Ka’upulehu Fish Parentage Summary

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NOAA CORAL REEF CONSERVATION PROGRAM

Introduction

Well designed and effectively enforced Marine Protected Areas (MPA) have been proved to have many benefits which include maintaining coral cover, reducing the prevalence of coral disease, and increasing fish biomass. Effective MPAs have been proven to confer a spillover effect whereby fish travel from within the MPA to other areas. This spillover can occur through either emigration of adults and juveniles and through dispersal of larvae spawned by adults within the MPA. This study is designed to investigate the potential spillover effect from this latter mechanism, which is poorly understood in Hawai‘i, using methods that compare genetic relationships between adult fish and offspring similar to those used in studies elsewhere in the Pacific. The study is located at an existing MPA at Ka'ūpūlehu, North Kona, Hawai‘i Island. In 2000, Ka’upulehu was designated Fisheries Management Area (FMA) which does not allow for the taking of marine life for aquarium purposes and is currently a candidate for additional protection through establishment of a 10 year fishing moratorium on Ka'ūpūlehu’s coral reef through Hawai‘i’s state Administrative Rulemaking process. This larval parentage will investigate the genetic connectivity between populations of the Convict tang, commonly called manini (*Acanthurus triostegus*) within Ka’upulehu and the surrounding area to evaluate recruitment inside Ka'ūpūlehu FMA and spillover through larval dispersal. This information will be obtained by working closely with local fishermen and community members to improve understanding and acceptance of results. This information is vital to management of coral reefs in Hawai‘i to meet social and ecological objectives.

Methods

Project Location

Ka’upulehu FMA is locally referred to as a Fish Replenishment Area (FRA), one of nine such areas in a network within the West Hawai‘i Regional Fisheries Management Area. Ka'ūpūlehu FMA/FRA spans 3.6 miles of coastline in North Kona and includes the ahupua`a (historic land divisions) of Kūki‘o and Ka'ūpūlehu. The Northern boundary is located at 19º 51.011’ N; 156º 58.111’ W, with the Southern boundary of 19º 49.209’ N; 156º 00.132’ W. The existing MPA extends to 100 fathoms, while

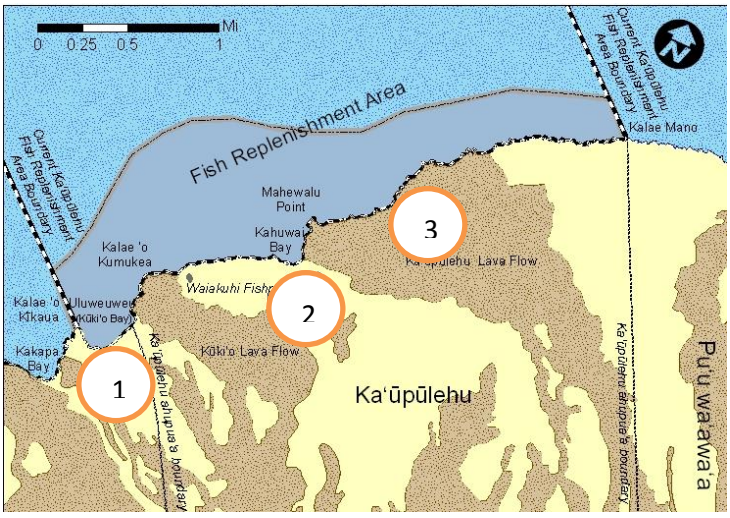

Figure 1 – The existing Ka'ūpūlehu FMA/FRA in North Kona on Hawai‘i Island includes the coastal extent of Kūki‘o and Ka'ūpūlehu and is bounded by the high water mark onshore to a seaward depth of 100 fathoms. The proposed Ka'ūpūlehu Marine Reserve shares these coastal boundaries but extends offshore to 20 fathoms. Three adult sampling sites (1-3) within Ka'ūpūlehu FMA are depicted and represent distinct habitat types and wave exposures.

the proposed fishing moratorium would be restricted to a 20 fathom depth. Within this area three sites have been selected for adult fish through consultation with local fishers and analysis of fisheries independent data collected by TNC's Marine Science Team. The Southern-most site is 19º 49.223' N, 155º 59.847' W, the second site is at 19º 49.854' N, 155º 59.307' W, and the final, and Northern most adult collection site 19° 50.23' N, 155° 58. 506'W. Each adult collection site is bounded at a depth of 20 feet to facilitate the preferred sampling method using throw nets while accounting for the preference for larger manini to congregate in 5-15 fsw. Sites 1 and 2 are located within distinct embayments while site 3 is along an exposed stretch of coastline. Site one at Uluweuweu Bay is characterized by a white sand and scattered coral substrate in depths <20 feet and is exposed to northwest swells. Site 2 at Kahuwai Bay is characterized by a sheltered black sand and patch reef substrate exposed to swells from the west. Site 3 at Keonenui Beach consists of a black sand and boulder habitat exposed to swells from southwest to north. All three sites transition into basalt and fringing coral reef habitat at depths between 20 and 100 feet. The juvenile fish are being collected on a broader distance along the coastline with a radiating distance of 25 km from the center of the proposed MPA.

Juvenile sampling sites are distributed more broadly with three sites located within Ka'ūpūlehu FMA, five sites to the north and two sites to the south. These sites were selected through consultation with additional fishers to focus on areas of known juvenile manini recruitment and were heavily focused on tidepool and sheltered shallow habitats with hardbottom substrate.

Sampling Design

We set our sampling targets based on successful research from the Great Barrier Reef, the Solomon Islands, and Papua New Guinea.

In general, 500-1000 adult samples comprising 30% of the adult population evenly distributed across three sites are desirable. For juvenile samples, up to 50 are desirable within the same geography as adult sampling, here defined at the Ka'ūpūlehu FMA. Fifty juvenile samples per site are ideal for coastal areas within 15 km of Ka'ūpūlehu FMA. One hundred juvenile samples per site are needed for sites 15-25 km from Ka'ūpūlehu FMA, and 150 juvenile samples are needed for sites >25km from Ka'ūpūlehu.

In order to approximate the expected number of adult manini per site, we first analyzed data collected by TNC's Marine Science Team during annual coral reef surveys from 2009-2011 (Appendix 1). Briefly, survey sites were randomly selected using ArcGIS software. At each survey site, divers identified, sized, and counted all individuals of all species of fish within two replicate 25x5 m belt transects. Using fish length and published size to weight conversions, fish biomass (weight) was calculated for each size class of fish for each species and summed to obtain total fish biomass. We combined this fisheries independent data with local fish consultations to select general areas for sampling as outlined in the previous section. We used

this preliminary information to pilot sampling methods in order to assess their viability (Appendix B). Once throw net sampling was determined to be viable in two of the three target areas, we conducted additional surveys targeting only manini in the 0-20 foot depth range. This snorkel survey method was adapted from our existing volunteer snorkel protocol and consisted of buddy pairs using a GPS to navigate between two fixed points and record the length (to the nearest cm) of all manini encountered within XXm of the invisible transect line. Buddy pairs maintained visual contact to avoid double counting individuals. We used these results to refine survey areas and update our target samples for sites 1 and 2. Large waves precluded a census at site 3, which will be surveyed in May, 2014.

We targeted juvenile sampling sites within 15 km of Ka'ūpūlehu FMA initially, with plans to expand this sampling focal area as time and resources permit. Juveniles are collected within discrete areas marked by GPS waypoints.

Sample Collection

Samples were collected from shore using non-lethal methods under a Special Activity Permit granted by the Hawai‘i Department of Land and Natural Resources. A priority for the specimen collections is the ability for the fish to return to the ocean with a minimal amount of stress to enhance their chances of survival. To accomplish this, various netting techniques are being employed, as detailed below. Upon capture of fish, all non-target species are immediately returned. Manini are transferred to 5 gallon buckets containing fresh seawater. Trained community members and/or TNC staff measure individual manini total length to the nearest tenth of a centimeter and remove an ~1 cm clip of fin tissue from the posterior dorsal fin rays. This fin clip is stored in a labelled vial containing buffer solution consisting of Dimethyl Sulfoxide (DMSO). Adult fish are handled to express sperm or eggs and identified as male, female, or undetermined. All fish are then immediate returned to the coastal waters in the area in which they were captured. In addition to sample number, total length (TL), and sex, sample teams record fishing effort, geographic coordinates using handheld Garmin GPS units, time of capture, number of manini individuals caught per throw, and bycatch using standard datasheets developed for the project. TNC staff have trained independent volunteer fishers to collect this information and provided them with sampling kits. The success of fully independent volunteers has be nominal, with 20 total samples collected in this way. We also contracted skilled fishers from Ka'ūpūlehu to collect samples with financial compensation. This has been much more successful, and most of the samples have been collected by contract fishers with and without TNC staff support. Adult samples (>17 cm TL) were obtained following peak spawning documented in April/May 2013 (Schemel personal communication) to provide ample opportunity for reproduction prior to sampling. Juvenile sampling was conducted opportunistically and targeted small (1.0 – 5.0 cm TL) individuals to reasonably account for young of year (YOY) from the spring 2013 spawning season. Sample vials are currently in storage and awaiting shipment to a genetics lab once sampling concludes in summer 2014.

Adult Sampling

The primary method of sampling adult fish utilizes a monofilament cast net 10-13 feet in diameter. Teams of skilled local fishers trained in sampling methods walk along the shoreline at incoming or high tides. Upon sighting schools of manini, fishers cast their nets to capture them. Successful net cast locations are recorded using GPS waypoints.

An interesting variation on this fishing method involves the use of a traditional Hawaiian method of Hukilau. Hukilau utilizes a net of dried ti leaves that hang vertically from a sturdy line. Two nets constructed for this project measure 125 and 75 feet long respectively. This fishing method was employed during two fishing days at site 2 – Kahuwai Bay. Teams of fishers and volunteers led by a lead fisher deploy these nets in a line outside of groups of manini and splash the water (pae pae) while moving the net (lau) to herd them into shallow exposed areas. Once manini have been herded into these areas they are caught with throw nets or if the fish aggregation is dense enough, scoop nets. A variation on this method utilized a monofilament gill net along with the lau to capture manini that attempted to breach the line of fishers – manini became entangled in the gill net and were removed by hand. Good coordination and a large amount of participants are required for this method to succeed, with multiple deployments of the hukilau net used strategically to herd individual schools of manini and to move groups of fish across Kahuwai Bay.

Juvenile Sampling

Juveniles were obtained using fine mesh scoop and cross nets. The scoop net method involved using a stick to prod juveniles out from beneath rocks in tidpools and into stationary scoop nets. This method was limited by fishers ability to flush and accurately predict the flight path of juvenile fish and was not sufficiently successful to justify continued effort.

Fine mesh cross net fishing involved 1-4 people predicting the exit of a group of fish then obstructing this exit with the cross-net. Fish were flushed into the net and scooped up from its perimeter. This fine mesh net does not get under the gill plate of most fish and is a benign method of sampling relatively vulnerable juvenile size classes (1.0 cm – 5.0 cm).

Another method of sampling juvenile *A. triostegus* has been using scoop nets in tide pools at night. Juvenile manini are usually asleep or very lethargic at night and unable to leave tidepools. This allows the fisher to locate the fish and collect them with the scoop net.

Information on the relative efficiency of adult and juvenile sampling methods is shared in the results section of this report.

Genetic and Parentage Analyses

DNA extractions (pending sampling completion)

Genomic DNA will be extracted from ~2mm^2 of fin tissue from each sample using the Qiagen (Valencia, CA) blood and tissue kit. A panel of 28 microsatellite loci will be amplified using the Type-it Microsatellite PCR kit (Qiagen) following the manufacturer's protocol with annealing temperatures ranging from 57 °C to 63 °C. Primers will be fluorescently labeled and pooled in multiple multiplex reactions with up to six loci per reaction. PCR products will be screened on an ABI 3730XL automated sequencer (Applied Biosystems). Allele sizes will be determined with the fragment analysis software Genemapper 3.7. A subset of the data will be tested for departure from Hardy-Weinberg equilibrium (HWE) by locus and over all loci using Genepop v 3.4, and 10,000 batches and 5,000 iterations will be employed to obtain standard errors below 0.01. We will then separate adult and juvenile genotypes and repeated the HWE tests by locus for each data set. We will use Microchecker v.2.2.3 to determine if deviations from HWE were due to null alleles. We will use Genepop v 3.4 to test for linkage disequilibrium among all locus pairs. Significance levels will be adjusted with sequential Bonferroni corrections for multiple tests with $p < 0.05$.

General genetic patterns

All adults and juveniles will be genotyped with a panel of 23 microsatellite markers. Statistics for each locus (number of alleles, number of genotyped individuals, observed and expected heterozygosities, F_{is} and an estimate of null allele frequency) for both data sets will be calculated. All 23 loci will be tested for the absence of significant departures from HWE in both data sets, the absence of null alleles for almost all loci confirmed from Microchecker v.2.2.3, absence of significant ($p < 0.05$) presence of null alleles in both data sets, and the absence of locus pairwise comparisons showing evidence of significant linkage disequilibrium prior to being used for parentage analyses.

Parentage assignments

Categorical allocation of parent-offspring relationships will beassessed based on a maximum likelihood approach implemented in the software program FAMOZ, which has recently been shown to provide accurate assignments when 20 or more polymorphic microsatellite loci are used. FAMOZ computes log of the odds ratio (LOD) scores for parent-offspring relationships and constructs statistical tests for parentage assignments. These tests are based on the simulation of offspring from genotyped parents (true pairs) and from allele frequencies estimated from the genetic dataset (false pairs) to construct statistical tests for parentage assignments. In the present study 10,000 simulated offspring will be generated from genotyped parents and allele frequencies. These simulations allow the inclusion of an error term to take into account genotyping error. We will use an error rate of 0.01% that minimizes statistical errors

associated with parentage tests. Minimum LOD score thresholds for accepting single-parent and two-parent assignments as being true will be defined as the intersection between the two distributions of LOD scores from simulated offspring (true versus false pairs) mentioned previously. LOD score threshold values will be 4.5 for single-parent assignments and 13.0 for two-parent assignments. This parameter set will be evaluated using the 'parentage test simulation' option to estimate the probability of excluding a true parent knowing that it was in the sample (Type I error) and the probability of assigning a false parent knowing that the true parent was not sampled (Type II error). Type I and II errors will be quantified.

All juveniles will be screened against the total pool of adult samples to identify parent-offspring relationships. Missing data distribution across loci will be quantified to the nearest tenth of one percent. We will exclude from further analyses juveniles assigned to only one parent that present two or more confirmed mismatches between their genotype and that of the assigned parents. For the remaining juveniles, the most likely parent (or parent pair) will be the only assigned parent (or parent pair).

Results

Although the final results of this study will consider genetic relationships along the north Kona and south Kohala coast based upon genetic analysis results, we have learned a great deal from our work to date.

Sampling Targets

The results of the manini census at sites 1 and 2 indicated a need to expand site 1 to include a larger portion of Uluweuweu Bay due to the relative absence of mature (>17 cm TL) manini encountered. Site 2 was determined to be sufficient to constitute a sample size of 150-300 adult manini, which is ideal for statistical analysis. This result indicates the importance of accompanying sampling with census data, which will be repeated for sites 1 (expanded to include all of Uluweuweu Bay) and 2 along with an initial survey of site 3 in 2013.

Ka'ūpūlehu Manini Census Survey Estimated Adult Manini Abundance

Site	Name	Depth zone (ft)	area (m2)	Avg Number/m2	Extrapolated Number	30% of estimated ADULT (>17cm) pop
1	Kahuwai Bay	0-20	215848	0.002611518	564	**169**
2	Uluweuweu Bay	0-20	148097	0.000316201	47	**14**

Table 1: Results of manini census conducted in August 2013. Note the strong variation in manini populations between sites 1 and 2. Results indicate the need to expand the geographic range of site 1 to include additional manini habitat.

Fishing Methods

Evaluation of multiple methods for both adult and juvenile sampling will inform our continued sampling work. Adult sampling showed a clear pattern of efficiency linked to gear type. Table 2

demonstrates the variability in CPUE by fishing method and location. Variability of throw net fishing is linked to a variety of factors including weather conditions (tide, wind, waves), skill of fisher teams, and additional fishing pressure present before sampling activities commence. Even the lowest throw net CPUE is an order of magnitude above hukilau efficiency.

Adult Manini Fishing Efficiency

Date	Site	**Fishing Gear**	Time (hr)	# of fishers	Effort (fisher hours)	Catch (# caught)	**CPUE**
7/30/2013	3*	**Throw**	1	1	0.98	7	**7.12**
7/31/2013	1 and 2	**Throw**	7	1	7	50	**7.14**
8/3/2013	2	**Hukilau**	2.5	40	100	9	**0.09**
8/25/2013	1 and 2	**Throw**	2.3	1	2.33	41	**17.57**
9/14/2013	2	**Hukilau**	3.5	22	77	27	**0.35**
9/14/2013	2	**Throw**	4.5	2	9	38	**4.22**

Table 2: Catch Per Unit Effort by Gear Type at adult sampling sites. Throw consists of fisher teams working independently. Hukilau constitutes large teams of fishers and volunteers using traditional nets. Site 3* was a scouting site at the north end of Ka'ūpūlehu FMA, but was determined to be too far outside of the area of focus to be viable as a sampling site. It is included for reference.

Hukilau was only conducted at site 2, but given the relatively high abundance of manini at this site, that is unlikely to be a factor for its low CPUE. The much higher efficiency of throw net teams does not tell the entire story however as hukilau events are a powerful opportunity to share information about this project and engage many hands in data collection. As such, we will continue to support them as an outreach vehicle for the larval parentage study, but will focus targeted fishing effort on throw net teams.

Juvenile sampling also benefited from experimentation with different methods. Although juvenile CPUE was not tracked as closely as adult sampling, participating fishers confirm that sampling juveniles in tidepools at night may be exponentially more efficient than during the day. This information was derived from two sampling trips to Kīholo Bay. The first occurred during daylight hours and focused on nearshore tidepool habitat. With concerted effort using scoop nets, fishers and TNC staff were able to collect two fish clip samples over a 3 hour period.

Subsequent to this sampling effort, one TNC staff and one fisher revisited the same location a month later, this time in the evening. Using the same type of scoop net methods, over fifty fish were caught and sampled during a one hour period, indicating the potential for this method. Fifty samples is a sufficient number of juvenile samples for this location based on the experimental design of this study. These methods are being replicated in other coastal areas, and fishers are learning the best tide and ocean conditions in which to employ them. To date we have a full sample size of juveniles from the Kīholo Bay site, but the rapidity with which juvenile samples can be collected is encouraging.

Sampling Summary

Since fishing began in August 2013, 488 total fin clips have been collected (Table 3). Based on size frequency, most of the samples collected at site 2 are at or near the reproductive size for manini, while most of the samples collected at site 1 are smaller than reproductive size. Based on our observations of fish in both of these areas, this is not surprising, but it does raise an important question with regard to the reproductive viability of these two populations. The reproductive size of manini may be highly variable with location (Schemel, Water Resources Conference 2014), and fishers are able to express sperm or eggs from non-reproductive manini at site 1. Although our effort is now focused on expanding this site to collect more manini >17cm, we will include smaller samples in our shipment in case manini are spawning at a smaller size. If this is the case, it will have far reaching implications for the scale at which size based fisheries management is effective in Hawai‘i.

Acanthurus triostegus collected specimens

Fish Size (cm) Bins	Kahuwai Bay	Kalaemano	Kiholo	South Uluweuweu	Grand Total
0.1-4.9	15	0	25	1	41
5.0-9.9	1	0	78	5	84
10.0-14.9	75	4	20	146	245
15.0-16.9	101	2	1	14	118
17.0-19.9	23	1	0	8	32
20.0-24.0	5	0	0	0	5
Total	220	7	124	174	525

Table 3: Fish sizing bins on the left margin with the various sampling sites on the top row.

Conclusion

We have successfully implemented a collaborative project working with fishers to understand the potential benefits of MPA’s in Hawai‘i through the process of larval spillover and have innovated methods for rapid assessment of resource fish populations and live capture through a truly collaborative engagement. We’ve revived a traditional fishing method to engage community members and fishers in our research. We’ve determined the most efficient methods

for collecting adult and juvenile samples, and we've learned a great deal about fishing in general from working closely with skilled local fishers. However, more work remains.

We will be replicating and expanding our census monitoring to further refine our sampling design. Collection of both adult and juvenile samples currently underway will be continued until sampling targets are met. Further testing of non-lethal fishing gears such as modified spears will be conducted through closely controlled trials. Above all, we will continue to share our results and obtain information from community members in real time through active participation in this study.

An additional research question that this study will inform is the potential for a moratorium on fishing to affect CPUE using both modern and traditional methods. Upon successful completion of the current project, we will have established a useful baseline against which to measure experimental replication in 5-10 years. We anticipate that it will be much easier to obtain samples following reductions in fishing pressure and are excited about the implications for traditional fishing gear to be studied alongside monofilament nets. It may be that the advent of monofilament so improved fishing technology that traditional methods were replaced, however, we may find that the depletion of abundance associated with multiple anthropogenic impacts on coral reefs rendered traditional fishing methods less viable. Although it is speculative until the studies can be complete, we look forward to understanding how the efficiency of traditional fishing methods changes as fish abundance increases in a fully protected area in west Hawai‘i and are grateful for the opportunity to begin this work.

Acknowledgements

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Appendix A: TNC Survey Methods and Data Analysis

The overarching goal of TNC's marine monitoring program is to detect change in the biological community over time on specific reef areas around the main Hawaiian Islands. In addition to detecting temporal change, the marine monitoring program seeks to provide data that can be used to compare coral reef areas with other reef ecosystems across the state and beyond. Such comparisons can provide a context within which to understand any observed changes. Thus, survey design and sampling protocols were specifically chosen to provide the greatest likelihood of compatibility with other monitoring efforts currently underway in Hawai‘i.

TNC’s marine monitoring team conducted all benthic and fish surveys at Puakō. Members of the monitoring team have hundreds of hours of experience conducting underwater surveys of coral reefs, and provide regular monitoring for numerous sites around the main Hawaiian Islands.

Survey Sites

The survey area at Ka‘ūpūlehu was delineated in ArcGIS (Figure B.1). The survey area covered approximately 1.2 km of coastline and included coral reef habitat between 3 and 15 m deep. Twenty-seven randomly generated sites were surveyed by divers deployed from a small boat. The survey team navigated to each predetermined site using a Garmin GPS unit. Once on site, the survey team descended directly to the bottom, where divers established two transect start points approximately 10 m apart. From each start-point, divers deployed a 25-m transect line along a predetermined compass heading, parallel to each other.

Benthic Community Surveys

Benthic surveys were not designed to collect comprehensive biodiversity data. Instead, surveys were designed to collect quantitative data on specific taxa, primarily individual coral species, algae at higher taxonomic resolution (*e.g.*, red, green, brown, turf, crustose coralline, etc.), and abiotic substratum type when the bottom was something other than hard substratum.

At each survey site, benthic photographs were collected at 1-m intervals along one of the two 25-m transect lines. Photographs were taken with a Canon G11 camera mounted on a 0.8-m long monopod, resulting in images that covered approximately 0.8 x 0.6 m of the bottom. Prior to photographing each

transect, the camera was white balanced to improve photograph quality. A 5-cm scale bar marked in 1-cm increments was included in all photographs.

Each photograph was imported into Adobe Photoshop CS5 where its color, contrast, and tone were autobalanced to improve photo quality prior to analysis using the Coral Point Count program with Excel extension (CPCe) developed by the National Coral Reef Institute (Kohler and Gill 2006). Using CPCe, 30 random points were overlaid on each digital photograph, and

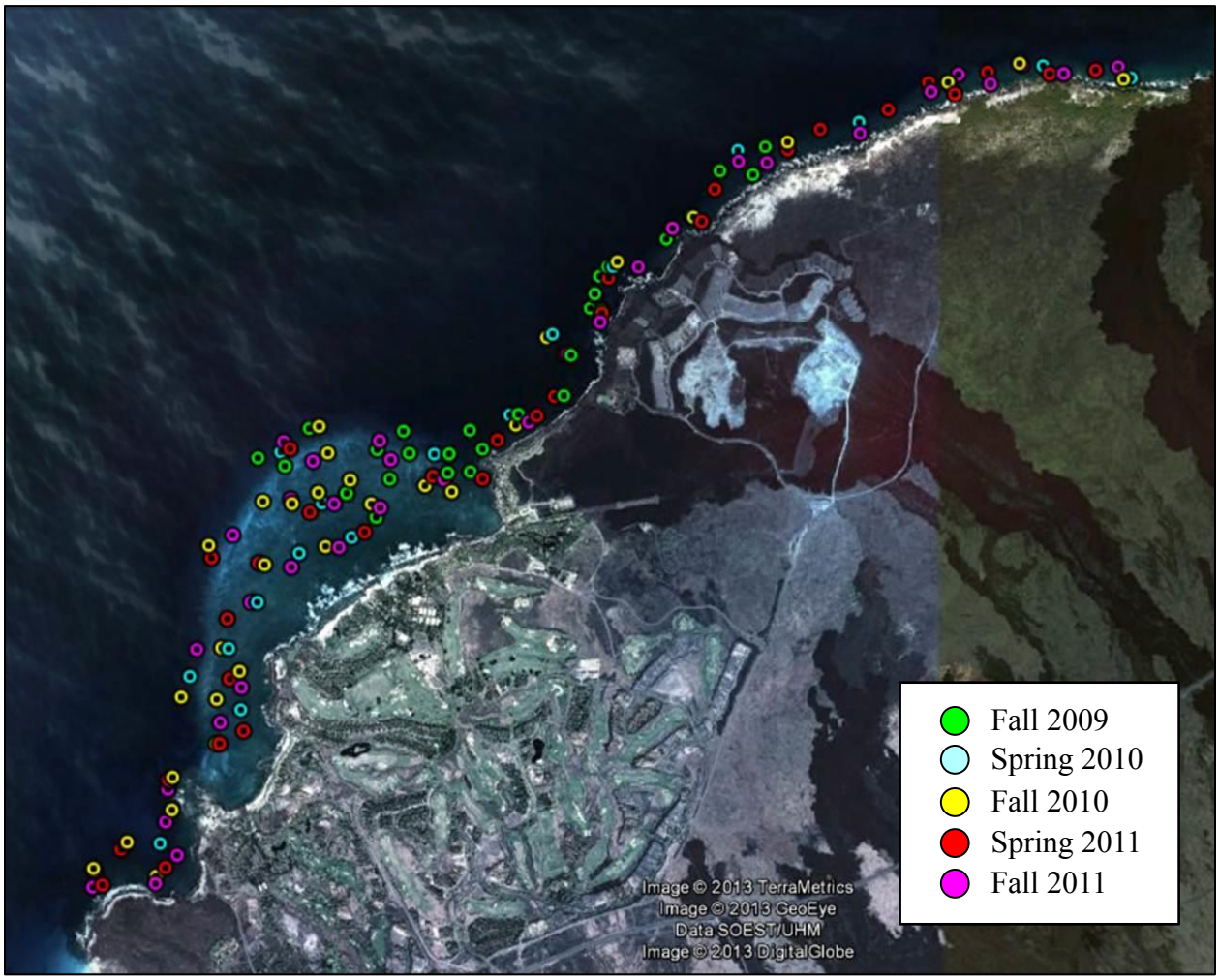

Figure B.1. Kaʻūpūlehu reef with the 148 randomly generated marine monitoring sites surveyed during Fall 2009, Spring and Fall 2010, and Spring and Fall 2011.

the benthic component under each point was identified to the lowest possible taxonomic level. To reduce observer variability, all photographs were processed by a single individual. The raw point data from all photographs on a transect line were combined to calculate the percent cover of each benthic component for the entire belt transect.

Data on coral colony size and density were collected *in situ* by a single diver. All coral colonies whose center lay within a 0.25 meter-square quadrat were identified to the lowest taxonomic level and their longest dimension measured using a plastic ruler. To improve efficiency in water, colonies were binned into the following size categories: <1cm, >1-2 cm, >2-5 cm, >5-10 cm, >10-20 cm, >20-40 cm, >40-80 cm, >80-160 cm, >160 cm. Colonies were individually distinguished by a variety of factors including color and morphology, but most importantly tissue and or skeletal boundary separation. Most colonies were distinguishable based on these parameters. However, at some sites, *Porites compressa* was extensive and grew in large amalgamated beds, which did not allow for reliable colony delineation in the time available. At these sites, the presence of *P. compressa* was noted, but colonies were not delineated or sized. Other species present in the quadrats were delineated and sized as described above. As many 0.25 meter-square quadrats as possible were haphazardly surveyed along one of the 25-m transect lines in time available time (~20-25 minutes). This resulted in from 4-20 quadrats surveyed at each survey site, depending upon the density of corals at the site.

Fish Community Surveys

All fish within or passing through a 5 m wide belt along each of the two 25 m transects deployed at each survey site were identified to species and sized into 5 cm bins (*i.e.*, 0-5 cm, >5-10 cm, >10-15 cm, etc.) Divers moved slowly along the transects, taking between 10 and 15 minutes to complete each belt survey. This method closely corresponds with that used by Dr. Alan Friedlander and colleagues for the "Fish Habitat Utilization Study" (FHUS), and provides comparable data. Details of their method and results of those surveys are given in a number of recent publications (Friedlander *et al.* 2006, Friedlander *et al.* 2007a, 2007b).

A 5-minute timed swim was conducted after divers completed surveying the 25-m transect lines. For the timed swims, the two fish surveyors swam approximately 5 m apart and visually censused all fish larger than 15 cm within or passing through a 5 m wide column (centered on the surveyor) extending from the ocean bottom to the surface. Divers communicated with each other to ensure that each fish was censused by only one surveyor (*i.e.*, fish were not double counted). All fish were identified to the lowest possible taxonomic level and sized into 5 cm bins.

Timed swims where aligned on depth contours. Short stretches of increased water depth or non-hard bottom habitat were quickly traversed by divers. If longer stretches of non-hard bottom or a significant change in depth was encountered, divers altered course to maintain a relatively constant depth and to avoid swimming into extensive areas of non-hard bottom habitat.

Data Analysis

Individual fish biomass (wet weight of fish per m^2 of reef area) was calculated from estimated lengths using size to weight conversion parameters from FishBase (Froese and Pauly, 2010) or the Hawai'i Cooperative Fisheries Research Unit (HCFRU) at the University of Hawai'i (UH). For analyses among survey sites, fish survey data were pooled into several broad categories, including: (1) all fishes, excluding manta rays; (2) target fishes[1], which are reef species targeted or regularly harvested by fishers (Table B.1); (3) prime spawners[2], which are target fishes larger than 70% of the maximum size reported for the species; and (4) non-target fishes, which are

Table B.1. The resource fish targeted by fishers in Hawai'i included as "Target Fish" for this report.

Surgeonfishes (Acanthuridae)	Apex
Acanthurus achilles	*Aphareus furca*
Acanthurus blochii	*Aprion virescens*
Acanthurus dussumieri	All Priacanthidae (big-eyes)
Acanthurus leucopareius	All Sphyraenidae (barracuda)

[1] Nearly all fish species are taken by some fishers at some time in Hawai'i, therefore designating a fish species as either 'targeted' or 'non-targeted' is oftentimes difficult. These two groupings are intended to represent the high and low ends of the fishing pressure continuum. The majority of fish biomass at most sites is comprised of species that fall somewhere in the middle of this continuum, and these species were not included in either group for this analysis.

[2] Large target fishes are generally heavily targeted by fishers. In addition, fishes at the high end of their size range tend to be a disproportionately important component of total stock breeding potential due to greater fecundity of large individuals, and higher survivorship of larvae produced by large fishes (Williams *et al.* 2008). Therefore 'prime spawner' biomass is likely to be a good indicator of fishing impacts, and represents an important component of ecological function (*i.e.*, population breeding potential).

Acanthurus nigroris

Acanthurus olivaceus

Acanthurus triostegus

Acanthurus xanthopterus

Ctenochaetus spp.

Naso spp.

Goatfishes (Mullidae)

All

Jacks (Carangidae)

All

Wrasses (Labridae)

Bodianus albotaeniatus

Cheilio inermis

Coris flavovittata

Coris gaimard

Iniistius spp.

Oxycheilinus unifasciatus

Thalassoma ballieui

Thalassoma purpureum

Soldier/Squirrelfishes(Holocentridae)

Myripristis spp.

Sargocentron spiniferum

Sargocentron tiere

Others

Chanos chanos

Cirrhitus pinnulatus

Monotaxis grandoculis

Parrotfishes (Scaridae)

All

species not targeted by fishers to any significant degree. Non-target taxa included: non-target wrasses (all wrasse species other than those listed in Table B.1); non-target surgeonfishes (*Acanthurus nigrofuscus* and *A. nigricans*); hawkfishes (all species except the stocky hawkfish, *Cirrhitus pinnulatus*); triggerfishes excluding planktivores; corallivorous butterflyfishes (*Chaetodon multicinctus, C. ornatissimus, C. quadrimaculatus* and *C. unimaculatus*); and benthic damselfishes (all *Plectroglyphidodon* and *Stegastes* species). In addition, data were pooled by family for parrotfish and

target surgeonfish. Those abundant and conspicuous fishes provide important ecosystem services (*i.e.*, herbivory).

Benthic and fish communities were examined using the suite of non-parametric multivariate procedures included in the PRIMER statistical software package (Plymouth Routines in Multivariate Ecological Research) (Clarke and Warwick 2001). These procedures have gained widespread use for analyzing marine ecological community data, and have significant advantages over standard parametric procedures (see Clarke 1993 for additional information).

Prior to analysis, percent cover data for each benthic category were square-root transformed and a Bray-Curtis similarity matrix generated (Clarke and Warrick 2001, Clarke and Gorley 2006). Non-metric multidimensional scaling (nMDS) plots were generated to explore patterns (Clarke and Gorley 2006) in benthic composition.

As with the benthic community data, fish biomass data at all sites were square-root transformed and a Bray-Curtis similarity matrix generated (Clarke and Warrick 2001, Clarke and Gorley 2006) prior to analysis in PRIMER. Non-metric multidimensional scaling (nMDS) plots were generated to explore patterns (Clarke and Gorley 2006) in fish community structure.

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Appendix B: Adult Sampling Pilot

Ka'ūpūlehu Larval Parentage Planning Field Assessment April 24, 2013

Present: Brandon, Kekaulike, Chad

Method: Throw net from shore

Area: Kumukeu – beach in front of 4 seasons – approx. ¼ mi. shoreline

Conditions: NW Swell @ 2 feet, Full sun, onshore breeze 5-10 kt.

Total time fishing: **1 hr 13 min** (12:12 PM – 1:35 PM)

Total net throws: **8**

Total catch: **33 manini** (8 > L50); 8 kupipi; 1 nenue; 1 `uouoa; 1 aholehole (mature); 1 poʻopaʻa; 0 uhu

CPUE**:** *Manini* = **Total**: 4.125/throw; 27.12/hour;

>L50 = 1/throw; 6.58/hour

PREVIOUS SUMMARY

Kaʻūpūlehu Larval Parentage Planning Field Assessment April 10, 2013

Present: Bart Wilcox, Kekaulike Tomich, Chad Wiggins

Method: Throw net from shore

Area: Uluweuweu Bay/Kahuwai approx. ¾ mi. shoreline

Conditions: NW Swell @ 5 feet, cloudy water in sandy areas, very rough at Kahuwai

Total time fishing: **3 hrs 12 min** (10:30 AM – 3:22PM w/ 40 minute break)

Total net throws: **8**

Total catch: **3 uhu** (1 > L50); **14 manini** (3 > L50); 6 kupipi; 2 nenue; 1 kikakapu

QUESTION: *If we pick a haremic species could we sample a smaller total number of the male population and still get a high proportion of the total breeding populations? For instance if there are 100 uhu, but only 10 males (90 females), could sampling 2 males be the rough equivalent of sampling 18 females from the same two harems?*

Observations:

- several (7-15) large parrotfish from multiple species observed at Uluweuweu, but not able to capture w/ net;
- multiple schools of mixed size manini present along entire shoreline of Uluweuweu (5-20 individuals/school)
- manini and uhu move in to feed on limu at high tide = best chance for catching large numbers
- manini at Kahuwai Bay seem less wary than manini at Uluweuweu (Bart obs.)
- no uhu observed at Kahuwai during 30 minutes of fishing
- manini schools move short distance away if spooked but may return
- multiple locations at Uluweuweu are candidates for crossnet/hukilau to capture entire schools of uhu/manini
- turtle population necessitates caution when throwing and may make cross netting difficult
- throw net fishermen at kahuwai caught large numbers (~50) of large reproductive mullet (breeding stock according to Bart)

Suggested Next Steps:

- Purchase throw net and spend entire tidal cycle in one suitable location to fully test feasibility as fishing method
- Investigate permits for cross net and small mesh bait net
- Discuss potentially scaling back number of sites/adjusting areas to realistically ensure adequate coverage of population