Culture of the Aquatic Plant *Egeria densa* in a Closed System

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Final Report for Contract No. 021065
Submitted to Division of Aquaculture, Florida Department of Agriculture and Consumer Services

December 31, 2015

Technical Report #167
Harbor Branch Oceanographic Institute
at Florida Atlantic University

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FLORIDA ATLANTIC UNIVERSITY
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Executive Summary

Egeria densa, also known as (Brazilian) Elodea, Anacharis, and "oxygenating plant", is important for Florida's aquarium trade, sold for use in aquaria and water gardens, but there is insufficient supply to meet demand. Egeria is mostly collected from the wild where it can be confused with Hydrilla. Growers in Florida have had some success cultivating Egeria; the growing season is November to April, with slow summer growth. Production in the winter does not keep up with demand that is highest at that time of the year.

The goal of this project was to determine the optimal light and temperature conditions for growth of Egeria under closed-system aquaculture and to transfer that information to Florida's aquaculture industry. The objectives of this project were:

1. Determine growth rates year-round at controlled temperatures under natural light levels which will vary during the year (e.g., winter vs. summer)
2. Determine optimal light and temperature conditions for growth of Egeria under closed-system aquaculture
3. Explain and transfer the project results and their applications to Florida aquaculturists via the project final report and a hands-on demonstration workshop at Harbor Branch.

To determine the effect of light and temperature conditions on the growth of Egeria under Florida aquaculture conditions, a three (light) x four (temperature) factorial experiment was conducted under winter (December to February), spring (March to April), and summer (May to June) conditions. For each experiment, three light levels were provided with neutral-density shade cloth: full sunlight, 50% sunlight, and 25% light; four temperature regimes were maintained in heated- and chilled-water raceways: 15°C, 20°C, 25°C, and 30°C. Each treatment was replicated three times, totaling 36 aquaria for each experiment. Metrics to assess growth of Egeria in these experiments were increases in biomass, number of branches, and total stem length elongation.

In all three experiments, Egeria plants survived, and indeed, grew under all light and temperature treatments. Of the two environmental factors examined, temperature consistently had a stronger influence on growth of Egeria than light, with growth higher at warmer temperatures (25°C and 30°C) than cooler ones (15°C and 20°C). Quite surprisingly, this project clearly demonstrated that high temperature (30°C) did not reduce growth. Nor did high light levels (100% ambient light). The influence of light was seasonally dependent, with best growth at 100% ambient light in the winter, but equivalent at all three light levels in the summer.

The results of this project indicate that Egeria in a closed system is able to grow well at higher temperatures than previously thought; indeed, growth was higher at warmer temperatures (25 °C and at 30 °C) than at cooler ones (15 °C and at 20 °C). Year-round production in a closed system should be achievable at a commercial scale in Florida. Further improvements in the system are likely with other modifications, such as optimization of nutrients and pH/carbon dioxide. Future research to identify the optimal conditions for cultivating Egeria will be conducted in the HBOI Egeria experimental closed system, with further collaboration and outreach with growers throughout the state.

Introduction

After tropical fish, aquatic plants are the most valuable product of the Florida aquaculture industry (FDACS 2012). *Egeria densa* is one of the most popular aquarium plants in the trade, sold under various names, including (Brazilian) Elodea, Anacharis, and "oxygenating plant". This popularity is due to the lush appearance of its dark green leaves and stems and tolerance of a wide range of conditions in aquaria. Egeria has become the top-selling aquatic plant for use in aquaria as "oxygenators" and increasingly popular in water gardening.

Egeria densa has been the number one selling aquarium plant in the U.S. by far, with a peak market (water gardening and aquarium plants) of as many as 100,000 plus bunches sold per week in the U.S. (McLane and Sutton 2008). The wholesale price of a bunch of Egeria ranges from $0.50 to $0.85 (Pat Faehnle, Aquatic Systems and Resources, pers. comm.). Retail prices are highly variable, depending on quantity and shipping costs. For aquarium use, currently a bunch of Egeria typically retails for about $3.00 (Brandon McLane, Florida Aquatic Nurseries, pers. comm.) to $5.00 (Petco 2015).

Egeria densa is a submersed member of the Hydrocharitaceae family native to South America (Yarrow et al. 2009). In the United States, this species occurs from New York south to Florida and west to California and Oregon (Flora of North America Editorial Committee 2000). This species was introduced into Florida by the aquarium trade (Blackburn et al. 1969) and grows rooted or free-floating in streams, ponds, and lakes throughout the southern U.S. (Kay and Hoyle 1999). Egeria is typically wild collected from those areas and in the field is often confused with Hydrilla, which it closely resembles, and with which it often occurs in mixed stands (Kay and Hoyle 1999).

In Florida, "*Egeria densa* is a bread and butter plant for both the aquarium and water garden market" (Pierre LePochat, WaterScapes Aquatic Plant Nursery, pers. comm.). Despite Egeria's importance in Florida's aquarium trade, supply is insufficient to meet demand. A lack of sufficient supply, especially in the months of March-May when demand peaks each year, and quality issues is limiting the use of Egeria in both Florida's aquarium (Brandon McLane, Florida Aquatic Nurseries, pers. comm.) and water gardening businesses (Pat Faehnle, Aquatic Systems Resources, pers. comm.). Traditional wild supplies have declined, perhaps due to over-harvesting and/or competition with Hydrilla.

Commercial cultivation of Egeria is ongoing in Florida. Plants in cultivation are likely all a male clone, reproducing vegetatively (Flora of North America Editorial Committee 2000) and easily propagated by cuttings. One aquatic plant grower, Florida Aquatic Nurseries, produced about 120,000 units in 2012 (estimated retail value of $360,000) with about 15% of that cultivated in tanks, the rest from wild collection. Efforts at culturing Egeria in Florida have had some success: the growing season is November to April, with slow growth in the summer, and production in the winter does not keep up with demand (Brandon McLane, Florida Aquatic Nurseries, pers. comm.). The most likely reason for this reported seasonality is that growth is reduced as a function of increasing temperature and/or light in the summer.

The need to develop closed-system culture techniques for *Egeria densa* was recognized by the following Research and Development Priorities described in the 2014-2015 Request for

Statements of Interest and the 2013-2014 Florida Aquaculture Plan (http://www.floridaaquaculture.com/publications/aquaplan.pdf), under “Technologies”:

- Develop closed system culture techniques for the aquatic plant *Egeria densa*
- Improve aquatic plant production techniques for new and existing species that will improve growth rate and production system design and operation

The development of these techniques will provide a more consistent year-round supply, improve quality, and reduce costs of Egeria production in Florida.

Previous research by Barko and Smart (1981) demonstrated that Egeria grows at similar rates over the temperature range of 16 to 28°C, but declines at higher temperatures. In that work, as would be the case in natural populations, light intensity co-varied during the year with temperature. Recent research (Riis et al. 2012) demonstrated the response to light on growth may be more important to growth than that of temperature. The current project built on this previous research and focused on determining growth rates year-round at controlled temperatures under natural light levels that vary during the year. These conditions are immediately relatable to outdoor tank cultures employed by the industry (Brandon McLane, Florida Aquatic Nurseries, pers. comm.).

Objectives

The goal of this project was to determine the optimal light and temperature conditions for growth of Egeria under closed-system aquaculture and to transfer that information to Florida's aquaculture industry. The objectives of this project were:

1. Determine growth rates year-round at controlled temperatures under natural light levels which will vary during the year (e.g., winter vs. summer)
2. Determine optimal light and temperature conditions for growth of Egeria under closed-system aquaculture
3. Explain and transfer the project results and their applications to Florida aquaculturists via the project final report and a hands-on demonstration workshop at Harbor Branch.

Methods and Procedures

To determine the effect of light and temperature conditions on the growth of Egeria under Florida aquaculture conditions, a three (light) x four (temperature) factorial experiment was conducted under winter (December to February), spring (March to April), and summer (May to June) conditions. For each experiment, three light levels were provided with neutral-density shade cloth: full sunlight, 50% sunlight, and 25% light; four temperature regimes (maintained in heated- /chilled-water raceways): 15°C, 20°C, 25°C, and 30°C. Each treatment was replicated three times, totaling 36 aquaria for each experiment.

The schematic in Figure 1 details the experimental system set up in Harbor Branch's Aquaculture facility. Egeria plants were grown in tanks (16" x 18" x 16") made of polyethylene, with a volume of 20 gal. The tanks were placed in four fiberglass troughs (96" x 36" x 12"; Fig. 2), which functioned as water temperature baths, each of which was maintained at one of the four

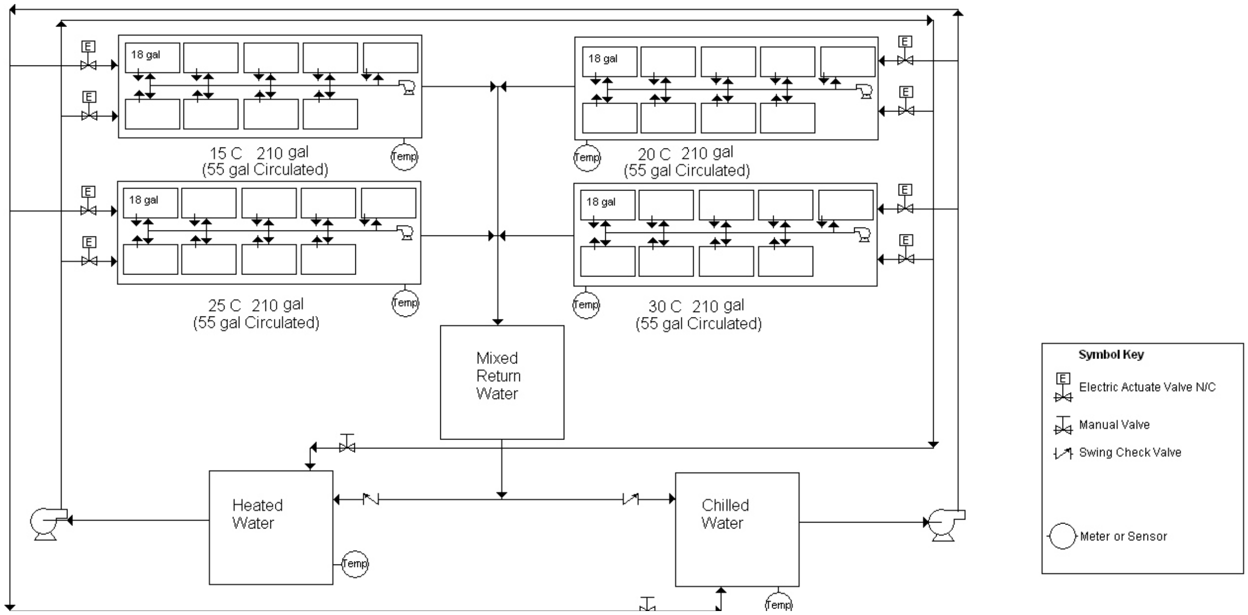

Figure 1. Process diagram depicting experimental apparatus for determination of optimum temperature and light requirements for production of *Egeria densa*.

experimental temperatures (15°C, 20°C, 25°C, and 30°C). Temperature in each trough was maintained within ± 1°C by the addition of cold and hot system water from two separate loops controlled by solenoid valves and a temperature controller. From these loops, each tank received continuously aerated well water at a flow rate of 2-3 gal per min. That movement of water was to keep the temperature uniform in each of the experimental tanks within each trough. Water flowed out of the tanks into the troughs and then was mixed in the return water to the two reservoirs to complete the closed loop for temperature control. The troughs were set up underneath two raceway covers (10’ x 20’ x 8’), made of translucent polyethylene covers to help regulate the temperatures in the experimental tanks (Fig. 3). The initial freshwater and any make-up water needed during the experiments were provided by the facility’s shallow freshwater well. Carbon dioxide gas was trickled into the system as needed to avoid high pHs (>9) that can occur during the day due to photosynthesis. Temperature, pH, and dissolved oxygen were monitored daily in each tank.

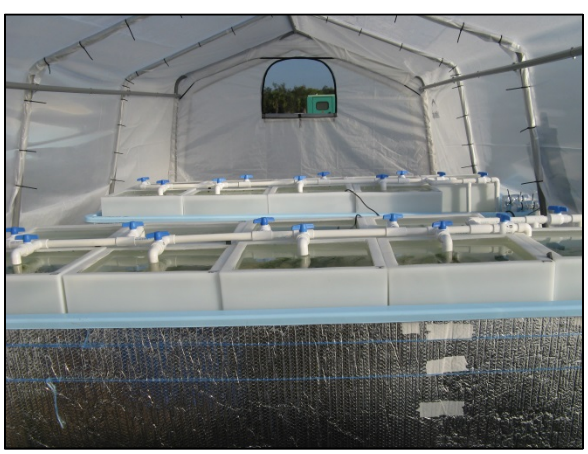

Figure 2. Experimental tanks were set up in four fiberglass troughs, one for each one of four temperatures.

Figure 3. The troughs were set up underneath two translucent raceway covers to help regulate temperatures.

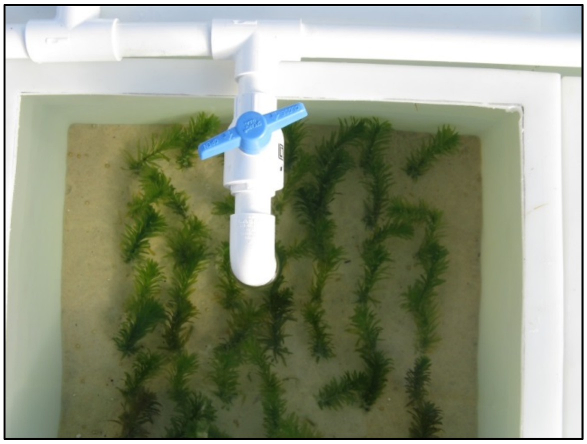

Figure 4. Each experimental tank was planted with 49 Egeria plants, each cut to 8 cm tall.

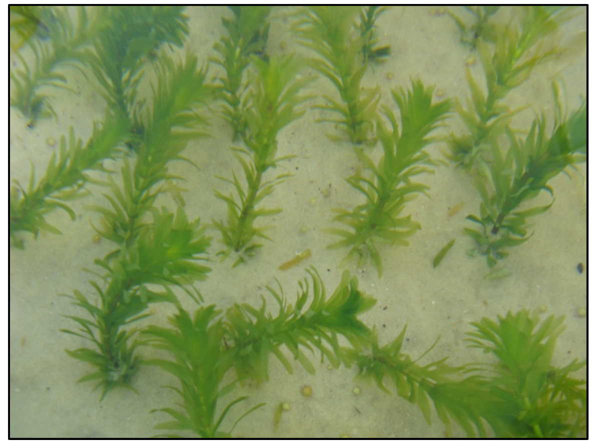

Figure 5. The sand substrate in the tanks was fertilized with Osmocote® Plus 15-9-12 (2 g per kg of sand).

For each of the three experiments, a new batch of plants (all provided by Florida Aquatic Nurseries), sand substrate, and fertilizer were used. Each tank was planted with 49 plants, each cut to 8 cm tall, a day or two after delivery of the plants, with about one third of the length in the sand sediment (ca. 13.5 cm = 6 inches deep) to which controlled slow-release fertilizer (2 g Osmocote® Plus, 15-9-12/kg of sand) had been added (Figs. 4, 5). During a brief acclimation period (prior to applying light and temperature treatments), the temperature in all four troughs (i.e., all tanks) was set at 22.5 C, which is the mid-point of the experimental treatments, and near the presumed optimal range (based on literature above) for the growth of Egeria.

The three seasonal three x four (light x temperature) factorial experiments were conducted on the following dates:

- Experiment 1 (winter conditions): On December 17, 2014, Egeria plants were received from Florida Aquatic Nurseries. Egeria was planted on December 19, with the intent of providing a two-week acclimation period prior to the initiation of the light and temperature treatments. However, acclimation was extended to three weeks due to a problem with one of our temperature controllers. During this pre-treatment period, the plants acclimated well, with no significant mortality or other issues. The plants immediately began to grow, which led us to conclude that we could minimize the acclimation period in future experiments. The first experiment began on January 10, 2015, and ran for a month with all tanks harvested on February 11, 2015.
- Experiment 2 (spring conditions): Originally, we had planned only two experiments (winter, summer) but given how well the first experiment went, and the confirmation that we can obtain significant results in the ca. 1 month, we decided to do an additional experiment that would enhance the output of our project by adding additional temporal (seasonal) resolution to our work. This batch of plants was received on March 19, 2015, and planted on March 20, 2015. Per above, acclimation was minimized (1 day), prior to applying the light and temperature treatments. The experiment ran for one month, with harvest on April 21, 2015.

- Experiment 3 (summer conditions): Plants were received from Florida Aquatic Nurseries on May 7, 2015, planted in the experimental tanks on May 8, 2015, and acclimated for several days. Light and temperature treatments were initiated on May 12, 2015, and terminated on June 19, 2015.

Metrics to assess growth of Egeria in these experiments were increases in biomass, number of branches, and total stem elongation. In each experiment, measurements were made on a subset of 10 plants randomly selected from each tank. For each of those 10 plants, the maximal height, number of branches, and length of all stems were measured. Those plants were then frozen in individual, labelled bags, as was the remaining biomass of the plants from each tank. Subsequently, all plants (both the 10 individual plants and the pooled sample from each tank) were dried at 80 °C and weighed.

Data for each of the three metrics are reported as mean and standard deviation (SD), with $n = 3$ for each temperature and light combinations in each experiment. Two-way ANOVA (SAS, GLM Procedure) was used to analyze the effects of temperature, light, and their interaction on growth. When statistically significant differences ($P < 0.05$) were found with ANOVA, Tukey's Studentized Range Test (HSD) was used to determine significant differences ($\alpha = 0.05$) among treatment levels.

Results

Experiment 1

Biomass

Under winter conditions, both temperature and light highly significantly (ANOVA, P <0.0001) affected the mean biomass growth of Egeria (Fig. 6). The interaction between temperature and light was not significant (ANOVA, P = 0.087). Among temperature levels, the mean biomass at 25 °C and 30 °C was significantly higher than at 20 °C which was significantly higher than at 15 °C (Tukey, P < 0.05). Among light levels, the mean biomass at 100% ambient light was significantly higher than at 50% ambient light which was significantly higher than at 25% ambient light (Tukey, P < 0.05). The lowest mean biomass (14.74± 2.54 g dry weight) was at 15 °C and 25% ambient light and the highest mean biomass (48.77 ± 5.27 g dry weight) was at 25 °C and 100% ambient light.

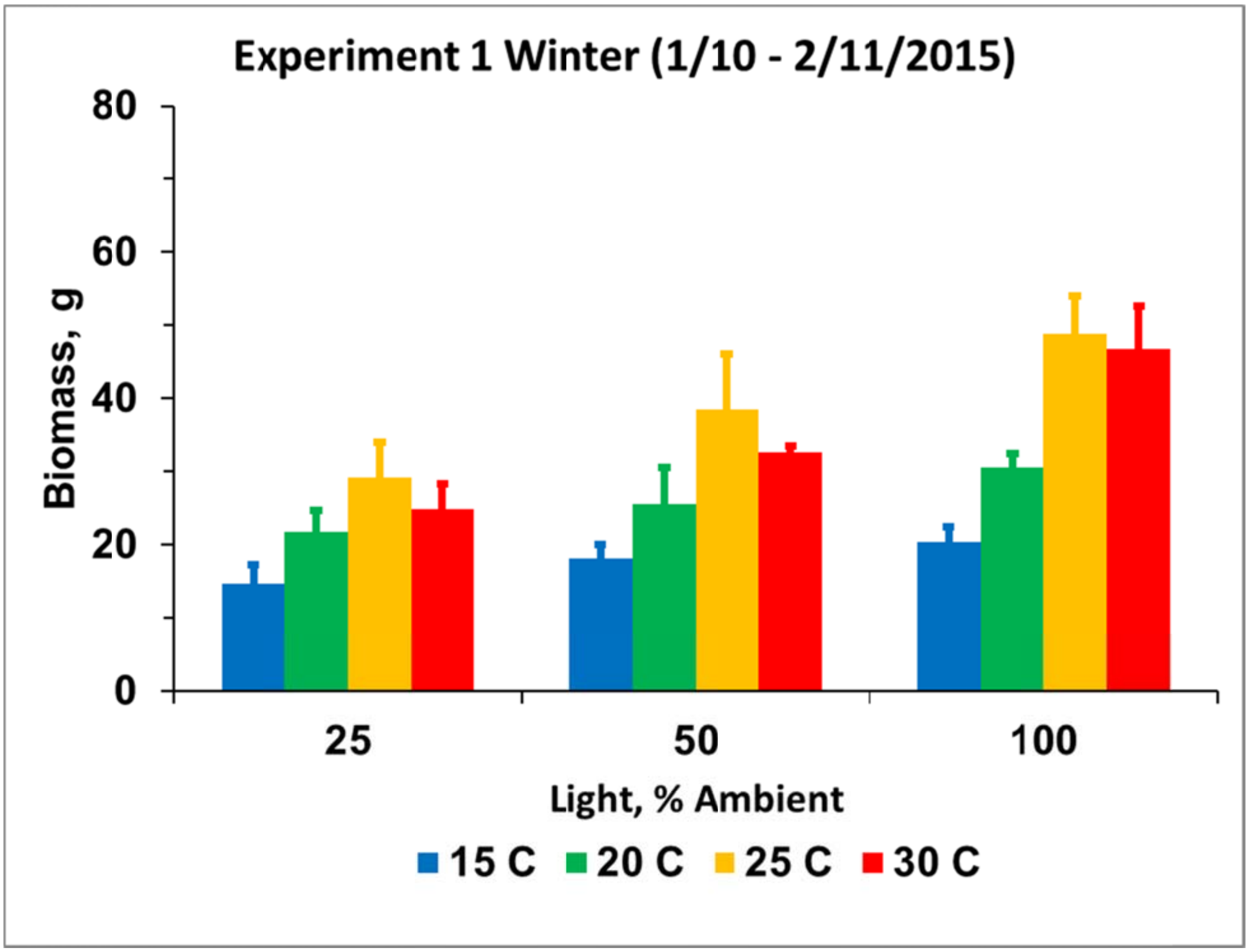

Fig. 6 Experiment 1: Mean (± SD) biomass growth (g dry weight) of *Egeria* as a function of temperature (15°C, 20°C, 25°C, and 30°C) and light (25%, 50%, and 100% ambient sunlight), n = 3 for each light and temperature combination, under winter conditions (January 10 – February 11, 2015).

Number of Branches

Both temperature and light also highly significantly (ANOVA, $P < 0.0001$) affected the mean number of branches of Egeria (Fig. 7). The interaction between temperature and light was also highly significant (ANOVA, $P < 0.0001$). Among temperature levels, the mean number of branches at 25 °C and 30 °C was significantly higher than at 20 °C which was significantly higher than at 15 °C (Tukey, $P < 0.05$). Among light levels, the mean number of branches at 100% ambient light was significantly higher than at 50% ambient light which was significantly higher than at 25% ambient light (Tukey, $P < 0.05$). The lowest mean number of branches (2.97 $\pm$ 0.15) was at 15 °C and 25% ambient light and the highest mean number of branches (9.60 $\pm$ 1.21) was at 30 °C and 100% ambient light.

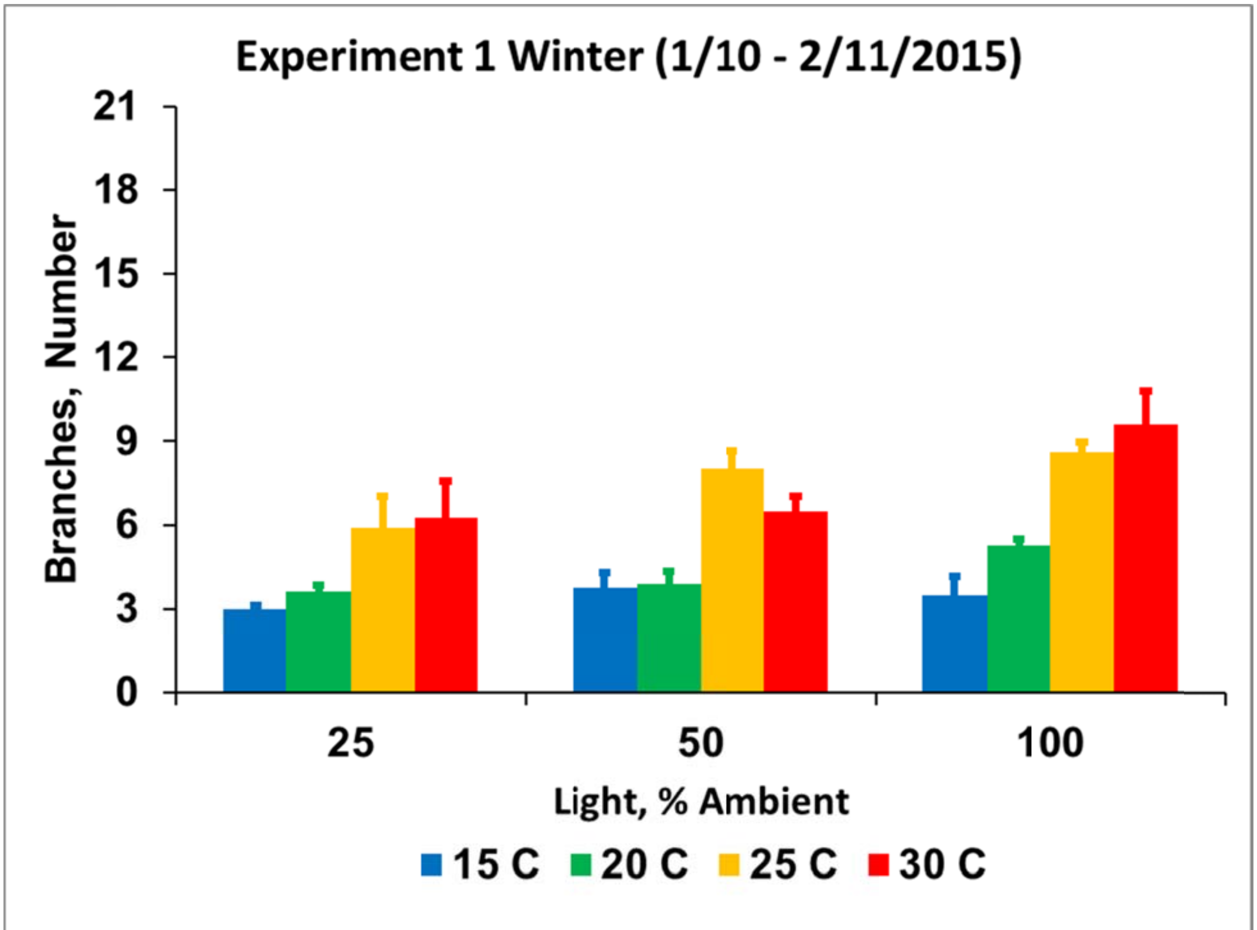

Fig. 7 Experiment 1: Mean ($\pm$ SD) number of branches of *Egeria* as a function of temperature (15°C, 20°C, 25°C, and 30°C) and light (25%, 50%, and 100% ambient sunlight), n = 3 for each light and temperature combination, under winter conditions (January 10 – February 11, 2015).

Total Stem Length

Both temperature and light highly significantly (ANOVA, $P < 0.0001$) also affected the mean total stem length of Egeria (Fig. 8). The interaction between temperature and light was also highly significant (ANOVA, $P = 0.0008$). Among temperature levels, the mean total stem length at 25 °C was significantly higher than at 30 °C was significantly higher than at 20 °C which was significantly higher than at 15 °C (Tukey, $P < 0.05$). Among light levels, the mean total stem length at 100% ambient light was significantly higher than at 50% ambient light which was significantly higher than at 25% ambient light (Tukey, $P < 0.05$). The lowest mean total stem length (59.08 $\pm$ 5.18) was at 15 °C and 25% ambient light and the highest mean total stem length (240.55 $\pm$ 28.38) was at 25 °C and 100% ambient light.

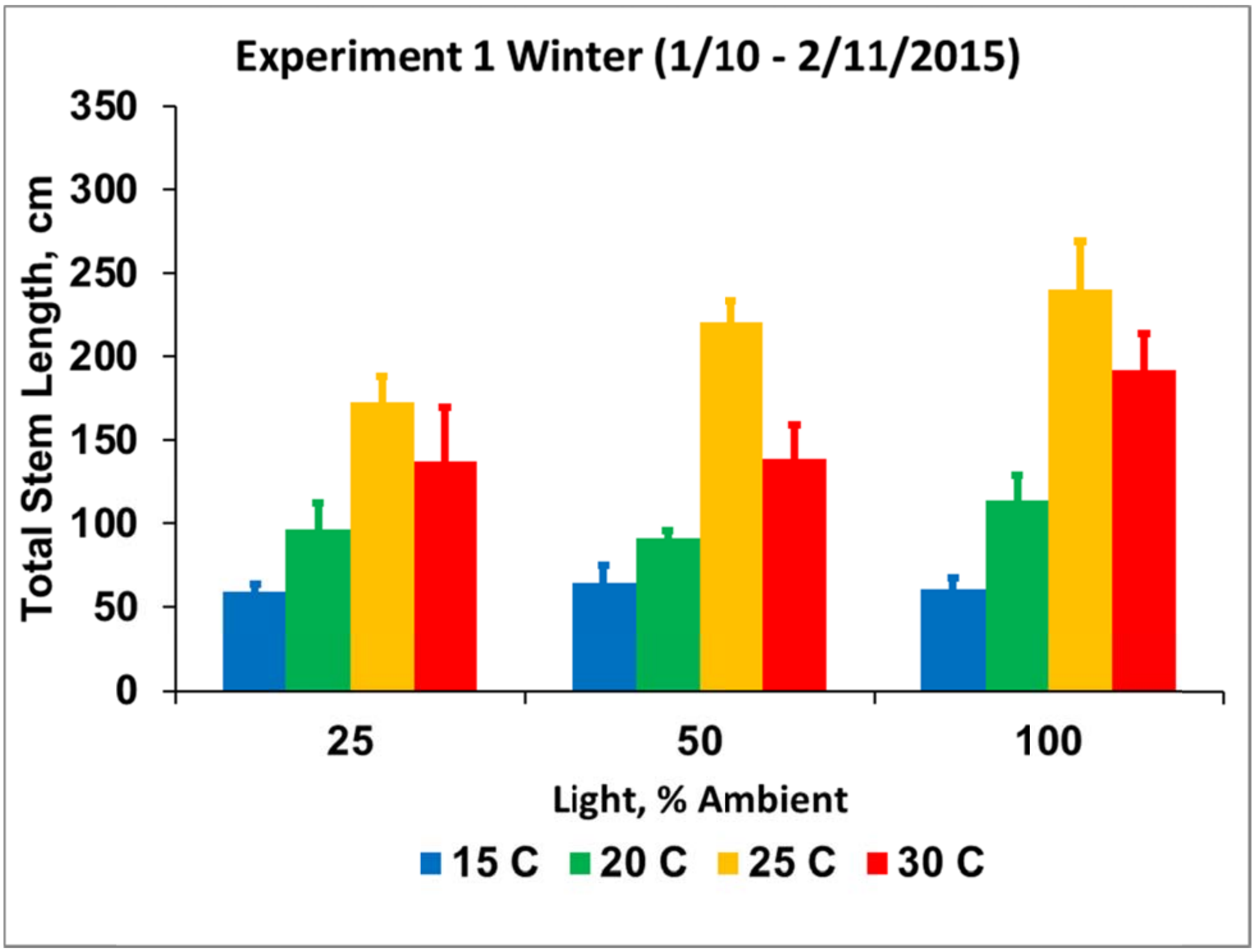

Fig. 8 Experiment 1: Mean ($\pm$ SD) total stem length (cm) of *Egeria* as a function of temperature (15°C, 20°C, 25°C, and 30°C) and light (25%, 50%, and 100% ambient sunlight), n = 3 for each light and temperature combination, under winter conditions (January 10 – February 11, 2015).

Experiment 2

Biomass

Under spring conditions, both temperature and light highly significantly (ANOVA, $P < 0.0001$) affected the mean biomass growth of Egeria (Fig. 9). The interaction between temperature and light was also highly significant (ANOVA, $P < 0.0001$). Among temperature levels, the mean biomass at 25 °C was significantly higher than at 30 °C which was significantly higher than at 20 °C which was significantly higher than at 15 °C (Tukey, $P < 0.05$). Among light levels, the mean biomass at 100% ambient light was significantly higher than at 50% ambient light and 25% ambient light (Tukey, $P < 0.05$). The lowest mean biomass (10.51$\pm$ 1.17 g dry weight) was at 15 °C and 50% ambient light and the highest mean biomass (51.33 $\pm$ 2.06 g dry weight) was at 25 °C and 100% ambient light.

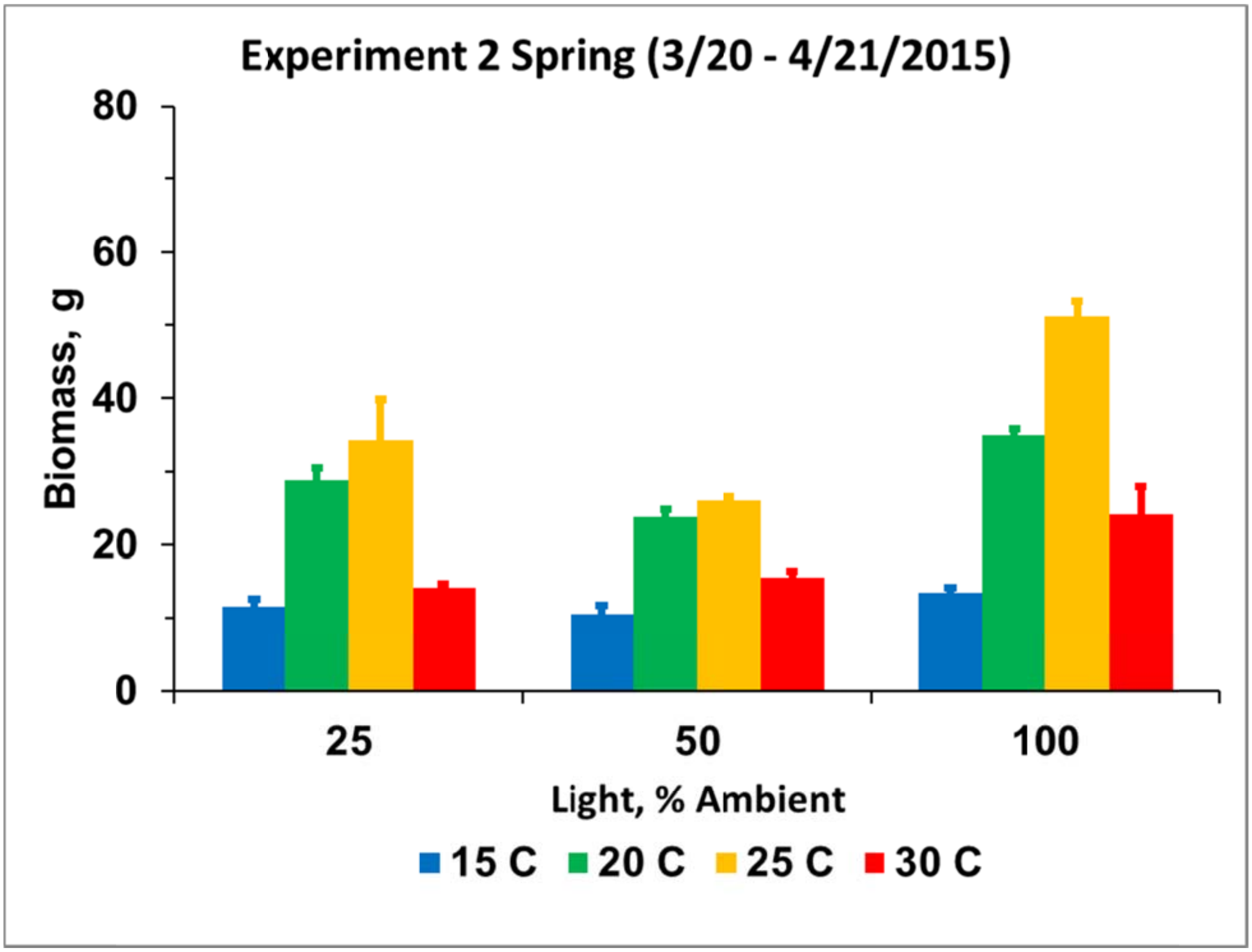

Fig. 9 Experiment 2: Mean ($\pm$ SD) biomass growth (g dry weight) of *Egeria* as a function of temperature (15°C, 20°C, 25°C, and 30°C) and light (25%, 50%, and 100% ambient sunlight), n = 3 for each light and temperature combination, under spring conditions (March 20 – April 21, 2015).

Number of Branches

Both temperature and light also highly significantly (ANOVA, P <0.0001) affected the mean number of branches of Egeria (Fig. 10). The interaction between temperature and light was also highly significant (ANOVA, P <0.0001). Among temperature levels, the mean number of branches at 25 °C and 30 °C was significantly higher than at 20 °C which was significantly higher than at 15 °C (Tukey, P < 0.05). Among light levels, the mean number of branches at 100% ambient light was significantly higher than at 50% and 25% ambient light (Tukey, P < 0.05). The lowest mean number of branches (2.60 ± 0.20) was at 15 °C and 50% ambient light and the highest mean number of branches (8.33± 0.75) was at 25 °C and 100% ambient light.

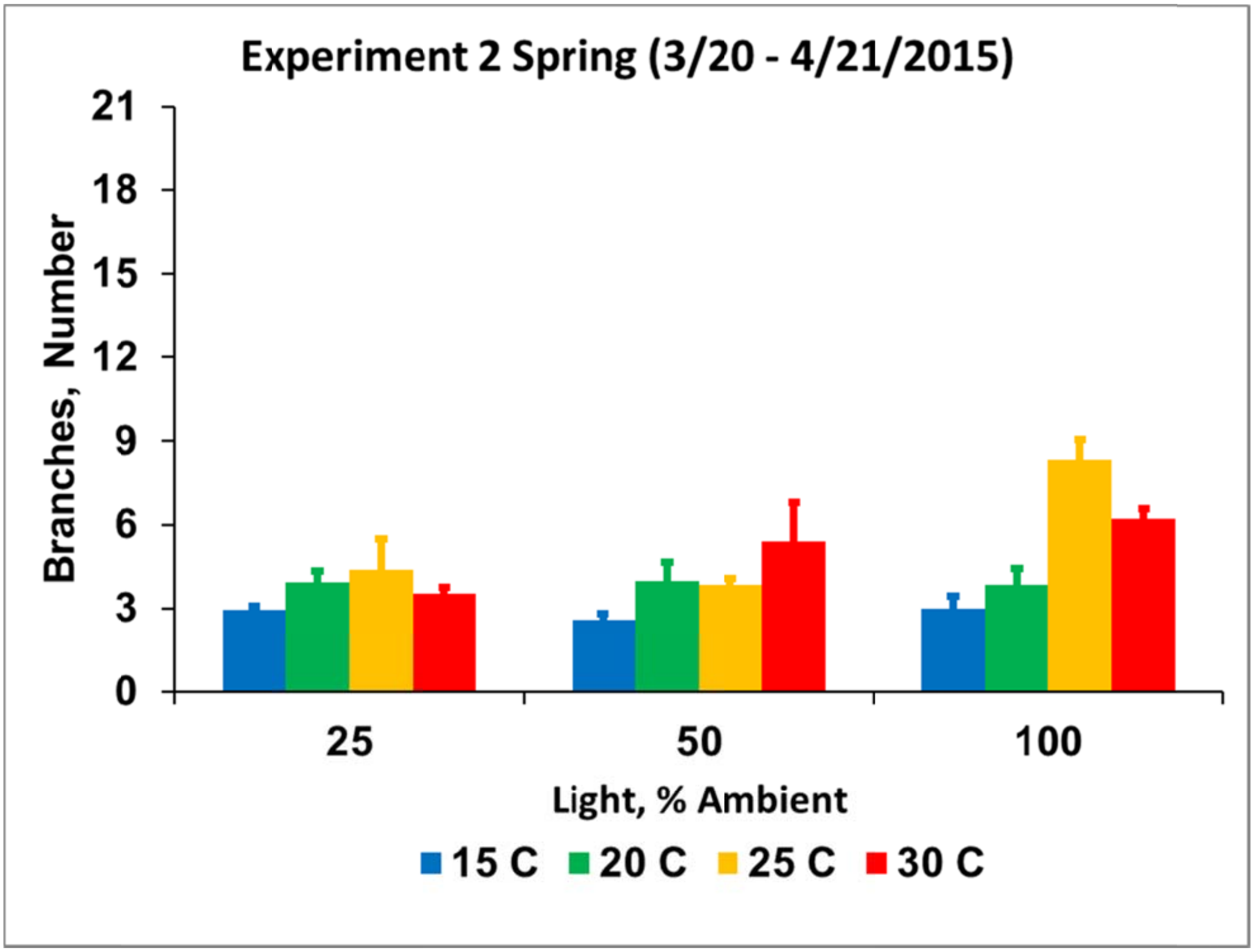

Fig. 10 Experiment 2: Mean (± SD) number of branches of *Egeria* as a function of temperature (15°C, 20°C, 25°C, and 30°C) and light (25%, 50%, and 100% ambient sunlight), n = 3 for each light and temperature combination, under spring conditions (March 20 – April 21, 2015).

Total Stem Length

Temperature highly significantly (ANOVA, $P < 0.0001$) affected the mean total stem length of Egeria, while light did so much less significantly (ANOVA, $P = 0.042$; Fig. 11). The interaction between temperature and light was also significant (ANOVA, $P = 0.005$). Among temperature levels, the mean total stem length at 25 °C was significantly higher than at 30 °C which was significantly higher than at 20 °C which was significantly higher than at 15 °C (Tukey, $P < 0.05$). Among light levels, the mean total stem length at 100% ambient light was significantly higher than at 25% ambient light; the intermediate mean total stem length at 50% ambient light was not significantly different higher than that at 25% and 100% ambient light (Tukey, $P > 0.05$). The lowest mean total stem length (33.39 $\pm$ 4.11) was at 15 °C and 100% ambient light and the highest mean total stem length (180.67 $\pm$ 17.02) was at 25 °C and 100% ambient light.

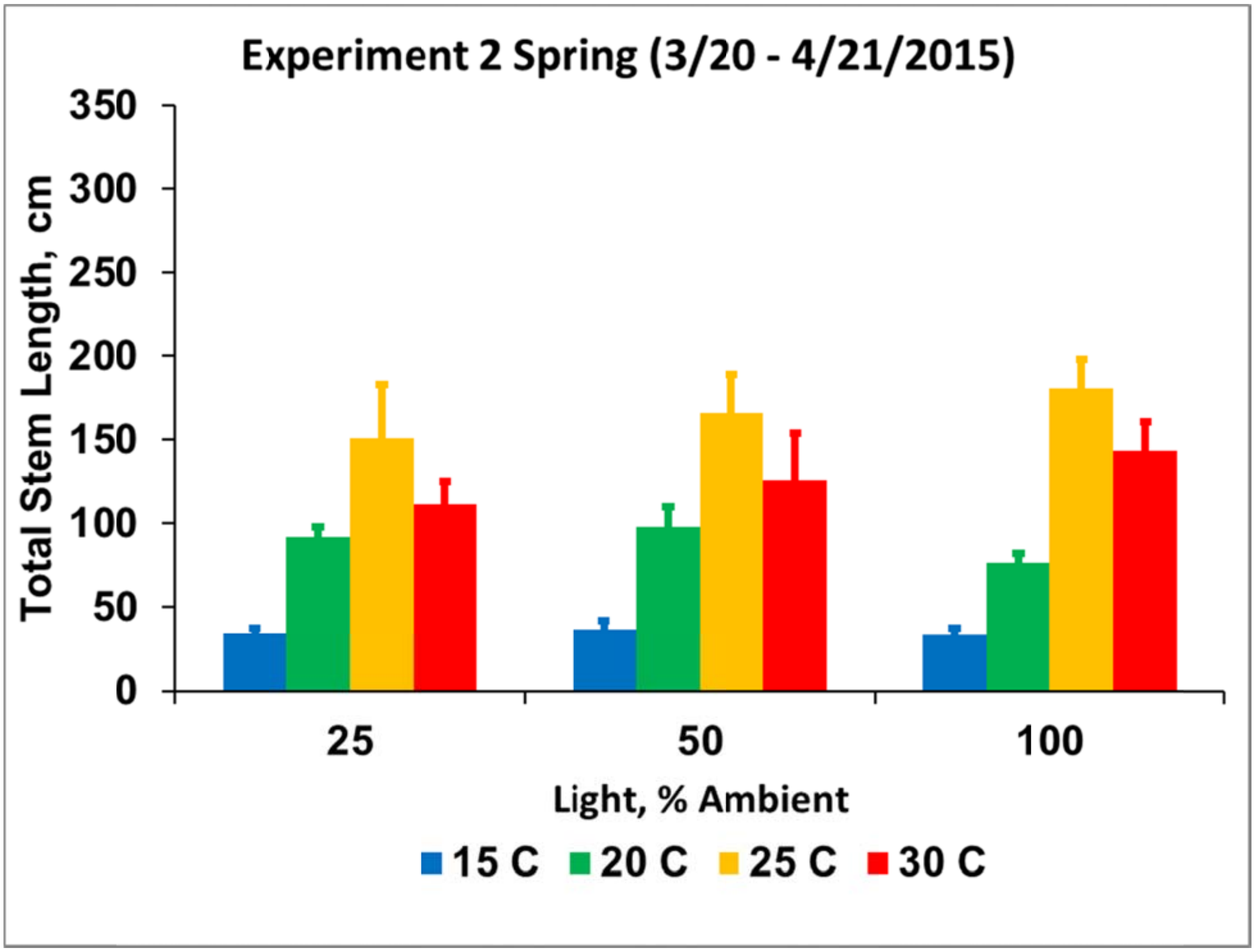

Fig. 11 Experiment 2: Mean ($\pm$ SD) total stem length (cm) of *Egeria* as a function of temperature (15°C, 20°C, 25°C, and 30°C) and light (25%, 50%, and 100% ambient sunlight), n = 3 for each light and temperature combination, under spring conditions (March 20 – April 21, 2015).

Experiment 3

Biomass

Under summer conditions, temperature highly significantly (ANOVA, P <0.0001) affected the mean biomass growth of Egeria (Fig. 12). But both light and the interaction between temperature and light were not significant (ANOVA, P = 0.413 and 0.982, respectively). Among temperature levels, the mean biomass at 30 °C was significantly higher than at 25 °C which was significantly higher than at 15 °C and 20 °C (Tukey, P < 0.05). The lowest mean biomass (12.52 ± 1.20 g dry weight) was at 15 °C and 50% ambient light and the highest mean biomass (65.51+ 11.60 g dry weight) was at 30 °C and 25% ambient light.

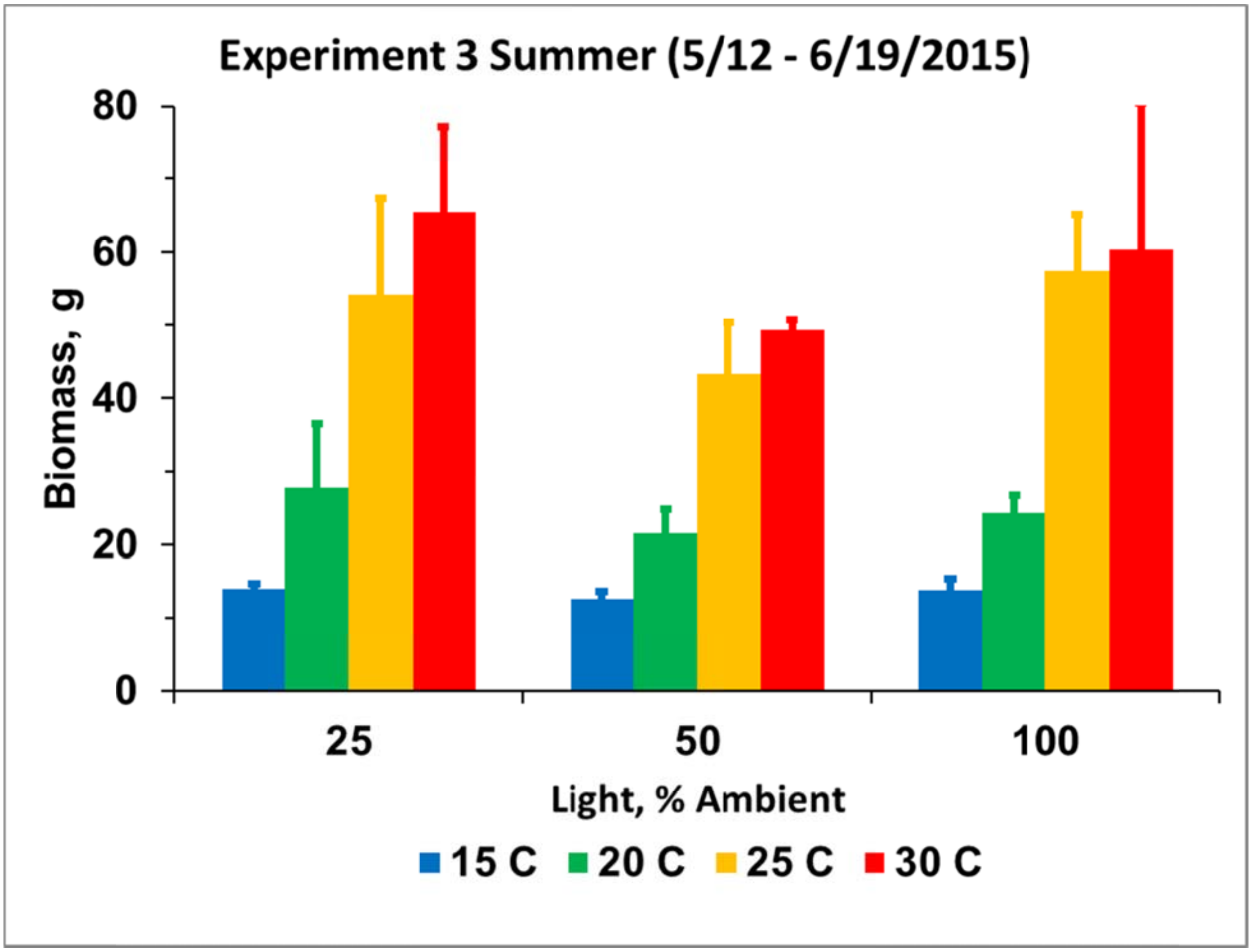

Fig. 12 Experiment 3: Mean (± SD) biomass growth (g dry weight) of *Egeria* as a function of temperature (15°C, 20°C, 25°C, and 30°C) and light (25%, 50%, and 100% ambient sunlight), n = 3 for each light and temperature combination, under summer conditions (May 12 – June 19, 2015).

Number of Branches

Temperature also highly significantly (ANOVA, P <0.0001) affected the mean number of branches of Egeria (Fig. 13). Both light and the interaction between temperature and light were not significant (ANOVA, P = 0.537 and 0.157, respectively). Among temperature levels, the mean number of branches at 30 °C was significantly higher than at 25 °C which was significantly higher than at 20 °C which was significantly higher than at 15 °C (Tukey, P < 0.05). The lowest mean number of branches (2.73 + 0.21) was at 15 °C and 25% ambient light and the highest mean number of branches (15.97 + 4.16) was at 30 °C and 50% ambient light.

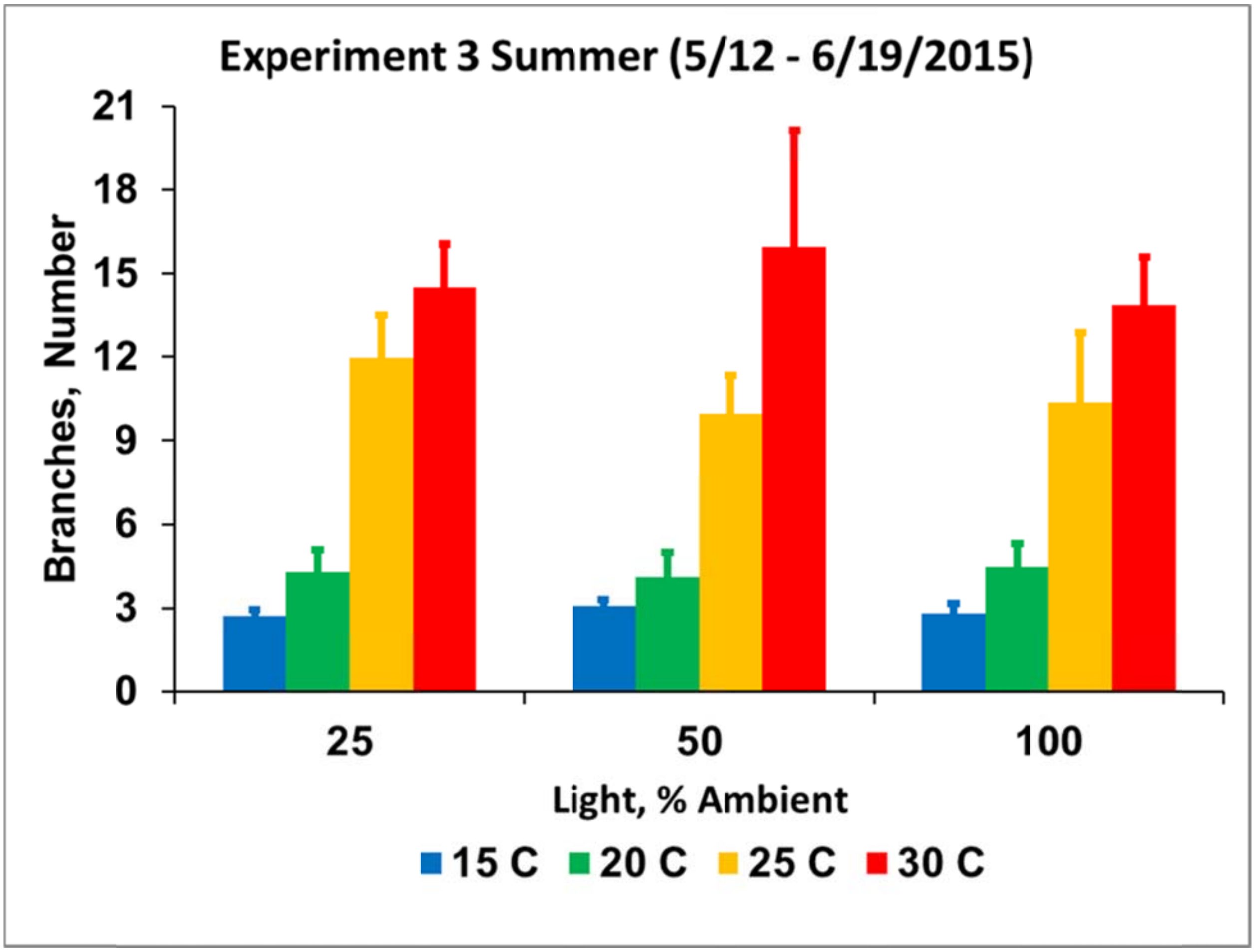

Fig. 13 Experiment 3: Mean (+ SD) number of branches of *Egeria* as a function of temperature (15°C, 20°C, 25°C, and 30°C) and light (25%, 50%, and 100% ambient sunlight), n = 3 for each light and temperature combination, under summer conditions (May 12 – June 19, 2015).

Total Stem Length

Temperature also highly significantly (ANOVA, P <0.0001) affected the mean total stem length of Egeria (Fig. 14). Light did not significantly (ANOVA, P = 0.154) affect the mean total stem length. The interaction between temperature and light was significant (ANOVA, P = 0.032). Among temperature levels, the difference in mean total stem length at 30 °C was significantly higher than at 25 °C which was significantly higher than at 20 °C and 15 °C (Tukey, P < 0.05). The lowest mean total stem length (29.03 ± 3.70) was at 15 °C and 100% ambient light and the highest mean total branch length (333.47 ± 62.68) was at 30 °C and 25% ambient light.

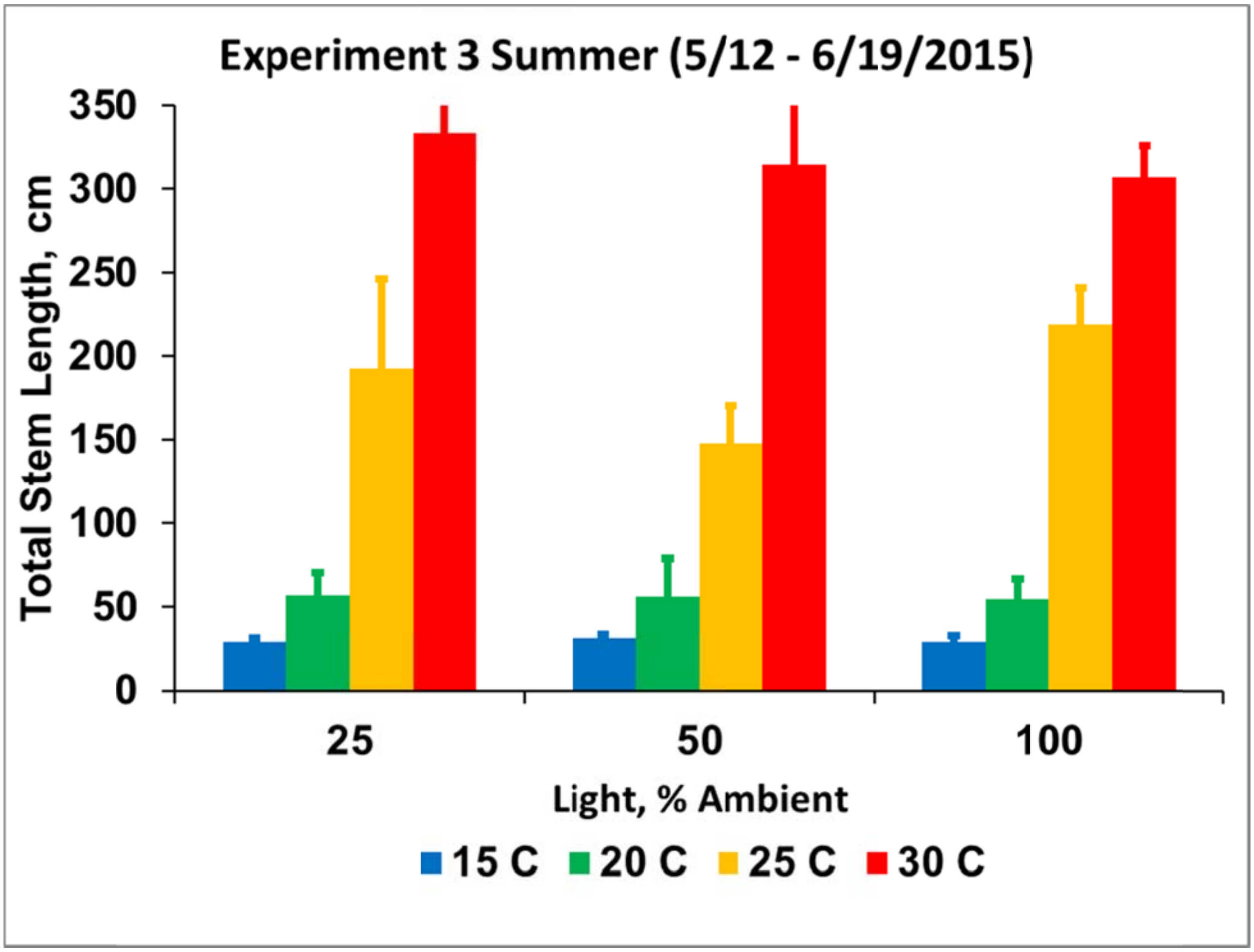

Fig. 14 Experiment 3: Mean (± SD) total stem length (cm) of *Egeria* as a function of temperature (15°C, 20°C, 25°C, and 30°C) and light (25%, 50%, and 100% ambient sunlight), n = 3 for each light and temperature combination, under summer conditions (May 12 – June 19, 2015).

Demonstration Workshop

On October 21, 2015, we hosted a combined workshop on two projects funded by grants from the Aquaculture Review Council and Florida Department of Agriculture and Consumer Services. The workshop consisted of short presentations by the researchers involved with each project and a tour of their culture systems and other HBOI Aquaculture facilities. There were 25 participants from around the state, including members of the Florida aquaculture industry and people interested in developing new aquaculture operations.

Susan Laramore reported on *Establishing Environmental Mineral Guidelines for Low Salinity Shrimp Culture* and discussed how mineral ratios affect shrimp production in low salinity culture operations and what minimum and maximum concentrations are for calcium, magnesium, and potassium in low salinity shrimp culture operations. Dennis Hanisak reported on the *Egeria Culture in Recirculating Systems* and discussed how farmers can increase production of the aquatic plant Egeria by manipulating light and temperature conditions and how to culture Egeria in a closed system for year-round production.

Tours were then conducted in both the shrimp and Egeria facilities, with an emphasis on system designs and other "tricks of the trade" and also the Integrated Multitrophic Aquaculture (IMTA) facilities which integrate plants and animals, an approach with broad applications in Florida. The Egeria tour was led by Paul Wills and Chris Robinson, who designed, operated, and maintained the Egeria facility for the project.

The announcement flyer, agenda, and participant list are provided in the Appendix 2. In addition to that outreach event and this final report, the PIs are happy to answer questions and provide guidance on how to transfer the information we have learned about the closed system cultivation to anyone interested in developing such systems to advance Florida aquaculture.

Discussion

Egeria is the most important aquatic plant in Florida aquaculture, yet its sales have plummeted in the last ten years due to a reduction in wild harvest and only partial success in its cultivation. As previously described, commercial efforts at culturing Egeria in Florida have found good growth from November to April, but slow growth in the summer. Thus, it is reasonable to hypothesize that the production of Egeria from May to October is most likely limited by warmer temperatures, or possibly higher light.

This project addressed that hypothesis by measuring growth in outdoor tanks as a function of four temperatures and three light levels that spanned the annual range likely to be experienced in Egeria cultivation systems in Florida, as evident by measurements at two aquatic plant nurseries in the state (Appendix 1). At the first nursery, Florida Aquatic Nursery in Davie, Florida, temperature conditions were very close to the ranges (15-30 °C) used in this project's experiments. The second nursery, WaterScapes Aquatic Plant Nursery, had more elevated temperatures, exceeding 30 °C in the first part of the summer. The differences between the two sites are likely due to differences in ambient air temperature, as well as in water flow and circulation in the two systems.

In the three experiments conducted during the year, which ranged from low-temperature and low-light winter conditions to high temperature and high-light summer conditions, Egeria plants survived, and indeed, grew under all light and temperature treatments. Quite surprisingly, this project clearly demonstrated that high temperature (30°C) did not reduce growth. Nor did high light levels (100% ambient light). Of the two environmental factors examined, temperature consistently was found to have a stronger influence on growth of Egeria than light. These results were true for all three performance metrics assessed: biomass, numbers of branches, and total stem length.

Growth based on dry biomass is generally the best metric to evaluate production of a plant. Biomass in Experiment 1 (winter conditions) and Experiment 3 (summer conditions), biomass growth significantly increased as temperature increased from 15 °C to 25 °C, with no significant decline in biomass production at 30 °C. In Experiment 2 (spring conditions), biomass growth significantly increased as temperature increased from 15 °C to 25 °C, but then significantly declined at 30 °C, being statistically equivalent to that at 15 °C. It is unclear as to why growth in biomass was reduced at 30 °C in the spring, contrary to the other two seasons. The importance of light to the productivity of Egeria is most evident in Experiment 1 (winter conditions), when the growth of Egeria significantly increased from 25 to 100% of ambient light, and when photoperiod (daily number of hours of light) was at the minimum. In Experiment 2 (spring conditions) and Experiment 3 (summer conditions), growth was similar at all light treatments, indicating growth of Egeria in this cultivation system was saturated at 25% ambient or lower. The highest growth was observed in Experiment 3, when there was the highest ambient light and longest photoperiod.

Growth based on number of branches produced consistently showed more branching at warmer temperatures (25 °C and 30 °C) than at cooler temperatures (15 °C and 20 °C). In Experiment 1 (winter conditions) and Experiment 2 (spring conditions), the mean number of branches at 25 °C and 30 °C was significantly higher than at 20 °C which was significantly higher than at 15 °C. Surprisingly, in Experiment 3 (summer), the mean number of branches was at 30 °C, followed in sequence by 25 °C, 20 °C, and at 15 °C. The amount of available light in both Experiment 1 (winter conditions) and Experiment 2 (spring conditions) was important with maximal branching observed at 100% ambient light. In Experiment 3, when there was the highest ambient light and longest photoperiod, there was the highest number of branches and no difference in the number of branches among the three light treatments, an indication that branching was saturated at 25% ambient light or lower.

Growth based on total stem length produced also consistently was higher at warmer temperatures (25 °C and 30 °C) than at cooler temperatures (15 °C and 20 °C). In Experiment 1 (winter conditions) and Experiment 2 (spring conditions), the mean total stem length at 25 °C was significantly higher at 30 °C which was significantly higher than at 20 °C which was significantly higher than at 15 °C. As with the number of branches, in Experiment 3 (summer), the mean total stem length was highest at 30 °C, followed in sequence by 25 °C, 20 °C, and at 15 °C. The amount of available light in both Experiment 1 (winter conditions) and Experiment 2 (spring conditions) was important with maximal branching observed at 100% ambient light. In Experiment 3, there was the highest total stem length and no difference among the three light treatments, again consistent with the pattern seen with the number of branches.

Figure 15. Egeria readily flowered in the experimental system, but only at 25°C, in winter and spring.

Another interesting observation, although not one of the three primary metrics, was flowering. Regardless of the light treatment, plants at 25 °C, and only at the temperature, flowered (Figure 15). Flowering occurred primarily during the winter, less so in spring, and not in the summer. This observation suggests that flowering in this species is controlled by temperature and photoperiod.

In summary, the results of this project indicate that Egeria in a closed system is able to grow well at higher temperatures than previously thought; indeed, growth was higher at warmer temperatures (25 °C and at 30 °C) than at cooler ones (15 °C and at 20 °C). Year-round production in a closed system should be achievable at a commercial scale in Florida. Further improvements in the system are likely with other modifications, such as optimization of nutrients and pH/carbon dioxide. Future research to identify the optimal conditions for cultivating Egeria will be conducted in the HBOI Egeria experimental closed system, with further collaboration and outreach with growers throughout the state.

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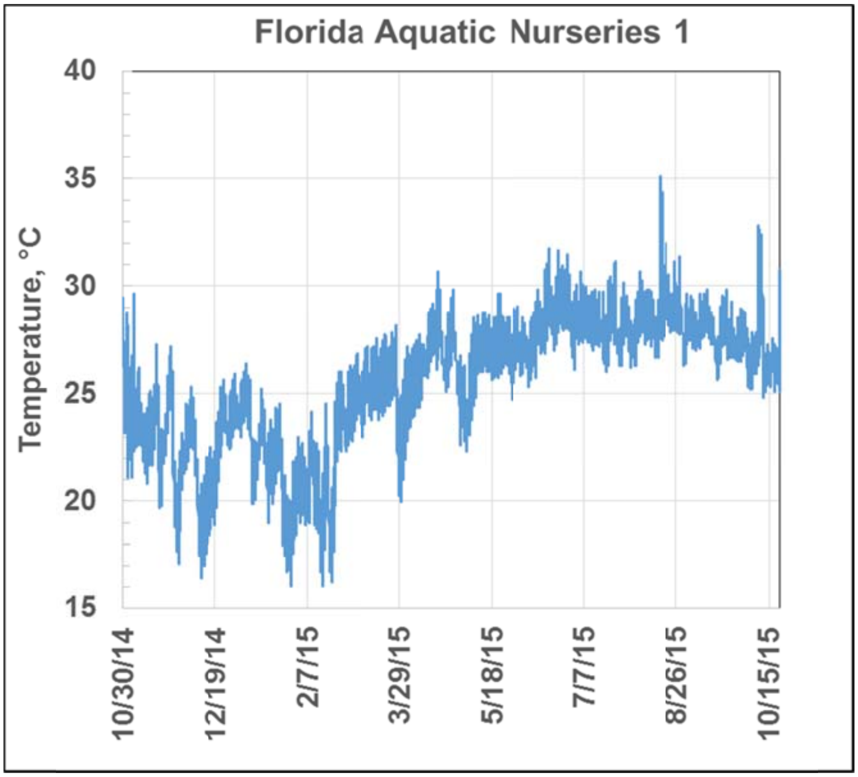
Acknowledgements

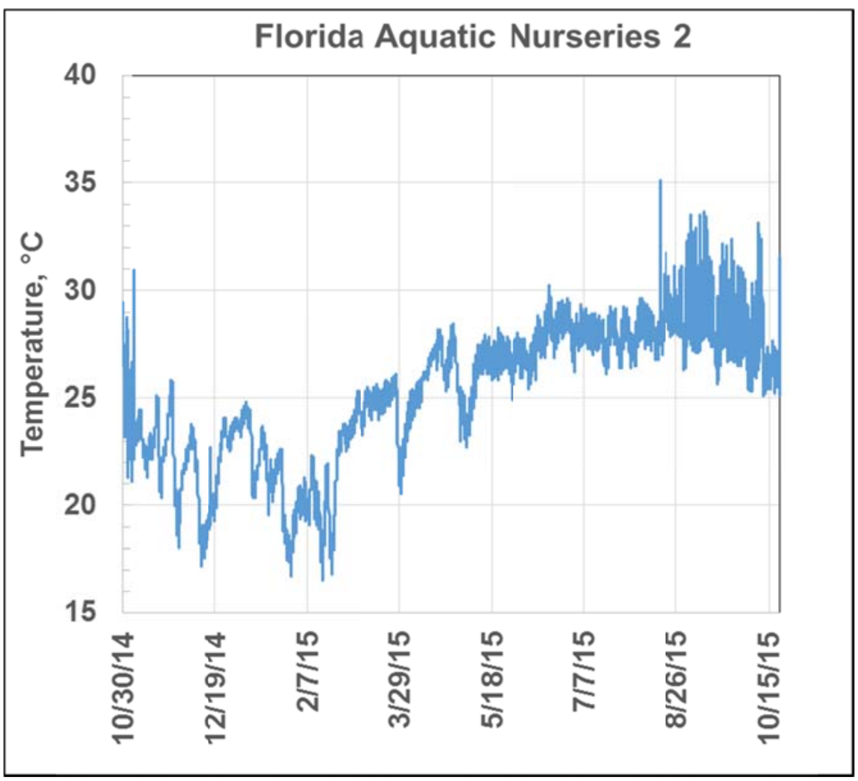
We thank Chris Robinson, Richard Mulroy, John Hart, and Patrick Monaghan for their excellent technical support; Brandon McLane (Florida Aquatic Nurseries) and Pierre LePochat (WaterScapes Aquatic Plant Nursery) for their technical guidance and industry perspective and allowing us to deploy temperature dataloggers in their farms so we could determine seasonal patterns and annual ranges; and Brandon McLane (Florida Aquatic Nurseries) for kindly donating Egeria plants for the experiments. Funding for this research was provided by the Aquaculture Review Council, Florida Department of Agriculture and Consumer Services, and the *Florida Aquaculture* Specialty License Plate, administered by the Harbor Branch Oceanographic Institution Foundation.

Appendix 1

Annual Water Temperature Data at Two Commercial Aquatic Plant Farms in Florida: Florida Aquatic Nursery, in Davie, Florida, and WaterScapes Aquatic Plant Nursery, in Seffner, Florida

At both sites, temperature was recorded at hourly intervals for a year, at two depths, with Hobo® dataloggers, ones approximately mid-depth, and a second near bottom.


Florida Aquatic Nurseries 1
40
35
30
25
20
15
Temperature, °C
10/30/14
12/19/14
2/7/15
3/29/15
5/18/15
7/7/15
8/26/15
10/15/15


Florida Aquatic Nurseries 2
40
35
30
25
20
15
Temperature, °C
10/30/14
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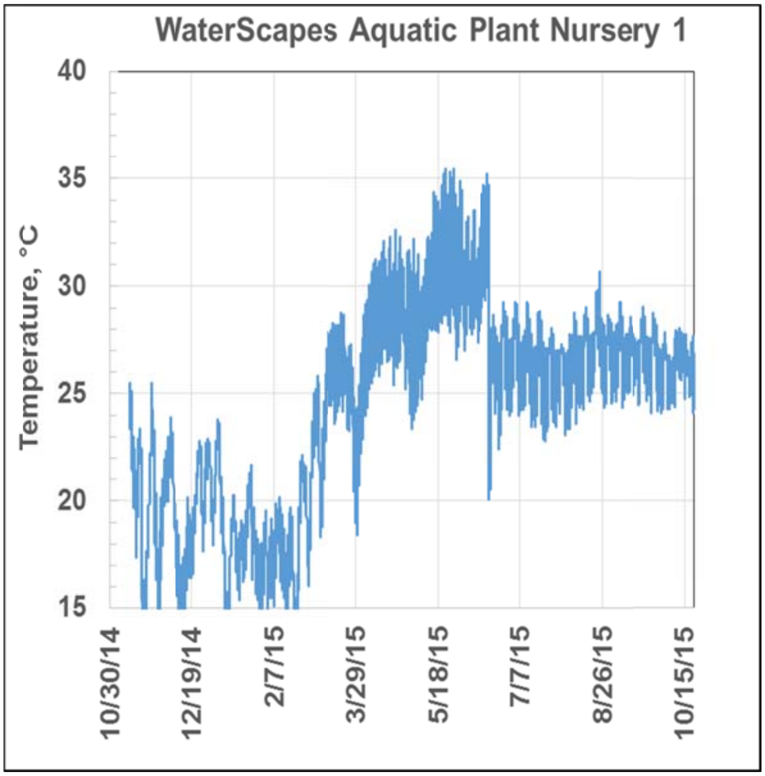
10/15/15


WaterScapes Aquatic Plant Nursery 1
40
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Temperature, °C
10/30/14
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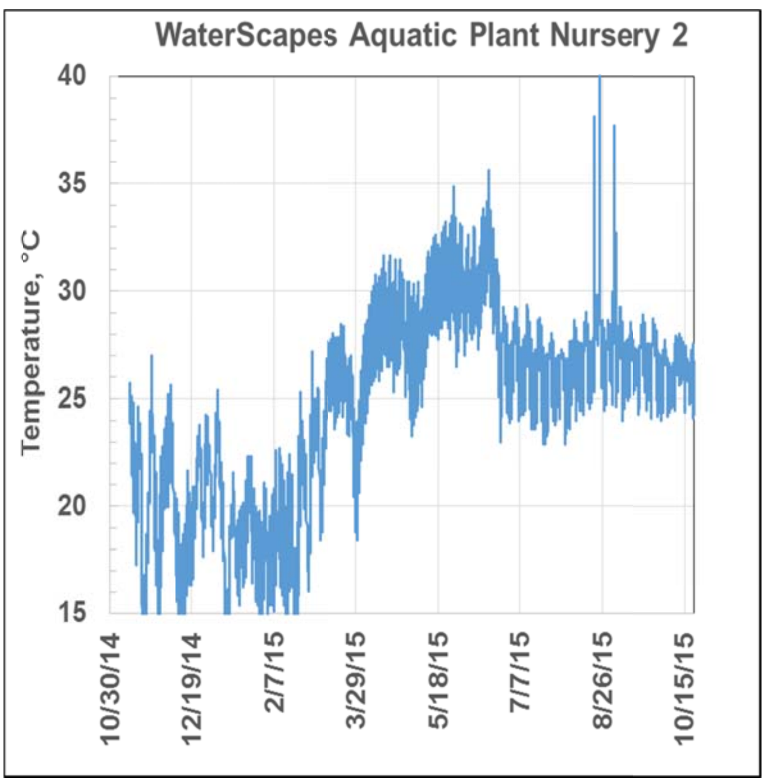
WaterScapes Aquatic Plant Nursery 2
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Temperature, °C
10/30/14
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2/7/15
3/29/15
5/18/15
7/7/15
8/26/15
10/15/15

Appendix 2

Demonstration Workshop: Egeria Culture & Low Salinity Shrimp Culture Operations

Workshop Announcement

Workshop Agenda

Workshop Participants

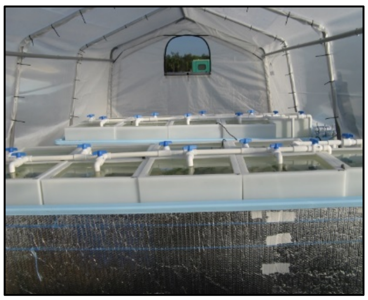
HARBOR BRANCH

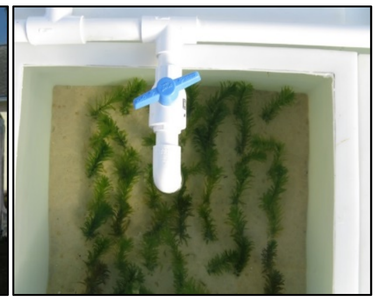
FLORIDA ATLANTIC UNIVERSITY®

Ocean Science for a Better World®

Egeria Culture & Low Salinity Shrimp Culture Operations

A combined workshop on two projects funded by grants from the Aquaculture Review Council and Florida Department of Agriculture and Consumer Services will be held at Harbor Branch Oceanographic Institute (HBOI) at Florida Atlantic University in Fort Pierce, Florida, on **Wednesday, October 21, 2015, from 1:00-4:15 p.m.** The workshop will consist of short presentations by the researchers involved with each project and a tour of their culture systems and other HBOI Aquaculture facilities.





Egeria Culture in Recirculating Systems

- Learn how to increase production of the aquatic plant Egeria by manipulating light and temperature conditions
- Learn how to culture Egeria in a closed system for year-round production



Establishing Environmental Mineral Guidelines for Low Salinity Shrimp Culture

- Learn how mineral ratios affect shrimp production in low salinity culture operations
- Learn what minimum and maximum concentrations are for calcium, magnesium, and potassium in low salinity shrimp culture operations

The workshop will be free-of-charge, but registration by October 16 is required. To register, please call 772-242-2506, and ask for Jill Sunderland. Attendance is limited to 30 participants. For directions, see: http://www.fau.edu/hboi/about/location.php.

HARBOR BRANCH

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Egeria Culture & Low Salinity Shrimp Culture Operations

Workshop Agenda

October 21, 2015, 1:00-4:15 p.m.

Welcome

Dr. Megan Davis, Interim Executive Director, FAU Harbor Branch

Establishing Environmental Mineral Guidelines for Low Salinity Shrimp Culture

- **Presentation – Dr. Susan Laramore, FAU Harbor Branch**
- **Questions & Discussion**

Egeria Culture in Recirculating Systems

- **Presentation – Dr. Dennis Hanisak & Dr. Paul Wills, FAU Harbor Branch**
- **Questions & Discussion**

Tours

- **Shrimp Culture**
- **Egeria and Other Plant Culture**

Egeria Culture & Low Salinity Shrimp Culture Operations
Workshop Participants

Tim Armstrong, Eat Your Yard Jax
Ronny Boyd, B & B Fish
Trever Boyd, B & B Fish
Nick Chyczewski, Satellite High School
Joe Clayton, FDAC - Division of Aquaculture
Rey, Diaz, US Tilapia
Pat Faehnle, Aquatic Resources
Holly Freer, Indian River State College
Brittanie Gloyd, FDAC - Division of Aquaculture
Joel Graves, Eat Your Yard Jax
George Habib, US Tilapia
Susan Kopec, Fish-A-Ponics
Grayson Kyte, Indian River State College
Pierre LePochat, WaterScapes Aquatic Plant Nursery
Brandon McLane, Florida Aquatic Nursery
Alexandria Pickard, FDAC - Division of Aquaculture
Andrew Richard, FDAC - Division of Aquaculture
Serina Rocco, FDAC - Division of Aquaculture
Heather Rossi, Indian River State College
Joe Scott, Satellite High School
Alberto Torres, US Tilapia
Huy Tran, Apopka Aquaponics Farm
Cindy Vlahogeorge
John Vlahogeorge
Rafael Zelaya, Fresh Shrimp USA