I. Project Title: Sea Urchin Herbivory in Hawaiian Shallow Water Ecosystems
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   Grant Number: NOA06NOS4260200
   Date: November 30, 2007

II. Executive Summary
   Sea urchins play a pivotal role in coral reef ecosystems. For example, an under-abundance of urchins can lead to conditions in which algae grow to dominate, thus tipping the ecosystem balance away from coral-dominated conditions. This kind of threat to the health of a reef ecosystem, from reduced urchin populations, has in particular been observed and extensively documented in the Caribbean. In Hawai‘i, we have many species of herbivorous sea urchins, yet, their collective ecological role in keeping the balance between coral- and algal-dominated systems is poorly understood. Now that many Hawaiian reefs are suffering from an overgrowth of introduced non-native algal species, there is an urgent need to understand the biology and ecology of these benthic herbivores to assess their potential importance within our reef ecosystems.

   To explore this issue, we took a three-step approach: 1) evaluation of biodiversity of the common sea urchin species in Hawai‘i; 2) determination of their abundance and distribution on multiple reefs around the island of Hawai‘i; and 3) assessment of feeding ecology of the common sea urchin species in aquaria and the field

III. Purpose
   A. Detailed description of the resource management problem(s) to be addressed
   Hawaiian coral reef ecosystems are a scientific treasure trove of rich biodiversity, and they are also the treasured foundation of a tourism-based economy. Sea urchins may play a vital role in the maintenance of this valuable biodiversity, as important herbivores and participants in the ecosystem balance on reefs. For example, an under-abundance of urchins can lead to conditions in which algae grow to dominate, thus tipping the ecosystem balance away from coral-dominated conditions (reviewed by Birkeland 1989; Knowlton 2001a). This kind of threat to the health of a reef ecosystem, from reduced urchin populations, has in particular been observed and extensively documented in the Caribbean (Lessios et al. 1984; Hughes 1994).

   Specifically, algal abundance responded quickly and positively to the removal of the herbivorous activities of Diadema antILLarum, following mass mortality of this long-spined urchin (Lessios 1988). In correlation with this increasing algal growth, coral percent cover markedly declined (Shulman & Robertson 1996), and such degraded conditions on many Caribbean coral reefs have continued to persist (Hughes 1994; Williams & Polunin 2001; Lessios 2005). Recent incidences of recovery in D. antILLarum populations have led to corresponding reductions in macroalgal cover and increased coral recruitment (Edmunds & Carpenter 2001; Carpenter & Edmunds 2006). Such recoveries may have been aided by the herbivorous activities of other sea urchins (Knowlton 2001b). In particular, the expansion of grazing ranges by Tripneustes urchins may have helped to create benthic conditions once again favorable for successful D. antILLarum
recruitment (Woodley 1999; Moses & Bonem 2001). Together, increased populations of both *Diadema* and *Tripneustes* urchins may be synergistically generating sufficient levels of herbivory to initiate ecological reversal to a coral-dominated state for reefs in the Caribbean. Thus, the biology and ecology of multiple species of urchins is intimately connected with the delicate ecological balance of coral reef health.

Many Hawaiian reefs are suffering from an overgrowth of introduced non-native algal species. For example, following its intentional introduction in 1974, populations of *Hypnea musciformis* have bloomed on some of Maui’s reefs; twenty thousand pounds per week of this marine algae have been reported to wash up on beaches there (Alien & Invasive Algae in Hawai‘i HCRI website). Similarly, the tourist-centered beaches of Waikiki on the island of O‘ahu are now frequently fouled by washes of the invasive and especially destructive *Gracilaria salicornia* (Alien & Invasive Algae in Hawai‘i HCRI website). The most widespread introduced alga, found on all of the Hawaiian Islands, is *Acanthophora spicifera* which out-competes native algal species and thus reduces algal diversity on Hawaiian reefs (Alien & Invasive Algae in Hawai‘i HCRI website). These invasive seaweeds are also gradually increasing in abundance and distribution at a number of shallow reef locations on the island of Hawai‘i (Alien & Invasive Algae in Hawai‘i HCRI website; Jessop unpubl.). The most famous case of a shift toward macroalgal dominance is at Kane‘ohe Bay on O‘ahu, where abundance of native *Dictyosphaeria cavernosa* increased dramatically in the 1960s. While nutrient enrichment from sewage outfalls has been implicated as a significant causative factor for this shift, continued persistence of high *D. cavernosa* abundance, even after sewage outfall cessation, may instead be due to ecological interactions involving herbivory: Reduced herbivore populations plus herbivore preference for introduced algal species such as *A. spicifera* may have freed *D. cavernosa* from growth control (Stimson *et al.* 2001).

Despite a species-rich community of herbivorous urchins, and their potential importance within reef ecosystems, there is a paucity of studies specific to their biology and ecology in Hawai‘i. A notable exception is *Tripneustes gratilla*, commonly called the Collector Urchin, whose potential for biological control over macroalgae on Hawaiian reefs has been well researched, primarily under the Hawai‘i Coral Reef Initiative (HCRI) Research Program. Specifically, it has been found that *T. gratilla* does consume invasive and alien algae, and populations of these urchins at high densities (1 per m²) have the potential to significantly affect macroalgal abundance and distribution (Stimson 2004). Indeed, research efforts regarding *T. gratilla* in Hawai‘i are now focusing on the aquaculture of these urchins, for artificial enhancement of their populations on reefs suffering from algal overgrowth (Hunter 2005).

However, two of the most common urchins on Hawaiian reefs, *Echinothrix calamaris* and *Echinothrix diadema*, collectively called Wana, have been investigated relatively little, either in Hawai‘i or elsewhere. These two species of long-spined urchins can be difficult to distinguish since they are anatomically remarkably similar; they also utilize the same habitat, appearing in mixed groups on reefs (Mortensen 1940). Despite being both morphologically and ecologically similar, it has been found that these co-existing urchins are genetically distinct (Lessios, personal communication). Moreover, this recent examination of their relatedness using mitochondrial DNA supports the existence of several genetically distinct clades throughout the Indo-West Pacific, which may represent cryptic species (Lessios, personal communication). However, the genetic identities and exact number of the different *Echinothrix* clades present in Hawai‘i, as well as their abundances and distributions, are currently unknown.

Quantification of biodiversity is a necessary first step for conservation efforts, especially
for keystone species such as sea urchins. That is, due to the potential for only small changes in their populations to affect large changes within the overall reef ecosystem, knowledge of the basic natural history and biology of urchin species is needed for successful coral reef conservation efforts (Dayton 2003; Soule et al. 2005). This could be especially true for the relatively abundant *Echinothrix* urchins of Hawai‘i, which may represent a biological and ecological analogue to their very similar relative on Caribbean reefs, *D. antillarum*. While *Echinothrix* urchins in Hawai‘i currently enjoy non-endangered status, they are subject to some fisheries collection pressures, in addition to other possible anthropogenic threats. Some anecdotal reports suggest that abundances of *Echinothrix* urchins have precipitously declined within the past generation (*i.e.* within the past fifty to seventy-five years; Jessop unpubl.). Ideally, studies of these urchins should be undertaken before more significant disturbances to their populations are witnessed, as occurred with *D. antillarum* urchins in the Caribbean. Thus, an investigation of *Echinothrix* biodiversity, demographics, and ecological roles is urgently needed, particularly since *Echinothrix* urchins may be important allies to *T. gratilla* in the biological control of macroalgae. Specifically, the impacts of *Echinothrix* herbivory in Hawaiian shallow water ecosystems are in need of thorough evaluation, in order to establish best reef management and conservation plans.

**B. Detailed description of the question(s) asked to answer the resource management problem(s)**

1) **Biodiversity**
   What species of *Echinothrix* sea urchins are present in Hawai‘i? How can they be distinguished?

2) **Population Status & Dynamics**
   What are the current abundances and distribution patterns of *Echinothrix* and *T. gratilla* in Hawai‘i? Are there interspecific differences in habitat partitioning? What correlations exist between urchin and algal populations?

3) **Herbivory & Ecological Roles**
   What are the relative feeding rates of *Echinothrix* and *T. gratilla* on the invasive alga *Gracilaria salicornia*, under no-choice experimental conditions? Under natural conditions, are there interspecific differences in trophic level; are these urchins true herbivores?

**C. Objectives to answer each question**

1) **Biodiversity**
   We plan to address these conservation research needs, by documenting the level of biodiversity present in *Echinothrix* sea urchins of Hawai‘i. We will simultaneously resolve this biodiversity with both genetics and morphology, to determine whether diversity of form in this genus is a result of numerous polymorphisms and/or plasticity, or does indeed correspond to genetically unique clades. Thus, we will document the morphological and genetic biodiversity of *Echinothrix* urchins in Hawai‘i, and provide a first step toward unifying molecular data with morphotype in this genus.

2) **Population Status & Dynamics**
   In order to provide baseline data regarding their population sizes, the current abundances and distribution patterns of *Echinothrix* urchins in Hawai‘i will be determined. These data, combined with reef profile observations, will also be useful for characterizing differences in habitat partitioning between *T. gratilla* and *Echinothrix* species, and for analyzing correlations
between urchin densities, urchin community diversity, and macroalgal abundance. For all surveys, abundance and distribution data for both *Echinothrix* and *T. gratilla* urchins will be correlated with benthic habitat characteristics (i.e. percent coral and algal cover), in order to investigate the ecology and relative importance of urchins in Hawaiian coral reef ecosystems. Thus, our research program will profile reef habitats, as well as possible correlations between algal parameters on reefs and urchin herbivory.

These ecological surveys will also include data collection on behavioral differences between *Echinothrix* and *T. gratilla* urchins, in regards to spatial habitat partitioning. Knowledge of such partitioning may be important for understanding how different urchin species successfully co-exist despite all functioning as generalist herbivores. We hypothesize that *T. gratilla* and the different *Echinothrix* species each take advantage of distinct microhabitats, by which they collectively exert greater and more comprehensive impacts on algal populations throughout the reef. For example, one fine-scale mechanism by which a phase shift to macroalgal dominance on reefs could be initially instigated is when certain microhabitat types preferred by macroalgal propagules are no longer grazed (e.g. as with the disappearance of *D. antillarum* in the Caribbean). Thus, we likewise hypothesize that *Echinothrix* species serve as allies in the control of algal populations, by occupying different microhabitat niches than those favored by *T. gratilla*.

3) Herbivory & Ecological Roles

We plan to further investigate the ecological roles of *Echinothrix*, by determining feeding preferences and rates for members of this genus, using both *in situ* investigations and laboratory experiments. Potential consumption of invasive algal species by *Echinothrix* will constitute a focus of these investigations. Feeding preferences *in situ* will be investigated using analysis of stable isotopes of urchin muscle tissues, in relation to surrounding algal food sources. Isotopic analysis will provide a means of identifying the food sources that are actually nutritionally utilized by *Echinothrix* and *T. gratilla* urchins, as opposed to just what food sources are consumed. We hypothesize that the feeding habits of *Echinothrix* urchins may be sufficiently different from *T. gratilla* to cause different, and perhaps synergistic, effects on the abundance and diversity of reef algal communities. To what extent *Echinothrix* urchins will select invasive algae species as food and/or may achieve significant reductions in invasive algal populations will be quantified using laboratory experiments that manipulate food availability.

IV. Approach

1) Biodiversity

A total of 88 specimens of *Echinothrix* have so far been collected, from over a dozen different sites around the island of Hawai‘i. For each specimen, data and photographs of morphological parameters have been obtained, and specimens have been appropriately preserved for later additional and finer micro-morphological analysis. Tissues from these specimens, for molecular work, were also preserved. The genomic DNA from all specimens has been extracted, and the mitochondrial marker of gene ATPase8/6 has been successfully amplified and sequenced. Using these DNA sequence data, the genetic clade of each specimen has been determined via phylogenetic analysis. Collection of more detailed morphometric data is ongoing.

2) Population Status & Dynamics

In early summer 2007, we collected preliminary survey data which was used to optimize ecological survey data collection methodologies in the subtidal. We have since completed ecological surveys of three sites: two in East Hawai‘i and one in West Hawai‘i. These sites were
selected in collaboration with DAR. These surveys, conducted on scuba, consist of at least six 50-m transects at each site, along which all *Echinothrix* species and *T. gratilla* are counted within eight 2x5-m strip transects. Also, within each strip transect, the dominant habitat type is recorded, and the type of microhabitat occupied by the first three urchins of each species encountered is recorded. The size class, and whether native and/or invasive macroalgae is present within 1-m² of these urchins, is also recorded. Benthic characteristics and depth are quantified using a point-intercept method, in sixteen 1-m² quadrats along the same 50-m transect line.

In collaboration with DAR, we are also conducting ecological surveys in the shallow intertidal, to document invasive algae and urchin abundances. These surveys, conducted by wading and/or snorkeling, are being conducted in East Hawai‘i in areas impacted by invasive algae. Surveys consist of haphazardly placed 1-m² quadrats (separated by 10-m or more, with at least 50 quadrats per site), in which the GPS location, percent cover of invasive algae, and number of *Echinothrix* species and *T. gratilla* are recorded.

3) Herbivory & Ecological Roles
We have completed five replicates of two-week feeding experiments, to investigate sea urchin feeding rates on the invasive algal species *Gracilaria salicornia*. This invasive algal species has been chosen for study due to their salient presence and increasing dominance in some shallow-water communities around the Island of Hawai‘i. Specifically, feeding rates of *E. calamaris*, *E. diadema*, and *T. gratilla* urchins on *G. salicornia* were measured, under no-choice conditions in aquaria. Sea urchins were collected from Onekahakaha Beach Park, East Hawai‘i, and starved in tanks with aeration for a minimum of three days prior to the start of experiments. At the outset of each experiment, four replicate sea urchins of each species were placed in randomly assigned 10-gallon aerated tanks that were housed within a constant-temperature water-bath. The invasive algae *G. salicornia* was freshly collected on the first day of experiment, cleaned of debris, spun in a salad spinner to remove excess water, and divided into 50-gram allotments with accurately measured wet weights. These allotments of algae were placed in each urchin tank. Algae were replaced with fresh allotments every 2-3 days during the experiment, to ensure that the algae remained palatable to the urchins. Wet weights were measured in a similar fashion as above before and after these exchanges, to quantify the amount of *G. salicornia* consumed by urchins. In addition, wet weights were converted to dry weights, using a 60 degree-C oven, and linear regression. Control tanks that contained algae but no urchins were included in the experiment to account for changes in weight not due to herbivory. Sizes of the urchins were assessed by measuring the average test diameter and wet weight before and after the feeding experiments.

We have completed a preliminary in situ investigation of interspecific differences in urchin trophic position, using stable isotope analysis. This investigation consists of measurements of ^15^Nitrogen and ^13^Carbon enrichment, of three specimens each of three different urchin species (*E. calamaris*, *E. diadema*, and *T. gratilla*), from two different sites in East Hawai‘i. In order to assess trophic position relative to locally available marine algae, isotope measurements of a rhodophyte algal species were also made from each site.

V. Results
1) Biodiversity
Three sets of morphological features have been found that can be used to reliably distinguish the two nominal species of *Echinothrix*. All 88 collected specimens were identified to
species-level using these sets of physical characteristics. For these specimens, all mitochondrial ATPase8/6 sequence differences were base-pair substitutions, with no insertion or deletions. There were a total of 48 different haplotypes. A cladogram from these DNA sequence data shows that, for the two deepest genetic clades, the set of physical characters that I had used to distinguish *Echinodermis* species is indicative of separate genetic identity: all *E. diadema* are monophyletic, as are all *E. calamaris* specimens (Figure 1). However, there is no support for additional genetic divergence beyond these two currently recognized species, in Hawai‘i. Furthermore, at least in Hawai‘i, color morphs possess indistinguishable haplotypes, and so are not likely candidates for cryptic sibling species. Instead, color variation in Hawaiian *E. calamaris* is the result of polymorphisms and/or plasticity. Therefore, Hawai‘i appears to be home to only the two nominal species of *Echinodermis*, and a reliable key for telling these species apart has been described.

**Figure 1**: Bootstrap (20,000 replicates) Consensus Cladogram: 88 *Echinodermis* specimens from Hawai‘i, 539 base pairs from Lysine t-RNA ATPase8/6 region of mitochondrial DNA, Maximum-likelihood distances (Evolution model: Tamura-Nei 1993, plus gamma correction), Neighbor-Joining algorithm (Paup* 4.0b10), Rooted with (3) species of the genus *Diadema*.  

6
2) Population Status & Dynamics
Preliminary subtidal data indicate that the urchin species *E. diadema* may be more abundant in shallower areas, whereas *E. calamaris*, as well as *T. gratilla* may be most abundant at mid-range depths (Figures 2-4). Data analyses of completed surveys are ongoing.

![Figure 2: Urchin densities at each isobath for *E. calamaris*](image)

![Figure 3: for *E. diadema*](image)

![Figure 4: for *T. gratilla*.](image)
3) Herbivory & Ecological Roles

Our experimental feeding trial data support the hypothesis that *T. gratilla* and *Echinothrix* species exhibit similar feeding rates (Figure 5; about 4 grams/day per urchin, considering each urchin weighs on average about 100 grams) on the Hawaiian invasive algae *G. salicornia*, under no-choice experimental conditions. Data variances are high due to large differences in feeding behavior between individual urchins of the same species, despite randomized tank assignments. In some of the one-week trials, *E. calamaris* urchins consumed significantly more *G. salicornia* algae (Figure 6). However, interspecific difference in consumption did not persist in all replicates; Mean feeding rates on *G. salicornia* significantly differed between trials (Figure 6). There were no significant differences in water temperature, salinity, or mean urchin size between replicates, but seasonal differences may be a confounding factor.

![Figure 5: Total mean rate of no-choice *G. salicornia* consumption per day normalized by urchin weight, by species.](image)

![Figure 6: Rates of no-choice *G. salicornia* consumption per day normalized by urchin weight, by species and replicate.](image)

The $\delta^{15}$Nitrogen ratio of a consumer’s tissue provides a measure of what has been assimilated from the diet over time. The isotope ratios we measured for *T. gratilla* and *Echinothrix* (Fig. 7) reflect trophic positions that may be higher than values expected for a true
herbivore, but additional data collection and analyses are ongoing. However, our data support the hypothesis that these co-existing sea urchin species do not partition food resources; there are no significant interspecific difference in $\delta^{15}$Nitrogen signatures.

Figure 7: $\delta^{15}$Nitrogen ($^{15}$N:$^{14}$N), by species and site.

In conclusion: With grazing rates that are significant and similar, no evidence for in situ partitioning of food resources, and possible differences in spatial distributions on reefs, our results suggest that *Echinoderm* species may be important allies with *T. gratilla* in the control of algae in Hawai‘i. These results should be useful to conservation managers working to understand and prevent coral reef degradation and macroalgal phase shifts in Hawai‘i.

VIII. Dissemination of Project results:

A. How the projects results have been, and will be, disseminated

Progress of this project has been presented at the first HCRI meeting in January 2007, the second HCRI meeting in August 2007, and at an informal management-focused meeting at DAR East Hawai‘i. Outside of Hawai‘i, Holly Jessop has presented this work at the 12th Annual Echinoderm Meeting in New Hampshire, an EPA Fellowship meeting in Washington, D.C, a seminar at the Smithsonian Tropical Research Institute in Panama, and the Western Society of Naturalists Annual Meeting. In addition, undergraduate assistant Mino Kahananui has given two seminars, and with another undergraduate student Camille Barnett a display about the project was made available at a public outreach event, “Ocean Day Hawai‘i”. Undergraduate students Camille Barnett and Nate Olson presented a poster on this HCRI research program at the EPSCoR Hawai‘i meeting in Waikoloa, HI. Holly Jessop plans to give both oral and poster presentations at the upcoming meeting of the Society for Integrative & Comparative Biology. In addition, a web resource about the sea urchins of Hawai‘i and this research program has been developed, and work to provide additional content is ongoing (http://www.mare.hawaii.edu/urchins). Other future presentations include an oral presentation by undergraduate assistant Kim Morishige for the Keahola program in December 2007, a public outreach talk at the Mokupapapa Discovery center by Holly Jessop in December 2007, and public outreach Reef Talks by Holly Jessop in February and March 2008. A single-page double-sided brochure is currently being created, for distribution at the upcoming public presentations. Finally, we plan to publish our research results in peer-reviewed international journals, attend
additional national and international conferences during Summer 2008, and publish at least one article for the popular media.

**B. List of publications, workshops, and presentations**

- September 2006 – Holly Jessop, EPA Fellows Conference, poster paper
- December 2006 – Mino Kahananui, Keahola Seminar, oral presentation
- January 2007 – Holly Jessop, HCRI Quarterly Meeting, oral presentation
- April 2007 – Mino Kahananui, Keahola Seminar, oral presentation
- April 2007 – “Ocean Day Hawai‘i” interactive display
- June 2007 – Holly Jessop, Smithsonian Tropical Research Institute Seminar, oral presentation
- August 2007 – Holly Jessop, HCRI Quarterly Meeting, oral presentation
- November 2007 – Holly Jessop, Western Society of Naturalists Conference, oral and poster presentations
- November 2007 – Camille Barnett & Nate Olson, EPSCoR Hawai‘i Conference, Waikoloa, HI, poster presentation
- December 2007 – Kim Morishige, Keahola Seminar, oral presentation
- December 2007 – Holly Jessop, Mokupapapa Discovery Center, Hilo, oral presentation for the public
- January 2008 – Holly Jessop, Society for Integrative & Comparative Biology Conference, oral and poster presentations
- February 2008 – Holly Jessop, Reef Talk, Waimea, HI, oral presentation for the public
- March 2008 – Holly Jessop, Reef Talk, Kailua-Kona, HI, oral presentation for the public

**C. Partnerships established with agencies or organizations**

This research program involves collaborations with Dr. Bob Nishimoto’s team at DAR East Hawai‘i, Dr. H.A. Lessios of the Smithsonian Tropical Research Institute, EPSCoR, Keahola STEM program, and numerous University of Hawai‘i undergraduate student volunteers.