I. Alien Algae on Hawaii's Reefs: Distributional Changes and Ecological Responses,

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Project FINAL REPORT FY 2003

II. Abstract

Statewide surveys found invasive alien algae at 67 of 81 sites, an increase of 11% since 1999, indicating that these invasive species have spread. *Acanthophora spicifera* was found throughout the Main Hawaiian Islands but the distributions of the four other invasive species (*Gracilaria salicornia, Kappaphycus spp., Hypnea musciformis,* and *Avrainvillea amadelpha*) remain limited to particular islands or embayments. Sexually reproductive *Acanthophora spicifera* was found commonly but was rare in the other four alien algae species.

Alien algal abundance was negatively correlated with water clarity, coral cover, sea urchin abundance, and herbivorous fish biomass. Regrowth of *Kappaphy*cus spp. in experimental removal plots, urchins appears to be held in check for up to 12 months by grazing of the sea urchin, *Tripneustes gratilla*.

In *Kappaphycus sp*p., tissue nitrogen and phosphorous were correlated with water column concentrations of nitrate and phosphate. However, *Kappaphycus* growth does not appear to be enhanced in nutrient treatments, suggesting that the potential for spread and blooms of this species will not be limited just to eutrophic areas.

Currently, there is insufficient information in the published literature on the biological characteristics of most of the invasive algae to determine trends or patterns that may influence invasibility.

Four community volunteer events in 2003 resulted in a total removal of over 35 tons of *Gracilaria salicornia* from the Waikiki Marine Life Conservation District. Numerous outreach activities were conducted and products developed (PowerPoint presentations, underwater ID cards, posters, and a community sourcebook/curriculum guide) during this project and in collaboration with other grant-funded activities.

III. Executive Summary

- Resurveys of the abundance of alien and native algae and herbivores (fish and invertebrates) were conducted at 81 sites statewide.
- Detailed maps of alien algal species throughout the State of Hawaii were generated to illustrate current distributions and rates of expansion and invasion of new areas.
- Reproductive status of each alien algal species was assessed at each site
- Species diversity estimates at heavily impacted were compared to control sites to determine how alien algae are affecting native reef ecosystems.
- Effectiveness of experimental removal plots, and the influence of herbivores and nutrients on regrowth were assessed; water column and tissue nutrients were measured relative to *Kappaphycus* abundance in Kaneohe Bay.

- Global database of invasive algae and flow chart of biological attributes contributing to invasiveness was designed and implemented.
- Public awareness of the threats of alien algae to Hawaii's reefs was enhanced.

IV. Purpose

- Assess impacts of alien algae on native reef species in 2003 compared to 1999 surveys and provide detailed maps of current distributions and rates of expansion and invasion of new areas.
- Determine if alien algae are spreading primarily through sexual or asexual reproduction.
- Quantify effect of aliens on native reef ecosystems.
- Improve management capacity for current alien algae problems on O'ahu
- Improve education & outreach capability
- Compile a global database on invasive macroalgae

V. Approach

The research team conducted resurveys of 80+ sites statewide previously surveyed in 1999-2000, to generate and update alien algae distribution maps. Graduate Assistants (Ms. Jennifer Smith, Cheryl Squair, Kim Peyton (UH-Botany) and Mr. Eric Conklin (UH-Zoology)) conducted field surveys on Oahu, Kauai, Molokai, Maui, Molokini, and the Big Island. This involved quantitative assessments of benthic reef communities & grazers (urchins, mollusks & fish) at each site as well as recording temperature, salinity, horizontal visibility, habitat protection, presence/absence of turtles, presence/absence of seagrass, and the extent of coral-alien algal interactions. Reproductive status of each alien species was ascertained when encountered.

Detailed distribution maps of alien and invasive algal species throughout the State of Hawaii were generated using ARC-View GIS. These maps illustrate current distributions and rates of expansion and invasion of new areas.

Two undergraduate students (Rebecca Most and Thomas Sauvage) continued monthly distributional surveys shore and analyses of experimental alien algal removal plots on Oahu's south. The effectiveness of manual removal of alien algal biomass was evaluated in ¼ m^2 experimental plots. Monthly photoquad surveys were conducted on 15 plots from which *Gracilaria salicornia* has been removed at Waikiki. A multi-factorial experiment was conducted using 72 experimental *Kappaphycus* sp. removal plots testing the additional influences of herbivory (in fish exclusion cages) and nutrient limitation (by addition of fertilizers) along three replicate transects at each of three reef sites in Kaneohe Bay.

Tools for effective community education and outreach were developed and tested in a number of venues. Four volunteer alien algae removal events were conducted at Waikiki MLCD during the past 12 months and two were held at the Hilton Hawaiian Village

beach. Numerous workshops were conducted for managers, teachers and community groups. Education and outreach materials were developed, produced, and/or distributed including underwater ID cards, brochures, posters, resin embedded specimens, a field guide, and lesson plans for K-12 teachers.

Detailed Methodologies:

Distribution Maps

Purpose
To map the current distribution of alien macroalgae in Hawaii and correlate with fish and invertebrate grazer abundance.

Materials
- Quadrats (¼ m^2)
- Transect lines
- UW slates

Deployment
1. Place approximately five (5) ¼ m^2 quadrats randomly throughout the shallow subtidal and intertidal regions to assess non-indigenous algae species abundances.

Data Collection
1. Conduct a general survey and reconnaissance of the area by snorkeling for approximately one hour at a depth of no more than 3 meters.
2. Survey benthic algae (*i.e.,* relative abundance, habitat type, date, temperature, salinity, GPS coordinates, dominant herbivore community, depth).
3. Count all fish that are observed within an estimated cylinder of set radius centered on the observer. The default radius is 5 meters, though at some sites visibility may restrict the counts to areas as small as a 2m radius.
4. Conduct randomly chosen replicate fish counts so they are representative of the area in which the algal surveys were conducted.
5. Compile a species list of all herbivores seen to ensure that species not recoded in the fish counts were recorded as being present at the site.

Data Analysis
1. Following each of the surveys, plot alien species distributions and relative abundance using ArcView GIS maps for each of the islands.
2. These maps can then be compared with those generated from the historical data compiled from the Bishop Museum's database. This information highlights which of the alien species have been most successful at dispersing between islands and where blooms currently exist. This information can help identify sites that may be

particularly vulnerable to invasion.

Deposit voucher specimens of algae at the Bishop Museum for future reference. Enter all relevant collection information including depth found, habitat type and date collected into the museum's current database. This database is readily available to managers.

Macroalgae Removal

Purpose

To determine the short- and long-term effectiveness of alien macroalga removal.

Materials:

- Transect line
- 8" stainless steel spikes (to mark corners of plots)
- Double strung ¼ m² quadrat with 36 intersection points
- Metal forceps
- Plastic bags
- Hand nets

Field Preparation

1. Establish three (3) 30m transects on the reef flat (1-2m depth). Transects were placed approximately 20 m seaward of the memorial wall extending towards the reef crest, running parallel to each other and approximately 25 m apart.
2. Five points were randomly selected on each transect where paired ¼ m^2 plots were permanently marked on either side of the transect with stainless steel pins.
3. Percent cover was measured by snorkelers using the point intercept method with a double-strung quadrat using a total of 36 intersections. In instances where canopy and understory algae were present, a single intersection would be assigned two or more points depending on the number of species encountered, therefore, some quadrats had more than 36 points sampled. Surveys were conducted in November 2002.

Deployment

1. Snorkelers hand-picked Alien alga (taking care to not disturb/remove native alga) and placed all biomass into a large, plastic bag while simultaneously fanning the water with a hand net to capture all fragments generated while removing larger pieces of the alga.
2. When all or most of the large visible biomass was removed, forceps were used to scrape small attachment points from the substratum.

Data Collection

1. Snorkelers recorded the type of habitat and the total amount of time required to remove all alien alga biomass per quadrat.
2. Upon returning to the shore wet weights of the removed alien alga measured for each plot.
3. Surveys were repeated for 12 months following the initial removal of alien alga to determine rates of regrowth

Data Analysis

1. Linear regression was used to test for relationships between removal time, biomass and percent cover.
2. Regrowth rates of the alien alga were evaluated.
3. Biodiversity changes during regrowth was assessed using the Shannon-Weiner Biodiversity Index (H').

Macroalgae Volunteer Removal Events

Purpose

To determine whether large scale removal efforts might be effective in controlling alien algal abundance and to raise community awareness about the threats of alien algae on Hawaii's reefs. The methodology described below was developed specifically for removal of alien algae from this particular location in terms of depth, exposure, and shore access, and participation of trained algal taxonomists for identification and sorting of alien from native seaweeds. Special use permits must be obtained from DLNR-DAR and City/County before events of this type are held.

Materials:

- Volunteer sign in and release sheets
- Burlap or rice bags
- Cable ties
- Boogie boards/bungie cords
- Weigh scale and replacement batteries
- Easel and paper for recording biomass weights
- Scissors, pens, paperweights
- Dumpster for transport of algal biomass to greenwaste recycler
- Rakes, shovels
- Educational brochures and posters
- T-shirts, snacks, and water for participants

Preparation

1. Participants were initially contacted through nongovernmental organization email lists (The Nature Conservancy, Reef Check), university classes, campus clubs, the University of Hawai'i Dive Safety Office, canoe clubs, Waikiki Aquarium volunteers, and by word-of-mouth.
2. T-shirts were printed and provided to each volunteer; refreshments and lunches were arranged through local vendors.
3. Informational brochures and posters were prepared and displayed on tables at the clean-up site for passersby and participants alike.
4. Permits for removal of algae from the MLCD (Marine Life Conservation District) were obtained from the Hawai'i Department of Land and Natural Resources; permits for use of the assembly area were obtained from the City and County of Honolulu's Parks Department.

Deployment

1. Participants signed the permit and contact information sheets as they arrived at the site.
2. Participants were organized into five workgroups: SCUBA divers, snorkelers, beach workers, bag carriers, and sorters/weighers.
3. University of Hawai'i certified research divers collected floating algae from the trench area (~3-4 m depth) about 80 m from shore.
4. Divers stuffed the algae into burlap bags that were then closed with a plastic zip tie.
5. Snorkelers retrieved the filled bags from the divers and secured them to boogie boards with elastic cords.
6. Bags were then floated on the boogie boards over the shallow reef flat to the beach.
7. From the beach, bags were carried to a concrete ramp to drain and the boogie boards passed back to the waiting snorkelers.
8. Workers also collected and bagged alien alga from the beach wash. A small mesh seine net was used to haul floating algae from the shallow nearshore area up onto the beach where it was bagged and carried to the sorting area; this method of collection was very effective in removing large amounts of algae in a fairly short time.
9. After draining for a timed interval of 5 minutes, bags were weighed and then emptied onto tarps in the sorting area. Researchers and trained students removed select

native algae that were mixed with the alien alga thalli and returned the native species to the reef.

10. The alien algal biomass was transferred to a dumpster that was later delivered to a local green-waste recycling company for processing into composting tea.
11. Some of the alien algae biomass was provided to farmers directly for test use as compost in taro agriculture.

Data Analysis

1. Numbers of volunteer participants and weight of biomass removed during each event were recorded.

Nutrient Enrichment

Purpose

To elevate the local concentration of fertilizer nutrients in field experiments.

Materials

- Fifteen (15), 20 cm diameter unglazed terra cotta clay flower pots
- marine epoxy
- 15 ft^2 PVC sheet, ¼ inch thick
- rough grit sand paper
- electric circular sander
- skill saw
- ¼ inch drill bits
- 1200 large (1 ft.) cable ties
- 15 rubber stoppers
- 1 funnel
- 220 kg of United Horticultural Supplies Turf Supreme Fertilizer (11% NH_3, 10% $N0_3$, 9% PO_4)
- Steel wool scrub pads
- 144, 1 gallon Ziploc storage bags

Deployment:

1. Cut PVC into 25 x 25 cm squares and drill one hole into each corner.
2. Rough sand the upper surface of all PVC squares to allow for easy attachment of pots.

3. Invert and secure the 20 cm diameter unglazed terra cotta clay flower pots with epoxy to PVC holding plates.
4. After the epoxy is dry, flip over and using funnel, fill unglazed clay flower pots with 1.4 kg of United Horticultural Supply turf fertilizer (11% NH_4, 10% NO_3, 7% P_2O_5).
5. Attach the flower pots to the benthos within 0.5 m of the settlement tiles using the large cable ties.

Data Collection

1. SCUBA or free divers retrieve the pots each month. Empty the pots, clean the surface using scrub pads and refill them with fertilizer.

2. In order to quantify enrichment levels take water samples for analysis on transects moving away from the pots on four sampling dates immediately following enrichment. Collect water samples via SCUBA using sterile 50 cc syringes at 0, 0.25, 0.50 and 1m distances away from the pots.

3. Collect ambient water samples at the same depth (10 m) but outside (> 5 m) of the experimental area.

4. Upon returning to the surface, transfer the water samples to acid washed 100 ml polypropylene bottles, place on ice, and store in freezer prior to analysis..

Laboratory Analysis

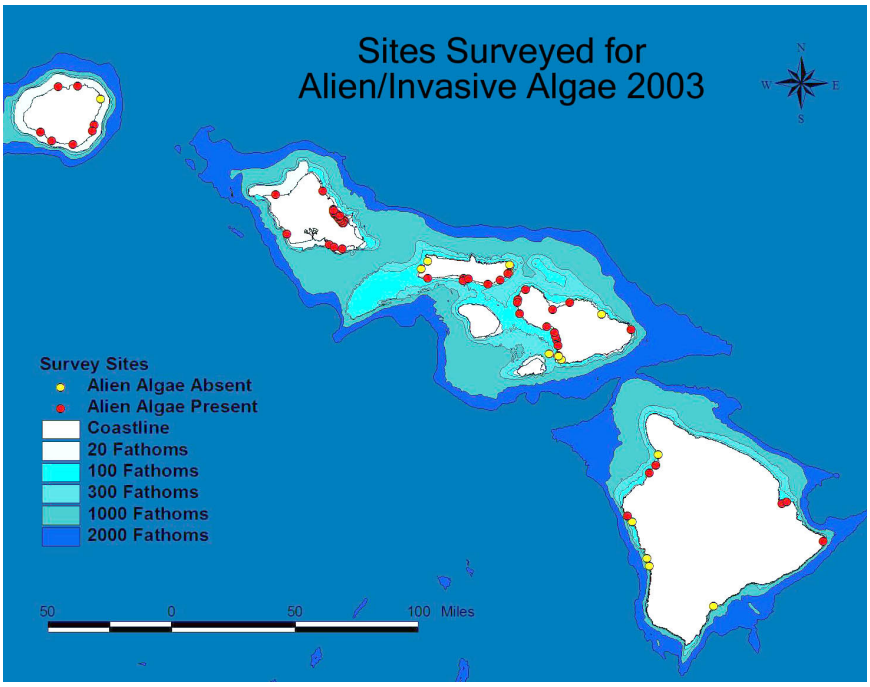
1. Analyze the samples for nitrate, ammonium and phosphate concentrations using a Technicon Autoanalyzer II at Analytical Services, University of Hawaii.

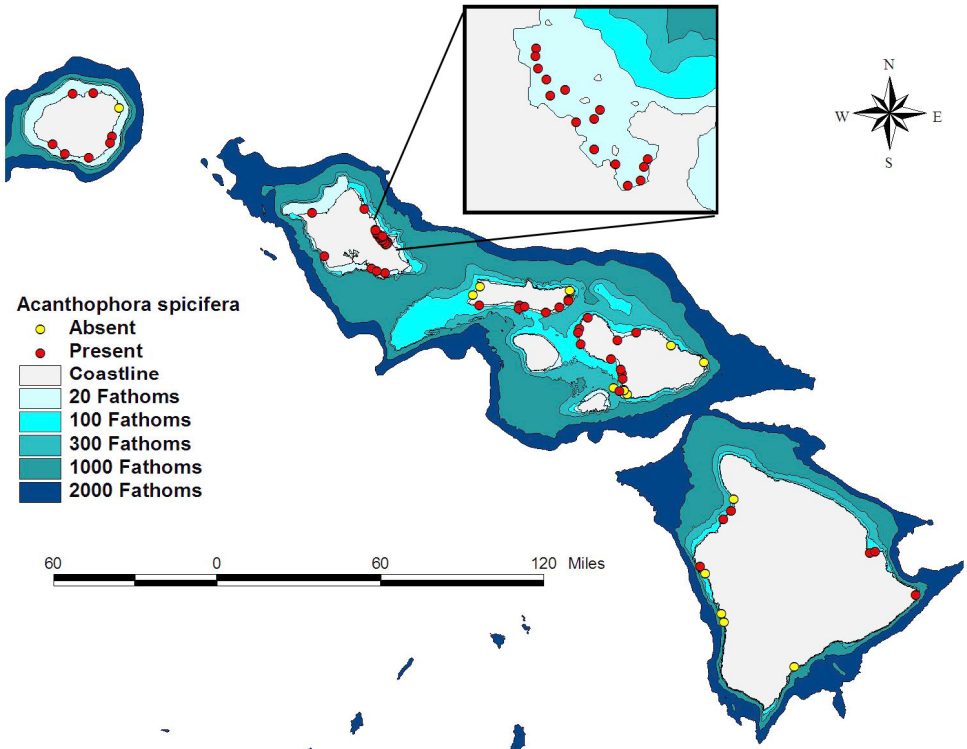
Data Analysis

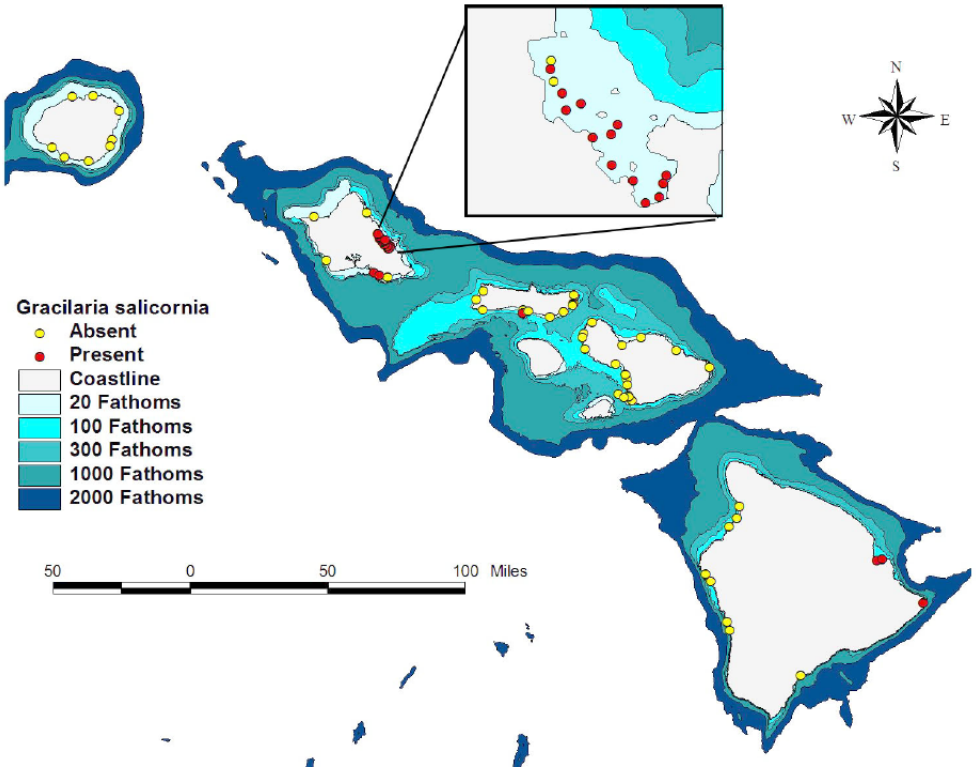
1. Nutrient enrichment of experimental area
    a. To determine if significant nutrient enrichment occurred above measurable background nutrient concentrations, compare results of water samples collected outside of the experimental area (ambient) and the samples collected at 0.5 m from the pots.
    b. Check data for normality and homoscedasticity.
    c. If above assumptions are met then perform a two-way analysis of variance (ANOVA) on each nutrient using treatment (ambient vs. enriched) as a fixed factor and date as a random factor.
    d. Examine results to determine if significant enrichment occurred.
2. Extent of enrichment:
    a. Perform a three-way analysis of variance on each nutrient data set using distance as a fixed effect and date and block as random effects.
    b. If distance is significant examine Tukey's pairwise comparisons (with a family error rate of 0.05) to identify which distances are different from one another.

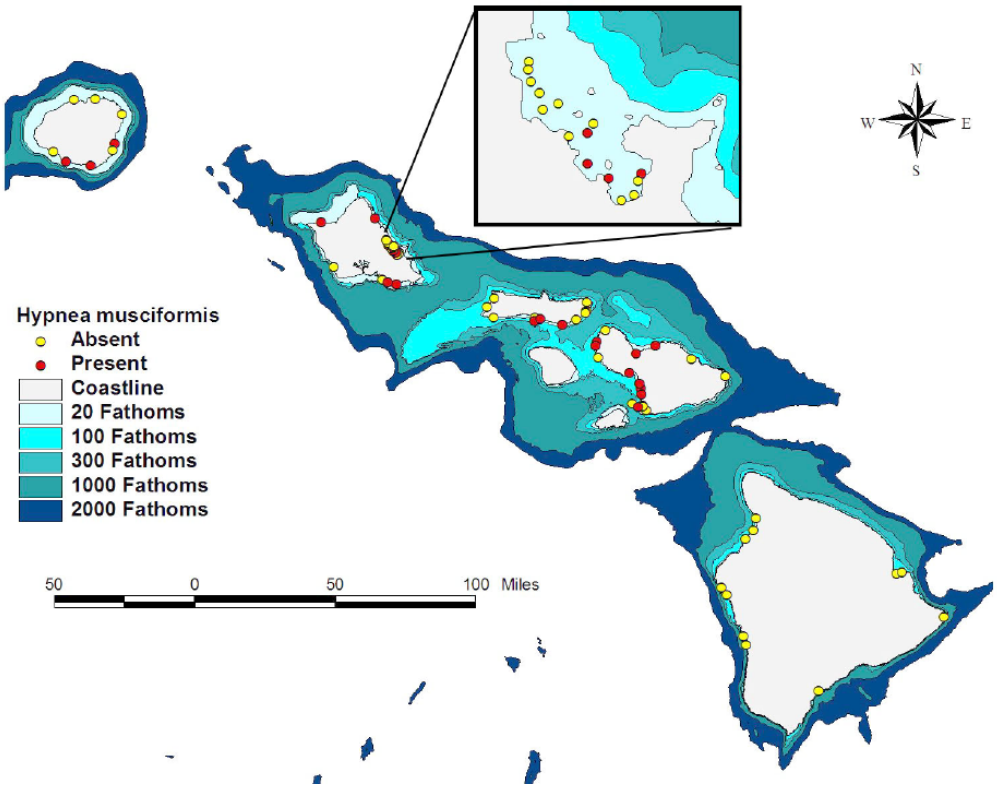
VI. Findings

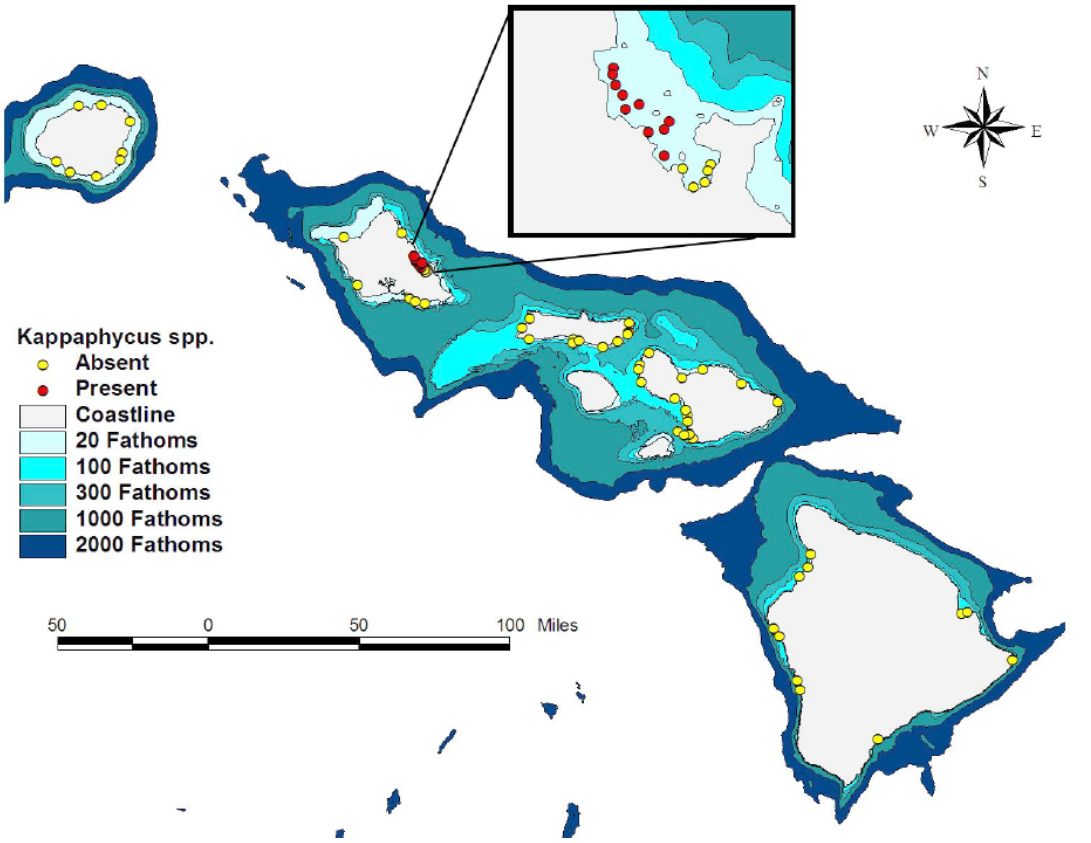
Over 480 man-hours were spent conducting quantitative field surveys on five islands. Detailed GIS maps of state-wide alien algae distributions in 2003 are illustrated below and provided on CD.

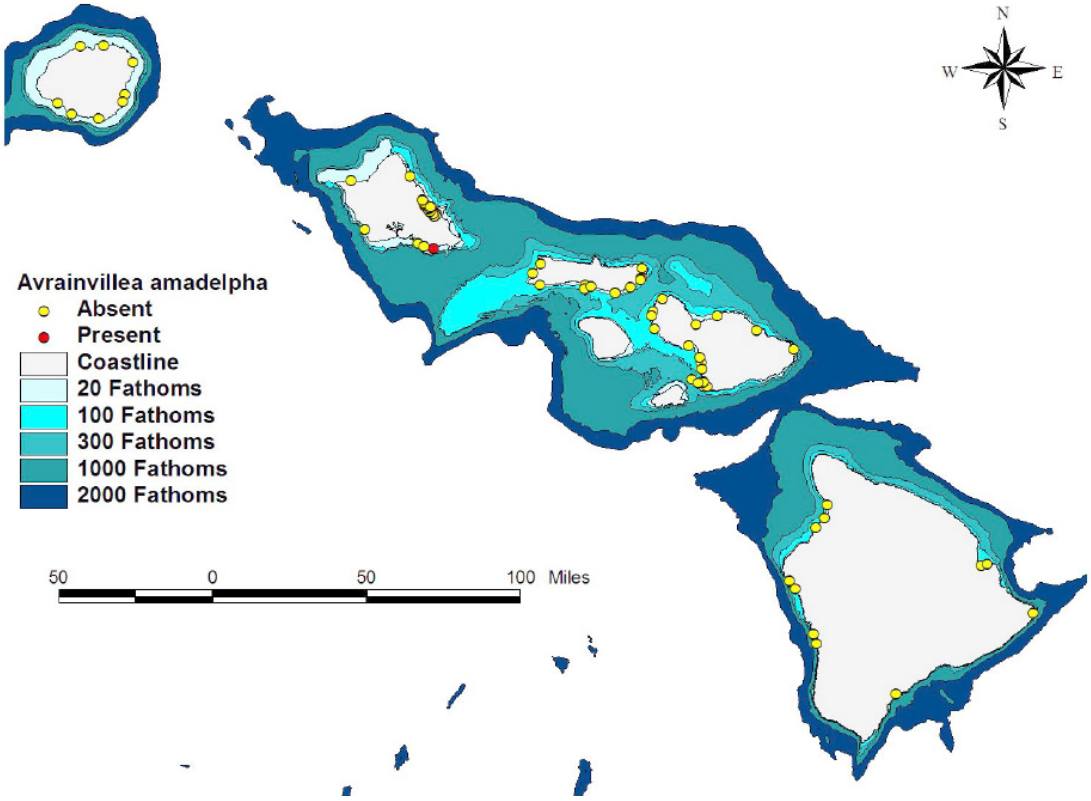




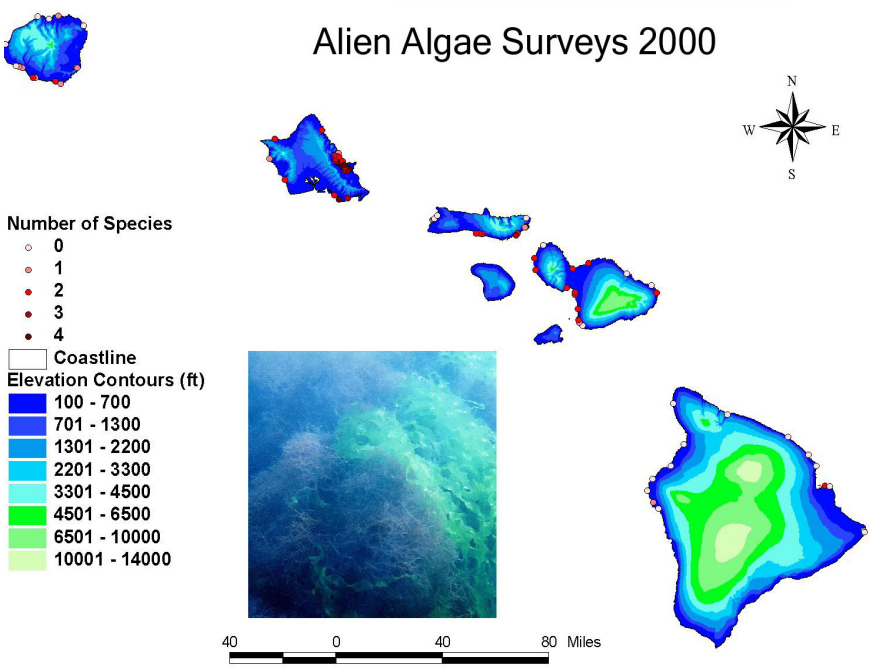

N
W
E
S
Gracilaria salicornia
Absent
Present
Coastline
20 Fathoms
100 Fathoms
300 Fathoms
1000 Fathoms
2000 Fathoms
50 0 50 100 Miles

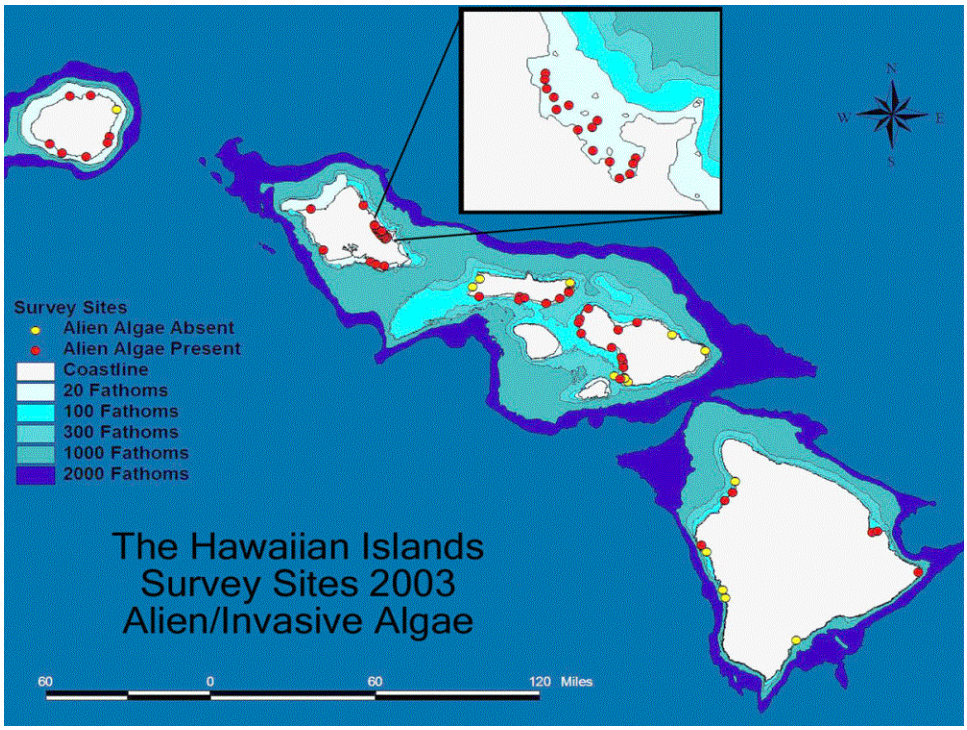

N
W
E
S
Hypnea musciformis
Absent
Present
Coastline
20 Fathoms
100 Fathoms
300 Fathoms
1000 Fathoms
2000 Fathoms
50 0 50 100 Miles


N
W
E
S
Kappaphycus spp.
Absent
Present
Coastline
20 Fathoms
100 Fathoms
300 Fathoms
1000 Fathoms
2000 Fathoms
50 0 50 100 Miles

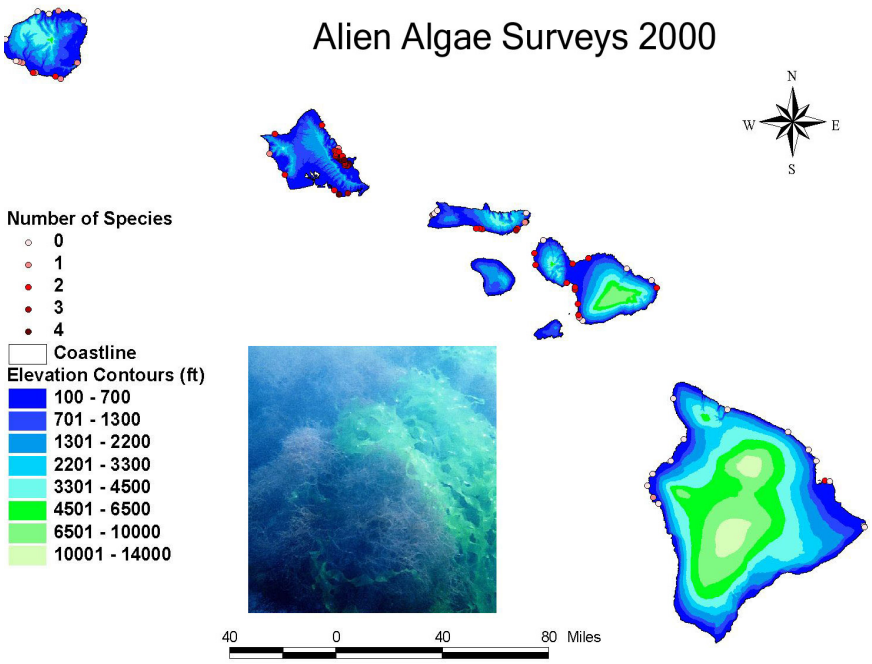

N
W
E
S
Avrainvillea amadelpha
Absent
Present
Coastline
20 Fathoms
100 Fathoms
300 Fathoms
1000 Fathoms
2000 Fathoms
50 0 50 100 Miles

Alien algae were absent from 23 of the sites surveyed in 1999/2000 but only 14 of these same sites were free of alien algae in 2003.





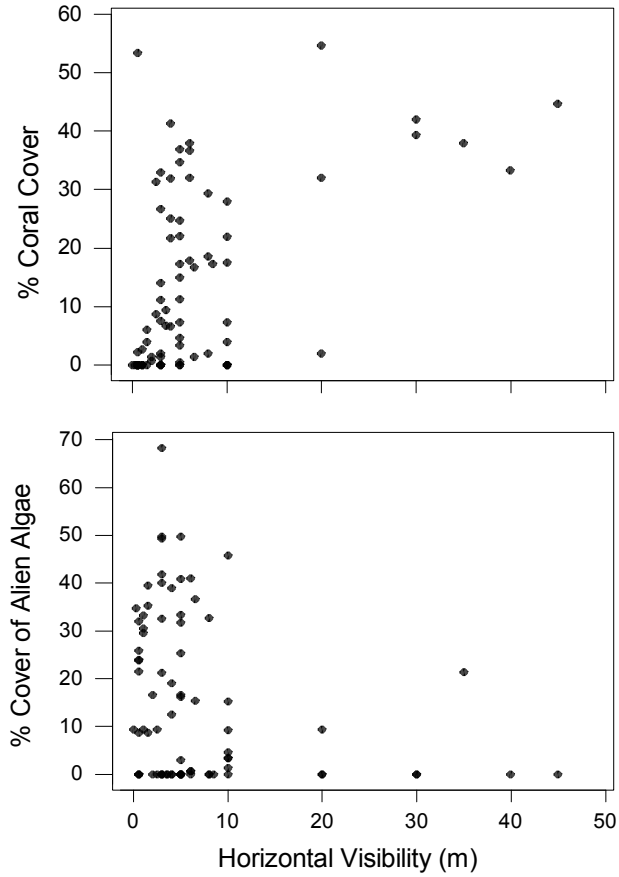
Alien algae were absent from 23 of the sites surveyed in 1999/2000 but only 14 of the sites were free of alien algae in 2003.



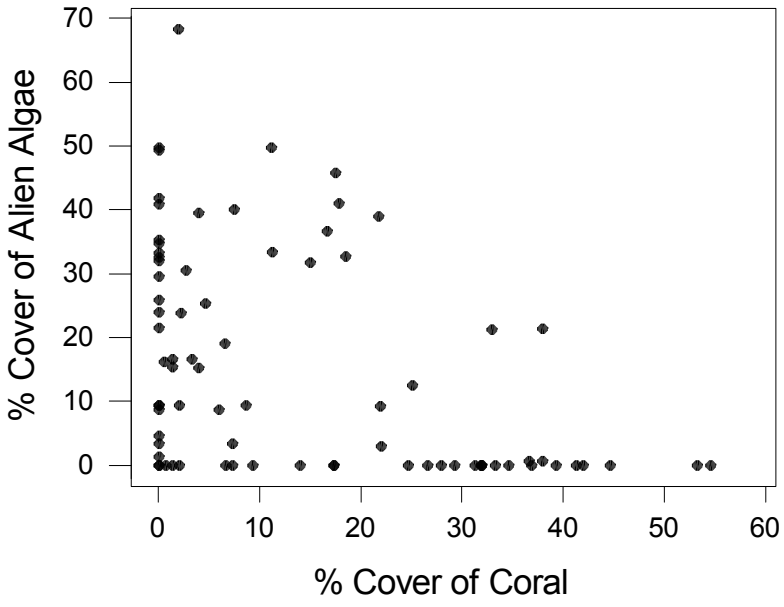
Acanthophora spicifera was the most broadly distributed alien algal species in 2003, recorded at 59% of the survey sites, including all sites on Oahu. *Hypnea musciformis* was found at 32% of survey sites, but it was not recorded on Hawaii Island. The mat-forming *Gracilaria salicornia* was found at 24% of sites, most of those in Kaneohe Bay; it was not recorded from Maui or Kauai. *Kappaphycus* sp. remains limited to Kaneohe Bay on Oahu, occurring at 10 of the 15 sites in the Bay. *Avrainvillea amadelpha* was found at only a single survey site on South Oahu, but has been reported by a member of our research group (K. Peyton, pers. comm.) to occur at Hanauma Bay.

Sexually reproductive *Acanthophora spicifera* was found at nearly all sites where this algae was recorded. Fertile *Kappaphycus spp.* was found at 3 of the 15 survey sites in Kaneohe Bay. No other sexually reproductive individuals of alien algae were identified.

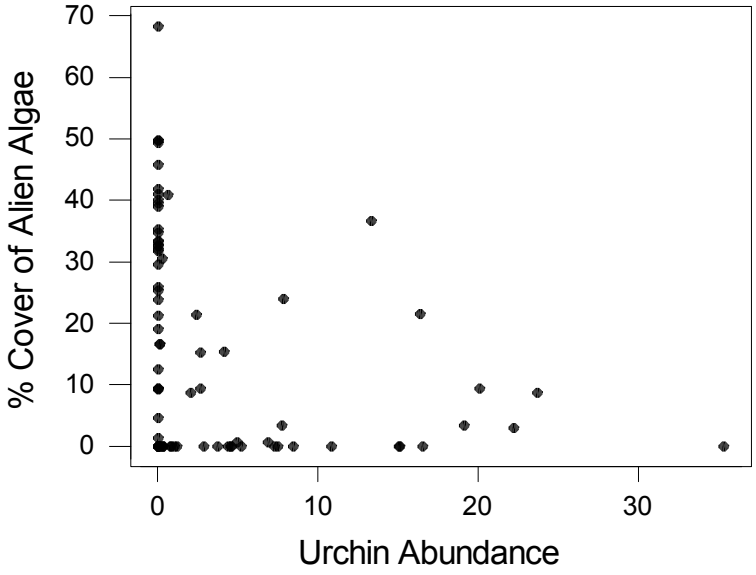
Other patterns that emerged from the surveys were that water clarity as estimated by horizontal visibility was positively correlated with coral cover but negatively correlated with algal cover.

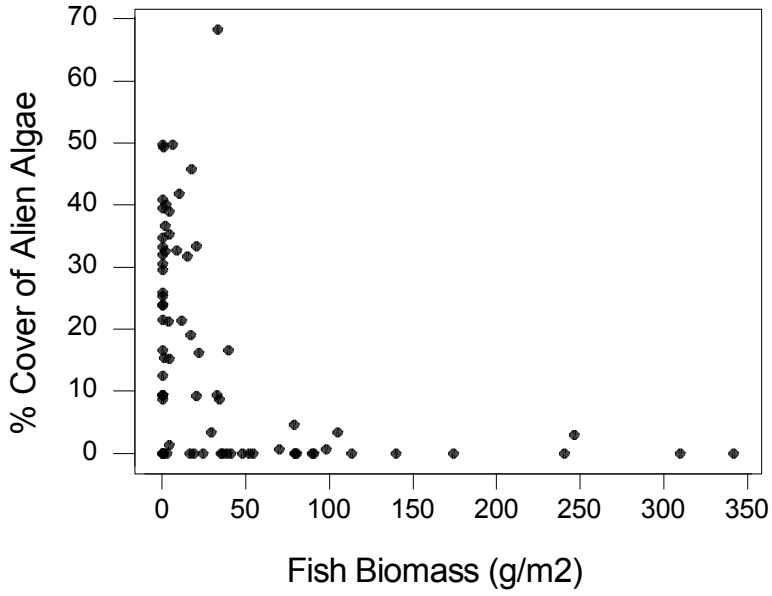

% Coral Cover
0
10
20
30
40
50
60
% Cover of Alien Algae
0
10
20
30
40
50
60
70
0
10
20
30
40
50
Horizontal Visibility (m)

Coral cover was negatively correlated with abundance of alien algae:

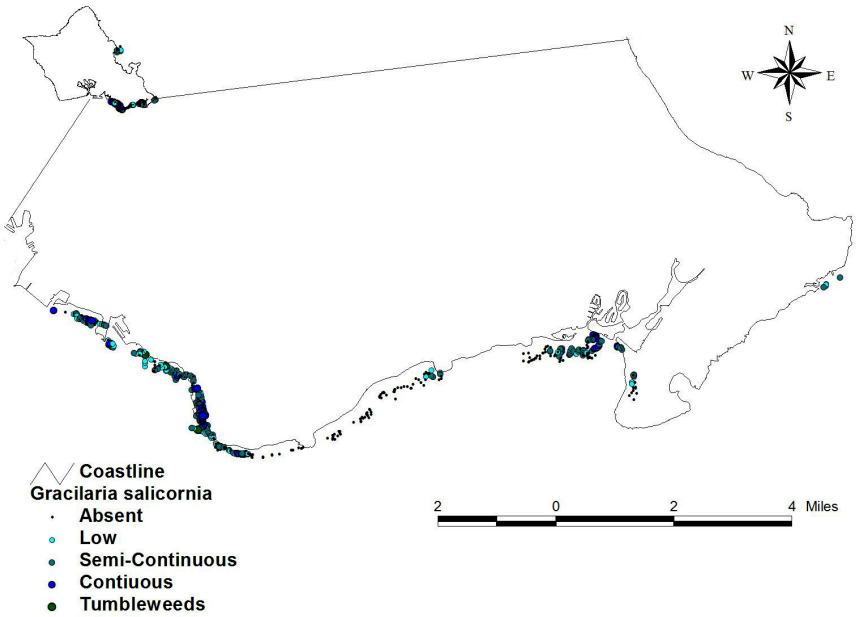


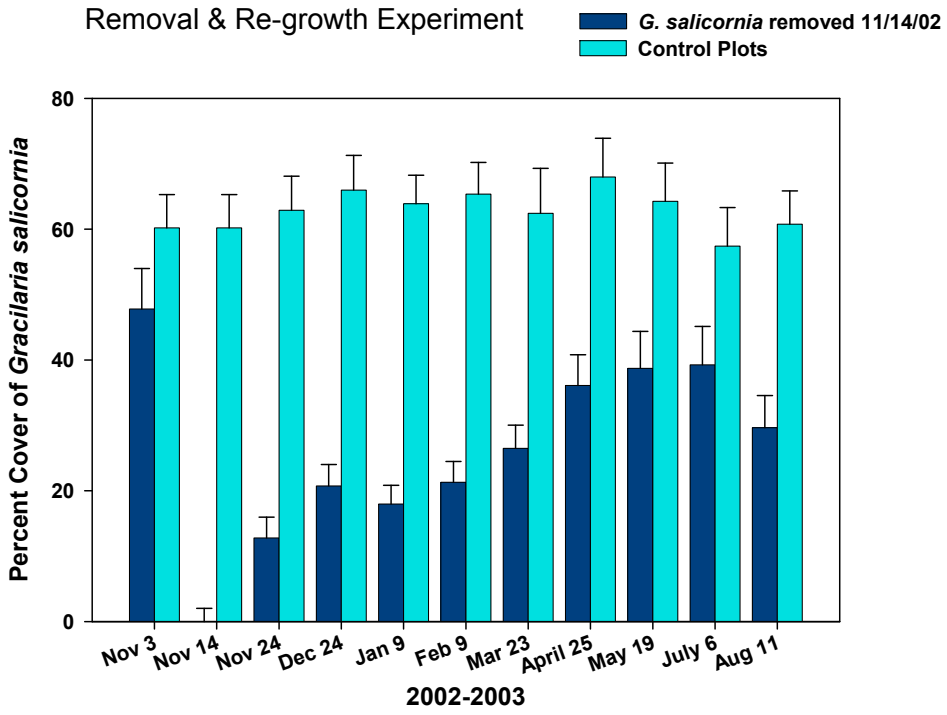
Alien algal abundance was negatively correlated with urchin abundance and fish biomass:



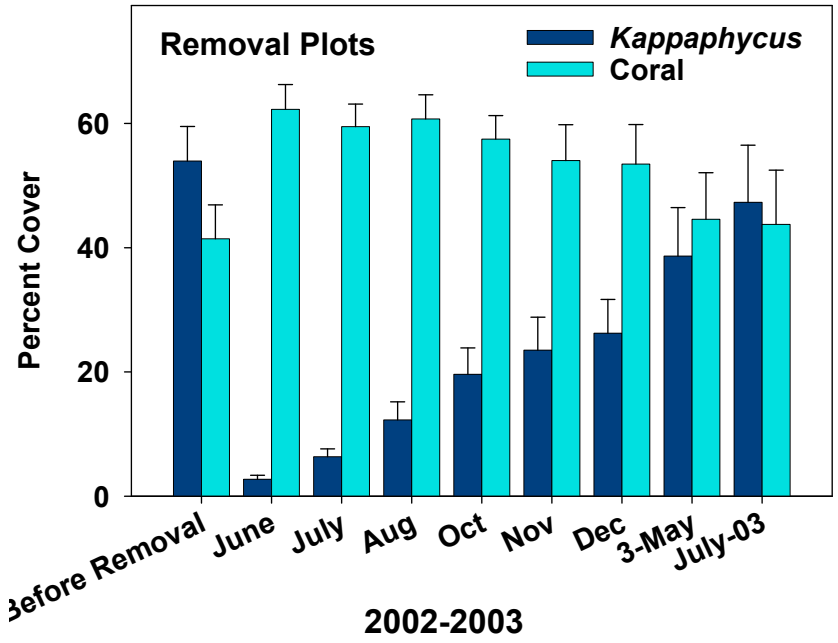


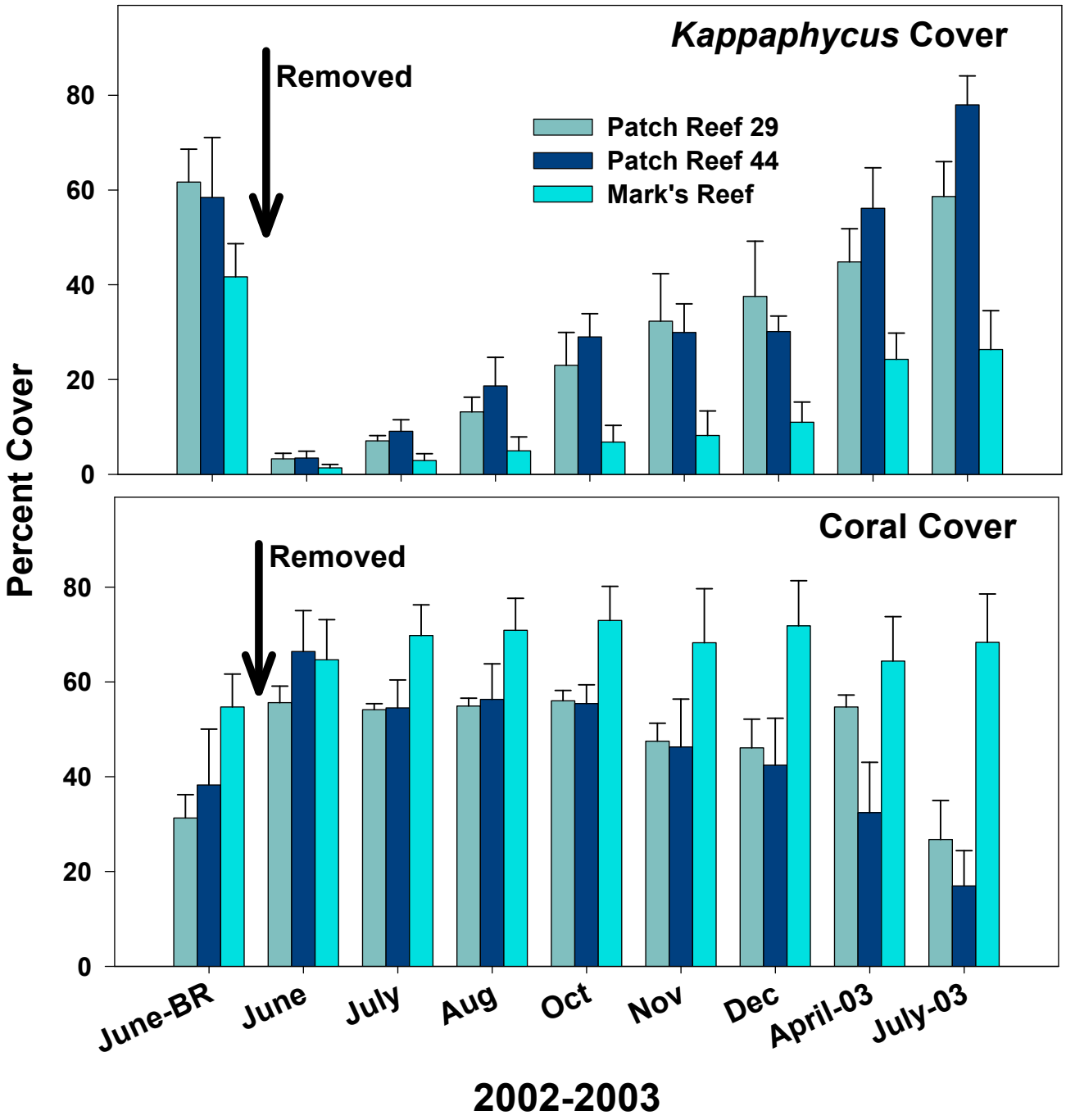
This alien algae population continues to spread and regrowth in removal plots is rapid.



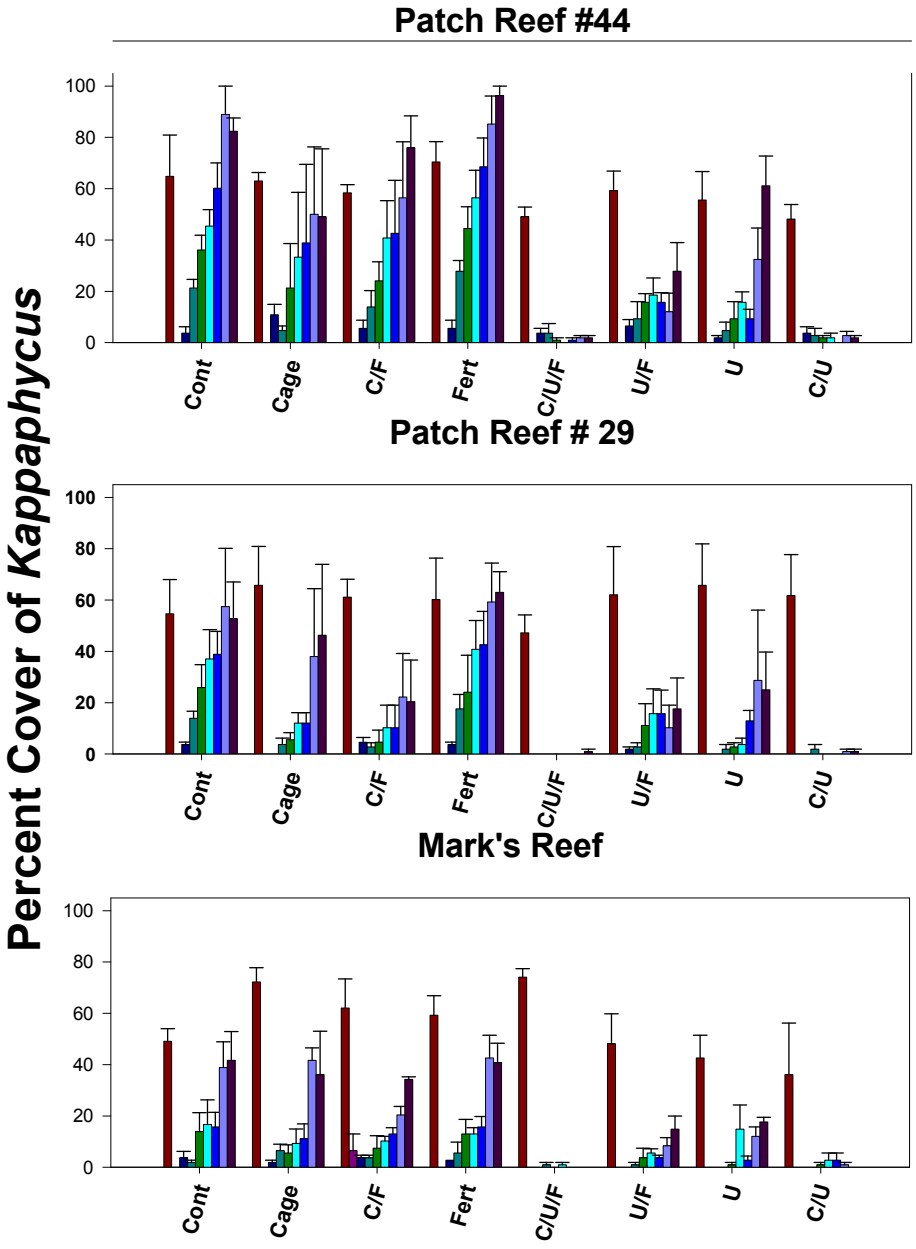


Methods in addition to manual removal will be needed to control *Gracilaria sallicornia* growth on reefs in Waikiki. Similarly, the percent cover of *Kappaphycus* regrew to nearly pre-removal abundance over a period of one year.

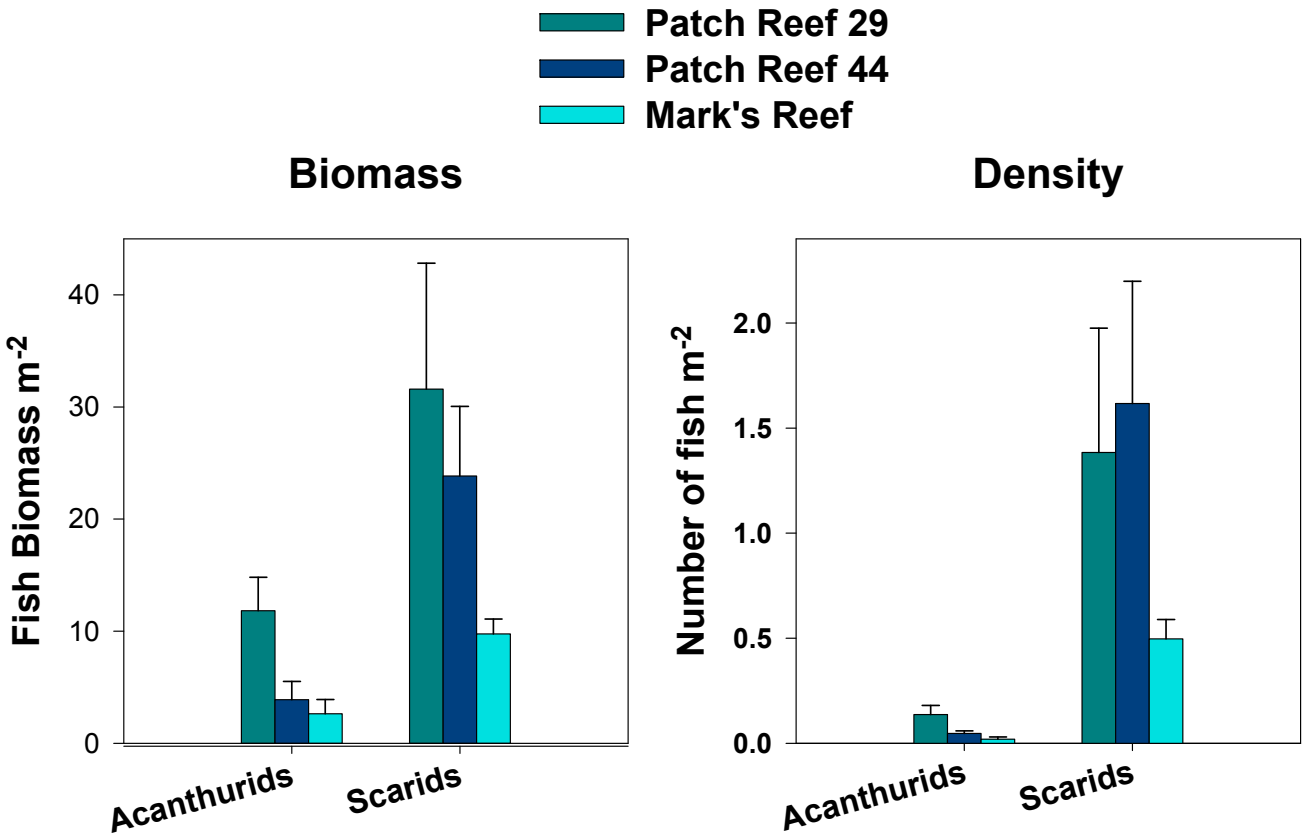



Kappaphycus Cover
Removed
Patch Reef 29
Patch Reef 44
Mark's Reef
Coral Cover
Removed
Percent Cover
0
20
40
60
80
June-BR
June
July
Aug
Oct
Nov
Dec
April-03
July-03
2002-2003

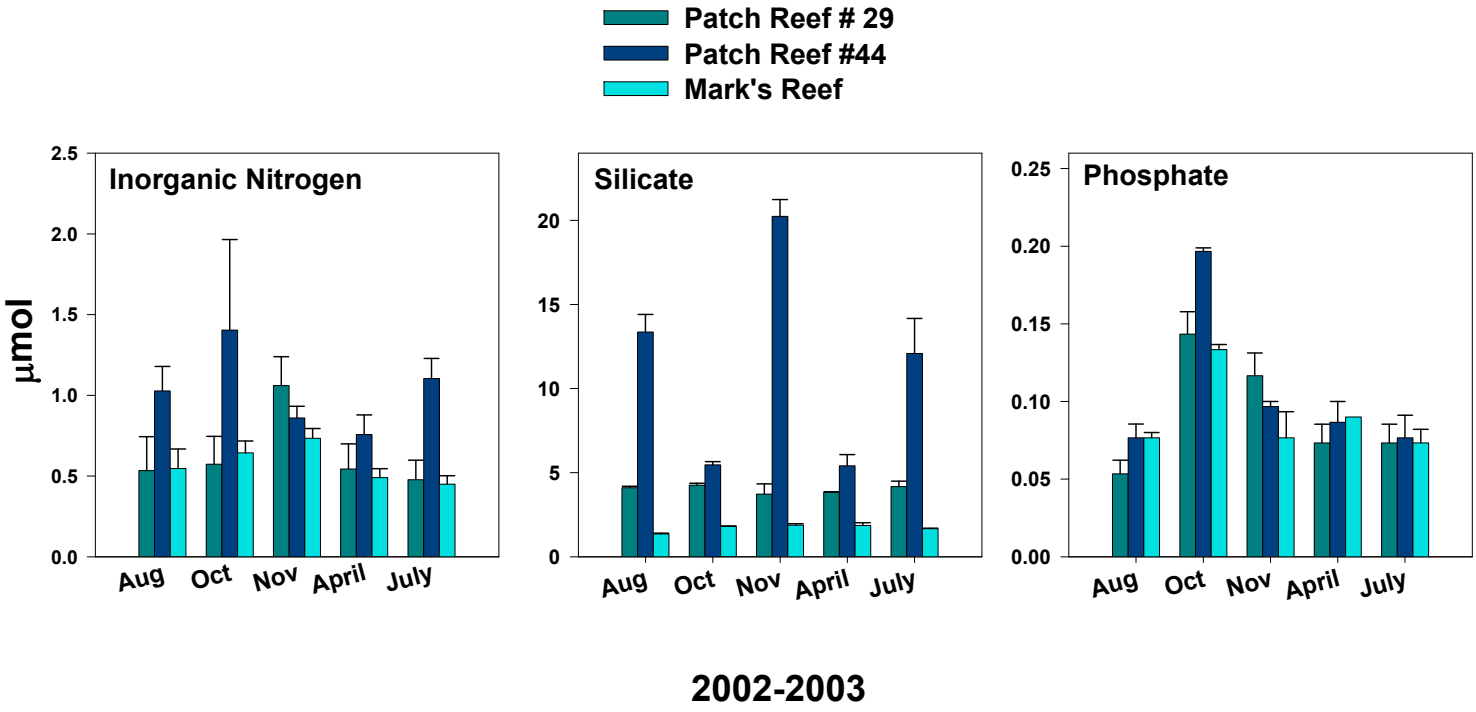
Monthly photoquad surveys and analyses of 72 experimental alien algal removal plots in Kaneohe Bay were completed. Regrowth of alien *Kappaphycus* spp. appears to be held in check by grazing of the urchin, *Tripneustes gratilla*. *Kappaphycus* growth does not appear to be enhanced in nutrient treatments, suggesting that the potential for spread and blooms of this species will not be limited just to eutrophic areas.



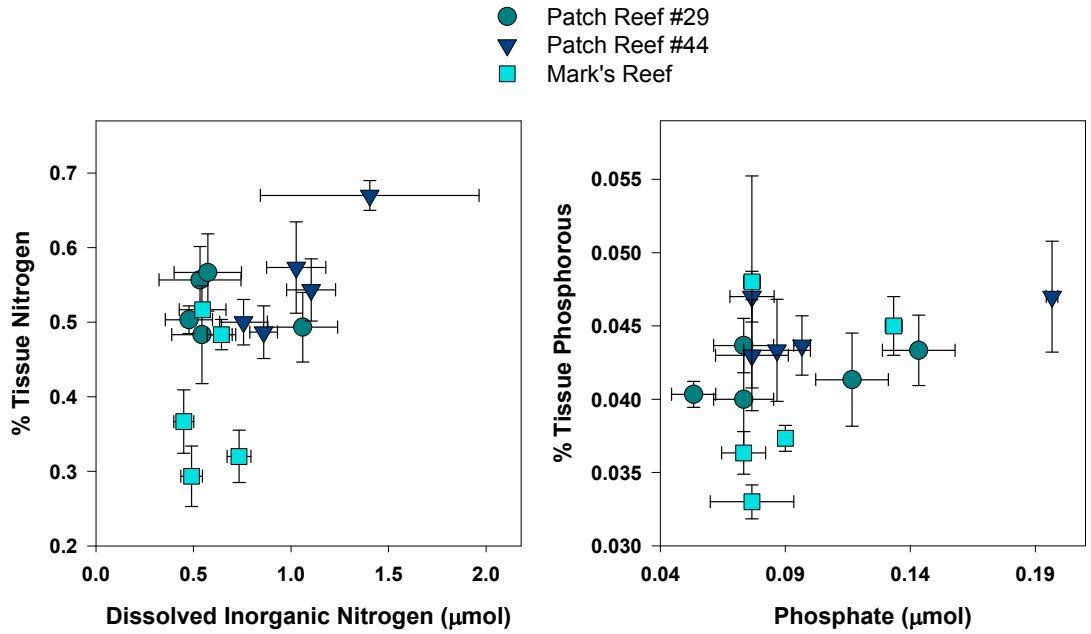
Herbivore abundance was negatively correlated with *Kappaphycus* abundance; fish herbivore biomass and density were lowest at Mark's Reef, which had the lowest amount of alien *Kappaphycus*.



Water column nutrients showed interesting trends over the one year study period, with highest levels of inorganic nitrogen and silicates being significantly higher at the reef (Patch Reef #44) with the highest levels of *Kappaphycus* abundance and regrowth rates.



Tissue nitrogen and phosphorous were significantly correlated with water column nitrate and phosphate levels, respectively.



invasive marine algae was constructed using the Microsoft program ACCESS©. One hundred and sixty-one records have been entered into the database to date. Currently, the database is still in development and testing phase. The small number of records in the database makes it difficult to draw conclusions that apply globally. However, based on the information currently in the database, a number of preliminary patterns can be observed.

With respect to the broad geographical distribution of invasions, the following table outlines the invasion occurrences for the records in the database (geographic areas are based on those defined in the "ASFA database user guide[1]."")

Geographic Area	Count	Percent	Regional Summary
Northeast Atlantic	25	15.53	
Northwest Atlantic	8	4.97	
Southwest Atlantic	1	0.62	65.22%
Mediterranean	71	44.10	
Northeast Pacific	20	12.42	
Eastern Antarctic	21	13.04	34.78%
Undetermined	15	9.32	
Total	161	100.00	

The "Undetermined" occurrences are primarily for the country of Australia. Depending on the exact location of the invasion, the coastline may fall into either the Eastern Antarctic or the Southwest Pacific. Consequently, the undetermined were occurrences were included with the other Pacific/Antarctic occurrences.

With respect to the taxonomic distribution of invaders, the following table outlines the number of invaders in each major taxonomic grouping for each of the three main macroalgal Divisions.

	Chlorophyta	Rhodophyta	Phaeophyta
Classes	2	1	2
Orders	4	12	11
Families	6	23	4
Genera	10	59	24
Species	22	106	34

Currently, there is insufficient information in the published record regarding the biological traits of many of the invasive algae to be able to determine trends or patterns that influence invasibility. More information needs to be entered into the database before trends in any of the three categories can be identified and a flowchart outlining invasive characteristics be completed.

[1] Alexander, Cheryl, 1980. Aquatic sciences and fisheries abstracts database user guide. National Oceanic and Atmospheric Administration, U. S. Dept. of Commerce. Rockville, MD

Public Outreach and Community Awareness

- **Printed materials**
  - brochures, fliers, posters, and t-shirts at volunteer events

- **Informational presentations**
  - Hanauma Bay Educational Seminar, Hawaii Aquatics Conference, CGAPS, MEGIS, ARCS, HIMB, LCC, DLNR, US ANSTF meeting, State Capitol, Hawaii Hotels Association, Kailua Bay Advisory Council; Workshops conducted for Sea Grant Reef Watchers and the National Park Service (Kona)

- **Community Sourcebook and K-12 Curriculum**—in review; beta-tested by 19 teachers at summer workshop

- **Clean-up events--**training of volunteers in algal identification and removal; media coverage; seven events conducted at Waikiki Marine Life Conservation District have utilized over 500 volunteers and removed over 35 tons of alien algae

Clean-up Event:	Volunteers	Trench	Beach	Total
24-Aug-02	62	1,995.20	411.60	2,406.80
21-Sep-02	82	2,693.10	391.40	3,084.50
16-Nov-02	105	2,648.40	2,344.80	4,993.20
1-Feb-03	118	2,478.40	3,128.60	5,607.00
5-Apr-03	120	2,762.70	1,648.00	4,410.70
17-May-03	54	2,034.64	2,447.70	4,482.34
13-Sep-03	220	--	--	6,609.90 kg
Total to date:	500+	14,612.44	10,372.10	31,594.44 kg 35 tons

Need for future work

- Examine other removal options e.g. mechanical suction device, urchin biocontrol
- Investigate effectiveness of native species enhancement after alien algae removal
- Continue to develop and promote outer island awareness of alien algae issues
- Continue to conduct volunteer clean-up events

VII. Evaluation

The proposed goals and objectives of this project were met as outlined in Section VI above in compliance with the contracted scope of service for the following tasks:

- Resurvey the abundance of alien and native algae and herbivores (fish and invertebrates) at 81 sites statewide.
- Generate detailed maps of alien algal species throughout the State of Hawaii to illustrate current distributions and rates of expansion and invasion of new areas.
- Assess reproductive status of each alien algal species at each site
- Compare heavily impacted areas to control sites to determine how alien algae are affecting native reef ecosystems.
- Determine effectiveness of experimental removal plots, and the influence of herbivores and nutrients on regrowth; measure water column and tissue nutrients relative to *Kappaphycus* abundance in Kaneohe Bay.
- Compile global database of invasive algae and design flow chart of biological attributes contributing to invasiveness.
- Develop training programs and educational materials regarding the control of alien and invasive algae species.

esults from this project were presented at 3rd International Marine Bioinvasions Conference on March 16-19, 2003:

- The impact of invasive algae on biodiversity and coral cover in Hawaii, Jennifer Smith.
- Developing a research-based management protocol for the invasive alga, *Kappaphycus alvarezii*, in Hawaii. Eric Conklin.
- Overview of alien algae issues in Hawaii. Cheryl Squair
- Removal and control of *Gracilaria salicornia* on Waikiki reef. Thomas Sauvage and Rebecca Most.
- Building community awareness and involvement in alien species eradication. Cynthia Hunter

Results were also presented by Jennifer Smith at the Annual Phycological Society of America on June, 2003 (Best Paper award), Hawaii Aquatics Conference-November, 2003, Western Society of Naturalists-November, 2003 (Best Paper award), Global Aquaculture Society Meeting-January 2004 (invited paper) and ASLO-February, 2004.

Two manuscripts have been prepared and submitted to scientific journals (included on CD report):

Smith, J.E., C.L. Hunter, E. Conklin, R. Most, T. Sauvage, C. Squair, and C.M. Smith. in press. The ecology of the invasive red alga *Gracilaria salicornia* (C. Agardh) E.Y. Dawson on O'ahu, Hawai'i. Pacific Science.

Smith, J.E., C.L. Hunter, E. Conklin, R. Most, T. Sauvage, C. Squair, and C.M. Smith Invasive Species on Coral Reefs: A New Threat to Diversity and Coral Health? in review. Science

In addition, education and outreach presentations have been made to over two dozen community groups, university classes, and the US Aquatic Nuisance Species Task Force. The website has been updated and includes the project description, maps of species distributions, site photographs, and links to other alien species sites: **http://www.botany.hawaii.edu/GradStud/smith/JENHOME.htm**

VIII. Signature of Principle Investigator

Cynthia L. Hunter, Ph.D. Date