Undaria pinnatifida: testing different methods of removal and the re-growth potential of an invasive kelp

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Abstract

Undaria pinnatifida is a kelp native to the shores of Japan, Korea, and China that has recently invaded the coastal waters of California. Discovered in Los Angeles harbors in 2000 this invader quickly expanded northward to Monterey Bay. It quickly became well established and thrived in Monterey Harbor. The invasion of *Undaria pinnatifida* presents a possible threat to native communities, particularly if it expands onto the open coast of Monterey Bay, which seems inevitable. We tested the re-growth potential of *Undaria* after manually removing the thallus either by cutting above or below the meristem, the primary zone of growth.

Introduction

Species Background

Undaria pinnatifida is a species of kelp indigenous to the shores of Japan, Korea, and China (Silva et al. 2002). It has been inadvertently introduced to the Atlantic coast of Europe and Mediterranean Sea (France, Spain, and England), Tasmania, New Zealand, Argentina, and Mexico (Zabin et al. 2009, Aguilar-Rosas et al. 2004). *Undaria pinnatifida* is a relatively small (<2.5 m long), brown alga possessing a small holdfast with numerous haptera, a moderately stiff stipe with a pinnate, wing-like blade, and at maturity a very distinctive and highly ruffled sporophyll just above the holdfast (Figure 1). The biphasic life cycle consists of a microscopic (haploid) gametophyte and macroscopic (diploid) sporophyte that persists for 2-6 months then dies (Thornber et al. 2004).

Possible explanations for the global dispersal of *Undaria* include three factors: (1) *Undaria* thrives in sheltered harbors and near many boats, and it may disperse by attaching to boats in the harbor (i.e. hull-fouling), (2) *Undaria*, which is highly fecund, can reproduce year-round in non-native habitats, which increases the chance of success in multiple transfers (Aguilar-Rosas et al. 2004, Thornber et al. 2004), and (3) *Undaria* has a high tolerance for a wide range of temperatures and salinity (Aguilar-Rosas et al. 2004).

Commonly known as wakame, *Undaria* is commercially grown in Asia for human consumption. In other parts of the world, *Undaria* is considered a fast-growing, undesirable invasive species. First discovered along the Pacific Coast of North America (Los Angeles) in March of 2000, *Undaria* rapidly spread northward to Monterey Bay by the summer of 2001 (Silva et al. 2002). In spring 2009 it was found as far north as San Francisco, occupying two harbors east of the Golden Gate Bridge (Zabin et al. 2009).

Figure 1. The Asian kelp *Undaria pinnatifida* attached to an experimental rack.

<u>Research Focus</u>

Undaria pinnatifida is an invasive species in Monterey Bay National Marine Sanctuary and efforts are underway to manage its spread. Effective management benefits from scientific evidence supporting the efficacy of potential management strategies. For example, what is the effectiveness of manually removing the thallus (i.e. main body) of *Undaria* either above or below the meristem (i.e. the main zone of growth)? This question serves as the focus of this research project.

When considering potential management strategies to control the growth and spread of *Undaria*, removal methods must be tested. It is important to understand *Undaria*'s growth patterns and ability to re-grow if it is not completely removed from the substrate. For example, if the blade were cut off, would the detached blade continue to grow? Would the holdfast, now lacking a blade, survive? Since the meristem is located basally, will survival and potential re-growth depend upon the presence of the meristem?

The meristem on *Undaria* is a dark area on the stipe between the holdfast and blade. Since the presence or absence of the meristem will likely impact the alga's ability to continue to grow, we conducted an experiment to follow the fate of both pieces of *Undaria* after cutting the thallus near the meristem. By cutting either above or below the meristem, we could track changes in both pieces to determine whether a blade with or without its meristem would grow, and if a holdfast would grow with or without its meristem. These data would then be used to inform manual removal strategies to control the growth and spread of *Undaria* in Monterey Harbor.

Materials and Methods

Two racks were built to support individual *Undaria* and keep samples in similar environmental conditions (e.g., depth and sunlight) throughout the experiment. The racks were attached to the floating dock and suspended 0.6 m below the surface, which kept samples well away from benthic herbivores (there are essentially no swimming herbivores in the harbor). The racks had two functions: (1) retain samples of *Undaria* and both cut portions and (2) provide easy access to the samples from the dock for repeated measurements.

<u>Construction and Placement of Sample Racks</u>

A one-meter piece of lumber (2 inch by 2 inch pine) served as the main axis for each rack. A 1.9 m long PVC pipe was screwed onto the main axis, creating a T shape (Figure 2).

Figure 2. One of the racks with samples *Undaria pinnatifida* attached.

The main axis was attached to the floating dock using a bolt and wing nut and the PVC rack was submerged about 60 cm below the surface. The rack could not be permanently attached to the dock because it needed to come to the surface for measuring purposes. A block of wood with a protruding bolt was nailed to the side of the dock and served as the anchor point for the main axis. A hole in the main axis about 8 cm from the top allowed the bolt to slide through. A wing nut held the entire rack in place but allowed the rack to be easily detached from the dock and brought to the surface to make measurements.

The function of the PVC pipe was to provide a means of attaching *Undaria* to the rack. 24 holes (12 on each rack) were drilled straight through the pipe 12.5 cm apart from each other. There were two methods in which the *Undaria* was attached; however, each method was used for a specific cut of kelp (holdfast or cut blade). Either a cable tie would loop through the PVC hole and hold down the stipe (between the holdfast and sporophyll) or a cable tie would puncture through the stipe of the blade and then loop through the PVC hole, thereby securing the cut blade to the PVC.

Once these racks were constructed they were placed on a floating dock off of Old Fisherman's Wharf in an area that had been approved by the harbormaster (H-tier). The floating dock moves up and down with the tide, so the rack remained at a constant depth 24 hr a day.

Divers collected samples of *Undaria* used in the experiment from the bottom of the harbor, in an area about 20 m from the experimental site. Divers collected whole *Undaria* of similar size and in relatively equivalent reproductive stages (juveniles were targeted). From the dozens of intact *Undaria* brought to the surface, 12 were haphazardly selected based on their total length, condition, and reproductive status (33-70 cm, healthy, and pre-reproductive, respectively).

Six of these *Undaria* were fastened to each rack, placed in every other hole and left for a week to acclimate.

<u>Cutting the thallus</u>

Cuts of the thallus were made in one of two places, either above or below the meristem. Along with the control, this represented five treatments:

The control (1)
A cut *below* the meristem producing:

a. A blade with a meristem (2)
b. A holdfast with no meristem (3)

A cut *above* the meristem producing:

c. A blade with no meristem (4)
d. A holdfast with a meristem (5)

Four controls were left undamaged. The remaining holes on the PVC pipe were filled with one of the possible cut pieces of *Undaria* (i.e. treatments 2, 3, 4, or 5). The cuts were made after a week of acclimation and confirmation that the *Undaria* was alive. The cuts were made using a putty knife, which made a clean cut.

After making the cuts subsequent measurements and data were collected as follows:

1. Total length (cm), which differed based on the treatment. For controls it was from base of the stipe to tip of the blade, for blades it was from the cut to the tip of the blade, and for holdfasts it was from the based of the stipe to the cut. This was done with a cloth measuring tape.
2. Stipe width, measured in mm. This was done with a caliper and measured according to the particular cut. For example, the treatments that had the blade, width would be measured between the sporophyll and the start of the blade, whereas, the treatments that had only a holdfast, width was measured just before the sporophyll.

3. The status of the sporophyll, from absent in juveniles to fully mature in reproductive adults.
4. A qualitative estimate of deterioration, making notes as to what showed signs of deterioration.

Observations began June 23, 2010 and ended August 2, 2010. During the first three weeks we checked the racks twice a week, but increased that to three times a week as the *Undaria* began to grow more quickly. We sampled the racks a total of 10 times over 48 days. During this time, 9 *Undaria* were lost and not replaced.

To measure the *Undaria* treatments, the racks were pulled up individually and placed on a hard surface so the *Undaria* could spread out. We measured total length and stipe width and the presence/absence of a sporophyll was noted, as were signs of deterioration or anything remarkable. After each piece was carefully measured and recorded, we took a digital picture of each *Undaria*. These pictures served as a permanent record of each individual and allowed visual comparisons through time. Then racks were replaced slowly back into the water (to avoid ripping *Undaria* from the rack) and re-fastened to the floating dock with the washer and wing nut.

Holes on the racks were numbered consecutively 1-12 (rack 1) and 13-24 (rack 2). Below is a list of what was attached at each hole. *Undaria* were left in place for one week prior to applying the treatment (cuts), and were originally placed in every other hole, thereby allowing the cut piece (i.e. the "other piece") to be fastened adjacent to the already attached holdfast.

1. Control
2. – empty
3. Cut Below
4. Cut below's other piece (from 3)
5. Cut Above
6. Cut above's other piece (from 5)
7. Control
8. – empty
9. Cut Above
10. Cut above's other piece (from 9)
11. Cut Below
12. Cut below's other piece (from 11)
13. Control
14. – empty
15. Cut Below
16. Cut below's other piece (from 15)
17. Cut Above
18. Cut above's other piece (from 17)
19. Control
20. – empty
21. Cut Below
22. Cut below's other piece (from 21)
23. Cut Above
24. Cut above's other piece (from 23)

Results

The primary focus of the experimental treatment was to determine whether a holdfast that lost the blade would continue to grow under two specific situations: when the meristem remained intact and when the meristem was removed with the blade (cut above and cut below, respectively). We hypothesized that the cut pieces lacking the meristem would not grow. Alternatively, cut pieces retaining the meristem would continue to grow and potentially develop a sporophyll. A secondary focus was to determine the fate of the detached blade, both without the meristem (cut above) and with the meristem intact (cut below). Current manual removal methods call for divers to bring the thallus to the surface for disposal in a landfill, however if the cut pieces were not viable, this material could be released into the harbor to senesce or be consumed by herbivores and detritivores.

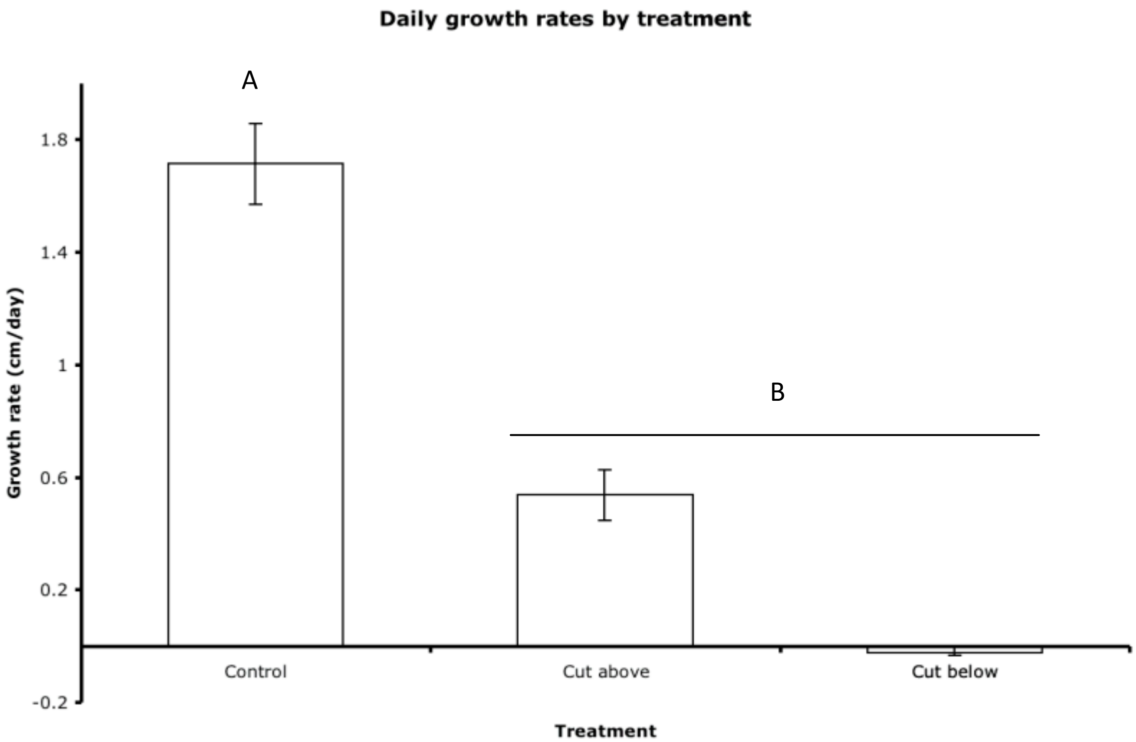

Figure 3. Mean growth rates (cm/day) for three *Undaria* treatments: control (undamaged), cut above (blade removed but meristem remains) and cut below (blade and meristem removed). Growth was significantly higher for control *Undaria* compared to those either cut above or below the meristem.

When comparing daily growth rates, it should be noted that "growth" is relative, since as the *Undaria* grew (addition of tissue at the base) it also eroded at the tip (oldest tissue). Thus, growth as measured by total length is a net change, where growth and loss are occurring simultaneously. Daily growth rates (total length in cm per day) were compared for three treatment categories (control, cut above, and cut below) using a single-factor ANOVA, which indicated the mean daily growth rates were significantly different ($P<0.001$, Figure 3). Control *Undaria* grew an average of 1.71 cm/d (0.144 SEM), which was significantly higher than the other two treatments. *Undaria* cut above the meristem grew an average of 0.54 cm/d (0.089), which was not unexpected since the meristem remained intact. This treatment is similar to wholesale removal of the blade by an herbivore. *Undaria* cut below the meristem either did not grow or decreased in size, yielding an average growth rate of -0.02 cm/d (0.014). These *Undaria* were not expected to show signs of growth since the meristem was absent.

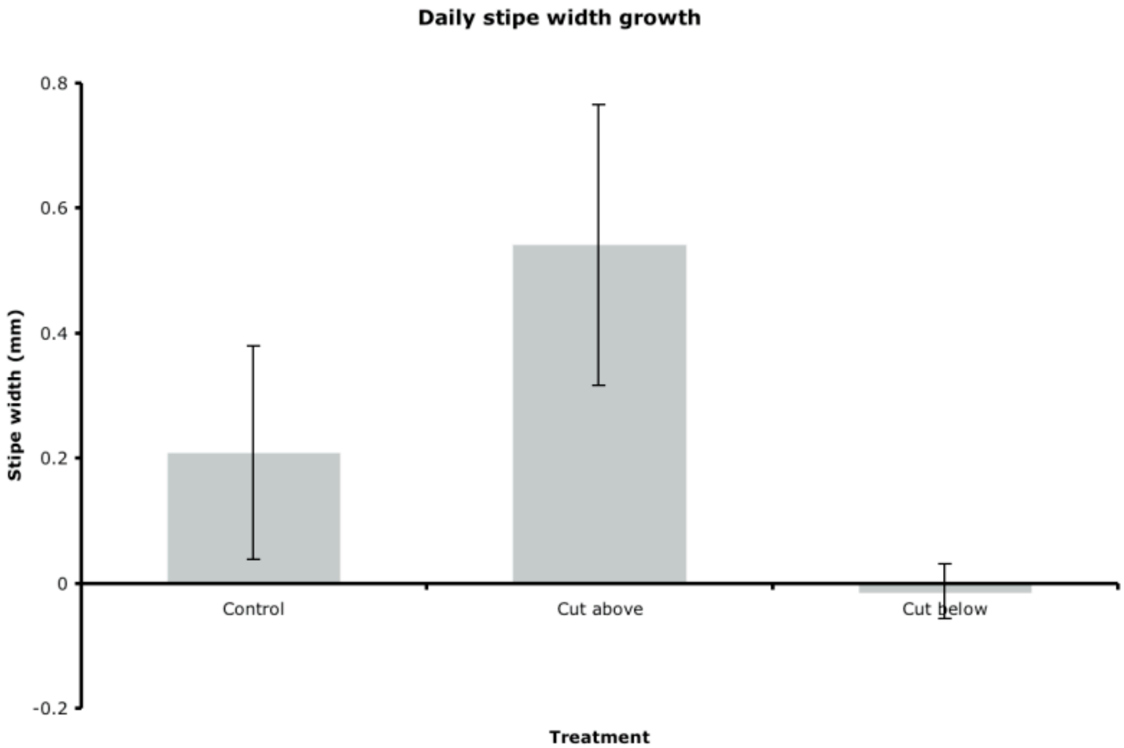

Figure 4. Average daily increase in stipe width for three *Undaria* treatments: control (undamaged), cut above (blade removed but meristem remains) and cut below (blade and meristem removed). Differences were not significant (ANOVA, P=0.102)

The average daily growth of the stipe width, measured in mm, was calculated for each of the three sample categories – control, above cut, and below cut for each replicate (Figure 4). Control *Undaria* stipe widths increased an average 0.21 mm/d (0.171). *Undaria* cut above the meristem increased in width an average of 0.54 mm/d (0.225), which was the largest of the three treatments. *Undaria* cut below the meristem decreased in width an average of -0.01 mm/d (0.044). An ANOVA test indicated there was no significant difference in stipe growth among the treatments (P=0.102).

Blades that were attached to the racks were fragile. Although part of the experimental design was to track the fate of blades with and without the meristem intact, most of the blades were too fragile to remain attached to the rack and fell off during measurements. Qualitatively, none of the blades with a meristem showed signs of growth. Blades lacking a meristem began to senesce almost immediately.

Discussion

Cutting the thallus of *Undaria* above the meristem is not an effective means of manual removal since the remaining holdfast and intact meristem can continue to grow. Although none of the treatments were left in the harbor long enough to re-grow a blade or develop a sporophyll, the potential exists. Total length of control *Undaria* increased, on average, 1.7 cm per day, which was 3x greater than holdfasts with a meristem but lacking a blade (cut above meristem), and holdfasts lacking a meristem (cut below) decreased an average of 0.02 cm per day, showing no signs of growth whatsoever. It appears that while growth can continue if a cut is made above the meristem, the catastrophic loss of the blade either reduces the rate of growth, perhaps through a reduction in nutrients and metabolic products necessary for growth, or the rate of growth for the remaining tissue is inherently lower than the blade. It should be noted that after cell addition at the meristem, there is continued cell elongation as these cells mature, which also contributes to total length of the thallus. However, measurements of total length account for both the addition and expansion of new cells, as well as the loss of older cells near the apex, due either to senescence or abrasion, or even herbivory.

The stipe width of control *Undaria* increased, on average, 0.21 mm per day, which was less than half the rate of holdfasts with a meristem but lacking a blade (cut above meristem, 0.54 mm per day). Holdfasts lacking a meristem (cut below) decreased an average of 0.01 mm per day. The growth of the controls was not significantly different from the experimental treatments. It is unclear why the width increased more for the treatment cut above the meristem than for the undamaged control.

The results of this experiment refute the null hypothesis that cut *Undaria* would not be able to grow. However, there was a difference between the treatments: holdfasts retaining the meristem could grow, but those lacking the meristem senesced. This pattern was expected for the meristem to grow up from the holdfast rather than down from the blade. Cellular growth happens in specific directions and for different purposes, and the cellular growth for *Undaria* grows up from the holdfast into a blade. The next step for this project could be a deeper look into other factors and/or variables affecting growth and invasion success, such as temperature, depth, water movement, and nutrient availability.

Improvements

We had many misfortunes with the racks and the techniques used to tie the *Undaria* to them. For the racks we used wood as the link between the dock and the *Undaria*, which proved to be problematic. The wood swelled prohibiting the wood to fit easily over the screw. The screws rusted and the screw on the second rack fell out of the bolted block of wood. Thus, on extraction of the second rack we would have to pull out the entire screw along with the rack. The manner in which we tied the *Undaria* to the racks also leaves room for great improvement. We used cable ties that worked well when the holdfast was present, because the holdfast offered a grip for the rest of the sample. But in the cases where only the blade was present we punctured a hole through the stipe with the cable tie and used that same cable tie to tie to the rack. The weight of the blade caused the cable tie to rip right through the stipe. Many blades were ripped off the rack during simple extraction from the water. We tried to tie a larger portion of the blade with a twisty-tie (ones used on loafs of bread), but to no avail. We lost several samples within the first few weeks of recording due to this quandary. A new method of attaching the *Undaria*, specifically the blades, would greatly improve the project.

Conclusions

If the situation ever called for removal of *Undaria* in the western part of the United States (primarily California), the research from this project would provide information on how to effectively remove the species. Cutting below the meristem was enough to cause decomposition in both pieces (without re-growth). Cutting above the meristem still allows the holdfast to grow and potentially develop a sporophyll, which undermines the purpose of the removal effort. Since removing the thallus below the meristem is faster than removing the entire thallus, this method is faster than current techniques and reduces disturbance to the substrate. Speeding up the process maximizes diver time under water, a considerable logistical constraint to the management of *Undaria* in Monterey Harbor.

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