Basil, *Ocimum basilicum*, yield in northern latitudinal aquaponic growing conditions

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Abstract
The need for yield research is increasing with the popularity of aquaponic food production systems where plants and fish are grown together in a recirculating system. Our objective was to compare the yield of three basil cultivars (“Elenora,” “Genovese,” “Nufar,” *Ocimum basilicum*) in four configurations (greenhouses: floating raft DWS, ebb & flow, A-frame; warehouse: floating raft; 1–8 tank replications/treatment) as well as a soilless control to establish a baseline for aquaponic producers in northern latitudes. We also tested whether basil yield varied among fish types: yellow perch, *Perca flavescens*, goldfish, *Carassius auratus*, tilapia, *Oreochromis* spp., and koi, *Cyprinus carpio*. Fish tank water temperature, nitrite, and alkalinity levels differed significantly over seasons (summer, winter). There was a significant decrease in basil production in all aquaponic systems that used koi (greenhouse, warehouse). No significant difference in basil yield was found among greenhouse and aquaponic systems for perch, tilapia, or goldfish nor among the basil “Elenora,” “Genovese,” and “Nufar.” Considerable variation occurred in yield although most of it occurred in fresh weights, being reduced significantly in dry weights. The effects of growing koi fish on reducing aquaponic...
production of basil is noteworthy, although future research is needed to the exact cause.

**KEYWORDS**
goldfish, Carassius auratus, greenhouse production, koi, Cyprinus carpio, tilapia, Oreochromis spp., yellow perch, Perca flavescens, warehouse production

1 | INTRODUCTION

Aquaponics is a method of production that combines aquaculture and hydroponics into closed loop circulating or noncirculating systems (Rakocy, Bailey, Shultz, & Danaher, 2007). The wastewater in aquaculture production is substituted for the nutrient solution used in hydroponic production. Occasionally the wastewater may have macro-nutrients (P, K; Nicoletto et al., 2018) or micronutrients (Fe; Abbey, Anderson, Yue, Short, et al., 2019) added in when levels are deficient for plant growth. Once the nutrients are absorbed by the plants in the hydroponic production, the clean water is returned to the aquaculture production, finishing the loop. Aquaponic systems have captured industry interest by having the potential to lower waste and increase production of locally grown produce and fish (Rakocy et al., 2007) when compared with conventional systems. Alternative methods of crop production and urban agriculture, which include aquaponic production, have grown by over 30% since 1990 (Siegner, Sowerwine, & Acey, 2018). However, finding the optimal combination of plant, aquaculture, and system design with increased efficiency is a primary challenge, particularly for growers in northern latitudes because of higher energy (heat, lighting) costs.

Aquaponics is of growing interest for greenhouse and warehouse producers to grow local, sustainable produce, and fish in northern latitudes (Abbey, Anderson, Yue, Short, et al., 2019; Abbey, Anderson, Yue, Schermann, et al., 2019). Short, Yue, Anderson, Russell, and Phelps (2017) surveyed consumers for their knowledge of aquaponic products. A minority of consumers (~33%) were aware of aquaponics as an environmentally friendly production system, although price points were perceived to be an issue (Short et al., 2017). Thus, marketing campaigns to educate consumers were delineated to be necessary for consumer acceptance. In a subsequent experimental auction, the informational impact of aquaponic production methods on consumers’ willingness-to-pay (WTP) for lettuce was tested (Short et al., 2018). Most consumers had similar WTP for both aquaponic and soil grown lettuce.

Commonly grown crops in aquaponics are leafy greens (lettuce), herbs (basil), and small fruits, such as strawberries (Abbey, Anderson, Yue, Short, et al., 2019; Abbey, Anderson, Yue, Schermann, et al., 2019; Love et al., 2015; Maucieri et al., 2018). Recent studies found that year-round production of aquaponic-grown, day-neutral strawberries, and lettuce in greenhouses and warehouses in northern latitudes could be accomplished (Abbey, Anderson, Yue, Short, et al., 2019; Abbey, Anderson, Yue, Schermann, et al., 2019).

Basil, Ocimum basilicum L. (Lamiaceae), is one of the top herb crops in the aquaponic industry (Love et al., 2015). It has some of the ideal qualities for aquaponic production, such as rapid growth, high fresh market value, no need for pollinators for harvest, and a compact growth habit. For all their benefits, aquaponic systems are limited to being adaptable for specific types of plant. Aquaponic crops must have a fast production time or “turn around” and/or high profitability in order to make up for the cost of electricity and heating year-round in northern latitudes (Abbey, Anderson, Yue, Short, et al., 2019; Abbey, Anderson, Yue, Schermann, et al., 2019; Love et al., 2015). Ideally, the plant should be harvestable without the need for additional pollinators because it is especially challenging to successfully introduce pollinators to controlled environment production like a greenhouse or warehouse. Basil also has the benefit of having a shallow, fibrous root system that can adapt to aquaponic production (Putievsky &
Galambosi, 1999) and because only the top half of the plant is harvested for consumption, basil avoids potential contamination with the aquaponic recirculating nutrient-rich water.

Basil is a tender, subtropical (warm-season) herbaceous annual that grows best between 18 and 21°C day temperatures (Putievsky & Galambosi, 1999; Ball Seed Company, 2019). Commonly grown types are categorized based on leaf size, plant shape, and/or flavors, for example, large-leaved basils (for example, lettuce leaf, sweet, and Italian basils) and small-leaved types (dwarf, bush, spicy, or lemon basils) (University of California, Davis, 2005). Although the most commonly cultivated species is *O. basilicum*, several other species are also grown because they have different volatile oils and flavors, for example, lemon, lime basils, *Ocimum americanum*, holy basil, *Ocimum sanctum*, clove basil, *Ocimum gratissimum*, Greek basil, *Ocimum minimum*, and interspecific hybrids of Greek columnar and Thai lemon basil types, *Ocimum × citriodorum*, African blue basil, *Ocimum kilimandscharicum × O. basilicum*, and spice basil and Sweet Dani basils, *O. basilicum × O. americanum* (North Carolina State University Extension Publications, 1995; Wikipedia, 2019). Although different species and cultivars have different growth habits, the most popular type in the U.S. is large-leaved sweet basil (i.e., “Genovese” types), which will grow approximately 60–80 cm in height (Succop & Newman, 1997) with an average fresh weight/plant of 90–98 g (Halva, 1987; Nykänen, 1989). The leaves (either as single or multi-cut harvests) are the most commonly harvested part of the plant for culinary use, although seeds are also harvested for culinary purposes (Simon, Reiss-Bubenheim, Joly, & Charles, 1992; University of California, Davis, 2005); in greenhouses, it is commonly grown in hydroponic systems. Overhead irrigation often causes damage to the foliage and has the potential to spread disease. However, this is not an issue for aquaponic or hydroponic production because the roots are washed continuously with a nutrient solution (Succop & Newman, 1997).

Nitrogen (N) is the most important nutrient to increase basil yield. Specifically, increases in nitrate nitrogen (NO₃⁻) rather than ammoniacal forms (NH₄⁺) significantly increases both the fresh and dry yield of sweet basil (Halva, 1987). Changes in phosphorus (P) and potassium (K) fertilization do not significantly impact basil yield (Lupatsch & Kissil, 1998). Basil’s reliance on N for high yield suits the N-rich environment of aquaponic production because there is often an excess in the system (Wongkiew, Hu, Chandran, Lee, & Khanal, 2017). The N levels in aquaponic systems can vary up to 40% depending on fish species, density, feed, and efficiency of the biofilter conversion rates (Wongkiew et al., 2017). Thus, it is important for aquaponic growers to enhance production conditions in order to maximize basil yield (Lupatsch & Kissil, 1998; Schneider et al., 2004; Van Rijn, 2013).

Basil is primarily seed-propagated, although it can be propagated vegetatively using stem tip cuttings. At least one vegetative basil cultivar exists on the market, that is, nonflowering, columnar Pesto Perpetuo, *Ocimum × citriodorum* (US Plant Patent No. 16,260; Monrovia Nursery, 2020). Initial seed germination and growth is 14–28 days for true leaves to appear. Once established, growth is rapid, needing only another ~28 days to reach full maturity (Ball Seed Company, 2019). Because of this growth cycle, producers can either direct sow seed into anchoring containers or use transplants for more rapid aquaponic production. Basil is typically harvested by cutting the entire plant at the first set of true leaves approximately 10–12 cm above the ground (single cut); although multicut by harvesting leaves separately can also be done. Generally, in aquaponic production selling entire plants with the root system is not recommended in aquaponic systems because of the potential for contamination from fish water. Some producers in warmer climates cut higher in order to later harvest the regrowth but that yield will be smaller than the original growth and can only be done 2–3 times before needing to replace the plants (Lupatsch & Kissil, 1998; Schneider et al., 2004; Van Rijn, 2013).

The U.S. is the largest producer and importer of basil in the world (Republic of South Africa, Dept. of Agriculture, Forestry, & Fisheries Department, 2012). Basil imports to the U.S. increased 6× from 1980 to 2000 (Furth, 2001) and, since 2000, spice imports as a whole have increased 400% (United States, Dept. of Agriculture, Economic Research Service, 2017). Given the continued popularity of basil, as well as reduced travel and post-harvest time, this crop is an excellent cash crop candidate for aquaponic production systems. Basil leaves are easily damaged and can only be stored at temperatures of 10°C for short periods of time, making postharvest care and shipping of fresh basil a challenge especially in colder northern latitudes (Succop & Newman, 1997). This challenge is an opportunity for local aquaponic producers to take advantage of their proximity to markets (Love et al., 2015).
The objective of this study is to create a baseline for potential yield of basil in the year-round aquaponic production systems of northern latitudes. The relevance of a northern latitude influences the duration of available sunlight (day length or photoperiod) throughout the year that is transmitted through the glazing materials of the greenhouses at 45°N lat. as well as the annual change in the angle of the sun which influences shadowing, particularly in winter months in the north (Nelson, 2012). This factor would not be relevant for warehouse aquaponic treatments because these are in an enclosed building without transparent glazing. The null hypotheses tested in this experiment include: H10: There is no difference in yield among basil produced aquaponically and those grown in soilless medium; H20: There is no difference in yield among basil grown with different fish in aquaponic production. Furthermore, H30: There is no difference in yield between basil grown with different aquaponic treatments.

2 | MATERIALS AND METHODS

2.1 | Genotypes tested

Cultivars were chosen based on popularity in aquaponic production and the fresh market. Three basil cultivars (pelleted seed) were grown in this experiment: “Elenora” (a 65-day, intermediate type with downy mildew resistance; Seed Lot No. 52780), “Genovese” Compact Improved (a 74-day, large-leaf, classic Italian type; Seed Lot No. 48383), and “Nufar” (a 77-day, large-leaf Italian type with Fusarium-resistance, bred for field and greenhouse production; Organic Seed Lot No. 48702). Pelleted seed lots were acquired from Johnny’s Selected Seeds (Fairfield, ME). Varying numbers of experimental units (replications) were included in each treatment because of space limitations; the specifics are delineated within each treatment.

The basil experiment was conducted for a 13-month period (January 2016–February 2017); aquaponics research was conducted in the Minneapolis–Saint Paul Metropolitan area, State of Minnesota, USA, St. Paul Campus of the University of Minnesota (44°59’17.8”N, −93°10’51.6”W). Basil seeds were sown in 288 plug trays filled with soilless medium, that is, pasteurized Berger BM2 Germination Mix (Berger Peat Moss, Saint-Modeste, Quebec, Canada) or in 3.81 cm diameter cylinder Trock rockwool plugs (medium grade, 4CF, 30/PL; Therm-O-Rock East, Inc., New Eagle, PA). Plug conditions for germination followed the guidelines for basil plugs (Ball Seed Company, 2019). An intermittent mist system, at a mist frequency of 10 min intervals (mist nozzles, reverse osmosis water) during 06:00 a.m.–10:00 p.m. with a 7 s. Duration, was used for seed germination in a glass greenhouse (21/21°C, day/night, 16 hr; 06:00 a.m.–10:00 p.m.) with lighting at a minimum set point of 150 μmol/m²/s (Anderson et al., 2011). Once they reached the true leaf stage, seedlings at the same stage of development were transplanted into rockwool plugs for the aquaponic systems, whereas those in the germination mix were transplanted into a soilless medium. A pretest of transplantable seedling fresh weight (FW of entire seedlings—roots, epicotyl, cotyledons and true leaf) analysis of variance (ANOVA) showed no significance difference among cultivars (p = .305). Thus, the pooled mean ± SD (66.59 ± 24.81 mg) was used as a uniform FW for all transplanted seedlings. The initial FW of <1 g/seedling would be negligible on commercial grower scales and was, thus, ignored in final plant harvest FW determinations, similar to previous hydroponic basil yield studies (Walters & Currey, 2015, 2018).

Four fish species were grown in the greenhouse and warehouse aquaponic facilities at the University of Minnesota: goldfish, Carassius auratus, koi, Cyprinus carpio, tilapia, Oreochromis spp., and yellow perch, Perca flavescens and were purchased as fingerlings at various time points (2014–2016), depending on their availability (Abbey, Anderson, Yue, Short, et al., 2019; Abbey, Anderson, Yue, Schermann, et al., 2019). PetSmart (Roseville, MN) was the source for the goldfish, purchased in March 2014. Koi were purchased at Tangletown Gardens (Plato, MN) in February 2016. The tilapia were grown by Arrowhead Fisheries, LLC (Canon City, CO) and shipped in January, 2015. Yellow perch were bought from Will Allen Farms, Growing Power (Milwaukee, WI) in March 2015. Once the biofilters were established in all aquaponic treatments (see below), all fish types were slowly acclimated in a quarantine tank before being placed in each system.
2.2 | Experimental setup

Our experimental setup was identical to that used for aquaponic strawberry (Abbey, Anderson, Yue, Short, et al., 2019) and lettuce production (Abbey, Anderson, Yue, Schermann, et al., 2019). Aquaponic systems were hand-built or installed in a staggered fashion which meant corollary timing for fish purchase and arrival (see above) as well as starting each aquaponic system (treatments). There were five environmental systems (treatments; the four aquaponic treatments are illustrated in Figure 1): (a) soilless medium (control; a non-aquaponic system), (b) floating raft deep water culture (DWC; Figure 1c), (c) A-frame ebb and flow (Figure 1a), (d) tray ebb and flow (Figure 1b), and (e) warehouse (Figure 1d). Treatment (e) was in a warehouse, whereas the others (a–d) were conducted in greenhouses. Koi fish were grown in treatments (c) and (e), yellow perch were used in treatment (b), whereas both goldfish and tilapia were produced in treatment (d).

Each cultivar treatment was equally randomized (randomized block design) throughout each system (Abbey, Anderson, Yue, Short, et al., 2019; Abbey, Anderson, Yue, Schermann, et al., 2019), with the exception of the A-frame ebb and flow treatments which were randomized by each polyvinyl chloride (PVC) pipeline of plants (cf. Figure 1, Abbey, Anderson, Yue, Short, et al., 2019). Experimental units were unbalanced among systems because of differences in available space and limitations of each growing system. For all treatments (aquaponic and soilless medium), the basil plants were grown in coordination with day neutral strawberries (“Albion,” “Portola,” and “Evie 2,” Fragaria ×ananassa; Abbey, Anderson, Yue, Short, et al., 2019) and lettuce (“Rex,” “Skyphos,” “Salanova Series Mix,” Lactuca sativa; Abbey, Anderson, Yue, Schermann, et al., 2019). The number of lettuce (Abbey, Anderson, Yue, Schermann, et al., 2019) and strawberry plants (Abbey, Anderson, Yue, Short, et al., 2019) per treatment were previously reported but are included herein in calculating the total number of plants/tank for each aquaponic treatment. The number of experimental units ranged from: (a) 32 plants/cultivar of each crop × 9 cultivars (all crops) = 288 total of all plants in the soilless medium (control; a non-aquaponic system) which were grown in square 754 cm³ plastic pots in carrier trays (Landmark Plastic, Akron, OH) filled with Sunshine LC8 soilless potting medium (Sun Gro Horticulture, Agawam, MA) and spaced at a density of 20 plants/m²; (b) 12 plants/tank (4 basil + 4 lettuce + 4 strawberries/tank) × 8 tanks = 72 total of all plants in the floating raft DWC spaced at a density of 9.03 plants/m²; (c) 144 plants/tank (8 basil + 8 lettuce + 8 strawberries per tube × 6 tubes/A-frame) × 2 tanks = 288 total of all plants in the A-frame ebb and flow spaced at a density of 18 plants/m²; (d) 108 plants/tank (9 basil + 9 lettuce + 9 strawberries per tub) × 4 tubs/tank) × 2 tanks = 216 total of all plants in the tray ebb and flow spaced at a density of 8 plants/m²; (e) 90 plants/tank (30 basil + 30 lettuce + 30 strawberries/tank) equally divided between two growing tubs × 1 tank = 90 total of all plants in the warehouse spaced at a density of 15 plants/m². In treatments where the number of plants grown/tank was not equally divisible by the number of cultivars/crop (n = 3), then the plants for each cultivar were randomly chosen.

Fish feeding and monitoring occurred throughout the experimentation period at daily intervals of 08.00 a.m.–09.00 a.m. and 03.00 p.m.–04.00 p.m. Personnel washed their hand thoroughly prior to entering each greenhouse or warehouse. Upon entering, personnel walked on the foot baths filled with quaternary ammonium chloride salts (Green-Shield®) on the floor to disinfect their shoes. All aquaponic tanks were checked to make sure that the water was flowing in each system and being aerated. If issues with a pump arose, fish feeding ceased and the fish/water quality was closely monitored and mechanically aerated until the issue was fixed. Plants were also visually inspected daily for nutrient deficiencies and insect or disease infestations. Prior to fish feeding, water quality was measured in 50% of the tanks each day with the remainder tested the next day; this alternation of testing continued throughout the experiment. Measurements were recorded for equilibrium water temperature, air temperature was recorded automatically with an Argus Controller (Surrey, British Columbia, Canada), oxygen level (>6 ppm), pH (6.5–7.5 range with an optimum of 7), nitrates (<0.75 ppm), nitrites (<0.75 ppm), ammonia (<0.75 ppm), alkalinity, or water hardness (ppm GH/KH, GH—general hardness, KH—carbonate hardness; API GH/KH Test Kit, https://apifishcare.com/pdfs/products-us/ gh-kg-test-kit/api-gh-&-kh-test-kit-instruction-manual.pdf) and total dissolved solids or TDS (ppt). If ammonia,
nitrites, or nitrates exceeded 0.75 ppm, water flow to the biofilter was checked to ensure it was cycling (emptying and filling); fish feeding ceased for the following day. Fish were then fed and checked for clinical signs of pain or distress, such as reduced/increased breathing, darkening of the skin, altered swimming behavior (listlessness, surface breathing, loss of equilibrium), aggression, reduced feeding, and infections (sores). If any dead fish were found, they were removed, sealed in plastic bags, and frozen immediately prior to disposal in accordance with our Institutional Animal Care and Use Committee protocol, 1610-34203A. Any dead leaves or plant residue were removed from the tanks and growing areas daily.

2.3 | Environmental conditions

2.3.1 | Insect control

We controlled insects in all greenhouses and the warehouse via a combination of biocontrol methods including yellow sticky tape and cards, the release of biocontrol agents, and registered fungicides. Yellow sticky cards
were positioned on metal holding wires to catch flying insects, placed intermittently throughout the crop; yellow “Stiky Tape” (15.24 cm in 161.5 m length rolls; Arbico Organics, Oro Valley, AZ) was positioned above and/or below the growing areas to catch additional insects. Several types of mites (Amblyseius andersoni, Amblyseius cucumeris, Amblyseius swirskii, Neoseiulus fallacis, Galendromus occidentalis, Neoseiulus californicus, Phytoseiulus persimilis; Beneficial Insectary, Redding, CA and Rincon Vitova, Ventura, CA) were released rotationally for biocontrol in all greenhouse and warehouse growing facilities during the experiment for spider-mite, Tetranychus urticae, white fly, Trialeurodes vaporariorum, and thrip, Thysanoptera spp. control (Abbey, Anderson, Yue, Short, et al., 2019; Abbey, Anderson, Yue, Schermann, et al., 2019).

2.3.2 | Soiless medium (control)

A soilless medium treatment (control) was both included and excluded as a type of “fish” treatment for comparative purposes, based on previous aquaponics research in strawberries and lettuce (Abbey, Anderson, Yue, Short, et al., 2019; Abbey, Anderson, Yue, Schermann, et al., 2019). Basil production occurred in greenhouse environmental conditions of 24.4 ± 3.0/18.3 ± 1.5°C day/night daily integral and a 16 hr photoperiod (06.00 a.m.–10.00 p.m.; long days). Supplemental lighting was supplied during winter months and cloudy days by 400 W high-pressure sodium-high-intensity discharge (HPS-HID) lamps, at a minimum of 150 μmol/m²/s at plant level (Abbey, Anderson, Yue, Short, et al., 2019; Abbey, Anderson, Yue, Schermann, et al., 2019). The computerized greenhouse was in the St. Paul campus Plant Growth Facilities (University of Minnesota, St. Paul, MN) as previously described (Abbey, Anderson, Yue, Short, et al., 2019; Abbey, Anderson, Yue, Schermann, et al., 2019).

Basil seedlings (germination mix) were transplanted into square 754 cm³ plastic pots (Landmark Plastic, Akron, OH) using Sunshine LC8 soilless potting medium (Sun Gro Horticulture, Agawam, MA), when they had at least one true leaf. Fertigation water was applied twice daily, between 07:00 a.m.–08:00 a.m. and 4:00 p.m.–5:00 p.m., using a constant liquid feed of 125 ppm N supplied from a water-soluble 20N–4.4P–16.6K fertilizer (Scotts, Marysville, OH). Monthly rotational fungicide drenches were administered, as applied successfully in aquaponic lettuce and strawberry production (Abbey, Anderson, Yue, Short, et al., 2019; Abbey, Anderson, Yue, Schermann, et al., 2019).

2.3.3 | Floating raft DWC (yellow perch)

This floating raft DWC treatment was in an aquaponic greenhouse with a 23.6 ± 0.8°C daily integral, based on a temperature setpoint of 23.5°C (Figure 1c). Identical photoperiod or light duration (16 hr photoperiod; 06.00 a.m.–10.00 p.m.; long days) and light quantity (min. 150 μmol/m²/s at plant level) were also supplied during winter months and cloudy days and biocontrol methods, as instituted in the soilless medium treatment, were also used. In this and all other subsequent aquaponic treatments in greenhouses and the warehouse, electric generators served as the electrical power backup system for all aquaponic setups. The floating raft DWC system had eight aluminum tanks (193 × 77.5 × 75 cm, l × w × h with 6.5 cm thick walls) for fish and plant production (Figure 1c). Each tank had a volumetric capacity of 696.516 L (184 gals.), although the tanks were filled with ~378.541 L (~100 gals.) to allow for floating rafts on top of the surface as well as preventing fish escape. The same tanks and volumes were also used in the A-frame ebb and flow and the tray ebb and flow systems. A floating raft system (2/tank; 60 × 60 × 5.5 cm, Owens Corning FOAMULAR 150, R-10 insulation sheathing; Owens Corning Co., Toledo, OH) was used in each tank; the water volume/tank was ~550 L or 0.55 m³. Each fish tank had two connected biofilters using plastic, hemispherical barrels (68 × 47 × 26 cm; cut in half lengthwise). Both biofilters for each fish tank were filled with 2 cm diameter granite gravel (Hedberg Aggregates, Stillwater, MN) and inoculated with ammonium chloride (1 g/biofilter; Hawkins Chemical Co., Roseville, MN). A low density (~25–30 fingerlings/tank) of goldfish was used to start the
biological filter in the gravel; these were removed several months later and replaced with yellow perch (150–30 fingerlings/tank) as the tested fish species for this treatment.

A magnetic drive pump (Danner Supreme 700 GPH, Danner, Islandia, NY) lifted water to the biofilter tanks at a rate of ~4 L/min. Each outflow had valves, and was split between the two biofilter tanks and a third outlet that discharged directly to the fish tank for additional aeration and circulation. Each biofilter tank had an automatic bell siphon that permitted the water levels in the gravel to rise from a low point of ~2 cm depth to a maxima of ~15 cm. At the maxima, the bell siphon would start and the water would drain into the fish tank, creating an ebb and flow in the gravel. Plant spacing on each raft was a max. of 16 plants in a 4 × 4 grid, each plant could be grown in a 12 cm diameter net cup (Hydrofarm Central, Grand Prairie, TX) filled with rockwool to anchor the basil roots. Water quality was routinely monitored (5 d/week, excluding weekends) (Abbey, Anderson, Yue, Short, et al., 2019; Abbey, Anderson, Yue, Schermann, et al., 2019). The daily temperature integral was 22.3 ± 0.9°C. Yellow perch were grown in this system for the duration of the experiment at varying densities (20–30 fish/tank), depending on age (Sorensen, Robinson, & Roth-Krosnoski, 2015). This treatment had 30 perch/tank. The perch were fed a Zeigler Finfish Bronze (Florida Tropical Fish Farmer’s Coop Store), starting first with the 3 mm pellets containing 35% min crude protein, 5% min crude fat, 4% max crude fiber (https://ftffacoop.com/zeigler/949-bronze-floating-3mm-1-8-33lb-bag.html), followed by the larger 5 mm pellets with the same percentages of crude protein, crude fat, and crude fiber (https://ftffacoop.com/zeigler/950-bronze-floating-5mm-3-16-33lb-bag.html) as the perch aged. The feeding rates followed the recommendations of Hart, Garling, and Malison (2006). Each fish tank received, on average, 50 mg of fish feed/day (regardless of pellet size) unless fish death occurred. For this and all other types of fish grown in the aquaponic treatments, the fish feeding rates were a maintenance diet to primarily provide a nitrogen source for the plants.

Tray ebb and flow (goldfish, tilapia): For this greenhouse, the daily integral was 21.7 ± 0.4°C with a temperature set-point of 21.5°C (Abbey, Anderson, Yue, Short, et al., 2019; Abbey, Anderson, Yue, Schermann, et al., 2019). Identical photoperiod or light duration (16 hr photoperiod; 06.00 a.m.–10.00 p.m.; long days) and light quantity (min 150 µmol/m²/s at plant level) were also supplied during winter months and cloudy days and biocontrol methods, as instituted in the soilless medium treatment, were also used.

One fish tank (aluminum; identical specifications as for the floating raft DWC, A-frame ebb and flow systems) was used for each of two independent, galvanized steel frame, adjustable shelving rack systems (reps.). Ammonium carbonate was used in both this treatment and in the A-frame ebb and flow systems to start the biofilters in 8–10 cm diameter lava rock (D-Rock Center, New Brighton, MN) to produce ~1 mg/L ammonia with an initial start of goldfish. The goldfish (90/tank) were fed tropical flake food from Pentair Aquatic Ecosystems (#ZTF25; https://pentairaes.com/flake-food.html) containing “15% min fat, 2% max fiber, 12% max moisture, 8% max ash.” Each fish tank received, on average, 50 mg of fish feed (regardless of pellet size) unless fish death occurred. Prior to the planting of basil, one fish tank was later replaced with tilapia, while goldfish remained in the other tank. All fish started as fingerlings and continued at varying densities (20–30 fish/tank), depending on fish age. As fingerlings, the tilapia were first fed starter crumble pellet size #2 (mm; http://www.skrettingusa.com/collections/frontpage/products/starter-crumble) containing 52% min protein, 16% min oil, 9% max moisture, 3% max fiber, 12% max. ash, and 18.2% digestible energy. This feed was followed by 1.5 mm, then 2.5 mm and finally 3.5 mm sized Skretting pellets as the fish grew, all of which contained 35% min protein, 7% min oil, 9% max moisture, 5% max fiber, 9% max ash, and 13% digestible energy (https://www.skrettingusa.com/products/koi-color_?_pos=1&_sid=f48d983c4&_ss=r). Each fish tank received, on average, 50 mg of fish feed (regardless of pellet size) unless fish death occurred.

Both systems had two shelves/rack (Figure 1b; cf. Figure 2; Abbey, Anderson, Yue, Short, et al., 2019). Two tubs/shelf were specially designed for the system (123 × 94 × 18 cm; Polytank Co., Litchfield, MN), each of which could hold six 50.8 × 25.4 cm (10 in. × 20 in.) web trays into which separate plug trays (50 s or 72 s) were inserted for the plants. The upper shelf in each rack system was exposed to natural and supplemental lighting (HPS HID lights), whereas the second shelf has supplemental light-emitting diode (LED) lighting supplied by either Sunshine Systems GrowPan (450–470, 630 nm; 300 W; Sunshine Systems, LLC, Wheeling, IL) or Green Power LED (450–470, 660 nm; 300 W; 152 × 12 cm; 110v strips; Royal Philips N.V., Andover, MA) (Figure 1b). One plastic, rectangular
tub (123 × 186 × 18 cm; Polytank Co., Litchfield, MN) served as a biofilter for each tank and was filled with 3–4 cm diameter lava rock. The biofilter tubs were placed onto concrete flooring.

A-frame ebb and flow (Koi): This treatment was in a greenhouse with a 21.7 ± 0.4°C daily integral with a temperature set-point of 21.5°C (Abbey, Anderson, Yue, Short, et al., 2019), although daily temperatures were 23.5 ± 0.9°C. Identical photoperiod or light duration (16 hr photoperiod; 06.00 a.m.–10.00 p.m.; long days) and light quantity (min 150 μmol/m²/s at plant level) were also supplied during winter months and cloudy days and biocontrol methods, as instituted in the soilless medium treatment, were also used.

Koi were grown in this treatment at varying densities (20–30 fish/tank), with decreasing numbers as fish grew in size to avoid overcrowding and induce stress. All koi were fed the same feed as the tilapia (see above); the rates varied (50 mg/tank), based on weekly fish sizes and densities. The two tanks in this greenhouse each fed separate A-frame ebb and flow systems (Figure 1a; cf. Figure 1; Abbey, Anderson, Yue, Short, et al., 2019). Water moved from the fish tank to the biofilter via airlift pumps; a Danner Supreme 700 GPH mag drive pump lifted the water to the A-frame lines from the biofilter and drained back to the respective fish tank. Four plastic, hemispherical tanks (68 × 47 × 26 cm) served as biofilters. They were mounted below each A-frame and filled with 3–4 cm diameter lava rock.

2.3.4 Warehouse (koi)

The warehouse treatment was a retrofitted walk-in cooler (7.19 m × 4.87 m × 2.74 m) located in the Plant Growth Facilities headhouse basement, near the greenhouse treatments. This warehouse had galvanized interior walls with a type F5 (Fantastically Fun Fresh Food Factory) commercial system from Nelson and Pade Company (http://aquaponics.com/; Montello, WI). The F5 system comprises one, 416 L fish tank with separate biofilters and two 0.9 m × 1.5 m plastic tubs for the floating rafts in which the basil was grown (Figure 1d). This treatment had a total of 32 koi fish/tank; feeding rates were the same as the other treatments (50 mg/tank/day). There were 15–5.8 cm net pots/raft and 2 rafts/tub that allowed for a maximum capacity of 90, tightly spaced plants to be grown in this system. An LED lighting system was used which consisted of a triple-band light (bar) above the plants, and telescoped vertically as plant height increased (Agrivolution LLC, http://www.agrivolution.co/; South Windsor, CT). The LED system was necessary as use of HPS-HID lamps would have severely overheated the warehouse and exceed the capacity of the cooling system. These LEDs had single-chip diodes emitting blue, green, and red light with full photosynthetically active radiation (PAR = 400–700 nm). Identical photoperiod or light duration (16 hr photoperiod; 06.00 a.m.–10.00 p.m.; long days) and light quantity (min. 150 μmol/m²/s at plant level) were also supplied year-round as well as biocontrol methods, as instituted in the soilless medium treatment, were also used. Supplemental cooling was used to maintain an average growing temperature of 20–21°C day/night.

2.4 Data collection

Basil plants had a single cut (1×) at each harvest at marketable maturity (Univ. of California, Davis, 2005). Each plant was harvested with a cut at the top of the soilless media or rockwool plug to divide it into the marketable top and the roots (bottom). Harvests were every ~4 weeks. (summer) to every ~6 weeks (winter); all treatments had at least 6 harvests. Harvests for each treatment occurred in the same week.

The difference in production time between summer (~4 weeks) and winter (~6 weeks) seasons is because of the reduced duration of available sunlight at northern latitudes (such as 45°N lat. herein) during winter as well as the lowered angle of the sun in the horizon (Nelson, 2012). At noon on the winter solstice (22 December), the angle of elevation from the horizon of the sun at 45°N lat. is 21.5°. Low sun angles (0° to 45°) such as this confer less intense sunlight. This significant difference in sun angle, even with supplemental lighting, reduces available light during the
winter months at 45° N lat. For plant production (Nelson, 2012), causing an increase in the production time as noted. In contrast, at noon on the summer solstice (22 June), the angle of elevation from the horizon of the sun at 45° N lat. = 68.5°, a difference of 47° from 22 December.

Above-ground portions were weighed (fresh weight, g/plant) and then put into a high-temperature oven (77°C) (Hotpack, Philadelphia, PA) for 7 days. Once drying was complete, each basil plant was removed from the oven and weighed again to obtain dry weights (g/plant). Final yields are reported as g/m² as well as g/plant.

2.5 Statistical analyses

We used a generalized linear mixed model test using non-normal distributions (GLIMMIX) in the analysis of fresh and dry weights. The analysis was conducted using a split-plot design using the type of fish as the treatment and cultivars as the variable. Treatments involving Koi (A-frame ebb and flow and warehouse) were analyzed separately. Dependent variables in this analysis were fresh and dry weights.

Summer weight gain for the plants over each 4-week period production period was based on the total feeding amount of 2,800 mg feed/tank in each treatment, whereas the winter weight gain for the plants during the 6-week winter production period was based on 2,100 mg feed/tank. While it might appear that the winter production period’s higher feeding total of two additional weeks, in comparison with the summer period, provides more potential nutrients for the plants; it must be emphasized that cold, darker winters in northern latitudes translate into decreased rates of nutrient uptake and plant growth. While the temperatures kept within the day/night setpoints standard error, conversion of ammonia into nitrate N occurs slower in the winter periods than during the summer which also means that less nitrate N is available for plant uptake and growth than in the summer production time period (Faust & Will, 2016). Thus, longer winter production periods in northern latitudes are needed to realize the same potential in quicker forcing regimes in summer.

The GLIMMIX determined the correlation between data that were fit to a specified statistical model (Abbey, Anderson, Yue, Short, et al., 2019). Common non-normal distribution models were tested and the lognormal distribution was found to have the lowest Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC) and, therefore, the best fit. A GLIMMIX assumption was a normal distribution of random effects, which fit this experiment. All statistical analyses were conducted using the software SAS v.25 (Cary, NC).

3 RESULTS AND DISCUSSION

ANOVA for fish water temperature were not significant for treatments (p = .057) and tanks (p = .987), whereas season did differ significantly (p ≤ .001; Table 1). Nearly all the interactions for water temperature were not significant, with the exception of treatment × season interaction (p ≤ .001; Table 1). Water temperatures were higher in the summer (22.43–25.11°C) when compared with winter (17.11–22.36°C), although only significantly different in the warehouse (Table 2), most likely because of the warehouse being underground in a modified cooler.

pH values (ANOVA) were not significantly differing over any main effect (treatment, season, or tank) or their interactions (Table 1). Mean pH values (pooled within treatments) ranged from pH = 7.19 (ebb and flow) to 8.32 (floating raft DWS; Table 2). These data illustrate the effectiveness of monitoring and pH changes instituted during the daily fish maintenance.

Ammonia was primarily not significant among all main effects and most interactions; only treatment × season was significant (p ≤ .001; Table 1). Mean ammonia levels (pooled for seasons) ranged from 0.4 ppm (A-frame) to 0.78 ppm (floating raft DWS; Table 2) and did not differ in significance.

Nitrite levels were very highly significant for treatments and season as well as treatment × season (p ≤ .001; Table 1). Summer nitrite levels ranged from 0.11 ppm (warehouse) to 3.49 ppm (A-frame; Table 2) but did not differ
in significance. In contrast, most winter nitrite levels (ranging from 0.67 to 7.3 ppm for floating raft DWS and A-frame, respectively; Table 2) were significantly higher than in summer; the only exception was the floating raft DWS treatment which did not differ seasonally. In the summer, biofilters were equally efficient in reducing the nitrite levels; greater and significantly different nitrite levels occurred among treatments in the winter (Table 2). This indicates that biofilters differed in efficiency among treatments as well as over seasons (Table 2). The accumulation of nitrites in winter is common in greenhouses because of a decreased rate of nitrification (Nelson, 2012).

### Table 1

| Effects                  | Temperature (°C) | pH     | Ammonia (ppm) | Nitrite (ppm) | Nitrate (ppm) | Alkalinity (ppm GH/KH) | TDS (ppt) |
|--------------------------|------------------|--------|---------------|---------------|----------------|------------------------|-----------|
| Trmt                     | 0.057 ns         | 0.202 ns | 0.422 ns      | ≤0.001***     | ≤0.001***      | ≤0.001***              | 0.598 ns  |
| Season                   | ≤0.001***        | 0.146 ns | 0.608 ns      | ≤0.001***     | 0.386 ns       | ≤0.001***              | ≤0.001*** |
| Tank                     | 0.987 ns         | 0.847 ns | 0.090 ns      | 0.632 ns      | 0.989 ns       | 0.126 ns               | 0.290 ns  |
| Trmt × season            | ≤0.001***        | 0.398 ns | ≤0.001***     | ≤0.001***     | 0.243 ns       | ≤0.001***              | 0.514 ns  |
| Trmt × tank              | 0.510 ns         | 0.825 ns | 0.544 ns      | 0.689 ns      | 1.000 ns       | 0.013**                | 0.378 ns  |
| Season × tank            | 0.406 ns         | 0.842 ns | 0.362 ns      | 0.614 ns      | 0.710 ns       | 0.584 ns               | 0.439 ns  |
| Trmt × season × tank     | .985 ns          | 0.837 ns | 0.119 ns      | 0.436 ns      | 0.239 ns       | 0.221 ns               | 0.124 ns  |

Abbreviation: ns, not significant.
**P ≤ 0.01.
***P ≤ 0.001.

### Table 2

| Treatment       | Season  | Temp (°C) | pH     | Ammonia (ppm) | Nitrite (ppm) | Nitrate (ppm) | Alkalinity (ppm GH/KH) | TDS (ppt) |
|-----------------|---------|-----------|--------|---------------|---------------|----------------|------------------------|-----------|
| Floating raft DWS | Summer  | 22.43 b   | 4.9 a  | 46.72 bc      | 59.79 c       | –              | 1.27 a                 |           |
|                 | Winter  | 22.04 b   | 0.67 a | 31.15 b       | 5.55 a        | 2.22 b         | –                      |           |
| Pooled          | 8.32 a  | 0.78 a    | 22.42 a|               |               |                |                        |           |
| Ebb and flow    | Summer  | 24.24 b   | 2.54 a | 31.15 b       | 5.55 a        | 2.22 b         | –                      |           |
|                 | Winter  | 22.36 b   | 7.25 b | 31.15 b       | 5.55 a        | 2.22 b         | –                      |           |
| Pooled          | 7.19 a  | 0.48 a    | 70.77 b|               |               |                |                        |           |
| A-frame         | Summer  | 24.19 b   | 3.49 a | 35.8 b        | 4.65 a        | 2.47 b         | –                      |           |
|                 | Winter  | 22.17 b   | 7.30 b | 4.65 a        | 2.47 b        | –              | –                      |           |
| Pooled          | 7.20 a  | 0.40 a    | 70.77 b|               |               |                |                        |           |
| Warehouse       | Summer  | 25.11 b   | 0.11 a | 53.88 c       | 46.18 bc      | –              | 1.20 a                 |           |
|                 | Winter  | 17.11 a   | 3.17 b | 46.18 bc      | –             |                |                        |           |
| Pooled          | 7.56 a  | 0.42 a    | 72.94 b|               |               |                |                        |           |

Note: Tanks within treatments were not significant; thus all means are pooled for tanks. Mean separations within columns based on Tukey's honestly significant difference (HSD) test at α = .05. – indicates too many missing data points.
Nitrate levels were significantly different for treatments \((p \leq 0.001)\) but not any other main effect or interaction (Table 1). Mean nitrate levels in the floating raft DWS (22.42 ppm) were significantly lower than all other treatments (Table 2). It is unclear what caused the difference of nitrates in the floating raft DWS, although the tank water surface was always covered by floating rafts, unlike the other treatment tanks which remained uncovered.

Alkalinity levels differed significantly for treatment \((p \leq 0.001)\) and season \((p \leq 0.001)\) as well as two interactions (treatment \(\times\) season, treatment \(\times\) tank; Table 1). Mean alkalinity levels were predominantly significantly lower in the winter than in the summer season (Table 2). All mean alkalinity levels \((4.65 - 59.79\) ppm GH/KH; Table 2) were within the acceptable range of 40–65 ppm GH/KH for greenhouse irrigation water (Getter, 2015); only levels >150 ppm GH/KH would have required treatment.

TDS were only significant for season \((p \leq 0.001)\) in the ANOVAs. Significantly higher mean TDS levels occurred in the winter \((2.22 - 2.47\) ppt) than summer \((1.04 - 1.27\) ppt; Table 2). None of these values were high enough to interfere with water flower and filtration in the systems because sediments were routinely removed as well as line and drain cleanouts.

ANOVA indicated that both fresh and dry basil weights were significantly different \((p \leq 0.001)\) for the factors in the systems: Aquaponic Locations by Greenhouse; Fish, Soilless Medium Included; as well as Fish without Soilless Medium Included (Table 3). In contrast, the factor of Basil Cultivars was not significantly different \((p \geq 0.05)\) for both fresh and dry weights (Table 3) and data from all three basil cultivars were pooled.

Mean fresh weights of basil ranged from 144 g/m\(^2\) \((9.6\) g/plant) in the warehouse with koi to 1,390 g/m\(^2\) \((69.5\) g/plant) in the soilless medium treatment (Table 4). The A-frame ebb and flow treatments with koi had the second-lowest average fresh weight of 23.9 g/plant, although its 446.9 g/m\(^2\) was rate was the third lowest because the plant number/ m\(^2\) differed from other treatments (Table 4). Fresh weight standard deviations from the mean were not consistently lower than the means, for example, the Floating raft DWC with yellow perch was 577.9 ± 623.9 g/m\(^2\) \((64.0 ± 69.1\) g/plant) \((\text{mean ± SD})\) and three other treatments had increased variation (Tray ebb and flow with goldfish; A-frame ebb and flow with Koi; Tray ebb and flow with tilapia; Table 4).

Consistent with mean fresh weights, mean dry weights were lowest in the warehouse/koi treatment 22.5 g/m\(^2\) \((1.5\) g/plant) and highest in the soilless medium 162 g/m\(^2\) \((8.14\) g/plant; Table 4). Dry weight standard deviations were lower in more treatment than fresh weights, with the exception of the A-frame ebb and flower (Koi) and Tray ebb and flow (tilapia) treatments (Table 4). Thus, the variation (SD) in the fresh weights was primarily because of differences in water content.

Although there were not any significant differences among basil cultivars for either fresh or dry weights (Table 3), “Nufar” had elevated levels of fresh and dry weights in comparison with the other two cultivars (Table 5). Basil harvests were a single cut that included leaves and stems, so it is not known if the lack of differences among cultivars is attributable to leaf and/or stem (internode length or diameter) biomass differences.
While the soilless medium (non-aquaponic) treatment had the highest fresh (69.5 g/plant) and dry (8.14 g/plant; Table 4) weights of basil production, in both instances there was at least one comparable aquaponic treatment for both fresh and dry weights. The floating raft DWC with yellow perch produced an average of 64.0 g/plant fresh weight (Table 4) while the Tray ebb and flow with goldfish had a mean dry weight of 7.68 g/plant (Table 4). The higher basil yields of 64.0 g/plant in the Floating raft DWC with yellow perch (Table 4) to the next best two treatments of Tray ebb and flow with goldfish (59.6 g/plant) and Tray ebb and flow with tilapia (52.4 g/plant) over a 4- (summer) and 6-week (winter) season provide a baseline range of expected yield in aquaponic systems. Yield expectations of aquaponic growers should be based on this range.

These differences in basil yield among aquaponic treatments (Table 4) is not entirely based on fish feed differences. Feed totals for the perch in the floating raft DWC treatment over the 4- and 6-week production seasonal periods were 2,800 and 4,200 mg/tank which meant the 30 fish/tank consumed, on average, a total of 93.2 and 140 mg during those production periods, respectively. These levels are the same for the tilapia in the tray ebb and flow tank and the koi A-frame tanks when there were 30 fish/tank. The other tanks with 32 koi (warehouse) and 90 goldfish (tray ebb and flow) meant totals of 31/46.6 mg/fish and (summer/winter) and 87.5 and 131.2 mg/fish, respectively.

### TABLE 4  Mean ± SD of fresh and dry weights (g/m²; g/plant) of basil grown in soilless medium as well as aquaponic greenhouse and warehouse treatments and four fish types

| Measurement       | Treatment          | Fish types | g/m²      | g/plant  |
|-------------------|--------------------|------------|-----------|----------|
| Fresh weight**    | Soilless medium    | —          | 1,390.0 ± 498.0 | 69.5 ± 24.9 |
|                   | Floating raft DWC  | Yellow perch | 577.9 ± 623.9 | 64.0 ± 69.1 |
|                   | Tray ebb and flow  | Goldfish   | 447.0 ± 339.0 | 59.6 ± 45.2 |
|                   | A-frame ebb and flow | Koi      | 4,469.3 ± 770.4 | 23.9 ± 41.2 |
|                   | Tray ebb and flow  | Tilapia    | 393.0 ± 478.5 | 52.4 ± 63.8 |
|                   | Warehouse          | Koi        | 144.0 ± 181.5 | 9.6 ± 12.1  |
| Dry weight**      | Soilless medium    | —          | 162.0 ± 72.0 | 8.1 ± 3.6  |
|                   | Floating raft DWC  | Yellow perch | 53.3 ± 52.4 | 5.0 ± 3.8  |
|                   | Tray ebb and flow  | Goldfish   | 57.8 ± 39.0 | 7.7 ± 5.2  |
|                   | A-frame ebb and flow | Koi      | 39.3 ± 69.2 | 2.1 ± 3.7  |
|                   | Tray ebb and flow  | Tilapia    | 42.0 ± 54.8 | 5.6 ± 7.3  |
|                   | Warehouse          | Koi        | 22.5 ± 27.0 | 1.5 ± 1.8  |

Note: Means are subdivided by location and fish treatment, including the soilless medium, and are the average of all harvests during the production period.
**p ≤ 0.01.

### TABLE 5  Mean ± SD of fresh and dry weights g/m² (g/plant) of basil “Elenora,” “Genovese,” and “Nufar”

| Measurement       | Cultivar    | g/m²      | g/plant  |
|-------------------|-------------|-----------|----------|
| Fresh weight (g/plant) | “Elenora”   | 651.0 ± 564.2 | 46.5 ± 40.3 |
|                   | “Genovese”  | 666.4 ± 746.2 | 47.6 ± 53.3 |
|                   | “Nufar”     | 805.0 ± 938.0 | 57.5 ± 67.0 |
| Dry weight (g/plant) | “Elenora”   | 75.6 ± 65.8 | 5.4 ± 4.7  |
|                   | “Genovese”  | 70.0 ± 78.4 | 5.0 ± 5.6  |
|                   | “Nufar”     | 86.8 ± 95.2 | 6.2 ± 6.8  |

Note: g/m² calculated as the mean number of plants/m² for all treatments = 14.0 plants/m².
No visible effects to the plant roots in any of the treatments, particularly the floating raft DWC treatment, were observed during the duration of this experiment. This was based on visual observations of roots in all aquaponic treatments on a weekly basis as the floating rafts or individual net pots were lifted up for observation of root damage. Earlier experimentation with different plant types had shown mild to severe root feeding on another herb crop, that is, dill (*Anethum graveolens*), but no such damage occurred with basil. While we saw no visible signs of root feeding or damage by the fish in the floating raft DWC treatment, it cannot be ruled out. This is one of the inherent challenges of testing different aquaponic systems because not all provide direct fish and plant root system interactions.

Aquaponic production of basil in northern latitudes is not significantly different than greenhouse production of sweet basil elsewhere (Table 6; note that yields are listed as g/plant because most citations did not provide m² area of production). For example, “Genovese” produced mean fresh weights of 47.6 g/plant (Table 5), compared with 27.1–64.1 g/plant in hydroponic greenhouses (Table 6) (Kiferle et al., 2013; Walters & Currey, 2015). “Nufar,” produced an average of 57.5 g/plant in our experiment (Table 5) whilst Walters and Currey (2015) only reported 43.7 g/plant (Table 6). Other, unnamed basil cultivars in hydroponic production (15–63 g/plant with varying fertilizer regimes; Table 3) were similar in range to both “Genovese” and “Nufar,” although one reported aquaponic system by Rackoczy, et al. (2007) had significantly lower production fresh weights (6–25 g/plant; Table 3). Therefore, basil growth was not reduced by the aquaponic growing environment because there was no difference in yield between some aquaponic treatments and the control (soilless medium), a typical greenhouse production environment. These yields overall were lower than production under 24-hr. lighting of 102.4 g/plant (Skrubis & Markakis, 1976). However, this can be reasonably attributed to the slow growth of production in a northern latitude because of reduced day lengths and light intensities as well as cooler temperatures.

The basil cultivars tested also had the same (nonsignificant) yield across treatments (Table 3). The significant differences, as indicated by the analysis, are between the different kinds of fish used or the environmental changes created by using different aquaponic systems, particularly in the warehouse where the temperatures had to be maintained significantly lower than in the greenhouses because of the heat buildup from electric motors. Thus, the fresh weight of basil cultivars in the warehouse with Koi fish (9.6 g/plant; Table 4) were significantly lower than all other aquaponic systems (23.9–64.0 g/plant; Table 4) and/or the soilless medium control treatment (69.5 g/plant; Table 4). Dry weights followed similar trends with the fresh weights although dry weights are significantly lower in

| Basil cultivars and growing conditions | Fresh weight (g/plant) | Dry weight (g/plant) | Citation |
|----------------------------------------|------------------------|----------------------|----------|
| Unknown cv. Hydroponic greenhouse, various fertilizer regimes | 15–63 g (early yield) | — | Hochmuth, Davis, Laughlin and Simonne (2003) |
| Unknown cvs.; aquaponics, outdoor production | 6, 18, 25 g (field, batch, staggered harvests) | — | Rakocy et al. (2007) |
| “Genovese;” hydroponic greenhouse | 34.3 | 2.6 | Walters and Currey (2015) |
| “Genovese;” hydroponic greenhouse | 27.1–64.1 | 2.96–6.03 | Kiferle, Maggini, and Pardossi (2013) |
| “Nufar;” hydroponic greenhouse | 43.7 | 3.6 | Walters and Currey (2015) |
| “Nufar;” hydroponic greenhouse | 30.5 | 2.5 | Walters and Currey (2018) |
| “Nufar;” hydroponic greenhouse; harvested at week 14 | 96.6 | 9.6 | Saha, Monroe, and Day (2016) |
| “Nufar;” aquaponic greenhouse | 150.2 | 15.9 | Saha et al. (2016) |

Note: Because most articles did not report the number of plants/m² this is not reported.
all cases than fresh weights, as would be expected because of water removal (Hochmuth et al., 2003; Rakocy et al., 2007; Succop & Newman, 1997; Walters & Currey, 2015).

There was a significant difference in both fresh and dry weight yields ($p \leq .01$; Table 4) when basil was grown with different fish as the source of nutrients in the tested aquaponic systems (treatments) (Table 3). Fresh weight yield was lowest in the systems using koi, with an average fresh weight of 23.9 g/plant in the A-frame ebb and flow treatment and 9.6 g/plant in the warehouse system (Table 4); similar trends were found for the dry weights. The A-frame ebb and flow koi system was in the same greenhouse as both the Tray ebb and flow tilapia and Tray ebb and flow goldfish systems, so it is unlikely that the reduced plant growth is attributable to variation in temperature or disease/insect pressures. It is unlikely that the ebb and flow part of the A-frame system design interfered with plant growth as the goldfish and tilapia ebb and flow tray systems (with the same design for water transfer) had yields similar to the control (Table 4). Other factors could have contributed to these differences, for example, light levels, although the species of fish is the most likely cause of these differences in basil production.

The warehouse DWC system with koi had the lowest average fresh and dry weight/plant of any system, yielding only a fifth of fresh and dry weights (Table 4). It is unlikely that the DWC system itself contributed to low yield in the greenhouse given that fish feeding levels were comparable among treatments and yield in the Floating raft DWC with yellow perch were not statistically different from the soilless medium control (Table 4). Similarly, LED lighting was also used in the rack systems with goldfish and tilapia and did not impact basil growth in a previous study (Bochenek & Fällström, 2015).

It is more likely that the cooler air temperatures of the warehouse (20–21°C, constant day/night), contributed to stunted growth. This is in contrast to the water temperature which did not differ significantly from those in the other treatments (Table 2), thus not affecting the fish. Although basil grows at ~21°C day temperatures (Ball Seed Company, 2019; Putievsky & Galambosi, 1999), 27°C is optimal (Pogany, Bell, & Kirch, 1968). Indeed, greenhouse temperatures (reported earlier) exceeded warehouse temperatures throughout the experiment, where <21°C has a strong negative impact on growth and decreases basil yield significantly (Hiltunen & Holm, 1999). The use of koi in the system may have had a negative impact on plant size as well. If the warehouse was the only aquaponic system to have lower yield then cooler temperatures could be pointed to as the culprit. However, because the Warehouse with Koi and the A-frame ebb and flow with Koi both suffered fresh weight yield losses, Koi may be having a negative impact on basil growth. This interaction seems to be specific to basil in that the lettuce and strawberries grown in conjunction with the basil had no significant reduction in yield because of Koi (Abbey, Anderson, Yue, Short, et al., 2019; Abbey, Anderson, Yue, Schermann, et al., 2019).

Mild leaf chlorosis (slight bleaching) of the lower basil leaves was observed in all aquaponic treatments (data not shown), especially in the treatments with Koi fish, and was most likely caused by N deficiency. This was indicated by low nitrate N levels in the water of all aquaponic treatments (Table 2). Supplemental N feeding may be required, although this has not been reported previously in aquaponic systems. Iron deficiency because of water movements in aquaponic systems that prevents a sufficient amount of chelated iron from coming in contact with roots (Brüggemann, Maas-Kantel, & Moog, 1993) has been noted in strawberry plants grown in the same systems (Abbey, Anderson, Yue, Short, et al., 2019). We advise aquaponic growers to supplement N or other deficient nutrients into their system as needed for optimal growth.

Because there was an impact of the fish, particularly koi, used in the aquaponic production system on yield (fresh and dry weights, Table 4) this could translate into a difference in volatile compounds within the basil. We did not analyze the basil for oil content or quality and do not know whether aquaponic systems are any better or worse for the production of basil for use in an essential oil production. Future research should also be devoted to studying the effects of koi in aquaponic systems growing basil. Perhaps there are also nutritional differences and/or consumer sensory evaluation differences (in taste, texture, color, flavor) among basil grown in aquaponics. Future studies will provide answers to potential effects of aquaponics on these issues in aquaponic basil production.

ABBEY ET AL.
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CONFLICT OF INTEREST
The authors declare no potential conflict of interest.

AUTHOR CONTRIBUTION
Marie Abbey conducted the research and wrote the article; Neil O. Anderson and Chengyan Yue were the PIs, oversaw the research, and helped with writing and editing the article; Michele Schermann, Nicholas Phelps, Paul Venturelli, and Zata Vickers were co-PIs on the grants and helped with advisement of the research and editing of the article.

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