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Assessing the Invasiveness of the Non-Native Kelp Undaria pinnatifida in Monterey Harbor and Implications for Its Management

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To the SEP Faculty:

Invasive species have been documented to be detrimental to native communities, often through outcompeting native species for resources such as light, space, and nutrients. Due to the harmful effects of invasives, these species are often well-documented, and the invasive kelp *Undaria pinnatifida* is no exception. *U. pinnatifida* is the only federally-declared invasive kelp, and has spread along the west coast of North America from its native range in Asia. In Central California, *U. pinnatifida* is a species of concern, and programs such as the *Undaria* Control and Removal Program organized by the Monterey Bay National Marine Sanctuary, seek to monitor, control and understand the spread and impacts of this species. Though much work has been devoted to mapping and monitoring *U. pinnatifida*'s spread along the California coast, very few studies have examined the reproductive physiology of this species. The reproductive physiology of a species determines its ability to complete its life cycle and survive in a particular location, and in kelps is often determined by abiotic factors such as temperature and nutrient availability. In response to this issue, my scientific inquiry honors capstone project posed the following questions:

1. What is the relative monthly reproductive (zoospore) output of *U. pinnatifida* and will there be a difference in sporophyte production between two temperature treatments throughout the year?
2. What is the importance of nitrate availability and temperature on *U. pinnatifida* microscopic stage development?
3. Is there a relationship between reproductive output and adult sporophyll size or vegetative blade length?

In order to address the first two study questions I cultured *U. pinnatifida* zoospores monthly for a year under variable temperature and nitrate (a limiting nutrient in marine systems) treatments in the lab to see how or if reproductive success (microscopic stage production, particularly the microscopic sporophyte) was affected. To address the final study question, I collected field data size measurements, such as blade length and sporophyll size, and took reproductive tissue samples from live individuals in Monterey Harbor. Data collected on *U. pinnatifida* by Brynn Hooton from Moss Landing Marine Labs for her masters' thesis were also utilized.

This project was designed to be useful to local policy-makers, students and faculty at marine research laboratories near invasion sites, and the other major stakeholders present in Monterey Harbor: boat owners and species who are possibly being excluded by *U. pinnatifida* invasion. Boat owners in particular need to receive information about the importance of de-fouling boat hulls, particularly if they are going from Monterey Harbor to another harbor along the coast, as boat hulls are viewed as the most likely method of transportation for *U. pinnatifida* along the west coast.

The analysis of these data is primarily intended to inform current and future policies regarding the removal and control of *U. pinnatifida*, particularly in California. Before starting this project, I had some preconceptions that my results would indicate that *U. pinnatifida* is a condition-flexible alga which allows it to colonize and dominate in locations with conditions different than

its native range. I collected a variety of data, in an attempt to either prove or disprove this preconception. The simplest way I worked toward avoiding bias in my data was to wait until I was finished collecting it before I analyzed or graphically presented it, thereby decreasing the temptation to skew data in a certain way. In the end, certain elements of my project supported my original hypothesis about the reproductive physiology of this species, while other elements were inconclusive.

I have many values associated with this project that have influenced the way I designed and collected data. I have spent many years studying and being interested in ocean health and the multitude of threats to it. Though invasive species are an often overlooked threat to oceans, it is particularly relevant to the Monterey Peninsula with regards to the *U. pinnatifida* monitoring and removal program. Invasive species are often cited to be detrimental to native species, and this project was designed to assess one aspect of this species' invasive nature. Although *U. pinnatifida* is a species of concern for outcompeting native species where it occurs, the actual impacts to native species is not well understood; I believe more information on this species is needed in order to understand the specific threat posed to native species in Monterey Harbor.

Sincerely,

Sarah Jeffries

Assessing the invasiveness of the non-native kelp *Undaria pinnatifida* in Monterey Harbor and implications for its management

A Capstone Project

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Abstract

The annual subtidal alga *Undaria pinnatifida* has been federally declared an invasive species and has spread rapidly across the globe from its native range in northern Asia. The ability of this alga to complete its life cycle determines its success in a particular location, and several abiotic factors have been found to be important in determining reproductive success in kelps. Laboratory zoospore culture experiments were conducted monthly to test the effects of temperature and nitrate concentrations on microscopic stage production throughout a year. Cultures were grown under two temperatures (12, 18º C) monthly and three nitrate concentrations (1,5,10 μmol) three months during the year. Each month sporophytes were produced in both temperatures, and densities were either higher in the 18ºC treatment or there was no difference between temperatures. Sporophytes were also produced in all nitrate treatments, but there was no consistent nitrate effect observed. Finally, field measurements and sporophyll punches were obtained to test the relationship between zoospore output and the physical features sporophyll size and blade length for *U. pinnatifida* in Monterey Harbor. These results revealed a non-linear relationship for individual plants, and a linear relationship at a population (average) level. Visual predictors of the reproductive status of an individual that can be used *before it is removed* (trauma can cause zoospore release) is essential to the success of programs seeking to avoid the further spread of this species, such as the Monterey Bay National Marine Sanctuary *Undaria* program. Alternately, information regarding abiotic influences on *U. pinnatifida* is important as microscopic stage production is vital in supporting future cohorts. Similar culture experiments have been conducted on other native central California kelp species, a majority of which were unable to produce sporophytes in all treatments. This suggests that *U. pinnatifida* is a condition-flexible alga whose reproductive physiology allows it to enter and thrive in new areas. The methods developed and used in this study should be implemented in other locations throughout *U. pinnatifida*'s range in order to inform local management efforts and provide a more complete understanding of the ability of this species to continue to spread.

Introduction

Undaria pinnatifida is an annual subtidal kelp species (of the family Alariaceae) indigenous to Japan, northern China and Korea (NIMPIS 2002, Silva et al. 2002), which has spread throughout the world's oceans over the past two decades (Casas et al. 2004, Zabin et al. 2009). In its native Japan, *U. pinnatifida* is farmed and sold commercially as a food source known as wakame (Silva et al. 2002). Wakame is one of the most popular seaweeds in Japan and East Asia, and is prepared in dishes such as wakame soup and seaweed salad (Yamanaka and Akiyama 1993, Silva et al. 2002). It may be due to this economic value that *U. pinnatifida* has been transported across the globe for cultivation, in combination with other factors such as accidental transport in aquaculture or as fouling on boats (Silva et al. 2002).

It is because of the rapid spread of *U. pinnatifida,* along with its ability to establish itself, thrive and dominate in new locations, that it has been declared one of the "100 worst invasive species" by the Global Invasive Species Database (Lowe et al. 2000), and classified as the only invasive kelp (Ruiz et al. 2000). In California, *U. pinnatifida* was first introduced to Los Angeles Harbor (Cabrillo Beach) in 2000 and has been found primarily in harbors, including Port Hueneme (2000), Santa Barbara (2001), Monterey (2001) and most recently San Francisco (2009) (Silva et al. 2002, Zabin et al. 2009). It has spread rapidly up the coast of California and has dominated in locations where it was introduced.

Invasive species can be detrimental to native communities (Alpert et al. 2000). According to the National Invasive Species Council (NISC), an invasive species is defined as "an alien species whose introduction does or is likely to cause economic or environmental harm or harm to human health" (NISC 2008). The "invasiveness" of a species is defined as the traits that make a species likely to invade (Alpert 2000). Many studies have found that sources of disturbance, natural or human-caused, play an important role in the establishment of invasive species (Alpert et al. 2000, Casas et al. 2004, Valentine and Johnson 2003). *U. pinnatifida* is an opportunistic species that takes advantage of frequent disturbance in areas such as harbors to establish itself in native

communities and may crowd out native seaweeds in several locations worldwide (Hay and Luckens 1987, Zabin et al. 2009). *Undaria pinnatifida* growth, seasonality and life history in both native and invaded environments has been studied extensively, though only Thornber et al. (2004) and Kohtio (2008) have specifically conducted studies on California populations in Santa Barbara and Monterey harbors, respectively. Studying the life histories of invasive species is important to understanding how native communities can be affected by them.

The life history of a kelp begins with a diploid macroscopic sporophyte that releases haploid microscopic zoospores, which settle onto the substrate and germinate (Hay and Luckens 1987). Germinated zoospores develop into male and female gametophytes, which, in *U. pinnatifida*, have been found to be extremely tolerant of a large range of temperatures (Hay and Luckens 1987, Thornber et al. 2004, Kohtio 2008). *U. pinnatifida* has a unique life history in that its growth and reproduction are cued by temperature, unlike other kelps which are primarily cued by daylength. This unique adaptation may have evolved due to the reliable water temperature shifts throughout the year in its native range of the Sea of Japan (Saito 1975, Stuart et al. 1999).

It is likely due to their high temperature tolerance, that *U. pinnatifida* gametophytes are present during the summer months rather than the winter months, unlike all other kelps, and can lay dormant for long periods of time (Saito 1975, Kohtio 2008). Finally, these male and female gametophytes will fertilize one another and develop into microscopic sporophytes, which will go through stages of cell division to eventually become the macroscopic sporophyte (Hay and Luckens 1987). The macroscopic *U. pinnatifida* sporophyte consists of a vegetative blade bisected by a midrib, and a reproductive sporophyll; the sporophyll attaches to the substrate by a holdfast (Pfister 1994, Stuart et al. 1999). The life history of the macroscopic kelp sporophyte consists of rapid vegetative growth during the first portion of its life, followed by a period of senescence where the blade rots away and sugars created during photosynthesis are pulled from the blade back into the sporophyll, allowing reproduction to become the main focus of resources for the kelp (Pfister 1992, Kohtio 2008).

As a federally declared invasive species and one of the "100 worst invasive species" cited by the Global Invasive Species Database (Lowe 2000), management of *U. pinnatifida* falls under the jurisdiction of the National Invasive Species Council (NISC). The NISC was created by Executive Order (EO) 13112 of 1999. This council is co-chaired by the Secretaries of the Interior, Agriculture and Commerce. NISC members include the Secretaries of Transportation, State, Defense, Homeland Security, Treasury, Health and Human Services, the Administrators of the Environmental Protection Agency and the National Aeronautics and Space Administration, the director of the US Agency for International Development and the US Trade Representative. EO 13112 charged this newly created council with the planning, coordination and overall leadership of invasive species programs across the country (NISC 2008). The *U. pinnatifida* removal program organized by the Monterey Bay National Marine Sanctuary (MBNMS) is one such program. This program organizes volunteers in a series of *U. pinnatifida* removal events in Monterey Harbor and several locations within San Francisco Bay (MBNMS 2008). Though this program is a concentrated effort by many institutions including the Monterey Bay National Marine Sanctuary, Elkhorn Slough Estuarine Research Reserve, California Department of Fish and Game, the City of Monterey, Moss Landing Marine Labs, the University of California at Santa Cruz, and many generations of volunteers, the effectiveness of such a removal regiment has not been sufficiently established (Lonhart 2003). It has been proposed that complete eradication of populations of *U. pinnatifida* may be impossible due to the vast number of zoospores released by a single kelp sporophyte throughout its lifetime, the rapid growth of both the microscopic and macroscopic stages, and the ability of the microscopic gametophytes to lay dormant for prolonged periods of time (Saito 1975, Silva et al. 2002, Kohtio 2008).

The purpose of this study was to increase understanding of the population dynamics and reproductive ability of this species in order to inform control and removal programs such as the one organized by the MBNMS about indicators of reproductive status pre-removal. The laboratory portion of this study followed the life history of *U. pinnatifida* microscopic stages through development under different temperature and nutrient

(nitrate) regimes, monthly throughout the year. The effects of different nitrate concentrations and temperature regimes were examined in order to (1) test the sensitivities of this species to different ambient conditions at different points throughout the year, (2) to identify period(s) of peak reproduction, and (3) to test if there is an elastic seasonal response to temperature evidenced by differences in sporophyte production between temperature treatments.

Methods

Objective 1: What is the relative monthly reproductivity of *U. pinnatifida* and will there be a monthly difference in sporophyte production between two temperature treatments throughout the year?

> Hypothesis 1: There will be a difference in reproductive output of *U. pinnatifida* between months.
> H_o: Reproductive output of *U. pinnatifida* will be constant between months.
>
> Hypothesis 1: There will be a difference in sporophyte production between temperature treatments throughout the year.
> H_o: There will be no difference in sporophyte production between temperature treatments.

The reproductive capabilities of *U. pinnatifida* in response to water temperature were tested monthly throughout the year. It was also assessed whether *U. pinnatifida* sporophytes are produced in different densities between warmer (18°C) and cooler (12°C) temperature treatments throughout the year.

Using reproductive tissue taken from individuals harvested from Monterey Harbor each month, laboratory cultures of *U. pinnatifida* zoospores were created. When mature (reproductive) adults were present in the harbor, the sporophylls of these individuals were brought back to the lab for spore release. In order to control diatom and bacterial growth in culture, sporophylls were rinsed in a 1% iodine solution for approximately 30 seconds before being scrubbed and placed between dry newspaper sheets. The cleaned sporophylls were stored in a dark room at 10°C for approximately 8-12 hours. After storage in the cold room, 3 hole punch size plugs from each sporophyll were cultured separately in the 12°C incubator in order to quantify zoospore output. Punches were left in the petri dishes for 24 hours before being removed, and zoospore settlement counts were

made (10 fields of view at 400x magnification) and averaged to represent zoospore output (adapted from Kinlan et al. 2003).

The remainder of the sporophylls was submerged in room temperature sterile seawater which induced zoospore release from the sori (zoospore-producing tissue). The sporophylls remained in the seawater for 45-60 minutes after which time the spore solution was strained through 10μm mesh to remove extraneous particles and to break up clumps of zoospores. Number of zoospores per mL solution were approximated using a medical hemocytometer at 400x magnification and variable amounts of spore solution was added to 14 Petri dishes (7 per temperature treatment) to reach the desired density of zoospores per dish (~60,000 spores per dish) using sterile seawater for dilution. These dishes were placed into 12°C and 18°C incubators and allowed to sit for 3 hours (after 3 hours, most viable zoospores had settled to the bottom of the Petri dishes). After 3 hours, the sterile seawater in the dishes was replaced with enriched seawater (Provasoli) containing 10μmol nitrate (a saturating amount in central California).

The zoospore cultures were counted for zoospore settlement after 24 hours, and after 48 hours the same cultures were counted to approximate spore germination. One week after setting up the cultures, the dishes were counted for gametophyte stage survivorship and densities of these were recorded. After the 1-week survivorship counts, the dishes were monitored for sporophyte production, but no further density counts were taken until sporophytes were found. Once sporophytes were seen in a dish, sporophyte density counts were taken after one week. In the second week after sporophytes are found, density counts were taken again. After final density measurements were made, dishes were discarded.

All recorded density measurements were converted from per field of view to per mm^2 to be useful in analysis. Within months, average densities per mm^2 of soral tissue were calculated and 2-sample t-tests were run between sporophyte production densities in the 12°C and 18°C incubators to test for significant differences

between temperatures. For an *in situ* comparison to laboratory temperature values (12ºC and 18ºC), a temperature logger (StowAway Tidbit) was placed in the outer harbor for the latter portion of this study.

Objective 2: What is the importance of nitrate availability and temperature on *U. pinnatifida* microscopic stage production?

> Hypothesis 1: There will be an effect of nitrate availability on *U. pinnatifida* microscopic stages and fertilization success.
> H_o: There will be no effect of nitrate availability on *U. pinnatifida* microscopic stages and fertilization success.
>
> Hypothesis 2: There will be an effect of temperature on *U. pinnatifida* microscopic stages and fertilization success.
> H_o: There will be no effect of temperature on *U. pinnatifida* microscopic stages and fertilization success.

The role played by nitrate concentrations and different ambient temperatures in inhibiting or supporting production of the microscopic stages of *U. pinnatifida* (zoospore settlement, germination, gametophyte and sporophyte production) was assessed.

Culturing methods and setup were similar to those described above, except there were three nutrient treatments (1, 5, 10µmol nitrate) with 7 replicates each per temperature treatment (12°C, 18°C), making a total of 42 dishes for this experiment.

Microscope methods were also similar to those detailed above, but all stage counts consisted of 15 fields of view at 400x magnification. The entire multi-nutrient treatment experiment was conducted three times in order to attempt to eliminate unrepresentative sources of error.

All recorded density measurements were converted to per field of view to per mm^2. Two-way ANOVAs were used to test for significant differences in microscopic stage production between temperature and nutrient treatments with Fisher's LSD post hoc comparisons tests to identify sources of significance.

Objective 3: Is there a relationship between reproductive output and adult sporophyll size or vegetative blade length?

Hypothesis 1: There is a relationship between zoospore output and sporophyll size.
H_o: There is no relationship between zoospore output and sporophyll size.

Hypothesis 2: There is a relationship between zoospore output and vegetative blade length.
H_o: There is no relationship between zoospore output and vegetative blade length.

The relationship between zoospore output (an indicator of reproductive ability) was compared to sporophyll size and vegetative blade length using field data to search for a visual indicator of reproductive ability in mature *U. pinnatifida* individuals.

Random *U. pinnatifida* adults were measured *in situ* for sporophyll size (length and width) and vegetative blade length, and a sample of soral tissue from each individual was taken to estimate zoospore output. Soral punches were obtained using a single-hole punch, and punches were separated into sections of a 7-day pill container before being taken back to the lab and cultured using the same methods outlined above under Objective 1. These data were collected for three months (July to September, 2010). Individual reproductive output (found by culturing soral punches as detailed under objective 1) was compared to individual blade length and sporophyll size (found by using length and width measurements and calculating the area of an ellipse). Also, average monthly reproductive output (data collected under objective 1) was compared to average monthly blade length of reproductive individuals from the harbor (data collected by Brynn Hooton from MLML for her masters' thesis).

Linear regressions with r^2 values were created to detect a relationship between individual blade length/sporophyll size and individual zoospore output, and between average monthly blade length and average monthly zoospore output.

Results

Monthly Reproductivity

Measurements for zoospore output changed during the course of this study; from November 2009 to May 2010, zoospore measurements were made using hemocytometer counts to approximate number (thousands) of zoospores per mL of zoospore solution. From June to October 2010, sporophyll punches were cultured and used to approximate number of spores (thousands) per mm^2 of soral tissue. Reproductive output of *U. pinnatifida* in Monterey Harbor (measured in thousands per mL/mm^2) varied monthly from November 2009 to October 2010, with two distinct peaks in March-May and September 2010 (Figure 1). No reproductive adults were found in the harbor between December 2009 and February 2010, therefore zoospore culture experiments could not be conducted during that period, and zoospore output was zero for those months.

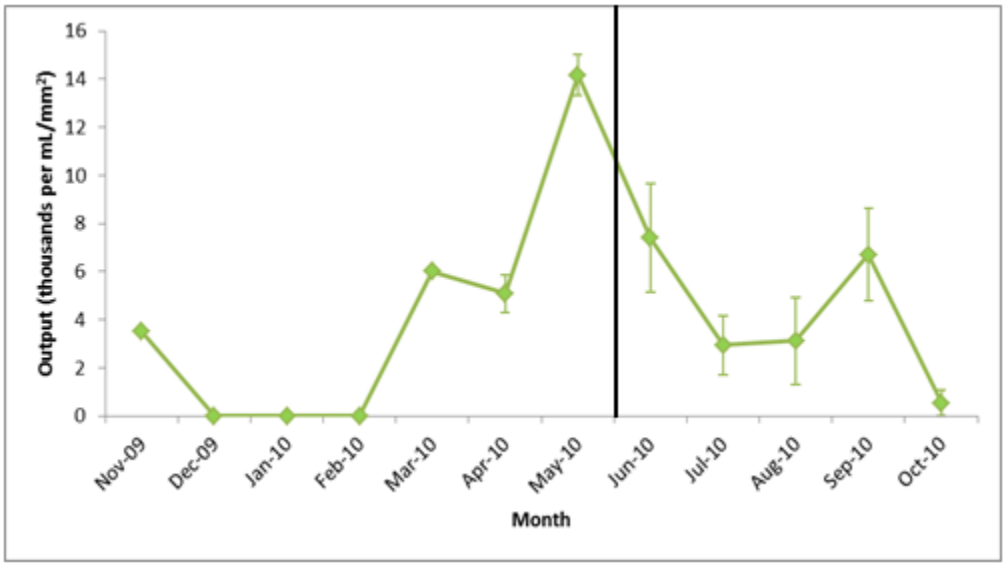


Figure 1: Monthly zoospore output measured as thousands per mL from November 2009 to May 2010, and thousands per mm^2 of soral tissue from June 2010 to October 2010, the change in measurement indicated by the bold line. Error bars are ± standard error (SE).

Water temperatures in Monterey Harbor at 0.5m water depth (Figure 2) were taken from March-December 2010. The measured water temperatures ranged between the two laboratory treatment temperatures (12°C and 18°C), averaging at ~16°C. Temperatures dropped below 12° in May and November-December 2010 but never

exceeded 18°C. This field data verifies that the laboratory temperature settings are accurate representations of minimum and maximum temperatures that *U. pinnatifida* experiences in Monterey Harbor.

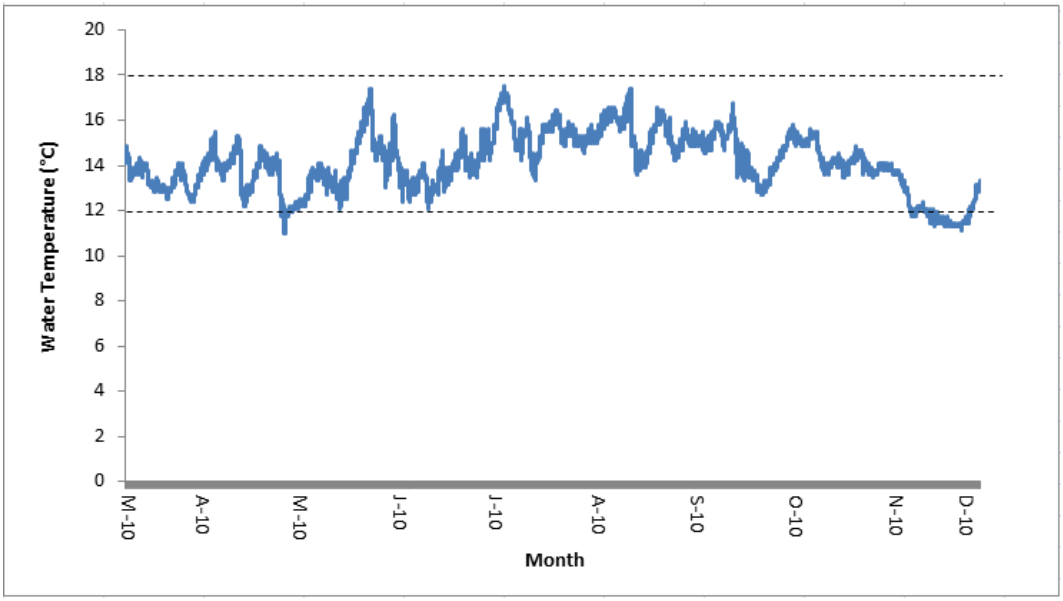


Figure 2: Ocean temperatures in Monterey Harbor between March 2010 and December 2010 at 0.5m water depth. Dashed lines indicate laboratory temperature settings.

Sporophytes were produced in the lab in both temperature treatments in all months when reproductive individuals were present at the study site. These experiments either yielded significantly higher sporophyte densities in the warmer temperature (April, July, September and October 2010) or a non-significant difference between the two temperature treatments (November 2009, March, May, June and August 2010); sporophyte densities were never greater in the 12°C treatment. In addition, sporophytes were observed in the 18°C treatment an average of a week earlier than sporophytes in the 12°C treatment.

Table 1: Average sporophyte production densities for each temperature treatment throughout the year with their respective standard error (SE) values. P-values were calculated using 2-sample t-tests, and significant values are highlighted in red.

Month	Nov-09	Dec-09	Jan-10	Feb-10	Mar-10	Apr-10
12°C density ± SE	8.54 ± 1.27	0	0	0	12.44 ± 2.17	0.94 ± 0.31
18°C density ± SE	4.75 ± 1.04	0	0	0	11.18 ± 2.08	8.08 ± 2.22
p-value	0.206	N/A	N/A	N/A	0.224	**0.008**

Month	May-10	Jun-10	Jul-10	Aug-10	Sep-10	Oct-10
12°C density ± SE	0.88 ± 0.24	7.22 ± 2.02	4.28 ± 0.36	3.7 ± 1.06	1.43 ± 0.19	2.23 ± 0.42
18°C density ± SE	2.63 ± 0.38	6.27 ± 1.13	9.65 ± 1.88	9.46 ± 3.03	9.78 ± 1.99	19.37 ± 2.18
p-value	0.232	0.955	**0.024**	0.086	**<0.001**	**0.002**

Though both zoospore output (per mL/mm^2) and sporophyte production (per mm^2) varied monthly, there was no obvious relationship of zoospore output with either temperature (Figure 3). Sporophyte production densities were higher in the 18°C treatment, but there was greater variation in the relationship between output and sporophyte density in that temperature, resulting in a linear relationship that explained only 0.09% of the variation. Sporophyte production densities were generally lower in the 12°C treatment, but there was slightly less variation in the relationship between output and sporophyte density in that temperature, resulting in a linear relationship that explained 4.4% of the variation.

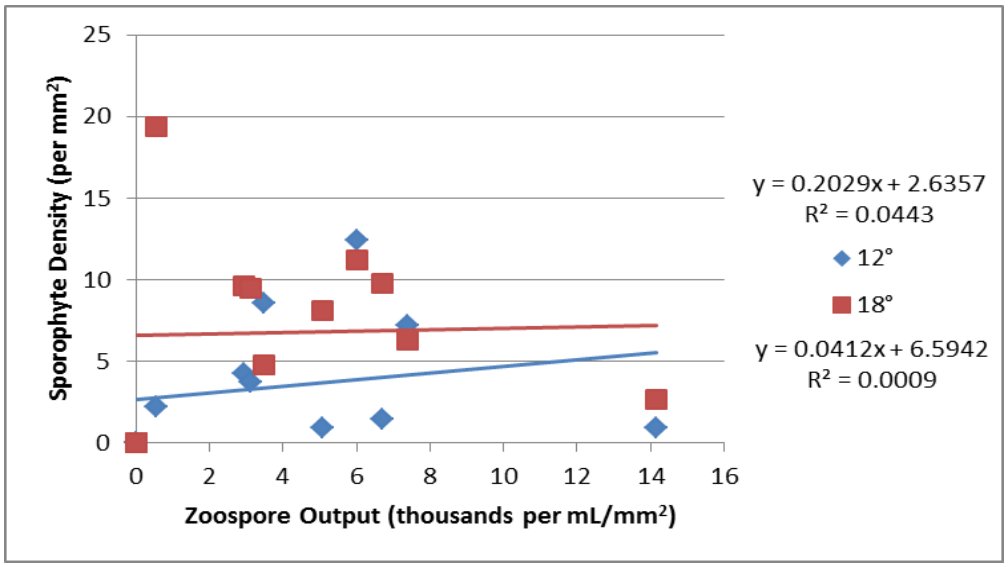


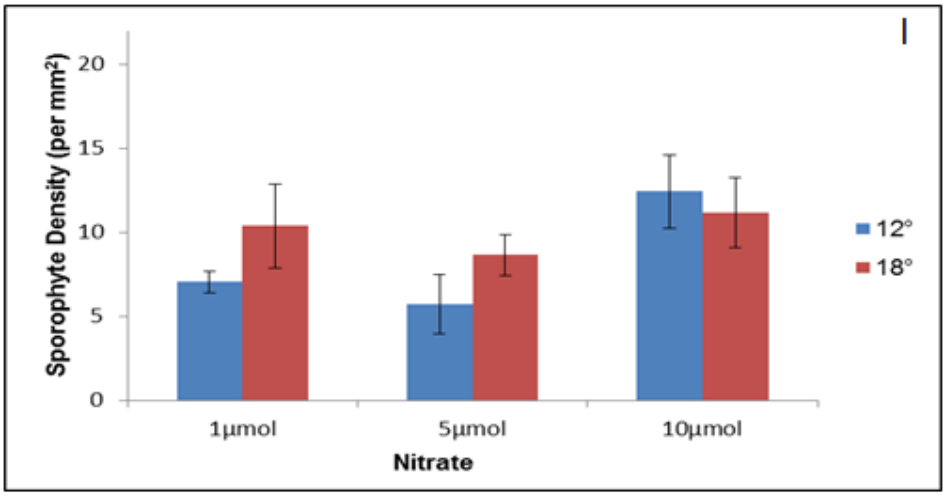
Figure 3: Scatter plot showing the relationship between zoospore output and sporophyte production densities in the 12°C (top equation and r^2 value) and 18°C (bottom equation and r^2 value) temperature treatments.

Temperature-Nitrate Effects

Production densities for the three nitrate-temperature experiment trials were averaged together for the early microscopic stages (zoospore settlement, zoospore germination, and gametophyte survivorship). Averaged

values resulted in no significant differences between any of the treatments for any stage (Table 2). Temperature and nitrate concentrations were shown to have no significant impact on production of these life history stages for *U. pinnatifida*.

Between the three trials, significant temperature and nitrate effects on sporophyte production were observed, but displayed no consistent pattern between the trials (Figure 4). Two-way ANOVAs revealed variation in the source of nitrate treatment significance between experiment repetitions. Trials 1 and 2 displayed significant nitrate effects; trial 1 showed a significant difference within the 12° treatment between the 1 and 10µmol and the 5 and 10µmol treatments, and trial 2 showed a significant difference within the 12° treatment between the 1 and 10µmol treatments. Trial 3 was the only trial of the three to display a significant temperature effect. However, when sporophyte densities for the three experiments were averaged together, there was no significant overall temperature or nitrate effect (Table 2). The interaction between temperature and nitrate availability on sporophyte production was also non-significant (Table 3).



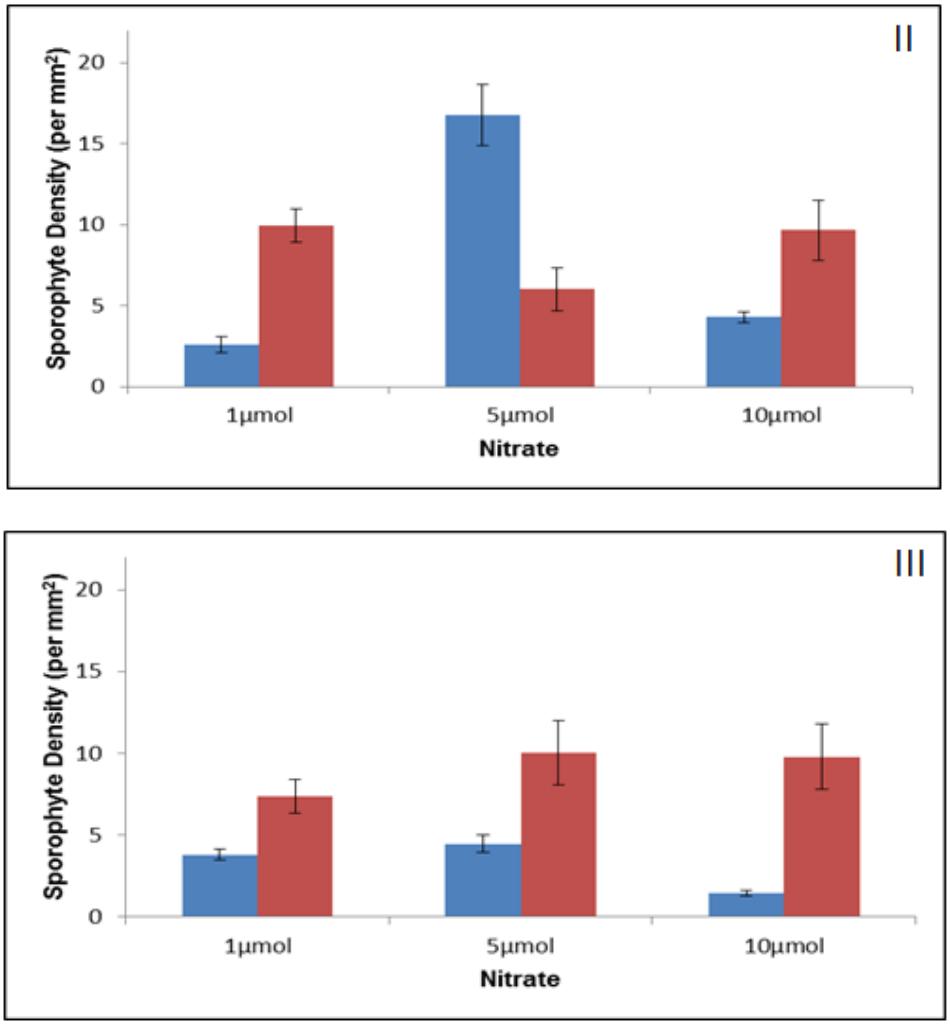


Figure 4: Nitrate-Temperature experiment was repeated three times throughout the year. Numbers in upper right corner attach to p-values in table 2. Error bars are ± SE.

Table 2: Significant p-values are highlighted in red. "Nitrate effect" are the significant differences within a temperature treatment due to nitrate availability. Values in parentheses indicate the source of significance according to a Fishers LSD post hoc comparisons test. "Temperature effect" are the significant differences within a nutrient treatment due to temperature.

Microscopic Stage	Temperature Effect	Nitrate Effect
Settlement	0.602	0.916
Germination	0.583	0.678
Survivorship	0.491	0.458
Sporophyte I	0.195	**0.042** (1-10, 5-10)
Sporophyte II	0.011	**0.026** (1-10)

Sporophyte III	**<0.001**	0.427
Sporophyte Average	0.165	0.730

Table 3: ANOVA on the effects of temperature and nitrate concentrations on sporophyte production in *Undaria pinnatifida*

Source	df	Mean Square	F	Sig.
Temp	1	33.341	2.187	**.165**
Nitrate	2	4.931	.323	**.730**
Temp * Nitrate	2	13.767	.903	**.431**
Error	12	15.245		

Reproductive Output vs. Physical Features

A linear regression comparing individual zoospore output and individual vegetative blade length/sporophyll size explained only 5.8% of the variation in this relationship, while a polynomial regression explained 16.5% of the variation. According to the curve of a fitted polynomial regression of sporophyll size vs. zoospore output, and the increase in r^2 value, there is a size where the sporophylls in this study were most reproductive- from about 30-50cm^2- and sporophylls that were either smaller or larger were less reproductive (Figure 5).

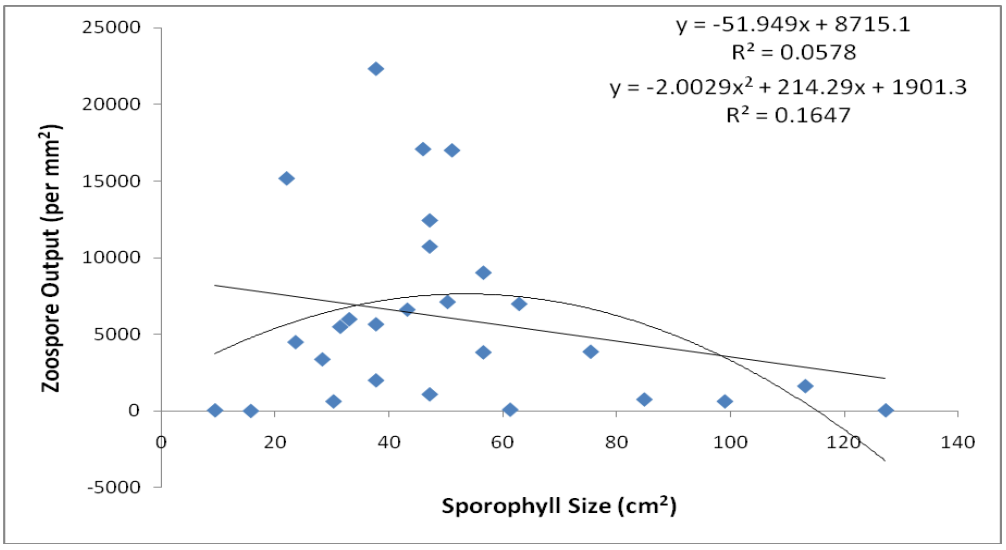


Figure 5: Linear and polynomial regressions of individual sporophyll size vs. individual zoospore output. The linear regression equation and r^2 value are on top of the upper right corner, and the polynomial regression equation and r^2 value are on the bottom of the upper right corner.

The linear regression for blade length vs. output accounted for only 17.1%, but zoospore output was maximized at a blade length of 80-100cm, where individuals with longer or shorter blades were less reproductive. Both regressions are plotted below but, unlike the regressions for sporophyll size and zoospore output, the polynomial regression followed the same trajectory as the linear regression, and explained the same amount of variation (Figure 6).

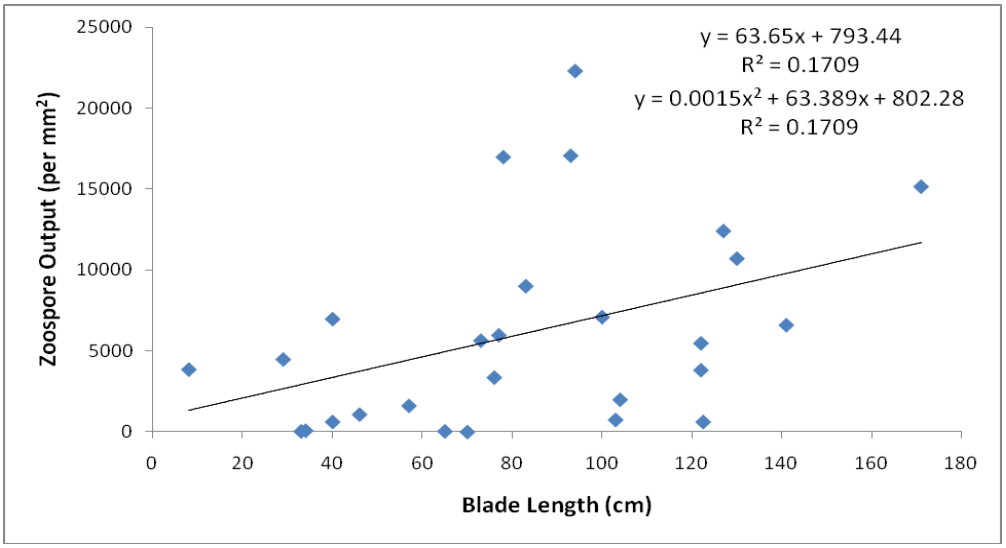


Figure 6: Linear and polynomial regressions of individual blade length vs. individual zoospore output represented the same line and the same amount of variation. The linear regression equation and r^2 value are on top of the upper right corner, and the polynomial regression equation and r^2 value are on the bottom of the upper right corner.

However, a comparison of many reproductive individuals from Monterey Harbor revealed a stronger relationship between *average* monthly zoospore output (data collected from Objective 1) and *average* monthly blade length of many individuals from Monterey Harbor (data collected by Brynn Hooton- MLML) than for either individual-based regression. The linear regression for this data explained 59.1% of the variation in this relationship and the polynomial regression explained 64.2% of the variation (Figure 7). When average monthly blade length and zoospore output were plotted as a function of time, similar patterns were seen. Both output and blade length peaked in spring (March-May) and fall (September) 2010, and crashed in winter (January) 2010 (Figure 8).

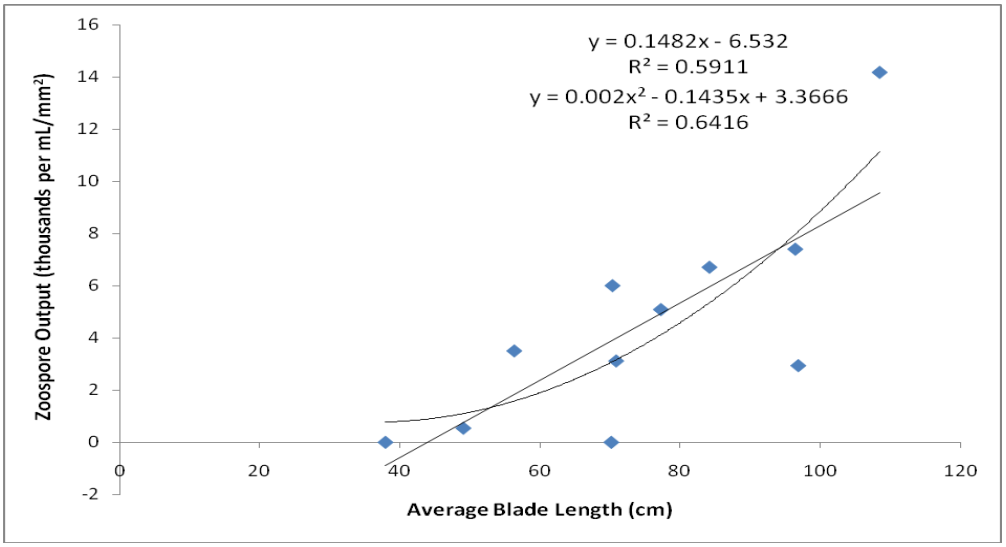


Figure 7: Linear and polynomial regressions of average monthly blade length vs. average monthly zoospore output. The linear regression equation and r^2 value are on top of the upper right corner, and the polynomial regression equation and r^2 value are on the bottom of the upper right corner.

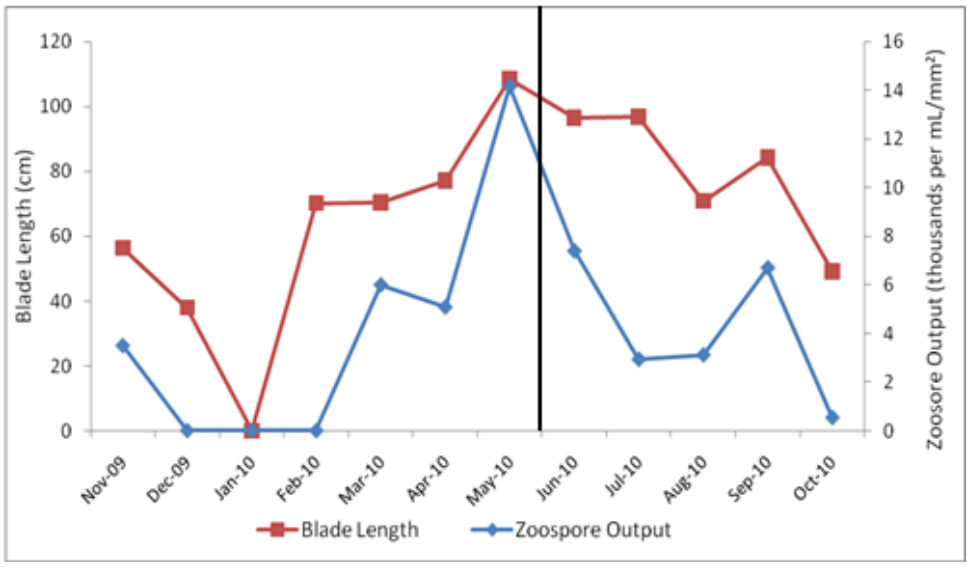


Figure 8: Average monthly blade length for adult individuals (primary y-axis) vs. average monthly zoospore output (secondary y-axis)**.** Bold line indicates switch from measuring zoospores as thousands per mL to thousands per mm^2.

Discussion

This study demonstrated that *Undaria pinnatifida* from Monterey Harbor had reproductive output values that fluctuated between months, peaking in spring (March-May) and fall (September). Microscopic sporophytes

were produced in two significantly different temperatures throughout the year, and more sporophytes were produced in the warmer temperature when there was an effect of temperature on sporophyte production. Microscopic sporophytes were also produced in significantly different nitrate treatments, but nitrate effects were varied and there was no overall effect observed. Finally, comparisons between physical features of *U. pinnatifida* and its reproductive output yielded extremely weak relationships ($r^2<0.2$) on an individual plant level. However, comparisons between many individuals' monthly averaged zoospore output and blade length revealed a stronger relationship ($r^2>0.59$), and averaged blade length and zoospore output tracked each other on a temporal scale.

Monthly Reproductivity

The alternate hypothesis that there will be a difference in reproductive output between months can be accepted as variation in zoospore output was seen throughout the study period. Two peaks in reproductivity were observed in May and September; these peaks in zoospore output coincided with juvenile recruitment pulses observed in Santa Barbara Harbor by Thornber et al. (2004). In this experiment, the authors found that *U. pinnatifida* recruitment pulses followed a drop in water temperature of approximately 4°C. Similar drops in temperature were seen in May and September in Monterey Harbor (Figure 2) suggesting that this species synchronizes reproductive effort with seasonal water temperature changes.

Cultures conducted in two different temperature treatments yielded significant differences in sporophyte production in four out of nine months. In all of these months, sporophyte production was significantly higher in the 18° treatment, though sporophytes were present in both treatments each month. The alternate hypothesis that there will be a difference in sporophyte production between temperatures can be accepted. Kohtio (2008) found that germination of *U. pinnatifida* occurred in greater densities in a warmer (18°C) temperature treatment, and less in a cooler treatment (12°C); she also found germination occurred more quickly in the warmer temperature.

The more numerous and rapid production of microscopic stages (germinated zoospore- Kohtio 2008, microscopic sporophyte- current study) in warm temperatures suggests that this is an adaptive trait which allows *U. pinnatifida* to deal with stressful temperature conditions. This temperature flexibility may prove to be a key aspect of this species' invasiveness that allows for range expansion both north (into cooler waters) and south (into warmer waters). This experiment did not test the entire temperature range of this species, so a more exact prediction of the range expansion capabilities of *U. pinnatifida* cannot be made based on its temperature tolerances, but it can be inferred that invasion in warmer southern locations will occur more quickly as this species produces microscopic stages at an elevated rate in warm temperature conditions.

Temperature-Nitrate Effects

The effects of nitrate on microscopic stage production yielded variable results. Nitrate effects on sporophyte production were inconsistent, and there was no significant nitrate effect for any of the other microscopic stages (zoospore settlement, zoospore germination or gametophyte survivorship), thus the alternate hypothesis (that there is an effect of nitrate on microscopic stages of *U. pinnatifida*) must be rejected. The expected outcome for this type of experiment would be to observe the greatest density of sporophytes in the 10μmol nitrate treatment (a saturating amount for central California), fewer in the 5μmol treatment, and the least in the 1μmol treatment (a limiting amount of nitrate). Dayton (1985) and Deysher and Dean (1986) stated that nitrogen is important to reproduction and recruitment, and is generally interconnected with other abiotic factors; because of this interconnection, the role of nitrate availability is rarely tested independently of other factors such as temperature. In this experiment, *U. pinnatifida* did not display the expected reaction to nitrate concentrations, independent of temperature. This result suggests that nitrate (a limiting resource in marine systems) is not the primary driver of reproductive success in this species; nitrate effects appear to be secondary to temperature, which had a consistent effect on microscopic stage production.

The 18°C treatment produced more sporophytes when there was a difference between temperatures. Zoospore settlement, zoospore germination, and gametophyte survivorship were not significantly affected by variable temperature conditions, and so the alternate hypothesis (that there is a temperature effect) can be accepted for sporophyte production, but must be rejected for the other microscopic stages. Deysher and Dean (1986) found that, contrary to previous studies' findings, *Macrocystis pyrifera* sporophytes could be produced in extremely high temperatures (<20°C) when light levels were high, as they were in the present experiment. These parallel results suggest that reproductive success in kelps is primarily driven by temperature rather than nitrate availability.

Reproductive Output vs. Physical Features

The linear relationship between sporophyll size and zoospore output was found to be negative and weak (Figure 5), implying that sporophyll size is not a good proxy for spore output in this species, and the alternative hypothesis that there is a relationship between zoospore output and sporophyll size must be rejected. However, a polynomial regression accounts for more of the variation in this relationship, showing that this relationship is non-linear, with peak reproductive sporophyll size being 30-50cm^2, not the maximum observed size, as was expected (Figure 5).

The linear relationship between vegetative blade length and zoospore output was also found to be weak though positive (Figure 6); the alternative hypothesis that there is a relationship between zoospore output and blade length must be rejected. However, a similar pattern as with sporophyll size can be seen though was not reflected by the polynomial regression; peak reproduction was at a blade length of 80-100cm, less than the maximum observed. Reed (1987) and Pfister (1992) found that sporophyll biomass in *Macrocystis pyrifera* and *Alaria nana* respectively declined sharply with vegetative tissue loss, and there was a strong correlation between zoospore output and sporophyll biomass (Reed 1987). Though the present study found no strong

relationship between zoospore output and vegetative blade length, it can be implied that vegetative tissue represents resources that are used in reproduction.

Implications for Management

The results of this study inform both ongoing management efforts and future policy decisions. *Removal* programs focused on this species are primarily interested in the status of individual plants and identifying invasion "hotspots." Information on individual plant physiology is of interest to this type of program, such as relationships between reproductive output and physical characteristics. On the other hand, *monitoring* programs are interested in the state of the entire population: how many individuals comprise it and how the size of the population is changing over time. Information on the entire population such as how abiotic factors affect recruitment is of interest to this type of program.

The *Undaria* program funded by the Monterey Bay National Marine Sanctuary is both a removal and monitoring program, with a greater emphasis on removal. Information that can help members of the removal program predict the reproductive status of an individual *before it is removed* (trauma to the plant can cause zoospore release) is critical to the success of programs seeking to avoid the further spread of this species. As is the case with most invasive species management programs, the MBNMS *Undaria* program is primarily carried out by volunteers with limited or no scientific training. This creates a greater need for program simplicity and efficiency. This study provides information about which *U. pinnatifida* individuals are the most reproductive (by looking at simple and obvious visual parameters) and therefore which individuals should be prioritized for removal.

While control and removal programs focus on the macroscopic individual, knowledge of the microscopic stages should also be of interest to these programs as understanding the effects of key abiotic factors on the reproductive stages leading to the macroscopic individual is essential to long-term success of such a program.

This type of data is of more interest to monitoring programs rather than removal programs as it relates to future cohorts and the continuation of the population. This study has shown that *Undaria pinnatifida* has a large tolerance for temperature variation, able to successfully reproduce in two significantly different temperatures. This temperature tolerance suggests that this species has the ability to continue to spread along the California coast or further. Experiments on the nitrate requirements of this species revealed little effect (or an effect that lacked consistency and was therefore undetected) suggesting that this species is somewhat unaffected by variable levels of nitrate. Seasonal changes in nitrate (such as during the rainy season or upwelling events) cannot be used as a reliable predictor for population dynamics such as reproductive or recruitment pulses.

While the results of this study are specific to *U. pinnatifida* in Monterey Harbor, the methods herein can be used in other locations. *U. pinnatifida* is clearly a condition-flexible alga, whose reproductive physiology most likely varies by location; the results of this study reflect the temperature tolerance and nitrate requirements of this species at the present study location. However, the methods adapted and used in this study for testing the importance of temperature and nitrate to the microscopic stages of *U. pinnatifida* should be conducted in other locations throughout *U. pinnatifida*'s range in order to detect differences in reproductive physiology by location. These location-specific data will inform management efforts at each location, as they do for efforts in Monterey Harbor, and will provide a more complete idea of the ability of this species to continue to spread outside its current range.

Future Directions

This experiment explored the importance of temperature to reproductive success. Therefore future experiments should include a gradient of temperature treatments that extend below 12° and over 18°C. Understanding the true effect of temperature on reproduction and discovering temperature thresholds for reproduction in *U. pinnatifida* requires a more robust temperature treatment array. Other studies might compare

average zoospore output with population sporophyll size or test other physical attributes such as sporophyll surface area or biomass for individual plant relationships with output. The importance of visual indicators of reproductive status to *U. pinnatifida* removal programs can't be overemphasized, and a number of these indicators working in conjunction would lead to a much more efficient volunteer removal effort.

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