Final Report

March 2006 to March 2008

Project Title: **Larval Connectivity and Fish Population Replenishment in a Network of Marine Managed Areas for Tropical Aquarium Fisheries**

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Principal Investigator's Institution: Oregon State University

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Introduction

The **goal** of this study was to understand the biological ability of MPAs within MMA networks to replenish fish populations. Scientists have long believed that protected areas produce "spillover" (post-settlement emigration) or "seeding" (larval export and connectivity) that help to maintain fished populations outside the protected areas, and this research aimed to determine the degree to which seeding occurs in a network of MMAs off West Hawai'i (the Kohala-Kona coast of the Big Island). Professional collectors capture aquarium fish near these MMAs, and a further objective, should the research show beneficial effects of the 1999 changes in the management regime for the MPA-MMA network, was to demonstrate the value of MPAs to the aquarium industry, and thereby develop industry support for MPAs.

In particular, more detailed research **objectives** were to:

(1) **Determine the level of larval connectivity** among MMAs, and between MMAs and nearby fished areas, by studying the population genetics of a key species. This information will aid our understanding of how, and to what degree, FRAs "seed" other reserves and fished areas within the network of MMAs.

(2) **Measure the effectiveness of the MMA network** by monitoring the recruitment, abundance, and catch of target species under certain management regimes in a given proximity to one anther. The target fish species (especially yellow tang) are professionally collected for the aquarium industry, and the study will measure the effectiveness of Fish Replenishment Areas (FRAs, areas where collecting is prohibited) within the network in terms of, first, whether or not they improve recruitment of fish species harvested for aquaria, and second, the degree to which aquarium collecting is enhanced outside the FRAs.

We expect that the information on connectivity, and factors bearing on connectivity, will also be useful to MMA planners when deciding on management regimes for marine management areas both within and beyond the Hawaiian archipelago. A further objective is to develop capacity to conduct science locally, and to engage and educate stakeholders in the effectiveness, and factors bearing on the effectiveness, of MMAs.

I. Report on the progress and the most recent status for each task and deliverable listed under "Activity, Tasks, Timeline" [Lead Legend: OSU=Oregon State University (Hixon et al.); UH=University of Hawai'i (Beets et al.); WSU=Washington State University (Tissot et al.); DAR=Hawai'i Dept of Aquatic Resources (Walsh)]:

March '06 — Meet with key representatives of three components of the target audience (scientists: Sara Peck of Hawai'i Sea Grant; managers: Dr. William Walsh of the Hawai'i Department of Aquatic Resources; conservationists: Dr. Stephen Thompson of the Cascadia Conservation Trust) to solicit feedback for final design of research (OSU):

Status: COMPLETED: All were consulted and approved of our research design with enthusiasm.

March-May '06 — Optimization of 9 microsatellite DNA primers (OSU):

Status: COMPLETED: We have optimized PCR conditions for 15 of the 28 microsatellite loci that have been developed (see APPENDIX A: Methodological Details of Genetic Analysis).

March-May '06 — Prepare field logistics & data management (UH & WSU):

Status: COMPLETED: The 2006 field season (May-September) is now complete.

June 30, 2006 — First Quarterly Report:

Status: COMPLETED: At CI's request, the first quarterly report was folded into the second quarterly (de facto semi-annual) report.

April-August '06 — Field collection of DNA samples (OSU):

Status: COMPLETED: See III below.

May-December '06 Meet with target audience, review additional potential deliverables, identify funding needs for those deliverables and seek funding:

Status: COMPLETED: (1) Dr. William Walsh (DAR) is serving as liaison with the Marine Aquarium Council (MAC). On 28 November 2006, there was a meeting to determine whether DAR's management of the aquarium fishery in West Hawai'i met MAC's standards for "Area Certification" (see www.aquariumcouncil.org). This process is still underway. We are also sending our progress reports to Mr. Paul Holthus, the Executive Director and President of MAC. MAC has also endorsed our proposals for additional funding (see APPENDIX B: Letter of Endorsement).

(2) The Cascadia Conservation Trust has agreed to provide funding and logistic support for the second year of this project (2007 field season).

(3) We have been awarded two additional grants to continue this project beyond this CI grant: (i) Effects of habitat and predation on the effectiveness of an MPA network in Hawaii to replenish the aquarium fish, *Zebrasoma flavescens*. NOAA Habitat Conservation Program. Tissot and Ortiz. $40,000. 7/1/2007-12/31/2008. (ii) Effects of a marine protected area network on the aquarium fishery in Hawai`i. WSU Vancouver Mini-Grant. Tissot. $3,200. 6/1/2007-12/31/2007. (iii) An award from Hawai'i Sea Grant to Beets.

(4) We have also submitted two additional proposals to continue this project beyond this CI grant: (i) Conserving Hawaii`s aquarium reef fish. Disney Foundation. Tissot and Walsh. $20,000. 7/1/2007-5/1/2009. (ii) Developing a cooperative fishery research program in Hawai`i. National Fish & Wildlife Foundation. Tissot and Walsh. $58,755. 8/16/2007-8/15/2009.

May '06 - March '07 Lab analysis of DNA samples (OSU):

Status: COMPLETED: See III below.

May-Sep '06 Field monitoring of recruitment (UH & WSU):

Status: COMPLETED: See III below.

September 30, 2006 Second Quarterly Report:

Status: COMPLETED: Incorporated in this report.

June-Sep '06 Field monitoring of fish-habitat relationships (WSU):

Status: COMPLETED: See III below.

Oct-Jan '07 Field monitoring of aquarium catch (UH, WSU, DAR):

Status: COMPLETED: See III below.

Oct-Mar '07 Data analysis, production and distribution of outreach materials; preparation and submission of manuscripts; engage target audience

for preparation of paper, and prepare paper; continued meetings with stakeholders (coastal community, fishing community, marine resource managers) in Hawai'i (OSU, WSU, UH, DAR); distribution of paper internationally (MMAS):

Status: NEARLY COMPLETED: See III below:

(1) Final statistical analyses of genetics data are in progress, as are ongoing compilations of field ecological data.

(2) We arranged to present our findings to the Hawaiian community via public meetings sponsored by the University of Hawai'i Sea Grant Program.

(3) Delisse Ortiz (WSU) presented an update of her research, entitled "Effects of habitat and predator abundance on recruitment of yellow tangs (*Zebrasoma flavescens*) in Hawai'i," at the November 2006 annual meeting Western Society of Naturalists meeting in Redmond, WA.

(4) Drs. Tissot and Walsh wrote a review entitled "Community-Based Management in West Hawai`i."

December 31, 2006 — Third Quarterly Report:

Status: COMPLETED.

Jan-Mar '07 — Identify needs for future funding and submit proposals to continue long-term project, including collaboration with social scientists:

Status: COMPLETED:

(1) The Cascadia Conservation Trust provided funding and logistic support for the second year of this project (2007 field season).

(2) We were awarded two additional grants to continue this project beyond this CI grant: (i) Effects of habitat and predation on the effectiveness of an MPA network in Hawaii to replenish the aquarium fish, *Zebrasoma flavescens*. NOAA Habitat Conservation Program. Tissot and Ortiz. $40,000. 7/1/2007-12/31/2008. (ii) Effects of a marine protected area network on the aquarium fishery in Hawai`i. WSU Vancouver Mini-Grant. Tissot. $3,200. 6/1/2007-12/31/2007. (iii) An award from Hawai'i Sea Grant to Beets.

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(4) Drs. Hixon (OSU) and Tissot (WSU) joined a working group at the National Center for Ecological Analysis and Synthesis (2006-2008), entitled "Governance Feasibility of Marine Ecosystem-

Based Management: A Comparative Analysis." The focus is to explore how to successfully scale-up marine reserves on coral reefs in developing nations from existing individual reserves to effective networks. Hawai'i, in particular our study system, is seen as a case study in a developed nation for comparative purposes.

II. Anticipated Success in Fulfilling Tasks and Deliverables within Project Period:

We were successful in completing our deliverables within the extended project period.

III. Results:

***A. Genetic Sample Collection (2006)*:** During the summer of 2006, we successfully collected fin tissue samples (posterior dorsal fin) for genetic analyses of larval dispersal and self-recruitment from over 1,300 yellow tang, as planned. The sampling design was as follows:

(1) *potential parents* (target n = 50 fish per site): One-time samples of adults from sites encircling the entire Island of Hawai'i: all 6 Kohala-Kona (West Hawai'i) sites (3 Fish Replenishment Area MMAs ["FRA"] and 3 unprotected reference sites ["open"]), 3 NE Hawai'i sites, and 2 SE Hawai'i sites.

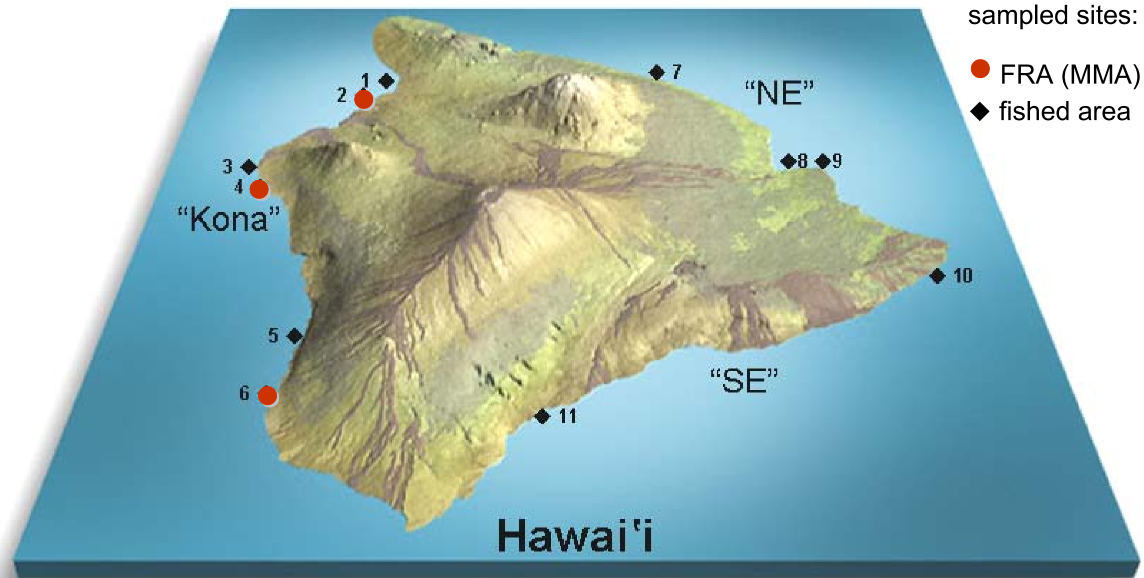
(2) *potential offspring* (target n = 30 fish per site per month): Monthly (June, July, August 2006) samples of recruits from all 6 Kona (West Hawai'i) sites, comprising the study network of MMAs.

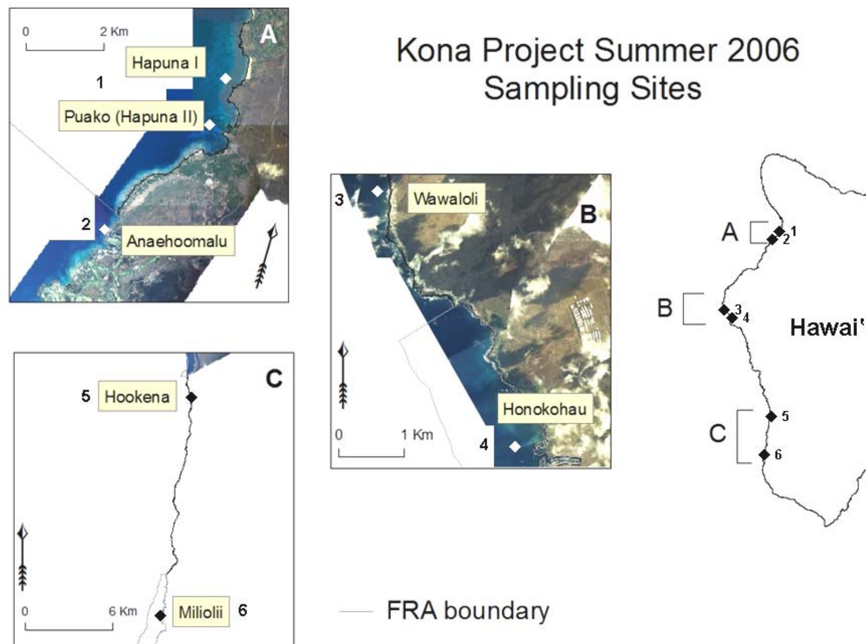
(3) *supplemental fish*: Juveniles at all sites to supplement recruit samples as needed (i.e., fish that settled during the spring as opposed to the summer target period).

Because many of the supplemental fish were not needed, we focused our analyses on recruits (potential offspring) and adults (potential parents), totaling 1,167 individuals genotyped.

The "regions" in the following table and maps are technically defined in Hawai'i as: "Kona north" = south Kohala (NW Big Island), "Kona central" = north Kona (W), "Kona south" = south Kona (SW), "NE Hawai'i" = Hanakua and Hilo (NE), and "SE Hawai'i" = Puna (SE):

Site (see map)	**Site Name**	**Region**	**Treatment**	**Number genotyped** **recruits** <50mm TL	**adults** ≥150mm TL	**Total**
1	Puako	Kona north	open	73	70	143
2	A-Bay	Kona north	FRA	58	75	133
3	Wawaloli	Kona central	open	133	50	183
4	Honokohau	Kona central	FRA	123	63	186
5	Hookena	Kona south	open	68	68	136
6	Milolii	Kona south	FRA	67	60	127
7	Laupahoehoe	NE Hawai'i	open	0	0	0
8	Puhi Bay	NE Hawai'i	open	45	0	45
9	Richardson's	NE Hawai'i	open	0	50	50
10	Isaac Hale	SE Hawai'i	open	18	51	69
11	Punaluu	SE Hawai'i	open	0	95	95
			Subtotal:	585	582	
					Total:	1167





***B. Genetic Analyses (2007)*:** Tissue samples from 1,167 yellow tang were processed in six steps: (1) extracting the genomic DNA (i.e., isolating the DNA from the rest of the tissue); (2) amplifying each specific region (locus) of the DNA (via the Polymerase Chain Reaction or PCR); (3) incorporating fluorescently labeled dye into the PCR product so that it can be read by a sequencing machine; (4) genotyping samples (i.e., PCR products are pooled and run through a capillary sequencing machine to determine their size); (5) scoring the resulting data; and (6) statistically analyzing the data to test for larval connectivity and self-recruitment (see APPENDIX A: Methodological Details of Genetic Analysis). We have optimized steps 1-4 such that 96 samples can now be processed every 1 to 1.5 weeks.

We tested several DNA extraction protocols and chose an approach that was relatively inexpensive and fast. We optimized PCR conditions for 15 of 28 microsatellite loci (7 of the 28 loci were dropped due to departures from Hardy-Weinberg Equilibrium, and an additional 6 loci were dropped due to scoring, stuttering, or pooling complications).

Besides the 1,167 fish genotyped so far, an additional 50 individuals, which scored poorly, will also be re-run to ensure accurate genotyping. Additionally, a random selection of 45 individuals that scored cleanly will be re-run in order to calculate a study-specific error rate. We will then complete all statistical analyses by the end of this no-cost extension. The focus to date has been to complete genotyping at all individuals rather than stopping and re-analyzing the data every quarter. This decision was made to expedite the completion of the data set so that final analyses can be started and completed as soon as possible.

In a preliminary analysis comparing *only* the central Kona coast (Honokohau, FRA site 4 on map) and Hilo (Richardson Ocean Center and Puhi Bay, open sites 8 and 9 on map), the overall observed heterozygosity (H_{Overall}) was 0.80 (indicating high levels of polymorphism) with a mean allelic richness of 12.54 (i.e., approximately 13 alleles per locus). The two Honokohau sampling dates (early and late July 2006) and the two Hilo sites were then combined to bolster sample size. F_{ST} between the two locations was calculated using the program FSTAT, which calculates an unbiased estimator of F_{ST} as well as significance (Goudet 2001). *F*-statistics were developed by Wright (1969) to partition heterozygote deficiency into a within- and an among-population component. They are widely used to assess levels of genetic differentiation among natural populations.

When all 15 loci were included in the analysis, the overall value of F_{ST} was 0.002 and was not significant (95% CI: -.001 – 0.004). However, 5 of the 15 loci had positive F_{ST} values (as well as significant *P*-values from exact tests of genic differentiation). The analysis was subsequently re-run with only these 5 loci, and the overall F_{ST} was then 0.008 and significant (95% CI: .004-.011). After accounting for the fairly high levels of heterozygosity using the method of Hedrick (1999), the overall F_{ST} was actually 0.04. This value of F_{ST} is quite substantial given the long pelagic larval duration (about 60 days) and small geographic scale (about 185 km from location to location along the coast) of this comparison. Importantly, this F_{ST} value is quite close to the value needed to successfully employ assignment tests with a very low percentage of incorrect assignments (Manel et al. 2005). These 5 loci also appear show a pattern of isolation by distance (although the number of sites is currently too low to warrant any conclusions).

These initial analyses indicate that certain loci appear to be much better at discriminating between populations than others. Although this general phenomenon has been known for some time (Banks et al. 2003), this outcome allowed us to increase the signal-to-noise ratio such that it may be possible to utilize assignment tests to determine regional patterns and sources of recruitment.

After all 1,200 fish are processed, it will be vital to determine which combination of loci demonstrates the greatest degree of differentiation between populations. If the same 5 loci appear to provide the greatest power for discriminating among populations, then it may be worthwhile to screen additional loci to provide better resolution. However, if different combinations of loci provide better discriminating power, then the current set of loci can be employed, and an assignment testing approach using various combinations of loci to discriminate among populations will be used. In any case, now that our methods are

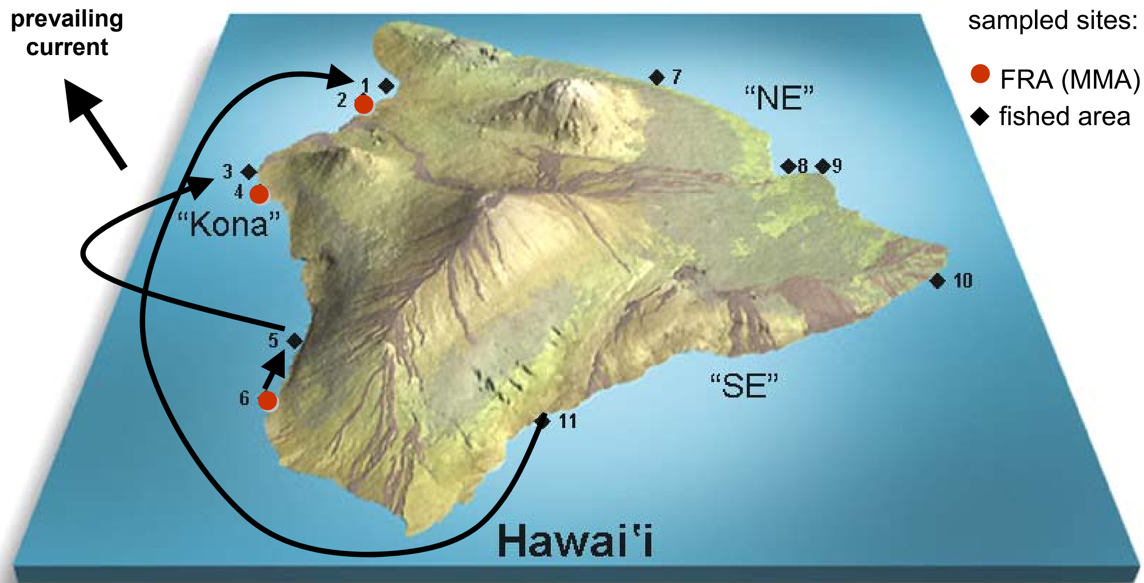
optimized, the remaining genetic samples will be processed relatively quickly. We additionally plan the following analyses:

- Calculate effective population size (proportion of the spawning population successfully contributing to the next generation).
- Test the sweepstakes hypothesis (differential spatial and temporal reproductive success).
- Test for isolation by distance (larval connectivity decreasing with increasing distance between populations).
- Compare genetic diversity between FRAs and fished sites, given that intensive fishing can reduce genetic diversity (Hauser et al. 2002 PNAS 99:11742).
- Measure relatedness among sampled fish (including parent-offspring pairs and sibships).

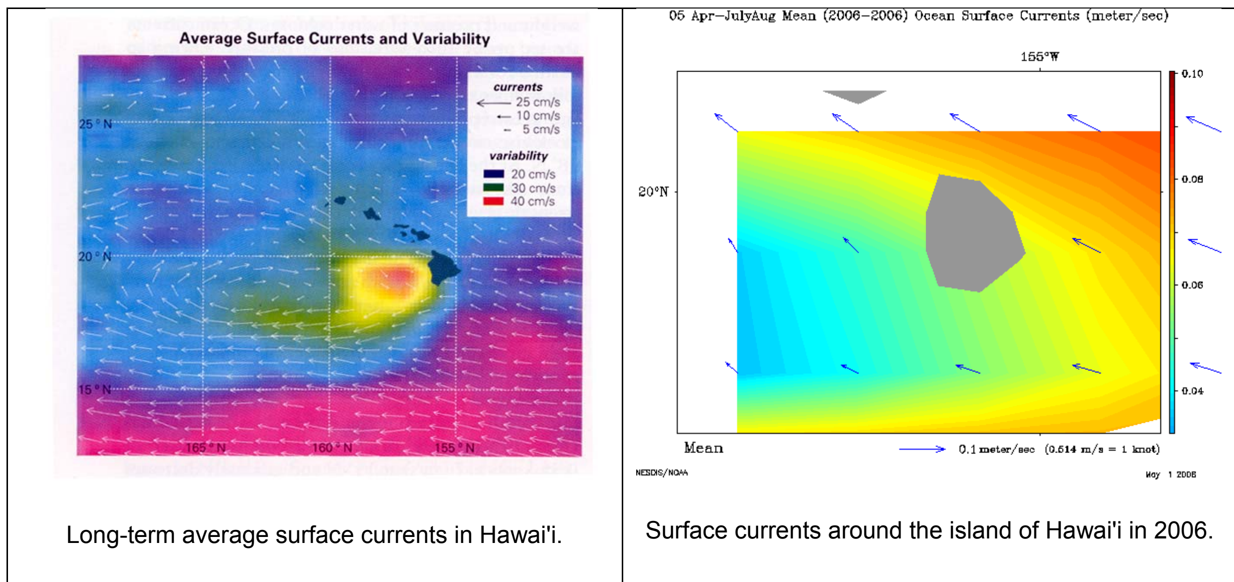
In addition to these analyses, a few recently developed techniques will also be used to resolve fine scale genetic structure. One such technique uses assignment tests applied in a moving window over a sampled study area (Manel et al. 2007). This procedure calculates the probability of observing an individual's multilocus genotype across the sampled area. This approach allows for the possibility of detecting cryptic population structure as well as a clearer insight into patterns of gene flow. Another novel technique we will employ is decomposed pairwise regression analyses (Koizumi et al. 2006). This method allows one to examine points on an isolation-by-distance graph and determine which evolutionary forces contribute most strongly to the observed pattern. This method is also useful for examining and potentially explaining outliers on isolation-by-distance graphs. Lastly, we will also run a partial-Mantel test with ecological and geographic variables. After applying some form of model selection (probably an Akaike Information Criterion), we will be able to determine what variables are correlated with the observed population genetic structure (e.g., various distance matrices, oceanographic variables, and demographic data).

C. Genetic Analyses (2008):

Putative parent-offspring pairs: Through the end of the funding period, we have found three putative parent-offspring pairs that share alleles at all 15 loci as well as a further 8 putative parent-offspring pairs that share alleles at 14 loci but have to be re-genotyped before further conclusions can be drawn. The three putative parent-offspring pairs that shared an allele at all 15 loci all had adults (parents) from populations that were south of where the recruits (offspring) were sampled, indicating northward dispersal (see maps below). One 172-mm adult from Milolii (location 6) was the parent of a 39-mm recruit found at Hookeena (location 5), which represents an along-shore dispersal distance of 15.2 km. A 154-mm adult from Hookeena (location 5) was the parent of an 36-mm recruit found at Wawaloli (location 3), which represents an along-shore dispersal distance of 48.95 km. One 156-mm adult from Punaluu (location 11) was the parent of a 42-mm recruit found at Puako (location 1), which represents an along-shore dispersal distance of 184 km. Consistent with these northward dispersal patterns is the fact that the prevailing surface current in the region in 2006 (as well as the long-term mean) flowed toward the northwest (satellite data from OSCAR: Ocean Surface Current Analyses-Real time, courtesy of NOAA):



Larval dispersal patterns inferred from 3 putative parent-offspring pairs sampled in 2006 (see table on page 5 for sample site information).

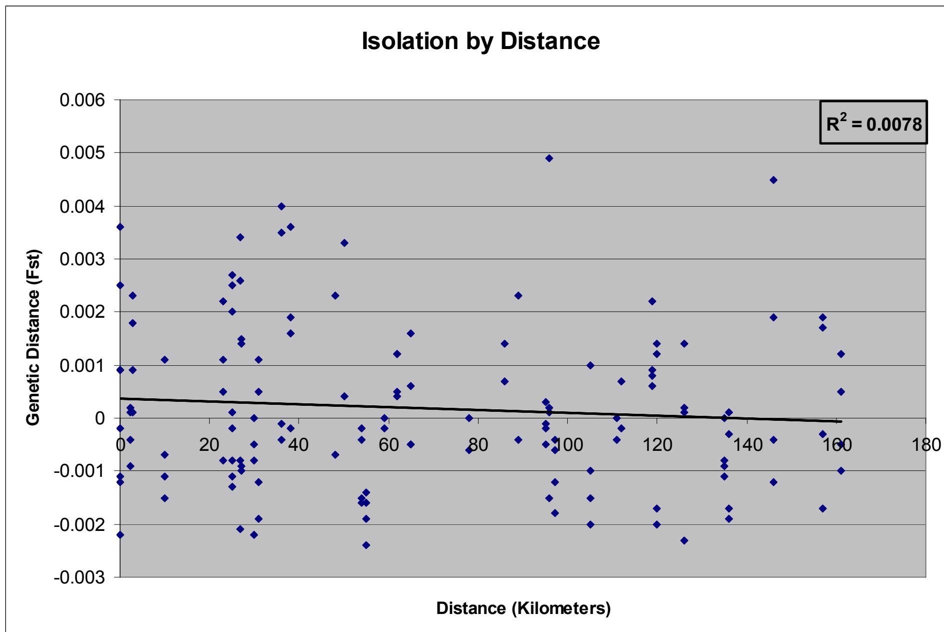


Long-term average surface currents in Hawai'i.

Surface currents around the island of Hawai'i in 2006.

Genetic Differentiation: Pair-wise F_{ST} values were calculated to examine patterns of genetic differentiation among sites. All tests demonstrate very low levels of genetic differentiation, which suggest moderate to high levels of connectivity among sites over long time scales.

Isolation by Distance: There appears to be no evidence for genetic differentiation being correlated with geographic distance (aka isolation by distance). Preliminary analyses suggest that there may be spatial correlations with oceanographic current data, but higher resolution data will be needed to explore this possibility.



Isolation by Resistance: To test whether FRAs influence patterns of genetic diversity, resistance values (i.e., "distance" based on environmental variables, see McRae and Beier 2007) were assigned to the three study FRAs. Resistance values were based on FRA location (because all parent-offspring dispersal was northward, the southern FRAs were assigned greater values) and fish age class (because adults are assumed to have greater genetic variation than recruits, adults were assigned greater values). Approximately 30% of the variation in genetic distance was explained by the two southern FRAs: Honokohau and Milolii. The third study FRA ("A-bay") did not contribute to this pattern. This result suggests that some FRAs may comprise sites of greater genetic variation, though further analyses are required to explore this possibility.

***D. Additional Field Work (2006)*:** Four supplemental studies were conducted during the summer of 2006:

(1) **Temperature loggers**, recording water temperate every half hour 24/7, were deployed at each of the six Kona sites throughout the summer of 2006. Temperature data will be examined for patterns that may correlate with larval settlement events.

(2) **Recruitment surveys** (25m x 2m transect counts) were conducted at 6 sites in NE Hawai'i and one site in SE Hawai'i (105 transects total). Yellow tang were present at only 2 of these sites on the relatively rocky "East Side." Of 121 yellow tang recruits counted, 119 were observed in finger coral (*Porites compressa*), only 2 were observed in rubble, and no recruits were observed in rock habitat. For the Kohala-Kona coast, recruitment will be estimated post-hoc as catch-per-unit effort during sampling of recruits for genetic analyses.

(3) **Predator distributions** were examined by deployment of an automated time-lapsing digital video camera. This approach proved useful for monitoring predator distributions in the absence of divers at two plots within the Honokohau FRA for 2 days each during July 2006. The camera recorded 2 sec every 30 sec for 0.5-6 hr per deployment, for a total of ca.13 hr of video.

video camera deployed on reef

Preliminary results revealed predator visitation to be 4 times as frequent at one plot compared to the other, indicating highly variable visitation rates:

Descriptive statistics of automated video censuses of piscivorous reef fishes. Data are mean (SE).

	Site-H21	Site-H22
Number of video days:	2	2
Video duration (hr / day):	2.290 (2.410)	4.000 (0.219)
Visits per video-hour:		
Caranx melampygus	0.031 (.0430)	0.032 (0.045)
Cephalopholis argus	0.0274 (0.033)	0.270 (0.150)
Fistularia commersoni	0.028 (0.039)	0.000
Aulostomus chinensis	0.000	0.002 (0.003)
All predators	2.500 (0.707)	10.000 (5.650)

(4) **Fish-habitat relationships** were examined during May to August 2006 to complete an on-going subproject. Fourteen existing $78m^2$ plots were re-surveyed at two FRAs, Keei and Honokohau (n= 7 plots /site) using a stationary point count method (Bohnsack and Bannerot 1986 *NOAA Technical Report* NMFS 41). Each site was monitored 7 times during the summer. All plots within a site were surveyed on the same day, and sampling of both sites occurred within a 48-hour period whenever possible. Fish were identified, counted, and sized

within each plot. The plots were randomly distributed along a spatial gradient of settler habitat (high, moderate, and low, based on *Porites* finger-coral cover) at each site. We have submitted a manuscript from this study, currently in review in Marine Ecology Progress Series: Ortiz, D. and B. N. Tissot. Ontogenetic patterns of habitat use by reef fish in an MPA network: a multi-scaled remote sensing and in-situ approach.

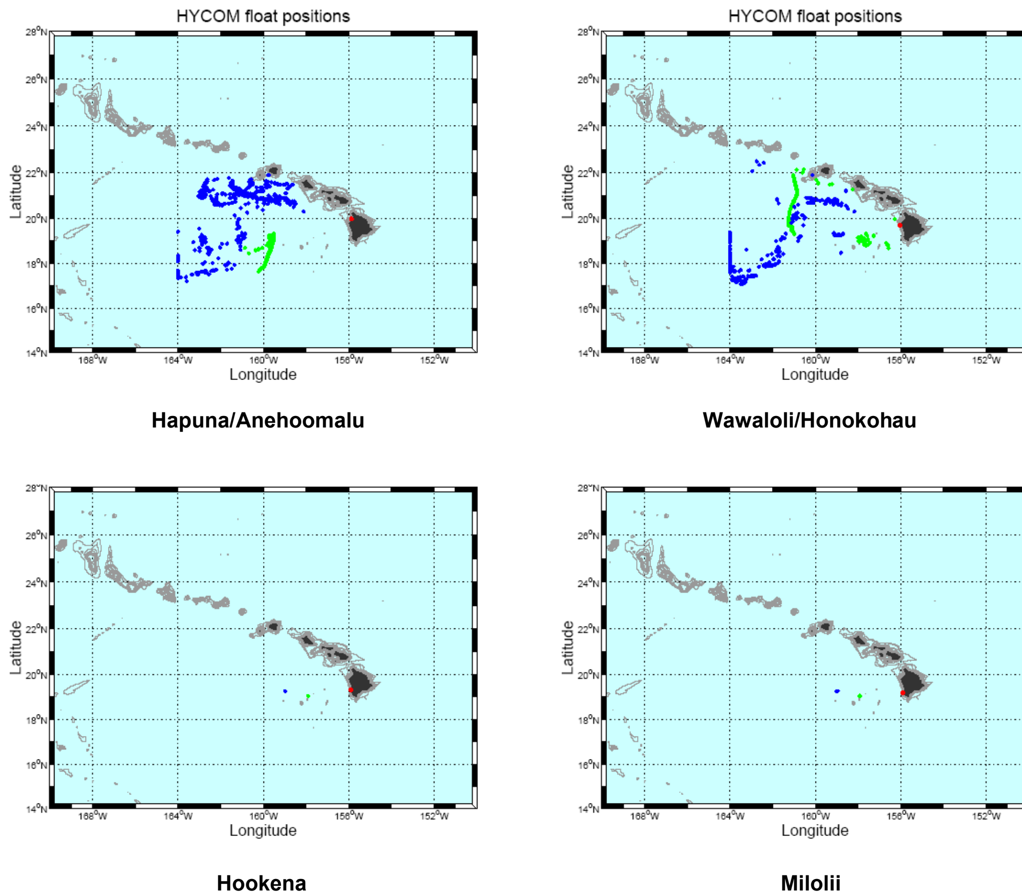
Note that "Additional Field Work" studies (3) and (4) comprise part of the doctoral dissertation of Delisse Ortiz (WSU).

E. Oceanographic Modeling**:** We are working with Dr. Yanli Jia of the University of Hawai'i at Manoa's International Pacific Research Center to employ the three-dimensional, high-resolution HYbrid Coordinate Ocean Model (HYCOM, hycom.rsmas.miami.edu) to simulate "virtual drifters" that will provide a null model for larval dispersal, i.e., larvae drifting as passive particles from their natal populations. HYCOM is a general ocean circulation model that evolved from the Miami Isopycnic-Coordinate Ocean Model (MICOM). HYCOM has become one of the premier ocean circulation models, having been subjected to various validation studies. These simulations will ultimately be compared to actual patterns of larval dispersal derived from the genetic analyses.

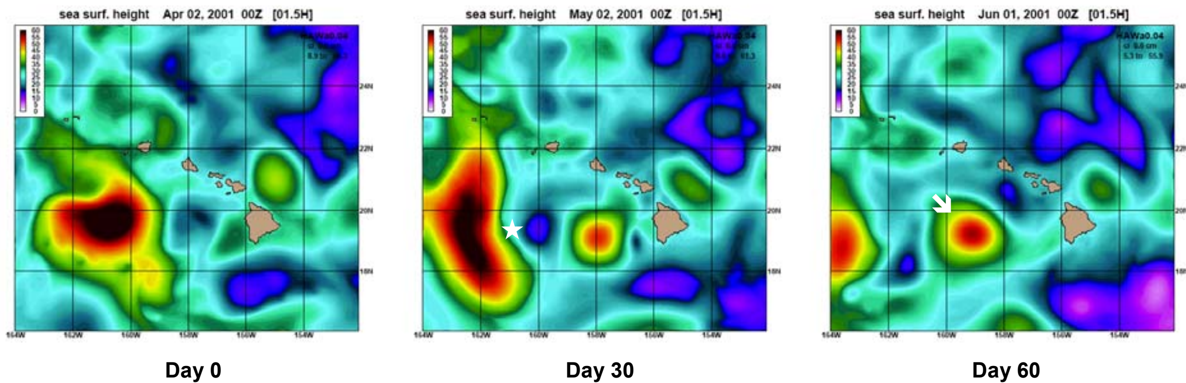
The map below shows the average year-round surface currents (arrows) and their variability (colors) in the Hawaiian archipelago. The high variability west of the Big Island is due to sporadic formation of gyres, which may retain larvae nearshore. Importantly, HYCOM makes use of actual oceanographic data from specific months. Simulations have been completed for spawning occurring monthly during April-July 2001 (the latest year with empirical data available for running the model), corresponding to settlement during June-September, at all our study sites around the Big Island (see map on page 5 for site numbers and maps on following pages for drifter simulation results). Unfortunately, the turbulent velocity option for HYCOM is not yet functioning on the UH computer, so results to date are deterministic, lacking random diffusion.

After the 60-day pelagic larval duration of yellow tang, it is clear that passive drifters released along the Kona coast do not return to the Big Island, but rather disperse up the Hawaiian archipelago (sites 1-4) and/or out to sea (sites 1-6). Simulations for the two northernmost Kona sites suggest that north Kona may seed other islands as far north as Kauai. Simulations for the two southernmost Kona sites show larvae becoming trapped in the center of an offshore anti-cyclonic eddy. In contrast, most drifters released elsewhere around the Big Island drifted up-island or out to sea (site 7) or ran aground immediately (site 10) or both (sites 8, 9, and 11). However, in a single simulation, there was local retention to settlement day 60 from June spawning at site 7 (Laupahoehoe). Ironically, that site has the least yellow tang habitat of all sites and we were able to sample only one yellow tang at that site, an adult.

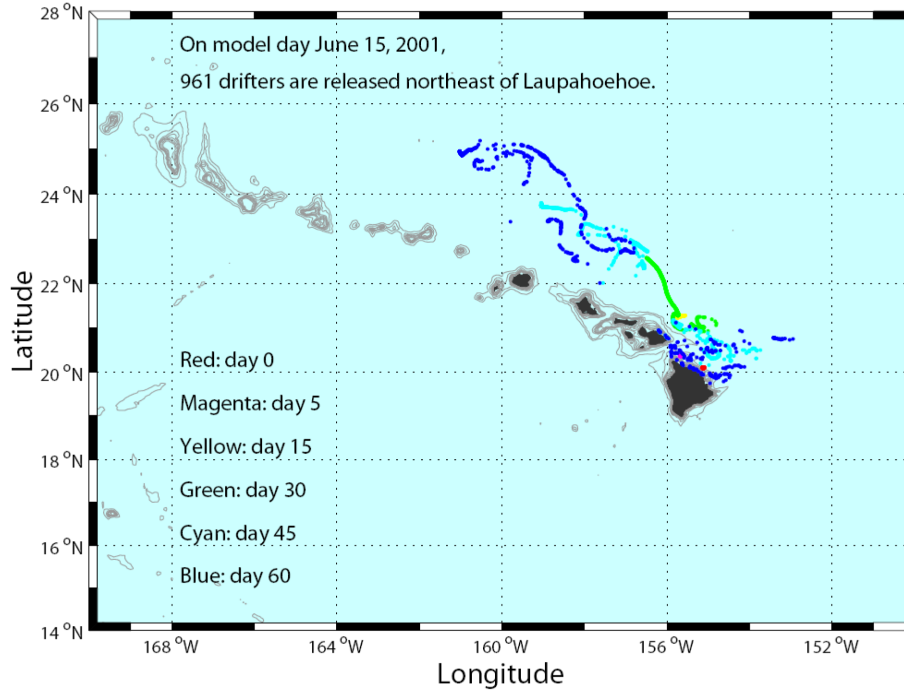
Importantly, these preliminary results imply that passive larvae spawned from the Kohala-Kona coast would not seed the Big Island. If the genetic data show that larvae settling at the Big Island are from the Big Island, then the implication will be that larval behavior (rather than passive drift) retains larval fish near the island.



Locations of virtual-drifter "larvae" at 0 days (red dots), 30 days (green dots), and 60 days (blue dots) after being "spawned" from 4 sites along the Kohala-Kona coast of the Big Island in April 2001. These simulations did not include turbulent mixing, so there is far less scatter than is realistic. 164°W is a hard boundary in the model.

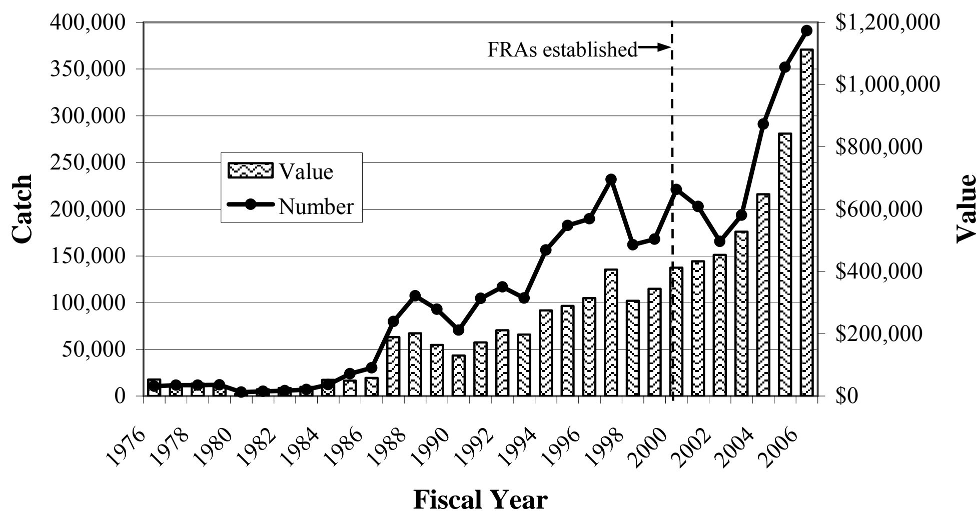


Empirically measured sea-surface height contours during the simulations illustrated above and below. Red circles indicate clockwise (anti-cyclonic) eddies and blue circles indicate counter-clockwise (cyclonic) eddies. Comparing the drifter simulations with these plots shows how virtual larvae from the two northern Kona sites (Hapuna/Anehoomalu and Wawaloli/Honokohau) were initially advected to the northwest, then many were pulled between two counter-rotating eddies which acted much like intermeshing gears (indicated by the star). In contrast, virtual larvae from the two southern Kona sites (Hookena and Milolii), were advected into the center of an anti-cyclonic eddy (indicated by the arrow).



Locations of virtual-drifter "larvae" from day 0 ("spawning" in red) to day 60 ("settlement" in blue) in the only simulation where larvae were in the vicinity of the Big Island at the time of settlement, indicating the possibility of self-recruitment due solely to passive dispersal at this particular time (June 2001) and place (Laupahoehoe).

F. Monitoring of Aquarium Fishery**:** Dr. Walsh continues to monitor the West Hawai'i aquarium fishery on behalf of the Hawai'i Department of Aquatic Resources. The graph below shows that both the catch and value of the yellow-tang fishery has increased substantially since the implementation of the Fishery Replenishment Areas (FRAs):



IV. A brief description of data collected or developed in this quarter under the grant. Any metadata submitted should use the Ecological Metadata Data Language (EML) format (http://knb.ecoinformatics.org/software/eml/):

Please see III above. CI has notified the PI that data files are not requested at this time.

V. Concerns or Difficulties:

(1) Field work for this project proved challenging, as we were starting from scratch in terms of securing equipment and supplies and refining our field protocol.

(2) Sea conditions on the NE and SE coasts of Hawai'i, posed major logistic challenges for completing field work in these regions. This situation was expected, and intramural funds from Oregon State University provided an inflatable boat so we could avoid potentially dangerous beach dives on these exposed coasts.

(3) Housing in Hawai'i presents a very expensive challenge to this project. Fortunately, Dr. Thompson of the Cascadia Conservation Trust, provided his private home and condominium for use by project personnel, but this temporary solution will be unavailable in the future. We have secured supplemental funds from the University of Hawai'i Sea Grant Program and elsewhere for housing support for continuing this project past the CI grant.

Attach the following materials (if applicable):

a. Two copies of any completed materials: Attached with the September 2006 progress report was a draft PowerPoint presentation, entitled "Microsatellites: introduction to applied and theoretical methodology," which includes information that will be part of a white paper to "provide step-by-step guidance on how to apply the approach, including development of partnerships with available marine genetics labs (a list of which will be included)" (from "Deliverables").

b. Any data collected or developed that has not been previously delivered to MMAS program, CI-MMAS (include metadata in the EML format): CI has notified the PI that this material is not requested at this time.

APPENDIX A
Methodological Details of Genetic Analyses

Sample Storage:

All yellow tang fin-clip samples were stored in 1.5mL microcentrifuge tubes in a -20°C freezer. Samples from the June 2006 collection were preserved temporarily in a solution of 85.81% ethanol, 8.97% isopropyl, and 4.31% methanol. These samples were transferred to 95 % non-denatured ethanol upon arrival to the lab. Subsequent testing found that this storage solution has in no way interfered with genotyping. All additional samples were immediately preserved in 95% non-denatured ethanol.

Extraction:

Samples were initially extracted using a conservative procedure: approximately 1cm of fin tissue was placed in 150µL of extraction buffer (75 mM NaCl; 25 mM EDTA; 1% SDS) with 2µL proteinase K (20 mg/ml) and placed in a 55°C water bath for two hours. Ammonium acetate (7.5 M) was subsequently added (70% by volume) and the samples were centrifuged until a pellet was formed. The resulting supernatant was subjected to a standard isopropanol precipitation (Sambrook and Russell 2001). Samples from this extraction procedure were amplified and run out on a gel for several loci. All tests were successful, once PCR conditions were optimized, indicating that this extraction procedure works well.

Given the length of time (ca. 8 hours) and low volume (30 individuals) involved with the above extraction procedure, faster procedures were tested. A Chelex 100 (Bio-Rad Laboritories) was tested and modified for our purposes. The advantage of this procedure was that 96 individuals could be extracted at a time and the entire procedure took no longer than 6 hours. This extraction method proved to very robust and yielded high quality genomic DNA for genotyping. The Chelex extraction protocol follows:

(1) Sterilize scoopula by dipping in bleach solution and rinsing consecutively in the 2 beakers of sterile water. Dry with Kimwipe.

(2) For 25 samples: 0.25 g Chelex 100, up to 5 mL sterile water, 50 µL ProK.

(3) Using (re-)sterilized scoopula, add sterile small stirbar to Chelex-ProK mix.

(4) Place tube on stir plate and stir rapidly.

(5) Sterilize razor blade by above method. Use blade to cut about ½ inch off of a 1000 µL pipette tip. The bore should be wide enough to allow easy pickup of Chelex-ProK solution.

(6) While solution is stirring and suspended, pipette 200 µL Chelex-ProK for each sample. Place sample tubes/96-well plate on ice.

(7) Using sterile forceps, pull a small piece of tissue (about 1 cm) off of each sample. Place tissue into well containing Chelex-ProK solution.

(8) Between samples, sterilize forceps in bleach, rinse with sterile water, and dry with Kimwipes. (Kimwipes can be reused several times.)

(9) Cap plate with strip caps (or use sealing tape). Vortex.

(10) Place plate in PCR machine on 56°C hold cycle for 2 hours. Vortex afterward.

(11) Boil in PCR machine for 8 minutes. Vortex. Use or store in -20°C until ready for PCR.

Polymerase Chain Reaction (PCR):

Prior to the start of this project, multiple primers for 28 microsatellite loci made specifically for yellow tang were obtained from Genetic Identification Services (GIS), and subsequently we have obtained primer sequences for 15 additional loci from Jeffrey Eble (University of Hawai'i at Manoa). Optimal annealing temperatures were determined for 24 of these loci using an MJ research PTC-200 gradient thermal cycler. Due to the large number of loci of unknown quality, individual labeled primers were not ordered for each locus. Rather, an M-13 (5'-GACTATGGGCGTGAGTGCAT) tailing technique was employed to attach fluorescently labeled dyes to any locus (Boutin-Ganache *et al.* 2001, Schuelke 2000). We did not actually use an M-13 tail, but rather a short sequence from a jellyfish that is unlikely to be homologous to anything within the yellow tang genome. Currently there are four fluorescent dyes (FAM, VIC, NED, HEX) available from ABI that can be employed for genotyping (regardless of whether the M13 technique is used). After annealing temperatures were optimized, the next step was to optimize primer concentrations. This step was performed in conjunction with the next step, as it was necessary to view the PCR product after genotyping to determine whether peak heights were too high or low. PCR conditions:

Reagent	Vol (µl)
HOH	6.075
10x buffer	1.5
25mM MgCl2	1.2
800 µM each dntp	3.75
1µM tailed primer (Forward)	0.15
10µM untailed primer (Reverse)	0.1125
10µM labled oligo (Dye)	0.1125
template	2
5µ/µl taq	0.1
total	15

cycles	temp	time
1x	95°C	4 min
30x	94°C	30 sec
	55°C	40 sec
	72°C	40 sec
1x	72°C	10 min
	4°C	∞

Genotyping:

All samples were genotyped at the Center for Gene Research and Biotechnology at Oregon State University. Samples were run on an ABI 3100 capillary sequencer and data viewed with GENOTYPER software. We genotyped and scored 22 loci run against a preliminary sample of 8 fish each. From these initial tests, the best performing loci were chosen and different color dyes were tested to facilitate pooling. Sets of 4 loci were then pooled at varying dilutions to optimize peak heights and minimize bleed-through. After

pooling, concentrations were optimized, and a set of 24 fish was genotyped with 22 loci. Results from this analysis were used to determine whether any of the loci might contain null alleles and to determine whether any of the loci had non-overlapping size ranges, so that 2 loci could be used with the same color dye at the same time. The results were very encouraging as the quality of the genotyping was very high (i.e., the loci looked "clean"). All loci scored very cleanly, showed little bleed-through, and multiplexed well with one another. Analysis of the 22 loci suggested that 16 of them were in Hardy-Weinberg Equilibrium (HWE), a critical assumption of microsatellite analyses. The programs GENEPOP (Goudet 2001) showed that the average heterozygosity per locus was approximately 0.62, indicating moderate levels of polymorphism.

However, after subsequently running the putative 16 loci against 190 fish, it was determined that 5 of the loci were in fact out of HWE. The cause for the departure from HWE was attributed to null alleles by the program MICROCHECKER (van Oosterhout *et al.* 2006). These 5 loci were therefore dropped and 6 additional loci were screened against the same 190 samples. Out of the 6 loci screened, 4 were found to meet our criteria of ease of scoring, reliability, and lack of null alleles, so we ultimately worked with 15 loci. Because we used the M13 tailing technique, we were able to switch dye colors among loci as needed (Boutin-Ganache *et al.* 2001, Schuelke 2000). This allowed us to fit 2 non-overlapping loci within each primer set, despite the availability of only 4 dye colors.

The final set of 15 loci was tested for deviations from HWE using the program GENEPOP (Raymond and Rousset 1995). The program was run with a dememorization number of 2000, followed by 1000 batches and 1000 iterations per batch. To minimize chances of a Whalund Effect (mistakenly pooling genetically distinct populations), the 190 samples were split into 4 sampling locales and dates (Honokohau early July, Honokohau late July, Richardson's, and Puhi Bay). Three loci were marginally out of HWE, two for Honokohau in late July and one for Richardson's. Given that these loci performed well for the other putative populations, we conclude that this heterozygote deficiency was not caused by null alleles. All 15 loci were also screened for the presence of linkage disequilibrium using GENEPOP. There was no evidence for linkage disequilibrium among loci after a Bonferonni correction. This result indicated that the loci were independent of one another.

Loci Used:

The 15 loci used in this study were conveniently pooled into three sets of 5 loci each. This was performed in order to minimize genotyping costs (costs are determined per sample well irrespective of how many loci are in a particular well.) The 15 loci were pooled according to the following specifications:

Dye set	Locus Name	Dye	Volume (µL)
P	C3	FAM	3
P	B105	VIC	3
P	yt105	PET	4
P	yt108	NED	3
P	yt111	FAM	3
P	H_2O		6

D	D103	FAM	3
D	D118	VIC	3
D	C113	VIC	3
D	D126	NED	3.5
D	D5	PET	4
D	H_2O		6
A	B4	FAM	4
A	yt113	VIC	2
A	B119	NED	3.5
A	D113	PET	4.5
A	yt116	PET	4
A	H_2O		5

After being pooled the loci were subsequently genotyped (see above) and scored with GENOTYPER software. All samples were scored initially following well established protocols for microsatellite scoring. All genotypic data were then exported and run through a Microsoft Excel macro to bin alleles. Bins were established by the careful observation of allele size ranges from the first two hundred samples. All alleles at all loci were binned according to the following specifications:

Allele Bins for Loci:

From = Lower bound (size in base-pairs (bp)) for a particular allele
To = Upper bound (size in bp) for a particular allele
Value = Size (in bp) assigned to an allele that falls within a “From” and “To” range.

P Set			**D Set**			**A Set**		
C3			**D103**			**B4**		
From	To	Value	From	To	Value	From	To	Value
306.5	309.99	308	171	174.99	173	196	199.99	198
310.5	313.5	312	175	178.99	177	200	203.99	202
314.5	318.5	316	183	186.99	185	204	207.99	206
319.5	322.99	320	187	190.99	189	208	211.99	210
324	326.99	324	191	194.99	193	212	215.99	214
328	331.5	328	195	198.99	197	216	219.99	218
332.5	334.99	333	199	202.99	201	220	223.99	222
336	338.5	337	203	206.99	205	228	231.99	230
339.5	342.99	341	207	210.99	209	232	235.99	234
344	346.99	345	211	214.99	213	**yt113**		
347.5	350.5	349	215	218.99	217	From	To	Value
351.5	354.5	353	219	222.99	221	178	181.99	180
B105			223	226.99	225	182	185.99	184
From	To	Value	227	230.99	229	186	189.99	188
270	272	271	231	234.99	233	190	193.99	192
278	281.5	279	235	238.99	237	194	197.99	196
282	283.99	283	239	242.99	241	198	201.99	200
284	286	285	**D118**			202	205.99	204
yt105			From	To	Value	206	209.99	208
From	To	Value	149	152.99	151	210	213.99	212

224	225.99	225
236	237.99	237
238	239.99	239
242	243.99	243
244	245.99	245
246	247.99	247
248	249.99	249
250	251.99	251
252	253.99	253
254	255.99	255
256	257.99	257
258	259.99	259
262	263.99	263
264	265.99	265
266	267.99	267
268	269.99	269
276	277.99	277
280	281.99	281

yt108

From	To	Value
130	131.99	131
141	142.99	142
145	146.99	146
150	151.99	151
154	155.99	155
156	157.99	157
160	161.99	161
162	163.99	163
164	165.99	165
166	167.99	167
168	169.99	169
188	189.99	189

yt111

From	To	Value
198	199.99	199
200	201.99	201
202	203.99	203
204	205.99	205
206	207.99	207
209	210.99	210
211	212.99	212
213	214.99	214
215	216.99	216
224	226.5	225
228	229.99	229
232	233.99	233

153	156.99	155
158	161.99	160
162	165.99	164
166	169.99	168
170	173.99	172
174	177.99	176
178	181.99	180
186	189.99	188
190	193.99	192

C113

From	To	Value
274	277.99	276
283	286.99	285
287	290.99	289
291	294.99	293
295	298.99	297
299	302.99	301
303	306.99	305
307	310.99	309
311	314.99	313
315	318.99	317
327	330.99	329
335	339.99	337
340	343.99	342
345	347.99	346
348	350.99	349

D126

From	To	Value
144	147.99	146
148	151.99	150
153	156.99	155
157	160.99	159
161	164.99	163
165	168.99	167
169	172.99	171
173	176.99	175
177	180.99	179
181	184.99	183
185	188.99	187
189	192.99	191
193	196.99	195
197	200.99	199
201	204.99	203
205	208.99	207
209	212.99	211

D5

From	To	Value
135	137	136
144	147.99	146
148	151.99	150
152	155.99	154

214	217.99	216
218	221.99	220
222	225.99	224
226	229.99	228
230	233.99	232
238	241.99	240
242	245.99	244
246	249.99	248
349	352.99	351
353	356.99	355
357	360.99	359

B119

From	To	Value
167	170.99	169
171	174.99	173
175	178.99	177
179	182.99	181
183	186.99	185
187	190.99	189
191	194.99	193
195	198.99	197
199	202.99	201
203	206.99	205
207	210.99	209
211	214.99	213
215	218.99	217
219	222.99	221
223	226.99	225
227	230.99	229
231	234.99	233
235	238.99	237
239	242.99	241
243	246.99	245
247	250.99	249
251	254.99	253
255	258.99	257
261	263.99	262
264	267.99	266
268	271.99	270
272	275.99	274
276	279.99	278
288	291.99	290

D113

From	To	Value
251	254.99	253
263	266.99	265
267	270.99	269
271	274.99	273
275	278.99	277
279	282.99	281
283	286.99	285

156	159.99	158	287	290.99	289
160	163.99	162	291	294.99	293
164	167.99	166	295	298.99	297
168	171.99	170	299	302.99	301
172	175.99	174	303	306.99	305
176	179.99	178	308	311.99	310
180	183.99	182	313	316.99	315
184	187.99	186	317	320.99	319
188	191.99	190	321	324.99	323
192	195.99	194	325	328.99	327
196	199.99	198	329	332.99	331
200	203.99	202	333	336.99	335
204	207.99	206	337	340.99	339
			341	344.99	343
			345	348.99	347
			349	352.99	351
			355	358.99	357
			yt116		
			From	To	Value
			167	168.99	168
			175	176.99	176
			177	178.99	178
			179	180.99	180
			181	182.99	182
			185	186.99	186
			187	188.99	188

After being binned, the data were printed out and re-checked against the GENOTYPER output to verify that the alleles had been scored correctly and to verify that the alleles had been binned correctly. All data were then formatted and entered into the program CONVERT (Glaubitz, 2004) to create a GENEPOP (Raymond & Rousset, 1995) file for subsequent analysis.

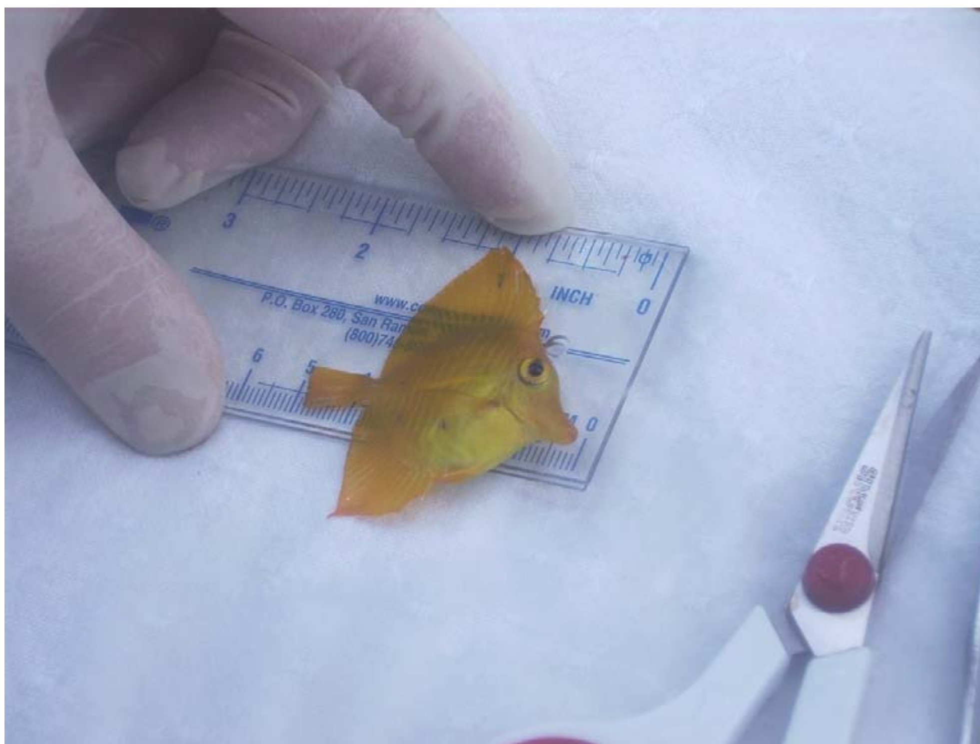
Microsatellite Characterization:

Christie and Eble (submitted) have collaborated to publish their combined studies regarding the isolation and characterization of 23 microsatellite loci in yellow tang:

Table 1 Characterization of 23 microsatellite loci isolated from the yellow tang *Zebrasom flavescens* (N=90).

Locus	Primer sequence (5'-3') and fluorescent dye	Repeat motif	k §	Allele size range (bp)	H_O †	H_E ‡	GenBank Accession no.
Zefl 01	**F:** GGATGGGACTGATTTGAAG-PET **R:** GATGCTGCTGTGCTGAAT	$(TAGA)_9$	17	136-206	0.87	0.84	
Zefl 02	**F:** ATGGCTGTTTGCCTTGTTTAG-FAM **R:** TGGAACGAGAGAAAAATCAGG	$(TAGA)_9$	17	173-241	0.83	0.88	
Zefl 03	**F:** TTGTGCAATTTAGTGCTTCAG-VIC **R:** CTTCCAAGGTCATCTGAGTGT	$(TAGA)_{10}$	25	238-418	0.66**	0.93	
Zefl 04	**F:** GAAGCAGAAACACAACGATG-FAM **R**: AGTTCCGTAAAGGATGGTGA	$(TAGA)_8$	13	206-300	0.38**	0.82	
Zefl 05	**F:** CCTCACTTCACTTCACCTCT-VIC **R:** CACCTACTCCCCAGACTTC	$(TAGA)_9$	24	257-353	0.80**	0.92	
Zefl 06	**F:** CCCTGAAAATGTAAACCTTG-NED **R:** ATTGTTGTCTGTGTTGATGTG	$(TAGA)_8$	19	235-315	0.75*	0.83	
Zefl 07	**F:** CGTTCTCAGTTTTCTGCTGT-PET **R:** CTTTCGCTCACACTTGTTG	$(TAGA)_{11}$	24	253-357	0.92	0.89	
Zefl 08	**F:** AGGACAAGAGAAGGCAGAGAC-VIC **R:** CCGCACTGAGAGAGAAAAATA	$(TAGA)_9$	10	151-191	0.70	0.81	
Zefl 09	**F:** GCATACAGAGGATTACAGATG-NED **R**: AGAACAGAGGCAGAAGATACT	$(TAGA)_8$	17	146-212	0.94	0.90	
Zefl 10	**F:** TTTACCGACTGACTGTATGCT-FAM **R:** AGGAAAGAGACTGAGACATCTG	$(GTCA)_8$	9	198-234	0.67	0.68	
Zefl 11	**F:** CGAACACACAATGCTCACTAA-PET **R:** AAACAAACCAATCACCAGTTG	$(GTCA)_9$	6	150-198	0.15**	0.77	
Zefl 12	**F:** GCACCGCTCAAGTGTGTT-VIC **R**: AGGCAAAATGGCAGCATA	$(GTCA)_{10}$	4	271-285	0.28	0.29	
Zefl 13	**F:** GAGGGCTGAAATGAACAT-FAM **R:** ATCTGGCTGACTGTCTCTG	$(GTCA)_9$	8	237-355	0.66*	0.81	
Zefl 14	**F:** TTAGGAACAGAGGAGTATTGAG-NED **R:** GGCTTTACCCTTCAGATAAG	$(GTCA)_{10}$	29	169-289	0.85	0.92	
Zefl 15	**F:** TGGAACGGAAAACATTACC-FAM **R:** GGGTGAGAATCTGTGGTGT	$(TACA)_8$	12	308-354	0.90	0.84	
Zefl 16	**F:** AAGATGCCATAGCGTTCAC-FAM **R**: GCAAAAACAAACCACAACC	$(TACA)_7$	8	132-178	0.54**	0.84	
Zefl 17	**F:** TGCTTGTATGGGTTCTGCAC-VIC **R**: CTGGCTCGCAGTTATTCTCC	$(TACA)_7$	15	276-348	0.82	0.82	
Zefl 18	**F:** AGAAGCAAGCAGAGAAGGA-PET **R**: ACATCGCAGACAGTGAGTG	$(TACA)_{10}$	7	230-279	0.38	0.76	
Zefl 19	**F:** CAGCATGCAGGAATACACAG-PET **R:** CATTGCCAGTATAAATGAGCATC	$(CT)_{19}$	14	238-278	0.9	0.866	
Zefl 20	**F:** CTTTCACAGGAGGTGACAGTGG-NED **R:** TGTTCGGCTGCACTCTGC	$(CT)_{23}$	7	146-165	0.66*	0.724	
Zefl 21	**F:** GGTGAGATCGGGACTTTGC-FAM **R:** CAGTTGTGGATGCAGTGAGC	$(CA)_{14}$	8	203-231	0.82	0.858	
Zefl 22	**F:** AGCTTCAGGCCTGTGTGG-PET **R:** CAAACCTCCAAACCATTTGC	$(GGAT)_{11}$	19	182-250	0.916	0.92	
Zefl 23	**F:** GTGGGCTACTGAAGTGTTGC-NED **R:** GCTCTTCTCCAAATCCAAGG	$(CTT)_8$	6	172-184	0.656	0.68	

§ number of allleles
† observed heterozygosity
‡ expected heterozygosity
* denotes significant departure from HWE at 0.05 level
** denotes significant departure from HWE after Bonferroni correction



APPENDIX B
Letter of Endorsement from the Marine Aquarium Council
(next page)

April 23, 2007

Michelle Pico
National Fish & Wildlife Foundation
1120 Connecticut Ave., NW Suite 900
Washington, D.C. 20036

Dear Ms. Pico,

I am pleased to write this letter of support for the aquarium fishery research and conservation project proposed by Dr. Brian Tissot of Washington State University. The Island of Hawai`i has been recognized as a good example of how multiple stakeholders can work together to support a sustainable aquarium fishery. The network of marine protected areas (MPAs) located in West Hawai`i provides an excellent opportunity to involve aquarium fisherman in cooperative research that could improve relationships between fisherman and scientists and provide valuable information on how MPAs influence fishing.

The Marine Aquarium Council (MAC) is an international, not-for-profit organization that brings marine aquarium animal collectors, exporters, importers and retailers together with aquarium keepers, public aquariums, conservation organizations and government agencies. Our mission is to conserve coral reefs and other marine ecosystems by creating standards and certification for those engaged in the collection and care of ornamental marine life from reef to aquarium. The information from the study proposed by Dr. Tissot could provide valuable information to MAC and by extension to a wide variety of aquarium fisherman in Hawai`i and around the world.

To that end I support the proposed Hawai`i aquarium fishery research and conservation project and urge you to give this proposal serious consideration.

Sincerely,

Paul Holthus
Executive Director and President
Marine Aquarium Council
email: paul.holthus@aquariumcouncil.org
website: www.aquariumcouncil.org

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LITERATURE CITED

Banks MA, Eichert W, Olsen JB (2003) Which genetic loci have greater population assignment power? *Bioinformatics* **19**, 1436-1438.

Boutin-Ganache I, Raposo M, Raymond M, Deschepper CF (2001) M13-tailed primers improve the readability and usability of microsatellite analyses performed with two different allele-sizing methods. *Biotechniques* **31**, 24-28.

Christie MR, Eble JA (submitted) Isolation and characterization of 23 microsatellite loci in the yellow tang, *Zebrasoma flavescens* (Pisces: Acanthuridae). *Molecular Ecology Resources*.

Glaubitz JC (2004) CONVERT: A user-friendly program to reformat diploid genotypic data for commonly used population genetic software packages. *Molecular Ecology Notes* **4**, 309-310.

Goudet J (2001) FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9.3). *Available from http://www.unil.ch/izea/softwares/fstat.html. Updated from Goudet (1995).*

Hedrick PW (1999) Perspective: Highly variable loci and their interpretation in evolution and conservation. *Evolution* **53**, 313-318.

Koizumi I, Yamamoto S, Maekawa K (2006) Decomposed pairwise regression analysis of genetic and geographic distances reveals a metapopulation structure of stream-dwelling Dolly Varden charr. *Molecular Ecology* **15**, 3175-3189.

Manel S, Berthoud E, Bellemain M, *et al.* (2007) A new individual-based spatial approach for identifying genetic discontinuities in natural populations. *Molecular Ecology* In Press.

Manel S, Gaggiotti OE, Waples RS (2005) Assignment methods: matching biological questions techniques with appropriate. *Trends in Ecology & Evolution* **20**, 136-142.

McRae BH, Beier P (2007) Circuit theory predicts gene flow in plant and animal populations. *Proceedings of the National Academy of Sciences of the United States of America* **104**, 19885-19890.

Raymond M, Rousset F (1995) GENEPOP, version 1.2: population genetics software for exact tests and ecumenicism. *Journal of Heredity* **86**, 248-249.

Sambrook J, Russell DW (2001) *Molecular Cloning: A Laboratory Manual*, 3rd edn. Cold Springs Harbor Laboratory Press, Cold Springs Harbor, N.Y.

Schuelke M (2000) An economic method for the fluorescent labeling of PCR fragments. *Nature Biotechnology* **18**, 233-234.

van Oosterhout C, Weetman D, Hutchinson WF (2006) Estimation and adjustment of microsatellite null alleles in nonequilibrium populations. *Molecular Ecology Notes* **6**, 255-256.

Wright S (1969) *Evolution and the genetics of populations.* University of Chicago Press.