THE EFFECTS OF DAILY LIGHT INTEGRAL ON THE GROWTH AND DEVELOPMENT OF HYDROPONICALLY GROWN BABY LEAF VEGETABLES

A Report

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Master of Professional Studies

by

Charles Glenn Gagne

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ABSTRACT

Baby leaf vegetables have recently increased in popularity among consumers worldwide. Greenhouses and plant factories are increasingly being used to produce baby leaf vegetables using hydroponic technology, however, the economics of these growing systems hinge on year-round production, high yields, and short growth cycles. The optimization of light quality, quantity, and photoperiod is essential to meeting these production targets. Little research has been done to understand the effects of these lighting characteristics, especially quantity, on plants grown specifically as baby leaf vegetables. Therefore, the objective of this study was to investigate the effects of daily light integral, or the cumulative amount of light delivered at the plant level each day, on the growth and development of four species of hydroponically grown baby leaf vegetables: collards (*Brassica oleracea* L. 'Flash'), lettuce (*Lactuca sativa* L. 'Sulu'), mizuna (*Brassica rapa* L. var. *japonica*), and pac choi (*Brassica rapa* L. var. *chinensis* 'Red Pac'). A controlled environment chamber was used to conduct one experimental cycle which consisted of five runs. In each run, the chamber, which had two blocks each containing four hydroponic tubs, was set to one of five DLI treatments (6, 12, 18, 24, and 30 mol m^{-2} d^{-1}). Three destructive harvests were completed during each lighting treatment by removing one float from each of the eight hydroponic ponds four, eight, and twelve days after Day 0. Incremental increases in daily light integral continued to positively impact fresh weight and dry weight in baby leaf vegetables up to 30 mol m^{-2} d^{-1}, although for lettuce and collards declining biomass benefits were found above 24 mol m^{-2} d^{-1}. Additionally, incremental increases in daily light integral were shown to have some significant impacts on leaf height, leaf width, leaf area, and leaf number, however, responses varied by species and the positive impacts of these four morphological characteristics rarely continued past the 24 mol m^{-2} d^{-1} treatment. The results of

this study could be used in the optimization of light intensity, in conjunction with light quality and photoperiod, in similar research or commercial settings with the goal of increasing yields, shortening growth cycles, and enhancing the ability to produce year-round.

BIOGRAPHICAL SKETCH

Charles G. Gagne was born in Wauwatosa, Wisconsin just outside of Milwaukee, Wisconsin. Charles has always had a passion for solving problems and for the environment. This led him to pursue a civil engineering degree at Vanderbilt University in Nashville, Tennessee with the goal of solving large environmental problems. After graduation, Charles started a career in engineering consulting with Kimley-Horn & Associates in Los Angeles, California. After two years of working in this field, Charles decided to change career paths and thus began his journey in controlled environment agriculture.

While in high school, Charles toured and then became a volunteer at Growing Power, a diverse urban farm in Milwaukee, Wisconsin. This was where he got his first experiences with urban farming as well as hydroponics and aquaponics. A few years later, Charles took an opportunity with World Wide Opportunities on Organic Farms (WWOOF) to work on a small organic farm in central Sweden. Together, these experiences helped to spark a passion in Charles for agriculture and more specifically urban, indoor agriculture. Thus, when deciding to change career paths, controlled environment agriculture was on the top of his list.

His communication with Soledad Almeida of Cornell University's Office of Professional Programs team and Dr. Neil S. Mattson of Cornell's controlled environment agriculture group ultimately led to his acceptance into a Master of Professional Studies degree program in the field of Horticulture at Cornell. His experience in this program has allowed him to gain the knowledge and skills needed to have a fulfilling career in controlled environment agriculture.

ACKNOWLEDGEMENTS

Firstly, I would like to earnestly thank my advisor Dr. Neil S. Mattson for believing in me and giving me the opportunity to have this amazing experience. He has dedicated extensive time and effort to mentor me and assist me in the design and implementation of my research project. He has also been instrumental in helping me network and meet potential employers. I am extremely grateful for everything that he has given me and I look forward to continuing our relationship long into the future.

Secondly, I would like to thank David de Villiers for his time and guidance during the design phase of my research project. His depth of knowledge and years of experience were enormously valuable. I would also like to thank him for his thoughtfulness and his continuous curiosity which allowed me to see my work in ways I never would have before.

Thirdly, I would like to thank the controlled environment agriculture group at Cornell University. Specifically, I would like to thank Kendra Hutchins and Matt Moghaddam for their help in making sure our entire group always has everything it needs to run smoothly. Additionally, I would like to thank my fellow graduate students (Jacob Holley, Dylan Kovach, Masaki Kurosaki, Jonathan Allred, Maya Ezzeddine, Renyuan Mi, Olu Roberts, and Jiaqi Xia) for their friendship, assistance, and advice over the last year. I wouldn't be where I am today without them.

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CEA	Controlled environment agriculture
CO_2	Carbon dioxide
DLI	Daily light integral
HPS	High-pressure sodium
LED	Light-emitting diode
PAR	Photosynthetically active radiation

Introduction

Baby leaf vegetables are those harvested after the development of true leaves, but before the eight true leaf stage (Di Gioia, et al., 2017). Principally consumed as raw products, baby leaf vegetables are gaining popularity among consumers worldwide as they represent a good source of minerals, vitamins and phytochemicals of considerable antioxidant potential (Subhasree, et al., 2009). Today, salad mixes include a mix of six to eight baby leaf vegetables that are flavorful, nutritious, and visually interesting. The main crop types grown for baby leaf salad mix are arugula, beet, kale, lettuce, mustard, pac choi, and spinach (Thornton, et al., 2015). Many of the cultivars used for baby leaf salad green production were bred for full head maturity, and only recently have seed companies begun to release cultivars bred specifically for baby leaf salad production. Cultivars developed for baby leaf production have good leaf shape, color, and texture, as well as thicker leaves for extended shelf life (Thornton, et al., 2015). Additionally, harvesting crops at the baby leaf size allows for greater efficiency with higher percentage of usable product, easier and faster processing, more attractive presentation in packaging because of the 3-D structure, and minimal oxidation (stem browning) due to smaller stem diameter (Martínez-Sánchez et al., 2012).

Controlled environment agriculture (CEA) is the modification of the natural environment to achieve optimum plant growth (Jensen, 2002). Hydroponics is a common technology used in CEA that involves growing plants in nutrient solutions with or without the use of an artificial medium to provide mechanical support. It is highly productive, conservative of water and land, and protective of the environment (Jensen, 1997). Pesticides are often not used in CEA due to lack of effective registered materials and consumer interest in more natural products. Pest and disease control still occurs, but typically focuses on the use of beneficial insects, microbial-based products, and environmental control. Greenhouses and plant factories are commonly used in CEA along with hydroponic technology to grow crops indoors. Indoor production allows for the control of light, water, plant nutrition, air and root temperatures, humidity, and carbon dioxide. The control of these variables allows for successive generations of crops to be grown in the same environmental conditions, resulting in more predictable crop yields (Ferentinos, 2000). This precise control of the growing conditions and predictable crop yields allow for significantly higher productivity compared to conventional field agriculture; however, indoor production has high capital and operational costs compared to conventional field agriculture. Additionally, indoor production allows for the strategic placement of production facilities near major population centers or regional transportation routes which can greatly reduce costs and emissions related to transportation. The most recent United States Department of Agriculture census indicates that the number of large- and small-scale farms producing vegetables and fresh cut herbs under glass or other protection in the United States has increased from 8,750 in 2012 to 10,849 in 2017 (USDA NASS, 2019). This increase in the number of farms using indoor growing technology sheds light into the growing importance of indoor agriculture.

Today, baby leaf vegetables are increasingly being produced in greenhouses and plant factories using hydroponic technology. This growing system is advantageous as products grown near major population centers are becoming ever more popular to consumers. However, the economics of using this growing system to produce baby leaf vegetables hinge on year-round production, high yields, and short growth cycles all of which are greatly benefited from the optimization of light quality, quantity, and photoperiod. Light quality refers to the spectral distribution of light whereas light quantity refers to the number of photons of photosynthetically active radiation (PAR) delivered. Light quantity can be measured as light intensity which is the instantaneous amount of light, or daily light integral (DLI) which is the cumulative amount of light delivered each day. Photoperiod refers to the number of hours of light in each 24-hour period. In plant factories, artificial lighting is used to provide light and light quality, quantity, and photoperiod can be adjusted. However, in greenhouses both supplemental lighting and shade curtains are often used to manage these light factors. The optimization of these lighting factors is crucial to the success of indoor baby leaf vegetable production as it is well known that growth and development are regulated by light quality, intensity, and photoperiod (Kang, 2013) and consistent year-round production is difficult to realize without consistent light integrals (Both, 1998).

Irradiance, or light intensity, is the primary factor affecting the relative growth rate of plants (Gent, 2014). Additionally, it is well known that, within bounds, an increase in light quantity leads to an increase in photosynthesis and crop biomass (Mattson, 2017). Specifically, a linear relationship has been shown between shoot dry weight and DLI in greenhouse grown butterhead lettuce (Albright, et al., 2000) and, similarly, a linear increase in dry weight with increasing DLI has been shown in young lettuce seedlings grown in a growth chamber (Kitaya, et al., 1998). Sole-source lighting and supplemental lighting are commonly used in plant factories and greenhouses, respectively, to meet light intensity and photoperiod – and thus light quantity – targets. The optimal light quantity, represented as daily light integral, varies by species. For example, the target daily light integral for greenhouse grown head lettuce is 17 mol m^{-2} d^{-1}, whereas the target daily light integral for greenhouse grown cucumbers, peppers, and tomatoes are all above 30 mol m^{-2} d^{-1}, and the target daily light integral for greenhouse grown strawberries is above 20 mol m^{-2} d^{-1} (Mattson, 2017). A 2012 study from the University of Arizona showed that yield of baby leaf lettuce was correlated with DLI, but that yield did not increase at DLI greater than ~20 mol m^{-2} d^{-1} (Kroggel, et al., 2012). Additionally, high-pressure sodium (HPS) lamps are commonly used as supplemental lighting sources and have historically been instrumental for year-round crop production in greenhouses. They are commonly used due to the high radiant emission, low price, long life span, high light emission, and high electrical efficiency (Pinho, 2011). However, HPS lamps do not allow for control of light spectrum. Light-emitting diodes (LEDs) have increasingly been adopted for horticulture lighting due to higher electrical efficiency, lower maintenance costs, and in some cases, the ability to adjust light spectrum. From a practical standpoint, crop yield, nutrient composition, flowering, plant height, and tolerance to biotic and abiotic stresses, among others, can be modulated by light (Pocock, 2015). A 2009 study found that the fresh weight, dry weight, stem length, leaf length, and leaf width of baby leaf lettuce all increased significantly with a supplemental far red treatment compared to a white light control (Li and Kubota, 2009). Thus, both HPS lamps and LEDs are commonly used in greenhouses and plant factories and can provide different benefits to the grower, however, the main role of supplemental and artificial lighting is to provide optimal light intensity so that consistent, year-round production can be achieved.

Much research has been done to understand the effects of light quality, quantity, and photoperiod for mature lettuce and other leafy greens, however, much less research has been done to understand the effects of these light characteristics, especially quantity, for plants grown specifically as baby leaf vegetables. Therefore, the objective of this study was to investigate the effects of daily light integral on the growth of hydroponically grown baby leaf vegetables. The results of this study could be used in the optimization of light intensity, in conjunction with light quality and photoperiod, in similar research or commercial settings. Understanding the effects of daily light integral on the growth of baby leaf vegetables could help to increase yields, shorten growth cycles, and enhance the ability to produce year-round. This could ultimately help growers increase their production capacity in the long run.

Materials and Methods

In this experiment, the response of baby leaf vegetables – grown hydroponically in deep water culture within a controlled environment chamber – to daily light integral was examined. Four leafy vegetable species; collards (*Brassica oleracea* L. 'Flash'), lettuce (*Lactuca sativa* L. 'Sulu'), mizuna (*Brassica rapa* L. var. *japonica*), and pac choi (*Brassica rapa* L. var. *chinensis* 'Red Pac') (Figure 1) were purchased from Johnny's Selected Seeds company (Winslow, ME USA); lot numbers were 46542, 60827, 58278, and 60249, respectively. Species were chosen for their presence in baby leaf salad mixes, similarity in germination rate and hours to germination (in a preliminary experiment), and for their diversity in color, texture, and leaf shape. Collards was chosen for its smooth, circular leaves and dark green color. Lettuce was chosen for its bright lime green color, 3-D texture, and upright habit. Mizuna was chosen for its bright green color and fringed leaves. Pac choi was chosen for its dark red/purple color and curved, oval-shaped leaves.

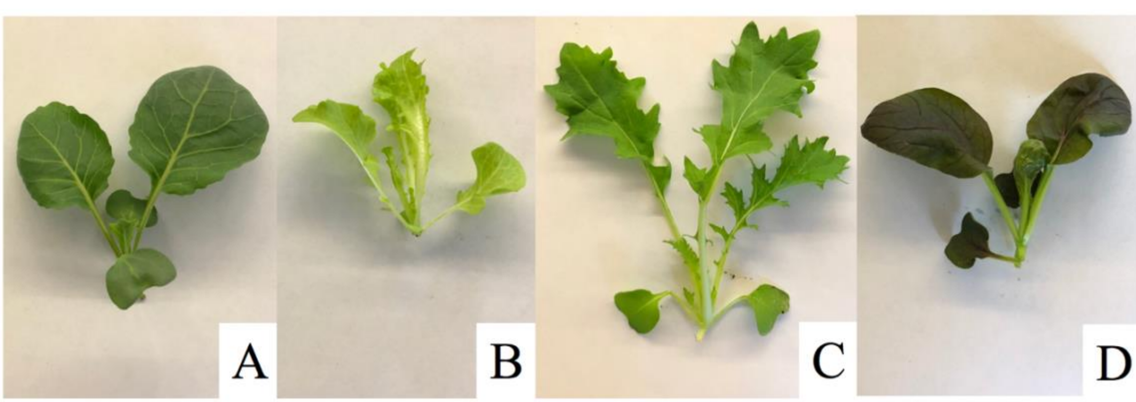

Figure 1. Images of leafy vegetable species utilized in this experiment where A, B, C, and D represent collards, lettuce, mizuna, and pac choi.

Plant Production System

A controlled environment chamber measuring 2.5 m wide by 3.0 m long with a ceiling height of 2.1 m located at the Kenneth Post Laboratory on Cornell University's main campus (Ithaca, NY) (Figure 2) was used for this experiment. A 1.2 by 2.4 m polyethylene table (Structural Plastics Corporation, Holly, MI, USA) supported by polyvinyl chloride legs with a height of 0.9 m from floor to table top was set up within the chamber. Eight, 29 L plastic tubs (Tablecraft Products) were placed on the table and used as hydroponic ponds. Each tub was covered with a polystyrene covering containing three 21.0 by 13.3 cm cutouts (to float forty cell polystyrene floats) and two 2.5 cm diameter cutouts. The eight tubs were organized into a randomized complete block design with four tubs acting as one block. Within each of the blocks, each of the four leafy green species was randomly assigned to one of the four hydroponic ponds. Within each hydroponic pond one forty cell, 20.3 by 12.7 cm polystyrene float was placed in each of the three rectangular cutouts (Figure 3). The two circular cutouts were used to provide space for the air stone to enter the nutrient solution and to draw samples from, respectively. The nutrient solution utilized for these experiments was Jack's Hydroponic 5-12-26 (JR Peters Inc., Allentown, PA, USA) and calcium nitrate both prepared with reverse osmosis water. Each tub was filled with 28 L of reverse osmosis water and 0.28 L each of 1:100 concentration Jack's Hydroponic 5-12-26 and calcium nitrate in the method described by Mattson and Peters (2014) resulting in 150 mg L^{-1} nitrogen. The tubs were topped off with nutrient solution to maintain this volume throughout the experiment. The pH was adjusted to 5.8 ± 0.4 using potassium hydroxide and sulfuric acid. Electrical conductivity and temperature of the nutrient solution were not controlled, but were measured to be 1736 ± 74 μS cm^{-1} and 24 ± 0.7 °C, respectively, for the duration of the experiment. Air was supplied to the nutrient solution with air pumps (General

Hydroponics) and air stones. The ambient carbon dioxide concentration and relative humidity in the growth chamber were not controlled, but were determined to be approximately 751 ± 53 ppm CO_2 and 34 ± 8.7 %, respectively, for the duration of the experiment. Air temperature in the chamber was maintained at 24 to 25 °C.

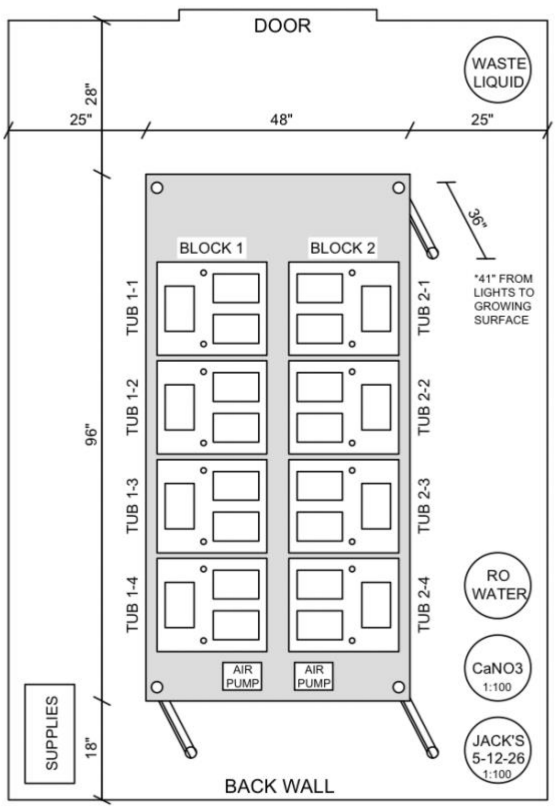

Figure 2. Growth chamber layout.

Figure 3. Eight tubs organized in randomized complete block design (top) and single pond float layout (bottom).

Lighting Treatment

The photoperiod was fixed at 20 hours and the lighting source was T5 fluorescent lamps (Sylvania, Wilmington, MA, USA). Daily light integral treatments were 6, 12, 18, 24, and 30 mol m^{-2} d^{-1} at plant level. Four light maps were taken before each treatment with 1 bank, 2 banks, 3 banks, and 4 banks turned on, respectively. A light meter with accompanying quantum sensor (LI-COR, Inc.) was used to determine instantaneous light intensity at 15 evenly spaced locations (Figure 4) across the growing area at plant level for each light map. The 15 light intensities were then averaged for each light map and the number of hours of illumination needed from each light bank to meet the target daily light integral was calculated. A shade cloth was hung beneath the fluorescent lights (Figure 5) for the 6 mol m^{-2} d^{-1} treatment. Light treatments were randomly assigned to one of five treatment periods which took place between October 2018 to April 2019.

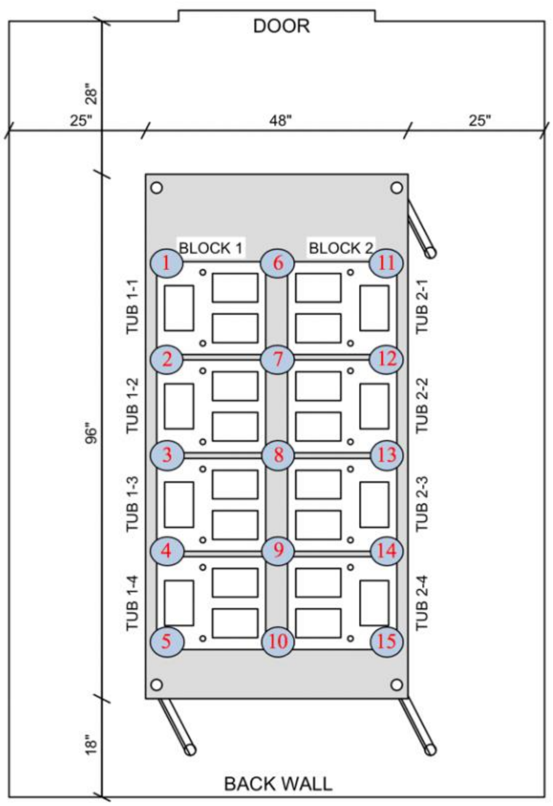

Figure 4. Fifteen sample locations for instantaneous light intensity.

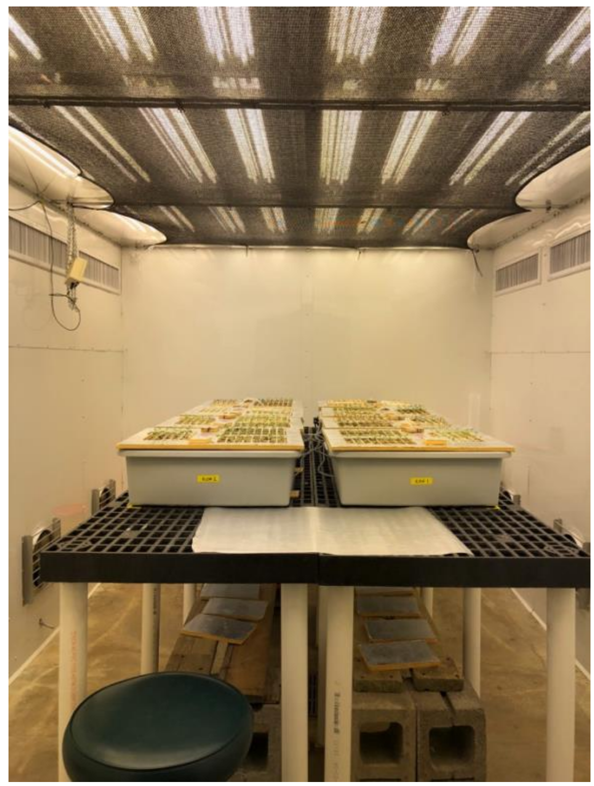

Figure 5. Black shade cloth hung beneath fluorescent lamps in growth chamber.

Germination Procedure

Seeds were single seeded by hand into individual cells of forty cell, 20.3 by 12.7 cm polystyrene floats filled with a commercial peat-lite germination mix (LM-1, Lambert Peat Moss, Rivière-Ouelle, Canada) resulting in a density of 1550 plants m^{-2}. Prior to seeding, the substrate was incorporated with reverse osmosis water at a ratio of 1:1 (RO water: peat-lite mix) by weight and since the substrate already contained moisture this led to a final moisture content ratio of 3:1 (water: substrate). Each baby leaf vegetable species was seeded into six, forty cell floats (24 floats total) and then individually wrapped in a one-gallon plastic storage bag (C & S Wholesale Grocers, Inc.) sealed with a twist tie to maintain moisture during germination. The floats were then organized into eight groups of three floats containing seeds of the same species and placed into eight propagation domes (Curtis Wagner Plastics Corp.) and then covered by a light restrictive flat (TO plastics) (Figure 6). The eight trays were placed at random on wooden planks located 43 cm from the floor within the growth chamber (Figure 7). The wooden planks were located 48 cm and 66 cm below the table top and the growing surface, respectively.

Preliminary experiments were conducted to determine hours to germination for each species under the germination conditions used for this experiment. Hours to germination was determined by the time from seeding to when 95% of seedlings had pushed through the soil surface. This yielded germination times of 47 hours for mizuna and pac choi and 54 hours for lettuce and collards. All 24 floats were seeded at the same time and then the six mizuna floats and the six pac choi floats were floated 47 hours after seeding, whereas the six lettuce floats and the six collard floats were floated after an additional seven hours (54 hours after seeding). The day that the plants were floated was considered Day 0.

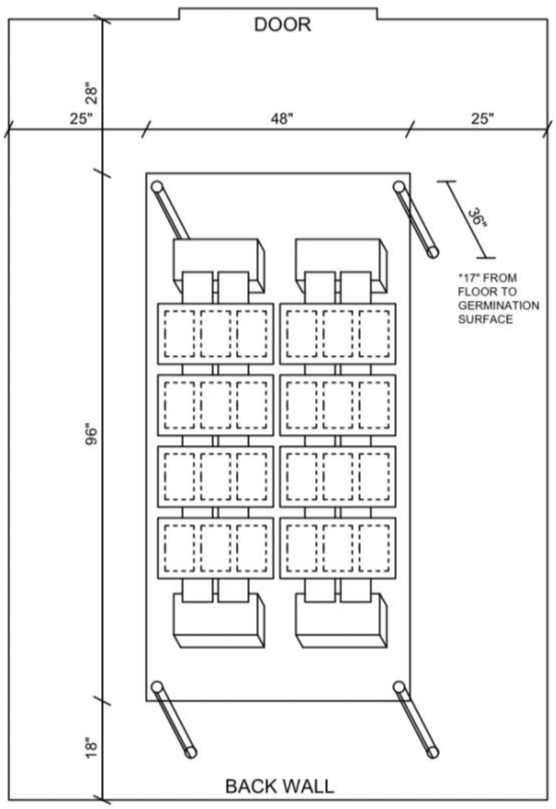

Figure 6. Schematic of below-table germination set-up.

Figure 7. Three floats individually wrapped and placed in propagation dome (top) and layout of germinating plants (bottom).

Harvest Procedure

Three destructive harvests were completed during each lighting treatment by removing one float from each of the eight hydroponic ponds four, eight, and twelve days after Day 0. Each of the three floats in each pond was randomly assigned a harvest day (four, eight, or twelve). During each of the three harvests, each of the eight floats were individually removed from the nutrient solution and the non-germinated cells were noted. The plants around the edges were removed – to account for edge-effects – by cutting the stem at the media line and taking the fresh weight of the group of outer plants using a precision balance with a guaranteed repeatability of 0.01 g (Mettler Toledo, PM4600). Plants from the inner 18 cells were then individually cut and weighed and placed into paper bags for drying. Five plants from the inner cells were randomly selected to be photographed and then each plant was measured for tallest leaf height, widest leaf width, total leaf number, and total leaf area. Leaf height was determined for each plant by measuring the tallest leaf from the point the leaf meets the stem to the tip of the leaf using a digital caliper (Mitutoyo, Kawasaki, Japan). Leaf width was determined for each plant by measuring the width of the widest leaf at the widest point using a digital caliper. The leaf number was determined for each plant by counting the number of unfurled true leaves. The total leaf area was determined for each plant by summing the leaf area of every leaf from the plant (including cotyledons) using an area machine (LI-COR, Inc., LI-3100). The plants were then put into individual paper bags and dried. Plants were dried for a minimum of 72 hours at 70 C in a mechanical convection oven (Thermo Fisher Scientific, Waltham, MA). The dried plants were then individually weighed using a precision balance with guaranteed repeatability of 0.001 g (Mettler Toledo, MS1003S). Additionally, a Day 0 harvest was completed by harvesting two floats of each species after their respective hours of germination in order to understand the fresh and dry weights as well as the leaf heights, widths, areas, and numbers of the plants at the time of floating.

Experimental Design and Statistical Analysis

One experimental cycle was conducted consisting of five runs. In each run, a single walk-in chamber, which had two blocks each containing four hydroponic tubs, was set to one of five DLI treatments. Within each species and block for each of three harvest dates per lighting treatment, plant fresh weight and dry weight data were taken from ca. 18 plants (from each germinated plant in the 18 inner cells) and all additional data were taken from the 5 randomly selected plants. All statistical analyses were completed in RStudio version 1.1.456 (R Core Team, 2018). Linear mixed effects models were estimated separately for each species for each response (fresh weight, dry weight, leaf height, leaf width, leaf area, and leaf number) with fixed effects of time, treatment, $time^2$, time x treatment, and $time^2$ x treatment, and random effects of block and block x time using the lme4 package (Bates et al., 2015). Fixed effects tests from each mixed model are reported, effects are deemed significant when $P \leq 0.05$. Post-hoc comparisons of the treatments for each day were estimated using the emmeans package (Lenth, 2019). Results were plotted using the ggplot2 package (Wickham, 2016).

Results

<u>*Fresh Weight*</u>

Fresh weight per plant for collards, lettuce, mizuna, and pac choi increased quadratically between Day 0 and Day 12 for each of the five DLI treatments (6, 12, 18, 24, and 30 mol m^{-2} d^{-1}) (Figure 8). For collards, lettuce, and pac choi there was a statistically significant difference ($p<0.0001$ for nearly all pairs) in the average fresh weight per plant at Day 12 between each of the DLI treatments. For mizuna, there was a statistically significant difference ($p<0.0001$) in the average fresh weight per plant at Day 12 between each of the DLI treatments except for between the 18 and 24 mol m^{-2} d^{-1} treatments ($p=0.0832$). At Day 8, there were statistically significant differences in the average fresh weight per plant between some of the DLI treatments for collards, lettuce, and pac choi. However, at Day 4, there were no statistically significant differences in the average fresh weight per plant between any of the DLI treatments for any of the species. Letters representing mean separation comparisons at Day 4 and Day 8 were not included in Figure 8 to avoid clutter.

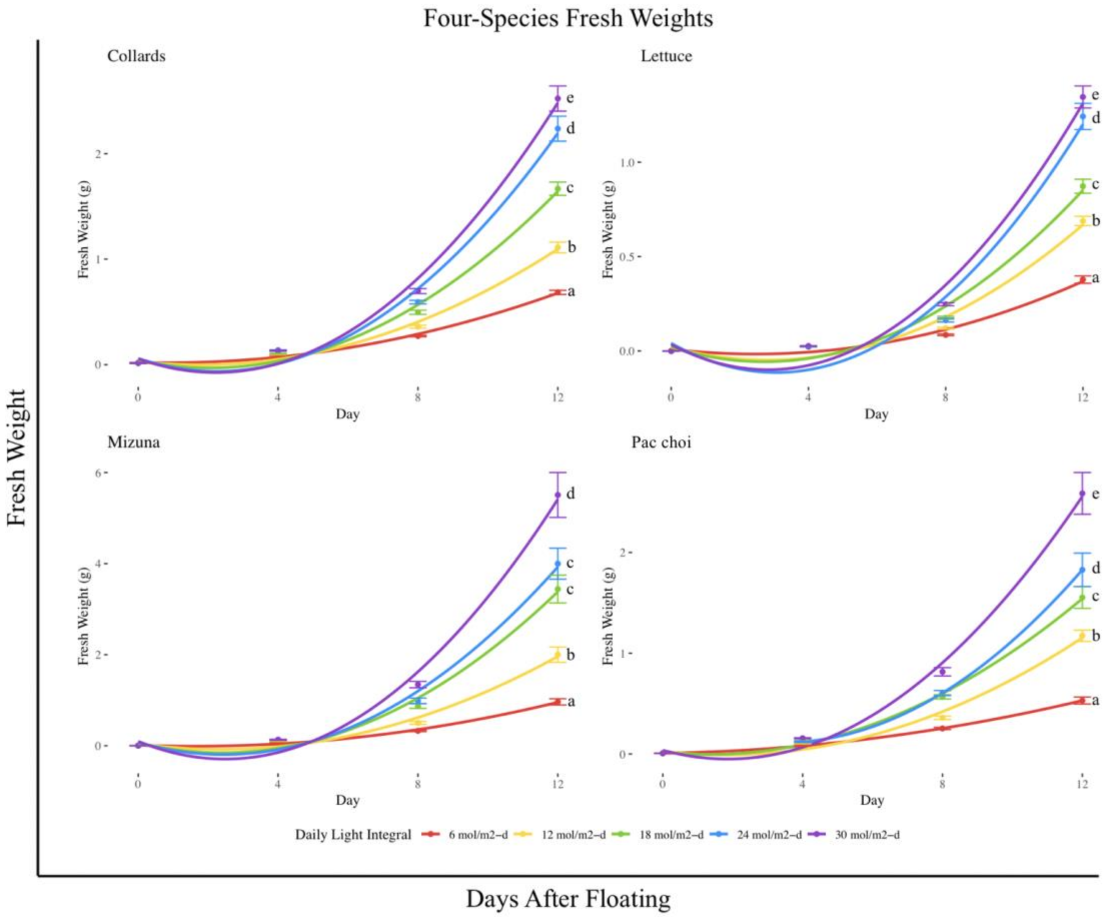

Figure 8. Average fresh weight per plant of collards, lettuce, mizuna, and pac choi under five DLI treatments of 6, 12, 18, 24, and 30 mol $m^{-2}d^{-1}$. Significant quadratic regressions for each species and DLI over time were found. Data represents mean ± SE of ca. 36 plants (ca. 18 plants per block per species per treatment). Letters represent mean separation comparison at Day 12 using Tukey's HSD at α = 0.05.

To estimate potential commercial yields of larger crop stands, Table 1 notes the calculated fresh weight in terms of kg per m^2 on Day 12 for collards, lettuce, mizuna, and pac choi for each of the five DLI treatments (6, 12, 18, 24, and 30 mol m^{-2} d^{-1}). This assumes a plant spacing of 1550 plants per m^2 (1 plant per in^2) and uses the average fresh weight per plant for each species from each of the five treatments. For each of the four species there continued to be an increase in yield up to the 30 mol m^{-2} d^{-1} treatment, however, for lettuce and collards a relatively small yield increase was noted between the 24 and 30 mol m^{-2} d^{-1} treatments.

Fresh Weight (kg m^{-2})					
DLI (mol $m^{-2}d^{-1}$)					
Species	**6**	**12**	**18**	**24**	**30**
Collards	1.1^{a}	1.7^{b}	2.6^{c}	3.5^{d}	3.9^{e}
Lettuce	0.6^{a}	1.1^{b}	1.4^{c}	1.9^{d}	2.1^{e}
Mizuna	1.5^{a}	3.1^{b}	5.3^{c}	6.2^{c}	8.5^{d}
Pac choi	0.8^{a}	1.8^{b}	2.4^{c}	2.8^{d}	4.0^{e}

Table 1. Fresh weight in kg per m^2 of collards, lettuce, mizuna, and pac choi under five DLI treatments of 6, 12, 18, 24, and 30 mol $m^{-2}d^{-1}$. Assuming a plant spacing of 1550 plants per m^2 (1 plant per in^2). Data were calculated from mean ± SE of ca. 36 plants (ca. 18 plants per block per species per treatment). Letters represent mean separation comparison at Day 12 using Tukey's HSD at $\alpha = 0.05$.

Dry Weight

Dry weight per plant for collards, lettuce, mizuna, and pac choi increased quadratically between Day 0 and Day 12 for each of the five DLI treatments (6, 12, 18, 24, and 30 mol m^{-2} d^{-1}) (Figure 9). For collards, there was a statistically significant difference ($p<0.0001$ for all pairs except $p=0.0165$ between the 24 and 30 mol m^{-2} d^{-1} treatments) in the average dry weight per plant at Day 12 between each of the DLI treatments. For lettuce, there was a statistically significant difference ($p<0.0001$) in the average dry weight per plant at Day 12 between each of the DLI treatments except for between the 24 and 30 mol m^{-2} d^{-1} treatments ($p=0.9966$). For mizuna and pac choi, there was a statistically significant difference ($p<0.0001$ for all pairs except $p=0.0064$ for mizuna between the 6 and 12 mol m^{-2} d^{-1} treatments) in the average dry weight per plant at Day 12 between each of the DLI treatments except for between the 18 and 24 mol m^{-2} d^{-1} treatments for both species ($p=0.1053$ and $p=0.0645$, respectively). At Day 8, there were statistically significant differences in the average dry weight per plant between some of the DLI treatments for each of the four species. Additionally, at Day 4, there were no statistically significant differences in the average dry weight per plant between any of the DLI treatments for collards, mizuna, and pac choi, however, for lettuce there were statistically significant differences in the average dry weight per plant at Day 4 between some of the DLI treatments. Letters representing mean separation comparisons at Day 4 and Day 8 were not included in Figure 9 to avoid clutter.

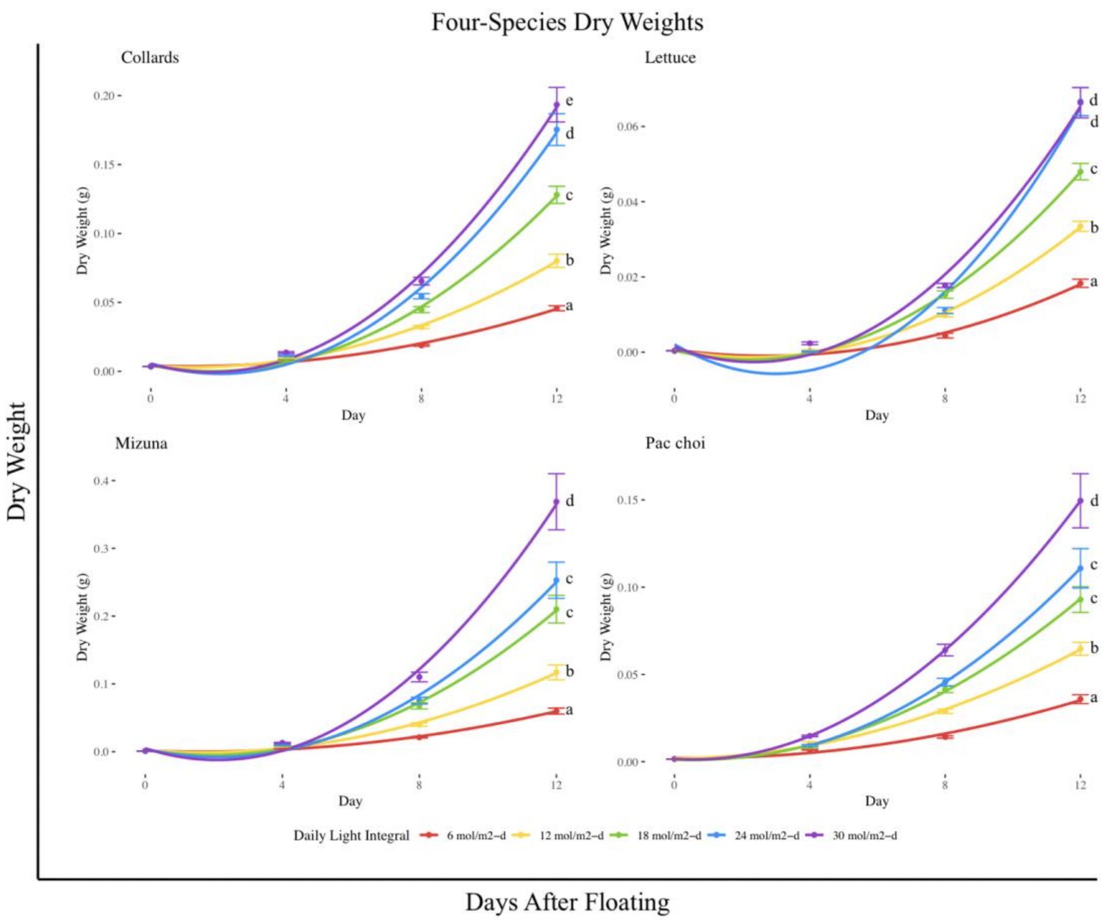

Figure 9. Average dry weight per plant of collards, lettuce, mizuna, and pac choi under five DLI treatments of 6, 12, 18, 24, and 30 mol m^{-2} d^{-1}. Significant quadratic regressions for each species and DLI over time were found. Data represents mean ± SE of ca. 36 plants (ca. 18 plants per block per species per treatment). Letters represent mean separation comparison at Day 12 using Tukey's HSD at $\alpha = 0.05$.

Leaf Height

The tallest leaf height per plant for collards, lettuce, mizuna, and pac choi increased quadratically between Day 0 and Day 12 for each of the five DLI treatments (6, 12, 18, 24, and 30 mol m^{-2} d^{-1}) (Figure 10). For collards, there was a statistically significant difference ($p<0.0001$) in the tallest leaf height per plant at Day 12 between each of the DLI treatments except for between the 6 and 12 mol m^{-2} d^{-1} and the 24 and 30 mol m^{-2} d^{-1} treatments, respectively ($p=0.2548$ and $p=0.6867$, respectively). For lettuce, there was no statistically significant difference in tallest leaf height per plant at Day 12 between any of the DLI treatments. For Mizuna, there was no statistically significant difference in the tallest leaf height per plant at Day 12 between the 6, 12 and 18 mol m^{-2} d^{-1} treatments ($p>0.5$) or the 24 and 30 mol m^{-2} d^{-1} treatments ($p=0.9973$), respectively. For pac choi, there was a statistically significant difference ($p<0.0001$) in the tallest leaf height per plant at Day 12 between each of the DLI treatments except for between the 12 and 18 mol m^{-2} d^{-1} and the 24 and 30 mol m^{-2} d^{-1} treatments, respectively ($p=0.9099$ and $p=0.4719$, respectively). At Day 8, there were statistically significant differences in the tallest leaf height per plant between some of the DLI treatments for collards, lettuce, and pac choi. Additionally, at Day 4, there were no statistically significant differences in the tallest leaf height per plant between any of the DLI treatments for collards, mizuna, and pac choi, however, for lettuce there was a statistically significant difference in the tallest leaf height per plant at Day 4 between the 6 and 12 mol m^{-2} d^{-1} treatments ($p=0.0448$). Letters representing mean separation comparisons at Day 4 and Day 8 were not included in Figure 10 to avoid clutter.

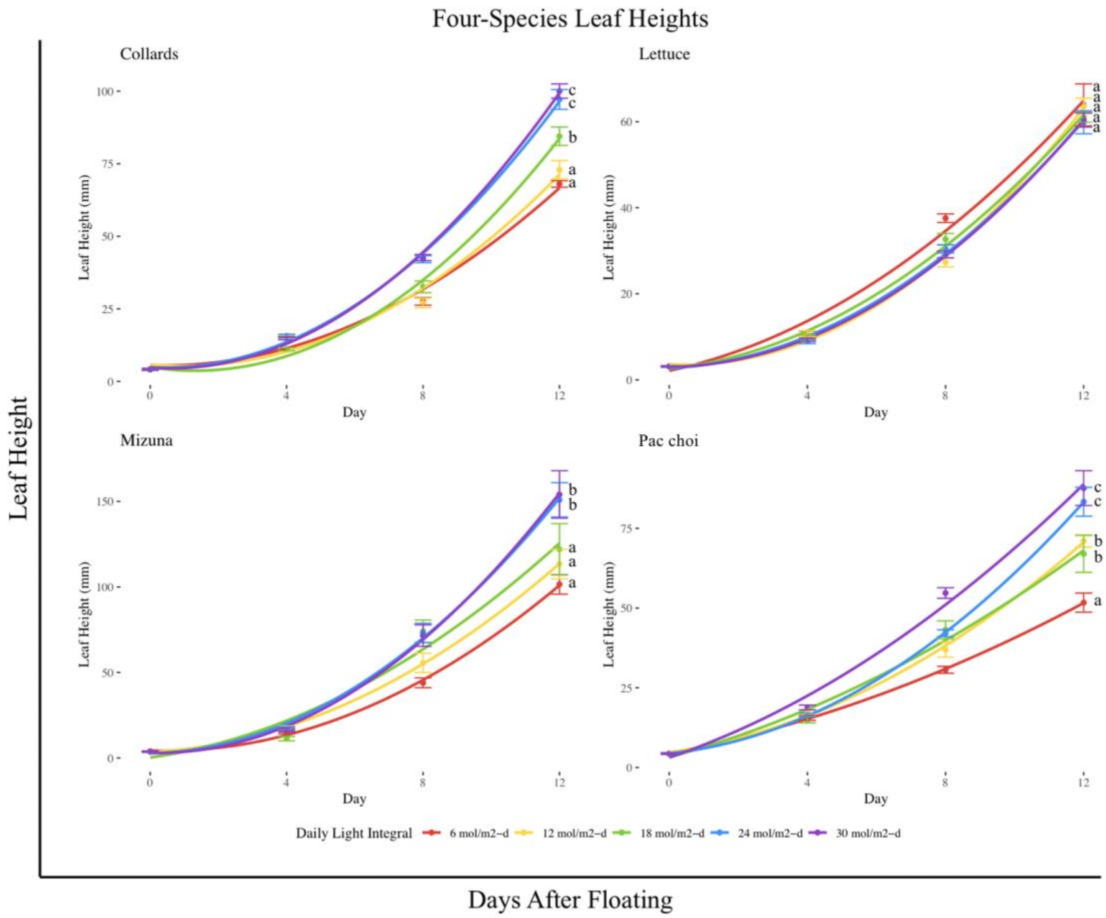

Figure 10. Tallest leaf height per plant of collards, lettuce, mizuna, and pac choi under five DLI treatments of 6, 12, 18, 24, and 30 mol m^{-2} d^{-1}. Significant quadratic regressions for each species and DLI over time were found. Data represents mean ± SE of ca. 36 plants (ca. 18 plants per block per species per treatment). Letters represent mean separation comparison at Day 12 using Tukey's HSD at α = 0.05.

Leaf Width

Widest leaf width per plant for collards, lettuce, mizuna, and pac choi increased quadratically between Day 0 and Day 12 for each of the five DLI treatments (6, 12, 18, 24, and 30 mol m^{-2} d^{-1}) (Figure 11). For collards, there was a statistically significant difference ($p<0.0001$) in the widest leaf width per plant at Day 12 between each of the DLI treatments except for between the 6 and 12 mol m^{-2} d^{-1} and the 24 and 30 mol m^{-2} d^{-1} treatments, respectively ($p=0.6196$ and $p=0.8787$, respectively). For lettuce, there were no statistically significant differences in the widest leaf width per plant at Day 12 between any of the DLI treatments. For Mizuna, there was no statistically significant difference in the widest leaf width per plant at Day 12 between the 6, 12 and 18 mol m^{-2} d^{-1} treatments ($p>0.05$) or the 24 and 30 mol m^{-2} d^{-1} treatments ($p=0.5147$), respectively. For pac choi, there was a statistically significant difference ($p<0.0001$) in the widest leaf width per plant at Day 12 between each of the DLI treatments except for between the 12 and 18 mol m^{-2} d^{-1} treatments or the 24 and 30 mol m^{-2} d^{-1} treatments, respectively ($p=0.9897$ and $p=0.7507$, respectively. At Day 8, there were statistically significant differences in the widest leaf width per plant between some of the DLI treatments for collards and pac choi. Additionally, at Day 4, there were no statistically significant differences in the widest leaf width per plant between any of the DLI treatments for collards, mizuna, and lettuce, however, for pac choi there was a statistically significant difference in the widest leaf width per plant at Day 4 between the 24 and 30 mol m^{-2} d^{-1} treatments ($p=0.0008$). Letters representing mean separation comparisons at Day 4 and Day 8 were not included in Figure 11 to avoid clutter.

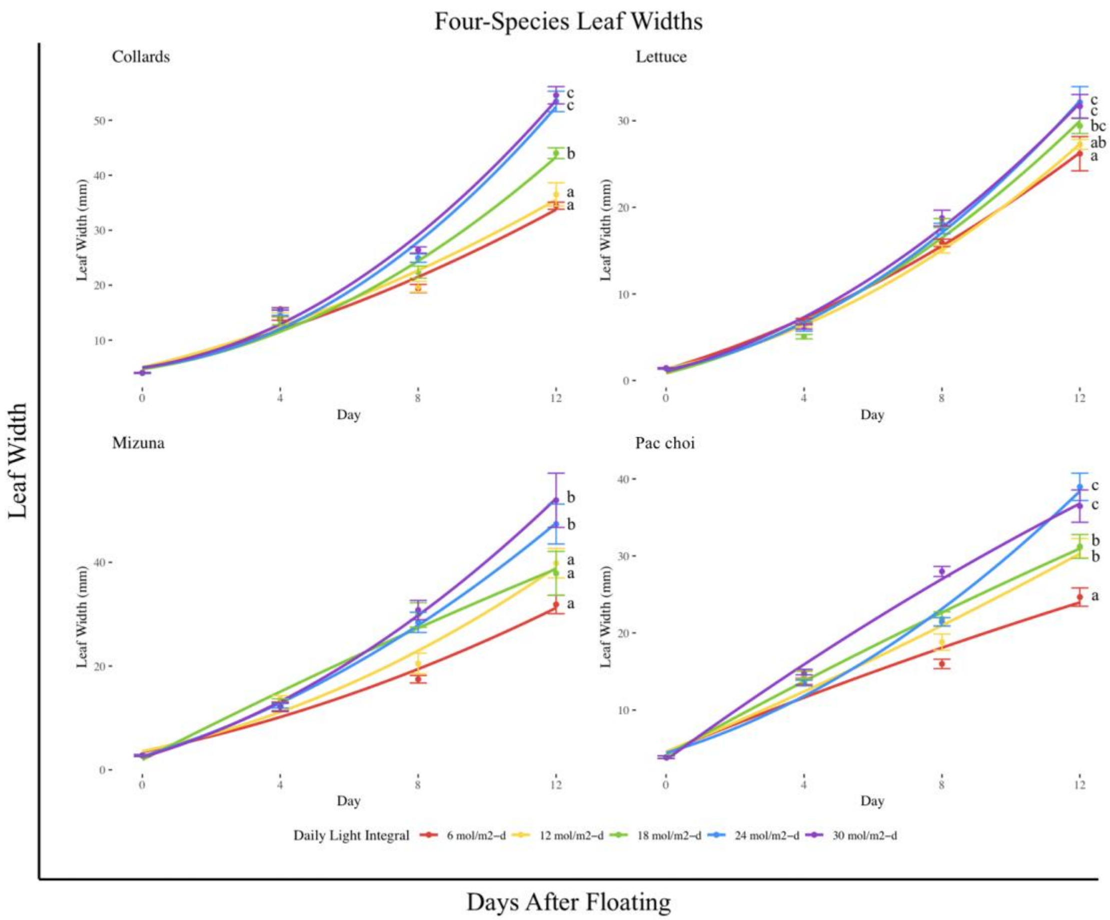

Figure 11. Widest leaf width per plant of collards, lettuce, mizuna, and pac choi under five DLI treatments of 6, 12, 18, 24, and 30 mol m^{-2} d^{-1}. Significant quadratic regressions for each species and DLI over time were found. Data represents mean ± SE of ca. 36 plants (ca. 18 plants per block per species per treatment). Letters represent mean separation comparison at Day 12 using Tukey's HSD at α = 0.05.

Leaf Area

Total leaf area per plant for collards, lettuce, mizuna, and pac choi increased quadratically between Day 0 and Day 12 for each of the five DLI treatments (6, 12, 18, 24, and 30 mol m^{-2} d^{-1}) (Figure 12). For collards, there was a statistically significant difference ($p<0.0001$ for all pairs except $p=0.0136$ between the 24 and 30 mol m^{-2} d^{-1} treatments) in the total leaf area per plant at Day 12 between each of the DLI treatments except for between the 6 and 12 mol m^{-2} d^{-1} treatments ($p=0.1344$). For lettuce and pac choi, there was no statistically significant difference in the total leaf area per plant at Day 12 between the 12 and 18 mol m^{-2} d^{-1} or the 24 and 30 mol m^{-2} d^{-1} treatments, respectively, for either species. For mizuna, there were no statistically significant differences in the total leaf area per plant at Day 12 between any of the DLI treatments. At Day 8, there were no statistically significant differences in the total leaf area per plant between any of the DLI treatments for any of the species except for lettuce which showed a statistically significant difference in the total leaf area per plant between the 18 and 24 mol m^{-2} d^{-1} treatments ($p=0.0336$). Additionally, at Day 4, there were no statistically significant differences in the total leaf area per plant between any of the DLI treatments for any of the species. Letters representing mean separation comparisons at Day 4 and Day 8 were not included in Figure 12 to avoid clutter.

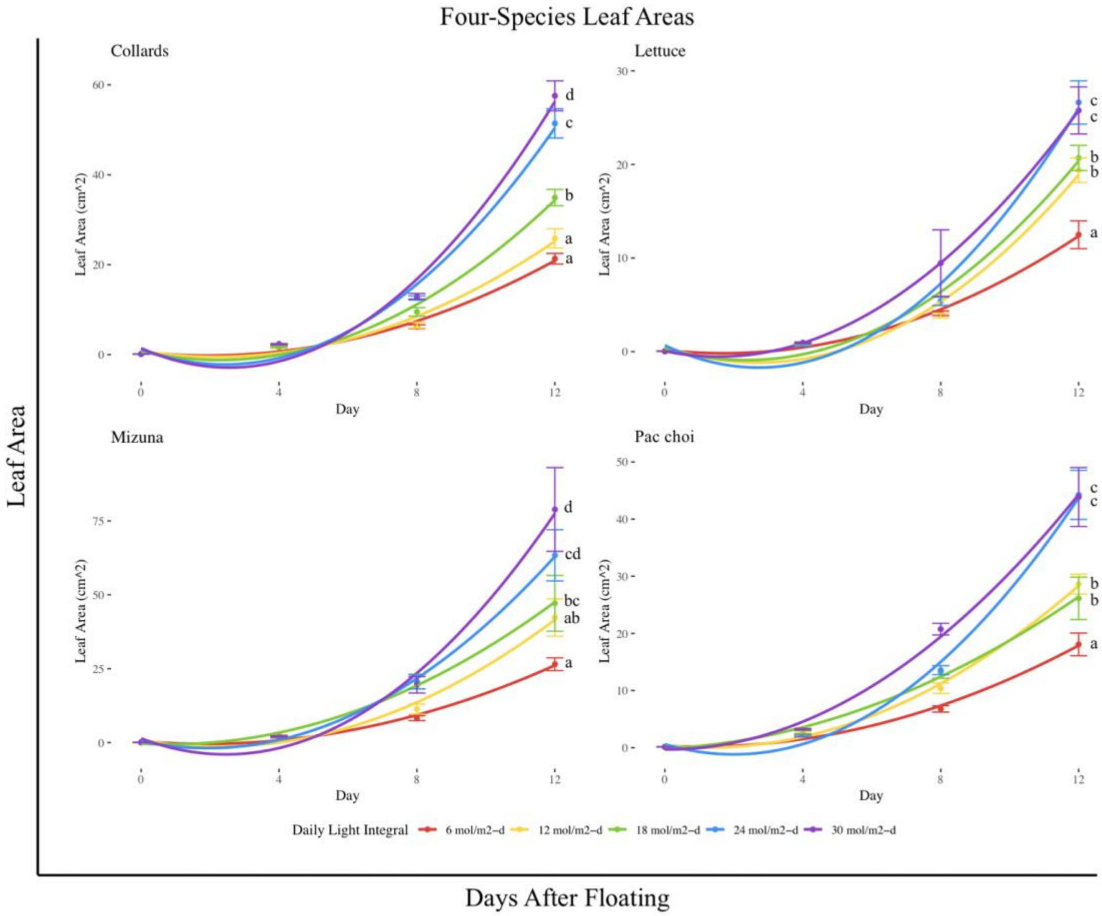

Figure 12. Total leaf area per plant of collards, lettuce, mizuna, and pac choi under five DLI treatments of 6, 12, 18, 24, and 30 mol m^{-2} d^{-1}. Significant quadratic regressions for each species and DLI over time were found. Data represents mean ± SE of ca. 36 plants (ca. 18 plants per block per species per treatment). Letters represent mean separation comparison at Day 12 using Tukey's HSD at $\alpha = 0.05$.

Leaf Number

Total leaf number per plant for collards, lettuce, mizuna, and pac choi increased quadratically between Day 0 and Day 12 for each of the five DLI treatments (6, 12, 18, 24, and 30 mol m^{-2} d^{-1}) (Figure 13). For collards, there was no statistically significant difference in the total leaf number per plant at Day 12 between the 6, 12, and 18 mol m^{-2} d^{-1} treatments. For lettuce, mizuna, and pac choi at Day 12, there was an overall pattern of increasing leaf number as DLI increased, but some of the light treatments were not significantly different from each other.

Additionally, there were statistically significant differences in the total leaf number per plant at both Day 8 and at Day 4 between some of the DLI treatments for collards, lettuce, and pac choi. Letters representing mean separation comparisons at Day 4 and Day 8 were not included in Figure 13 to avoid clutter.

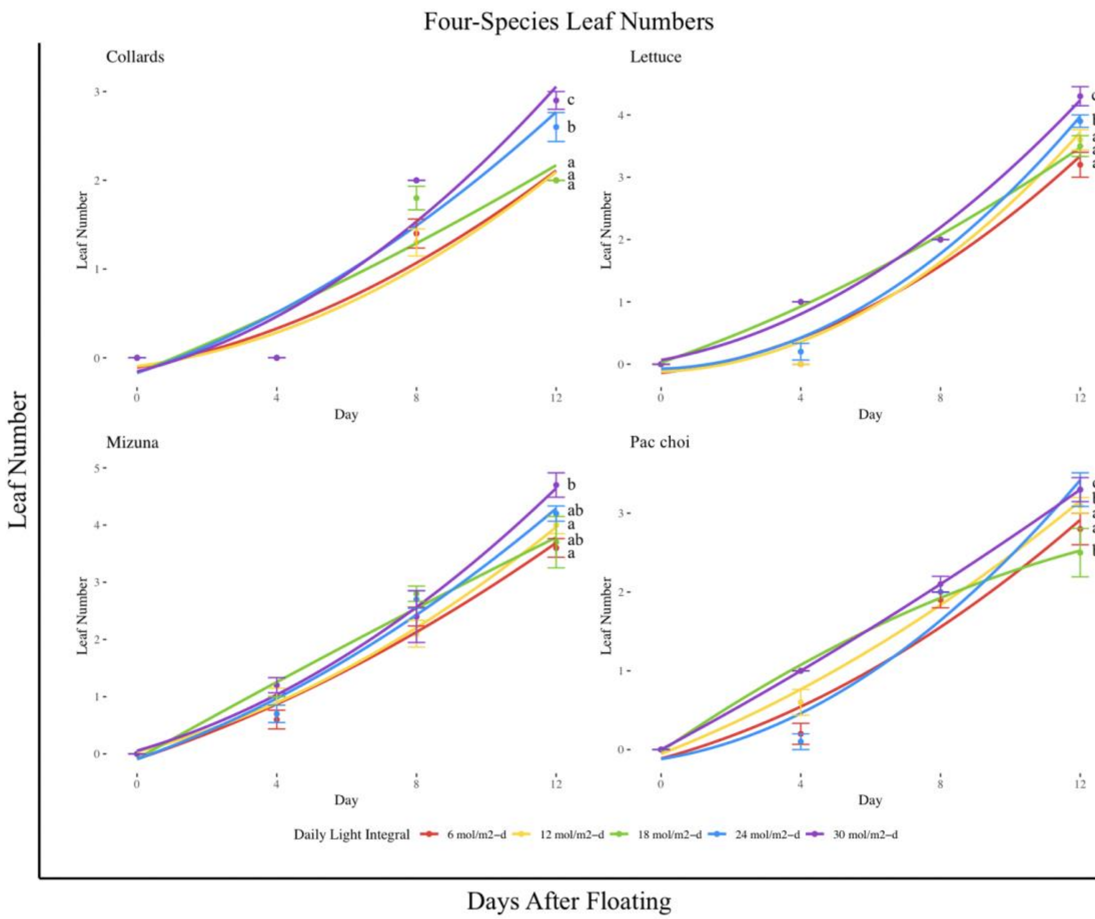

Figure 13. Leaf number per plant of collards, lettuce, mizuna, and pac choi under five DLI treatments of 6, 12, 18, 24, and 30 mol m^{-2} d^{-1}. Significant quadratic regressions for each species and DLI over time were found. Data represents mean ± SE of ca. 36 plants (ca. 18 plants per block per species per treatment). Letters represent mean separation comparison at Day 12 using Tukey's HSD at α = 0.05.

Discussion

Recommendations for optimal daily light integral for the growth and development of baby leaf vegetables have not been previously reported in the scientific literature. However, a linear relationship has been shown between shoot dry weight and DLI in greenhouse grown butterhead lettuce (Albright, et al., 2000) and a similar linear increase in dry weight with increasing DLI has been shown in young lettuce seedlings grown in a growth chamber (Kitaya, et al., 1998). Additionally, a 2012 study from the University of Arizona showed that yield of baby leaf lettuce was correlated with DLI, but that yield did not increase at DLI greater than ~20 mol m^{-2} d^{-1} (Kroggel, et al., 2012). In the current experiment, it was shown that incremental increases in daily light integral even up to 30 mol m^{-2} d^{-1} resulted in significant increases in fresh weight and dry weight in each of the four species of baby leaf vegetables studied. However, even though a maximum daily light integral at which plant biomass did not further increase was not determined, it has been shown that incremental increases in daily light integral will continue to positively impact yield in baby leaf vegetables up to 30 mol m^{-2} d^{-1}, although for lettuce and collards declining biomass benefits were found above 24 mol m^{-2} d^{-1}.

In this experiment, it was also shown that incremental increases in daily light integral had some significant impacts on leaf height and leaf width for some of the species studied. Collards, mizuna, and pac choi all showed generally increasing leaf heights and leaf widths with increasing DLI, however, there were no significant increases in either characteristic between the 24 and 30 mol m^{-2} d^{-1} light treatments for these species. In contrast, lettuce showed no significant differences in leaf height or leaf width between any of the five lighting treatments. Typically, baby leaf vegetables are characterized by their leaf height – along with number of true leaves and days to maturity – with the typical harvestable leaf height often being described as ~10 cm (or ~4 inches) (Thornton, et al., 2015; Ryder, 2002). This experiment shows that for some species of baby leaf vegetables, incremental increases in daily light integral can have some significant, positive effects on leaf height thus potentially shortening days to reach harvestable height. However, other factors may have a greater impact on leaf height and leaf width than daily light integral, such as leaf temperature and days from seeding. In our experiment, this was especially apparent in lettuce which exhibited no impact on leaf height or leaf width by daily light integral. Interestingly in lettuce, leaf height and leaf width, along with fresh weight, dry weight, and stem length, were shown to increase significantly when white light was supplemented with far red light (Li and Kubota, 2009). Thus, the relative lack of far red light in our experiment conducted under T5 white fluorescent fixtures may explain the lack of observed leaf morphology responses in lettuce. Regarding temperature, a study by Jan Bensink (1971) found a light-temperature relationship whereby the greatest effects of light on leaf width are found at high temperature, whereas for leaf height, light intensity effects are greatest at low temperature. This study proposes that there is a critical light intensity above which leaf growth will be enhanced by raising temperature, and below which it is decreased (Bensink, 1971). Thus, daily light integral may be used to control leaf height and leaf width for some baby leaf vegetable species, but other characteristics such as temperature and light quality may have more significant impacts on these characteristics than DLI. These characteristics were not studied in this experiment.

Regarding leaf area, this experiment showed some significant impact of incremental increases in daily light integral on total leaf area per plant for some of the species. Specifically, collards, lettuce, and pac choi showed generally increasing total leaf area per plant with increasing DLI, however, collards was the only species that showed a significant impact of DLI above 24 mol m^{-2} d^{-1}. For mizuna, there were no significant impacts on total leaf area per plant between any of the incremental DLI levels. It appears that increasing DLI has an impact on leaf area in baby leaf vegetables, however, research has shown that other treatment characteristics such as light quality can also have an impact on leaf area. Specifically, one study showed that 17 days after seeding the leaf area of red leaf lettuce plants treated with red light was 33% greater than plants under fluorescent lights, but that the leaf area of plants treated with blue light was 9% less than plants under fluorescent lights (Johkan, et al., 2010). Light quality impacts on leaf area were not studied as part of this experiment.

The current experiment also showed an overall pattern of increasing leaf number as DLI increased, but some of the light treatments were not significantly different from each other for lettuce, mizuna, and pac choi. For collards, no impact on leaf number was seen as DLI increased. Although it appears that increasing DLI has some impact on leaf number in baby leaf vegetables, other research has shown that there are other treatment characteristics such as temperature that can also have an impact on leaf number. For example, research has shown that leaf production in lettuce increases with temperatures at all light intensities (Bensink, 1971).

Daily light integral may also have other benefits on baby leaf vegetables other than the specific growth characteristics measured in this experiment. For example, variations in amount and intensity of light influence the nutritional composition of leafy greens, especially their ascorbic acid (Salunkhe & Desai, 1998). However, a study of greenhouse grown baby leaf lettuce and komatsuna found that DLI did not have an effect on anthocyanin, total phenolics, carotenoid, or ascorbic acid concentrations (Kroggel, et al., 2012). More research is needed to fully understand the effects of daily light integral on the nutritional content of baby leaf vegetables.

In our experiment, DLI responses were studied only for one crop cycle per DLI treatment. Further work should add further repetitions to fully validate the results of this experiment. Additionally, this experiment could be expanded upon in a number of ways including increasing the range of DLI treatments, measuring the effects on nutrient composition, exploring the effects on other baby leaf vegetable species, or varying the seeding density.

Conclusion

To our knowledge, this present study is the most comprehensive overview of the effects of daily light integral on the growth and development of baby leaf vegetables. In the current experiment, it was shown that incremental increases in daily light integral between 6 and 30 mol m^{-2} d^{-1} resulted in significant increases in fresh weight and dry weight in each of the four species of baby leaf vegetables studied. Additionally, for each of the four species there continued to be an increase in yield up to the 30 mol m^{-2} d^{-1} treatment, however, for lettuce and collards a relatively small yield increase was noted between the 24 and 30 mol m^{-2} d^{-1} treatments. The current experiment has also shown that incremental increases in daily light integral have some significant impacts on leaf height, leaf width, leaf area, and leaf number, but that responses vary by species. Also, positive impacts to these four morphological characteristics rarely continued past the 24 mol m^{-2} d^{-1} treatment. Future work should be conducted to further validate these results through multiple replications. Increasing the range of daily light integrals in future studies could aid growers in determining optimal DLI levels for different species of baby leaf vegetables.

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