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The effects of sea level fluctuations on coral reef fishes: Genetic differences between outer reef and lagoon inhabiting wrasses (Genus *Halichoeres*)

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The effects of sea level fluctuations on coral reef fishes: Genetic differences between outer reef and lagoon inhabiting wrasses (Genus *Halichoeres*)

by

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Thesis

Presented to the Faculty of the Graduate School of

The University of Texas at Austin

in Partial Fulfillment

of the Requirements

for the Degree of

Master of Science in Marine Science

The University of Texas at Austin

December 2011

Dedication

This work is dedicated to my family and friends. Without their strong support and understanding I would not have found the strength to pursue what I love to do. I would especially like to extend my thanks to my parents for always being understanding and supportive, and to my sisters Cori and Cami for pushing me to achieve my goals. I also want to thank the other graduate students in Port Aransas for helping me create so many good memories during my time here.

Acknowledgements

This thesis would not be possible without the input and help from my committee members, Dr. Luiz Rocha, Dr. Deana Erdner, and Dr. Benjamin Walther. Dr. Luiz Rocha took me on as a student without any previous knowledge on the laboratory techniques or subject matter, and without his guidance and patience I would not be completing this thesis. My lab mate Moisés Bernal also deserves recognition for all the help he has given me in running samples for this project, giving professional input, and editing manuscript drafts. This project would not have been possible without the help of many collaborators over the years. I would like to thank the Papahanaumokuakea Marine National Monument, US Fish and Wildlife Service, Hawaii Department of Land and Natural Resources, Coral Reef Research Foundation, and Pulu Keeling National Park for coordinating research activities and permitting procedures. The following individuals helped with logistics and field work: the crew of the NOAA Ship "Hi'ialakai", B. Bowen, T. Daly-Engel, C. Bird, M. Craig, R. Kosaki, S. Karl, C. Meyer, M. Gaither, D. R. Robertson, M. Iacchei, Z. Szabo, J. Drew, D. Pence, Y. Papastamatiou, B. Victor, J. H. Choat, R. "Greenie" Thorn, P. Colin, L. Colin, M. Mesubed, E. Basilius, J. Zamzow, J. Earle, R. Pyle, L. Sorensen, J. Drew, J. Robinson, J. Mortimer, B. Yeeting, and K. Flanagan. Financial support was provided by the University of Texas Marine Science Institute, US National Science Foundation Grants to B. W. B. and R. J. T. (OCE-0454873, OCE-0453167, OCE-0623678, and OCE-0929031) and to the UH EECB program (OCE-0232016), in conjunction with the HIMB-NWHI Coral Reef Research Partnership (NMSP MOA 2005-008/6682) and Cocos and Christmas Island collection support from National Geographic Society grant to M. Craig and L. Rocha (8208-07). I also thank the staff of the HIMB and UT Austin Core Facilities for sequencing.

Abstract

The effects of sea level fluctuations on coral reef fishes: Genetic differences between outer reef and lagoon inhabiting wrasses (Genus *Halichoeres*)

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Sea levels fluctuated following glacial cycles during the Pleistocene, reaching approximately 115-130m below current sea levels in the Indian and Pacific Oceans during the last glacial maximum 17,000 years before present. The effects of these sea level fluctuations on population structure have been shown in many near-shore marine taxa, revealing several common patterns. However, the underlying mechanisms behind these observed patterns are largely unknown. Drops in sea level affect the distribution of shallow marine biota, exposing the continental shelf on a global scale, and displacing coral reef habitat to steep slopes where shelf breaks are shallow. In these circumstances, we expect that species inhabiting lagoons should show reduced genetic diversity relative to species inhabiting more stable outer reefs. Here, I tested this expectation on the scale of an entire ocean-basin with four wrasses (genus *Halichoeres*): *H. claudia* (N=194, with

ocean-wide distribution) and *H. ornatissimus* (N=346, a Hawaiian endemic) inhabit seaward reef slopes, whereas *H. trimaculatus* (N=239) and *H. margaritaceus* (N= 118) inhabit lagoons and shallow habitats throughout the Pacific. Two mitochondrial markers (cytochrome oxidase I and control region) were sequenced to resolve population structure and history of each species. Haplotype and nucleotide diversity were similar among all four species. The outer reef species showed significantly less population structure, consistent with longer pelagic larval durations and a historically stable population. Mismatch distributions and significant negative Fu's F values indicate Pleistocene population expansion for all species, and (contrary to expectations) reduced genetic diversity in the outer slope species. These data indicate that lagoonal species may persist through the loss of habitat, but are restricted to isolated refugia during lower sea level stands, which may inflate genetic diversity during high sea levels. Outer reef slope species on the other hand have homogeneous and well-connected populations through their entire ranges regardless of sea level fluctuations. These findings contradict the hypothesis that shallow species are less genetically diverse as a consequence of glacial cycles.

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Chapter 1

Introduction: The response of marine organisms to Pleistocene sea level changes in the Indo-Pacific

Rising sea levels caused by climate change have become a heavily studied topic. Ascending seas are expected to cause problems for coastal areas worldwide with some projections estimating that 7 to 70 million people will have to be relocated, and up to $1 trillion value in goods and resources that lie less than 1m above current sea levels will be lost (Milne 2008, Stern 2007). Small island nations within the Coral Triangle, where the Indian and Pacific Oceans meet, are expected to be heavily impacted by the rising oceans (McLeod et al. 2010). Aside from the highly publicized socio-economic effects of higher water, there will also be considerable ecological impacts. We may gain a better understanding of how marine systems within the Indo-Pacific region will adjust to current day sea level changes by looking at the most recent glaciation events.

During the Pleistocene (2.6mya – 11.7 kya) sea levels fluctuated due to climactic oscillations, culminating in global sea levels 115 to 130m below current values during the last glacial maximum (LGM) approximately 17,000 to 19,000 years before present (YBP; Fairbanks 1989, Voris 2000, Hanebuth et al. 2000, Clark et al. 2009). Sea level estimates are greatly impacted by subsidence and tectonic uplifting, yet results from studies conducted across the Indian (West Indian Ocean: Camoin et al. 2004; Maldives: Fürstenau et al. 2010) and Pacific Oceans (Tahiti: Bard et al. 1996; Indonesia: Hanebuth et al. 2000; Papua New Guinea: Chappell & Polach 1991) all indicate relatively uniform heights across both ocean basins.

During the LGM the Coral Triangle was heavily impacted by the presence of the shallow Sunda and Sahul shelves, which connected several islands in the area and the nearby Asian continent (Voris 2000). It should be noted that sea levels reached their lowest point only for short periods during Pleistocene glaciations (approximately 6% of the time during the last 250kya), but they remained at intermediate depths of less than 40m below present day levels for the majority of Pleistocene glaciations keeping the Sunda and Sahul barriers effectively operating in this region (Voris 2000). These barriers restrict connections between the Indian and Pacific oceans, and are thought to be important in generating biodiversity within the Coral Triangle during sea level lows (Rocha & Bowen 2008).

After the LGM, sea levels did not rise linearly over time. Rather, they showed a pattern of several rapid pulses (Fairbanks 1989). Melt water pulse 1a (MWP-1a) occurred ~14,500 years ago when the Western Antarctic Ice Sheet (WAIS) retreated (Clark et al. 2009). Evidence of the MWP-1a event is seen in the Atlantic (Fairbanks 1989), the Pacific (Bard et al. 1996, Hanebuth et al. 2000, Tanabe et al. 2010), and the Indian Ocean (Camoin et al. 2004, Fürstenau et al. 2010). A second rapid sea level rise (MWP-1b) has also been described in the Atlantic, Indian and Pacific Oceans (Fairbanks 1989, Cabioch et al. 2003, Fürstenau et al. 2010) occurring ~11,000 years before present. MWP-1b is more controversial though, as evidence is lacking for it in the West Indian Ocean (Camoin et al. 2004), Tahiti (Bard et al. 1996), and Japan (Tanabe et al. 2010). It can be difficult to disentangle tectonic uplift and subsidence from the effects of MWP-1b, and if MWP-1b did occur it is possible that it was not as severe as originally described

(Tanabe et al. 2010). Regardless of the magnitude of the MWPs, they had several implications for marine biota.

During sea level lows ecological aspects of many organisms can be altered in several ways: 1) qualitative changes are those associated with habitat destruction or the alteration of the quality of habitat where an organism lives, 2) quantitative changes alter the total habitat area, and 3) climatic changes associated with changes in sea surface temperatures (SST, Paulay 1990). However, temperature fluctuations during the LGM were not predicted to impact coral within the tropical Pacific (Paulay 1989), and are considered to be negligible in terms of altered ecosystem dynamics (Paulay 1990, but also see Stanley & Campbell 1981). Furthermore, Paulay (1990) argued that qualitative effects have a greater influence than quantitative effects. Despite possible qualitative and quantitative changes during the LGM, it has been noted that many marine organisms were not heavily impacted, and few became extinct (Wise & Schopf 1981, Valentine & Jablonski 1991).

Although most species managed to avoid extinction during sea level lows, new species may have evolved due to the formation of barriers (Rocha & Bowen 2008), and population parameters were likely affected in many species (e.g. Fauvelot et al 2003). Therefore the goal of this chapter is to review how glacial sea level fluctuations during the Pleistocene affected various groups of marine organisms in the Indian and Pacific Oceans and to gain insights into how species might adjust to present day rising sea levels. While there are several repeating genetic patterns seen in this region (lack of structure, structure associated with the Sunda Shelf barrier, or structure shown on small spatial

scales), this review will discuss relationships between sea level change and both speciation and population structuring for various taxa using relevant case studies to highlight patterns observed in each group.

1-A: Invertebrates

Corals

The majority of studies that examine the biotic impact of Pleistocene glaciations involve species living in close proximity to coral reefs. Coral reefs add structural complexity and create habitat for a wide array of organisms. Understanding how these reefs have been impacted by sea level change is critical for understanding the entire ecosystem they support. While many studies have examined fossil corals in the Atlantic, the Indo-Pacific region is only beginning to be explored (Shen et al. 2010).

Corals provide a unique system due to the abundance of their fossilized remains. These remnants of older reefs can provide information on coral species composition and turnover through time, as well as isotopic proxies for sea level change and SST. This ability to view species composition in space and time can directly address questions about paleo-community ecology. Interestingly, when community composition was examined in fossil corals off Papua New Guinea, both species richness and community composition were found to be stable over a period of 95,000 years during the Pleistocene (Pandolfi 1996, 1999), despite the occurrence of several periods of sea level fluctuations and variations in SST (up to 6°C) during that time span. Pandolfi (1996) found spatial,

but not temporal, variation in the coral communities, suggesting that local environmental factors such as freshwater input played significant roles in community assembly.

Additional studies at the Ryukyu Islands near Japan found similar results. Coral taxonomic composition changed slowly during the Pleistocene at these reefs (Humblet et al. 2009). One interesting finding in this island group is that the overall total species and generic richness of Pleistocene taxa was not less than that of present day reefs in this region (Humblet et al. 2009), which indicates that coral community composition did not change dramatically during the Pleistocene, and consistent structurally complex habitats for reef-dependant species were maintained.

This observed stasis could easily be explained by slow rates of sea level change: rapid rises would presumably "drown" corals while slower rates of sea level rise would allow corals to progressively accrete upwards and colonize newly available shallow habitat (Chappell & Polach 1991). Yet sea levels did not always rise steadily during the Pleistocene, as demonstrated by evidence for the MWP-1a and MWP-1b events (Fairbanks 1989). The effect of MWP-1a was a rapid rise in sea levels, at the rate of 40-50mm/year (Hanebuth et al. 2000), much faster than the rising rate of ~8mm/year (Fairbanks 1989, Bard et al. 1990) during the remaining time since the LGM. This rapid rise outpaced the ability of corals to vertically accrete, and as a result some reefs "drowned", or fell below the critical depth needed to maintain growth (Webster et al. 2004, Beaman et al. 2008). Direct evidence for this can be seen at old Hawaiian reefs, which are currently 150m below sea level (Webster et al. 2004, Faichney et al. 2011). Radiocarbon dating of these corals found that growth ceased at the same time period as

MWP-1a (Webster et al. 2004). Evidence of MWPs in the morphology of ancient reefs can also be seen in the Maldives (Fürstenau et al. 2010), and the Great Barrier Reef (GBR; Beaman et al. 2008).

Sea level rise, whether abrupt or steady, only accounts for ~10% of the Pleistocene, meaning the majority of the epoch experienced either steady or falling sea levels (Pandolfi & Greenstein 2007). Therefore it is imperative to also examine the effects of falling sea levels (Tager et al. 2010). The species composition of one lowstand reef off Papua New Guinea was examined across a 244,000 year interval (between 130kya – 374kya; Tager et al. 2010). These reefs show taxonomic changes in community composition through time, possibly due to responses to changing wave energy regimes, or in response to limited dispersal ability of corals during sea level lows (Tager et al. 2010). Although results from this study are preliminary in terms of lowstand coral reefs, they show that while community composition of lowstand coral reefs change over time, reef habitats can persist during glacial maxima (Tager et al. 2010) and therefore provide habitat for other reef-associated species.

Mollusks

The phylum Mollusca includes a large variety of organisms from bivalves and gastropods to cephalopods. Like corals, certain mollusks (including bivalves and some gastropods) have left substantial fossilized records of their distribution during the Pleistocene. These fossils provide a geological record of species distributions. Unlike fossil coral studies, which simply examine changes in coral communities through time

(Pandolfi 1996), one bivalve study examined species presence as it related to habitat availability (Paulay 1990). This study examined the effect of sea level cycles on bivalves inhabiting lagoons and outer reefs, and found a difference in the fossil abundance between the two groups (Paulay 1990). Species adapted to shallow, sandy habitats like those found in lagoons disappeared during times of sea level lows due to a drying of shallow lagoons that leads to local population declines (Paulay 1990). These species did not go completely extinct, however, and the author postulated that individuals could have found refuge on continental margins in the West Pacific and then re-colonized oceanic lagoons as the sea levels rose again (Paulay 1990). Unfortunately the majority of fossils used in this study were restricted to currently shallow areas. Fossils left during sea level lows are difficult to collect at current sea levels because of sampling difficulties at depth (Valentine & Jablonski 1993, Marko 2004), and therefore another method of investigating the impacts of sea levels must be used.

The fossil record is not the only source of information about past demographic events. Although not as informative for pinpointing geographic distributions of species during sea level fluctuations, population genetic approaches should reflect historic patterns of structure and diversification. These methods can also be used to examine similar questions as previous fossil studies, such as the historical dissimilarity between species adapted to distinct habitats. One such study found significant genetic structuring between two similarly distributed Indo-Pacific gastropods, and concluded that the differences may have arisen due to habitat preferences (Crandall et al. 2008a). Both species examined showed a history of population expansion, but the species that inhabits

inner reef flats showed a much more rapid population expansion since the LGM, possibly due to a rapid increase in available habitat as sea levels rose and flooded lagoons that were previously dry (Crandall et al. 2008a).

Further studies have considered genetic differences between ecologically diverse oceanic and continental *Echinolittorina* snails in the Indo-Pacific, finding more genetic structuring among continental species (species only distributed on continental shelves), and no genetic structuring among oceanic species (species only distributed on oceanic islands; Reid et al. 2006). The discrepancy in observed patterns between the two species-associated habitats is most likely due to ecological differences between oceanic species and continental species (Reid et al. 2006). Continental species exhibit a large genetic break between the Indian and Pacific Oceans most likely caused by the exposure of the Sunda shelf barrier as sea levels fell (Reid et al. 2006). Interestingly, species inhabiting oceanic islands were not affected by this large barrier (Reid et al. 2006).

Genetic structuring between populations from these two oceanic basins is the most common result of Indo-Pacific marine population genetic studies, and similar patterns have been reported in several unrelated bivalves (Lind et al. 2007, Kochzius & Nuryanto 2008), and gastropods (Reid et al. 2006, Imron et al. 2007, Crandall et al. 2008a). The Sunda Shelf biogeographic barrier may have been critical for speciation in the Indo-Pacific (Rocha & Bowen 2008), and this barrier has been pointed to as a possible mechanism driving allopatric partitioning of groups of certain gastropods (Frey & Vermeij 2008). The pattern of interspecific or intraspecific genetic diversity abutting

the Sunda shelf barrier is also common in many other groups of species, as discussed below.

Crustaceans

Crustaceans constitute an extremely diverse group of approximately 42,000 species in the marine environment (Ruppert et al. 2004). They have also been the subject of several biogeographical studies in the Indian and Pacific Oceans. Unlike the majority of studies examining historic patterns of coral and mollusk diversity, most studies on crustaceans use population genetic techniques rather than fossil evidence. While several studies have found a major genetic break between the Pacific and Indian Oceans (e.g. Lavery et al. 1996), many of these studies also examined fine-scale population structure within the Indo-Pacific junction. This is unique for this group of organisms, as many other invertebrate studies have only addressed larger scale patterns.

Genetic sequence variation in stomatopods from the Coral Triangle suggest long histories of limited connectivity across relatively small spatial scales dating back to Pleistocene glaciations (Barber et al. 2006). The geographical break among populations was found to be associated with limited transport of larvae across the Maluku and Flores seas within the Coral Triangle (Barber et al. 2006). Another study examining fine scale structure among Australian populations of caridean shrimp found a large genetic break across the Torres Strait, which was expected based on the emergence of the Sahul Shelf during sea level lows (Voris 2000, Haig et al. 2010). Structuring of both of these species

reflects the smaller scale patterns operating within the Coral Triangle, although species-specific responses are likely (Barber et al. 2006).

Another group of crustaceans, the barnacles, have also been studied within the Coral Triangle. Cryptic speciation, attributed to sea level fluctuations, has been suggested for the barnacle *Chthamalus malayensis* (Tsang et al. 2008) and is also likely to have occurred in *Tetraclita squamosa* (Chan et al. 2007a,b). This process of speciation may have been facilitated by Pleistocene sea level changes, with contemporary oceanographic currents that maintain divergence (Tsang et al. 2008). Each clade, however, had its own unique population expansion signature, with the most recent expansion occurring in the Indo-Malay region, suggesting distinct evolutionary histories of each population (Tsang et al. 2008).

Population structuring of crustaceans has also been explored within the Indian Ocean itself, with conflicting results. The mud crab *Scylla serrata* shows genetic structuring consistent with many other studies that found major breaks between Indian and Pacific Ocean populations, but also with a third distinct population in Northwest Australia (Fratini et al. 2010). However, within the Western Indian Ocean there is no detectable population structure (Fratini et al. 2010). In contrast, the mangrove crab *Neosarmatium meinerti* shows structuring between populations of the Eastern coast of Africa and the oceanic islands within the Western Indian Ocean (Ragionieri et al. 2009, 2010). Consistent with the coastal populations from both aforementioned studies, the giant tiger prawn *Panaeus monodon* shows no structure along the East African coast (Benzie et al. 2002). However, the absence of sampling this species from oceanic islands

in the Indian Ocean precludes comparisons with other crustacean studies. The genetic structuring of the tiger prawn also suggests possible refuge areas in Southeast Africa, and along the Australian coast during sea level lows (Benzie et al. 2002). As will be seen in other groups, identifying putative refuge areas as well as the ecological reasons why species show different structuring patterns is a crucial step in understanding the impacts of sea level change within the Indian and Pacific Oceans.

Echinoderms

By examining seastars that occupy two different habitats and their associated ectoparasites, Crandall and colleagues (2008b) attempted to gain further insight into genetic structure for this species complex in the Indo-Pacific. These authors found discordant patterns not only between both seastars, but also between two parasites that differ in their host specificity. The conclusion was that the differences were caused mainly by one seastar (*Protoreaster nodosus*) having lower genetic diversity due to a sharp population bottleneck. This species is strongly associated with lagoon habitats and therefore was most likely extirpated from shallow habitats during sea level lows (Crandall et al. 2008b). The other seastar in the study (*Linckia laevigata*), which specializes on outer reef habitats, showed the typical pattern of population divergence across the Coral Triangle, likely driven by isolation during sea level lows (Crandall et al. 2008b, Kochzius et al. 2009). The difference between the genetic structure of the parasites involved in this study were most likely due to their degree of host specificity (Crandall et al. 2008b). Comparative phylogeographic studies focusing on ecological

differences, such as the one just described, are at the forefront of understanding the broad patterns of geological changes.

Early genetic studies within the Coral Triangle focused on several seastars (Williams & Benzie 1998, Benzie 1999). These studies were some of the first to document genetic breaks between Indian Ocean and Pacific Ocean populations. Prior to these efforts it was thought that species with large distributions most likely did not exhibit strong genetic structuring due to a high dispersal ability (Briggs 1974). In addition, sea urchins have also been studied within this region, but with contrasting results. Several species of *Eucidaris* (Lessios et al. 1999) and *Diadema* (Lessios et al. 2001) show the general trend of a major break between the Indian and Pacific Oceans, but species of the genus *Tripneustes* (Lessios et al. 2003), as well as *Diadema setosum* (Lessios et al. 2001), do not show any structure between the ocean basins. It was these findings that initiated questions about life history characteristics or ecological situations that could have caused the differential response of species to sea level fluctuations. This is what has led to recent studies that are designed specifically to find the underlying causes of this phylogeographic structuring (e.g. Fauvelot & Planes 2002, Fauvelot et al. 2003, Thacker 2004, Crandall et al. 2008b).

Porifera

In comparison to other invertebrate phyla, the literature concerning the historic presence and phylogeography of Porifera is relatively scarce. Although many studies have examined genetic structure in the Atlantic and Mediterranean (e.g. Lopez-Lengentil

& Pawlik 2009, Debiasse et al. 2010, Xavier et al. 2010), sponge genetics are somewhat of a mystery in the Indo-Pacific. The few studies that have been done in the Indo-Pacific have shown interesting results when compared to studies of other sessile invertebrates.

Sponge phylogeographic patterns highlight the importance of a short pelagic larval duration. Present day distributions of various Porifera show high genetic structure among locations, with differentiation often arising to levels indicating cryptic species (e.g. Muricy et al. 1996). The number of migrants between populations has been estimated in some species to be less than one individual per generation (Wörheide et al. 2008). Due to this limited connectivity, many sponge distributions have been heavily impacted by sea level fluctuations.

Most of the sponge studies in the Coral Triangle region are focused around the GBR. *Leucetta chagosensis* is a calcareous sponge with a wide distribution encompassing the Indian and Pacific Oceans. Large-scale divergence has been found between the two ocean basins as expected from many of the aforementioned studies (Wörheide et al. 2008). In addition, *L. chagosensis* exhibits small scale genetic structuring, a common phenomenon in sponges. Along the GBR *L. chagonsensis* separates into two divergent clades, representing a northern population and a southern population (Wörheide et al. 2002). The recognition that sea level drops coincided with shifting oceanographic currents led the authors to hypothesize that these two populations represent two local refugia for this species, one at the Queensland Plateau in the Northern GBR, and the other in the southern limit of the GBR (Wörheide et al. 2002). Similarly, several sponges of the genus *Hymeniacidon* also show small scale structuring off Japan,

where during sea level lows parts of the Seto Inland Sea were isolated from the Pacific (Hoshino et al. 2008). Nevertheless this structure was not seen in another study in the GBR of the sponge *Pericharax heteroraphis*, which suggests that sponges as a whole did not respond uniformly to sea level fluctuations (Bentlage & Wörheide 2007).

Although data for Indo-Pacific sponges are limited, these observed patterns show that sponge phylogeographies may be much more variable than initially thought, and that they may be susceptible to changes in sea levels on small scales (although see Hoshino et al. 2008 for an example of a global species with little genetic differentiation). Sponge phylogeography in the Indo-Pacific will benefit from future studies that include more species, locations, and different habitats (e.g. sponges with different depth distributions).

1-B: Vertebrates

Marine Reptiles

The marine reptiles that have been included in Coral Triangle studies include both shallow water marine snakes and sea turtles. Neither of these groups is heavily represented in the literature for this region and there is much to be learned about them. Marine snakes are strongly associated with shallow waters and do not disperse across deep water habitats except during stochastic events (Lukoschek et al. 2008), a characteristic that is ideal for studying the effects of sea-level impacts. The olive sea snake *Aipysurus laevis* is distributed across the northern portion of Australia. Two studies examining different parts of the snake's genome found a concordant pattern

whereby the Western Australian population was ancestral and endured several sea-level fluctuations, while populations in the Gulf of Carpentaria and the GBR were recent migrants from the western population and have only been in place since the LGM (Lukoschek et al. 2007, 2008). Much like the previously discussed taxa, these two populations (Western Australia and GBR) are genetically distinct between the two ocean basins (Lukoschek et al. 2007, 2008).

Data from the sea snake *Cerberus rynchops* reveal a more detailed picture on historical demographics around the Coral Triangle. Throughout its range *C. rynchops* separates into four distinct populations, all of which were likely the result of sea level fluctuations (Alfaro et al. 2004). As the Sunda Shelf barrier became exposed, the amount of total coastal area shrank allowing some populations of *C. rynchops* to mix and some to become separated leaving distinct populations in the Philippines, around Thailand, the Greater Sunda Islands and the rest of the Indian Ocean including Myanmar (Alfaro et al. 2004). The results of this study indicate that sea snakes are highly impacted by availability of shallow habitat, and therefore are susceptible to local extirpations or population mixing as sea levels change.

Sea turtles are typically highly migratory and are unlike any of the previous organisms mentioned in that they are not restricted to shallow habitats. This migratory ability in addition to strong natal homing (reviewed for all species in Bowen & Karl 2007) and recent population bottlenecks caused by fishing and bycatch practices make it difficult to detect Pleistocene effects on sea turtles. It is possible that lowered sea levels would restrict the amount of sandy beaches necessary for breeding, leaving the few

remaining beaches (and their unique genetic populations) susceptible to stochastic events that could cause population bottlenecks (Hatase et al. 2002). Furthermore, populations during the LGM would have been restricted to lower latitudes, as eggs would not be viable at higher latitudes due to a decrease in ambient air temperature. Evidence for this has been found in Japanese populations of loggerhead turtles (*Caretta caretta*), where each nesting colony exhibits a signature of population expansion (Hatase et al. 2002).

Overall, marine reptiles are an underrepresented group in the literature examining Pleistocene sea level effects. While further studies on sea snakes may reveal patterns associated with the LGM, past sea turtle demographics are concealed by their pelagic adult migrations coupled with beach nesting site fidelity in females, as well as recent fishing effects. Therefore the effects of the LGM on sea turtle populations may remain obscured unless extensive fossil evidence is found.

Marine Mammals

Marine mammals comprise cetaceans (dolphins and whales), pinnipeds (seals and sea lions), sea otters, and sirenians (manatees and dugongs). Cetaceans are not limited to shallow water habitat and many species migrate long distances to breed, thereby confounding any use of genetic approaches to determine effects of Pleistocene glaciations. Although not specifically limited to the Coral Triangle region, changes in SST during glacial periods may have allowed species to migrate between anti-tropically distributed habitats. Thus, it is thought that Pleistocene or earlier glaciations may have influenced speciation in several cetaceans (Fordyce & Barnes 1994, Hare et al. 2002).

Pinnipeds evolved during the Oligocene, mainly have an anti-tropical distribution, and are conspicuously absent from the central and northern Indian Ocean (Deméré et al. 2003). In the Pacific, the only tropical species is the Hawaiian monk seal (*Monachus schaunislandi*). This species has extremely low genetic diversity due to overharvesting (Kretzmann et al. 1997, Schultz et al. 2009) and it is unknown how sea level fluctuations affected its population numbers. Like cetaceans, glaciations during the Pleistocene may have facilitated movement in anti-tropically distributed species and may have played a role in the evolutionary history of this order (Deméré et al. 2003).

There are only four extant species of sirenians today, and only one of them is present in the Indian and Pacific Oceans, the dugong (*Dugong dugong*). Dugongs are strongly associated with coastal habitats, and therefore are likely to have been impacted by sea level fluctuations. Historic variation in dugong movement patterns are not well understood, although it is thought that dugongs are of continental origin and migrated from the Indian Ocean east towards the Pacific (Domning & Furusawa 1994). Dugong populations in Northern Australia show signs of mixing between two distinct populations, which presumably arose during the Pleistocene when a land bridge connected Northern Australia with Papua New Guinea (McDonald 2005). Furthermore, these populations were all distinct from Asian populations, suggesting high genetic differentiation in this species (McDonald 2005). A similar study in Thailand suggested that dugongs from the Andaman Sea founded populations in the Gulf of Thailand following sea level rises (Palmer 2004). While these studies show that dugongs have been impacted by

Pleistocene sea level fluctuations, comprehensive studies that examine population dynamics of dugongs across their entire range have yet to be completed.

Fish

The dynamics and population structuring of many fish populations have revealed diverse patterns of connectivity ranging from a lack of structuring at the scale of entire ocean basins (e.g. Bowen et al. 2001, Craig et al. 2007, Reece et al. 2010) to complex local and regional geographic structuring (e.g. Nelson et al. 2000, Timm & Kochzius 2008), to non-geographic structuring (e.g. Horne et al. 2008, Evans et al. 2010). A consistent challenge in fish population genetics is to tease out contemporary from historic patterns, which can be done using molecular markers that evolve at different rates. Once this is done, the numerous studies of fish offer something lacking in most of the previously mentioned groups: comparative phylogeographic studies with closely related species, which I highlight below.

The Coral Triangle has been the central topic of several studies examining the false clownfish, *Amphiprion ocellaris*. Two studies have been conducted using different mitochondrial markers, and both studies have revealed several interesting patterns. Primarily, this demersal egg-laying species shows fine scale geographic genetic structuring, but also shows evidence of Pleistocene glacial effects (Nelson et al. 2000, Timm & Kochzius 2008). Genetic diversity signatures in Nelson et al. (2000) suggest that this species was isolated on the Sunda Shelf edge during sea level lows and subsequently re-colonized shelf habitats as sea levels rose. The study by Timm and

Kochzius (2008) took a more refined look at this species with a faster mutating mitochondrial region and found distinct populations at four locations within the Indo-Malay Archipelago. Further, some of these locations contained mixed genetic signatures from multiple ancient populations, which may have been separated during sea level lows and became connected later (Timm & Kochzius 2008). Both studies conclude that the observed genetic structuring in this species is the result of both historic and present day factors such as geographic distance and oceanic currents.

While *A. ocellaris* is strictly associated with shallow waters and coral habitat, species that are not so strongly associated to coral reefs have also shown evidence of glacial impacts. Several species in the genus *Naso* have been used in comparative phylogeographic studies, and with consistent results (Klanten et al. 2007, Horne et al. 2008). Three closely related species (*N. vlamingii, N. brevirostris*, and *N. unicornis*) were examined between two studies, and none showed geographic structure. However, two species (*N. vlamingii*, and *N. brevirostris*) showed temporal genetic structure and evidence of present day mixing of populations that were presumably separated during the Pleistocene (Klanten et al. 2007, Horne et al. 2008). Even though *N. unicornis* did not show a similar pattern, it may consist of one remnant population that survived Pleistocene sea level fluctuations, thus explaining its lack of evidence for past structure (Horne et al. 2008). Despite these species not being as closely associated with coral reefs as other examined species, the congruent observed patterns suggest they respond to similar ecological and evolutionary influences and not stochastic mutational processes (Horne et al. 2008).

One obligate coral reef fish group that has been closely examined at spatial scales similar to the *Naso* studies is the parrotfish (Subfamily Scarinae). Both *Scarus psittacus* and *S. rubroviolaceus* show similar patterns of a highly mixed central Indo-Pacific population with genetic divergence found at the periphery of each species range (Fitzpatrick et al. 2011, Winters et al. 2010). High present day connectivity is also seen in the related *S. ghobban* in the Western Indian Ocean, which shows evidence of temporal structure but complete present day homogeneity of populations (Visram et al. 2010). In contrast, an ecologically similar parrotfish *Chlorurus sordidus* shows distinct ocean basin structuring between the Indian Ocean and Pacific Oceans reminiscent of Pleistocene barriers (Bay et al. 2004). However *C. sordidus* shows no structure over large distances throughout the Pacific (Bay et al. 2004), suggesting similar dispersal abilities to previously mentioned parrotfish studies. The reasons why these and other similar species show more or less genetic structuring across the Indo-Pacific are unknown.

In an attempt to gain insight into the specific factors that cause these discrepancies, habitat preference and reproductive strategies were considered across a variety of coral reef fish in French Polynesia (Fauvelot & Planes 2002, Fauvelot et al. 2003). The authors found correlations between genetic diversity and habitat preferences (lagoon versus outer reef) but no significant correlations with reproductive strategy (demersal versus pelagic eggs; Fauvelot et al. 2003). It is hypothesized that during sea level lows lagoon habitats were exposed and dried up, causing extirpation of strictly lagoon species. This extirpation resulted in lower genetic diversities and signatures of

population expansion when sea levels rose and the lagoons were subsequently re-colonized (Fauvelot & Planes 2002, Fauvelot et al. 2003). This habitat preference hypothesis has been validated by another study that examined a different set of species (Gobies) across several South Pacific Islands (Thacker 2004). To date, these are the only studies that have examined specific ecological and evolutionary differences between groups of fishes to try to determine which factors lead a species to be highly impacted by sea level fluctuations. However, they provide intriguing hypotheses about the factors that promote species resilience during environmental changes.

Future Directions

Work undertaken during the last few decades has laid the foundation for the study of organismal responses to sea level fluctuations, yet many questions remain unanswered. While fossil evidence has been useful in some groups, the majority of our knowledge comes from both intraspecific and higher level genetic differences between biogeographic areas. The author feels that most future knowledge of this topic will come from population genetics and phylogeography, as fossils can be extremely difficult to obtain in large numbers for most groups.

To summarize the current knowledge, several patterns have been observed. Genetic structure between populations has been detected at all scales. This includes isolated populations that are relatively geographically close to one another, such as in crustaceans, populations that are structured between the Indian and Pacific Oceans, a pattern observed in most groups, as well as cases where no or very small genetic structure

is found across the entire range of a species (e.g. Craig et al. 2007, Reece et al. 2010). We are also beginning to understand how specific traits influence a species demographic history, which has been seen in habitat preference studies (Fauvelot & Planes 2002, Fauvelot et al. 2003, Thacker 2004) and in symbiotic relationships (Crandall et al. 2008b).

However, it is this last point regarding species-specific traits influencing demographic history that is relatively unknown. Future studies can fill this gap by taking a multi-species comparative approach in order to test between different traits, and not just examine patterns among a single species. Using molecular techniques, future studies can add to the ecological knowledge of sea level fluctuations by distinguishing between historic and current influences over modern genetic signatures. While there are also some gaps in the knowledge for some groups (e.g. sponges, marine snakes), certain general patterns are evident in many marine phyla. Comparative studies that couple ecological or evolutionary traits among species are necessary to advance the field. Not only will these comparative studies reveal patterns associated with sea level fluctuations allowing responses to future dynamic sea levels to be projected, but extensive genetic mapping of many species may also reveal cryptic species (as in sponges) and specific management units that should be targeted for conservation efforts.

Chapter 2

Surviving sea level lows: The difference between living in shallow lagoons versus outer reef slopes in Indo-Pacific Wrasses (Genus *Halichoeres*)

Introduction

Glacial cycles and associated sea level changes during the Pleistocene have historically affected the distribution of plants and animals in both terrestrial and marine environments (Wallace 1881, Molengraaff & Weber 1921). Sea levels dropped as much as 120m during the last glacial maxium approximately 17,000 years ago, exposing large areas of continental shelf (Fairbanks 1989, Voris 2000), restricting reefs to vertical or steep slopes on oceanic islands and reducing, drastically changing, or eliminating the habitat for shallow water species (Bellwood & Wainwright 2002). During these events, lagoon species may have undergone population bottlenecks or local extirpation (Fauvelot et al. 2003), while species adapted to the outer reef edges could use deeper reefs as refuges (Craig et al. 2007). As a result, population genetic patterns should be closely linked to habitat preference. Fluctuations in habitat area in the Pleistocene due to sea level change are suspected to affect population abundance in several groups, including marine gastropods (Crandall et al. 2008a), crabs (He et al. 2010), fish (Janko et al. 2007), and corals (Woodroffe et al. 2010).

Habitat preferences differ markedly among closely related species of wrasses (genus *Halichoeres*; family Labridae). The sister species *H. ornatissimus* and the recently described *H. claudia* are usually found on the outer margins of reefs (Gosline 1965, Randall & Rocha 2009). *H. claudia* spans the Indo-Pacific, from Cocos-Keeling in

the eastern Indian Ocean, to French Polynesia and the Line Islands in the Central Pacific, and north to Indonesia, whereas *H. ornatissimus* is restricted to the Hawaiian Islands (Randall & Rocha 2009). Two other wrasses of the same genus occupy a range similar to *H. claudia*: *H. trimaculatus* and *H. margaritaceus*. However, these latter two species occupy shallower habitats in lagoons and reef flats. *H. trimaculatus* is found in shallow lagoons and bays among sand, rubble, and small coral heads as shallow as a few centimeters, and rarely deeper than 10m (Randall et al. 1997). *H. margaritaceus* is commonly found in shallow reefs and rocky shores exposed to wave surge within the top 3m of the water column (Randall et al. 1997, Allen 2003).

Previous studies have examined genetic diversity in lagoon and outer reef fish and found that lagoon species have less genetic diversity than outer reef species (Fauvelot et al. 2003, Thacker 2004). In each case these conclusions were based on sampling one or a few locations. However, to completely understand the genetic structure of a species it is preferable to sample the entire range (Winters et al. 2010). Therefore the goal of this study was to assess the genetic consequences of sea level changes on lagoon and outer reef fishes sampled across the Pacific.

Here I address two questions in the context of range-wide phylogeography: 1) what is the genetic (mtDNA) diversity of each species across their range, and 2) have historical factors differentially influenced the genetic diversity and population structure of species with different habitat preferences? I hypothesize that sea level lows resulted in large population bottlenecks for lagoon species which should cause decreased genetic diversity when compared to outer reef species. To address these questions I used two

mtDNA segments, cytochrome oxidase I (CO1) and the control region (CR). These markers were chosen so that the results could be directly compared to previous studies and examine different temporal regimes with the more conservative CO1 and the more rapidly evolving (non-coding) CR segment.

Methods

Sampling and extraction

Samples of *Halichoeres claudia* (n=194, 5 locations), *H. ornatissimus* (n=346, 12 locations), *H. trimaculatus* (n=239, 7 locations), and *H. margaritaceus* (n=118, 3 locations) were taken from 21 locations in the Indian and Pacific Oceans (Fig 1). All samples were collected using pole spears while SCUBA diving or snorkeling between 2006 and 2009. Fin clips or gill tissue was subsequently taken from each individual and stored in either 95% ethanol or 20% salt saturated DMSO (Seutin et al. 1991). DNA was later extracted using the "Hot-Shot" method described in Meeker et al. (2007) and stored at 10°C before PCR amplification.

Laboratory Procedures

Both CO1 and CR were amplified for all specimens. CO1 segments were amplified with primers BOL-F1 (5' TCA ACY AAT CAY AAA GAT ATY GGC AC 3') and BOL-R1 (5' ACT TCY GGG TGR CCR AAR AAT CA 3') (Ward et al. 2005). Each 25µl reaction contained approximately 10ng DNA, 3.5mM $MgCl_2$, 1x buffer, 0.18 µM of each primer, 2.5mM DNTP, and 2 units of GoTaq DNA Polymerase (Promega). Polymerase chain reactions were conducted using a temperature profile of a one minute denaturing step at 95°C, followed by a 30 second annealing step at 45-52°C depending on

the species, and completed with an extension of 45 seconds at 72°C, for 32 cycles. Samples were then purified and sequenced using the BOL-F1 primer. CR segments were amplified with primers CRA (5' TTC CAC CTC TAA CTC CCA AAG CTA G 3') and CRE (5'CCT GAA GTA GGA ACC AGA TG 3') (Lee et al. 1995). Temperature profiles for PCR amplification were similar to CO1 except annealing temperature was 52-55°C depending on the species. PCR amplifications for both loci included aliquots without genomic DNA (negative control) to detect possible contamination. PCR products were verified with 1.5% agarose gel electrophoresis using GelStart Nucleic Acid Stain (Cambrex Bio Science). Amplicons were then purified and sequenced at the ICMB Core Facilities, University of Texas at Austin, using an ABI 3730 automated sequencer (Applied Biosciences). Samples were sequenced using the forward primer, and rare or questionable haplotypes were sequenced in both directions.

Population Genetic Analyses

Sequences were aligned using Geneious 5.0.2 (Biomatters). Haplotype and nucleotide diversity (*h* and π respectively; Nei 1987) were calculated in Arlequin 3.5.1.2 (Excoffier & Lischer 2010). Pairwise Φ_{st} values between all sampling locations were calculated with 1,000 runs in Arlequin. Population subdivisions were detected with an analysis of molecular variation (AMOVA) (Excoffier et al. 1992), and isolation by distance was analyzed using a Mantel test with 1000 permutations performed in Arlequin. The Mantel test compared straight-line distance between locations (km) to pairwise Φ_{st} values. Migrate 3.1.4 (Beerli & Palczewski 2010) was applied to estimate the overall strength and direction of migration between populations. For each species 10 runs of

Migrate were performed, each consisting of 10 short chains of 100,000 steps, and one long chain of 1,000,000 steps, sampling every 100 increments for each. In addition 10 heated chains with varying temperatures (1-10) were used, with a swapping interval set to 1. Migrate runs were then averaged and checked for convergence.

Phylogeographic and Coalescent Analyses

The most appropriate nucleotide substitution models were chosen based on maximum likelihood scores under the AIC approach in Modeltest 3.7 (Posada & Crandall 1998). Coalescent ages were estimated with BEAST 1.6.1 (Drummond et al. 2002) using the most appropriate substitution model with a population expansion model which was verified by mismatch distributions. A strict mutation rate of 3% per million years (MY) between lineages was employed for CO1 (Lessios 2008) and 9% per MY between lineages for CR. The mutation rate for CO1 is based on published literature values for trans-isthmian wrasses which range from 2.3%/MY to 3.4%/MY (Table 3 in Lessios 2008). The mutation rate for CR is based on the pairwise divergence of a trans-isthmian pair of damselfish, *Chromis atrilobata* (Accession numbers: EF489847.1- EF489848.1) and *Chromis multilineata* (Accession numbers:EF489843.1-EF489844.1) using the software MEGA 4 (Tamura et al. 2007), assuming 3 million years divergence (Lessios 2008). Each BEAST run was conducted with a chain length of 10,000,000. Runs were repeated until the effective sample size was larger than 200. Multiple runs were then combined using LogCombiner 1.6.1 and then viewed using Tracer 1.5. CR mutation rates are controversial in fishes, and this rate should be cautiously regarded as a first-order approximation.

Minimum spanning networks (MSNs) were constructed in TCS 1.21 (Clement et al. 2000). For CO1 the connection limit was set to 95%, while for CR a fixed connection limit was set at 50 steps. MSNs were redrawn in Adobe Illustrator CS5 following procedure outlined by Templeton et al. (1992).

Population Expansion and Neutrality

Sequences were checked for neutrality using Fu's F (Fu 1997) implemented in Arlequin using 1000 simulated samples. Pairwise mismatch distributions (Harpending 1994) were also constructed comparing observed and expected mismatch distributions under a demographic expansion model in Arlequin.

Results

Genetic Diversity

A 526-559bp segment of CO1, depending on the species, was obtained from 787 individuals, as well as a 252-343bp segment of the CR from 723 individuals. Slope inhabitant *Halichoeres claudia* had 31 haplotypes for CO1 and 109 haplotypes for CR with 18 and 90 unique haplotypes (observed in single individuals), respectively (Fig 2 & 3). The minimum spanning network for CO1 shows a star pattern with the majority of individuals (65%) belonging to one haplotype (Fig 2). Overall haplotype diversity was 0.572 ± 0.0492 for CO1, and 0.996 ± 0.0017 for CR. Nucleotide diversity was 0.00175 ± 0.001349 for CO1 and 0.0256 ± 0.0135 for CR. Slope inhabitant *H. ornatissimus* was sampled from 13 locations across the Hawaiian archipelago, plus adjacent Johnston Atoll. CO1 had 34 haplotypes (22 unique), and CR had 165 haplotypes (117 unique; Fig 2 & 3). Similar to *H. claudia,* CO1 minimum spanning networks for *H. ornatissimus* show a star

like pattern dominated by a single haplotype (Fig 2). Overall haplotype diversity was 0.407 ± 0.0361 for CO1 and 0.981 ± 0.0039 for CR. Nucleotide diversity was 0.00108 ± 0.001 for CO1 and 0.0268 ± 0.014 for CR. Lagoon inhabitant *H. trimaculatus* had 32 haplotypes for CO1 (26 unique), and 100 haplotypes for CR (78 unique; Fig 2 & 3). The CO1 minimum spanning network for *H. trimaculatus* shows three common haplotypes, each separated by one transition (Fig 2). Overall haplotype diversity was 0.754 ± 0.0165 for CO1 and 0.967 ± 0.0071 for CR. Nucleotide diversity was 0.00576 ± 0.00334 for CO1 and 0.0268 ± 0.014 for CR. Lagoon inhabitant *H. margaritaceus* had 27 haplotypes for CO1 (18 unique), and 75 haplotypes for CR (66 unique; Fig 2 & 3). Overall haplotype diversity was 0.821 ± 0.0296 for CO1 and 0.994 ± 0.0032 for CR. Nucleotide diversities were 0.00219 ± 0.00155 for CO1 and 0.040 ± 0.02 for CR. Haplotype diversity and nucleotide diversities by sampling locations are provided in Tables 1 and 2 and a visual representation of overall haplotype diversity between species is provided in Figure 4.

Population Structure and Migration

For widespread slope species *H. claudia* 93% of the variance was within populations for CO1 and 87% for CR. All pairwise differences were significant for the Marquesas (Φ_{ST} = 0.074-0.183; Table 3). In addition, CR showed significant structure for Moorea when compared to all other locations (Φ_{ST} = 0.183-0.251; Table 3). For Hawaiian slope species *H. ornatissimus* (endemic to the Hawaiian Archipelago and adjacent Johston Atoll) AMOVA revealed that 99% of genetic variation was within populations for both markers. None of the pairwise Φ_{ST} values were significant within

the Hawaiian archipelago (P>0.05; Table 4). When Hawaiian locations are compared to Johnston Atoll (1400 km southwest of Hawaii), most pairwise Φ_{ST} values were low but significant (Φ_{ST} = 0.001-0.097; Table 4). For lagoon species *H. trimaculatus* AMOVA indicates 83-90% of the variation within populations, and for *H. margaritaceus*, AMOVA indicates 72-75% of the variation within populations. Lagoon species *H. trimaculatus* shows significant spatial structuring as well with Φ_{ST} values ranging from 0.019-0.495 (Table 5). For this species, Moorea and Fiji are significantly differentiated from all locations except each other. All six pairwise Φ_{ST} comparisons are significant for *H. margaritaceus* (Φ_{ST} =0.155-0.419, Table 6). For both lagoon and slope species, all Mantel tests failed to show a significant relationship between pairwise Φ_{ST} and distance (r= -0.349 – 0.269, P>0.424).

Migrate results for each species did not converge on any specific point. This may be due to equal migration between all locations, or due to our study species not meeting assumptions of the method. Therefore our results from Migrate (not shown) are inconclusive.

Neutrality and Population Expansion

All Fu's F values were significant (*P*<0.001) and ranged from -31.71 to -14.55 (Tables 1 and 2). These values result from an excess of rare haplotypes, and indicate selection or population expansion (Fu 1997). Comparing the observed to simulated distribution of pairwise differences under a population expansion model (Harpending 1994, Rogers 1995) failed to reject the model of sudden expansion in all species (*P*>0.13), except for CO1 in *H. trimaculatus* (*P*= 0.03, Fig. 5).

Coalescent Estimates

Based on a 3% per million year mutation rate for CO1 and a 9% per million year mutation rate for CR, I was able to estimate time to most recent common ancestor (TMRCA) within each species. For CO1, slope species *H. claudia* and *H. ornatissimus* coalesce approximately 137,000 years before present (ybp; 95% CI 54,000- 246,000 ybp) and 139,000ybp (95% CI 37,000 – 299,000 ybp), respectively. For CR, the same species coalesce at approximately 277,000 ybp (95%CI 198,000- 366,000 ybp), and 299,000 ybp (95% CI 220,000- 385,000 ybp). These two sister species have only recently been described as distinct taxa (Randall & Rocha 2009), so an additional BEAST analysis was run to determine the TMRCA between species. CO1 coalesces at 223,000 ybp (95% CI 71,000 – 390,000 ybp), while CR coalesces to 346,000ybp (95% CI 234,000 – 420,000 ybp). Lagoon species (*H. trimaculatus* and *H. margaritaceus*) coalesce to the late Pleistocene for both markers. *H. trimaculatus* coalesces at 350,000 ybp (95% CI 150,000 – 577,000 ybp) for CO1 and 484,000 ybp (95% CI 297,000 – 693,000 ybp) for CR. Similarly, *H. margaritaceus* coalesces to 107,000ybp for CO1 (95% CI 34,000 – 211,000 ybp) and 399,000ybp for CR (95% CI 210,000 – 616,000 ybp). Even though most of the 95% confidence intervals overlap for both markers these are approximations that should be interpreted with caution. However, all values agree on a late Pleistocene (<500,000 yr) coalescence.

Discussion

This survey of four wrasses with two mtDNA sequences was designed to assess connectivity and population history. The expectation, based on previous research, was

that lagoon wrasses would have shallow histories and perhaps more recent connections than species on the outer reef slope. The results show the opposite; the lagoon wrasses show strong population structure and older population histories than their slope congeners. The range-wide study design allows additional inferences to be made about overall patterns of connectivity, and the generality of site-specific findings. Prior to discussing these results, I mention three caveats relevant to this study:

1) *H. margaritaceus* was only collected at three locations due to either scarcity at sample locations or logistical limitations. As these three locations do not represent the full species range in the central and West Pacific, results from *H. margaritaceus* should be regarded as provisional.

2) Clock rates for CO1 seem to cluster well among short-lived reef fishes (Table 3 in Lessios 2008) but control region rates are more controversial and probably more variable. With this uncertainty in mind, I caution against the over-interpretation of coalescence times and effective population sizes. However, the rate estimate of 9%/MY is close to the estimate of 10%/MY used for marine angelfishes (Pomacanthidae; Bowen et al. 2006).

3) The second caveat leads to the observation that coalescence times from CR are two or three times greater than CO1 in three of the four species. For this reason I limit conclusions to a generic late Pleistocene timeframe rather than specific glacial events. The disparity between CR and CO1 results can be explained by several phenomena beyond the scope of this paper, but I note here that a higher mutation rate for CR, as has been suggested for other fish species (reviewed in

Bowen et al. 2006), would likely bring the two loci into closer alignment for coalescence times.

Population Expansion and Neutrality

Sea level variations due to Pleistocene glaciations have been used to explain genetic architecture in several reef fishes (Fauvelot et al. 2003, Bay et al. 2004, Thacker 2004, Klanten et al. 2007,Winters et al. 2010, Gaither et al. 2011). The results of these studies are variable and lagoon/slope comparisons generally involved a single area or species. The goal of this study was to examine the effects of Pleistocene glaciations on lagoon and outer reef species across a large spatial scale. All species show relatively high haplotype diversity (*h*) and low nucleotide diversity (π, Fig 4). This genetic signature can be attributed to rapid population expansion after a reduction in effective population size (Grant & Bowen 1998). In support of this finding, Fu's Fs values indicate a significant excess of rare haplotypes (Fu 1997), consistent with population expansion, although this can be subject to other interpretations (see below). All mismatch distributions, except for CO1 of *H. trimaculatus*, failed to reject a simulated population expansion model, providing additional evidence for a population expansion in all four species. These results are similar to previous studies on coral reef fish that have shown temporal, rather than geographic structuring between populations (Fauvelot et al. 2003, Bay et al. 2004, Thacker 2004, Klanten et al. 2007,Winters et al. 2010). Coalescence times are consistent with population expansions associated with Pleistocene glaciations, although not the most recent glacial maxima.

The finding of similar levels of genetic diversity (Tables 1 & 2, Fig. 4) was unanticipated, as we expected lagoon species to have low haplotype and nucleotide diversities consistent with fluctuating population sizes (Grant & Bowen 1998, Fauvelot et al. 2003). Contrary to our expectations, lagoon species either exhibited similar (CR data) or greater (CO1 data) haplotype diversity than outer shelf species (Fig 4). CR values are near saturation (approaching h=1), so greater emphasis is placed on genetic diversity estimates obtained from CO1 data. There are several possible scenarios that can explain these observations.

High genetic diversity in lagoon species

Previous studies of genetic diversity in South Pacific reef fishes found evidence of population bottlenecks in both lagoon and outer reef species, but generally lower genetic diversity in lagoon species (Fauvelot et al. 2003, Thacker 2004). These studies concluded that population bottlenecks were possibly due to reduced reef area, a higher degree of habitat disturbance, and possibly complete extirpation from some areas during intervals of low sea level (Fauvelot et al. 2003). The results of this study are not consistent with the previous studies in that the lagoon species examined here exhibit higher genetic diversity than outer reef species (Fig 4).

The CO1 networks for lagoon species *H. trimaculatus* contain three haplotype clusters (84% of all individuals, Fig 2c), but even though a strong genetic structure is evident, a phylogeographic signal is lacking as each of these clusters contains representatives from each location. This pattern is consistent with previous findings where species ranges may have been sundered during sea level lows and then

subsequently reconnected (Klanten et al. 2007, Crandall et al. 2008a, Gaither et al. 2011). Although the pattern is not as distinct with *H. margaritaceus*, its CO1 haplotype network shows several clusters anchored by a common haplotype (Fig 2d). Isolated refuges may have existed for lagoon species during sea level lows, depending on the bathymetry of their geographic range, which then could have mixed as they re-colonized habitats during sea level rises. This would result in the non-geographically structured haplotype networks observed in this study.

It is also possible that with a lack of lagoon habitat these species may be able to survive in small patches of sandy, low energy habitat, even without a lagoon. These species have been observed outside of lagoons in sandy areas protected from high wave energy. Both lagoon species show bimodal mismatch distributions in the CR, which can have several interpretations. Primarily this signature is associated with fluctuating population sizes which is certainly possible given the multiple sea level fluctuations during the Pleistocene. However, this bimodal signature can also be interpreted as a signal of isolated populations that have recently come back into contact (Horne et al. 2008), which supports the concept of isolated refugia. Either interpretation leads to the hypothesis that lagoon wrasses have survived either in currently deep lagoons, wide continental margins, or the lee (protected) coastlines of oceanic islands during sea level lows (Paulay 1990).

Outer Reef Species Diversity

In addition to the unexpected high levels of diversity in lagoon species, another unexpected result of this study was the incongruence between markers for the outer reef

species. Control region diversity levels were nearly saturated making interspecific comparisons difficult, while the CO1 region showed lower haplotype diversity for both *H. ornatissimus* and *H. claudia*. These results can be interpreted several different ways. Not only can a negative Fu's F statistic (Fu 1997) show possible population expansion, but it can also indicate selection. CO1 is a protein coding region of the mitochondrial DNA, whereas CR is a non-coding region, invoking the possibility that selection is occurring. The effects of any selective sweep effecting CO1, though, would also be present in the CR, and the lack of this signal in both markers leads to the conclusion that selection is not defining the mtDNA findings in these species.

Another possible explanation is that these outer reef species experienced a population bottleneck, indicated by the negative Fu's F values and the star pattern of the minimum spanning network, and then underwent a spatial expansion. Previous studies have employed both temporal and spatial factors to describe genetic diversity patterns (Bay et al. 2004). A spatial expansion following Pleistocene glaciations therefore may not have been observed in the CR data due to a mutation rate which is approximately three to four times higher than CO1. A recent spatial expansion could also explain the isolation by distance mantel tests, which were not significant for the outer reef species ($r = -0.349$-0.027, $p > 0.424$).

Slope species *H. claudia* and *H. ornatissimus* have a relatively long pelagic larval duration (PLD) of approximately 40 days (Victor 1986) that may allow greater dispersal and rapid colonization of new habitats. Notably the lagoon species in this study have a shorter PLD (*H. margaritaceus* 21.7 days, *H. trimaculatus* 26.8 days; Victor 1986). This

may explain why *H. ornatissimus* is the sole representative of the genus in the Hawaiian archipelago. This would also explain why Indian Ocean populations are not distinct from Pacific populations in *H. claudia*, a pattern observed in other fish species with high dispersal ability (Horne et al. 2008, Gaither et al. 2010, DiBattista et al. 2011, Eble et al 2011, Reece et al. 2011). Although a large meta-analysis revealed that the relationship between PLD and population genetic structure is not as simple as intuition would indicate (Weersing & Toonen 2009), it is interesting that the lagoon species with shorter PLD are more genetically structured, consistent with earlier theories of reef fish connectivity (Doherty et al. 1995).

Given this genetic evidence for high dispersal ability, I suggest that both *H. ornatissimus* and *H. claudia* have been able to undergo rapid population expansions across their ranges, as indicated by the CO1 haplotype networks (Fig. 2). This signature of population expansion likely resulted after a population bottleneck associated with Pleistocene glaciations. Reduced effective population sizes during the Pleistocene would result in the lower genetic diversity indices observed in these slope species.

Conclusions

Sea level fluctuations during the Pleistocene reduced overall reef area and severely influenced reef lagoon habitat. Reduced habitat area may result in a reduced genetic diversity for lagoon species in comparison to outer reef species, at specific sites where lagoon species may have been extirpated. However, this study found no significant difference in genetic diversity between lagoon species and outer reef species, while examining the question at a large spatial scale. All species seem to have been

effected by population bottlenecks during the Pleistocene. During sea level lows it is possible that multiple refugia and lower dispersal allowed some populations of lagoon species to become isolated from one another either on continental margins or remote island chains, and subsequently reconnect, which resulted in the observed high genetic diversity and strongly structured haplotype networks. This conclusion is supported by bimodal mismatch distributions and observed non-geographical clade patterns in the haplotype networks for lagoon species. The contrasting pattern for outer reef species can be explained by population expansion and a long pelagic larval duration. Finally, I cannot rule out that the idiosyncrasies of individual species may explain some of the results (Toonen et al. 2011). Ultimately the magnitude of the effects of Pleistocene glaciations most likely will be specific and unique based on each species particular life history, ecological, and demographic attributes.

Tables

Site	N	N_h	*h*	π	Fu's F
H. claudia					
Marquesas	42, **32**	9, **30**	0.617 ± 0.076, **0.994 ± 0.011**	0.002 ± 0.002, **0.025 ± 0.014**	-3.49, **-25.06**
Moorea	18, **27**	3, **18**	0.216 ± 0.124, **0.954 ± 0.025**	0.000 ± 0.001, **0.013 ± 0.008**	-1.74, **-11.28**
Kiribati	31, **31**	10, **27**	0.546 ± 0.108, **0.991 ± 0.010**	0.002 ± 0.001, **0.023 ± 0.012**	-7.48, **-21.26**
Cocos-Keeling	32, **26**	8, **23**	0.595 ± 0.094, **0.991 ± 0.013**	0.001 ± 0.001, **0.029 ± 0.016**	-5.04, **-13.71**
Christmas Island	26, **23**	10, **21**	0.671 ± 0.103, **0.992 ± 0.015**	0.002 ± 0.001, **0.023 ± 0.013**	-8.64, **-15.19**
H. ornatissimus					
Big Island	71, **72**	16, **56**	0.502 ± 0.073, **0.982 ± 0.009**	0.001 ± 0.001, **0.015 ± 0.009**	-19.23, **-25.91**
Maui	6, **6**	2, **6**	0.333 ± 0.215, **1.000 ± 0.096**	0.001 ± 0.001, **0.021 ± 0.013**	0.00, **-2.03**
Oahu	17, **15**	3, **14**	0.228 ± 0.130, **0.990 ± 0.028**	0.001 ± 0.001, **0.013 ± 0.008**	-0.96, **-11.61**
Kauai	32, **29**	7, **28**	0.393 ± 0.109, **0.998 ± 0.010**	0.001 ± 0.001, **0.020 ± 0.011**	-4.55, **-25.51**
Kaula Rock	17, **20**	6, **18**	0.515 ± 0.145, **0.984 ± 0.024**	0.001 ± 0.001, **0.015 ± 0.008**	-3.77, **-15.37**
Niihau	27, **25**	4, **21**	0.214 ± 0.103, **0.983 ± 0.017**	0.000 ± 0.001, **0.030 ± 0.016**	-3.21, **-10.18**
Nihoa	7, **6**	1, **5**	0.000 ± 0.000, **0.933 ± 0.122**	0.000 ± 0.000, **0.011 ± 0.007**	0.00, **-1.46**
Necker	15, **19**	5, **16**	0.476 ± 0.155, **0.983 ± 0.022**	0.001 ± 0.001, **0.014 ± 0.008**	-2.17, **-11.64**
FFS	46, **46**	9, **33**	0.356 ± 0.091, **0.965 ± 0.018**	0.001 ± 0.001, **0.014 ± 0.008**	-6.74, **-26.05**
Pearl & Hermes	35, **37**	6, **28**	0.316 ± 0.101, **0.978 ± 0.014**	0.001 ± 0.001, **0.014 ± 0.008**	-4.09, **-25.42**
Kure	3, **4**	1, **4**	0.000 ± 0.000, **1.000 ± 0.177**	0.000 ± 0.000, **0.019 ± 0.014**	0.00, **-0.52**
Johnston Atoll	35, **30**	8, **20**	0.610 ± 0.083, **0.949 ± 0.024**	0.001 ± 0.001, **0.014 ± 0.008**	-4.77, **-12.65**

Table 1: Summary statistics for slope species based on sample location – Control region values are in bold, and follow CO1 values. Number of individuals (N), number of haplotypes (N_h), haplotype diversity (h), nucleotide diversity (π), and Fu's F statistic are given for each location sampled.

Site	N	N_h	*h*	π	Fu's F
H. trimaculatus					
Moorea	31, **20**	5, **14**	0.574 ± 0.067, **0.947 ± 0.034**	0.001 ± 0.001, **0.021 ± 0.012**	-1.37, **-4.87**
Palmyra	26, **19**	7, **13**	0.563 ± 0.108, **0.906 ± 0.060**	0.001 ± 0.001, **0.021 ± 0.012**	-3.73, **-3.82**
Kiribati	34, **31**	8, **19**	0.611 ± 0.093, **0.875 ± 0.056**	0.002 ± 0.001, **0.017 ± 0.010**	-3.19, **-9.01**
KWAJ	35, **28**	8, **24**	0.726 ± 0.050, **0.987 ± 0.014**	0.002 ± 0.001, **0.032 ± 0.017**	-3.21, **-13.31**
Fiji	31, **22**	11, **13**	0.783 ± 0.054, **0.866 ± 0.066**	0.002 ± 0.002, **0.018 ± 0.010**	-7.17, **-3.74**
Palau	34, **33**	9, **24**	0.788 ± 0.039, **0.958 ± 0.023**	0.002 ± 0.002, **0.031 ± 0.017**	-3.98, **-9.88**
Cocos-Keeling	27, **24**	5, **18**	0.607 ± 0.087, **0.967 ± 0.023**	0.002 ± 0.001, **0.029 ± 0.016**	-0.77, **-6.31**
H. margaritaceus					
Kiribati	19, **17**	6, **14**	0.771 ± 0.062, **0.971 ± 0.032**	0.005 ± 0.003, **0.030 ± 0.016**	0.71, **-3.16**
KWAJ	39, **36**	10, **32**	0.749 ± 0.054, **0.989 ± 0.012**	0.005 ± 0.003, **0.041 ± 0.021**	-1.03, **-15.06**
Fiji	50, **37**	19, **33**	0.706 ± 0.072, **0.993 ± 0.009**	0.004 ± 0.002, **0.027 ± 0.014**	-12.77, **-22.58**

Table 2: Summary statistics for lagoon species based on sample location – Control region values are in bold, and follow CO1 values. Number of individuals (N), number of haplotypes (N_h), haplotype diversity (h), nucleotide diversity (π), and Fu's F statistic are given for each location sampled.

Sampling Location	Marquesas	Kiribati	Moorea	Cocos-Keeling	Christmas Island
Marquesas	-	0.109*	0.183*	0.102*	0.128*
Kiribati	0.074*	-	0.228*	0.008	0.014
Moorea	0.100*	-0.009	-	0.216*	0.251*
Cocos-Keeling	0.120*	0.015	0.026	-	-0.005
Christmas Island	0.082*	0.001	0.001	0.023*	-

Table 3: Pairwise Φ_{ST} values for *H. claudia* based on sampling location –

Values for CO1 are given below the diagonal, while CR values are above.

*: Significant at P=0.05

Sampling Location	Big Island	FFS	Pearl & Hermes	Kure	Oahu	Maui	Kaula Rock	Niihau	Necker	Nihoa	Kauai	Johnston Atoll
Big Island	-	-0.008	-0.011	-0.036	-0.021	0.038	-0.013	0.016	-0.014	0.031	-0.004	0.060*
FFS	-0.003	-	-0.007	-0.007	-0.022	0.045	-0.014	0.021	-0.004	0.063	-0.002	0.071*
Pearl & Hermes	-0.011	-0.003	-	-0.008	-0.016	0.043	-0.011	0.019	0.006	0.070	-0.004	0.061*
Kure	-0.187	-0.190	-0.188	-	-0.031	-0.019	-0.037	-0.078	-0.044	-0.003	-0.054	0.019
Oahu	-0.016	-0.018	-0.028	-0.195	-	-0.031	-0.021	-0.007	-0.023	0.067	-0.016	0.050*
Maui	-0.030	-0.030	-0.011	-0.154	-0.003	-	0.052	-0.017	0.044	0.057	0.006	0.165*
Kaula Rock	-0.011	-0.006	-0.018	-0.178	-0.026	-0.022	-	0.008	-0.028	0.018	-0.023	0.042*
Niihau	-0.008	-0.005	-0.005	-0.198	-0.011	0.030	0.010	-	0.003	-0.005	-0.005	0.055*
Necker	-0.003	-0.003	0.000	-0.174	-0.023	-0.022	-0.031	0.020	-	-0.012	-0.018	0.048*
Nihoa	-0.065	-0.064	-0.064	0.000	-0.064	0.028	-0.048	-0.072	-0.041	-	0.006	0.097*
Kauai	-0.001	0.001	-0.005	-0.184	-0.024	-0.024	-0.012	-0.005	-0.010	-0.060	-	0.047*
Johnston Atoll	0.057*	0.057*	0.062*	-0.128	0.047	0.01	0.056*	0.060*	0.058*	-0.011	0.053*	-

Table 4: Pairwise Φ_{ST} values for *H. ornatissimus* based on sampling location – Values for CO1 are given below the diagonal, while CR values are above. FFS= French Frigate Shoals. *: Significant at P=0.05

Sampling Location	Palau	KWAJ	Palmyra	Cocos	Kiribati	Moorea	Fiji
Palau	-	-0.013	0.040	0.004	0.124*	0.090*	0.071*
KWAJ	0.021	-	0.021	-0.001	0.010*	0.084*	0.065*
Palmyra	0.149*	0.045	-	0.017	-0.007	0.207*	0.206*
Cocos	0.033	-0.007	0.016	-	0.080*	0.150*	0.137*
Kiribati	0.083*	0.019	-0.007	-0.013	-	0.335*	0.337*
Moorea	0.138*	0.300*	0.495*	0.342*	0.383*	-	-0.008
Fiji	0.063*	0.188*	0.365*	0.226*	0.278*	-0.004	-

Table 5: Pairwise Φ_{ST} values for *H. trimaculatus* based on sampling location – Values for CO1 are given below the diagonal, while CR values are above.

*: Significant at P=0.05

Sampling Location	Kiribati	Fiji	KWAJ
Kiribati	-	0.304*	0.246*
Fiji	0.419*	-	0.208*
KWAJ	0.305*	0.155*	-

Table 6: Pairwise Φ_{ST} values for *H. margaritaceus* based on sampling location – Values for CO1 are given below the diagonal, while CR values are above.

*: Significant at P=0.05

Figures

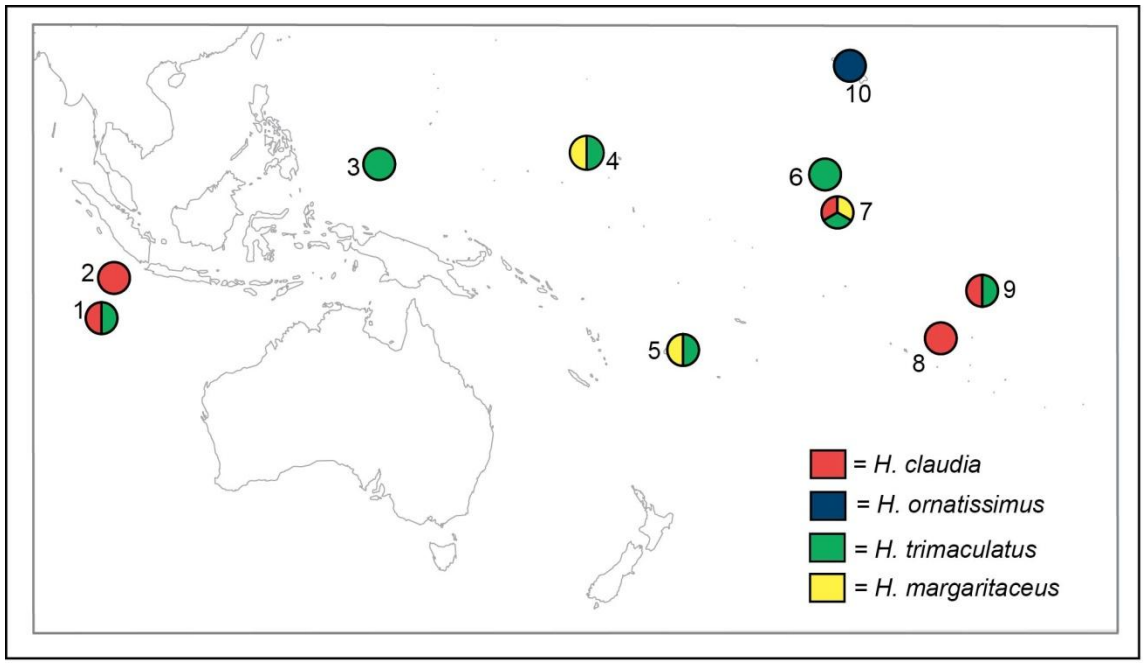

Figure 1: Map of the Indo-Pacific where samples were taken –

1) Cocos-Keeling Island, 2) Christmas Island, 3) Palau, 4) Kwajalein, Marshall Islands, 5) Fiji, 6) Palmyra, 7) Kiribati, 8) Moorea, 9) Marquesas, 10) Hawaiian Archipelago. Colors in the pie charts indicate species sampled at each location. Specific locations within the Hawaiian Archipelago can be seen in Table 1.

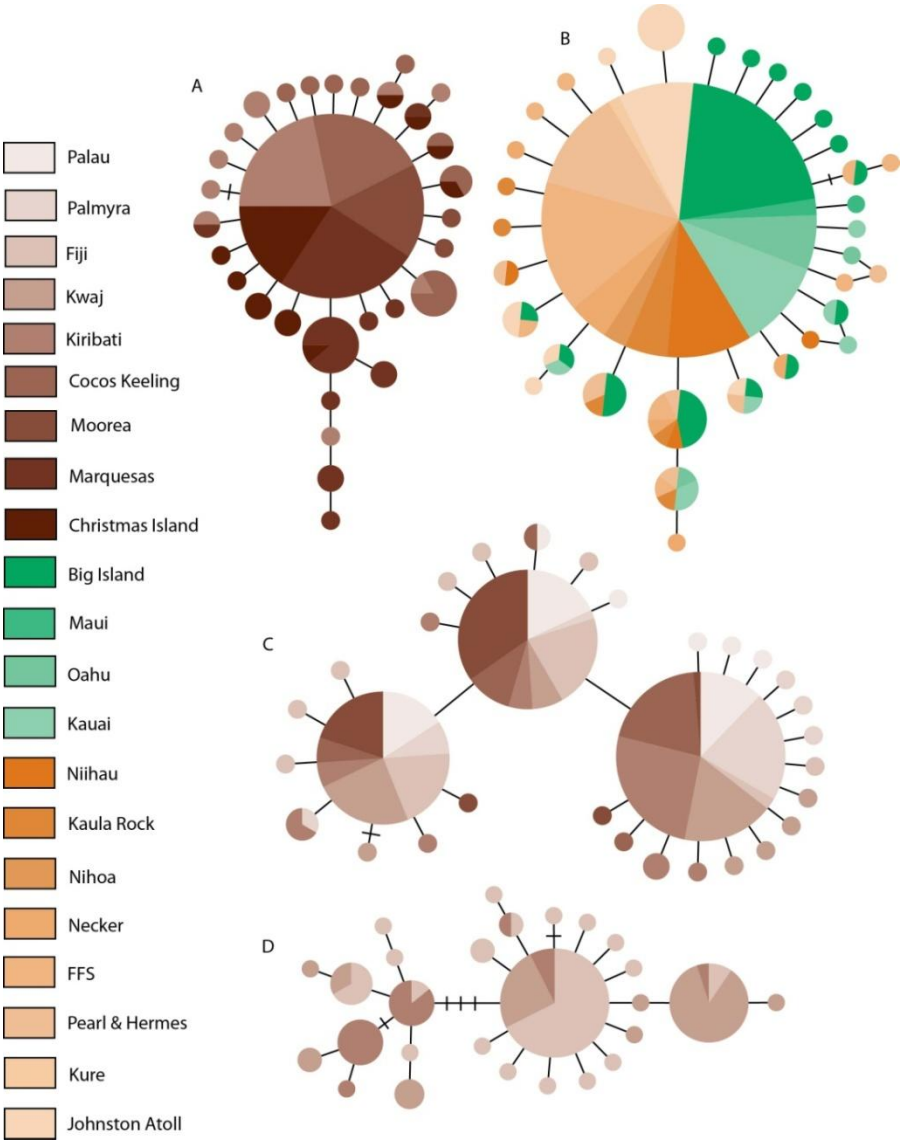

Figure 2: CO1 Haplotype networks –

A) *H. claudia* (slope), B) *H. ornatissimus* (slope), C) *H. trimaculatus* (lagoon), and D) *H. margaritaceus* (lagoon). For *H. ornatissimus* the haplotypes are shown based on location, green colors representing the main Hawaiian Islands and orange colors representing the Northwest Hawaiian Islands. Horizontal bars represent mutational steps between haplotypes.

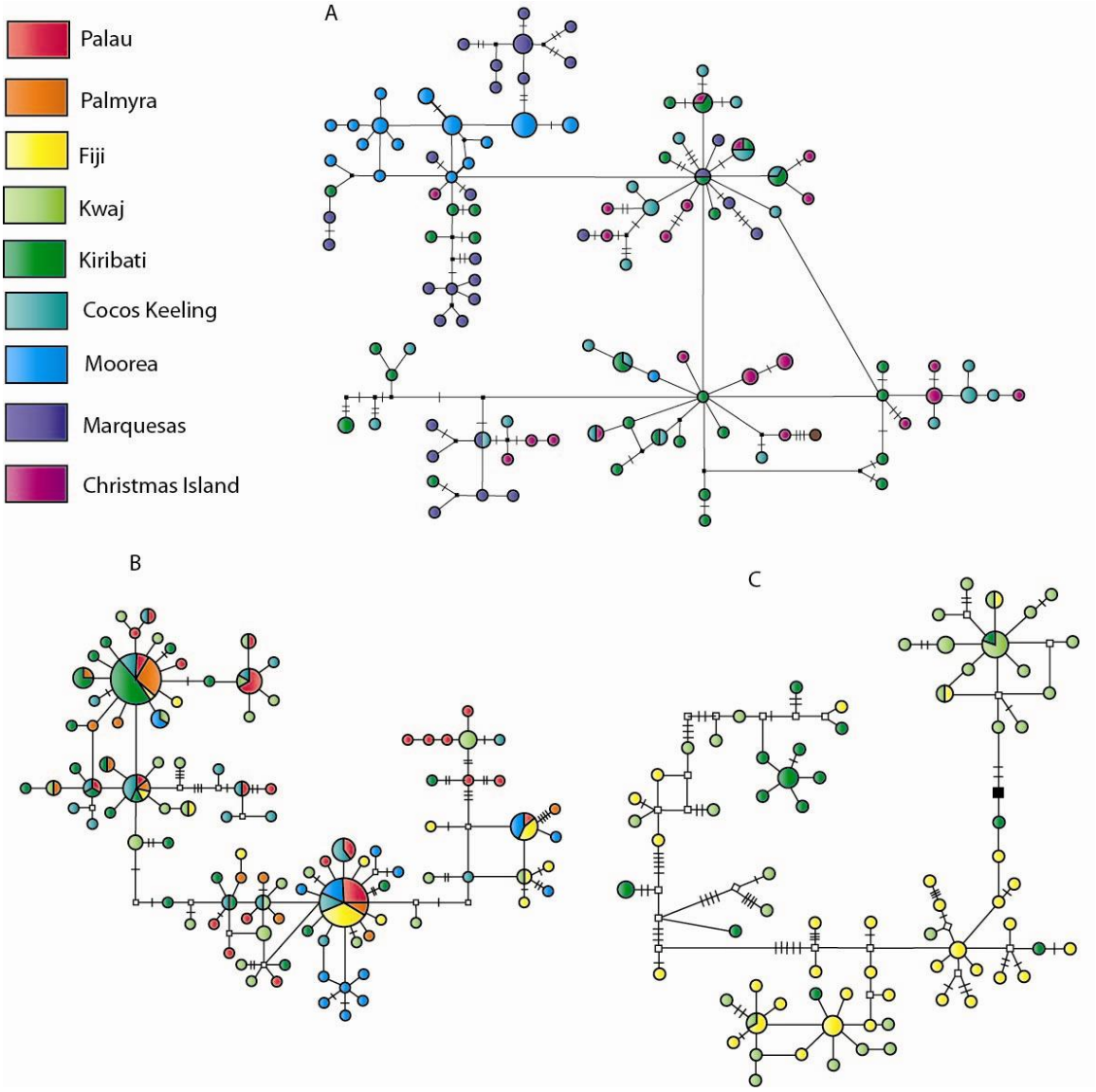

Figure 3: Haplotype networks for the control region –

A) *Halichoeres claudia*, B) *H. trimaculatus*, and C) *H. margaritaceus*. *H. ornatissimus* is not shown. Horizontal bars and open squares represent single missing haplotypes while black squares represent 10 missing haplotypes.

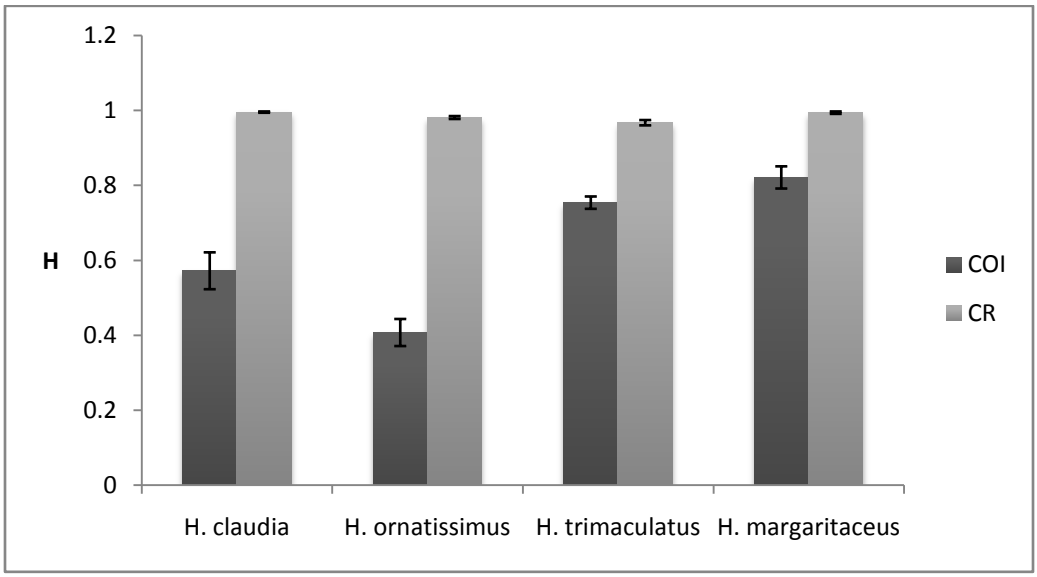

Figure 4: Haplotype diversities –

Haplotype diversities are given for each of four *Halichoeres* species, and for each marker. COI (dark grey) indicates diversities for cytochrome oxidase subunit1 fragment, CR (light grey) indicates diversities for the control region fragement.

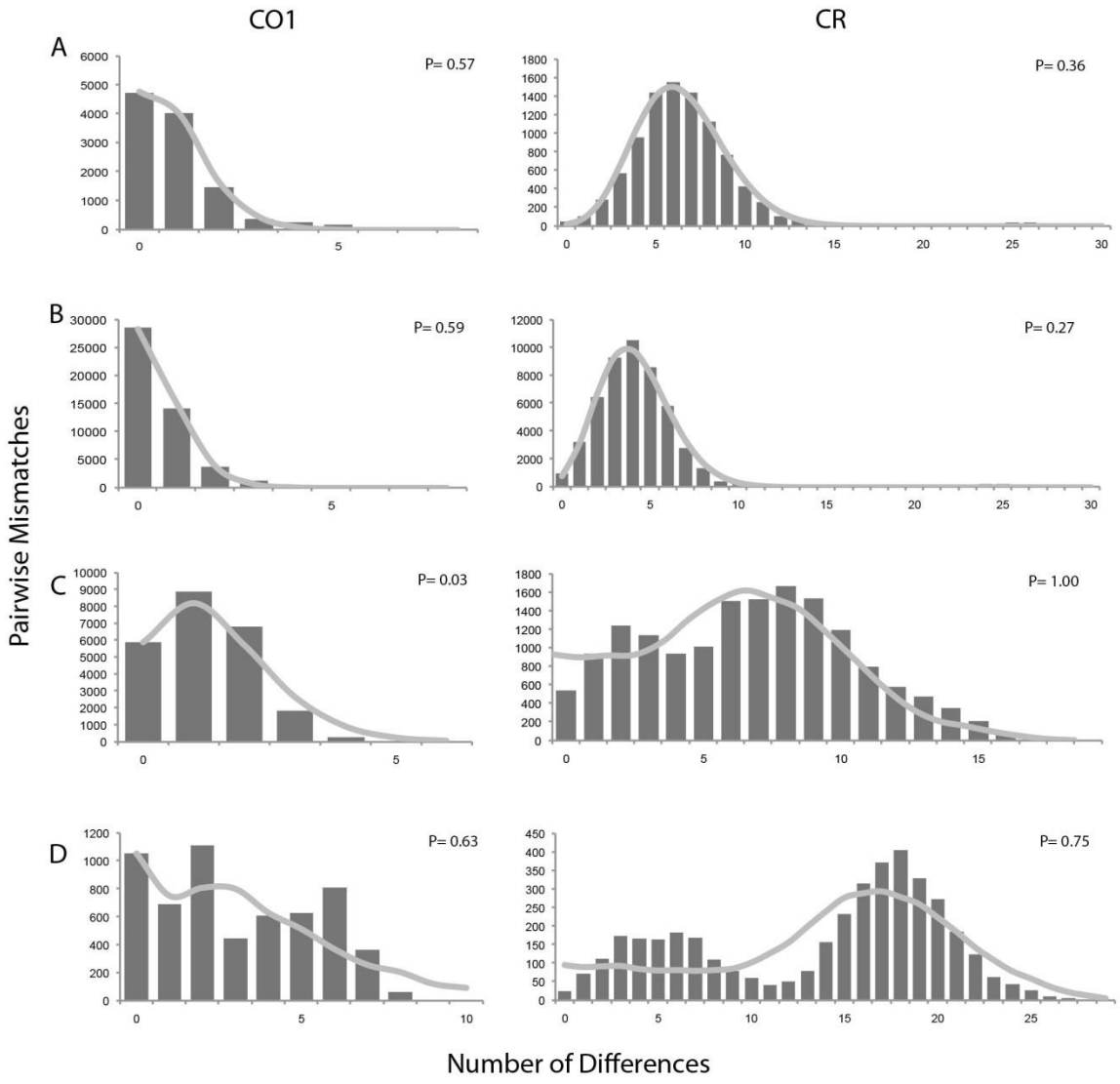

Figure 5: Simulated and observed mismatch distributions for each species and each marker –

Observed mismatch distributions are represented by the bar graphs, while the curves represent the simulated mismatch distribution under a population expansion model. P-values are reported for each marker. A) *Halichoeres claudia*, B) *H. ornatissimus*, C) *H. trimaculatus*, and D) *H. margaritaceus*.

Chapter 3

Summary and Conclusions

Present day sea level fluctuations due to global warming have the potential to displace a large amount of people and cause economic losses in the upcoming century. The rising water level will also have ecological implications. By studying the biological impacts that sea level fluctuations had during the Pleistocene we may gain a better understanding of impending changes.

During the Pleistocene sea levels fluctuated several times, with the LGM occurring approximately 17,000 years before present. During this LGM sea levels were as much as 130m below present, revealing several land barriers between the Indian and Pacific Oceans, and restricting coral reef habitats to steep continental slopes. Impacts of this have been seen in many marine taxa that are associated with coral reefs and other near-shore habitats. While these impacts are apparent in a wide array of species from distantly related genera, families, and even phyla, some general patterns have been found across taxonomic groups.

The majority of these data comes from genetic differences at the population level due to a scarcity of fossils for most taxa. The general patterns that have been found include a lack of geographic genetic structuring across the entire Indo-Pacific, a strong break between the two ocean basins, and more fine scale structuring among geographically close populations. The latter case is mostly species specific, where as the first two patterns have been found in several groups. However, the mechanistic reasons

why one species may show a lack of structure across their entire range and another closely related species shows large genetic breaks are still largely unknown.

Comparing the genetic history of closely related species can give us insight into what processes or mechanisms are behind the variety of observed patterns. Several studies of parrotfish and surgeonfish have seen similar responses to sea level fluctuations, suggesting common historical influences, and not just stochastic variation in genetic structure. Fossil evidence from bivalves has suggested that habitat associations may play a large role in how sea level fluctuations will affect a species, as those inhabiting shallow lagoons were extirpated during sea level lows, whereas slope species have maintained relatively stable populations (Paulay 1990). This pattern has been corroborated in both sea stars (Barber et al. 2006) and several reef fishes (Fauvelot et al. 2003, Thacker 2004), albeit on limited spatial scales.

The goal of the present study was to examine the genetic difference between two lagoon-inhabiting and two outer reef wrasses of the genus *Halichoeres*. Counter to previous results I found either similar genetic diversity or even larger genetic diversity in lagoon species compared to outer reef species depending on which loci was examined. Traditionally, lagoon species have shown lower genetic diversity due to large population bottlenecks associated with extirpation following sea level drops. However, in this study I found several lines of evidence suggesting isolation in multiple refugia followed by range expansion for lagoon species.

During sea level lows all species involved experienced population bottlenecks, and while the outer reef species seem to have maintained population connectivity, lagoon

species were isolated in several different refugia and accumulated genetic differences. Once the sea levels rose, these populations of lagoon species re-connected and re-colonized shallow lagoons in their ranges. This mixing of populations from different refugia raised the genetic diversity seen today in this group.

This study suggests that population dynamics of shallow water species may be more complex than previously thought. All lagoon inhabiting species were extirpated from associated habitats during sea level lows and later re-colonized as sea levels rose. However, I have shown that what occurred between extirpation and re-colonization may vary between species. The pattern of multiple isolated refuges during sea level lows observed in this study contrasts to previous studies involving species with similar habitat requirements. This could represent an uncommon response to sea level lows that is not seen in other species. Alternatively the species range encompassing design of this study may have revealed historic demographic pattern that were not seen in previous studies due to their geographically limited sampling designs. Overall this study contributes to current scientific knowledge by suggesting that there were multiple refugia during sea level lows for lagoon species, and that these refugia may have been isolated from one another until sea levels rose again.

While these patterns may be unique to the examined species complex, this study demonstrates that not all species are as heavily impacted during sea level lows as previously reported. Ultimately, each species may react to changing sea levels differently, but future comparative studies between closely related taxa may reveal specific characteristics causing the observed patterns that are reported here.

Appendix

ABBREVIATIONS:

π..... Nucleotide diversity

AMOVA……Analysis of molecular variation

CO1……..Cytochrome oxidase 1

CR…….....Control region

FFS……..……French Frigate Shoals

GBR…….Great Barrier Reef

H…….Haplotype diversity

KWAJ……...Kwajalein, Marshall Islands

LGM…….. Last glacial maximum

MSN…….Minimum spanning network

MWP……...Melt water pulse

MY……....Million years

SST……. Sea surface temperature

TMRCA……Time to most recent common ancestor

WAIS…….Western Antarctic ice sheet

YBP……...Years before present

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