KA PILINA O NĀ HĀʻUKEʻUKE

A Study of the Size and Genetic Connectivity of the Culturally Significant Sea Urchin (*Colobocentrotus atratus)* in Hawaiʻi

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I certify that I have read this thesis and that, in my opinion, it is satisfactory in scope and quality as a thesis for the degree of Bachelor of Science in Global Environmental Science.

THESIS ADVISOR

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For my dad, my son, and all my family and friends.

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ABSTRACT

Overharvesting without proper regulation is a common and growing problem for many Hawaiian invertebrates. This paper reports differences in size and the population genetic structure of the intertidal marine urchin species *Colobocentrotus atratus,* also known as *Hā'uke'uke,* from the islands of Kaua'i, O'ahu, Maui and the Big Island of Hawai'i. Individuals from the northern shores of each island were significantly larger (in terms of length, width and height) than congeners on the southern shores of the same island. Based on mitochondrial cytochrome oxidase subunit 1 (COI) sequences, genetic differentiation among sampling sites on each island were uniformly low (pairwise F_{ST} = 0.01 to 0.08), with only Maui and O'ahu being significantly differentiated. In contrast, exact tests of population differentiation revealed that none of the populations are drawn from the same gene pool, and that although *hā'uke'uke* populations in Hawai'i currently exhibit high gene flow, the populations are not freely interbreeding. Thus, any massive loss of individuals from a population (such as overharvest) would have major impacts for the remaining individuals on that island. We recommend these results on *Hā'uke'uke* population structure be used to inform better management practices for this important cultural resource.

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1.0 INTRODUCTION

1.1 Geographic Context

The Hawaiian Islands, located in the northern Pacific Ocean, are the most isolated archipelago in the world (DLNR, 2013). The Main Hawaiian Islands consist of a chain of eight islands separated by oceanic channels, located approximately 3700 km from the continental United States (the nearest continent). Their nearest nation neighbor is Kiribati, which is ~1600 km away (Western Pacific Regional Fishery Management Council, 2019). The Main Hawaiian Islands vary in size from ~45 square miles (Kahoʻolawe) to over 4,000 square miles (Hawaiʻi Island), for a state total area of 6,423 square miles (Morgan, 1996, Juvik and Juvik, 1998). A location map is provided in Figure 1.

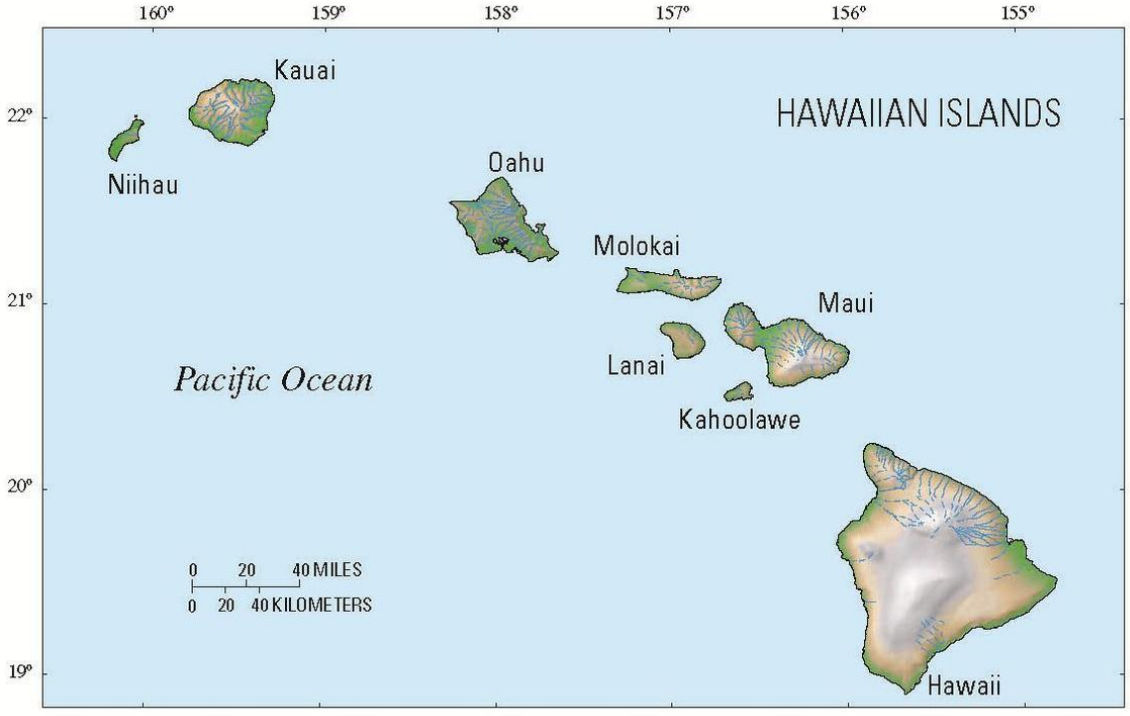


Figure 1.1. Map of the Main Hawaiian Islands (USGS, 2018).

1.2 Population genetics

Population genetics is a field of biology that studies the genetic variation within populations over space and time. Alleles are alternative forms of the same locus or gene, and a genotype is the set of alleles, one from the mother and one from the father, carried by an individual.

Population genetics is the study of how allele and genotype frequencies are distributed among individuals at different geographic locations, and how these frequencies change from generation to generation. (e.g., Virtual Genetics Education Centre, 2019). Population genetics can be used to monitor target species in ecologically sensitive areas to understand the structure of those populations and to estimate the degree of exchange (connectivity) among them. Understanding the genetic connectivity of separate populations of a species can aid in the development of proper management plans for that species (Bohonak, 1999). Thus, population genetics is an effective management tool that has been applied widely in fields as diverse as wildlife management (e.g., Gompert, 2012**)** and pest control (e.g., Desvars-Larrive et al, 2019).

Population genetics have also been applied to marine invertebrates. In Hawai‘i, Toonen et al. (2011) used population genetics to show that most Hawaiian coral reef species have limited exchange among each of the main Hawaiian Islands, and argued that each island should be managed separately and independently of the others. However, current federal and State of Hawai‘i guidelines treat the entire archipelago as a single unit and do not have island-specific management strategies, as recommended by Toonen

(2011). Therefore, overharvesting intertidal species without proper regulation is a common and growing problem for many Hawaiian invertebrates.

1.3 Hāʻukeʻuke

Hāʻukeʻuke (*Colobocentrotus atratus)* is a marine invertebrate species of sea urchin, also known as shingle or helmet urchins. They lack the typical spines associated with most sea urchins; instead they have armored plates which protect them from crashing waves (e.g., Santos and Flammang, 2008, Lawrence, 2013). Their tube feet help secure them to the substrate (Figure 1.2). *Hāʻukeʻuke* inhabit wave-swept, rocky intertidal shores throughout the Hawaiian Archipelago. Adults are sessile, while the larval phase is planktonic. Although the species has a wide distribution and adults do not move far from their homes, it is unclear how far individuals can disperse during their planktonic larval phase.

Figure 1.2. Hāʻukeʻuke specimens.

There are currently no regulations on harvesting *hā'uke'uke* in Hawai'i and no governmental permits are required to study them. However, Hawaiian cultural protocol emphasizes the importance of asking permission from the *'āina* (land) before harvesting. *Hā'uke'uke* is mentioned in the *Kumulipo* (the Hawaiian creation chant) as one of the first organisms to be born (Figure 1.3)

0022. Hanau ka Hawa'e, o ka Wana-ku kana keiki, puka
Born was the smooth sea urchin, his child the long-spiked came forth
0023. Hanau ka Ha'uke'uke, o ka 'Uhalula kana keiki, puka
Born was the ring-shaped sea urchin, his child the thin-spiked came forth
0024. Hanau ka Pi'oe, o ka Pipi kana keiki, puka
Born was the barnacle, his child the pearl oyster came forth
0025. Hanau ka Papaua, o ka 'Olepe kana keiki, puka
Born was the mother-of-pearl, his child the oyster came forth
0026. Hanau ka Nahawele, o ka Unauna kana keiki, puka
Born was the mussel, his child the hermit crab came forth
0027. Hanau ka Makaiauli, o ka 'Opihi kana keiki, puka
Born was the big limpet, his child the small limpet came forth
0028. Hanau ka Leho, o ka Puleholeho kana keiki, puka
Born was the cowry, his child the small cowry came forth
0029. Hanau ka Naka, o ke Kupekala kana keiki, puka
Born was the naka shellfish, the rock oyster his child came forth
0030. Hanau ka Makaloa, o ka Pupu'awa kana keiki, puka
Born was the drupa shellfish, his child the bitter white shell fish came forth
0031. Hanau ka 'Ole, o ka 'Ole'ole kana keiki, puka
Born was the conch shell, his child the small conch shell came forth
0032. Hanau ka Pipipi, o ke Kupe'e kana keiki, puka
Born was the nerita shellfish, the sand-burrowing shellfish his child came forth
0033. Hanau ka Wi, o ke Kiki kana keiki, puka
Born was the fresh water shellfish, his child the small fresh water shellfish came forth

Figure 1.3. Excerpt from the *Kumulipo* (First Era, Second Verse), translated by Beckwith (1951).

Thus, *Hā'uke'uke* is culturally important in Hawai'i. It is recreationally harvested for food, medicine and fish bait. *Hā'uke'uke* is prized for the delicious, sweet tasting "meat" (*uni*) of its gonadal tissues (Parrish et al., 1990). It is used medicinally to

treat skin irritations, such as Ringworm and rashes (Kaaiakamanu and Akina, 1922), and is also used as fish bait (Titcomb et al., 1978).

Hāʻukeʻuke can be either beneficial or competitors to ʻopihi (limpet) depending on their population density because they inhabit the same area and may consume the same prey (algae). An overabundance of *hāʻukeʻuke* prevents ʻopihi larvae from settling, but at lower densities *hāʻukeʻuke* graze macroalgae that inhibit ʻopihi grazing, and both species benefit as a result (Bird, 2006). Unlike ʻopihi, the *hāʻukeʻuke* population tends to be very stable, since they are longer-lived, found in abundance, and have planktonic larvae that are capable of dispersing long distnaces.

1.4 Goals

This research is the first population genetic analysis of *hāʻukeʻuke* (*C. atratus*) in Hawaiʻi. It aims at generating new knowledge about *hāʻukeʻuke* population structure, in order better inform effective management plans.

This study has three key goals: (1) Characterize size differences among populations of *hāʻukeʻuke* throughout the Main Hawaiian Islands; (2) Elucidate the population genetic structure of *hāʻukeʻuke* throughout the Main Hawaiian Islands; and (3) Interpret the above results to recommend better management practices for this important cultural resource.

2. METHODS

2.1 Sample sites

Hā'uke'uke individuals were collected from eight sites along the northern and southern shorelines of four islands within the Main Hawaiian Island chain (Figure 2.1). To simplify site labels, the islands were delineated, from southeast to northwest, as follows: BI (Hawai'i Island), M (Maui), O (O'ahu), and K (Kaua'i). Within an island, sites were designated as either North (N) or South (S). Only one location was selected in the north and south coast of each island, so all site labels are unique identifiers. No samples were taken from the eastern or western shores. To enable reproducibility of this analysis, the GPS latitude and longitude of each site was recorded. The sampling locations are listed in Table 2.1. and shown in Figure 2.1

Table 2.1. Sample sites for *hā'uke'uke* collection, listed from south to north.

Island	Location	Site Label	GPS longitude and latitude
Hawai'i	Ka Lae	BIS	18º54'58''N, 155º40'05''W
Hawai'i	Mahukona	BIN	20º11'09''N, 155º54'07''W
Maui	Keoneoio	MS	20º35'00''N, 156º24'45''W

Maui	Ho‘okipa	MN	20º56’11’’N, 156º21’20’’W
O‘ahu	Black Point	OS	21º15’19’’N, 157º47’36’’W
O‘ahu	Laniakea	ON	21º37’11’’N, 158º05’08’’W
Kaua‘i	Poipu	KS	21º52’18’’N, 159º27’00’’W
Kaua‘i	Princeville	KN	22º13’18’’N, 159º29’53’’W

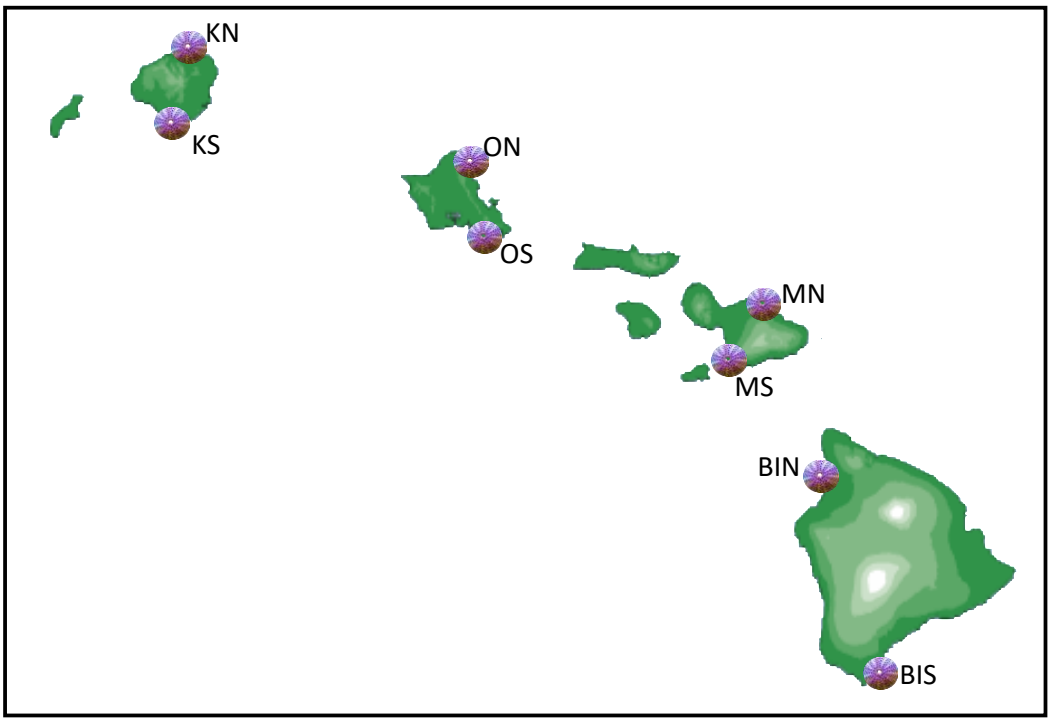


Figure 2.1. Map of sample site locations. Site labels are defined in text (see previous paragraph).

2.2 Sample collection and protocol

At each of the 8 sites, 24 whole animals were measured, for a total sample size of 192 individuals. Samples were randomly selected and measured on site during a weeklong period in March 2014. Samples were collected during that same week, except for the Oahu samples which were collected in April 2014.

Because there are currently no regulations on harvesting *hāʻukeʻuke* in Hawaiʻi, no governmental permits were required. In addition, following Hawaiian cultural protocol, prior to collecting any samples, we chanted an *oli* (chant) on site to ask permission from place, organism, and ancestors. We also ceremonially presented *hoʻokupu* (offerings) to the site and its organisms. All collections were conducted with a *pono* (righteous) mindset. After collections, the organisms were returned to the environment with *hoʻokupu* (when possible), and a *mele* (chant or song) was sung to thank the place, organism, and ancestors.

2.3 Size measurements

On location, each individual *hāʻukeʻuke* test (shell) was measured for height, length, and width. All populations were measured during the same week to exclude any seasonal reproductive size differences. To standardize the measurement protocol, length and width are respectively defined as the longer and shorter of the two horizontal measurements, regardless of orientation. A caliper was used to measure the height of each individual at the center, with height defined as the distance from mouth to anus. Two-sample t-tests (unequal variances) were performed to assess the statistical

significance of mean differences between (1) populations from the north vs. south shore of each island, as well as (2) between different island groups.

2.4 PCR and sequencing

On location, peristomal tissue was removed using a sterile razor blade. These tissue samples were preserved in RNAlater for 24 hours, then transferred to a laboratory freezer (-20ºC) for storage until all islands were sampled. Once all fieldwork was completed, the stored peristomal tissue samples were removed from the preservative and rinsed with deionized (DI) water. Genomic DNA was then extracted from the tissue samples using a QIAGEN DNeasy extraction kit. After extraction, DNA concentrations were measured using a NanoDrop. Concentrations in excess of 50 nanograms per microliter (ng / μL) were diluted with either elution buffer or sterile deionized water, to standardize concentrations and facilitate visualization of the DNA.

One mitochondrial DNA locus, Cytochrome Oxidase subunit I (mtDNA COI), was amplified using polymerase chain reaction (PCR). We used the COI primers, developed by Dr. Rob Toonen:

F1: 5'-CCTCGAATGAAAAACATGAGC-3' and

R1: 5'-AACTGTGAACATGTGGTGTGC-3'. Twenty microliter PCRs were performed using 10 μL of 2x Bioline Biomix Red, 1 μL of 5 μM F1 primer, 1 μL of 5 μM R1 primer, 7 μL of sterile deionized water, and 1 μL of template DNA at ~5-50 ng/μL. The PCRs were run for one cycle with a 4 min, 94 ºC denaturation step, a 1 min 30 s, 48 ºC annealing step, and a 2 min, 72 ºC elongation step on a Bio-Rad T100 Thermal Cycler. Thirty-five additional cycles were run with the following parameters: 30 s at 94 ºC, 45 s

at 48 ºC, and 45 s at 72 ºC, concluding with a final 10 min elongation step at 72 ºC with a hold at 4 ºC.

PCR products were cleaned using the QIAquick PCR Purification Kit protocol. Because the concentrations of products differed, normalization was conducted using the SequalPrep Normalization Plate Kit protocol. Purified DNA fragments were sequenced using the Sanger method by the Genomics Core Facility at the University of Hawaii John A. Burns School of Medicine.

2.5 Post-sequencing protocol

Sequences were visually inspected for quality. Sequences with noisy or weak signal traces were marked as failed reactions and were not considered during analysis. High-quality sequences were aligned using Clustal in the GENEIOUS software package, and base pair differences among individuals were identified as unique haplotypes. Using the ARLEQUIN v3.5 software package, an analysis of molecular variance (AMOVA) was conducted to determine the overall population genetic structure. Unique haplotypes were analyzed in two ways: by island and by site. A haplotype network was then created with the Network Publisher V2.1.2.5

Finally, an exact test of population differentiation was run to determine whether populations deviate from expectations under panmixia (random mating). This test compares haplotype frequencies among sites and asks how likely is that distribution if the populations mix each generation (Goudet et al. 1996). The exact test reveals how complex population structure can be, even when far from fixation as measured by F-statistics (Wright, 1965), which has important implications for management plans.

3.0 RESULTS

3.1 Urchin size

In this study, 24 individuals from each of 8 *hā'uke'uke* populations on the north and south shores of 4 islands were sampled for a total sample size of 192 individuals. The lengths, widths and heights of each animal were measured. However, all measurements of the O'ahu North Shore population were subsequently lost, reducing the sample size to 168 individuals. These size measurements are reported in Table 3.1.

Table 3.1. (a) Length, width, and height measurements of each individual (raw data). (b) Mean values of length, width, and height for each site. O'ahu North (ON) shore measurements were lost.

(a) Length, width, and height measurements of each individual.

Individual	Site	Length (mm)	Width (mm)	Height (mm)
1	BIS	49.18	43.61	21.11
2	BIS	48.98	42.51	20.83
3	BIS	51.62	44.06	22.98
4	BIS	46.13	44.00	19.50
5	BIS	52.65	42.93	20.05
6	BIS	49.98	43.23	20.61
7	BIS	47.25	38.23	19.64
8	BIS	42.23	36.98	16.24
9	BIS	51.09	44.25	20.80
10	BIS	44.77	34.34	18.98
11	BIS	50.82	45.64	19.63
12	BIS	53.13	44.38	21.75
13	BIS	54.75	46.00	21.11
14	BIS	46.77	40.45	18.70
15	BIS	48.96	41.94	22.07
16	BIS	47.29	40.01	16.37
17	BIS	42.54	36.16	18.66
18	BIS	42.49	35.55	17.14
19	BIS	45.33	37.71	17.38

20	BIS	37.74	35.90	15.64
21	BIS	45.26	40.80	19.35
22	BIS	40.82	34.43	15.03
23	BIS	39.89	33.42	17.14
24	BIS	39.80	31.24	14.19
1	BIN	41.10	35.20	15.50
2	BIN	46.90	40.84	19.11
3	BIN	50.98	48.17	18.62
4	BIN	49.45	47.15	19.25
5	BIN	56.43	52.08	20.47
6	BIN	53.39	49.62	18.46
7	BIN	45.35	42.93	17.04
8	BIN	54.35	49.17	20.13
9	BIN	54.00	52.82	21.43
10	BIN	52.99	44.92	20.06
11	BIN	49.93	48.28	18.17
12	BIN	52.23	45.73	18.21
13	BIN	53.31	46.92	19.88
14	BIN	49.27	48.97	18.07
15	BIN	50.65	47.23	20.32
16	BIN	59.25	52.21	21.95
17	BIN	54.53	50.52	21.23
18	BIN	59.35	55.75	20.52
19	BIN	70.82	63.22	24.98
20	BIN	54.37	50.74	21.44
21	BIN	57.29	52.49	21.64
22	BIN	58.48	57.79	21.20
23	BIN	52.81	51.10	19.39
24	BIN	58.85	54.17	20.50
1	MS	36.32	29.81	13.45
2	MS	42.91	37.80	17.24
3	MS	42.90	37.00	17.24
4	MS	34.70	31.53	16.89
5	MS	37.02	33.09	18.95
6	MS	43.61	40.80	18.94
7	MS	53.10	46.13	22.23
8	MS	34.10	28.57	14.52
9	MS	36.20	30.96	15.94
10	MS	40.22	36.95	18.75

11	MS	37.20	32.71	18.27
12	MS	38.03	33.70	16.60
13	MS	39.06	33.99	16.77
14	MS	56.43	51.24	23.28
15	MS	42.51	38.93	16.04
16	MS	40.88	36.02	16.03
17	MS	51.59	45.02	20.85
18	MS	46.07	37.47	17.19
19	MS	40.18	36.88	18.09
20	MS	38.86	36.75	17.49
21	MS	35.65	30.26	14.41
22	MS	34.29	27.58	13.56
23	MS	37.64	34.21	17.34
24	MS	37.05	31.54	14.88
1	MN	61.88	54.18	21.17
2	MN	42.52	37.22	17.04
3	MN	49.38	43.47	18.33
4	MN	60.80	56.54	21.30
5	MN	41.50	35.49	17.60
6	MN	47.42	41.68	19.48
7	MN	42.35	36.86	16.94
8	MN	54.46	45.08	20.31
9	MN	42.74	36.67	18.30
10	MN	49.45	46.37	20.00
11	MN	56.59	51.02	22.49
12	MN	48.21	42.48	17.81
13	MN	44.40	36.86	17.93
14	MN	41.59	36.98	16.66
15	MN	41.95	38.10	18.21
16	MN	41.32	35.95	17.97
17	MN	48.67	46.26	18.50
18	MN	47.51	40.72	18.70
19	MN	41.42	36.73	16.11
20	MN	39.49	34.72	15.81
21	MN	41.03	36.81	15.94
22	MN	48.09	43.29	18.17
23	MN	43.45	38.19	20.05
24	MN	37.15	33.56	16.56
1	OS	44.21	33.17	17.74

2	OS	66.6	35.56	24.77
3	OS	50.48	36.95	21.61
4	OS	52.86	37.37	18.71
5	OS	45.14	37.98	17.77
6	OS	54.7	38.61	19.5
7	OS	51.99	39.43	18.72
8	OS	47.56	39.51	18.81
9	OS	50.9	39.54	19.37
10	OS	58.8	40.96	23.98
11	OS	58.23	41.19	21.5
12	OS	55.16	42.28	20.32
13	OS	37.29	43.25	14.87
14	OS	42.04	44.47	16.06
15	OS	42.2	45.97	17.48
16	OS	43.5	46.4	17.05
17	OS	40.51	47.17	15.88
18	OS	41.4	47.36	16.58
19	OS	46.58	49.55	18.7
20	OS	46.18	50.07	17.69
21	OS	54.13	51.85	21.76
22	OS	43.15	54.03	17.57
23	OS	59.46	57.51	23.03
24	OS	47.89	60.77	18.45
1	KS	40.87	38.96	19.02
2	KS	50.37	45.06	21.48
3	KS	49.75	45.02	18.12
4	KS	37.26	32.95	15.12
5	KS	42.72	40.04	17.97
6	KS	47.41	42.26	19.14
7	KS	47.39	45.92	19.08
8	KS	36.12	34.82	13.35
9	KS	44.72	43.13	18.54
10	KS	42.05	38.52	18.40
11	KS	34.54	30.56	14.31
12	KS	41.29	39.39	16.33
13	KS	36.64	39.31	16.84
14	KS	41.49	36.17	19.96
15	KS	44.40	48.82	18.44
16	KS	43.05	38.92	17.77

17	KS	39.54	35.54	15.28
18	KS	44.32	37.94	16.91
19	KS	47.56	45.59	24.65
20	KS	28.14	34.59	14.46
21	KS	43.01	42.64	18.89
22	KS	42.54	40.30	19.69
23	KS	39.47	31.95	14.11
24	KS	40.34	38.77	17.64
1	KN	57.89	48.34	22.52
2	KN	61.54	55.48	22.80
3	KN	52.11	44.91	20.06
4	KN	66.49	62.33	25.17
5	KN	56.61	51.54	20.24
6	KN	49.86	43.65	19.64
7	KN	54.36	45.08	19.63
8	KN	56.97	51.50	22.27
9	KN	57.59	53.63	22.53
10	KN	45.85	39.58	17.44
11	KN	45.64	42.21	16.79
12	KN	58.22	53.44	22.21
13	KN	64.43	57.09	25.64
14	KN	55.67	50.37	20.77
15	KN	59.23	49.83	18.66
16	KN	69.69	63.21	33.34
17	KN	68.61	65.61	31.93
18	KN	64.60	61.80	22.63
19	KN	40.65	37.60	16.25
20	KN	57.31	50.73	22.67
21	KN	49.44	45.70	19.31
22	KN	50.55	48.85	18.02
23	KN	56.00	52.66	24.20
24	KN	62.74	57.83	24.10

(b) Mean length, width, and height measurements for each site, with minimum and maximum values shown in italics.

Site Label	**Mean Length (mm)**	**Mean Width (mm)**	**Mean Height (mm)**
BIS	46.64	39.91	18.95
BIN	53.59	49.50	19.90

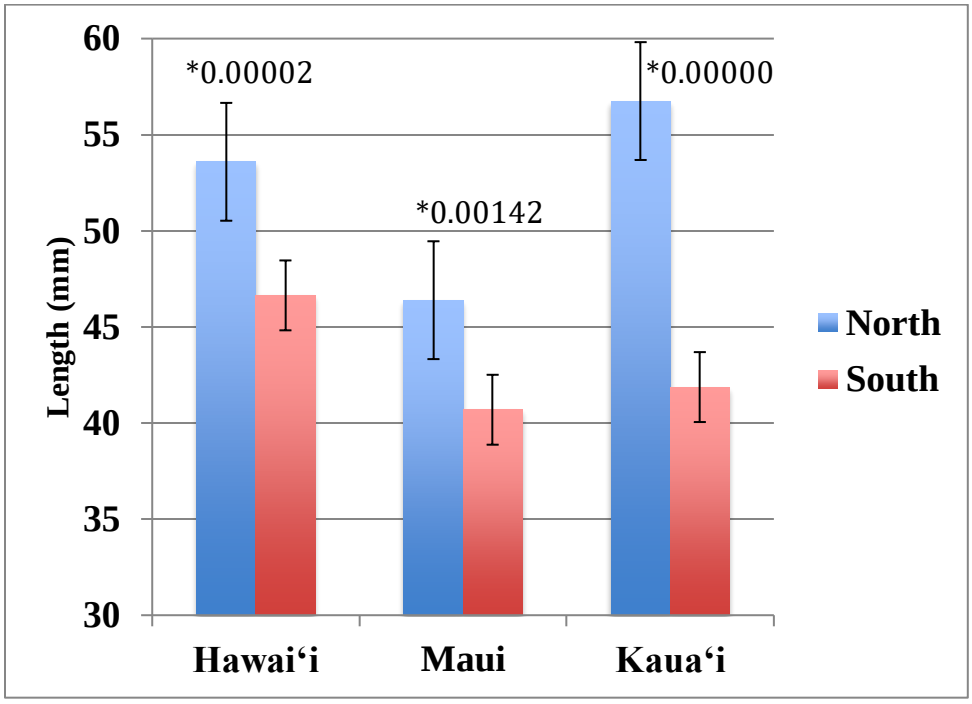
MS	*40.69 min*	*35.79 min*	*17.29 min*
MN	46.39	41.05	18.39
OS	49.21	44.21	19.08
KS	41.87	39.47	17.73
KN	*56.75 max*	*51.37 max*	*22.03 max*

Mean sizes of urchins for each island range from 40.69 to 56.75 mm in length, 35.79 to 51.37 mm in width, and 17.29 to 22.03 mm in height. All three mean minimum values are associated with the Maui South site, whereas all three mean maximum values are associated with the Kauaʻi North site. A possible explanation for these results is presented below (see Discussion Section 4.1)

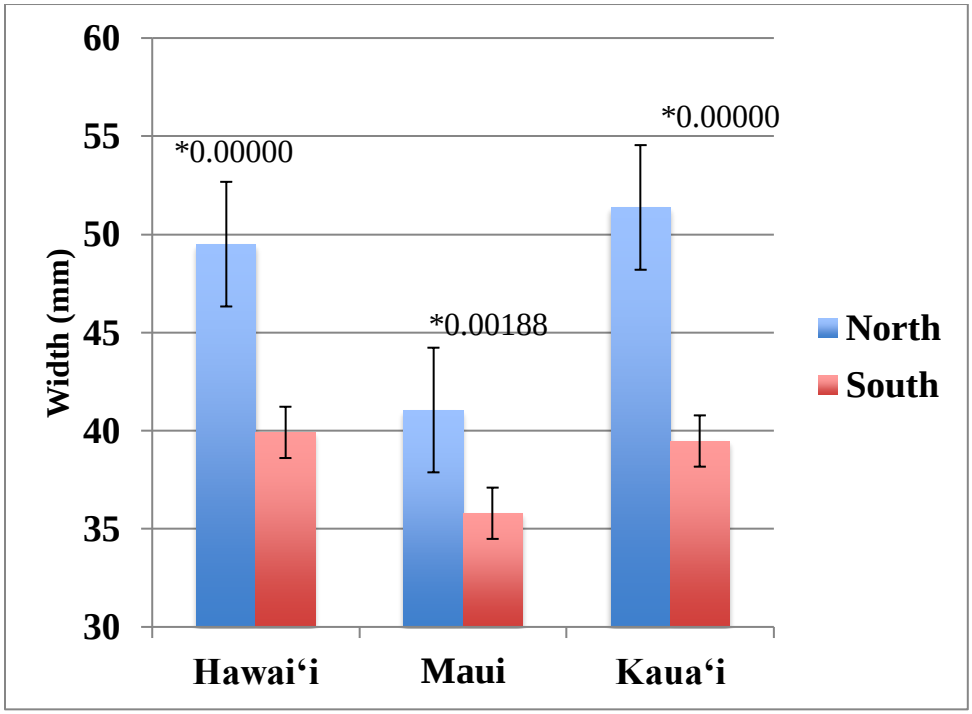
3.1.1. Size comparison between north and south shore populations within each island.

For the three islands from which north and south shore measurements are available, the north shore samples showed a consistently greater mean length, width, and height compared with south shore samples from the same island (Figure 3.1). These differences were statistically significant in all comparisons (two-tailed t-test with unequal variances. $p < 0.05$).

A) Length



B) Width



C) Height

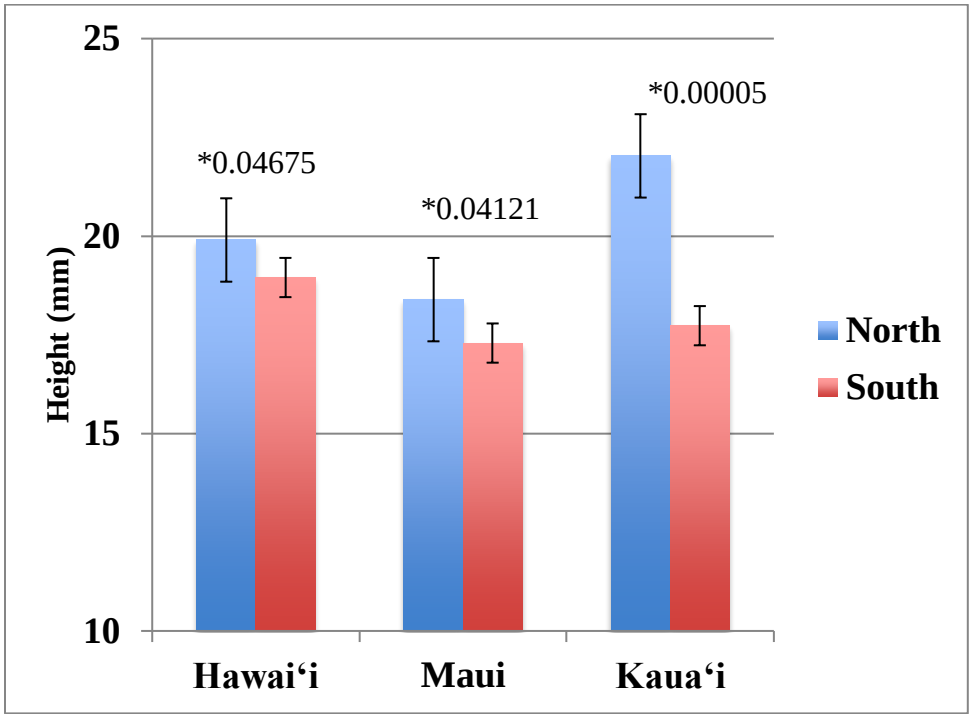


Figure 3.1. Comparison between mean morphological measurements between the north vs. south shores of three islands: (A) length, (B) width and (C) height (Oahu excluded due to missing Northshore data). Error bars represent standard error of the mean. Asterisks (*) denote statistical significance at $p < 0.05$.

3.1.2. Size comparison among south shore populations across islands.

The length, width, and height of urchins were also compared among the different islands. Because the differences between the South and North Populations within each island were found to be statistically significant (as shown in Figure 3.1), and because the O‘ahu North Shore data was lost, we decided to compare the size of urchins from south shores only, for consistency. To determine statistical significance, two-tailed t-tests for each pair of islands were again performed. Significant differences among these six pairwise comparisons are shown in Table 3.2.

Table 3.2. P-values of two-tailed t-tests for (a) length, (b) width and (c) height of samples from the south shore of each of 4 Hawaiian Islands. Significant values ($p < 0.05$) are bolded and marked with asterisks (*).

(a) Length

Island	Hawai‘i	Maui	O‘ahu	Kaua‘i
Hawai‘i	--			
Maui	**0.0004***	--		
O‘ahu	0.1532	**0.0001***	--	
Kaua‘i	**0.0014***	0.4617	**0.0002***	--

(b) Width

Island	Hawai‘i	Maui	O‘ahu	Kaua‘i
Hawai‘i	--			
Maui	**0.0074***	--		
O‘ahu	**0.0154***	**0.000***	--	
Kaua‘i	0.7376	**0.0193***	**0.0094***	--

(c) Height

Island	Hawai‘i	Maui	O‘ahu	Kaua‘i
Hawai‘i	--			
Maui	**0.0206***	--		
O‘ahu	0.8604	**0.0174***	--	
Kaua‘i	0.0910	0.5464	0.0744	--

South shore mean length showed significant differences among four of six island pairs: Hawaii-Maui, Hawaii-Kauai, Maui-Oahu and Oahu-Kauai (Table 3.2a). All but one island pair (Hawaii-Kauai) showed significant differences in mean width (Table 3.2b). In contrast, only two significant differences were found among islands for mean height: Hawai‘i-Maui and O‘ahu Maui (Table 3.2c).

3.2 Haplotype network

A total of 42 unique haplotypes were identified among the *hā‘uke‘uke* populations studied. We observed a haplotype diversity of $h = 0.828$ and a nucleotide diversity of $\pi =$

0.00422. The haplotype network, shown in Figure 3.2, reveals three common haplotypes (H_25, H_7 and H_6), shown in large yellow circles. These three haplotypes occur in all four islands, with the two most common haplotypes (H_25 and H_7) occurring in all eight populations.

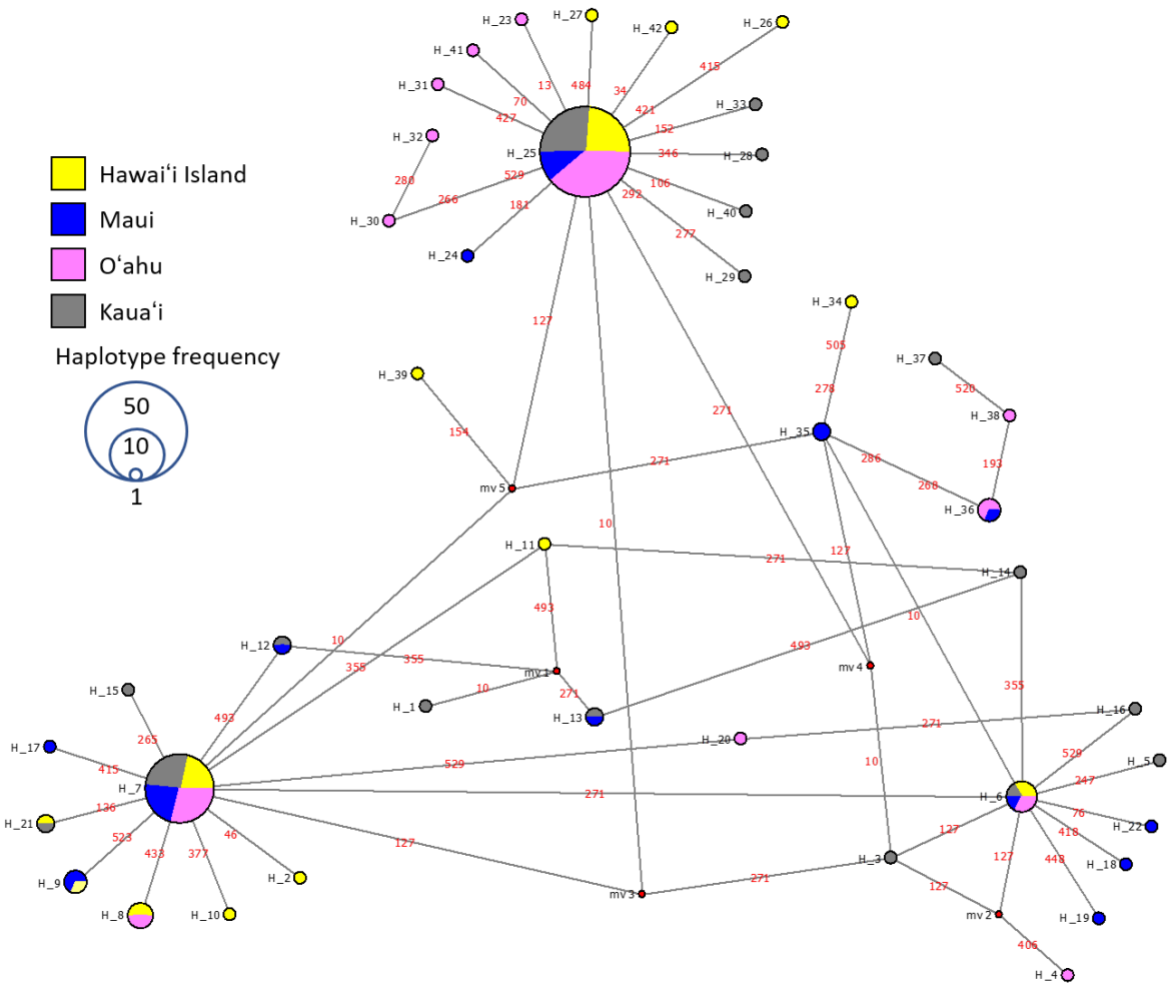


Figure 3.2. Haplotype network for *hā'uke'uke* in the Main Hawaiian Islands. Each circle represents a unique haplotype (n = 42). The red circles represent mutations that are presumed to exist but were not sampled in this study. The red numbers report the position where the nucleotide differs between connected haplotype sequences. The size of the circles is proportional to haplotype frequency, where the smallest circle represents one occurrence of a haplotype.

Table 3.3 reports the haplotype distribution by site. Both unique and total haplotypes are listed for each population. Island totals are also computed. We note that, by definition, (1) the number of unique haplotypes for an island will be less than or equal to the sum of the number of unique haplotypes for each population within that island (due to overlap); and (2) the number of total haplotypes for an island will be less than or equal to the sum of the number of total haplotypes for each population within that island, again due to overlap.

Table 3.3. Haplotype distribution by site. Island totals are listed in bold.

	BIN	BIS	**BI all**	MN	MS	**M all**	ON	OS	**O all**	KN	KS	**K all**
# Unique Haplo Types	3	5	**8**	5	1	**7**	7	1	**8**	6	5	**11**
# Total Haplo types	7	11	**14**	12	3	**13**	10	6	**13**	10	9	**17**
% Haplo type Represe ntation[1]	17%	26%	**33**%	29%	7%	**31**%	24%	14%	**31**%	24%	21%	**40**%

[1]% Haplotype representation is the total haplotypes represented in each population, shown as a percentage of the Hawaii total (42).

With 11 unique haplotypes, the island of Kauaʻi exhibits the greatest haplotype diversity compared with the rest of the Main Hawaiian Islands, which have 7 or 8 unique haplotypes each (Table 3.3). Moreover, Kauaʻi also has the greatest haplotype representation (40%), whereas the other islands only account for 31-33% of all

haplotypes. This is unexpected, given that Kauaʻi is the smallest island, so has the least amount of habitat space.

Comparing each sampling location, rather than island totals, the North Oʻahu population exhibits the highest haplotype diversity, with 7 unique haplotypes. The North Maui population accounts for the greatest number of total haplotypes (12), which represents 29% of all haplotypes found in this study (Table 3.3).

3.3 Analysis of molecular variance

An analysis of molecular variance (AMOVA) was conducted to determine the overall population genetic structure using mitochondrial cytochrome oxidase subunit 1 (mtDNA COI) sequences in two ways. First, the sample sites were grouped by island, and the results are shown in Table 3.4. Very little structure was identified, with only Maui and Oʻahu showing a statistically significant difference ($p<0.01$). The corresponding F_{ST} (0.079) for this only significant compariosn is quite low, indicating that considerable gene flow occurs among *hāʻukeʻuke* populations on different islands.

Table 3.4: Population genetic structure among *hāʻukeʻuke* (*C. atratus*) populations across the main Hawaiian Islands (based on mtDNA COI sequences). Values above the diagonal are Pairwise F_{ST} values, with p-values for significance below. Significant values ($p < 0.05$) are bolded and marked with asterisks (*).

Site	Hawaiʻi	Maui	Oʻahu	Kauaʻi
Hawaiʻi	--	0.030	0.007	0.001
Maui	0.09	--	**0.079**	0.016
Oʻahu	0.24	**<0.01***	--	0.002
Kauaʻi	0.61	0.16	0.31	--

Secondly, we performed an analysis by site – that is, we calculated pairwise F_{ST} comparisons of all eight sites (Table 3.5). Only three of 28 site pairwise site comparisons showed a statistically significant difference ($p < 0.05$), and even in these cases, the corresponding F_{ST} values are also quite low (0.067 to 0.087). This indicates that considerable gene flow occurs among *hāʻukeʻuke* populations from different sites and supports the island-wide results found in Table 3.4. These results suggest that, given enough time, *hāʻukeʻuke* are able to disperse and mix throughout the Main Hawaiian Islands.

Table 3.5: Population genetic structure among *hāʻukeʻuke* (*C. atratus*) populations across all sampling sites across the main Hawaiian Islands (based on mtDNA COI sequences). Values above the diagonal are pairwise F_{ST} values, and below the diagonal are p-values. Significant values ($p < 0.05$) are bolded and marked with asterisks (*).

Site	BIN	BIS	MN	MS	OS	ON	KN	KS
BIN	--	0.064	**0.087**	0.065	-0.024	-0.022	-0.009	-0.003
BIS	0.07	--	-0.022	-0.127	0.048	**0.067**	-0.001	-0.001
MN	**0.02***	0.72	--	-0.105	0.055	**0.073**	0.005	0.003
MS	0.18	0.96	0.94	--	0.019	0.030	-0.059	-0.062
OS	0.72	0.09	0.06	0.32	--	-0.033	-0.017	-0.014
ON	0.76	**0.05***	**0.03***	0.29	0.91	--	-0.011	-0.017
KN	0.45	0.38	0.32	0.63	0.61	0.54	--	-0.035
KS	0.35	0.37	0.34	0.69	0.56	0.62	0.94	--

3.4 Exact Test of Population Differentiation

An exact test of population differentiation was run to determine whether populations deviate from panmixia (random mating). Results are presented in Table 3.6, with statistically significant divergence from an interbreeding gene pool determined at p

< 0.05 following family-wise error correction for multiple tests (Yekutieli & Benjamini, 1999). Interestingly, these results reveal that every comparison but one (BIS to MS) is significantly different than all others. Except for this single case, all populations deviate from random mating, which indicates that *hā'uke'uke* are absolutely not mixing every generation. This implies that although there is much gene flow, as determined by the AMOVA results (Tables 3.4 & 3.5), the mixing takes generations of time to occur, and has important management implications, which are discussed at length in Section 4 below.

Table 3.6: Exact test of population differentiation (p-values) for *hā'uke'uke* (*C. atratus*) populations sampled across the main Hawaiian Islands based on mtDNA COI sequences. Samples that deviate significantly ($p < 0.05$) from expected gene frequencies under panmixia (random mating) are bolded and marked with asterisks (*).

Site	BIN	BIS	MN	MS	OS	ON	KN	KS
BIN	--							
BIS	**0.000***	--						
MN	**0.000***	**0.003***	--					
MS	**0.006***	0.347	**0.038***	--				
OS	**0.000***	**0.000***	**0.000***	**0.023***	--			
ON	**0.000***	**0.000***	**0.000***	**0.001***	**0.000***	--		
KN	**0.000***	**0.001***	**0.000***	**0.024***	**0.000***	**0.000***	--	
KS	**0.000***	**0.006***	**0.000***	**0.048***	**0.000***	**0.000***	**0.000***	--

4.0 DISCUSSION

This study had three key goals: (1) Characterize any size differences among *hā'uke'uke* populations throughout the Main Hawaiian Islands; (2) Elucidate the population genetic structure of *hā'uke'uke* throughout the Main Hawaiian Islands; and (3)

Interpret the above results to recommend better management practices for this important cultural resource. Each of these goals is discussed individually below.

4.1 Urchin Size

The morphological measurements (Section 3.1) showed three different statistically significant patterns in length, width and/or height among *hāʻukeʻuke* populations.

a) North vs. South Differences

For each island, the north shore samples showed statistically greater mean lengths, widths, and heights compared with south shore samples from the same island. Height measurements were the least different, but all were statistically significant ($p < 0.05$). Given that all site measurements were done during the same week, size differences are likely attributed to differences in conditions that each (North and South) shore uniquely provides.

The north shores of the main Hawaiian Islands are all oriented in a similar northwesterly direction (Figure 1.1), and thus tend to experience a similar pattern of less frequent, more powerful waves. In contrast, the south shores of all islands tend to experience more frequent, but less powerful wave action. Within an island, the north vs. south shore conditions can be very different (Vitousek, S. et al., 2009).

This north vs. south shore difference in wave conditions could account for the smaller sized *hāʻukeʻuke* observed on the south facing shores. More frequent, less

powerful wave action could put a continous stressor on an organism, which could reduce growth rate, whereas less frequent, more powerful wave action could allow for a longer growing period without a stressor.

The more powerful waves which characterize the north shore could also inhibit larval settlement or increase rates of dislogement and mortality. Thus, there may be less competition for resources on the north shore, resulting in fewer, larger *hāʻukeʻuke*. Although not measured, it was observed that the southern shores of each island were generally more populated than the northern shores at the particular sample sites chosen.

b) Extreme measurements on Maui and Kauai

Our results showed that the smallest urchins occur on the Maui South site, whereas the largest are found on North Kauaʻi. One possible explanation for this difference could be related to the specific characteristics of the sites. The Kauai North site had high wave action and a small population, suggesting that competition may be reduced and more food may have been available. In contrast, the Maui South site was a small tide pool area, with limited food supply. Therefore, we hypothesize the size differences are likely to result from site characteristics, as opposed to the island locations.

c) Inter-island islands (based on south shore data)

The south shore populations showed similar patterns of length, width and height among islands: the maximum values on all three measurements were found on Oahu, the minimum on Maui, with Big Island and Kauaʻi intermediate (Table 3.1b). However, not all differences between islands were statistically significant (Figure 3.1a-c). In particular,

the heights of urchins among populations on different islands were least likely to show significant differences. The relative consistency of heights across islands may be related to the timing of survey because the survey was conducted directly after the spawning period, when the heights would be expected to be relatively low or may be constrained by adaptation to their wave-dominated habitats.

4.2 Population genetic structure

The results obtained in this study are the first population genetic analysis of *hāʻukeʻuke* (*C. atratus*) in Hawaiʻi. Our analysis yielded a high haplotype diversity (83%) and a low nucleotide diversity (0.4%), which is similar in magnitude to many other marine invertebrates studies in Hawaiʻi to date (Toonen et al. 2011). AMOVA results on both island-wide and site-specific populations identified little significant population genetic structure (Tables 3.4 and 3.5). Only a few pairings showed statistically significant differences and, even in these cases, the corresponding F_{ST} values were quite low. This indicates little genetic structure, no barriers to dispersal, and high gene flow among *hāʻukeʻuke* populations from different sites. These results suggest that, given enough time, *hāʻukeʻuke* are able to disperse and mix throughout the Main Hawaiian Islands.

Interestingly, the exact test of population differentiation showed that, except for a single case (BIS to MS), all populations deviate from random mating among populations (Table 3.6). This result indicates that the populations are not interbreeding, and although there is high gene flow between populations, this mixing is done slowly, over generations of time. Interestingly, the only pair of sites that meet expectations of random mating are those which also show the highest level of connectivity in a recent model of larval

dispersal for the Hawaiian Archipelago (Wren et al., 2016). This unequivocally indicates that *hā'uke'uke* populations are not mixing in every generation. Such limited exchange among sites could be explained by inconsistent and periodic larval dispersal among sites (Wren et al., 2016; Conklin et al., 2018), low survival of individuals that colonize sites far from where they were spawned (e.g., Strathmann et al., 1981) or reproductive failure due to different spawning times as outlined below.

Hā'uke'uke spawn twice a year, generally in early winter and early summer (Bertelmann, 2011). Ocean currents differ throughout the year with winter and summer having drastic differences between the north and south shores in Hawai'i. During the winter months (and some fall months), Hawai'i receives the peak of the north Pacific swell, which could produce ocean waves in excess of 7 m (Vitousek et al., 2009). During the summer months, Hawai'i receives the peak of the southern swell, which is generated further from Hawai'i than the north Pacific swell, but can still produce wave heights of 2.5–3.0 m (Vitousek et al., 2009). The direction of swells and currents during *hā'uke'uke* spawning periods could be a key factor in the timing and likelihood of dispersal for larvae among populations. Larval dispersal models (Wren et al. 2016) indicate the chances of successful recruitment to a neighbor island is already low, but if differences in size or spawning timing among populations are adaptive such that dispersal to a new island results in decreased settlement success, survivorship and/or spawning success, then an already rare event becomes considerably rarer.

4.3 Management Implications

In this population study, we were interested in highlighting the genetic connectivity of the *hāʻukeʻuke* species for management purposes. This was done to test the common assumption that proper management plans can be developed for species by just surveying one population. The population genetic structure revealed that although *hāʻukeʻuke* populations in Hawaiʻi currently exhibit high gene flow, overharvest of individuals from a population could have major impacts for the remaining individuals, even within islands, let alone between them.

The simplest explanation for the high gene flow, yet low random mating, among populations is that *hāʻukeʻuke* do not disperse to neighbor islands with any frequency. Populations are able to mix over generations of time with the appropriate conditions (i.e., dispersal capacity due to ocean wave or current intensity), but dispersal among sites is low enough that the gene pools do not mix entirely. These results highlight that *hāʻukeʻuke* have the potential to become endangered due to human impacts, particularly if managed as a single large stock across all islands (as is currently the case in Hawaiʻi). For instance, if a population was devastated due to overharvesting for human consumption, the area could not repopulate on its own right away. It would take generations of time for a larva from another population to find its way to the devastated population. Even so, when the new larvae arrive, they may not be able to settle due to an overgrowth of algae because of a lack of grazers, and the loss of *hāʻukeʻuke* from a site has cascading effects on other ecologically and culturally important species like *ʻopihi* that cannot colonize a site if overgrown with macroalgae (Bird 2006). Therefore, a management plan specific to each island that restricts

overharvesting should be developed to continue the successful growth and sustainable harvest of this intertidal marine invertebrate species. Although we do not have fine-scale samples from many sites around individual islands, the data also show that the greatest differences in size and many of the most significant pairwise exact tests are between north and south shore populations on each island as opposed to between the islands. These results suggest future study should examine many sites within each island (e.g, Conklin et al. 2018; Coleman 2019) to determine the appropriate scale for resource management that will ensure sustainability for the future.

5.0 CONCLUSION

The results obtained in this study are the first population genetic analysis of *hāʻukeʻuke* (*C. atratus*) in Hawaiʻi. This study of eight *hāʻukeʻuke* populations on four Hawaiian Islands yields several key conclusions. Differences in size between the north and south shore populations within an island are greater than the size differences among south shore populations of different islands. Although it is unclear as to why there are size differences among the south shore populations among islands, north vs. south shore size differences may be attributed to shore-specific wave action patterns. The population genetic analyses reveal low population structure consistent with a high rate of gene flow among populations, but exact tests of population differentiation indicate that virtually every site is a distinct breeding population. Thus, *hāʻukeʻuke* populations exhibit surprisingly low migration among sites: that is, it takes generations of time for the aforementioned level of gene flow. Therefore, a generalized management plan based on a single archipelago stock for the entire state of Hawaiʻi is not appropriate. Instead of state-

wide, given the within-island differences in breeding populations, this work provides more support for traditional *moku*-scale management units with restrictions specific to each population implemented for the protection and sustainable harvest of this important cultural resource.

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