POPULATION GENETIC AND PHYLOGEOGRAPHIC INSIGHTS INTO THE PHYLLOSOMAL ODYSSEY

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DEDICATION

This dissertation is dedicated to my family; notably, my grandma, Faustina (Florence) Olivo, whose love and commitment to family first have always been an inspiration to me; my nana, Mary Iacchei, whose exuberant approach to life reminds me to enjoy every moment; my grandpa, Mario Olivo and my poppy John Iacchei, both of whom left this world too soon, but whose presence and life lessons have remained constant. My grandfather's heeding of the ocean's power resonates with me each time I am in the field. My great aunt, Norma Pacia, and her son, my cousin Phillip Pacia, who welcomed me into their lives as a son, and will always be my California family. Though Norma sadly departed us shortly before I completed this dissertation, I know she'll be glad I finally, "got the hell 'outa school!"

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ABSTRACT

The spatial and temporal scale of genetic connectivity influences ecological and evolutionary processes ranging from metapopulation dynamics to species diversification. The majority of marine species maintain genetic connectivity through pelagic propagules, and propagule duration is hypothesized to limit dispersal potential. This dissertation aims to determine the scales of genetic connectivity when propagule duration is not limiting, and elucidate the factors driving specific patterns. Spiny lobsters (Decapoda; Palinuridae) have one of the longest pelagic durations, with some phyllosoma wandering the wine-dark sea for over a year. To gain insight into this odyssey, I combine mitochondrial DNA sequences and nuclear microsatellites to determine the patterns of genetic connectivity in three *Panulirus* lobster species. At the broadest geographic extent, I observe significant genetic structure in *Panulirus penicillatus* (Olivier, 1791) at multiple spatial scales throughout its distribution from the Red Sea to the East Pacific Ocean. Population subdivision corresponds frequently with provincial biogeographic boundaries, and a potential species-level disjunction occurs across the East Pacific Barrier. Notably, certain sites are highly isolated within broader regions of minimal genetic discontinuity. To determine whether regional patterns are generalizable between species, I compare *P. penicillatus* results from Hawai‘i to patterns in the Hawaiian endemic, *P. marginatus* (Quoy & Gaimard, 1825). I document greater genetic structuring for *P. marginatus* than the broadly distributed *P. penicillatus*; however, the specific connectivity patterns are not generalizable between species. Finally, I use a seascape genetics approach to understand genetic structuring in *P. interruptus* (Randall, 1840) from Point Concepcion, California to Bahía Magdalena, Mexico. By combining kinship analyses with traditional *F*-statistics, I find specific sites within the species distribution are highly differentiated.

The magnitude of differentiation is strongly positively related to the proportion of kin at each site, and both are related to the proximity of each site to upwelling regions. In conclusion, even marine species with limitless propagule dispersal potential encounter barriers to gene flow on small spatial and temporal scales. Patterns of genetic connectivity are site and species-specific, and may be driven by larval behavior or site-specific oceanographic processes (i.e., upwelling). This knowledge informs the prudent management of these valuable marine resources.

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CHAPTER I

INTRODUCTION

Identifying the drivers of larval dispersal patterns and connectivity in marine populations is essential both to understand marine metapopulation dynamics and to successfully manage marine species and populations (Palumbi 2004, Kritzer & Sale 2006, Fogarty & Botsford 2007). The majority of marine taxa have a biphasic lifestyle, with sedentary adults, but a pelagic larval stage that persists in the water column from a few minutes to multiple years (Thorson 1950, Strathmann 1987, McEdward 1995). This larval phase enables individuals that may not move at all as adults to produce offspring that are capable of settling thousands of kilometers away. An intuitive expectation is the duration of the larvae in the water column will positively correlate with the distance those larvae disperse, and in turn, the scale at which populations are connected (Kinlan & Gaines 2003, Shanks et al. 2003). However, as the number of studies investigating correlations between pelagic larval duration (PLD), dispersal distance, and the genetic structure of populations has increased, the evidence has not supported these expectations (reviewed in Bradbury et al. 2008, Shanks 2009, Weersing & Toonen 2009, Riginos et al. 2011, Selkoe & Toonen 2011).

In general, there is a bimodal distribution in the dispersal distances of larvae with one peak below 1 km and another above 20 km. These peaks typically correspond to a bimodal distribution in PLD, with some species in the water column for less than 10 hours, and the rest with PLD greater than 24 hours (Shanks et al. 2003, Shanks 2009). Below the lower PLD threshold, the correlation between PLD, dispersal, and F_{ST} is strong: larvae in the water column for less than 10 hours tend rarely to disperse farther than 1 km, and in turn have higher levels of genetic structure.

Above the 24 hour threshold, the relationship is less clear: PLD can exceed four years (Strathmann & Strathmann 2007), dispersal distance varies from meters to 1000s of kilometers, and genetic structure ranges from low to high, with an overall correlation between PLD and dispersal distance of R^2=0.5 (Shanks 2009), and between PLD and genetic structure of R^2=0.3 (Weersing & Toonen 2009, Selkoe & Toonen 2011). The majority of larvae do not disperse as far as their PLD predicts, whether dispersal distance is estimated by a passive particle model (Shanks et al. 2003, Shanks 2009) or Lagrangian dispersal model (Siegel et al. 2003, Shanks 2009). Newer biophysical models have successfully predicted dispersal patterns by matching genetic connectivity estimates in some situations, often by incorporating environmental variables and/or larval behaviors hypothesized to reduce larval dispersal (Gilg & Hilbish 2003, Baums et al. 2006, Cowen et al. 2006, Galindo et al. 2006, White et al. 2010, Rivera et al. 2011, Foster et al. 2012). Larvae may fail to realize their full dispersal potential for any number of reasons, including biogeographic barriers (Barber et al. 2000, 2002, Crandall et al. 2008, Gaither et al. 2010, but see Lessios & Robertson 2006, and review in Riginos et al. 2011), contemporary oceanographic currents (Shulman & Bermingham 1995, Baums et al. 2005, 2006, White et al. 2010), larval behavior (Jones et al. 1999, 2005, Leis 2006; Montgomery et al. 2006, Toonen & Tyre 2007), ecological barriers (Rocha et al. 2005, Selkoe et al. 2010), and even anthropogenic effects (Puritz & Toonen 2011).

The majority of species in these studies, including the meta-analytical reviews, have pelagic larval durations less than ~60 days, with only rare instances of species with truly long larval durations (>120 days). This likely reflects the infrequent occurrence of extremely long PLDs in nature, with less than 5% of marine invertebrates estimated to have a PLD > 12 weeks (Thorson 1950, Jeffs et al. 2005), and similar values expected for marine fishes.

Scheltema (1971) named these long-lived, pelagic larval dispersers "teleplanic"; larvae that originate in the continental-shelf benthos, but are often found in the open ocean due to their long larval duration that provides a means for dispersal over very long distances. Teleplanic larvae have been hypothesized not only to allow the colonization of new regions, but also to sustain gene flow across a species' full range (Scheltema 1971). But does this long distance dispersal occur frequently enough to maintain both genetic connectivity over long temporal scales and ecological population connectivity in the short term? Shanks (2009) removed data on teleplanic larvae included in an earlier review (Shanks et al. 2003), stating that "while such long-distance dispersal would clearly affect population connectivity, the low number of larvae successfully dispersing such long distances would have little impact on the maintenance of populations."

Here I use genetic data to address this fundamental question regarding pelagic larval dispersal as proposed by Shanks (2009): do marine species with teleplanic larvae have sufficiently high levels of gene flow to sustain population connectivity on both short and long time scales throughout their full species distributions? Furthermore, is there an upper threshold PLD level, similar to the lower threshold revealed by recent reviews, over which pelagic larvae will overcome any of the aforementioned barriers to dispersal?

To answer these questions, I investigated the patterns of population genetic connectivity in three species of spiny lobsters within the genus *Panulirus*, each of which have pelagic larval durations exceeding 180 days (Phillips 2006). For each species, I surveyed the full extent of the species' range, as well as examined genetic connectivity across smaller spatial scales within geographic regions of the broader species distribution.

The spiny lobsters (Panuliridae) are an ideal group for studying the effects of extremely long larval duration on metapopulation dynamics. Most spiny lobsters have broad geographic ranges, with little movement as adults, despite their large relative size for mobile invertebrates (Phillips et al. 2006). The large range sizes of spiny lobsters are likely maintained by their extremely long-lived pelagic larva, which have been collected over 1500km from coastal waters (Johnson 1956, 1960, Jeffs et al. 2005). Spiny lobsters have a two-phase larval stage: the initial phyllosoma stage that undergoes multiple molts while at large in the pelagic environment and a final puerulus stage, which settles into juvenile habitat at the end of the phyllosoma's pelagic journey. The combined PLD of these two phases ranges from 90 days to over 540 days depending on species (Phillips et al. 2006).

These two larval phases are hardly passive or moving at the whims of the prevailing ocean currents. Spiny lobsters are known to have relatively large larvae capable of dynamic movement. Both the phyllosoma and puerulus stages show evidence of active movement in the pelagos, with phyllosoma exhibiting diel vertical migrations as well as horizontal movements (Kittaka 1994, Chiswell & Booth 1999, Phillips et al. 2006, Butler et al. 2011), and pueruli demonstrating rapid swimming, navigation toward the coast, and habitat settlement preferences (Serfling & Ford 1975, Jeffs et al. 2005, Phillips 2006). Pringle (1986) found both geographic and depth stratification of different stages of both phyllosoma and pueruli of *Panulirus interruptus* collected during yearly larval tows in the California Current Ecosystem, suggesting this species undergoes active ontogenetic shifts in larval depth preference. Most recently, Butler et al. (2011) provide experimental evidence for both diel vertical migration and ontogenetic shifts in phototaxic response in larvae of *Panulirus argus*.

Butler et al. (2011) demonstrated that when these ontogenetic shifts in behavior are incorporated into Lagrangian dispersal models, the expected dispersal distances of *P. argus* dramatically decreases.

The prevalence of dynamic larval behavior across a number of species suggests the long PLD in these species may only rarely disperse larvae across the full species range. According to "Johnson's model" (Menzies & Kerrigan 1979) of lobster larval dispersal, the majority of lobster phyllosoma and pueruli are retained locally in gyres or eddies near the coastline, and recruitment is dependent on local production. Johnson (1971, 1974) hypothesized that larvae found far offshore, past these local entrainment features, had a much lower probability of survival, and therefore represented a loss from their site of origin, rather than a potential recruit to a distant site. If true, long PLDs may have evolved for reasons other than to colonize unoccupied habitat. For example, Strathmann et al. (2002) propose that some species have evolved a long PLD to avoid predation during the larval phase, rather than to facilitate broad dispersal of larvae. The extended PLD may enable larvae to disperse far offshore, into a pelagic environment that is favorable for the survival of unprotected larval-stage individuals (Strathmann et al. 2002).

The majority of genetic research addressing this question for species with PLD > 120 days are population genetic and phylogenetic surveys of spiny lobsters (Menzies 1980, Shaklee & Samollow 1984, Seeb et al. 1990, Glaholt & Seeb 1992, Brasher et al. 1992, Ovenden et al. 1992, Silberman et al. 1994, Thompson et al. 1996, Sarver et al. 1998, Johnson & Wernham 1999, Perez-Enriquez et al. 2001, Tolley et al. 2005, Gopal et al. 2006, Inoue et al. 2007, Mathee et al. 2007, Palero et al. 2008, Babbucci et al. 2010, Chow et al. 2011, but for other species see Reece et al. 2010, 2011).

For the most part, genetic investigations of both lobsters and other species with teleplanic larvae have identified minimal population structuring across broad geographic scales (Ovenden et al. 1992; Silberman et al. 1994, Thompson et al. 1996, Tolley et al. 2005, García-Rodríguez & Perez-Enriquez 2006, Inoue et al. 2007, García-Rodríguez & Perez-Enriquez 2008, Reece et al. 2011). When genetic discontinuities have been observed in species with long PLD, they have invariably corresponded with known biogeographic barriers, or oceanographic transitions (Palero et al. 2008, Babbucci et al. 2010, Chow et al. 2011). However, there has been some evidence of local recruitment (Silberman & Walsh 1994, Johnson & Wernham 1999), as well as genetic differentiation corresponding to species-level distinctions across some spiny lobster species' distributions (Sarver et al. 1998, Gopal et al. 2006, Chow et al. 2011, Tourinho et al. 2012).

Here, I investigate whether marine species with teleplanic larvae maintain population genetic connectivity throughout their full species distributions using three different species of spiny lobsters at multiple geographic scales. In chapter two, I examine genetic connectivity using mitochondrial DNA (mtDNA) sequence data at the broadest spatial scale, throughout the distribution of *Panulirus penicillatus* (Olivier, 1791) from the Red Sea, across the Indian Ocean, the Western and Central Pacific Oceans and across to the East Pacific Ocean. In chapters three and four, I examine the species distributions (chapter 3) and patterns of genetic connectivity in mtDNA (chapter 4) of *Panulirus penicillatus* at a smaller spatial scale (the Hawaiian Archipelago). I compare these data to those of a congeneric species, *Panulirus marginatus* (Quoy & Gaimard, 1825) that is sympatric with *P. penicillatus* over this geographic extent, but is endemic to the Hawaiian Islands.

In chapters five and six, I conduct a deeper investigation into the genetic structuring of a third species, *Panulirus interruptus* (Randall, 1840), across the majority of its species distribution along the West Coast of North and Central America from Monterey Bay, CA to Bahia Magdalena, Mexico (excluding the small population in the Gulf of California). Chapter five addresses the development of eight microsatellite genetic markers to detect finer spatial genetic partitioning in *P. interruptus*. Chapter six combines mitochondrial DNA (mtDNA) sequence data and seven of the eight nuclear microsatellites (nDNA) to examine population connectivity in this species, and also proposes a new analysis mechanism that uses microsatellite data to gain deeper understanding into population genetic structuring in marine species. Through these combined studies, I broaden our knowledge of the natural history of the pelagic phase of spiny lobster life, and I directly assess the generality of the relationship between pelagic larval duration and dispersal scale in teleplanic larval dispersers. In addition to a fundamental understanding of population connectivity in 'teleplanic' dispersers, this knowledge contributes to the scientific foundation of prudent management of these valuable marine resources.

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CHAPTER II

TESTING PROVINCIAL BOUNDARIES FOR PHYLLOSOMA: RANGE-WIDE PHYLOGEOGRAPHY OF THE PRONGHORN SPINY LOBSTER *PANULIRUS PENICILLATUS*

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Abstract

Biogeographic provinces provide a framework for understanding the origin, distribution, and evolution of species. Here we present the first range-wide phylogeographic study of a species distributed across all three major biogeographic boundaries in the tropical Indo-Pacific. The pronghorn spiny lobster, *Panulirus penicillatus* has the broadest species range of all spiny lobsters, extending from the Red Sea to the East Pacific and occurring in seven of the nine tropical Indo-Pacific biogeographic provinces. We applied the mtDNA cytochrome *c* oxidase subunit I gene (COI) to 26 locations (N=671) from across the full extent of the species distribution, and added 6 locations (N=54) from public databases to our analyses. Results indicate *Panulirus penicillatus* is significantly differentiated across the Indo-Pacific (global Φ_{ST} = 0.175, $P < 0.001$), despite a nine-month pelagic larval duration. There is significant differentiation (Φ_{CT} = 0.69, P=0.011) among three regions defined by the major Indo-Pacific biogeographic barriers (Western Indian Ocean, Western and Central Pacific, East Pacific). The greatest barrier to gene flow is Darwin's 'impassable' East Pacific barrier (Φ_{CT} = 0.847, P=0.039). There is no genetic break across the Indo-Pacific Barrier (Φ_{CT} = 0.00032, P=0.259); however, there is significant genetic structure within the West/Central Pacific (global Φ_{ST} = 0.0198, P = 0.00001). There is also significant differentiation between sampling locations in the warm-temperate Sino-Japanese province and almost all sites in the tropical Indo-Pacific (pairwise Φ_{ST} = 0.045–0.835, $P < 0.05$). These results illustrate that population subdivision matches biogeographic provinces in this broadly dispersed species and can serve as a framework to test hypothesis of population connectivity in species with more restricted ranges.

Introduction

Biogeographic provinces are defined by habitat discontinuities and high (>10%) levels of endemism (Briggs, 1974; Brown & Lomolino, 1998; Briggs & Bowen, 2012, 2013), and provide a framework for understanding the origin, distribution, and evolution of species. Across the Indo-Pacific basin, there are nine tropical biogeographic provinces including the Red Sea province, with an additional seven adjacent warm-temperate provinces at the higher latitudes in the region (Fig. 2 in Briggs & Bowen 2013). The number of species found in each of these provinces varies by an order of magnitude for the most well described and categorized taxa (fishes), ranging from approximately 139 fish species in the Easter Island province to over 2,600 species at multiple locations in the well-known center of species diversity, the Coral or East Indies Triangle (Randall & Cea, 2011; Allen & Erdmann, 2012). While many species have range sizes that overlap adjacent provinces, few species maintain populations across all of the tropical or warm temperate provinces. Randall (1998) notes that 492 species of fishes are distributed from the Western Indian Ocean province (WIO) across the vast Indo-Polynesian province to the Hawaiian province, the Line Islands, or French Polynesia. However, this number drops to 12 species of fishes that are common to the Hawaiian province and the other peripheral regions of the Indo-Pacific, Easter Island and the Red Sea. Few coastal reef-associated species have the dispersal ability to transcend multiple biogeographic barriers, with the exception of the moray eels that are aided by a leptocephalus larva that can persist for up to ten months in pelagic environments (Castle, 1965; Brothers & Thresher, 1985; Reece *et al.*, 2011).

Among reef-associated invertebrates, the species with the greatest dispersal potential are the spiny lobsters in the genus *Panulirus*. There are a minimum of 15 *Panulirus sp*. in the Indo-West Pacific; seven of which have ranges that extend across at least two of the biogeographic provinces in the region, and four (*P. ornatus, P. penicillatus, P. polyphagus, P. versicolor*) of which can be found across the Indo-Polynesian (IP), West Indian Ocean (WIO), and Red Sea provinces. The pronghorn lobster, *Panulirus penicillatus* (Olivier, 1791) is the most broadly distributed *Panulirus sp*. and occurs in the Sino-Japanese warm-temperate province, as well as seven of the nine tropical Indo-Pacific provinces: Red Sea, WIO, IP, Hawaiian, Marquesas, Panamanian, and Galápagos Provinces (Holthuis & Loesch, 1967; George & Main, 1967; Holthuis, 1991). This distribution encompasses two of the three highest regions of endemism in the Pacific (Hawai‘i and the Marquesas Islands), and bridges all three major biogeographic barriers in the Indo-Pacific Region: the break between Red Sea (Klausewitz, 1989; Righton *et al.*, 1996) and Indian Ocean; the Indo-Pacific Barrier (Briggs, 1974), most often associated with the Sunda Shelf between Asia and Australasia (Fleminger, 1986; Barber *et al.*, 2006); and the East Pacific Barrier (EPB), the 4000-7000km expanse of deep water that separates the Eastern Pacific from the Central Pacific (Ekman, 1953).

Panulirus penicillatus exhibits strong habitat specificity and is generally restricted to shallow forereefs less than 10m depth (Pitcher, 1993). Termed an 'oceanic' species by George (1974), *P. penicillatus* are most commonly found in windward surf zones of oceanic reefs, where clear, clean, and highly oxygenated water due to high wave energy maintain the species' preferred environment (Pitcher, 1993). The pronghorn lobster supports valuable local and artisanal fisheries throughout its distribution, although catch rates vary greatly by location (Pitcher, 1993). At the East Pacific end of the range, abundances are high enough to support commercial fisheries in the Revillagigedos Islands in Mexico and in the Galápagos Islands (Holthuis & Loesch, 1967, George & Main, 1967; Pitcher, 1993; Bustamante, 1999), although catch rates in the Galápagos Islands have declined recently (Hearn & Murillo 2008).

Panulirus penicillatus is thought to maintain such a vast distribution across the Indo-Pacific basin through a phyllosoma larval stage that has been found in the middle of the Pacific Ocean, as far as 3,500-4,000 km from the nearest potential source in the Galápagos Islands (Johnson, 1974). Johnson (1968) estimated the pelagic larval duration (PLD) of *P. penicillatus* at greater than seven to eight months based on field-collected phyllosoma. Subsequently, the complete larval development was estimated as 8.3 – 9.4 months for cultured phyllosoma (Matsuda, 2006). Both of these PLD estimates are much greater than the hypothesized 50-81 day time period required for passive transport across the EPB under strong El Niño conditions, and even surpass the 100-155 day time period estimated under normal conditions (Wyrtki *et al.*, 1981; Richmond, 1990). The phyllosoma larval stage may provide a mechanism to maintain gene flow across Darwin's 'impassable' marine dispersal barrier for shallow water species (Darwin, 1872), and in turn, would designate *P. penicillatus* a truly 'trans-Pacific' species (Briggs, 1961).

In contrast to this expectation, recent genetic evidence indicates the Eastern and Western Pacific *P. penicillatus* represent two distinct populations with no ongoing gene flow across the EPB (Chow *et al.*, 2011). Body color also varies across regions: in the East Pacific, *P. penicillatus* is known as the "red lobster" (Holthuis & Loesch, 1967; Holthuis, 1991), whereas throughout the rest of the species distribution, body color varies from brownish-green to yellow-green to blue-black (Holthuis, 1991; George, 2006), and the longitudinal stripes on the legs also vary in color and thickness (Iacchei, pers obs.). In addition, McWilliam (1995) observed regional differentiation in morphology of the posterolateral sternal spines of *P. penicillatus* phyllosoma.

Here we analyze mtDNA sequence data from *P. penicillatus* to assess species' response to biogeographic divisions. We sample throughout the Indo-Pacific, including the Red Sea and the East Pacific to assess the relative magnitude of population subdivision across the three major biogeographic barriers, as well as the influence of weaker provincial boundaries on genetic structure in *P. penicillatus*. We combine the first genetic data collected for this species from the Panamanian province with previously published data from the Galápagos Province (Chow *et al.* 2011) to provide a robust evaluation of the EP barrier to gene flow for *P. penicillatus*. To our knowledge, this is the first study to evaluate all three major biogeographic divisions within the tropical Indo-Pacific provinces using a single species, and provides the broadest marine phylogeographic coverage for a species in this region to date (Keyse *et al.*, 2013).

Methods

Sample Collection

We collected 697 *Panulirus penicillatus* individuals from 26 sites (Fig. 2.1, Table 2.1). Lobsters were collected by hand while free diving or scuba diving, obtained from commercial and recreational fishers as detailed in Chapter 3, or purchased at local fish markets. Tissue samples were preserved in either 20% dimethyl sulfoxide salt-saturated buffer (Seutin *et al.*, 1991; Gaither *et al.*, 2011a) or 95% ethanol, and stored at room temperature until extracted.

In addition to the samples we collected, we incorporate 54 individual sequences obtained from GenBank into our analyses. These sequences were obtained from lobsters collected at nine locations; three sites (New Caledonia, Chesterfield Islands, Fiji) that augmented our samples, and six sites (India, Japan, Indonesia, Torres Strait, Tuamotu Archipelago, Galápagos Islands) that added locations as well as samples to our dataset. The GenBank accession numbers and trimmed sequence for each of the individuals we used off of GenBank in our analysis will be made available on Dryad.

DNA Extraction, PCR, and Sequencing

Genomic DNA was isolated using either a modified HotSHOT method (Truet *et al.*, 2000, Meeker *et al.*, 2007) or a DNeasy Animal Tissue kit (Qiagen Inc., Valencia, CA, USA) following manufacturer instructions. We amplified a 460 bp fragment of COI using species-specific primers PpenCOI-F (5'-GCTGGAATAGTGGGGACCTC-3') and PpenCOI-R (5'-GCTTCTGACCGACCGTAACT-3') designed from GenBank sequence #AF339468 (Ptacek *et al.*, 2001) using PRIMER3 (Rozen & Skaletsky, 2000).

Polymerase chain reactions (PCRs) for each individual were performed in 20µl aliquots containing 5-50 ng of genomic DNA, 0.125 µM each of forward and reverse primer, 0.75x Bovine Serum Albumin (BSA), 10µl of 2x Biomix Red (Bioline), and sterile deionized water to volume. A Bio-Rad d Thermal Cycler (Bio-Rad Laboratories, Hercules, CA, USA) was used for all PCR reactions. The PCR protocol consisted of an initial denaturation step of 95 °C for 4 min, 35 cycles of denaturation (95°C for 30 sec), annealing (56°C for 30 sec), and extension (72°C for 30 sec), and a final extension step (72°C for 10 min). PCR products were purified with 0.75 units of Exonuclease I and 0.5 units of Fast Alkaline Phosphatase (ExoFAP, Fermentas) per 7.5 µl of PCR product, and incubated at 37°C for 60 min, followed by deactivation at 85°C for 15 min. Purified PCR products were all sequenced in the forward direction with an ABI 3730XL or an ABI 3130XL capillary sequencer (Applied Biosystems, Foster City, CA). Each unique haplotype and all sequences with ambiguous nucleotide calls were also sequenced in reverse to confirm sequence identity. Sequences were edited, aligned, and trimmed to a uniform size using GENEIOUS Pro v. 4.8.5 (Biomatters Ltd., Auckland, New Zealand). The alignment did not contain any indels or frameshift mutations, and was free from stop codons. All unique haplotypes were identified using the Haplotype Collapser and Converter option in FaBox 1.35 (http://131.204.120.103/srsantos/fabox/; accessed: 05-27-13), and will be deposited in GenBank.

Data Analysis

Summary statistics

We calculated nucleotide (π) and haplotype diversity (h) described in Nei (1987) using ARLEQUIN 3.5 (Excoffier *et al.*, 2010). Effective number of alleles ($1/(1-h)$) as described by Jost (2008) was calculated by hand.

We constructed a median-joining network (Bandelt *et al.*, 1999) using the program NETWORK 4.6.0.0 (http://www.Fluxus-engineering.com/network_terms.htm) to visualize the frequencies, spatial distributions, and relationships among haplotypes.

Phylogeographic structure

To investigate the spatial scale of genetic connectivity, we conducted an analysis of molecular variance (AMOVA) in ARLEQUIN 3.5 (Excoffier *et al.*, 2010). We tested for broad-scale regional differentiation across the range using barriers between each ocean basin and the East Pacific Barrier as a priori regional divides, giving us four major regions: the Red Sea, the Indian Ocean, the Western and Central Pacific, and the East Pacific. To examine the magnitude of differentiation among barriers, we also tested the differentiation across neighboring regions, comparing just the Red Sea to the Indian Ocean, Indian Ocean to the West/Central Pacific, and West/Central Pacific to the East Pacific. We also determined the level of genetic differentiation between sites by calculating pairwise Φ_{ST} between all sites with $N > 5$ in ARLEQUIN. Φ_{ST} is an analogue of Wright's F_{ST} that incorporates a model of sequence evolution (Excoffier *et al.*, 1992). Using jModelTest2 (Darriba *et al.*, 2012; Guindon & Gascuel 2003), we determined that the Tamura & Nei (1993) with a Ti/Tv ratio of 7.273 and gamma parameter of 1.550 was the most appropriate model of sequence evolution implemented in ARLEQUIN. Global Φ_{ST}, regional Φ_{CT} and each pairwise population Φ_{ST} were tested for significance with 100,000 permutations. To correct for inherent bias when conducting multiple comparisons, we implemented a false discovery rate (FDR) correction (Benjamini, 2006) to adjust the critical *P*-value for each pairwise site comparison.

Results

Summary statistics

We resolved 460 bp of COI in 669 lobsters from 31 locations across the Pacific Ocean and Indian Ocean, which includes the 54 GenBank sequences. Based on results in chapter four, we combined five distinct islands within the Northwestern Hawaiian Islands into a single Northwestern Hawaiian Island site (NWHI), and we combined five distinct islands within the Main Hawaiian Islands into a single Main Hawaiian Island Site (MHI) for this broad-scale biogeographic analysis. Unfortunately, there were problems resolving the Red Sea sequences in time for this dissertation deadline, so these are not included here. A few other sites were also collected too late for these analyses: Revillagigedos Islands, Rota, and American Samoa. Just before turning in this dissertation, four new samples from the Red Sea, 17 samples from Wake Atoll, and a few samples from Okinawa Japan were added to this dataset. These are not listed in the table, but will be included in the updated analysis submitted to the Journal of Biogeography.

The 669 sequences we resolved contain 181 unique haplotypes, 118 of which (65.2%) are singletons. The median joining network (Fig. 2.2) is dominated by one common haplotype that represents 193 individuals (28.8% of the individuals in the study), and is observed at 15 of 20 grouped locations in the study. This most common haplotype is absent from the East Pacific sites, but is present in all Indo-Pacific sites where $N > 5$, with the exception of Japan. The second most abundant haplotype represents 56 individuals (8.3%) at all Indo-Pacific sites where $N > 5$, with the exception of Fiji. This haplotype is also not detected in the East Pacific. The majority of haplotypes are differentiated by one substitution, with a maximum of three base pairs separating most closely related haplotypes from individuals in the Indian and Western/Central Pacific Ocean basins. The mean haplotype diversity is $h = 0.87$.

There is a mean of 11.7 effective haplotypes, and these range from 3.75 in the Tuamotus to 34.97 in Japan. Nucleotide diversity ranges from $\pi = 0.003$ to $\pi = 0.013$, with of $\pi = 0.005$ for locations where N > 1. Table 2.1 lists number of individuals sequenced (N), haplotype diversity (h), effective number of haplotypes (h_{eff}), and nucleotide diversity (π) for each site. Supplementary figure S2.1 duplicates figure 2.2, but with all sites where samples were collected identified. Supplementary figure S2.2 is a full, unedited network with all sites and all connections between haplotypes depicted.

Phylogeographic structure

Global Φ_{ST} (0.175) is significant ($P < 0.000005$). When dividing the dataset into three major groups (Indian Ocean, Western and Central Pacific, East Pacific) using ocean basin and the well-documented East Pacific Barrier as a priori divisions, there is significant regional differentiation ($\Phi_{CT} = 0.69$, P=0.011). East Pacific and Western/Central Pacific regions have strong, significant regional differentiation ($\Phi_{CT} = 0.847$, P=0.039), whereas there is no significant differentiation between the Indian Ocean and the Western/Central Pacific Ocean ($\Phi_{CT} = 0.00032$, P=0.259, Table 2.2). There is significant genetic structure within the West/Central Pacific region as a whole ($\Phi_{ST} = 0.0198$, $P = 0.00001$), as well as regional differentiation when sites are grouped into archipelagos as in Table 2.1 ($\Phi_{CT} = 0.013$, P=0.023). Across the species' distribution, 42 of 105 pairwise Φ_{ST} comparisons (40%) were significant (Table 2.3), with significant Φ_{ST} values ranging from 0.012 to 0.960, and a mean pairwise Φ_{ST} value for significant comparisons of 0.528. After correcting for false discovery rate (FDR), 32 of the 42 comparisons remain significant (Table 2.3).

Discussion

Our range-wide phylogeographic survey of the green or tufted spiny lobster, *Panulirus penicillatus* reveals the highest levels of genetic structure detected in any spiny lobster to date ($\Phi_{ST} = 0.175$, $P < 0.001$). Despite an estimated 9-month pelagic larval duration (Johnson, 1968; Matsuda, 2006), *P. penicillatus* shows significant genetic differentiation across a well-known biogeographic barrier (the EPB). There also appears to be restricted gene flow between the tropical Indo-Pacific provinces and the single warm–temperate province occupied by *P. penicillatus* (Sino-Japanese province). Most surprisingly, although *P. penicillatus* has relatively high gene flow across the Indo-Polynesian Province, there are a few locations within the center of this range that appear to be relatively isolated (Table 2.3). Below, we examine each of these regional barriers in light of biogeographic theory and hypotheses regarding the evolution and radiation of this species.

East Pacific Barrier

The 4,000 to 7,000 km expanse of open ocean separating the Eastern Tropical Pacific (ETP) and the Central Pacific (CP) known as the East Pacific Barrier (EPB, Ekman 1953) was long thought to be an 'impassable' dispersal barrier for nearshore marine species (Darwin, 1872; Mayr, 1954), and may have been established as of 65 mya (Grigg & Hey, 1992). Subsequently, Briggs (1960, 1961) documented 13 coastal fish species that were found on both sides of the EPB, and that number has since increased to at least 54 (Leis, 1984). Similar distributions on either side of the EPB have been documented for a variety of taxa (reviewed in Robertson et al. 2004), including corals (Glynn & Ault, 2000), mollusks (Vermeij, 1987), sea urchins (Ekman, 1953; Lessios *et al.*, 1998, 1999, 2003) and polychaetes (Fauchald, 1977).

These species distributions may be relics of pan-Tethyan species that were connected to the Caribbean through the Central American corridor (McCoy & Heck, 1976), or they have been established and maintained by dispersal from the IWP more recently, after the last Pleistocene glacial maxima (Dana, 1975; Glynn & Wellington, 1983; Cortes, 1997; Bowen & Karl, 2007). Phylogenetic and population genetic studies indicate a significant barrier to gene flow between the IWP and the EP for the reef building coral *Porites lobata* (Forsman, 2003; Baums *et al.*, 2012). However, studies of both echinoderms (Lessios *et al.*, 1998) and fishes (Rosenblatt & Waples, 1986; Lessios & Robertson, 2006; Fitzpatrick *et al.*, 2011) indicate contemporary gene flow across the EPB for many species. For example, a survey of 20 'transpacific' fish species found that 18 either shared haplotypes between the ETP the CP or had haplotypes that differed by only a few base pairs, indicating the EPB is crossed at least sporadically by propagules that survive to reproduce on the other side (Lessios & Robertson, 2006). However, the magnitude, timing, and direction of gene flow varied from species to species with no discernable pattern (Lessios & Robertson, 2006).

In contrast to these fishes, *P. penicillatus* shares no haplotypes between any of our sampling locations separated by the EPB (Fig. 2.2). Minimum separation between haplotypes is 8bp, and there are significant, large Φ_{ST} values between all pairwise comparisons across the EPB (Table 2.3). Our findings corroborate the genetic results of Chow *et al.* (2011) who suggested that the EPB is a significant barrier to gene flow for *P. penicillatus*. Furthermore, these data support the morphological distinction of the populations in the ETP, and affirm the hypothesis by George (2006) that dark-reddish brown form of *P. penicillatus* found there, *P. penicillatus* 'red' represents a Panamanian basin regional endemic. Ekman's (1953) EPB functions as a major barrier to dispersal for *P. penicillatus*, as hypothesized by George (2006).

Both Johnson (1974) and Chow *et al.* (2011) found phyllosoma of *P. penicillatus* in the North and South Equatorial currents as far as 3,500 - 4,500 km west of the Galápagos Islands. Chow *et al.* (2011) also found phyllosoma of *P. gracilis*, another EP lobster, at the same larval tow stations as *P. penicillatus*, but no Western Pacific species' phyllosoma have ever been found in the EPB. If phyllosoma do successfully cross the EPB, these data indicate it is likely from East to West, which would match the predominant direction of gene flow for at least 10 fish species (Lessios & Robertson, 2006; Fitzpatrick *et al.*, 2011).

Indo-Pacific Barrier

The Indo-Pacific Barrier (IPB) is a widely recognized disjunction in marine faunal distributions between the Indian and Pacific Ocean basins (Briggs, 1974). This prominent barrier to dispersal for many marine species has fluctuated in magnitude and geographic extent through time, because it formed through the repeated lowering of sea levels during glacial cycles in the Pleistocene. Most often, the IPB is synonymous with the Sunda Shelf between Australia and Asia (Fleminger, 1986; Barber *et al.*, 2006), although the specific location is debated (Hobbs *et al.*, 2009). At the height of the Pleistocene glaciations, sea level at the Sunda Shelf dropped as low as 120 m below present depths, creating a nearly complete barrier between the two oceans (Voris, 2000; Naish *et al.*, 2009). This loss of shallow water habitat, combined with strong upwelling in the region (Galloway & Kemp, 1981; Fleminger, 1986; Voris, 2000; Naish *et al.*, 2009), likely created a barrier to dispersal between ocean basins and reduced population sizes of shallow-depth fauna on both sides of the IPB. Gaither *et al.* (2010) identified 18 species of marine fishes and invertebrates that were sampled across the IPB and found that 15 of 18 (83%) had significant genetic structure defined by the IPB.

Gaither *et al.* (2010) add two *Lutjanus sp.* to the total with their study; one of which (*Lutjanus fulvus*) exhibits genetic differentiation across the IPB, and the other (*L. kasmira*) does not, and subsequently, Gaither *et al.* (2011b) show significant structure across the IPB for *Cephalopholus argus*. Carpenter *et al.* (2011) and Barber *et al.* (2011) examine concordant phylogeographic breaks within the Coral Triangle for both marine invertebrates and fishes. Both studies identify few species that maintain high gene flow across the IPB. Exceptional species include the trumpetfish *Aulostomus chinensis* (Bowen *et al.*, 2001), the surgeonfishes *Naso brevirostris* (Horne *et al.*, 2008) and *N. hexacanthus* (Horne & van Herwerden, 2013), the sea urchins, *Diadema savignyi* (Lessios *et al.*, 2001) and *Tripneustes gratilla* (Lessios *et al.*, 2003), and the marine snail, *Nerita plicata* (Crandall *et al*., 2008). These few species that maintain connectivity across the IPB preclude any generalization of mechanisms by which some species are able to maintain gene flow.

Here, we add the pronghorn lobster *P. penicillatus* to the list of species that does not have a significant genetic break between the Indian and Pacific Oceans (Φ_{CT} = 0.00032, *P*=0.259, Table 2.2). While four of 13 specific pairwise Φ_{ST} comparisons between the WIO and the Central and West Pacific (CWP) sites were significant, there is no regional differentiation or specific area of the CWP that contains all of the differentiated sites, and the magnitude of the pairwise comparisons is no greater than other statistically significant comparisons within the CWP (Table 2.3). Although the extremely long (9 month) pelagic larval duration in *P. penicillatus* would intuitively be the mechanism that maintains connectivity across biogeographic boundaries, in the case of the IPB, it is possible that the adult lobsters have maintained connectivity between these two oceans.

The strong upwelling regimes around the Sunda Shelf likely would have restricted dispersal of lobster phyllosoma larvae, increasing population subdivision in this region, as has been demonstrated in the conspecific *P. interruptus* (Iacchei *et al.*, 2013). However, *P. penicillatus* adults prefer shallow windward surf zones with high wave energy (George, 1974; Pitcher, 1994), which are likely common in areas of high upwelling in the region. Tagged *P. penicillatus* adults move on average less than 2km (Prescott, 1988), but they are known to move into very shallow (< 0.5 m) surge channels on the reef crest and reef flat to forage at night (Pitcher, 1993). This affinity for shallow, scoured shorelines may have allowed adult *P. penicillatus* to persist across broader areas of the Sunda Shelf during glacial cycles than other invertebrates or fishes that have more reef-specific requirements, and in turn, maintain population connectivity across the IPB.

Sino-Japanese Province

After the EPB and the RSIO, the most significant regional differentiation we observed for *P. penicillatus* is between the tropical IWP and the temperate Sino-Japanese Province (SNP). The SNP begins on the oceanic side of Japan at Cape Inubo in the North and continues south to, but not including the Amami Islands at the northern end of the Ryuku Archipelago. On the mainland side, it extends from the tip of the Korean Peninsula and at Hamada in the Sea of Japan (Briggs & Bowen, 2012). The Sino-Japanese province represents the northernmost end of the *P. penicillatus* species range, and is the only warm-temperate province where *P. penicillatus* occurs. While not identified as one of the major biogeographic barriers for species distributed across the IWP, this may be because only a few species have been studied across the boundary between the Sino-Japanese and the Indo-Polynesian provinces.

The most well-studied island in this transition zone, Taiwan, has fish fauna on the northwestern coast of the island that correspond to the warm-temperate mainland coast, but fauna on the southeastern coast which are tropical in origin (Shao *et al.*, 1999; Briggs & Bowen, 2012). These tropical reef communities are likely maintained by the warm, northerly-flowing Kuroshio Current, and their persistence may depend on the magnitude and location of the current through time (Ujiie *et al.*, 2003).

For *P. penicillatus*, we see significant genetic differentiation between Japan and almost all other sampled sites, with the exception of the Tuamotu Archipelago (N=6). Significant pairwise Φ_{ST} values between Japan and the IWP sites ranged form 0.047 to 0.261, and in general, were greater than other significant comparisons in the study, with the exception of comparisons across the EPB or the RSIO barriers (Table 2.3). Our results corroborate findings from earlier phylogeographic studies that included sites in Japan in addition to tropical IWP sites, although they may not have specifically been testing for a barrier to gene flow in this transition zone. For the lollyfish *Holothuria atra*, Skillings *et al.* (2011) identified significant genetic differentiation between Okinawa, Japan and all 17 sites sampled in the IWP. There was also significant genetic differentiation between Ogasawara, Japan and 13 of the 17 IWP sites sampled. Additionally, BARRIER analysis placed two of the top three genetic barriers in the region for *H. atra* between the Japanese Island chains and the sample sites in the central Pacific (Skillings *et al.*, 2011). Similarly, for yellow tang *Zebrasoma flavescens,* Eble *et al.* (2011) found significant genetic differentiation between Chichi-Jima, Japan and all 18 of their IWP sample locations for both mitochondrial and 14 nuclear microsatellite markers.

Fitzpatrick *et al.* (2011) document significant genetic differentiation between Taiwan and 15 of 18 sites across the IWP using 15 nuclear microsatellite markers, although the magnitude of F_{ST} was no greater than between sites within the IWP. However, Craig *et al.* (2007) find no differentiation between Okinawa and 10 other sites in the IWP for *Myripristis berndti*, and Reece *et al.* (2011) find no structure between Taiwan and any IWP site for two moray eel species. Though still limited in number, the majority of these phylogeographic surveys that have sampled the Sino-Japanese province indicate there is likely restricted gene flow or isolated relic populations for many species in this temperate/tropical transition zone, and this should be a focus of future phylogeographic investigations.

Indo-Polynesian, Hawai'i, and Marquesas Provinces

The largest and most well studies biogeographic province in the *P. penicillatus* distribution is the IWP, which contains the IP province, the Hawaiian Province and the Marquesas Province (Briggs & Bowen, 2013). The massive expanse of coral reef atolls and island archipelagos within these provinces extend over half the circumference of the globe, and consist of patchy reef habitats that are arranged such that there is no oceanic gap greater than 800km from the westernmost edge at the IPB to the Society Islands in the east (Schultz *et al.*, 2008). Most reefs throughout the region can be colonized within typical pelagic larval durations of reef fish and invertebrates (Mora *et al.*, 2012). This hypothesis of highly connected metapopulations has been corroborated by biophysical dispersal models (Treml *et al.*, 2008; Kool *et al.*, 2011), population genetic and phylogenetic surveys of invertebrates (Lessios *et al.*, 2003; Crandall *et al.*, 2008) and fishes (Craig *et al.*, 2007; Gaither *et al.*, 2010; Gaither *et al.*, 2011b, c; DiBattista *et al.*, 2011; Eble *et al.*, 2011), and coupling dispersal models with genetic data (Crandall *et al.*, 2012).

High levels of endemism among shore fishes (25%; Hourigan & Reese, 1987; Randall, 1998) and reef invertebrates (34%; Kay & Palumbi, 1987) in the Hawaiian Province, and high levels of endemism among shore fishes in the Marquesas Province (11.6%; Randall, 1998), indicate that these peripheral areas may be highly isolated from the IP province. Biophysical models also predict the isolation of Hawai‘i and the Marquesas (Treml *et al.*, 2008), and historical sea level changes do not alter the mean distance between sites across the Central Pacific (Baums *et al.*, 2013). To date, genetic results have been split on the Marquesas, with three fishes showing evidence of strong differentiation (Planes & Fauvelot, 2002; Gaither *et al.,* 2010), and two that show no differentiation (Craig *et al.* 2007; Schultz *et al.* 2007). The isolation of Hawai‘i is better supported, with 14 out of 18 broadly distributed species from multiple taxonomic groups showing a significant genetic divergence of Hawai‘i from other IWP locales (reviewed in Gaither *et al.* 2011c).

There is significant genetic structure across the IP, Hawai‘i and Marquesas provinces for *P. penicillatus*, with both significant global Φ_{ST} (0.0198, $P < 0.01$) for all the sites in those three provinces combined, as well as regional differentiation when sites are grouped into archipelagos designated in Table 2.1 ($\Phi_{CT} = 0.013$, P=0.023). The magnitude of the differentiation across this region is not as great as the biogeographic breaks discussed previously; however, there are specific sites that seem to be driving the pattern. For example, unlike some of the fish species surveyed, *P. penicillatus* in the Marquesas are not significantly differentiated from Hawai‘i or any of the sites in the IP province. The Northwestern Hawaiian Islands (NWHI) are the most commonly differentiated location, showing significant structure in pairwise comparisons with the Main Hawaiian Islands (MHI), Sarigan and Pagan in the Commonwealth of the Northern Marianas Archipelago (CNMI), and Fiji.

Sarigan and Pagan are also genetically distinct from Western Samoa, which is distinct from Fiji and MHI. This pattern seems to match the dispersal pathways predicted by Treml *et al.* (2008) with the isolation of the most northern atolls, the NWHI of Hawai‘i, and the CNMI. However, neither Hawai‘i, nor the CNMI is differentiated from French Polynesia, New Caledonia, or the Line Islands, which is not consistent with the model predictions. The model also does not predict differentiation between Western Samoa and Fiji, despite the fact that the maximum dispersal times modeled are only 20-25% of the pelagic larval duration for *P. penicillatus*. Interestingly, both Fiji and Western Samoa are located in the center of the IWP, and would not be expected to be genetically distinct from other centrally located sites. However, similar to our findings here, Drew *et al.* (2008) document the genetic distinction of Fiji for five fishes based on color morph variation. Selkoe *et al.* 2010, Iacchei *et al.* (2013) and Iacchei *et al.* (in review, chapter 4) document similar site-specific, rather than regional, patterns of genetic differentiation in two other *Panulirus* spp. that were hypothesized to be genetically homogenous across their respective species ranges based on their long (>6 month) PLD. In isolation, it is possible that these significant pairwise comparisons may be noise or statistical artifacts, but as more species are surveyed across this geographic range, it will be informative to focus on some of these site-specific comparisons across species, especially for sites such as the NWHI and CNMI, which have both been set aside as large marine ecosystem reserves.

Conclusion

Our genetic survey of the pronghorn spiny lobster *Panulirus penicillatus* indicates that even a species with an extremely long pelagic larval duration (~9 months) and one of the broadest species distributions in the sea is subject to the same barriers to gene flow that have facilitated speciation in other taxa. While genetic connectivity for *P. penicillatus* is unimpeded by the ephemeral Indo-Pacific barrier to gene flow, the East Pacific Barrier notably restricts it. Additionally, we detect a genetic break between the tropical Indo-Pacific biogeographic province sites and the warm-temperate Sino-Japanese Province, which has not been a traditional focus for phylogeographic structure, but appears prevalent as more taxa are examined. The long pelagic larval durations and broad species distributions of many *Panulirus* spp. is incongruous with the fact that approximately half of the species in the Pacific are archipelagic endemics or regionally restricted species (Holthuis, 1991). These species have likely evolved mechanisms to increase local retention within their restricted ranges, as indicated by Butler *et al.* (2011) for *P. argus*, by Pringle (1986) and Iacchei *et al.* (2013) for *P. interruptus*, and by Polovina *et al.* (1995), George (2006), and Iacchei *et al.* (Chapter 4) for *P. marginatus*. Here, our data indicates that even the broadly distributed *P. penicillatus* may have evolved similar mechanisms for larval retention, especially the East Pacific sub-populations, which may warrant subspecies or species level designation for George's (2006) *P. penicillatus* 'red'.

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Table 2.1. Summary statistics for 751 *Panulirus penicillatus* mtDNA cytochrome *c* oxidase subunit I sequences obtained from 32 sites. The following information is listed from westernmost to easternmost collection sites: total number of individuals sequenced (N), haplotype diversity (*h*), effective number of haplotypes (h_{eff}) and nucleotide diversity (π). "NC" designates sites for which h_{eff} could not be calculated because $h = 1$. Sites are grouped by region and/or within archipelagos for specific analyses. Sites marked with an asterisk include some sequences from GenBank. Sequences for sites with only N listed were not able to be included in time for this chapter, but will be in the paper submitted to the Journal of Biogeography.

Region/ Archipelago	Collection Site (Abbreviation)	N	*h*	h_{eff}	π
Red Sea	Saudi Arabia (SAUD)	2	-	-	-
Indian Ocean	Zanzibar (ZANZ)	16	0.9231	13.00	0.005
	Seychelles Islands (SECH)	4	-	-	-
	India (INDI)*	2	1.00	nc	0.007
Central Indo-Pacific	Indonesia (INDO)*	4	1.00	nc	0.005
	Japan (JAPA)*	15	0.97	34.97	0.006
	Torres Strait, Australia (TORRE)*	1	1.00	nc	0.000
Marianas Archipelago	Rota (ROTA)	26	-	-	-
	Saipan (SAIP)	4	1.00	nc	0.003
	Sarigan Island (SARI)	36	0.85	6.70	0.004
	Pagan Island (PAGA)	50	0.91	11.14	0.005
New Caledonia	Chesterfield Islands (CHEST)*	18	0.97	30.58	0.007
	New Caledonia (NCAL)*	33	0.91	11.12	0.005
	Fiji (FIJI)*	18	0.79	4.69	0.003
Northwestern Hawaiian Islands	Pearl and Hermes Reef (PEAR)	30	0.91	11.15	0.006
	Lisianski Island (LISI)	18	0.96	25.51	0.013
	Maro Reef (MARO)	5	1.00	nc	0.012
	French Frigate Shoals (FREN)	44	0.90	10.17	0.006
	Mokumanamana (NECK)	5	0.90	10.00	0.005
Main Hawaiian Islands	Kaua'i (KAUA)	52	0.89	9.02	0.007
	Moloka'i (MOLO)	21	0.81	5.25	0.007
	Lāna'i (LANA)	35	0.85	6.47	0.004
	Maui (MAUI)	24	0.75	4.06	0.006
	Hawai'i (HAWA)	47	0.89	8.79	0.008
Samoas	Western Samoa (WSAM)	37	0.91	11.48	0.006
	American Samoa (ASAM)	23	-	-	-
Line Islands	Palmyra Atoll (PALM)	51	0.88	8.29	0.004
	Kiritimati (KIRI)	63	0.92	12.85	0.005
French Polynesia	Tuamotu Archipelago (TUAM)*	6	0.73	3.75	0.004
	Marquesas Islands (MARQ)	35	0.93	13.83	0.005
East Pacific	Galápagos Islands (GALA)*	10	0.78	4.50	0.005
	Revillagigedos Islands, Mexico (REVI)	16	-	-	-

Table 2.2. AMOVA results showing degrees of freedom (df), sum of squares (SS), variance components (Var), Φ-statistics (Φ_{CT}) and *P*-values (*P*) for each regional biogeographic barrier tested for *Panulirus penicillatus*.

Among groups	**d.f.**	**SS**	**Var**	**Φ_{CT}**	***P***
Indian Ocean, Western/Central Pacific, East Pacific	2	127.851	2.5724	0.6902	0.011
Indian Ocean and Western/Central Pacific	1	1.439	0.0004	0.0003	0.259
Western/Central Pacific and East Pacific	1	126.362	6.3469	0.8466	0.039

Table 2.3. Pairwise population genetic structure results for a 460 bp fragment of the mtDNA cytochrome *c* oxidase subunit I region (COI). Φ_{ST} is below the diagonal and *P*-values are above the diagonal. Shaded boxes below the diagonal indicate significant differences at $P < 0.05$. Asterisks indicate comparisons that were no longer significant after correcting for False Discovery Rate ($P > 0.01$). Sites are grouped by ocean basin, and by island archipelago within the West and Central Pacific Ocean. Only sites where N > 5 are included. SAUD = Saudi Arabia; ZANZ = Zanzibar, Tanzania; JAPA = Japan; SARI = Sarigan Island; PAGA = Pagan Island; CHES = Chesterfield Islands; NCAL = New Caledonia; FIJI = Fiji; WSAM = Western Samoa; PALM = Palmyra Atoll; KIRI = Kiritimati; TUAM = Tuamotu Archipelago; MARQ = Marquesas Islands; NWHI = Northwestern Hawaiian Islands; MHI = Main eight Hawaiian Islands; GALA = Galápagos Islands.

		Central and Western Pacific Ocean															
		Red Sea	Indian Ocean	Marianas Archipelago			New Caledonia				Line Islands		French Polynesia		Hawaiian Archipelago		East Pacific
		SAUD	ZANZ	JAPA	SARI	PAGA	CHES	NCAL	FIJI	WSAM	PALM	KIRI	TUAM	MARQ	NWHI	MHI	GALA
Red Sea	SAUD		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Indian Ocean	ZANZ	-		0.087	1.000	0.054	0.301	0.068	0.007	0.454	0.049	0.259	0.392	0.121	0.273	0.037	0.000
-	JAPA	-	0.045		1.000	0.000	0.003	0.000	0.000	0.015	0.000	0.002	0.064	0.000	0.003	0.000	0.000
Marianas Archipelago	SARI	-	**0.071**	**0.198**		0.292	0.188	0.217	0.662	0.010	0.208	0.053	0.493	0.400	0.006	0.253	0.000
	PAGA	-	0.036	**0.147**	0.002		0.401	0.820	0.348	0.031	0.209	0.223	0.727	0.798	0.028	0.529	0.000
New Caledonia	CHES	-	0.008	**0.081**	0.010	0.000		0.751	0.151	0.580	0.334	0.789	0.919	0.828	0.557	0.246	0.000
	NCAL	-	0.042	**0.143**	0.007	-0.009	-0.013		0.174	0.099	0.101	0.341	0.603	0.877	0.092	0.338	0.000
	FIJI	-	**0.117**	**0.261**	-0.008	0.003	0.019	0.016		0.003	0.081	0.027	0.100	0.198	0.005	0.101	0.000
	WSAM	-	-0.002	**0.047**	**0.041**	**0.021***	-0.006	0.016	**0.080**		0.081	0.751	0.853	0.158	0.911	0.033	0.000
Line Islands	PALM	-	**0.042***	**0.142**	0.006	0.005	0.003	0.015	0.026	0.014		0.285	0.638	0.306	0.059	0.318	0.000
	KIRI	-	0.008	**0.075**	0.016	0.003	-0.010	0.001	**0.037***	-0.006	0.002		0.880	0.560	0.899	0.217	0.000
French Polynesia	TUAM	-	0.006	0.063	-0.006	-0.029	-0.054	-0.020	0.060	-0.045	-0.023	-0.044		0.563	0.895	0.757	0.000
	MARQ	-	0.027	**0.133**	0.000	-0.008	-0.015	-0.014	0.013	0.010	0.003	-0.003	-0.016		0.243	0.367	0.000
Hawaiian Archipelago	NWHI	-	0.007	**0.063**	**0.031**	**0.014***	-0.004	0.013	**0.058**	-0.008	0.011	-0.005	-0.044	0.004		0.008	0.000
	MHI	-	**0.041***	**0.150**	0.003	-0.002	0.006	0.001	0.018	**0.017***	0.001	0.002	-0.033	0.000	**0.012**		0.000
East Pacific	GALA	-	**0.851**	**0.835**	**0.871**	**0.848**	**0.820**	**0.853**	**0.885**	**0.836**	**0.861**	**0.837**	**0.868**	**0.847**	**0.832**	**0.865**	

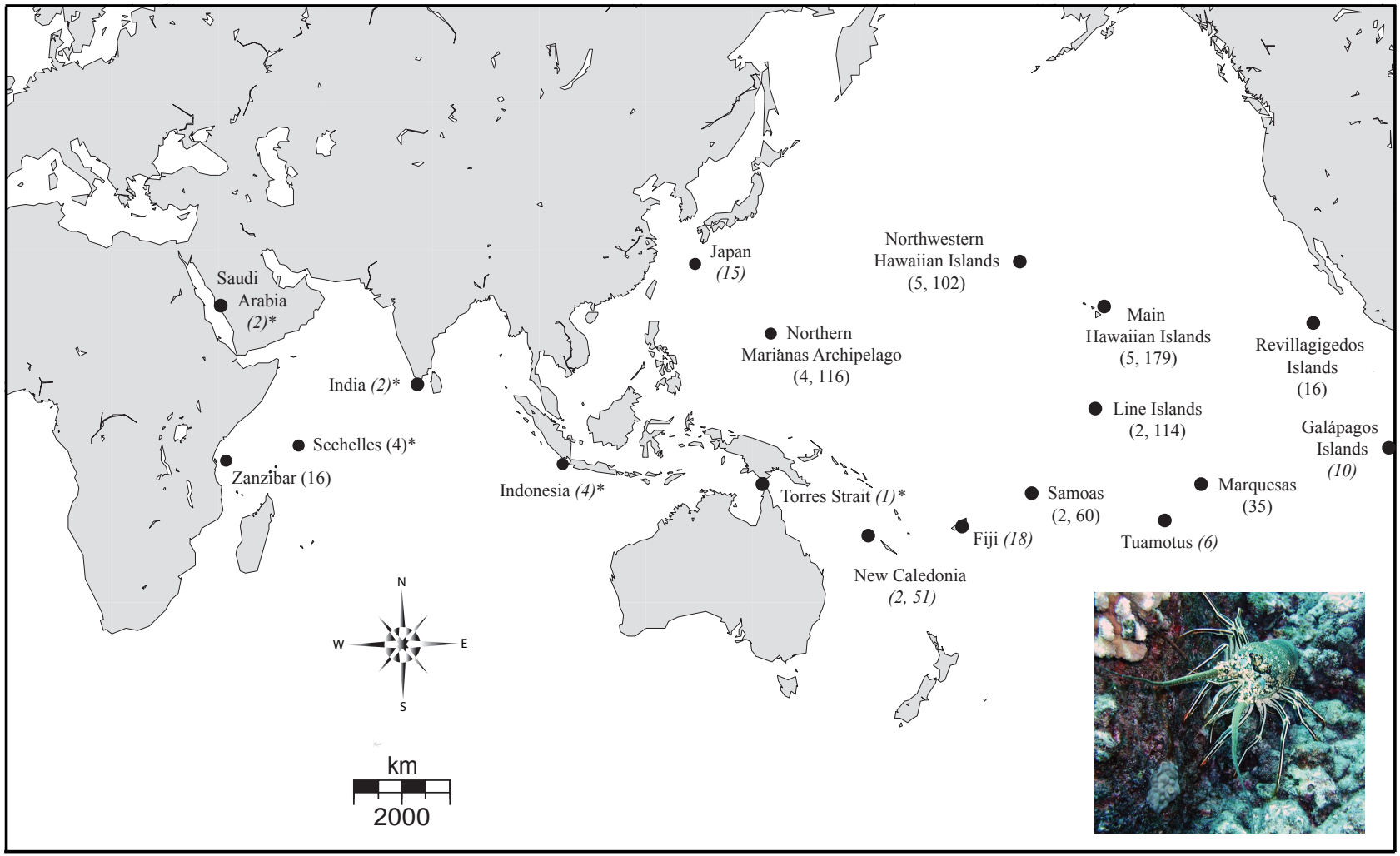


Figure 2.1. Collection locations and sample sizes for *Panulirus penicillatus* across the Indo-Pacific. For locations with two numbers in parentheses, the first number is the total number of islands sampled in that group of islands, and the second number is the total number of individuals sequenced across all of the islands in that group. Sample numbers that are italicized identify sites where some or all of the sequences were obtained from publicly available databases. Locations marked with an asterisk were not included in population level analyses, with the exception of Saudi Arabia, as the only Red Sea location.

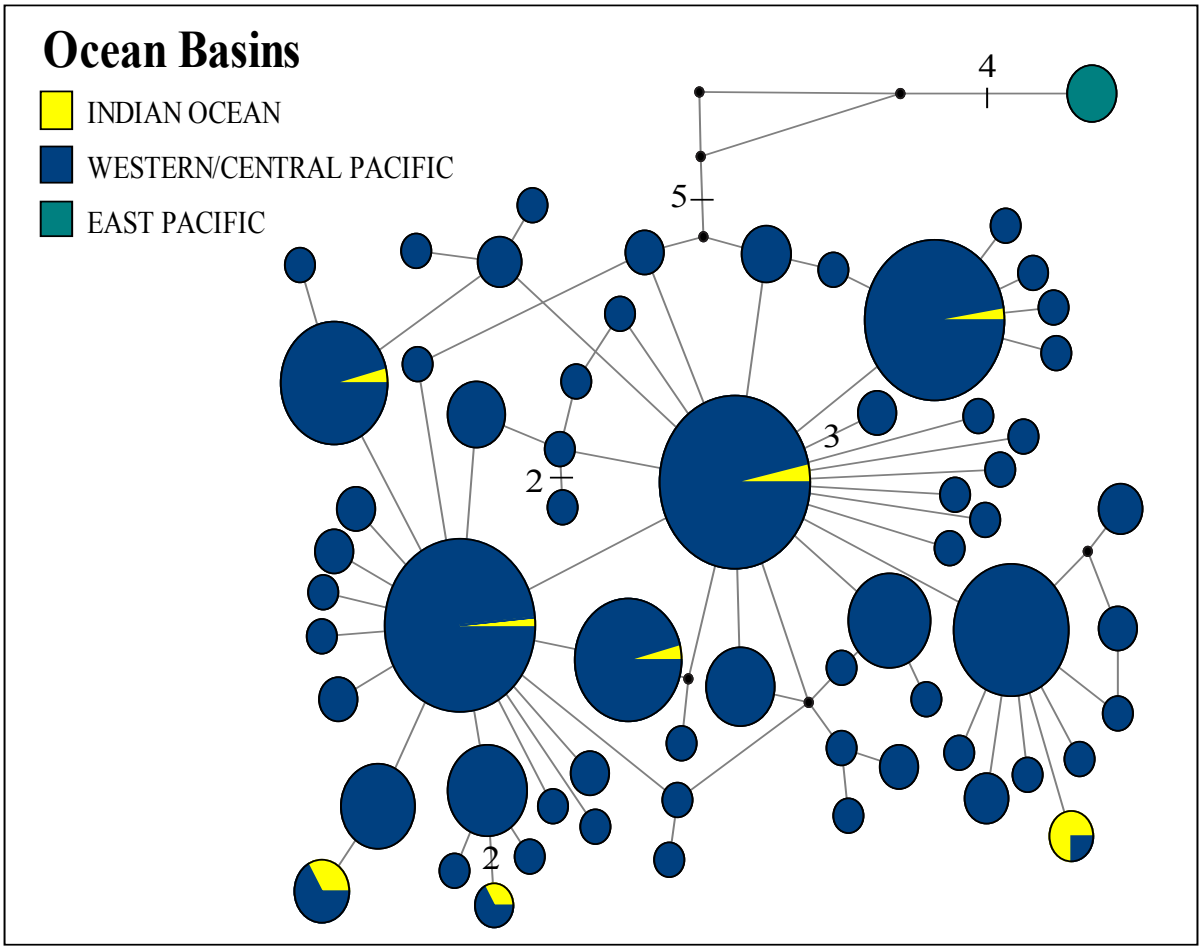
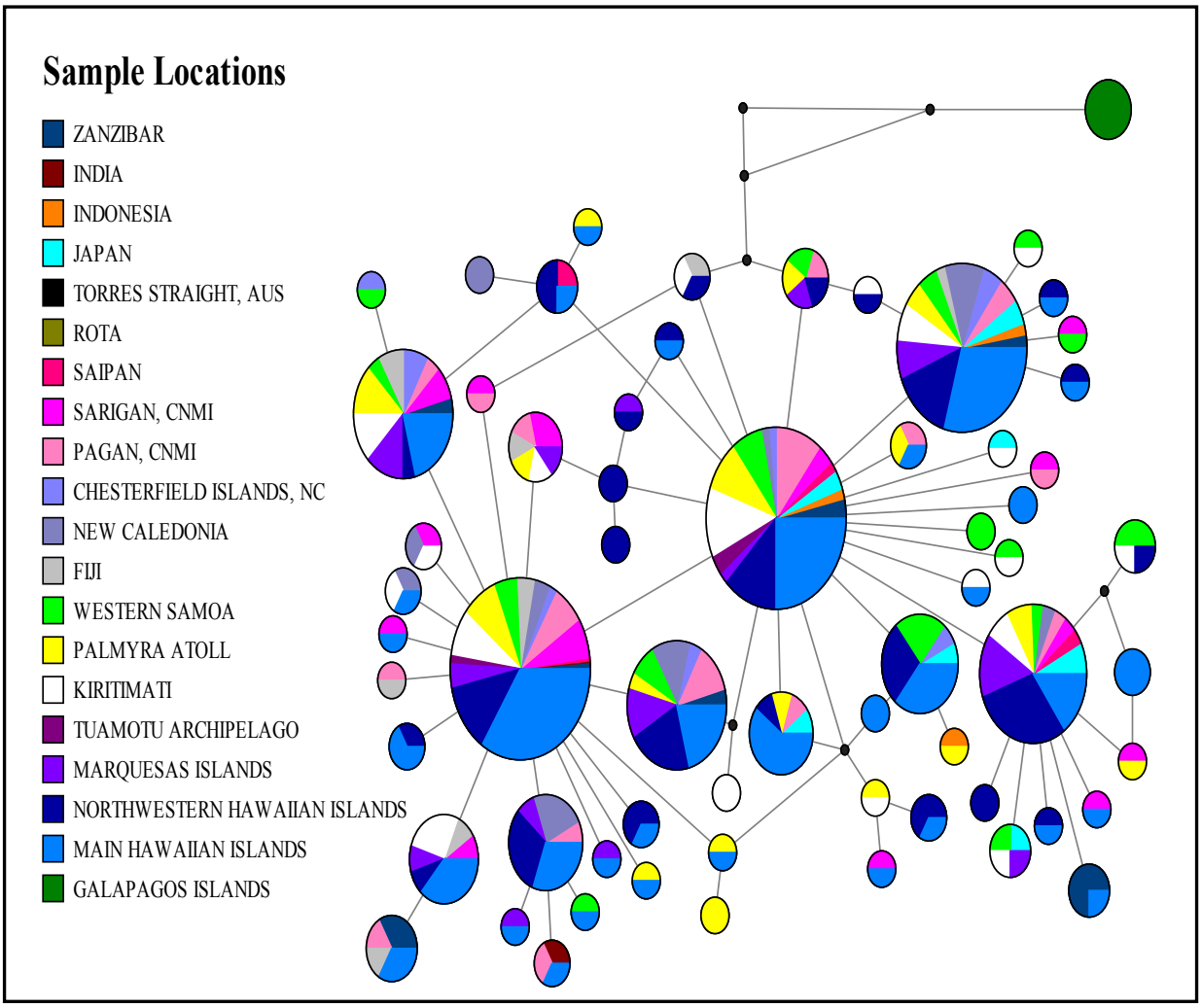


Figure 2.2. Median-joining network for *Panulirus penicillatus* mtDNA, constructed using 460 base pairs of cytochrome *c* oxidase subunit I (COI) from each of 671 individuals in the program NETWORK 4.6.0.0. Each circle is a unique haplotype proportional in size to the number of individuals with that haplotype. The two largest circles represent 193 and 56 individuals. The smallest circle represents two individuals: there are 118 singletons in the dataset, but these have been omitted for ease of visualization. A full, unedited network is included in the supplementary material (Fig. S2.2). Colors correspond to one of the three major biogeographic regions sampled – the Red Sea is not included here. A similar network, with all sampling locations colored differently is included in the supplementary material (Fig. S2.1). Lines connecting haplotypes represent a single base pair difference between haplotypes, with numbers of differences listed when N > 1.

Supplemental Information



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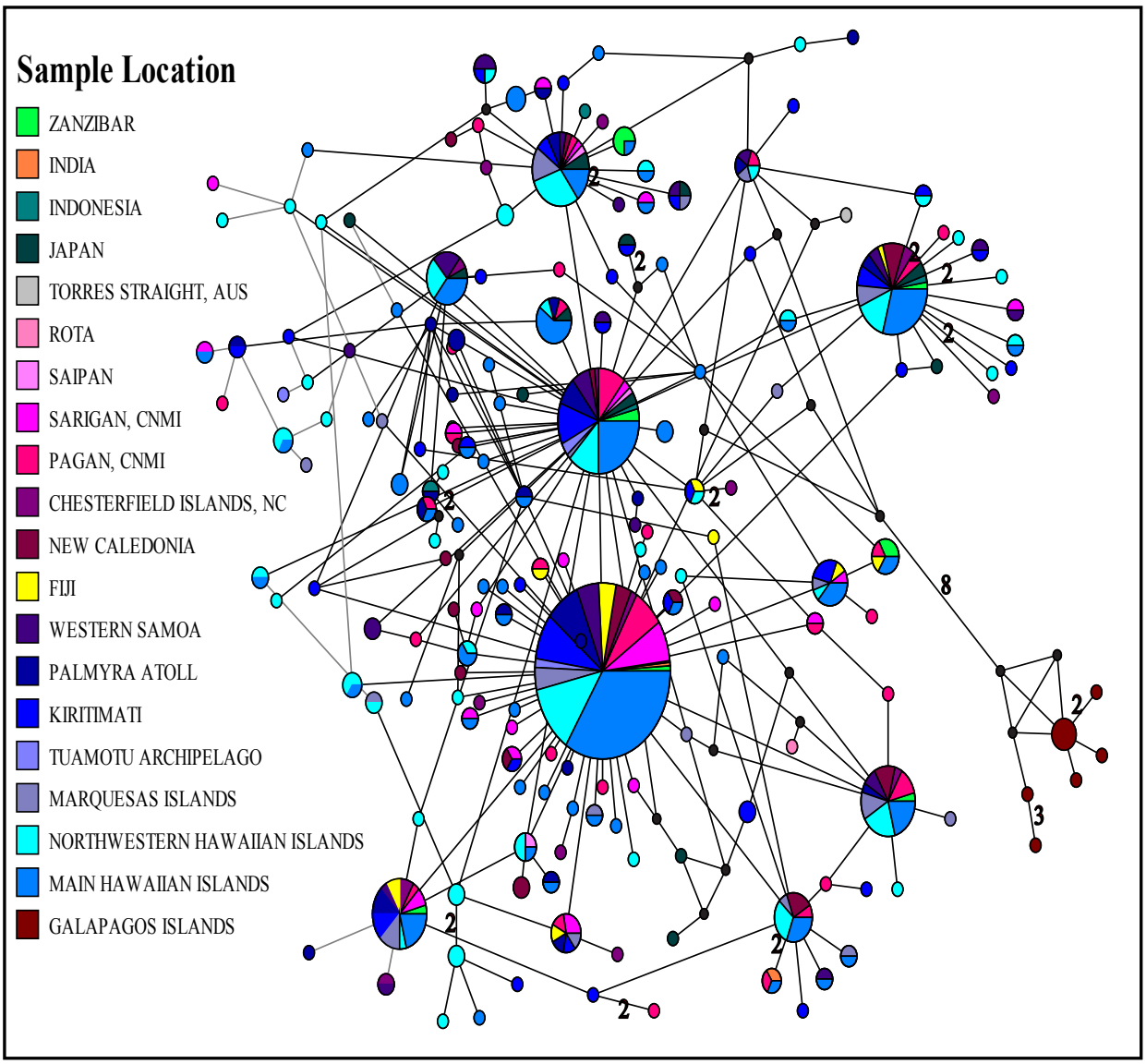

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CHAPTER III

CAVERNS, COMPRESSED AIR, AND CRUSTACEAN CONNECTIVITY: INSIGHTS INTO HAWAIIAN SPINY LOBSTER POPULATIONS

Published as:

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Abstract

Since the arrival of the first Polynesian voyagers to the Hawaiian archipelago, spiny lobsters have proved to be valuable fisheries species in Hawai‘i. However, the combination of long-term commercial and recreational fisheries for this species, and changing environmental conditions, have led to declining catch rates since the 1950s. The Papahānaumokuākea Marine National Monument (PMNM), established in 2006, now provides an extensive harvest refuge area for lobster species in the Northwest Hawaiian Islands (NWHI). This study aims to investigate the potential for the PMNM to rejuvenate lobster populations and fisheries in the Main Hawaiian Islands (MHI) by using mitochondrial DNA markers to examine how lobster populations are related throughout the Hawaiian archipelago. State and federal agencies, dive shops, and recreational and commercial lobster fishermen all collaborated to obtain genetic samples across the ~2500km stretch of ocean spanned by the State of Hawai‘i. Over 1249 tissue samples from 15 islands, atolls, reefs and banks throughout the archipelago were collected prior to 2011. In the process of collecting samples for genetic analysis, I observed a significant shift in abundance of lobster species between the MHI and the NWHI. *Panulirus marginatus* is the most abundant spiny lobster (73%) in the NWHI catch, while *P. penicillatus* (88%) dominates the MHI catch (Fisher's exact test, $P << 0.001$). I also observed *P. marginatus* living at shallower depths in the NWHI where they would not be found in the NWHI. This could be due to thermal regime, habitat availability, species competition, or fishing effects.

Introduction

The overall goal of this dissertation chapter combined with chapter four is to determine the scales of population connectivity in the two congeneric species of spiny lobsters in the Hawaiian Archipelago, *Panulirus marginatus*, and *P. penicillatus*, using mitochondrial DNA sequence variation, as well as to generate hypotheses regarding what may drive the pattern in gene flow. These data will enable managers to determine whether the recently established Papahānaumokuākea Marine National Monument (PMNM) has the potential to rejuvenate lobster stocks in the Hawaiian Archipelago, or if additional reserves will be needed to sustain populations in the Main Hawaiian Islands (MHI). In this chapter, I discuss the methods utilized to collect samples throughout the species range, and the subsequent data I was able to obtain through the characterization of these collections. Due to the nature of the collections, I had a unique opportunity to document species life history traits and characterize species distributions throughout the archipelago. This data, in turn, informs the hypotheses on population connectivity patterns in these species described in chapter four.

Methods

Sampling

Lobsters were collected using three distinct methods: by hand while SCUBA diving or free diving, with standard Fathoms Plus® commercial lobster traps on-board both large and small research vessels, and through collaborations with commercial and recreational fishers. To cover such a broad geographical area and depth gradient for sample collection, the assistance of a number of organizations was employed: two National Oceanic and Atmospheric Association vessels (RV Hi‘ialakai and RV Oscar Elton Sette), six dive shops located throughout the state of Hawai‘i, five Hawai‘i Division of Aquatic Resource (DAR) Offices, five commercial fishermen, and over 20 recreational divers/fishermen, in addition to University of Hawai‘i scientific divers. Whenever possible, carapace length, sex, and a GPS coordinate of sampling locations were recorded. However, with the large diversity of sampling personnel, it was not always possible to obtain all of this information for each specimen. All samples collected by scientists employed non-lethal sampling protocols as outlined in Skillings and Toonen (2013). In all sampling instances, a single leg or antenna segment was taken for a tissue sample and lobsters were returned to the site of capture (with the exception of commercial and recreational fishers, who retained all legal lobsters). Removal of a walking leg is a standard sampling technique for crustacean genetic surveys because these appendages typically grow back during the next molt (Mykles 2001).

The tissue samples were taken in the field and stored in either 20% dimethyl sulfoxide salt-saturated buffer (Seutin et al. 1991, Gaither et al. 2011) or 95% ethanol at room temperature. At all locations in the Northwestern Hawaiian Islands where > 50 samples have been collected (particularly, Necker Island, Gardner Pinnacles, Maro Reef, and Laysan Island where sample sizes are >> 50), the samples were obtained from the National Oceanic and Atmospheric Association (NOAA), National Marine Fisheries Service (NMFS) annual lobster tagging cruises, prior to the establishment of the Papahānaumokuākea Marine National Monument (PMNM) in 2006.

Laboratory procedures

Extractions, amplification, and sequencing protocols are detailed in chapter four. Briefly, genomic DNA was extracted from each tissue sample using the DNeasy Animal Tissue kits (Qiagen Inc., Valencia, CA, USA) following the manufacturer's protocol. For *Panulirus marginatus*, a 662 bp region of mtDNA cytochrome oxidase II (COII) was amplified for each sample using the polymerase chain reaction (PCR; Saiki et al. 1988) and a standard PCR protocol (Palumbi 1996) on a Bio-Rad iCycler Thermal Cycler (Bio-Rad Laboratories, Hercules, CA, USA). For *Panulirus penicillatus*, a 460 bp region of the cytochrome oxidase I (COI) region of the mitochondrial DNA was amplified using the same procedures described above. Purified PCR products were sequenced in the forward direction with an ABI 3730XL or an ABI 3130XL capillary sequencer (Applied Biosystems, Foster City, CA). Sequences were edited, aligned, and trimmed to a uniform size using GENEIOUS Pro v. 4.8.5 (Biomatters Ltd., Aukland, New Zealand).

Results

Sampling

Prior to 2011, 967 *P. marginatus*, and 282 *P. penicillatus* have been collected from 15 islands/atolls throughout the Hawaiian Archipelago (Table 3.1). Subsequently, 67 *P. marginatus*, and 12 *P. penicillatus* have been collected and added to the dataset of sequences in chapter four. However, these samples and the effort to collect them is not included in this analysis. In both the NWHI and MHI, 49% of lobsters captured were female (Table 3.2), which was not significantly different than the expected 1:1 ratio (NWHI: $\chi^2 = 0.184$, df = 1, $P = 0.668$; MHI: $\chi^2 = 0.039$, df = 1, $P = 0.844$). Since there is a ban on taking female lobsters in the MHI, the sex ratio for lobsters that can be legally harvested in the MHI (>3.25 inches carapace length (CL)) was examined. For individuals in this legal size class, 45% of lobsters in the NWHI were female (Table 3.2), which was significantly different than the expected 1:1 ratio ($\chi^2 = 4.111$, df = 1, $P = 0.043$). In the MHI, 39% of lobsters captured were female (Table 3.2), but this deviation was not significant due to lower sample size ($\chi^2 = 3.125$, df = 1, $P = 0.077$).

Specimens of at least one of the two lobster species were successfully collected while snorkeling or SCUBA diving at all islands/atolls in the Hawaiian Archipelago with the exception of Gardner Pinnacles and Nihoa Island. From 2006 through 2009, one of the authors (MI) was involved in dive collection trips resulting in 11,091 diver-hours searching for lobsters: 4453 hours in the MHI, and 6638 hours in the NWHI. A best-guess estimate places the total diver-hours spent from 2006 to 2010 searching for lobsters at close to 20,000. Diving depths ranged from 0 to 30m, with a mean depth of 14m (45ft), and median of 13m (43ft).

In the Main Hawaiian Islands (MHI), snorkeling or SCUBA diving was used to collect 24 *P. marginatus* and 175 *P. penicillatus*. In the Northwest Hawaiian Islands (NWHI), 275 *P. marginatus* and 102 *P. penicillatus* were collected while snorkelling or SCUBA diving (Table 3.2, Fig. 3.1). This is a highly significant shift in the species abundances across the two regions, with roughly three-fold more *P. marginatus* than *P. penicillatus* collected in the NWHI, whereas roughly seven-fold more *P. penicillatus* than *P. marginatus* were sampled in the MHI (Fisher's exact test, $P << 0.001$).

Trapping was not as widely utilized as snorkeling and SCUBA diving for lobsters due to difficulty in obtaining scientific collecting permits to fish with the Fathoms Plus® commercial traps. Lobsters of at least one of the two species were succesfully collected with traps at six islands/atolls in the Hawaiian Archipelago, and traps were fished unsuccesfully at four additional islands/atolls. Trapping depths ranged from 10m (33ft) to 117m (384ft), with a mean depth of 42m (139ft), and a median of 35m (114ft). The vast majority of lobster samples obtained through trapping were *P. marginatus* (59 samples in the MHI; 609 in the NWHI; Table 3.2, Fig. 3.1) obtained from the annual NOAA/NMFS lobster stock assessment and tagging cruises that visited the NWHI each summer. Trapping proved unsuccessful for collecting *P. penicillatus* samples (3 samples in the MHI; 2 in the NWHI; Table 3.2, Fig. 3.1).

All samples collected in the Northwestern Hawaiian Islands were collected by University of Hawai‘i or NOAA/NMFS biologists. In the MHI, though, both commercial and recreational fishers assisted in the sample collection. Figure 3.2 shows a distribution of samples collected in the MHI according to the stakeholder group that collected them.

From 2006 to 2010, 261 lobster samples have been collected in the MHI: 83 *P. marginatus*, and 178 *P. penicillatus* (Table 3.1). Of these, 59% (72 *P. marginatus*, 81 *P. penicillatus*) were collected by commercial fishers, 15% (4 *P. marginatus*, 35 *P. penicillatus*) by recreational fishers, and 26% (7 *P. marginatus*, 62 *P. penicillatus*) were collected by University of Hawai‘i scientists (Table 3.2, Fig. 3.2).

Laboratory procedures

DNA has been extracted from a total of 449 *P. marginatus* individuals from 14 islands/atolls, and 227 *P. penicillatus* individuals from 8 islands/atolls. Details on the resulting sequences produced and the genetic data analysis are presented in chapter four.

Discussion

Data on size, sex, and depth distributions are not usually recorded or published in marine phylogeographic or population genetic studies because these data are generally not available or relevant to the research questions being addressed. However, the collection of morphology, sex, fishery, and distribution data in addition to tissue samples for this genetic analysis has revealed interesting ecological patterns. For example, anecdotal evidence suggests that *Panulirus penicillatus* will not readily enter traps (Holthuis 1991), but no study to date has documented this phenomenon with experimental trap catch reports. Here, we report trap data (Fig. 3.1) that corroborates this hypothesis. Of the 673 spiny lobsters trapped in total for this study, only 5 (0.7%) were *P. penicillatus*. This result may be explained partially by the fact that the majority of lobsters (90.8%) were caught in the NWHI and at deeper depths, where our data suggests *P. marginatus* is the more abundant species.

However, even in traps that were fished at shallower depths in the MHI, where *P. penicillatus* makes up 68.2% of the total spiny lobster catch, *P. penicillatus* only comprised 1.6% of the trap catch. These data demonstrate that while *P. marginatus* will readily enter baited traps, this method of capture is relatively unsuccessful for *P. penicillatus*. Holthuis (1991) argues that the majority of tropical spiny lobster species will not enter traps, with the exception of *P. marginatus* in Hawai‘i, and *P. pascuensis*, which is endemic to Easter Island. In contrast, temperate lobster species support lucrative commercial trap fisheries in the North Atlantic, East Pacific, Western and Central Pacific, and South Africa (Holthuis 1991). Physical, ecological, or behavioral drivers of these differences in trapability are unclear.

The recording of the sex of each individual lobster when possible allowed a test of sex ratio differences in the MHI and the NWHI. The NWHI have been closed to lobster fishing since 2000 (Botsford et al. 2002), but recreational and commercial lobster fishing still occur throughout the MHI. In 2006, legislation was passed banning all take of female lobsters in the MHI (previously males and females >3.25in CL could be taken). All of the lobsters collected in the MHI for this study were obtained after the ban on female lobster take was implemented. While the sex-ratio in the MHI prior to this ban is undocumented, 49% of lobsters collected in the MHI in this study were female, with this percentage decreasing to 39% when only the lobsters of legal size (>3.25 in. CL) are considered (Table 3.2). This compares with 45% of lobsters >3.25 in. CL that were female in the NWHI, where there is currenlty no fishing (Table 3.2). This divergence from a 50:50 sex ratio for legal-sized lobsters in both the MHI and NWHI may be due to a number of factors, including differences in species composition and growth rates, bank-specific growth-rate differences (O’Malley and MacDonald 2009), or illegal and undocumented poaching.

The discrpency in patterns seen in the MHI and NWHI could also be due to differences in sample size between legal-sized lobsters in the MHI and in the NWHI (Table 3.2). However, the consequences of the ban on taking of female lobsters in the MHI deserves further consideration. The continued monitoring of the ratios of female and male spiny lobsters in the MHI would provide valuable data on the effect of the State's regulation on population dynamics of spiny lobsters here. If these management regulations continue to skew the sex ratio of lobsters so that the the majority of males are small, there is the potential that female fecundity will become sperm-limited, which in turn could limit overall egg production as has been demonstrated in the congeneric *P argus* as well as *Jasus edwardsii* (MacDiarmid and Butler 1998). This in turn would stymy any hope for stock recovery of spiny lobsters in the MHI.

One of the most interesting and unexpected results obtained from this sampling effort is the significant shift in abundance of lobster species between the MHI and the NWHI. *Panulirus marginatus* is the most abundant spiny lobster (73%) in the NWHI catch, while *P. penicillatus* dominates the MHI catch (88%). *Panulirus marginatus* is known to inhabit shallow depths down to 143m, and *P. penicillatus* inhabits 0 to 4m depth (Holthuis 1991), but in the MHI, it is rare to find *P. marginatus* shallower than 15m. The same pattern was expected in the NWHI; however, at multiple atolls, *P. marginatus* were commonly found in 1- 3m of water on patch reefs in calm areas inside of the atoll. *Panulirus penicillatus* in the MHI is commonly found in shallow depths, but can be as deep as 12m in areas of high wave action. In the NWHI, *P. penicillatus* were much less common, and where found, were restricted to a much narrower depth range (5m maximum). It is clear that *P. marginatus* is able to thrive at shallow depths; however, the species may have a low tolerance for turbidity and wave action.

In the NWHI, the only *P. marginatus* found in very shallow (< 3m) waters were found in calmer areas inside of atolls, where they were protected from high wave energy. However, even on the forereef, where no such protection is available, they could be found as shallow as 7 or 8m. Shallow areas with low wave energy may be relatively rare in the main eight Hawaiian Islands, so the species depth range has shifted deeper in the MHI. Alternatively, they may prefer cooler water temperatures and therefore need to move deeper waters in the MHI as do a number of fish species (Chave and Mundy 1994), because surface water temperature in the MHI is warmer. It is also remotely possible that heavy fishing pressure through time has removed most of the shallow-water individuals, or shifted the species distribution as a whole. *Panulirus penicillatus* is morphologically adapted to survive in high energy environments, with legs that are both longer and greater in diameter than individuals of the same CL in other spiny lobster species (Iacchei, pers. obs.). Areas with high wave action may provide a natural harvest refuge for this species in the MHI, but are more rare in the NWHI (hence the lower numbers there), although there is unlikely to be such a large discrepancy in the amount of shallow water habitat between the NWHI and the MHI. Further experiments would be required to test the hypothesis that *P. marginatus* outcompetes *P. penicillatus* where they co-occur, but is excluded from high energy environments, where *P. penicillatus* is the only spiny lobster species present. Alternatively, the two species may have originally colonized the Hawaiian Archipelago from opposite ends of the chain, and only had limited success expanding to the far opposite ends. This hypothesis will be tested to the extent possible using a phylogenetic framework with the genetic data obtained in this study. However, given the presence of both lobster species at all islands/atolls in the archipelago, it is more likely that the differences in abundance and distribution are driven by ecological factors.

The genetic analyses of the *P. marginatus* and *P. penicillatus* mtDNA sequences obtained from these collected tissue samples are discussed in chapter four. These genetic data contribute to the growing number of population genetic surveys of congeneric species in Hawai‘i (i.e. Bird et al. 2007, Craig et al. 2010, DiBattista et al. 2011, Skillings et al. in press), leading to a broader understanding of the factors driving meta-population dynamics in the Hawaiian Archipelago. Knowledge of spiny lobster connectivity patterns will provide valuable insight into whether the protection of the PMNM will allow the rejuvenation of lobster stocks in both the NWHI and the MHI, or if further actions will be required to protect MHI lobster stocks. This added ecological component illustrates the importance of and the additional insight that can be gained by recording ecological data during the collection of tissue samples for phylogeographic and population genetic studies, rather than just blindly treating each organism as, “simply a bag of DNA” (Donahue, pers. com.).

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Skillings DJ, Bird CE, Toonen RJ. 2013. Comparative population structure of two edible Indo-Pacific coral reef sea cucumbers (Echinodermata: Holothuroidea). Bulletin of Marine Science. Accepted pending revisions.

Table 3.1. Total number of lobster tissue samples collected prior to 2011 from each island, atoll, and bank in Hawai‘i listed from southernmost to the northernmost collection site. Not all samples were used in the genetic analysis in chapter four. However, subsequent to this synthesis, 67 samples of *P. marginatus* and 12 samples of *P. penicillatus* were added to the genetic analyses.

Collection Site (Hawaiian name)	*Panulirus marginatus*	*Panulirus penicillatus*
Hawai‘i	1	47
Maui Nui Complex	17	84
O‘ahu	63	0
Kaua‘i	2	47
Nihoa Island	0	0
Necker Island (Mokumanamana)	161	5
French Frigate Shoals (Kānemiloha‘i)	13	37
Gardner Pinnacles (Pūhāhonu)	125	0
Maro Reef (Nalukākala)	232	5
Laysan Island (Kauō)	126	1
Lisianski Island (Papa‘āpoho)	50	21
Pearl and Hermes Reef (Holoikauaua)	67	30
Midway Atoll (Pihemanu Kauihelani)	53	4
Kure Atoll (Mokupāpapa)	57	1

Table 3.2. *Panulirus marginatus* and *P. penicillatus* tissue collections by sampling method, stakeholder group, sex, and size category (sub-legal versus legal-sized). Numbers listed are totals collected prior to 2011 from the Main Hawaiian Islands (MHI) and the Northwest Hawaiian Islands (NWHI).

		Panulirus marginatus		***Panulirus penicillatus***	
		MHI	**NWHI**	**MHI**	**NWHI**
Catch methods	**Snorkel/dive**	24	275	175	102
	Trap	59	609	3	2
	Commercial Fishers	72	N/A	81	N/A
	Recreational Fishers	4	N/A	35	N/A
	Scientists	7	884	62	104
Males	**Males**	3	276	50	1
	Sub-legal males	0	72	9	0
	Legal-sized males	3	204	41	1
Females	**Females**	4	266	46	0
	Sub-legal females	4	101	18	0
	Legal-sized females	0	165	28	0

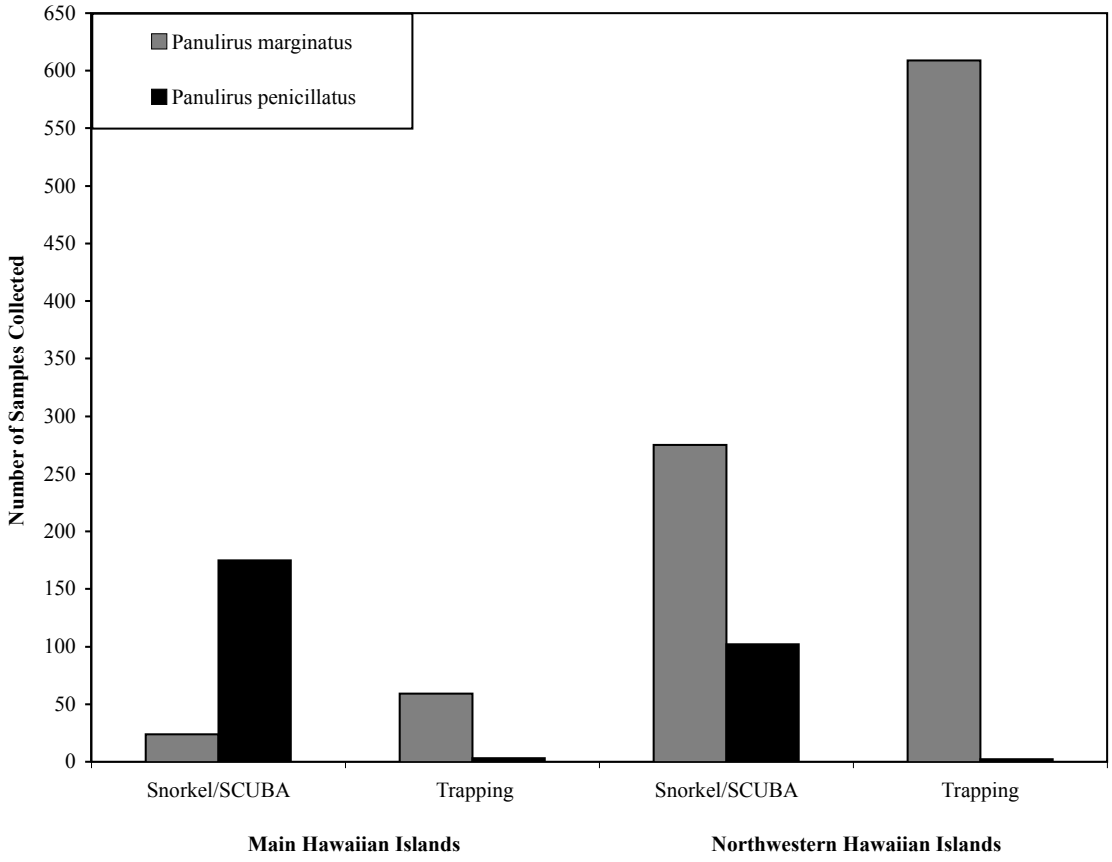

Figure 3.1. Number of lobster tissue samples collected per species in the Main Hawaiian Islands and the Northwest Hawaiian Islands by methodology.

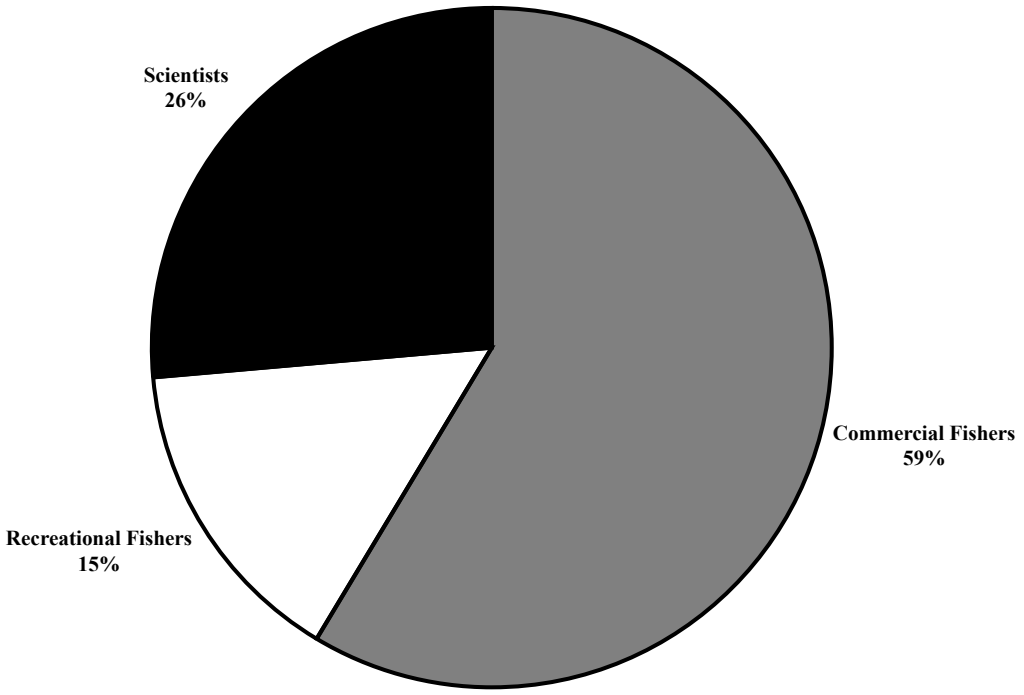

Figure 3.2. Percentage of lobster tissue samples collected in the Main Hawaiian Islands by stakeholder groups.

CHAPTER IV

AFTER THE GOLD RUSH: POPULATION STRUCTURE OF SPINY LOBSTERS IN HAWAI‘I FOLLOWING A FISHERY CLOSURE AND IMPLICATIONS FOR CONTEMPORARY SPATIAL MANAGEMENT

Submitted to a special issue on Indo-Pacific marine connectivity as:

Iacchei M, O’Malley JM, Toonen RJ. 2014. After the gold rush: population structure of spiny lobsters in Hawai‘i following a fishery closure, and implications for contemporary spatial management. Bulletin of Marine Science.

Abstract

We compare mitochondrial genetic data for two spiny lobsters in Hawai‘i with different geographic ranges and histories of fishing pressure. *Panulirus marginatus* is endemic to Hawai‘i, and experienced a short intense fishery in the Northwestern Hawaiian Islands (NWHI), whereas lobsters in the Main Hawaiian Islands (MHI) were fished with less annual effort but over a greater time period. Populations show significant overall structure (F_{ST} = 0.0037, P = 0.007; D_{est_Chao} = 0.137), with regional differentiation (F_{CT} = 0.002, P = 0.047) between the MHI and the NWHI, and 3.5 times greater effective migration from the NWHI to the MHI. Haplotype diversity is not significantly different among regions ($F_{2, 8}$ = 3.740, P = 0.071); however, nucleotide diversity is significantly higher at the primary NWHI fishery banks (0.030) than in the MHI (0.026, Tukey's P = 0.013). In contrast, *P. penicillatus* is found across the tropical Indo-West Pacific and the Red Sea, was not targeted by the NWHI fishery, but also has experienced long-term exploitation in the MHI. *Panulirus penicillatus* has no significant overall population structure in Hawai‘i (F_{ST} = 0.0083, P = 0.063; D_{est_Chao} = 0.278), although regional differentiation (F_{CT} = 0.0076, P = 0.0083) between the MHI and the NHWI is significant. In contrast to *P. marginatus*, *P. penicillatus* has over a 500-fold higher effective migration rate from the MHI to the NWHI. Neither haplotype nor nucleotide diversity were significantly different between regions for *P. penicillatus*. While neither species has suffered a loss of genetic diversity from fishing, our results highlight that only by incorporating knowledge of fishing history with genetic connectivity data can we understand the most beneficial management strategy for each of these species. Neither exemplar species nor specific suites of traits are reliable predictors of the spatial scales of management.

Introduction

There has been increasing interest in establishing effective marine protected areas for species conservation and management to meet the goals of ecosystem-based management (Browman and Stergiou 2004, 2005, Gerber et al. 2007, Beger et al. in press). An essential data requirement for evaluating whether marine protected areas are properly sized and located for effective species management is detailed information regarding connectivity among disjunct habitats (e.g., Botsford et al. 2001, Botsford et al. 2003, Halpern and Warner 2003, Palumbi 2004, Cowen et al. 2006). The size and number of connected populations, and frequency of larval influxes can profoundly influence the density and persistence of individuals at specific locales (MacArthur and Wilson 1963, Stacey and Taper 1992, Sale and Kritzer 2003). This fact is notably pertinent for benthic marine invertebrates living in remote island chains, where isolated locations are more likely related through larval dispersal than adult movement. Unfortunately, the pelagic developmental period of most invertebrate larva, coupled with a small size and often the ability to swim to either avoid or take advantage of the prevailing oceanographic currents, make larvae extremely difficult to track (reviewed by Levin 2006). The poor understanding of population connectivity in organisms with a biphasic life cycle and pelagic larval development has confounded the management of these marine species (Carr et al. 2003, López-Duarte et al. 2012).

A profound example of this the Northwestern Hawaiian Islands (NWHI) lobster fishery, which began in 1976, and principally targeted the endemic Hawaiian spiny lobster, *Panulirus marginatus* (Quoy & Gaimard, 1825), as well as the scaly slipper lobster, *Scyllarides squammosus* (H. Milne-Edwards, 1837).

The Hawaiian Archipelago consists of the Main Hawaiian Islands (MHI, Hawai‘i to Ni‘ihau), and the NWHI, a series of islands, reefs, seamounts, and atolls (hereafter referred to as banks) that extend approximately 2000 km across the subtropical Pacific (Fig. 4.1). From 1976 to 2000, this NWHI lobster fishery reported landings of spiny and slipper lobsters totaling 11 million individuals (Table 1 in Schultz et al. 2011). Landings peaked within seven to nine years (1983-1985; DiNardo et al. 2001), during which time it was Hawaii's most valuable demersal fishery (Polovina 1993). However, both reported landings (DiNardo et al. 2001) and catch per unit effort (CPUE; Fig. 2 in O'Malley 2009) steadily declined over the next decade (1986–1995). Despite almost yearly stock assessments and a variety of management measures, the National Marine Fisheries Service (NMFS) ultimately closed the fishery in 2000 because of increasing uncertainty in population and stock assessment models, particularly with regard to spatial heterogeneity and the assumption of synchronous dynamics among bank-specific stocks (Botsford et al. 2002). Since the closure of the fishery, there has been no evidence of recovery of either species (O'Malley 2009, 2011). In 2006, the entire NWHI region was protected as the Papahānaumokuākea Marine National Monument (PMNM). The non-extraction nature of the PMNM ensures that lobster fishing will not likely resume in the NWHI.

Extensive subsequent research has justified the concerns of NMFS lobster fishery managers. O'Malley presents evidence for both spatial and temporal differences in growth rates of *P. marginatus* (2009) and *S. squammosus* (2011) across the three primary banks targeted by the fishery (Necker Island, Maro Reef, and Gardner Pinnacles), and an additional location closed to fishing throughout most of the fishery's duration (Laysan Island). The results of these studies indicate that both spiny and slipper lobsters at each of these islands or atolls are experiencing different environmental conditions and/or varied prey regimes (O'Malley et al. 2012).

Less well known is whether lobsters at each of the islands and atolls in the Hawaiian Archipelago are genetically isolated populations that are locally adapting to these bank-specific dynamics, or if this is one single population with phenotypically plastic traits that vary from bank to bank. Quantitative estimates of population connectivity are required to develop models that more accurately represent island/bank specific population dynamics. Results from tagging studies indicate that adult lobsters are unable or unwilling to traverse the deep-water channels between banks. Not one of approximately 85,000-tagged lobsters was recaptured on a different bank (O'Malley and Walsh 2013). On average, tagged adults of both *P. marginatus* and *S. squammosus* move less than 1km from their initial tagging location, even after five years at liberty (O'Malley and Walsh 2013). Therefore, if the different islands and atolls are forming a single panmictic population or even a structured metapopulation, the gene flow between islands is maintained through the pelagic larval phase.

Spiny and slipper lobsters have some of the longest larval durations of any taxa (three to six months, and six months to over one year, respectively; Lavalli and Spanier 2007, Phillips et al. 2006). Such long pelagic durations make larval dispersal patterns extremely difficult to predict, but intuitively, species with larval durations of this length are expected to be panmictic across broad geographic ranges (Shanks et al. 2003, Siegel et al. 2003). More recent analyses suggest only a weak relationship exists between larval duration and the degree of population structure across a species' range (Shanks 2009, Weersing and Toonen 2009, Riginos et al. 2011, Selkoe and Toonen 2011).

Accordingly, although many previous genetic studies on spiny lobsters have found high levels of gene flow across broad geographic scales (Shaklee and Samollow 1980, Ovenden et al. 1992, Tolley et al. 2005, García-Rodríguez and Perez-Enriquez 2006, Inoue et al. 2007, García-Rodríguez and Perez-Enriquez 2008), indications of localized recruitment despite an 8-12 month larval duration have been demonstrated in a few *Panulirus sp.* (Silberman and Walsh 1994, Johnson and Wernham1999, Iacchei et al. 2013).

Dispersal patterns of Hawaiian lobster larvae are not well known, although hypotheses have been derived from offshore distributions of phyllosoma of *P. marginatus* and *P. penicillatus* (Olivier, 1791), the two spiny lobster species in Hawai‘i (i.e., Johnson 1968, Polovina and Moffit 1995), as well as from settlement patterns of pueruli (MacDonald 1986), and Lagrangian dispersal kernel modeling (Polovina et al. 1999, Kobayashi 2006). Pollock (1992) proposed phyllosoma mix together in the Pacific subtropical gyre and remain there for up to four years before recruiting to Hawaiian reefs, while MacDonald (1986) contended that larvae are retained around the archipelago for shorter time periods before settlement. Polovina et al. (1999) concluded that phyllosoma move with the predominant currents in a southeasterly direction until reaching Necker Island, at which time they travel southwest. Genetic studies using allozymes to examine population differentiation indicate that prior to the opening of the NWHI fishery, spiny lobsters were genetically homogeneous throughout the archipelago (Shaklee and Samollow 1980), but did not yield any hypotheses on direction of gene flow. After the peak of exploitation in the NWHI, Seeb et al. (1990) studied *P. marginatus* populations at just Maro Reef and Necker Island (the two most heavily fished banks), and found a significant difference between these banks at one of seven allozyme loci, but no significant differences among locations for the other six.

While allozyme studies have proven useful for population delineation, they have limited resolution especially in cases of fine scale population structure. DNA sequence data contain higher levels of variability that often allow the distinction of populations that are not resolved with allozyme markers (Avise 1994).

Here we evaluate the scale of population connectivity for the most heavily targeted species in the NWHI lobster fishery, the endemic Hawaiian spiny lobster, *P. marginatus,* using mtDNA cytochrome subunit *c* oxidase I (COI). We expand the scope of previous studies to encompass 13 islands and atolls throughout the Hawaiian Archipelago. We compare the pattern of genetic differentiation to that of a second spiny lobster species found in Hawai‘i, the broadly distributed congener, *P. penicillatus*, which has been steadily fished in the Main Hawaiian Islands (MHI), but was a negligible portion of the NWHI catch. Our goals for this study are threefold: (1) determine the patterns of population genetic differentiation for both of these species in Hawai‘i; (2) compare patterns of genetic connectivity between these two species with similar evolutionary histories and ecological requirements, but vastly different distribution ranges, and (3) assess whether fishing has had a detectable impact on the neutral genetic variability in these two species. These data will enable fisheries managers to determine whether the closure of the NWHI lobster fishery and the establishment of the PMNM in the NWHI have the potential to rejuvenate lobster stocks in the Hawaiian Archipelago. In addition, the results will address the utility of using exemplar species to set management regimes for similar taxa.

Methods

Collection

We collected *Panulirus marginatus* and *P. penicillatus* tissue specimens using three distinct methods: by hand, with Fathoms Plus (San Diego, California) lobster traps, and from commercial and recreational fishers (detailed in Iacchei and Toonen in press). All specimens were collected non-lethally by removing a small piece of an antenna or a leg segment, and lobsters were returned to the site of capture (with the exception of commercial and recreational fishers, who retained all legal-sized lobsters). Whenever possible, carapace length, sex, and a GPS coordinate of sampling locations were recorded. For *Panulirus marginatus*, we collected and sequenced a total of 564 individuals from 13 islands and atolls throughout the Hawaiian Archipelago. Our southernmost *P. marginatus* samples were obtained from Maui, while our northernmost were from Kure Atoll (Fig. 4.1, Table 4.1). For *Panulirus penicillatus*, we collected and sequenced a total of 281 individuals from 10 islands or atolls in Hawai‘i, with the southernmost site, Hawai‘i Island, and the northernmost, Pearl and Hermes Reef (Fig. 4.1, Table 4.1). Tissue samples were preserved in either 20% dimethyl sulfoxide salt-saturated buffer (Seutin et al. 1991, Gaither et al. 2011) or 95% ethanol, and stored at room temperature until extracted.

DNA Extraction, PCR, and Sequencing

Genomic DNA was isolated using DNeasy Animal Tissue kits (Qiagen Inc., Valencia, CA, USA) or the modified HotSHOT method (Truet et al. 2000, Meeker et al. 2007). For *P. penicillatus*, we amplified a 460 bp fragment of COI using species-specific primers PpenCOI-F (5’-GCTGGAATAGTGGGGACCTC-3’) and PpenCOI-R (5’-GCTTCTGACCGACCGTAACT-3’) designed from GenBank sequence #AF339468 (Ptacek et al. 2001).

For *P. marginatus*, we amplified a 662bp fragment of cytochrome *c* oxidase subunit II gene (COII) using species-specific primers PmarCOII-F (5'-GCTGGAATAGTGGGGACCTC-3') and PmarCOII-R (5'-GCTTCTGACCGACCGTAACT-3') designed from the *Panulirus japonicas* mitochondrial genome, GenBank sequence #NC_005251.1 (Yamauchi et al. 2002). Both sets of primers were designed using PRIMER3 (Rozen and Skaletsky 2000). COII was used for *P. marginatus* instead of COI (the common mtDNA marker used for intraspecific studies of spiny lobsters) because we consistently found double peaks in the electropherograms of *P. marginatus* COI sequences. We assumed these were the result of a nuclear DNA pseudogene, which are known to occur commonly in crustaceans (Williams & Knowlton 2001, Buhay 2009, Calvignac et al. 2011), and would confound our analyses.

For both species, polymerase chain reactions (PCRs) for each individual were performed in 20µl aliquots containing 5-50 ng of genomic DNA, 0.125 µM each of forward and reverse primer, 0.75x Bovine Serum Albumin (BSA), 10µl of 2x Biomix Red (Bioline), and sterile deionized water to volume. A Bio-Rad iCycler Thermal Cycler (Bio-Rad Laboratories, Hercules, CA, USA) was used for all PCR reactions. The PCR protocol was the same for both species, and consisted of an initial denaturation step of 95 °C for 4 min, 35 cycles of denaturation (95°C for 30 sec), annealing (56°C for 30 sec), and extension (72°C for 30 sec), and a final extension step (72°C for 10 min). PCR products were purified with 0.75 units of Exonuclease I and 0.5 units of Fast Alkaline Phosphatase (ExoFAP, Fermentas) per 7.5 µl of PCR product, and incubated at 37°C for 60 min, followed by deactivation at 85°C for 15 min. Purified PCR products were sequenced in the forward direction with an ABI 3730XL or an ABI 3130XL capillary sequencer (Applied Biosystems, Foster City, CA). Sequences with ambiguous nucleotide calls and all unique haplotypes were also sequenced in reverse to confirm sequence identity.

Sequences were edited, aligned, and trimmed to a uniform size using GENEIOUS Pro v. 4.8.5 (Biomatters Ltd., Auckland, New Zealand). Neither the *P. marginatus* nor the *P. penicillatus* alignment contained any frameshift mutations, stop codons, or indels.

Data Analysis

Summary statistics

We calculated nucleotide (π) and haplotype diversity (h) described in Nei (1987) using ARLEQUIN 3.5 (Excoffier et al. 2010). Effective number of alleles ($1/(1-h)$) as described by Jost (2008) was calculated by hand. We constructed a median-joining network (Bandelt et al. 1999) using the program NETWORK 4.6.0.0 (http://www.Fluxus-engineering.com/network_terms.htm) to visualize the frequencies, spatial distributions, and relationships among haplotypes.

Spatial scale of genetic connectivity

To investigate the spatial scale of genetic connectivity in these species, we conducted an analysis of molecular variance (AMOVA) in ARLEQUIN 3.5 (Excoffier et al. 2010). In the AMOVA framework, we tested for genetic differentiation across the entire Hawaiian Archipelago, as well as between two regions of the archipelago, the MHI and the NWHI. These regions were chosen a priori based on regional genetic differentiation found in other species (reviewed in Toonen et al. 2011), as well as contemporary fisheries management regimes. We specifically wanted to test the hypothesis of whether the protected NWHI could be a source of propagules to rejuvenate lobster stocks in the MHI. We also determined the level of genetic differentiation between sites by calculating pairwise Φ_{ST} in ARLEQUIN. Φ_{ST} is an analogue of Wright's F_{ST} that incorporates a model of sequence evolution (Excoffier et al. 1992).

Using jModelTest2 (Guindon and Gascuel 2003, Darriba et al. 2012), we determined the most appropriate model of sequence evolution to implement in ARLEQUIN for each species and genetic marker. For *P. marginatus* COII, we used the algorithm of Tamura and Nei (1993) with a Ti/Tv ratio of 8.731 and gamma parameter of 1.467. For *P. penicillatus* COI we used the same algorithm with a Ti/Tv ratio of 7.273 and gamma parameter of 1.550. For both species, global Φ_{ST}, regional Φ_{CT} and each pairwise population Φ_{ST} were tested for significance with 100,000 permutations. To correct for inherent bias when conducting multiple comparisons, we implemented a false discovery rate (FDR) correction (Benjamini 2006) to adjust the critical *P*-value for each pairwise site comparison. We also conducted exact tests of population differentiation (Raymond and Rousset 1995, Goudet et al. 1996) in ARLEQUIN. For this test, we used 100,000 Markov chains, with 10,000 de-memorization steps and a *P*-value = 0.05. Due to the high mean within population heterozygosity of both COII in *P. marginatus* and COI in *P. penicillatus*, we also calculated D_{est_Chao} (Jost 2008) as an absolute measure of differentiation between sites. While D_{est_Chao} does not account for the genetic distance between haplotypes, it is less susceptible to biases caused by genetic diversity (Bird et al. 2011), whereas the magnitude of F_{ST}/Φ_{ST} is inversely proportional to the within population expected heterozygosity (Hedrick 2005, Meirmans 2006, Jost 2008). For a more detailed explanation of the statistical properties of D_{est_Chao}, see Bird et al. (2011) and Skillings et al. (this issue). D_{est_Chao} was calculated with GENODIVE 2.0b20 (Meirmans and Van Tienderen 2004).

Effective migration rates

To test whether larval production from protected lobsters in the PMNM has the potential to be exported to the MHI, for each lobster species we calculated a Bayesian coalescent-based migration rate (N_em) between the MHI and the NWHI and a region-specific mutation parameter (θ) for each of the two regions using MIGRATE-N 3.3.2 (Beerli 2006, 2009, Beerli and Palczewski 2010). The Ti/Tv ratios and gamma values were the same as listed above, and we determined the mtDNA sequence variation using the respective gamma values and three regions of the mtDNA sequence. For both species, we used a sequencing error rate of 0.001 and kept θ constant for the initial calculation of $F_{ST.}$ A windowed exponential prior was used for θ and migration, and the respective minimum, mean, maximum, and delta values were determined for each factor by trial runs with shorter Markov chain lengths. We ran three independent replicates of a Bayesian MCMC search strategy for each species, with a Markov chain length of 1,000,000, sampled once every 100 iterations, and with a 500,000-chain burn-in. Program defaults were used for all other settings.

Impacts of fishing on genetic diversity

To assess the potential effects of fishing on neutral genetic diversity in *P. marginatus*, we conducted one-way ANOVAs followed by Tukey's post-hoc tests for three regions of different fishing pressure: the southern NWHI (Necker Island, Gardner Pinnacles, Maro Reef), the MHI (Maui, O'ahu, Kaua'i), and the central and northern NWHI (Laysan Island, Lisianski Island, Pearl and Hermes Reef, Midway Atoll, and Kure Atoll).

The southern NWHI has undergone a quarter-century (1975-2000) of short, but very intensive fishing, the MHI have been fished intensively for over a century, but with lower instantaneous levels of effort than the NWHI fishery sites, and the central and northern NWHI have been fished through time, though not as intensively as the NWHI or MHI fishery areas. For *P. penicillatus*, we conducted t-tests comparing the MHI with the NWHI, because *P. penicillatus* have been taken in the MHI for over a century (Morris 1968), but were rarely captured by traps in the NWHI fishery (Uchida et al. 1980).

Haplotype diversities were rarefied to 30 (*P. marginatus*) and 15 (*P. penicillatus*) individuals using the program CONTRIB 1.2 (Petit et al. 1998) in order to account for increasing haplotype diversity with larger sample sizes. For both species, statistical analyses were conducted on haplotype diversities that were logit transformed in order to maintain equivalent variances among treatments. After logit transformation, variances were homogenous for all tests (Levene's test, $P > 0.05$). All statistical analyses were conducted in SPSS 17.0.

Results

Panulirus marginatus

Summary statistics

We resolved 662 bp of COII for a total of 564 lobsters across 13 islands/atolls throughout the Hawaiian Archipelago, yielding 282 mtDNA haplotypes. The median joining network (Fig. 4.2) portrays two common haplotypes that are separated by five bp and represent 56 and 53 individuals respectively. Each of these haplotypes is found in 12 out of 13 sites in the study (neither was found at Lāna‘i), and combined these haplotypes represent 19.3% of the individuals in the study.

A third haplotype is separated by five bp through intermediate haplotypes from one of the dominant haplotypes (53) and by nine bp from the other (56). It represents only 16 individuals (2.8% of those sampled). Most of the haplotypes in this study differ by only one base pair from their closest linked haplotype, although some differ by as many as four base pairs. Overall, the median joining network is dominated by three distinct starburst patterns off of the three central haplotypes, although there is additional complex structure and linkages within the network. As evidenced by the network, haplotype diversity is high, averaging $h = 0.977$, and ranging from $h = 0.93$ in Maui to $h = 1.00$ at Lānaʻi and French Frigate Shoals, although each of these sites is represented by less than 10 individuals. This range in haplotype diversities translates to a broad range of effective number of haplotypes, from a low of 14.77 (Maui) to a high of 147.06 (Pearl and Hermes Atoll) when Lānaʻi and French Frigate Shoals are excluded. The mean number of effective haplotypes is 58.53. Nucleotide diversity has a mean of $\pi = 0.029$, and ranges from $\pi = 0.025$ at Kauaʻi to $\pi = 0.035$ at Lānaʻi. The number of individuals sequenced (N), haplotype diversity (h), effective number of haplotypes (h_{eff}), and nucleotide diversity (π) are listed for each site in Table 4.1.

Spatial scale of genetic connectivity

Global F_{ST} is low (0.0037), but statistically significant ($P = 0.007$), whereas global Φ_{ST} is -0.0016, and not statistically significant ($P = 0.635$). The distinction between the MHI and NWHI regions follows a similar pattern, with low, but statistically significant genetic structure as evidenced by F_{CT} (0.002, P=0.047), but no significant genetic structure between these regions using Φ_{CT} (0.003, P=0.105). Of 55 pairwise F_{ST} comparisons, 13 (23.6%) are statistically significant at $P \leq 0.05$, and they range in magnitude from 0.007 to 0.020 (Table 4.2).

In contrast, the only statistically significant pairwise Φ_{ST} comparison is between Kaua‘i and Pearl and Hermes Atoll (Φ_{ST} =0.023, P=0.044, Table 4.2). After correcting for FDR, no pairwise comparisons for either F_{ST} or Φ_{ST} remain significant. The overall exact test of population differentiation is significant ($P < 0.000005$), and there are 19 (34.5%) statistically significant comparisons (Table S4.1). In general, the significantly different pairwise site comparisons from the exact tests of differentiation match those of the significant pairwise F_{ST} comparisons, not the predominantly non-significant pairwise Φ_{ST} results (Table 4.2, S4.1). Overall D_{est_Chao} is 0.137, almost two orders of magnitude greater than global F_{ST} (0.0037), as expected given the high within-population mean diversity (Bird et al. 2011). Most of the pairwise D_{est_Chao} comparisons are also much greater in magnitude than pairwise F_{ST} or Φ_{ST} values, and range up to 0.564 (Table 4.3). A test for significance is not yet available for D_{est_Chao}.

Effective migration rates

MIGRATE results indicate high effective migration rates (N_em) from both the MHI to the NWHI and from the NWHI to the MHI, but there is an almost 3.5 fold higher effective migration rate from the NWHI to the MHI than in the other direction (Table 4.4). All posterior probability distributions are unimodal curves. Both components of effective migration differ between regions: θ in the NWHI is approximately 2.5 times greater than θ in the MHI, and the migration rate from the NWHI to the MHI is almost 9x greater than from the MHI to the NWHI (Table 4.4).

Impacts of fishing on genetic diversity

There is no significant difference in rarefied haplotype diversity among regions ($F_{2, 8} = 3.740$, $P = 0.071$), but rarefied haplotype diversity is greatest at the heavily fished banks of the NWHI (0.961: Necker Island, Maro Reef, Gardner Pinnacles), followed by the lightly fished banks in the NWHI (0.956: Laysan Island, Lisianski Island, Pearl and Hermes Reef, Midway Island, Kure Atoll), and the MHI (0.946: Maui, O‘ahu, Kaua‘i).

There is an overall significant difference in nucleotide diversity among banks with different fishing pressure ($F_{2, 8} = 7.567$, $P = 0.014$). Tukey’s post-hoc tests show that nucleotide diversity is significantly greater in the heavily fished area of the NWHI (0.030) than in the MHI (0.026, P=0.013). Differences in nucleotide diversity between the lightly fished (0.029) and the heavily fished (0.030) banks of the NWHI are not significant (P=0.337), nor are differences between the MHI (0.026) and the lightly fished banks of the NWHI (0.029, P=0.058).

Panulirus penicillatus

Summary statistics

We resolved 460 bp of COI in 281 lobsters across 10 islands/atolls in Hawai‘i, which yielded 85 unique haplotypes among these 281 individuals. The median joining network (Fig. 4.3) is dominated by one common haplotype that represents 90 individuals (32.0% of the individuals in the study), and is found at eight of 10 sites in the study (not found at Maro Reef (N=5) and Necker Island (N=5)). This haplotype is separated by one bp from the next most common haplotype representing 21 individuals (7.5%), and also found at the same eight of 10 sites. The rest of the haplotypes are predominantly branched off of one of these two haplotypes in a starburst pattern (Fig. 4.3).

The majority of haplotypes are connected by one base pair, with a maximum of two base pairs separating neighboring haplotypes. Haplotype diversity is moderate, ranging from $h = 0.75$ at Maui to $h = 1.00$ at Maro Reef (N=5), with mean of $h = 0.89$. There is a mean of 10.05 effective haplotypes, and these range from 4.06 at Maui to 25.51 at Lisianski Island, excluding Maro Reef. Nucleotide diversity is low, ranging from $\pi = 0.004$ to $\pi = 0.013$ with a mean of $\pi = 0.007$. Table 4.1 lists number of individuals sequenced (N), haplotype diversity (h), effective number of haplotypes (h_{eff}), and nucleotide diversity (π) for each site.

Spatial scale of genetic connectivity

Global F_{ST} (0.0083) and global Φ_{ST} (0.0097) are similar in magnitude, and neither is significant (F_{ST} $P = 0.063$; Φ_{ST} $P = 0.054$). However, the regional separation between the MHI and NWHI is low, but statistically significant for both F_{CT} (0.0076, P=0.0083) and Φ_{CT} (0.011, P=0.023). The only statistically significant pairwise F_{ST} comparison is between Maui and Lisianski Atoll (F_{ST}=0.055, P=0.029, Table 4.5), and this is no longer significant after correction for FDR. There are four statistically significant pairwise Φ_{ST} comparisons: Lānaʻi and Lisisanki Atoll (Φ_{ST}=0.043, P=0.035), Lānaʻi and Pearl and Hermes Atoll (Φ_{ST}=0.044, P=0.014), Kauaʻi and Lisianski Atoll (Φ_{ST}=0.040, P=0.023), and Kauaʻi and Pearl and Hermes Atoll (Φ_{ST}=0.036, P=0.012) (Table 4.5). None of these comparisons are significant after FDR correction. The exact tests of population differentiation are significant (P=0.021), and Maui versus Lisianski Atoll and Lānaʻi versus Pearl and Hermes Atoll are the only two significantly different site pair comparisons (Table S4.1).

As described above, one of each of these pairs is significant in the pairwise F_{ST} comparisons, and one is significant in the pairwise Φ_{ST} comparisons. Overall D_{est_Chao} was 0.278, which is more than an order of magnitude greater than global F_{ST} (0.008). Most of the pairwise D_{est_Chao} comparisons are also much greater in magnitude than pairwise F_{ST} or Φ_{ST} values, and range up to 0.339 (Table 4.3).

Effective migration rates

MIGRATE results indicate effective migration rates (N_em) greater than one from both the MHI to the NWHI and from the NWHI to the MHI, but *P. penicillatus* showed the opposite gene flow pattern relative to *P. marginatus*, with over a 500-fold higher effective migration rate from the MHI to the NWHI than in the other direction (Table 4.4). All posterior probability distributions are unimodal curves, although θ from the NWHI has a small peak, but a broad range of equally probable values. Both components of effective migration differ between regions: θ in the NWHI is approximately four times greater than θ in the MHI, and the migration rate from the MHI to the NWHI is almost 125x greater than from the MHI to the NWHI (Table 4.4).

Impacts of fishing on genetic diversity

Mean rarefied haplotype diversity in the NWHI (0.8931), where there has been little to no historical fishing pressure on *P. penicillatus*, is higher than in the MHI (0.8696), but not significant ($t = -2.073$, $df=6$, $P = 0.084$). Similarly, mean nucleotide diversity is slightly higher in the NWHI (0.0083) than in the MHI (0.0064), but not significant ($t = -1.002$, $df=6$, $P = 0.355$).

Discussion

Spiny lobsters constitute valuable fisheries wherever they are found, leaving them vulnerable to overharvesting, despite seemingly large population sizes. Additionally, spiny lobsters such as *P. marginatus* and *P. penicillatus* in Hawai‘i share life history characteristics that include a biphasic life cycle, a small adult home range, and an extremely long (> 6 months) pelagic larval duration that are typical of species that rely on their pelagic larval phase to maintain population connectivity across broadly dispersed habitats. Species with these characteristics are notoriously difficult to manage because the local larval supply is potentially decoupled from local larval production (e.g., Caley et al. 1996, Cowen and Sponaugle 2009). Here, we elucidate contrasting patterns of gene flow in these two species within the Hawaiian Archipelago using mtDNA data. This data, combined with information on historical and contemporary patterns of fishing activity in Hawai‘i, provides insight into whether the PMNM, which was not specifically created for fisheries management, may serve as a useful management tool to enhance depleted lobster stocks in the MHI (Morris 1968).

Spatial genetic structure and the PMNM as a larval source for the MHI

Panulirus marginatus

We see a surprising amount of genetic structure in *P. marginatus* for a species with an estimated 12-month pelagic larval duration (Polovina & Moffitt 1995). There is significant structure between the NWHI and the MHI for *P. marginatus* ($F_{ST} = 0.037$, $P = 0.007$), indicating limited exchange between these two regions. Additionally, mean D_{est_Chao} between regions (0.2013) is greater than the mean among sites in the NWHI (0.060) as well as the overall mean (0.1325), but is lower than the comparisons among MHI sites (0.254).

Most likely, these patterns are driven by the isolation of Kaua‘i, which had significant pairwise F_{ST} values for seven of 10 comparisons, and D_{est_Chao} ranging from 0.102 to 0.508 across all comparisons. Although no pairwise tests were significant after correcting for multiple comparisons, it is notable that seven of the 13 significant comparisons at $P = 0.05$ included Kaua‘i. Two other sites (Maui, Pearl and Hermes Atoll) also show significant differentiation from the rest of the chain, while the majority of sites show little to no genetic differentiation.

This finding resembles a pattern documented by Iacchei et al. (2013) in a congeneric species, *P. interruptus*. In Baja California, five sites were significantly differentiated from most other collection sites throughout the species' distribution, while locations at the opposite ends of the geographic range from one another showed no genetic differentiation from one another. Iacchei et al. (2013) showed that these specific sites had significantly higher proportions of kin, likely as a result of either localized recruitment or coordinated larval delivery, which may be due to persistent upwelling regimes at those specific sites.

These site-specific patterns are often only evident when multiple species are compared over the same geographic scale (e.g., Selkoe et al. 2010, Toonen et al. 2011). In the Hawaiian Archipelago, Kaua‘i has been genetically isolated from either the NWHI (14/20) or the rest of the MHI (12/21) in 26/41 comparisons that were possible across 27 species studied to date (Toonen et al. 2011). The genetic distinction of Kaua‘i holds across a variety of taxa that have substantially different life history strategies, ecologies and evolutionary histories. A dispersal barrier shared across species with such diverse biological characteristics suggests that the primary drivers of Kauai's isolation are shared physical drivers.

Geographic distance is can be ruled out as a possibility, because there are much greater distances between banks in the NWHI. However, not enough is known about the near shore current dynamics to determine whether realized oceanographic distance (White et al. 2010) is large between Kaua‘i and the rest of the archipelago. Channel depth (Schultz et al. 2008), current velocity and/or direction (Bird et al. 2007), or nearshore oceanographic gyres (Christie et al. 2010, Fox et al. 2012) may all serve as isolating or retentive factors in Hawai‘i, but do not obviously match genetic patterns reported to date (Toonen et al. 2011). Regardless, evidence is accumulating (Selkoe et al. 2010, Toonen et al. 2011, Iacchei et al. 2013) that a location-specific rather than species-specific focus may provide more fruitful insights into connectivity drivers, and these drivers should be investigated more closely in the Hawaiian Archipelago.

The modest barrier to gene flow between the NWHI and MHI indicates little exchange occurs between the regions, but occasional dispersal opens the possibility that populations in the NWHI may, over generations of time, aid in the rejuvenation of populations in the MHI and vice-versa. The median joining network shows that two of the three major haplotypes in the network are found at all sites except Lāna‘i (N=3), and the third major haplotype is found in both the MHI and the NWHI, but is missing from Maui, Laysan, and Lisianski Islands. Additionally, the results from the MIGRATE analysis show relatively high rates of effective migration (N_em) both from the MHI to the NWHI and vice-versa, though the N_em from the NWHI to the MHI is more than double (2.5x) the reverse. *Panulirus marginatus* is notable in being the first species surveyed to date across this region with greater effective migration rates from the NWHI to the MHI rather than the reverse (Toonen et al. 2011).

Although discrepancy in effective migration rate is mostly driven by *m,* which is nine times greater from the NWHI to the MHI, θ was also 3.5x greater in the NWHI than in the MHI (Table 4.4). The larger θ in the NWHI matches observations of *P. marginatus* abundances during collection surveys (Chapter 3), and commercial landings from the NWHI fishery (DiNiardo and Marshall 2001) were also consistently much higher than any historical landings in the MHI (Morris 1968, Skillman and Ito 1981). The highest CPUEs in the archipelago occur at Necker Island, Maro Reef, and Gardner Pinnacles in the NWHI (Uchida and Tagami 1984, DiNardo and Marshall 2001). These three banks contain extensive habitats of intermediate relief that sustain larger abundances of *P. marginatus* juveniles (Parrish and Polovina 1994). MacDonald (1986) also documented higher settlement rates of pueruli in the NWHI than in the MHI; therefore, elevated θ in the NWHI is supported by greater abundances of *P. marginatus* at three major life history stages (pueruli, juveniles, and adults).

The higher effective migration rates from the NWHI to the MHI were also driven by a difference in *m*, which was nine times greater from the NWHI to the MHI. This matches trajectories of *P. marginatus* phyllosoma predicted by a Lagrangian particle dispersal model designed for this species (Polovina et al. 1999). Polovina et al. (1999) concluded that the dominant directional transport of *P. marginatus* phyllosoma was from the northwest to the southeast to Necker Island, and from Necker Island to the southwest. Necker Island is uniquely positioned to receive larvae from both the northern and southern ends of the range, and also had a high self-recruitment rate (32-37% on average, Table 2 in Polovina et al. 1999). The combination of influx of recruits from the northern end of the NWHI plus high local retention was the likely reason for the higher lobster abundance at Necker Island, hence the consistently higher fishery production there.

However, Necker Island was also predicted to export a large proportion of phyllosoma to the south, with 31-41% of the phyllosoma within settlement range of Oahu after 12 months (Table 2 in Polovina et al. 1999). In general, all of the four locations studied (Midway Atoll, Maro Reef, Necker Island, O‘ahu Island) were predicted to receive a higher proportion of phyllosoma from banks to the north or through larval retention than they were from locations to the south (Polovina et al. 1999).

The spatial distribution of historical fishing pressure in the NWHI in combination with asymmetric migration rates between the NWHI and the MHI may also influence lobster abundances in the MHI. If CPUE is considered an accurate proxy for abundance, prior to intensive harvest in the NWHI lobster fishery, CPUE at Necker Island (4.72) and Maro Reef (4.04) were fairly similar, and 2x - 94x the CPUE at other reefs in the NWHI (Table 1 in Uchida et al. 1980). By the time the fishery was closed in 2000, the CPUE at all of the banks in the NHWHI declined, but at Necker Island and Maro Reef, CPUE declined to approximately 1 and 1.4 respectively (Fig. 2 in O’Malley 2009), which represents a five-fold decline at Necker Island in just 25 years. Compounding this decline in abundance, the fishery selectively removed the largest lobsters with the greatest reproductive output (DeMartini et al. 2003). The migration rates from our genetic data indicate that there are higher levels of effective migration from the NWHI to the MHI (19-23,00 effective migrants per generation, Table 4.4) than there are from the MHI to the NWHI (9-366 effective migrants per generation, Table 4.4). The number of effective migrants may be influenced by many factors, so values should not be taken literally, nor compared between species; however, relative magnitudes within a species are informative.

The coalescent migration results in this case are also corroborated by the modeled phyllosoma dispersal trajectories that predict 19-28% of phyllosoma released from Maro Reef and 31-41% of phyllosoma from Necker Island are transported to Oahu, whereas Oahu is only predicted to export 1-3% of its phyllosoma to Maro Reef, and 16-24% to Necker Island (Polovina et al. 1999). No larvae are predicted to make it to Oahu from Midway Atoll or vice versa (Polovina et al. 1999). Although this is a limited representation of the total number of banks in the NWHI, based on this data, the NWHI banks in the middle of the archipelago provide more recruits to the MHI than the banks at the far northwest, and the overall contribution to the MHI from the NWHI is greater than from the MHI to the NWHI. Both sets of data indicate that the banks in the NWHI likely influence stock dynamics in the MHI.

Despite the relatively high rate of gene flow we report from the NWHI to the MHI, and the potential for larval export from the NWHI to the MHI (Polovina et al. 1999), any anticipation that the now protected PMNM will rapidly replenish MHI stocks of *P. marginatus* is probably misguided. Eight years after the closure of the NWHI lobster fishery, fishery-independent monitoring data indicated that CPUE remained consistently low since the fishery closed, with 2008 CPUE equaling 0.80 at Necker Island, 0.29 at Gardner Pinnacles, and 0.75 at Maro Reef (O'Malley 2009, Schultz et al. 2011). Given that these were reliably the most productive banks in the Hawaiian Archipelago, if these locations do not recover their pre-exploitation densities, it is unlikely that there will be enough recruits from the NWHI to contribute to stock enhancement in the MHI, unless reproductive output increases solely based on increasing sizes of females (i.e., Birkeland and Dayton 2005). Our genetic data indicated a maximum of 2300 effective migrants per generation from the NWHI to the MHI over evolutionary time scales.

With an estimated three-year generation time (Uchida and Tagami 1984), this number of effective migrants translates into a best-case scenario average of approximately 750 individuals from the NWHI recruiting and surviving to reproduce in the MHI each year, with a worst-case scenario average of six individuals per year. Additionally, the estimates from the genetic data are taken from pre-exploitation population abundances in the NWHI, which were larger than present abundance, based on CPUE. While only six individuals per year is sufficient to maintain gene flow between these regions, even 750 individuals recruiting and surviving to reproduce in the MHI each year would not rapidly rebuild stocks in the MHI.

Panulirus penicillatus

While there is still some potential over a longer time period for *P. marginatus* stocks to rebound after the closure of the NWHI lobster fishery, *P. penicillatus* stocks in the MHI are unlikely to benefit from the establishment of the PMNM at all. Negligible numbers of *P. penicillatus* were removed during the NWHI fishery due to the incongruence between the shallow, rough water habitat preferences of *P. penicillatus* (George 2006) and the depths permitted for the NWHI lobster fishery (18m and deeper, Parrish and Polovina 1994). Hence, the protection of the PMNM would not have directly reduced harvested levels of *P. penicillatus*, so reproductive output will likely remain the same (based solely on the perceived impact fishing would have had on the population).

Although the two dominant haplotypes are shared across both regions (Fig. 4.3), there is significant structure between the NWHI and the MHI for *P. penicillatus* F_{CT} (0.0076, P=0.0083) and Φ_{CT} (0.011, P=0.023), indicating that there is limited exchange between these two regions.

Notably, all significant pairwise comparisons for both F_{ST} and Φ_{ST} are between sites located in different regions (MHI vs. NWHI). Means of both within-region D_{est_Chao} values are below zero, whereas the mean between MHI and NWHI sites (0.072), while low, is greater than the within region comparisons and the overall mean (0.0321).

In addition, our MIGRATE results indicate that effective migration rate is largely asymmetrical, with an over 500-fold higher effective migration rate from the MHI to the NWHI than in the opposite direction. The majority of this difference is due to mutation/migration, with a 125x greater estimated *m* from the MHI to the NWHI, although θ was also four times greater in the NWHI. The difference in θ did not match our observations of *P. penicillatus* abundances during collection surveys (Iacchei and Toonen in press), as there were much greater numbers of *P. penicillatus* in the MHI than the NHWI. This is also counter to distributions of *P. penicillatus* phyllosoma, which were numerically dominant (over *P. marginatus*) in larval tows near O‘ahu, but were not present in tows east of French Frigate Shoals or off of Midway Atoll (Johnson 1968). The higher rate of *m* from the MHI to the NWHI also does not match the Lagrangian dispersal model predictions of Polovina et al. (1999), which may be due to differences in the timing and or habitat where larvae are released for each species. Although the accuracy of values estimated in coalescent analyses of migration remain debated, hundred-fold differences in magnitude are likely informative (Waples and Gaggiotti 2006). The *P. penicillatus* results do match effective migration rates for other species in the Hawaiian Archipelago, where almost all species surveyed to date have shown equal or greater effective migration to the NWHI from the MHI (Bird et al. 2007, Timmers et al. 2011, Dibattista et al. 2011, Baums et al. 2013, Concepcion et al. in press, *Holothuria atra* in Skillings et al. in press).

Effects of fishing pressure on genetic diversity

Intensive fishing pressure on populations has the potential to reduce genetic variation, alter population subdivision, and induce selection on specific genes or traits (reviewed in Carvalho and Hauser 1994, Allendorf et al. 2008). The NWHI lobster fishery rapidly expanded from almost zero harvest to industrial harvest levels (16 vessels, 1000 traps hauled/vessel day, 1000 metric tons/year) in approximately six years (Clarke et al. 1992, Schultz et al. 2011). With increased fishing pressure, CPUE of both spiny and slipper lobsters decreased almost five-fold from their pre-fished levels (DeMartini et al 2003, Fig. 2 in O'Malley 2009, Schultz et al. 2011). Median body size at sexual maturity also declined (Polovina 1989); a response to exploitation documented in other spiny lobster fisheries (Pollock 1995a, b, Melville-Smith and de Lestang 2006). This is often thought to be a temporary phenotypically plastic response (e.g., Melville-Smith and de Lestang 2006), although it may also be an evolutionary response to the high level of exploitation (Allendorf et al. 2008). In addition, size-specific fecundity increased at Necker Island over two subsequent time periods, which provides additional evidence of a compensatory response to fishing (DeMartini et al. 2003). The most rigorous test of fishing effects on genetic diversity requires genetic data from pre-exploitation to current exploitation levels (Coltman et al. 2003, Kuparinen and Merilla 2007, Allendorf et al 2008, Hauser and Carvalho 2008). Because no such historical data was available, we used spatial disparity in fishing effort to investigate the effects on nucleotide and haplotype diversity, with the knowledge that many factors may vary spatially as well that could influence these values.

In *P. marginatus*, there was no significant difference in haplotype diversity; however, nucleotide diversity was significantly higher at the heavily fished banks in the NWHI than it was in the MHI. The less-targeted banks in the NWHI had intermediate haplotype and nucleotide diversity to the MHI and heavily fished banks in the NWHI, although these differences were not significant. There was no significant difference in either nucleotide or haplotype for *P. penicillatus* between the MHI and the NWHI, which is not surprising given the relative insensitivity of these metrics to all but the strongest bottlenecks (Allendorf 1986, Allendorf et al. 2008, Gaither et al. 2010).

The higher diversity levels of *P. marginatus* in the center of the chain most likely reflect the larger historical N_e of those banks, rather than a detectable effect of fishing. CPUE prior to exploitation in the NWHI (Uchida and Tagami 1984), extent of deep water habitat to support juveniles (Parrish and Polovina 1994), and pueruli recruitment abundances (MacDonald 1986) all peak at the banks in the center of the NWHI chain. Although recruitment supply is not necessarily a limiting factor for adult lobster abundance at banks in the center of the Hawaiian Archipelago (Parrish and Polovina 1994), only these banks are predicted to receive recruits from both the northern and southern ends of the chain (Polovina et al. 1999), which could increase diversity in the central region. In addition, the center of the archipelago has a much greater potential to receive recruits from Johnston Atoll than other regions in the chain (Kobayashi 2006), further increasing diversity there. These higher diversity levels may be driven by bank-specific dynamics that are affecting populations of multiple species, as similar patterns of elevated diversity have been observed at banks in the center of the chain for species such as the Hawaiian grouper, *Epinephelus quernus* (Rivera et al. 2004, Rivera et al. 2011), the Hawaiian limpets, *Cellana sp.* (Bird et al. 2007), and the lollyfish, *Holothuria atra* (Skillings et al. 2011).

Even if differences in nucleotide diversity are being driven by fishing, rather than *Ne*, it still may be too soon to detect these genetic effects (Palero et al. 2011), especially given the three-year generation time of *P. marginatus* (Uchida and Tagami 1984), and the rapid and recent removal of individuals through the fishery. All of our tissue samples are from six to eight years after the fishery closed, so we are sampling, at maximum, 30-35 years after the short, but intense fishery began in the NWHI. Based on typical spiny lobster life histories (Phillips and Melville-Smith 2006), our samples are likely either individuals that escaped the fishery through a size refuge or trap avoidance, or they are the newest recruits to the fishery, and the offspring of the lobsters that escaped the fishery.

If we are detecting the effects of fishing, the increase in both haplotype and nucleotide diversity at the fished sites is likely due to both the size-selective nature of the harvest, as well as the removal of large berried females in the initial years of harvest until the federal Fisheries Management Plan (FMP) for spiny lobsters in the Western Pacific Region was established in 1983 (Western Pacific Regional Fishery Management Council 1982). Although handling and release mortality was high (DiNardo et al. 2001), and the fishery eventually became a retain-all-catch fishery, the size selective nature of the fishery likely resulted in the removal of more large adults than juveniles. The effects of size-selective harvesting are numerous and varied (Fenberg and Roy 2007); however, one of the most profound influences on population dynamics is that the removal of the largest individuals exponentially decreases reproductive output by removing the most fit individuals (MacDiarmid and Butler 1999, reviewed in Birkeland and Dayton 2005, Law 2007). Theoretically, it is possible that removal of the largest individuals allows reproductive output to be more evenly distributed across a larger number of individuals, effectively increasing N_e while potentially decreasing overall fitness of the population (Ryman et al. 1981, Karl 2008).

This pattern would be exacerbated if those large individuals also had the majority of successfully recruiting offspring (Hedgecock 1986, 1994a, b, Beldade et al. 2012). Both Polovina (1989) and DeMartini et al. (2003) provide evidence for this compensatory reproductive output by smaller individuals at Necker Island, where densities decreased the most over the course of the fishery. *P. marginatus* at Necker Island had a 36% increase in size-specific fecundity over the course of the fishing period (Polovina 1989, DeMartini et al. 2003). As the fishery progressed, female *P. marginatus* produced both more eggs and larger eggs at smaller sizes, and by the fishery's closure, small females were dominating the reproductive output of Necker Island (DeMartini et al. 2003).

Alternatively, the reduction in local density may also simply allow for an increased proportion of migrants recruiting to Necker Island instead of local recruits, as hypothesized in exploited populations of Tasmanian black-lip abalone, *Haliotis rubra* (Miller et al. 2009). Necker is predicted to receive a high proportion of self-recruits, but also receives recruits from both northern and southern banks as well (Polovina et al. 1999), and reduction of local production may have increased the proportion of recruits from these other areas. This hypothesis is supported by genetic data showing a distinction between Maro Reef and Necker Island in one of seven allozyme loci just after peak exploitation (Seeb et al. 1990), and the subsequent lack of differentiation between Necker Island and most other banks in the archipelago observed in this study. However, it does not match the high gene flow across the archipelago observed in allozyme data prior to exploitation (Shaklee and Samollow 1980). It is more likely that Necker Island has maintained relatively high levels of gene flow with other banks in the archipelago throughout the exploitation period, although reproductive output from there likely declined.

The lack of genetic differentiation of Necker Island from all other banks with the exception of Maui and Kaua‘i provides evidence that this is not a genetically isolated population being impacted by harvest (Coltman et al. 2003, Allendorf et al. 2008). However, relatively short and intense harvest of individuals at Necker Island clearly impacted reproductive dynamics (Polovina 1989, DeMartini et al. 2003), and the potential negative effects of such intensive harvest on genetic diversity have been documented in a number of marine species (Hauser et al. 2002, Hutchinson et al. 2003, Perez-Rusafa et al. 2006, Schultz et al. 2009, but see Fenberg et al. 2010, Palero et al. 2011). Based on the cumulative evidence presented here, it is more likely that bank-specific characteristics, such as location within the archipelago and habitat, are driving the increased diversity at Necker Island rather than fishery-induced impacts. However, given the importance of maintaining genetic variation for adaptive potential (Fisher 1930, Frankham 1996, Reed and Frankham 2003, Bell and Okamura 2005), the patterns reported here warrant further investigation into the possible fishery effect on genetic diversity in *P. marginatus* using samples collected many generations after the fishery closure.

Conclusion

With the growing demand for ecosystem-based, as opposed to single species-based, management there has been a search for generalizations to set the spatial scale of management regimes for multiple species. However, mounting evidence has shown that characteristics such as pelagic larval duration (Shanks 2009, Weersing and Toonen 2009, Riginos et al. 2011, Selkoe and Toonen 2011), developmental mode (Mercier et al. 2013), and species distribution range (Lester et al. 2007) cannot reliably predict scales of genetic connectivity. Broad taxonomic category may provide some insight (Bradbury et al. 2008, Riginos et al. 2011); however, even within these taxonomic groups, species that are congeneric and ecologically similar have shown very different patterns of genetic differentiation across their co-distributed range (Rocha et al. 2002, Bird et al. 2007, Crandall et al. 2008, Gaither et al. 2010, Skillings et al. in press).

Here our mtDNA survey of two spiny lobster species *P. marginatus* and *P. penicillatus* throughout the Hawaiian Archipelago bolsters the idea that neither exemplar species nor specific suites of traits are reliable predictors of the spatial scales of management. Both spiny lobster species have extremely long (> 6 month) pelagic larval durations, but both show significant population structure across the Hawaiian Archipelago. As in the case of the endemic Hawaiian limpets (Bird et al. 2007) and two *Holothuria spp*. in Hawai‘i (Skillings et al. in press), we caution that the genetic data of one species of *Panulirus* in Hawai‘i provide little to no information on the best management strategy for the other. Instead, only by combining data on gene flow patterns in each species with information on historical and contemporary harvest regimes can we begin to derive the optimal management strategies for fisheries species.

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Table 4.1. Summary statistics for *Panulirus marginatus* cytochrome *c* oxidase subunit II gene (COII) and *Panulirus penicillatus* cytochrome *c* oxidase subunit I gene (COI) listed from southernmost collection site to the northernmost location in the Hawaiian Archipelago. Included are the total number of individuals sequenced (N), haplotype diversity (h), effective number of haplotypes (h_{eff}) and nucleotide diversity (π) for each species. Locations where h_{eff} = nc are sites where h_{eff} could not be calculated because h is 1. Locations where dashes are present represent sites where no lobsters of that particular species were found.

Collection Site (Abbreviation)	*Panulirus marginatus*				*Panulirus penicillatus*			
	N	h	h_{eff}	π	N	h	h_{eff}	π
Hawai‘i (Hawa)	-	-	-	-	47	0.89	8.79	0.008
Maui (Maui)	34	0.93	14.77	0.026	24	0.75	4.06	0.006
Lāna‘i (Lana)	2	1.00	nc	0.035	35	0.85	6.47	0.004
Moloka‘i (Molo)	-	-	-	-	21	0.81	5.25	0.007
O‘ahu (OAHU)	54	0.98	62.11	0.027	-	-	-	-
Kaua‘i (Kaua)	50	0.94	16.34	0.025	52	0.89	9.02	0.007
Necker Island (Neck)	58	0.99	75.19	0.030	5	0.90	10.00	0.005
French Frigate Shoals (Fren)	7	1.00	nc	0.028	44	0.90	10.17	0.006
Gardner Pinnacles (Gard)	58	0.99	75.19	0.032	-	-	-	-
Maro Reef (Maro)	56	0.99	90.91	0.029	5	1.00	nc	0.012
Laysan Island (Lays)	57	0.98	63.69	0.029	-	-	-	-
Lisianski Island (Lisi)	47	0.97	34.84	0.027	18	0.96	25.51	0.013
Pearl and Hermes Atoll (Pear)	49	0.99	147.06	0.028	30	0.91	11.15	0.006
Midway Atoll (Midw)	41	0.96	28.25	0.031	-	-	-	-
Kure Atoll (Kure)	51	0.97	35.46	0.029	-	-	-	-

Table 4.2. *Panulirus marginatus* pairwise population structure results for a 662 base pair fragment of the mitochondrial DNA cytochrome *c* oxidase subunit II gene. F_{ST} is below the diagonal and Φ_{ST} is above the diagonal. Shaded comparisons are significant at $P < 0.05$. No comparisons were significant after correcting for false discovery rate. Site abbreviations are defined in Table 4.1. Only sites with N > 10 are included.

Region		**Main Hawaiian Islands**			**Northwestern Hawaiian Islands**							
	Site	Maui	Oahu	Kaua	Neck	Gard	Maro	Lays	Lisi	Pear	Midw	Kure
Main Hawaiian Islands	Maui	-	-0.008	0.002	-0.006	-0.007	-0.012	-0.007	-0.007	-0.001	-0.007	-0.006
	Oahu	0.013	-	0.009	0.007	-0.006	-0.006	0.003	0.003	0.018	0.008	-0.007
	Kaua	0.019	0.007	-	0.014	0.009	0.004	0.007	0.009	0.023	-0.001	0.000
Northwestern Hawaiian Islands	Neck	0.015	0.000	0.009	-	-0.004	-0.003	-0.012	-0.005	-0.002	0.001	0.002
	Gard	0.008	0.000	0.014	-0.002	-	-0.007	-0.005	-0.003	0.011	0.001	-0.008
	Maro	0.007	-0.002	0.016	-0.001	-0.004	-	-0.007	-0.004	-0.002	-0.007	-0.009
	Lays	0.010	0.000	0.010	-0.004	-0.003	-0.003	-	-0.007	-0.002	0.000	-0.001
	Lisi	0.008	0.004	0.017	0.004	0.000	-0.001	0.001	-	-0.002	0.001	-0.001
	Pear	0.021	0.000	0.018	0.001	0.001	0.000	0.002	0.007	-	-0.002	0.009
	Midw	0.015	0.002	0.005	0.002	0.004	0.003	0.000	0.005	0.008	-	-0.004
	Kure	0.012	0.001	0.006	0.002	0.002	0.003	0.002	0.006	0.006	0.004	-

Table 4.3. Pairwise D_{est_Chao} for Panulirus marginatus COII (below the diagonal) and Panulirus penicillatus COI (above the diagonal) for site pairs where N >10 for both locations. D_{est_Chao} ranges between zero and one, and represents an absolute percent difference between two sites in terms of haplotype composition. Comparisons where absolute differentiation between sites > 10% are shaded grey with values in bold. Site abbreviations are detailed in Table 4.1.

Region		Main Hawaiian Islands						Northwestern Hawaiian Islands								
	Site	Hawa	Maui	Lana	Molo	Oahu	Kaua	Neck	Fren	Gard	Maro	Lays	Lisi	Pear	Midw	Kure
Main Hawaiian Islands	Hawa		0.046	-0.038	-0.043	-	-0.013	-	-0.012	-	-	-	0.096	0.032	-	-
	Maui	-		-0.005	-0.053	-	0.022	-	0.073	-	-	-	**0.339**	**0.155**	-	-
	Lana	-	-		-0.026	-	-0.036	-	-0.058	-	-	-	0.074	0.049	-	-
	Molo	-	-	-		-	-0.001	-	0.037	-	-	-	**0.219**	0.091	-	-
	Oahu	-	**0.309**	-	-		-	-	-	-	-	-	-	-	-	-
	Kaua	-	**0.279**	-	-	**0.175**		-	-0.082	-	-	-	0.069	-0.009	-	-
Northwestern Hawaiian Islands	Neck	-	**0.359**	-	-	0	**0.231**		-	-	-	-	-	-	-	-
	Fren	-	-	-	-	-	-	-		-	-	-	-0.039	-0.015	-	-
	Gard	-	**0.196**	-	-	0.022	**0.361**	-0.161	-		-	-	-	-	-	-
	Maro	-	**0.17**	-	-	-0.122	**0.436**	-0.062	-	-0.29		-	-	-	-	-
	Lays	-	**0.242**	-	-	-0.003	**0.26**	-0.253	-	-0.232	-0.244		-	-	-	-
	Lisi	-	**0.155**	-	-	**0.19**	**0.356**	**0.196**	-	-0.014	-0.034	0.057		0.027	-	-
	Pear	-	**0.564**	-	-	0.009	**0.508**	0.091	-	0.091	0.02	**0.172**	**0.388**		-	-
	Midw	-	**0.276**	-	-	0.086	**0.102**	0.065	-	**0.152**	**0.135**	0.009	**0.141**	**0.362**		-
	Kure	-	**0.253**	-	-	0.049	**0.132**	0.088	-	**0.104**	**0.162**	0.075	**0.223**	**0.338**	**0.112**	

Table 4.4. Effective migration rate estimates (N_em) for *Panulirus marginatus* and *P. penicillatus* from the Papahānaumokuākea Marine National Monument (PMNM; encompassing the Northwestern Hawaiian Islands) to the Main Hawaiian Islands (MHI), and comparative rates from the MHI to the PMNM. Reported for each species and direction are the mode, plus the 95% highest posterior density (HPD) of (N_em) based on a Bayesian MCMC simulation in MIGRATE-N 3.3.2. To estimate N_em, the value of m calculated by MIGRATE was multiplied by θ of the destination location. Also reported are the mode and 95% HPD of individual m and θ values used to calculate N_em.

	Effective Migration (N_em)		**Migration & Mutation (m)**		**N_e (θ)**	
Species	**From PMNM**	**To PMNM**	**From PMNM**	**To PMNM**	**PMNM**	**MHI**
Panulirus marginatus	487.19 (18.70 - 2,298.67)	142.17 (9.02 - 366.08)	15,032 (6,680 - 29,880)	1,707 (240 - 3,200)	0.083 (0.038 - 0.114)	0.032 (0.003 - 0.077)
Panulirus penicillatus	1.03 (0.00 - 320.39)	539.41 (28.96 - 16,986.34)	67 (0 - 11,333)	8,333 (1,200 - 85,733)	0.065 (0.024 - 0.198)	0.015 (0.004 - 0.028)

Table 4.5. *Panulirus penicillatus* pairwise population structure results for a 460 base pair fragment of the mitochondrial DNA cytochrome *c* oxidase subunit I gene. F_{ST} is below the diagonal and Φ_{ST} is above the diagonal. Shaded comparisons are significant at $P < 0.05$. No comparisons were significant after correcting for a false discovery rate. Site abbreviations are defined in Table 4.1. Only sites with N > 10 are included here.

Region		**Main Hawaiian Islands**					**Northwestern Hawaiian Islands**		
	Site	Hawa	Maui	Lana	Molo	Kaua	Fren	Lisi	Pear
Main Hawaiian Islands	Hawa	-	-0.006	-0.001	-0.014	0.003	0.001	0.024	0.014
	Maui	0.009	-	-0.020	-0.012	-0.008	-0.008	0.039	0.026
	Lana	-0.006	-0.002	-	0.003	-0.008	-0.007	0.043	0.044
	Molo	-0.008	-0.015	-0.006	-	0.000	-0.005	0.024	0.008
	Kaua	-0.002	0.004	-0.006	-0.001	-	-0.001	0.040	0.036
Northwestern Hawaiian Islands	Fren	-0.001	0.014	-0.009	0.005	-0.010	-	0.006	0.015
	Lisi	0.009	0.055	0.009	0.028	0.006	-0.002	-	0.018
	Pear	0.004	0.030	0.007	0.014	-0.001	-0.002	0.002	-

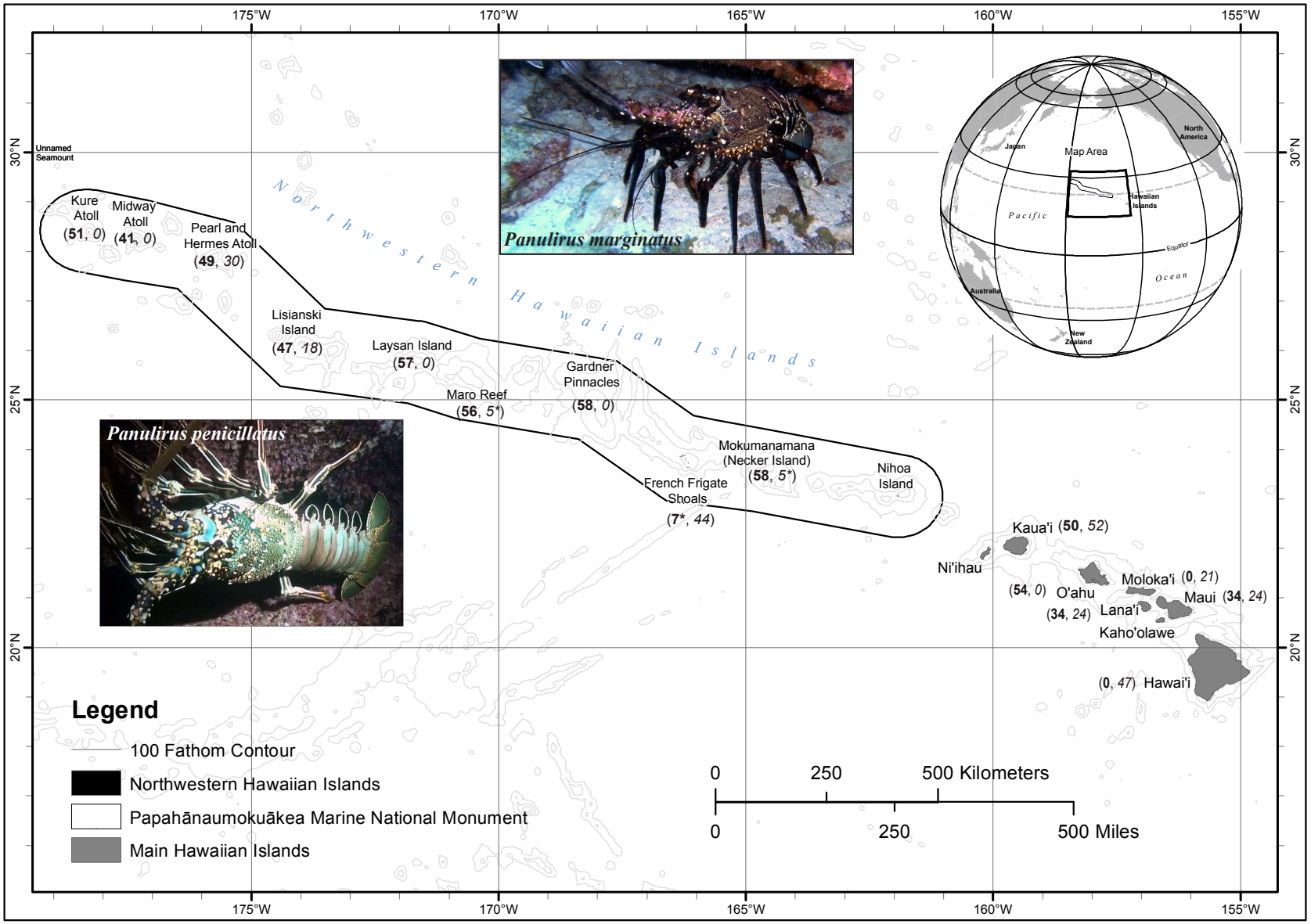
175°W
170°W
165°W
160°W
155°W
30°N
25°N
20°N
Unnamed Seamount
Kure Atoll (51, 0)
Midway Atoll (41, 0)
Pearl and Hermes Atoll (49, 30)
Lisianski Island (47, 18)
Laysan Island (57, 0)
Maro Reef (56, 5*)
Gardner Pinnacles (58, 0)
Mokumanamana (Necker Island) (58, 5*)
French Frigate Shoals (7*, 44)
Nihoa Island
Ni'ihau (54, 0)
Kaua'i (50, 52)
O'ahu (34, 24)
Moloka'i (0, 21)
Maui (34, 24)
Lana'i
Kaho'olawe
(0, 47) Hawai'i
Northwestern Hawaiian Islands
Panulirus marginatus
Panulirus penicillatus
Map Area
Japan
North America
Pacific
Hawaiian Islands
Equator
Ocean
Australia
New Zealand
Legend
100 Fathom Contour
Northwestern Hawaiian Islands
Papahānaumokuākea Marine National Monument
Main Hawaiian Islands
0 250 500 Kilometers
0 250 500 Miles

Figure 4.1. Map of lobster specimen collection locations and number of individuals of *Panulirus marginatus* (bold font) and *Panulirus penicillatus* (italicized font) sampled at each location. Due to low sample size, site/species combinations identified with an asterisk were not included in the pairwise population analysis, but were included in the overall analysis of genetic diversity, as well as in the median joining networks. The distinction between the Main Hawaiian Islands (MHI) and the Northwest Hawaiian Islands (NWHI) is also identified in the figure: note that the Papahānaumokuākea Marine National Monument encompasses all of the NWHI locations, but not the MHI sites. Photo credit: *Panulirus marginatus*, in the upper right hand corner – Nyssa Silbiger; *Panulirus penicillatus*, in the lower left hand corner – Terry Lilley.

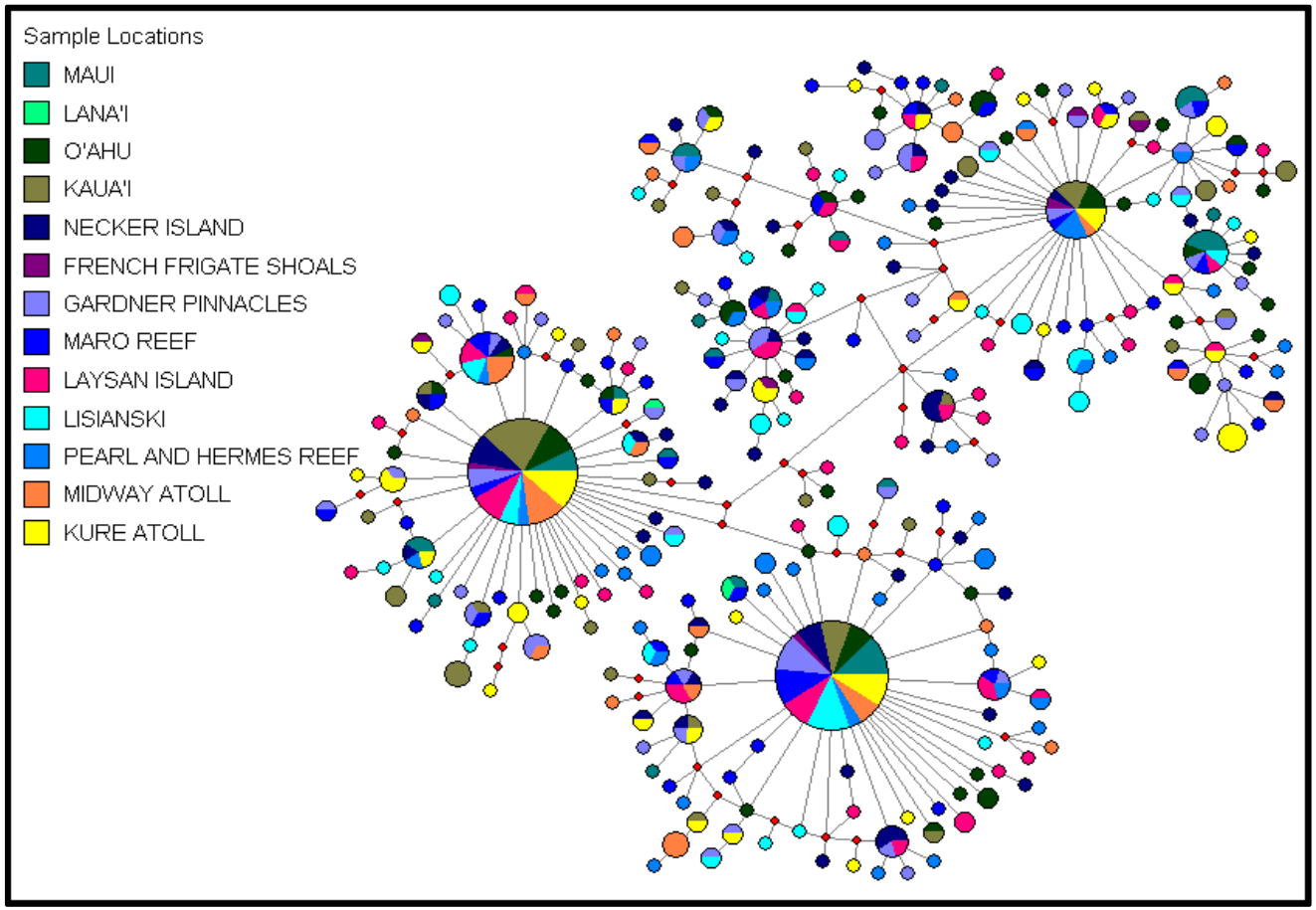

Figure 4.2. Median-joining network for *Panulirus marginatus* mtDNA, constructed using 662 base pairs of cytochrome *c* oxidase subunit II (COII) from each of 564 individuals in the program NETWORK. Each circle represents a unique haplotype proportional in size to the number of individuals with that haplotype. The smallest circle represents one individual, and the two largest circles represent 56 and 53 individuals. Lines represent a single base pair difference between haplotypes, with crossing lines each representing one additional difference. Colors correspond to one of 13 locations where the individual haplotypes were found (see key, Fig. 4.1, Table 4.1).

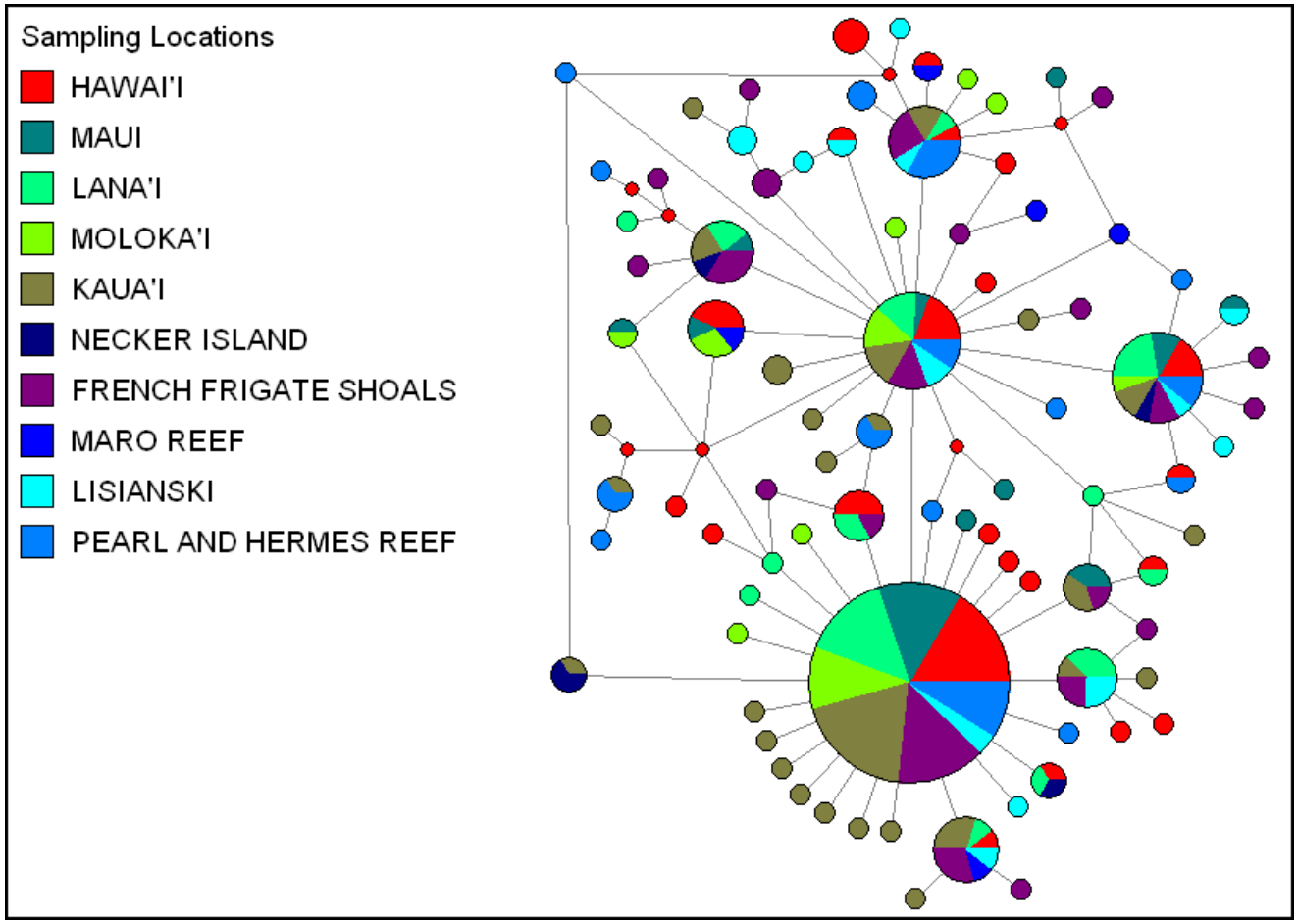

Figure 4.3. Median-joining network for *Panulirus penicillatus* mtDNA, constructed using 460 base pairs of cytochrome *c* oxidase subunit I (COI) from 281 individuals in the program NETWORK. Each circle represents a unique haplotype proportional in size to the number of individuals with that haplotype. The smallest circle represents one individual, and the largest represents 90 individuals. Lines represent a single base pair difference between haplotypes, with crossing lines each representing one additional difference. Colors correspond to one of 10 locations where the individual haplotypes were found (see key, Fig. 4.1, Table 4.1).

Supplemental Information

Table S4.1. *P*-values from pairwise exact tests of population differentiation for *Panulirus marginatus* cytochrome *c* oxidase subunit II mitochondrial DNA gene (below the diagonal) and for *Panulirus penicillatus* cytochrome *c* oxidase subunit I mitochondrial DNA gene (above the diagonal). *P*-values <0.05 are shaded grey with values in bold. Only sites with N > 10 are included. Site abbreviations are defined in Table 4.1.

Region		**Main Hawaiian Islands**						**Northwest Hawaiian Islands**								
	Site	Hawa	Maui	Lana	Molo	Oahu	Kaua	Neck	Fren	Gard	Maro	Lays	Lisi	Pear	Midw	Kure
Main Hawaiian Islands	Hawa		0.49	0.55	0.78	-	0.10	-	0.11	-	-	-	0.10	0.07	-	-
	Maui	-		0.35	0.69	-	0.93	-	0.55	-	-	-	**0.04**	0.06	-	-
	Lana	-	-		0.22	-	0.74	-	0.88	-	-	-	0.19	**0.05**	-	-
	Molo	-	-	-		-	0.59	-	0.30	-	-	-	0.09	0.12	-	-
	Oahu	-	0.07	-	-		-	-	-	-	-	-	-	-	-	-
	Kaua	-	**0.00**	-	-	**0.05**		-	0.93	-	-	-	0.27	0.35	-	-
Northwest Hawaiian Islands	Neck	-	0.11	-	-	0.13	0.06		-	-	-	-	-	-	-	-
	Fren	-	-	-	-	-	-	-		-	-	-	0.35	0.13	-	-
	Gard	-	0.57	-	-	0.17	**0.01**	0.85	-		-	-	-	-	-	-
	Maro	-	0.86	-	-	0.80	**0.02**	0.70	-	0.95		-	-	-	-	-
	Lays	-	0.18	-	-	0.14	**0.01**	0.91	-	0.88	0.94		-	-	-	-
	Lisi	-	**0.04**	-	-	**0.03**	**0.00**	**0.05**	-	0.36	0.46	0.16		0.15	-	-
	Pear	-	**0.02**	-	-	0.22	**0.00**	0.29	-	0.34	0.54	0.11	**0.02**		-	-
	Midw	-	**0.00**	-	-	0.12	**0.01**	0.43	-	0.26	0.52	0.36	0.06	0.06		-
	Kure	-	**0.02**	-	-	0.08	**0.02**	0.10	-	0.16	0.14	0.06	**0.00**	**0.02**	**0.02**	

CHAPTER V

CHARACTERIZATION OF EIGHT POLYMORPHIC MICROSATELLITE LOCI FOR THE CALIFORNIA SPINY LOBSTER, *PANULIRUS INTERRUPTUS* AND CROSS-AMPLIFICATION IN OTHER ACHELATE LOBSTERS

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Contribution

Although Tal Ben-Horin is listed as the first author on this paper, we split the work evenly, with each of us contributing four microsatellite primers. Specifically, my portion of the work was to screen the microsatellite library developed by Robert Toonen and Thien Mai, and successfully implement the first four primers listed: Pin10, Pin29L, Pin189, Pin244 (lines 84-87). I also collected the lobsters and egg masses used to test for Mendelian inheritance (lines 116-118), and conducted all of the cross-species testing for all eight markers (lines 136-142, Table 5.2). Each author wrote and edited their respective sections of the paper. I have decided to include this short chapter because the development of these markers (in addition to the *Panulirus interruptus*-specific cytochrome *c* oxidase subunit I (COI) marker) directly facilitates the work in chapter six.

Abstract

Microsatellite sequences were isolated from both non-enriched and enriched genomic libraries of California spiny lobster, *Panulirus interruptus*. Eight consistently amplifying, scorable and polymorphic loci were characterized for 79 individuals collected at Santa Cruz and San Clemente Islands, California, and tested for cross-species amplification in four closely related *Panulirus spp.*, as well as four other species of the order Achelata. The number of alleles observed per locus ranged from three to 54 and observed heterozygosities ranged from 0.57 to 0.98. Quality control testing shows that all loci were reliably scorable, independently segregating, inherited in Mendelian ratios, and had low to moderate (≤ 14.4%) frequencies of null alleles and high statistical power for detecting fine scale genetic structure.

Introduction

The California or red spiny lobster, *Panulirus interruptus*, occurs in shallow, rocky coastal areas from Monterey Bay, California to Manzanillo, Mexico, with a small, isolated population in the northern Sea of Cortez (Barsky 2001); however, the majority of the population occurs between Point Conception, California and Bahia Magdalena, Mexico (Duffy 1973; Pringle 1986). There is significant potential for individuals to disperse across much of this range. After hatching in nearshore waters phyllosome larvae spend seven to nine months drifting in prevailing currents before metamorphosis to the puerulus stage (Serfling and Ford 1974; Engle 1979; Booth and Phillips 1994). Following metamorphosis, pelagic puerulus larvae remain in surface waters for an additional two and a half months before settling to inshore benthic habitats (Shaw 1986). Due to lucrative commercial and recreational fisheries for this species in the United States and Mexico, and the potential impact of these fisheries on the sustainability of lobster populations outside regional management jurisdictions, there is considerable interest in understanding patterns of dispersal and connectivity (Perez-Enriquez *et al.* 2001; Iacchei *et al.* 2005). Here we report development of polymorphic microsatellite markers to assess gene flow and infer patterns of connectivity in this species.

Methods, Results, and Discussion

Microsatellites were drawn from two different sources. First, a series of 15 potential loci were developed following the Microsatellites for Ecologists protocol (Toonen 1997) as described in Toonen *et al.* (2004). From roughly 2500 colonies in the initial library, 56 colonies positive for microsatellite-containing inserts were isolated and sequenced, yielding 15 putative microsatellite loci. Of these, 11 contained perfect dinucleotide repeats and four contained compound dinucleotide and/or trinucleotide repeats. The remaining sequences lacked sufficient flanking sequence around the repeat motifs to design primers. PRIMER 3 (Rozen and Skaletsky 1996) was used to develop primers, and unlabelled primers were ordered from Operon Inc., Alameda, CA, USA. These 15 primer sets were tested on 5 individuals from each of 5 sites (Anacapa Island, CA, Santa Catalina Island, CA San Clemente Island, CA San Diego, CA, and Bahia Tortugas, Mexico), and four of the loci were found to be polymorphic, amplify consistently, and be reliably scorable (Pin10, Pin29L, Pin189, Pin244).

The other polymorphic loci were developed from four microsatellite-enriched libraries constructed by Genetic Identification Services (GIS: Chatsworth, CA, USA) using pooled genomic DNA extracted from three adult *P. interruptus* collected off Isla Vista Reef, CA. DNA was isolated from leg tissue stored in 70% ethanol using a phenol-chloroform-isoamyl alcohol protocol followed by isopropanol precipitation (after Toonen 1997). Isolated DNA pellets were washed with 70% ethanol and re-suspended with 50µl of sterile 1X TE buffer, pH 7.2. Methods for DNA library construction, enrichment and screening followed Jones *et al.* (2002). A total of 34, 16, 16, and 34 colonies positive for microsatellite inserts were isolated and sequenced from $(GA)_{15}$, $(CATC)_8$, $(TACA)_8$,and $(TAGA)_8$ enriched libraries, respectively yielding 19, 6, 5, and 14 putative microsatellite loci.

PCR primer pairs were designed for these 44 loci using DESIGNERPCR version 1.03 (Research Genetics, Inc.) and unlabeled primer pairs were ordered from Sigma Proligo, St. Louis, MO, USA. Polymerase chain reactions were performed in 10µl volumes containing 20ng template DNA, 1x Colorless GoTaq Reaction Buffer (containing 1.5mM $MgCl_2$ pH 8.5; Promega Corp, Madison, WI, USA), 2µM dNTPs, 6µM forward primer, 6µM reverse primer, and 0.01U/µl GoTaq polymerase (Promega Corp.) using a Bio-Rad DNA Engine Dyad Peltier Thermal Cycler (Bio-Rad Laboratories, Hercules, CA, USA). PCR conditions were as follows: 3min at 94°C, followed by 35 cycles of 40 seconds at 94°C, 40 seconds at annealing temperature (see table 5.1 for each locus), 40 seconds at 72°C, and a final extension step of 30 minutes at 72°C. Fourteen primer pairs amplified products of appropriate size and were selected for further study.

For genotyping, PCR amplification was carried out using the conditions described above with forward primers fluorescently labeled with WellRed D2, D3, or D4 dye (Beckman-Coulter Inc., Fullerton, CA, USA; see table 5.1 for each primer label). PCR products were sized on a Beckman-Coulter CEQ 8000 capillary sequencer (Beckman-Coulter Inc.). Size markers (Size Standard 400; Beckman-Coulter Inc.) were included in the lane with PCR products and alleles were scored using a CEQ 8000 genetic analysis system (Beckman-Coulter Inc.). Four loci were found to be consistently amplifying, scorable and polymorphic (PinA5, PinA102r, PinA110, PinD110).

These eight loci were tested for conformation to Mendelian expectations using four *P. interruptus* mothers collected at Santa Catalina Island, California and ≥ 8 attached offspring per mother. A composite chi-squared analysis was used to test for deviations from expected equal inheritance of maternal, heterozygote alleles by offspring. When maternal genotypes were found to be homozygous, we tested for obligate inheritance of the maternal allele in all genotyped offspring. All eight loci passed the composite chi-squared test ($\alpha = 0.05$) and, when applicable, displayed obligate inheritance of maternal homozygote alleles.

Here we report the combined results (Table 5.1) from two population samples consisting of 79 adult *P. interruptus* collected from Santa Cruz Island and San Clemente Island, California in 2006 and 2007. MICRO-CHECKER (van Oosterhout *et al.* 2004) found no evidence of scoring errors due to stuttering or large-allele dropout. FREENA (Chapuis and Estoup 2007) estimated low (<10%) to moderate (10-15%) frequencies of null alleles in all loci except PinA5 and Pin10. The presence of low to moderate frequencies of null alleles in these loci is not surprising as this is commonly observed in a variety of abundant marine invertebrate species (Kaukinen 2004). Five loci had significant heterozygote deficiencies (tested in ARLEQUIN 3.11; Excoffier *et al.* 1995). The severity of the deficits (F_{IS}; FSTAT 1.2; Goudet 1995) at all loci showed a very strong positive correlation with their average frequency of null alleles estimated in FREENA ($\rho = 0.98$, $P < 0.001$). This suggests that observed heterozygote deficits result from low to moderate frequencies of null alleles. There was no significant evidence for linkage disequilibrium among pairs of loci following corrections for multiple comparisons (FSTAT 1.2; Goudet 1995).

Cross-amplification was tested in four congeneric species in the *Panulirus* genus, as well as four phylogenetically distant achelate lobsters (Table 5.2). Of the eight loci tested, five cross-amplified in other lobster species and were found to be polymorphic. All scored alleles in these samples were within the size range of scored alleles observed in *P. interruptus*. One locus, Pin189, was found to be monomorphic in the phylogenetically distant *Parribacus antarcticus*, and the scored allele in these samples was outside the size range of alleles found in *P. interruptus*. Cross-amplification was otherwise only observed in *Panulirus spp*.

The capacity for these loci to detect fine-scale patterns of population structure was evaluated using the simulation-based power analysis POWSIM (Ryman & Palm 2006). Simulation results estimate 94% statistical power in detecting fine-scale population structure (F_{ST} = 0.01) with 30 individuals sampled from each subpopulation. Statistical power is increased to 99% when sampling is increased to 50 individuals from each subpopulation. Measures of population genetic structure, such as F_{ST}, may be overestimated in the presence of null alleles when populations are substantially differentiated. This bias diminishes as gene flow between populations increases (Chapuis and Estoup 2007). Larval exchange among populations of *Panulirus interruptus* is potentially extensive, and consequently, patterns of genetic differentiation are likely to be subtle. These eight loci therefore provide sufficient variability and statistical power to address this fine-scale and complex population structure, providing a useful set of molecular markers to address questions of connectivity in this highly valuable fishery species.

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Table 5.1. Locus name, repeat motif, dye label, primer sequence, annealing temperature of the PCR reaction (T_a), number of individuals successfully genotyped out of an initial sample size of 79 (N), observed number (N_a) and size range of alleles, observed (H_O) and expected (H_E) heterozygosity, Hardy-Weinberg Equilibrium P-value (†locus is out of Hardy Weinberg Equilibrium), null allele frequency, F_{IS}, and GenBank accession # for each locus.

Locus name	Repeat motif	Well Red dye	Primer Sequence (5′-3′)	T_a (°C)	N	N_a	Allele size range	H_O	H_E	P_{HWE}	Null freq.	F_{is}	GenBank accession#
PinA5	(CT)29	D4	GC A A ATT CT CT C A A GGA A GGTT GGC A C GAT AT AC A CTT G GTTT G	56	79	25	116–178	0.97	0.93	0.315	0.000	−0.048	EU735032
PinA102r	(GA)34	D4	T GC A AC AC A GT GGT AT CT CT A A AC GTT C CT AT C AC ATT TT CTT G	48	79	22	271–323	0.86	0.93	0.001†	0.028	0.072	EU735033
PinA110	(CT)26	D3	C C AC GA G A CCA A GGA A ACT A C GA AT GC TT GT GTC GC T A ACT G	54	79	3	170–174	0.57	0.58	0.918	0.002	0.010	EU735034
PinD110	(GATA)13(GA)7	D4	GG G A ATCT CT A A GGA AT GT TC G AGC C A A A GC C A C GA C A A G	60	63	49	243–409	0.68	0.97	0.000†	0.144	0.302	EU735035
Pin29L	(GT)22	D3	TTT ATC C GC AT G GAT G A C GC AGAT GG G GT C CCA A GGT GT G	60	79	44	189–363	0.85	0.97	0.000†	0.057	0.129	EU735036
Pin189	(CA)18	D4	AACACCCCTCCTACCCCC C CC CAT C A A A ACCT CT G GA C	60	79	27	261–321	0.87	0.94	0.036†	0.028	0.073	EU735037
Pin10	CTA)16	D3	C GA AT CAT GGC T GTT C GA GG GGA CT C A GT G GT CT GT A ATT GT	62	79	19	126–182	0.90	0.92	0.601	0.000	0.021	EU735038
Pin244	(GT)36	D2	TC A GT G GA T GA A GGTT A C G AC A GCC TTT CT GT GGA ATT A	56	79	54	93–327	0.90	0.97	0.006†	0.036	0.076	EU735039

Table 5.2. Cross-amplification of the eight microsatellite loci in eight additional lobster species. The number of individuals tested for each species is indicated by the number in (). Numbers in columns indicate the number of samples amplifying polymorphic products of comparable size to that amplified from *P. interruptus*. ○ indicates no amplification of any products. * indicates amplification of monomorphic products outside the size range of products amplified from *P. interruptus*.

	PinA5	**PinA102r**	**PinA110**	**PinD110**	**Pin29L**	**Pin189**	**Pin10**	**Pin244**
Panulirus marginatus (4)	○	3	○	2	4	4	○	○
Arctides regalis (4)	○	○	○	○	○	○	○	○
Scyllarides haanii (4)	○	○	○	○	○	○	○	○
Panulirus penicillatus (4)	○	○	○	○	3	4	4	○
Panulirus inflatus (1)	○	○	○	○	○	○	○	○
Parribacus antarcticus (4)	○	○	○	○	○	2*	○	○
Scyllarides squammosus (4)	○	○	○	○	○	○	○	○
Panulirus versicolor (2)	○	○	○	○	2	○	○	○

CHAPTER VI

COMBINED ANALYSES OF KINSHIP AND F_{ST} SUGGEST POTENTIAL DRIVERS OF CHAOTIC GENETIC PATCHINESS IN HIGH GENE-FLOW POPULATIONS

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Abstract

We combine kinship estimates with traditional *F*-statistics to explain contemporary drivers of population genetic differentiation despite high gene flow. We investigate range-wide population genetic structure of the California spiny (or red rock) lobster (*Panulirus interruptus*), and find slight, but significant global population differentiation in mtDNA (Φ_{ST} = 0.006, P = 0.001; D_{est_Chao} = 0.025) and seven nuclear microsatellites (F_{ST} = 0.004, P < 0.001; D_{est_Chao} = 0.03), despite the species' 240 to 330 day pelagic larval duration. Significant population structure does not correlate with distance between sampling locations, and pairwise F_{ST} between adjacent sites often exceeds that among geographically distant locations. This result would typically be interpreted as unexplainable, chaotic genetic patchiness. However, kinship levels differ significantly among sites (Pseudo-$F_{(16, 988)}$ = 1.39, P = 0.001), and ten of 17 sample sites have significantly greater numbers of kin than expected by chance (P < 0.05). Moreover, a higher proportion of kin within sites strongly correlates with greater genetic differentiation among sites (D_{est_Chao}, R^2 = 0.66, P < 0.005). Sites with elevated mean kinship were geographically proximate to regions of high upwelling intensity (R^2 = 0.41, P = 0.0009). These results indicate *Panulirus interruptus* does not maintain a single homogenous population, despite extreme dispersal potential. Instead, these lobsters appear to either have substantial localized recruitment or maintain planktonic larval cohesiveness whereby siblings more likely settle together than disperse across sites. More broadly, our results contribute to a growing number of studies showing that low F_{ST} and high family structure across populations can coexist, illuminating the foundations of cryptic genetic patterns and the nature of marine dispersal.

Introduction

Determining the temporal and spatial scales of dispersal and gene flow across species' ranges is essential for effective conservation and management. *F*-statistics (Wright 1943), and their analogues (e.g. Nei 1973; Weir & Cockerham 1984; Excoffier *et al.* 1992; Hedrick 1999) have been the workhorses in this regard for over 65 years. However, as both the number and diversity of genetic markers have increased, so has the demand for analyses that can complement fixation indices and extend our understanding of genetic data beyond the single marker, two allele system pioneered by Wright (1943). Coalescent simulations (Kingman 1982) have emerged as the most informative techniques for distinguishing between historical and contemporary drivers of population differentiation (Tavare *et al.* 1997; Hey & Wakeley 1997; Rosenberg & Nordborg 2002; Rozas *et al.* 2003; Drummond *et al.* 2005; Hickerson *et al.* 2006; Hey & Nielsen 2007, Eldon & Wakeley 2009). By incorporating data from multiple nucleotide sequence-based markers, these equilibrium-independent analyses can isolate the effects of effective population size, demographic history, migration, mutation and drift summarized by F_{ST} (Hart & Marko 2010; Marko & Hart 2011, 2012). However, for fragment length data such as that generated by microsatellite markers, a number of alternative approaches have been advanced that can add insight into what is driving F_{ST} patterns (reviewed in Hedgecock *et al.* 2007; Lowe & Allendorf 2010).

One underutilized approach is the coupling of indirect metrics of gene flow (e.g. *F*-statistics, D_{est_Chao}) with more direct measures such as kinship or parentage analyses (e.g. Loiselle *et al.* 1995; Selkoe *et al.* 2006; Buston *et al.* 2009; Christie *et al.* 2010; Palsbøll *et al.* 2010).

Broadly speaking, kinship analyses provide an index of the relative relatedness of all genotyped individuals in a dataset, and parentage is a distinct case of kinship whereby the most likely parents of individual juveniles are identified (Vekemans & Hardy 2004; Jones & Arden 2003; reviewed in Blouin 2003; Jones *et al.* 2010). Kinship coefficients (also known as coefficients of coancestry) are widely interpreted as the probability of identity by descent of the genes, but they are more properly defined as "ratios of differences of probabilities of identity in state" (Hardy & Vekemans 2002, p. 23) from homologous genes sampled randomly from each pair of individuals (Rousset 2002; Hardy & Vekemans 2002; Blouin 2003; Vekemans & Hardy 2004).

By comparison, *F*-statistics and D_{est_Chao} are often blind to the relatedness of individuals; different population samples with the same kinship structure can have very different levels of genetic differentiation among them and vice versa. By assessing how alleles are shared among individuals, kinship analyses can elucidate which locations have comparatively little ongoing genetic exchange in situations where low F_{ST} values suggest high contemporary population connectivity. This clarification is important because such inferences can in fact be due to historically high migration rates, effective population sizes, or measurement error (Hart & Marko 2010; Marko & Hart 2011, 2012; Faurby & Barber 2012).

Direct evidence of coancestry between individuals provides a particularly valuable complement to *F*-statistics when it is not possible to derive other independent estimates of demographic connectivity such as through the tagging and tracking of adults or larvae (e.g. Bellquist *et al.* 2008; Meyer *et al.* 2009; Cartamil *et al.* 2011; Carson *et al.* 2011; López-Duarte *et al.* 2012; reviewed in Lowe & Allendorf 2010).

In marine systems, the majority of taxa have relatively sedentary adults, but a pelagic larval stage that persists in the water column from a few minutes to over a year (Thorson 1950; Strathmann 1987; McEdward 1995). These larvae are notoriously difficult to track directly (Levin 2006), but the time that larvae spend in the open ocean has led to the intuitive expectation that the majority of marine species have high levels of gene flow (Hedgecock *et al.* 2007). However, the preponderance of recent indirect genetic evidence, based mostly on *F*-statistics, indicates that there is generally a weak relationship between dispersal potential inferred from pelagic larval duration (PLD) and genetic structure (reviewed in Bradbury *et al.* 2008; Shanks 2009; Weersing & Toonen 2009; Riginos *et al.* 2011; Selkoe & Toonen 2011). Furthermore, it is generally overlooked that indirect gene flow via multi-generational stepping stone dispersal at small scales can mimic direct gene flow across large scales (Puebla *et al.* 2012). The relatively few studies that have directly measured larval dispersal through larval tagging or parentage analyses have bolstered the claim that many larvae have limited dispersal, and often recruit back to their region of origin (Jones *et al.* 2005; Gerlach *et al.* 2007; Planes *et al.* 2009; López-Duarte *et al.* 2012). Due to the nature of these direct (kinship/parentage) versus indirect (*F*-statistics) measures of population connectivity, it is possible that direct analyses may identify recruitment patterns that cannot be detected using traditional *F*-statistics (Waples & Gaggiotti 2006; Saenz-Agueldo *et al.* 2009; Palsbøll *et al.* 2010). For example, Christie *et al.* (2010) found little genetic differentiation (max F_{ST} = 0.0097) among populations of bicolor damselfish (*Stegastes partitus*) in Exuma Sound, Bahamas, but parentage analysis identified high levels of self-recruitment at two of the eleven sampled locations. The direct identification of parent/offspring pairs resulted in very different management advice for this species than interpretation based on the F_{ST} data alone.

To date, most marine kinship studies have understandably focused on parent-offspring identification in reef fishes with fairly short larval durations. Here we show kinship analyses can also be useful at the opposite end of the potential dispersal continuum: the California spiny, or red rock lobster, *Panulirus interruptus* (Randall, 1840), spends at least the first eight months of its life in the plankton, during which time it can presumably disperse across its entire geographic range. Species without barriers to dispersal are expected to exhibit no detectable neutral genetic population structure. Here we use the California spiny lobster as a model to test the intuitive assumption of genetic homogeneity in species with extended PLD. We demonstrate the utility of individual-based estimates of genetic exchange in the interpretation of connectivity based on *F*-statistics.

Methods

Study system

The California spiny or red rock lobster, *Panulirus interruptus,* exhibits high site fidelity during its adult phase (Withy-Allen 2010), but has a two-phase pelagic larval stage with a total PLD of eight to eleven months (Johnson 1956, 1960; Serfling & Ford 1975). The initial phyllosoma stage undergoes multiple molts to produce 11 vertically and geographically stratified stages in the pelagic environment (Johnson 1960; Pringle 1986). The eleventh phyllosoma stage molts into the final puerulus stage, which settles into the lobster's preferred juvenile habitat. *P. interruptus* can be found across a 1400 km Pacific coast range from Monterey Bay, CA (though very rare north of Point Concepcion) to Bahia Magdalena, Mexico. Throughout its geographic distribution, *P. interruptus* plays an important ecological role in structuring both kelp forest and intertidal communities (Tegner & Levin 1983; Robles 1987; Lafferty 2004).

Spiny lobsters are also a valuable commercial and recreational fisheries species in both Mexico and the USA with a combined value of over $39 million from the most recent estimates (Chávez & Gorostieta 2010; Porzio 2012).

Sample collection and DNA extraction

We collected tissue samples from 1102 *P. interruptus* individuals across 17 sites located throughout the entire Pacific coastal range from Point Conception, CA in the north to Bahia Magdalena (BMG), Baja California Sur, Mexico in the south (Fig. 6.1, Table 6.1). Samples were collected non-lethally by removing a small piece of an antenna or a leg segment from each lobster. Lobsters were either captured by hand while scuba diving or obtained from commercial fishermen. Tissue samples were preserved in 95% ethanol, and stored at room temperature until extracted. DNA was isolated using a standard salting out protocol (Sunnucks & Hales 1996), a rapid-boil technique (Valsecchi 1998), or DNeasy Animal Tissue kits (Qiagen, Inc., Valencia, CA, USA).

Mitochondrial DNA (mtDNA)

We amplified a 494 bp fragment of cytochrome *c* oxidase subunit I gene (COI) using species-specific primers PintCOI-F (5'-GCTTGAGCTGGAATGGTAGG-3') and PintCOI-R (5'-CACTTCCTTCTTTGATCCC-3'), which were designed from GenBank sequence #AF339460 (Ptacek *et al.* 2001) using PRIMER3 (Rozen & Skaletsky 2000). Polymerase chain reactions (PCRs) for each sample were performed in a 20µl reaction containing 10µl of 2x Biomix Red (Bioline, Taunton, MA, USA), 0.125 µM each of forward and reverse primer, 5-50 ng of genomic DNA, and 0.75X Bovine Serum Albumin (BSA).

PCR was carried out on a Bio-Rad Mycycler Thermal Cycler (Bio-Rad Laboratories Hercules, CA, USA), with an initial denaturation step of 95 °C for 4 min, 35 cycles of denaturation (95°C for 30 sec), annealing (56°C for 30 sec), and extension (72°C for 30 sec), followed by a final extension step of 72°C for 10 min. PCR products were treated with 0.75 units of Exonuclease I and 0.5 units of Fast Alkaline Phosphatase (ExoFAP, Thermo Fisher Scientific, Waltham, MA, USA) per 7.5 µl of PCR product, and incubated at 37°C for 60 min, followed by deactivation at 85°C for 10 min. Purified DNA fragments were sequenced in the forward direction with fluorescently-labeled dideoxy terminators either on an ABI 3730XL capillary sequencer (Applied Biosystems, Foster City, CA) by the Advanced Studies of Genomics, Proteomics and Bioinformatics (ASGPB) Center at the University of Hawai‘i at Mānoa or an ABI 3130XL Genetic Analyzer (Applied Biosystems) at the Hawai‘i Institute of Marine Biology EPSCoR Sequencing Facility. Unique sequences and sequences with ambiguous nucleotide calls were also sequenced in reverse to confirm sequence identity. Sequences were edited, aligned, and trimmed to a uniform size using SEQUENCHER 4.8b (GeneCodes Corporation, Ann Arbor, MI, USA). The alignment did not contain any indels or frameshift mutations.

We calculated nucleotide (π) and haplotype diversity (h) for each sampling site as described in Nei (1987) using ARLEQUIN 3.5 (Excoffier *et al.* 2005). To visualize relationships between individual sequences, we constructed a median-joining network (Bandelt *et al.* 1999) using NETWORK 4.6.0.0 (http://www. Fluxus-engineering.com/network_terms.htm). We investigated population structure using an analysis of molecular variance (AMOVA) as implemented in ARLEQUIN. We used an analogue of Wright's F_{ST}, (Φ_{ST}), which incorporates a model of sequence evolution, for both our complete dataset and for pairwise population comparisons (Weir & Cockerham 1984; Excoffier *et al.* 1992).

Using jModelTest2 (Guindon & Gascuel 2003; Darriba *et al.* 2012), we determined that the Tamura & Nei (1993) with a Ti/Tv ratio of 11.2 and gamma parameter of 2.1 was the most appropriate model of sequence evolution implemented in ARLEQUIN. Global Φ_{ST}, and each pairwise population Φ_{ST} were tested for significance with 100,000 permutations. Due to the high heterozygosity in cytochrome *c* oxidase subunit I gene (COI) in *P. interruptus*, we also calculated D_{est_Chao} as an absolute measure of differentiation between sites. The magnitude of F_{ST} is inversely proportional to heterozygosity (Hedrick 2005; Meirmans 2006; Jost 2008), while D_{est_Chao} is less susceptible to biases caused by genetic diversity (Bird *et al.* 2011). For mtDNA, D_{est_Chao} (Jost 2008) was calculated with SPADE (Chao & Shen 2009). Mantel tests using linearized F_{ST} [F_{ST} /(1- F_{ST})] and the natural log of Euclidean distance (Rousset 1997) were conducted in GenoDive 2.0b20 (Meirmans & Van Tienderen 2004) to test for isolation by distance (IBD) among all sample sites, as well as subsets of sites across the range (California sites, Mexico sites, all island sites, all mainland/continental sites).

Microsatellite DNA

Seven previously developed microsatellites (A5, A102r, A110, Pin29L, Pin189, Pin10, Pin244) (Ben-Horin *et al.* 2009) were amplified by PCR in a10µL reaction containing 1x GoTaq Reaction Buffer (with 1.5mM $MgCl_2$ pH 8.5; Promega Corp., Madison, WI, USA), 2µM total dNTPs, 0.1 U GoTaq polymerase (Promega Corp.), 6 µM each of forward and reverse primer, and 20 ng of genomic DNA. PCR was carried out using a Bio-Rad DNA Engine Dyad Thermal Cycler with the following conditions: an initial denaturation step of 95°C for 3 min, 35 cycles of denaturation (94°C for 40 sec), annealing at primer-specific temperatures (for 40 sec; see Table 1 in Ben-Horin *et al.* 2009), and extension (72°C for 40 sec), and a final extension of 72°C for 30 min.

Forward primers were fluorescently labeled with WellRed D2, D3, or D4 dye (Beckman Coulter Inc., Fullerton, CA, USA; see Table 1 in Ben-Horin *et al.* 2009 for primer labels). Microsatellite PCR products were sized on a Beckman Coulter CEQ 8000 capillary sequencer with a 400bp size standard (Beckman Coulter Inc.). Alleles were scored with a CEQ 8000 genetic analysis system (Beckman Coulter Inc.).

Microsatellite quality control (Hardy-Weinberg Equilibrium, linkage equilibrium, scoring errors) followed Selkoe & Toonen (2006) as detailed in Ben-Horin *et al.* (2009), but expanded across all sample locations and loci. Additionally, null allele frequency was re-calculated with *ML-Relate* (Kalinowski *et al.* 2006) to enable significance testing. We calculated observed (H_o) and expected (H_e) heterozygosity at each location, as well as allelic richness (A), rarified to 25 individuals, using the Excel Microsatellite Toolkit 3.1.1 (Park 2001) and FSTAT (Goudet 2001) respectively. We also calculated the effective number of alleles at each locus in GenoDive. Overall genetic structure was analyzed using an AMOVA framework (Excoffier *et al.* 1992) implemented in GenoDive assuming the infinite allele model, with F_{ST} equivalent to Weir & Cockeram's θ (1984). Consistent with mtDNA (COI), microsatellite allelic diversity was likewise high, so we calculated D_{est_Chao} (Jost 2008) in addition to F_{ST}. GenoDive was used to calculate pairwise F_{ST} and D_{est_Chao} for all sampling location pairs, and F_{ST} was tested for significance using100,000 permutations. Patterns of IBD were investigated for microsatellite data in GenoDive as described above for mtDNA. The program GESTE 2.0 (Foll & Gaggiotti 2006) was used to calculate local $F_{ST,}$ a site-specific metric of allelic differentiation that accounts for the non-independence inherent in multiple comparisons (Balding & Nichols 1995; Hudson 1998).

Kinship

In order to further examine the factors driving the observed F_{ST} and D_{est_Chao} patterns, we calculated kinship coefficients (Loiselle *et al.* 1995) for each pair of individuals in GenoDive. These coefficients are based on the probability of identity of two alleles for each pair of homologous genes compared between each pair of individuals. Kinship was estimated with respect to the allele frequencies for the full dataset, so these coefficients provide an index of relative relatedness between each pair of individuals. In order to determine whether individuals collected at the same location were more closely related to each other than individuals collected at different locations, we conducted an AMOVA on the kinship coefficients. This approach compared the variation of within-population kinship coefficients with the variation in among-population kinship coefficients using the PERMANOVA+ 1.0.2 software add-on as implemented in PRIMER 6 (Clarke & Warwick 2001), following Stat *et al.* (2011) and Padilla-Gamiño *et al.* (2012). Specifically, the kinship covariance matrix created in GenoDive was loaded into PRIMER6 as a Correlation Resemblance Matrix. A simple 1-way analysis of variance (termed PERMANOVA in PRIMER6) was conducted with 10,000 unrestricted permutations of the raw data and Type III sums of squared differences. Significance is determined by evaluating a pseudo-F value (Clarke & Warwick 2001) based on the F-distribution, which is not to be confused with Wright's *F*-statistics.

To investigate site-specific patterns in kinship, we counted the number of closely related individuals within each site where the kinship coefficient was greater than or equal to the equivalent of that expected for quarter-siblings (0.047). Following Selkoe *et al.*'s (2006) and Buston *et al.*'s. (2009) analysis of relatedness approach, we binned our counts of closely related lobsters according to specific levels of kinship.

We used the Loiselle *et al.* (1995) coancestry coefficients (full-sib = 0.25, half-sib = 0.125) to generate the following bins: "nearly identical", 0.570 > k > 0.375; "full-sib", 0.375 > k > 0.1875; "half-sib", 0.1875 > k > 0.09375; and "quarter-sib", 0.09375 > k > 0.047. These bounds represent the midpoints between the coancestry coefficients in Loiselle *et al.* (1995), and we use quarter-sib as a short-hand to represent half of the level of co-ancestry as half-sibs. The nearly identical bin represents comparisons above full-sibs, and is based on our kinship coefficient distribution for comparisons of individuals to themselves. We tested multiple bin sizes and divisions and found our results to be robust to these changes. To test for an overabundance of closely related lobsters within sites, we implemented a permutation test (10,000 replicates) where the lobsters were randomly assigned to sites and the observed number of closely related individuals was compared to the null distribution for each site. We calculated the maximum likelihood estimate of relatedness (r) in *ML-Relate*, following the scale for the index of relatedness (full-sibs, r = 0.5; half-sibs = 0.25) to be able to compare our kinship results with a relatedness index. We also tested the relationship of the mean pairwise F_{ST}, mean pairwise D_{est_Chao}, and local F_{ST} for each site versus the proportion of closely related lobsters at that site (summed across all four sibship categories for the Loiselle et al. (1995) metric, and across half-sibs and full-sibs in *ML-Relate*).

Upwelling

We identified hotspots of genetic differentiation by calculating mean D_{est_Chao} for each sampling location. We interpolated these values beyond the specific sites we sampled using an inverse weighted distance (IWD) algorithm in the Spatial Analyst extension in ArcGIS 10.

To test the hypothesis that upwelling is a potential driver of both increased kinship, and in turn, site-specific genetic structure for *P. interruptus* in this region, we overlaid on this map known areas of consistent upwelling in Baja California, Mexico (as identified by Zaytsev *et al.* (2003)). We tested the relationship between the mean kinship at a site and that site's closest distance to an area of persistent upwelling. For sites within the Southern California Bight (SCB), where there are no areas of persistent upwelling, either the distance to Point Conception or to the edge of the northernmost upwelling area in Baja California, Mexico was used, whichever was shorter. The southernmost sites in the range were measured to an upwelling area just off of Bahia Magdalena (BMG), which was identified by Zaytsev *et al.* (2003), but is not included in our figure. Negative distances represent sites that are located within upwelling regions, and are measured from their location to the nearest edge of the upwelling zone. Both kinship and upwelling regression analyses were performed in SPSS 17.0.

Results

Mitochondrial DNA (mtDNA)

We sequenced COI for 931 individuals across 17 sites, which yielded 238 haplotypes. Haplotype diversity (h) was high, ranging from 0.88 to 0.95 (8.3-20 effective haplotypes), with a mean of 0.92 (12.5 effective haplotypes). In contrast, nucleotide diversity (π) was relatively low, ranging from 0.005 to 0.018, with a mean of 0.010. The number of individuals sequenced (N), haplotype diversity (h), effective number of haplotypes (h_{eff}), and nucleotide diversity (π) for each site are listed in Table 6.1.

The median-joining network (Fig. 6.2) reveals two dominant haplotypes differing by one nucleotide substitute, and present at all sample sites. The most numerous haplotype was found in 235 individuals (25% of individuals sequenced), the second most numerous haplotype was found in 92 individuals (10%). The haplotype network was characterized by a starburst pattern, with the majority of remaining haplotypes differentiated by one to two base pairs from the dominant haplotypes. 80 haplotypes were represented in only two individuals and there were 131 singletons across all 17 sites; the removal of singletons did not impact the overall structure of the haplotype network (Fig. 6.2), so they were omitted for ease of visualization. A full, unedited network is included in figure S6.3.

Global Φ_{ST} (0.006) was low but significant ($P = 0.001$). Statistically significant pairwise Φ_{ST} values were 0.01 - 0.04, and in general D_{est_Chao} values were an order of magnitude higher than Φ_{ST}, for both global D_{est_Chao} (0.025), and for each of the pairwise comparisons, ranging up to 0.300 (Table 6.2). Among pairwise Φ_{ST} comparisons, 26 of 136 (19%, Table 6.2) were significant at $P < 0.05$; however, after correcting for the false discovery rate (Benjamini *et al.* 2006), no pairwise comparisons were significant ($P < 0.00035$, Table 6.2).

There was no pattern of isolation by distance (Wright 1943) at any scale in the dataset. This was true whether we examined all sampling locations together, or for any of the specific sub-regions: all Mexico sampling locations, California locations (Southern California Bight), only islands, and only continental sites (Table S6.1).

Microsatellite DNA

We scored 989 individuals across 17 sites for seven nuclear microsatellite loci, with five to 104 alleles per locus, which translated to between two and 23 effective alleles per locus (Table S6.2). The genotyping error rate, determined by re-genotyping 74 individuals at each locus ranged from 0.0-4.1%, with an average of 2.3% overall (Table S6.2). We identified two pairs of individuals with identical genotypes and removed one individual of each pair from the data set in order to eliminate the possibility that the same individual was sampled two times. In both cases, identical genotypes were identified within a sampling site. The expected chances of observing true identical twins in this study ranged from 1×10^{-42} to 4.39×10^{-26}, while our observed rate was substantially greater, 2 in 989 specimens.

Rarefied allelic richness was similar among sites, and ranged from 16.19 to 18.51, while the effective number of alleles ranged from 12.05 to 15.98 (Table 6.1). Expected heterozygosity (H_e) was between 0.86 and 0.90, while observed heterozygosity (H_o) exhibited a slightly wider range from 0.76 to 0.87 (Table 6.1). Tests for linkage disequilibrium (LD) were significant in 20 of 349 comparisons (~6%) after correcting for multiple tests, and there were no locus-specific patterns. The three sample sites with the highest percentage of LD comparisons correspond to the three sites with the highest levels of kinship, as reported below, suggesting kinship may be high enough at these sites to produce a signal of LD. In general, however, LD is a weak test of family structure, and we found no site-specific patterns across the rest of the sites in the study.

There were significant deviations from Hardy-Weinberg equilibrium (HWE) in 46 of 119 (~39%) comparisons after correcting for multiple comparisons, but again no sample-specific patterns were observed. MICRO-CHECKER 2.2.3 (Van Oosterhout *et al.* 2004) found no evidence of scoring errors due to large allele dropout or stutter; however, six out of seven markers showed patterns consistent with null alleles, which are the likely cause of the deviations from HWE (see Ben-Horin *et al.* 2009). Although the frequencies of these null alleles are low (1.20 – 6.63% across loci, Table S6.2), we wanted to be sure they would not affect our results. To test for the impact of null alleles on our results, we used FreeNA (Chapuis & Estoup 2007) to generate alleles for the dataset where nulls were expected, re-analyzed the data, and our subsequent results and conclusions remained the same. Therefore, we present only analyses with the full original dataset.

Significant partitioning of the samples among locations was detected in the microsatellite loci with both a global fixation test ($F_{ST} = 0.004$, $P < 0.001$) and a global genetic differentiation test ($D_{est\_Chao} = 0.03$, $P < 0.0005$). Global partitioning was also detected in analyses of individual loci, with a significant global F_{ST} (0.002 – 0.011, Table S6.2) and global D_{est_Chao} (0.030 – 0.114) at each individual locus, except A110.

We ran pairwise F_{ST} and D_{est_Chao} comparisons for each marker individually, as well as jackknifing across markers, and found the results were fairly consistent throughout these comparisons. Significant pairwise F_{ST} comparisons among sampling sites using the microsatellite loci were fairly low, ranging from 0.002 to 0.015, but 71 of 136 comparisons (52%, Table 6.2) remained significant after correcting for the false discovery rate (Benjamini *et al.* 2006).

As in our mtDNA results, pairwise D_{est_Chao} comparisons generally were an order of magnitude higher than each respective pairwise F_{ST} comparison, and ranged from -0.021 to 0.128 (Table 6.2). The higher magnitude of D_{est_Chao} compared F_{ST} to matches the expectation when heterozygosity is ~0.9, as it is in this study (Bird *et al.* 2011).

The sites with the highest mean pairwise F_{ST} and D_{est_Chao} values were in the northern and central Baja California, Mexico region (Puerto Nuevo, Punta Banda, Punta Baja, and Bahia Tortugas). These sites also stood out by having the highest proportion of significant differences when compared to the other sites. Three of these four sites also had the highest local F_{ST} values (PBDA, BTG, and PTN). There was no pattern of isolation by distance in the dataset, whether we examined all sampling locations together, or for any of the specific sub-regions, as described for the mtDNA results (Table S6.1).

Kinship

Following the exclusion of lobster specimens with identical genotypes, as well as self-comparisons, kinship coefficients ranged from -0.155 to 0.570 (Fig. 6.3). The overall mean kinship, which is expected to be zero, was -0.000025 ± 0.00008SE. There was a disparity, however, between the within-site mean kinship (0.003 ± 0.0004) and the among-site mean kinship (-0.0002 ± 0.00009) (Fig. 6.3). Mean within-site maximum likelihood estimates of relatedness (*r,* from *ML-Relate*) ranged from 0.034 to 0.057 (Table S6.3), and were significantly correlated with mean kinship values (k) at each site (Table S6.3, Pearson R = 0.92, $P < 0.0005$). Kinship coefficients were significantly greater for within-site than for among-site comparisons (Pseudo-$F_{(16, 988)} = 1.39$, $P = 0.001$).

In total, 10 of 17 sites had significantly greater numbers of closely related pairs of individuals than expected by chance ($P < 0.05$) in at least one of four kinship categories: five sites had an excess of individuals in the "nearly identical" category ($0.570 > k > 0.375$), five sites had an excess in the "full-sib" group ($0.375 > k > 0.1875$), five sites had an excess in the "half-sib" category ($0.1875 > k > 0.09375$), and four sites had an excess in the "quarter-sib" bin ($0.09375 > k > 0.047$) (Fig. 6.4, Table S6.3). The proportion of kin in each site was significantly related to mean pairwise F_{ST} ($R^2 = 0.669$; $F_{(1, 16)} = 30.333$, $P < 0.0005$) and D_{est_Chao} ($R^2 = 0.658$; $F_{(1, 16)} = 28.885$, $P < 0.0005$; Fig. 6.5) for each site as well as to local F_{ST} ($R^2 = 0.243$; $F_{(1, 16)} = 4.825$, $P = 0.044$; Fig. 6.5). These findings are consistent across kinship classes. When we removed the lowest level of kinship (examining only kinship levels equivalent to "nearly identical", "full-sib", and "half-sib"), the relationship of both F_{ST} ($R^2 = 0.672$; $F_{(1, 16)} = 30.741$, $P < 0.0005$) and D_{est_Chao} ($R^2 = 0.658$; $F_{(1, 16)} = 28.887$, $P < 0.0005$) versus the proportion of kin stayed approximately the same, while the relationship of local F_{ST} versus the proportion of kin strengthened ($R^2 = 0.303$; $F_{(1, 16)} = 6.526$, $P = 0.022$). The relationships of mean pairwise F_{ST}, D_{est_Chao}, and local F_{ST} versus the proportion of related individuals (r, full and half-sibs) from *ML-Relate* were all significant and stronger than the relationships between these summary statistics and proportion of kin (Fig. S6.1).

Upwelling

Regions containing persistent, strong upwelling regimes, both across seasons within a year and across all years from 1996 to 2002 were traced from figure 14 in Zaytsev *et al*. (2003), and are depicted by dotted lines in figure 6.6a. Three of the four upwelling regions overlap the geographic range of our study and contain four of our sampling locations: CRDO, PTN, PBDA, and BTG (Fig. 6.6a). Three of these four sites (PTN, PBDA, BTG) contain one of the highest four values for mean D_{est_Chao}, mean local F_{ST}, and mean kinship. The IWD function applied to this data extrapolates these site-specific metrics across the entire study area, including the unintended extrapolation of D_{est_Chao} values to ocean areas where adult lobsters do not live, but phyllosoma may be present. Along the Baja California coastal areas containing adult lobsters, all three of the areas with the highest genetic differentiation (D_{est_Chao}) overlap the regions of strong upwelling intensity (Fig. 6.6a). The results for mean kinship and local F_{ST} were similar, though not shown. Across the geographic extent of our study, we see a significant relationship (R^2 = 0.407; $F_{(1, 16)} = 10.296$, $P = 0.006$) between mean kinship at each site and the closest distance between each site and the edge of an upwelling zone ((Mean Kinship + 1) = -0.005ln (Distance to Upwelling + 100km) + 1.028, Fig. 6.6b). This relationship holds for mean relatedness (r) versus distance from upwelling as well (Fig. S6.2).

Discussion

There is accumulating evidence from multiple approaches that larvae rarely reach their full dispersal potential, resulting in a paradigm shift away from the perception that most marine populations are genetically homogenous across broad geographic scales (Jones *et al*. 1999; Swearer *et al.* 1999; Mora & Sale 2002; Swearer *et al.* 2002; Grantham *et al.* 2003; Taylor & Hellberg 2003; Marko 2004; Cowen *et al.* 2006; Becker *et al.* 2007; López-Duarte *et al.* 2012). However, this evidence has come exclusively from species with short to modest larval periods (1-60 days). Even among species with modest PLD, there are just a few striking examples of broad populations with no genetic substructure across their full range. For example, reef fishes *Myripristis jacobis* in the Atlantic Ocean (Bowen *et al.* 2006), *Lutjanus kasmira* in the Central Pacific and Eastern Indian Oceans (Gaither *et al.* 2010), and *Acanthurus nigrofuscus* in the Pacific (Eble *et al.* 2011) all exhibit genetic homogeneity across thousands of kilometers (up to 12,000 km). Dispersal potential is assumed to be great in species with very long PLDs (> 120 days), and population genetic surveys of such species to date have revealed little population structuring across broad geographic scales (Ovenden *et al.* 1992; Silberman *et al.* 1994; Thompson *et al.* 1996; Tolley *et al.* 2005; García-Rodríguez & Perez-Enriquez 2006; Inoue *et al.* 2007; Horne *et al.* 2008; Reece *et al.* 2011). When genetic discontinuities have been observed in species with long PLD, they have invariably corresponded with known biogeographic barriers, or oceanographic transitions (Palero *et al.* 2008; Babbucci *et al.* 2010; Chow *et al.* 2011).

In contrast to the intuitive expectation that *P. interruptus*, with a minimum PLD of 240 days, would be genetically homogenous across its entire 1400 km range along the west coast of North America, we found slight, but significant genetic structuring among several sampling locations throughout Mexico and Southern California (Table 6.2). This finding contrasts with previous work that did not detect population structure in *P. interruptus* throughout Baja California, Mexico using mtDNA RFLPs (García-Rodríguez & Perez-Enriquez 2006). Notably, lobsters do not exhibit a genetic break across Punta Eugenia, a faunal boundary for rocky intertidal species (Valentine 1966; Blanchette *et al.* 2008; Gaines *et al.* 2009) and a phylogenetic break for a number of coastal fishes (Bernardi *et al.* 2003). Nor does the overall pattern of genetic differentiation in *P. interruptus* correspond to the Northern, Central, and Southern regional population subdivision within Baja predicted by Perez-Enriquez *et al.* (2001). Rather, genetically differentiated sites are nested within a greater area of undifferentiated sites (Fig. 6.6a). Specifically, some sites exhibit no genetic differentiation across the 1400 km species range, whereas other sites are differentiated from the majority of sampled sites, and there is no signal of isolation by distance in either the mtDNA or the nuclear microsatellite markers across multiple spatial scales (Table S6.1). Similar patterns of genetic differentiation among proximate sites have been shown in species with shorter larval developmental periods (reviews by Larson & Julian 1999; Hedgecock *et al.* 2007; Riginos *et al.* 2011; Toonen & Grosberg 2011), but have not been reported for a species with such a long PLD as this one. Additionally, *P. interruptus* has equivalent or greater levels of genetic substructure than other species occurring in this same region (Selkoe *et al.* 2010), despite a PLD that is an order of magnitude higher.

The term chaotic genetic patchiness was coined (Johnson & Black 1982; 1984) to describe ephemeral, finely spatio-temporal patterns of genetic structure generated by variation in the larval pool, recruitment, and natural selection, that are counteracted in the long-term by dispersal and gene-flow (Toonen & Grosberg 2011). Much of the difficulty in interpreting these unexpected patterns in genetic differentiation is due to the nature of F_{ST} as a summary statistic. Significant structure among populations may be a result of differences in effective population size (and corresponding genetic drift), demographic or colonization history, migration, or some combination of these factors, especially for populations that may not have reached migration-drift equilibrium. Direct interpretation of summary statistics (F_{ST}, D_{est_Chao}) in the context of gene flow can be problematic (reviewed by Lowe & Allendorf 2010; Hart & Marko 2010; Marko & Hart 2011; Bird *et al*. 2011; Karl *et al*. 2012), especially in species such as *P. interruptus*, with highly fecund individuals and a potential for reproductive skew (Eldon & Wakeley 2009). For the many marine species with high fecundity and a type III survivorship curve, an independent test can help determine whether gene flow is the primary driver resulting in the observed population structure (Lowe & Allendorf 2010; Hart & Marko 2010).

Here, kinship (Loiselle *et al.* 1995) enriches our understanding of the drivers underlying significant differences in F_{ST} among sites. The pattern of chaotic genetic patchiness in *P. interruptus* evident in the F_{ST} and D_{est_Chao} analyses seems to be primarily a result of the non-random occurrence of closely related lobsters within sample sites. Across all sites, lobsters were more closely related within sites than between sites, and at the majority of sites, we found significantly greater than expected levels of kinship between adult lobsters (Fig. 6.4).

Moreover, the proportion of kin found at each site accounts for the majority of the variation in the sites' genetic differentiation: the most greatly differentiated sampling sites have the highest proportion of kin (Fig. 6.5).

One potential scenario that could generate high proportions of kin within sites is recruitment pulses of related individuals. The simplest explanation for this phenomenon is that larvae released together, stay together throughout dispersal and recruitment (kin aggregation). However, this pattern would also result from extreme differential reproductive success among individuals, so that a recruiting cohort is entirely made up of offspring from only a few individuals (sweepstakes recruitment, Hedgecock 1986; Hedgecock 1994a, b). Sweepstakes recruitment could generate the star-shaped pattern of our mtDNA haplotype network (Fig. 6.2, Fig. S6.3), although this pattern could also be indicative of a recent population expansion. Previous kinship analyses have detected high levels of relatedness within cohorts of larval recruits in both fishes (Planes *et al.* 2002; Pujolar *et al.* 2006; Selkoe *et al.* 2006; Buston *et al.* 2009; Bernardi *et al.* 2012) and invertebrates (Veliz *et al.* 2006), which supports both the hypothesis of kin aggregation throughout development and/or the hypothesis of sweepstakes reproduction. Unfortunately, we could not directly test these alternatives in *P. interruptus* because we did not have samples of new recruits. Nevertheless, given the size selectivity of lobster traps, and the intense fishing pressure for lobster depressing the age range (Iacchei *et al.* 2005; Kay & Wilson 2012), it is possible that our samples are largely made up of single year classes consisting of closely related individuals recruiting together by one of these aforementioned mechanisms. To our knowledge, this study is the first documented case of kin aggregation in the adult population of a marine species with planktonic larvae, although kin aggregation in recruits has been reported.

Previous studies that have looked at only kin relationships among adults, rather than among cohorts of recruits, have found no evidence of kin aggregation in marine species (Avise & Shapiro 1986; Kolm *et al.* 2005; Buston *et al.* 2007; Palm *et al.* 2008; Andrews *et al.* 2010, Berry *et al.* 2012). Consequently, kin aggregation is generally assumed to be a transient phenomenon limited to newly settled recruits, with little detectable signal in adult populations due to multiple source populations of recruits, changes in reproductive success, and differential juvenile mortality (Kordos & Burton 1993; Moberg & Burton 2000; Flowers *et al.* 2002; Planes *et al.* 2002; Selkoe *et al.* 2006; Buston *et al.* 2009).

High levels of within-site kinship could also be driven by a temporally stable pattern of self-recruitment, either through larval retention or larval dispersal with subsequent recruitment back to the natal site. The prospect that larvae stay in the plankton for 240-330 days and return to settle near their site of release seems unlikely at first. However, the site-specific kinship patterns in our data match theoretical predictions for a species that has evolved a long PLD to avoid predation during the larval phase rather than to facilitate broad dispersal of larvae (Strathmann *et al.* 2002). The extended PLD may enable phyllosoma to disperse far offshore, into a pelagic environment that is favorable for the survival of unprotected larval-stage individuals (Strathmann *et al.* 2002). Late-stage lobster larvae (pueruli) are fast swimmers (Serfling & Ford 1975), and may utilize strong upwelling regimes to return and settle near their natal site after dispersing offshore. If this behavior is selectively advantageous, we would expect to observe enhanced local recruitment regardless of PLD. Furthermore, local recruitment should be more pronounced at sites with strong, persistent upwelling (Fig. 6.6b).

The four sites with the highest proportion of kin in Baja California, Mexico are located in areas of persistent upwelling (Zaytsev *et al.* 2003, Fig. 6.6a). Although upwelling was initially proposed as a mechanism for advecting recruits away from coastal areas (Roughgarden *et al.* 1988), subsequent studies have questioned that prediction (e.g. Shanks & Brink 2005; Morgan *et al.* 2009; Morgan & Fisher 2010; reviewed in Shanks & Shearman 2009). Genetic studies have proven equivocal in this regard: some have found reduced genetic substructure between populations in years with greater cumulative upwelling (e.g. Flowers *et al.* 2002; Barshis *et al.* 2011), and others have found upwelling dynamics to be insufficient to explain temporal and spatial genetic patterns (e.g. Toonen & Grosberg 2011). Passive larval dispersal models indicate that the effects of upwelling may depend on whether larvae stay near the surface (upwelling advects larvae offshore), or undergo regular migrations to depth (upwelling delivers larvae to the coast) (Byers & Pringle 2006; Marta-Almeida *et al.* 2006).

Spiny lobsters are known to have relatively large larvae capable of dynamic movement. Both the phyllosoma and puerulus stages show evidence of active movement in the pelagos, with phyllosoma exhibiting diel vertical migrations as well as horizontal movements (Kittaka 1994; Chiswell & Booth 1999; Phillips 2006; Butler *et al.* 2011), and pueruli demonstrating rapid swimming, navigation toward the coast, and habitat settlement preferences (Serfling & Ford 1975; Jeffs *et al.* 2005; Phillips *et al.* 2006). In *P. interruptus* specifically, Pringle (1986) found both geographic and depth stratification of different stages of both phyllosoma and pueruli collected during yearly larval tows in the California Current Ecosystem, suggesting active ontogenetic shifts in larval depth preference.

Incorporating such larval behaviors into biophysical models of dispersal in a congeneric species, *P. argus*, resulted in a 60% or greater decrease in the average distance a larva is predicted to settle from its release site compared with simulations of larvae that remain on the surface (Butler *et al.* 2011). Lobsters in the California Current Ecosystem rely on kelp forest habitat for survival: *P. interruptus* has much higher relative survival rates in the presence of kelp than in surrounding areas where kelp is absent (Mai & Hovel 2007). Given the ephemeral dynamics of kelp forest habitat (Reed *et al.* 2006), and the high variability in ocean conditions in this region, these lobsters may have evolved similarly complex behaviors as their congeners to increase successful local recruitment despite their extremely long PLD (e.g. Shanks & Eckert 2005).

Conclusion

Here we present a novel approach to understand contemporary drivers of population differentiation in systems with high gene flow. In isolation, the population-level data present a commonly documented scenario among marine species: no evidence for any particular regional separation or isolation by distance patterns, but low and significant pairwise differences among populations. While the agreement between nuclear and mitochondrial markers confirms the results are not due to statistical artifact, the nature of *F*-statistics leaves us without a clear indication of what is driving the pattern of genetic differentiation. The addition of kinship analyses reveals how alleles are shared between individuals, rather than just among populations, and provides an independent test of the hypothesis that population genetic structure as measured by *F*-statistics is a result of population connectivity. In this case, the majority of locations contained an excess of closely related individuals.

This supports the inference that either self-recruitment or some form of coordinated larval delivery is driving population-level genetic differences in a species that would be expected to be broadly dispersive throughout its range, given its extremely long pelagic larval duration. In combination with regional oceanographic data and larval dispersal behavior, kinship analyses provide evidence for a mechanism of differentiation in an otherwise murky population genetic dataset. The ability to directly test hypotheses about what drives population genetic substructure in high gene flow species by independent means, such as kinship or coalescent analyses provides greater confidence in the underlying causes of population substructure than summary statistics alone. As the ease of developing greater numbers of genetic markers increases, individual-based analyses such as relative kinship indices can provide a valuable complement for understanding patterns in traditional population genetics datasets.

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Table 6.1. Summary statistics for *Panulirus interruptus* listed from northernmost to southernmost collection sites: total number of individuals sequenced for 7 microsatellites (N, nDNA) and mtDNA Cytochrome c oxidase subunit I (N, mtDNA). For mtDNA: haplotype diversity (h), effective number of haplotypes (heff) and nucleotide diversity (π). For nDNA microsatellites: rarefied allelic richness (AR), effective number of alleles (Aeff), observed (Ho) and expected (He) heterozygosity.

Collection Site (Abbreviation)	**N**		***h***	***h_{eff}***	**π**	**AR**	**A_{eff}**	**H_o**	**H_e**
	nDNA	**mtDNA**							
Carpinteria (CARP)	74	57	0.94	16.67	0.011	18.46	15.98	0.83	0.89
San Miguel Island (SMI)	76	60	0.93	14.29	0.007	18.11	14.59	0.81	0.88
Santa Cruz Island (SCI)	54	53	0.92	12.50	0.005	18.48	15.51	0.84	0.89
Malibu (MLBU)	71	68	0.93	14.29	0.018	17.86	15.37	0.87	0.90
Santa Catalina Island (SCAT)	81	56	0.94	16.68	0.008	17.46	14.74	0.83	0.88
San Nicholas Island (SNI)	38	36	0.94	16.67	0.006	16.66	12.22	0.76	0.86
San Clemente Island (CLEM)	25	63	0.92	12.50	0.008	18.14	12.37	0.87	0.89
Islas Coronados (CRDO)	63	61	0.94	16.67	0.012	18.42	15.78	0.84	0.89
Puerto Nuevo (PTN)	57	56	0.91	11.11	0.007	16.83	13.65	0.86	0.88
Punta Banda (PBDA)	47	38	0.94	16.67	0.009	16.19	12.05	0.78	0.88
Punta Baja (PBJ)	94	70	0.88	8.33	0.009	17.85	15.06	0.86	0.88
Isla Guadalupe (IGP)	69	79	0.91	11.11	0.011	17.20	14.02	0.82	0.88
Laguna Ojo de Liebre (ODL)	42	55	0.91	11.11	0.008	18.51	15.34	0.83	0.88
Punta Eugenia (PEU)	45	45	0.93	14.29	0.012	17.36	13.39	0.82	0.89
Bahia Tortugas (BTG)	40	47	0.93	14.29	0.012	16.83	12.66	0.81	0.88
Punta Abreojos (ABRE)	66	42	0.88	8.33	0.009	17.44	14.96	0.84	0.89
Bahia Magdalena (BMG)	47	45	0.95	20.00	0.012	17.92	15.01	0.86	0.88

Table 6.2. *Panulirus interruptus* pairwise population structure results for (a) seven microsatellite loci and (b) a 494 bp fragment of the mtDNA cytochrome *c* oxidase subunit I region (COI). F_{ST} (ϕ_{ST} for COI) is below the diagonal and Jost's D_{est_Chao} is above the diagonal. Shaded boxes indicate significant differences at $P < 0.05$ for both nDNA and mtDNA. For nDNA, comparisons marked with *, **, or ‡ are significant after a false discovery correction is applied. * indicates $P < 0.01$, and ** indicates $P < 0.0005$. For mtDNA, no comparisons were significant after correcting for false discovery, although some were significant at $P < 0.01$ and 0.0005, and indicated as such. CARP = Carpenteria, CA; SMI = San Miguel Island, CA; SCI = Santa Cruz Island, CA; MLBU = Malibu, CA; SCAT = Santa Catalina Island; SNI = San Nicholas Island, CA; CLEM = San Clemente Island, CA; CRDO = Islas Coronados, Mx; PTN = Puerto Nuevo, Mx; PBDA = Punta Banda, Mx; PBJ = Punta Baja, Mx; IGP = Isla Guadalupe, Mx; ODL = Laguna Ojo de Liebre, Mx; PEU = Punta Eugenia, Mx; BTG = Bahia Tortugas, Mx; ABRE = Punta Abreojos, Mx; BMG = Bahia Magdalena, Mx.

(a)	CARP	SMI	SCI	MLBU	SCAT	SNI	CLEM	CRDO	PTN	PBDA	PBJ	IGP	ODL	PEU	BTG	ABRE	BMG
CARP	-	0.035	0.011	0.013	0.016	0.030	0.028	-0.011	0.048	0.066	0.033	0.032	0.002	0.027	0.053	0.017	0.018
SMI	0.005*	-	0.013	0.030	0.026	0.031	0.016	0.019	0.063	0.073	0.035	0.003	-0.006	0.037	0.071	0.010	-0.008
SCI	0.001	0.002	-	0.008	0.028	0.048	0.014	0.006	0.043	0.049	0.025	0.006	-0.002	0.024	0.060	0.009	0.011
MLBU	0.001	0.004*	0.001	-	0.014	0.036	-0.005	-0.005	0.015	0.067	0.023	0.002	0.005	0.018	0.042	0	0.012
SCAT	0.002	0.004*	0.004*	0.002	-	-0.001	0.026	-0.005	0.041	0.089	0.040	0.029	0.007	0.034	0.029	0.013	0.023
SNI	0.004‡	0.005‡	0.007*	0.005‡	0	-	0.038	0.021	0.052	0.093	0.054	0.032	0.011	0.027	0.030	0.032	0.017
CLEM	0.003	0.002	0.002	-0.001	0.003	0.005	-	-0.009	0.056	0.073	0.035	0.010	-0.012	0.002	0.073	0.019	-0.005
CRDO	-0.001	0.002‡	0.001	-0.001	-0.001	0.003	-0.001	-	0.028	0.059	0.013	0.001	-0.012	0.008	0.037	-0.005	0.003
PTN	0.006**	0.009**	0.005*	0.002	0.006**	0.007*	0.007‡	0.004‡	-	0.104	0.046	0.026	0.023	0.058	0.077	0.031	0.044
PBDA	0.008**	0.010**	0.006*	0.008**	0.012**	0.013**	0.009*	0.008**	0.014**	-	0.104	0.066	0.075	0.069	0.128	0.081	0.070
PBJ	0.004**	0.005**	0.003‡	0.003*	0.005**	0.008**	0.005‡	0.002	0.006**	0.014**	-	0.016	0.013	0.045	0.079	0.025	0.012
IGP	0.004*	0	0.001	0	0.004*	0.005‡	0.001	0	0.004‡	0.009**	0.002‡	-	-0.006	0.030	0.065	0.001	-0.002
ODL	0	-0.001	0	0.001	0.001	0.002	-0.002	-0.001	0.003	0.010**	0.002	-0.001	-	0.005	0.047	-0.021	-0.02
PEU	0.003	0.005*	0.003	0.002	0.004*	0.004	0	0.001	0.007**	0.009**	0.006**	0.004‡	0.001	-	0.019	0.023	0.025
BTG	0.007*	0.010**	0.008**	0.005*	0.004‡	0.004	0.009*	0.005‡	0.010**	0.017**	0.011**	0.009*	0.006‡	0.002	-	0.051	0.064
ABRE	0.002	0.001	0.001	0	0.002	0.005‡	0.002	-0.001	0.004*	0.011**	0.003*	0	-0.003	0.003	0.007*	-	0.003
BMG	0.002	-0.001	0.001	0.001	0.003‡	0.002	-0.001	0	0.006*	0.009**	0.002	0	-0.003	0.003‡	0.008**	0	-

(b)	CARP	SMI	SCI	MLBU	SCAT	SNI	CLEM	CRDO	PTN	PBDA	PBJ	IGP	ODL	PEU	BTG	ABRE	BMG
CARP	-	0.003	0.016	0.018	0	0.118	0	0.067	0.004	0.005	0.081	0	0.015	0	0	0.039	0
SMI	0	-	0.124	0.022	0	0.214	0	0.032	0.020	0.011	0.057	0.008	0.058	0	0	0.003	0
SCI	-0.002	0.005	-	0.108	0.100	0	0.025	0.209	0.072	0.027	0.143	0.068	0	0.005	0	0.096	0.093
MLBU	0.009	0.008	0.015	-	0	0.189	0	0.033	0.037	0.033	0.072	0.024	0.055	0	0	0.016	0
SCAT	0.007	0.002	0.005	0.001	-	0.144	0	0.044	0.024	0	0.072	0	0.057	0	0	0.021	0
SNI	0.003	0.006	-0.002	0.013	0.002	-	0.117	0.300	0.174	0.068	0.253	0.164	0.054	0.081	0.070	0.190	0.171
CLEM	0.001	0.002	0.007	0.011	0.013	0.009	-	0	0	0	0.025	0	0	0	0	0	0
CRDO	-0.002	0.003	-0.001	0.006	0	0.002	0.002	-	0.080	0.135	0.090	0.038	0.165	0	0.017	0.06	0
PTN	0.018	0.003	0.024*	0.015	0.007	0.021	0.013	0.018	-	0.018	0.033	0	0.023	0	0	0	0.008
PBDA	0.001	0.003	0.005	0.007	-0.007	0.003	0.014	-0.005	0.013	-	0.126	0.042	0	0	0	0.031	0.015
PBJ	0.019	0.022	0.008	0.013	0.001	0.010	0.03*	0.008	0.034*	0.002	-	0.022	0.098	0.009	0.003	0	0.072
IGP	-0.003	0.001	-0.002	0.006	-0.003	-0.002	0.002	-0.003	0.012	-0.005	0.008	-	0.048	0	0	0	0
ODL	-0.002	-0.005	-0.005	0.002	-0.003	-0.001	-0.003	-0.002	0.004	-0.003	0.009	-0.005	-	0	0	0.027	0.073
PEU	0.002	0	0	0.005	0	0.004	-0.006	0.001	0.001	0.005	0.015	0.001	-0.007	-	0	0	0
BTG	0.003	0.002	-0.002	0.006	0	0.004	-0.004	0.001	0.005	0.005	0.012	0.001	-0.006	-0.021	-	0	0
ABRE	0.002	-0.002	0.008	0.004	0.004	0.001	-0.003	0.005	0.003	0.009	0.020	0.002	-0.005	-0.005	-0.003	-	0.023
BMG	0.018	0.025	0.014	0.022	0.006	0.014	0.043**	0.008	0.040*	-0.005	0.002	0.006	0.017	0.031*	0.029	0.035*	-

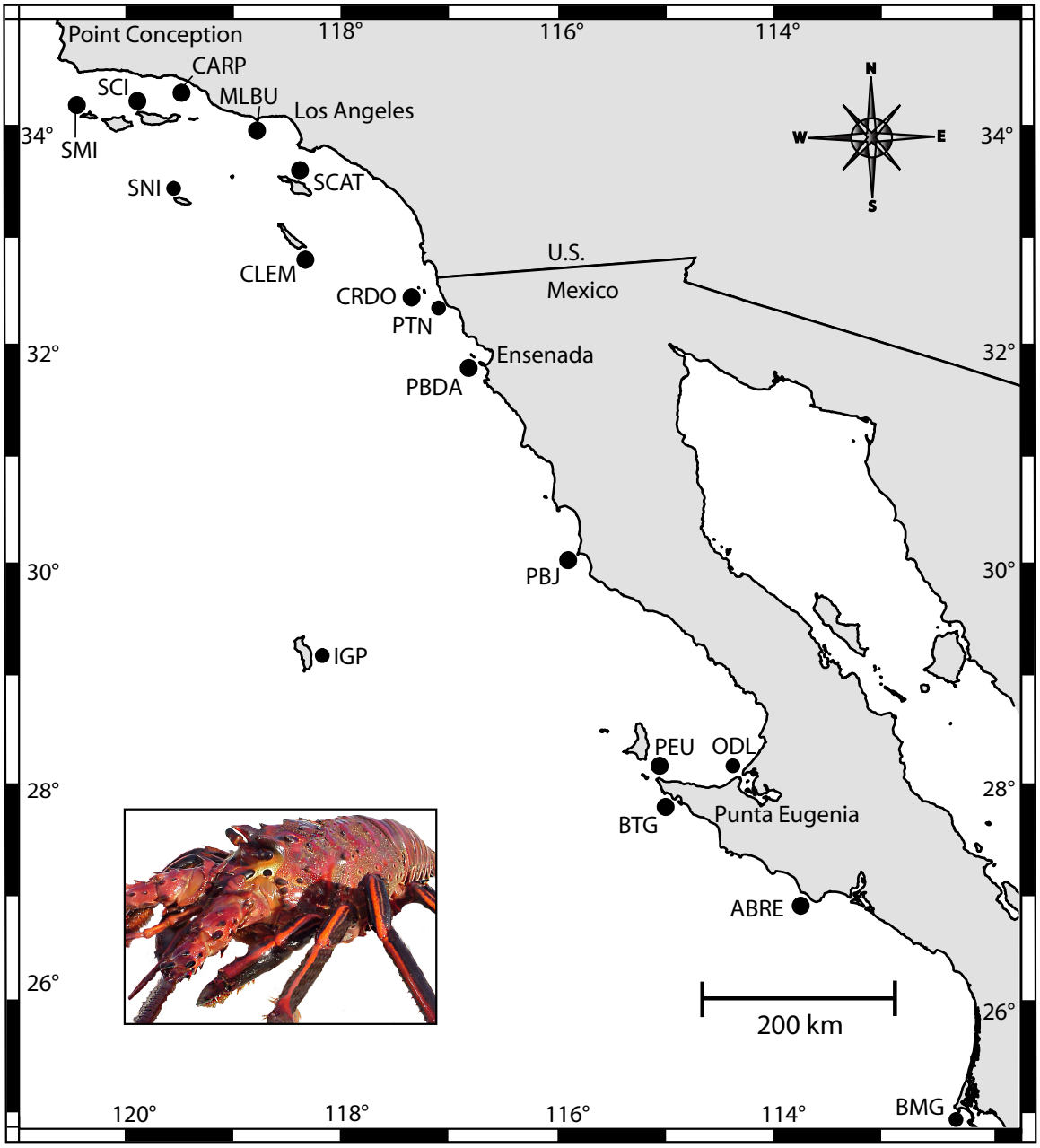

Figure 6.1. Map of lobster specimen collection locations in the Southern California Bight and Baja California, Mexico. CARP = Carpenteria, CA; SMI = San Miguel Island, CA; SCI = Santa Cruz Island, CA; MLBU = Malibu, CA; SCAT = Santa Catalina Island, CA; SNI = San Nicholas Island, CA; CLEM = San Clemente Island, CA; CRDO = Islas Coronados, Mx; PTN = Puerto Nuevo, Mx; PBDA = Punta Banda, Mx; PBJ = Punta Baja, Mx; IGP = Isla Guadalupe, Mx; ODL = Laguna Ojo de Liebre, Mx; PEU = Punta Eugenia, Mx; BTG = Bahia Tortugas, Mx; ABRE = Punta Abreojos, Mx; BMG = Bahia Magdalena, Mx. Photo credit: Patrick W. Robinson.

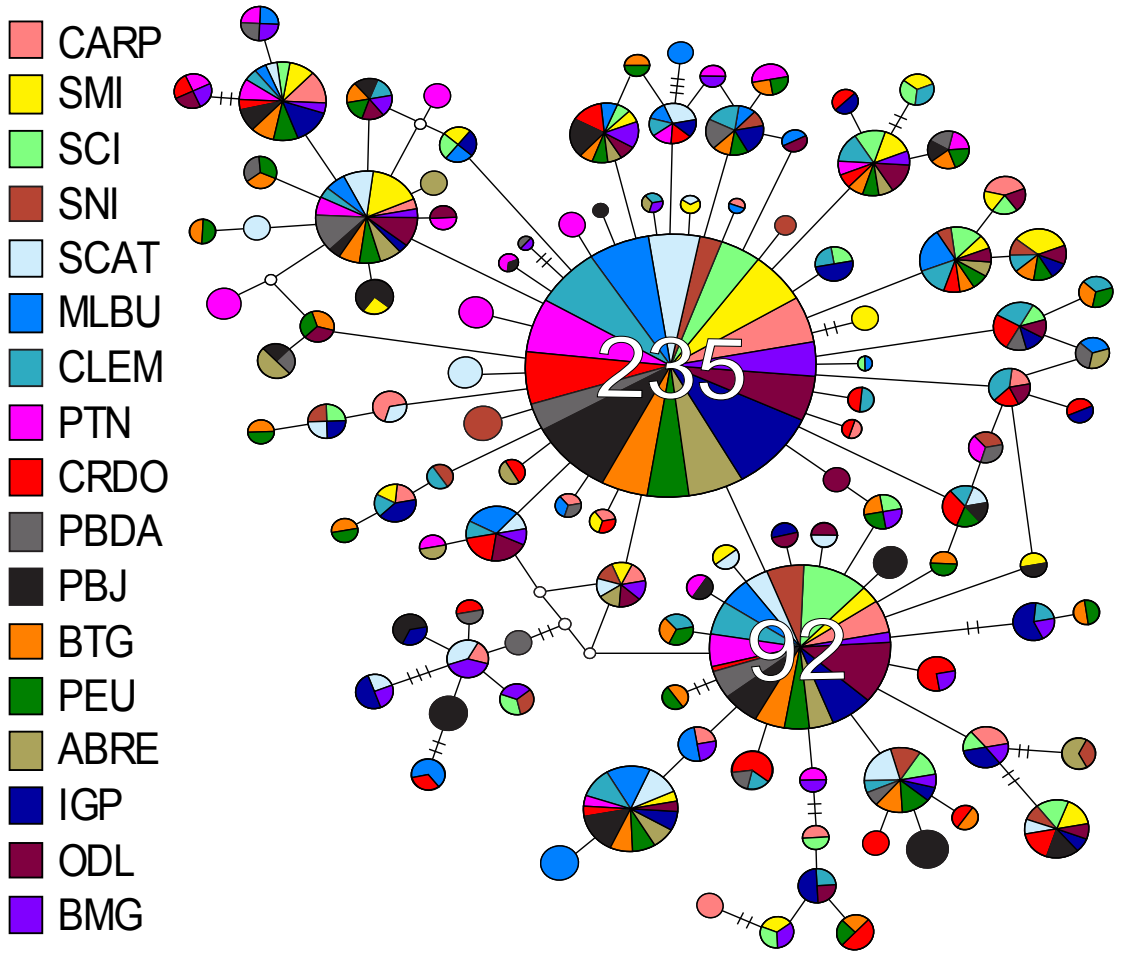

Figure 6.2. Median-joining network for *Panulirus interruptus* mtDNA, constructed using 454 base pairs of cytochrome *c* oxidase subunit I (COI) from each of 931 individuals in the program NETWORK 4.6.0.0. Each circle is a unique haplotype proportional in size to the number of individuals with that haplotype. The two largest circles represent 235 and 95 individuals. The smallest circle represents two individuals: there are 131 singletons in the dataset, but these have been omitted for ease of visualization. A full, unedited network is included in the supplementary material (Fig. S6.3). Colors correspond to one of 17 locations where the individual haplotypes were found (see key, Fig. 6.1, Table 6.1). Lines connecting haplotypes represent a single base pair difference between haplotypes, with crossing lines representing additional differences.

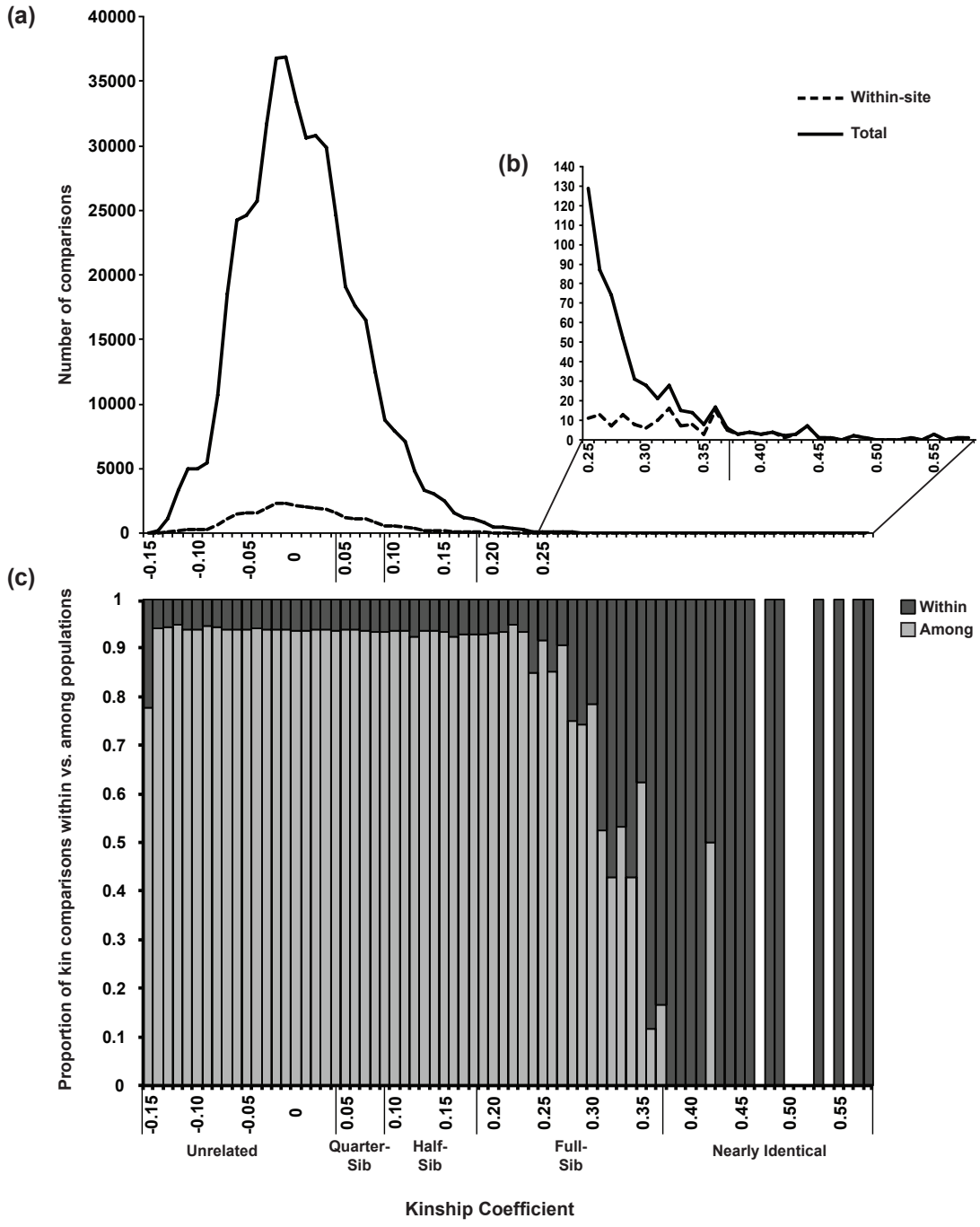
(a)
Number of comparisons
40000
35000
30000
25000
20000
15000
10000
5000
0
-0.15
-0.10
-0.05
0
0.05
0.10
0.15
0.20
0.25
Within-site
Total
(b)
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
0.25
0.30
0.35
0.40
0.45
0.50
0.55
(c)
Proportion of kin comparisons within vs. among populations
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Within
Among
-0.15
-0.10
-0.05
0
0.05
0.10
0.15
0.20
0.25
0.30
0.35
0.40
0.45
0.50
0.55
Unrelated
Quarter-Sib
Half-Sib
Full-Sib
Nearly Identical
Kinship Coefficient

Figure 6.3. (a) Line graph depicting the total number of kinship (k) pairwise comparisons for each 0.01 bin of kinship (solid line, total N = 457,652), and the number of within-site kinship comparisons (dashed line, total N = 30,914). (b) Inset of (a), depicting the total number of kinship comparisons (solid line) and the number of within-site comparisons (dashed line) from 0.25 to 0.57 on a separate y-axis that ranges from 0 to 140 comparisons. The majority of these kinship comparisons are between individuals sampled at the same location. (c) Distribution of kinship coefficients divided into 0.01 bins and colored by the proportion of within-site (dark grey) versus among-site (light grey) comparisons within each 0.01 division. White bars represent levels of kinship that were not found in the dataset. Bars on the x-axis represent the divisions between unrelated and related individuals and between each of the four kinship categories we analyzed: "quarter-sib", $0.047 < k < 0.09375$; "half-sib", $0.09375 < k < 0.1875$; "full-sib", $0.1875 < k < 0.375$; and "nearly identical", $0.375 < k < 0.570$.

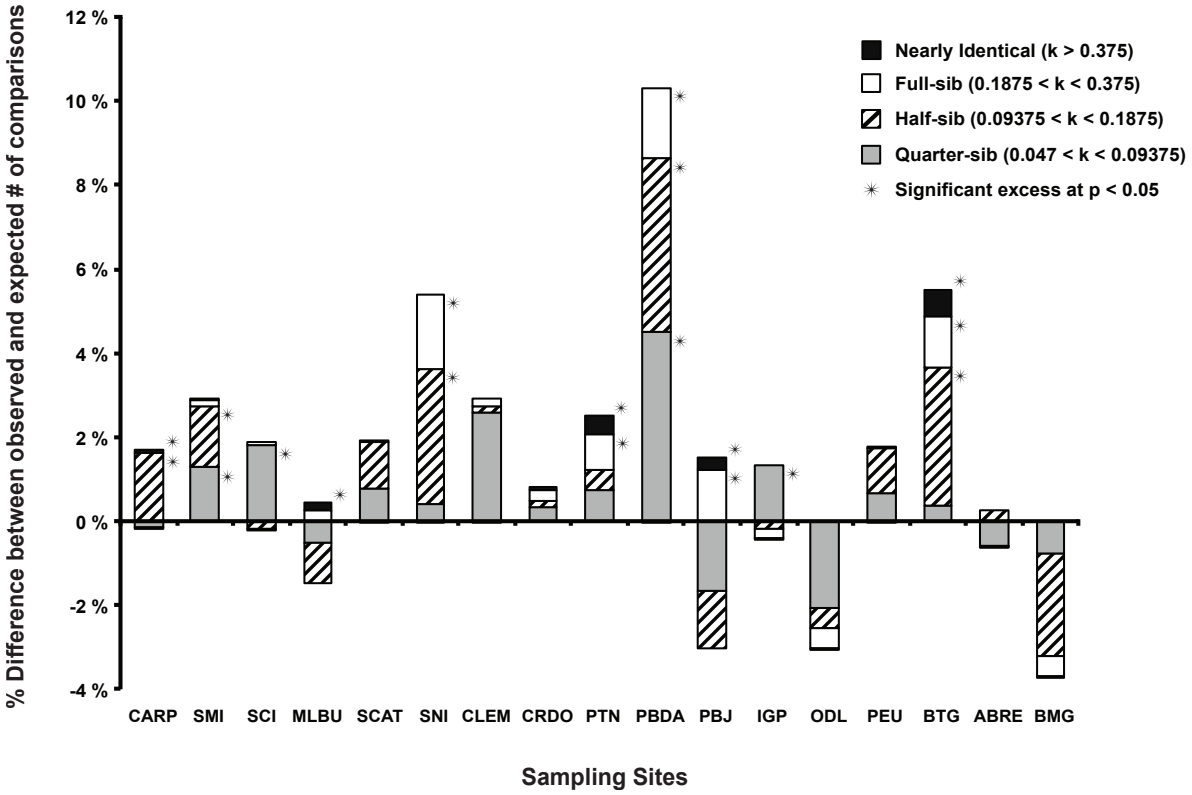

Figure 6.4. Histogram of the proportion of kinship observed for each site that is in excess of expected levels due to chance in four kinship categories: "quarter-sib", $0.047 < k < 0.09375$; "half-sib", $0.09375 < k < 0.1875$; "full-sib", $0.1875 < k < 0.375$; and "nearly identical", $0.375 < k < 0.570$. Asterisks indicate significant ($P < 0.05$) differences between the observed and expected proportion of kinship comparisons within that category at that site. Expected kinship levels were constructed using 10,000 permutations of all kinship values across all sites without replacement to generate the distribution of kinship values that should occur if individuals were randomly distributed among sites. The nearly identical bin represents comparisons above full-sibs, and is based on our kinship coefficient distribution for comparisons of individuals to themselves. Counts of the actual number of pairwise comparisons at each site that fell within each kinship category are listed in Table S6.3.

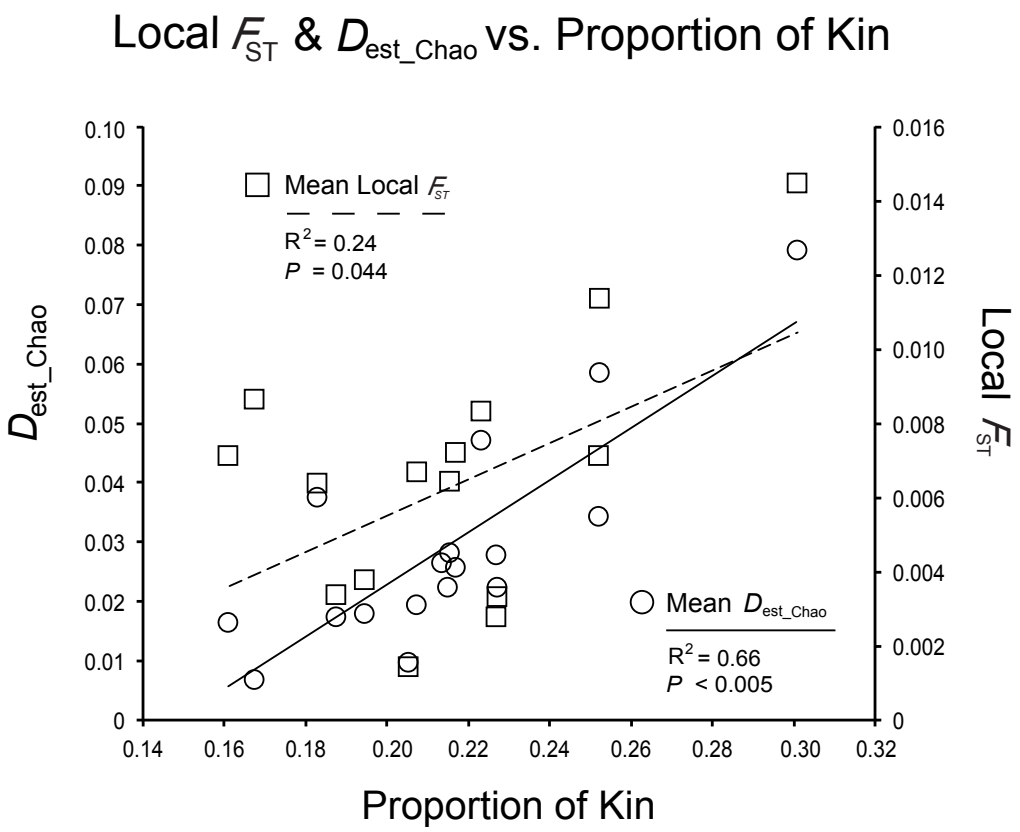

Figure 6.5. Linear regression of mean pairwise D_{est_Chao} (circles, solid line) and local F_{ST} (squares, dashed line) for seven microsatellite loci. Both metrics were regressed on the proportion of within-site kinship comparisons at each site that are significantly ($P < 0.05$) greater than k = 0.047 (the lower boundary of the four designated kinship categories).

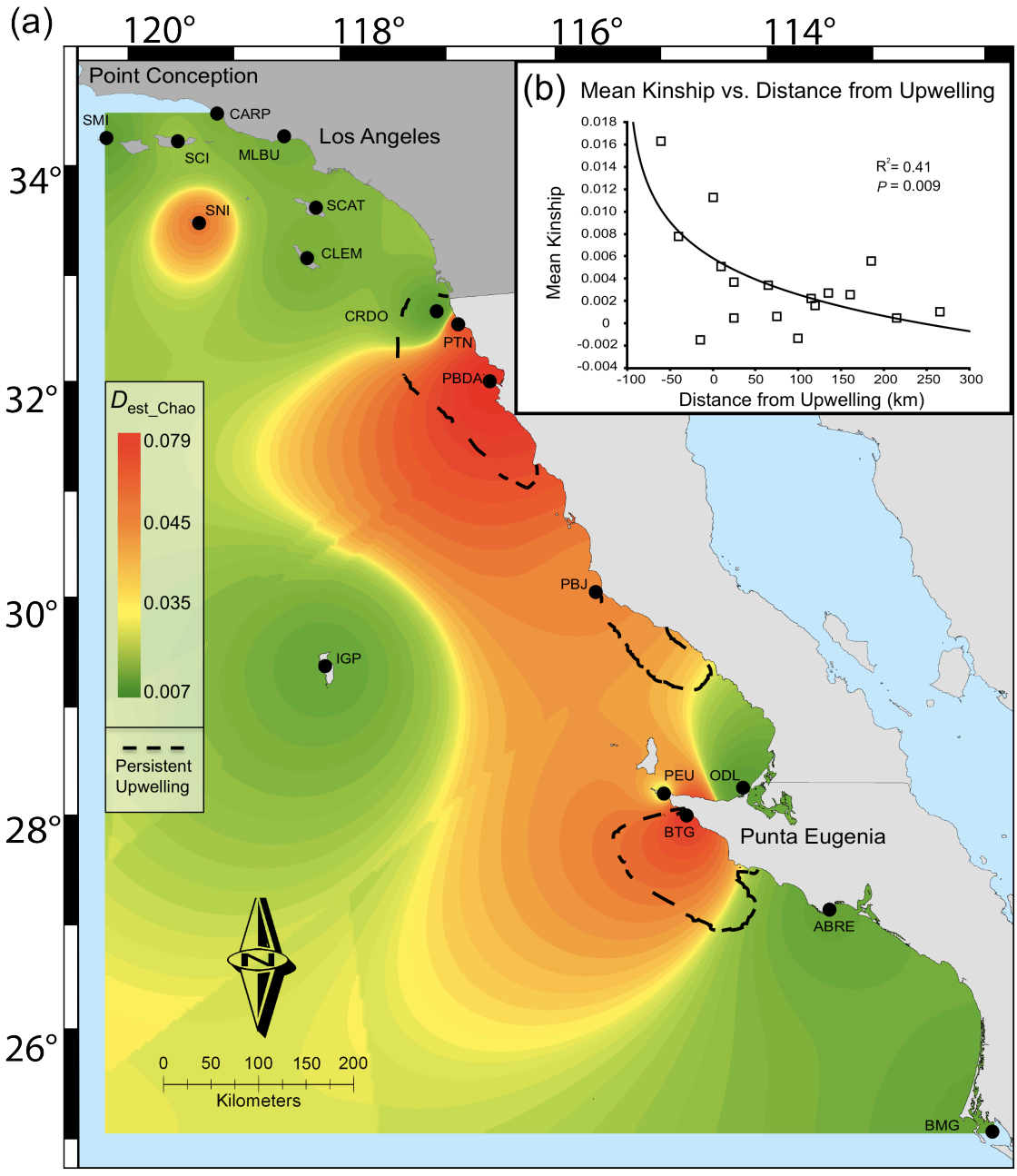
(a)
120°
118°
116°
114°
34°
32°
30°
28°
26°
Point Conception
SMI
CARP
SCI
MLBU
Los Angeles
SNI
SCAT
CLEM
CRDO
PTN
PBDA
PBJ
IGP
PEU
ODL
BTG
Punta Eugenia
ABRE
BMG
D_{est_Chao}
0.079
0.045
0.035
0.007
Persistent Upwelling
N
0 50 100 150 200
Kilometers
(b) Mean Kinship vs. Distance from Upwelling
Mean Kinship
0.018
0.016
0.014
0.012
0.010
0.008
0.006
0.004
0.002
0
-0.002
-0.004
R^2= 0.41
P = 0.009
-100 -50 0 50 100 150 200 250 300
Distance from Upwelling (km)

Figure 6.6. (a) Map of lobster specimen collection locations overlaid with an inverse weighted distance (IWD) interpolation of mean D_{est_Chao} values at each site created using the Spatial Analyst extension in ArcGIS 10. Red coloration represents areas of greatest genetic differentiation, green represents areas of little genetic differentiation, and yellow and orange represent the graded values between these extremes. Mean kinship values at each site showed the same pattern as D_{est_Chao}, with the highest kinship in red, and lowest in green. Dashed lines circle areas of consistently high upwelling intensity (adapted from Zaytsev *et al.* 2003). There are also areas of high upwelling intensity at Point Conception, and one south of BMG that are not captured by this map. (b) Log-linear regression of mean kinship at each site on the distance (km) to the nearest edge of an area of high upwelling intensity (from (a)).

Supplemental Information

Table S6.1. Isolation by distance (IBD) results from Mantel tests conducted in GenoDive 2.0b20 to examine patterns of genetic differentiation across multiple spatial scales based on sampling locations: All sites, only California sites, only Mexico sites, only continental sites, and only island sites. Data reported include the marker used (Marker), the metric used (Metric), the IBD equation (Equation), the number of sites included in the analysis (N-sites), the error sum of squares (Error SS), the total sum of squares (Total SS), the r-squared value (R^2), and the *P*-value (*P*-value). Although there is one significant comparison (*), this was not significant after correcting for multiple comparisons.

Sample Sites	Marker	Metric	Equation	N-sites	Error SS	Total SS	R^2	*P*-value
All Sites	Microsatellites	Jost D_{est_Chao}	Y = 0.051 + (-0.004 * X)	17	0.125	0.126	0.009	0.198
		F_{ST}	Y = 0.007 + (-0.000 * X)	17	0.002	0.002	0.011	0.177
	Cytochrome Oxidase I	Jost D_{est_Chao}	Y = 0.063 + (-0.006 * X)	17	1.094	1.097	0.003	0.330
		Φ_{ST}	Y = -0.002 + (0.001 * X)	17	0.014	0.014	0.012	0.154
California Sites Only	Microsatellites	Jost D_{est_Chao}	Y = 0.022 + (0.000 * X)	7	0.004	0.004	0	0.470
		F_{ST}	Y = 0.002 + (0.000 * X)	7	0	0	0.002	0.401
	Cytochrome Oxidase I	Jost D_{est_Chao}	Y = 0.052 + (0.002 * X)	7	0.168	0.168	0	0.416
		Φ_{ST}	Y = 0.006 + (0.000 * X)	7	0.001	0.001	0	0.477
Mexico Sites Only	Microsatellites	Jost D_{est_Chao}	Y = 0.071 + (-0.006 * X)	10	0.065	0.066	0.017	0.266
		F_{ST}	Y = 0.009 + (-0.001 * X)	10	0.001	0.001	0.018	0.256
	Cytochrome Oxidase I	Jost D_{est_Chao}	Y = -0.009 + (0.003 * X)	10	0.227	0.227	0.001	0.433
		Φ_{ST}	Y = -0.010 + (0.003 * X)	10	0.007	0.007	0.039	0.126
Continental Sites Only	Microsatellites	Jost D_{est_Chao}	Y = 0.070 + (-0.005 * X)	10	0.058	0.058	0.014	0.282
		F_{ST}	Y = 0.009 + (-0.001 * X)	10	0.001	0.001	0.014	0.278
	Cytochrome Oxidase I	Jost D_{est_Chao}	Y = -0.073 + (0.013 * X)	10	0.162	0.167	0.030	0.178
		Φ_{ST}	Y = -0.015 + (0.004 * X)	10	0.007	0.007	0.075	0.063
Island Sites Only	Microsatellites	Jost D_{est_Chao}	Y = 0.057 + (-0.008 * X)	7	0.004	0.005	0.094	0.108
		F_{ST}	Y = 0.008 + (-0.001 * X)	7	0	0	0.093	0.111
	Cytochrome Oxidase I	Jost D_{est_Chao}	Y = -0.050 + (0.026 * X)	7	0.301	0.306	0.017	0.300
		Φ_{ST}	Y = 0.020 + (-0.003 * X)	7	0	0	0.258	0.022*

Table S6.2. Locus-specific diversity indices and F_{ST} values as calculated in GenoDive for each of the seven nuclear microsatellite loci, and null allele frequency for each microsatellite locus as calculated in *ML-Relate*. Metrics included are the total number of alleles for each locus (N), effective number of alleles for each locus (A_{eff}), observed heterozygosity (Ho), within-population heterozygosity (Hs), total heterozygosity (Ht), F_{ST} (F_{ST}), significance test for F_{ST} (*P*-value (F_{ST})) based on 100,000 permutations in GenoDive, percentage of genotyping error (% Genotyping Error), percentage of null alleles (% Null Alleles), and the *P*-value from a Monte-Carlo randomization test (20,000 randomizations) of whether the null-allele frequency is significant (*P*-value (NA)).

Locus	N	A_{eff}	Ho	Hs	Ht	F_{ST}	*P*-value (F_{ST})	% Genotyping Error	% Null Alleles	*P*-value (NA)
A110	5	2.203	0.497	0.552	0.552	0.001	0.296	0.00%	3.69%	0.0035
A5	35	11.412	0.943	0.921	0.924	0.003	0.002	1.35%	0.00%	0.3612
A102r	37	13.084	0.875	0.933	0.935	0.003	0.010	4.05%	3.10%	0.0001
pin244	104	23.301	0.909	0.967	0.970	0.004	< 0.0005	2.70%	2.95%	< 0.00005
pin29L	75	23.036	0.842	0.967	0.969	0.002	0.002	4.05%	6.63%	< 0.00005
pin10	35	10.137	0.897	0.911	0.920	0.011	< 0.0005	1.35%	1.20%	0.0098
pin189	41	14.852	0.858	0.943	0.944	0.002	0.023	2.70%	4.60%	< 0.00005

Table S6.3. Comparison of *ML-Relate* calculations of relatedness (*r*) and GenoDive calculations of kinship for each sampling location. Counts of Relatedness (*r*) represent the number of individual pairwise comparisons that had the lowest log-likelihood score for each category, as calculated in *ML-Relate*: UR (most likely unrelated), HS (most likely half-sibs), FS (most likely full-sibs), PO (most likely parent-offspring). Counts of Kinship represent the number of pairwise comparisons that fell within the categories of kinship as described in the text: unrelated (UN), $k < 0.047$; quarter-sib (QS), $0.047 < k < 0.09375$; half-sib (HS), $0.09375 < k < 0.1875$; full-sib (FS), $0.1875 < k < 0.375$; and nearly identical (NI), $k > 0.375$. The Total Number of Pairwise Comparisons is the same for both metrics. Also reported are the mean site-specific values of relatedness (*r*), as calculated by taking the mean value of all maximum likelihood estimates of (*r*) for all pairwise individual comparisons within a site (*ML-Relate* Mean (*r*)), and the mean values of the Loiselle *et al.* 1995 coancestry metric, as calculated in GenoDive (Loiselle Mean Kin). Site-specific mean (*r*) and mean kin are significantly correlated (Pearson $R = 0.92$, $P < 0.0005$).

	Counts of Relatedness (*r*)				**Counts of Kinship**					**Total Number**	***ML-Relate***	**Loiselle**
Site	**UR**	**HS**	**FS**	**PO**	**UR**	**QS**	**HS**	**FS**	**NI**	**of Comparisons**	**Mean (*r*)**	**Mean Kin**
CARP	2415	278	8	0	2125	350	212	12	2	2701	0.0364	0.0025
SMI	2522	312	16	0	2203	410	218	18	1	2850	0.0396	0.0035
SCI	1289	135	7	0	1124	213	86	8	0	1431	0.0355	0.0016
MLBU	2243	220	22	0	2019	312	131	18	5	2485	0.0375	0.0005
SCAT	2860	358	22	0	2537	449	237	17	0	3240	0.0433	0.0027
SNI	613	82	7	1	526	95	66	16	0	703	0.0524	0.0056
CLEM	267	32	1	0	232	47	19	2	0	300	0.0378	0.0022
CRDO	1770	174	10	0	1552	262	124	15	1	1954	0.0361	-0.0014
PTN	1400	167	29	0	1240	221	107	21	7	1596	0.0504	0.0078
PBDA	902	167	12	0	756	190	112	23	0	1081	0.0575	0.0163
PBJ	3882	396	93	0	3573	499	212	74	13	4371	0.0439	0.0051
IGP	2092	241	13	0	1860	338	142	6	0	2346	0.0404	0.0010
ODL	782	77	2	0	717	95	49	0	0	861	0.0336	-0.0013
PE	858	128	4	0	777	136	72	5	0	990	0.0417	0.0037
BTG	668	97	15	0	583	105	74	13	5	780	0.0543	0.0112
ABRE	1910	227	8	0	1728	268	139	10	0	2145	0.0391	0.0006
BMG	960	118	3	0	907	133	41	0	0	1081	0.0352	0.0005

Table S6.4. Comparison of *ML-Relate* calculations of relatedness (*r*) and GenoDive calculations of kinship for each sampling location. Counts of Relatedness (*r*) represent the number of individual pairwise comparisons that had the lowest log-likelihood score for each category, as calculated in *ML-Relate*: UR (most likely unrelated), HS (most likely half-sibs), FS (most likely full-sibs), PO (most likely parent-offspring). Counts of Kinship represent the number of pairwise comparisons that fell within the categories of kinship as described in the text: unrelated (UN), $k < 0.047$; quarter-sibs (QS), $0.047 < k < 0.09375$; half-sibs (HS), $0.09375 < k < 0.1875$; full-sibs (FS), $0.1875 < k < 0.375$; and nearly identicals (NI), $k > 0.375$. The Total Number of Pairwise Comparisons is the same for both metrics. Also reported are the mean site-specific values of relatedness (*r*), as calculated by taking the mean value of all maximum likelihood estimates of (*r*) for all pairwise individual comparisons within a site (*ML-Relate* Mean (*r*)), and the mean values of the Loiselle *et al.* 1995 coancestry metric, as calculated in GenoDive (Loiselle Mean Kin). Site-specific mean (*r*) and mean kin are significantly correlated (Pearson $R = 0.92$, $P < 0.0005$).

	Counts of Relatedness (*r*)				**Counts of Kinship**					**Total Number**	***ML-Relate***	**Loiselle**
Site	**UR**	**HS**	**FS**	**PO**	**UR**	**QS**	**HS**	**FS**	**NI**	**of Comparisons**	**Mean (*r*)**	**Mean Kin**
CARP	2415	278	8	0	2125	350	212	12	2	2701	0.0364	0.0025
SMI	2522	312	16	0	2203	410	218	18	1	2850	0.0396	0.0035
SCI	1289	135	7	0	1124	213	86	8	0	1431	0.0355	0.0016
MLBU	2243	220	22	0	2019	312	131	18	5	2485	0.0375	0.0005
SCAT	2860	358	22	0	2537	449	237	17	0	3240	0.0433	0.0027
SNI	613	82	7	1	526	95	66	16	0	703	0.0524	0.0056
CLEM	267	32	1	0	232	47	19	2	0	300	0.0378	0.0022
CRDO	1770	174	10	0	1552	262	124	15	1	1954	0.0361	-0.0014
PTN	1400	167	29	0	1240	221	107	21	7	1596	0.0504	0.0078
PBDA	902	167	12	0	756	190	112	23	0	1081	0.0575	0.0163
PBJ	3882	396	93	0	3573	499	212	74	13	4371	0.0439	0.0051
IGP	2092	241	13	0	1860	338	142	6	0	2346	0.0404	0.0010
ODL	782	77	2	0	717	95	49	0	0	861	0.0336	-0.0013
PE	858	128	4	0	777	136	72	5	0	990	0.0417	0.0037
BTG	668	97	15	0	583	105	74	13	5	780	0.0543	0.0112
ABRE	1910	227	8	0	1728	268	139	10	0	2145	0.0391	0.0006
BMG	960	118	3	0	907	133	41	0	0	1081	0.0352	0.0005

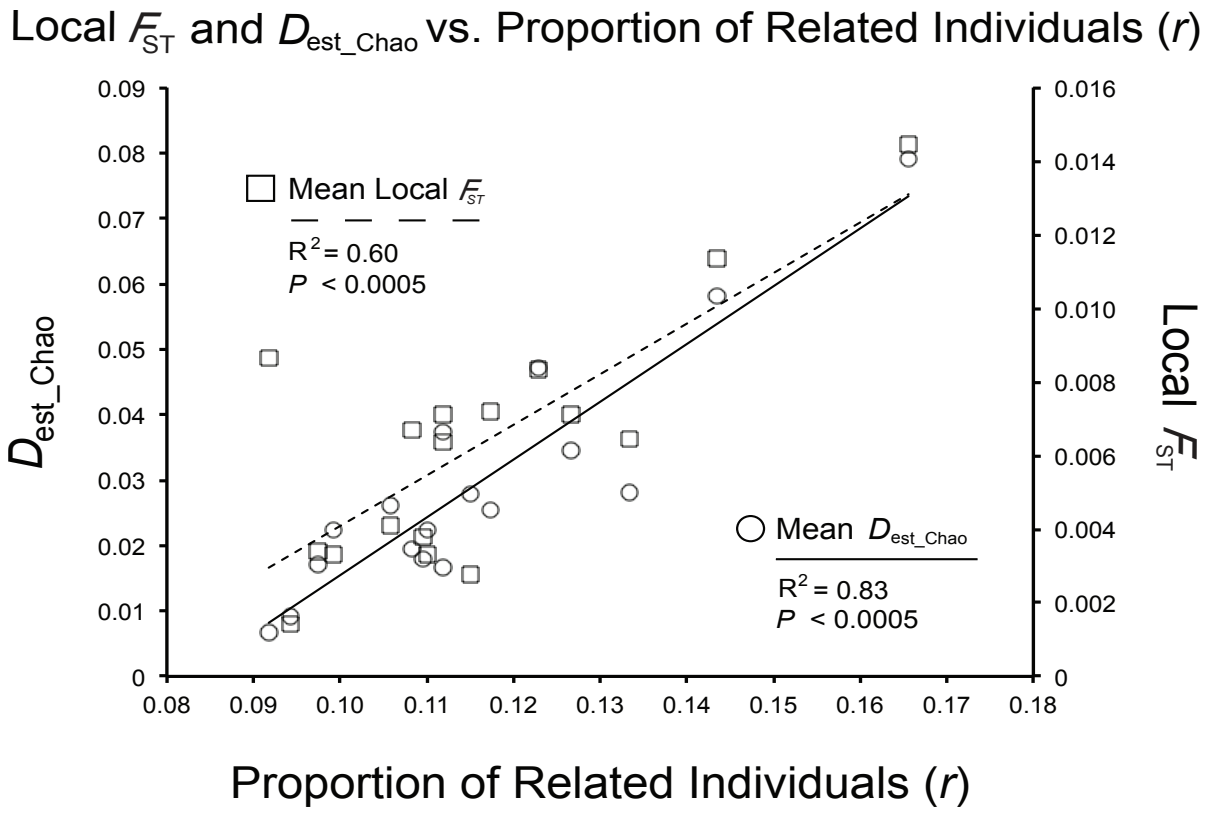

Figure S6.1. Linear regression of mean pairwise local F_{ST} (squares, dashed line) and D_{est_Chao} (circles, solid line) at each site for seven microsatellite loci. Both metrics were regressed on the proportion of combined within-site half (r = 0.25) and full (r = 0.5) sibs as calculated using a maximum likelihood estimation of relatedness (r) in ML-Relate. Only comparisons where half or full sibs were determined to be both a significant ($P < 0.05$) and the most likely relationship were included. The linear regression of F_{ST} versus proportion of related individuals, though not shown here, is also significant ($R^2 = 0.82$, $P < 0.0005$).

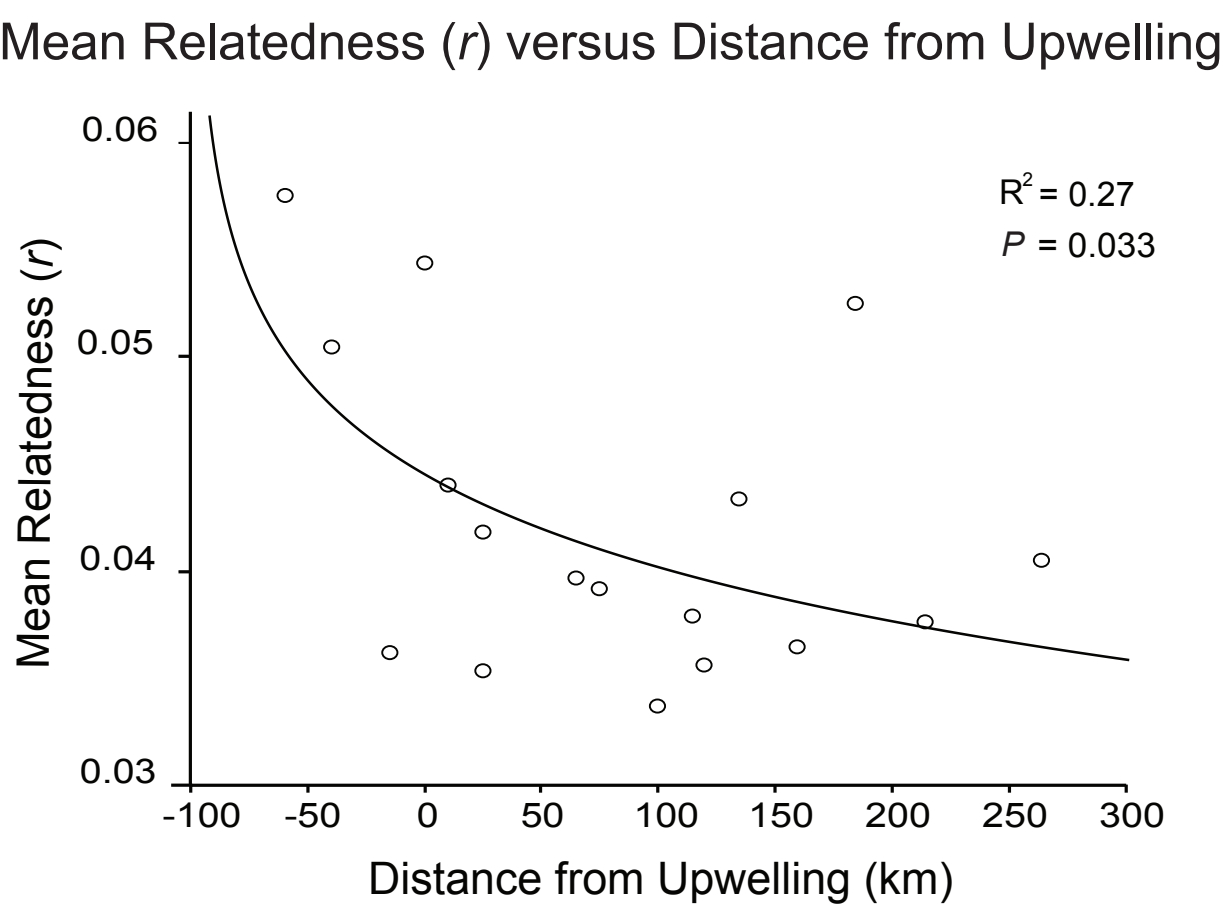

Figure S6.2. Log-linear regression of mean relatedness (r) at each site on the distance (km) to the nearest edge of an area of high upwelling intensity (from Fig. 6.6a).

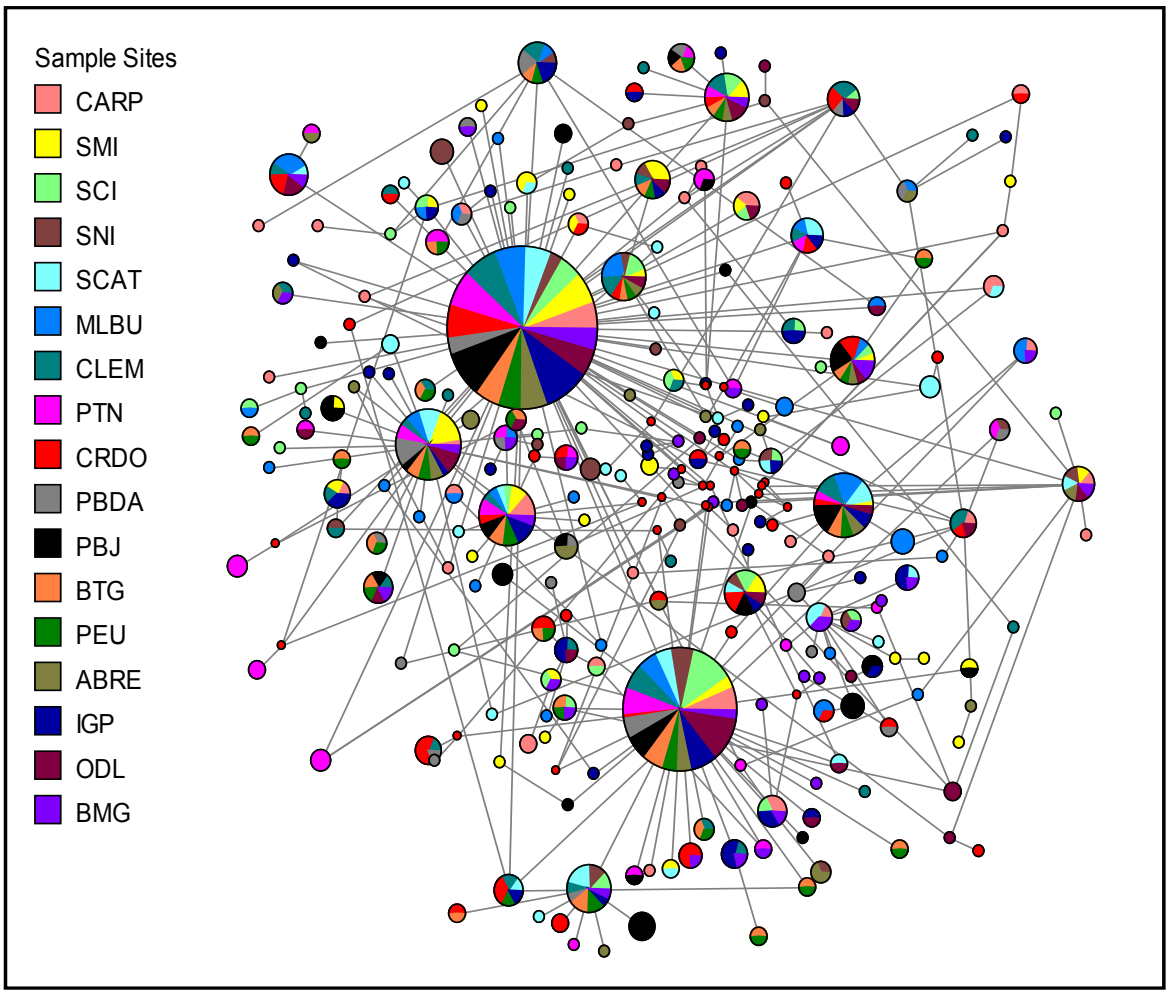

Figure S6.3. Median-joining network for Panulirus interruptus mtDNA, constructed using 454 base pairs of cytochrome *c* oxidase subunit I (COI) from each of 931 individuals in the program NETWORK. Each circle is a unique haplotype proportional in size to the number of individuals with that haplotype. The two largest circles represent 235 and 95 individuals. The smallest circle represents one individual. Colors correspond to one of 17 locations where the individual haplotypes were found (see key, Fig. 6.1, Table 6.1). Lines here are not representative of distance between haplotypes, although most haplotypes only differ by one base pair (see Fig. 6.2).

CHAPTER VII

SUMMARY AND SYNTHESIS: NOT ALL THOSE WHO WANDER ARE LOST

Summary

When Odysseus returns to the house of Aeolus, after nearly arriving at his Ithaca home, Aeolus and his family ask, "How came you here Odysseus? What hostile power assailed you? With care we sent you forth, to let you reach your land and home or anywhere you pleased." (Homer, trans by Palmer 2003). This metaphorically describes the goal of this dissertation, which is to gain a richer understanding of the phyllosomal odyssey: the maximum distances traveled, the frequency of exchange between locations, the proportion of larvae that settle close to their site of release, and the factors that influence these parameters. In this dissertation, I examined the scales of population genetic connectivity in three species of spiny lobsters across their full species distributions: *Panulirus penicillatus* from the Red Sea to the East Pacific Ocean, *Panulirus marginatus* across the Hawaiian Archipelago, and *Panulirus interruptus* from Point Concepcion, California to Bahía Magdalena, Baja California, Mexico. Each of these species in the genus *Panulirus* has a pelagic larval duration (PLD) exceeding 180 days (Phillips 2006), which is near the upper extreme for PLDs in the sea (Thorson 1950). Through these genetic surveys, my aim was to understand more clearly the role of the teleplanic phyllosoma larval phase in maintaining population connectivity for these species. Teleplanic larvae originate in the continental-shelf benthos, but are often found in the open ocean due to a long larval duration that provides a means for dispersal over vast distances (Scheltema 1971). These larva have been hypothesized not only to allow the colonization of new regions in a random, waif-like manner, but also to sustain gene flow across a full species distribution over longer periods of time (Scheltema 1971).

Alternatively, Johnson (1971, 1974) proposed that the larvae found far offshore, past local entrainment features, had a much lower probability of survival, and therefore represented a loss from their site of origin, rather than a potential recruit to a distant site. Finally, Strathmann et al. (2002) hypothesize that the long PLD did not evolve to facilitate the broad dispersal of larvae, but rather to avoid predation during the larval phase. In this case, the larvae found far offshore would be temporarily residing in an environment that is favorable to the survival of unprotected larval-stage individuals. Within this framework of hypotheses, I was fundamentally interested in addressing two questions regarding the dispersal patterns of teleplanic larvae across the wine-dark seas:

1. Despite the recent evidence for only a weak link between PLD and dispersal distance for PLD < ~60 days, is there an upper threshold PLD level, over which pelagic larvae are able to overcome dispersal barriers and maintain connectivity across broad geographic scales?

2. Do marine species with teleplanic larvae have sufficiently high levels of gene flow to sustain population connectivity on both short and long time scales throughout their full species distributions?

In the second line of the poem, "All that is Gold Does Not Glitter," Tolkien (1954) writes, "Not all those who wander are lost." Although the phyllosomal odyssey is long, likely tumultuous, and seemingly aimless, my data suggest that not all the larvae far adrift are lost, as suggested by Johnson (1971, 1974). I first examine genetic connectivity using mitochondrial DNA (mtDNA) sequence data for *Panulirus penicillatus* (Olivier, 1791) across the full extent of its species distribution. *Panulirus penicillatus* has the broadest distribution of any spiny lobster species, and is found from the Red Sea, across the Indian Ocean, the Western and Central Pacific Oceans and across to a number of islands in the East Pacific Ocean. This species served as a litmus test for my first question: Is there an upper threshold PLD level, over which pelagic larvae could overcome all dispersal barriers? *Panulirus penicillatus* maintains high levels of gene flow across broad extents of the species range. This includes high connectivity across the Indo-Pacific biogeographic barrier, which limits species distributions for many reef organisms and serves as a strong biogeographic barrier for others. However, *P. penicillatus* was significantly differentiated across its range, with the highest global Φ_{ST} value recorded for a Panulirid to date. Of particular note, there has likely been no recent gene flow across the East Pacific Barrier, which Darwin (1872) deemed 'impassable' for marine organisms. Based on my data, the East Pacific *Panulirus penicillatus* 'red,' as designated by George (2006) deserves species-level recognition, distinct from the Indo-West Pacific *P. penicillatus*. In addition to the genetic divide at the East Pacific Barrier, I found significant genetic breaks between the Sino-Japanese Province and the rest of the tropical Indo-Pacific, as well as relative genetic isolation of a number of specific sites within the Indo-Polynesian province, while other sites within this province are well connected to locations throughout the species distribution.

This data refutes the hypothesis that there is an upper threshold above which no barrier to gene flow is maintained. *Panulirus penicillatus* exhibits significant structure at a number of locations as well as strong regional barriers throughout its range despite an estimated nine-month PLD. Particularly for the East Pacific Barrier, it may be only the occasional random, 'lost' phyllosoma that manages to cross, leading to isolation of these populations over time.

Although not as far removed as the East Pacific populations, one isolated region of the *P. penicillatus* range is the Hawaiian Archipelago. *Panulirus penicillatus* sites in the Northwestern Hawaiian Islands were significantly genetically differentiated from a number of sites throughout the Indo-Pacific. There is a second species of *Panulirus* in Hawai'i, *P. marginatus* (Quoy & Gaimard, 1825), which is endemic to the Hawaiian Islands. I compared the patterns of genetic connectivity between these two congeners across their sympatric distribution in Hawai'i to determine whether the same processes restricting the distribution of *P. marginatus* to the Hawaiian Archipelago were also isolating populations of *P. penicillatus* there. Although each species shows genetic structure between the Main Hawaiian Islands and the Northwest Hawaiian Islands, there was not significant differentiation for *P. penicillatus* across the archipelago as a whole, and almost all of the genetic structure for *P. penicillatus* is driven by isolation of two of the northernmost atolls. In contrast, *P. marginatus* has significant genetic structure across the Hawaiian archipelago, which is mostly driven by sites in the Main Hawaiian Islands, especially Kaua'i and Maui. My data from Hawai'i indicate that the phyllosoma larvae of *P. marginatus* disperse across the full species distribution, although at a higher frequency between specific locations than between others.

There was higher overall genetic differentiation for the endemic *P. marginatus* across its full species range than there was for *P. penicillatus* at an outpost of its range; however, there were no other cross-species or location-specific generalizations that could be gleaned from this comparison. Each species showed a unique pattern of genetic exchange.

My data from the investigation of *Panulirus interruptus* connectivity patterns across southern California and Pacific Baja California, Mexico serves as the most convincing evidence that the teleplanic phyllosoma are truly on an odyssey: traveling for many a rosy-fingered dawn, often covering long distances, but with some also getting caught up along the way, many dying, and many eventually making it back to their place of origin. The mtDNA data from *P. interruptus* provides evidence that specific sites are more genetically distinct from all other sites throughout the species range, but with no discernable pattern to what may be driving this differentiation.

In both *P. marginatus* and *P. penicillatus* datasets, I also observed high levels of connectivity between some sites even at the far ends of each of the species distributions, but I documented other sites that were inexplicably isolated. For *P. interruptus*, I delved deeper into the problem by adding seven microsatellites to the analysis. The benefit of this was not necessarily the higher diversity which may lead one to believe I am looking at a shorter time-scale; my mtDNA marker already had ~95% haplotype diversity. Rather, multiple markers, each with high diversity, allowed me to assess relationships between individuals, rather than populations. This additional data provided evidence that the sites with the greatest level of differentiation from other sites also had the highest proportion of kin within that site. The most closely related individuals were more frequently found at the same location, rather than at different sites.

The elevated levels of kinship at specific sites could be driven by localized recruitment, or by timed settlement of related individuals (i.e. larvae that are released together stay with each other throughout the odyssey and settle together in one location). To further understand what processes may be driving either of these phenomena at specific sites but not others, I examined oceanographic phenomena. The majority of the genetically isolated sites occurred within Baja California, Mexico, where there is a much stronger and more consistent upwelling regime, rather than in the Southern California Bight, where upwelling is almost non-existent. The proportion of kin at a site was positively correlated with the proximity of a site to an upwelling center: the closer to an upwelling center, the higher the proportion of kin.

The prospect that larvae stay in the plankton for 240-330 days and return to settle near their site of release, or stay together as a group of related individuals and settle in the same location seems unlikely at first. However, the site-specific kinship patterns in my data match theoretical predictions for a species that has evolved a long PLD to avoid predation during the larval phase rather than to facilitate broad dispersal of larvae (Strathmann *et al.* 2002). The extended PLD may enable phyllosoma to disperse far offshore, into a pelagic environment that is favorable for the survival of unprotected larval-stage individuals (Strathmann *et al.* 2002). If this behavior is selectively advantageous, we should expect to observe enhanced local recruitment regardless of PLD. Future studies should consider what other factors might facilitate local retention of larvae, or cause larvae to settle out at one location.

Synthesis

Through these combined studies of three spiny lobsters species with PLDs > 180 days, I broaden our knowledge of the natural history of the pelagic phase of spiny lobster life, and I directly assess the generality of the relationship between pelagic larval duration and dispersal scale in teleplanic larval dispersers. A few overall themes can be gleaned from this dissertation:

1. **There does not appear to be an upper PLD threshold above which any biogeographical barrier may be crossed.** All three spiny lobsters have PLD >180 days, yet all three have significant genetic structure (though varied in magnitude) across their species distributions. The longest stretch of pelagic ocean the phyllosoma of any of these species would have to cross is the 'impassable' East Pacific Barrier (EPB). Passive particle transport models predict that the EPB would be traversed in 50-81 days under strong El Niño conditions and in 100-155 days under normal conditions (Wyrtki et al. 1981, Richmond 1990). A PLD of 180 days is greater than both of these estimates, and for *P. penicillatus*, the actual PLD is estimated to be between 8.3 and 9.4 months (Matsuda 2006), which is almost double the maximum number of days required to cross the EPB under normal conditions. Based on the genetic evidence here, *P. penicillatus* has crossed the EPB in the past, but it does not occur often, and may not have occurred over recent ecological time scales. This demonstrates that other factors beyond PLD may limit dispersal distance to much smaller spatial scales than PLD predicts.

2. **Marine species with teleplanic larvae do have sufficiently high levels of gene flow to sustain population connectivity throughout much of their species distributions, if not all.** For all three species, there were high levels of connectivity across large geographic scales. For example, in both *P. marginatus* and *P. penicillatus*, despite significant regional differentiation between the Main Hawaiian Islands and the Northwest Hawaiian Islands, some sites in each geographic region were undifferentiated from sites in the geographic region at the other end of the archipelago. Similarly, for example, the Marquesas, one of the most isolated archipelagos in the Pacific, was undifferentiated from either of the *P. penicillatus* collection sites in the Marianas Archipelago, or even from Zanzibar in the far Western Indian Ocean. For *P. interruptus*, there was no differentiation between some of the northernmost Channel Island sites in California, and Bahía Magdalena, at the southern extent of the species range. In all three species, sites at the extreme ends of the species range showed evidence of high levels of gene flow between them.

3. **Site-specific patterns were apparent within species.** Despite the lack of genetic differentiation between some sites located at opposite ends of the distribution of each species, sites that were close together were often the most genetically distinct. There were no patterns of isolation by distance for any of the study species at any spatial scale, nor many generalizable regional patterns. While there were signals of regional isolation, especially for *P. penicillatus*, in general, most of the patterns I observed were site-specific. Within each study, there were certain places that were genetically differentiated from all or most all other sites, and I tested upwelling as one mechanism potentially driving these patterns in *P. interruptus*. For tropical species (*P. marginatus* and *P. penicillatus*), upwelling is less likely to play a strong role: future studies should examine potential isolating factors in the tropics based on genetic data collected to date.

4. **Species-specific patterns were evident, even across the same sites.** While within species, site-specific patterns were evident; the same sites did not exhibit the same patterns of connectivity between species when I looked at sympatric distributions of *Panulirus marginatus* and *P. penicillatus* in Hawai‘i. This difference indicates that any species of interest should be looked at individually, and that species cannot serve as proxies to infer patterns of genetic connectivity in other species. Different connectivity patterns have been documented for congeneric species in other species suites both in Hawai‘i and elsewhere (Rocha et al. 2002, Bird et al. 2007, Crandall et al. 2008, Gaither et al. 2010, Skillings et al. in press), limiting the extent of inference that can be made from a genetic survey of one species. However, there is potential to derive regional generalizations when enough species are examined (i.e. Toonen et al. 2011).

5. **Additional data provides additional insight.** In Greek, "Odysseus" means, "trouble," and Homer documents plenty of trouble for Odysseus on that first odyssey (Homer, trans by Palmer 2003). It is not surprising then that, like any dissertation, there were many obstacles to completing this investigation of the phyllosomal odyssey, including difficulties finding and collecting lobsters, obtaining permits, finding markers that work consistently and reliably, and discerning reality from the seeming genetic chaos. The most frustrating, yet intriguing dataset came from *Panulirus interruptus*; and the addition of the microsatellite markers allowed me to take a novel approach and investigate kin relationships, rather than just compare population means. This analysis methodology enabled me to explain otherwise indiscernible patterns in the data, and then led me to seek a phenomenon driving the increased kinship at some sites, but not others, and I discovered that upwelling may be a strong component. With the advent of next generation sequencing technology and the ease of generating numerous genetic markers for a study system, this is the approach that should be taken in the immediate future. Until we are able to obtain whole genomes for every organism in our studies; kinship, and where possible, parentage analyses provide much better insights into questions of population connectivity, especially on shorter time scales. By obtaining this type of genetic data at finer spatial scales and coupling it with oceanographic or ecological data (which is also being collected in droves), we should be able to move towards more of a process-based understanding of connectivity patterns, which in turn, will allow for greater predictive capability.

Fisheries Management Implications

In addition to a fundamental understanding of population connectivity in teleplanic dispersers, the data generated in this dissertation should contribute to the scientific foundation of prudent management of these valuable marine species. There are a number of implications of these data for fisheries management, depending on the scale under consideration, and the species of interest:

1. From the three species examined in this dissertation, the one broad recommendation I would give about how population genetic data can be useful for fishery management is that these studies can certainly aid in management decisions, but they need to be designed to answer exactly the question of interest. This may sound logical, but expense, time, and human nature dictate that we make generalizations among species or across sites for a suite of species to facilitate management implementation. Considering all of the information generated to date, and the approaches currently available, this approach will not usually be fruitful. The results for one species are only likely to be informative for other species across genetic barriers that are already well known from information on species distributions or oceanographic phenomena (i.e., the East Pacific Barrier), and there are exceptions to even these (i.e., *P. penicillatus* across the Indo-Pacific Barrier). While surveys of 50 or more species may provide insight into shared barriers across a region (i.e., Toonen et al. 2011), if there are specific management goals for certain species or specific Marine Protected Area designs that are under consideration, I would recommend that those specific species and those specific sites be studied to understand the potential genetic connectivity patterns.

As chapters three and four indicate, even conspecific lobster species that are sympatric to the extent that they share dens together in some locations do not have generalizable patterns of connectivity across Hawai‘i at the spatial scales relevant to management. And my data for *Panulirus interruptus* in chapter six indicate that even sites sampled relatively closely to one another (e.g., Punta Eugenia and Laguna Ojo de Liebre) have very different interconnectedness with other sites across the species range depending on the oceanographic features they are influenced by. The relevant spatial scale at which these factors shift is yet to be determined, but it is safe to say that management plans can benefit greatly from genetic data, as long as the precise species and sites under consideration are analyzed explicitly.

2. *Panulirus penicillatus* across the Pacific: The most obvious recommendation from this data is that the East Pacific populations and the rest of the distribution should be managed as two separate species. This is likely occurring already, with the Galapagos fishery being managed within the islands and the Revillagigedos likely managed locally as well (based on the way other fisheries in Mexico are managed). One consideration here would be what the status of those populations would be going forward, if indeed they are a separate species. Future work (including the samples I have not yet added to this study) will reveal the level of population subdivision within the East Pacific, and those results may warrant a closer look at the vulnerability status of those populations/species. Also of note is the distinction of the samples from Japan.

More in-depth research will be required to determine the scale at which this population is subdivided, though it is likely that all of these sites are managed by specific provinces within Japan. Because this is the case for most countries, which likely manage at the countrywide level, some of the more relevant results of this study for management are the vast stretches of islands over which there is no genetic structure. For the countries included in this study, it is notable that many of them may benefit from cooperatively managing their marine resources with their island neighbors (at least *P. penicillatus* from this study). For countries interested in this approach, future studies could specifically investigate migration rates between multiple island nations to determine potential source and sink locations if designing multi-country management plans.

3. *Panulirus marginatus* and *Panulirus penicillatus* in Hawai‘i: As discussed in chapters three and four, the investigation of these two species across the Hawaiian Archipelago was revealing in the sense of the lack of a general management scheme that could be derived for both species given the current genetic data. What is clear is that the Papahānaumokuākea Marine National Monument (PMNM) is not assisting with the management of either of these species. Although there is high gene flow throughout the archipelago for both of these species, the magnitude of flow is mostly to the Northwest Hawaiian Islands (NWHI) for *Panulirus penicillatus*, not from the PMNM to the main Hawaiian Islands (MHI). For *P. marginatus*, the magnitude is not as drastically different and the dominant direction is less clear, but the stocks in the NWHI have failed to recover after the short, intensive fishery there, so the overall reproductive output reaching the MHI is likely low regardless of the magnitude and direction of gene flow.

Of the genetic structure I did find in the archipelago, for both species, the MHI and NHWI appear to be genetically distinct. The most differentiated sites for *P. marginatus* are in the MHI (Kaua‘i and Maui), and although I could not obtain samples from Hawai‘i in time for this dissertation, I suspect that island may be similarly distinct. This suggests that management and protection of these species should focus on the MHI, and a prudent approach would be to focus on each island individually for this species. This is a study system where the addition of more markers as in chapter six for *P. interruptus* would likely add significantly to our understanding of gene flow in this archipelago. An additional result of interest for management that came out of this research is the skewed sex ratio of spiny lobsters in the MHI from a 50/50 ratio to a female-biased ratio, especially in the legal-sized lobsters. Whether this is a result of small sample size, or a consequence of the ban on taking of female lobsters in the MHI deserves further consideration. If these management regulations continue to skew the sex ratio of lobsters so that the the majority of males are small, there is the potential that female fecundity will become sperm-limited, which in turn could limit overall egg production as observed in the congeneric *P argus* as well as *Jasus edwardsii* (MacDiarmid and Butler 1998). This in turn would stymy any hope for recovery of spiny lobster stocks in the MHI.

4. *Panulirus interruptus* in California and Mexico: Of the three study systems in this dissertation, this one has the best match between management regime and connectivity regime. For the most part, the sites I sampled in California (with the exception of San Nicholas Island) are fairly well connected to other sampled sites throughout the region, and there were no sites besides San Nicholas Island that had elevated levels of kinship.

There was significant genetic structure in the microsatellite data between some locations within the Southern California Bight, but the magnitude of differentiation was lower than throughout the rest of the range. This bodes well for management in California, which currently treats on all locations in California as one stock. In addition, California has already established a network of Marine Protected Areas (MPAs) that are located proximate to many of the study sites included here. In Mexico, where I observed greater differentiation, and high levels of kinship at certain locations, it would be prudent to manage this species on a site-specific basis, cognizant that many of the recruits at a site are derived from lobsters at that location. Mexico has already adopted this management strategy, with lobster (as well as abalone and conch) fisheries managed on a local scale through fisheries cooperatives. One area that should be explored further is cooperation between these two nations, as my data indicates there is no genetic break between California, USA and Baja California, Mexico: the USA/Mexico border poses no difficulty for lobsters to cross. In light of this, these nations would likely benefit from managing this stock cooperatively. One area of research underway is an investigation of the rates and direction of *P. interruptus* gene flow across the USA/Mexico border. Preliminary results suggest that the magnitude of gene flow is greater from Mexico to the USA, which would also match catch rate data and other anecdotal observations. If there are higher levels of gene flow from Mexico to the United States, and in turn, the fisheries in the USA depend on reproduction of lobsters in Mexico, then California lobster fisheries will benefit if that information is incorporated into management plans.

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