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Watercress (Nasturtium officinale) production utilizing brook trout (Salvelinus fontinalis) flow-through aquaculture effluent

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Watercress (*Nasturtium officinale*) Production Utilizing Brook Trout (*Salvelinus fontinalis*) Flow-through Aquaculture Effluent

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ABSTRACT

Watercress (*Nasturtium officinale*) Production Utilizing
Brook Trout (*Salvelinus fontinalis*) Flow-through Aquaculture Effluent

Erika Nichole Smith

Dissolved nitrogen (N) and phosphorus (P) present in flow-through aquaculture effluent can pose the risk of eutrophication to receiving streams when discharged from flow-through systems. One potential solution to prevent nutrient loading is the establishment of an integrated system that cultures green plants in the effluent. The objectives of this research were to determine watercress' (*Nasturtium officinale*) growth and nutrient contents in both a hydroponic controlled environment and a flow-through aquaponic production system utilizing brook trout (*Salvelinus fontinalis*) aquaculture effluent; and to evaluate various treatments to determine the best cultural conditions for watercress in the aquaponic system for optimization as a nutrient recovery option for and value-added by-product to fish production. A 6-week long hydroponic and three 12-week long aquaponic experiments were conducted to meet these objectives. The hydroponic experiment studied the effects of light intensity and nutrient solution concentration and the aquaponic experiments studied the effects of water velocity, plant density, growing media, location, and season on watercress growth and nutrient contents. Whole plants were sampled for growth data (fresh weights, lengths, and dry weights) and dried tissue was analyzed for total N and P content. All experiments were randomized complete block (RCB) designs with three replications per treatment. Growth and nutrient data were analyzed separately and all significance was determined using SAS software. Data from the hydroponic experiment indicated that watercress growth and nutrient contents were greatest in the intermediate light intensity. The half-strength Hoagland's nutrient solution treatment resulted in significantly longer plants but had no significance on fresh weight or nutrient content versus the full-strength nutrient solution treatment. Overall, results from the aquaponic experiments provided that watercress growth was significantly greater when grown in the high water velocity, high plant density, paper growing medium, Aquaponic Production Greenhouse (APG), and spring season treatments. These treatments also resulted in greater nutrient contents in dry tissue, with the exception of greater nutrient contents in plants grown during the winter season. Nutrient sufficiency ranges may or may not have been met in the various experiments which suggest that the effluent may be nutrient limiting at times. In conclusion, watercress production is possible utilizing brook trout flow-through aquaculture effluent. The risk of nutrient loading from the system studied is insignificant because watercress growth and nutrient contents were not significant among treatments exposed and not exposed to effluent. Therefore, the focus of this integrated watercress and trout production system becomes a sustainable agriculture versus a phytoremediation approach that takes advantage of resources already available. Watercress could also serve as a secondary marketable crop for farmers to potentially increase farm income.

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LIST OF SYMBOLS / NOMENCLATURE

1. ARF – Aquaculture Research Facility
2. APG – Aquaponic Production Greenhouse
3. ANOVA – Analysis of Variance
4. BCF – Bio-concentration Factor
5. BMP – Best Management Practices
6. CAAP – Concentrated Aquatic Animal Production
7. N – Nitrogen
8. P – Phosphorus
9. TSS – Total Suspended Solids
10. ELG – Effluent Limit Guidelines
11. EPA – Environmental Protection Agency
12. NFT – Nutrient Film Technique
13. NPDES – National Pollutant Discharge Elimination System
14. NRCCE – National Research Center for Coal and Energy
15. OLSB – Off-line Settling Basin
16. RCB – Randomized Complete Block
17. RMF – Reymann Memorial Farm
18. PAR – Photosynthetically Active Radiation
19. PEITC - 2-phen(yl)ethyl-isothiocyanate
20. WVDEP – West Virginia Department of Environmental Protection
21. BWQ – Baseline Water Quality
22. WV – West Virginia
23. WVU – West Virginia University
24. US – United States

INTRODUCTION

The aquaculture, or concentrated aquatic animal production (CAAP), industry is under increasing pressure to reduce the concentration and amount of aquaculture effluent that is released into the environment from aquaculture systems. Aquaculture effluent contains nutrient waste generated from production. When these nutrients are discharged from aquaculture systems, they can result in nutrient loading of natural water bodies and lead to environmental degradation. In the absence of treatment, pollutant loadings from individual CAAP facilities can contribute up to several thousand pounds of nitrogen (N) and phosphorus (P) and up to several million pounds of total suspended solids (TSS) per year (EPA, 2004).

Effluent limitation guidelines (ELGs) have been established by the Environmental Protection Agency (EPA) regarding flow-through, recirculating, or net pen aquaculture systems that directly discharge wastewater into the nation's waters (EPA, 2004). CAAP facilities qualify as point sources and are required under the Clean Water Act to obtain a National Pollutant Discharge Elimination System (NPDES) permit to regulate the amount of soluble solids and nutrients discharged into the nation's waters (EPA, 2004).

Systems yielding over 9,090 kg (harvest weight) of aquatic animals annually and feeding over 2,272 kg of food during a calendar month of maximum feeding (major limiting factor) are required to obtain a NPDES permit (EPA, 2004). Systems producing and feeding less than these amounts are not currently required to obtain a permit. Due to potential environmental degradation from any aquaculture system, regulations are also likely to be implemented in the future on systems yielding under the current

guidelines.

At the state level, Antidegradation Implementation Procedures established by the West Virginia Department of Environmental Protection (WVDEP), under Titles 60, CSR 5 and 46, CSR 1, require baseline water quality (BWQ) assessments for receiving water segments for any new or expanded operation that wants permit coverage. If BWQ has not been previously established, it is the responsibility of the regulated entity to conduct the assessment according to the proper procedures set forth by the WVDEP (WVDEP, 2001).

The number of operations selling and/or distributing fish and/or eggs in West Virginia (WV) increased from 25 in 2003 to 31 in 2004. The number of operations in the United States (US) increased from 545 in 2003 to 610 in 2004 (NASS, 2005). The total value of all trout sales (fish and eggs) in 20 selected states, including WV, totaled $68.7 million in 2004, a 7% increase from 2003 (NASS, 2005). Statistics reveal growth of the aquaculture industry in both the nation and WV, which has an aquaculture output of about $2 million annually (Semmens, 2003). This current trend indicates a valid concern for protecting the environment and providing the aquaculture industry with cost-effective methods to manage effluent to ensure compliance, now and in the future.

One such cost-effective method to recover nutrients and positively utilize aquaculture effluent is the development of sustainable, integrated aquaponic systems that cultivate green plants in effluent to remove nutrients. Many studies have looked at integrated aquaponic systems to address the aquaculture effluent issue in recirculating systems, but not as a management option for flow-through systems.

This research involves an integrated system that evaluates the production of

watercress, *Nasturtium officinale* R.Br, and its ability to recover nutrients from brook trout, *Salvelinus fontinalis* (Mitchill), flow-through aquaculture effluent by utilizing the phytoremediation process versus dilution or discharge. There were two main roles of watercress in this integrated agriculture research: to act as a bio-filter and recover N and P from effluent to prevent nutrient loading of the receiving stream and to potentially increase aquaculture industry income by serving as a value-added, secondary marketable crop that utilizes resources (i.e. irrigation, fertilizer) already available.

Figure 1 shows the general layout of the research location at the West Virginia University (WVU) Reymann Memorial Farm (RMF) in Wardensville (Hardy County), WV where the aquaponic experiments took place. The aquaculture research facility (ARF) currently feeds less than 2,272 kg of feed during any month raises and does not fall under the NPDES permit requirement. Quiescent zones in addition to an off-line settling basin (OLSB) and other best management practices (BMPs) are currently used to manage solids and effluent generated from fish production.

Results from this research may provide fish farmers with a pro-active, preventative, cost-effective, and sustainable method of managing flow-through aquaculture effluent. If successful, this research could potentially aid in alleviating environmental degradation and the pressures that currently face the CAAP industry in WV, the nation, and world wide.

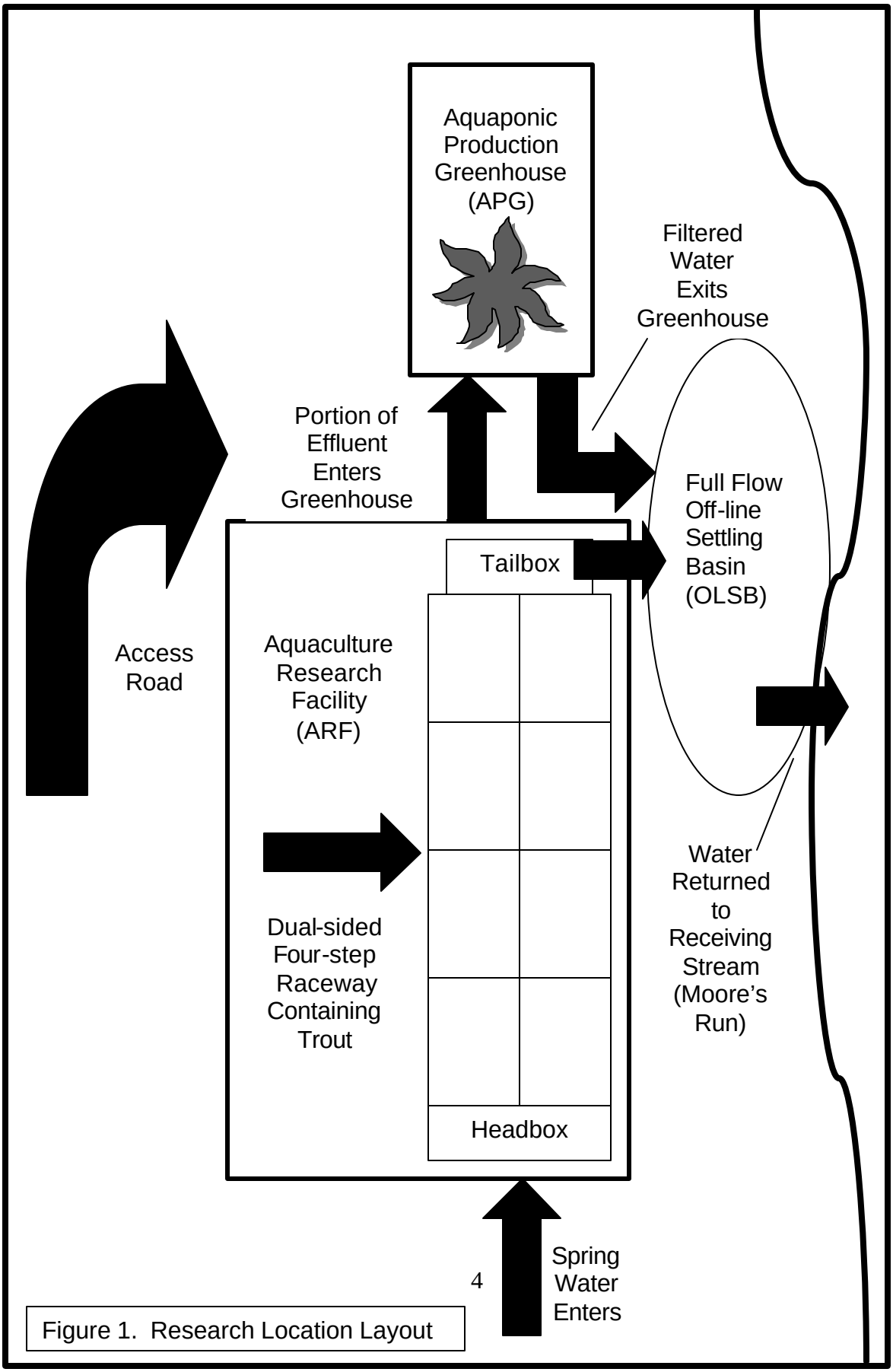

Figure 1. Research Location Layout

LITERATURE REVIEW

Watercress *(Nasturtium officinale* R.Br.)

Watercress is in the family Brassicaceae (Cruciferae), the Mustard Family, which consists of about 350 genera and over 3000 species. Some commonly known plants in this family include broccoli, bok choy, cabbage, cauliflower, and onion. Brassicaceae members share a suite of glycosinolate compounds, known as mustard oils, which are characteristic in identification of the family (Texas A & M Univ., 2004).

Watercress dates back to the 1st Century A.D. and is one of the oldest known green vegetables consumed by humans. It is used as a salad green, garnish, steamed vegetable, and medicinal herb (Howard, 1976). Watercress is characterized by its tangy, peppery flavor. The plant is very nutritious with plant constituents including beta carotene (Vit A), aspartic acid (Vit C), calcium, folic acid, iron, iodine, and phosphorous. It also contains arginine, glycine, lysine, tryptophan, the antioxidant a-tocopherol, and a chemo-preventative of several tobacco specific carcinogens, 2-phen(yl)ethyl-isothiocyanate (PEITC), which is also the primary flavoring component of the plant (Palaniswamy and McAvoy, 2001).

Watercress is an aquatic, perennial herb native to Europe and naturalized in the United States. It lives in and obtains its nourishment from water, is not considered to have a high nutrient demand, and little is known about the need or effectiveness of fertilizer in growing beds (Seelig, 1974). It can grow in cool streams, near springs, or in moist soil on stream banks, but grows best in running water. Watercress growth is dependent on water velocity. The higher the nitrogen content of the water source, the

smaller the flow required for a given size bed. A large flow of water is needed to supply other nutrients and protect plants from freezing (Seelig, 1974). The water supply should contain greater than 2ppm of nitrate from larger springs and even greater levels for smaller springs to support profitable beds (Shear, 1968). The sufficiency ranges for N and P contents based on watercress new leaf samples taken in the middle of the growing season are 4.2 to 6.0% N and 0.7 to 1.3% P (Mills et al, 1996).

Watercress can tolerate a range of light conditions from partial shade to full sun. Production is reportedly heavier in summer months when more daylight promotes growth (Seelig, 1974). If all other conditions are in proper supply, aquatic plants saturate photosynthesis between 300 to 1000 µmol m^{-2} s^{-1}, with a good target range between 200 to 500 µmol m^{-2} s^{-1}. Light intensities below 100 µmol m^{-2} s^{-1} are considered low light and aquatic plants have a minimum compensation point required between 15 to 85 µmol m^{-2} s^{-1} to stay alive (Pushak, 1997). Watercress is reported to prefer a soil pH within the range of 4.3 to 8.3 (Simon et al, 1984).

Watercress has smooth, creeping or freely floating, stems with adventitious roots forming at each node, typically below water. Leaves are compound with 3 to11 round or oblong leaflets. Small white flowers, with the corolla in the shape of a cross, develop in elongated racemes and bloom from April to June. Fruits are siliques that are 1.27 to 2.54 cm (0.5 to 1 in) long with 2 seeds per locule.

Commercial watercress is propagated via seed, shoot tip cuttings, and tissue culture. Seed propagation is the preferred method due to the increased risk of spreading turnip mosaic virus, a common disease of watercress when propagating via shoot tip cuttings (Palaniswamy and McAvoy, 2001). One of the most serious pests of

commercial watercress is *Armadillidium vulgare,* or sow bug, which eats underwater leaves and chews through stems. One method of sow bug control includes crop rotation (Seelig, 1974). Other known diseases and pests include algae, duckweed, crook rot disease, *Cercospora* sp. (chlorotic leaf spot), yellow spot virus, *Plutella xylostella* (diamond back moth), *Gammarus pulex* (a terrestrial arthropod pest), *Steneotarsonemus pallidus* (cyclamen mites), liver flukes, and several aphid species (Palaniswamy and McAvoy, 2001). Maintaining a dense growth of watercress is one of the most effective ways to control weeds (Seelig, 1974).

Watercress seed is typically sown in gravel beds with germination occurring in 5 to10 days. The cool season crop grows best with day temperatures of 20 to 25°C and night temperature of 15 to 20°C, but can still grow successfully up to 28 °C. The minimum temperature required to sustain a commercial system is 10 °C (The Growing Edge, 2002).

Plants are harvested when they reach a height of 18 cm (7 in) around 35 days (in summer) and 50 days (in spring and fall) or 6 to 7 weeks after sowing (Palaniswamy and McAvoy, 2001). Subsequent harvesting is done at 15 to 30 day intervals. A sharp object is used to cut the stems 15 to 20 cm (6 to 8 in) below the tip of the plant and plants are submerged in water until harvest is complete. Adventitious roots decrease market value, therefore, only the above water portion of the plants are harvested. The plants are rinsed clean, 20 to 30 stems are collected in bunches and tied close to the top, and the bottoms of the stems are trimmed evenly to 10 to 15 cm (4 to 6 in) (Seelig, 1974). The average yield per cutting is about 2500 bunches per 93 m^2 (1000 ft^2) of well-

established growing beds (Shear, 1968).

Bunches typically sell for $1 to $3, depending on the market, with higher prices usually obtained in winter months (The Growing Edge, 2002).

Watercress is perishable and should be shipped or marketed directly after harvest. Watercress bunches are left loose or bagged and placed in lined containers and separated by layers of ice. The crop should be kept at 0°C and 90 to 95% relative humidity during storage and marketing (Seelig, 1974).

Watercress was chosen for this study because it has been previously used in remediation efforts and is indigenous to the WVU RMF flora, but more importantly because it is an aquatic plant naturally well-suited to hydroponic production and relatively easy to grow. It prefers cool (12 to 20 °C), moving water like the conditions found in natural springs and used for trout production. An on-site natural spring supplies the WVU RMF with water for aquaculture production and other farm demands.

Aquaculture

Aquaculture is the cultivation of marketable freshwater and marine plants or animals via three methods: flow-through, pond, and re-circulating systems. Effluent is any substance, particularly a liquid, which enters the environment from a point source. Effluent from aquaculture systems often have high N and P contents which is detrimental to the environment because these nutrients contribute to eutrophication (Adler et al., 2000). Eutrophication is an enrichment of a water body by nutrients (primarily N and P) that results in an excessive growth of phytoplankton, algae, or vascular plants. As these organisms die, oxygen in the water is consumed, leading to

oxygen depletion which adversely effects aquatic life and can lead to death.

Flow-through aquaculture systems create large volumes of effluent carrying relatively dilute nutrients that are difficult to treat (Heinen et al.,1996). Flow-through systems typically have higher flow rates and lower nutrient concentrations than pond and recirculating systems. In addition to dilute soluble nutrients, flow-through effluents often contain suspended solids which add to its nutrient content.

N and P present in soluble waste released in fish urine and across the gills and solid waste from feces and undigested food become suspended in solution as water travels through the raceway. Quiescent zones are located at the end of each raceway and serve as settling areas for the majority of solids. Ideally, these zones are cleaned daily to remove accumulated solids; however, when this occurs, some solid waste is re-suspended resulting in waste streams that are typically higher in N and P (Avault, 1996).

Figure 1 displays the dual-sided, four-step, flow-through "raceway" system at the WVU RMF Aquaculture Research Facility (ARF) which utilizes water from a high-yielding spring to raise trout. This is the flow-through system that provided effluent for the aquaponic experiments in this research.

Hydroponics

Hydroponics is a soil-less method of growing plants which includes water culture (water and dissolved nutrients) and substrate culture (inert media, water, and dissolved nutrients) (Acquaah, 2002). Examples of inert media include oasis cubes, which are made from a foam-based material typically used in the floral industry, and horticultural

rockwool, which consists of melted basalt rock and chalk spun into fibers.

Hydroponic systems are beneficial because they concentrate crop production into smaller areas than those required in the field without compromising yield. This is accomplished by providing high levels of nutrients and water to plants (Univ. of the Virgin Islands, no date). Some examples of hydroponic techniques available include ebb-and-flow and floating systems (Acquaah, 2002).

Hydroponic watercress is grown commercially following the cultural conditions described in the watercress section above. Systems are typically based on large outdoor gravel beds or nutrient film technique (NFT) channels filled with water 2.54 to 5.08 cm (1 to 2 in) deep. One study reports that NFT sub-systems are less efficient at removing nutrients from fish effluent and producing good plant biomass and yields than either gravel bed or floating hydroponic sub-systems (Lennard et al, 2004). Nutrient solution is flooded into the system and generally re-circulated to limit environmental impacts. Yields of 1.5 to 2.0 kg/m²/month have been obtained in summer from protected systems and 500 g/m²/month is common in winter (The Growing Edge, 2002).

Limitations to hydroponic systems include costs associated with the continual need for nutrients to be artificially supplied through the irrigation water and the potential for environmental degradation from nutrient discharges in non-recirculating systems.

Aquaponics

Aquaponics (aquaculture plus hydroponics) is the simultaneous culture of marketable fish and plants. Nutrients from fish production acts as fertilizer to provide essential nutrients, like N and P, to plants which use the nutrients for growth.

Simultaneously, plants serve as a bio-filter to remove some nutrients before it's reused or discharged from the system.

The role of nitrifying bacteria, present in growing beds and in association with plant roots, in the nutrient cycling process is critical and without them the conversion of ammonia (toxic to plants and fish) present in effluent to nitrate (form available to plants) would not take place (Diver, 2006). In Step 1 of the nitrification process, *Nitrosomonas* spp. oxidize ammonium into nitrite and in Step 2, *Nitrobacter* spp. transform nitrite to nitrate (Mills et al, 1996).

Researchers and growers have turned aquaponics into a working model of sustainable food production. Aquaponics supports sustainable food production by: turning by-products from one system into nutrients for another system, establishing a polyculture that increases crop diversity and yields multiple products, re-using natural resources (i.e. water), generating local food production, and supporting the local economy (Diver, 2006).

Watercress has been grown as a bio-filter in an aquaponic system utilizing trout farm effluent for production. This system grew watercress in a pond using floating frames (C.W. Johnson, unpublished data). Watercress was found to flourish on the effluent alone without the addition of other nutrients for growth and it effectively removed suspended solids and many of the nutrients produced by the fish. The North Carolina Division of Environmental Management conducted tests on the effluent above and below these ponds and found that 93% of the solids were removed, ammonia was reduced by 74%, P showed a decline of 50%, and the biological oxygen demand decreased by 58% (C.W. Johnson, unpublished data).

Another example of watercress' use in aquaponics is a watercress-crayfish polyculture system that used effluent from a trout hatchery to grow watercress. Watercress removed nutrients from the water for growth, which resulted in clean water for crayfish production, and served as an easy food source for the crayfish diet (Rundquist, 1976).

Watercress is capable of recovering nutrients from trout effluent in a low volume flow, high nutrient concentration pond environment and in a polyculture system used to generate multiple aquaculture crops. This research evaluated an integrated flow-through system to determine if watercress could obtain nutrients and grow in trout effluent in a high volume flow, low nutrient concentration environment. Instead of gravel beds, a floating raft system was used, which allowed any suspended solids to settle out and provide a substrate for rhizobacteia within the system.

Phytoremediation

Phytoremediation is the use of green plants in the removal of contaminants, toxins, and wastes from soil and water. The primary concerns in this study are the nutrient concentrations (nitrogen and phosphorus) present in the aquaculture effluent.

One example of how phytoremediation has been used to successfully recover nutrients from aquaculture effluent is a study that evaluated an aquaponic system that integrated the production of lettuce to uptake nutrients from rainbow trout effluent in a recirculating system. The objective of this research was to remove >95% of the phosphorus in the effluent while producing a marketable crop, which they achieved

(Adler, 1998). Watercress has also been used in other phytoremediation efforts. Several studies have looked at watercress and its ability to accumulate contaminants such as chromium, perchlorate, thallium, and zinc from soil and water at affected sites.

In this study, watercress will be grown in flow-through aquaculture effluent to determine if it is able to use nutrients from the effluent to meet its growth requirements, while also producing cleaner water for discharge from the system

EXPERIMENTAL OBJECTIVES

This project involved multiple disciplines including horticulture, aquaculture, and environmental engineering to address the issues of plant production, fish production, and water quality, respectively. The horticulture research involved two objectives: 1) to determine watercress growth and nutrient contents in a hydroponic controlled environment experiment and a flow-through aquaponic system and 2) to evaluate various treatments based on growth and nutrient data to determine the best cultural conditions for watercress in the aquaponic system for optimization as a nutrient recovery option and value-added product for fish production.

The objectives of the environmental engineering researchers working on this project were to measure the water quality prior to, during, and after fish production and after watercress production to determine the nutrient concentrations of the water and how the water was affected by the fish and plants. For further information on the water quality outcomes of this combined research, please refer to Dyer (2006).

Two types of experiments were conducted to achieve the horticulture objectives. A hydroponic experiment took place at the WVU Davis College of Agriculture Forestry and Consumer Sciences (DCAFCS) and evaluated the effects of light intensity and nutrient solution concentration on watercress growth and nutrient contents in controlled environments. Whole plant fresh weight, length, and dry weight measurements were taken to determine growth and dried plant tissue was analyzed for N and P content. This experiment was designed to supply baseline values for watercress growth and nutrient contents in a controlled environment with optimum cultural conditions. These

results are intended to support results from the aquaponic experiments with regards to light intensity and nutrient concentrations.

Aquaponic experiments were conducted at the WVU Reymann Memorial Farm (RMF) Aquaculture Research Facility (ARF) and Aquaponic Production Greenhouse (APG). The effects of water velocity, plant density, growing medium, location, and season on watercress growth and nutrient contents were evaluated. Growth and nutrient data collection was the same as for the hydroponic studies. These studies should supply values on watercress growth and nutrient contents with regards to the above variables in a flow-through aquaponic system and semi-controlled environment.

Aquaponic experiments should provide preliminary data that will allow future researchers or growers to optimize cultural conditions for watercress production in an integrated, flow-through aquaponic system to meet nutrient demands and achieve a harvestable crop. If successful, aquaponic watercress may prove to be a value-added, by-product of trout production.

MATERIALS & METHODS

Aquaculture System

Water is gravity-fed from a spring located on the farm through a series of 31 cm (12 in) pipes and transported approximately 183 m (600 ft) to the raceway. It enters the raceway's headbox where it is aerated before it flows through the raceway by gravity. Approximately 25.23 L s^{-1} (400 gpm) of water flows into the raceway creating a water velocity of 0.91 cm s^{-1} (0.030 ft s^{-1}).

The amount of fish per tank varies, but there are typically about 5000 fish in the system. Fish are fed Zeigler Gold Floating 3.0 MM[1] at a rate that supports full growth potential to maintain 318 to 454 kg (700 to 1000 lb) of fish per tank. Fish were periodically removed to keep weight within or below this range (Semmens, personal communication).

Effluent exits the raceway at the tailbox and flows through a 31 cm (12 in) pipe to the OLSB. During the aquaponic experiments a portion of the water was diverted to either a head tank and/or supply manifolds which supplied effluent to experimental channels before flowing to the OLSB by pipe. The full flow OLSB is adjacent to the ARF and functions as a polishing pond to remove additional nutrients and settle additional solids before it discharges the effluent into the receiving stream, Moore's Run.

[1] ZEIGLER BROS., INC., Gardners, PA 17324

Hydroponic Experiment

Watercress seed was sown in coarse vermiculite[2] and placed in a mistbed at the WVU Greenhouse for germination and initial growth. Seedlings were watered with tap water only and no fertilizer was added during this time. After 20d, watercress seedlings with at least one set of true leaves forming were transferred from the vermiculite, rinsed with de-ionized water, and placed in 500mL flasks containing either aerated full-strength (100%) or half-strength (50%) Hoagland's complete nutrient solution or the control (de-ionized water) (Hoagland et al., 1936). The nutrient solution recipe was described in Reed (2006) and consisted of the following: 1 mM KH_2PO_4, 5 mM KNO_3, 5 mM $Ca(NO_3)_2$ * $4H_2O$, 2 mM $MgSO_4$ * $7H_20$, 11.8 µM $MnSO_4$ * H_2O, 0.7 µM $ZnSO_4$ * $7H_2O$, 0.32 µM $CuSO_4$ * $5H_2O$, 0.16 µM $(NH_4)_6Mo_7O_{24}$ * $4H_2O$, 46.3 µM H_3BO_3, 5 µM Sequestrene 330 (10% Fe) and 1N KOH to adjust pH to 6.3 using a Corning pH meter 430[3]. The only modification to this recipe was the use of 5 µM Iron Chelate DP (10% Fe), which is the same formulation as Sequestrene 330, just sold under a different name. Aeration was supplied by Tetra Whisper® air pumps[4] and plastic airlines with pinholes in the end.

A hydroponic experiment was conducted at the WVU DCAFCS to evaluate watercress growth and nutrient contents under ideal conditions. Half of the experiment was ran under a low (50 ± 10 µmol m^{-2} s^{-1}) light intensity in a Percival Incubator[5] where lighting was supplied by cool white fluorescent lamps. The other half of the experiment

[2] Therm-O-Rock East, Inc., New Eagle, PA 15067
[3] Corning Inc., Science Products Division, One Riverfront Plaza, Corning, NY 14831
[4] Tetra Holding (US), Inc., 3001 Commerce Street, Blacksburg, VA 24060-6671
[5] Percival Scientific, Inc., 505 Research Drive, Perry, IA 50220

was ran under an intermediate (450 ± µmol m^{-2} s^{-1}) light intensity in a Sherer CEL 34-7 Growth Chamber[6] where lighting was supplied by cool white fluorescent lamps and incandescent bulbs. Light intensity is a measure of the amount of photosynthetically active radiation (PAR) in the visible light spectrum of 400 to 700nm, which represents the range that plants are able to use for photosynthesis.

The two light intensities were selected based on watercress' light requirement information and to coincide with the average light intensities found in the ARF (low PAR) and APG (intermediate PAR) at the WVU RMF where the aquaponic experiments were conducted. Light intensities were confirmed by an AccuPAR model LP-80 PAR/LAI Ceptometer[7]. Photoperiods in both experiments consisted of a 16-hr light:8-hr dark cycle and temperatures in both chambers were maintained at a 23:18ºC day-night cycle with 50% relative humidity.

Seedlings were placed in modified foam stoppers which were inserted into the mouths of 500mL flasks containing the designated nutrient solutions filled to the 500 mL level to ensure that roots were fully immersed in solution. All flasks were wrapped in aluminum foil to maintain iron in solution and reduce algal growth. One seedling was placed in each flask and arranged in a randomized complete block (RCB) design with three replications of each treatment per block for a total of 27 flasks per experiment.

Nutrient solutions were changed 14d from the initiation of the experiment and every 7d thereafter for a total of 6 weeks. Sampling occurred every two weeks for six weeks for a total of three samplings. One plant from each treatment in each block was

[6] Sherer Inc., Marshall, MI, USA
[7] DECAGON, 950 NE Nelson Court, Pullman, WA 99163

randomly selected and measured every two weeks and whole plant fresh weights and lengths were recorded. On the last sample date (Week 6), the plants sampled were placed in a drying oven at 75ºC for 24hr, and then dry weights were recorded. Whole plant dried samples were ground using a ceramic mortar and pestle, placed in the oven for a second drying, then stored in a -80ºC freezer until nutrient analysis.

Aquaponic Experiments

Experimental beds used to culture watercress in the aquaponic experiments were constructed of 0.64 cm (0.25 in) plywood, insulated with polystyrene panels, and lined with a heavy-duty black plastic pond liner. Each bed measured 2.44 m (8 ft) long x 1.22 m (4 ft) wide and contained three channels which each measured 2.44 m (8 ft) long x 0.36 m (15 in) wide. Each channel had its own water inflow (set at a designated velocity treatment) and water outflow (with a 15 cm (6 in) standpipe) and contained three floating rafts for a total of nine rafts per bed. The rafts were designed for this system and were constructed from 2.54 cm (1 in) PVC and 1.91 cm (0.5 in) plastic poultry netting and measured 74 cm (29 in) long x 36 cm (14 in) wide each. A HOBO Microstation Datalogger[8] with two 2-bit temperature sensors and two photosynthetically active radiation (PAR) sensors were used to monitor and collect data on air temperature and light intensity during the experiments.

Summer 2005 (ARF)

This experiment took place in the Aquaculture Research Facility (ARF) during the

[8] Ben Meadows Company, PO Box 5277, Janesville, WI 53547-5277

summer of 2005 and ran from late June to mid September. Effluent was pumped from the tailbox of the raceway to a 3785 liter head tank. Water from the head tank was diverted to a series of experimental channels via a supply manifold. There were 11 experimental beds with three channels per bed for a total of 33 experimental channels. Three channels remained empty to serve as a control for the environmental engineers to determine if the nutrient removal was in fact due to watercress versus some other phenomenon. Only 30 channels actually contained plants and a single bed (containing three channels) was placed at the headbox of the raceway to serve as a control. This bed received spring water prior to entering the raceway and did not contain nutrients generated from fish production. The effluent from the outtake of all other channels was piped to the OLSB before discharge into the receiving stream. Figure 2 shows the experiment layout for Summer 2005 in the ARF.

A 12-week, 3 x 3 x 3 factorial, RCB design experiment evaluated the effects of three different water velocities, plant densities, and growing media on watercress growth and nutrient content. High, medium, and low water velocity treatments were set at 0.61 cm s^{-1} (0.02 ft s^{-1}), 0.30 cm s^{-1} (0.01 ft s^{-1}), and 0.06 cm s^{-1} (0.002 ft s^{-1}), respectively. Plant density treatments consisted of low, medium, and high plant densities of 0.02, 0.04, and 0.08 plants cm^{-2} (50, 100, and 200 plants per raft (each raft measured 2619.35 cm^2), respectively. Hydroponic growing media treatments included a single-ply white paper medium[9], Isolatek mineral wool bulk insulation product[10]

[9] SCOTT PAPER LIMITED, P.O. Box 1500, Streetsville, Ontario
[10] Isolatek International, Stanhope, NJ 07874

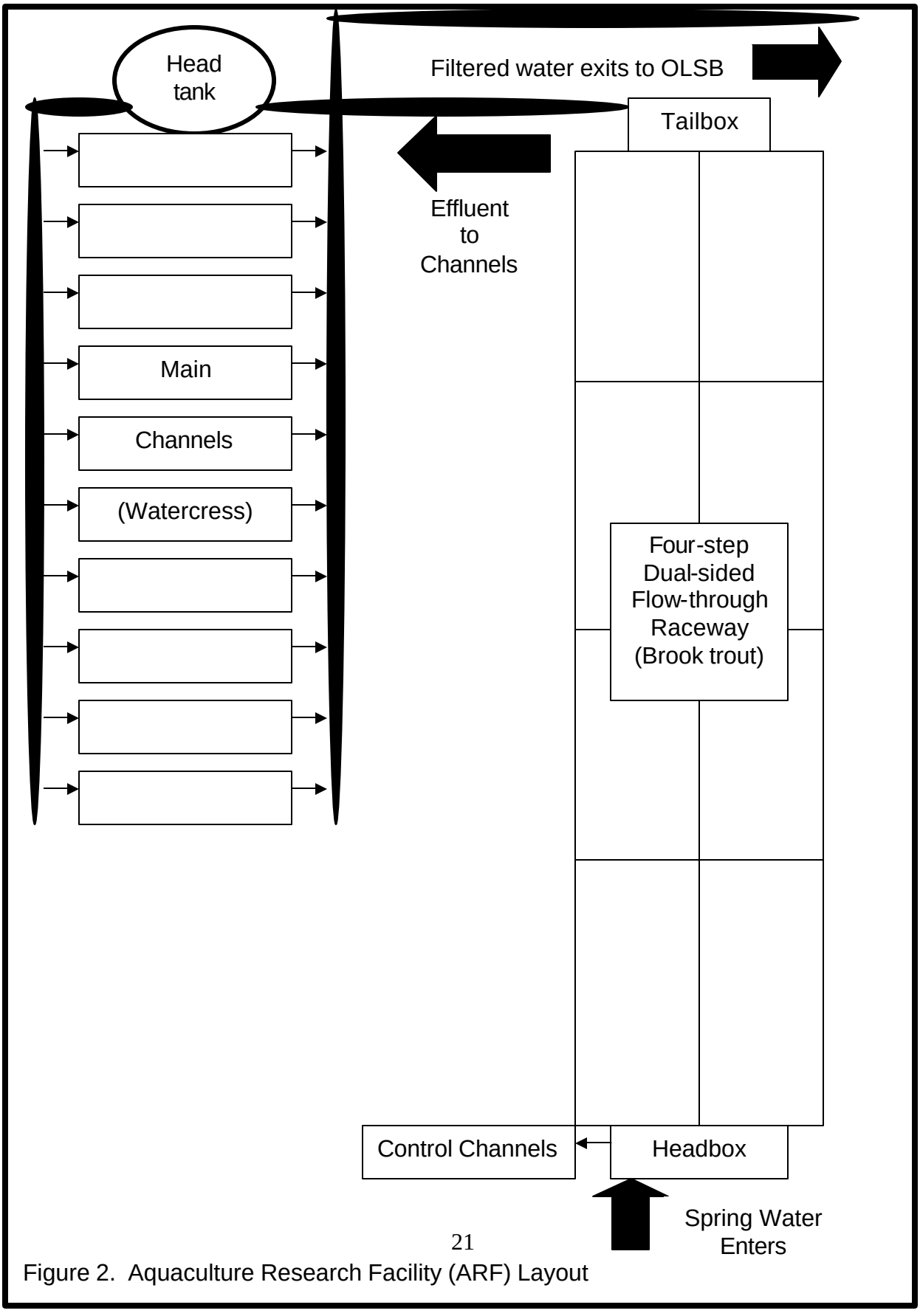

Figure 2. Aquaculture Research Facility (ARF) Layout

(horticultural rockwool), and 4 cm (1.5 in) oasis rootcubes cubes[11]. Each factorial treatment combination was replicated three times. The control channels incorporated all three water velocities and growing media used in the main channels, but only the medium plant density due to replication limitations.

During the experiment, plant samples were collected four times at three week intervals. Three plants, representing a single sample, were taken from each raft and whole plant fresh weights and plant lengths were recorded. Sample criteria required plants to have at least two sets of true leaves and a collective fresh weight of at least 600 mg. The rafts were systematically rotated within their respective channels at the end of each sampling to account for nutrient fluctuations within the channels. Samples were placed in brown paper bags and transported to WVU and placed in a drying oven as above, and dry weights were recorded.

Samples were prepared and stored as above until analysis for total N and P content. Whole plant fresh weight and length averages were taken to provide growth data on a per plant basis while dried samples used for nutrient analysis contained all three plants to ensure enough tissue to meet detection limits.

Winter and Spring 2006 (APG)

Additional funding provided for construction of a new greenhouse, so the Winter and Spring 2006 experiments took place in the Aquaponic Production Greenhouse (APG) instead of the ARF. Due to an opaque roof covering in the ARF and associated low light intensities, a decision was made to conduct subsequent aquaponic

[11] Hummert International, 4500 Earth City Expressway, Earth City, MO 63045

experiments in the APG. The winter experiment ran from mid December to early March and the spring experiment ran from mid March to early June.

The 15 m (48 ft) long x 8 m (25 ft) wide double-layer polyethylene greenhouse with roll-up side walls and polycarbonate end walls was constructed due east of the ARF. The greenhouse was not equipped with a formal heating and cooling system, so it basically served as a protective, semi-controlled environment structure for crop production. Ventilation was achieved by rolling up the side walls, a vent fan, and vent. Lumite 52 x 52 mesh screening[12] was attached to the side walls to allow for ventilation while also reducing pest populations.

Based on observations from the Summer 2005 experiment and limited space in the APG, the medium velocity, medium density, and rockwool and oasis media treatments were eliminated. Figure 3 depicts the general layout of the Winter and Spring 2006 experiments in the APG. These experiments were conducted simultaneously with other experiments. Water from trout production exited the tailbox of the raceway inside the ARF and was pumped to main and sub-main manifolds inside the greenhouse which supplied experimental beds with effluent. Four beds in the center of the APG were dedicated to watercress production (Figure 3). Each bed contained three channels each for a total of twelve experimental channels with the same dimensions described above. Effluent flowed through pipes to the OLSB before discharge into the receiving stream.

Twelve-week, 2 x 2 factorial, RCB design experiments evaluated the effects of

[12] Lumite Inc., 2100c Atlanta Hwy., Gainesville, GA 30504

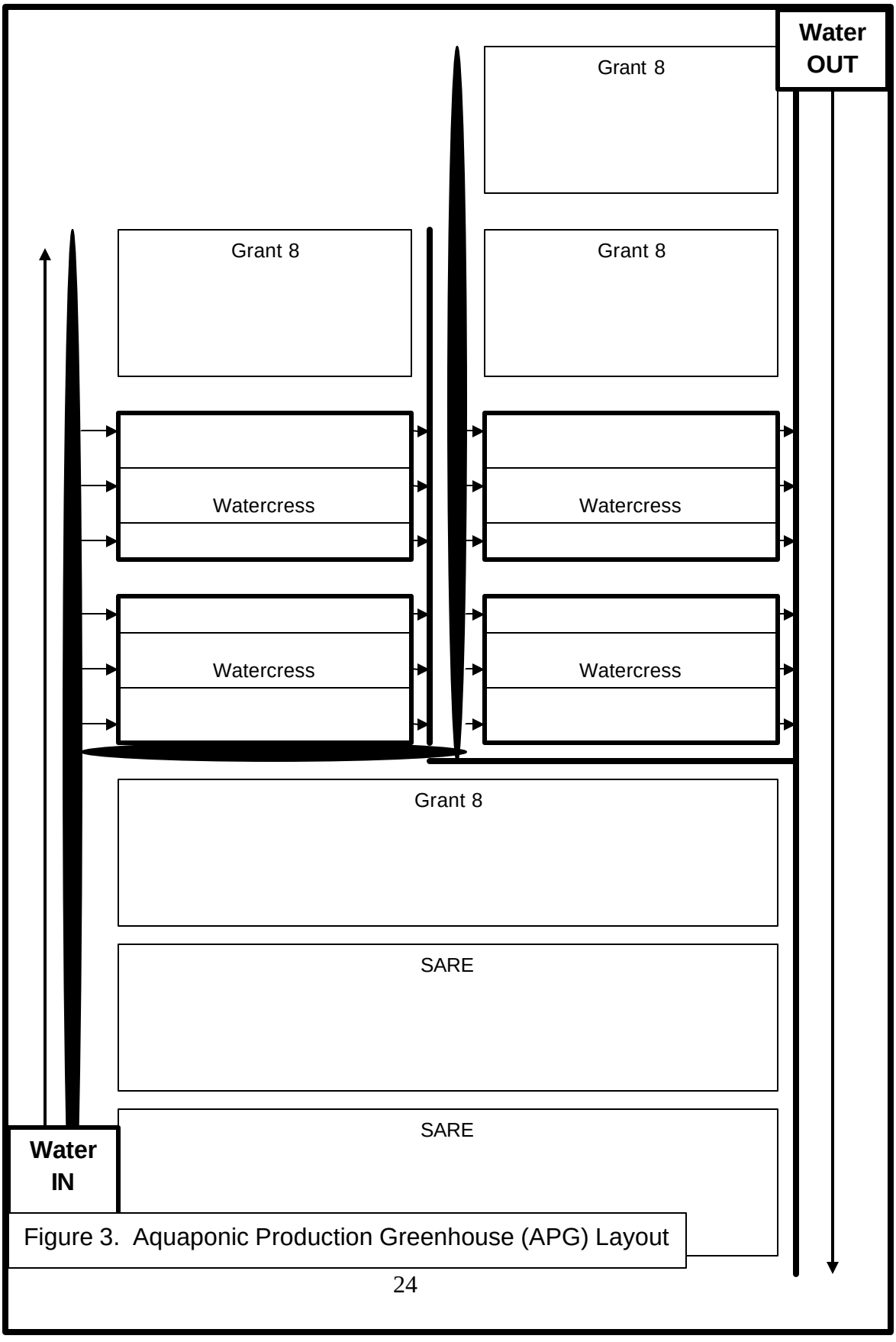

Figure 3. Aquaponic Production Greenhouse (APG) Layout

two different water velocities and plant densities on watercress' growth and nutrient content. The low and high velocities and plant densities used were the same as those in the Summer 2005 experiment above. Watercress was direct-seeded on paper medium. There was no control in these experiments because there was no way to allow for an experimental channel in the greenhouse that contained spring water only, since all water entering the greenhouse originated from the raceway and contained nutrients from fish production.

Sample criteria and sampling procedure used were the same as that for the Summer 2005 experiment above. Dried plants were also treated the same until nutrient analysis.

Plant Tissue Analysis

A minimum dry weight criterion was established for each element prior to analysis to ensure detection limits were met. Based on technician and equipment recommendations, dried samples had to weigh greater than or equal to 150 mg to be included in the data set for nitrogen analysis (R. Weaver, personal communication, 2006). Dried samples had to weigh greater than or equal to 200 mg to be included in the data set for phosphorus analysis (K. Stewart, personal communication, 2006). Total nitrogen was determined using a LECO TruSpec CHN-S (carbon, hydrogen, nitrogen, and sulfur) analyzer[13] for all but thirteen samples. Those samples were sent to the WVU Chemical Engineering Lab and analyzed for nitrogen content by gas chromatography when the TruSpec was undergoing maintenance.

[13] LECO Corporation, 3000 Lakeview Ave., St. Joseph, MI 49085

Samples analyzed for phosphorus were sent to the National Research Center for Coal and Energy (NRCEE) Analytical Lab. Total phosphorus was determined using a Varian ICP-OES[14] (inductively coupled plasma optical emission spectrometer).

Data Collection and Statistical Analysis

Whole plants (roots and shoots) were collected for growth and nutrient data and analysis for all experiments. Whole plant fresh weights and plant lengths (distance from root tip to shoot tip) were recorded at sample time and whole plant dry weights were recorded after drying.

Separate statistical programs were created for growth and nutrient data for each experiment and analyzed separately for analysis of variance using the SAS General Linear Model. Type III SS and Tukey's Studentized Range (HSD) test were used for significant means and contrast statements were used to determine if trends were linear or quadratic.

Growth data only represents the last nine weeks of each aquaponic experiment because plants did not meet the sampling criteria during the first three weeks of each experiment. Nutrient data only represents the last sampling (Week 6) of the hydroponic experiment and the last six weeks of each aquaponic experiment because it wasn't until then that there was consistently enough dry tissue among the treatments to meet plant tissue analysis criteria.

[14] Varian Instruments, 2700 Mitchell Dr., Walnut Creek, CA 94598

RESULTS & DISCUSSION

Hydroponic Experiment

Growth results are based on whole plant (roots and shoots) length and fresh weight means that were recorded at each sampling. Nutrient results are based on whole plant dry weight means that were recorded for the last sampling only (Week 6) due to inadequate amounts of dry tissue for analysis at Weeks 2 and 4.

Growth Data

Light intensity, sample date, and nutrient solution concentration had significant effects on watercress length and fresh weight (Appendices 1 & 2, respectively). Plants grown under the intermediate light intensity (450 ±10 PAR) were significantly longer and weighed significantly more than those grown under the low light intensity (50 ± 10 PAR) (Table 1).

There was a linear relationship between sample date and watercress length and fresh weight. Plants sampled at Week 6 were significantly longer and weighed significantly more than those sampled at Week 2. Plants sampled at Week 4 were not significantly different than those sampled at Week 2 or Week 6 with regard to length, however, they did weigh significantly less than those sampled at Week 6, but were not significantly different in weight from those sampled at Week 2 (Table 1).

There was a quadratic relationship between nutrient solution and watercress length and a linear relationship between nutrient solution and watercress fresh weight. Plants grown in half-strength Hoagland's nutrient solution were significantly longer than

those grown in the full-strength solution and the control (de-ionized water). Plants grown in the full-strength solution were significantly longer than those grown in the control. Plants grown in the half- and full-strength solutions weighed significantly more than those grown in the control, but there was no significant difference between fresh weights of plants grown in the half- and full-strength solutions (Table 1).

Plants were significantly longer and weighed significantly more when grown under intermediate light intensities (450 ± 10 PAR) than those grown under low light intensities (50 ± 10 PAR) because there was more PAR available for plants to use for photosynthesis. Pushak (1997) reported that aquatic plants have a saturation range between 300 to 1000 µmol m^{-2} s^{-1}, with a good target range between 200 to 500 µmol m^{-2} s^{-1} and the intermediate PAR value used in this experiment falls within that range. Pushak (1997) also reported that light intensities below 100 µmol m^{-2} s^{-1} are considered low light and aquatic plants have a minimum compensation point required between 15 to 85 µmol m^{-2} s^{-1} to stay alive. The low PAR value used in this experiment falls within that range, so it was enough to keep the plants alive, but photosynthesis was reduced resulting in poor growth exhibited by plants that were significantly shorter and weighed significantly less than plants in the intermediate PAR treatment.

Plants in this experiment continued to grow for the duration of the experiment, apparent by the linear increase in length and fresh weight over time, although not always significant between samplings.

Seelig (1974) reported that watercress is not considered to have a high nutrient demand. The half-strength Hoagland's nutrient solution resulted in significantly longer plants for both PAR (50 and 450 ± 10) treatments; however there was no significant

Table 1 Effects of Light intensity, Sample Date, and Nutrient Solution on Watercress Length and Fresh Weight in a Controlled Environment

Treatment	Length (cm)[2,3,4]	Fresh Weight (mg)[2,3,4]	N
Light intensity			
Low (50 ± 10 PAR)	10.43 a	683 a	27
Intermediate (450 ± 10 PAR)	17.94 b	8432 b	27
Significance[1]	*	**	
Sample Date (# of weeks)			
7/24/2006 (2)	5.58 a	76 a	18
8/7/2006 (4)	13.47 ab	1021 a	18
8/21/2006 (6)	23.50 b	12577 b	18
Linear	***	***	
Quadratic	ns	ns	
Significance[1]	***	***	
Nutrient Solution			
Full-strength Hoagland's	17.31 a	7844 a	18
Half-strength Hoagland's	25.25 b	5830 a	18
Control (de-ionized water)	0.00 c	0.00 b	18
Linear	***	**	
Quadratic	***	*	
Significance[1]	***	**	

[1]ns=not significant, * = significant at 5% level, ** = significant at 1% level, *** = significant at 0.1% level;

[2]Means based on whole plant (root and shoots) samples; means represent a single plant

[3]Means transformed for analysis using (y+0.5)^0.5; means reported are non-transformed

[4]Means in a column followed by the same letter are not significant from each other according to Tukey's Studentized (HSD) Range test

difference between plants in the half- and full-strength solutions regarding fresh weight.

Greater plant lengths in the half-strength solution early on suggests that the nutrient solution was providing essential nutrients for seedling root establishment and initial stem elongation without presenting a nutrient overload to the young plants. This provided plants grown in half-strength solution treatments with a head start in length. As plants continued to grow, the full-strength solution became more desirable for watercress nutritional demands, allowing plants in these treatments to catch up with plants in the half-strength solution which suggests why no significant difference for fresh weight occurred between these treatments.

Figure 4 represents the interaction of light intensity and nutrient solution concentration (non significant interaction) on watercress length at Week 6 only. Figure 5 represents the significant ($P < 0.05$) interaction of light intensity and nutrient solution concentration on watercress dry weight at Week 6 only. These figures serve as a reference for light intensity and nutrient solution concentration data between the Spring 2006 - Location Comparison aquaponic experiment growth data at Week 6 (below) and the hydroponic experiment growth data at Week 6.

Nutrient Data

Light intensity and nutrient solution concentration had significant effects on watercress total nitrogen (N) content (Appendix 3). Plants in intermediate PAR treatments had significantly more N in dry tissue than those in low PAR treatments. There was a linear relationship between nutrient solution and watercress total N content. Plants grown in full-strength Hoagland's nutrient solution had significantly

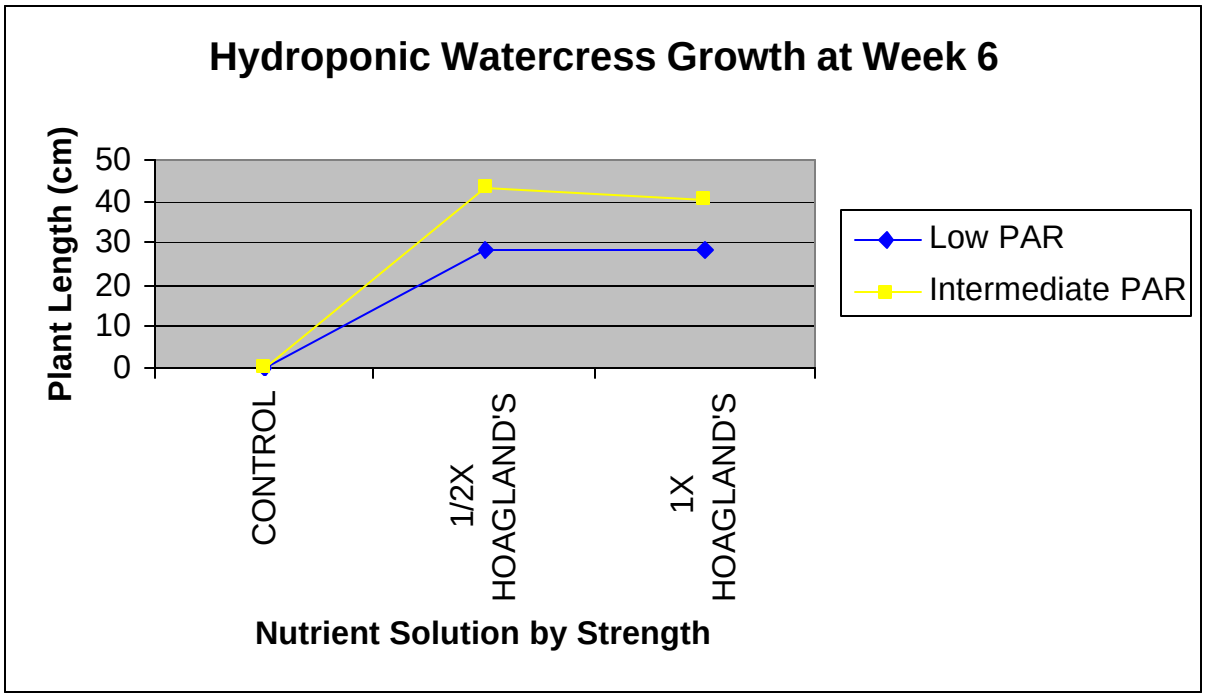

Figure 4. Hydroponic Watercress Growth at Week 6 - Length

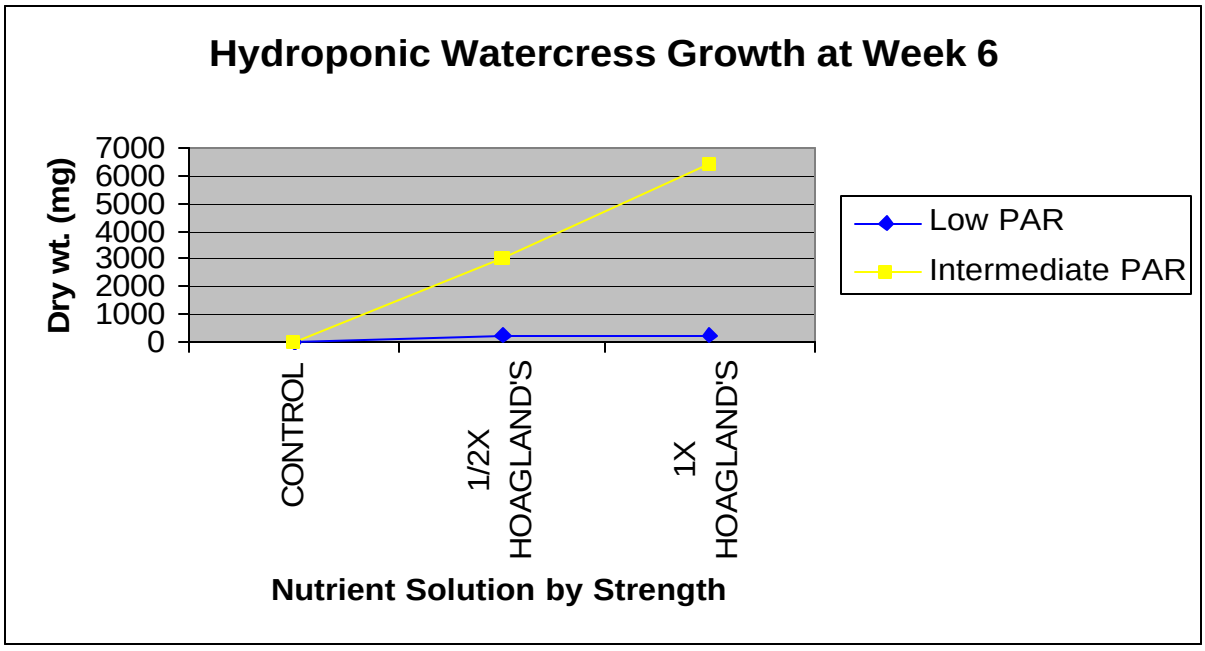

Figure 5. Hydroponic Watercress Growth at Week 6 – Dry Weight

more N in dry tissue than those grown in the control. Plants grown in the half-strength solution were not significantly different from either the full-strength solution or control with regard to N content (Table 2).

There was no significant difference in phosphorus (P) content among light intensity or nutrient solution concentration treatments in the hydroponic experiment (Appendix 4).

The intermediate PAR treatment produced plants with significantly more N in dry tissue than those grown under low PAR because plants were able to photosynthesize more, resulting in greater nutrient uptake. As expected, plants in the control treatments did not survive in either light treatment due to the absence of nutrients in the de-ionized water. The full-strength solution contained significantly more N in dry tissue than the control because N was actually present and available for uptake.

Mills et al. (1996) reported that watercress' sufficiency range for N is 4.2 to 6.0%. Based on %N means in Table 2, the sufficiency range was never achieved in any light intensity or nutrient solution treatment in this hydroponic experiment. The reported sufficiency range may not be an accurate comparison for watercress N contents in this system because the range came from analysis of new leaves sampled in the middle of the growing season. Samples used for analysis in this research came from whole plants, including roots and shoots, sampled at six weeks which could have affected the means and does not truly allow for a direct comparison with the reported sufficiency ranges. Janick (1986) reported that typical plant N contents are 2.5 to 4.5% of the dry weight for fully expanded leaves, which is lower than the values quoted by Mills. However, N contents in this experiment did not fall within this range either.

Table 2 Effects of Light intensity and Nutrient Solution on Watercress Total Nitrogen Content in a Controlled Environment

Treatment	%N[2,3,4]	N
Light Intensity		
Low (50 ± 10 PAR)	0.60 a	9
Intermediate (450 ± 10 PAR)	1.94 b	9
Significance[1]	*	
Nutrient Solution		
Full-strength Hoagland's	2.29 a	6
Half-strength Hoagland's	1.52 ab	6
Control (de-ionized water)	0.00 b	6
Significance[1]	*	
Linear	**	
Quadratic	ns	

[1]ns = not significant, * = significant at the 5% level, ** = significant at the 1% level, *** = significant at the 0.1% level

[2]Means based on whole plant (roots and shoots) samples; means represent a composite sample of three plants

[3]Means were transformed for analysis using (y+0.5)^0.5; means reported are non-transformed

[4]Means in a column followed by the same letter are not significant from each other according to Tukey's Studentized Range (HSD) test

Aquaponic Experiments

Three, 12-week long, aquaponic experiments were conducted. The first experiment was conducted during the summer (June to September) of 2005 in the Aquaculture Research Facility (ARF). The second and third experiments were conducted during the winter (December to March) and spring (March to June) of 2006 in the Aquaponic Production Greenhouse (APG).

Whole plant length, fresh weight, and dry weight data were collected and recorded for all experiments. Whole plant length and dry weight means were used for statistical analysis.

During the Summer 2005 experiment in the ARF, there was an aphid infestation on some of the plants in several rafts in various channels at Week 9. A 20:1 horticultural soap:water solution was made and applied to infested plants to suffocate the aphids and prevent further damage.

By Week 12, there was no longer an aphid issue; however, a cabbage worm (*Pieris rapae* (Linneaus)) infestation was discovered. The cabbage worm larva had defoliated some of the plants in several rafts in various channels, so pest damage was random, but primarily within the first replication. Some plants were not able to be sampled due to damage from both pests, but no raft lost all of its plants and Week 12 signified the end of the experiment, so this did not become a major experimental issue.

Pests did not become an issue or affect data collection in the APG during the second and third aquaponic experiments. This was probably due to lower seasonal ambient air temperatures, the fine mesh screen that was installed on the sides of the

greenhouse, and less weed establishment within the greenhouse versus the ARF.

Summer 2005 (ARF)

Growth Data

Water velocity, growing media, and sample date had significant effects on watercress length and dry weight (Appendices 5 & 6, respectively). There was a linear relationship between water velocity and watercress length and dry weight. Plants were significantly longer and weighed significantly more in the medium (0.30 cm s^{-1}) and high (0.61 cm s^{-1}) velocity treatments than the low (0.061 cm s^{-1}) velocity treatments. There was no significant difference between length and dry weight in the medium and high velocity treatments (Table 3).

There was a quadratic relationship between growing medium and watercress length and dry weight. For plant length, the paper medium produced significantly longer plants than the rockwool and oasis media. The rockwool medium produced significantly longer plants than the oasis medium. For dry weight, there was no significant difference between plants grown in the paper and rockwool media, but plants grown in these media weighed significantly more than plants grown in oasis medium (Table 3).

Replication (Rep) had a significant effect on watercress dry weight (Appendix 6). There was a linear relationship between replication and watercress dry weight. Plants in Rep 1 treatments weighed significantly less than plants in Rep 3 treatments, but plants in Rep 2 treatments were not significantly different from plants in either Rep 1 or Rep 3 treatments with regard to dry weight (Table 3).

There was a linear relationship between sample date and watercress length and

dry weight. A significant increase in length and dry weight occurred between samplings at Weeks 6, 9, and 12 (Table 3).

The higher velocity treatments allowed for plant roots to be exposed to greater amounts of nutrients and potentially more dissolved oxygen and resulted in significantly longer plants that weighed significantly more than those in low velocity treatments (Appendix 7

The paper medium treatments resulted in significantly longer plants than the rockwool and oasis media treatments because plant roots were able to penetrate the thin paper barrier easier, which provided greater contact of plant roots with the effluent earlier in the plants life cycle, giving plants a head start on elongation. Plants grown in rockwool were longer than those grown in oasis because plant roots were able to penetrate through the rockwool fibers better than the dense oasis for greater contact of plant roots with the effluent. Watercress grown on the oasis medium were not able to easily penetrate the dense texture of the medium, resulting in severely limited root exposure to the effluent which caused significantly reduced plant growth as compared to the other treatments. The paper and rockwool media were not significantly different with regard to dry weight which indicates that plants in the rockwool medium were able to accumulate as much biomass as plants in the paper medium. The absorbency of the rockwool may have provided an increased reserve of nutrients to the plants despite less contact of roots with the effluent.

The significance of replication (Rep) regarding mean dry weight corresponds to the aphid and cabbage worm infestations observed during the last two samplings of this

Table 3 Effects of Water Velocity, Growing Media, Replication, and Sample Date on Watercress Length & Dry Weight – Summer 2005 (ARF)

Treatment	Length (cm)[2,3]	Dry Weight (mg)[2,3]	N
Velocity (cm s^{-1})			
Low (0.06)	34.31 a	90.74 a	81
Medium (0.30)	41.04 b	143.09 b	81
High (0.61)	44.72 b	171.73 b	81
Significance[1]	***	***	
Linear	***	***	
Quadratic	Ns	ns	
Media			
Paper	62.63 a	212.72 a	81
Rockwool	53.19 b	182.84 a	81
Oasis	4.25 c	10.00 b	81
Significance[1]	***	***	
Linear	***	***	
Quadratic	***	***	
Replication #			
1	36.62 a	110.12 a	81
2	41.82 a	134.20 ab	81
3	41.63 a	161.23 b	81
Significance[1]	Ns	*	
Linear	Ns	***	
Quadratic	Ns	ns	
Sample Date (# of weeks)			
8/5/2005 (6)	19.27 a	21.73 a	81
8/26/2005 (9)	42.69 b	134.69 b	81
9/16/2005 (12)	58.12 c	249.14 c	81
Significance[1]	***	***	
Linear	***	**	
Quadratic	Ns	ns	

[1]ns = not significant, * = significant at 5% level, ** = significant at 1% level, *** = significant at the 0.1% level

[2]Means based on whole plant (roots and shoots) samples; Means represent a single plant

[3]Means in a column followed by the same letter are not significant from each other according to Tukey's Studentized Range (HSD) test

experiment. Rep 1 was affected the most by the infestations than any other replication. Rep 2 was affected by the infestations more than Rep 3, which was the replication least affected by the pests. No significant difference was seen in length because stems were left intact and upright, but heavy defoliation occurred during the last sampling, which contributed to the reduction in dry weights, particularly in Rep 1.

The significant increase in watercress length and dry weight at each sampling indicates a positive growth curve.

Nutrient Data

Water velocity, growing media, and sample date had significant effects on watercress total N content (Appendix 8). There was a linear relationship between water velocity and watercress N content. Plants grown in medium and high velocity treatments had significantly more N in dry tissue than those grown in low velocity treatments, but there was no significant difference between medium and high velocity treatments with regard to N content (Table 4).

There was a quadratic relationship between growing media and watercress total N content. Plants grown in rockwool and paper media had significantly more N in dry tissue than those grown in oasis medium. There was no significant difference between paper and rockwool media regarding N content (Table 4).

Plants sampled at Week 9 had significantly less N in dry tissue than those sampled at Week 12 (Table 4).

The factorial interaction between water velocity and growing media treatments had a significant effect on watercress total P content (Appendix 9). Plants grown in high

Table 4 Effects of Water Velocity, Growing Media, and Sample Date on Watercress Total Nitrogen Content - Summer 2005 (ARF)

Treatment	%N[2,3]	N
Velocity (cm s^{-1})		
Low (0.06)	1.36 a	54
Medium (0.30)	1.85 b	54
High (0.61)	1.78 b	54
Significance[1]	*	
Linear	*	
Quadratic	ns	
Media		
Paper	2.43 a	54
Rockwool	2.41 a	54
Oasis	0.15 b	54
Significance[1]	***	
Linear	***	
Quadratic	***	
Sample Date (# of weeks)		
8/26/2005 (9)	1.27 a	81
9/16/2005 (12)	2.05 b	81
Significance[1]	***	

[1]ns = not significant, * = significant at the 5% level, ** = significant at the 1% level, *** = significant at the 0.1% level

[2]Means based on whole plant (roots and shoots) samples; means represent a composite sample of three plants

[3]Means in a column followed by the same letter are not significant from each other according to Tukey's Studentized Range (HSD) test

velocity treatments on paper medium had significantly more P in dry tissue than plants grown in low velocity treatments on rockwool medium. All velocity and oasis medium treatment combinations had significantly less P in dry tissue than any other treatment combination. All other velocity and paper or rockwool media treatment combinations were not significantly different from each other (Table 5).

Effluent concentrations of N and P that entered all experimental channels were based on raceway tailbox inorganic N and P water quality measurements quantified by environmental engineers working on this project (Dyer, 2006). Water samples were not taken at the inflow of each channel, so the concentration of effluent entering each channel was assumed to be the same for all treatments. However, within each channel, the faster velocities provided plants with more N and P in the same amount of time (mg/L/3wks) as the low velocity treatments (Appendix 7). Seelig (1974) reported that N content of the water source and water flow are both important considerations in aquatic plant production. Since N contents of the effluent were low, a larger flow was required to meet nutritional demands. This supports why watercress N contents were greater in the medium and high velocity treatments.

Janick (1986) reported that roots must be supplied with oxygen in all hydroponic systems. Logically, the faster velocities would have provided plants with more dissolved oxygen, although not part of the water quality measurements. Warm water cannot hold as much dissolved oxygen as cold water. Water temperatures in the low velocity channels were several degrees warmer than those in the higher velocity channels, which supports the theory that less oxygen was available in the low velocity treatments. Low velocity channels also resulted in less watercress growth, which allowed for growth

Table 5 Effects of the Factorial Combination of Water Velocity and Growing Media on Watercress Total Phosphorus Content – Summer 2005 (ARF)

Treatment Combination (3 x 3)		%P[2,3]	N
Velocity (cm s^{-1})	Media		
Low (0.06)	Paper	0.58 ab	18
	Rockwool	0.35 b	18
	Oasis	0.00 c	18
Medium (0.30)	Paper	0.62 ab	18
	Rockwool	0.58 ab	18
	Oasis	0.03 c	18
High (0.61)	Paper	0.63 a	18
	Rockwool	0.62 ab	18
	Oasis	0.03 c	18
Significance[1]		**	

[1]ns = not significant, * = significant at the 5% level, ** = significant at the 1% level, *** = significant at the 0.1% level

[2]Means based on whole plant (roots and shoots) samples; means represent a composite sample of three plants

[3]Means in a column followed by the same letter are not significant from each other according to t-test for paired comparisons, where t = 0.27

of undesirable species like algae and duckweed, which could have contributed to the eutrophic conditions this research aimed to avoid.

Plants grown in the paper and rockwool media contained significantly more N in their tissue than the oasis medium because the seedlings' roots were able to penetrate the thin paper barrier and rockwool fibers easier than the dense oasis cubes for greater access to N and oxygen present in the effluent for nutrient uptake.

The significant increase in N content from Week 9 to Week 12 indicates that plants continued to uptake N between these samplings. Based on reported sufficiency ranges for watercress N content, %N means for this experiment do not fall within that range or the reported typical plant N range. This suggests that the effluent did not contain enough N to meet watercress' N requirement and indicates the potential for N deficiency according to the cultural conditions in the ARF in the Summer 2005 experiment. There were no obvious signs of nutrient deficiency. The only deficiency observed was etiolation due to the low light intensity within the ARF.

The factorial interaction favoring the high water velocity and paper medium treatment combination for significantly greater watercress P contents also relates to higher P concentrations present in high velocity channels and greater access of plant roots with the effluent due to the thin paper barrier. The paper media is also more cost effective for the grower than rockwool or oasis and it's bio-degradable. Mills et al. (1997) reported sufficiency range for watercress P content is 0.7 to 1.3%. Mean values for %P did not fall within this reported range; however, Janick (1986) reported that the optimum leaf P concentration of a typical plant is 0.2 to 0.3%. The mean %P contents for the factorial combinations of medium and high velocities and paper and rockwool

media in this experiment were above this range. The effluent may or may not have contained enough P to meet watercress' P requirement and may or may not have been P limiting, depending on which range is considered acceptable. No obvious signs of P deficiency were observed and the only deficiency observed was etiolation due to the low light intensity within the ARF.

The sufficiency ranges reported by Mills et al. (1996) may not be an accurate comparison for watercress nutrient contents in this system because the ranges came from analysis of new leaves sampled in the middle of the growing season. Samples used for analysis in this research came from whole plants, including roots and shoots, and were sampled throughout the growing season which could have affected the means and does not truly allow for a direct comparison with the reported sufficiency ranges. It is likely that the reported watercress ranges came from commercially grown watercress that was heavily fertilized contributing to the higher ranges given for N and P.

Insufficient watercress nutrient contents could also be attributed to nutrients settling out in the channels before watercress is able to use them. One suggestion is to lower the standpipe to 7.62 cm (3 in), which is slightly higher than commercial depth, so roots are able to take advantage of the nutrients which may be present in solids at the bottom of the channels. Another option to ensure that sufficient nutrient requirements are met would be the addition of supplemental nutrients, preferably in the form of a water soluble organic fertilizer or from the application of solids removed from the quiescent zones during cleaning. This could potentially improve growth and even reduce the amount of time required to achieve a harvestable crop. In contrast, if watercress did not utilize all of the supplemental nutrients, then nutrient loading of the

environment could result, which is a conflict of interest since this is the issue that's trying to be avoided in the first place.

Potential nutrient loading via the addition of supplemental nutrients is a delicate situation that needs to be addressed and considered carefully. Local residents have been harvesting and consuming watercress from natural springs in the area where this research occurred for generations and these plants are not intentionally exposed to supplemental fertilizer. Watercress grown in aquaculture effluent should have the added benefit of higher nutrient concentrations than if grown in spring water alone. The added production costs and environmental risks associated with fertilizer additions probably would not improve the system as a whole, especially since watercress is cited as not having a high nutrient demand (Seeling, 1974).

Summer 2005 (ARF) - Control Comparison

This comparison looks at the control channels placed at the headbox of the raceway (containing spring water only) and channels from each replication in the main experimental channels (containing aquaculture effluent) that contained only the medium plant density treatments.

Growth Data

Water velocity, growing media, and sample date had significant effects on watercress length and dry weight (Appendices 10 and 11, respectively).

There was a linear relationship between water velocity and watercress length and dry weight in both the control and main channels. Plants were significantly longer

and weighed significantly more in high velocity versus low velocity treatments. There was no significant difference in watercress length or dry weight between medium velocity and either low or high velocity treatments (Table 6).

There was a quadratic relationship between growing media and watercress length and a linear relationship between growing media and watercress dry weight in both the control and main channels. Plants were significantly longer and weighed significantly more in the paper and rockwool media versus the oasis medium, but there was no significant difference between the paper and rockwool media regarding length or dry weight (Table 6).

There was a linear relationship between sample date and watercress length and dry weight in both the control and main channels. There was a significant increase in watercress length and dry weight between samplings at Weeks 6, 9, and 12 (Table 6).

The objective of this comparison was to see if there was a difference in the growth of plants exposed to effluent (main channels) and those that were not (control channels), which is distinguished by 'Rep' in the data. In Appendices 10 and 11, you can see that 'Rep' was not significant for length or dry weight. Even though length and dry weight means were greater for plants exposed to effluent, they were not significantly different from the length and dry weight means of plants grown in spring water only. As mentioned in the literature review (aquaculture section), flow-through systems typically have lower nutrient concentrations than pond or re-circulating systems. The lack of significance between plants grown in effluent versus spring water in this system, which is small in comparison to other aquaculture systems, suggests that watercress grows as well in the spring water as it does in aquaculture effluent. This may not be

Table 6 Effects of Water Velocity, Growing Media, and Sample Date on Watercress Length and Dry Weight - Summer 2005 (ARF) – Control Comparison

Treatment	Length (cm)[2,3]	Dry Weight (mg)[2,3]	N
Velocity (cm s^{-1})			
Low (0.06)	28.93 a	72.50 a	36
Medium (0.30)	37.53 ab	148.89 ab	36
High (0.61)	43.25 b	195.28 b	36
Significance[1]	**	**	
Linear	***	***	
Quadratic	ns	ns	
Media			
Paper	56.46 a	234.72 a	36
Rockwool	47.95 a	170.56 a	36
Oasis	5.29 b	11.39 b	36
Significance[1]	***	***	
Linear	***	***	
Quadratic	***	ns	
Sample Date (# weeks)			
8/5/2005 (6)	17.23 a	23.33 a	36
8/26/2005 (9)	39.93 b	138.33 b	36
9/16/2005 (12)	52.55 c	255.00 c	36
Significance[1]	***	***	
Linear	***	***	
Quadratic	ns	ns	

[1]ns=not significant, * = significant at 5% level, ** = significant at 1% level, *** = significant at 0.1% level

[2]Means based on whole plant (roots and shoots) samples; means represent a single plant

[3]Means in a column followed by the same letter are not significant from each other according to Tukey's Studentized Range (HSD) test

true for larger systems that may produce effluent with significantly higher nutrient concentrations than the source water.

The significant increase in watercress length and dry weight at each sampling indicates a positive growth curve.

Nutrient Data

Growing media and sample date had significant effects on watercress total N content (Appendix 12). There was a quadratic relationship between growing media and watercress total N content in both the control and main channels. Plants in the paper and rockwool media treatments had significantly more N in dry tissue than those in the oasis medium treatments, but were not significantly different from each other with regard to N content. Plants sampled in both the control and main channels at Week 9 had significantly less N in dry tissue than those sampled at Week 12 (Table 7).

Water velocity and growing media had a significant effect on watercress total P content (Appendix 13). There was a linear relationship between water velocity and watercress total P content in both the control and main channels. Plants grown in high velocity treatments had significantly more P in dry tissue than those grown in low velocity treatments. Plants grown in medium velocity treatments were not significantly different from either the low or high velocity treatments with regard to P content. There was a quadratic relationship between growing media and watercress total P content in both the control and main channels. Plants grown in paper and rockwool media had significantly more P in dry tissue than those grown in oasis medium. There was no

significant difference in P content between paper and rockwool media treatments (Table 7).

Similar to the growth results, the objective of this comparison was to see if there was a difference in the nutrient contents of watercress in treatments exposed to effluent (main channels) and those that were not (control channels), which is distinguished by 'Rep' in the data. In Appendices 12 and 13, 'Rep' is not significant. Although watercress grown in the main channels contained slightly more N and P in dry tissue than those grown in the control channels, these amounts were not significant. This indicates that the nutrient contribution of the effluent is insignificant because watercress N and P contents were approximately the same whether grown in spring water or in effluent.

Water quality data provided by the project environmental engineers also supports this finding. Nutrient concentrations of the spring water sampled in the headbox prior to trout production were very dilute (less than 1 mg/L (1ppm) N and P) and concentrations remained dilute in samples taken from the tailbox after production. Only small increases in N and P concentrations, based on water quality testing, were seen in the tailbox and some results even showed a decline or no change at all in nutrient concentrations after fish production (Dyer, 2006).

Thus, based on this comparison, the function and purpose of this integrated system becomes water re-use and production of a secondary marketable crop versus nutrient recovery. Watercress is able to recover nutrients from this system, but since the amount of nutrients in the effluent is insignificant, the threat of nutrient loading and associated environmental impacts is unlikely from this aquaculture system as is.

Table 7 Effects of Water Velocity, Growing Media, and Sample Date on Watercress Total Nitrogen and Phosphorus Content – Summer 2005 (ARF) – Control Comparison

Treatment	%N[2,3]	%P[2,3]	N
Velocity (cm s^{-1})			
Low (0.06)	1.18 a	0.29 a	24
Medium (0.30)	1.81 a	0.36 ab	24
High (0.61)	1.63 a	0.43 b	24
Significance[1]	ns	*	
Linear	ns	**	
Quadratic	ns	ns	
Media			
Paper	1.96 a	0.56 a	24
Rockwool	2.43 a	0.50 a	24
Oasis	0.22 b	0.02 b	24
Significance[1]	***	***	
Linear	***	***	
Quadratic	***	***	
Sample Date (# weeks)			
8/26/2005 (9)	1.26 a	0.35 a	36
9/16/2005 (12)	1.82 b	0.37 a	36
Significance[1]	*	ns	

[1]ns = not significant, * = significant at the 5% level, ** = significant at the 1% level, *** = significant at the 0.1% level

[2]Means based on whole plant (roots and shoots) samples; means represent a composite sample of three plants

[3]Means in a column followed by the same letter are not significant from each other according to Tukey's Studentized Range (HSD) test

Winter 2006 (APG)

This experiment and the Spring 2006 experiment study of the effects of low and high water velocities and low and high plant densities on watercress growth and nutrient contents in the Aquaponic Production Greenhouse (APG).

Growth Data

Sample date had a significant effect on watercress length (Appendix 14). There was a quadratic relationship between sample date and watercress length. Plant length significantly increased between samplings at Weeks 6, 9, and 12 (Table 8).

Water velocity and sample date had significant effects on watercress dry weight (Appendix 15). Plants grown in high velocity treatments weighed significantly more than those grown in low velocity treatments. There was a linear relationship between sample date and watercress dry weight. Dry weight significantly increased between samplings at Weeks 6, 9, and 12 (Table 8).

Seelig (1974) reported that watercress grows best in flowing water. The high velocity studied in all of the aquaponic experiments had a flow rate about ten times greater than the low velocity studied. This allowed for plant roots to be exposed to greater amounts of nutrients and oxygen in high velocity treatments in the same amount of time as those in low velocity treatments and resulted in plants that weighed significantly more (Appendix 7). Since it was winter and ambient air temperatures were colder, effluent in the high velocity channels probably insulated plants better than in the low velocity channels because the greater flows kept the water in the high velocity channels from freezing.

Table 8 Effects of Water Velocity and Sample Date on Watercress Length and Dry Weight - Winter 2006 (APG)

Treatment	Length (cm)[2,3]	Dry Weight (mg)[2,3]	N
Velocity (cm s^{-1})			
Low (0.06)	14.55 a	209.20 a	54
High (0.61)	16.32 a	302.35 b	54
Significance[1]	ns	*	
Sample Date (# of weeks)			
1/22/2006 (6)	0.51 a	1.57 a	36
2/19/2006 (9)	18.48 b	187.04 b	36
3/4/2006 (12)	27.21 c	578.70 c	36
Significance[1]	***	***	
Linear	***	***	
Quadratic	***	*	

[1]ns=not significant, * = significant at 5% level, ** = significant at 1% level, *** = significant at 0.1% level

[2]Means based on whole plant (root and shoots) samples; means represent a single plant

[3]Means in a column followed by the same letter are not significant from each other according to Tukey's Studentized Range (HSD) Test

The significant increase in watercress length and dry weight at each sampling indicates a positive growth curve.

Nutrient Data

Sample date had a significant effect on watercress total N content (Appendix 16). There was a significant increase in watercress total N content between samplings (Table 9).

There was no significant difference in total P content among any treatments in the Winter 2006 experiment (Appendix 17).

The significant increase in N content from Week 9 to Week 12 indicates that plants continued to uptake N between these samplings. The mean total N content at Week 12 (4.33 %N) fell within the sufficiency ranges reported by Mills et al. (2006) and Janick (1986), which suggests that N concentrations of the effluent during this time may be sufficient in meeting watercress' N requirement. Again, the reported sufficiency ranges for watercress specifically may not serve as an accurate comparison with this experiment due to the different types of tissue sampled and the different life stages of watercress at the time of sampling.

Winter 2006 – Location Comparison

Since space limitations in the APG did not allow for a control bed (containing spring water only), a bed was set up in the ARF to study the effect of location during the Winter and Spring 2006 experiments to determine if light intensity was significant. This comparison studies the factorial combination of low water velocity and high plant density

Table 9 Effect of Sample Date on Watercress Total Nitrogen Content - Winter 2006 (APG)

Treatment	%N[2,3]	N
Sample Date		
2/19/2006 (9)	2.50 a	36
3/4/2006 (12)	4.33 b	36
Significance[1]	***	

[1]ns = not significant, * = significant at the 5% level, ** = significant at the 1% level, *** = significant at the 0.1% level

[2]Means based on whole plant (roots and shoots) samples; means represent a composite sample of three plants

[3]Means in a column followed by the same letter are not significant from each other according to Tukey's Studentized Range (HSD) Test

treatments only on watercress growth and nutrient contents in the ARF (low PAR) versus the APG (intermediate PAR) in the Winter 2006 experiment.

Growth Data

Location and sample date had significant effects on watercress length and dry weight (Appendix 18 and 19, respectively). Plants grown in the APG were significantly longer and weighed significantly more than those grown in the ARF. There was a linear relationship between sample date and watercress length and dry weight. Plant length and dry weight significantly increased between samplings at Weeks 6, 9, and 12 (Table 10).

Plants grown in the APG location had a significant increase in length and dry weight because the greenhouse provided greater light intensities and ambient air temperatures than the ARF which aided photosynthesis and promoted growth. PAR values and ambient air temperatures for the APG and the ARF can be found in Dyer (2006). Plants did not grow at all in the ARF during the winter which resulted in zeroes for growth. which is attributed to lower light intensities and ambient air temperatures during the winter.

The significant increase in watercress length and dry weight at each sampling indicates a positive growth curve for plants grown in the APG.

Nutrient Data

Location and sample date had significant effects on watercress total N content (Appendix 20). Plants grown in the APG had significantly more N in dry tissue than

Table 10 Effects of Experiment Location and Sample Date on Watercress Length and Dry Weight - Winter 2006 - Location Comparison

Treatment	Length (cm)[2,3,4]	Dry Weight (mg)[2,3,4]	N
Location			
ARF	0.00 a	0.00 a	27
APG	14.79 b	207.41 b	27
Significance[1]	***	***	
Sample Date (# of weeks)			
1/22/2006 (6)	0.67 a	0.93 a	18
2/19/2006 (9)	9.09 b	72.59 a	18
3/4/2006 (12)	12.43 b	237.59 b	18
Significance[1]	***	***	
Linear	***	***	
Quadratic	ns	ns	

[1]ns=not significant, * = significant at 5% level, ** = significant at 1% level, *** = significant at 0.1% level

[2]Means based on whole plant (root and shoots) samples; means represent a single plant

[3]Means in a column followed by the same letter are not significant from each other according to Tukey's Studentized Range (HSD) Test

[4]Means transformed for analysis using (y+0.5)^0.5; means reported are non-transformed

those grown in the ARF. Watercress total N content significantly increased between samplings at Weeks 9 and 12 (Table 11).

Location had a significant effect on watercress total P content (Appendix 21). Plants grown in the APG had significantly more P in dry tissue than those grown in the ARF (Table 11).

Nutrient contents were significantly greater in the APG versus the ARF due to higher PAR values present in the APG that promoted photosynthesis, growth, and nutrient uptake at that location. Plants did not grow at all in the ARF during the winter resulting in zeroes for nutrient contents, which is attributed to low light intensities and air temperatures during the winter which prevented germination. The mean %N of plants grown in the APG did not fall within the reported sufficiency range for watercress, but did fall within the typical plant N range. which suggests that effluent N concentrations may or may not have been limiting during the winter in the APG depending which range is considered acceptable. The mean %P of plants grown in the APG did fall within the reported sufficiency range for watercress and was above the typical plant range which suggests that effluent P concentrations were sufficient during the winter in the APG.

The significant increase in N content from Week 9 to Week 12 indicates that plants continued to uptake N between these samplings in the APG.

Spring 2006 (APG)

Growth Data

Water velocity, plant density, and sample date had significant effects on watercress plant length (Appendix 22). Plants grown in high velocity treatments were

Table 11 Effects of Experiment Location and Sample Date on Watercress Total Nitrogen and Phosphorus Content - Winter 2006 - Location Comparison

Treatment	%N[2,3,4]	%P[2,3]	N
Location			
ARF	0.00 a	0.00 a	18
APG	3.44 b	0.72 b	18
Significance[1]	***	***	
Sample Date (# of weeks)			
2/19/2006 (9)	1.25 a	0.37 a	18
3/4/2006 (12)	2.19 b	0.35 a	18
Significance[1]	*	ns	

[1]ns = not significant, * = significant at the 5% level, ** = significant at the 1% level, *** = significant at the 0.1% level

[2]Means based on whole plant (roots and shoots) samples; means represent a composite sample of three plants

[3]Means in a column followed by the same letter are not significant from each other according to Tukey's Studentized Range (HSD) Test

[4]Means transformed for analysis using (y+0.5)^0.5; means reported are non-transformed

significantly longer than those grown in low velocity treatments. Plants grown in high density treatments were significantly longer than those grown in low density treatments. There was a quadratic relationship between sample date and watercress length. Plant length significantly increased between samplings at Weeks 6, 9, and 12 (Table 12).

Water velocity and sample date had significant effects on watercress dry weight (Appendix 23). Plants grown in high velocity treatments weighed significantly more than those grown in low velocity treatments. There was a linear relationship between sample date and watercress dry weight. Plants sampled at Week 12 weighed significantly more than plants sampled at Weeks 6 and 9, however there was no significant difference in dry weight between plants sampled at Weeks 6 and 9 (Table 12).

The high velocity treatments allowed for plant roots to be exposed to greater amounts of nutrients and potentially more dissolved oxygen which resulted in plants that were significantly longer and weighed significantly more (Appendix 7).

Plants in high density treatments were significantly longer because of a greater leaf area index in the upper leaf canopy which provided a greater area for photosynthesis and subsequent elongation to occur. High density treatments were also more efficient than low density treatments for this system because they took advantage of the entire available growing area. Seelig (1974) reported that maintaining a high plant density aids in weed (i.e. algae, duckweed) reduction in watercress production. Reducing weeds would also decrease nutrient competition and potential oxygen depletion. Another aquaponic study also found that the highest okra production was found at a high plant density (Rakocy et al, 2004).

Table 12 Effects of Water Velocity, Plant Density, and Sample Date on Watercress Length and Dry Weight - Spring 2006 (ARF)

Treatment	Length (cm)[2,3]	Dry Weight (mg)[2,3]	N
Velocity (cm s^{-1})			
Low (0.06)	16.45 a	126.50 a	54
High (0.61)	26.97 b	619.20 b	54
Significance[1]	***	***	
Density (#plants/cm^2)			
Low (0.02)	17.69 a	276.30 a	54
High (0.08)	25.74 b	469.40 a	54
Significance[1]	***	ns	
Sample Date (# weeks)			
4/22/2006 (6)	8.36 a	21.60 a	36
5/11/2006 (9)	16.44 b	118.00 a	36
6/2/2006 (12)	40.33 c	979.10 b	36
Significance[1]	***	***	
Linear	***	***	
Quadratic	***	**	

[1]ns=not significant, * = significant at 5% level, ** = significant at 1% level, *** = significant at 0.1% level

[2]Means based on whole plant (root and shoots) samples; means represent a single plant

[3]Means in a column followed by the same letter are not significant from each other according to Tukey's Studentized Range (HSD) Test

The significant increase in watercress length and dry weight throughout the Spring 2006 experiment indicates a positive growth curve, although not always significant for dry weight between samplings.

Nutrient Data

Water velocity and replication (Rep) had significant effects on watercress total N content (Appendix 24). Plants grown in high velocity treatments contained significantly more N in dry tissue than those grown in low velocity treatments. There was a linear relationship between replication and watercress total N content. Plants grown in Rep 1 had significantly more N in dry tissue that those grown in Rep 3. Plants grown in Rep 2 were not significantly different with regard to N content from those grown in Rep 1 or Rep 3 (Table 13).

Water velocity and plant density had significant effects on watercress total P content (Appendix 25). Plants grown in high velocity treatments contained significantly more P in dry tissue than those grown in low velocity treatments. Plants grown in high density treatments contained significantly more P in dry tissue than those grown in low density treatments (Table 13).

The high velocity treatments provided plants with more N and P and dissolved oxygen in the same amount of time as the low velocity treatments (Appendix 7). This led to increased growth and subsequent nutrient uptake in the high velocity versus low velocity treatments. Seelig (1974) reported that N content of the water source and water flow are both important considerations in aquatic plant production. Since N contents of the effluent were low, a larger flow was required to meet nutritional

Table 13 Effects of Water Velocity, Plant Density, and Replication on Watercress Total Nitrogen and Phosphorus Contents - Spring 2006 (APG)

Treatment	%N[2,3]	%P[2,3]	N
Velocity (cm s^{-1})			
Low (0.06)	1.20 a	0.26 a	36
High (0.61)	2.31 b	0.37 b	36
Significance[1]	***	*	
Density (#plants cm^{-2})			
Low (0.02)	1.59 a	0.26 a	36
High (0.08)	1.91 a	0.37 b	36
Significance[1]	ns	*	
Rep #			
1	2.26 a	0.33 a	24
2	1.60 ab	0.32 a	24
3	1.40 b	0.29 a	24
Significance[1]	*	ns	
Linear	*	ns	
Quadratic	ns	ns	

[1]ns = not significant, * = significant at the 5% level, ** = significant at the 1% level, *** = significant at the 0.1% level

[2]Means based on whole plant (roots and shoots) samples; means represent a composite sample of three plants

[3]Means in a column followed by the same letter are not significant from each other according to Tukey's Studentized Range (HSD) Test

demands, which also supports why watercress N contents were greater in the high velocity treatment.

The high plant density treatments resulted in plants with significantly greater P contents because there was less competition for P among undesirable species within those experimental channels. Plants in the high density treatments grew better enabling them to shade out algae and duckweed that would have competed for nutrients and could have also led to eutrophic conditions.

The mean %N and %P contents for this experiment did not fall within the sufficiency ranges reported for watercress, however, the %P contents fell with the P range reported for plants in general. This suggests that the effluent nutrient concentrations in the Spring 2006 experiment were not sufficient to meet watercress' N requirement and the potential for N deficiency existed. The effluent may or may not have been sufficient to meet watercress' P requirement depending on which range is considered acceptable. Plants grown in the APG showed no obvious signs of deficiency and plants reached a harvestable size in the same amount of time a harvest would occur commercially (six weeks). The suggestions listed above under the Summer 2005 experiment, lowering the standpipe and application of supplemental nutrients, are also applied here to potentially achieve watercress N sufficiency range. The same concerns also apply and more research needs to be conducted to determine optimal nutrient recommendations, if any, for this system.

Replication (Rep) was significant regarding N content due to channel spatial arrangement and PAR fluctuations within the APG. PAR was manually measured with the ceptometer mentioned above (hydroponic experiment section) to determine site

specific PAR values within the greenhouse versus general PAR readings obtained from the datalogger. Rep 1 was closer to the east end wall of the greenhouse than Rep 3 and PAR values were higher in this area. Watercress' performance under different light intensities and cultural requirements stated in the literature review (watercress section) suggest that watercress growth would be greater in the higher PAR areas of the greenhouse. This would result in increased N contents in treatments in the high PAR areas due to increased photosynthesis, growth, and nutrient uptake.

Spring 2006 – Location Comparison

This comparison studies the factorial combination of low water velocity and high plant density treatments only on watercress growth and nutrient contents in the ARF (low PAR) versus the APG (intermediate PAR) in the Spring 2006 experiment.

Growth Data

Sample date had a significant effect on watercress length (Appendix 26). There was a quadratic relationship between sample date and watercress length. Plant length significantly increased between samplings at Weeks 6, 9, and 12 (Table 14).

Location and sample date had significant effects on watercress dry weight (Appendix 27). Plants grown in the APG weighed significantly more than those grown in the ARF. There was a linear relationship between sample date and watercress dry weight. Dry weight significantly increased between Week 12 and Weeks 6 and 9, however there was no significant difference in dry weight between Weeks 6 and 9 (Table 14).

Table 14 Effects of Experiment Location and Sample Date on Watercress Length and Dry Weight - Spring 2006 - Location Comparison

Treatment	Length (cm)[2,3]	Dry Weight (mg)[2,3]	N
Location			
ARF	20.98 a	39.88 a	27
APG	22.09 a	179.26 b	27
Significance[1]	ns	***	
Sample Date (# weeks)			
4/22/2006 (6)	5.04 a	14.07 a	18
5/11/2006 (9)	16.19 b	66.11 a	18
6/2/2006 (12)	43.37 c	248.52 b	18
Significance[1]	***	***	
Linear	***	***	
Quadratic	***	ns	

[1]ns=not significant, * = significant at 5% level, ** = significant at 1% level, *** = significant at 0.1% level

[2]Means based on whole plant (root and shoots) samples; means represent a single plant

[3]Means in a column followed by the same letter are not significant from each other according to Tukey's Studentized Range (HSD) Test

Plants grown in the APG location had a significant increase in dry weight because the greenhouse allowed for greater light intensities and ambient air temperatures than the ARF which aided photosynthesis and promoted growth. PAR values and ambient air temperatures for the APG and the ARF can be found in Dyer (2006).

Plants grown in the spring in both locations continued to grow for the duration of the experiment, apparent by the increase in length and dry weight over time, although not always significant for dry weight between samplings.

The Spring 2006 - Location Comparison experiment is the only comparison that can be used to compare aquaponic growth data with hydroponic growth data. Since the hydroponic experiment only ran for six weeks, only data from Week 6 of the Spring 2006 – Location Comparison experiment can be compared because it is the only aquaponic experiment that represents data for both low and intermediate PAR environments and where cultural environmental conditions (photoperiod, ambient air temperature, pH, etc.) were similar to those used in the hydroponic experiment.

At Week 6, length and dry weight means were zero in the ARF (low PAR). Watercress was growing in the ARF at this time, but samples did not meet the sampling criteria (Data Collection and Statistical Analysis section). In reference to Figures 4 and 5, watercress grown in the hydroponic experiment under low PAR was about 20 times longer and weighed about 200 to 300 times more in the half- and full-strength Hoagland's nutrient solutions, respectively, than in effluent at Week 6. This suggests that watercress length and dry weight was greater at Week 6 when grown in a hydroponic nutrient solution versus flow-through aquaculture effluent under low PAR.

At Week 6, length and dry weight means were 10.07 cm and 84.55 mg, respectively, in the APG (intermediate PAR). In reference to Figures 4 and 5, watercress grown in the hydroponic experiment under intermediate PAR was about 4 times longer and weighed about 35 to 75 times more in the half- and full-strength Hoagland's nutrient solution, respectively, than in effluent at Week 6. This suggests that watercress length and dry weight was greater at Week 6 when grown in a hydroponic nutrient solution versus flow-through aquaculture effluent under intermediate PAR.

The greater lengths and dry weights found from plants grown in a hydroponic nutrient solution versus aquaculture effluent suggest that the effluent was nutrient limiting and potentially prevented watercress from reaching its growth potential, thus limiting its ability for effluent nutrient recovery and potentially for a secondary marketable crop. Although plants grew better under intermediate light intensities in a hydroponic nutrient solution versus aquaculture effluent, they may have grown too well and this kind of growth may not be desirable from a commercial perspective. At six weeks, the thicker stems and larger leaves of plants grown in Hoagland's were not as appetizing as plants grown in effluent, which resembled what one would purchase in a market.

Nutrient Data

Location had a significant effect on watercress N and P contents (Appendices 28 and 29, respectively). Plants grown in the APG contained significantly more N and P in

dry tissue than those grown in the ARF (Table 15).

Nutrient contents were significantly greater in the APG versus the ARF due to higher PAR values present in the APG that promoted photosynthesis, growth, and nutrient uptake at that location. Plants did grow in the ARF in the spring experiment, but there was not enough dry tissue to meet the criteria for N analysis, which resulted in zeroes for watercress N content for treatments in the ARF. Dry tissue samples from the ARF were only analyzed for total P content.

The mean %N contents in both locations and %P contents in the ARF for this experiment comparison do not fall within the sufficiency ranges reported for watercress, however mean %P content in the APG did fall with the reported typical plant P range. This suggests that the effluent nutrient concentrations were not sufficient to meet watercress' N requirement, however may or may not have been sufficient in meeting watercress' P requirement, depending on which range is considered acceptable.

The Spring 2006 - Location Comparison experiment is the only comparison that could be used to extrapolate aquaponic nutrient data with hydroponic nutrient data because it is the only aquaponic experiment that represents data for both low and intermediate PAR and where cultural conditions were most similar to those used in the hydroponic experiment. Since the hydroponic experiment only ran for six weeks, only nutrient data from Week 6 of the Spring 2006 aquaponic experiment location comparison can be used here. Since there was not enough dry tissue for analysis (Plant Tissue Analysis section) in any aquaponic experiment at Week 6, aquaponic nutrient data cannot be compared with the hydroponic nutrient data. The lack of dry tissue for analysis at Week 6 also supports that effluent nutrient concentrations were

Table 15 Effect of Experiment Location on Watercress Total Nitrogen and Phosphorus Contents - Spring 2006 - Location Comparison

Treatment	%N[2,3,4]	%P[2,3]	N
Location			
ARF	0.00 a	0.14 a	18
APG	1.48 b	0.35 b	18
Significance[1]	***	**	

[1]ns = not significant, * = significant at the 5% level, ** = significant at the 1% level, *** = significantat the 0.1% level

[2]Means based on whole plant (roots and shoots) samples; means represent a composite sample of three plants

[3]Means in a column followed by the same letter are not significant from each other according to Tukey's Studentized Range (HSD) Test

[4]Means transformed for analysis using (y+0.5)^0.5; means reported are non-transformed

limiting for watercress, thus preventing watercress from reaching its growth potential and limiting its ability for effluent nutrient recovery and potentially for a secondary marketable crop.

Winter v. Spring 2006 – Season Comparison

In addition to the above significant effects reported for water velocity, plant density, and location on watercress growth and nutrient contents in Winter and Spring 2006 separately, an analysis was ran for plants grown in the APG only for Winter 2006 versus Spring 2006 to evaluate the effect of season on watercress growth and nutrient contents.

Season had a significant effect on watercress length, N content, and P content. Plants grown in the APG in Spring 2006 were significantly longer than those grown in the APG in Winter 2006 (Appendix 30). Plants grown in the APG in Winter 2006 had significantly more N and P in dry tissue than those grown in the APG in Spring 2006 (Appendices 31 and 32, respectively).

There are two explanations why plants were significantly longer in Spring 2006. First, the increased day length, higher PAR values, and higher air temperatures in the APG in the spring could have resulted in greater stem elongation. PAR values and ambient air temperatures for the Winter and Spring 2006 experiments can be found in Dyer (2006). Second, the effluent nutrient concentrations were lower during the spring experiment which could have resulted in plants with decreased biomass. This makes sense since there was no dry weight significance between the Winter and Spring 2006 experiments.

Plants in the Winter 2006 experiment contained significantly more N and P in dry tissue because N and P concentrations in the effluent were higher during this time than during the Spring 2006 experiment (Appendix 33). Seasonal water quality measurements can be found in Dyer (2006).

Watercress Yield and Profit Estimates

Based on the experimental results that promoted significant watercress growth and nutrient contents, estimated watercress potential yields and profits were calculated for this flow-through aquaculture system.

The watercress yield and profit estimates are based on a proposed watercress production system in the APG that uses the factorial combination of high velocity, high density, and paper medium treatments and either a single or double harvest staggered cropping system. No harvest treatments were conducted during the aquaponic experiments in this research and the following yield estimate and profit potential given for watercress are theoretical and based on the current growing area in the APG, commercial harvest schedules, and current market prices.

The following estimates are not an attempt to verify the actual yields or profits from this system, but rather theoretical attempts to show what this system is potentially capable of with regard to these topics. Further research needs to be conducted to determine and optimize the actual yields and profits from this system and those issues are intended to be addressed in subsequent research efforts.

Watercress Yield Estimate (Theoretical)

Channels = 0.93 m^2 (10 ft^2) * 3 channels per bed = 2.79 m^2 (bed)
2.79 m^2 (bed) * 14 beds (proposed system) = **39.06 m^2 total growing area**

Based on the Spring 2006, APG, factorial combination of high velocity and high density treatment means:
Avg. Fresh weight per plant = 0.48 g
0.48 g/plant * 20 plants/bunch = **9.6 g/bunch**

Based on Proposed 2nd Harvest System (see Watercress Profit Potential):
3 single harvests (630 bunches) + 43 double harvests (18,060 bunches) = **18, 690 bunches annually in a 39.06 m^2 growing area**

18, 690 bunches * 9.6 g/ bunch = 179, 424 g OR **179.4 kg watercress annually**

YIELD = $\frac{179.4\ kg}{39.06\ m^2}$ = **4.59 kg/m^2 annually (0.38 kg/m^2/month)**

Single Harvest System:

46 single harvests = 9660 bunches * 9.6 g/bunch = 92, 736 g OR **92.7 kg annually**

YIELD = $\frac{92.7\ kg}{39.06\ m^2}$ = **2.37 kg/m^2 annually (0.20 kg/m^2/month)**

Watercress Profit Potential from a Flow-Through Aquaponic System (Theoretical)

The APG (as is) can accommodate:
200 plants per raft * 3 rafts per channel = 600 plants per channel
3 channels per bed * 14 beds = 42 channels
600 plants * 42 channels = 25,200 plants (after ALL channels harvested once)
25,200 plants / 20 plants per bunch = 1260 bunches

MARKET PRICE = Anywhere from $1 to $3 per bunch (depending on the market)
(The Growing Edge, 2002)

Typical harvest is 6 wks from seed (but potential for **2nd harvest** every 3 wks.), so…

PROPOSED HARVEST SCHEDULE and PROFITABILITY:
Wk 0 - Sow 7 channels
Wk 1 - Sow 7 channels
Wk 2 - Sow 7 channels
Wk 3 - Sow 7 channels
Wk 4 - Sow 7 channels
Wk 5 - Sow 7 channels (All rafts in 42 channels sown at this time)
Wk 6 - *Harvest Wk0 (600 plants x 7 channels = 4200 plants = 210 bunches = $210 - $630 per weekly harvest)
Wk 7 - *Harvest Wk 1 ($210 - $630)
Wk 8 - *Harvest Wk 2 ($210 - $630)
Total Profit from 1st three single harvests = $630 - $1890
Wk 9 - Harvest Wk 3; **2nd harvest** Wk 0; **Clean-up & Re-seed 2nd harvest channels**
Wk 10 - Harvest Wk 4; **2nd harvest** Wk 1; **Clean-up & Re-seed 2nd harvest channels**
Wk 11 - Harvest Wk 5; **2nd harvest** Wk 2; **Clean-up & Re-seed 2nd harvest channels**
***END OF 1ST CYCLE = All 42 channels harvested + (3) 2ND harvests (21 channels)
TOTAL PROFIT FROM 1ST CYCLE (3 single harvests + 3 double harvests = **$1890 - $5670 for 12wks**)

DOUBLE HARVEST SYSTEM (6wks down time + 3 single harvests + 43 double harvests = 52 wks)
TOTAL ANNUAL PROFIT = $19,110 - $57330; Difference = $38,220

SINGLE HARVEST SYSTEM (6wks down time + 46 single harvests = 52 wks)
TOTAL ANNUAL PROFIT = $9660 – $28,980; Difference = $19,320

Shear (1968) reported per cutting yields of 2550 bunches per 93 m² of well established growing beds. According to the theoretical yield estimate above, if all channels were harvested once, this system would produce per cutting yields of 1260 bunches per 39.06 m² of growing beds. This is equivalent to 3000 bunches in 93 m² of growing bed, which is 450 bunches more than could be produced in the same area commercially. At $1 to $3 per bunch, this could amount to $450 to $1350 more per cutting from the proposed system versus a commercial system, depending on the market.

The Growing Edge (2002) reported yields of 1.5 to 2.0 kg/m²/month in summer from protected systems. According to the theoretical yield estimate above, this proposed system would produce 0.20 to 0.38 kg/m²/month from the single and double harvest systems respectively. Based on these values, our proposed system would yield less watercress in kg/m²/month than the reported system.

This presents a conflicting view of the proposed system in that according to one source this system yields more watercress, while according to the other source this system yields less watercress. Apparently it depends on whether yield is considered based on number of bunches or mass per unit area. Perhaps the addition of supplemental nutrients would improve the estimated yields from this proposed system, but this is an area of research that needs to be explored further.

CONCLUSIONS

This preliminary research provided useful data on watercress' growth potential and nutrient contents in both a controlled environment and an integrated flow-through aquaponic system subject to seasonal variations.

Overall, the hydroponic experiment concluded that watercress growth and nutrient contents are greater when grown under an intermediate light intensity and a half-strength nutrient solution provides increased elongation early in the life cycle.

The comparison of data from Week 6 of the Spring 2006 –Location Comparison aquaponic experiment with data from Week 6 of the hydroponic experiment suggests that watercress length and dry weights were greatest when grown in a hydroponic nutrient solution under intermediate PAR than when grown in aquaculture effluent under intermediate PAR. Greater watercress lengths and dry weights when grown in a hydroponic solution versus effluent induces the need for further research to determine if supplemental nutrient application is necessary within the aquaponic system to improve watercress growth and possibly nutrient recovery.

Overall, the aquaponic experiments, regardless of season or location, showed that watercress growth and nutrient contents were greatest in high velocity (0.61 cm s^{-1}), high plant density (0.08 plants cm^{-2}), and paper medium treatments in the current flow-through aquaponic system. Increased growth and nutrient contents in these treatments are attributed to greater contact of plant roots with effluent, greater nutrient availability, potentially greater dissolved oxygen availability, and shading of undesirable aquatic species that would have competed for nutrients and could have contributed to eutrophic

conditions within the experimental channels.

Based on location, watercress growth and nutrient contents were greatest in the Aquaponic Production Greenhouse (APG) under intermediate light intensities as opposed to the Aquaculture Research Facility (ARF) where low light intensities were present. Even without a heating or cooling system, the APG allowed for out-of-season production apparent by growth in the winter and summer (supported by data from additional experiments not included in this document). Watercress only grows naturally during the spring season and higher market values can be obtained out-of-season. No experiments were conducted during the autumn (late September to early December) to evaluate watercress' performance during that season. The water temperature created a microclimate within the experimental channels that provided insulation in the winter and cooling in the summer. The addition of a heating and cooling system to the APG could potentially improve growth and nutrient contents during the winter and summer.

Based on season, watercress growth was greatest during the spring experiment, yet nutrient contents were greatest during the winter experiment. Plant nutrient contents are dependent on nutrient concentrations of the effluent apparent from higher nutrient concentrations present during the winter.

Results from the comparison of watercress grown in effluent and those grown in spring water (no effluent) indicated that the nutrient contribution of the effluent was insignificant for watercress growth and nutrient contents. It was determined that the threat of nutrient loading and associated environmental impacts is unlikely from this aquaculture system as is due to the small size of the operation and insignificant nutrient concentrations. Thus, the function and purpose of this integrated system becomes

water re-use and production of a secondary marketable crop versus nutrient recovery, which becomes an added benefit of the system.

Further research is needed to determine other factors (i.e. water depth, harvest schedule, marketability, etc.) and exact biological concentration factors (BCFs) that could optimize watercress' use as a sustainable, secondary crop to supplement fish farm income and possible nutrient recovery option for flow-through aquaculture effluent.

Additional research is also needed to characterize other factors in this system, such as dissolved oxygen levels and the contribution of solids to nutrient concentration of the effluent and the nitrification process, to determine which microbes are present in effluent and their role in nitrification and nutrient removal, to establish water quality of the polishing pond and understand the interactions taking place there, and to study other crops that may be suited for production in this type of integrated system.

Steps have already been taken to address these issues and ongoing research aims to answer the questions that this preliminary research could not. In addition, watercress production could be applicable to other industries or situations besides the aquaculture industry. Operations such as nurseries or animal feed lots could possibly utilize watercress to recover nutrients from fertilizer or manure runoff, as an animal feed supplement, or as a food source for production of additional aquaculture species.

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APPENDIX

Appendix 1. Analysis of variance for the effects of light intensity, sample date, nutrient solution, and replication for watercress length in a controlled environment

Dependent Variable: Length

Source of Variation	DF	Type III SS	Mean Square	F Value	Pr > F
Light Intensity	1	10.3228167	10.3228167	4.35	0.0426
Sample Date	2	44.5114704	22.2557352	9.38	0.0004
Nutrient Solution	2	157.1666259	78.5833130	33.13	<.0001
Replication	2	1.9662259	0.9831130	0.41	0.6632

Appendix 2. Analysis of variance for the effects of light intensity, sample date, nutrient solution, and replication for watercress fresh weight in a controlled environment

Dependent Variable: Fresh weight

Source of Variation	DF	Type III SS	Mean Square	F Value	Pr > F
Light Intensity	1	20825.07782	20825.07782	11.07	0.0017
Sample Date	2	49470.43370	24735.21685	13.15	<.0001
Nutrient Solution	2	28883.54627	14441.77314	7.68	0.0013
Replication	2	1 436.57449	718.28725	0.38	0.6846

Appendix 3. Analysis of variance for the effects of light intensity, nutrient solution, and replication for watercress total nitrogen content in controlled environment

Dependent Variable: Nitrogen

Source of Variation	DF	Type III SS	Mean Square	F Value	Pr > F
Light Intensity	1	1.44500000	1.44500000	6.66	0.0241
Nutrient Solution	2	2.20390000	1.10195000	5.08	0.0252
Replication	2	0.41670000	0.20835000	0.96	0.4104

Appendix 4. Analysis of variance for the effects of light intensity, nutrient solution, and replication for watercress total phosphorus content in a controlled environment

Dependent Variable: Phosphorus

Source of Variation	DF	Type III SS	Mean Square	F Value	Pr > F
Light Intensity	1	0.00245000	0.00245000	0.02	0.8966
Nutrient Solution	2	1.07223333	0.53611667	3.85	0.0510
Replication	2	0.00333333	0.00166667	0.01	0.9881

Appendix 5. Analysis of variance for the effects of water velocity, plant density, growing medium, replication, and sample date for watercress length - Summer 2005 (ARF)

Dependent Variable: Length

Source of Variation	DF	Type III SS	Mean Square	F Value	Pr > F
Velocity (VEL)	2	4510.7935	2255.3967	8.67	0.0002
Density (DEN)	2	268.7342	134.3671	0.52	0.5975
Medium (MED)	2	159132.5925	79566.2962	305.69	<.0001
Replication	2	1406.6448	703.3224	2.70	0.0694
Sample Date	2	61989.8600	30994.9300	119.08	<.0001
VEL*DEN	4	723.4146	180.8537	0.69	0.5963
VEL*MED	4	1663.8433	415.9608	1.60	0.1760
DEN*MED	4	1414.5797	353.6449	1.36	0.2494
VEL*DEN*MED	8	1710.2954	213.7869	0.82	0.5845

Appendix 6. Analysis of variance for the effects of water velocity, plant density, growing medium, replication, and sample date for watercress dry weight - Summer 2005 (ARF)

Dependent Variable: Dry weight

Source of Variation	DF	Type III SS	Mean Square	F Value	Pr > F
Velocity (VEL)	2	273224.691	136612.346	9.08	0.0002
Density (DEN)	2	80669.136	40334.568	2.68	0.0709
Medium (MED)	2	1940217.284	970108.642	64.47	<.0001
Replication	2	105918.519	52959.259	3.52	0.0314
Sample Date	2	2094451.852	1047225.926	69.60	<.0001
VEL*DEN	4	59906.173	14976.543	1.00	0.4111
VEL*MED	4	120424.691	30106.173	2.00	0.0956
DEN*MED	4	111402.469	27850.617	1.85	0.1203
VEL*DEN*MED	8	102200.000	12775.000	0.85	0.5606

Appendix 7. Estimated channel concentrations of available nitrogen and phosphorus in mg/L per 3 wks based on tailbox nutrient concentrations and water velocity

Experiment:	Summer 2005		Winter 2006		Spring 2006	
Velocity:	LV	HV	LV	HV	LV	HV
Nitrogen (mg/L)						
Week 3	333.40	2778.30	374.22	3118.50	278.96	2324.70
Week 6	256.36	2211.30	251.75	2097.90	285.77	2381.40
Week 9	319.79	2664.90	347.00	2891.70	278.96	2324.70
Week 12	1544.50	12870.90	299.38	2494.80	238.14	1984.50
Phosphorus (mg/L)						
Week 3	401.44	3345.30	374.22	3118.50	333.40	2778.30
Week 6	340.20	2835.00	374.22	3118.50	340.20	2835.00
Week 9	312.98	2608.20	374.22	3118.50	340.20	2835.00
Week 12	394.63	3288.60	360.61	3005.10	326.59	2721.60

Appendix 8. Analysis of variance for the effects of water velocity, plant density, growing medium, replication, and sample date for watercress total nitrogen content - Summer 2005 (ARF)

Dependent Variable: Nitrogen

Source of Variation	DF	Type III SS	Mean Square	F Value	Pr > F
Velocity (VEL)	2	7.5286778	3.7643389	4.61	0.0116
Density (DEN)	2	1.8429370	0.9214685	1.13	0.3264
Medium (MED)	2	186.4200444	93.2100222	114.22	<.0001
Replication	2	1.1427704	0.5713852	0.70	0.4983
Sample Date	1	24.8199265	24.8199265	30.41	<.0001
VEL*DEN	4	1.4099852	0.3524963	0.43	0.7854
VEL*MED	4	6.6840222	1.6710056	2.05	0.0914
DEN*MED	4	4.7321519	1.1830380	1.45	0.2213
VEL*DEN*MED	8	4.0790481	0.5098810	0.62	0.7558

Appendix 9. Analysis of variance for the effects of water velocity, plant density, growing medium, replication, and sample date for watercress total phosphorus content - Summer 2005 (ARF)

Dependent Variable: Phosphorus

Source of Variation	DF	Type III SS	Mean Square	F Value	Pr > F
Velocity (VEL)	2	0.42534198	0.21267099	10.49	<.0001
Density (DEN)	2	0.02391235	0.01195617	0.59	0.5560
Medium (MED)	2	10.89474938	5.44737469	268.67	<.0001
Replication	2	0.01841605	0.00920802	0.45	0.6360
Sample Date	1	0.05013889	0.05013889	2.47	0.1182
VEL*DEN	4	0.13278395	0.03319599	1.64	0.1686
VEL*MED	4	0.37526914	0.09381728	4.63	0.0016
DEN*MED	4	0.06099877	0.01524969	0.75	0.5583
VEL*DEN*MED	8	0.03151605	0.00393951	0.19	0.9913

Appendix 10. Analysis of variance for the effects of water velocity, growing medium, replication, and sample date for watercress length - Summer 2005 (ARF) - Control comparison

Dependent Variable: Length

Source of Variation	DF	Type III SS	Mean Square	F Value	Pr > F
Velocity (VEL)	2	3737.79602	1 868.89801	6.28	0.0027
Medium (MED)	2	54145.71931	27072.85965	91.03	<.0001
Replication	3	1617.11040	539.03680	1.81	0.1502
Sample Date	2	23066.85171	11533.42585	38.78	<.0001
VEL*MED	4	1842.21637	460.55409	1.55	0.1946

Appendix 11. Analysis of variance for the effects of water velocity, growing medium, replication, and sample date for watercress dry weight - Summer 2005 (ARF) - Control comparison

Dependent Variable: Dry weight

Source of Variation	DF	Type III SS	Mean Square	F Value	Pr > F
Velocity (VEL)	2	276738.8889	138369.4444	7.04	0.0014
Medium (MED)	2	951950.0000	475975.0000	24.20	<.0001
Replication	3	31718.5185	10572.8395	0.54	0.6577
Sample Date	2	966066.6667	483033.3333	24.56	<.0001
VEL*MED	4	157377.7778	39344.4444	2.00	0.1008

Appendix 12. Analysis of variance for the effects of water velocity, growing medium, replication, and sample date for watercress total nitrogen content - Summer 2005 (ARF) - Control comparison

Dependent Variable: Nitrogen

Source of Variation	DF	Type III SS	Mean Square	F Value	Pr > F
Velocity (VEL)	2	5.07308611	2.53654306	2.49	0.0914
Medium (MED)	2	65.32441111	32.66220556	32.10	<.0001
Replication	3	5.16110417	1.72036806	1.69	0.1788
Sample Date	1	5.59451250	5.59451250	5.50	0.0224
VEL*MED	4	1.23528889	0.30882222	0.30	0.8745

Appendix 13. Analysis of variance for the effects of water velocity, growing medium, replication, and sample date for watercress total phosphorus content - Summer 2005 (ARF) - Control comparison

Dependent Variable: Phosphorus

Source of Variation	DF	Type III SS	Mean Square	F Value	Pr > F
Velocity (VEL)	2	0.23521111	0.11760556	3.99	0.0238
Medium (MED)	2	4.13951111	2.06975556	70.14	<.0001
Replication	3	0.03751528	0.01250509	0.42	0.7366
Sample Date	1	0.01003472	0.01003472	0.34	0.5620
VEL*MED	4	0.07283889	0.01820972	0.62	0.6520

Appendix 14. Analysis of variance for the effects of water velocity, plant density, replication, and sample date for watercress length - Winter 2006 (APG)

Dependent Variable: Length

Source of Variation	DF	Type III SS	Mean Square	F Value	Pr > F
Velocity (VEL)	1	84.71225	84.71225	3.26	0.0738
Density (DEN)	1	26.17638	26.17638	1.01	0.3176
Replication	2	24.08294	12.04147	0.46	0.6301
Sample Date	2	13421.70804	6710.85402	258.64	<.0001
VEL*DEN	1	6.56627	6.56627	0.25	0.6160

Appendix 15. Analysis of variance for the effects of water velocity, plant density, replication, and sample date for watercress dry weight - Winter 2006 (APG)

Dependent Variable: Dry Weight

Source of Variation	DF	Type III SS	Mean Square	F Value	Pr > F
Velocity (VEL)	1	234271.319	234271.319	5.74	0.0184
Density (DEN)	1	45.448	45.448	0.00	0.9734
Replication	2	60767.141	30383.571	0.74	0.4775
Sample Date	2	6250532.759	3125266.380	76.60	<.0001
VEL*DEN	1	642.111	642.111	0.02	0.9004

Appendix 16. Analysis of variance for the effects of water velocity, plant density, replication, and sample date for watercress total nitrogen content - Winter 2006 (APG)

Dependent Variable: Nitrogen

Source of Variation	DF	Type III SS	Mean Square	F Value	Pr > F
Velocity (VEL)	1	236560251	236560251	1.36	0.2479
Density (DEN)	1	235235280	235235280	1.35	0.2492
Replication	2	135625712	67812856	0.39	0.6788
Sample Date	1	6030655488	6030655488	34.65	<.0001
VEL*DEN	1	2717558	2717558	0.02	0.9009

Appendix 17. Analysis of variance for the effects of water velocity, plant density, replication, and sample date for watercress total phosphorus content - Winter 2006 (APG)

Dependent Variable: Phosphorus

Source of Variation	DF	Type III SS	Mean Square	F Value	Pr > F
Velocity (VEL)	1	578888.000	578888.000	0.08	0.7756
Density (DEN)	1	2945973.556	2945973.556	0.42	0.5208
Replication	2	2722385.250	1361192.625	0.19	0.8253
Sample Date	1	962809.389	962809.389	0.14	0.7133
VEL*DEN	1	887556.056	887556.056	0.13	0.7242

Appendix 18. Analysis of variance for the effects of experiment location, replication, and sample date for watercress length - Winter 2006 - Location comparison

Dependent Variable: Length

Source of Variation	DF	Type III SS	Mean Square	F Value	Pr > F
Location	1	101.5170667	101.5170667	97.34	<.0001
Replication	2	0.1054333	0.0527167	0.05	0.9508
Sample Date	2	40.9080111	20.4540056	19.61	<.0001

Appendix 19. Analysis of variance for the effects of experiment location, replication, and sample date for watercress dry weight - Winter 2006 - Location comparison

Dependent Variable: Dry Weight

Source of Variation	DF	Type III SS	Mean Square	F Value	Pr > F
Location	1	1515.906150	1515.906150	58.51	<.0001
Replication	2	34.762633	17.381317	0.67	0.5160
Sample Date	2	883.081411	441.540706	17.04	<.0001

Appendix 20. Analysis of variance for the effects of experiment location, replication, and sample date for watercress total nitrogen content - Winter 2006 - Location comparison

Dependent Variable: Nitrogen

Source of Variation	DF	Type III SS	Mean Square	F Value	Pr > F
Location	1	13.73937778	13.73937778	128.56	<.0001
Replication	2	0.36562222	0.18281111	1.71	0.1974
Sample Date	1	0.75111111	0.75111111	7.03	0.0125

Appendix 21. Analysis of variance for the effects of experiment location, replication, and sample date for watercress total phosphorus content - Winter 2006 - Location comparison

Dependent Variable: Phosphorus

Source of Variation	DF	Type III SS	Mean Square	F Value	Pr > F
Location	1	462959772.3	462959772.3	86.93	<.0001
Replication	2	25444863.2	12722431.6	2.39	0.1084
Sample Date	1	328520.0	328520.0	0.06	0.8055

Appendix 22. Analysis of variance for the effects of water velocity, plant density, replication, and sample date for watercress length - Spring 2006 (APG)

Dependent Variable: Length

Source of Variation	DF	Type III SS	Mean Square	F Value	Pr > F
Velocity (VEL)	1	2988.94246	2988.94246	39.98	<.0001
Density (DEN)	1	1750.79468	1750.79468	23.42	<.0001
Replication	2	215.50156	107.75078	1.44	0.2415
Sample Date	2	19903.65826	9951.82913	133.11	<.0001
VEL*DEN	1	279.68926	279.68926	3.74	0.0559

Appendix 23. Analysis of variance for the effects of water velocity, plant density, replication, and sample date for watercress dry weight - Spring 2006 (APG)

Dependent Variable: Dry Weight

Source of Variation	DF	Type III SS	Mean Square	F Value	Pr > F
Velocity (VEL)	1	6553116.99	6553116.99	19.15	<.0001
Density (DEN)	1	1007271.46	1007271.46	2.94	0.0893
Replilcation	2	65029.09	32514.55	0.10	0.9094
Sample Date	2	20011323.16	10005661.58	29.24	<.0001
VEL*DEN	1	207742.01	207742.01	0.61	0.4378

Appendix 24. Analysis of variance for the effects of water velocity, plant density, replication, and sample date for watercress total nitrogen content - Spring 2006 (APG)

Dependent Variable: Nitrogen

Source of Variation	DF	Type III SS	Mean Square	F Value	Pr > F
Velocity (VEL)	1	2221200118	2221200118	17.49	<.0001
Density (DEN)	1	189384722	189384722	1.49	0.2264
Replication	2	959551373	479775687	3.78	0.0280
Sample Date	1	388238401	388238401	3.06	0.0851
VEL*DEN	1	111611760	111611760	0.88	0.3519

Appendix 25. Analysis of variance for the effects of water velocity, plant density, replication, and sample date for watercress total phosphorus content - Spring 2006 (APG)

Dependent Variable: Phosphorus

Source of Variation	DF	Type III SS	Mean Square	F Value	Pr > F
Velocity (VEL)	1	23156280.89	23156280.89	4.51	0.0376
Density (DEN)	1	23006805.56	23006805.56	4.48	0.0382
Replication	2	2458904.69	1229452.35	0.24	0.7879
Sample Date	1	780416.89	780416.89	0.15	0.6980
VEL*DEN	1	11284416.89	11284416.89	2.20	0.1432

Appendix 26. Analysis of variance for the effects of experiment location, replication, and sample date for watercress length - Spring 2006 - Location comparison

Dependent Variable: Length

Source of Variation	DF	Type III SS	Mean Square	F Value	Pr > F
Location	1	16.66667	16.66667	0.40	0.5291
Replication	2	121.22416	60.61208	1.46	0.2420
Sample Date	2	13996.19416	6998.09708	168.76	<.0001

Appendix 27. Analysis of variance for the effects of experiment location, replication, and sample date for watercress dry weight - Spring 2006 - Location comparison

Dependent Variable: Dry Weight

Source of Variation	DF	Type III SS	Mean Square	F Value	Pr > F
Location	1	262272.7399	262272.7399	18.40	<.0001
Replication	2	27592.0798	13796.0399	0.97	0.3871
Sample Date	2	545671.3058	272835.6529	19.15	<.0001

Appendix 28. Analysis of variance for the effects of experiment location, replication, and sample date for watercress total nitrogen content - Spring 2006 - Location comparison

Dependent Variable: Nitrogen

Source of Variation	DF	Type III SS	Mean Square	F Value	Pr > F
Location	1	3.45340278	3.45340278	42.63	<.0001
Replication	2	0.04602222	0.02301111	0.28	0.7547
Sample Date	1	0.33446944	0.33446944	4.13	0.0508

Appendix 29. Analysis of variance for the effects of experiment location, replication, and sample date for watercress total phosphorus content - Spring 2006 - Location comparison

Dependent Variable: Phosphorus

Source of Variation	DF	Type III SS	Mean Square	F Value	Pr > F
Location	1	42499534.03	42499534.03	10.63	0.0027
Replication	2	4852312.39	2426156.19	0.61	0.5516
Sample Date	1	5439001.36	5439001.36	1.36	0.2525

Appendix 30. Analysis of variance for the effects of season, water velocity, plant density, replication, and sample date for watercress length - Winter v. Spring 2006 (APG) - Season comparison

Dependent Variable: Length

Source of Variation	DF	Type III SS	Mean Square	F Value	Pr > F
Season (SEA)	1	2128.22944	2128.22944	35.27	<.0001
Velocity (VEL)	1	2040.01720	2040.01720	33.81	<.0001
Density (DEN)	1	1102.56370	1102.56370	18.27	<.0001
Replication	2	111.67579	55.83789	0.93	0.3980
Sample Date	2	31216.43104	15608.21552	258.70	<.0001
VEL*DEN	1	100.27319	100.27319	1.66	0.1988
SEA*VEL*DEN	3	1894.02719	631.34240	10.46	<.0001

Appendix 31. Analysis of variance for the effects of season, water velocity, plant density, replication, and sample date for watercress dry weight - Winter v. Spring 2006 - season comparison

Dependent Variable: Dry weight

Source of Variation	DF	Type III SS	Mean Square	F Value	Pr > F
Season (SEA)	1	740458.43	740458.43	3.71	0.0553
Velocity (VEL)	1	4632729.00	4632729.00	23.24	<.0001
Density (DEN)	1	510424.44	510424.44	2.56	0.1111
Replication	2	1198.39	599.20	0.00	0.9970
Sample Date	2	24023890.03	12011945.02	60.26	<.0001
VEL*DEN	1	115741.67	115741.67	0.58	0.4470
SEA*VEL*DEN	3	2744194.22	914731.41	4.59	0.0039

Appendix 32. Analysis of variance for the effect of season, water velocity, plant density, replication, and sample date for watercress total nitrogen content - Winter v. Spring 2006 - season comparison

Dependent Variable: Nitrogen

Source of Variation	DF	Type III SS	Mean Square	F Value	Pr > F
Season (SEA)	1	92.04803403	92.04803403	49.83	<.0001
Velocity (VEL)	1	19.30870069	19.30870069	10.45	0.0015
Density (DEN)	1	4.12428403	4.12428403	2.23	0.1375
Replication	2	13.01392639	6.50696319	3.52	0.0323
Sample Date	1	41.20570069	41.20570069	22.31	<.0001
VEL*DEN	1	0.43670069	0.43670069	0.24	0.6276
SEA*VEL*DEN	3	4.87307431	1.62435810	0.88	0.4536

Appendix 33. Analysis of Variance for the effects of season, water velocity, plant density, replication, and sample date for watercress total phosphorus content - Winter v. Spring 2006 - season comparison

Dependent Variable: Phosphorus

Source of Variation	DF	Type III SS	Mean Square	F Value	Pr > F
Season (SEA)	1	4.20933611	4.20933611	123.51	<.0001
Velocity (VEL)	1	0.32871111	0.32871111	9.64	0.0023
Density (DEN)	1	0.14951111	0.14951111	4.39	0.0381
Replication	2	0.02987639	0.01493819	0.44	0.6461
Sample Date	1	0.14694444	0.14694444	4.31	0.0398
VEL*DEN	1	0.06934444	0.06934444	2.03	0.1561
SEA*VEL*DEN	3	0.07565278	0.02521759	0.74	0.5301

CURRICULUM VITAE

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