# Development of methods leading to outplanting native reef algae.

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**Abstract.** Invasive algae are the principal threat to the health of native coastal ecosystems in Hawai'i. Healthy reefs sustain substantial populations of herbivorous reef fish and urchins. As numbers of herbivores have declined, resource managers employ tools that simulate massive herbivore grazing (Supersucker) or the release of generalist grazers (*Tripneustes gratilla*). We proposed a new tool that will lead to outplanting the dominant species of reef algae into places where community based cleanups will remove invasive biomass in Waikīkī and He'eia fishpond coastal regions. In Summer 2013, *Sargassum* spp. (limu kala) plants were collected and invertedly hung to allow for external fertilization to occur and resulting zygotes to settle on tiles that lined the bottom of the water bath. After 12 weeks of growth, plants grew to ~2 cm tall, demonstrating the potential for 'seeding' juvenile limu kala on substrata. In early 2014 we will repeat and refine this effort with 200 five $cm^2$ limestone tiles $site^{-1}$ seeded by hanging 20 fertile adults from a PVC rack placed in a 500 L, 1.5 $m^2$ fiberglass filled with flowing, filtered, low nutrient seawater in full sun. After 7 days, adults will be removed; seeded tiles will remain in the outdoor mesocosm with aeration and filtered seawater for ca. 2 months before outplanting. Outplanting in Waikīkī and He'eia fishpond is expected to enhance limu kala abundance leading to direct interactions with invasive algae and potential recruitment of other native species.

**Introduction.** Though the consequences of phase shifts are well known ecologically, phase shifts in Hawai'i have also resulted in loss of culturally significant marine native species. The reefs of the Waikīkī Marine Life Conservation District (MLCD) (*1-4*) and Kāne'ohe Bay (*2, 3*) are prime examples of well-studied native-dominated ecosystems now overwhelmed by invasive algae such as *Gracilaria salicornia* (*4, 5*). Impacts from *G. salicornia* include doubled sediment loading (*6*), the shading of many understory plants and potential reductions in numbers of native fish (*7*) and other diversity losses (*8*).

Recent surveys of the Waikīkī reef area, surprisingly, documented large ~100 $m^2$ patches of *Sargassum* spp. (limu kala), which had diminished markedly following the introduction of *G. salicornia*. This recent re-expansion of limu kala in Waikīkī suggests that the time is right to aggressively outplant natives. Outside of the He'eia Fishpond, where limu kala was once abundant, this alga is present but now less abundant. Historic changes in the Kāne'ohe Bay watershed may have made the environment less favorable. However, limu kala's persistence in Waikīkī and He'eia fuels interest in re-establishing this alga in both locations.

New steps and more accelerated efforts are urgently needed to restore native flora to these sites. Thus, over the short term, I propose to develop methods to use reproductive limu kala from source populations in Waikīkī and He'eia respectively, to seed limestone tiles with juveniles that will allow us to outplant to areas where these reef plants were once abundant (Figs. 1 and 2). Explicitly, our first step is to test: Will juveniles survive as assayed by several measures and remain attached to limestone tiles one week after outplanting?

## Methods.

<u>Rehabilitation of historic populations of limu kala: developing out-planting techniques:</u> Initial trials in outdoor mesocosms have successfully seeded limestone tiles with limu kala germlings:

- From a PVC rack in a 500 L fiberglass mesocosm, 20 fertile limu kala adults were hung over 400, 5 $cm^2$ limestone tiles per site.
- Mesocosms had flowing, filtered, low nutrient seawater .
- Gametes were shed in the water for external fertilization (*9*).
- After one week, adults were removed and seeded tiles remained in the outdoor mesocosm with aeration and filtered seawater for ca. 2 months.
- Every seven days, the tiles were randomly selected to monitor status, density, photosynthetic rates and growth for germlings > 1 cm tall.
- For my next cycle, plants will be allowed to grow to ~2 cm (primary axis). 200 tiles will be outplanted in Waikīkī and 200 tiles will be out-planted at He'eia. Remaining tiles (from each site) will be kept in the mesocosm system for further study of germling development

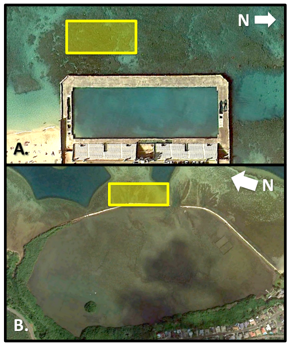


Figure 1. A. Waikīkī (south shore) experimental site. B. He'eia (Windward) experimental site.

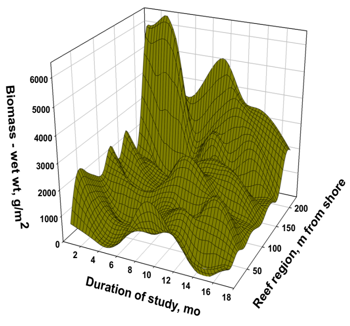


Figure 2. Limu kala biomass on the reef at the Natatorium, 12/67 - 4/69, prior to the introduction of G. salicornia (4).

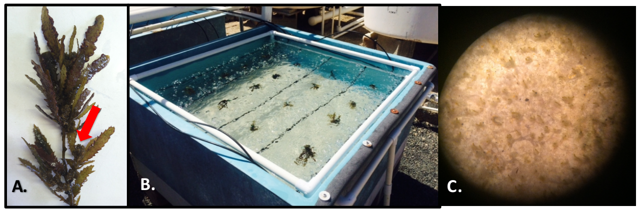


Figure 3. A. Adult S. aquifolium (limu kala); arrow points to a receptacle housing gametes. B. Outdoor mesocosm with reproductive limu kala hanging above limestone tiles. C. Limu kala germlings seen through a dissecting scope 4 weeks after initial settlement.

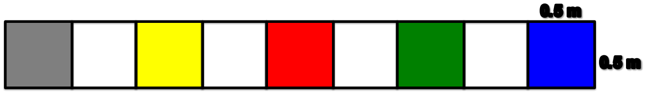


Figure 4. Rectangular factorial plot. Legend: [grey] cleared treatment; [yellow] reference treatment; [red] outplant reference treatment; [green] Limu kala cleared treatment (n=20); [blue] Limu kala uncleared treatment (n=20); [white] buffer zone. All treatments and buffer zones are 0.5 $m^2$.

<u>Rectangular Factorial Plots</u>: Following Smith et al. (*8*), we will use a randomized factorial block method (Fig. 4 below) at two locales (Fig. 1):

- Five (4.5m x 0.5 m) replicate factorial plots will be established in each site (Fig. 1).
- Each block will be laid out in areas dominated by *G. salicornia* and sited to insure similar flow regimes, perpendicular to plots. Treatments will be randomly arranged within each block. Outplants and removed biomass will be randomly placed in a 0.5 $m^2$ area divided into 25 equal units.
- The five treatments per plot include (Fig. 4):
  - i) unmanipulated reference.
  - ii) cleared treatment (all biomass removed from substrate).
  - iii) manual removal of invasives @ 20 subplot sites (unmanipulated treatment).
  - iv) 20 limu kala tiles (1960s density outplanted in cleared area).
  - v) 20 limu kala tiles outplanted with only invasive biomass directly surrounding the plants removed.
- Monthly measurements over 20 months, after initial outplanting, will be made to assess success. Two tiles / treatment / site will be removed to measure the size of outplants, number of limu kala / tile, and rates of photosynthesis for these plants.
- The Kahala reef will be monitored periodically, as a reference site.

**Expected Outcomes.**

- Initial expected outcomes are that juveniles will remain attached to limestone tiles and grow in stature.
- Intermediate expected outcomes include the survival of juveniles when in direct competition with invasive algae.
- Over the long term, the outplanting of limu kala will likely encourage the growth of other native species as well as the return of native fish and invertebrates that consume native plants.
- Finally, the success of outplanting approaches will establish robust methods that may be used during reef ecosystem restoration along other reefs with native species, here and elsewhere in the Pacific Islands.

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