchialine fauna (Fleischer & McIntosh, 2001; Price & Clague, 2002; Craft et al., 2008; Santos & Weese, 2011), freshwater species likely derive from a succession of singular events. This is consistent with the hypothesis that the arrival of freshwater species to the Hawaiian Islands corresponded to rare and independent colonization events, based on the observation that the endemic fishes belong to five different genera derived from distinct groups found outside of the archipelago (McDowall, 2003). Albeit we cannot rule out that the two recovered clades in the neighbour-joining tree of E. sandwicensis (Fig. S2) may represent two colonization events, we consider it more likely that these clades are the result of ancestral polymorphism of the colonizing populations, or of post-colonization divergence within Hawai‘i.

We also found that tmrca estimates for stream species did not track island age, but rather fit into two periods of colonization: the Mid-Pliocene for N. granosa and the Pleistocene for A. stamineus, E. sandwicensis and S. stimpsoni (Fig. 3). This suggests that most of the endemic stream fishes did not colonize the archipelago until after all the extant high islands except the island of Hawai‘i had emerged. Thus, nearly any island, or multiple islands, could have been colonized first. As the estimated colonization time for N. granosa spans the period when O‘ahu was forming and Kaua‘i was fully emerged, one or both of these islands could have served as the source of dispersal to younger islands. As has been observed elsewhere (Carine, 2005; Kim et al., 2008; Salvo et al., 2010), evidence of discrete periods of colonization conforms to predictions of the colonization window hypothesis. Although further analysis of additional species might yield a record of continuous colonization (Price & Clague, 2002; Cook et al., 2010), colonization of the Hawaiian Islands by freshwater fauna appears to have proceeded according to temporally dynamic but time-specific ecological opportunities (Carine, 2005) reflecting the coincidence of suitable habitat during island ontogeny and individual species requirements.

The noteworthy coincidence of the estimated tmrca for the three stream fishes raises the possibility that large-scale climate fluctuations may have influenced community assembly across the Hawaiian Islands. The importance of climatic events (e.g. sea level changes) also has been noted for the assembly of endemic flora on Macaronesian and Mediterranean islands (Carine, 2005; Kim et al., 2008; Salvo et al., 2010). Estimated tmrca for the three fishes coincides with the Mid-Pleistocene Transition between 1.25 and 0.7 mya, which was an epoch encompassing dramatic changes in sea level and water temperatures (Clark et al., 2006; Fig. 3). This period has been more commonly associated with isolation and lineage divergence across the Indo-Pacific (Barber et al., 2002; Crandall et al., 2010; Kokita & Nohara, 2010; Lord et al., 2012), but it is possible that shifts in temperature and pressure gradients could also have favoured long-distance transport of pelagic larvae (Clark et al., 2006; Filippelli & Flores, 2009).

Inferring the tmrca using fossil-calibrated gene trees may overestimate divergence times because the approach does not correct for gene divergence predating speciation (Edwards & Beerli, 2000). Accordingly, analysis of species trees reflecting variation over multiple gene regions could improve tmrca estimates. Nonetheless, the date of the crown node is a conservative (i.e. upper time limit) approximation for colonization because dispersal might have occurred at any time along the branch between the crown node of the colonizing species’ haplotypes and its sister species’ common ancestor. Thus, with the exception of A. stamineus and its sister species A. guamensis (Lindstrom et al., 2012) – for which we estimated dispersal from their ancestral area between 0.672 and 1.314 mya (95% HPD: 0.265–1.149 and 0.566–2.199, respectively) – outgroups are distant and the closest relative is unknown or extinct for most of the Hawaiian taxa (Price &
Crandall et al., 2002; McDowall, 2003; Cowie & Holland, 2008). Hence, the date of the crown node is the best approximation as an upper time limit for colonization and usually preferred over other divergence estimates (Price & Clague, 2002).

Island age, population expansion and genetic variation

Our findings corroborate evidence of demographic expansion in amphidromous species elsewhere in the Indo-Pacific (Crandall et al., 2010; Hoareau et al., 2012; Chabarria et al., 2014). Prior studies have found genetic signatures indicating that estimated times of the most recent population expansions are associated with Pleistocene sea level fluctuations (Crandall et al., 2010; Hoareau et al., 2012; Lord et al., 2012; Chabarria et al., 2014). However, we found that the magnitude of expansions was strikingly variable, implying that demographic histories may vary independently across a species’ range and among codistributed species (Hoareau et al., 2012; Lord et al., 2012). Similarly, we found that estimates of time since the last demographic expansion and the magnitude of expansions were highly variable among species and islands and that S. stimpsoni has not undergone a significant expansion (Fig. 4, Table 1). Additionally, in three of the four species we examined, the most recent demographic expansion, either calculated with our estimated phylogenetic rate or with commonly used universal fish mitochondrial rates, appears to have occurred well after colonization, which indicates that estimates of colonization times based on demographic analyses of mismatch distribution may be grossly underestimated (Cook et al., 2008). These findings reinforce the perspective that colonization and subsequent expansion can be distinct processes shaped by different factors, and illustrate how demographic analyses are not a reliable proxy of colonization times. Furthermore, time estimates of population-level processes, such as demographic expansions, must be considered with caution because they might be overestimated due to time-dependency of phylogenetic substitution rates (Ho et al., 2005).

According to the range of substitution rates and mean time estimates (Table S4), it appears that the last demographic expansions of all species occurred within the last 350 000 years. This was an epoch of cyclical climatic change leading towards warmer and wetter conditions (Sheldon, 2006), and therefore consistent with demographic and geographic expansion as these circumstances favour the development of aquatic habitats. However, variation in each species signature of expansion, and consequently in genetic diversity, is suggestive of differential responses to climatic or other environmental change. For example, our estimates indicate that N. granosa underwent significant island-scale demographic expansions well prior to those exhibited by stream fishes, and although estimated times since the most recent expansion were similar, differences in magnitude were detected among islands. We detected ~17- to 20-fold increases in \( N_e \) on the islands of Moloka‘i, Maui and Hawai‘i, whereas the effective population on Kaua‘i barely doubled in size during the same time interval (Fig. 5). This suggests that the ontogeny of younger islands facilitated early colonization, expansion and diversification of \( N. \) granosa in the archipelago, which is consistent with ecological preferences of \( N. \) granosa (e.g. rocky substrates) being skewed to habitats found on younger islands (Hodges, 1992). Evidence of an ecological preference is further reflected in the observed pattern of genetic diversity, which was highest on Hawai‘i and negatively correlated with island age. Fishes, on the other hand, exhibited a trend of higher diversity on older islands, which is consistent with the expectation that time is a predictor of diversity (Parent & Crespi, 2006). Thus, the discordant patterns observed among species suggest that genetic diversity may only conform to island age when the distribution and prevalence of suitable habitat track time since formation. It remains possible, however, that post-colonization demographic changes, including expansion, bottlenecks and extinction followed by recolonization – all of which are expected in a highly stochastic volcanic landscape (Valenti et al., 2014) – could have erased or reshaped signatures of genetic diversity attributable to more ancient phenomena.

Additionally, observed patterns of genetic differentiation of amphidromous fauna among islands did not reflect island age. Rather, we detected remarkably low levels of differentiation in all four species, and little concordance among species or islands. Parallel genetic breaks only occurred in \( A. \) stamineus and \( E. \) sandwicensis between Hawai‘i and O‘ahu and between Hawai‘i and Moloka‘i (Table S3). This contrasts with prior work showing that a majority of species with marine dispersing larvae exhibit common barriers to dispersal between Kaua‘i and O‘ahu and between Maui and Hawai‘i (Toonen et al., 2011).

The very recent and nearly simultaneous expansions of \( A. \) stamineus and \( E. \) sandwicensis during the late Pleistocene also favours the hypothesis that factors other than geology shape the structure and diversity of oceanic island stream communities. Following the premise that ecological parallels promote parallel demographic histories, \( A. \) stamineus and \( S. \) stimpsoni might be expected to exhibit comparable demographic histories, as both utilize similar adult habitat. However, trophic ecology and life history could be comparable or stronger determinants of demographic history. Unlike \( A. \) stamineus and \( E. \) sandwicensis (Kido et al., 1993), \( S. \) stimpsoni is a diatom specialist (Kido, 1996), which allows \( S. \) stimpsoni to persist in streams that do not support either of the other species (Schoenfuss et al., 2004; Julius et al., 2005). Prior work also has shown that
S. stimpsoni is obligately amphidromous (Hogan et al., unpublished data), whereas A. stamineus is facultatively amphidromous (Hogan et al., 2014). Eleotris sandwicensis may also be facultatively amphidromous, as are Eleotris elsewhere (Huey et al., 2014). Although these associations merit consideration, further work will be necessary to better ascertain drivers of demographic history among Hawaiian stream fishes. We particularly encourage that work be done to determine whether local stochastic factors moderate the influence of broader parallels on demographic change over time.

Comparison of colonization and demographic histories across a suite of endemic species suggests that ecological factors shape the origins and assembly of oceanic island streams communities. Further constraining tmrca estimates could offer additional perspective on the timing and tempo of community assembly (Edwards &Beerli, 2000). For example, identifying and accounting for sister taxa could improve the accuracy and robustness of colonization time estimates. Prior studies of widely distributed and well-sampled Macrobachium crustaceans (Liu et al., 2007) and Indo-Pacific Sicydiinae gobies (Taillebois et al., 2014) would provide a foundation for exploring the value of this approach. Examining additional species would help confirm that our findings are representative of conditions in the Hawaiian Islands, and similarly, analyses of other archipelagos would show whether parallel processes govern global patterns of biodiversity in oceanic island streams.

Acknowledgments

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References


## Supporting information

Additional Supporting Information may be found online in the supporting information tab for this article:

**Appendix S1** Molecular methods: PCR amplification and sequencing.

**Table S1** Cytochrome *c* oxidase *I* and cytochrome *b* gene sequences of the *Neritina granosa* and the gobioid fishes data sets.

**Table S2** Fossil calibration points used in the molecular clock analyses for the *Neritina granosa* and the gobioid fishes data sets.

**Table S3** Genetic differentiation (*θ*<sub>ST</sub>) between islands for all species studied.

**Table S4** Time in thousands of years since the last demographic expansion.

**Figure S1** Rarefaction curves calculated for the accumulated number of haplotypes sampled for each of the four native Hawaiian freshwater species and island.

**Figure S2** Neighbour-joining trees inferred for the unique haplotypes of *Neritina granosa* (*coi*), *Awaous stamineus* (*cytB*), *Eleotris sandwicensis* (*cytB*) and *Sicyopterus stimpsoni* (*coi*). Only bootstrap values of ≥ 70 are shown.

**Figure S3** Bayesian skyline plots estimated in BEAST 1.8.0 for each species and island.

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