I. Shifting the Competitive Advantage toward Native Species on Hawaii’s Reefs,
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II. Abstract

Over the past 50 years, over 20 species on marine algae have been introduced onto Hawaii’s reefs. Previous research has demonstrated that a subset of these species have become highly invasive on Hawaiian reefs and are rapidly expanding at the expense of coral cover and native species biodiversity. The goal of this project was to develop methods by which reefs impacted by these invasive species could be restored to their natural state. Experimental plots established on the reef in Waikiki were cleared of the invasive *Gracilaria salicornia* and followed over time to determine the ability of native algae to resist alien invasion following removal of the invasive alga. Data from these plots suggest that regardless of the abundance or species composition of the native algal community, the invasive alga can re-establish and regain dominance within a short time span. Native “collector urchins”, *Tripneustes gratilla*, have been shown in previous research to be highly effective biocontrol agents for controlling invasive algal growth. The abundance of these urchins on most impacted reefs is quite low, however, and a source of large numbers of urchins for use in further experiments and potential widespread use as biocontrol agents is needed. Protocols for rearing urchins through final larval stages were developed and continuing research hopes to develop equally successful techniques to rear larvae through metamorphosis and juvenile development.

III. Executive Summary

Over the past 50 years, over 20 species on marine algae have been introduced onto Hawaii’s reefs. Previous research has demonstrated that a subset of these species have become highly invasive on Hawaiian reefs and are rapidly expanding at the expense of coral cover and native species biodiversity. The goal of this project was to develop methods by which reefs impacted by these invasive species could be restored to their natural state. Restoration methods examined were focused on shifting the competitive advantage towards native reef communities by propagating and replanting native algae and corals and enhancing sea urchin populations in areas that have become overgrown by alien algae.

In order to assess the ability of native algal communities to resist invasion by alien species, experimental plots were established on the reef in Waikiki, cleared of the invasive *Gracilaria salicornia*, and followed over two years. The abundance of *G. salicornia* remained fairly constant at approximately 60% in the control plots, while the cover was reduced to zero in the removal plots. *G. salicornia* cover quickly recovered, however, and the communities seen in the removal plots converged with those of the control plots. Analysis of the data with respect to the presence and abundance of native species revealed that regardless of the abundance or species composition of the native algal community, the invasive alga can re-establish and regain dominance within a short
time span. Without a means of controlling the regrowth of alien algae, either through decreasing nutrient inputs, increasing herbivory, or the involvement of community groups “weeding” selected reef areas, it is unlikely that replanting of native species will be a successful means of restoring native communities.

Native “collector urchins”, *Tripneustes gratilla*, have been shown in previous research to be highly effective biocontrol agents for controlling invasive algal growth. The abundance of these urchins on most impacted reefs is quite low, however, and a source of large numbers of urchins for use in further experiments and potential widespread use as biocontrol agents is needed. Protocols for the rearing urchins through final larval stages were developed and continuing research hopes to develop equally successful techniques to rear larvae through metamorphosis and juvenile development. Once large numbers of these urchins are available through culturing, large-scale trials of their efficacy as biocontrol agents will be conducted in concert with the use of a recently developed mechanical suction device capable of removing large quantities of alien algae from Hawaii’s reefs.

With the development of the mechanical suction device and the pending culture of urchins, resource managers should in the near future have a suite of tools available to address the impacts of alien algae on Hawaii’s reefs. Community-based cleanups continue to be effective at removing algae from certain areas, and community involvement in restoring and maintaining native algal populations should be cultivated. The combination of the mechanical removal device with the use of cultured urchins is a promising tool for use in areas not easily accessible to community activity and will hopefully soon be experimentally tested.

**IV. Purpose**

**IV. A.** There were no significant impediments to research.

**IV. B. Approach**

- Investigate the ability of native algae to resist invasion by alien species
- Develop methods for the culture of native collector urchins, *Tripneustes gratilla*, for use in biocontrol experiments
- Conduct small-scale community-based cleanups
- Develop education and outreach materials

**V. Approach**

**V. A. Work to be Performed**

**Replanting of Native Algae**

- Established 30 permanent plots on the reef in front of the Waikiki Natatorium, cleared all of the alien algae out of half of the plots, and monitored the plots monthly by image analysis of digital photoquadrats

**Urchin Rearing**

- Conducted multiple spawning trials, and reared urchin larvae while examining factors including species and quantities of microalgal food sources, temperature, larval density, water treatment and quality, handling methods and antibiotic use
Community Education, Outreach and Volunteer Training

- Organized alien algae clean-up events with multi-agency partnerships
- Produced education and outreach materials for distribution to the community
- Conducted workshops with community groups to provide information and hands-on experience with alien algal issues
- Presented research findings at numerous scientific, management and community meetings

V. A. Project Management

Dr. Hunter was the principal investigator in charge of the project. Eric Conklin was the graduate assistant in charge of conducting the culture research and overseeing the work of the undergraduate employees, Rebecca Most and Thomas Sauvage. Ms. Most and Mr. Sauvage conducted the field surveys in Waikiki and assisted with the culture work. Nadiera Sukhraj, Signe Opheim, and Eric Co assisted with culturing activities.

VI. Findings

VI. A. Actual Findings

- **Replanting of Native Algae:** The analysis of several plots cleared of alien algae in Waikiki shows that the invasive *Gracilaria salicornia* regrows fairly rapidly following removal (Figure 1). Analysis of community structure reveals that within 16 months of *G. salicornia* removal, benthic communities are nearly indistinguishable from pre-removal communities heavily dominated by alien species (Figure 2). The re-establishment of these alien-dominated communities occurs irregardless of the presence, abundance, or species composition on the native community (Figure 3).
Figure 1: Percent cover over time (±1 SE) of the most abundant algal species in 15 control and 15 removal plots located on the reef in front of the Waikiki Aquarium. In plots from which the alien G. salicornia and Acanthophora spicifera were removed, the G. salicornia recovers quickly from removal, except for a brief period during which A. spicifera dominates. In control plots, G. salicornia levels remain consistently high throughout. Native algal species Sargassum polyphyllum and G. coronopifolia occur at low abundance throughout.
Figure 2: Multidimensional scaling (MDS) plot of the benthic community structure of the 15 removal plots. The point at 11/3/2002 represents the pre-removal community, with 11/4/2002 representing the first of roughly monthly surveys of post-removal community structure. Despite an initial large change in community structure, it can be seen that post-removal community structure becomes increasingly similar to the pre-removal community dominated by *G. salicornia*. 
Figure 3: Change in percent cover of *G. salicornia* (±1 SE) plotted against the percent cover of native algal species (*Sargassum, Microdictyon*, and *Dictyosphaeria*), an alien species (*A. spicifera*), and species richness and diversity (±1 SE). If the presence and abundance of native species or increased species diversity reduced the rate at which *G. salicornia* re-invaded plots following removal, we’d expect to see a the change in percent cover of *G. salicornia* at or below zero with increased native species abundance, richness, and diversity. Instead, *G. salicornia* increases regardless of the presence, abundance, or species composition on the native community.
• **Urchin Rearing:** Methods were developed to successfully spawn *T. gratilla*, collect gametes, fertilize eggs, and rear hatched larvae through larval development until they were competent to settle. Several spawning and rearing trials were developed to determine the effect of adult size on gamete production (Figure 4-5), the concentrations of eggs and sperm that led to optimal fertilization success (Figure 6-7), and to develop larval feeding and handling techniques that could successfully rear larvae through all stages of larval development (Figure 8-9).

**Figure 4:** The quantity of sperm (A) and eggs (B) collected from adult urchins against size of the urchins (±1 SE). Individual variation was more important than test size in determining the quantity of gametes obtained, indicating that broodstock may consist of any adult individuals irrespective of age and size.

![Graph showing quantity of sperm and eggs collected](image)

**Figure 5:** Photographs of the induction of spawning by the injection of KCl into the gonads (A) and the production (B) and collection (C) of eggs from a female urchin.

![Photographs of spawning induction](image)
Figure 6: The percent fertilization success (±1 SE) of varying concentrations of sperm (μl of sperm per ml of water) mixed with a fixed quantity of eggs. Sperm concentrations above 150 µl/ml produced polyspermy and abnormal development in qualitative trials. Results of trials indicate that a broad range of sperm concentrations can produce high fertilization success rates.

Figure 7: Photographs of fertilized urchin eggs (A) and embryos undergoing their first (B) and second (C) cell divisions.
Figure 8: Post-oral arm length (±1 SE) of larval sea urchins against age in days after fertilization. Growth of urchin larvae is fairly constant throughout larval development until reaching the size and developmental stage necessary for metamorphosis at 30-40 days after fertilization.

Figure 9: Photographs of larval T gratilla in the blastula stage (A: hatching to 2-3 days post-fertilization), two-arm pluteus stage (B: ~4 days post-fertilization to 21-28 days) and multi-arm pluteus stage (C: ~28 days post-fertilization to metamorphosis).
Community Education, Outreach and Volunteer Training

Community Presentations and Workshops
- Mauna Lua Bay Algae Workshop
- DOE Teachers Workshop
- Invasion of the Reef Snatchers Family Fun Day alien algae cleanup and informational booth
- Continued community-based cleanups in Waikiki as well as a cleanup at the Hawaii Institute of Marine Biology with the teachers of the Malama Program
- Informational presentations to the Malama Program and Habilitat

Public Education and Outreach Materials
- "Looking at Limu" educational curriculum
  - LAL G 9-12 Curriculum,
  - LAL Community Workshop
  - LAL Tool Kit (attached)
- “Hawaiian Marine Life: Alien Seaweeds” waterproof ID cards

VI. B. Significant Problems
This project was very successful at developing techniques to rear larval sea urchins. However, this development took nearly the entire year of the current project, which meant that only recently were we able to get large numbers of individuals to reach competency to settle. Within this limited time frame, we have not yet been able to develop similarly effective techniques for moving large numbers of larvae through metamorphosis into the juvenile phase.

VI. C. Need for Future Work
- Continue research on culture techniques to produce large numbers of adults that can be used in large-scale experiments
- Conduct large-scale experiments using cultured urchins to determine the efficacy and impact of using T. gratilla as biocontrol agents
- Conduct field trials with the newly developed “Super Sucker” device for mechanical removal of large quantities of alien algae from Hawai`i’s reefs
- Culture large numbers of native algal species that be used in large-scale restoration efforts
- Produce an experimentally supported management plan for the control of alien algae species incorporating the mechanical removal of algae from extensive reef areas, augmenting native herbivore populations through urchin additions to control the regrowth of alien species, and the restoration of native algal communities by planting cultured native algae.

VII. Evaluation

VII. A. The goal of this project were either met or carried as far as they could be within the one year time frame of the current project. The feasibility of replanting of native species given current conditions was assessed and it was concluded that
until the issues of nutrient inputs and herbivore reductions are addressed, communities will return to domination by alien algae within a relatively short time frame. Significant progress was made on the culturing of *T. gratilla* and continuing research will build upon these findings to develop full culture protocols.

**VII. B.** Presentations of research findings have been given at Hawaii Invasive Species Council meetings, Coordinating Group on Alien Pest Species meetings, quarterly reports to the Hawaii Coral Reef Initiative, Hawaii Conservation Conference, and the 10th International Coral Reef Symposium. Waterproof identification cards have been widely distributed to the public. The “Looking at Limu” curriculum has been circulated amongst educators who have been asked to comment upon the content and delivery.

**VIII. Signature of Principal Investigator**

Cynthia L. Hunter, Ph.D.  

Date