LONG-LINE CULTURE OF RED SEAWEED IN THE PACIFIC NORTHWEST

By

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A Thesis Presented to

The Faculty of California State Polytechnic University, Humboldt

In Partial Fulfillment of the Requirements for the Degree

Master of Science in Natural Resources: Fisheries

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May 2023

ABSTRACT

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The goal of this study was to adapt open-water rope culture techniques to the native red seaweed *Devaleraea mollis* to support a new seaweed aquaculture industry in Humboldt Bay, California. The specific objectives for this study were to: 1) evaluate the growth of *D. mollis* cultivated at different depths and seasons (fall/winter, spring/summer), 2) estimate nutrients removed by *D. mollis* from the water, and 3) measure heavy metals and pesticides to determine potential health risk upon ingestion. Bundles of seaweed were inserted into 3 m long weighted vertical lines attached to two horizontal long-lines suspended by floats. Two four-month trials (September to December 2020 and April to July 2021) were compared. Long-lines in Trial 1 were seeded on the same date, and data was pooled from both long-lines. Data was analyzed separately for each long-line in Trial 2 as the lines were seeded on different dates. Both depth ($p<0.001$) and month ($p<0.002$, two-way ANOVA, Tukey HSD test) had significant effects on wet weight for the first trial with depths 0 m and 1 m and months November and December producing the best growth. For Trial 2 only depth was significant ($p=0.006$) for long-line 1 with the 0 m depth producing the best growth while both depth ($p<0.001$) and month ($p=0.006$) significantly affected wet weight for long-line 2 with 0 m and the month of May

producing the best growth. Maximum growth rate from Trial 1 was 0.21 g/day, and 0.19 g/day from Trial 2. A total of 1.20 kg of carbon, 0.12 kg of nitrogen, and 0.02 kg of phosphorus were removed from the water by the seaweed produced in this study. All pesticides were found to be undetectable, and all heavy metals were either undetectable or below action levels with the exception of manganese for 3 m in Trial 1 and 2 m and 3 m in Trial 2 (21.0 mg/kg, 77.6 mg/kg, 93.3 mg/kg respectively). Results from this study suggest optimal growth occurs in the winter and early spring at no more than 1 m in depth, *D. mollis*'s potential for nutrient bioextraction, and that *D. mollis* grown in Humboldt Bay poses low risk to consumers for heavy metals and pesticides.

ACKNOWLEDGEMENTS

This research would not have been possible without the support of the California State University's Agricultural Research Institute, Cal Poly Humboldt's Sponsored Programs, Humboldt Marine & Coastal Science Institute Award, the Malcom Oliphant Marine Science Scholarship, and the Richmond Rod and Gun Club Award. I would also like to acknowledge the staff of GreenWave – in particular Karen Gray Geisler who not only navigated the two-year permitting and approval process and helped physically build the farm, but also planted the seed that allowed Cal Poly Humboldt's ProvidenSea seaweed farm and consequently this study to exist. Thank you to Drs. Rafael Cuevas Uribe, Jose Marin Jarrin, and Frederick Zechman for serving as members of my thesis committee and for their knowledge and mentorship. Also thank you to the staff of the Humboldt Bay Harbor, Recreation, and Conservation District for their approval, support, and allowing use of their landing craft and personnel for deploying farm equipment. Thank you to Steven Monk, Kyle Weis, and Grant Eberle for acting as boat operators, being knowledgeable on use of equipment, biofouling species ID, and for helping maintaining the tank-based nursery IMTA system. Finally, sincere thanks to my crew mates and helpers Nicole Matonak, Sarah DeJesus, Jasmine Martin, Matt Wolder, Katie Terhaar, Noah Jenkins, Nick Isola, and my family for all of their help and support.

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ANOVA – analysis of variance

C – carbon

˚C – degrees Celsius

CeNCOOS – central and northern California ocean observing system

cm – centimeter

CTD – “conductivity, temperature, depth” sonde

CUTI – coastal upwelling transport index

d – day

DW – dry weight

˚F – degrees Fahrenheit

ft – feet

FW – fresh weight, wet weight

g – grams

GC-MS/MS – gas chromatography tandem mass spectrometry

ICP-MS – inductively coupled plasma mass spectrometry

ICP-OES – inductively coupled plasma optical emission spectrometry

IMTA – integrated multitrophic system

in – inch

kg – kilogram

km - kilometer

LC-MS/MS – liquid chromatography tandem mass spectrometry

L – liter

lb - pound

LL – long-line

m - meter

MHHW – mean higher high water

MHW – mean high water

MLLW – mean lower low water

MLW – mean low water

N – nitrogen

NOAA – National Oceanic and Atmospheric Administration

P – phosphorus

PAR – photosynthetic active radiation

pg – picogram

psu – practical salinity units

s – second

TML – Cal Poly Humboldt Telonicher Marine Laboratory

UL – Tolerable weekly upper limit

µg – microgram

µmol – micromole

INTRODUCTION

Since ancient times, people in coastal locations have used seaweeds as food, fertilizer, and animal feed (Indergaard and Minsaas, 1991; Grote, 2017). Over time people discovered seaweeds contain valuable phycocolloids such as agar, carrageenan, and alginate. They are currently also being marketed as a health food as they can be high in protein (up to 35% dry weight), vitamins, minerals, and antioxidants bringing along added health benefits like cancer cell suppression and disease prevention (Fleurence, 1999; Grote, 2017). Interest in using seaweeds for various applications in the pharmaceutical, cosmetics, food, fertilizer, animal feed, biofuel, and chemical compound industries is on the rise (Indergaard and Minsaas, 1991; Edwards and Dring, 2011; WHO, 2011; FAO, 2014). However, wild harvest in the USA has dropped in recent years, and the country will need to increase its aquaculture production in order to meet this increasing demand without depending too heavily on importing its seaweed from others (FAO, 2023).

As much as 95% of seaweed that is consumed and processed in the USA is imported from major producing countries such as China, Indonesia, Republic of Korea, the Philippines, and Japan costing the USA an estimated $229 million annually (Piconi et al., 2020; McKinley Research Group, 2021). These countries account for over 98% of global seaweed production, whereas the USA accounts for <0.04% (FAO, 2020; NMFS, 2020). Cultivation of seaweed dates back hundreds of years in coastal Asian countries, while it is a relatively new industry in the USA. However, interest in seaweed cultivation

in the USA is on the rise as people are becoming aware of the many benefits seaweeds have to offer (Kim et al., 2019). USA seaweed aquaculture production has increased 12.5 times from 2017 to 2021, and shows promise to become a major contributor to USA aquaculture (NMFS, 2020; FAO, 2023). Unfortunately, strict permitting and regulations in California can make entry into an aquaculture business difficult. However, the Humboldt Bay Harbor, Recreation, and Conservation District (harbor district) obtained state and federal authorizations to establish pre-permitted sites for aquaculture of shellfish and native red seaweed species in the *Chondracanthus*, *Gracilaria*, *Palmaria*, and *Porphyra* genera in order to encourage the growth of the local aquaculture industry in the bay.

One such species is *Devaleraea mollis* (Pacific dulse), previously known as *Palmaria mollis*. It is a pseudo-perennial species with fronds that are usually deep red in color, and are palmate in shape due to deeply divided lobes radiating from the middle of the fronds (Werner and Dring, 2011). Primary fronds also show marginal proliferations that can produce new fronds. The fronds can reach up to 40 cm long, and grow from a small discoid holdfast and very short stipe. The fronds of *D. mollis* can also present different morphologies – some being more flattened and "ribbon-like" while other individuals take on a more "bushy" appearance of finely dissected fronds with numerous narrow divisions. Morphology may be environmentally influenced with bushy individuals being associated with sheltered, silty areas (Werner and Dring, 2011). This species can be found in the low intertidal and upper subtidal zones usually down to 10 m with a maximum depth of 20 m from the Bering Sea to northern California on rocky substrates

or growing as an epiphyte on other seaweed species (Demetropoulos and Langdon, 2004b, Werner and Dring, 2011). Sites that have a moderate to strong current that are semi-exposed or sheltered are preferred. The lifecycle of *D. mollis* consists of gametophyte and tetrasporophyte phases with tetrasporophytes and male gametophytes appearing visually indistinguishable. After a tetrasporophyte releases its spores, they will settle and develop into male and female gametophytes. The male gametophytes are visually indistinguishable from tetrasporophytes, and take a year to mature. Female gametophytes are microscopic, and must be fertilized by males from the previous year within a few days or they will die off. Should a female be fertilized, a new tetrasporophyte will grow on top of and completely engulf it (Werner and Dring, 2011).

There are several reasons why *D. mollis* would make a good candidate for cultivation in Humboldt Bay. *Devaleraea mollis* has a similar life history to the closely related *Palmaria palmata* (Dulse), therefore, it can be cultivated using the same methods as its popular Atlantic cousin. This similarity opens up literature on *P. palmata* that can be useful guidelines for *D. mollis.* Another reason for selecting *D. mollis* for cultivation is that it can propagate vegetatively via fragmentation besides by spores, thus eliminating the need and expense of operating a seaweed hatchery to culture it from spores for farmers who do not have access to such a facility (Werner and Dring, 2011). *Devaleraea mollis* is a native species high in nutrients and protein making it a good candidate as a product in the health food market as well as a sustainably produced high-quality abalone feed (Indergaard and Minsaas, 1991; Demetropoulos and Langdon, 2004d; Grote, 2017; Wulffson, 2020). The aquaculture industry is also developing domesticated *D. mollis*

strains and unique cultivars (e.g., Oregon State University's patented "bacon flavored" strain) (Floyd, 2015).

Cultivating *D. mollis* could also provide beneficial ecosystem services. One such benefit of seaweed culture can be local ocean deacidification. In 2021, global anthropogenic CO_2 emissions added 36.3 Gt of CO_2 to the atmosphere – about 30% of which is absorbed by the world's oceans (Gruber et al., 2019; IEA, 2022). As CO_2 dissolves it reacts with water molecules to form carbonic acid (H_2CO_3) before dissociating into hydrogen ions (H^+) and bicarbonate ions (HCO_3^-) lowering the pH of seawater. The acidifying of seawater can adversely affect calcifying organisms such as shellfish, an important industry in Humboldt Bay (Kurihara, 2008). *Devaleraea mollis* is a species of seaweed that is able to utilize dissolved inorganic carbon in both forms of CO_2 and HCO_3^- by dehydrating to CO_2 and OH^-, and has the potential to be a good candidate for carbon sequestration (Demetropoulos and Langdon, 2004ad).

A second benefit to farming *D. mollis* in Humboldt Bay can be improved water quality by lowering the amount of nitrogen and phosphorus in the water (Levin, 1991; Evans and Langdon, 2000). Nutrients, particularly those containing nitrogen and phosphorus are important for producing new growth. However, water high in nutrients can lead to eutrophic conditions which can cause blooms of phytoplankton that can shade out other aquatic plants and macroalgae, increase risk of hypoxia, and harmful algal blooms that emit toxins (Kim et al., 2014). The improvement of water quality provided by this alga may also compliment the shellfish aquaculture already operating in the bay as bivalves produce ammonia as a waste product. *Devaleraea mollis* can more readily

uptake ammonia compared to nitrate as a nitrogen source, and can potentially improve water quality in close vicinity to a shellfish farm (Demetropoulos and Langdon, 2004c).

Because of the many benefits *D. mollis* can offer, and that it is an approved species in the harbor district lease permit, it was selected as the subject of this study. The main goal of this project was to assess *D. mollis* as a candidate for open-water long-line cultivation in Humboldt Bay with one application that this assessment supports the bay's developing seaweed aquaculture industry. In 2020, California State Polytechnic University, Humboldt (Cal Poly Humboldt), in collaboration with GreenWave, a non-profit organization offering training and support to new seaweed farmers, obtained a lease from the harbor district to install a commercially licensed pilot seaweed farm in one of the pre-permitted sites. This study involved cultivating *D. mollis* on long-lines at this farm site, and had three primary objectives:

1. Evaluate the growth of *D. mollis* along various depths and across different seasons.
2. Estimate the amount of carbon, nitrogen, and phosphorus *D. mollis* removed from the surrounding waters.
3. Analyze tissue samples for heavy metals and pesticides to determine if seaweed grown in Humboldt Bay is safe for human consumption.

MATERIALS AND METHODS

Study Site

The study was conducted in Humboldt Bay, CA where Cal Poly Humboldt has leased a 0.5-acre section of a pre-permitted subtidal lease area (Figure 1) from the harbor district for a commercially licensed pilot seaweed farm. Humboldt Bay is a protected embayment with a mouth that remains open to tidal exchange continuously, and is composed of four major compartments: South Bay, Entrance Bay, the Main Channel, and Arcata Bay. South Bay and Arcata Bay are located at either end of Humboldt Bay consisting mainly of shallow, intertidal and subtidal mud flats. Arcata Bay also hosts the majority of the oyster aquaculture production in the bay. Main Channel and Entrance Bay are deep as they are dredged to allow for shipping traffic (Sutula et al., 2007; Swanson, 2015). The entrance of the bay has mean tidal range (MHW to MLW) of 1.49 m (4.89 ft), and a diurnal range (MHHW to MLLW) of 2.09 m (6.86 ft) (NGS, 2023). Water parameters of Humboldt Bay are influenced more by nearshore water. Its contributing watershed is small (562 km^2) with no major river inputs, and has a tidal prism up to 54% of MHHW (Barnhart et al., 1992; Schlosser and Eicher, 2012). However, despite having a large tidal prism this water may not be replaced completely by new water if nearshore currents aren't strong enough to remove it all away from the mouth of the bay before the start of the next flood tide (Barnhart et al., 1992; Swanson, 2015).

Humboldt Bay has a temperate coastal climate with an average air temperature of 11.7˚C (53˚F) and receives on average 96.5 cm (38 in) of rain annually. However, only 64 cm (25.2 in) of rain occurred during the study period as it took place during the 2020 to 2023 triple-dip La Niña event (NWS, 2023). Rain events can cause temporary, episodic dilution of bay water, but does not tend to result in vertical stratification of salinity, temperature, or nutrients due to mixing of the water within Humboldt Bay. Instead, horizontal gradients occur across the bay for salinity during wet winter periods, and then for temperature during warm summer periods (Gast and Skeesick, 1964; Swanson, 2015).

An important factor for productivity in the bay is upwelling. During the spring and summer, winds and currents close to shore are from the north, which along with the rotation of the Earth, causes water to move south and west, bringing up nutrient-rich bottom waters to the surface. These upwelled waters may take roughly three days for to enter the bay (Swanson, 2015). During winter and early spring, runoff from increased precipitation becomes the main contributor of nutrients (Barnhart et al., 1992). Second to upwelling and runoff, wastewater from the Arcata and Eureka wastewater treatment plants acts as a year-round source of nutrients. These plants used to be a major contributor of nitrogen for Humboldt Bay, but after improvements to both facilities, nitrogen in treated wastewater was greatly reduced. This may have played a part in the nitrogen limited production seen in the bay today (Pequegnat and Butler, 1981; Barnhart et al., 1992).

Data collection began on August 25, 2020 and was completed on July 30, 2021. The location of the farm is 40º48’32.2”N 124º11’12.9”W in the Samoa Channel (Figure 2) Site depth ranges from 3.5 m to 4.2 m (11.7 to 13.6 ft) MLLW with a bottom substrate consisting of mud and shells. The nearby Samoa shoreline protects the site from nearshore wave action, and minimizes wind-generated waves. By being situated above the confluence of the Samoa and Eureka channels, the site avoids stronger currents and wave action produced by the converging waters.

Study System Design

In this study, we grew *Devaleraea mollis,* which had been collected locally from the intertidal zone adjacent to the Cal Poly Humboldt Telonicher Marine Laboratory (from now on referred to as TML) in Trinidad, CA. Originally, *Gracilariopsis andersonii* was also intended to be a test subject to compare to *D. mollis,* but due to concerns over whether the species collected by Cal Poly Humboldt was the native *G. andersonii* or the invasive look-a-like *Agarophyton vermiculophyllum* (formerly known as *Gracilaria vermicullphylla*) the seaweed was eliminated from the project to prevent culture and spread of non-native species. *Devaleraea mollis* was propagated in a land-based Integrated Multitrophic Aquaculture (IMTA) nursery system with sablefish (*Anoplopoma fimbria*) at the TML until they were out-planted for grow-out at the study site (Mele et al., 2019).

The project consisted of two trials or growing seasons (late August 2020 - December 2020 to test fall/winter, and Late March 2021 - July 2021 to test

spring/summer) each four months long to see how different times of year affect growth. It was originally intended that there would be three four-month trials so as to encompass the entire year to create a growth profile for *D. mollis*, but due to the COVID pandemic and delays in permitting there was only time to conduct two seasons.

The seaweed was cultured using long-lines (Figure 3). This method consists of single lines suspended by floats and buoys at the surface while being secured to the bottom substrate with anchors. The long-lines paralleled the shoreline to reduce drag caused by the current. On August 21, 2020 two long-lines were installed at the farm site, each with a 107 m (350 ft) long surface line attached to a large mooring buoy at both ends of the line, and suspended with 24-26 crab floats. Four 45.4 kg (100 lbs) steel Danforth anchors were deployed, one attached at each end of a long-line. "Pig tails" consisting of a crab float and yellow polypropylene rope were attached to each anchor to allow a boat to adjust the anchors when needed. Blue Steel™ ½ inch 3-strand rope (Continental Western Corporation, San Leandro, CA, USA) was selected for the surface line due to its polypropylene's high strength, abrasion and UV resistance, and its ability to float. Long-lines were installed using the harbor district's landing craft. Six regulatory marker buoys that follow U.S. Coast Guard recommendations with 90.7 kg (200 lbs) concrete block anchors were installed every 91.4 m (300 ft) around the perimeter of the farm, and each was outfitted with a flashing light and signage stating "Danger Aquafarm Submerged Lines" to make the seaweed farm more visible to vessels.

Prior to outplanting, *D. mollis* fronds were weighed and sorted into 15 g bundles, and then inserted between the lay of the dropper lines on August 25, 2020 for Trial 1 and

March 23 and April 6, 2021 for Trial 2 (Figure 4). The tension of the rope strands was used to keep the seaweed bundles in place. The seaweed bundles were secured to each dropper at 0, 1, 2, and 3 m in order to test the effect of depth on growth. It should be noted that "0 m" was actually located about 20 cm below the water instead of directly at the surface due to knotting and line splicing. Each dropper consisted of a 2.5 cm (⅜ in) 3-strand nylon rope with a 1.1 kg (2.4 lbs) concrete weight attached to the bottom to keep the line vertical in the water column (Figure 3). Nylon was selected for the droppers because it is prone to sinking, and the softness of its fibers makes for a better grow-out substrate for seaweed compared to other types of line (Werner and Dring 2011). The seeded droppers were stored overnight in tanks of the IMTA nursery system until they were deployed the next morning on August 26th, 2020 for Trial 1, and March 24 and April 7, 2021 for Trial 2. The long-lines in Trial 2 are separated in time because only half of the total number of droppers were able to be seeded on March 23rd, 2021. High winds prevented another visit to seed long-line 2 until two weeks later. Each long-line had 50 dropper lines attached to the main line spaced two meters apart to avoid entanglement, and each dropper had a length of just over three meters.

Data Collection

Growth of *D. mollis* was measured by taking samples once a month. Samples consisted of collecting 6 droppers (3 per long-line) randomly selected without replacement. Because seaweed could break loose and float away with the current over time, it was important that sampled droppers have all their bundles intact so that there

would be data for all 4 depths. To do this while still using random selection, the previous week's presence/absence data would inform which droppers may still have all 4 bundles, and those dropper IDs would be entered into a random generator for selection. The bundles were removed from the droppers, placed into labeled bags, and transported on ice to the marine laboratory. There each bundle was photographed, visually inspected with notes made on appearance, cleaned of any fouling organisms, and then wet weight was determined. Fouling was removed by cleaning with toothbrushes and washing with fresh water. Coupled with growth, crop retention was measured by recording the presence or absence of bundles on the dropper lines on a weekly basis weather permitting. A bundle was considered present if any amount of seaweed was visible at a given depth location, even if a bundle experienced some frond breakage as there is no way to quantify that loss. A bundle was considered absent if no seaweed visibly remained at a given depth location on the line. Each dropper on both long lines were pulled to the surface, and bundles were visually inspected at each depth for their presence and overall condition. After the final month's samples were collected for each grow-out season, *D. mollis* on remaining droppers was harvested, its presence/absence was determined, and was weighed.

Tissue samples were collected for testing carbon and nutrients, heavy metals, and pesticide from the IMTA nursery at the start of each trial to act as a baseline, and from the total harvest at the end of each trial. After tissue samples were cleaned of any fouling, samples of the same depth were combined for each month. Tissue samples were then either shipped fresh or after having been stored at 6.6˚C (20˚F) until shipped to the University of Missouri's Agricultural Experiment Station Chemical Laboratories

(Columbia, MO) and AGQ Labs (Oxnard, CA) for analysis. A water sample was collected weekly from just below the surface in a clean plastic ziplock bag at the farm site during grow-out, transported on ice to the TML, and tested for ammonia, nitrate, nitrite, and phosphorus with a handheld Hach DR 900 Multiparameter Colorimeter and the associated Hach testing procedures: ammonia (method 10031), nitrite (method 8153), nitrate (method 10020), phosphorous (method 8180).

Environmental data was also collected across depths and seasons to determine their correlation with changes in growth, nutrients, and contaminants. Current velocity data was obtained from the North Bay Channel at Samoa Channel current station. Water visibility was measured using a 20 cm secchi disc, and pH was measured with a PC60 Premium Multiparameter Pocket Tester (Apera Instruments, Columbus, OH). A CastAway CTD (SonTek, San Diego, CA) was used to measure seawater temperature and salinity gradients across the entirety of the water column. A YSI Model 55 Dissolved Oxygen Instrument (YSI, Yellow Springs, OH) measured seawater temperature, salinity, and dissolved oxygen between 0.5 m to 1 m in depth (limited due to cord length). Photosynthetically active radiation (PAR light) was measured with a LI-193SA Spherical Underwater Quantum Sensor (LI-COR®, Lincoln, NE). Each long line had four HOBO MX2202 temperature and light data loggers (Onset®, Bourne, MA) stationed at it, one for each 0 m, 1 m, 2 m, and 3 m depth to detect temperature differences the seaweeds may be experiencing across time. HOBO light data was not used due to fouling of the light sensor. Upwelling data was obtained from the CUTI index database.

Data Analysis

Daily growth rates were calculated from the data collected from each trial. Daily growth rates (D) are expressed as wet weight gained per day, and were calculated as follows:

$$D = (X - X_0)/t$$

Where X is the final weight measurement, X_0 is the initial weight measurement, and t is the total time in days.

Growth of *D. mollis* was analyzed graphically for trends across depths and time by plotting the average wet weights for each depth obtained from each month of sampling. A two-way analysis of variance (ANOVA) was used to test for the effect that depth and month, and their interaction had on wet weight using the aov() function in base-R for trial 1 (both long-lines), trial 2 long-line1, and trial 2 long-line 2. Trial 2's long-lines were tested separately as they were seeded at different times. If there were significant results, a post-hoc TukeyHSD test was performed using the TukeyHSD() function in base R to make pair-wise comparisons between the different treatments. The best model's residuals were graphically analyzed to determine if the assumptions of normality and homogeneity of variance were met. Two-way ANOVA and TukeyHSD were also performed on PAR, water temperature, and salinity data to determine if depth and month had an effect. The statistical results for PAR, water temperature, and salinity measurements were then compared to growth trends.

Along with the growth, the presence or absence of bundles on the droppers is an important factor to consider when it comes to the size of harvest. Crop loss was measured by calculating the percent loss (%loss) as follows:

$$\%loss = \sum_{i,T}$$

Where $\sum$ is the sum of all missing bundles for depth i in trial T. Because there are 100 bundles per depth, this sum translates directly to a percent.

Tissue samples sent to AGQ Labs were dried at 80˚C and analyzed using an elemental analyzer and inductively coupled plasma optical emission spectrometry (ICP-OES) to determine what fraction of *D. mollis* tissue was made up of carbon, nitrogen, and phosphorus which can be used to determine how much of each of these three elements this species of red macroalgae can sequester from its environment. Total amount of a given element removed from the water (tot_x) was calculated by multiplying the total *D. mollis* net biomass dry weight including both harvest and sample weights (N) by the percent element mass in the sample that was reported on the lab results (%nm).

$$tot_x = N/\%nm$$

Nutrient removal rates were then calculated for carbon, nitrogen, and phosphorous to evaluate the potential for removal by *D. mollis* using the following equation:

$$RR = W_{LL} \bullet 0.15 \bullet G_i \bullet (\%nm/100)$$

where RR is the removal rate, W_{LL} is the net wet weight per long-line, 0.15 is the conversion factor to convert wet weight to dry weight assuming 85% moisture, and G_i is daily growth rate for depth i.

Tissue samples sent to AGQ Labs were also tested for 584 pesticide contaminants measured in mg/kg using gas chromatography – tandem mass spectrometry (GC-MS/MS) and liquid chromatography – tandem mass spectrometry (LC-MS/MS), and tissue samples sent to University of Missouri ESCL underwent inductively coupled plasma mass spectrometry ICP-MS analysis for the following eight heavy metals: Arsenic, Cadmium, Chromium, Copper, Manganese, Mercury, Lead, and Zinc measured in mg/kg.

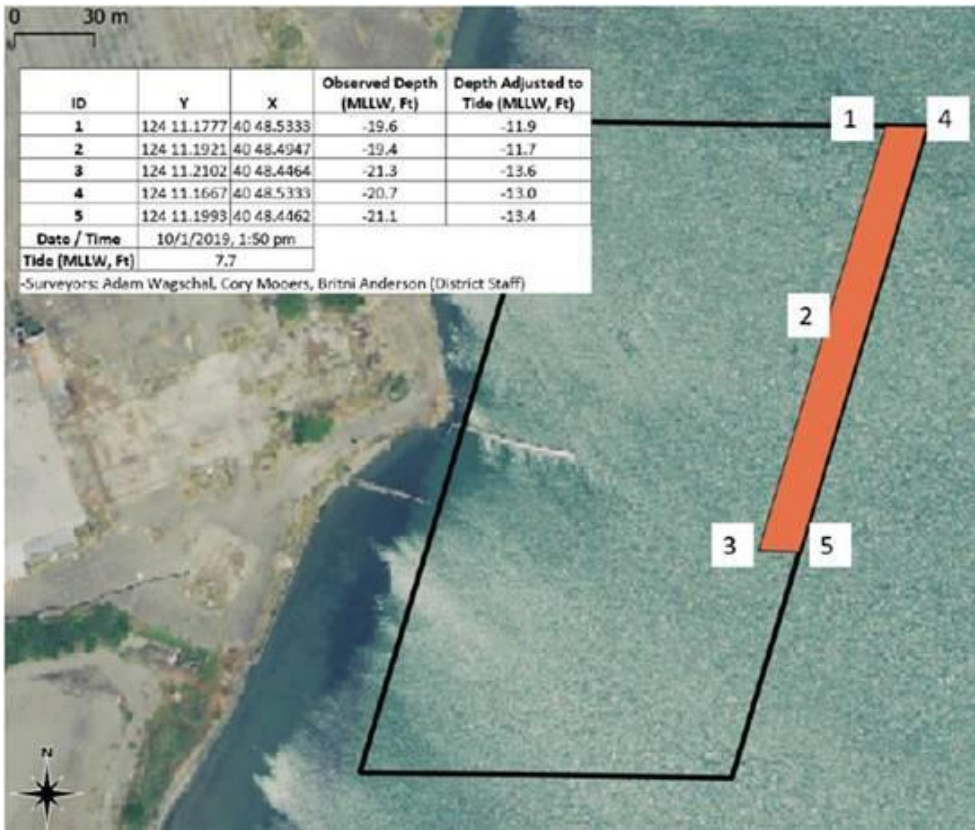

Figure 1. Black outline indicates harbor district pre-permitted lease area. Red rectangle indicates the farm site lease area containing the two long-lines. Image courtesy of Adam Wagschal.

Figure 2. Location of farm site in Samoa Channel marked by star symbol. Image courtesy of Adam Wagschal.

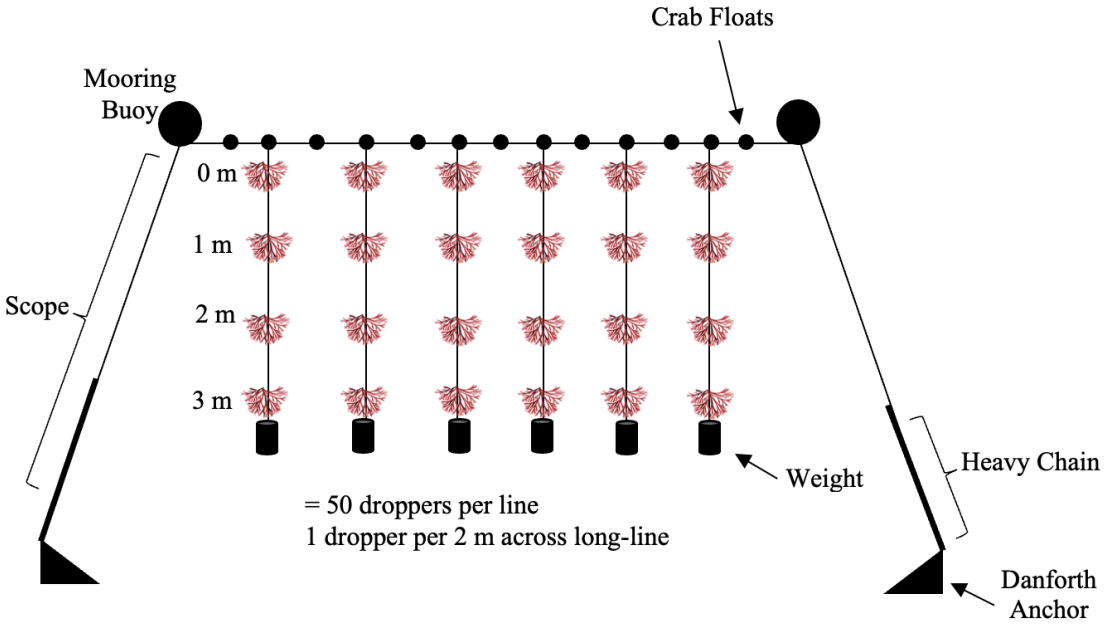

Figure 3. Illustration of long-line layout. Diagram not to scale.

Figure 4. Illustration on inserting *D. mollis* into droppers: (a) twist line in opposite direction of natural twist to open up the strands of the line, (b) insert seaweed and twist in natural direction to close strands around the seaweed.

RESULTS

Growth and Crop Retention

Trial 1 (August 26 – December 18) resulted in a total wet weight of 17.42 kg (38.4 lbs) of dulse including both the amount from collected samples and the amount harvested at the end of the grow-out period. Subtracting the initial 6 kg (13.2 lbs) of seed dulse the trial began with results in a net wet weight of 11.42 kg (25.2 lbs). Trial 2 March 27 – July 16 and April 7 – July 30) resulted in a similar amount with a total wet weight of 17.15 kg (37.8 lbs), and a net wet weight of 11.15 kg (24.6 lbs). However, due to the different out-planting times in Trial 2, the long-lines did not contribute equally to the total as they did in Trial 1. Long-line 1 produced 10.71 kg (23.6 lbs) of the total wet weight while long-line 2 produced only 6.44 kg (14.2 lbs).

Depths 0 m and 1 m tended to have higher mean daily growth rates compared to depths 2 m and 3 m (Table 1). Trial 1 and Trial 2 long-line 1 had similar mean daily growth rates of 0.21 g/day and 0.19 g/day respectively. Trial 2 long-line 2 which was out-planted two weeks later than Trial 2 long-line 1 had the lowest mean daily growth rates across depths.

Depth in Trial 1 had a significant effect on wet weight (two-way ANOVA, $F_{3,87}$ =10.664, p<0.001). Pair-wise comparison revealed 0 m had significantly more biomass than both the 2 m (TukeyHSD, p=0.003) and the 3 m growth (p<0.001), and the 1 m had significantly more biomass than the 3 m growth (p=0.002). Month was also found to be a

significant factor in wet weight of *D. mollis* during Trial 1 ($F_{3,87}$ =5.617, p=0.002) with September having significantly less biomass compared to both November (p=0.036) and December (p=0.005) while December had more biomass compared to October (p=0.02). This is reflected in Figure 5 as the trends for the 0 m and 1 m break away from the 2 m and 3 m as time moves forward. The interaction between depth and month were not significant for Trial 1 ($F_{9,87}$ =1.045, p=0.413). For Trial 2 long-line 1 depth ($F_{3, 51}$=4.697, p=0.006) was a significant factor for wet weight with the 0 m having significantly more biomass than the 2 m (p=0.002) and the 3 m (p=0.025) as shown with the large spike in growth (Figure 6). The interaction between depth and month ($F_{9,51}$=3.558, p=0.003) was also significant with May 0 m having significantly more biomass than all other month and depth combinations: April 0 m (p=0.003), April 1 m (p=0.002), April 2 m (p<0.001), April 3 m (p=0.007), May 1 m (p=0.004), May 2 m (p<0.001) May 3 m (p<0.001), June 0 m (p<0.001), June 1 m (p=0.005), June 2 m (p<0.001), June 3 m (p<0.001), July 0 m (p=0.003), July 1 m (p<0.001), July 2 m (p<0.001), and July 3 m (p<0.001). Trial 2 long-line 2 presents similar growth trends as long-line 1 just at a smaller scale (Figure 7). Just as with long-line 1, depth is a significant factor ($F_{3,47}$=9.228, p<0.001) for wet weight on long-line 2 with 0 m having more biomass compared to 1 m (p=0.037), 2 m (p<0.001), and 3 m (p<0.001). However, month is also significant for Trial 2 long-line 2 ($F_{3,47}$=4.679, p=0.006) with the drop in wet weight from May to June being more significantly pronounced (p=0.005). The interaction between depth and month was not significant ($F_{9,47}$=2.175, p=0.514).

In terms of crop retention, all depths experienced a loss of nearly 50% of bundles by the end of Trial 1 (Figure 8). However, Trial 2 experienced a different trend with a steady increase of crop loss as depth increased with depths 0 m and 1 m having the greatest crop retention (Figure 9).

While fouling was not a factor that was measured in this study, it was observed that fouling increased with increasing depth. Fouling was heavier in Trial 2 than in Trial 1, with fouling increasing from spring to summer while a decrease in fouling was observed during November in Trial 1 (Figures 10, 11, 12). Fouling during fall consisted mainly of hydroids and sediment. Barnacles and sediment dominated spring and summer. Besides these, other organisms that occurred on the lines and *D. mollis* included other macroalgae (e.g., red filamentous algae, *Ulva* spp.), colonial tunicates, amphipods and isopods, nudibranchs and their egg cases, skeleton shrimp, worms and their sticky fibers, rock crabs, kelp crabs, and small juvenile fishes.

Water Quality

During the fall to early winter grow-out season, secchi depth ranged from 1.1 m to 3.3 m with a mean of 1.8 ± 0.6 m. YSI water temperature varied widely from 9.9˚C to 18.6˚C decreasing through time, with a mean of 12.6 ± 2.3˚C. CastAway CTD data revealed that month has a significant effect on water temperature at the farm site (two-way ANOVA, $F_{3,39}$=10.82, p<0.001) while the effect of depth ($F_{3,39}$=1.04, p=0.388) and the interaction between depth and month ($F_{9,39}$=1.214, p=0.332) were not significant. The

month of December had a significantly lower average temperature than September (TukeyHSD, $p<0.001$), October ($p<0.001$), and November ($p=0.040$) (Figure 13). YSI salinity ranged from 31.8 psu to 33.2 psu with a mean of 32. ± 0.3 psu. CastAway CTD data revealed that month has a significant effect on salinity ($F_{3,39}=18.061$, $p<0.001$) as well as the interaction between depth and month ($F_{9,39}=7.665$, $p<0.001$), but depth is not a significant factor ($F_{3,39}=2.981$, $p=0.06$). The month of September had a significantly lower salinity than October ($p<0.001$) and November ($p=0.001$). December also had a significantly lower salinity than October ($p<0.001$) and November ($p=0.007$). The interaction between September 0 m was significantly lower than all other depth and month combinations of Trial 1 ($p<0.001$ per combination) (Figure 15). pH ranged from 8.01 to 8.12 varying little with a mean of 8.07 ± 0.04. Dissolved oxygen ranged from 1.57 mg/L to 9.70 mg/L with a mean of 6.63 ± 2.92 mg/L. Both depth ($F_{3,43}=17.002$, $p<0.001$) and month ($F_{3,43}=8.482$, $p<0.001$) during Trial 1 had a significant effect on PAR intensity, but the interaction between depth and month was not significant ($F_{9,43}=1.221$, $p=0.322$). Pair-wise comparison revealed that 0 m depth had significantly higher PAR light intensity than 1 m ($p=0.010$), 2 m ($p<0.001$), and 3 m ($p<0.001$). Depth 1 m also had significantly higher PAR light intensity than 3 m ($p<0.011$). Pair-wise comparison also revealed that the month of October had a significantly higher PAR light intensity than both November ($p=0.012$) and December ($p<0.001$).

The spring and summer growth-out season experienced lower average visibility than in Trial 1 with secchi depth ranging from 0.85 m to 2.65 m with a mean of 1.58 ±

0.45 m. YSI water temperature ranged from 11.0˚C to 18.7˚C increasing through time with a mean 14.54 ± 2.20˚C. CastAway CTD data revealed that month has a significant effect on water temperature (two-way ANOVA, $F_{3,39}$=8.705, p<0.001) with April being significantly cooler than July (TukeyHSD, p<0.001) while depth ($F_{3,39}$=0.445, p=0.722) and the interaction between depth and month ($F_{9,39}$=0.023, p=1.000) were not significant factors (Figure 14). Salinity ranged from 31.6 psu to 33.5 psu with a mean of 33.0 ± 0.4 psu. CastAway CTD data revealed that month has a significant effect on salinity ($F_{3,39}$=26.587, p<0.001), but depth ($F_{3,39}$=0.807, p=0.498) and the interaction between depth and month ($F_{9,39}$=0.194, p=0.993) did not have a significant effect. The month of April was significantly less saline than May (p<0.001), June (p<0.001), and July (p<0.001) (Figure 16). pH ranged from 7.77 to 8.28 with a mean 8.05 ± 0.13. Dissolved oxygen ranged from 6.10 mg/L to 11.25 mg/L with a mean of 7.83 ± 1.25 mg/L. Depth was the only significant factor affecting PAR light intensity during Trial 2 ($F_{3,59}$=34.826, p<0.001). Depth 0 m had significantly higher PAR light intensity than 1 m (p=0.002), 2 m (p<0.001), and 3 m (p<0.001). Depth 1 m also had significantly higher PAR light intensity than 2 m (p=0.003) and 3 m (p<0.001). Interaction between depth and month in Trial 2 was not significant ($F_{9,59}$=0.478, p=0.882). Average light intensity was lower for Trial 2 and experienced greater percent light reduction than in Trial 1 (Table 2, Figures 17 and 18).

Seawater nitrate values remained low (≤1.4 mg/L) through the study. Phosphorus was also low (≤0.5 mg/L). Ammonia was not detected (0 mg/L) while nitrite fluctuated

throughout the study (0 mg/L to 10 mg/L) (Figures 19 and 20). The coastal upwelling season of 2020 concluded in October, and resumed in February 2021 increasing through to the end of the study (Figure 21).

Carbon, Nitrogen, and Phosphorus Analysis

Average moisture level for samples submitted was 85.4 g per 100 g. Lab results stated that carbon made up 36.4% dry sample biomass, nitrogen made up 3.76% dry sample biomass, and phosphorus made up 0.52% of dry sample biomass resulting in approximately 1.20 kg (2.64 lbs) of carbon, 0.12 kg (0.26 lbs) of nitrogen, and 0.02 kg (0.04 lbs) of phosphorus sequestered from the bay across the total 8 months of the experiment. Removal rates for carbon, nitrogen, and phosphorus are presented in Table 3.

Heavy Metal and Pesticide Analysis

Trial 2 depths 2 m and 3 m for manganese exceeded the tolerable upper intake limit (UL) as did initial IMTA nursery samples for manganese and zinc. Trial 2 depths 0 m and 1 m exceed the Malaysian UL for arsenic, but are under ULs for Norway and Hong Kong (Tables 4, 5). All other values from the tissue analysis of the eight heavy metals were under their corresponding UL.

Unfortunately, statistical analysis could not be performed on heavy metals as only one sampling event occurred per trial; however, the concentration values in Tables 2 and 3 revealed a trend occurring across seasons that could be descriptively analyzed. Chromium, copper, lead, manganese, and zinc all tend to display increasing

concentrations with increasing depth in both trials with clearly defined difference between depths 0 m to 1 m and 2 m to 3 m for Trial 2. Interestingly, the trend for arsenic and cadmium was opposite with decreasing concentration of the metals with increasing depth again showing a defined difference between depths 0 m to 1 m and 2 m to 3 m in Trial 2. Concentrations of heavy metals also appear to increase during the spring and summer as compared to fall and winter with the exception of mercury for which no values greater than 0.1 mg/kg were recorded for either depth or season. Manganese had the highest concentrations for 2 m and 3 m depths during Trial 2 with an 8 to 15-fold increase compared to its 0 m and 1 m concentrations. From 584 pesticide contaminants tested (see list in Appendix), no positive results were found. Limit of quantification was 0.01 mg/kg.

Table 1. Mean daily growth rates ± SD (g/day) for dulse at depths of 0 m, 1 m, 2 m, and 3 m (for Trial 1 both long-lines (LL) n = 22 per depth, for Trial 2 n = 12 per depth for each LL).

Depth (m)	Trial 1 Both LL	Trial 2 LL 1	Trial 2 LL 2
0	0.21 ± 0.99	0.19 ± 0.88	-0.02 ± 0.43
1	0.10 ± 0.47	-0.04 ± 0.36	-0.09 ± 0.12
2	-0.05 ± 0.81	-0.21 ± 0.20	-0.2 ± 0.10
3	-0.17 ± 0.10	-0.14 ± 0.66	-0.33 ± 0.10

*Negative values due to frond breakage.

Table 2. Mean PAR values per depth and trial ± SD (μmol/m^2/s), range, and percent reduction in light intensity from the surface to corresponding depth.

Trial	Depth	Minimum	Maximum	Average	% Light Reduction
Trial 1	0 m	21.5	2619	931.1 ± 587.4	
	1 m	15.9	1193.5	570.3 ± 312.1	38.8
	2 m	8.8	647.0	319.7 ± 179.6	65.7
	3 m	5.1	54.8	185.5 ± 98.0	80.1
Trial 2	0 m	101.4	1167	668.3 ± 323.8	
	1 m	67.9	754.5	431.7 ± 201.2	35.4
	2 m	32.9	350.2	210.0 ± 108.9	68.6
	3 m	14.4	203.6	100.9 ± 55.4	84.9

Table 3. Removal rates for carbon, nitrogen, phosphorous (g $LL^{-1}d^{-1}$) by net wet weight of *D. mollis* tissue produced per Trial. Only positive growth rates were used to calculate removal rates.

Trial	Element	Depth 0 m	Depth 1 m
Trial 1, Both LL	C removal rate	65.5	31.2
	N removal rate	6.8	3.2
	P removal rate	0.9	0.44
Trial 2, LL1	C removal rate	80.0	
	N removal rate	8.3	
	P removal rate	1.1	

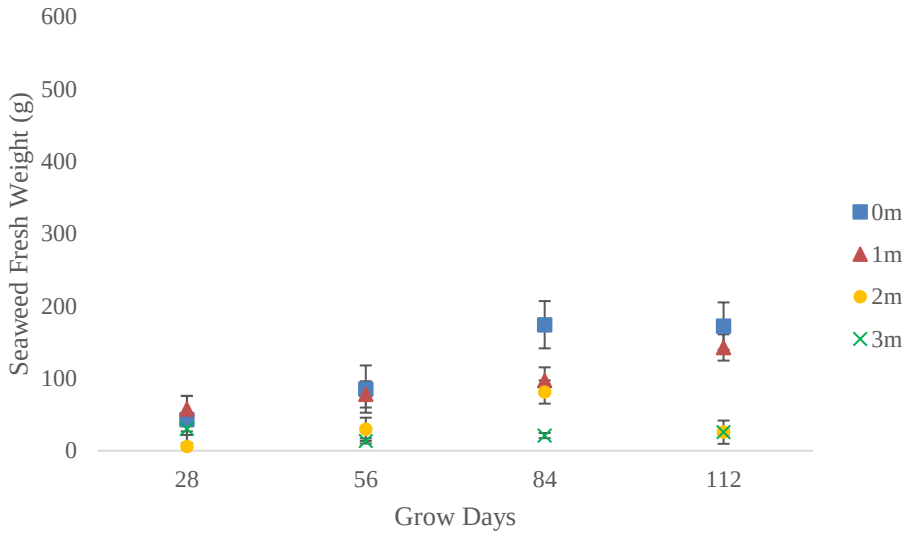

Figure 5. Trial 1 growth trends for dulse in grams ± SE in Humboldt Bay for the months of September (28 days), October (56 days), November (84 days), and December (112 days) of 2020 for four different depths on both long-lines (LL) combined (with n = 6 per depth per month).

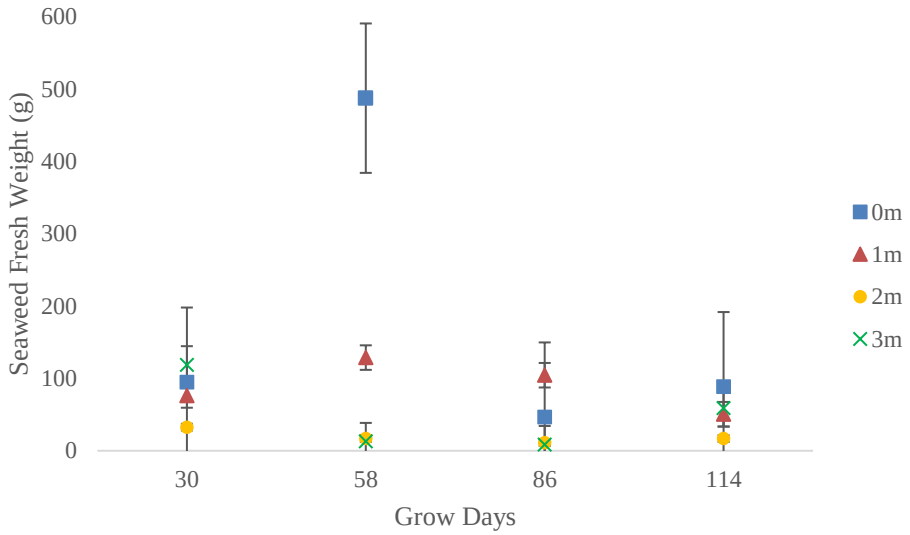

Figure 6. Trial 2 growth trends for dulse in grams ± SE in Humboldt Bay for the months of April (30 days), May (58 days), June (86 days), and July (114 days) of 2022 for four different depths on long-line 1 (LL1) (*n* = 3 per depth per month).

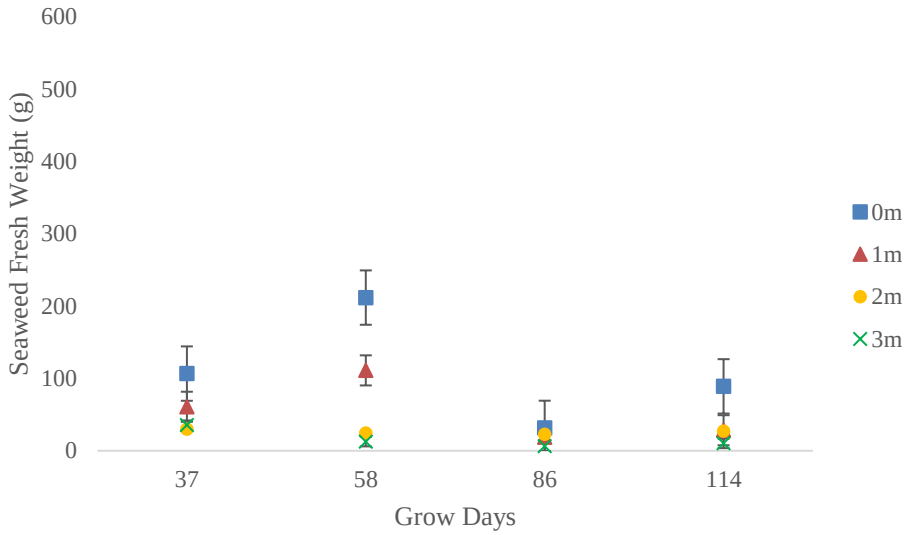

Figure 7. Trial 2 growth trends for dulse in grams ± SE in Humboldt Bay for the months of April (37 days), May (58 days), June (86 days), and July (114 days) of 2022 for four different depths on long-line 2 (LL2) (*n* = 3 per depth per month).

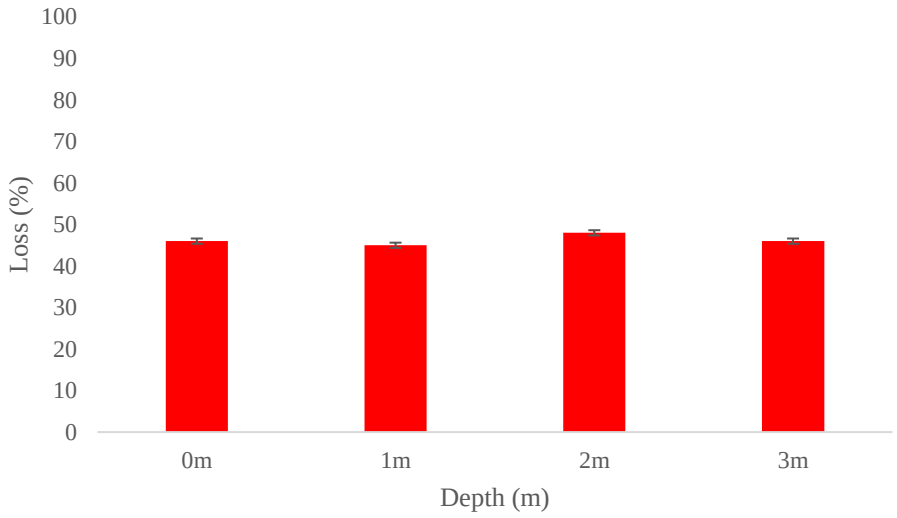

Figure 8. Percent crop loss ± SE for depths 0 m, 1 m, 2 m, 3 m with both long-lines combined from September to December 2020 (n=100 per depth).

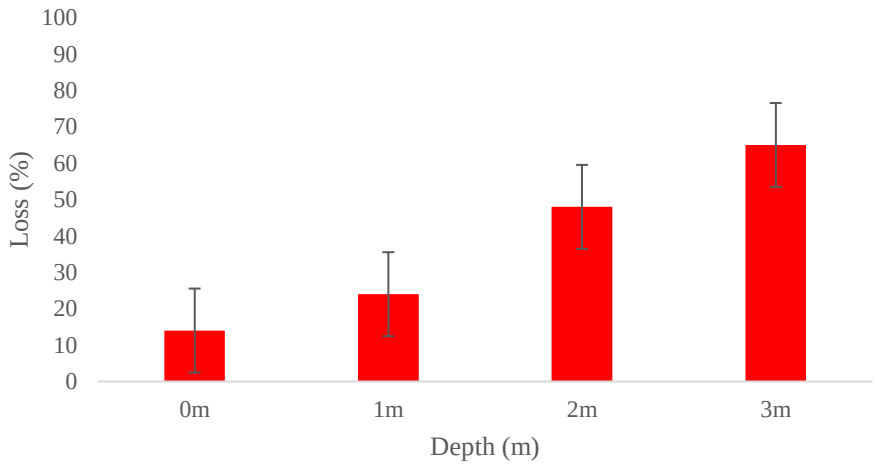

Figure 9. Percent crop loss ± SE for depths 0 m, 1 m, 2 m, 3 m with both long-lines combined from late March to late July 2021 (n=100 per depth).

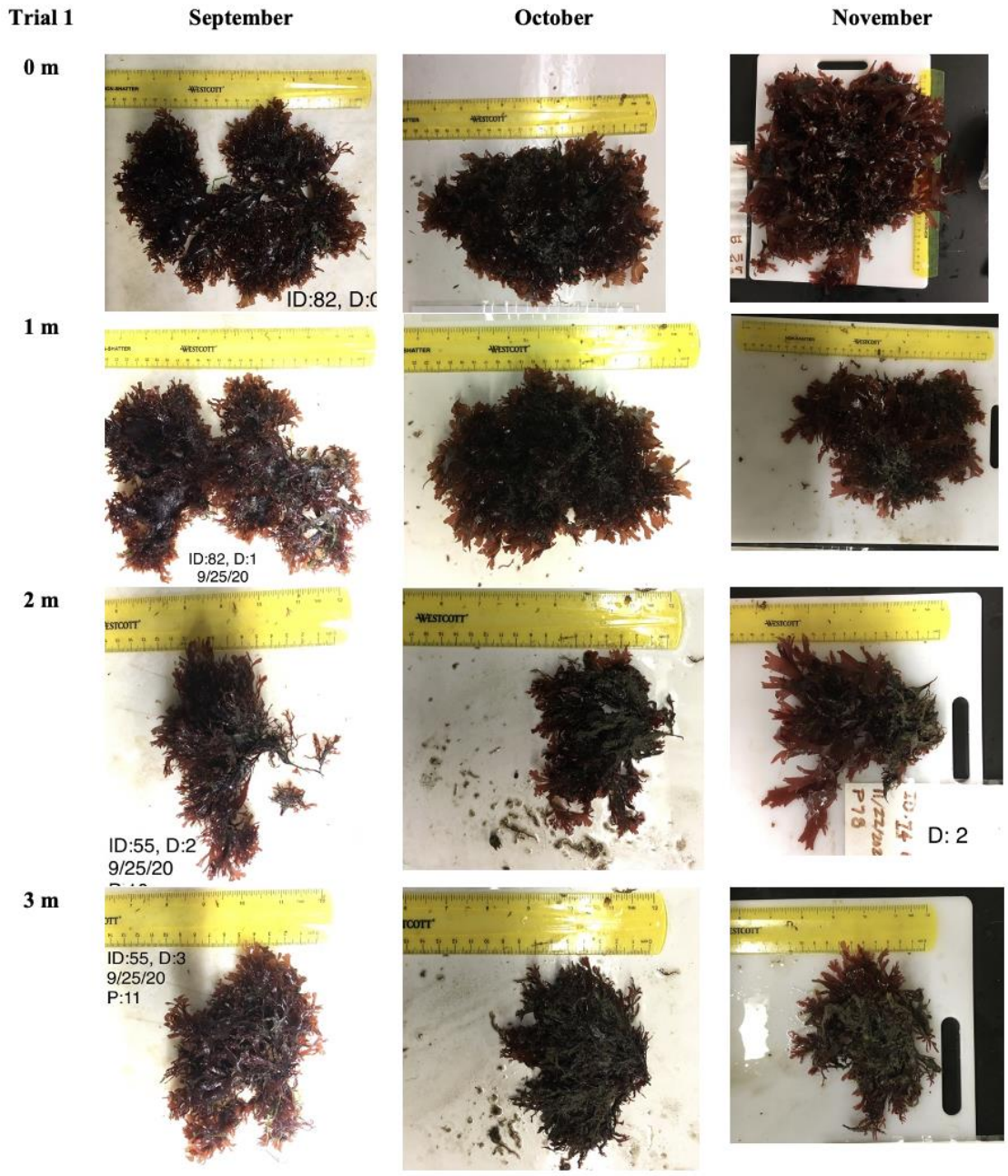

Figure 10. Trial 1 progression of fouling by month and depth on *D. mollis* grown in Humboldt Bay. December is excluded as no notes or photos were taken of samples on harvest day. Photos of monthly samples were selected that most closely resembled the average state of the seaweed on both long-lines.

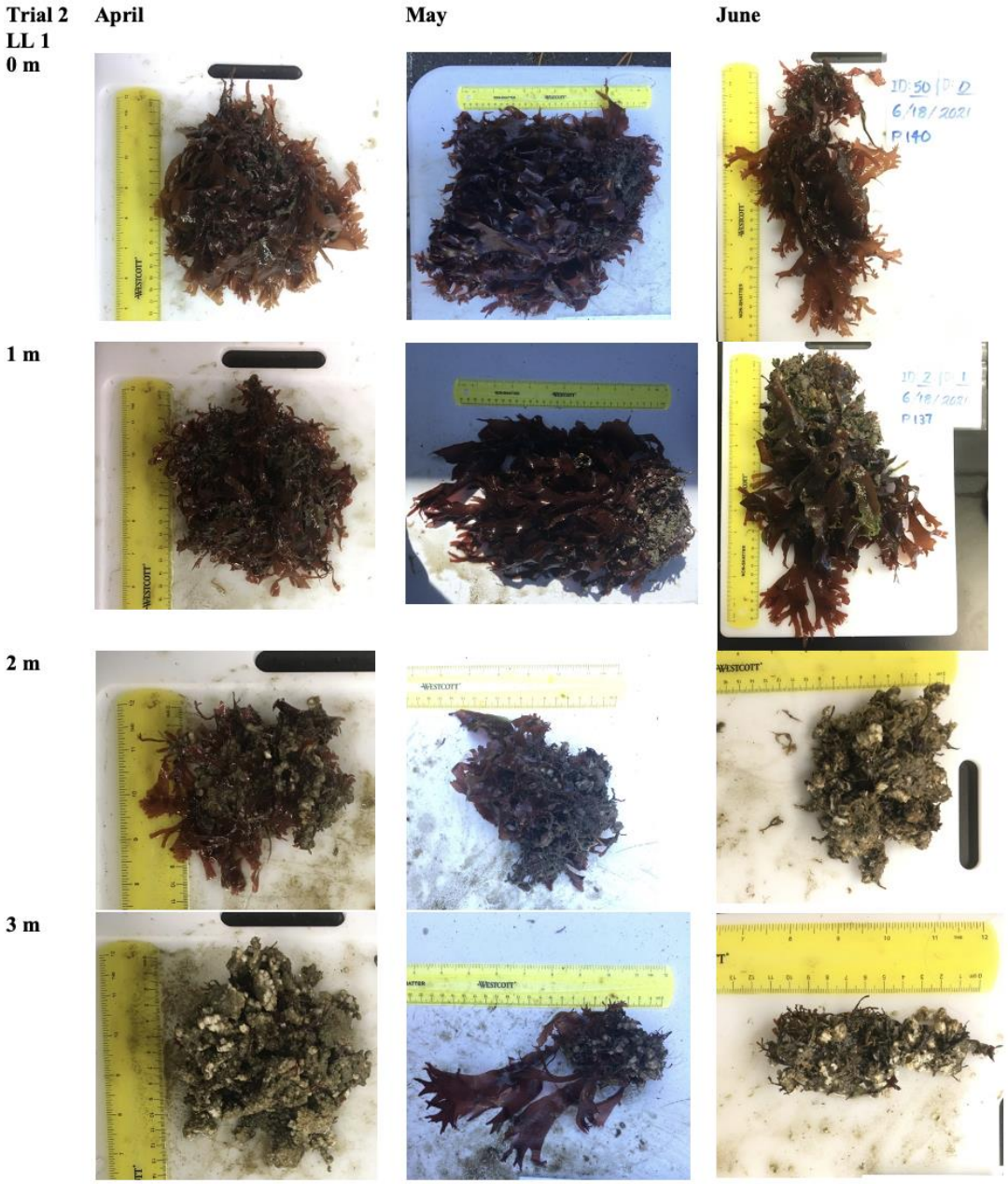

Figure 11. Trial 2 progression of fouling by month and depth on *D. mollis* grown in Humboldt Bay. July is excluded as no notes or photos were taken of samples on harvest day. Photos of monthly samples were selected that most closely resembled the average state of the seaweed on long-line 1.

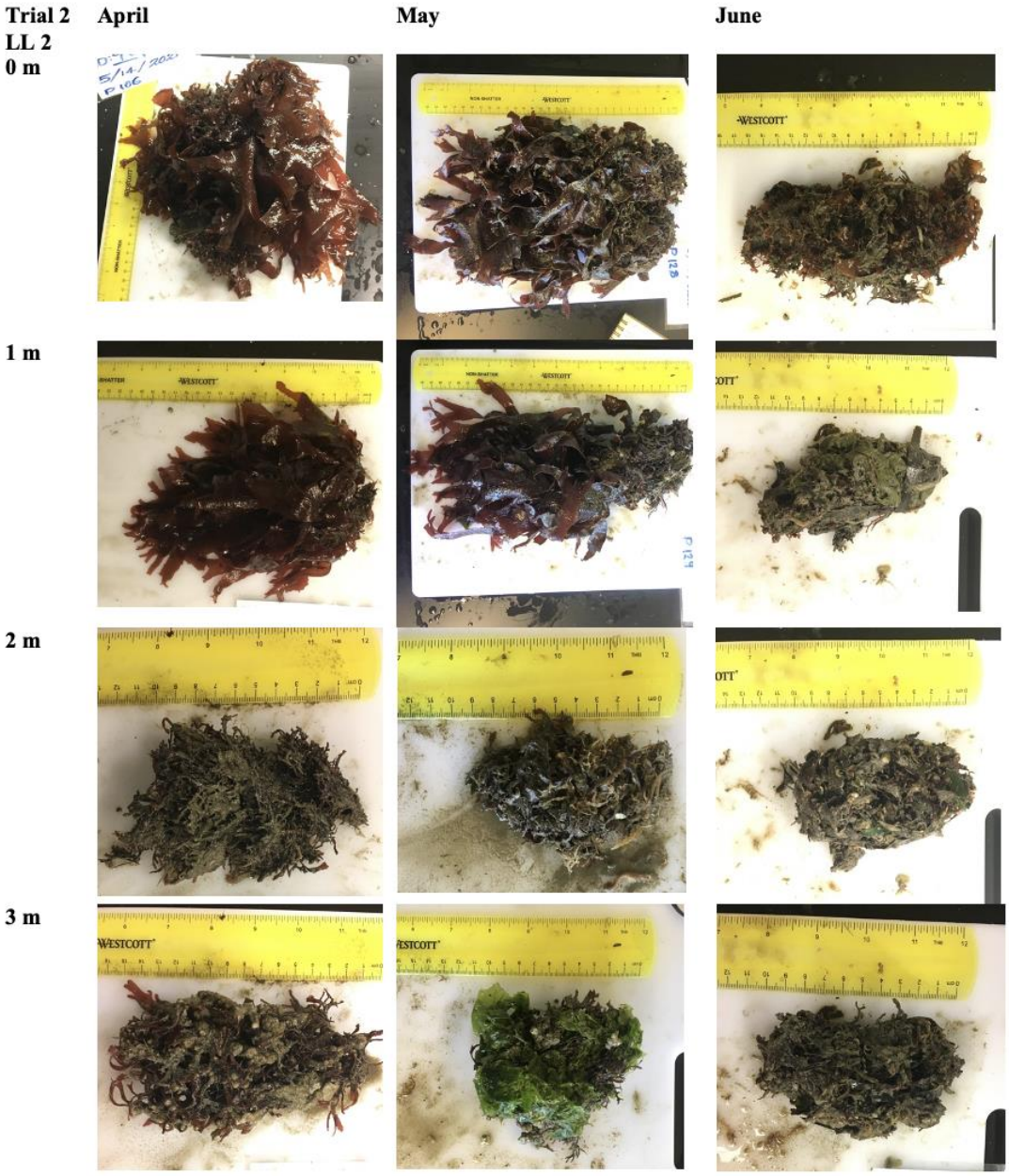

Figure 12. Trial 2 progression of fouling by month and depth on *D. mollis* grown in Humboldt Bay. July is excluded as no notes or photos were taken of samples on harvest day. Photos of monthly samples were selected that most closely resembled the average state of the seaweed on long-line 2

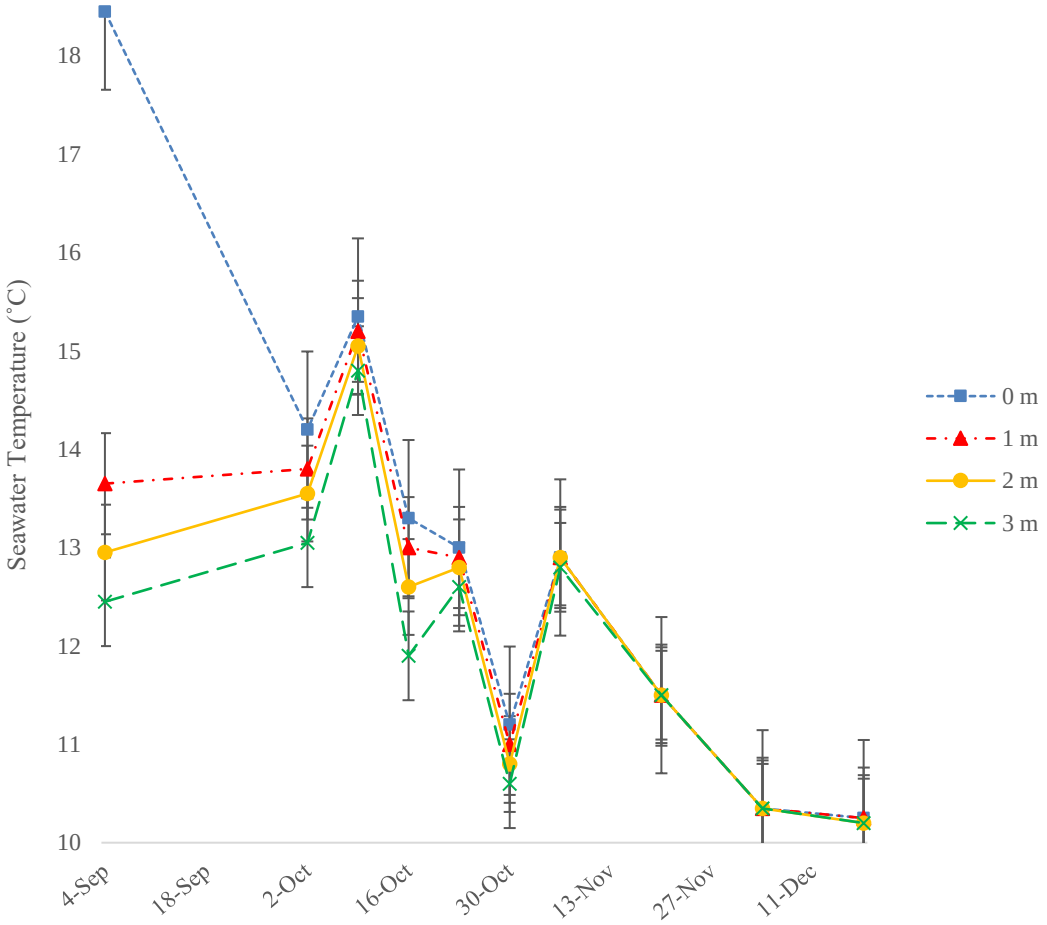

Figure 13. Mean water temperature ± SE per depth from Trial 1 (n=64).

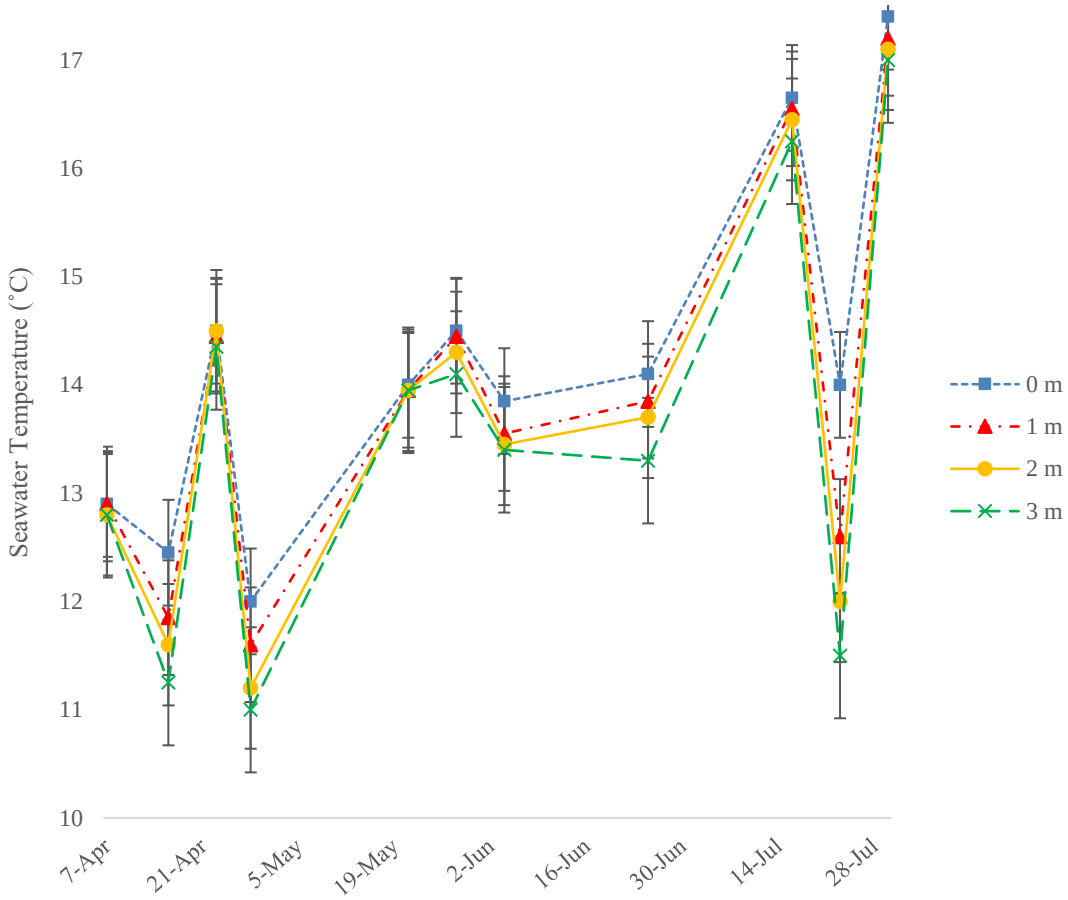

Figure 14. Mean water temperature ± SE per depth from Trial 2 (n=72).

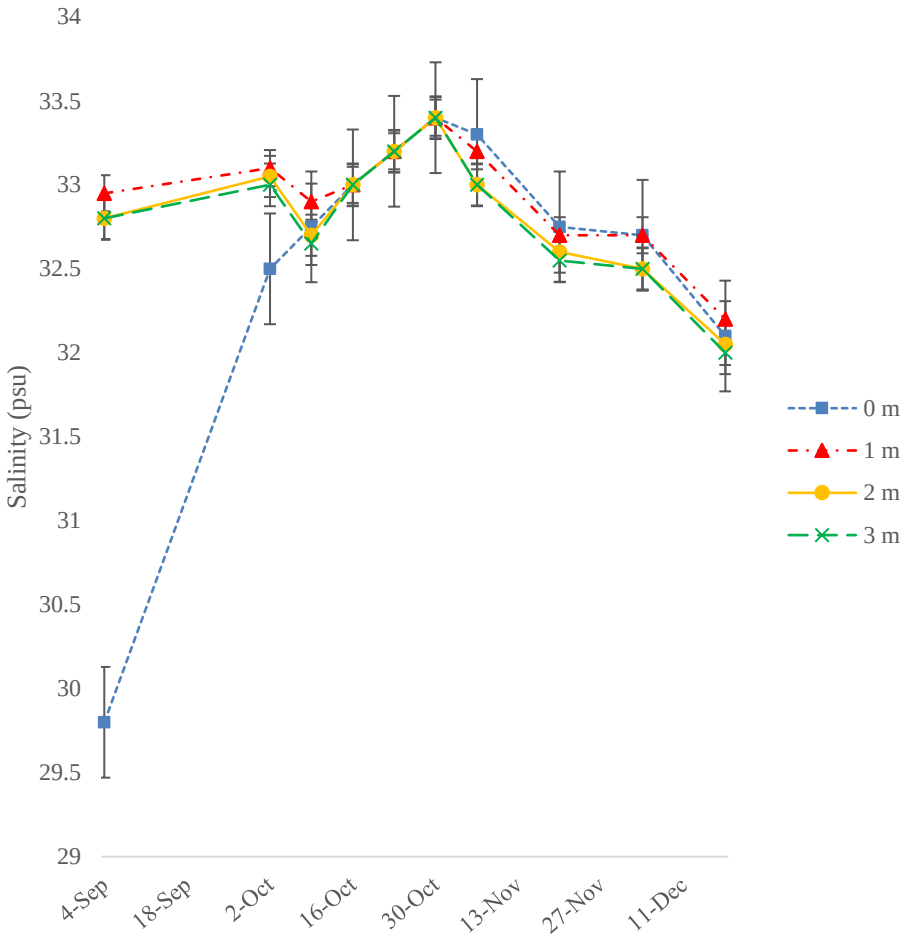

Figure 15. Mean water salinity ± SE per depth from Trial 1 (n=64).

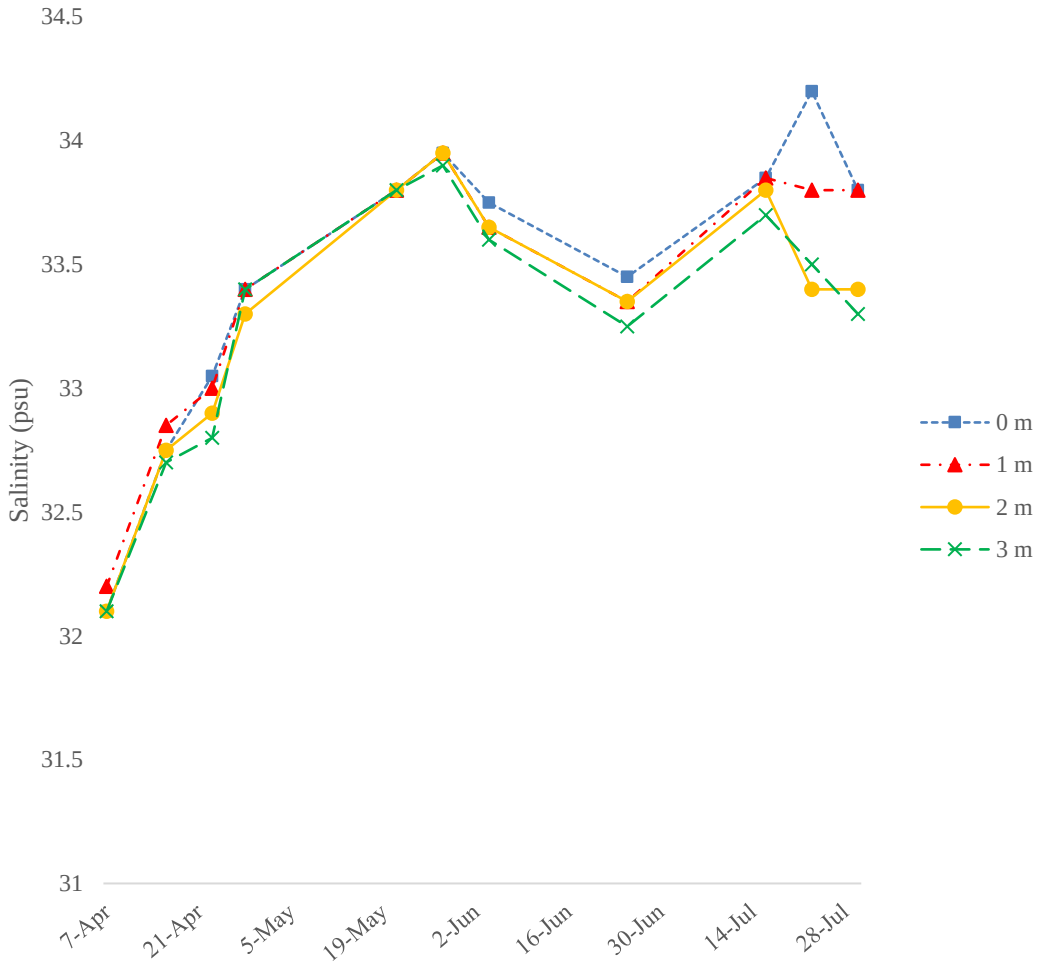

Figure 16. Mean water salinity ± SE per depth from Trial 2 (n=72).

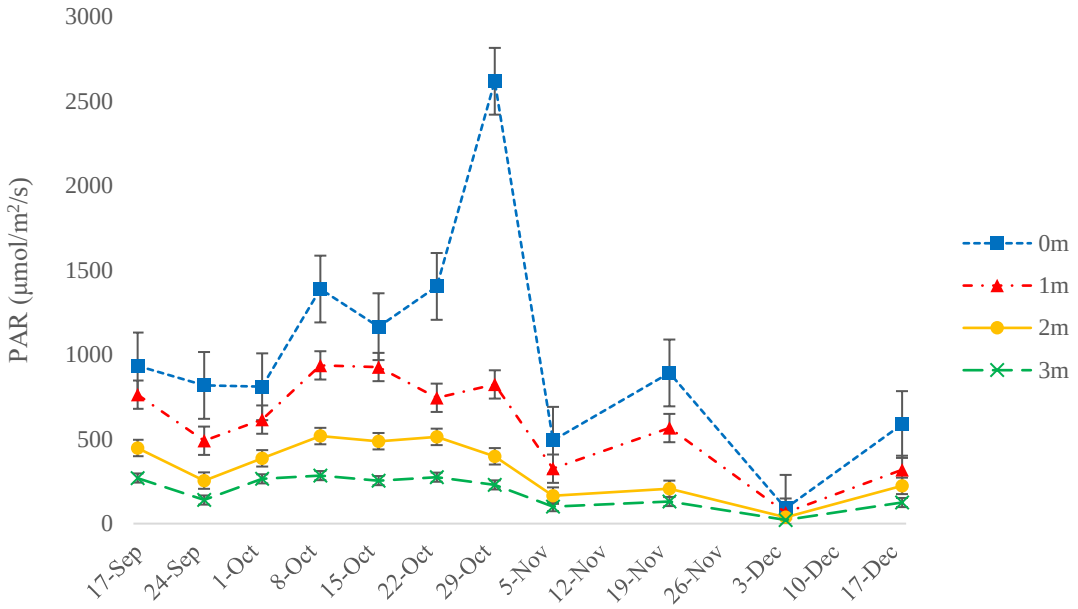

Figure 17. Mean PAR irradiance ± SE for Trial 1 at four different depths: 0 m, 1 m, 2 m, and 3 m (n=20 per depth).

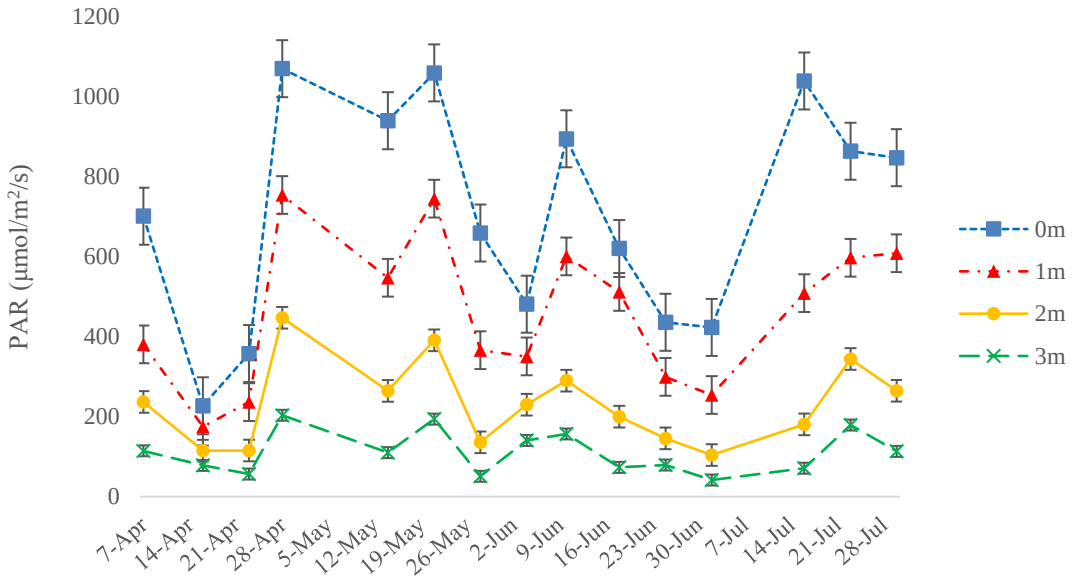

Figure 18. Mean PAR irradiance ± SE for Trial 2 at four different depths: 0 m, 1 m, 2 m, and 3 m (n=25 per depth).

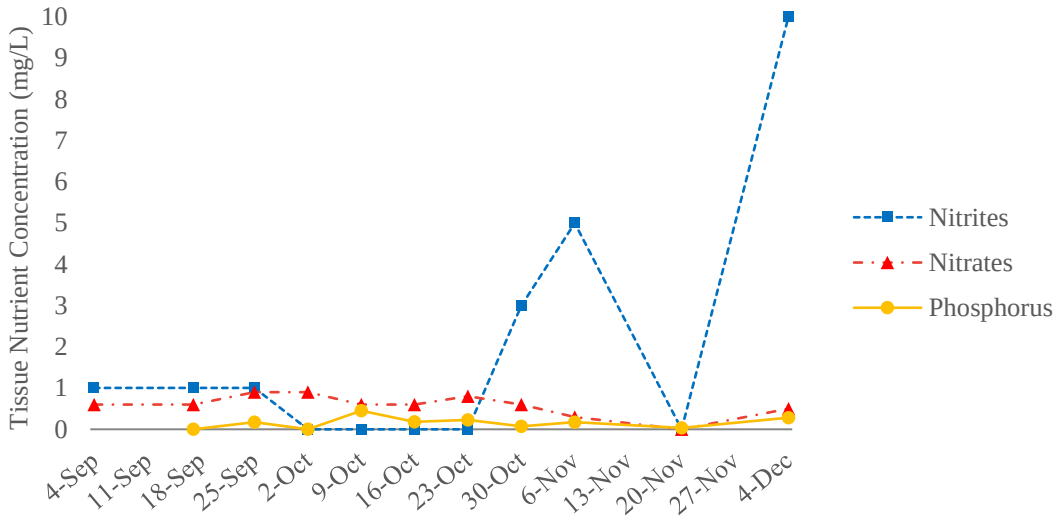

Figure 19. Trial 1 water quality measurements for nitrite, nitrate, and phosphate. Ammonia is excluded as it was not detected by the test used. Error bars not included as each point represents a single measurement (n=11 per nutrient).

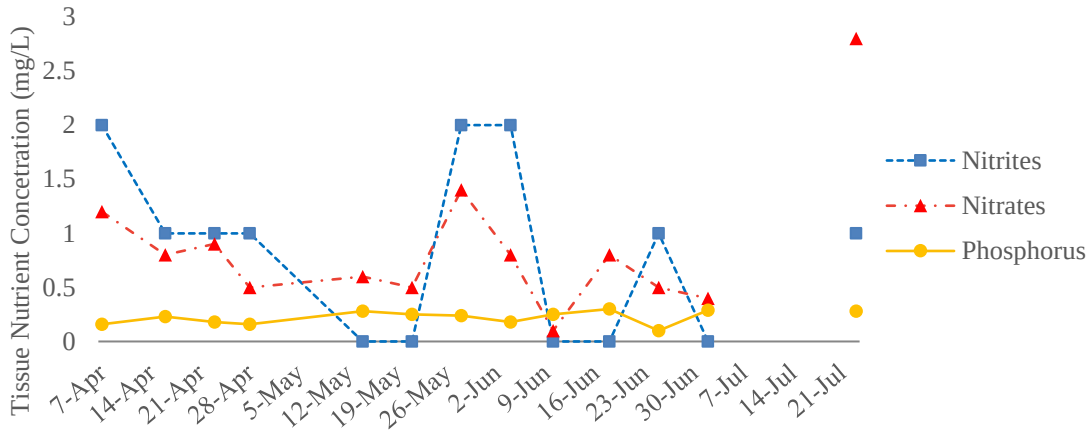

Figure 20. Trial 2 water quality measurements for nitrite, nitrate, and phosphate. Ammonia is excluded as it was not detected by the test used. Error bars not included as each point represents a single measurement (n=13 per nutrient).

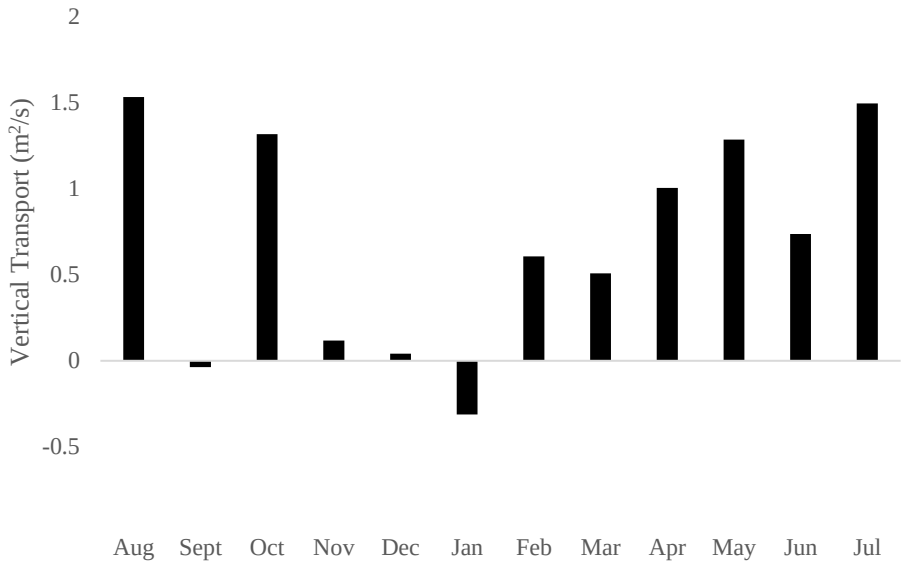

Figure 21. Average monthly upwelling estimates obtained from the CUTI upwelling index for latitude 41˚ N on the northern California coast (Jacox et al. 2018; PEFL, 2023).

Table 4. Lab results for Trial 1 heavy metals in *D. mollis* tissue samples from various depths. Depth heavy metal values expressed as mg/kg. Tolerable Intake Upper Limits (UL) are expressed as either mg/kg or mg/day.

Depth (m)	$Arsenic_{Total}$	Cadmium	Chromium	Copper	Lead	Manganese	Mercury	Zinc
Nursery	0.038	0.035	0.113	1.09	0.071	3.78	< 0.01	18.1
0	0.48	0.07	0.10	1.11	0.05	3.82	< 0.1	5.11
1	0.56	0.07	0.46	1.48	0.12	7.72	< 0.1	7.23
2	0.29	0.06	0.39	1.63	0.08	7.68	< 0.1	5.88
3	0.35	0.05	1.11	1.95	0.21	21.0	< 0.1	5.66
UL	1-10 mg/kg[1]	3.0 mg/kg[2]	N/A	10 mg/day[3]	3.0 mg/kg[2]	11 mg/day[4]	0.1 mg/kg[2]	40 mg/day[5]

[1]Regulatory limits for total arsenic in seafood for Norway (4 mg/kg fish), Hong Kong (6 mg/kg fish, 10 mg/kg shellfish), and Malaysia (1 mg/kg fish) (Chew, 1996; Smith et al., 2010), [2]For food supplements (seaweed not specified) with exception of Cadmium where food supplements are made exclusively or mainly of seaweed (European Commission, 2006), [3]NIH, 2022b (seaweed not specified), [4]NIH, 2021 (seaweed not specified), [5]NIH, 2022d (seaweed not specified).

Table 5. Lab results for Trial 2 heavy metals in dulse tissue samples from various depths. Depth heavy metal values expressed as mg/kg, and Tolerable Intake Upper Limits (UL) are expressed as either mg/kg or mg/day.

Depth (m)	$Arsenic_{Total}$	Cadmium	Chromium	Copper	Lead	Manganese	Mercury	Zinc
Nursery	0.083	0.075	0.120	2.85	0.307	28.2	< 0.1	43.3
0	1.30	0.175	0.275	1.13	0.064	9.77	< 0.1	3.98
1	1.25	0.145	0.183	1.35	0.047	6.27	< 0.1	3.71
2	0.364	0.073	0.979	2.81	0.323	77.6	0.028	14.2
3	0.282	0.075	0.494	3.53	0.356	92.2	0.043	19.0
UL	1-10 mg/kg[1]	3.0 mg/kg[2]	N/A	10 mg/day[3]	3.0 mg/kg[2]	11 mg/day[4]	0.1 mg/kg[2]	40 mg/day[5]

[1]Regulatory limits for total arsenic in seafood for Malaysia (1 mg/kg fish), Norway (4 mg/kg fish), and Hong Kong (6 mg/kg fish, 10 mg/kg shellfish) (Chew, 1996), [2]For food supplements (seaweed not specified) with exception of Cadmium where food supplements are made exclusively or mainly of seaweed (European Commission, 2006), [3]NIH, 2022b (seaweed not specified), [4]NIH, 2021 (seaweed not specified), [5]NIH, 2022d (seaweed not specified).

DISCUSSION

Growth and Crop Retention

It was found that depth and month both affect the biomass of *D. mollis* in an open-water system in Humboldt Bay. This may be explained by how growth correlates with PAR light, seawater temperature, and nutrient fluctuations. Statistical depth and month results for PAR light reflect a similar pattern displayed by depth and month results for wet weight. Both light and growth decreased with increase in depth with 0 m and 1 m performing better than the 2 m and 3 m depths for both trials (Figures 5, 6, 7, 17, 18). Higher irradiance during May 2021 (Figure 18) correlates with the increase in wet weight occurring at the same time (Figures 6 and 7). Interestingly, noticeable increase in growth occurred during lower PAR light intensities as irradiance decreased during November and December 2020. This may be that low PAR light intensities can trigger reproduction in *D. mollis* (Werner and Dring, 2011). Potentially, the growth could be in preparation for spore production, but temperature may have also contributed to the growth in late fall and winter.

During September and early October 2020, as in June and July 2021, temperatures reached up to 18˚C (Figures 13 and 14), a temperature considered stressful in a study with *P. palmata,* and while no significant difference in growth rate was found in temperature experiments for the Oregon State University C-3 *D. mollis* strain (at 14˚C, 16˚C, and 18˚C) there was a decreasing trend observed in growth at 18˚C

(Morgan and Simpson, 1981; Demetropoulos and Langdon, 2004b). However, cooler temperatures may have encouraged an increase in growth during November and December 2020. In land-based tank cultivation experiments, Demetropoulos and Langdon (2004d) found that at lower light intensities, such as those observed in late fall and winter in Humboldt Bay, *D. mollis* grows best at approximately 12˚C, which is similar to the average temperature of Trial 1 Demetropoulos and Langdon (2004bd) also found that temperatures from 14˚C to 16˚C (again, similar to the average temperature of Trial 2) are ideal growing conditions for *D. mollis* grown under higher light intensities such as those occurring during spring and summer.

Increase in nutrients, particularly nitrogen and phosphorus, correlate with increase in growth (Demetropoulos and Langdon, 2004c; Martinez et al., 2006). Trial 2 experienced an increase of nutrients in water samples compared to Trial 1 due to seasonal upwelling (Figure 21). Maximal growth at 0 m depth was reached in the month of May similar to findings in Martinez et al. (2006) correlating with an increase in upwelling. Amid fluctuation in coastal upwelling events, increased productivity can reduce nutrient levels during the summer (Martinez et al., 2006). Nitrogen and phosphorous in water samples began decreasing during mid-June 2021 (Figure 20). In late November to early December 2020 there was a minimal uptick in nitrogen and phosphorus when storm events began increasing freshwater input into the bay. However, there was probably less nutrients from run-off due to the drier La Niña conditions experienced that winter.

Typically, salinity is another important factor to the growth of seaweed in general; however, reduced freshwater input may have also contributed to the relativity stable salinity and pH measurements throughout the entirety of the study only decreasing slightly during the wetter winter season. Average salinity in Trial 1 and pH of both trials observed in this study were within acceptable ranges (30 ± 1 to 32 psu; 7.78 to 8.15 pH) for growth for *D. mollis* (Davis, 1980; Demetropoulos and Langdon, 2004ab).

While this project demonstrates that *D. mollis* can be cultivated in Humboldt Bay, it also shows that successful harvests depend on avoiding or minimizing the major issues plaguing this study – fouling and crop loss. Fouling not only down-grades the quality of the seaweed produced, but in excessive amounts it can be a major contributor to loss of the crop as it can severely reduce or prevent all together the algae's ability to photosynthesize. If mostly or fully engulfed, over time the tissue will weaken and break off the line. Loss can occur by other means as well. Longer fronds were more likely to detach in the current from increased drag or while being handled during inspection (Kim et al., 2014). Werner and Dring (2011) also found this to be true in their studies while developing a cultivation manual for *P. palmata* stating that fronds greater than 40 cm or 100 g had a much higher chance of being detached. This study's design may also have partially contributed to seaweed loss as the section of the thallus that was pinched between the lay of the dropper line can bleach, die, and then break as it isn't exposed to the sun. This was observed in a study conducted off the coast of Spain in a similar dropper long-line system growing *P. palmata* (Martinez et al., 2006). While fouling and

loss can have an effect on *D. mollis* in the bay, choosing the correct grow-out season, depth, and harvest period can greatly improve the quality, growth, and retention of the seaweed.

Firstly, the time of year *D. mollis* is cultivated can greatly reduce or increase fouling of the crop. In Figure 5, wet weight increased at all depths during November. It was also observed that much of the fouling that accumulated over September and October began to die off leaving cleaner long-lines and seaweed on the farm (Figure 10). This die off of fouling species could be attributed to the reduction in light, water temperature, nutrients, and a very slight drop in salinity during the late fall and winter season. Werner and Dring (2011) also found fall and winter to be an optimal time to out-plant for this very reason. While *D. mollis* can grow slower in these conditions as well, the farm site mean water temp of around 12˚C at this time year allowed for optimal growth at the corresponding lower light levels (Figure 13) (Demetropoulos and Langdon, 2004d). This growth could potentially be sustained throughout the winter based on what has been observed during Trial 1 and data collected on water conditions via the CUTI index and Humboldt Bay CeNCOOS station (Figure 21, 22); however, January through March was not tested in this study and therefore I could not verify that this is true. The month of May in Trial 2 produced the greatest growth at the 0 m depth and sustained growth for 1 m depth. This may be due to the increase in light, temperature, and nutrients. Unfortunately, because of these better growing conditions, fouling occurred earlier on in Trial 2 than in Trial 1, with most of the 3 m depth bundles being encrusted in barnacles 3 to 4 weeks in, and the 0 m and 1 m bundles becoming

affected by the start of June (Figures 11 and 12). The long-lines themselves were also heavily fouled and sagging under the extra weight. Quality of the fronds reduced during the summer due to a combination of factors: higher water temperatures, competition for nutrients, and loss of frond surface area due to fouling, which affected photosynthesis. Similar findings have been observed on studies of *P. palmata* (Martinez et al., 2006; Werner and Dring, 2011). Therefore, the best time to cultivate *D. mollis* in an open-water system in Humboldt Bay could be from late fall to early spring, although grow out can be extended to the end of May to take advantage of the fast growth, but should be harvested before the seaweed is fouled.

Time of year can be important in considering when to outplant fouling risk aside. While fouling during spring can easily out compete small starter bundles, nutrient fluxes come into play as well. These changes in nutrients could explain the unequal contributions of long-line 1 and long-line 2 during Trial 2. This will be explained in more detail in the following section.

Secondly, the depth at which *D. mollis* is grown in Humboldt Bay can affect how heavily it could be fouled and lost. It has been observed in other studies that growth tends to decrease with increasing depth just as it did in this study (Pansch, 2007; Kim et al., 2014). Usually this is attributed to a decrease in light intensity as it is filtered out of the water. Proximity of seaweed to the bay floor is also known to increase fouling and grazing (Pansch, 2007). Trial 1 saw equal crop retention across all depths (Figure 8) while crop retention decreased with an increase in depth in Trial 2 (Figure 9). These trends could be affected by the decreased light as mentioned before, but fouling is

probably the major contributor as both crop retention and fouling share similar trends (Figures 8, 9, 10, 11, 12).

During the spring and summer, there was heavy fouling to the point that many 2 m bundles and nearly all 3 m bundles had been completely engulfed by fouling species like barnacles resulting in prevention of growth or loss of the bundles entirely. Mean PAR was also surprisingly lower in Trial 2 compared to Trial 1, further hindering the growth of the two lowest bundles. However, this lower light during spring and summer could be affected by several factors from time of day be it a foggy morning instead of a sunny afternoon to a cloud passing overhead while PAR measurements were in progress. Water visibility was also lower during the summer than the fall and winter leading to a further reduction of PAR reaching the bottom. Because 2 m and 3 m bundles in Trial 1 were not engulfed in fouling and benefitted from slightly increased light penetration they did not lose nearly as many bundles as in the spring and summer. But they did still experience lower growth rates as their Trial 2 counterparts did (Table 1). This loss and fouling can also explain the negative growth rates calculated for both trials as it has in other studies (Martinez et al., 2006; Kim et al., 2014).

Thirdly, fouling and loss could be further reduced by shortening the time period between harvests. Four months of cultivation on long-lines in Humboldt Bay seems to be too long a timeframe as it allows fouling to build up and bundles to become too large and more prone to detachment. Based on personal observation, I would suggest that one month would be a more suitable duration as there is less time for fouling species to establish themselves and bundles are not at as high a risk of detachment but also still of

a harvestable size – an observation also made by Martinez et al. (2006). Werner and Dring (2011) did not specify an exact period of time for harvest of *P. palmata*, but rather opted to recommend harvesting fronds as they come to size, whether that be on a regular basis or leaving them in over winter. Just as long as fronds were harvested before they become fouled.

Other methods to prevent loss unrelated to fouling could be a type of containment system. Martinez et al. (2006) experimented with bags attached to their droppers containing fronds of *P. palmata*, and found that it solved the problem of losing seaweed material though it did reduce light. Three prototype containment systems were constructed to be tested during the winter period of January through March, but Covid-19 lockdown measures and permit delays caused the containment experiment to be canceled. While bags or cages may have merit during the wintertime, they may not be appropriate for spring and summer deployment as they may only provide more substrate for fouling species to attach to. Research on this topic will need to be conducted to see if this would be a good strategy to retain more of the crop.

Carbon, Nitrogen, and Phosphorus Analysis

While *D. mollis*'s primary use is for human consumption, it can also provide ecosystem services as an environmental biofilter during its grow-out phase (Demetropoulos and Langdon, 2004d; Wulffson, 2020). Seaweeds have the ability to sequester various elements such as carbon, nitrogen, and phosphorus needed for photosynthesis and new growth. Because of this seaweed farms around the world are

being tested as a method to combat climate change and excessive nutrient loading from sources such as sewage, agriculture, and other forms of aquaculture (Levin, 1991; Evans and Langdon, 2000; Baruah et al., 2006). Amounts of these nutrients extracted from Humboldt Bay during this study could be used to explore *D. mollis*'s potential as a biofilter.

In terms of tissue carbon and nutrient sequestration, the thallus tissue of *D. mollis* grown in Humboldt Bay tends to have higher than average carbon, nitrogen, and phosphorus concentrations for macroalgae according to a study that examined literature values for 46 species of macroalgae though the species weren't specified (Duarte, 1992). Duarte (1992) found that typically 8.9% to 48.4% of dry weight (DW) is made up of carbon with a mean of 25% (compared to 36.4% C in this study) while nitrogen composes 0.4% to 4.4% DW with a mean of 1.9% (3.76% N in this study) and phosphorus with a range of 0.01% to 0.45% and mean of 0.10% (0.52% in this study). When comparing *D. mollis* to other common domestically farmed species, it also tends to have higher than average concentration of carbon, nitrogen, and phosphorus (Table 6). It should be noted that in this current study *D. mollis* tissue concentration is based off of one measurement each for carbon, nitrogen, and phosphorus, and that tissue nutrient levels can vary through time. Nevertheless, other studies at Oregon State University have demonstrated that *D. mollis* is effective at removing nutrients from rich aquaculture effluents (Levin, 1991; Evans and Langdon, 2000). It was also found that *D. mollis* grown in Humboldt Bay reflects similar carbon sequestration results from another west coast land-based farm that found their crop removed 0.45 kg (1 lb) of

carbon for every 1.81 kg (4 lbs) of dry seaweed with this study removing 0.68 kg (1.5 lbs) of carbon per 1.81 kg dry seaweed (Oregon Seaweed, 2022).

Primary sources of nutrients for Humboldt Bay alternate with time of year which can affect the concentrations of carbon, nitrogen, and phosphorus in the thallus depending if they are being gathered and stored during times of plenty or metabolized when nutrients are limited (Thomas and Harrison, 1985; Demetropoulos and Langdon, 2004b). During late fall and winter, moderate amounts of nitrogen and phosphorus is supplied by runoff entering the bay from increase storm precipitation and potentially dislodged plant and fouling species (Barnhart et al., 1992). This was observed to an extent on the farm though the increase in nitrogen and phosphorus was slight. As mentioned before, this may be due to less runoff from a drier than average winter. Later on, spring upwelling caused by northern winds along the coast produce a higher amount of nutrients as runoff episodes decrease. During this time nitrate (NO_3^-) will be moderate to high in nearshore and bay entrance waters, but will be low in the upper bay due to plant and algae production and denitrification (Barnhart et al., 1992). Phosphate (PO_4^{3-}) tends to exhibit a different pattern having a higher concentration in the upper bay and decreasing down the bay to the entrance and nearshore waters. PO_4^{3-} also decreases at high tide and increases at low tide in general as it is mainly sourced from bay sediments and wastewater (Burton and Liss, 1976; Pequegnat and Butler, 1981; Barnhart et al., 1992). Nitrogen in the form of ammonia (NH_4^+) is also low in nearshore waters, and is sourced mostly from wastewater and oyster farms (Barnhart et al., 1992). Municipal wastewater was once a major nutrient contributor to Humboldt Bay year-

round until it was diverted through the Arcata Marsh in mid-1980's resulting in reduced productivity measured by chlorophyll concentration (Pequegnat and Butler, 1981; Pequegnat, 1988).

Using these observed nutrient trends as described above and tracking upwelling can help determine optimal out-planting dates for *D. mollis* in Humboldt Bay. Nitrogen in form of nitrites began increasing starting in November 2020 and increasing a second time in December 2020 before the end of the first trial (Figure 19). This increase correlates with increased November sample tissue mass (Figure 5). Water quality measurements beginning April 7th, 2021 revealed a decrease in nitrogen in form of nitrites and nitrates, which could explain the poor performance of Long-line 2 (Figure 20). Long-line 1 may have been out-planted when there was more nitrogen available in the bay allowing those bundles to grow and store excess nutrients that could be utilized during the nitrogen-limited span of April to mid to late May. Long-line 2 bundles, on the other hand, may have been exposed to lower amounts of nitrogen from the beginning resulting in slow growth. Unfortunately, a water sample was not collected from Trial 2 Long-line 1 out-planting to verify this.

Heavy Metal and Pesticide Analysis

The trend for chromium, copper, lead, manganese, and zinc could be explained by the proximity of the seaweed to the bay floor as sediment can contain high amounts of heavy metals, nutrients, and other toxins (Roleda et al., 2019). A study in Malaysia on the levels of metals in seawater and sediment found higher concentrations in water

near the bottom compared to water near the surface (Noor et al., 2015). Besides absorbing metals from the surrounding water, the lower 2 m and 3 m bundles may have been directly exposed to metals in the sediment at very low tides, especially when the tension of the long-lines loosened and sagged. These lower bundles may have rested on or near the bottom as they tended to be covered in more sediment and fouling than 0 m and 1 m bundles. This can happen more so during spring and summer as the average water level tends to be lower than in winter (Figure 23), and could have contributed to the distinctively higher concentrations for 2 m and 3 m bundles during Trial 2 as compared to Trial 1. Fine sediment may also have been thrown up from the bottom and settled on the fronds as the bottom substrate at the farm site consisted of mud and shells (Werner and Dring, 2011). It is unknown why arsenic and cadmium do not also reflect this pattern of increasing concentration with increasing depth in this study, especially since these two elements have reportedly exhibited such a pattern during a similar study in Norway for *Saccharina latissima* (Wang et al., 2022). However, Zon et al. (2020) found that cadmium can be absorbed by microplastics, and because many microplastics are less dense than water, they'll float in the water column. Han et al. (2020) also found that increased photosynthesis correlates with an increase in cadmium uptake and bioaccumulation. No studies were found that suggest these same possible mechanisms for arsenic. Overall higher concentrations across depths for Trial 2 may also have been influenced by upwelling as deep ocean water is transported into the bay. Several other studies have correlated increased cadmium levels in other coastal environments, mussels, and the brown seaweeds *Macrocystis pyrifera* and *S. latissima* with upwelling

(Van Geen et al., 1992; van Geen and Husby, 1996; Lares et al., 2002; Takesue et al., 2004; Valdéz et al., 2008; Wang et al., 2022). Mercury's low concentration across depths may suggest that *D. mollis* has a low affinity for this heavy metal. Seaweed in general tends to exhibit low concentrations of mercury for several species including Atlantic dulse, *P. palmata* (Sears and Battaglia, 1990; Roleda et al., 2019).

While nearly all metal concentrations were below their associated ULs, the exceedance of manganese and zinc may be misleading as their ULs are in units of mg/day while data are in units of mg/kg. The toxicity of arsenic in *D. mollis* may also be misrepresented as most studies tend to measure total arsenic, as was done for this study, while most regulations are focused on inorganic forms of arsenic as these reported to be much more toxic than organic arsenic. Most regulations state a general UL of 0.3 µg/kg for inorganic arsenic, but France stated an UL of 3 mg/kg specifically for inorganic arsenic in seaweed (EPA, 1995; ASTDR, 2007; CEVA, 2014). Fortunately, inorganic arsenic tends to make up a small fraction of total arsenic, but proportions can vary among species and locations (Andrewes et al., 2004; Roleda et al., 2019). For example, tissue analysis on bull kelp (*Nereocystis luetkeana*) grown in Humboldt Bay revealed only 0.38% of total arsenic was inorganic (K. Gray Geisler, pers. Comm., 2023). Chromium lacks a UL as it was concluded to have no negative affects when ingested in high amount in food and supplements, but caution should be used as research on high intakes of chromium is limited (NIH, 2022a). Nevertheless, exposure to these metals and others can be controlled by the amount and frequency *D. mollis* grown in Humboldt Bay is consumed, especially since seaweed tends to be eaten

in small amounts in general. Estimated average consumption of seaweed for Japanese is 4 g/adult per day, for Chinese it is 5.2 g/adult per day, and 8.5 g/adult per day for South Korean (Roleda et al., 2019). However, there is no estimated average consumption for Europeans or North Americans. Monteiro et al. (2019) assumed a 5 g/adult per day diet for a 60 kg adult for their health risk assessment performed for the European Food Safety Authority (EFSA), and found that the seven brown and green seaweeds under review did not exceed unsafe levels of heavy metals at this amount. To reflect the potential amount of heavy metals that could be consumed in a more reasonable portion size of *D. mollis* compared to the kilogram portion of most of the ULs, 5 g portions were calculated based on the highest heavy metal concentration recorded during grow-out. The resulting 5 g portion (DW) could include 6.5 µg total arsenic, 0.875 µg cadmium, 5.55 µg chromium, 17.65 µg copper, 1.78 µg lead, 0.461 mg manganese, 0.5 µg mercury, and 0.095 mg of zinc – all very low amounts, especially if consumed only once a week.

Another way to reduce heavy metals in *D. mollis* is by washing and cooking it (Monteiro et al., 2019; Roleda et al., 2019). It should be noted that as part of the procedure of preparing tissue samples for this study that fronds were briefly washed in freshwater to remove biofouling. This may have influenced the values of metals during analysis to read lower than they would have without washing.

While the group of metals evaluated in this study contains the four of most concern in seafood (arsenic, cadmium, lead, and mercury), there are still many other elements and toxins that this study did not look into that could pose a health risk to

humans in excessive amounts. As mentioned before, inorganic arsenic can be highly toxic, and would be important to discern the proportion of total arsenic in Humboldt Bay grown *D. mollis* that is inorganic. Iodine is another important element for human health that seaweed can provide, but that can adversely affect the thyroid gland in excess (NIH, 2022c). Other important toxins that were also not covered in this study are dioxin-like compounds (dioxins, furans, and dioxin-like PCBs) which are anthropogenically produced chemical by-products from many industries including lumber mills and their associated conical burners, an industry with a prominent history in Humboldt Bay (Department of Environmental Science and Management, 2011). Humboldt Bay was officially designated as impaired with dioxins and PCBs in 2006 (California State Water Resources Control Board, 2006). Although recent analysis of these chemicals from bull kelp grown in the same Humboldt Bay system found that values of dioxins and PCBs were below the general Tolerable Weekly Intake of 14 pg WHO-TEQ/kg body weight (European Commission, 2006; K. Gray Geisler, pers. Comm., 2023). There is currently no dioxin UL for seaweed.

Table 6. Common seaweed species farmed in the U.S.A. and associated nutrient tissue contents and growth rates.

Species	Common name	C (% DW)	N (% DW)	P (% DW)	Growth rate (g FW/day)	Production system	Reference
Devaleraea mollis	Pacific Dulse	36.4	3.76	0.52	0.10-0.21	Inshore long-line droppers	Current study
		40.6-44.4	2.64-5.27	0.61-0.84	2.1-16.2%d^{-1}	Tanks with nutrient additions	Demetropoulos & Langdon, 2004c
Palmaria palmata	Dulse	33.21	5.87	0.75		Tank culture	Tremblay-Gratton et al., 2018
			1.28-2.25	0.183	0.104-0.7	Nearshore long-line droppers, bags, some fertilizer	Martinez et al., 2006
Saccharina latissima	Sugar Kelp	23.9-31.4	2.23-3.24			Wild	Gevaert et al., 2001
			0.49-3.67	0.05-0.82	0.0089-0.0121	Long-line	Marinho et al., 2015
		~24%	~0.8			Common garden	Umanzor & Stephens, 2023
Nereocystis luetkeana	Bull Kelp	4.23	0.46			Inshore long-line array	K. Gray Geisler, pers. Comm., 2023
Alaria marginata	Ribbon Kelp, Winged Kelp	~32.5	~1.5			Common garden	Umanzor & Stephens, 2023
Ulva lactuca	Sea Lettuce	31.68	4.41	0.26		Tank culture	Tremblay-Gratton et al., 2018

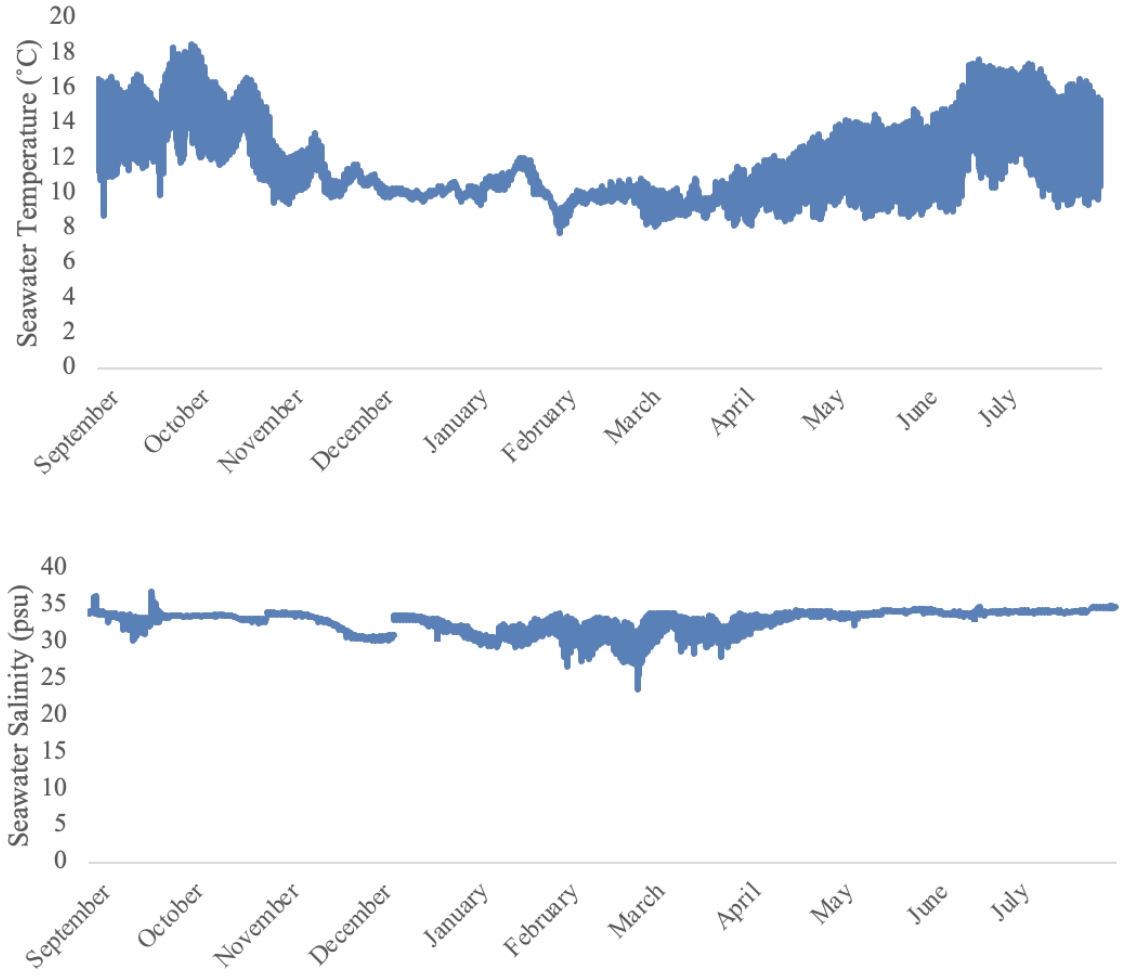

Figure 22. Seawater trends in temperature and practical salinity from data collected by Central and Northern California Ocean Observing System (CeNCOOS) Humboldt/Chevron Dock Station. Data spans the duration of the study from August 26, 2020 to July 31, 2021 (Cal Poly Humboldt, 2022).

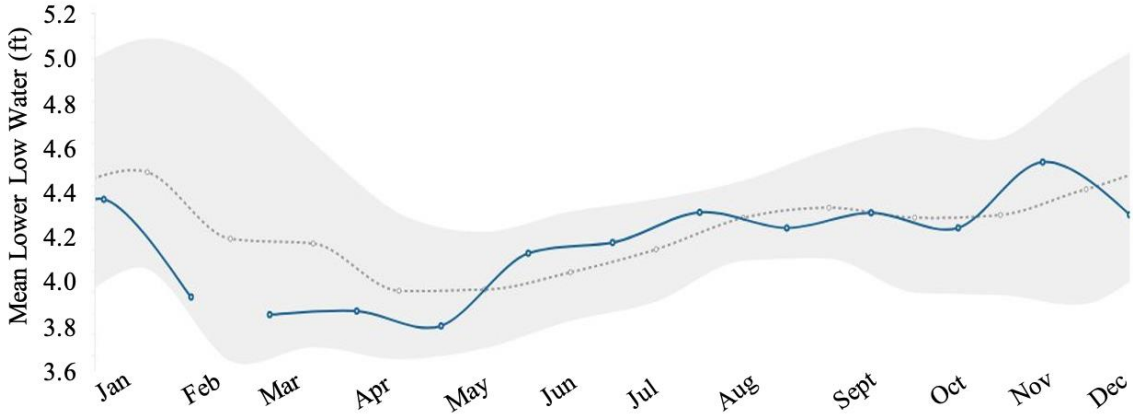

Figure 23. Mean water level for year 2021 for Humboldt Bay from CeNCOOS North Spit, Humboldt Bay HBYC1 Station. Solid blue line indicates the mean from 2021. Dotted gray line indicates the mean from all past recorded years. Grey area indicates range of water level values. Image courtesy of NOAA (2022).

RECOMMENDATIONS

The results of this study show that *D. mollis* can be successfully grown in Humboldt Bay, but the major problems to a farmer are biofouling and crop loss. The following recommendations can reduce the effects of these issues. Firstly, optimal grow-out for this species is from late fall through early spring. Harvesting before June will reduce the amount of fouling on the seaweed and avoiding reduced summer nutrient levels as productivity increases in the bay, leading to higher quality fronds. While the grow-out season can be extended to the start of June, successful out-planting in spring may depend on monitoring nutrient levels in the water to determine when and when not to out-plant small starter bundles as upwelling trends can vary year to year. For year-round production of *D. mollis*, cultivation vegetatively in a tank system would be more practical as there is more control over fouling, nutrients, and lighting, and it would require less starting material than long-lines. Further research should also be conducted on whether trimming *D. mollis* for multiple harvests would still allow for continued thallus regeneration during a nutrient-limited time period from older tissue that experienced an upwelling event prior. Winter and early spring water quality parameters appear good for grow-out as lower temperatures, nutrients, and PAR levels inhibit growth of biofouling species. However, this part of the year was not tested in this study and should be considered for future studies. Also, four-month grow-out periods between harvests were deemed too long. One-month grow-out between harvests is recommended as it increases the number of harvests in a growing season (thereby increasing farm

production), reduces crop loss by increasing the chance larger bundles can be harvested before the current removes them from the line, and reduces fouling as there is less time for fouling species to become established without compromising bundle size. Future studies for regrowth should explore the ability to perform multiple harvests via trimming compared to replacement with new frond tissue. Research cultivating *D. mollis* produced from spore-inoculated twine compared to vegetative propagation should also be pursued to study crop retention.

It was also found that *D. mollis* grows best down to 1 m of depth. This depth range produced the most growth in terms of wet weight, was the least fouled, had the healthiest fronds, and had the greatest crop retention. Eliminating dropper line length below 1 m at this site also eliminates higher heavy metal concentrations in tissue from sediment exposure during very low tides. Grazing can also be reduced since seaweed predators on the bay floor cannot climb the shorter 1 m long dropper lines as compared to the 3 m long dropper lines in this study. It is recommended that bundle density and shading effects be considered for future studies as these can directly impact the amount of *D. mollis* that can be produced.

While *D. mollis* is a great way to add important nutrients, protein, and minerals to the diet, its ability to sequester toxins should also be taken into account. *Devaleraea mollis* grown in Humboldt Bay tends to be low in heavy metals and pesticides, but there are ways to further reduce one's exposure when consuming this seaweed. Shorter grow-out intervals reduce the time that the tissue can accumulate toxic materials while washing, cooking, and smaller portion sizes can lower the amount ingested post-harvest.

Future studies should evaluate other elements and toxic substances that could impact human health not included in this study such as inorganic arsenic, iodine, and dioxins.

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APPENDIX

Appendix: Results for pesticide residue analysis. All values expressed as mg/kg. Since results were identical for both trials, this appendix will contain only one analytical report for both Trials 1 and 2.

Technique: GC-MS/MS

Parameter	**Result**	**LOQ**	**Parameter**	**Result**	**LOQ**
2,4,6-Trichloroanisole	<0.010	0.010	Heptachlor (Sum)	<0.010	0.010
2,4,6-Trichlorophenol	<0.010	0.010	Heptachlor Epoxide A	<0.010	0.010
2-phenylphenol (SP)	<0.010	0.010	Heptachlor Epoxide B	<0.010	0.010
g-hydroxyquinoline (SP)	<0.010	0.010	Heptenophos	<0.010	0.010
Acetochlor	<0.010	0.010	Hexachlorobenzene	<0.010	0.010
Acrinathrin	<0.010	0.010	Hexachlorobutadiene	<0.010	0.010
Alachlor	<0.010	0.010	Hexaconazole	<0.010	0.010
Aldrin	<0.010	0.010	Iodofenphos	<0.010	0.010
Alpha Endosulfan	<0.010	0.010	Iprobenfos	<0.010	0.010
Alpha-HCH	<0.010	0.010	Iprodione	<0.010	0.010
Ametryn	<0.010	0.010	Iprovalicarb	<0.010	0.010
Anthraquinone	<0.010	0.010	Isazofos	<0.010	0.010
Atrazine	<0.010	0.010	Isofenphos	<0.010	0.010

Parameter	Result	LOQ	Parameter	Result	LOQ
Beflubutamid	<0.010	0.010	Isofenphos-methyl	<0.010	0.010
Benalaxyl	<0.010	0.010	Kresoxim-methyl	<0.010	0.010
Benfluralin	<0.010	0.010	Lambda-Cyhalothrin	<0.010	0.010
Beta Endosulfan	<0.010	0.010	Lindane	<0.010	0.010
Beta-HCH	<0.010	0.010	Malaoxon	<0.010	0.010
Bifenazate	<0.010	0.010	Malathion (SP)	<0.010	0.010
Bifenox	<0.010	0.010	Malathion (Sum)	<0.010	0.010
Bifenthrin	<0.010	0.010	Mefenpyr Diethyl	<0.010	0.010
Biphenyl	<0.010	0.010	Mepronil	<0.010	0.010
Bitertanol	<0.010	0.010	Metalazul-M (Mafenoxam)	<0.010	0.010
Bromophos-ethyl	<0.010	0.010	Methacrifos	<0.010	0.010
Bromophos-methyl	<0.010	0.010	Methidathion	<0.010	0.010
Bromopropylate	<0.010	0.010	Methoxychlor	<0.010	0.010
Bupirimate	<0.010	0.010	Metribuzin	<0.010	0.010
Captan	<0.010	0.010	Mevinphos	<0.010	0.010
Captan (Sum)	<0.010	0.010	Mirex	<0.010	0.010
Carbophenothion	<0.010	0.010	Molinate	<0.010	0.010
Chinomethionat	<0.010	0.010	Myclobutanil	<0.010	0.010
Chlordane (Sum)	<0.010	0.010	Naled	<0.010	0.010
Chlorfenapyr	<0.010	0.010	Naled (Sum)	<0.010	0.010

Parameter	Result	LOQ	Parameter	Result	LOQ
Chlorfenson	<0.010	0.010	Napropamide	<0.010	0.010
Chlorfenvinphos	<0.010	0.010	Nitrofen	<0.010	0.010
Chlormephos	<0.010	0.010	Nitrothal Isopropyl	<0.010	0.010
Chlorobenzilate+Chloro Propylate	<0.010	0.010	Nuarimol	<0.010	0.010
Chlorothalonil	<0.010	0.010	o,p'-DDD	<0.010	0.010
Chlorotoluron	<0.010	0.010	o,p'-DDE	<0.010	0.010
Chlorpropham	<0.010	0.010	Ofurace	<0.010	0.010
Chlorpyrifos	<0.010	0.010	Oxadixyl	<0.010	0.010
Chlorpyrifos-methyl	<0.010	0.010	Oxychlordan	<0.010	0.010
Chlorthal-dimethyl	<0.010	0.010	Oxyfluorfen	<0.010	0.010
Chlorthion	<0.010	0.010	p,p'-DDT	<0.010	0.010
Chlozolinate	<0.010	0.010	p,p'-DDE	<0.010	0.010
Cinidon-ethyl	<0.010	0.010	Paraoxon Methyl	<0.010	0.010
Cis-Chlordane	<0.010	0.010	Paraoxon-ethyl	<0.010	0.010
Cyfluthrin	<0.010	0.010	Parathion Methyl (SP)	<0.010	0.010
Cyproconazole	<0.010	0.010	Parathion Methyl (Sum)	<0.010	0.010
Cyprodinil	<0.010	0.010	Parathion-ethyl	<0.010	0.010
DDD-pp+DDT-op	<0.010	0.010	Parathion-ethyl (Sum)	<0.010	0.010
DDT (Sum)	<0.010	0.010	Penconazole	<0.010	0.010

Parameter	Result	LOQ	Parameter	Result	LOQ
DEET	<0.010	0.010	Pendimethalin	<0.010	0.010
Delta-HCH	<0.010	0.010	Pentachloroaniline	<0.010	0.010
Deltamethrin	<0.010	0.010	Pentachloroanisole	<0.010	0.010
Desethyl atrazine	<0.010	0.010	Pentachlorobenzene	<0.010	0.010
Diafenthiuron	<0.010	0.010	Pentachlorobenzonitrile	<0.010	0.010
Diazinon	<0.010	0.010	Pentachlorophenol	<0.010	0.010
Dichlobenil	<0.010	0.010	Permethrin	<0.010	0.010
Dichlofenthion	<0.010	0.010	Phenthoate	<0.010	0.010
Diclobutrazol	<0.010	0.010	Phorate	<0.010	0.010
Dicioran	<0.010	0.010	Phosalone	<0.010	0.010
Dicofol (Sum)	<0.010	0.010	Phtalimide	<0.010	0.010
Dicofol o,p’	<0.010	0.010	Piperonyl butoxide	<0.010	0.010
Dicofol p,p’	<0.010	0.010	Pirimiphos-ethyl	<0.010	0.010
Dicrotophos	<0.010	0.010	Pirimiphos-methyl	<0.010	0.010
Dieldrin	<0.010	0.010	Prochloraz (Sum)	<0.010	0.010
Dieldrin (Sum)	<0.010	0.010	Procymidone	<0.010	0.010
Difenoconazole	<0.010	0.010	Profenophos	<0.010	0.010
Diflufenican	<0.010	0.010	Profluralin	<0.010	0.010
Dimefox	<0.010	0.010	Prometryn	<0.010	0.010
Dimoxystrobin	<0.010	0.010	Propazine	<0.010	0.010

Parameter	Result	LOQ	Parameter	Result	LOQ
Diniconazole	<0.010	0.010	Propetamphos	<0.010	0.010
Dinobuton	<0.010	0.010	Propyzamide	<0.010	0.010
Diphenylamine	<0.010	0.010	Prothiofos	<0.010	0.010
Disulfoton (SP)	<0.010	0.010	Pyrazophos	<0.010	0.010
Disulfoton (Sum)	<0.010	0.010	Pyridaben	<0.010	0.010
Disulfoton Sulfone	<0.010	0.010	Pyridaphenthion	<0.010	0.010
Disulfoton Sulfoxide	<0.010	0.010	Pyrifenox	<0.010	0.010
Ditalimfos	<0.010	0.010	Pyrimethanil	<0.010	0.010
Endosulfan (Sum)	<0.010	0.010	Pyriproxyfen	<0.010	0.010
Endosulfan-Sulphate	<0.010	0.010	Quinalphos	<0.010	0.010
Endrin	<0.010	0.010	Quintozene	<0.010	0.010
EPN	<0.010	0.010	Quintozene (Sum)	<0.010	0.010
Epsilon-HCH	<0.010	0.010	Silthiofam	<0.010	0.010
EPTC	<0.010	0.010	Simazine	<0.010	0.010
Ethalfluralin	<0.010	0.010	Tau-Fluvalinate	<0.010	0.010
Ethion	<0.010	0.010	Tebuconazole	<0.010	0.010
Ethofumesate (SP)	<0.010	0.010	Tebufenpyrad	<0.010	0.010
Ethoprophos	<0.010	0.010	Tecnazene	<0.010	0.010
Etridiazole	<0.010	0.010	Tefluthrin	<0.010	0.010
Etrimfos	<0.010	0.010	Terbacil	<0.010	0.010

Parameter	Result	LOQ	Parameter	Result	LOQ
Fenarimol	<0.010	0.010	Terbumeton	<0.010	0.010
Fenazaquin	<0.010	0.010	Terbuthylazine	<0.010	0.010
Fenchlorphos (SP)	<0.010	0.010	Terbuthylazine Desethyl	<0.010	0.010
Fenchlorphos (Sum)	<0.010	0.010	Terbutryn	<0.010	0.010
Fenchlorphos Oxon	<0.010	0.010	Tetrachlorvinphos	<0.010	0.010
Fenitrothion	<0.010	0.010	Tetraconazole	<0.010	0.010
Fenpropathrin	<0.010	0.010	Tetradifon	<0.010	0.010
Fenson	<0.010	0.010	Tetrahydrophthalimide	<0.010	0.010
Fenthion (SP)	<0.010	0.010	(THPI) Tetramethrin	<0.010	0.010
Fenthion Oxon	<0.010	0.010	Tetrasul	<0.010	0.010
Fenvalerate	<0.010	0.010	Thiometon	<0.010	0.010
Flucythrinate	<0.010	0.010	Tolclofos-methyl	<0.010	0.010
Flumetralin	<0.010	0.010	Trans-Chlordane	<0.010	0.010
Fluopicolide	<0.010	0.010	Transfluthrin	<0.010	0.010
Fluopyram	<0.010	0.010	Triadimefon	<0.010	0.010
Fluotrimazole	<0.010	0.010	Triadimenole	<0.010	0.010
Flurtamone	<0.010	0.010	Tri-allate	<0.010	0.010
Folpet	<0.010	0.010	Triamiphos	<0.010	0.010
Folpet (Sum)	<0.010	0.010	Trifluralin	<0.010	0.010

Parameter	Result	LOQ	Parameter	Result	LOQ
Fonofos	<0.010	0.010	Uniconazole	<0.010	0.010
Furalaxyl	<0.010	0.010	Vinclozolin	<0.010	0.010
Heptachlor (SP)	<0.010	0.010	Zeta-cypermethrin	<0.010	0.010

Technique: LC-MS/MS

Parameter	Result	LOQ	Parameter	Result	LOQ
Methiocarb Sulfoxide	<0.010	0.010	Glyphosate	<0.010	0.010
3-OH carbofuran (SQ)	<0.010	0.010	Halosulfuron methyl	<0.010	0.010
Abamectin	<0.010	0.010	Haloxyfop	<0.010	0.010
Acephate	<0.010	0.010	Haloxyfop (Sum)	<0.010	0.010
Acequinocyl	<0.010	0.010	Haloxyfop-2-ethoxyethyl	<0.010	0.010
Acetamiprid	<0.010	0.010	Haloxyfop-methyl (SP)	<0.010	0.010
Acibenzolar-S-methyl (SP)	<0.010	0.010	Hexaflumuron	<0.010	0.010
Aldicarb (SP)	<0.010	0.010	Hexazinone	<0.010	0.010
Aldicarb (Sum)	<0.010	0.010	Hexythiazox	<0.010	0.010
Aldicarb Sulfone	<0.010	0.010	Imazalil	<0.010	0.010
Aldicarb Sulfoxide	<0.010	0.010	Imazapic	<0.010	0.010
Ametoctradin	<0.010	0.010	Imazapyr	<0.010	0.010
Aminocarb	<0.010	0.010	Imidacloprid	<0.010	0.010

Parameter	Result	LOQ	Parameter	Result	LOQ
Amitraz (SP)	<0.010	0.010	Indaziflam	<0.010	0.010
Atrazine Desisopropyl	<0.010	0.010	Indoxacarb	<0.010	0.010
Azaconazole	<0.010	0.010	Iodosulfuron-methyl (SP)	<0.010	0.010
Azadirachtin	<0.010	0.010	Ioxynil (SP)	<0.010	0.010
Azamethiphos	<0.010	0.010	Isocarbophos	<0.010	0.010
Azimsulfuron	<0.010	0.010	Isoprocarb	<0.010	0.010
Azinphos-ethyl	<0.010	0.010	Isoprothiolane	<0.010	0.010
Azinphos-methyl	<0.010	0.010	Isoproturon	<0.010	0.010
Azocyclotin and Cyhexatin (SQ)	<0.010	0.010	Isoxaben	<0.010	0.010
Azoxystrobin	<0.010	0.010	Isoxathion	<0.010	0.010
Ben-Carb-TPM (Sum)	<0.010	0.010	Ivermectin	<0.010	0.010
Bendiocarb	<0.010	0.010	Lenacil	<0.010	0.010
Bentazone (SP)	<0.010	0.010	Linuron	<0.010	0.010
Bentazones-methyl	<0.010	0.010	Lufenuron	<0.010	0.010
Benthiavalicarb	<0.010	0.010	Mandipropamid	<0.010	0.010
Bioallethrin	<0.010	0.010	MCPA (SP)	<0.010	0.010
Bixafen	<0.010	0.010	Mecarbam	<0.010	0.010
Boscalid	<0.010	0.010	Mepanipyrim	<0.010	0.010
Bromacil	<0.010	0.010	Meptyidinocap	<0.010	0.010

Parameter	Result	LOQ	Parameter	Result	LOQ
Bromoxynil (SP)	<0.010	0.010	Mesosulfuron-methyl	<0.010	0.010
Bromuconazole	<0.010	0.010	Mesotrione	<0.010	0.010
Buprofezin	<0.010	0.010	Metaflumizone	<0.010	0.010
Butachlor	<0.010	0.010	Metamitron	<0.010	0.010
Butocarboxim	<0.010	0.010	Metazachlor (SP)	<0.010	0.010
Butoxicarboxim Sulfoxide	<0.010	0.010	Metconazole	<0.010	0.010
Butralin	<0.010	0.010	Methabenzthiazuron	<0.010	0.010
Buturon	<0.010	0.010	Methamidophos	<0.010	0.010
Cadusafos	<0.010	0.010	Methiocarb (SP)	<0.010	0.010
Carbaryl	<0.010	0.010	Methiocarb (Sum)	<0.010	0.010
Carbendazim and Benomyl	<0.010	0.010	Methiocarb sulfone	<0.010	0.010
Carbetamide	<0.010	0.010	Methomyl	<0.010	0.010
Carbofuran (SP/SQ)	<0.010	0.010	Methomyl (Sum)	<0.010	0.010
Carboxin	<0.010	0.010	Methoprotryne	<0.010	0.010
Carfentrazone-ethyl (SP)	<0.010	0.010	Methoxyfenozide	<0.010	0.010
Chlorantraniliprole	<0.010	0.010	Metobromuron	<0.010	0.010
Chlorbromuron	<0.010	0.010	Metolachlor and S-Metolachlor	<0.010	0.010
Chlorfluazuron	<0.010	0.010	Metolcarb	<0.010	0.010

Parameter	Result	LOQ	Parameter	Result	LOQ
Chloridazon	<0.010	0.010	Metoxuron	<0.010	0.010
Chloroxuron	<0.010	0.010	Metrafenone	<0.010	0.010
Chlorsulfuron	<0.010	0.010	Metsulfuron-methyl	<0.010	0.010
Chlorthiophos	<0.010	0.010	Milbemectin SQ (Sum)	<0.010	0.010
Clethodim (SP)	<0.010	0.010	Milbemycin A3 (SQ)	<0.010	0.010
Clethodim Sulfoxide	<0.010	0.010	Milbemycin A4 (SQ)	<0.010	0.010
Clofentezine	<0.010	0.010	Monocrotophos	<0.010	0.010
Clomazone	<0.010	0.010	Monolinuron	<0.010	0.010
Clopyralid	<0.010	0.010	Monuron	<0.010	0.010
Clothianidin	<0.010	0.010	Neburon	<0.010	0.010
Coumaphos	<0.010	0.010	Nicosulfuron	<0.010	0.010
Crimidine	<0.010	0.010	Nitenpyram	<0.010	0.010
Cyanazine	<0.010	0.010	Norflurazon	<0.010	0.010
Cyantraniliprole	<0.010	0.010	Novaluron	<0.010	0.010
Cyazofamid	<0.010	0.010	Omethoate	<0.010	0.010
Cyclanilide	<0.010	0.010	Oryzalin	<0.010	0.010
Cycloate	<0.010	0.010	Oxadiargyl	<0.010	0.010
Cycloxydim (SP)	<0.010	0.010	Oxydiazon	<0.010	0.010
Cyenopyrafen	<0.010	0.010	Oxamyl	<0.010	0.010
Cyflufenamid	<0.010	0.010	Oxasulfuron	<0.010	0.010

Parameter	Result	LOQ	Parameter	Result	LOQ
Cyflumetofen	<0.010	0.010	Oxathiapiprolin	<0.010	0.010
Cyhalofop-butyl	<0.010	0.010	Oxycarboxin	<0.010	0.010
Cymoxanil	<0.010	0.010	Paclobutrazol	<0.010	0.010
Cyromazine	<0.010	0.010	Pencycuron	<0.010	0.010
Demeton S	<0.010	0.010	Penthiopyrad	<0.010	0.010
Demeton-S-methyl	<0.010	0.010	Phenmedipham	<0.010	0.010
Demeton-S-Methylsulfone	<0.010	0.010	Phorate (Sum)	<0.010	0.010
Demeton-S-sulfoxide	<0.010	0.010	Phorate Oxon	<0.010	0.010
Desmedipham	<0.010	0.010	Phorate Oxon Sulfone	<0.010	0.010
Desmetryn	<0.010	0.010	Phorate Oxon Sulfoxide	<0.010	0.010
Dialifos	<0.010	0.010	Phorate Sulfone	<0.010	0.010
Dichiofluanid	<0.010	0.010	Phorate Sulfoxide	<0.010	0.010
Dichormid	<0.010	0.010	Phosmet (SP)	<0.010	0.010
Dichloroprop	<0.010	0.010	Phosmet (Sum)	<0.010	0.010
Dichlorvos	<0.010	0.010	Phosmet oxon	<0.010	0.010
Diclofop (SP/SQ)	<0.010	0.010	Phosphamidon	<0.010	0.010
Diclofop (Sum)	<0.010	0.010	Phoxim	<0.010	0.010
Diclofop-methyl (SP/SQ)	<0.010	0.010	Picolinafen	<0.010	0.010
Diathofencarb	<0.010	0.010	Picoxystrobin	<0.010	0.010

Parameter	Result	LOQ	Parameter	Result	LOQ
Diflubenzuron	<0.010	0.010	Pinoxaden	<0.010	0.010
Dimefuron	<0.010	0.010	Pirimicarb	<0.010	0.010
dimethachlor	<0.010	0.010	Pirimicarb Desmethyl	<0.010	0.010
Dimethenamid-P	<0.010	0.010	Pirimicarb Desmethyl Formamide	<0.010	0.010
Dimethoate	<0.010	0.010	Prochloraz (SP)	<0.010	0.010
Dimethoate (Sum)	<0.010	0.010	Promecarb	<0.010	0.010
Dimethomorph	<0.010	0.010	Propachlor	<0.010	0.010
Dimethylaminosulfotoluidide (DMST)	<0.010	0.010	Propamocarb (SP)	<0.010	0.010
Dinotefuran	<0.010	0.010	Propanil	<0.010	0.010
Diuron	<0.010	0.010	Propaquizafop	<0.010	0.010
DNOC	<0.010	0.010	Propargite	<0.010	0.010
Dodemorph	<0.010	0.010	Propham	<0.010	0.010
Dodine	<0.010	0.010	Propiconazole	<0.010	0.010
Edifenphos	<0.010	0.010	Propoxur	<0.010	0.010
Emamectin	<0.010	0.010	Propinazid	<0.010	0.010
Epoxiconazole	<0.010	0.010	Prosulfocarb	<0.010	0.010
Ethaboxam	<0.010	0.010	Prosulfuron	<0.010	0.010
Ethiofencarb	<0.010	0.010	Prothioconazole	<0.010	0.010
Ethiofencarb sulfone	<0.010	0.010	Pimetrozine	<0.010	0.010

Parameter	Result	LOQ	Parameter	Result	LOQ
Ethiofencarb sulfoxide	<0.010	0.010	Pyracarbolid	<0.010	0.010
Ethiprole	<0.010	0.010	Pyraclostrobin	<0.010	0.010
Ethirimol	<0.010	0.010	Pyraflufen	<0.010	0.010
Ethoxyquin (SQ)	<0.010	0.010	Pyraflufen-ethyl (SP)	<0.010	0.010
Etofenprox	<0.010	0.010	Pyraflufen-ethyl (Sum)	<0.010	0.010
Etoxazole	<0.010	0.010	Pyridalyl	<0.010	0.010
Famoxadone	<0.010	0.010	Pyridate (SP)	<0.010	0.010
Fenamidone	<0.010	0.010	Quinchlorac	<0.010	0.010
Fenamiphos (SP)	<0.010	0.010	Quinoxyfen	<0.010	0.010
Fenamiphos (Sum)	<0.010	0.010	Quizolofop-ethyl (SP)	<0.010	0.010
Fenamiphos Sulfone	<0.010	0.010	Rimsulfuron	<0.010	0.010
Fenamiphos Sulphoxide	<0.010	0.010	Rotenone	<0.010	0.010
Fenbuconazole	<0.010	0.010	Saflufenacil (SP)	<0.010	0.010
Fenbitatin oxide	<0.010	0.010	Sebuthylazine	<0.010	0.010
Fenhexamid	<0.010	0.010	Sethoxydim	<0.010	0.010
Fenobucarb	<0.010	0.010	Spinetoram	<0.010	0.010
Fenoxycarb	<0.010	0.010	Spinosad	<0.010	0.010
Fenpiclonil	<0.010	0.010	Spirodiciofen	<0.010	0.010
Fenpropidin (SP)	<0.010	0.010	Spiromesifen	<0.010	0.010
Fenpropimorph	<0.010	0.010	Spirotetramat (SP)	<0.010	0.010

Parameter	Result	LOQ	Parameter	Result	LOQ
Fenpyrazamine	<0.010	0.010	Spirotetramat (Sum)	<0.010	0.010
Fenpyroximate	<0.010	0.010	Spirotetramat enol-glucoside	<0.010	0.010
Fensulfothion	<0.010	0.010	Spirotetramat-enol	<0.010	0.010
Fensulfothion Oxon	<0.010	0.010	Spirotetramat-ketohydroxy	<0.010	0.010
Fensulfothion Oxon Sulfone	<0.010	0.010	Spirotetramat-monohydroxy	<0.010	0.010
Fensilfotion Sulfone	<0.010	0.010	Spiroxamine	<0.010	0.010
Fenthion (Sum)	<0.010	0.010	Sulcotrione	<0.010	0.010
Fenthion Oxon Sulfone	<0.010	0.010	Sulfosulfuron	<0.010	0.010
Fenthion Oxon Sulfoxide	<0.010	0.010	Sulfotep	<0.010	0.010
Fenthion Sulfone	<0.010	0.010	Sulfoxaflor	<0.010	0.010
Fenthion Sulfoxide	<0.010	0.010	Tebufenozide	<0.010	0.010
Fentin (SP/SQ)	<0.010	0.010	Teflubenzuron	<0.010	0.010
Fenuron	<0.010	0.010	Tepraloxydim (SP)	<0.010	0.010
Fipronil (SP)	<0.010	0.010	Terbufos	<0.010	0.010
Fipronil (Sum)	<0.010	0.010	Terbufos (Sum)	<0.010	0.010
Fipronil Sulfide	<0.010	0.010	Terbufos sulfone	<0.010	0.010
Fipronil Sulfone	<0.010	0.010	Terbufos sulfoxide	<0.010	0.010
Flamprop	<0.010	0.010	TFNA	<0.010	0.010

Parameter	Result	LOQ	Parameter	Result	LOQ
Flazasulfuron	<0.010	0.010	TFNG	<0.010	0.010
Flonicamid (SP)	<0.010	0.010	Thiabendazole	<0.010	0.010
Flonicamid (Sum)	<0.010	0.010	Thiacloprid	<0.010	0.010
Florasulam	<0.010	0.010	Thiamethoxam	<0.010	0.010
Fluazifop-methyl (SP)	<0.010	0.010	Thidiazuron	<0.010	0.010
Fluazifop-P (SP)	<0.010	0.010	Thifensulfuron-methyl	<0.010	0.010
Fluazifop-P-butyl (SP)	<0.010	0.010	Thiobencarb	<0.010	0.010
Fluazinam	<0.010	0.010	Thiocyclam	<0.010	0.010
Flubendiamide	<0.010	0.010	Thiodicarb	<0.010	0.010
Fludioxonil	<0.010	0.010	Thiofanox	<0.010	0.010
Flufénacet	<0.010	0.010	Thiofanox sulfone	<0.010	0.010
Flufenacet (Sum)	<0.010	0.010	Thiofanox sulfoxide	<0.010	0.010
Flufenacet ESA	<0.010	0.010	Thiophanate-methyl	<0.010	0.010
Flufenacet OA	<0.010	0.010	Tolfenpyrad	<0.010	0.010
Flufenoxuron	<0.010	0.010	Tolylfluanid (SP)	<0.010	0.010
Flumioxazine	<0.010	0.010	Tolylfuanid (Sum)	<0.010	0.010
Fluometuron	<0.010	0.010	Triasulfuron	<0.010	0.010
Fluoxastrobin	<0.010	0.010	Triazophos	<0.010	0.010
Flupyradifuron	<0.010	0.010	Triazoxide	<0.010	0.010
Fluquinconazole	<0.010	0.010	Trichlofon	<0.010	0.010

Parameter	Result	LOQ	Parameter	Result	LOQ
Fluroxypyr (SP)	<0.010	0.010	Tricresyl phosphate	<0.010	0.010
Fluroxypyr-meptyl	<0.010	0.010	Tricyclazole	<0.010	0.010
Flusilazole	<0.010	0.010	Tridemorph	<0.010	0.010
Flutolanil	<0.010	0.010	Trifloxystrobin	<0.010	0.010
Flutriafol	<0.010	0.010	Triflumizole (SP)	<0.010	0.010
Fluxapyroxad	<0.010	0.010	Triflumazole (Sum)	<0.010	0.010
Foramsulfuron	<0.010	0.010	Triflumazole FM-6-1	<0.010	0.010
Forchlorfenuron	<0.010	0.010	Triflumuron	<0.010	0.010
Formetanate (SP)	<0.010	0.010	Triforine (SP)	<0.010	0.010
Formothion	<0.010	0.010	Triticomazole	<0.010	0.010
Fosthiazate	<0.010	0.010	Vamidothion	<0.010	0.010
Fuberidazole	<0.010	0.010	Zoxamide	<0.010	0.010

Technical Definitions/Informational Text:

ABAMECTIN: Sum of avermectin B1a, avermectin B1b and delta-8,9 isomer of avermectin B1a, expressed as avermectin B1a ALDICARB (Sum): Sum of aldicarb, its sulfoxide and its sulfone, expressed as aldicarb

ALDRIN AND DIELDRIN: Aldrin and Dieldrin combined expressed as Dieldrin (Sum)

ALPHA-HCH: Hexachlorocyclohexane (HCH), Alpha-isomer

AZOCYCLOTIN AND CYHEXATIN: Sum of Cyhexatin and Azocyclotin expressed as Cyhexatin

BENALAXYL: Including other mixtures of constituent isomers including benalaxyl-M (sum of isomers) Ben-Carb-TPM (Sum): sum of Benomyl-Carbendazim and Thiophanate-methyl (SP)

BENTHIAVALICARB: Benthiavalicarb-isopropyl(KIF-230 R-L) and its enantiomer (KIF-230 S-D) and its diastereomers(KIF-230 S-L and KIF-230 R-D), expressed as benthiavalicarb-isopropyl

BETA-HCH: Hexachlorocyclohexane (HCH), Beta-isomer

BIFENAZATE: Sum of bifenazate plus bifenazate-diazene expressed as bifenazate

BIFENTHRIN: Sum of isomers

BITERTANOL: Sum of isomers

BROMUCONAZOLE: Sum of diasteroisomers

CAPTAN (Sum): Sum of captan and THPI, expressed as Captan (Sum)

CARBENDAZIM AND BENOMYL: Sum of Benomyl and Carbendazim expressed as Carbendazim

CARBETAMIDE: Sum of carbetamide and its S isomer

CARBOXIN: Carboxin plus its metabolites carboxin sulfoxide and oxycarboxin (carboxin sulfone), expressed as carboxin

CHLORANTRANILIPROLE: DPX E-2Y45

CHLORDANE (Sum): Sum of cis- and trans-chlordane

CINIDON-ETHYL: Sum of cinidon ethyl and its E-isomer

CYFLUFENAMID: Sum of cyflufenamid (Z-isomer) and its E-isomer, expressed as cyflufenamid)

CYFLUTHRIN: Cyfluthrin including other mixtures of constituent isomers (sum of isomers)

DDT (Sum): Sum of p,p′-DDT, o,p′-DDT, p-p′-DDE and p,p′-TDE (DDD) expressed as DDT (Sum)

DEET: N, N-diethyl-m-toluamide

DELTAMETHRIN: Cis-deltamethrin

DICLOFOP: Sum Diclofop-methyl and Diclofop acid expressed as Diclofop-methyl

DICOFOL (Sum): Sum of p, p′ and o,p′ isomers

DIMETHOMORPH: Sum of isomers

DINICONAZOLE: Sum of isomers

DISULFOTON (Sum): Sum of Disulfoton (SP), Disulfoton Sulfoxide and Disulfoton Sulfone expressed as Disulfoton (Sum) DNOC: 2-methyl-4,6-dinitrophenol

EMAMECTIN: Emamectin benzoate B1a, expressed as emamectin

ENDOSULFAN (Sum): Sum of Alpha- and Beta-isomers and Endosulfan-Sulphate expresses as Endosulfan

EPN: O-ethyl-O- (4-nitrophenyl) phenylphosphothionate

EPTC: Ethyl dipropylthiocarbamate

FENAMIPHOS (sum): Sum of Fenamiphos (SP) and its sulphoxide and sulphone expressed as Fenamiphos

FENBUCONAZOLE: Sum of constituent enantiomers

FENCHLORPHOS (Sum): Sum of Fenchlorphos and Fenchlorphos Oxon expressed as Fenchlorphos

FENPROPIMORPH: Sum of isomers

FENTHION (Sum): Fenthion (SP) and its oxigen analogue, their Sulfoxides and Sulfone expressed as parent

FENVALERATE: Any ratio of constituent isomers (RR, SS, RS & SR) including Esfenvalerate

FIPRONIL (Sum): Sum Fipronil (SP) + Sulfone metabolite (MB46136) expressed as Fipronil

FLONICAMID (Sum): Sum Flonicamid (SP), TFNA and TFNG expressed as Flonicamid

FLUCYTHRINATE: Flucythrinate including other mixtures of constituent isomers (sum of isomers)

FLUFENACET (Sum): Sum of all compounds containing the N fluorophenyl-N-isopropyl moiety expressed as Flufenacet equivalent

FLUOXASTROBIN: Sum of fluoxastrobin and its Z-isomer

FOLPET (Sum): Sum of Folpet and Phtalimide, expressed as Folpet

HALOXIFOP (Sum). Sum of haloxyfop, its esters, salts and conjugates expressed as haloxyfop (sum of the R- and S- isomers at any ratio)

HEPTACHLOR (Sum): Sum of Heptachlor (SP) and Heptachlor Epoxide expressed as Heptachlor

Heptachlor Epoxide A is also referred to as trans-Heptachlor Epoxide

Heptachlor Epoxide B is also referred to as cis-Heptachlor Epoxide

IMAZALIL: Any ratio of constituent isomers

INDOXACARB: Sum of indoxacarb and its R enantiomer

LAMBDA-CYHALOTHRIN: Includes gamma-cyhalothrin (sum of R,S and S,R isomers)

LINDANE: Gamma-isomer of hexachlorocyclohexane (HCH)

LUFENURON: Any ratio of constituent isomers

MALATHION (Sum): Sum of Malathion (SP) and Malaoxon expressed as Malathion

MANDIPROPAMID: Any ratio of constituent isomers

MCPA: 4-Chloro-2-methylphenoxyacetic acid

MEPTYLDINOCAP: Sum of 2,4 DNOPC and 2,4 DNOP expressed as Meptyldinocap

METAFLUMIZONE: Sum of E- and Z- isomers

METCONAZOLE: Sum of isomers

METHIOCARB (Sum): Sum of Methiocarb (SP) and Methiocarb Sulfoxide and Sulfone, expressed as Methiocarb

METOLACHLOR AND S-METOLACHLOR: Metolachlor, including other mixtures of constituent isomers including S-Metolachlor (sum of isomers)

MEVINPHOS: Sum of E- and Z-isomers

MILBEMECTIN SQ (Sum): Sum of Milbemycin A4 (SQ) and Milbemycin A3 (SQ), expressed as Milbemectin SQ

Naled (Sum): sum of Naled (SP) and Dichlorvos

o, p'-DDD = TDE: Dichlorodiphenyldichloroethane

o, p'-DDE: Dichlorodiphenyldichloroethylene

p, p'- DDE: Dichlorodiphenyldichloroethylene

p, p'-DDT: Dichlorodiphenyltrichloroethane

PACLOBUTRAZOL: Sum of constituent isomers

PARATHION METHY (Sum): Sum of Parathion methyl (SP) and Paraoxon methyl expressed as Parathion Methyl

Parathion-ethyl (Sum): sum of Parathion-ethyl and Paraoxon-ethyl

PENCONAZOLE: Sum of constituent isomers

PERMETHRIN: Sum of isomers

PHORATE (Sum): Sum of phorate, its oxygen analogue and their sulfones expressed as Phorate

PHOSMET (Sum): Phosmet (SP) and Phosmet oxon expressed as Phosmet

PROCHLORAZ (Sum): Sum of prochloraz and its metabolites containing the 2,4,6-Trichlorophenol moiety expressed as Prochloraz

PROPACHLOR: Oxalinic derivate of propachlor, expressed as propachlor

PROPICONAZOLE: Sum of isomers

PROTHIOCONAZOLE: Prothioconazole-desthio (sum of isomers)

PYRAFLUFEN ETHYL (Sum): Sum of Pyraflufen Ethyl and Pyraflufen, expressed as Pyraflufen Ethyl

QUINTOZENE (Sum): Sum of quintozene and pentachloroaniline expressed as Quintozene

SPINETORAM: XDE-175

SPINOSAD: Sum of spinosyn A and spinosyn D

SPIROTETRAMAT (Sum): Spirotetramat (SP) and its 4 metabolites BYI08330-enol, BYI08330-ketohydroxy, BYI08330-monohydroxy, and BYI08330 enol-glucoside, expressed as Spirotetramat

SPIROXAMINE: Sum of isomers

SULFOXAFLOR: Sum of isomers

Terbufos (Sum): sum of Terbufos, Terbufos-sulfone, and Terbufos-sulfoxide

THIOBENCARB: 4-chlorobenzyl methyl sulfone

TOLYLFLUANID (Sum): Sum of Tolylfluanid (SP) and Dimethylaminosulfotoluidide expressed as Tolylfluanid

TRIADIMENOL: Any ratio of constituent isomers

TRIFLUMIZOLE (Sum): Triflumizole and metabolite FM-6-1(N-(4-chloro-2-trifluoromethylphenyl)-n-propoxyacetamidine), expressed as Triflumizole