Title
Genetic Assessment of the Population Connectivity of the Red Urchin (Strongylocentrotus franciscanus)

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Genetic Assessment of the Population Connectivity of the Red Urchin
(Strongylocentrotus franciscanus)

A Thesis submitted in partial satisfaction of the requirements for the degree Master of Science

in

Biology

by

Celeste Elizabeth Benham

Committee in charge:
Professor Ronald Burton, Chair
Professor Josh Kohn, Co-Chair
Professor Kaustuv Roy

2009
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Co-Chair

Chair

University of California, San Diego

2009
“All things are one thing and one thing is all things – plankton, a shimmering phosphorescence on the sea and the spinning planets and an expanding universe, all bound together by the elastic string of time. It is advisable to look from the tide pool to the stars and then back to the tide pool again.”

~John Steinbeck

Log from the Sea of Cortez
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Signature page</td>
<td>iii</td>
</tr>
<tr>
<td>Epigraph</td>
<td>iv</td>
</tr>
<tr>
<td>Table of Contents</td>
<td>v</td>
</tr>
<tr>
<td>List of Abbreviations</td>
<td>vi</td>
</tr>
<tr>
<td>List of Figures</td>
<td>vii</td>
</tr>
<tr>
<td>List of Tables</td>
<td>viii</td>
</tr>
<tr>
<td>Acknowledgements</td>
<td>ix</td>
</tr>
<tr>
<td>Abstract</td>
<td>x</td>
</tr>
<tr>
<td>Introduction</td>
<td>1</td>
</tr>
<tr>
<td>Materials and Methods</td>
<td>7</td>
</tr>
<tr>
<td>Results</td>
<td>10</td>
</tr>
<tr>
<td>Discussion</td>
<td>14</td>
</tr>
<tr>
<td>Conclusion</td>
<td>19</td>
</tr>
<tr>
<td>Further Work</td>
<td>20</td>
</tr>
<tr>
<td>Amplified Fragment Length Polymorphism (AFLP)</td>
<td>21</td>
</tr>
<tr>
<td>Figures</td>
<td>22</td>
</tr>
<tr>
<td>Tables</td>
<td>24</td>
</tr>
<tr>
<td>References</td>
<td>27</td>
</tr>
</tbody>
</table>
LIST OF ABBREVIATIONS

Locations:

LI    Louscoone Inlet
CR    Campbell River
PtA   Point Arena
M     Malibu
LJ    La Jolla
PtL   Point Loma

Other:

IBD   Isolation by distance
AFLP  Amplified Fragment Length Polymorphism
LIST OF FIGURES

Figure 1. Maps showing collection sites in California and British Colombia........22
Figure 2. Scatter plot showing Isolation by Distance (IBD). Distance in minimum
waterway in km. $F_{ST}/(1 - F_{ST})$ used as a measure of genetic distance........22
Figure 3. Pie graphs showing AMOVA calculations of variance.......................23
LIST OF TABLES

Table 1. Collection sites, collection years, and sample sizes………………. 24

Table 2. Microsatellite loci, including the number of alleles, expected heterozygosity, observed heterozygosity, inbreeding coefficient $F_{IS}$, and the estimated frequency of the null allele………………………….. 24

Table 3. Significance of exact G test for genotypic and genic differentiation for each population when compared across five loci and across all seven loci…….. 25

Table 4: Pairwise $F_{ST}$ values below the line, P-values above the line……………... 25

Table 5: Pairwise $\rho_{ST}$ values……………………………………………………………. 26

Table 6: Percent of genotypes that were assigned to their own population in a population assignment test…………………………………………………… 26

Table 7: Number of alleles in each locus……………………………………………… 26
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I would like to thank Eddie Kisfuludy, Dave Rudie, Peter Halmay, Erik Owen, Robert Juntz, Tom Ford, and Brian Meux for helping to collect urchins and/or providing urchin tissue. I would also like to thank Carolina Bonin, Kristen Gruenthal Ph. D., and Asako Yamamoto for all their help with lab work and data analysis. Kristina Miller Ph.D., Janine Supernault, and Liane Stenhouse from the Fishery and Oceans Canada Pacific Biological Station’s Molecular Genetics Laboratory were generous and helpful in sharing data from British Columbia populations. A special thanks to my committee Ronald Burton, Ph.D., Josh Kohn Ph.D., and Kaustuv Roy, Ph.D. Funding for this project was provided by California Sea Grant.
ABSTRACT OF THE THESIS

Genetic Assessment of the Population Connectivity of the Red Urchin
(Strongylocentrotus franciscanus)

by

Celeste Elizabeth Benham

Master of Science in Biology

University of California, San Diego, 2009

Professor Ronald Burton, Chair
Professor Josh Kohn, Co-Chair

A set of seven microsatellite genetic markers were used to examine the population connectivity of the red urchin, Strongylocentrotus franciscanus. I compared four locations in California and two locations in British Columbia (data previously published by Miller et al. 2004). I found significant genetic differentiation
between the British Columbia and the California populations, however among the California populations there were few indications of differentiation. I detected some differentiation between recruits and adults within one California population. These results show for the first time that, on a range wide scale, populations of red urchins are genetically divergent. However, on a regional scale, which may be more relevant to fisheries and marine reserve management, there does not seem to be strong genetic differentiation. These findings do not exclude the possibility that there may be substantial local recruitment within populations.
INTRODUCTION

Connectivity among populations of marine organisms is directly related to current conservation issues such as fisheries management and marine protected area effectiveness (Cowen et al. 2007). In recent years several methods have been used to address the issue of connectivity, including novel tagging techniques (Jones et al. 1999, 2005; Almany et al. 2007), the use of geochemical signatures in calcified structures (Becker et al. 2007), and advanced genetic techniques (Hellberg et al. 2002; Bentzen et al. 1996; Gruenthal and Burton 2005, 2007, 2008). Genetic studies are unique because they provide an indirect method of observing gene flow throughout the entire range of an organism. This study utilizes a genetic approach to assess the levels of connectivity among populations of the red sea urchin, *Strongylocentrotus franciscanus*, with the goal of providing a more detailed and powerful examination of the population genetic structure than was previously reported.

*S. franciscanus* is a long-lived benthic echinoderm that also has a very long larval duration. It has been noted that, in aquarium conditions, urchins can spend 7 to 19 weeks as a planktonic larvae prior to settlement (Strathmann 1978, 1987; Cameron and Schroeter 1980; Rowley 1989). In the wild, this long larval stage potentially allows an individual urchin to drift hundreds of miles before settling (Grantham et al. 2003), leading to the prediction that the populations are open and genetically homogeneous. This general assumption is supported by observations of larvae of near shore species in ocean gyre systems (Johnston 1960; Cowen 1985). It is also supported by genetic studies which conclude that broadcast spawning marine species
typically appear to be genetically homogeneous throughout their range (Addison et al. 2008; Shulman and Birmingham 1995).

However, an increasing number of studies indicate that many marine organisms may have more limited dispersal than these studies indicate (Warner and Cowen 2002). Genetic homogeneity can be maintained by as little as a few migrants per generation, so it is possible that small numbers of individuals actually disperse long distances (Hellberg et al. 2002). Mussels and reef fish are just a few examples of organisms whose populations have been shown to have substantial self-recruitment (Jones et al. 1999, 2005, Almany et al. 2007, Becker et al. 2007). Furthermore, it has been argued that the life history traits of near-shore fishes and benthic crustaceans have developed to maximize the likelihood of larval retention near parental populations (Shanks and Eckert 2005). If this is the case, the structure of urchin populations may resemble a metapopulation more than one large panmictic population (Kritzer and Sale 2004, 2006).

Red urchins are very important herbivores in kelp forest ecosystems along the west coast of North America, ranging from Baja California to Alaska. They have the ability to completely graze down entire kelp forests when they are starved of drift algae and when they are released from the pressures of predation by California spiny lobsters and sheephead fish (Dayton 1985). A population explosion of urchins around 1970 was associated with loss of kelp biomass leading to urchin barrens (Wheeler 1970). This aspect of urchin population biology must be considered for effective kelp forest management and marine reserve design. For example, one could ask the
question: How will creating a no take reserve affect the size of urchin populations, and how will this in turn affect kelp density? Understanding patterns of urchin recruitment is critical to answer this question. It has also been shown that juvenile red urchins tend to live under the spines of large adults, so refuges containing more large adults may increase the survival rate of recruits or other invertebrates (Tegner 1977, Rogers-Bennett 2001). Alternatively, if urchins were allowed to explode in population size within a reserve, they could potentially competitively exclude other herbivores, such as abalone (Karpov 2001). Currently an effort is underway in California to develop a set of marine reserves, through the Marine Life Protection Act Initiative. The Central Coast study region reserves have been selected, but the South and North Coast study region reserves are still being developed. The role that urchins will have to play in these reserves is an important consideration.

The commercial urchin fishery is relatively new to California, having only started in 1971 as a part of a National Marine Fisheries program aimed at taking advantage of under-utilized fisheries in Southern California, as well as reducing the destructive effects of sea urchin over-grazing of kelp beds (Annual Status of the Fisheries Report 2003). Since then it has become one of California’s most valuable fisheries, estimated to be worth over $30 million at its peak in 1991 (Cal. Fish and Game 2006). Although harvestable stocks have been in decline since 1990, consistent annual recruitment is thought to sustain the populations (Annual Status of the Fisheries Report 2003). As the fishery becomes increasingly dependent on recruitment to sustain harvests, fisheries managers need to know if there are distinct
sub-populations which should be managed differently (Andrew et al. 2002). Currently
the regulations for northern California and southern California are only slightly
different, based on minimum size limit and number of fishing days per year (Annual
Status of the Fisheries Report 2003). Managers also need to know possible source and
sink populations on a relevant time scale if they are to make effective regulatory
decisions, such as enforcing regional fishery closures. Genetic techniques are best
suited to answering questions about overall population differentiation (Cowen 2006),
whereas tagging and elemental tracing studies, coupled to biological-physical models
may be able to delineate specific larval sources and sinks (Jones et al. 1999, 2005;
Almany et al. 2007; Becker et al. 2007; Werner 2007).

Thus far, three previous studies have been published on the population genetics
of *S. franciscanus*, none of which provide a clear picture of the population structure
throughout their range. Moberg and Burton conducted an allozyme study which
concluded that, although some patchy differentiation was evident between
populations, there was no geographic pattern to the variation (Moberg et al. 2000). An
interesting aspect of this study was the employment of size stratified sampling as a
way of approximating age classes in order to assess within population genetic
variation and patterns of recruitment. The sizes were “recruits” which were 30 mm
less and were approximately 1-2 years old, and a somewhat arbitrary division between
“juveniles” at 31-60 mm and “adults” which were 60 mm and bigger. They showed
that the recruits had different allelic frequencies than the juveniles and adults from the
same population. Another study by Debenham et al. (2000) compared sequence data
from a 273 base pair region of the bindin gene and found that they could not reject the hypothesis that the red urchin is panmictic throughout its range from Southern California to Alaska. Lastly, Miller et al. (2006) also found little evidence of population differentiation among British Columbia populations ranging from the southern end of Alaska to the Washington border. Several studies on the population genetics of a sympatric species closely related to *S. franciscanus*, *Strongylocentrotus purpuratus*, have been published. Edmunds et al. (1996) found the purple urchin showed significant genetic subdivision in California using allozyme and mitochondrial DNA analyses. Flowers et al. (2002) found little evidence of reduced genetic variation in recruits relative to adult urchins.

This study builds on the work of Miller et al. (2004, 2006) using seven microsatellite markers that were developed for their study in British Columbia. Microsatellite markers were used in this study because the statistical power provided by using multiple independently segregating loci was hoped to provide a more sensitive analysis of the population structure over the entire range. Microsatellites are tandem repeats of one to six nucleotides found in the nuclear genome. Their benefits include high variability, easy sample preparation, and inexpensive development and use. However, complications of microsatellite markers include their high variability, which can lead to uncertainties in interpreting allele size due to homoplasy, and unclear mutational mechanisms (Selkoe 2006, Hellberg et al. 2002). In this study, we use 7 microsatellite markers to assess the connectivity of six populations of urchins ranging from British Columbia to California. We also employed size-stratified
sampling for three of the four California populations in this study (Moberg et al. 2000).
MATERIALS AND METHODS

Sample Collection:

*S. franciscanus* samples were collected from 4 locations in California from November 2007 to December 2008: Point Loma, La Jolla, Malibu, Point Arena (Table 1, Figure 1). The urchins were measured and the sizes were recorded so that the populations could be grouped into 3 size classes: small (≤ 30mm), medium (31-60 mm), and large (> 60mm). Gonadal tissue samples were either taken directly from the live urchin, or they were taken from pieces of roe that had been processed for human consumption, and they were stored at -80°C. The preservative used in the processing of uni, potassium aluminum sulphate (KAl(SO$_4$)$_2$), did not affect DNA extraction, PCR, or other downstream applications. DNA was extracted from the gonadal tissue using the Qiagen DNEasy Tissue kit (Quiagen Inc., Valencia, CA).

Microsatellite amplification:

The seven microsatellite primers that I used were developed by Miller et al. and published in 2004 (Sfr 06, Sfr 13, Sfr 14, Sfr 22, Sfr 34, Sfr 55, and Sfr 64). Forward primers were labeled with 6-FAM tags on the 5’ end for fluorescent visualization. The microsatellite loci were amplified using polymerase chain reaction in a BioRad thermalcycler (Bio-Rad Laboratories, Hercules, CA). Cycling conditions included an initial denaturation at 95° for 5 minutes, followed by 35-40 cycles of 95° for 30 seconds, 52° for 45 seconds, then 72° for 1 minute, and a final elongation step at 72° for 7 minutes. This final elongation step allowed the successful addition of the
plus-A tag, used to prevent one base pair stutter (Brownstein et al. 1996). Genotyping was performed using the Molecular Dynamics MegaBACE™ DNA sequencer, using ROX end labeled MegaBACE™ ET-500 Size Standard (GE Healthcare). The peaks were scored using the genotyping computer program GeneMarker® (Softgenetics LLC, State College, PA).

Statistical Analysis:

Allelic and genotypic frequency data were analyzed using the programs GENEPOP (Raymond and Rousset 1995, 2003) and Microsatellite Analyzer (MSA) (Dieringer and Schlötterer 2003). Conformance to Hardy-Weinberg equilibrium and linkage disequilibrium was tested using GENEPOP. G-tests for genotypic and genic differentiation between populations and size classes was calculated in GENEPOP (Genic differentiation is concerned only with the distribution of alleles, whereas genotypic distribution is concerned with the distribution of diploid genotypes). The frequency of the null allele was calculated using maximum likelihood in ML-nullfreq (Kalinowski and Taper 2006). Levels of significance across all populations were calculated in Chiperm (Posasa 2000). \( F_{ST} \) and \( \rho_{ST} \) were calculated in GENEPOP and in MSA. Significance of \( F_{ST} \) was calculated in MSA. Mantel tests (1000 permutations) were run in GENEPOP to assess whether there was a signal of Isolation by Distance (IBD). The parameter \( F_{ST} \) was standardized to \( F_{ST} / (1 - F_{ST}) \) and plotted against the distance between populations. An AMOVA (Analysis of Molecular Variance) was used to estimate within individual and among population
variance in GENALEX (Peakall and Smouse, 2005). A population assignment test was also run using GENALEX.

Six of the seven microsatellite markers used (Sfr 06, Sfr 13, Sfr 22, Sfr 34, Sfr 55, and Sfr 64) were also used by Miller et al. in their 2006 study of *S. franciscanus* in British Colombia and Alaska. We compared our data for 5 of these markers (Sfr 34 was left out due to difficulty aligning alleles from the two studies) to the allele frequencies observed at every location surveyed by Miller et al. 2006. We did this using a Chi-squared analysis whose significance is evaluated using Monte Carlo algorithm in the program Chiperm (Posada 2000). We also analyzed genotype data for two of the populations from the Miller et al. 2006 study, Campbell River and Louscoone Inlet, in all analyses mentioned above (Figure 1).
RESULTS

GENETIC VARIATION WITHIN SAMPLES

All seven microsatellite markers used were highly polymorphic, ranging from 19-206 alleles (Table 2). There were no significant differences between the number of alleles observed within each population (Table 7). There was no evidence of linkage disequilibrium between any of the loci accept for one pair, Sfr06 and Sfr22, at the Malibu location only. This is thought to be an anomaly because there were no repeated samples. Significant (p<0.05) heterozygote deficiencies were found at every locus, with estimates of null allele frequencies ranging from 0.032 to 0.09 (Table 2). By population, La Jolla was the most deviant from Hardy-Weinberg equilibrium, with every locus having significantly low heterozygote frequencies. Estimates of F_{IS} ranged from 0.05 for Sfr06 to 0.21 for Sfr34. Observed heterozygosity, F_{IS}, and F_{ST} were very similar to data published in Miller et al. 2006.

GENETIC VARIATION AMONG SAMPLES

A chi-squared analysis of observed allelic counts in every population (four California and thirteen British Columbia) showed that the populations were highly differentiated at every locus (p<0.006 for Sfr 06; p<0.001 for all other loci).

Global genotypic variation was insignificant between the California populations but it was highly significant between Campbell River and all the California populations and Louscoone Inlet and all the California populations (Table 3). Of the two Canadian populations, Louscoone Inlet was more divergent from the
California populations than Campbell River. When the California samples were compared across all seven loci, the global levels of differentiation were insignificant (Table 3). However, when populations are compared at individual loci, there were some significant differences between California populations. At locus Sfr 34, Point Loma was significantly differentiated from Point Arena (p=0.049), and at Sfr 64 Point Loma was significantly different from La Jolla and Malibu (p=0.053, p=0.036).

More populations showed significant genic differentiation than significant genotypic differentiation (Table 3). In an analysis with just 5 loci, the British Columbia populations were differentiated from the California populations, but the British Columbia populations were also differentiated from each other. Point Loma and Point Arena were also significantly different. This is interesting because they are the two furthest populations within California. When genic differentiation was compared across all seven loci for just the California populations, Point Loma was significantly different than Malibu and Point Arena, but not La Jolla. Malibu and La Jolla were also significantly different.

$F_{ST}$ values were low across all loci, as is expected for populations of broadcast spawning marine organisms. However, $F_{ST}$ was relatively high between Campbell River and Malibu, and between Louscoone Inlet and all the California populations (Table 4). $\rho_{ST}$ was also relatively high between Louscoone Inlet and all the California populations (Table 5). A Mantel test using $F_{ST}$ values for all six locations showed significant isolation by distance ($Pr(\text{correlation} > \text{observed correlation}) = 0.036$)
A test of isolation by distance among the California samples only did not show a significant correlation ($\Pr(\text{correlation} > \text{observed correlation}) = 0.925$).

In a population assignment test, Louscoone Inlet genotypes were assigned to Louscoone Inlet population 31% of the time (Table 6). Given that there were 6 populations, if they were randomly assorted, we would expect the genotypes to be assigned to the correct location 16.6% of the time. The other populations had lower percentages of correct assignments, suggesting that Louscoone Inlet is the most genetically distinct.

An AMOVA test of genetic variation using $F_{ST}$ showed 2% molecular variation among populations when all six populations were treated separately, but when the populations were pooled into two groups, one group of all California and another group of all British Columbia, there was 4% molecular variation among populations (Figure 3). When the four locations in California were compared there was 0% molecular variation was among populations, suggesting they are genetically homogeneous, and when the two British Columbia were compared there was less than 1% molecular variation. This group of tests shows that the variance between the two British Columbia populations and the four California populations is greater than the variance within each of these groups of populations.

Analysis of the size-stratified samples did not show any genotypic differentiation between recruit, juvenile, and adult size classes using a G-test of genotypic differentiation ($p$-values ranging from 0.342 to 0.853). However, using a G-test of genic differentiation the recruits and the adults from Point Loma were
significantly different (p = 0.027). Point Loma was the only location from which we had a large number of recruits, so it was impossible to make the same comparison in other locations.
DISCUSSION

Taken as a whole, the data presented here show that populations in California are genetically divergent from those in British Columbia. There is evidence of isolation by distance, but it is important to consider that there are only six populations in this study, and the populations are not evenly distributed throughout the range of *S. franciscanus*. Additional populations need to be included in the analysis for a more confident result. There was a weak and patchy detection of differentiation among California populations but no signal of isolation by distance, similar to Moberg and Burton et al. (2000). Considered along with the results of the $F_{ST}$, IBD, and AMOVA tests in this study, this slight differentiation is insufficient to definitively say that California populations are divergent from each other.

For the three populations from which we have size stratified samples, we found that juveniles and adults showed little differentiation, however recruits and adults showed significant *genic* differentiation in Point Loma. Between the size classes there were no major differences in the number of alleles per locus, only in the allele frequencies. This could mean that the larval pool is not well mixed geographically and consequently we are seeing a disproportionate number of recruits from a certain fertilization event. On the other hand, the lack of strong *genotypic* differentiation between recruit, juvenile, and adult populations could mean one of two things: either the populations are mostly self-recruiting, or the larval influx from various other locations is genetically heterogeneous. Since we saw little evidence of strong population differentiation on a small geographic scale, we infer that there can be many
successful fertilization events that supply successful recruits. In other words, there are multiple winners of sweepstakes recruitment, as Flowers and Burton (2002) concluded in their study of purple urchin recruitment patterns.

This study does not discern specific sources and sinks of urchin populations, but it does show that the sources and sinks are variable enough to prevent strong genetic differentiation within California. Although clear genetic differentiation between populations is only seen on a large scale, this study does not exclude the possibility that there may be strong local recruitment within populations. It only takes a few migrants per generation to maintain genetic homogeneity between populations, and even moderate gene flow can be indistinguishable from random mating (Palumbi 2003). Two recent papers on the California sea mussel *Mytilus californianus* show that even when there is genetic homogeneity over a range of thousands of miles, there still can be substantial self-recruitment to local populations. Addison et al. (2008) could not reject that California sea mussels are genetically homogeneous from Alaska to Baja California using a variety of genetic markers including allozymes, scnDNA markers and mitochondrial DNA sequences. However, in a paper by Becker et al., elemental fingerprinting was used as a tracking tool to determine sources of settled mussel larvae from two different sympatric mussel species that there can be strong asymmetric mixing (88% of the *M. californianus* larvae originated in the northern part of their study region) and also that there can be very high self recruitment to a region (*M. galloprovincialis* showed low site-specific recruitment but 40% self recruitment within regions). It is important to consider that this is a snapshot of the recruitment
within just one year, and that additional studies such as this are needed to integrate the data over appropriate temporal and special scales. It is likely that mussel recruitment patterns vary temporally, which could lead to the observed genetic homogeneity over many years.

The other population genetic studies of *S. franciscanus* discussed in this paper show some different patterns in genetic diversity than our results indicate. My results were not consistent with what Debenham et al. (2000) found using bindin gene sequences. This study may have found genetic differentiation across the whole range of *S. franciscanus* where Debenham et al. did not because of the use of multiple, highly variable genetic markers. Overall, my results were consistent with patterns found in Miller et al. (2006), as I did not show high levels of differentiation between the British Columbia samples. Comparing my study to Moberg and Burton’s 2000 study of red urchins, there are some consistencies, such as finding some differentiation between adults and recruits, but some differences, such as their observance of significant population heterogeneity (no geographic pattern) within California. I saw weak population differentiation in California, if any. It is unclear why different genetic markers can often lead to different results, but it has been suggested that in some cases certain markers may be subject to natural selection while others are not (Hellberg 2002). Microsatellite loci are generally assumed to be neutral genetic markers, although trinucleotide and hexanucleotide repeats don’t cause frameshift mutations so they could possibly be found in coding regions. In this study I used 6 di-nucleotide repeat loci and one tri-nucleotide repeat locus, Sfr 55. Another
confounding factor could be the high variability of our markers. Highly variable loci, such as Sfr 34 (206 alleles), generally provide less power unless the sample size is very large. However, tests run without Sfr 34 produced the same results as when it was included, so it does not seem that in this case Sfr34 is skewing the results. Furthermore, the consistent high variability of our markers shows that the population divergence between California and Alaska is not likely due to a past bottleneck in population size (Table 7).

Relatively high inbreeding coefficients, $F_{IS}$, and high estimates of null alleles found in this study could be attributed to a variety of different factors including laboratory artifacts, natural selection acting on the genetic markers, or unrecognized spatial or temporal structure within samples. Similar results were observed in Miller et al. (2006). Additionally, McCartney et al. developed a separate set of *S. franciscanus* primers, which were not used in this study, and also found heterozygote deficits (McCartney 2004). It is interesting to note that large, positive $F_{IS}$ values are commonly observed for broadcast spawning species in the marine environment, and furthermore, broadcast spawning organisms tend to have significantly higher $F_{IS}$ values than marine species with direct sperm transfer (Addision and Hart 2005). This correlation suggests that some aspect of broadcast spawning in the marine environment tends to cause greater deviations from Hardy-Weinberg equilibrium, in the form of heterozygote deficit, than we would intuitively expect for open populations. Addison and Hart propose that a likely explanation for this pattern could be the Wahlund effect (due to extensive within population genetic structure) or
possibly high variance in reproductive success, neither of which are definitive conclusions of this study. It may also be that there are higher rates of molecular evolution due to high fecundity, which might indirectly result in higher proportions of null alleles. Whatever the cause of the heterozygote deficiency in this study, it is worth noting heterozygote deficiencies are not uncommon for broadcast spawning marine organisms.
CONCLUSION

Advanced understanding of *S. franciscanus* connectivity is necessary because red urchins are a key player in kelp forest ecology and they support a lucrative fishery along the west coast of the United States and Canada. This study shows that there is significant population differentiation between urchins in California and in British Columbia, however within California there was minimal detection of significant differentiation. There was also some evidence for genetic differentiation between the different size classes of red urchins. For a more meaningful analysis of this size stratified data, it would be important to consider recruits from multiple locations over multiple years.

**Implications for Management:**

On an evolutionary time scale, there only seems to be strong differentiation over a large geographic area. However, on an ecological time scale, differing patterns of recruitment and demography may be observed over smaller distances, so it is important that fishery and marine protected area managers consider these variables as well as genetic information.
FURTHER WORK

I plan to genotype another set of northern California samples from Fort Bragg CA, which may provide additional insight into the variation within California populations. Additionally, I plan to analyze full genotype data from two to three other locations in British Columbia, especially those north of Louscoone Inlet, to further test for IBD.
AMPLIFIED FRAGMENT LENGTH POLYMORPHISM (AFLP)

AFLP genetic markers are highly variable, versatile, and low cost, making them attractive markers for ecological studies (Bensch 2005). An attempt was made to develop a set of >100 AFLP markers for *S. franciscanus* to supplement the microsatellite markers, as Gruenthal and Burton did for their 2008 study of black abalone, however the results were not repeatable and therefore could not be used. As many as 30% of the alleles did not consistently amplify when repeated for the same samples. I experimented with DNA extracted according to three different protocols, none of which was reproducible. DNA extracted with a phenol-chloroform extraction produced clear bands, however they were not repeatable. The Quiagen DNEasy® (Quiagen Inc., Valencia, CA) and Charge Switch® (Invitrogen Corp., Carlsbad CA) kits yielded DNA that only produced about 10% of the bands observed with the DNA extracted via phenol-chloroform extraction. Some possible explanations for the lack of reproducibility include high levels of genetic variation in *S. franciscanus*, poor quality DNA, or impure DNA. Two other publications have used AFLP markers to study sea urchins: one constructed a linkage map for use in hybrid cross experiments using *Strongylocentrotus intermedius* and *S. nudus* (Zhou 2006) and another described population differentiation between *Strongylocentrotus intermedius* and *S. nudus* (Zhou 2007).
FIGURES

Figure 1. Maps showing collection sites in California and British Colombia*

*British Columbia populations were presented in Miller et al. 2006

Figure 2. Isolation by distance. Distance in minimum waterway in km. $F_{ST}/(1 - F_{ST})$ used as a measure of genetic distance.
Percentages of Molecular Variance between all six individual populations

Among Pops: 2%
Among Indiv: 20%
Within Indiv: 78%

Percentages of Molecular Variance between the combined California and combined British Columbia populations, based on F-statistics

Among Pops: 4%
Among Indiv: 19%
Within Indiv: 77%

Percentages of Molecular Variance between only the populations in California

Among Pops: 0%
Among Indiv: 17%
Within Indiv: 83%

Percentages of Molecular Variance between only the two populations in British Columbia

Among Pops: <1%
Among Indiv: 26%
Within Indiv: 73%

Figure 3. Pie graphs showing AMOVA calculations of variance.
## TABLES

Table 1. Collection sites, collection years, and sample sizes.

<table>
<thead>
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<tr>
<td>La Jolla</td>
<td>California</td>
<td>2008</td>
<td>123</td>
<td>1</td>
<td>32</td>
<td>90</td>
</tr>
<tr>
<td>Malibu</td>
<td>California</td>
<td>2008</td>
<td>80</td>
<td>0</td>
<td>16</td>
<td>64</td>
</tr>
<tr>
<td>Point Arena</td>
<td>California</td>
<td>2008</td>
<td>85</td>
<td>2</td>
<td>7</td>
<td>76</td>
</tr>
<tr>
<td>Campbell River*</td>
<td>Colombia</td>
<td>2003</td>
<td>89</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>Louscoone Inlet*</td>
<td>British</td>
<td>2000-2003</td>
<td>95</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
</tr>
</tbody>
</table>

Table 2. Microsatellite loci, including the number of alleles, expected heterozygosity, observed heterozygosity, inbreeding coefficient $F_{IS}$, and the estimated frequency of the null allele.

<table>
<thead>
<tr>
<th>Locus*</th>
<th># of alleles</th>
<th>He</th>
<th>Ho</th>
<th>$F_{IS}$</th>
<th>Null</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sfr 06</td>
<td>20</td>
<td>0.87</td>
<td>0.81</td>
<td>0.05</td>
<td>0.032</td>
</tr>
<tr>
<td>Sfr 13</td>
<td>39</td>
<td>0.90</td>
<td>0.80</td>
<td>0.11</td>
<td>0.057</td>
</tr>
<tr>
<td>Sfr 14</td>
<td>25</td>
<td>0.88</td>
<td>0.79</td>
<td>0.12</td>
<td>0.056</td>
</tr>
<tr>
<td>Sfr 22</td>
<td>42</td>
<td>0.95</td>
<td>0.79</td>
<td>0.15</td>
<td>0.090</td>
</tr>
<tr>
<td>Sfr 34</td>
<td>71</td>
<td>0.99</td>
<td>0.80</td>
<td>0.21</td>
<td>0.063</td>
</tr>
<tr>
<td>Sfr 55</td>
<td>64</td>
<td>0.97</td>
<td>0.89</td>
<td>0.14</td>
<td>0.045</td>
</tr>
<tr>
<td>Sfr 64</td>
<td>19</td>
<td>0.83</td>
<td>0.73</td>
<td>0.11</td>
<td>0.069</td>
</tr>
</tbody>
</table>

*Primers from Miller et al. 2004
Table 3. Significance of exact G test for genotypic and genic differentiation for each population when compared across five loci (Sfr 06, Sfr13, Sfr 22, Sfr 55, Sfr 64) and across all seven loci (Sfr 06, Sfr13, Sfr 14, Sfr 22, Sfr 34, Sfr 55, Sfr 64).

<table>
<thead>
<tr>
<th>Population pair</th>
<th>Genotypic Differentiation</th>
<th>Genic Differentiation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5 Loci</td>
<td>7 Loci</td>
</tr>
<tr>
<td>Point Loma &amp; La Jolla</td>
<td>0.350</td>
<td>0.49</td>
</tr>
<tr>
<td>Point Loma &amp; Malibu</td>
<td>0.395</td>
<td>0.108</td>
</tr>
<tr>
<td>La Jolla &amp; Malibu</td>
<td>0.416</td>
<td>0.345</td>
</tr>
<tr>
<td>Point Loma &amp; Point Arena</td>
<td>0.158</td>
<td>0.072</td>
</tr>
<tr>
<td>La Jolla &amp; Point Arena</td>
<td>0.919</td>
<td>0.978</td>
</tr>
<tr>
<td>Malibu &amp; Point Arena</td>
<td>0.737</td>
<td>0.699</td>
</tr>
<tr>
<td>Point Loma &amp; Campbell River</td>
<td>0.013</td>
<td>*</td>
</tr>
<tr>
<td>La Jolla &amp; Campbell River</td>
<td>0.006</td>
<td>*</td>
</tr>
<tr>
<td>Malibu &amp; Campbell River</td>
<td>0.001</td>
<td>*</td>
</tr>
<tr>
<td>Point Arena &amp; Campbell River</td>
<td>0.040</td>
<td>*</td>
</tr>
<tr>
<td>Point Loma &amp; Louscoone Inlet</td>
<td>0.003</td>
<td>*</td>
</tr>
<tr>
<td>La Jolla &amp; Louscoone Inlet</td>
<td>0.001</td>
<td>*</td>
</tr>
<tr>
<td>Malibu &amp; Louscoone Inlet</td>
<td>0.000</td>
<td>*</td>
</tr>
<tr>
<td>Point Arena &amp; Louscoone Inlet</td>
<td>0.001</td>
<td>*</td>
</tr>
<tr>
<td>Campbell River &amp; Louscoone Inlet</td>
<td>0.089</td>
<td>-</td>
</tr>
</tbody>
</table>

* denotes significantly divergent populations

Table 4: Pairwise F_{ST} values below the line, P-values above the line

<table>
<thead>
<tr>
<th>Point Loma</th>
<th>La Jolla</th>
<th>Malibu</th>
<th>Point Arena</th>
<th>Campbell River</th>
<th>Louscoone Inlet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Point Loma</td>
<td>0.0573</td>
<td>0.097</td>
<td>0.1614</td>
<td>0.0849</td>
<td>0.0091*</td>
</tr>
<tr>
<td>La Jolla</td>
<td>0.001454</td>
<td>0.0703</td>
<td>0.9175</td>
<td>0.1105</td>
<td>0.0020*</td>
</tr>
<tr>
<td>Malibu</td>
<td>0.001434</td>
<td>0.00194</td>
<td>0.6137</td>
<td>0.0021*</td>
<td>0.0008*</td>
</tr>
<tr>
<td>Point Arena</td>
<td>0.001057</td>
<td>0.001572</td>
<td>0.000494</td>
<td>0.2521</td>
<td>0.0048*</td>
</tr>
<tr>
<td>Campbell River</td>
<td>0.001528</td>
<td>0.001562</td>
<td>0.000745</td>
<td>0.1919</td>
<td></td>
</tr>
<tr>
<td>Louscoone Inlet</td>
<td>0.002902</td>
<td>0.004126</td>
<td>0.005235</td>
<td>0.0011</td>
<td></td>
</tr>
</tbody>
</table>

* denotes significantly different F_{ST} values
Table 5: Pairwise $\rho_{ST}$ values

<table>
<thead>
<tr>
<th></th>
<th>Point Loma</th>
<th>La Jolla</th>
<th>Malibu</th>
<th>Point Arena</th>
<th>Campbell River</th>
<th>Louscoone Inlet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Point Loma</td>
<td>-0.0028</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>La Jolla</td>
<td>-0.0033</td>
<td>-0.0052</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Malibu</td>
<td>-0.0048</td>
<td>-0.0056</td>
<td>-0.0054</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Point Arena</td>
<td>-0.0029</td>
<td>0.0015</td>
<td>0.0011</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Campbell River</td>
<td>-0.0048</td>
<td>-0.0056</td>
<td>-0.0054</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>River</td>
<td>0.0015</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Louscoone Inlet</td>
<td>0.0324</td>
<td>0.0415</td>
<td>0.0376</td>
<td>0.0408</td>
<td>0.0187</td>
<td></td>
</tr>
</tbody>
</table>

Table 6: Percent of genotypes that were assigned to their own population in a population assignment test.

<table>
<thead>
<tr>
<th>Pop</th>
<th>Self Pop</th>
<th>Other Pop</th>
<th>Total</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Point Loma</td>
<td>21</td>
<td>129</td>
<td>150</td>
<td>14%</td>
</tr>
<tr>
<td>La Jolla</td>
<td>25</td>
<td>98</td>
<td>123</td>
<td>20%</td>
</tr>
<tr>
<td>Malibu</td>
<td>21</td>
<td>59</td>
<td>80</td>
<td>26%</td>
</tr>
<tr>
<td>Point Arena</td>
<td>20</td>
<td>65</td>
<td>85</td>
<td>24%</td>
</tr>
<tr>
<td>Campbell River</td>
<td>22</td>
<td>67</td>
<td>89</td>
<td>25%</td>
</tr>
<tr>
<td>Louscoone Inlet</td>
<td>29</td>
<td>66</td>
<td>95</td>
<td>31%</td>
</tr>
</tbody>
</table>

Table 7: Number of alleles at each locus, by location.

<table>
<thead>
<tr>
<th></th>
<th>Sfr 06</th>
<th>Sfr 13</th>
<th>Sfr 14</th>
<th>Sfr 22</th>
<th>Sfr 34</th>
<th>Sfr 55</th>
<th>Sfr 64</th>
</tr>
</thead>
<tbody>
<tr>
<td>TOTAL</td>
<td>17</td>
<td>37</td>
<td>25</td>
<td>39</td>
<td>206</td>
<td>63</td>
<td>18</td>
</tr>
<tr>
<td>Point Loma</td>
<td>150</td>
<td>14</td>
<td>25</td>
<td>19</td>
<td>31</td>
<td>126</td>
<td>53</td>
</tr>
<tr>
<td>La Jolla</td>
<td>123</td>
<td>12</td>
<td>19</td>
<td>20</td>
<td>34</td>
<td>116</td>
<td>53</td>
</tr>
<tr>
<td>Malibu</td>
<td>80</td>
<td>14</td>
<td>18</td>
<td>19</td>
<td>31</td>
<td>94</td>
<td>42</td>
</tr>
<tr>
<td>Point Arena</td>
<td>85</td>
<td>12</td>
<td>19</td>
<td>21</td>
<td>29</td>
<td>98</td>
<td>48</td>
</tr>
<tr>
<td>Campbell River</td>
<td>89</td>
<td>14</td>
<td>19</td>
<td>n/a</td>
<td>28</td>
<td>n/a</td>
<td>37</td>
</tr>
<tr>
<td>Louscoone Inlet</td>
<td>95</td>
<td>14</td>
<td>17</td>
<td>n/a</td>
<td>31</td>
<td>n/a</td>
<td>38</td>
</tr>
</tbody>
</table>
REFERENCES


Zhou ZC, Zhenmin Bao, Ying Dong, Shi Wang, Chongbo He, Weidong Liu, Limei Wang and Feng Zhu. 2006. AFLP linkage map of sea urchin constructed using an interspecific cross between Strongylocentrotus nudus (♀) and S. intermedius (♂) Aquaculture. 259 (1-4):56-65