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REEF FISH ENDEMISM AND GENETIC CONNECTIVITY: PHYLOGEOGRAPHY OF ENDEMIC DAMSELFISHES IN THE HAWAIIAN ARCHIPELAGO

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by

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Abstract

REEF FISH ENDEMISM AND GENETIC CONNECTIVITY:

PHYLOGEOGRAPHY OF ENDEMIC DAMSELFISHES IN THE HAWAIIAN ARCHIPELAGO

Kimberly A. Tenggardjaja

While it was once widely accepted that there is plentiful larval dispersal and high levels of connectivity in marine populations, studies detecting self-recruitment and local larval retention in reef fishes have revealed that not all marine organisms demonstrate broad-scale dispersal. Isolated oceanic islands with their abundance of endemic species serve as excellent systems for studying the factors that may be influencing dispersal in marine populations. Furthermore, understanding patterns of dispersal in endemic species is relevant to conservation and management efforts that seek to protect these unique and vulnerable species. This dissertation employed molecular techniques to analyze the phylogeography of damselfishes endemic to the Hawaiian Archipelago and to draw conclusions about patterns of connectivity in endemic reef fishes. The endemic damselfishes that have been surveyed throughout the archipelago thus far all exhibit population structure (Chapter 1). This is consistent with the assumption that the low dispersal ability of endemic species results in population structure. However, some Hawaiian endemic reef fishes lack genetic structure in the archipelago, so population structure may be characteristic of endemics only in certain taxonomic families, such as the Pomacentridae. In contrast to their

endemic counterparts, widespread damselfishes that have broad distributions across the Indo-Pacific show high levels of connectivity across the Hawaiian Archipelago (Chapter 2). This side-by-side comparison of widespread and endemic damselfishes lends support to a proposed correlation between range size and dispersal ability, at least within the spatial scale of the archipelago. These widespread damselfishes, together with several species of widespread reef fishes that lacked population structure in the Hawaiian Islands, show genetic differentiation across the spatial scale of the Indo-Pacific. Thus, at a larger spatial scale (between archipelagoes), the relationship between range size and dispersal ability is more tenuous. Patterns of connectivity even vary with spatial scale in the endemic damselfish *Chromis verater* found on shallow and mesophotic reefs in the Hawaiian Archipelago and Johnston Atoll (Chapter 3). The dispersal abilities of *C. verater* do not appear to limit it in terms of vertical connectivity (7-113 m in this study), but there are some restrictions to horizontal connectivity across the Hawaiian Archipelago (2600 km) and between the archipelago and Johnston Atoll (separated by 860 km). Overall, the results from this dissertation lend support to the assumption that the low dispersal ability of endemic species results in less connectivity and more population structure than in widespread species. However, it remains unclear whether this applies to all reef fish families or if it holds true in locations besides the Hawaiian Archipelago. Also, these results illustrate the importance of considering spatial scale and phylogenetic constraint when drawing conclusions about dispersal in reef fishes.

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Introduction

Owing to the lack of barriers in the ocean and the bipartite life cycle characteristic of most marine organisms, a widely accepted paradigm was that larvae passively drifted on ocean currents, potentially being transported long distances (Caley et al. 1996; Palumbi 1994). Consequently, high levels of larval dispersal were predicted for marine species, resulting in little genetic differentiation among populations (Shaklee 1984; Sale 1991; Palumbi 1997; Cowen et al. 2000). However, studies demonstrating self-recruitment and local larval retention in reef fishes have illustrated that not all marine organisms exhibit broad-scale dispersal (Jones et al. 1999; Swearer et al. 1999; Swearer et al. 2002; Jones et al. 2005; Almany et al. 2007; Jones et al. 2009). Accordingly, research interests have shifted toward understanding the factors that are influencing connectivity in marine systems.

Isolated oceanic islands serve as excellent settings for investigating questions related to dispersal and connectivity in marine organisms. In particular, rates of endemism are strikingly high at these locations. Since endemic species usually arise after long periods of isolated local reproduction, they are model study organisms for understanding dispersal (Swearer et al. 2002). Studying dispersal patterns in endemic species is useful not only for understanding the population biology of marine organisms, but also for informing conservation and management strategies.

Biodiversity is one of the key biological criteria for deciding which sites should be designated as marine protected areas (MPAs), and endemism contributes to the biodiversity value of potential MPA sites (Roberts et al. 2003). Due to their

restricted geographic range sizes, endemic species are vulnerable to disturbances and may be at greater risk of local extirpation than more widespread species (Hughes et al. 2002). Endemism is a ubiquitous feature of coral reefs, which are becoming increasingly threatened by human activity (Burke et al. 2007). Knowledge on connectivity patterns in endemic species can be incorporated into the design of networks of MPAs. Patterns of dispersal can lead to conclusions about the flow of adults and larvae into and out of MPAs (Palumbi 2003; Avise 2004). Knowledge of how much connectivity is occurring between populations is important in deciding the size, positioning, and number of MPAs (Mora and Sale 2002).

To improve our understanding of dispersal in marine systems and to contribute knowledge relevant to conservation, this dissertation investigated patterns of connectivity in endemic damselfish species in the Hawaiian Archipelago. Located about 3800 km from the nearest continent, it is one of the most isolated archipelagoes in the world and is one of the most famously cited examples for endemism in terrestrial flora and fauna. Additionally, the Hawaiian Archipelago boasts an abundance of endemic marine species. About 25% of its shore fishes are endemics (Randall 1998), and the archipelago is a hotspot for reef fish endemism (Allen 2008). The Hawaiian Archipelago is comprised of two regions: the nine Northwestern Hawaiian Islands (NWHI) and the eight main Hawaiian Islands (MHI). In 2006, the NWHI were designated as the Papahānaumokuākea Marine National Monument, one of the largest marine protected areas in the world and the largest in the U.S. Owing to their remote location, the virtually uninhabited NWHI represent a nearly pristine

ecosystem that has only been lightly fished (Friedlander and DeMartini 2002). About one third of the reef fishes in the NWHI are endemic species, making this region a biodiversity hotspot (Friedlander 2008). Of particular interest to the management of the Hawaiian Archipelago are the degree and direction of connectivity between the NWHI and the MHI, and I interpreted my dissertation research results in order to address these questions.

Since direct observation of larvae is impractical, this dissertation utilized genetic surveys, which investigate larval dispersal indirectly and offer a practical alternative for evaluating patterns of connectivity (Palumbi 2003). Genetic markers are well-suited for studying connectivity because even minor levels of gene flow are enough to prevent genetic differentiation between populations (Hellberg et al. 2002; Palumbi 2003). Shallow or recent population structure can provide insight into current population demography, indicating minimal exchange of migrants between populations, and major events that influenced the evolution of a species can be manifested as deep or historical population structure (Avise 2004). Additionally, genealogical concordance across co-distributed species provides strong support that historical regional processes have shaped species distributions (McMillan and Palumbi 1995; Avise 2000; Avise 2004), providing the basis for the comparative approach to phylogeography (Bermingham and Moritz 1998).

The three chapters of this dissertation provide insight into different aspects of connectivity and dispersal in Hawaiian endemic damselfishes. Chapter one is a comparative phylogeography of the endemic species *Abudefduf abdominalis*,

Chromis ovalis, and *Chromis verater* throughout the archipelago. This chapter evaluated the assumption that endemic species have low dispersal ability, which should be manifested as low levels of connectivity and greater genetic structure between populations. Furthermore, I interpreted these results in light of whether these species showed a relationship between range size and dispersal ability. Combining these results with those from previously studied endemic damselfishes *Dascyllus albisella* and *Stegastes marginatus*, I was able to compare five out of the eight endemic damselfishes in the Hawaiian Archipelago. All five species showed evidence of genetic differentiation in the archipelago, supporting the assumption that endemic species have less connectivity and low dispersal ability. A review of genetic surveys for endemic reef fishes in the Hawaiian Archipelago revealed that genetic structure in endemics may be characteristic of certain reef fish families, including the Pomacentridae.

Chapter two built upon the study in chapter one by adding genetic surveys for two widespread damselfishes found in the Hawaiian Archipelago, *Abudefduf vaigiensis* and *Chromis vanderbilti*. Since they have broad distributions across the Indo-Pacific, comparing the phylogeographic patterns in these two species with the three endemics from chapter one allowed for a more explicit evaluation of whether endemic species really exhibit more genetic structure than widespread species. Results were consistent with the expected trend for widespread species to exhibit more connectivity within the Hawaiian Archipelago, but it remains to be determined whether this trend is restricted to certain reef fish families. In addition to sampling the

widespread species throughout the Hawaiian Archipelago, specimens were also collected from other locations in the Indo-Pacific. As a result, I was able to analyze how patterns of connectivity in the widespread species varied with spatial scale. While *A. vaigiensis* and *C. vanderbilti* showed little genetic differentiation within the Hawaiian Archipelago, they both exhibited strong genetic structure when analyzed across their Indo-Pacific ranges. A similar pattern was detected when reviewing other widespread reef fishes surveyed at a fine scale throughout the archipelago as well as on a broader scale across the Indo-Pacific. As such, I concluded that using geographic range size as indicator of dispersal ability may be more reliable at smaller scales (within archipelago) than at larger scales (between archipelagoes).

Lastly, chapter three focused on connectivity in the endemic damselfish *C. verater* in the Hawaiian Archipelago and Johnston Atoll. This species is found on both shallow (< 30 m) and mesophotic reefs (30 – 150 m), and the sampling design for this chapter allowed for the evaluation of both vertical and horizontal connectivity in this species. While *C. verater* did not exhibit any genetic differentiation by depth, there was low yet significant genetic structure across its range. A genetic break was detected between the archipelago and Johnston Atoll, and within the archipelago, population structure was driven by samples from the island of Hawaii and Lisianski. This represents the first connectivity study on a mobile organism that spans both shallow and mesophotic depths and serves as an initial reference point for future connectivity studies on mesophotic fishes.

CHAPTER 1

Endemism and dispersal in the Hawaiian Archipelago: Comparative phylogeography of three endemic damselfishes

Abstract

Endemic species of remote oceanic islands provide an opportunity for investigating the proposed correlation between range size and dispersal ability. Given that these species are only found within a restricted geographic range, it is assumed that they have limited dispersal ability, and consequently this would be reflected in high population genetic structure. To assess this correlation at a small scale and to determine if it may be related to specific reef fish families, here we employ a phylogeographic survey of three Hawaiian endemic damselfishes: *Abudefduf abdominalis*, *Chromis ovalis*, and *Chromis verater*. Data from the mitochondrial markers cytochrome *b* and control region show that these species all exhibit genetic structure. Combining these results with data from a previous study on *Dascyllus albisella* and *Stegastes marginatus*, five of the eight damselfish species endemic to the Hawaiian Archipelago show evidence of genetic structure. This suggests that genetic structure may be characteristic of Hawaiian endemic damselfishes. Though individual patterns of connectivity varied, these five endemic damselfishes showed a trend of limited connectivity between the two regions (Northwestern Hawaiian Islands and main Hawaiian Islands) of the archipelago, providing direction for

management of marine resources to ensure connectivity of Hawaii's endemic marine species.

Introduction

Due to an apparent lack of barriers in the ocean and the potential for larvae to disperse long distances via ocean currents, the previously long-held paradigm has been that there is abundant connectivity and consequently little genetic differentiation between populations of marine organisms (Shaklee 1984; Sale 1991; Caley et al. 1996; Palumbi 1997; Cowen et al. 2000; Eble et al. 2011a). However, studies demonstrating self-recruitment and local larval retention indicate that not all marine organisms are exhibiting broad-scale larval dispersal (Jones et al. 1999; Swearer et al. 1999; Swearer et al. 2002; Jones et al. 2005; Almany et al. 2007; Jones et al. 2009). In these circumstances, research has shifted toward understanding the factors mediating connectivity in marine systems and whether there are general patterns related to phylogenetic groups, pelagic larval duration, ecology, or behavior (Lester et al. 2007; Bradbury et al. 2008; Reece et al. 2011; Selkoe and Toonen 2011). Nevertheless, generalizations have proven elusive.

Isolated oceanic islands provide an excellent opportunity for investigating dispersal in marine organisms. Rates of endemism are markedly high, and since endemic species are usually the products of long periods of isolated local recruitment and reproduction, they serve as model study organisms for understanding dispersal (Swearer et al. 2002). The general assumption has been that the constrained geographic range sizes of endemic species reflect their limited dispersal abilities.

However, retention-favorable traits are not common characteristics of oceanic endemics (Robertson 2001; Swearer et al. 2002). For instance, pelagic larval duration (PLD) is a life history trait that provides an intuitive gauge of dispersal, by the logic that more time spent in the plankton results in greater dispersal and connectivity (Waples 1987; Doherty et al. 1995; Shanks et al. 2003). However, endemic reef fishes do not show a trend toward shorter PLDs relative to widespread congeners, and some studies have shown the opposite (Victor and Wellington 2000; Robertson 2001; Lester et al. 2007).

While no diagnostic life history traits related to endemism have been identified, there is support for a positive correlation between dispersal ability and range size (Brothers and Thresher 1985; Lester and Ruttenberg 2005). Eble et al. (2009) sought to evaluate this relationship through a phylogeographic comparison in the Hawaiian Archipelago of three surgeonfishes (family Acanthuridae) with different geographic ranges. The Hawaiian endemic was predicted to exhibit less genetic connectivity (more genetic structure) than widespread members of the family. Results supported this hypothesis, with the endemic species demonstrating more, albeit weak, genetic structure than the two species with broader geographic distributions. Likewise, in a meta-analysis of tropical reef fishes, the relationship between range size and dispersal potential, as inferred from PLD, was found to vary between oceans, with a significant correlation demonstrated in the Indo-Pacific (Lester and Ruttenberg 2005). This relationship strengthened at higher taxonomic levels and was significant in the damselfishes (Pomacentridae), wrasses (Labridae), and butterflyfishes

(Chaetodonidae), indicating that phylogenetic affiliation is a component of this relationship.

Here we assess genetic connectivity across the Hawaiian Archipelago in three endemic damselfishes: *Abudefduf abdominalis, Chromis ovalis,* and *Chromis verater*. These three species have ranges that span the entire extent of the Hawaiian Archipelago, and *C. verater* is also found at Johnston Atoll, which is located about 860 km south of Hawaii. Since the marine fauna at Johnston Atoll is predominantly Hawaiian (Randall et al. 1985), species that occur at both locations are still referred to as Hawaiian endemics. The Hawaiian Archipelago is one of the most isolated archipelagoes in the world, boasting an abundance of endemic species, including 25% endemism for shore fishes (Randall 1998; Allen 2008). The archipelago comprises eight Main Hawaiian Islands (MHI), which are "high islands" of volcanic basaltic composition, and nine Northwestern Hawaiian Islands (NWHI), which are mostly "low islands" with coral reefs and sand banks overgrowing subsided basaltic foundations (Juvik et al. 1998). The NWHI constitute the Papahānaumokuākea Marine National Monument, one of the largest marine protected areas in the world and the largest in the U.S. The degree of connectivity between the NWHI and the MHI is of particular interest to the management of marine resources in the archipelago. The vast and uninhabited marine protected area (NWHI), adjacent to a large community that depends on the sea for nutrition (MHI), is postulated to have a spillover effect (Roberts et al. 2001; Goñi et al. 2008). Our fine-scale sampling

throughout the Hawaiian Islands can illustrate whether the NWHI have the potential to subsidize the overexploited reefs of the MHI.

Our study of endemic Hawaiian damselfishes is intended to build upon a previous survey of two endemic damselfishes (*Stegastes marginatus* and *Dascyllus albisella*). Ramon et al. (2008) found genetic structure in both species, in contrast to the majority of reef fishes surveyed across Hawaii, which show no structure within the archipelago [(Craig et al. 2007; Craig et al. 2010; DiBattista et al. 2011; Eble et al. 2011a) but see (Toonen et al. 2011)]. Furthermore, one of our study species, *C. verater*, is the subject of a separate report on connectivity between shallow and mesophotic (> 30 m) reef habitats (Chapter 3). No vertical (depth-related) structure was identified in this species, but the Hawaiian Archipelago was significantly differentiated from adjacent Johnston Atoll (cyt*b*: $\Phi_{ST} = 0.068$, $P < 0.001$; CR: $\Phi_{ST} = 0.116$, $P < 0.001$).

The three damselfishes surveyed for the current study were chosen because they are abundant throughout the entire archipelago and belong to the sister genera of Abudefdufinae and Chrominae (Cooper et al. 2009). This phylogenetic constraint should reduce variable traits among species. There are a total of eight Hawaiian endemic damselfishes, so utilizing results from the previous studies, we are able to examine phylogeographic patterns across five of these species. Given that two Hawaiian endemic damselfishes already show significant genetic structure, we would predict genetic differentiation across the ranges of *A. abdominalis*, *C. ovalis*, and *C. verater* as well, providing more support for a correlation between range size and

dispersal ability. Additionally, this finding may indicate that genetic differentiation is typical of Hawaiian endemic damselfishes.

Material and Methods

Tissue collection

Collections of 334 *A. abdominalis*, 412 *C. ovalis*, and 425 *C. verater* specimens (fin clips) were made at 13-15 locations across the Hawaiian Archipelago from 2009-2012 (Figure 1). An additional 47 *C. verater* specimens were collected at Johnston Atoll. Collections were made with pole spears or hand nets while snorkeling or on SCUBA.

DNA extraction, marker amplification, and sequencing

Tissue samples were preserved in salt-saturated DMSO (Seutin et al. 1991). All of the protocol for DNA extraction, marker amplification and sequencing are identical to those used in Chapter 3. Cytochrome *b* (cyt*b*) and mitochondrial control region (CR) sequences of *C. verater* generated for Chapter 3 were used in this study. Sequences were aligned and edited using GENEIOUS R6 (Biomatters, LTD, Auckland, NZ). Alignments of cyt*b* were unambiguous, while CR contained multiple indels of 1-2 bp. Unique haplotypes for each marker were identified in ARLEQUIN 3.5 (Excoffier and Lischer 2010).

Genetic diversity and population structure analyses

Haplotype diversity (h) and nucleotide diversity (π) were calculated in ARLEQUIN. Population structure was analyzed using analyses of molecular variance (AMOVAs) and population pairwise Φ_{ST} comparisons in ARLEQUIN. The Φ_{ST}

fixation index incorporates genetic distance and ranges from 0 to 1, with low values indicating a lack of genetic structure and high values indicating complete genetic differentiation. Significance of pairwise Φ_{ST} comparisons and AMOVA calculations was tested with 10,000 permutations, and to correct for multiple comparisons, a modified false discovery rate method was implemented (Benjamini and Yekutieli 2001). We determined the best model of sequence evolution for each marker in jMODELTEST 2 (Posada and Crandall 1998; Guindon and Gascuel 2003). Because the models identified by the Akaike information criterion were not available in ARLEQUIN, we selected the Tamura-Nei model (Tamura and Nei 1993). For *A. abdominalis*, populations at Gardner Pinnacles (N=1) and Nihoa (N=1) were not included in most analyses due to small sample sizes. However, these samples were included in haplotype networks. Parsimony-based haplotype networks for each marker were constructed in R using haploNet in the package PEGAS 0.5-1 (Paradis 2010). Haplotype frequencies used in these networks were calculated in ARLEQUIN.

To test for a signal of population expansion, Fu's *F*s test for neutrality and mismatch distributions were calculated in ARLEQUIN with 10,000 permutations (Rogers and Harpending 1992; Fu 1997). Significant negative F_s values indicate an excess of rare haplotypes, which can be a signal of selection or recent population expansion. For cyt*b* data, we fitted the population age parameter τ and pre and post-expansion population size parameters θ_0 and θ_1 to estimate the time to coalescence (Li 1977; Rogers and Harpending 1992). Time to coalescence was calculated with $\tau = 2\mu T$, where T is the age of the population in generations and μ is the annual fragment

mutation rate. Since the generation times of *A. abdominalis*, *C. ovalis*, and *C. verater* are unknown, we conditionally used a generation time of 3 years based on estimates in the damselfish *Chromis chromis* (Dulcic and Kraljevic 1995). A mutation rate of 2% per million years between lineages or 1% within lineages for cyt*b* was applied (Bowen et al. 2001).

To avoid making *a priori* assumptions about the locations of genetic barriers, we used BARRIER 2.2 (Manni et al. 2004), which employs a computational geometry approach to visualize genetic barriers in geographic space. The software implements Monmonier's maximum-difference algorithm to compare a distance matrix (e.g. matrix of pairwise Φ_{ST} values between locations) with a matrix of geographic distances. *A posteriori* AMOVAs subsequently were performed on population groupings inferred by BARRIER output. The barrier configurations with the greatest variation among groups (as measured by Φ_{CT}) are reported here.

Mantel tests were performed to test for isolation by distance. Mantel tests were run in the *vegan* package in R 3.0.2 software with 10,000 permutations, using matrices of pairwise Φ_{ST} values and geographic distance as calculated by the Geographic Distance Matrix Generator (Oksanen et al. 2013; Ersts 2014). Mantel tests were performed with matrices that included negative Φ_{ST} and with negative values converted to zeroes. If AMOVAs detected significant structure among groups comprised of more than one sample location, partial Mantel tests were run, incorporating a third dissimilarity matrix that took into account the regional structure.

Partial Mantel tests can help distinguish whether isolation by distance or regional population structure accounts for more genetic variance in data (Meirmans 2012).

Results

A 670 bp segment of the cyt*b* gene was sequenced for *A. abdominalis*, 660 bp for *C. ovalis*, and 719 bp for *C. verater*. For CR, 400 bp were sequenced for *A. abdominalis*, 388 for *C. ovalis*, and 394 bp for *C. verater*. Summary statistics for number of haplotypes (*H*), haplotype diversity (*h*), nucleotide diversity (π), and Fu's *Fs* are listed in Table 1. For *C. ovalis* and *C. verater*, overall haplotype diversity for cyt*b* was high with *h* = 0.9501 and 0.9077 respectively. Conversely, overall haplotype diversity for cyt*b* in *A. abdominalis* was lower with *h* = 0.5865. For CR, overall haplotype diversity approached saturation for all species with *h* = 0.9955-0.9997.

All three species had negative and significant individual Fu's *Fs* values for both mtDNA markers at most locations (Table 1). Overall Fu's *Fs* values for both markers were negative and significant for all species (cyt*b*: *Fs* = -25.6820 to -29.8590, CR: *Fs* = -23.4009 to -23.7039). Unimodal mismatch distributions in cyt*b* do not indicate significant deviation from a sudden demographic expansion model for any of the species. Based on a generation time of 3 years and a mutation rate of 2% per million years (1% within lineages), mismatch analyses indicate the time since coalescence to be on the order of 68,000 years for *A. abdominalis*, 249,000 years for *C. ovalis*, and 163,000 years for *C. verater* (Table 2). Since we used estimates for generation time and mutation rate, coalescence time calculations are approximations at best but are useful for comparisons between species in this study.

Overall estimates for Φ_{ST} varied by marker and by species (Table 3). For *A. abdominalis*, the Φ_{ST} based on cyt*b* was not significant (Φ_{ST} = 0.0063, *P* = 0.0911), but CR yielded weak yet significant genetic structure (Φ_{ST} = 0.0123, *P* = 0.0034). For *C. ovalis*, fixation indices for both markers showed weak but significant structure (cyt*b*: Φ_{ST} = 0.0121, *P* = 0.0047; CR: Φ_{ST} = 0.0059, *P* = 0.0370). *Chromis verater* had the highest significant Φ_{ST} values across its range of the Hawaiian Archipelago and Johnston Atoll (cyt*b*: Φ_{ST} = 0.0232, *P* < 0.0001; CR: Φ_{ST} = 0.0363, *P* < 0.0001). When analysis was limited to only the Hawaiian Archipelago, the fixation indices for *C. verater* dropped but remained significant (cyt*b*: Φ_{ST} = 0.0093, *P* = 0.0197; CR: Φ_{ST} = 0.0115, *P* = 0.0087).

Pairwise Φ_{ST} comparisons revealed different patterns of genetic structure among the sampling locations for each species (Table 4, Table 5, and Table 6). *Abudefduf abdominalis* had only 6 significant comparisons for cyt*b*, but 19 for CR with 7 of those including comparisons with Niihau (Φ_{ST} = 0.0495 – 0.0963), based on *N* = 8. BARRIER identified a genetic break between Necker and Niihau, and *a posteriori* AMOVAs confirmed this as a significant break in both markers (cyt*b*: Φ_{CT} = 0.0107, *P* = 0.0044; CR: Φ_{CT} = 0.0098, *P* = 0.0123).

Chromis ovalis had 18 significant comparisons for cyt*b* and 13 for CR, with Pearl and Hermes included in 9 and 4 of these comparisons, respectively. Since most of these comparisons involved populations east of Pearl and Hermes, *a posteriori* AMOVAs simulating a genetic break between Pearl and Hermes and Lisianski were run, which detected weak yet significant structure for both markers (cyt*b*: Φ_{CT} =

0.0192, $P = 0.0049$; CR: $\Phi_{CT} = 0.0096$, $P = 0.0287$). AMOVAs did not support any of the genetic breaks identified in BARRIER for this species.

Chromis verater showed significant differentiation of Johnston Atoll in most pairwise comparisons for cyt*b* and CR (Table 6). Within the Hawaiian Archipelago, the Big Island of Hawaii was significantly different in at least half of the pairwise comparisons for *C. verater* (6 for cyt*b*; 7 for CR). BARRIER detected a genetic break between Johnston Atoll and the Hawaiian Archipelago, which was supported by moderate Φ_{CT} values (cyt*b*: $\Phi_{ST} = 0.0679$, $P < 0.0001$; CR: $\Phi_{ST} = 0.1156$, $P < 0.0001$). Also, BARRIER identified a genetic break between Maui and the Big Island of Hawaii, and *a posteriori* AMOVAs confirmed this as a significant break (cyt*b*: $\Phi_{ST} = 0.0211$, $P = 0.0194$; CR: $\Phi_{ST} = 0.0352$, $P = 0.0045$).

In addition to looking at individual patterns of genetic structure among sampling locations, we compared the proportion of significant population pairwise Φ_{ST} comparisons: 1) within the NWHI, 2) within the MHI, and 3) between the NWHI and the MHI. The most significant comparisons occurred between locations in the NWHI and those in the MHI (Table 7).

Parsimony-based haplotype networks for each marker illustrated similar patterns in all species. Networks for cyt*b* were dominated by widely distributed common haplotypes (Figure 2). The network for *A. abdominalis*, which had the lowest haplotype diversity, was dominated by one common haplotype. *Chromis ovalis* and *C. verater*, which had similarly high haplotype diversities, had multiple common haplotypes in the networks. In all species, the most common haplotypes

were present at nearly every sampling location. In contrast, the networks for CR in all three species showed an abundance of haplotypes observed in single individuals, as expected with haplotype diversities $h > 0.99$ (Figure 3). While there did not appear to be much geographic clustering of haplotypes, the CR haplotype network for *C. verater* showed some grouping of Johnston Atoll haplotypes, which supports the genetic differentiation between these samples and Hawaiian samples (Figure 3c).

Applying the Mantel test for cyt*b*, *C. ovalis* and *C. verater* did not exhibit isolation by distance, but *A. abdominalis*, the species with the lowest overall population structure, had a significant signal ($r = 0.5308$, $P = 0.0003$). Since AMOVAs with *A. abdominalis* populations grouped into the NWHI and the MHI were significant for both markers, a partial Mantel test for cyt*b* was run accounting for this regional structure. The isolation by distance signal was weaker but still significant ($r = 0.4685$, $P = 0.0005$). For CR, no Mantel tests or partial Mantel tests were significant (data not shown).

Discussion

In accordance with the expected correlation between dispersal ability and range size, the Hawaiian endemic damselfishes *A. abdominalis*, *C. ovalis*, and *C. verater* all demonstrated evidence of genetic differentiation in their ranges. Although the species differed in terms of the specific patterns of connectivity among locations, in general, there was a trend toward more genetic structure between locations in the NWHI and the MHI, which has implications for the management of marine resources in the Hawaiian Archipelago. Additionally, the genetic breaks exhibited by each

species were concordant with previously identified barriers to dispersal in the archipelago, providing direction as to how management units should be defined.

Population structure of Hawaiian endemic damselfishes

Our genetic survey based on mitochondrial markers cyt*b* and CR revealed that these three endemic damselfishes exhibited low but significant population structure within their ranges. Very few migrants per generation are sufficient to prevent genetic differentiation between populations (Allendorf and Phelps 1981), so even weak genetic structure that is statistically significant indicates some restriction to gene flow (Planes 2002). For each species in this study, global Φ_{ST} values were significant within the Hawaiian Archipelago, and each species exhibited multiple significant pairwise Φ_{ST} comparisons for both markers. As previously mentioned, the only other Hawaiian endemic damselfishes for which genetic surveys have been conducted are *D. albisella* and *S. marginatus*. Similar to our results for *A. abdominalis*, *C. ovalis*, and *C. verater*, both of these species had multiple significant pairwise Φ_{ST} comparisons for the mitochondrial control region. Combining results for these two studies, five out of eight endemic damselfishes exhibit significant genetic structure, supporting the hypothesis that the restricted ranges of endemic species is representative of lower dispersal ability. Without data on the two remaining species *Chromis hanui*, *Chromis struhsakeri*, and *Plectroglyphidodon sindonis*, we cannot definitively conclude that all Hawaiian endemic damselfish species demonstrate population subdivision over their range, but results so far support this trend.

Anomalies in A. abdominalis

The cyt*b* results for *A. abdominalis* produced several differences from those of the two *Chromis* species: 1) a significant isolation by distance signal, 2) one common haplotype dominating the haplotype network, and 3) lower haplotype diversity. The high mutation rate and higher diversity of the CR may have masked these characteristics. While *A. abdominalis*, *C. ovalis*, and *C. verater* share similar life history traits, such as spawning seasonality, feeding behavior, and egg type, they differ in PLD. The PLD for *A. abdominalis* is 17-18 days, while the PLDs for *C. ovalis* and *C. verater* are estimated to be 30 days and as long as 3 months respectively. The isolation by distance signal for *A. abdominalis* may result from the shorter PLD and thus weaker dispersal (Shanks et al. 2003), but the relationship between PLD and dispersal distance remains controversial (Weersing and Toonen 2009; Mora et al. 2012; Treml et al. 2012). One notable result from our data sets is a rank order, wherein the species with the longest PLD (*C. verater*) has the most population structure and the species with the shortest PLD (*A. abdominalis*) has the least structure, exactly contrary to expectations.

In addition to PLD, the depth ranges for the *Chromis* species (5-199 m) differ from that of *A. abdominalis* (1-50 m). Sea level fluctuations during the Pleistocene resulted in loss of habitat and possible extirpations of shallow-water fauna. *Chromis ovalis* and *C. verater* may have retreated to refugia in the deeper parts of their depth range, while *A. abdominalis* may have been more susceptible to these changes in sea level (Fauvelot et al. 2003). The refugia populations of the *Chromis* species may have become genetically differentiated over time and subsequently reestablished

connectivity once sea levels rose, resulting in haplotype networks comprised of several common haplotypes. Conversely, in *A. abdominalis*, the network is dominated by a single haplotype, and its lower haplotype diversity may reflect a population bottleneck following sea level change and subsequent population expansion. Significant negative Fu's F_s values, unimodal mismatch distributions, and shallow coalescence times reinforce that all three species have experienced recent population expansions, possibly as the result of past fluctuations in climate and sea level.

Phylogeographic patterns of Hawaiian marine endemic reef fishes

Since multiple genetic surveys exist for Hawaiian endemic reef fishes, we can compare results to investigate further the relationship between range size and dispersal ability. Lester and Ruttenberg (2005) found a correlation between PLD and range size for certain reef fish families but not for others. The current study demonstrates that most Hawaiian endemic species in the Pomacentridae have genetic structure. The Hawaiian grouper, *Hyporthodus quernus*, is the only member of Serranidae that is endemic to the Hawaiian Archipelago and Johnston Atoll. Population pairwise comparisons for CR and nuclear microsatellite markers demonstrated low but significant structure within the Hawaiian Islands (Rivera et al. 2011). For contrast, the widespread grouper *Cephalopholis argus* showed no population structure from the central Pacific (Line Islands) to northeastern Australia, a distance of about 8000 km (Gaither et al. 2011a). In the surgeonfishes (Acanthuridae), the Hawaiian endemic *Ctenochaetus strigosus* exhibited low to moderate genetic structure in population pairwise comparisons for cyt*b* (Eble et al.

2009). The surgeonfish *Zebrasoma flavescens*, which occurs in the Northwest Pacific but is only abundant in the Hawaiian Archipelago, shows multiple population breaks within the archipelago (Eble et al. 2011b). In the same family, *Acanthurus nigroris*, recently reclassified as a Hawaiian endemic (Randall et al. 2011), showed low yet significant population structure in pairwise comparisons and a significant global Φ_{ST} value across its range, but this is driven by the Johnston Atoll specimens (DiBattista et al. 2011). In the wrasses (Labridae), *Halichoeres ornatissimus* only exhibited significant genetic differentiation in pairwise comparisons with Johnston Atoll and otherwise did not show significant structure within the Hawaiian Islands (Ludt et al. 2012). Hawaiian endemic butterflyfishes (Chaetonidae) also lacked population structure, with cyt*b* data revealing no genetic structure for *Chaetodon fremblii*, *Chaetodon miliaris*, or *Chaetodon multicinctus* (Craig et al. 2010). Though some Hawaiian (or North Pacific) endemics show structure and others do not, this should be interpreted against findings for widespread Indo-Pacific fishes that occur in Hawaii, which generally show a lack of population structure across this archipelago (Craig et al. 2007; Reece et al. 2010; Eble et al. 2011a; Gaither et al. 2011b; Andrews et al. 2014; Szabó et al. 2014).

Besides the Pomacentridae, genetic surveys of Hawaiian endemics are only available for one to three species within other reef fish families, making it difficult to draw robust conclusions regarding whether taxonomic family is a good predictor of the relationship between range size and dispersal ability. Superficially, there appears to be a trend in the families that have genetic data for more than one endemic species.

Genetic structure is seen in five endemic damselfishes and in three surgeonfishes, though structure in *A. nigroris* may be debatable. Meanwhile, the three endemic species in the Chaetonidae lacked genetic structure. Additional genetic surveys of Hawaiian endemic reef fishes would provide interesting perspective on whether there is consistency in the relationship between range size and dispersal ability at the family taxonomic level. Furthermore, evaluating this correlation truly requires side-by-side comparisons of both Hawaiian endemics and species that have widespread ranges.

Connectivity between the NWHI and the MHI and concordant genetic breaks in the Hawaiian Archipelago

While individual patterns of genetic connectivity among sampling locations varied by species, our study found that that there was more genetic structure between the NWHI and the MHI than within either region (Table 8). Additionally, AMOVAs for *A. abdominalis* exhibited a significant genetic break between these two regions (Table 4). Results for *D. albisella* and *S. marginatus* also supported this trend with 57% and 50% of respective significant pairwise comparisons occurring between the NWHI and the MHI (Ramon et al. 2008). Though *A. abdominalis*, *C. ovalis*, and *C. verater* demonstrated weak genetic structure, this still demonstrates some restriction to gene flow between these two regions. Since these species are only found in the Hawaiian Islands and Johnston Atoll, management plans should take into account spatial patterns of connectivity exhibited by endemic species, in order to preserve the unique biodiversity within this region.

Multispecies genetic surveys are useful for implementing ecosystem-based management and highlighting potential management units. This study detected several significant genetic breaks in the archipelago: 1) between the NWHI and the MHI (*A. abdominalis*), 2) east of Pearl and Hermes (*C. ovalis*), and 3) between Maui and the Big island of Hawaii (*C. verater*). These breaks are consistent with three previously identified barriers in the Hawaiian Archipelago. Toonen et al. (2011) compared genetic surveys of 27 taxonomically diverse species on Hawaiian coral reefs and found four concordant barriers to dispersal. Agreement between those breaks and the ones in our study contributes to the proposal that these barriers delineate potential zones of resource management (Toonen et al. 2011). Moreover, the consistency in genetic breaks across different taxonomic levels reinforces the conclusion that abiotic factors are likely responsible for limitations to connectivity within the archipelago.

Conclusions

Based on the results from this study and that of Ramon et al. (2008), the five Hawaiian endemic damselfishes surveyed to date exhibit genetic structure across their ranges. Since we predicted that these endemic species would demonstrate genetic differentiation as a result of their limited dispersal abilities, this finding supports a relationship between range size and dispersal ability. However, this would be more strongly supported if widespread damselfish species demonstrated lower genetic structure across the same geographic range as the endemic species. Our review of genetic surveys of Hawaiian endemic reef fishes indicates that the presence of genetic

structure in endemic species may be specific to particular taxonomic families, but again studies on more species would be needed to validate this claim. Since our study was limited to the Hawaiian Archipelago and Johnston Atoll, it is difficult to extend our conclusions to other archipelagos as place-specific abiotic factors (e.g. oceanography, geologic history) undoubtedly contribute to restricting the dispersal of endemic species. Consequently, we limited the interpretation of our results to trends that may be occurring among endemic reef fishes in the Hawaiian Archipelago.

Our results on the Hawaiian endemics *A. abdominalis*, *C. ovalis*, and *C. verater* not only reinforce the significance of previously identified genetic breaks in the Hawaiian Archipelago, but also illustrate a general trend in connectivity in Hawaiian endemic reef fishes. The preservation of marine biodiversity inherently calls for a better understanding of connectivity patterns in endemic species. The genetic structure between locations in the NWHI and the MHI in our study species and in Ramon et al. (2008) indicates that the protected status of the Papahānaumokuākea Marine National Monument may not replenish depleted reef resources in the MHI. Taking measures to ensure connectivity between protected areas in the MHI will aid in maintaining the biodiversity unique to this archipelago.

Tables

Table 1: Molecular diversity indices for *A. abdominalis*, *C. ovalis*, and *C. verater*. Number of individuals (*N*), number of haplotypes (*H*), nucleotide diversity (π), haplotype diversity (*h*), and Fu's Fs are listed for cyt*b* and CR. F_S values in bold are significant ($P < 0.05$). For *A. abdominalis*, populations at Gardner Pinnacles (*N*=1) and Nihoa (*N*=1) were not included in most analyses due to small sample sizes.

	N	***H***		***π***				***h***				**Fu's *Fs***	
Sample location		**cyt*b***	**CR**	**cyt*b***		**CR**		**cyt*b***		**CR**		**cyt*b***	**CR**
A. abdominalis													
Hawaiian Archipelago													
Kure	33	7	27	0.0014	± 0.0011	0.0358	± 0.0183	0.5833	± 0.0944	0.9867	± 0.0111	**-2.9312**	**-8.5606**
Midway	48	7	40	0.0012	± 0.0010	0.0380	± 0.0192	0.5408	± 0.0808	0.9920	± 0.0061	**-2.9243**	**-18.492**
Pearl and Hermes	29	7	27	0.0010	± 0.0009	0.0335	± 0.0173	0.5222	± 0.1084	0.9951	± 0.0106	**-4.3629**	**-13.351**
Lisianski	16	6	15	0.0011	± 0.0010	0.0333	± 0.0177	0.5417	± 0.1472	0.9917	± 0.0254	**-3.616**	**-4.5074**
Laysan	32	12	27	0.0018	± 0.0013	0.0351	± 0.0180	0.7157	± 0.0859	0.9839	± 0.0144	**-8.7456**	**-9.5195**
Maro Reef	30	11	25	0.0019	± 0.0014	0.0347	± 0.0179	0.7448	± 0.0821	0.9862	± 0.0129	**-6.9081**	**-7.9587**
French Frigate Shoals	29	11	28	0.0012	± 0.0010	0.0335	± 0.0173	0.6207	± 0.1055	0.9975	± 0.0099	**-10.288**	**-16.032**
Necker	20	7	20	0.0015	± 0.0011	0.0345	± 0.0181	0.6421	± 0.1176	1.0000	± 0.0158	**-3.6691**	**-9.9856**
Niihau	8	3	8	0.0007	± 0.0008	0.0248	± 0.0145	0.4643	± 0.2000	1.0000	± 0.0625	-0.999	-2.2287
Kauai	25	6	25	0.0010	± 0.0009	0.0393	± 0.0202	0.4267	± 0.1216	1.0000	± 0.0113	**-3.3803**	**-13.487**
Oahu	28	8	27	0.0015	± 0.0011	0.0337	± 0.0174	0.5423	± 0.1117	0.9974	± 0.0104	**-4.2214**	**-14.903**
Maui	28	10	24	0.0013	± 0.0010	0.0290	± 0.0151	0.6349	± 0.1043	0.9868	± 0.0141	**-8.3239**	**-9.5825**
Island of Hawaii	19	6	17	0.0012	± 0.0010	0.0343	± 0.0180	0.5380	± 0.1330	0.9883	± 0.0210	**-2.9396**	**-4.6294**
All of Hawaiian Archipelago	345	44	235	0.0013	± 0.0010	0.0343	± 0.0171	0.5865	± 0.0318	0.9955	± 0.0009	**-29.859**	**-23.704**

C. ovalis												
Hawaiian Archipelago												
Kure	29	22	29	0.0046	± 0.0028	0.0780	± 0.0391	0.9778	± 0.0153	1.0000	± 0.0091	**-20.4731**	**-10.9002**
Midway	38	27	38	0.0050	± 0.0029	0.0679	± 0.0339	0.9659	± 0.0177	1.0000	± 0.0060	**-25.2805**	**-19.8388**
Pearl and Hermes	37	20	36	0.0049	± 0.0029	0.0701	± 0.0349	0.9459	± 0.0182	0.9985	± 0.0067	**-11.9549**	**-15.0322**
Lisianski	4	3	4	0.0028	± 0.0024	0.0526	± 0.0355	0.8333	± 0.2224	1.0000	± 0.1768	0.0062	1.0580
Laysan	33	19	33	0.0040	± 0.0024	0.0691	± 0.0346	0.9015	± 0.0432	1.0000	± 0.0075	**-13.6652**	**-15.1002**
Maro Reef	28	18	28	0.0054	± 0.0032	0.0717	± 0.0361	0.9550	± 0.0237	1.0000	± 0.0095	**-10.4982**	**-10.8746**
Gardner Pinnacles	15	13	15	0.0057	± 0.0034	0.0756	± 0.0393	0.9714	± 0.0389	1.0000	± 0.0243	**-8.3469**	-3.2110
French Frigate Shoals	31	19	31	0.0048	± 0.0029	0.0691	± 0.0347	0.9613	± 0.0191	1.0000	± 0.0082	**-12.3126**	**-13.5519**
Necker	29	21	29	0.0054	± 0.0031	0.0689	± 0.0347	0.9286	± 0.0418	1.0000	± 0.0091	**-16.0795**	**-12.0406**
Nihoa	28	21	28	0.0043	± 0.0026	0.0748	± 0.0376	0.9418	± 0.0371	1.0000	± 0.0095	**-19.6826**	**-10.5918**
Niihau	20	16	19	0.0045	± 0.0027	0.0665	± 0.0340	0.9474	± 0.0435	0.9947	± 0.0178	**-12.9546**	**-4.1037**
Kauai	29	17	29	0.0043	± 0.0026	0.0724	± 0.0363	0.9360	± 0.0284	1.0000	± 0.0091	**-10.6489**	**-11.4865**
Oahu	31	19	31	0.0048	± 0.0028	0.0755	± 0.0378	0.9462	± 0.0253	1.0000	± 0.0082	**-12.3276**	**-12.4146**
Maui	29	21	28	0.0050	± 0.0029	0.0719	± 0.0361	0.9729	± 0.0173	0.9975	± 0.0099	**-17.0340**	**-8.8047**
Island of Hawaii	31	23	31	0.0053	± 0.0031	0.0667	± 0.0335	0.9699	± 0.0197	1.0000	± 0.0082	**-19.5568**	**-13.8980**
All of Hawaiian Archipelago	412	144	387	0.0049	± 0.0028	0.0681	± 0.0331	0.9501	± 0.0069	0.9997	± 0.0002	**-25.6820**	**-23.4292**
C. verater												
Hawaiian Archipelago												
Kure	6	6	6	0.0026	± 0.0020	0.0683	± 0.0405	1.0000	± 0.0962	1.0000	± 0.0962	**-4.5527**	0.3120
Midway	36	20	36	0.0035	± 0.0021	0.0760	± 0.0378	0.9190	± 0.0322	1.0000	± 0.0065	**-15.3135**	**-12.9842**
Pearl and Hermes	43	21	41	0.0032	± 0.0020	0.0817	± 0.0404	0.9313	± 0.0220	0.9978	± 0.0056	**-16.1515**	**-12.6525**
Lisianski	5	4	5	0.0022	± 0.0018	0.0688	± 0.0428	0.9000	± 0.1610	1.0000	± 0.1265	-1.4048	0.8051
Laysan	16	11	16	0.0029	± 0.0019	0.0783	± 0.0405	0.9083	± 0.0633	1.0000	± 0.0221	**-7.3192**	-1.7070

Gardner Pinnacles	12	6	12	0.0021	± 0.0015	0.0855	± 0.0452	0.8182	± 0.0840	1.0000	± 0.0340	**-2.0878**	-0.0851
French Frigate Shoals	39	18	38	0.0027	± 0.0018	0.0823	± 0.0408	0.8920	± 0.0306	0.9987	± 0.0062	**-13.6020**	**-14.4092**
Nihoa	36	20	36	0.0036	± 0.0022	0.0827	± 0.0411	0.9413	± 0.0229	1.0000	± 0.0065	**-14.7456**	**-15.1230**
Niihau	67	34	62	0.0038	± 0.0023	0.0822	± 0.0403	0.9439	± 0.0164	0.9973	± 0.0033	**-26.6171**	**-24.0938**
Kauai	30	21	27	0.0035	± 0.0022	0.0797	± 0.0399	0.9494	± 0.0276	0.9931	± 0.0105	**-19.9573**	-3.6324
Oahu	72	31	68	0.0029	± 0.0018	0.0828	± 0.0405	0.8901	± 0.0279	0.9984	± 0.0026	**-27.2002**	**-24.0863**
Maui	33	17	31	0.0031	± 0.0019	0.0797	± 0.0397	0.9072	± 0.0365	0.9962	± 0.0086	**-11.9743**	**-8.1731**
Island of Hawaii	30	14	30	0.0028	± 0.0018	0.0818	± 0.0409	0.8851	± 0.0425	1.0000	± 0.0086	**-8.3701**	**-11.0474**
All of Hawaiian Archipelago	425	104	392	0.0032	± 0.0020	0.0786	± 0.0380	0.9152	± 0.0083	0.9996	± 0.0002	**-26.4923**	**-23.4322**
Johnston Atoll													
Johnston Atoll	47	11	39	0.0025	± 0.0016	0.0598	± 0.0298	0.6920	± 0.0666	0.9880	± 0.0082	**-3.4506**	**-11.1614**
Johnston Atoll and Hawaiian Archipelago	472	109	431	0.0032	± 0.0019	0.0782	± 0.0378	0.9077	± 0.0089	0.9995	± 0.0002	**-26.4557**	**-23.4009**

Table 2: Estimates of tau (τ), pre and post-expansion theta (θ_0 and θ_1), and coalescence time in years (95% confidence limit of τ) for *A. abdominalis*, *C. ovalis*, and *C. verater*.

Species	**τ**	**θ₀**	**θ₁**	**Coalescence time (years ago)**
A. abdominalis	0.918	0	15.716	68,507 (15,746 - 127,537)
C. ovalis	3.297	0.035	154.375	249,773 (165,152 - 295,909)
C. verater	2.355	0.011	99999	163,769 (143,394 - 188,943)

Table 3: Analysis of molecular variance (AMOVAs) for *A. abdominalis*, *C. ovalis*, and *C. verater* with percent variation (% variation), fixation indices (Φ_{CT} and Φ_{ST}), and associated *P* values. "/" is used to separate different groupings of sampling locations. "/" is used to separate different groupings of sampling locations. Bold values are significant ($P < 0.05$). FFS = French Frigate Shoals.

		Cyt*b*						**CR**					
		Among groups			**Within populations**			**Among groups**			**Within populations**		
Species	**Groupings**	**% variation**	**Φ_{CT}**	***P* value**	**% variation**	**Φ_{ST}**	***P* value**	**% variation**	**Φ_{CT}**	***P* value**	**% variation**	**Φ_{ST}**	***P* value**
A. abdominalis	All samples				99.37	0.0063	0.0911				98.77	**0.0123**	**0.0034**
	Kure, Midway, Pearl & Hermes, Lisianski, Laysan, Maro Reef, FFS, Necker / Niihau, Kauai, Oahu, Maui, island of Hawaii	1.07	**0.0107**	**0.0044**	98.81	0.0119	0.081	0.98	**0.0098**	**0.0123**	98.26	**0.0175**	**0.0028**
C. ovalis	All samples				98.79	**0.0121**	**0.0047**				99.41	**0.0059**	**0.0370**
	Kure, Midway, Pearl & Hermes / Lisianski, Laysan, Maro Reef, FFS, Necker, Niihau, Kauai, Oahu, Maui, island of Hawaii	1.21	**0.0121**	**0.0338**	98.08	**0.0192**	**0.0049**	0.96	**0.0096**	**0.0287**	98.84	**0.0116**	**0.0368**

C. verater	**Johnston Atoll and Hawaiian Archipelago**							
	All samples	97.68	**0.0232**	**0.0000**		97.06	**0.0363**	**0.0000**
	Johnston Atoll / Hawaiian Archipelago	93.21	**0.0679**	**0.0000**		88.44	**0.1156**	**0.0000**
	Hawaiian Archipelago							
	All samples	99.07	**0.0093**	**0.0197**		98.85	**0.0115**	**0.0087**
	Island of Hawaii / rest of archipelago	97.89	**0.0211**	**0.0194**		96.48	**0.0352**	**0.0045**

Table 4: Population pairwise Φ_{ST} values for *A. abdominalis*. Cyt*b* below the diagonal and CR above. Bold denotes significant values (P <0.05) and * denotes significance after application of the false discovery rate ($P \leq 0.01$).

Location	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
1. Kure	-	-0.0095	**0.0316**	0.0254	-0.0046	0.0012	0.0104	0.0114	**0.0950**	-0.0070	**0.0299**	**0.0400***	0.0154
2. Midway	-0.0007	-	0.0147	0.0111	0.0007	-0.0088	0.0162	0.0102	**0.0495**	0.0009	0.0114	**0.0185**	0.0031
3. Pearl and Hermes	0.0104	-0.0129	-	0.0287	0.0135	0.0053	0.0031	-0.0084	**0.0963**	**0.0268**	**0.0265**	0.0160	0.0157
4. Lisianski	0.0059	-0.0086	-0.0217	-	**0.0415**	0.0099	0.0077	-0.0097	0.0379	0.0087	0.0165	0.0137	0.0029
5. Laysan	0.0068	0.0042	0.0010	-0.0044	-	0.0082	0.0017	0.0077	**0.1048***	-0.0008	**0.0247**	**0.0429***	0.0111
6. Maro Reef	-0.0025	0.0029	0.0049	-0.0054	0.0046	-	0.0148	0.0028	**0.0615**	0.0019	-0.0056	0.0035	-0.0029
7. French Frigate Shoals	0.0232	0.0129	-0.0089	-0.0170	0.0069	0.0087	-	-0.0101	**0.0945***	-0.0059	**0.0283**	**0.0245**	0.0073
8. Necker	0.0174	0.0051	-0.0082	-0.0273	0.0095	-0.0063	-0.0065	-	**0.0737**	-0.0005	0.0131	-0.0021	0.0087
9. Niihau	0.0125	0.0138	0.0033	-0.0121	-0.0111	-0.0203	-0.0372	-0.0028	-	**0.0543**	0.0343	0.0289	0.0171
10. Kauai	0.0161	0.0108	-0.0011	-0.0004	0.0004	-0.0028	0.0030	**0.0210**	-0.0042	-	0.0090	0.0184	-0.0033
11. Oahu	**0.0420***	**0.0340**	0.0160	0.0034	0.0150	0.0165	0.0010	0.0088	-0.0236	0.0125	-	0.0030	-0.0054
12. Maui	**0.0377**	**0.0261**	0.0141	-0.0050	0.0134	0.0063	0.0009	-0.0095	-0.0181	0.0064	-0.0047	-	0.0004
13. Island of Hawaii	0.0110	0.0217	0.0134	0.0069	0.0070	-0.0131	-0.0044	**0.0232**	-0.0468	-0.0089	0.0083	0.0104	-

Table 5: Population pairwise Φ_{ST} values for *C. ovalis*. Cyt*b* below the diagonal and CR above. Bold denotes significant values (P <0.05) and * denotes significance after application of the false discovery rate ($P \leq 0.01$).

Location	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**
1. Kure	-	0.0088	0.0043	0.0072	0.0077	0.0045	-0.0050	0.0086	**0.0320**	0.0073	**0.0260**	0.0045	0.0082	0.0018	0.0147
2. Midway	0.0062	-	0.0029	0.0344	0.0019	0.0046	-0.0033	-0.0066	**0.0379***	-0.0029	**0.0232**	-0.0017	0.0120	-0.0054	0.0134
3. Pearl and Hermes	**0.0275**	0.0034	-	0.0595	0.0139	0.0098	0.0090	0.0038	**0.0557***	0.0153	**0.0488***	-0.0107	**0.0220**	0.0120	**0.0283**
4. Lisianski	**0.1199**	0.0663	**0.1588**	-	-0.0212	-0.0022	-0.0217	0.0138	-0.0460	-0.0173	-0.0241	0.0508	-0.0269	-0.0336	-0.0374
5. Laysan	0.0022	0.0010	**0.0446***	0.0977	-	0.0004	-0.0075	-0.0014	0.0048	-0.0112	0.0026	0.0130	-0.0047	-0.0139	-0.0097
6. Maro Reef	0.0090	-0.0074	0.0198	0.0671	-0.0087	-	-0.0092	-0.0021	**0.0221**	-0.0120	0.0149	0.0076	0.0057	0.0010	0.0119
7. Gardner Pinnacles	0.0141	-0.0005	0.0317	0.0340	0.0033	-0.0093	-	-0.0155	0.0041	-0.0204	-0.0086	-0.0047	-0.0122	-0.0152	-0.0004
8. French Frigate Shoals	0.0197	-0.0087	0.0120	0.0942	0.0131	0.0018	0.0004	-	**0.0206**	-0.0122	0.0084	-0.0066	0.0007	-0.0062	0.0084
9. Necker	**0.0326**	0.0154	**0.0578***	0.0286	-0.0040	-0.0050	-0.0006	**0.0321**	-	0.0123	-0.0036	**0.0479***	0.0034	0.0029	0.0082
10. Nihoa	0.0234	0.0042	**0.0586***	0.0319	-0.0074	-0.0024	-0.0064	0.0119	0.0024	-	-0.0049	0.0061	-0.0095	-0.0125	-0.0036
11. Niihau	**0.0396**	0.0284	**0.0781***	0.0346	0.0092	0.0051	0.0162	**0.0467**	-0.0151	0.0026	-	**0.0416***	-0.0064	-0.0023	-0.0075
12. Kauai	0.0071	-0.0077	-0.0094	**0.1716**	0.0203	0.0063	0.0137	-0.0038	**0.0414**	**0.0364**	**0.0691***	-	0.0119	0.0042	**0.0255**
13. Oahu	0.0102	0.0030	**0.0419**	0.0346	-0.0163	-0.0053	0.0029	0.0145	-0.0072	-0.0033	-0.0027	0.0234	-	-0.0055	-0.0001
14. Maui	0.0208	-0.0026	**0.0431**	0.0404	-0.0047	-0.0008	-0.0166	0.0055	0.0035	-0.0047	0.0213	0.0217	0.0014	-	-0.0083
15. Island of Hawaii	0.0116	-0.0005	**0.0369**	0.0229	-0.0087	-0.0081	-0.0065	0.0071	-0.0043	-0.0086	-0.0091	0.0202	-0.0173	-0.0052	-

Table 6: Population pairwise Φ_{ST} values for *C .verater*. Cyt*b* below the diagonal and CR above. Bold denotes significant values (P <0.05) and * denotes significance after application of the false discovery rate ($P \leq 0.01$).

Location	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**
1. Kure	-	-0.0259	-0.0317	0.0116	-0.0169	-0.0355	-0.0113	0.0273	-0.0334	-0.0325	-0.0214	-0.0243	0.0180	**0.1985***
2. Midway	-0.0556	-	0.0102	-0.0081	-0.0125	-0.0071	0.0235	**0.0554***	-0.0001	-0.0120	0.0003	-0.0055	**0.0637***	**0.1465***
3. Pearl and Hermes	-0.0573	-0.0104	-	0.0334	0.0125	-0.0142	-0.0026	0.0126	-0.0043	-0.0007	0.0020	0.0027	0.0163	**0.1367***
4. Lisianski	**0.2098**	**0.1159**	**0.1436**	-	-0.0189	0.0084	0.0396	0.0843	0.0238	-0.0061	0.0263	0.0052	**0.1083**	**0.1916***
5. Laysan	-0.0140	-0.0086	0.0040	0.0528	-	0.0057	0.0326	**0.0708**	0.0035	-0.0016	0.0036	-0.0061	0.0792	**0.1285***
6. Gardner Pinnacles	-0.0051	-0.0101	-0.0187	**0.2970***	0.0312	-	-0.0155	0.0124	-0.0061	-0.0160	-0.0047	-0.0115	0.0052	**0.1591***
7. French Frigate Shoals	-0.0138	0.0118	-0.0027	**0.2386***	**0.0448**	-0.0371	-	0.0051	0.0015	0.0123	0.0089	0.0078	0.0096	**0.1257***
8. Nihoa	-0.0179	**0.0247**	0.0125	**0.1414**	0.0254	-0.0139	0.0104	-	**0.0277**	**0.0453**	**0.0394***	**0.0501***	-0.0048	**0.1799***
9. Niihau	-0.0520	0.0024	-0.0050	**0.1425***	0.0118	-0.0174	-0.0019	0.0089	-	-0.0022	-0.0010	0.0009	**0.0343**	**0.1291***
10. Kauai	-0.0490	-0.0078	-0.0102	**0.1577***	0.0055	-0.0145	0.0016	**0.0280**	-0.0023	-	-0.0037	-0.0065	**0.0555**	**0.1370***
11. Oahu	-0.0476	0.0003	-0.0061	**0.1861***	0.0146	-0.0100	0.0040	**0.0271***	-0.0013	-0.0079	-	-0.0108	**0.0521***	**0.1047***
12. Maui	-0.0536	-0.0050	-0.0093	**0.1569**	0.0137	0.0001	0.0104	**0.0351**	0.0026	-0.0060	-0.0072	-	**0.0621***	**0.1087***
13. Island of Hawaii	0.0104	**0.0416**	0.0215	**0.2449***	**0.0690**	-0.0231	-0.0061	-0.0009	0.0144	**0.0380**	**0.0367**	**0.0411**	-	**0.2140***
14. Johnston Atoll	0.0295	**0.0473***	**0.0699***	**0.2578**	**0.0591**	**0.0941**	**0.1045***	**0.1287***	**0.0743***	**0.0375**	**0.0651***	**0.0589***	**0.1563***	-

Table 7: Percentage of significant ($P < 0.05$) pairwise Φ_{ST} comparisons within the NWHI, within the MHI, and between the NWHI and MHI for *A. abdominalis*, *C. ovalis*, and *C. verater*, based on cyt*b* and CR sequence data.

	Cyt*b*				**CR**			
Species	**Total number of significant comparisons**	**Within NWHI**	**Within MHI**	**Between NWHI and MHI**	**Number of significant comparisons**	**Within NWHI**	**Within MHI**	**Between NWHI and MHI**
A. abdominalis	6	-	-	100%	19	11%	5%	84%
C. ovalis	18	44%	6%	50%	13	38%	15%	46%
C. verater	21	38%	14%	48%	12	17%	33%	50%

Figures

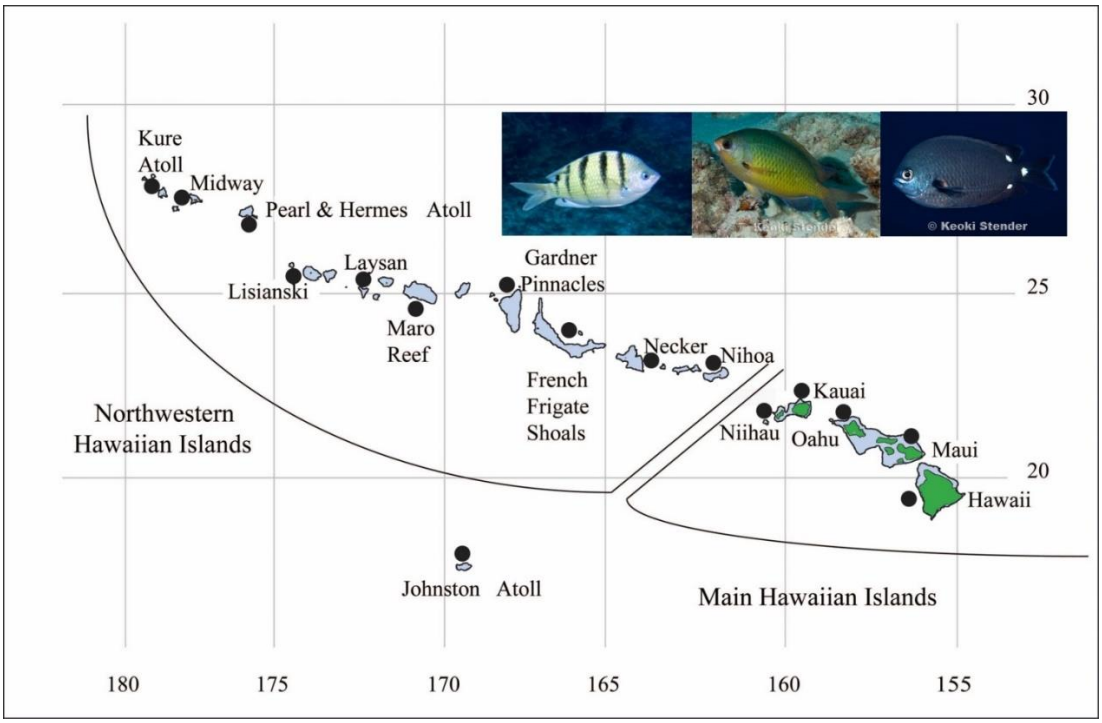

Figure 1: Map of collection locations in the Hawaiian Archipelago and Johnston Atoll for *A. abdominalis*, *C. ovalis*, and *C. verater* (photos left to right). Specimens of all species were collected at each location with the exception of Maro Reef and Johnston Atoll. No *C.* verater specimens were collected at Maro Reef, and only *C. verater* specimens were collected at Johnston Atoll. (Photo credit for *A. abdominalis*: Kim Tenggardjaja. Photo credit for *Chromis* species: Keoki Stender www.marinelifephotography.com)

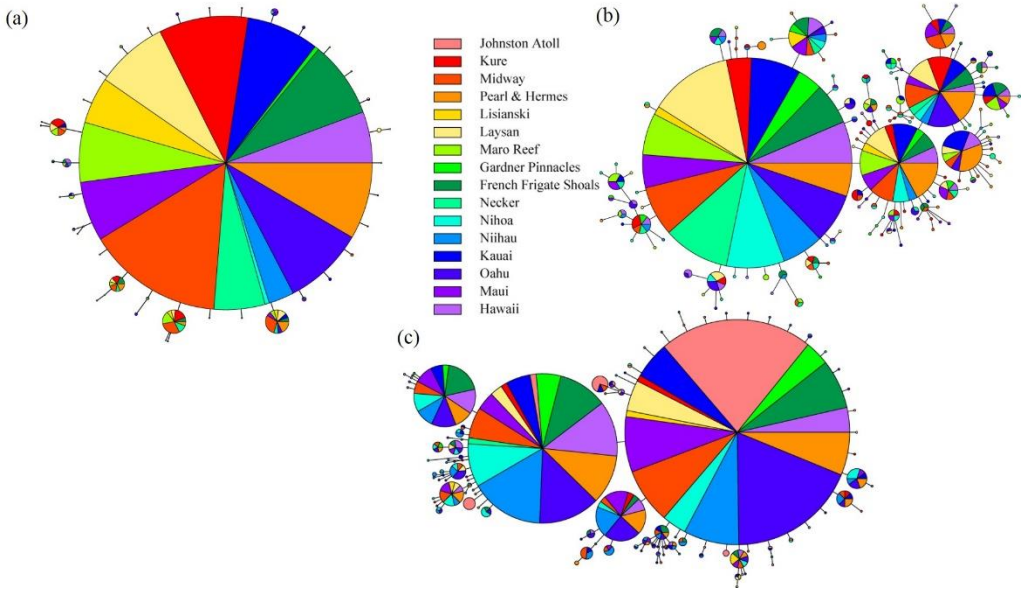

Figure 2: Parsimony-based haplotype networks using cytb sequence data for: (a) *A. abdominalis*, (b) *C. ovalis*, and (c) *C. verater*. Each circle represents a haplotype and is proportional to the frequency of that haplotype. Length of branches is proportional to number of mutations. Networks are color-coded by sampling location.

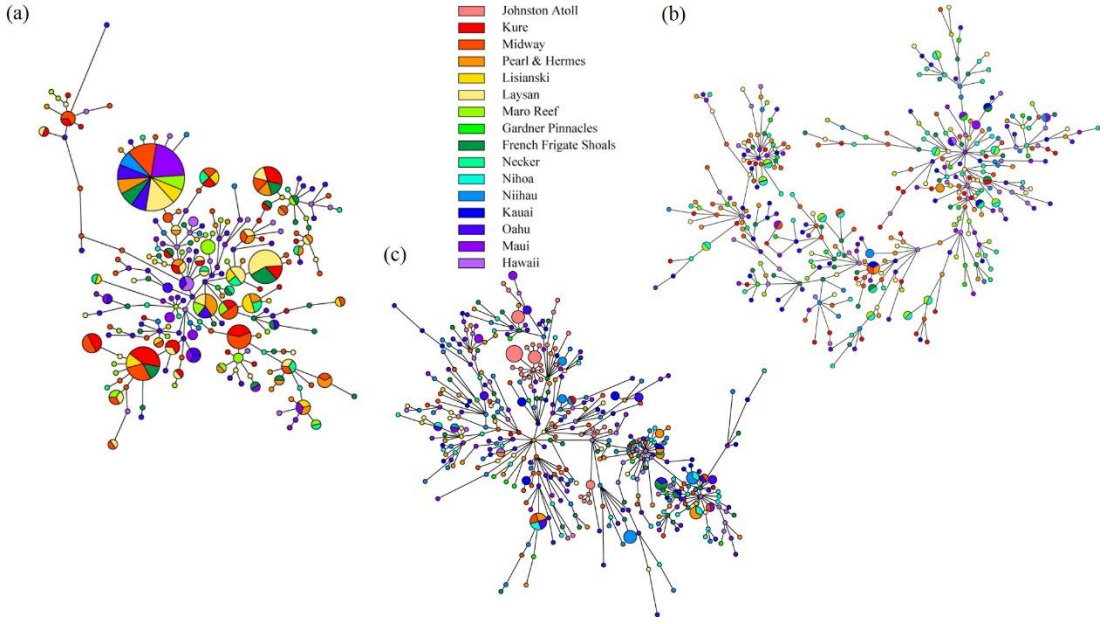

Figure 3: Parsimony-based haplotype networks using CR sequence data for: (a) *A. abdominalis*, (b) *C. ovalis*, and (c) *C. verater*. Each circle represents a haplotype and is proportional to the frequency of that haplotype. Length of branches is proportional to number of mutations. Networks are color-coded by sampling location.

CHAPTER 2

Biogeographic range size and dispersal: Comparative phylogeography of widespread and endemic damselfishes in the Hawaiian Archipelago

ABSTRACT

Aim

The Hawaiian Archipelago, one of the most remote archipelagoes in the world, is a hotspot for reef fish endemism. The restricted biogeographic range sizes of endemic species is interpreted to indicate low dispersal ability, whereas broad distributions of widespread species indicate high dispersal potential. Here we analyze mitochondrial sequence data for two widespread damselfish species (*Abudefduf vaigiensis* and *Chromis vanderbilti*) across the Hawaiian Archipelago and more broadly across the Indo-Pacific, and we compare results to those of five Hawaiian endemic damselfishes. We evaluate the assumption that widespread reef fishes show less genetic structure than endemic species and assess how patterns of connectivity vary across spatial scales. Additionally, we examine our results in light of the proposed relationship between range size and dispersal ability.

Location

The Hawaiian Archipelago (central Pacific Ocean)

Methods

Mitochondrial cytochrome *b* and control region sequence data were collected for *A. vaigiensis* (N = 277) and *C. vanderbilti* (N = 357) from across the Hawaiian Islands and across their broader distributions in the Indo-Pacific. Population genetic analyses

assessed patterns of population structure at two spatial scales: within the Hawaiian Archipelago and between archipelagoes. We compared results from these widespread species with those from a previous study on Hawaiian endemic damselfishes (*Abudefduf abdominalis*, *Chromis ovalis*, and *Chromis verater*) (Chapter 1).

Results

The widespread species *A. vaigiensis* and *C.vanderbilti* exhibit less population structure in the Hawaiian Archipelago than endemic species. Across the larger spatial scale of their Indo-Pacific ranges, both widespread damselfish species showed strong and significant population structure, with Hawaiian populations being genetically differentiated from most non-Hawaiian locations.

Main conclusions

Our comparison of widespread and endemic damselfish species is consistent with the expected trend for widespread species to exhibit more connectivity within the Hawaiian Archipelago, but this pattern may be restricted to certain reef fish families. In addition, widespread species in this study and previous studies, which had little to no population subdivision within archipelagoes, have shown strong genetic structure when analyzed across the broader Indo-Pacific. We conclude that geographic range size may be a better indicator of dispersal ability at smaller (within archipelago) than at larger spatial scales (between archipelagoes). Management should note that reef fishes unique to Hawaii seem to have less gene flow across the archipelago than Indo-Pacific species.

INTRODUCTION

Located roughly 3800 km from the nearest continent, the Hawaiian Archipelago is one of the most isolated archipelagoes in the world. Johnston Atoll, the closest land mass, is over 860 km away. Owing to its remote location, the Hawaiian Archipelago is home to an abundance of endemic species. Terrestrial flora and fauna in the archipelago are famously cited examples of endemism. Hawaiian honeycreepers, *Drosophila*, and silverswords are well-studied groups that rapidly speciated through adaptive radiation (Futuyma 1986). With respect to marine fauna, the Hawaiian Islands are a hotspot with 25% endemic reef fishes, the highest in the world (Randall 1998; Allen 2008). Most endemic Hawaiian fish species lack sister taxa within the archipelago but have Indo-Pacific counterparts (Springer 1982; Hourigan and Reese 1987; Mundy 2005). Similar to Hawaiian marine invertebrates, there are multiple endemics within a genus, but each endemic species appears to be derived separately from an Indo-Pacific ancestor (Kay and Palumbi 1987).

The scarcity of adaptive radiations in Hawaiian marine fauna has been attributed to the high dispersal potential of pelagic larvae (Hourigan and Reese 1987). This logic is based on the once widely held perception that populations of marine organisms were broadly open as a result of larvae drifting with ocean currents to distant locales (Palumbi 1994; Caley et al. 1996). Consequently, there should be high connectivity between populations, which would counter genetic drift and produce genetic homogeneity across populations of most marine organisms (Doherty et al. 1995; Shulman 1998). Some genetic surveys of reef fishes have revealed high levels

of connectivity and a lack of population structure across thousands to tens of thousands of kilometers [unicornfishes (Horne et al. 2008)], surgeonfishes [(Eble et al. 2009; DiBattista et al. 2011; Eble et al. 2011b)], moray eels [(Reece et al. 2010)], pygmy angelfishes [(Schultz et al. 2007)], and trumpetfishes [(Bowen et al. 2001)]). Other reef fish studies have shown low levels of gene flow and limited connectivity between populations. Among these are studies that have resulted in a paradigm shift in how we think about marine connectivity by indicating self-recruitment and local retention of larvae (Swearer et al. 2002; Jones et al. 2005; Almany et al. 2007; Jones et al. 2009). Reef fishes that lack a pelagic larval stage, such as *Pterapogon kauderni* and *Acanthochromis polyacanthus,* have been shown to exhibit genetic structure and little gene flow across their restricted geographic ranges (Planes et al. 2001; Vagelli et al. 2008).

Because of their narrow geographic distribution, endemic species provide opportunities for investigating connectivity and dispersal in marine populations. Endemic species essentially represent closed systems that are maintained by self-recruitment (Swearer et al. 2002). Their limited range size is interpreted as an outcome of low dispersal ability, compared to more broadly distributed species. Not only are studies on connectivity in endemic species important to the study of population biology in marine organisms, but they are also valuable for conservation and management of these species. Endemic species are vulnerable to disturbances and may be at greater risk of extinction than widespread species (Hughes et al., 2002 but

see Hawkins et al., 2000). Furthermore, endemic species contribute to the biodiversity value of a region, making them conservation priorities (Roberts et al. 2003).

Patterns of dispersal are highly relevant to the design of marine reserves (Roberts et al. 2003; Shanks et al. 2003) but are difficult to measure directly (Hellberg et al. 2002). While connectivity studies on individual species have yielded significant insight, as evidenced by the self-recruitment and larval retention studies, performing these types of assessments on all organisms within a region is not feasible. Utilizing certain traits as predictors of dispersal ability would facilitate the integration of knowledge regarding connectivity patterns into spatial marine planning.

Many life history traits have been investigated as indicators of dispersal ability, including reproductive characteristics, egg type, and perhaps the most debated, pelagic larval duration (PLD) (Doherty et al. 1995; Selkoe and Toonen 2011). Eble et al. (2009) explored the correlation between geographic range size and dispersal ability through a comparison of surgeonfishes in the Hawaiian Archipelago. Multiple life history traits have been found to correlate with range size (Brown et al. 1996), suggesting that population genetic structure may be more useful as an indicator of dispersal ability than individual life history traits. In their study, the three surgeonfish species had varying distributions, and in accordance with a correlation between dispersal ability and range size, the endemic species exhibited the most genetic structure (Eble et al. 2009).

Previous studies investigated this relationship in Hawaiian endemic damselfishes. As predicted based on a relationship between range size and dispersal

ability, Ramon et al. (2008) found significant population structure in *Dascyllus albisella* and *Stegastes marginatus*. Likewise, *Abudefduf abdominalis*, *Chromis ovalis*, *Chromis verater* all had genetic structure (Chapter 1), consistent with endemic species having low dispersal ability. Taking into consideration other genetic surveys of reef fishes in the Hawaiian Islands, we concluded that whether endemic species demonstrate genetic structure within this region may be dependent on the taxonomic family (Chapter 1). The life history traits of damselfishes may predispose them to showing genetic structure (Shulman 1998), and maybe this trend is not limited to endemic species. Thus, it is difficult to draw conclusions without knowing the patterns of connectivity in widespread damselfish species in the Hawaiian Islands.

The current study is a genetic survey of two widespread damselfish species, *Abudefduf vaigiensis* (Quoy and Paul 1825) and *Chromis vanderbilti* (Fowler 1941), and complements Chapter 1 by resolving patterns of genetic structure in widespread and endemic damselfish species across the Hawaiian Archipelago. Besides the study by Eble et al. (2009), explicit comparisons of population structure patterns in widespread and endemic reef fishes are sparse. A genetic survey of eight marine fishes in the Galapagos Islands found that the endemic species showed less genetic structure than the widespread species (Bernardi et al. 2014). While Bernardi et al. (2014) were examining how different life history traits and species ranges would affect population structure, the eight species were from several different reef fish families, so it is possible that phylogenetic effects may have influenced these results.

To control for differences in traits that may influence dispersal ability, we chose widespread species within the same genera as the study species in Chapter 1 (Abudefdufinae and Chrominae). *Abudefduf vaigiensis* has a vast range across the Indo-Pacific, extending longitudinally from the Red Sea and East Africa to the Line Islands and French Polynesia and latitudinally from southwestern Japan to the Great Barrier Reef. The PLD of this species is 17-20 days (Wellington and Victor 1989), which is very similar to that of its endemic counterpart *A. abdominalis*. The widespread *Chromis vanderbilti* occurs from northwestern Australia to Hawaii and French Polynesia. This species has a PLD of 30-32 days (Wellington and Victor 1989), which may be similar to the proposed PLD of 30 days for *C. ovalis* but is much shorter than the speculated PLD of three months for *C. verater* (Swerdloff 1970).

This study sought to test the prediction that widespread species exhibit less population structure than endemic species. We compared our population genetics results from two widespread damselfishes in the Hawaiian Archipelago with those of the three endemic species in Chapter 1. Additionally, utilizing specimens collected from non-Hawaiian locations, we analyze the genetic structure of the two widespread species at two spatial scales: 1) within the Hawaiian Archipelago (2600 km) and 2) between archipelagoes (4200-17,000 km). Comparing our results to those of previous genetic surveys of widespread reef fishes across the Indo-Pacific, we are able to draw some conclusions regarding connectivity patterns of reef fishes with broad distributions and the relationship between dispersal ability and range size. Lastly, we

utilize our comparison of widespread and endemic reef fishes to make inferences relevant to management, with special consideration given to patterns occurring in the Hawaiian Archipelago.

MATERIALS AND METHODS

Tissue collection

Within the Hawaiian Archipelago, collections of 211 *A. vaigiensis* and 324 *C. vanderbilti* specimens (fin clips) were made at 12-14 sites in the Northwestern Hawaiian Islands (NWHI) and the main Hawaiian Islands (MHI) from 2009-2012 (Figure1a). Additionally, non-Hawaiian specimens of 74 *A. vaigiensis* and 33 *C. vanderbilti* were obtained from sites in Australia, Chagos Archipelago, Cook Islands, Fiji, Madagascar, Moorea, Palmyra, and Saudi Arabia (Figure1b). Collections were made with pole spears or hand nets with SCUBA or while snorkeling. Specimens of the endemic species *A. abdominalis* (*N*=334), *C. ovalis* (*N*=412), and *C. verater* (*N*=472), previously analyzed in Chapter 1, also were used in this study.

DNA extraction, marker amplification, and sequencing

Tissue samples were preserved in salt-saturated DMSO (Seutin et al. 1991). All of the protocol for DNA extraction, marker amplification and sequencing are identical to those used in Chapter 3, with the exception of marker amplification for *C. vanderbilti*. Cytochrome *b* (cyt*b*) and mitochondrial control region (CR) sequences generated for Chapter 1 were used in this study. All *A. vaigiensis* and *C. vanderbilti* specimens were sequenced for these two markers. Since *C. vanderbilti* did not amplify well with the previously used primers, reverse primers were designed for

cyt*b*, CVcytbrvs1 (5'-AGTTGTCGGGATCTCCGAGAAGG-3'), and CR, CVdlooprvs (5'-CCAGGAATAGTTCACTYYGTGAAACC-3'). For *A. vaigiensis*, PCR protocols and cycling conditions for cyt*b* and CR were performed as described in Chapter 1. Sequences were aligned and edited using GENEIOUS R6 (Biomatters, LTD, Auckland, NZ). Alignments of cyt*b* were unambiguous, while CR contained multiple indels of 1-6 bp in length. Unique haplotypes were identified in ARLEQUIN 3.5 (Excoffier and Lischer 2010).

Genetic diversity and population structure analyses

Haplotype diversity (*h*) and nucleotide diversity (π) were calculated in ARLEQUIN. Population structure was analyzed using analyses of molecular variance (AMOVAs) and population pairwise Φ_{ST} comparisons in ARLEQUIN. The Φ_{ST} fixation index incorporates genetic distance and ranges from 0 to 1, with low values indicating a lack of genetic structure and high values indicating genetic differentiation. Significance of pairwise Φ_{ST} comparisons and AMOVA calculations were tested with 10,000 permutations, and to correct for multiple comparisons, a modified false discovery rate method was implemented (Benjamini and Yekutieli 2001). We determined the best model of sequence evolution for each marker in jMODELTEST 2 (Posada and Crandall 1998; Guindon and Gascuel 2003). Because the models identified by the Akaike information criterion were not available in ARLEQUIN, we selected the Tamura-Nei model as the closest available option (Tamura and Nei 1993). Populations of *A. vaigiensis* at Kure (*N*=1), Maro Reef (*N*=3), and Nihoa (*N*=3) were not included in population analyses due to small

sample sizes. However, these samples were included in haplotype networks. Parsimony-based haplotype networks for each marker were constructed in R using haploNet in the package PEGAS 0.5-1 (Paradis 2010). Haplotype frequencies used in these networks were calculated in ARLEQUIN. Fu's *F*s test for neutrality was run in ARLEQUIN with 10,000 permutations (Fu 1997). Significant negative *F*s values indicate an excess of rare haplotypes, which can be a signal of selection or recent population expansion.

To avoid making *a priori* assumptions about the possible locations of genetic barriers, we used BARRIER 2.2 (Manni et al. 2004), which employs a computational geometry approach to visualize where genetic barriers are located in geographic space. The software implements Monmonier's maximum-difference algorithm to compare a distance matrix (e.g. matrix of pairwise population ΦST values) with a matrix of geographic distances and identifies where genetic barriers are located geographically. *A posteriori* AMOVAs subsequently were performed on population groupings inferred by BARRIER output. The barrier configurations with the greatest variation among groups (as measured by Φ_{CT}) are reported here.

Mantel tests were performed to test for isolation by distance. Mantel tests were run in the *vegan* package in R with 10,000 permutations, using matrices of pairwise Φ_{ST} values and geographic distance as calculated by the Geographic Distance Matrix Generator (Oksanen et al. 2013; Ersts 2014). Mantel tests were performed with matrices that included negative Φ_{ST} and with negative values converted to zeroes.

RESULTS

A 648 bp segment of the cyt*b* gene was resolved for *A. vaigiensis* and a 697 bp segment for *C. vanderbilti*. For CR, 401 bp were resolved for *A. vaigiensis* and 329 bp for *C. vanderbilti*. Summary statistics for number of haplotypes (*H*), haplotype diversity (*h*), nucleotide diversity (π), and Fu's *Fs* are listed in Table 1. (See Table 1 in Chapter 1 for diversity indices for endemic species.) For the *Abudefduf* species, overall haplotype diversity for cyt*b* in the Hawaiian Archipelago was higher in the widespread *A. vaigiensis* (*h* = 0.8062) than in the endemic *A. abdominalis* (*h* = 0.5865). Overall cyt*b* haplotype diversity for *A. vaigiensis* from non-Hawaiian sites (*h* = 0.8915) was comparable to that within the Hawaiian Archipelago. Contrary to the *Abudefduf* species, haplotype diversity in the widespread *C. vanderbilti* (*h* = 0.5072) was lower than in the endemic *C. ovalis* and *C. verater* (*h* = 0.9501 and 0.9077). Haplotype diversity within non-Hawaiian sites for *C. vanderbilti* (*h* = 0.7027) was higher than within the Hawaiian Archipelago, but the low sample size for Moorea may have inflated this value. For CR, all species had high overall haplotype diversity (*h* = 0.8000 to 0.9997), with *C. vanderbilti* possessing the lowest value. All species had negative and significant Fu's *Fs* values for both markers at most locations in the Hawaiian Archipelago, except for CR in *A. vaigiensis*.

Parsimony-based haplotype networks for cyt*b* in all species were dominated by widely distributed common haplotypes (See Figure 2 for haplotype network based on all sampling locations for widespread species. Haplotype networks with only

Hawaiian sampling locations are in figures 4 and 5. All haplotype networks for endemic species can be found in Chapter 1). Networks for *A. abdominalis, A. vaigiensis,* and *C. vanderbilti* were dominated by one or two common haplotypes, whereas *C. ovalis* and *C. verater* had multiple common haplotypes in their networks. In all species, the most common haplotypes were present at nearly every sampling location in the Hawaiian Archipelago. For *A. vaigiensis,* the two haplotypes observed only in Moorea clustered together and branched off of the dominant haplotype. A haplotype observed only in Palmyra Atoll was separated from the dominant haplotype by 52 mutations. Haplotypes observed only in the Chagos Archipelago clustered together and were 49 mutations different from the dominant haplotype. One Madagascar specimen grouped with the Chagos Archipelago haplotypes. For *C. vanderbilti,* haplotypes observed only in Palmyra Atoll cluster together. The CR haplotype network for this species closely resembled the cyt*b* network with one dominant haplotype (Figure 3b). Haplotypes observed in the Cook Islands clustered together, including specimens from Palmyra Atoll. The networks for CR in the remaining four species mainly consisted of an abundance of low frequency haplotypes (Figure 3a for *A. vaigiensis* and see Chapter 1 for all endemic species). The network for *C. verater* showed some grouping of Johnston Atoll haplotypes. For *A. vaigiensis,* similar to the cyt*b* haplotype network, haplotypes unique to Moorea clustered together and were 18 mutations different from the next closest haplotype (Figure 3a). Haplotypes observed in Palmyra Atoll and the Chagos Archipelago were very divergent from the rest of the network (112 and 101 mutations). As in the cyt*b*

network, one specimen from Madagascar grouped with the Chagos Archipelago haplotypes.

For *A. vaigiensis*, both genetic markers revealed strong population structure between the Hawaiian Archipelago and non-Hawaiian sites for (cyt*b*: Φ_{CT} = 0.2497, *P* = 0.0065; CR: Φ_{CT} = 0.2184, *P* = 0.0023) (Table 2. See Chapter 1 for pairwise comparisons for endemic species). When non-Hawaiian sites were excluded from analyses, the overall estimate for Φ_{ST} across the Hawaiian Archipelago was not significant for cyt*b* (Φ_{ST} = -0.0045, *P* = 0.6297). Nonetheless, weak yet significant genetic structure across the archipelago was resolved by CR (Φ_{ST} = 0.0163, *P* = 0.0278). Likewise, the endemic *A. abdominalis* did not have a significant Φ_{ST} for cyt*b* (Φ_{ST} = 0.0057, *P* = 0.1175), but CR yielded weak but significant genetic structure (Φ_{ST} = 0.0126, *P* = 0.0047) comparable to that of its widespread counterpart *A. vaigiensis*. With respect to the significance of genetic breaks within the Hawaiian Archipelago, the *Abudefduf* species demonstrated different results. AMOVAs did not confirm genetic breaks identified by BARRIER for the widespread *A. vaigiensis*. However, a genetic break between Necker and Niihau identified in *A. abdominalis* was supported by *a posteriori* AMOVAs for both markers (cyt*b*: Φ_{CT} = 0.0125, *P* = 0.0050; CR: Φ_{CT} = 0.0113, *P* = 0.0080).

For the widespread species *C. vanderbilti*, both markers demonstrated strong population structure between the Hawaiian Archipelago and the non-Hawaiian sites (cyt*b*: Φ_{CT} = 0.5144, *P* = 0.0016; CR: Φ_{CT} = 0.3716, *P* = 0.0023) (Table 2). Within the Hawaiian sites, neither marker exhibited a significant overall Φ_{ST} value,

indicating a lack of genetic structure across the archipelago (cyt*b*: Φ_{ST} = 0.0009, *P* = 0.3968; CR: Φ_{ST} = -0.0017, *P* = 0.6533). In contrast, evidence of population structure was seen in the two endemic *Chromis* species. For *C. ovalis*, fixation indices for both markers showed weak but significant structure (cyt*b*: Φ_{ST} = 0.0121, *P* = 0.0047; CR: Φ_{ST} = 0.0059, *P* = 0.0370). This also was true within the Hawaiian Archipelago for *Chromis verater* (cyt*b*: Φ_{ST} = 0.0093, *P* = 0.0197; CR: Φ_{ST} = 0.0115, *P* = 0.0087). When Johnston Atoll was included in analyses for this species, Φ_{ST} values increased, but the resulting population structure remained weak (cyt*b*: Φ_{ST} = 0.0232, *P* < 0.0001; CR: Φ_{ST} = 0.0363, *P* < 0.0001). As for significant genetic breaks, *a posteriori* AMOVAs supported a genetic break identified by BARRIER between Johnston Atoll and the Hawaiian Archipelago (cyt*b*: Φ_{ST} = 0.0679, *P* < 0.0001; CR: Φ_{ST} = 0.1156, *P* < 0.0001). Within the archipelago, BARRIER identified a genetic break between Maui and the Big Island of Hawaii, which was confirmed by AMOVAs (cyt*b*: Φ_{ST} = 0.0211, *P* = 0.0194; CR: Φ_{ST} = 0.0352, *P* = 0.0045). For *C. ovalis*, *a posteriori* AMOVAs simulating a genetic break between Pearl and Hermes and Lisianski detected weak yet significant structure for both markers (cyt*b*: Φ_{CT} = 0.0192, *P* = 0.0049; CR: Φ_{CT} = 0.0096, *P* = 0.0287). For *C. vanderbilti*, no significant genetic breaks for both markers were detected in this widespread species.

Population pairwise Φ_{ST} comparisons for the widespread species demonstrated that Hawaiian sites were significantly different from most non-Hawaiian sites (Table 3 and Table 4). Confirming AMOVA results, this shows evidence of genetic structure across the broad distributions of *A. vaigiensis* and *C.*

vanderbilti. Similar to the endemic species *A. abdominalis*, *C. ovalis*, and *C. verater*, population pairwise Φ_{ST} comparisons revealed different patterns of genetic structure among the Hawaiian sampling sites for the widespread species. In *A. vaigiensis*, there were few significant comparisons for cyt*b*, and 5 of the 7 significant comparisons in CR involved the population of Oahu (Φ_{ST} = 0.0689 to 0.1155). For *C. vanderbilti*, there were only two significant comparisons in CR, and all four significant comparisons for cyt*b* involved the population at French Frigate Shoals (Φ_{ST} = 0.0328 to 0.0880).

To determine whether the widespread species exhibited more or less population structure than their endemic counterparts, we compared the percentage of significant pairwise Φ_{ST} comparisons in the Hawaiian Archipelago between congeneric species (Table 5). Both *A. vaigiensis* and *A. abdominalis* had similar percentages of significant comparisons for cyt*b*. However, the endemic *A. abdominalis* had a greater percentage for the CR data. For the *Chromis* species, the widespread *C. vanderbilti* had a lower percentage of significant pairwise comparisons than either of the endemic species. Additionally, we looked for general trends in the regions of the archipelago by comparing the proportion of significant population pairwise Φ_{ST} comparisons: 1) within the NWHI, 2) within the MHI, and 3) between the NWHI and the MHI (Table 5). With respect to these three categories, the species generally exhibited more significant comparisons between locations in the NWHI and those in the MHI.

Mantel tests for isolation by distance for the widespread species were performed: 1) across all non-Hawaiian and Hawaiian sites and 2) only across Hawaiian sites. For *A. vaigiensis*, Mantel tests across all non-Hawaiian sites and the Hawaiian Archipelago were not significant for either marker (data not shown). Mantel tests only including non-Hawaiian sites were not significant. Within the Hawaiian Archipelago, neither marker detected significant isolation by distance for *A. vaigiensis*. This was also the case for CR in *A. abdominalis*, but cyt*b* demonstrated a significant isolation by distance signal ($r = 0.4953$, $P < 0.0001$). Since AMOVAs with *A. abdominalis* populations grouped into the NWHI and the MHI were significant for both markers, a partial Mantel test was run with this regional structure. The isolation by distance signal weakened but still persisted ($r = 0.3750$, $P = 0.0030$).

For *C. vanderbilti*, Mantel tests were not significant across non-Hawaiian sites and the Hawaiian Archipelago for both markers (data not shown). When only non-Hawaiian sites were included, the Mantel tests were not significant for either marker (data not shown). Across only the Hawaiian sites, the Mantel test was not significant for cyt*b* ($r = -0.0837$, $P = 0.7095$) but was significant for CR ($r = 0.2576$, $P = 0.0409$). In contrast to *C. vanderbilti*, the endemic *C. ovalis* and *C. verater* did not exhibit significant isolation by distance for either marker.

DISCUSSION

The goals of this study were to compare the results of the widespread species with those of their endemic counterparts and to elucidate connectivity patterns that each widespread species was exhibiting across its larger range (4500-17,000+ km),

beyond the boundaries of the Hawaiian Archipelago (2600 km). Both *A. vaigiensis* and *C. vanderbilti* revealed significant levels of strong genetic structure across their widespread ranges and also strong genetic differentiation between the Hawaiian Islands and the non-Hawaiian sites. However, within the Hawaiian Archipelago, both widespread species generally showed little evidence of genetic structure in comparison to the endemic species.

Within-archipelago spatial scale: comparative phylogeography of widespread and endemic damselfishes across the Hawaiian Archipelago

The benefit of congeneric comparisons is the inherent phylogenetic constraint, which may reduce variability in traits that affect dispersal. For instance, larval swimming behavior may influence gene flow patterns in reef fish, and within the Pomacentridae, swimming ability of late pelagic stage larvae can have a 7.5-fold difference across species (Stobutzki 1998). Additionally, PLDs, which have been used as predictors of dispersal (Shanks et al. 2003; Lester and Ruttenberg 2005), tend to be similar within damselfish genera (Wellington and Victor 1989). Thus, congeneric comparisons aid in controlling this trait, which may influence dispersal ability (but PLD for *C. verater* may be unusually long – Swerdloff, 1970).

Nonetheless, there were variable characteristics in our congeneric pairings that we were not able to control. An important difference between the endemic and widespread *Abudefduf* species is that the widespread *A. vaigiensis* recently colonized the Hawaiian Archipelago. Although it is the most abundant *Abudefduf* in the Indo-Pacific, it was not reported in the Hawaiian Islands until about 1990 and is speculated

to have arrived there by rafting on flotsam (Hoover 2007). While our analyses detected little genetic structure in *A. vaigiensis*, they are not likely to meet the prerequisite assumption of equilibrium conditions. Furthermore, *A. vaigiensis* is interbreeding with the endemic *A. abdominalis* (Maruska and Peyton 2007) with a hybridization frequency of ~ 5-9% (Coleman et al. *submitted*), which also may be affecting the observed population structure of the widespread species. For the *Chromis* species, the widespread *C. vanderbilti* is notably smaller in body size (7 cm) than *C. ovalis* and *C. verater* (19-22 cm). Since body size has been correlated with range size (Luiz et al. 2013), we might expect that small-bodied species would have smaller ranges and thus less dispersal. However, we dismiss this trait as influencing our results because *C. vanderbilti*, despite being smaller in size, exhibited greater dispersal than the larger endemic *Chromis* species.

In Chapter 1, we assessed the relationship between dispersal ability and range size by evaluating phylogeographic patterns of three endemic Hawaiian damselfish species (*A. abdominalis*, *C. ovalis*, and *C. verater*). Combining data on *D. albisella* and *S. marginatus* from Ramon et al. (2008), we showed that five Hawaiian endemic damselfish species exhibit genetic structure within the archipelago. Assuming that the restricted biogeography of endemic species is a reflection of lower dispersal ability, we interpreted this finding of genetic structure as support for a relationship between dispersal ability and range size. We can evaluate this relationship more thoroughly with the results from the current study on the widespread damselfish species *A. vaigiensis* and *C. vanderbilti*.

For the *Abudefduf* species, more evidence of genetic structure was seen in the endemic *A. abdominalis* than the widespread *A. vaigiensis*. Both species exhibited weak yet significant population structure across the Hawaiian Archipelago for CR, but the endemic species had a greater percentage of significant pairwise comparisons than the widespread species. Moreover, while both genetic markers demonstrated a significant genetic break between Niihau and Necker in *A. abdominalis*, there were no concordant genetic breaks in the widespread *A. vaigiensis*. Although both species exhibited some population structure within the archipelago, the endemic *A. abdominalis* showed more genetic structure across analyses.

The situation was even more clear-cut with the *Chromis* species, as analyses did not detect consistent genetic structure in the widespread species, *C. vanderbilti*, while there was weak but significant population structure across the Hawaiian Archipelago for both endemic species, *C. ovalis* and *C. verater*. The widespread *Chromis* also had lower percentages of significant pairwise comparisons than either of its endemic counterparts. Furthermore, for each of the endemic species, there was concordance between the genetic markers in where genetic breaks were located (between Pearl and Hermes and Lisianski for *C. ovalis* and between Maui and the Big Island for *C. verater*). In contrast, there was no concordant support for genetic breaks in *C. vanderbilti*.

If the broader distributions of the widespread species are indicative of their greater dispersal ability, they should exhibit less or possibly no genetic structure when compared to their endemic counterparts. As described above, our congeneric

comparisons within the scale of the Hawaiian Archipelago are consistent with this prediction. Both of the widespread species had less evidence of genetic structure than their endemic counterparts. More generally speaking, when considering the seven phylogeographic surveys that have been conducted on damselfishes in the Hawaiian Archipelago, the widespread species exhibited less genetic structure than the five endemic species, similar to the study on three surgeonfish species (Eble et al. 2009). Thus, phylogeographic surveys of Pomacentridae and Acanthuridae in the Hawaiian Archipelago showed support for the relationship between range size and dispersal ability.

Between-archipelagoes spatial scale: patterns of connectivity in widespread reef fishes across the Indo-Pacific

We can evaluate the relationship between dispersal ability and range size further by comparing the results from previous genetic surveys that sampled widespread reef fishes throughout the Hawaiian Archipelago. Overall, widespread species from different reef fish families have shown little to no genetic structure in the Hawaiian Archipelago. Soldierfishes [genus *Myripristis* (Craig et al. 2007)], goatfishes [genus *Parupeneus* (Szabó et al. 2014)], surgeonfishes [genus *Acanthurus* (Eble et al. 2009)], snappers [genera *Lutjanus* (Gaither et al. 2010)], *Pristipomoides* (Gaither et al. 2011b), *Etelis* (Andrews et al. 2014)], and moray eels [genus *Gymnothorax* (Reece et al. 2010)] have lacked genetic structure within the archipelago. Similar to the widespread damselfish species in our study, there was some evidence of weak yet significant population differentiation within the

archipelago for the surgeonfish *Zebrasoma flavescens* (Eble et al. 2009; Eble et al. 2011b). It is remarkable that all of the widespread reef fishes surveyed thus far in Hawaii do not demonstrate much genetic structure at the spatial scale of the archipelago.

However, a different story emerges for several of these widespread species, when we zoom out to the larger spatial scale of their biogeographic ranges. Though they did not show consistent structure in the archipelago, *A. vaigiensis* and *C. vanderbilti* both exhibited strong and significant genetic structure across the Indo-Pacific range. This range spanned from as far east as the Cook Islands (4500+ km) for *C. vanderbilti* and even farther to Madgascar (17,000+ km) for *A. vaigiensis*. Likewise, there was evidence of strong genetic structure when a larger spatial scale was analyzed for *Myripristis berndti* (Craig et al. 2007), *Acanthurus nigrofuscus* (Eble et al. 2009), *Lutjanus kasmira* (Gaither et al. 2010), and *Parupeneus multifasciatus* (Szabó et al. 2014). Since the ranges of widespread reef fishes may span multiple biogeographic provinces, these species generally show concordance between phylogeography and biogeography.

Genetic surveys of widespread reef fishes performed at a small spatial scale within French Polynesia and at a broader scale across archipelagoes in the Indo-Pacific have illustrated a similar trend. A range-wide study of the Convict Surgeonfish (*Acanthurus triostegus*) revealed significant genetic partitioning across its wide distribution from East Africa to the Tropical Eastern Pacific but not within in the Society Archipelago (Planes et al. 1996; Planes and Fauvelot 2002). Along the

same lines, Bernardi et al. (2003) compared patterns of gene flow in the damselfish *Dascyllus trimaculatus* at multiple spatial scales throughout its range across the Indo-West Pacific. Within French Polynesia, no genetic differentiation was detected between the reefs of Moorea in the Society Archipelago or between Moorea and Rangiroa in the Tuamotu Archipelago. Conversely, at the larger scale of the Indo-West Pacific, there was reduced gene flow and significant genetic structure in *D. trimaculatus*. Limited genetic connectivity across broader ranges illustrates that long-distance migration is probably infrequent in many widespread reef fishes. Consequently, self-recruitment at an archipelago scale is implicated in maintaining these widely separated populations (Planes and Fauvelot 2002).

The design of effective marine protected areas (MPAs) should account for connectivity and dispersal ability of the organisms that are being protected (Roberts et al. 2003; Shanks et al. 2003). Though using distinguishing traits as indicators of dispersal ability (e.g. pelagic larval duration) would simplify the process of creating MPAs, debate continues over whether these traits accurately predict dispersal ability (Doherty et al. 1995; Shanks et al. 2003; Lester and Ruttenberg 2005; Lester et al. 2007; Weersing and Toonen 2009; Mora et al. 2012; Treml et al. 2012). We suggest that managers exercise caution in using geographic range size as an indicator of dispersal ability. Based on our findings, the relationship between dispersal ability and range size is most valid at a small spatial scale (e.g. within an archipelago) but seems to be less accurate when larger spatial scales (e.g. between archipelagoes, between ocean basins) are considered. As discussed in Chapter 1, even at small spatial scales,

the relationship between dispersal ability and range size may vary between reef fish families.

Nonetheless, these conclusions are based on studies that assessed genetic structure at a small spatial scale in one geographic location (i.e. within the Hawaiian Archipelago, within French Polynesia), in addition to sampling across the broader distribution of the widespread species. Would these widespread reef fishes still show a lack of genetic structure when surveyed across a small spatial scale in locations other than Hawaii and French Polynesia? Perhaps in a location with complex oceanography, such as the Indonesian Archipelago, these species would show genetic structure at a within-archipelago scale. For instance, *Dascyllus aruanus* has a broad distribution across the Indo-Pacific, and a genetic survey revealed significant population structure in the Coral Triangle (Raynal et al. 2014), which has been observed in several other reef fish species in this area (reviewed in Carpenter et al., 2011). Notably, this species also showed low levels of genetic structure between archipelagoes in French Polynesia but greater gene flow at smaller spatial scales within that region (Planes et al. 1993). While there has been no range-wide study on *D. aruanus*, the patterns of genetic structure demonstrated on these small spatial scales indicate that this species likely exhibits structure at the larger scale of its Indo-Pacific range. In the future, attempts to expand on the aforementioned range-wide surveys of widespread reef fishes by sampling at a small spatial scale in other archipelagoes will shed more light on whether the relationship between dispersal ability and range size depends on geography as well as taxonomy.

Implications for management within the Hawaiian Archipelago

The uninhabited NWHI constitute the Papahānaumokuākea Marine National Monument, one of the world's largest marine protected areas and the largest under U.S. jurisdiction. Given that this region occupies a large proportion (1700+ km) of the Hawaiian Archipelago (2600 km), there continues to be much interest in whether the protected waters of the Monument are subsidizing the depauperate reefs of the MHI. As described in Toonen et al. (2011), four concordant barriers to gene flow have been identified, and the three genetic breaks discussed in Chapter 1 matched these barriers. The presence and strength of these barriers varies with each surveyed species, so numerous mechanisms may be at play, driving these patterns across taxonomic groups. Even when limiting our scope only to reef fishes, individual phylogeographic patterns across the archipelago are still complex. As summarized in DiBattista et al. (2011), some but not all reef fish species show evidence of genetic differentiation between the NWHI and the MHI. Additionally, the five damselfish species in our current study showed evidence of limitations to connectivity between these two regions. Regardless of which mechanisms are behind it, evidence of genetic differentiation between the NWHI and the MHI in certain species but not others highlights the complexity of understanding whether the benefits of the Monument are being transferred to the MHI.

Our comparison of widespread and endemic damselfish species supports the previously noted trend that widespread Indo-Pacific reef fishes have higher levels of connectivity than endemic species within the boundaries of the archipelago (Eble et

al. 2009). Despite the presence of shared haplotypes across the archipelago in the endemic damselfishes (Chapter 1), evidence of genetic structure still reflects at least some restriction to gene flow (Planes and Fauvelot 2002), though it remains debatable how this applies to ecological timescales (Christie et al. 2010a). Given that the Hawaiian Archipelago has the highest percentage of reef fish endemism in the world (Randall 1998), management should take into consideration the evidence of endemic reef fishes exhibiting more structure than the more widely ranging Indo-Pacific species. Certain widespread species have been shown to be genetically distinct in Hawaii (this study, (Gaither et al. 2010; Eble et al. 2011a; Eble et al. 2011b; Gaither et al. 2011b)), and the possibility still exists that Hawaiian populations may receive sporadic dispersal from non-Hawaiian sites. Conversely, the persistence of populations of the endemic species is totally reliant on self-recruitment within the archipelago. Since endemic reef fishes show evidence of genetic structure in the Hawaiian Islands, management strategies to ensure connectivity between populations may serve to minimize the risk of local extirpation. Our finding that endemic reef fishes exhibit greater genetic structure than widespread species, combined with multiple species demonstrating limited connectivity between NWHI and MHI, emphasizes the importance of protecting endemic species in the overfished MHI. While MPAs are effective tools for fishery management, they are also valuable for protecting biodiversity (Hughes et al. 2002; Roberts et al. 2003).

TABLES

Table 1 Molecular diversity indices for *A. vaigiensis* and *C. vanderbilti*. Number of individuals (*N*), number of haplotypes (*H*), nucleotide diversity (π), haplotype diversity (*h*), and Fu's F_s are listed for cyt*b* and CR. F_S values in bold are significant ($P < 0.05$). For *A. vaigiensis*, populations at Kure (*N*=1), Maro Reef (*N*=3), and Nihoa (*N*=3) were not included in most analyses due to small sample sizes.

	N	*H*		π				*h*				Fu's *Fs*	
Sample location		**cyt*b***	**CR**	**cyt*b***		**CR**		**cyt*b***		**CR**		**cyt*b***	**CR**
A. vaigiensis													
Hawaiian Archipelago													
Midway	31	12	25	0.0028	± 0.0019	0.0400	± 0.0205	0.8344	± 0.0583	0.9849	± 0.0124	**-5.9065**	**-6.4127**
Pearl and Hermes	27	8	25	0.0017	± 0.0013	0.0394	± 0.0203	0.6695	± 0.0900	0.9943	± 0.0119	**-3.751**	**-8.3289**
Laysan	10	7	10	0.0035	± 0.0024	0.0453	± 0.0249	0.9333	± 0.0620	1.0000	± 0.0447	**-2.9055**	-2.1905
Gardner Pinnacles	19	8	18	0.0030	± 0.0020	0.0439	± 0.0229	0.8538	± 0.0537	0.9942	± 0.0193	-2.3999	**-5.4804**
French Frigate Shoals	31	9	27	0.0031	± 0.0020	0.0427	± 0.0218	0.8086	± 0.0454	0.9914	± 0.0104	-2.1093	**-9.0621**
Necker	5	4	5	0.0043	± 0.0032	0.0492	± 0.0309	0.9000	± 0.1610	1.0000	± 0.1265	-0.4448	0.4035
Niihau	8	6	8	0.0026	± 0.0020	0.0485	± 0.0275	0.8929	± 0.1113	1.0000	± 0.0625	**-3.0541**	-1.0941
Kauai	20	7	16	0.0019	± 0.0014	0.0379	± 0.0198	0.6421	± 0.1176	0.9684	± 0.0280	**-2.8003**	-2.4031
Oahu	16	9	12	0.0034	± 0.0022	0.0395	± 0.0209	0.8917	± 0.0603	0.9583	± 0.0363	**-3.7068**	-0.3567
Maui	17	7	15	0.0031	± 0.0021	0.0407	± 0.0214	0.8382	± 0.0634	0.9853	± 0.0252	-1.5222	-2.932
Island of Hawaii	19	9	17	0.0034	± 0.0022	0.0450	± 0.0235	0.8830	± 0.0461	0.9883	± 0.0210	**-3.1111**	-3.6302
All of Hawaiian Archipelago	203	24	107	0.0028	± 0.0018	0.0418	± 0.0208	0.8062	± 0.0212	0.9885	± 0.0019	**-13.381**	**-23.817**
Indo-Pacific													
Madagascar	3	3	3	0.0571	± 0.0432	0.2504	± 0.1878	1.0000	± 0.2722	1.0000	± 0.2722	2.49445	3.45531
Saudi Arabia	19	11	19	0.0029	± 0.0019	0.0573	± 0.0295	0.7895	± 0.0995	1.0000	± 0.0171	**-6.8279**	**-6.3751**
Chagos Archipelago	18	6	16	0.0033	± 0.0021	0.0363	± 0.0192	0.8301	± 0.0544	0.9869	± 0.0229	-0.2876	-3.9059
Australia	5	5	4	0.0049	± 0.0036	0.0476	± 0.0299	1.0000	± 0.1265	0.9000	± 0.1610	**-2.116**	2.5405

Fiji	5	4	5	0.0037	± 0.0028	0.0417	± 0.0264	0.9000	± 0.1610	1.0000	± 0.1265	-0.7012	0.20943
Moorea	22	2	9	0.0001	± 0.0003	0.0027	± 0.0021	0.0909	± 0.0809	0.6580	± 0.1142	-0.9568	**-6.4014**
Palmyra	2	1	1	0.0000	± 0.0000	0.0000	± 0.0000	0.0000	± 0.0000	0.0000	± 0.0000	-	-
All Indo-Pacific sites	74	27	57	0.0382	± 0.0188	0.1752	± 0.0847	0.8915	± 0.0260	0.9693	± 0.0146	3.95612	-2.7396
C. vanderbilti													
Hawaiian Archipelago													
Kure	23	5	11	0.0008	± 0.0008	0.0037	± 0.0027	0.4545	± 0.1234	0.6917	± 0.1095	**-2.4029**	**-8.6178**
Midway	32	8	14	0.0007	± 0.0007	0.0048	± 0.0033	0.4415	± 0.1094	0.8266	± 0.0626	**-7.2897**	**-10.0370**
Pearl and Hermes	27	8	17	0.0012	± 0.0010	0.0057	± 0.0038	0.6011	± 0.1062	0.9117	± 0.0464	**-5.0946**	**-15.9201**
Laysan	21	9	14	0.0014	± 0.0011	0.0058	± 0.0038	0.6810	± 0.1131	0.8667	± 0.0737	**-6.5805**	**-11.8248**
French Frigate Shoals	31	4	15	0.0005	± 0.0006	0.0043	± 0.0030	0.2946	± 0.1020	0.8452	± 0.0606	**-1.8481**	**-13.1844**
Necker	13	3	5	0.0004	± 0.0005	0.0038	± 0.0029	0.2949	± 0.1558	0.5385	± 0.1611	**-1.4015**	-1.2388
Nihoa	29	4	11	0.0007	± 0.0007	0.0046	± 0.0032	0.4803	± 0.0887	0.7537	± 0.0783	-1.1609	**-5.9951**
Niihau	23	7	13	0.0010	± 0.0009	0.0049	± 0.0034	0.5217	± 0.1241	0.8182	± 0.0820	**-4.7800**	**-10.2619**
Kauai	30	6	15	0.0010	± 0.0008	0.0039	± 0.0028	0.5287	± 0.0949	0.7862	± 0.0789	**-2.9449**	**-14.4522**
Oahu	33	6	16	0.0008	± 0.0008	0.0050	± 0.0034	0.4924	± 0.0943	0.8182	± 0.0667	**-3.2318**	**-13.1901**
Maui	30	8	14	0.0010	± 0.0009	0.0048	± 0.0033	0.5103	± 0.1088	0.7540	± 0.0849	**-5.7370**	**-10.4927**
Island of Hawaii	32	9	16	0.0013	± 0.0010	0.0050	± 0.0034	0.6976	± 0.0804	0.8569	± 0.0579	**-4.2024**	**-13.4015**
All of Hawaiian Archipelago	324	30	72	0.0009	± 0.0008	0.0047	± 0.0031	0.5072	± 0.0329	0.8000	± 0.0232	**-29.8940**	**-27.6530**
Indo-Pacific													
Cook Islands	18	8	12	0.0013	± 0.0010	0.0073	± 0.0047	0.6405	± 0.1300	0.9150	± 0.0521	**-5.9310**	**-7.3919**
Moorea	2	2	2	0.0014	± 0.0020	0.0183	± 0.0198	1.0000	± 0.5000	1.0000	± 0.5000	-	-
Palmyra	13	3	9	0.0010	± 0.0009	0.0091	± 0.0057	0.6026	± 0.0885	0.8718	± 0.0913	0.0493	**-3.5660**
All Indo-Pacific sites	33	11	22	0.0014	± 0.0011	0.0093	± 0.0055	0.7027	± 0.0754	0.9470	± 0.0249	**-8.6288**	**-18.0768**

Table 2 Analysis of molecular variance (AMOVAs) for congeneric groupings of widespread and endemic species: 1) widespread *A. vaigiensis* and endemic *A. abdominalis* and 2) widespread *C. vanderbilti* and endemic *C. ovalis* and *C. verater*. Percent variation (% variation), fixation indices (Φ_{CT} and Φ_{ST}), and associated *P* values are listed. "/" is used to separate different groupings of sampling locations. Bold values are significant ($P < 0.05$). FFS = French Frigate Shoals. Data for endemic species is from Chapter 1.

		Cyt*b*						**CR**					
		Among groups			**Within populations**			**Among groups**			**Within populations**		
Species	**Groupings**	**% variation**	**Φ_{CT}**	***P* value**	**% variation**	**Φ_{ST}**	***P* value**	**% variation**	**Φ_{CT}**	***P* value**	**% variation**	**Φ_{ST}**	***P* value**
Widespread													
A. vaigiensis	**Indo-Pacific locations and Hawaiian Archipelago**												
	All samples				18.82	**0.8118**	**0.0000**				36.13	**0.6387**	**0.0000**
	Madagascar, Saudi Arabia, Chagos Archipelago, Australia, Fiji, Moorea, Palmyra / Hawaiian Archipelago	24.97	**0.2497**	**0.0065**	16.11	**0.8389**	**0.0000**	21.84	**0.2184**	**0.0023**	33.32	**0.6668**	**0.0000**
	Hawaiian Archipelago												
	All samples				100	-0.0045	0.6297				98.37	**0.0163**	**0.0278**
	Midway, Pearl & Hermes, Laysan, Gardner Pinnacles, FFS, Necker, Niihau, Kauai / Oahu, Maui, island of Hawaii	0	-0.005	0.7829	100	-0.0074	0.6254	1.76	0.0176	0.0594	97.38	**0.0262**	**0.0290**
Endemic													
A. abdominalis	All samples				99.37	0.0063	0.0911				98.77	**0.0123**	**0.0034**
	Kure, Midway, Pearl & Hermes, Lisianski, Laysan, Maro Reef, FFS, Necker / Niihau,	1.07	**0.0107**	**0.0044**	98.81	0.0119	0.081	0.98	**0.0098**	**0.0123**	98.26	**0.0175**	**0.0028**

	Kauai, Oahu, Maui, island of Hawaii												
Widespread													
C. vanderbilti	**Indo-Pacific locations and Hawaiian Archipelago**												
	All samples				82.02	**0.1798**	**0.0000**				88.72	**0.1128**	**0.0000**
	Cook Islands, Moorea, Palmyra / Hawaiian Archipelago	51.44	**0.5144**	**0.0016**	47.49	**0.5251**	**0.0000**	61.74	**0.3716**	**0.0023**	37.16	**0.3826**	**0.0000**
	Hawaiian Archipelago												
	All samples				99.91	0.0009	0.3968				100	- 0.0017	0.6533
Endemic													
C. ovalis	All samples				98.79	**0.0121**	**0.0047**				99.41	**0.0059**	**0.0370**
	Kure, Midway, Pearl & Hermes / Lisianski, Laysan, Maro Reef, FFS, Necker, Niihau, Kauai, Oahu, Maui, island of Hawaii	1.21	**0.0121**	**0.0338**	98.08	**0.0192**	**0.0049**	0.96	**0.0096**	**0.0287**	98.84	**0.0116**	**0.0368**
C. verater	**Johnston Atoll and Hawaiian Archipelago**												
	All samples				97.68	**0.0232**	**0.0000**				97.06	**0.0363**	**0.0000**
	Johnston Atoll / Hawaiian Archipelago				93.21	**0.0679**	**0.0000**				88.44	**0.1156**	**0.0000**
	Hawaiian Archipelago												
	All samples				99.07	**0.0093**	**0.0197**				98.85	**0.0115**	**0.0087**
	Island of Hawaii / rest of archipelago				97.89	**0.0211**	**0.0194**				96.48	**0.0352**	**0.0045**

Table 3 Population pairwise Φ_{ST} values for *A. vaigiensis*. Cyt*b* below the diagonal and CR above. Bold denotes significant values (P <0.05) and * denotes significance after application of the false discovery rate ($P \leq 0.01$). Locations 1 – 7 are in the Indo-Pacific, and 8 – 18 are in the Hawaiian Archipelago.

Location	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**
1. Madagascar	-	0.3064	**0.7123**	0.1597	0.1818	**0.7641***	0.5932	0.4055	**0.4072**	0.2497	0.3422	**0.4151**	0.1241	0.1980	0.3855	**0.3624**	0.3586	0.3508
2. Saudi Arabia	**0.5398***	-	**0.8877***	0.0112	0.0352	**0.6546***	**0.8777***	**0.0792***	**0.0487***	**0.0407**	**0.0682***	**0.0522***	0.0531	0.0185	**0.0822***	**0.0617***	**0.0442**	0.0291
3. Chagos Archipelago	**0.7909***	**0.9657***	-	**0.9108***	**0.9134***	**0.9574***	**0.9421***	**0.9084***	**0.9092***	**0.9055***	**0.9046***	**0.9057***	**0.9078***	**0.9044***	**0.9130***	**0.9105***	**0.9096***	**0.9041***
4. Australia	0.1957	0.0071	**0.9598***	-	0.0163	**0.8582***	**0.9127**	0.0805	0.0526	0.0287	0.0602	0.0102	-0.0044	-0.0197	0.0674	0.0648	-0.0011	-0.0016
5. Fiji	**0.2201**	**0.1936***	**0.9625***	0.0415	-	**0.8780***	**0.9300**	**0.1462**	0.0681	0.0402	0.0952	0.0203	0.0782	0.0582	**0.1198**	**0.1366**	0.0374	0.0190
6. Moorea	**0.7383***	**0.7696***	**0.9833***	**0.8472***	**0.8920***	-	**0.9946***	**0.7076***	**0.7066***	**0.7964***	**0.7256***	**0.6808***	**0.8616***	**0.8085***	**0.7667***	**0.7516***	**0.7547***	**0.7252***
7. Palmyra	0.5605	**0.9702***	**0.9661***	**0.9562**	**0.9674**	**0.9985***	-	**0.9144***	**0.9149***	**0.9095**	**0.9085***	**0.9091***	**0.9116**	**0.9062**	**0.9225***	**0.9157***	**0.9142***	**0.9062***
8. Midway	**0.6188***	**0.0396***	**0.9663***	-0.0102	**0.2246***	**0.7337***	**0.9693***	-	0.0054	0.0390	-0.0067	**0.0280**	0.0199	-0.0127	-0.0100	**0.1012***	0.0307	0.0152
9. Pearl and Hermes	**0.6456***	0.0162	**0.9737***	0.0537	**0.1860**	**0.8209***	**0.9817***	**0.0471**	-	0.0200	0.0040	-0.0018	0.0359	-0.0023	-0.0061	**0.0836***	0.0186	-0.0106
10. Laysan	**0.3870***	0.0205	**0.9624***	-0.0905	0.0828	**0.8104***	**0.9652**	-0.0190	-0.0004	-	0.0328	0.0124	-0.0393	-0.0238	0.0292	0.0310	-0.0226	-0.0222
11 .Gardner Pinnacles	**0.5343***	**0.0622**	**0.9645***	-0.0312	0.1297	**0.7696***	**0.9679***	0.0003	0.0391	-0.0359	-	-0.0110	0.0073	-0.0409	-0.0139	**0.0945***	0.0148	0.0161
12 .French Frigate Shoals	**0.6130***	**0.0567**	**0.9645***	-0.0297	0.0560	**0.7249***	**0.9668***	**0.0350**	0.0080	-0.0350	-0.0205	-	0.0001	-0.0221	0.0136	**0.0689***	-0.0100	-0.0054
13. Necker	**0.2106**	**0.1004**	**0.9613***	-0.1719	0.1099	**0.8674***	**0.9612**	0.0147	**0.1511**	-0.0580	-0.0321	0.0063	-	-0.0165	0.0601	0.0408	-0.0451	-0.0156
14. Niihau	**0.3454**	-0.0053	**0.9652***	-0.0696	0.1499	**0.8617***	**0.9742**	-0.0508	-0.0045	-0.0872	-0.0629	-0.0374	-0.0436	-	-0.0298	0.0367	-0.0166	-0.0176
15. Kauai	**0.5806***	0.0051	**0.9713***	0.0065	0.2047	**0.8238***	**0.9800***	0.0041	-0.0223	-0.0328	0.0148	0.0035	0.0959	-0.0392	-	**0.1155***	0.0327	0.0140
16. Oahu	**0.4901**	**0.0443**	**0.9623***	-0.0759	0.1155	**0.7628***	**0.9639***	-0.0169	0.0307	-0.0567	-0.0457	-0.0208	-0.0808	-0.0723	-0.0029	-	0.0373	**0.0444**
17. Maui	**0.5085***	**0.0517**	**0.9639***	-0.0483	0.0873	**0.7742***	**0.9671***	0.0018	0.0231	-0.0451	-0.0437	-0.0286	-0.0474	-0.0649	0.0086	-0.0463	-	-0.0045
18. Island of Hawaii	**0.5251***	0.0259	**0.9627***	-0.0702	0.0840	**0.7469***	**0.9644***	0.0107	0.0022	-0.0378	0.0152	0.0055	-0.0174	-0.0218	-0.0131	-0.0116	0.0000	-

Table 4 Population pairwise Φ_{ST} values for *C. vanderbilti*. Cyt*b* below the diagonal and CR above. Bold denotes significant values (P <0.05) and * denotes significance after application of the false discovery rate ($P \leq 0.01$). Locations 1 – 3 are in the Indo-Pacific, and 4 – 15 are in the Hawaiian Archipelago.

Location	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**
1. Cook Islands	-	0.0914	**0.1772***	**0.4973***	**0.4755***	**0.4334***	**0.4216***	**0.4789***	**0.4472***	**0.4634***	**0.4417***	**0.4847***	**0.4765***	**0.4636***	**0.4503***
2. Moorea	0.0281	-	0.0731	**0.5857***	**0.5326***	**0.4609***	**0.4436***	**0.5545***	**0.5405***	**0.5137***	**0.4964***	**0.5650***	**0.5241***	**0.5116***	**0.4948***
3. Palmyra	**0.2462***	0.3216	-	**0.1867***	**0.1815***	**0.1425***	**0.1338***	**0.1846***	**0.1391**	**0.1677***	**0.1397***	**0.1836***	**0.1706***	**0.1583***	**0.1571***
4. Kure	**0.4813***	**0.5893**	**0.6251***	-	0.0039	-0.0100	-0.0041	0.0110	0.0033	0.0057	-0.0045	0.0058	-0.0010	-0.0114	-0.0064
5. Midway	**0.5512***	**0.6543***	**0.6708***	-0.0065	-	0.0020	-0.0008	0.0043	0.0131	**0.0231**	-0.0088	0.0189	**0.0224**	0.0104	0.0078
6. Pearl and Hermes	**0.4002***	**0.4572**	**0.5428***	-0.0119	0.0136	-	-0.0140	0.0108	-0.0025	0.0015	-0.0118	0.0055	-0.0010	-0.0097	-0.0099
7. Laysan	**0.3696***	0.3779	**0.5105***	-0.0168	0.0029	-0.0191	-	-0.0020	-0.0255	-0.0008	0.0007	-0.0072	0.0053	-0.0141	-0.0179
8. French Frigate Shoals	**0.6004***	**0.7346***	**0.7244***	-0.0103	-0.0085	0.0328	0.0287	-	-0.0038	-0.0060	0.0043	-0.0103	0.0104	0.0031	-0.0096
9. Necker	**0.5325***	**0.7357**	**0.6913***	-0.0191	-0.0394	-0.0121	-0.0155	-0.0001	-	-0.0118	-0.0044	-0.0048	-0.0031	-0.0051	-0.0194
10. Nihoa	**0.4269***	**0.5620**	**0.5974***	0.0088	0.0447	-0.0091	-0.0169	**0.0880***	0.0215	-	0.0159	-0.0140	-0.0018	-0.0132	-0.0141
11. Niihau	**0.4882***	**0.5674**	**0.6182***	-0.0167	-0.0044	-0.0127	-0.0002	-0.0015	-0.0223	0.0303	-	0.0090	-0.0020	0.0035	0.0013
12. Kauai	**0.4238***	**0.5143**	**0.5745***	-0.0180	0.0164	-0.0143	-0.0178	**0.0403**	-0.0078	-0.0179	0.0071	-	-0.0103	-0.0096	-0.0122
13. Oahu	**0.4351***	**0.5438**	**0.5912***	-0.0042	0.0275	-0.0097	-0.0122	**0.0592**	0.0009	-0.0203	0.0098	-0.0170	-	-0.0102	-0.0069
14. Maui	**0.4393***	**0.5229**	**0.5830***	-0.0176	0.0040	-0.0163	-0.0243	0.0230	-0.0198	-0.0098	-0.0015	-0.0172	-0.0124	-	-0.0144
15. Island of Hawaii	**0.3882***	**0.4261**	**0.5217***	-0.0111	0.0179	-0.0084	-0.0052	**0.0328**	-0.0043	0.0011	0.0103	-0.0086	-0.0017	-0.0020	-

Table 5 Number of sampling locations in the Hawaiian Archipelago, percentage of significant ($P < 0.05$) pairwise Φ_{ST} comparisons within the NWHI, within the MHI, and between the NWHI and MHI for *A. vaigiensis*, *A. abdominalis*, C. *vanderbilti*, *C. ovalis*, and *C. verater*, based on cyt*b* and CR sequence data. Data for endemic species is from Chapter 1.

			Cyt*b*				**CR**			
Species	**Distribution**	**No. of Hawaiian sampling locations**	**% significant pairwise**	**Within NWHI**	**Within MHI**	**Between NWHI and MHI**	**% significant pairwise**	**Within NWHI**	**Within MHI**	**Between NWHI and MHI**
A. vaigiensis	widespread	11	5%	100%	-	-	13%	14%	29%	57%
A. abdominalis	endemic	13	6%	-	-	100%	26%	10%	10%	80%
C. vanderbilti	widespread	12	6%	25%	-	75%	3%	50%	-	50%
C. ovalis	endemic	15	17%	44%	6%	50%	12%	38%	15%	46%
C. verater	endemic	13	23%	38%	14%	48%	13%	17%	33%	50%

FIGURES

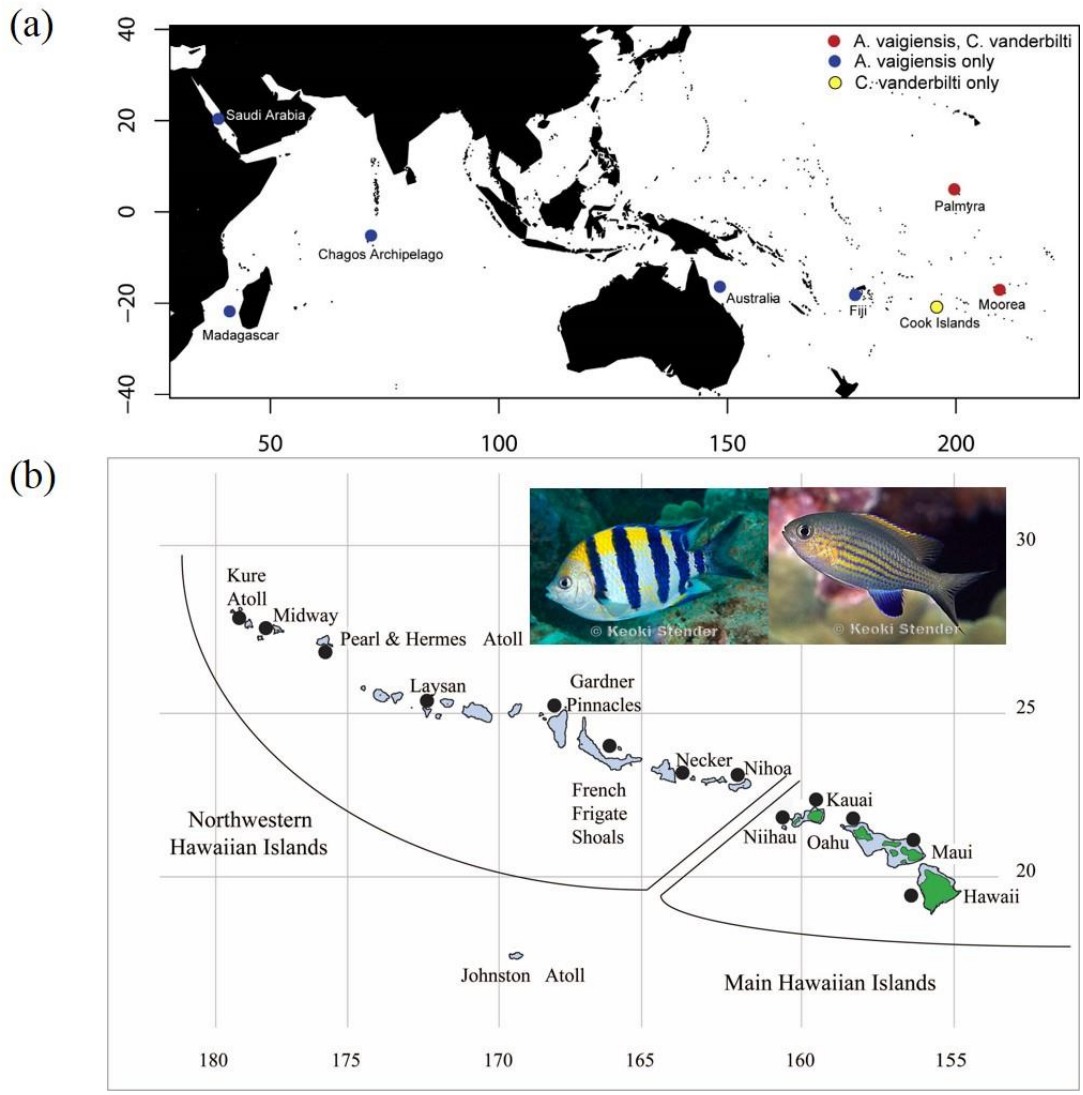

Figure 1 Maps of collection locations: (a) in the Indo-Pacific and (b) within the Hawaiian Archipelago for *A. vaigiensis* and *C. vanderbilti* (photos left to right). In (a), colors represent which species were collected at the Indo-Pacific locations. In the (b), specimens of both species were collected at each location with the exception of Gardner Pinnacles, where only *A. vaigiensis* were collected.

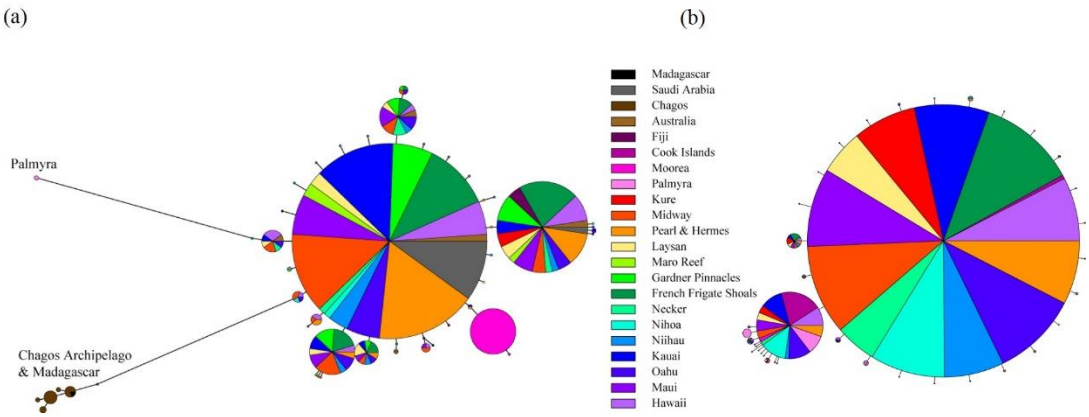

Figure 2 Parsimony-based haplotype networks using cyt*b* sequence data and all sampling locations for: (a) *A. vaigiensis* and (b) *C. vanderbilti*. Each circle represents a haplotype and is proportional to the frequency of that haplotype. Length of branches is proportional to number of mutations. Networks are color-coded by sampling location.

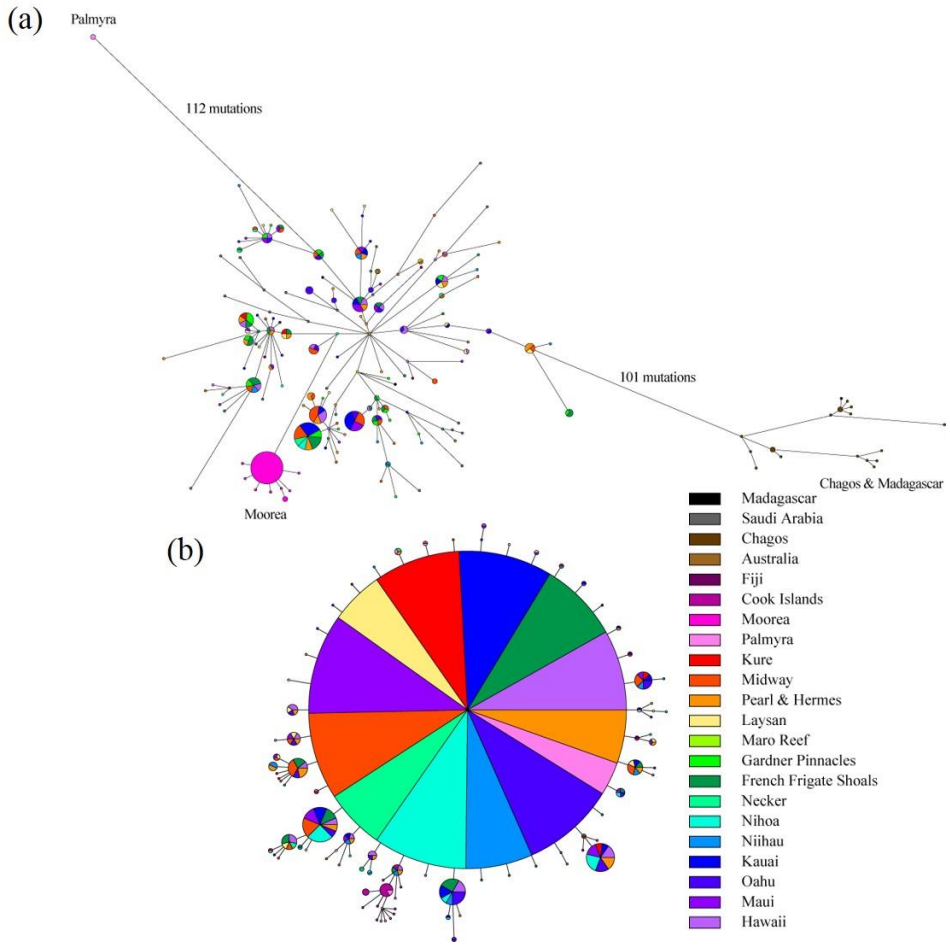

Figure 3 Parsimony-based haplotype networks using CR sequence data and all sampling locations for: (a) *A. vaigiensis* and (b) *C. vanderbilti*. Each circle represents a haplotype and is proportional to the frequency of that haplotype. Length of branches is proportional to number of mutations. Networks are color-coded by sampling location.

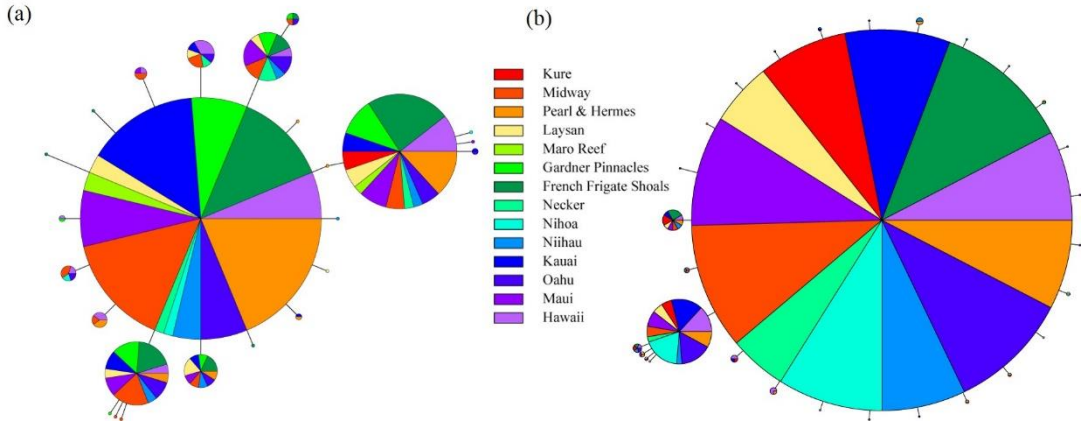

Figure 4 Parsimony-based haplotype networks using cyt*b* sequence data and Hawaiian sampling locations for: (a) *A. vaigiensis* and (b) *C. vanderbilti*. Each circle represents a haplotype and is proportional to the frequency of that haplotype. Length of branches is proportional to number of mutations. Networks are color-coded by sampling location.

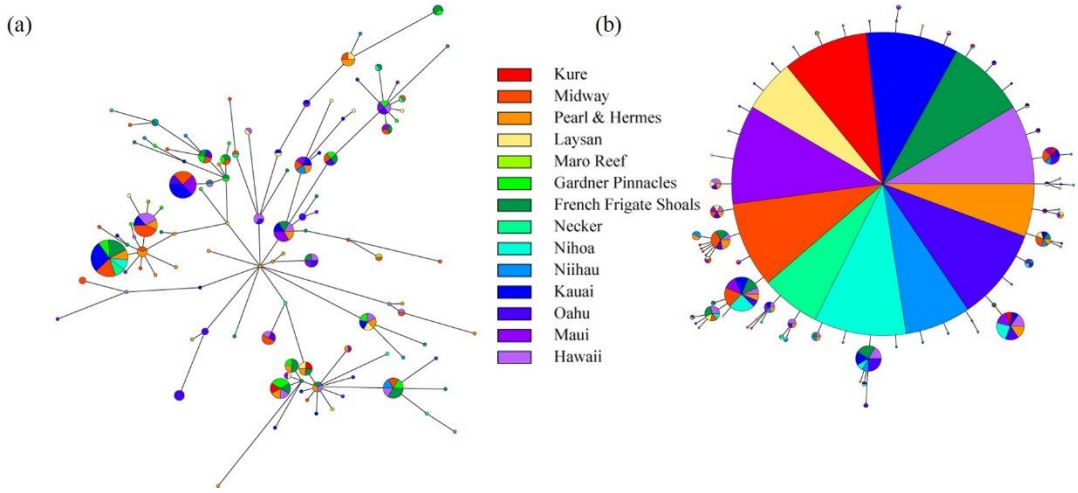

Figure 5 Parsimony-based haplotype networks using CR sequence data and Hawaiian sampling locations for: (a) *A. vaigiensis* and (b) *C. vanderbilti*. Each circle represents a haplotype and is proportional to the frequency of that haplotype. Length of branches is proportional to number of mutations. Networks are color-coded by sampling location.

CHAPTER 3

Vertical and horizontal connectivity in *Chromis verater*, an endemic damselfish found on shallow and mesophotic reefs in the Hawaiian Archipelago and adjacent Johnston Atoll

Abstract

Understanding vertical and horizontal connectivity is a major priority in research on mesophotic coral ecosystems (30-150 m). However, horizontal connectivity has been the focus of few studies, and data on vertical connectivity are limited to sessile benthic mesophotic organisms. Here we present results on patterns of vertical and horizontal connectivity in the Johnston-Hawaiian Islands endemic threespot damselfish, *Chromis verater*, based on 319 shallow specimens and 153 deep specimens. The mtDNA markers cytochrome *b* and control region were sequenced to analyze genetic structure: 1) between shallow (< 30 m) and mesophotic (30-150 m) populations and 2) across the species' geographic range. Additionally, the nuclear markers rhodopsin and internal transcribed spacer 2 of ribosomal DNA were sequenced to assess connectivity between shallow and mesophotic populations. There was no significant genetic differentiation by depth, implying high levels of vertical connectivity between shallow and deep aggregates of *C. verater*. Consequently, shallow and deep samples were combined by location for analyses of horizontal connectivity. We detected low but significant population structure across the Hawaiian archipelago (overall cytochrome *b*: $\Phi_{ST} = 0.009$, $P = 0.020$; control region:

Φ_{ST} = 0.012, P = 0.009) and a larger break between the archipelago and Johnston Atoll (cytochrome *b*: Φ_{ST} = 0.068, $P < 0.001$; control region: Φ_{ST} = 0.116, $P < 0.001$). The population structure within Hawaii was driven by samples from the island of Hawaii at the southeast end of the chain and Lisianski in the middle of the archipelago. The lack of vertical genetic structure supports the refugia hypothesis that deep reefs constitute a population reservoir for species that may be depleted in shallow reef habitats. These findings represent the first connectivity study on a mobile organism that spans shallow and mesophotic depths and provide a reference point for future connectivity studies on mesophotic fishes.

Introduction

The majority of coral reef ecosystems studied to date occur at depths shallower than 30 m, yet zooxanthellate corals can extend to depths of over 150 m (Kahng et al. 2010). Mesophotic coral ecosystems (MCEs or "deep reefs") make up this "twilight zone" of 30-150 m (Pyle 1996; Puglise et al. 2009). The establishment of MCEs depends on multiple factors, including light penetration, water temperature, and substrate availability (Puglise et al. 2009). In areas where shallow reefs thrive, strong thermoclines can prevent the development of mesophotic reefs (Bongaerts et al. 2010a), and the depth at which light is not sufficient to support zooxanthellae defines the lower limit of MCEs (Puglise et al. 2009; Kahng et al. 2010). The upper boundary of mesophotic reefs is based on the depth limit of conventional SCUBA diving (30-40 m) (Kahng et al. 2010). The mesophotic zone is divided into two categories: the upper mesophotic and the lower mesophotic. The upper mesophotic zone occurs between

30-60 m and comprises communities similar to those on shallow reefs (< 30 m) (Slattery et al. 2011). The lower mesophotic zone extends from 60-150 m and generally is inhabited by sponges, algae, and fishes that have adapted to these depths (Slattery et al. 2011).

Studies on vertical and horizontal connectivity have been highlighted as priorities in MCE research (Lesser et al. 2009; Puglise et al. 2009; Hinderstein et al. 2010; Kahng et al. 2014). One of the major motivations for understanding vertical connectivity is evaluating the possibility of whether mesophotic reefs can seed shallow reefs. As postulated in the "deep reef refugia" hypothesis, MCEs may act as a reproductive source that restocks depleted shallow reefs or a haven where shallow populations can escape adverse conditions (Glynn 1996; Bongaerts et al. 2010a; Hinderstein et al. 2010). Given the vulnerability of MCEs to anthropogenic effects that also plague shallow reefs, an additional motivation for studying connectivity in these ecosystems is to prevent the loss of potentially unique genetic diversity.

Vertical connectivity has been the primary emphasis of mesophotic genetic studies to date, with less focus on horizontal connectivity. Kahng et al. (2014) have summarized our current knowledge about connectivity in MCEs. First, there is a growing number of mesophotic studies that demonstrate limited vertical connectivity in sessile benthic organisms (Eytan et al. 2009; Bongaerts et al. 2010b; Costantini et al. 2011; van Oppen et al. 2011; Brazeau et al. 2013). This genetic structure has been suggested to be the result of adaptations to unique environmental conditions at

different depths (Eytan et al. 2009; Kahng et al. 2010; Prada and Hellberg 2012; Luck et al. 2013; Kahng et al. 2014). No generalized patterns have been observed for vertical connectivity in organisms that can move between shallow and mesophotic depths, because few studies of this nature exist. The second pattern that has arisen in MCE connectivity studies is that high levels of horizontal connectivity may be common for mesophotic organisms (Bongaerts et al. 2010b; Gaither et al. 2011b; Brazeau et al. 2013; Costantini et al. 2013; Andrews et al. 2014).

Here we present a genetic survey of the threespot chromis, *Chromis verater*, which inhabits shallow and deep reefs, using mitochondrial and nuclear markers. The pelagic larval duration of *C. verater* is not known but has been postulated to last as long as three months (Swerdloff 1970). The depth range of this species ranges from 7 m to beyond 150 m with a maximum recorded depth of 199 m (Mundy 2005), and it is usually sparse in shallow water and abundant at depths greater than 18 m (Swerdloff 1970; Randall 1998). The presence of higher numbers of juveniles in deeper water suggests that *C. verater* recruits in deeper habitats, occasionally migrating into shallower water later in life (Swerdloff 1970; Hoover 2007). This planktivorous damselfish is endemic to the Hawaiian Archipelago and adjacent Johnston Atoll, which is located about 860 km south of the archipelago. The Hawaiian Archipelago, which comprises the eight main Hawaiian Islands and the nine Northwestern Hawaiian Islands (NWHI) (Figure 1), is one of the few areas in the Pacific where progress is being made in MCE exploration. These ecosystems exhibit a patchy distribution throughout the archipelago, with better developed and deeper

MCEs occurring near the southern end (Rooney et al. 2010; Blyth-Skyrme et al. 2013). Large fish communities have been observed on some mesophotic reefs but, for unknown reasons, are absent from others (Boland et al. 2011). Surveys of MCEs in the NWHI revealed that 46% of reef fishes on mesophotic reefs are endemic species, in comparison to 21% endemism on shallow reefs in this region (DeMartini and Friedlander 2004; Kane et al. 2014). Thus, MCEs harbor fish communities that overlap with those on shallow reefs but also have unique attributes (Brokovich et al. 2008; Bejarano et al. 2014). Our results for *C. verater* can illuminate both the vertical and the horizontal aspects of connectivity in MCEs, as well as the phylogeography of this species.

Our study addresses two primary issues: 1) vertical connectivity between shallow and mesophotic populations of *C. verater* and 2) horizontal connectivity across mesophotic populations and also across the geographic range of this species. With respect to issue #1, we predict exchange between shallow reefs and MCEs based on the abundance of juveniles at depth, resulting in high vertical connectivity. With respect to issue #2, most reef fishes show no structure across the Hawaiian Archipelago, but the exceptions tend to be endemics (Eble et al. 2009; Rivera et al. 2011). In particular, a previous study on the endemic damselfishes *Dascyllus albisella* and *Stegastes marginatus* demonstrated genetic structure across the Hawaiian Archipelago and between the archipelago and Johnston Atoll (Ramon et al. 2008). Therefore, we predict *C. verater* to show horizontal genetic structure across this range.

Material and Methods

Tissue collection and ethics statement

Across the species range in the Hawaiian Archipelago and Johnston Atoll, 319 shallow and 153 mesophotic *C. verater* specimens (fin clips) were collected (Figure 1). Collections at 12 shallow sites were made with pole spears or hand nets with SCUBA or while snorkeling. Collections at 11 mesophotic sites were made using open-circuit technical diving, rebreather diving, and submersibles, and many of the mesophotic specimens (herein referred to as "deep specimens") were collected during research expeditions to explore MCEs in the Hawaiian Archipelago and Johnston Atoll. Although data is unavailable for exact depths at which most specimens were collected, shallow specimens were collected above 30 m, and deep specimens were collected depths below 30 m with a greatest depth of 113 m (Figure 2). All tissue collections were made under permits PMNM-2007-032, PMNM-2008-046, PMNM-2009-032L, PMNM-2009-044, PMNM-2011-025, and PMNM-2012-045, issued by the National Oceanic and Atmospheric Administration, the U.S. Fish and Wildlife Service, and the State of Hawaii Division of Aquatic Resources to BWB at the Hawaii Institute of Marine Biology. Tissue collections were made under protocol approved by the Institutional Animal Care and Use Committees at the University of California Santa Cruz and the University of Hawaii.

DNA extraction, marker amplification, and sequencing

Tissue specimens were preserved in salt-saturated DMSO (Seutin et al. 1991), and genomic DNA was extracted using the HotSHOT method (Meeker et al. 2007). Individuals were amplified for two mitochondrial markers: cytochrome *b* (cyt*b*) and control region (CR). Cyt*b* was amplified with primers GLUDG-5′ (5′-TGACTTGAARAACCAYCGTTG-3′, (Palumbi 1996) and H16460 (5′-CGAYCTTCGGATTACAAGACCG-3′, http://nmg.si.edu/bermlab.htm). CR was amplified with primers Pro-L (5′-CTACCTCCAACTCCCAAAGC-3′, (McMillan and Palumbi 1997) and CR-E (5′-CCTGA AGTAGGAACCAGATG-3′, (Lee et al. 1995). These markers were chosen so that our results could be compared to previous studies and in case the more variable non-coding CR would be able to resolve patterns not detected in cyt*b*.

To verify that any observed patterns were not restricted to the mitochondrial genome, subsets of the shallow (*N*=49) and mesophotic (*N*=45) specimens from the Hawaiian Archipelago were amplified for two nuclear markers: rhodopsin and internal transcribed spacer 2 of ribosomal DNA (ITS2). Rhodopsin was amplified according to published nested amplification protocols, using RHO-30F and RHO-319R for the first set of primers and Rod-F2x and Rod-R4n in the second (Sevilla et al. 2007). ITS2 was amplified following published protocols, using primers 5.8sr (5′-CTACGCCTGTCTGAGTGTC-3′) and 28s (5′-ATATGCTTAAATTCAGCGGG-3′) (Presa et al. 2002).

Polymerase chain reactions (PCRs) were performed in 14 µl reactions containing 1 µl of diluted DNA extract (one part DNA to 49 parts of nanopure water), 0.29 µl of each 10 µM primer, 7.14 µl of premixed PCR solution MangoMix™ (Bioline Inc., Springfield, NJ, USA), and 5.28 µl of nanopure water. PCR amplification of cyt*b* consisted of an initial denaturation at 94°C for 3 min, followed by 35 cycles of 45 s at 94°C, 1 min 15 s at 50°C, and 1 min 15 s at 72°C, with a final extension for 5 min at 72°C. PCR amplification of CR consisted of an initial denaturation at 94°C for 5 min, followed by 35 cycles of 30 s at 94°C, at 49°C, and at 72°C, with a final extension for 7 min at 72°C. After purification of PCR products following the manufacturer's protocol (Applied Biosystems, Foster City, CA, USA), sequencing was performed with the forward PCR primers on an ABI 3100 automated sequencer (Applied Biosystems, Foster City, CA, USA) at the University of California Berkeley's DNA Sequencing Facility. Sequences were aligned and edited using GENEIOUS R6 (Biomatters, LTD, Auckland, NZ). Alignments of cyt*b* and rhodopsin were unambiguous, while CR and ITS2 each contained multiple indels, which varied from 1-2 bp (control region) and 1-28 bp (ITS2) in length. For the nuclear markers, IUPAC ambiguity codes were used to score heterozygous individuals. Unique haplotypes for each marker were identified in ARLEQUIN and were uploaded to GenBank.

Genetic diversity and population structure analyses

Haplotype diversity (*h*) and nucleotide diversity (π) were calculated in ARLEQUIN 3.5 (Excoffier and Lischer 2010). Population structure was analyzed in

terms of vertical connectivity and horizontal connectivity, using analyses of molecular variance (AMOVAs) and population pairwise Φ_{ST} comparisons in ARLEQUIN. The Φ_{ST} fixation index incorporates genetic distance and ranges from 0 to 1, with low values indicating a lack of genetic structure and high values indicating genetic differentiation. Significance of pairwise Φ_{ST} comparisons and AMOVA calculations was tested with 10,000 permutations, and to correct for multiple comparisons, a modified false discovery rate method was implemented (Benjamini and Yekutieli 2001). We determined the best model of sequence evolution for each marker in jMODELTEST2 (Posada and Crandall 1998; Guindon and Gascuel 2003). Because the models identified by the Akaike information criterion were not available in ARLEQUIN, we selected the Tamura-Nei model as it was the most similar one available (Tamura and Nei 1993). Because Midway deep (N=2) and Necker deep (N=1) had small sample sizes, they were included in adjacent populations of Pearl and Hermes deep and French Frigate Shoals deep respectively, after establishing that they had closely related haplotypes to those at these adjacent sites. Parsimony-based haplotype networks for each marker were constructed in R using haploNet in the package PEGAS 0.5-1 (Paradis 2010). Haplotype frequencies used in these networks were calculated in ARLEQUIN.

For comparisons within the Hawaiian Archipelago, we wanted to rule out the possibility that the large sample size of shallow specimens (N=296) was overwhelming population structure due to the mesophotic specimens (N=129). To accomplish this, we sought to run AMOVAs with equal sample sizes for the shallow

and mesophotic specimens. The shallow dataset was randomly subsampled for 129 individuals to match the number of mesophotic specimens, and subsampling was replicated ten times. Then for both mitochondrial markers, AMOVAs were run with the full set of Hawaiian mesophotic specimens and each of the shallow subsample sets to determine whether there was significant genetic structure between shallow and deep.

To avoid making *a priori* assumptions about the possible locations of genetic barriers, we used BARRIER 2.2 (Manni et al. 2004), which employs a computational geometry approach to visualize where genetic barriers are located in geographic space. The software implements Monmonier's maximum-difference algorithm to compare a distance matrix (e.g. matrix of pairwise population Φ_{ST} values) with a matrix of geographic distances and identifies where genetic barriers are located geographically. *A posteriori* AMOVAs subsequently were performed on population groupings inferred by BARRIER output.

Mantel tests were performed to determine if there was significant isolation by distance. Mantel tests were run in the VEGAN package in R with 10,000 permutations, using matrices of pairwise Φ_{ST} values and geographic distance as calculated by the Geographic Distance Matrix Generator (Oksanen et al. 2013; Ersts 2014).

Results

A total of 719 bp of cyt*b* and 394 bp of CR were resolved for 319 shallow and 153 mesophotic *C. verater* specimens, including those from Johnston Atoll. Summary statistics for number of haplotypes (*H*), haplotype diversity (*h*), and nucleotide diversity (π) are listed in Table 1. Nucleotide diversity across shallow sites was similar to that across mesophotic sites for both markers (Table 1). For locations with shallow and mesophotic specimens (Johnston Atoll, French Frigate Shoals, Nihoa, Niihau, Oahu, and Maui), nucleotide diversity at shallow sites was comparable to that at mesophotic sites. Overall haplotype diversity was very high with $h = 0.9041$ to 0.9066 for cyt*b* and $h = 0.9994$ to 0.9997 for CR (Table 1). For cyt*b*, haplotype diversity for shallow Johnston Atoll ($h = 0.6245$) and deep Johnston Atoll ($h = 0.7645$) were lower than that of any site in the Hawaiian Archipelago ($h = 0.8182$-0.9722). Nearly every CR sequence was a unique haplotype, so haplotype diversity was even higher for this marker and had a narrower range across the various sites ($h = 0.9833$-1.0000). Haplotype diversity across shallow sites was similar to that across mesophotic sites for both markers (Table 1). For locations with shallow and mesophotic specimens, haplotype diversity at shallow sites was comparable to that at mesophotic sites.

The haplotype networks for cyt*b* and CR in *C. verater* do not illustrate clustering of haplotypes by depth (Figure 3 and Figure 4). In the network for cyt*b*, the three most common haplotypes comprise both shallow and mesophotic individuals.

Since nearly each CR sequence constituted a unique haplotype, the shape of this network is very different from that for cyt*b*. Nevertheless, there seems to be abundant intermixing of shallow and mesophotic specimens. In the supplementary material, the same haplotype networks are presented but are color-coded according to geographic sampling location (Figure 7 and Figure 8). Overall, haplotypes do not appear to group by geographic location, except for some clustering of Johnston Atoll haplotypes in the CR haplotype network.

A total of 442 bp of rhodopsin and 401 bp of ITS2 were sequenced for 49 shallow and 45 mesophotic *C. verater* specimens from the Hawaiian Archipelago. Summary statistics for number of haplotypes (*H*), haplotype diversity (*h*), and nucleotide diversity (π) for the nuclear markers are listed in Table 6. Nucleotide diversity across shallow sites was higher than that across mesophotic sites for both nuclear markers (Table 6). Haplotype diversity across shallow sites was similar to that across mesophotic sites (Table 6).

Similar to the haplotype networks for the mtDNA markers, the networks for rhodopsin and ITS2 do not show clustering of haplotypes by depth (Figure 5 and Figure 6). The network for rhodopsin is dominated by three common haplotypes, and the ITS2 network has one common haplotype. When color-coded by sampling location, in the network for ITS2, a few haplotypes comprised of individuals from Kauai, Lisianski, and Maui deep are divergent from the other haplotypes by 12

mutations (Figure 10). Nonetheless, the haplotype networks do not show clustering by geographic location (Figure 9 and Figure 10).

Vertical connectivity

To determine if there was significant genetic differentiation by depth in *C. verater*, first we ran an AMOVA separating all of the specimens from Johnston Atoll and the Hawaiian Archipelago into two groups: shallow and mesophotic. Neither cyt*b* nor CR demonstrated significant genetic structure between the shallow and mesophotic groups (cyt*b*: Φ_{ST} = 0.003, *P* = 0.079; CR: Φ_{ST} = 0.003, *P* = 0.103) (Table 2). In addition, when Johnston Atoll individuals were removed from the analysis, there was no evidence of significant structure by depth across the Hawaiian Archipelago (cyt*b*: Φ_{ST} = 0.001, *P* = 0.217; CR: Φ_{ST} = -0.00002, *P* = 0.366). Likewise, AMOVAs indicated no significant genetic structure between shallow and deep populations for either nuclear marker (rhodopsin: Φ_{ST} = -0.018, *P* = 0.779; ITS2: Φ_{ST} = -0.009, *P* = 0.482). For the mtDNA markers, AMOVAs also were performed for individual locations that had shallow and mesophotic individuals (Johnston Atoll, Pearl & Hermes, French Frigate Shoals, Nihoa, Niihau, Oahu, Maui), and none demonstrated significant population structure between shallow and mesophotic specimens (Table 2).

For comparisons within the Hawaiian Archipelago, we employed a subsampling procedure for the mtDNA markers to rule out the possibility that the large sample size of shallow specimens (*N*=296) was overwhelming population

structure due to the mesophotic specimens (*N*=129). Nine out of ten runs showed no evidence of population structure by depth (Table 3). One run indicated very weak structure that was nearly significant for cyt*b* (Φ_{ST} = 0.005, *P* = 0.061) and significant for control region (Φ_{ST} = 0.007, *P* = 0.044). Since there was a lack of significant vertical genetic structure in the majority of these runs, we did not perform additional subsampling runs for this data set.

Horizontal connectivity

We performed an AMOVA using all Johnston Atoll and Hawaiian locations without separating shallow and mesophotic individuals. We detected weak yet significant population structure for both cyt*b* and CR (cyt*b*: Φ_{ST} = 0.023, *P* < 0.00001; for CR: Φ_{ST} = 0.036, *P* < 0.00001) (Table 4). Pairwise comparisons revealed that Johnston Atoll likely was driving this structure, as it was significantly different from almost all locations in the Hawaiian Archipelago (Table 5). Also, BARRIER identified a genetic break between Johnston Atoll and the Hawaiian Archipelago. AMOVAs that were run with individuals grouped into these two regions confirmed that this break was significant (cyt*b*: Φ_{ST} = 0.068, *P* < 0.00001; for CR: Φ_{ST} = 0.116, *P* < 0.00001) (Table 4).

Specifically to test for connectivity across mesophotic sites, an AMOVA was performed across all Johnston Atoll and Hawaiian mesophotic sites, revealing low but significant structure (cyt*b*: Φ_{ST} = 0.035, *P* = 0.001; CR: Φ_{ST} = 0.032, *P* = 0.002) (Table 4). Again, Johnston Atoll specimens were driving this significant genetic

structure. When the analysis was run without Johnston Atoll, the population structure was not significant across the Hawaiian mesophotic sites (cyt*b*: Φ_{ST} = 0.013, *P* = 0.127; CR: Φ_{ST} = -0.001, *P* = 0.500).

To determine if there was population structure across the Hawaiian Archipelago, we ran AMOVAs with Johnston Atoll removed from the analyses. When all Hawaiian populations were included without distinguishing between shallow and deep specimens, the overall population structure was weak but significant (cyt*b*: Φ_{ST} = 0.009, *P* = 0.020; CR: Φ_{ST} = 0.012, *P* = 0.009) (Table 4). Population pairwise tests shed light on which populations could be responsible for this signal (Table 5). For cyt*b*, Lisianski was significantly different in all pairwise comparisons, except with the adjacent location at Laysan. When Lisianski was excluded from the AMOVA, the overall population structure across the archipelago was no longer significant for cyt*b* (Φ_{ST} = 0.004, *P* = 0.117) but remained significant for CR (Φ_{ST} = 0.011, *P* = 0.011). For both cyt*b* and CR, the island of Hawaii was significantly different in at least half of the comparisons (6 for cyt*b*; 7 for CR). In the analysis of the archipelago, BARRIER identified a genetic break between the island of Hawaii and the rest of the Hawaiian populations. Grouping individuals into these two regions in an AMOVA confirmed a significant break (cyt*b*: Φ_{ST} = 0.021, *P* = 0.019; CR: Φ_{ST} = 0.035, *P* = 0.004) (Table 4).

Mantel tests were performed combining shallow and deep specimens by location both with Johnston Atoll individuals and with only Hawaiian locations.

There was no evidence for isolation by distance across locations in the Hawaiian Archipelago (cyt*b*: r = 0.022, *P* = 0.400; CR: r = 0.010, *P* = 0.427). Even when Johnston Atoll was included in the analysis, there was no significant correlation between Φ_{ST} and geographic distance (cyt*b*: r = 0.031, *P* = 0.384; CR: r = 0.089, *P* = 0.243).

Discussion

This study represents the first attempt to assess: 1) horizontal connectivity across mesophotic populations and 2) vertical connectivity between shallow and mesophotic reefs in a species of reef fish. We acknowledge the shortcomings of low mesophotic sample sizes and uneven geographic sampling, which are due to the difficulty of collecting specimens at mesophotic depths. It would be premature to use these data on *C. verater* to make broad generalizations about connectivity patterns in mesophotic fishes, and caution should be exercised in extending these results to other types of fishes that occur at depth. Nevertheless, the results presented here serve as a case study of a reef fish species that spans shallow and mesophotic depths and provide an initial reference point for understanding connectivity in mobile mesophotic organisms.

Vertical connectivity

Using the mitochondrial markers cyt*b* and CR, we found high levels of genetic connectivity between shallow and mesophotic populations of *C. verater* in the Hawaiian Archipelago. Even for individual locations where shallow and mesophotic

individuals had been collected, there was no significant genetic differentiation by depth. For the Hawaiian Archipelago, the large number of shallow specimens (*N*=296) is not interfering with a signal of genetic structure from the mesophotic specimens (*N*=129). When analyses were run with equivalent sample sizes of shallow and mesophotic individuals, nine out of ten runs exhibited high levels of vertical connectivity. We dismissed the possibility that this trend was limited to the mitochondrial genome by sequencing a subset of specimens for nuclear markers rhodopsin and ITS2, which also failed to demonstrate genetic differentiation by depth.

Explicit collection depths were not available for most specimens, raising the possibility that the lack of vertical genetic structure is due to errors in categorizing specimens as shallow or mesophotic. However, most mesophotic specimens were collected during expeditions that specifically sought to explore deep reefs with open-circuit technical diving, rebreather diving, or submersibles, so we believe that this potential for error is minimal. To address this concern, future connectivity studies on reef fishes that span shallow and mesophotic reefs may want to consider a sampling approach that targets three depth categories, such as shallow (< 20 m), middle (20-40 m), and deep (40+ m). This would allow for comparison of the shallowest and deepest individuals, as well as a separate comparison of specimens that were collected near the threshold depth of 30 m. Execution of such a sampling strategy may be more difficult but could perhaps tease out more fine-scale vertical connectivity patterns than the sampling approach in our study.

The lack of genetic structure between shallow and mesophotic *C. verater* contrasts with a number of mesophotic studies that demonstrate limited vertical connectivity in sedentary benthic organisms, predominantly coral species (Kahng et al. 2014). Multiple coral species exhibit genetic partitioning by depth, with the deepest individuals often segregating as the most genetically distinct (Eytan et al. 2009; Bongaerts et al. 2010b; Costantini et al. 2011; van Oppen et al. 2011; Brazeau et al. 2013). This is likely the result of adaptation to environmental conditions specific to different depths (Prada and Hellberg 2012; Luck et al. 2013). While corals must rely on their gametes for dispersal potential, fishes also have the ability to disperse as juveniles/adults, which may contribute to the vertical genetic homogeneity in *C. verater*. Furthermore, *C. verater* is suspected to have a life history trait that would explain connectivity between populations at different depths. It has been suggested that *C. verater* larvae settle on deep reefs, gradually migrating inshore as they age (Swerdloff 1970).

With respect to the "deep reef refugia" hypothesis, the extensive vertical connectivity revealed by our results implies that mesophotic populations of *C. verater* are capable of replenishing shallow populations. If the ontogenetic shift hypothesis is valid for *C. verater*, then mesophotic populations already are serving as sources for replenishing shallow populations. So far, it appears that the ability for mesophotic populations to serve as "deep reef refugia" varies by site and by organism. For example, at Scott Reef in northwestern Australia, there was evidence of migration from deep (31-43 m) to shallow (25-27 m) colonies in the scleractinian coral

Seriatopora hystrix. Meanwhile, there was no evidence to support the "deep reef refugia" hypothesis at Yonge reef in northeastern Australia, where this species did not exhibit migration from deep to shallow colonies (van Oppen et al. 2011). Additional connectivity studies will elucidate whether these varied patterns extend to mesophotic fishes as well.

Horizontal connectivity across mesophotic reefs

For the analyses of horizontal connectivity using the mitochondrial markers, we made no distinction between shallow and mesophotic individuals, combining them per location. Nevertheless, an AMOVA run with only the mesophotic individuals did not indicate significant genetic structure across the Hawaiian Archipelago. The only anomaly with the mesophotic specimens was that the Lisianski population was significantly different in most pairwise comparisons for cyt*b*. Yet since there were no shallow individuals available for this location, it is not certain that this pattern is unique to mesophotic individuals.

There are no other genetic connectivity studies on mesophotic reef fishes with which to compare the results from *C. verater*. Comparisons could be made with genetic studies on some deepwater snappers (Gaither et al. 2011a; Gomes et al. 2012; Andrews et al. 2014), but these are not really equivalent comparisons because, while these species can be found at mesophotic depths, they are not necessarily associated with MCE habitat. Studies on mesophotic corals reveal mixed patterns of horizontal connectivity. The coral *S. hystrix* exhibited more genetic structure between depths

than horizontally across geographic locations (Bongaerts et al. 2010b). On the other hand, the mesophotic red coral *Corallium rubrum* demonstrated significant geographic genetic differentiation at multiple spatial scales, from tens of meters to hundreds of kilometers, illustrating limited horizontal connectivity (Costantini et al. 2013). Similarly, the scleractinian coral *Montastraea cavernosa* demonstrated low horizontal connectivity as well as genetic differentiation by depth (Brazeau et al. 2013). Since there was no evidence of genetic structure between depths in our study, the results from our phylogeographic analyses combining shallow and mesophotic individuals should reflect connectivity patterns across mesophotic reefs.

Phylogeography of a Johnston-Hawaiian Islands endemic

When shallow and mesophotic individuals were combined, the results indicate limitations to horizontal connectivity across the 860 km that separate Johnston Atoll and the Hawaiian Archipelago (cyt*b*: $\Phi_{ST} = 0.068$, $P < 0.001$; CR: $\Phi_{ST} = 0.116$, $P < 0.001$). This trend remained significant regardless of whether shallow, mesophotic, or shallow/mesophotic specimens were analyzed. The Johnston Atoll population was significantly differentiated from almost all of the Hawaiian locations in pairwise comparisons for both mitochondrial markers.

The genetic distinctiveness of Johnston Atoll populations in comparison to the Hawaiian Islands has been documented previously (DiBattista et al. 2011; Skillings et al. 2011), including in *Dascyllus albisella*, another Hawaiian Islands-Johnston endemic damselfish (Ramon et al. 2008). Based on oceanographic models, potential

dispersal corridors between Johnston Atoll and French Frigate Shoals in the mid-archipelago and Kauai in the main Hawaiian Islands (Kobayashi 2006). Johnston Atoll has been implicated as a stepping stone for colonization of the Hawaiian Archipelago (Gosline 1955; Kenyon 1992; Maragos et al. 2004; Rivera et al. 2011). Conversely, for some species, Johnston Atoll seems to act more as an outpost for Hawaiian fauna (DiBattista et al. 2011; Skillings et al. 2011). Cyt*b* and CR haplotype diversities for *C. verater* at Johnston Atoll are lower than at any Hawaiian site. Lower genetic diversity could be an artifact of a founder event, in which Johnston Atoll was colonized by a few individuals, or it could be indicative of a smaller population.

Within the Hawaiian Archipelago, a horizontal connectivity pattern for *C. verater* was the genetic divergence of the island of Hawaii. BARRIERS identified that a significant genetic break occurs between this sample and the rest of the archipelago, and this was supported by low but significant AMOVAs with both mitochondrial markers. This genetic break is concordant with one of the strongest marine barriers previously identified in the Hawaiian Archipelago and is believed to be based on oceanographic conditions (Toonen et al. 2011). The Alenuihaha Channel that separates Maui and the island of Hawaii is regarded by native navigators as some of the most dangerous waters in the archipelago, as indicated by the name which translates into “I’ll-end-you-ha-ha”. Winds channeled off the peaks of adjacent Maui (3000 m high) and the island of Hawaii (4100 m high) can be five times stronger than winds outside of the channel. The prevailing northeasterly trade winds produce cyclonic mesoscale eddies on the lee side of Hawaii (Dickey et al. 2008) that have

been reported to last as long as 60 days, sufficient for many reef fish larvae to complete their pelagic stage (Lobel and Robinson 1986). Christie et al. (2010b) posit that active behavior mechanisms allow larvae of the yellow tang, *Zebrasoma flavescens*, to extricate themselves from eddies and settle back on reefs. In that same study, virtual drifters released at 30 m depth stayed closer to the island of Hawaii than drifters released at sea surface level. If *C. verater* larvae recruit to deep reefs (> 30 m) as hypothesized, then they may complete their pelagic larval duration in these eddies, retained near the island of Hawaii. Notably, this explanation does not apply to Lisianski, the only other location in the archipelago to show a low but significant level of population differentiation. Lisianski, a small (1.5 km^2) flat outpost of coral reef habitat, lies 1676 km northwest of Honolulu (Figure 1). Explanations of genetic differentiation due to genetic drift or population size seem unlikely since the large Neva Shoals coral habitat (980 km^2) lies directly southeast of Lisianski. Instead, it is more likely that oceanographic conditions unknown to us are driving this trend at Lisianski.

Conclusions and implications for conservation

This genetic survey of *C. verater*, a reef fish found on both shallow and mesophotic reefs, constitutes the first glimpse of connectivity patterns for mobile organisms that inhabit MCEs. This species exhibits high connectivity between shallow (< 30 m) and mesophotic reefs (> 30 m) in the Hawaiian Archipelago and Johnston Atoll, while demonstrating weak genetic structure across this range. This

dichotomy between vertical and horizontal connectivity provides an interesting perspective on dispersal in endemic species. The restricted range sizes of endemic reef fishes is thought to be a reflection of their limited dispersal abilities (Eble et al. 2009). The lack of genetic structure between shallow and mesophotic specimens in our dataset indicate that *C. verater*'s dispersal abilities do not limit it in terms of vertical connectivity, a scale of 7-113 m in this study. However, vertical connectivity is on a much smaller scale than horizontal connectivity, which shows some limitations within the 2600 km of the Hawaiian Archipelago (2600 km) and between the archipelago and Johnston Atoll (separated by ~ 860 km).

Our study on connectivity in *C. verater* is relevant to emerging conservation issues for MCEs. Biodiversity hotspots are a focus for conservation efforts, and endemic species are a large component of regional biodiversity (Hughes et al. 2002; Roberts et al. 2003; Allen 2008). In the NWHI, endemic reef fishes were over twice as abundant on MCEs as on shallow reefs, illustrating the argument for protecting MCEs as potential biodiversity hotspots (Kane et al. 2014). Another motivation for protection of mesophotic reefs is that they provide shelter for small and juvenile fishes, possibly making this critical nursery habitat for reef fishes (Blyth-Skyrme et al. 2013). Our results indicate that there is a lot of exchange between shallow and mesophotic populations of *C. verater*, highlighting the link between these deep reefs and other parts of coral reef ecosystems. The high levels of vertical connectivity observed in our study lend support to the argument that MCEs serve an important

ecological role as habitat and refugia for populations that may be depleted in shallow habitats.

Tables

Table 1. MtDNA molecular diversity indices for shallow and mesophotic samples of *Chromis verater*. Number of individuals (*N*), number of haplotypes (*H*), nucleotide diversity (π), and haplotype diversity (*h*) are listed for cyt*b* and CR. Because Midway deep (*N*=2) and Necker deep (*N*=1) had small sample sizes, they were included in adjacent populations of Pearl and Hermes deep and French Frigate Shoals deep respectively for most population genetic analyses.

	Cyt*b*								CR							
	N		***H***		***π***		***h***		***N***		***H***		***π***		***h***	
Sample location	**shallow**	**deep**	**shallow**	**deep**	**shallow**	**deep**	**shallow**	**deep**	**shallow**	**deep**	**shallow**	**deep**	**shallow**	**deep**	**shallow**	**deep**
Hawaiian Archipelago																
Kure	6	-	6	-	0.0026± 0.0020	-	1.0000± 0.0962	-	6	-	6	-	0.0695± 0.0412	-	1.0000± 0.0962	-
Midway	34	-	18	-	0.0035± 0.0021	-	0.9091± 0.0353	-	34	-	34	-	0.0828± 0.0412	-	1.0000± 0.0071	-
Pearl and Hermes	30	15	18	10	0.0036± 0.0022	0.0027± 0.0018	0.9402± 0.0269	0.9238± 0.0530	30	15	30	14	0.0850± 0.0425	0.0890± 0.0461	1.0000± 0.0086	0.9905± 0.0281
Lisianski	-	5	-	4	-	0.0022± 0.0018	-	0.9000± 0.1610	-	5	-	5	-	0.0713± 0.0443	-	1.0000± 0.1265
Laysan	-	16	-	11	-	0.0029± 0.0019	-	0.9083± 0.0633	-	16	-	16	-	0.0839± 0.0433	-	1.0000± 0.0221
Gardner Pinnacles	12	-	6	-	0.0021± 0.0015	-	0.8182± 0.0840	-	12	-	12	-	0.0912± 0.0482	-	1.0000± 0.0340	-
French Frigate Shoals	30	9	13	8	0.0026± 0.0017	0.0035± 0.0023	0.8713± 0.0395	0.9722± 0.0640	30	9	29	9	0.0893± 0.0446	0.0889± 0.0487	0.9977± 0.0094	1.0000± 0.0524
Nihoa	32	4	18	4	0.0036± 0.0022	0.0044± 0.0034	0.9435± 0.0231	1.0000± 0.1768	32	4	32	4	0.0889± 0.0442	0.0959± 0.0637	1.0000± 0.0078	1.0000± 0.1768
Niihau	45	22	26	16	0.0040± 0.0024	0.0036± 0.0023	0.9424± 0.0224	0.961± 0.0260	45	22	41	22	0.0888± 0.0438	0.0885± 0.0447	0.9960± 0.0057	1.0000± 0.0137
Kauai	30	-	21	-	0.0035± 0.0022	-	0.9494± 0.0276	-	30	-	27	-	0.0844± 0.0422	-	0.9931± 0.0105	-
Oahu	31	41	16	22	0.0027± 0.0017	0.0032± 0.0020	0.8903± 0.0396	0.8915± 0.0416	31	41	28	41	0.0871± 0.0434	0.0921± 0.0455	0.9935± 0.0100	1.0000± 0.0054
Maui	16	17	10	10	0.0027± 0.0018	0.0033± 0.0021	0.9000± 0.0619	0.9191± 0.0438	16	17	14	17	0.0822± 0.0425	0.0881± 0.0452	0.9833± 0.0278	1.0000± 0.0202
Island of Hawaii	30	-	14	-	0.0028± 0.0018	-	0.8851± 0.0425	-	30	-	30	-	0.0862± 0.0430	-	1.0000± 0.0086	-
All of Hawaiian Archipelago	296	129	83	49	0.0032± 0.0020	0.0032± 0.0020	0.9131± 0.0102	0.9155± 0.0170	296	129	271	128	0.0792± 0.0384	0.0808± 0.0393	0.9993± 0.0004	0.9999± 0.0010
Johnston Atoll																
Johnston Atoll	23	24	7	8	0.0021± 0.0015	0.0029± 0.0019	0.6245± 0.1096	0.7645± 0.0765	23	24	21	22	0.0616± 0.0314	0.0651± 0.0330	0.9921± 0.0154	0.993± 0.0144

Table 2. Analysis of molecular variance (AMOVAs) for vertical connectivity in *Chromis verater*, using different groupings of populations. Percent variation within populations (% variation), fixation indices (Φ_{ST}), and associated *P* values are listed. "/" is used to separate different groupings of sampling locations. Bold values are significant ($P < 0.05$).

Groupings	Cyt*b*			CR		
	% variation within populations	**Φ_{ST}**	***P* value**	**% variation within populations**	**Φ_{ST}**	***P* value**
Johnston Atoll and Hawaiian Archipelago						
All shallow / all deep	99.71	0.0029	0.0786	99.74	0.0026	0.1032
Shallow and deep Johnston Atoll / Shallow and deep Hawaiian Archipelago	92.92	**0.0679**	**0.0001**	88.44	**0.1156**	**0.0000**
Shallow Johnston Atoll / shallow Hawaiian Archipelago	95.24	**0.0476**	**0.0019**	88.08	**0.1192**	**0.0000**
Deep Johnston Atoll / deep Hawaiian Archipelago	92.92	**0.0708**	**0.0001**	90.88	**0.0912**	**0.0000**
Shallow Johnston Atoll / deep Johnston Atoll	100.00	-0.0199	0.7048	100.00	-0.0245	0.9412
Hawaiian Archipelago						
All shallow / all deep	99.87	0.0013	0.2170	100.00	0.0000	0.3663
Shallow Pearl and Hermes / deep Pearl and Hermes	100.00	-0.0072	0.5495	100.00	-0.0008	0.3717
Shallow French Frigate Shoals / deep French Frigate Shoals	100.00	-0.0289	0.7680	100.00	-0.0411	0.9522
Shallow Nihoa / deep Nihoa	100.00	-0.0392	0.6353	100.00	-0.0375	0.6743
Shallow Niihau / deep Niihau	100.00	-0.0072	0.6930	100.00	-0.0177	0.9796
Shallow Oahu / deep Oahu	100.00	-0.0112	0.8643	100.00	-0.0087	0.7999
Shallow Maui / deep Maui	97.97	0.0203	0.1809	98.63	0.0137	0.2101

Table 3. AMOVAs for vertical connectivity runs with sets of shallow subsamples (N=129) in *Chromis verater*. AMOVAs were run with specimens divided into shallow individuals and deep individuals. Percent variation within populations (% variation), fixation indices (Φ_{ST}), and associated *P* values are listed. Bold values are significant ($P < 0.05$).

Subsample	Cyt*b*			CR		
	% variation within populations	**Φ_{ST}**	***P* value**	**% variation within populations**	**Φ_{ST}**	***P* value**
1	99.95	0.0005	n.s.	100	-0.0021	n.s.
2	99.46	0.0054	0.0610	99.29	**0.0071**	**0.0436**
3	99.82	0.0018	n.s.	100	-0.0012	n.s.
4	100	-0.0041	n.s.	100	-0.0023	n.s.
5	100	-0.0016	n.s.	100	-0.0027	n.s.
6	100	0.0000	n.s.	100	-0.0008	n.s.
7	99.89	0.0011	n.s.	100	-0.0023	n.s.
8	99.87	0.0013	n.s.	99.82	0.00178	n.s.
9	100	-0.0033	n.s.	100	-0.0017	n.s.
10	100	-0.0003	n.s.	100	-0.0016	n.s.

Table 4. AMOVAs for horizontal connectivity in *Chromis verater*, using different groupings of populations. "/" is used to separate different groupings of sampling locations. Percent variation within populations (% variation), fixation indices (Φ_{ST}), and associated *P* values are listed. "/" is used to separate different groupings of sampling locations. Bold values are significant ($P < 0.05$).

	Cyt*b*			**CR**		
Groupings	**% variation within populations**	**Φ_{ST}**	***P* value**	**% variation within populations**	**Φ_{ST}**	***P* value**
Johnston Atoll and Hawaiian Archipelago						
All (shallow and deep combined per sampling location)	97.6800	**0.0232**	**0.0000**	97.06	**0.0363**	**0.0000**
All deep	96.54	**0.0346**	**0.0015**	96.84	**0.0316**	**0.0025**
All shallow	98.88	**0.0112**	**0.0182**	97.10	**0.0290**	**0.0000**
Johnston Atoll / Hawaiian Archipelago	93.21	**0.0679**	**0.0000**	88.44	**0.1156**	**0.0000**
Hawaiian Archipelago						
All (shallow and deep combined per sampling location)	99.07	**0.0093**	**0.0197**	98.85	**0.0115**	**0.0087**
All deep	98.69	0.0131	0.1269	100.00	-0.0014	0.5002
All shallow	99.68	0.0032	0.2470	99.05	0.0095	0.0549
Island of Hawaii / rest of archipelago	97.89	**0.0211**	**0.0194**	96.48	**0.0352**	**0.0045**

Table 5. Population pairwise Φ_{ST} values for *Chromis verater*. Cyt*b* below the diagonal and CR above. Bold denotes significant values (P <0.05) and * denotes significance after application of the false discovery rate ($P \leq 0.01$).

Location	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**
1. Kure	-	-0.0259	-0.0317	0.0116	-0.0169	-0.0355	-0.0113	0.0273	-0.0334	-0.0325	-0.0214	-0.0243	0.0180	**0.1985***
2. Midway	-0.0556	-	0.0102	-0.0081	-0.0125	-0.0071	0.0235	**0.0554***	-0.0001	-0.0120	0.0003	-0.0055	**0.0637***	**0.1465***
3. Pearl and Hermes	-0.0573	-0.0104	-	0.0334	0.0125	-0.0142	-0.0026	0.0126	-0.0043	-0.0007	0.0020	0.0027	0.0163	**0.1367***
4. Lisianski	**0.2098**	**0.1159**	**0.1436**	-	-0.0189	0.0084	0.0396	0.0843	0.0238	-0.0061	0.0263	0.0052	**0.1083**	**0.1916***
5. Laysan	-0.0140	-0.0086	0.0040	0.0528	-	0.0057	0.0326	**0.0708**	0.0035	-0.0016	0.0036	-0.0061	0.0792	**0.1285***
6. Gardner Pinnacles	-0.0051	-0.0101	-0.0187	**0.2970***	0.0312	-	-0.0155	0.0124	-0.0061	-0.0160	-0.0047	-0.0115	0.0052	**0.1591***
7. French Frigate Shoals	-0.0138	0.0118	-0.0027	**0.2386***	**0.0448**	-0.0371	-	0.0051	0.0015	0.0123	0.0089	0.0078	0.0096	**0.1257***
8. Nihoa	-0.0179	**0.0247**	0.0125	**0.1414**	0.0254	-0.0139	0.0104	-	**0.0277**	**0.0453**	**0.0394***	**0.0501***	-0.0048	**0.1799***
9. Niihau	-0.0520	0.0024	-0.0050	**0.1425***	0.0118	-0.0174	-0.0019	0.0089	-	-0.0022	-0.0010	0.0009	**0.0343**	**0.1291***
10. Kauai	-0.0490	-0.0078	-0.0102	**0.1577***	0.0055	-0.0145	0.0016	**0.0280**	-0.0023	-	-0.0037	-0.0065	**0.0555**	**0.1370***
11. Oahu	-0.0476	0.0003	-0.0061	**0.1861***	0.0146	-0.0100	0.0040	**0.0271***	-0.0013	-0.0079	-	-0.0108	**0.0521***	**0.1047***
12. Maui	-0.0536	-0.0050	-0.0093	**0.1569**	0.0137	0.0001	0.0104	**0.0351**	0.0026	-0.0060	-0.0072	-	**0.0621***	**0.1087***
13. Island of Hawaii	0.0104	**0.0416**	0.0215	**0.2449***	**0.0690**	-0.0231	-0.0061	-0.0009	0.0144	**0.0380**	**0.0367**	**0.0411**	-	**0.2140***
14. Johnston Atoll	0.0295	**0.0473***	**0.0699***	**0.2578**	**0.0591**	**0.0941**	**0.1045***	**0.1287***	**0.0743***	**0.0375**	**0.0651***	**0.0589***	**0.1563***	-

Table 6. Nuclear molecular diversity indices for shallow and mesophotic samples of *Chromis verater*. Number of individuals (*N*), number of haplotypes (*H*), nucleotide diversity (π), and haplotype diversity (*h*) are listed for subsample of 94 individuals sequenced for rhodopsin and ITS2.

	Rhodopsin								ITS2							
	N		*H*		π		*h*		*N*		*H*		π		*h*	
Sample location	shallow	deep	shallow	deep	shallow	deep	shallow	deep	shallow	deep	shallow	deep	shallow	deep	shallow	deep
Hawaiian Archipelago																
Midway	-	2	-	1	-	0.0000± 0.0000	-	0.0000± 0.0000	-	2	-	1	-	0.0000± 0.0000	-	0.0000± 0.0000
Pearl and Hermes	-	7	-	4	-	0.0005± 0.0008	-	0.8095± 0.1298	-	7	-	2	-	0.0012± 0.0013	-	0.4762± 0.1713
Lisianski	-	3	-	1	-	0.0000± 0.0000	-	0.0000± 0.0000	-	3	-	2	-	0.1318± 0.0993	-	0.6667± 0.3143
Laysan	-	11	-	3	-	0.0005± 0.0007	-	0.6364± 0.0895	-	11	-	4	-	0.0068± 0.0044	-	0.6000± 0.1539
Niihau	8	5	4	3	0.0006 ± 0.0009	0.0000± 0.0000	0.7500 ± 0.1391	0.7000± 0.2184	8	5	1	2	0.0000 ± 0.0000	0.0000± 0.0000	0.0000 ± 0.0000	0.4000± 0.2373
Kauai	16	-	8	-	0.0015 ± 0.0014	-	0.8417 ± 0.0748	-	16	-	6	-	0.0967 ± 0.0496	-	0.6167 ± 0.1347	-
Oahu	12	4	8	2	0.0014 ± 0.0014	0.0000± 0.0000	0.8939 ± 0.0777	0.5000± 0.2652	12	4	2	2	0.0000 ± 0.0000	0.0000± 0.0000	0.1667 ± 0.1343	0.5000± 0.2652
Maui	-	13	-	5	-	0.0004± 0.0007	-	0.8333± 0.0597	-	13	-	5	-	0.0566± 0.0299	-	0.5385± 0.1611
Island of Hawaii	13	-	4	-	0.0005 ± 0.0007	-	0.7564 ± 0.0698	-	13	-	6	-	0.0107 ± 0.0063	-	0.7179 ± 0.1279	-
All of Hawaiian Archipelago	49	45	15	7	0.0011± 0.0011	0.0003± 0.0006	0.8129± 0.0378	0.7131± 0.0418	49	45	10	9	0.0409 ± 0.0205	0.0269 ± 0.0138	0.4600 ± 0.0884	0.4879± 0.0887

Figures

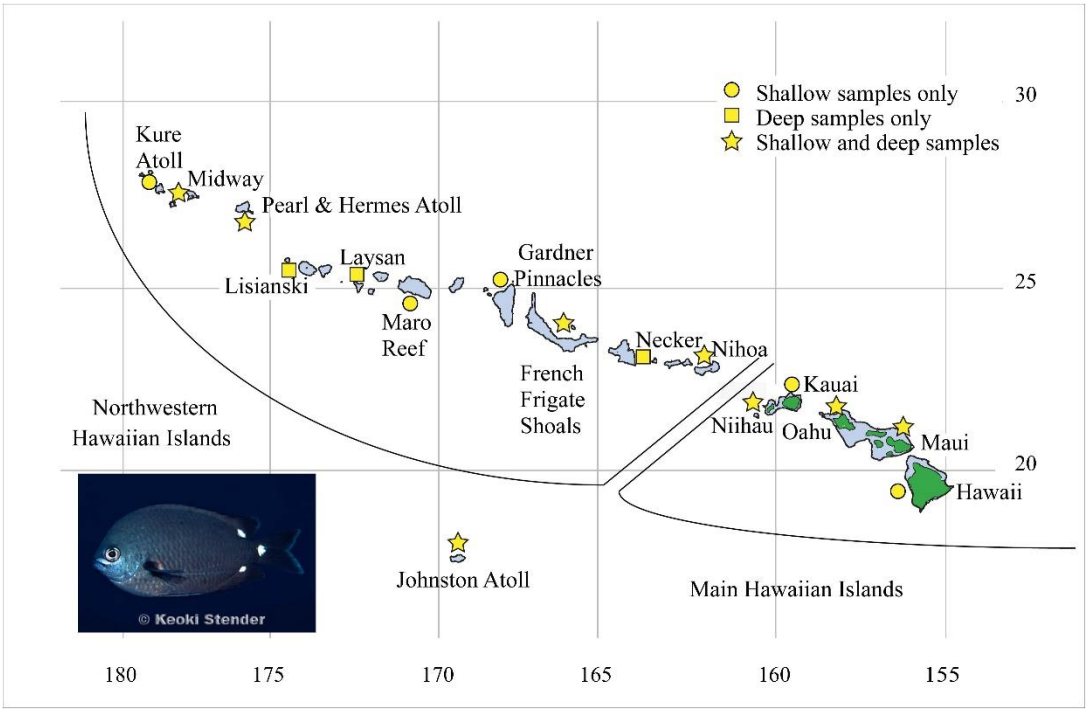

Figure 1. Map of collection locations. Collection locations for *Chromis verater*. Colors indicate whether shallow (red), mesophotic (blue), or both shallow and mesophotic (yellow) specimens were collected at the location. (Photo credit: Keoki Stender, www.marinelifephotography.com)

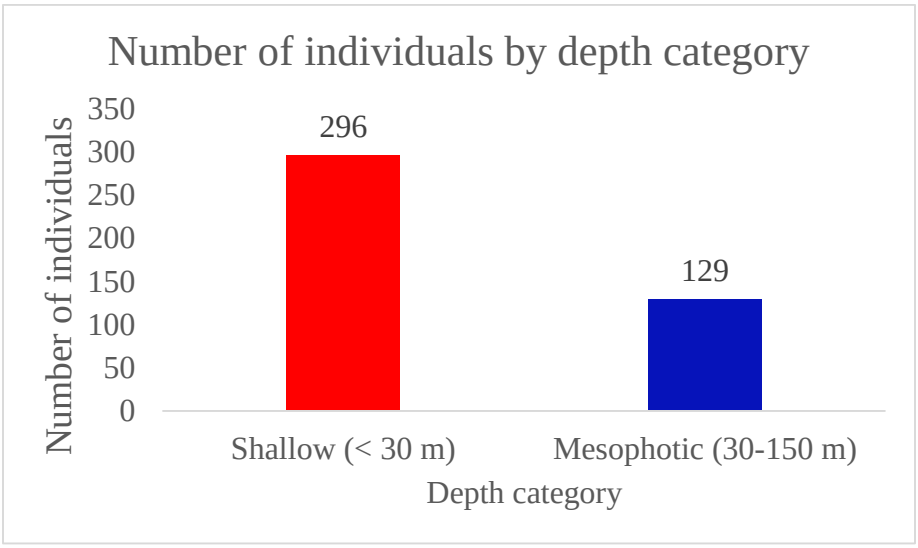

Figure 2. Plot of number of individuals from each depth category.

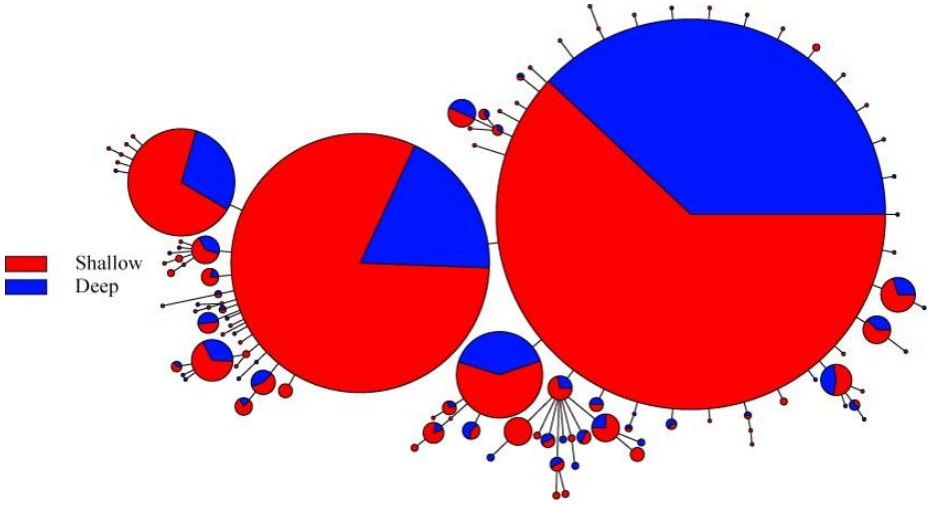

Figure 3. Cyt*b* haplotype network for *C. verater.* Parsimony-based network using cyt*b* sequence data and color-coded according to depth at which specimens were collected.

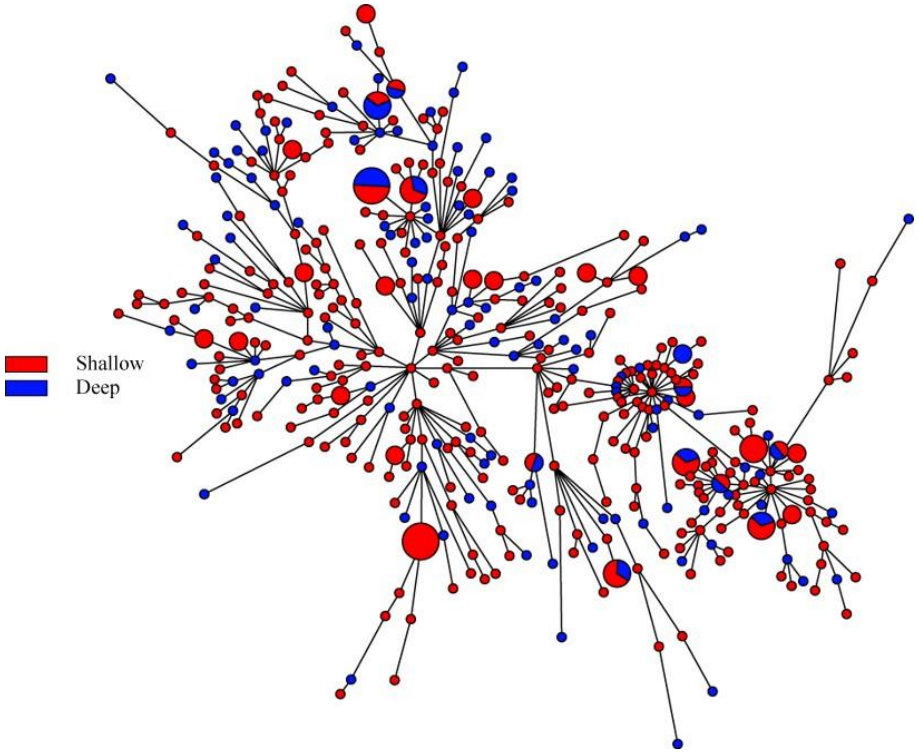

Figure 4. CR haplotype network for *C. verater.* Parsimony-based network using CR sequence data and color-coded according to depth at which specimens were collected.

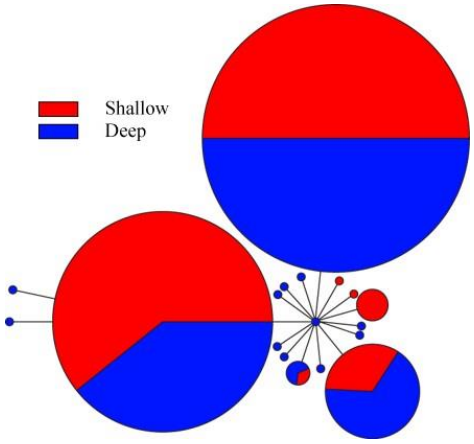

Figure 5. Rhodopsin haplotype network for *C. verater*. Parsimony-based network using rhodopsin sequence data for subsample of 94 specimens and color-coded according to depth at which specimens were collected.

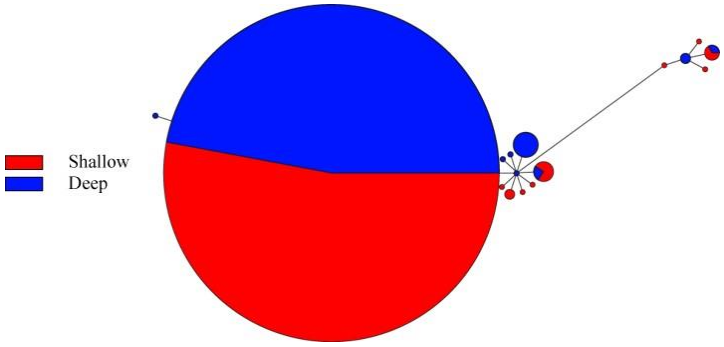

Figure 6. ITS2 haplotype network for *C. verater*. Parsimony-based network using ITS2 sequence data for subsample of 94 specimens and color-coded according to depth at which specimens were collected.

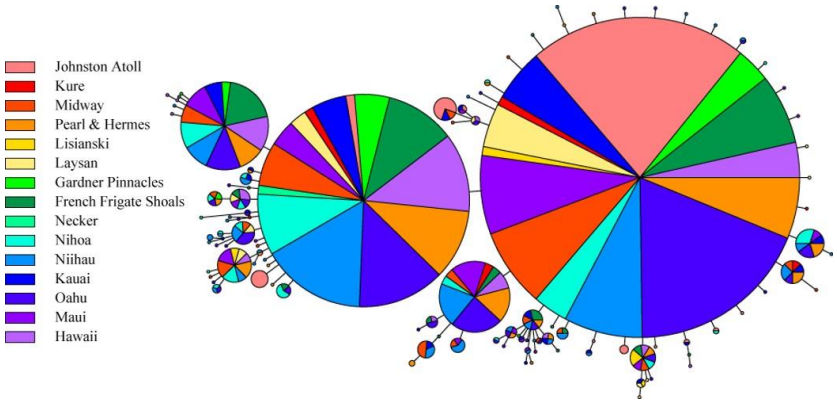

Figure 7. Cytb haplotype network for C. verater. Parsimony-based network using CR sequence data and color-coded by sampling location.

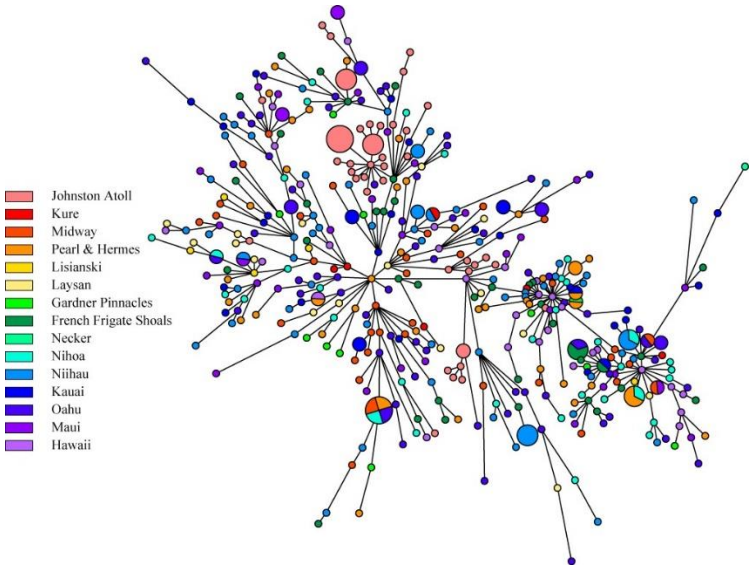

Figure 8. CR haplotype network for C. verater. Parsimony-based network using CR sequence data and color-coded by sampling location.

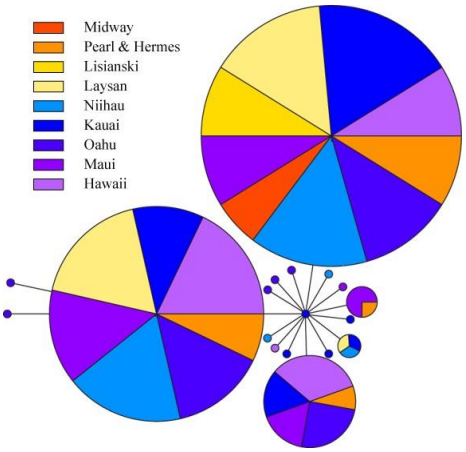

Figure 9. Rhodopsin haplotype network for *C. verater*. Parsimony-based network using rhodopsin sequence data for subsample of 94 specimens and color-coded by sampling location.

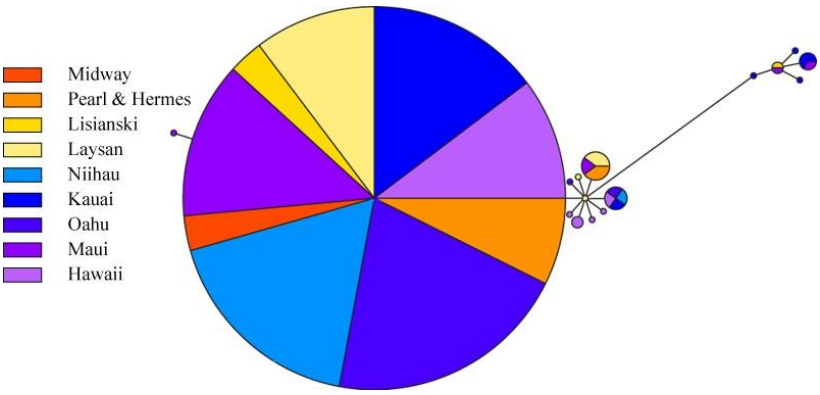

Figure 10. ITS2 haplotype network for *C. verater*. Parsimony-based network using ITS2 sequence data for subsample of 94 specimens and color-coded by sampling location.

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