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Comparison of Hydroponic Butterhead Lettuce Grown in Reject Water from a Reverse Osmosis System, Municipal Water, and Purified Water

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Abstract. **A main constraint of hydroponics is the need for high-quality water. This study evaluated the viability of reverse osmosis concentrate backflush, or reject water, as a water source for hydroponic lettuce production. We compared the growth of 'Casey' butterhead lettuce produced using nutrient film technique hydroponics with municipal (tap) water (TW), reverse osmosis (RO) water, and reject water from an RO system [wastewater (WW)]. Characterization of water sources showed the following: RO had trace minerals; TW had the greatest potassium (K), calcium (Ca), and magnesium (Mg); and WW had greatest sulfur (S), sodium (Na), and chloride (Cl). No water source had detectable heavy metals. Mineral and heavy metal tissue contents were determined at mid-harvest and end-harvest, and nutrient budgets for nitrogen (N), S, Ca, Mg, Na, and Cl were calculated. Water sources did not impact lettuce growth during the 4-week hydroponic production cycle based on leaf greenness, diameter, fresh weight, or dry weight. In the nutrient reservoirs, Ca and Mg increased over time and were greatest in TW. The Cl and Na levels in WW were 106 and 203 mg·L^{-1}, respectively, and the electrical conductivity (EC) of this treatment increased to 3.5 dS·m^{-1} after 4 weeks; however, plant water uptake, water use efficiency, and percent moisture loss were unaffected. In shoot tissue, lettuce grown with WW had less Ca at mid-harvest and less Mg at mid-harvest and end-harvest compared with other treatments, suggesting antagonization of uptake by high salt. Tissue S was not different across treatments; however, there were differences in reservoirs of the water sources as S accumulated more from WW. Tissue Na and Cl levels were different among the treatments, and higher amounts in nutrient reservoirs translated to higher uptake by lettuce. Although the heavy metal content in all water sources was <0.01 ppm, arsenic, lead, and chromium were measured in lettuce tissue at levels that exceed recommended limits in most instances. Our results indicated that a water source of RO reject water from municipal feed water is a viable irrigation option for short-term hydroponic production of butterhead lettuce; however, EC reduction in the nutrient solution would be necessary when continuing sequential cropping.**

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Pressing concerns such as food security, resource scarcity, and climate change require widespread adoption of sustainable crop production practices that minimize resource use compared with that of traditional farming methods (Dubbeling et al. 2019). Hydroponics is an agricultural production system that uses a liquid nutrient solution instead of a soil-based medium (Shrestha and Dunn 2017). Nutrient film technique (NFT) is a form of hydroponics that is popular for leafy greens production in which a thin film of water containing all essential plant nutrients flows through the roots in a covered channel with enough space for root development and an adequate supply of oxygen (Langenhoven 2016). Hydroponics offers the potential to produce consistent, premium, and high yields of crops when the nutrient supply is well-managed in a high-quality water source with appropriate levels of salt and minimal contaminants (Os et al. 2016). A constraint of hydroponics is the need for high-quality irrigation water and, ultimately, the lack of available freshwater (Sheikh 2006), especially as water becomes an increasingly limiting natural resource (Mehran et al. 2017). Municipal water is frequently used for hydroponics production, but plant growth can vary depending on the source; additionally, municipal water in hydroponic nutrient reservoirs must be changed frequently to avoid an accumulation of salts (Niu et al. 2018).

Many producers have turned to reverse osmosis (RO) water because its purity lends itself to hydroponics production (Mattson and Peters 2014). Reverse osmosis is a process by which pressured semi-permeable membranes separate water from its contaminants, and the contaminants are flushed off the membranes as wastewater (WW) (Connecticut Department of Public Health 2009). This process generates a significant amount of WW; 15% to 30% of the feed water that enters the system is lost, and the removed concentrate, or reject water, requires discharge or disposal (Environmental Protection Agency 2023). For example, Madison Water Treatment Facility, one of the first municipal water sources in Minnesota to use RO, reported that of the approximate 300 gallons of water per minute entering the RO system, 210 gallons of water pass through the RO membranes and approximately 90 gallons are considered reject water (Minnesota Department of Health Public Water Supply Unit 1999). Repurposing this large amount of WW, even for crop production, may extend water resources. However, RO reject water contains approximately four- to seven-times more contaminants compared with that in feed water (Yaqub et al. 2022). Concerns regarding the use of RO reject water in hydroponics production include a build-up of salt levels in nutrient reservoirs that reduce plant biomass production and cause leaf burn (Adhikari et al. 2019) and the accumulation of heavy metal contaminants in consumed plant tissue.

Williams and DeWolf (2022) found that the growth of 'New Red Fire' leaf lettuce (*Lactuca sativa*) and 'Elida' basil (*Ocimum basilicum*) plants grown with RO, municipal water (TW), and reject water from the RO system (WW) in recirculating NFT was not different across the three water sources. A key difference in initial water quality was increased Na (150 mg·L^{-1}) and Cl (70 mg·L^{-1}) in WW and increased Na and Cl in WW nutrient solutions compared with that of the other sources when the crops were harvested, increasing to 250 and 150 mg·L^{-1}, respectively, at 27 d after transplant (DAT). These results suggested that further research of WW from RO systems is warranted to determine whether the water could be repurposed for the safe production of short-term crops produced in hydroponics systems.

The objectives of this experiment were to evaluate the viability of RO reject water as a potential irrigation water source in hydroponics and, specifically, to measure the impact of the water source on plant yield, nutrient solution management, and nutrient budgets, and to evaluate heavy metal or other ion accumulations in plant tissue that would influence the nutrient content or food safety.

Materials and Methods

Experimental design and water treatments. Our experiment was a completely random design with three treatments of different water sources and three replications of each water source for a total of nine hydroponics channels and respective nutrient reservoirs; each channel (an experimental unit) contained 11 plants. The water treatments were municipal (tap) water (TW; Manhattan, KS, USA), RO water from treated municipal water in the Throckmorton Plant Sciences Center (TPSC) at Kansas State University (Manhattan, KS, USA); and RO concentrate backflush, or reject water (WW), from the TPSC water treatment system (Fig. 1). The TPSC water treatment process has a five-stage water treatment system consisting of a prefilter, carbon filter tank (HI-FLO22; Culligan, Rosemont, IL, USA) filled with granulated activated charcoal, water softener (High Efficiency Automatic Water Softener #01023115; Culligan), RO membranes (model E2-2; Culligan), and dual deionization tanks (Mixed Bed 1; Puretec, Oxnard, CA, USA). The feed water—municipal water, in this case—is first sent to a sediment filter that removes larger sediments such as dirt and debris by means of mechanical filtration. Next, the water passes through a carbon filter that acts to remove Cl and other contaminants that affect the smell, taste, and color of the water (Culligan 2023a; Dvorak and Skipton 2013). Then, the water softener reduces the overall hardness of the water by ion exchange between Ca and Mg ions in the water, and Na ions from NaCl are added to generate a salt solution held in a separate brine tank (Culligan 2023b). After the water has been pretreated, the pressurized feedwater is sent through semipermeable RO membranes to separate the contaminants from the water (Culligan 2021). The RO system is effective at removing protozoa, bacteria, viruses, and chemical contaminants, including metal ions, and aqueous salts (Centers for Disease Control and Prevention 2023). Finally, the feed water is sent to a mixed bed deionization tank to remove any additional mineral salts by using an ion exchange of strong acid cation resin and strong base anion resin beads mixed together with the cations and anions present in the water (Puretec 2021). The WW produced directly from the flush of concentrated solution from the RO membranes was collected while the RO system was in operation. Therefore, this WW used during this study was composed of the remaining contaminants from the feedwater that entered the RO system and had been pretreated by the prefilter, carbon filter, and softening system. The prefilter and the carbon filter hold contaminates until they are replaced, and the softening system is occasionally flushed.

Plant material and hydroponic system. On 1 Jun 2023, Casey butterhead lettuce (*Lactuca sativa*) seeds (Johnnys Selected Seeds, Winslow, ME, USA), a heat and tip burn-tolerant cultivar for summer production in controlled-environment agriculture, were sown as one seed per rockwool cube (36 × 36 × 40 mm; Grodan AO 36/40 Plug, Milton, ON, Canada). The seeds were watered as needed with municipal water (Manhattan, KS, USA). Beginning 7 Jun 2023, the seedlings were fertigated until they were placed in an NFT hydroponic system with 10 × 244 cm HydroCycle NFT channels (FarmTek, Dyersville, IA, USA). On 17 Jun 2023, 11 uniform seedlings were transplanted into each of the nine channels of the system. Each channel drained to its own 19-L nutrient reservoir (Menards green bucket; Eau Claire, WI, USA) calibrated to 16 L. Reservoir lids (Encore Plastics Corp., Cambridge, OH, USA) had a diameter of 30.5 cm with two holes drilled to accommodate a 323-cm nutrient solution delivery pipe from a submersible pump (Active Aqua AAPW160; Hydrofarm, Petaluma, CA, USA) and a 38-cm return line from the channel end to the tank.

On 17 Jun 2023, nutrient reservoirs were initially filled with 16 L from their respective water treatment and a fertilizer mix of 1 $g{\cdot}L^{-1}$ Peter's professional 5N–4.8P–21.6K (Dublin, OH, USA) and 0.65 $g{\cdot}L^{-1}$ Hi-Yield Calcium Nitrate 15.5N–0P–0K (Bonham, TX, USA). Afterward, each channel's reservoir was replenished weekly with 6 L of treatment water for a total of four additions. The last three of four water additions included two-thirds of the strength of the initial fertilizer amount. The pH and electrical conductivity (EC) of the reservoirs were tested daily using pH and EC pens (Bluelab Corp. Ltd., Tauranga, New Zealand), and 1 N H_2SO_4 acid solution or (rarely) a base solution of potassium hydroxide and potassium carbonate (General Hydroponics, Sebastopol, CA, USA) additions were made accordingly to target a pH range of 5.4 to 6.2. The plants were grown for 4 weeks from installation in the system, and the study was concluded on 13 Jul 2023.

Environmental data of temperature and light were recorded hourly by three HOBO sensors (Onset Corp., Cape Cod, MA, USA) placed on 19 Jun 2023. The average day temperature was 32 °C (89.6 °F). The average night temperature was 25.5 °C (77.9 °F). The high and low temperatures were 40.4 °C (104.8 °F) and 23.8 °C (74.9 °F), respectively. The average light intensity during the day was 232.8 $\mu mol{\cdot}m^{-2}{\cdot}s^{-1}$, with a high of 545.8 $\mu mol{\cdot}m^{-2}{\cdot}s^{-1}$ and a low of 3.82 $\mu mol{\cdot}m^{-2}{\cdot}s^{-1}$ (Thimijan and Heins 1983).

Data collection and analyses. Water sources were sampled four times (once during each week of the experiment). Water source samples were collected from the RO and TW supply lines that delivered water to TPSC's glass greenhouse range. A large tank was filled with WW obtained from TPSC's water treatment system on 6 Jun 2023, and used for subsequent nutrient reservoir additions and WW source sampling. In addition, 500-mL solution samples were collected from each of the nutrient reservoirs during each week of the experiment.

Plant growth data were collected at the middle (29 Jun; 12 DAT) and end (13 Jul; 26 DAT) of the cropping period. At mid-crop, three plants from the middle and each end of a channel were sampled for the experimental unit; at end-crop, four plants from every other opening in each channel were sampled for the experimental unit. Plant width was determined by averaging two perpendicular measurements of plant diameter. Leaf greenness was determined by averaging three readings per plant using a soil plant analysis development (SPAD) meter (Konica Minolta, Ramsey, NJ, USA). Fresh weight was measured and plants were

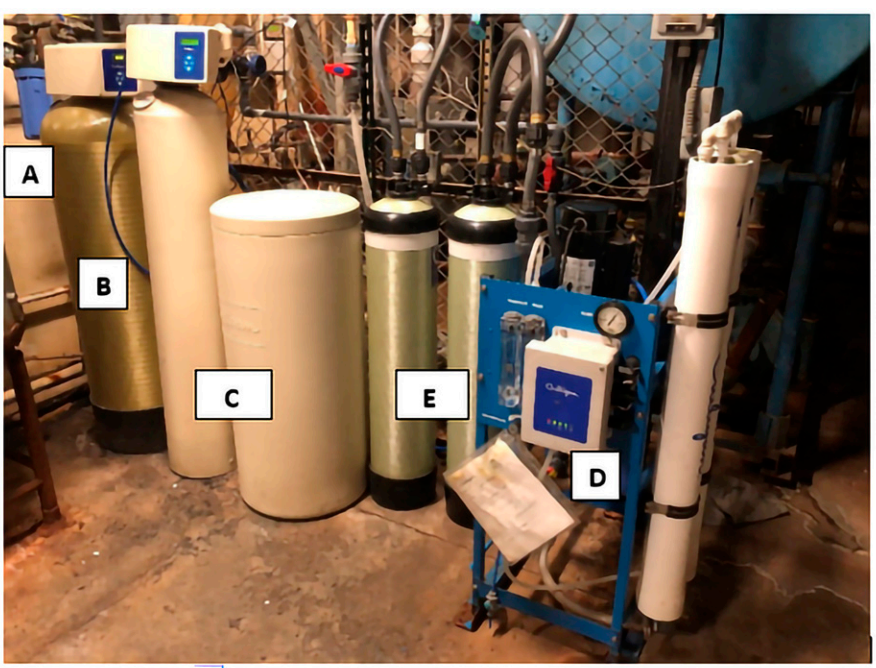

Fig. 1. The five-stage water treatment system from which the wastewater treatment was collected. Sediment filter (**A**), carbon filtration system (**B**) filled with granulated activated charcoal, water softener (**C**), reverse osmosis membranes (**D**), and dual deionization tanks (**E**).

rinsed in RO water; then, they were placed in a 70 °C forced-air oven for several days, and dry weights were measured. From these data, plant water uptake (g fresh weight/L water used), water uptake efficiency (WUE; g dry weight/L water used), and percent moisture [fresh weight − dry weight/fresh weight) × 100] were calculated for each treatment. Dried plants were ground to pass a 20-mesh screen for tissue analyses.

Nutrient analyses and nutrient budgets. Water and plant tissue samples were assayed by Kansas State University's Soil Testing Laboratory (Manhattan, KS, USA), except for the analysis of Na and tissue aluminum (Al), which were conducted by A & L Great Lakes Laboratories (Fort Wayne, IN, USA). In addition to Na, plant tissue was tested for N, phosphorus (P), potassium (K), Ca, Mg, S, iron (Fe), zinc (Zn), copper (Cu), manganese (Mn), Cl, chromium (Cr), lead (Pb), cadmium (Cd), and arsenic (As). Total N was analyzed via dry combustion (LECO TruSpec CN combustion analyzer; LECO Corp., St. Joseph, MI, USA). The Cl level was determined from calcium nitrate extraction. Nutrients P, K, Ca, Mg, Zn, Fe, Cu, Mn, and S and heavy metals Cr, Pb, Cd, and As were determined using a nitric-perchloric digest (Gieseking et al. 1935) and analyzed with ICP-OES (Model 5800; Agilent Technologies, Santa Clara, CA, USA). Then, Na and Al were analyzed via microwave hot acid extraction (SW846-2051A) and determined by inductively coupled atomic plasma (AOAC 985.01 run on Thermo iCAP 6500; Waltham, MA, USA). Elements in water samples (P, K, Ca, Mg, Zn, Fe, Cu, Mn, Zn, and S) were determined by ICP-OES (Model 5800; Agilent Technologies).

For nutrient budgets, the amounts of N, S, Ca, Mg, Na, and Cl that remained in the system were calculated by subtracting the total nutrients removed from the system from total nutrients added to the system and comparing to the estimated nutrients that remained in a channel's reservoir. The root dry weight was estimated by multiplying the shoot dry weight by a root-to-shoot ratio determined from data of hydroponic lettuce (Both et al. 1999). The root nutrient content was overestimated by multiplying the estimated root dry weight by the measured shoot tissue content.

Statistical analyses. All data were analyzed using a one-way analysis of variance with JMP Pro version 16 software (SAS Institute, Cary, NC, USA). When P>F was <0.05, the means were separated using Tukey's honest significant difference mean separation procedure.

Results and Discussion

Growth data. Hydroponic lettuce heads typically weigh approximately 140 g (Brechner et al. 2013); at end-harvest, all treatments in this study produced more than 140 g fresh weight. Niu et al. (2018) evaluated the growth of leaf lettuce 'Tropicana' grown in a recirculating NFT system and treatments comprising the supplementation of a base nutrient solution with one-third-strength nutrients in TW, RO water, or a high-salt water treatment with NaCl. They reported fresh weight yields of approximately 80 g, which was half of the fresh weight results of this study (Table 1), and dry weight yields of approximately 4.2 g, which was lower than the dry weight results of this study (Table 1). Yield differences can be attributed to cultivar, environment, and methodology characteristics. Niu et al. (2018) reported leaf greenness results of approximately 33 SPAD units, which was similar to the end-harvest leaf greenness measurements of this study (Table 1). Similar to our study, the length of their production cycle in recirculating NFT was approximately 4 weeks, and they did not observe growth differences in leaf lettuce based on their nutrient solution treatments (Niu et al. 2018).

Similar to results of Williams and DeWolf (2022), no growth differences occurred among lettuce plants grown with the treatment water sources based on leaf greenness, diameter, fresh weight, and dry weight at mid-harvest or end-harvest (Table 1) in the current study. Kim et al. (2008) reported similar results in that they observed no differences in the growth of romaine lettuce (*Lactuca sativa* L. 'Clemente') in their control and 15-d duration of fertilization with salt levels that ranged from 50 to 1000 mM, although long-term irrigation with 5 mM NaCl negatively affected lettuce growth. In contrast, Al-Maskri et al. (2010) found that salinity stress of 50 and 100 mM from NaCl for 50 d negatively affected the growth of 'Parris Island Cos' lettuce in a closed-recycled NFT system similar to the one used in our study.

Across the five studies referenced, differences in lettuce yield across salinity treatments primarily depended on the length of time during which plants were exposed to high salt levels. In general, 2 to 4 weeks of lettuce growth with increased salt levels in the recirculating solution did not result in measurable yield differences; however, beyond this length of time, growth was measurably affected. Because hydroponic lettuce typically has a production cycle of 4 weeks after transplant into recirculating culture, production without yield loss is possible.

Plant water use. Murray et al. (2021) reported that lettuce contains approximately 95% water, which was consistent with our results across treatments at both mid-harvest and end-harvest (Table 2). Increasing salt levels did not negatively affect the lettuce percent moisture after 2 or 4 weeks of production in recirculating culture.

Similar to yield, no differences among the water treatments occurred for plant water uptake, WUE, or percent moisture loss at either mid-harvest or end-harvest (Table 2). The values among all the treatments were similar, indicating that the water sources had little effect on plant water relations during the 4 weeks of production in the recirculating system. Our plant water uptake results were similar to those in a study that evaluated the growth of lettuce indoors under various light-emitting diode lighting (Pennisi et al. 2019) and determined that lettuce water use was within the range of 47 to 75 g fresh weight/L; however, our lettuce water use ranged from 55 to 65 g fresh weight/L (Table 2). Our WUE results were lower than those reported by Vetrano et al. (2020), who evaluated the use of gibberellic acid to increase the salt tolerance of 'Crispa' leaf lettuce grown at three different salt levels in a floating system and calculated the WUE as 4.3 to 4.6 g plant dry weight/L water.

Nutrient levels in water sources. The nutrient contents of the treatment water sources (Table 3) comprised different N, K, Ca, Mg, S, Cl, and Na levels. The WW had the greatest amounts of S, Cl, and Na. The TW had the greatest amounts of K, Ca, and Mg. The RO treatment had the smallest amount of all nutrients and Na. The RO system is expected to remove more than 98% of salt rejection, Cl, and other contaminants and total dissolved solids from TW (Culligan 2016), and only trace amounts of nutrients are present in the RO water source. Although As, Pb, Cr, and Cd were below our detectable limit of 0.01 $mg \cdot L^{-1}$ in the water sources (data not shown), trace amounts may accumulate over time in nutrient reservoirs; therefore, plant tissue should be further evaluated. The water sources did not supply N, P, K, Fe, Mn, Zn, or Cu in amounts that would influence the fertilization program (Table 3). The TW

Table 1. Leaf greenness, average diameter, fresh weight, and dry weight of 'Casey' butterhead lettuce plants measured at mid-harvest (week 2) and end-harvest (week 4) grown in nutrient film technique hydroponics with three water sources of reverse osmosis (RO), municipal [tap water (TW)], and reject water from RO system (WW) (n = 3).

Harvest[i]	Treatment	Leaf greenness (SPAD)	Avg diam (cm)	Fresh wt (g)	Dry wt (g)
Mid	RO	29.2	16.4	17.8	1.07
	TW	27.9	15.9	14.7	0.87
	WW	28.8	17.9	21.7	1.27
	P value[ii]	NS	NS	NS	NS
End	RO	31.5	26.5	211.4	6.84
	TW	28.8	26.4	157.8	5.39
	WW	30.1	25.7	199.0	6.18
	P value	NS	NS	NS	NS

[i] Mid-harvest comprised three samples per experimental unit, and end-harvest comprised four samples per experimental unit.
[ii] Analyzed using a one-way analysis of variance.
NS = not significant.

Table 2. Plant water uptake, water use efficiency, percent moisture loss at mid-harvest (week 2), and percent moisture loss at end-harvest (week 4) of 'Casey' butterhead lettuce plants grown with three water sources of reverse osmosis (RO), municipal water [tap water (TW)], and reject water from the RO system (WW) during 4 weeks in a recirculating nutrient film technique hydroponic system.

Treatment	Plant water uptake[i] (g FW/L)	Water use efficiency[ii] (g DW/L)	Percent moisture mid-harvest[iii] (%)	Percent moisture end-harvest[iii] (%)
RO	62.0	2.03	93.9	96.8
TW	54.6	1.92	94.0	96.5
WW	65.0	2.07	94.2	96.8
P value	NS[iv]	NS	NS	NS

[i] PWU (g FW/L) = g plant fresh weight/L water used. This incorporated mid-harvest and end-harvest fresh weights of plants produced in the system divided by the total water added to nutrient reservoirs.
[ii] WUE (g DW/L) = g plant dry weight/L water used. This incorporated mid-harvest and end-harvest dry weights of plants produced in the system divided by the total water added to nutrient reservoirs.
[iii] PM (%) = {[plant fresh weight (g) − plant dry weight (g)]/plant fresh weight (g)} × 100.
[iv] Analyzed using a one-way analysis of variance.
DW = dry weight; FW = fresh weight; NS = not significant.

contained 31 $mg{\cdot}L^{-1}$ Ca and 17 $mg{\cdot}L^{-1}$ Mg; however, much lower amounts were found in RO and WW. The WW and TW provided 57 and 17 $mg{\cdot}L^{-1}$ S, respectively, which are beneficial in the fertilizer program.

The most notable differences between the water sources were Cl and Na in WW of 106 and 203 $mg{\cdot}L^{-1}$, respectively, which were two-times and four-times the amount in the TW, respectively (Table 3). These levels have the potential to induce salinity stress in lettuce. All water sources, including WW, had levels of nitrate as N, sulfate as S, Fe, Mn, Zn, Cu, and Cl that were below the primary and secondary maximum contaminant levels (MCLs) enforced by the Environmental Protection Agency for drinking water (Environmental Protection Agency 2009) (Table 3). The primary MCLs pertain to health-related effects, and secondary MCLs refer to cosmetic (skin and teeth) or esthetic (taste, odor, and color) effects of the water source.

Nutrient solution management. The EC and pH were managed daily (Fig. 2). The target pH range was 5.4 to 6.2, and acid or base additions were performed depending on the daily pH measurements. Although alkalinity is <55 $mg{\cdot}L^{-1}$ in the municipal water source used for the TW and in the RO system that generated the WW, its pH typically exceeded 8.5 from municipal treatment (Kansas Department of Health and Environment 2024). Therefore, the pH of both TW and WW began near 7.0, and it initially climbed as they were brought within range over 4 d with acid additions. Base additions were needed for 3 d to increase the pH of the RO reservoirs to the target range. On 24 Jun 2023, 6 L of treatment water sources were added to the reservoirs, and this increased the pH of both TW and WW and decreased EC across treatments by diluting nutrients (Fig. 2). On 30 Jun, 5 Jul, and 10 Jul 2023, 6 L of the treatment water sources with two-thirds of the fertilizer recipe were added to the reservoirs. The EC and pH tended to change slightly in response to the additions (Fig. 1). Generally, the pH of the reservoirs increased after adding water and fertilizer. The EC generally decreased after the addition of water and nutrients, except at the end of the study, when EC trended upward for WW and RO treatments. The overall increase in the EC of the WW treatment can be attributed to the accumulation of salts in the reservoir. Niu et al. (2018) similarly found that the EC of the recirculating solution increased over time in TW and TW plus NaCl treatments; however, in contrast to our study, the pH of RO was highest at the end of their study. After 2 weeks of production in recirculating culture, the EC of their nutrient reservoirs peaked at 2.0. During our experiment, the EC of the WW nutrient reservoir reached 3.5 ± 0.53 $dS{\cdot}m^{-1}$ after 4 weeks of production in recirculating culture. The target EC for hydroponic lettuce production is 1.2 to 1.8 $dS{\cdot}m^{-1}$ (Sharma et al. 2018); however, lettuce grew well in the WW treatment with EC levels that exceeded this range for the duration of the experiment. However, continued production with this water source and increasing EC would inevitably impact lettuce yield without intervention to decrease EC or the elements that contribute to it.

Ion levels in recirculating nutrient solutions and shoot tissue. The N, P, and K levels were not different between the treatment nutrient solutions (Table 4). The N level declined over the course of the month of the experiment and was <10 $mg{\cdot}L^{-1}$ in all treatments at the end of the experiment (Fig. 3A); however, shoot tissue N concentrations of 4.8% to 5.0% at end-harvest (Table 5) were not deficient. The P, K, and Mn levels also decreased in the nutrient solutions over the course of the study (Table 4); this was attributed to their rapid uptake rates (Bugbee 2004; Mattson and van Iersel 2011). Similar to N, no deficiencies in plant tissue were observed for these nutrients (Table 5).

The water source did not result in meaningful differences in micronutrients of Fe, Mn, Zn, and Cu between treatments, although RO water had slightly more Fe and Zn at 3 DAT than that in TW or WW (Table 4). In plant tissue, all of these nutrients were within the sufficiency ranges across water treatments at both mid-harvest and end-harvest (Table 5); however, WW resulted in more Zn at mid-harvest than RO and more Cu at end-harvest than RO or TW. Aluminum in tissue was not different between water source treatments, but it increased from mid-harvest to end-harvest.

During the first 2 weeks of production, S levels in recirculating solution were different between water sources. The lower level of S in the RO nutrient solution compared with that in TW and WW during this period can be attributed not only to the higher content of S in the TW and WW water sources (Table 3) but also to the additions of H_2SO_4 for pH management of TW and WW during the first week of the study. As the experiment progressed, S differences between the water sources resulted in increased accumulation in the WW nutrient solution compared with that in RO and TW (Fig. 3B; Table 4); however, tissue concentrations were not different across treatments at mid-harvest or end-harvest (Table 5). Niu et al. (2018) found that S was highest in their tap water plus NaCl treatment after 3 weeks of production in recirculating culture compared

Table 3. Nutrient content of reverse osmosis (RO), municipal [tap water (TW)], and reject water from RO system (WW) water sources averaged from samples collected each of 4 weeks during production in nutrient film technique hydroponics system used to produce 'Casey' butterhead lettuce.

	Nutrient sources ($mg{\cdot}L^{-1}$)											
Treatment	N	P	K	Ca	Mg	S	Fe	Mn	Zn	Cu	Cl	Na
RO (n = 4)[i]	<0.01 b[ii]	<0.01	<0.01 c	0.09 b	0.05 b	0.08 c	<0.01	<0.01	0.02	0.01	8.54 c	0.15 c
TW (n = 4)	0.52 a	<0.01	8.57 a	30.7 a	17.3 a	26.1 b	<0.01	<0.01	0.02	0.02	56.0 b	49.2 b
WW (n = 4)	0.56 a	<0.01	3.89 b	1.06 b	0.57 b	57.2 a	<0.01	<0.01	0.01	0.01	106.1 a	202.7 a
P value	***	NS	***	***	***	***	NS	NS	NS	NS	***	***
EPA standard[iii]	10	NA	NA	NA	NA	250	0.3	0.05	5	1.3	250	NA

[i] n = total number of samples per treatment of the reported average.
[ii] Letters indicate the mean difference according to Tukey's honest significant difference multiple comparison procedure (a = 0.05).
[iii] Environmental Protection Agency primary or secondary maximum contaminant level for drinking water.
Ca = calcium; Cl = chloride; Cu = copper; EPA = Environmental Protection Agency; Fe = iron; K = potassium; Mg = magnesium; Mn = manganese; N = nitrogen; Na = sodium; NA = not available; P = phosphorus; S = sulfur; Zn = zinc.

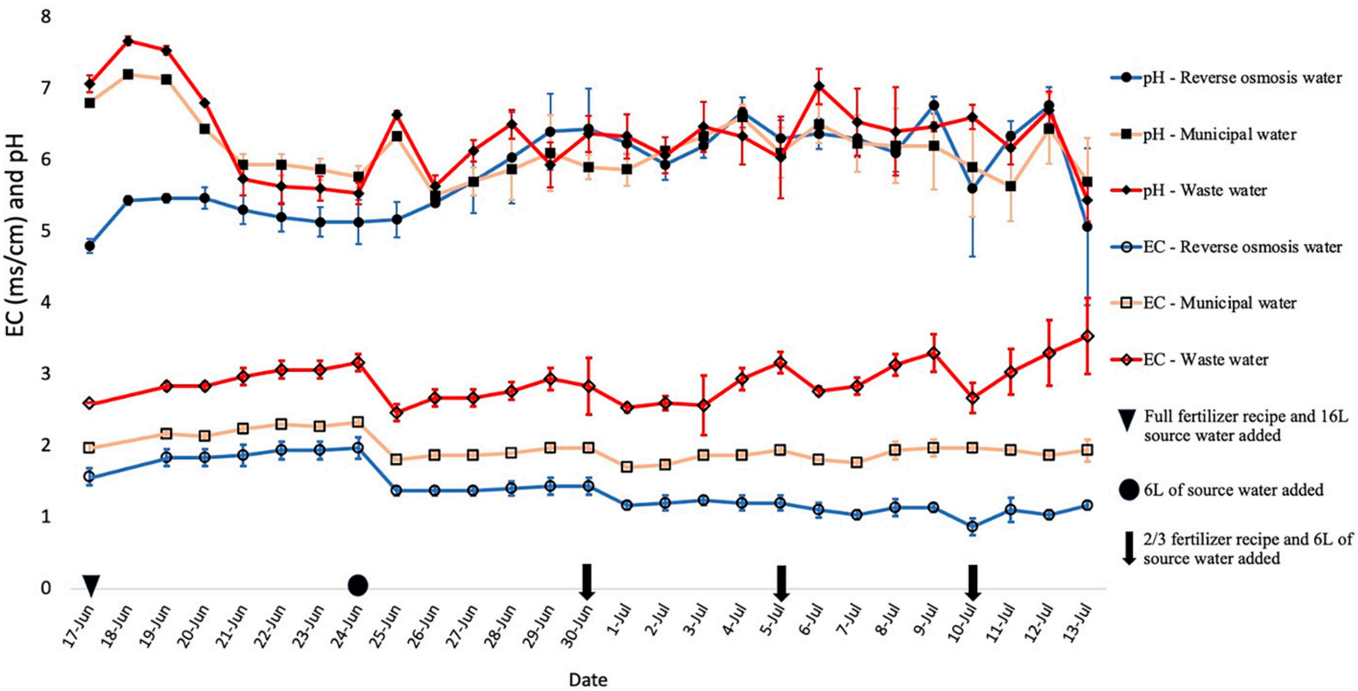

Fig. 2. The daily pH and electrical conductively (EC) levels as managed in water treatment reservoirs of municipal, reverse osmosis, and wastewater for 4 weeks. Symbols indicate additions made to the nutrient reservoirs.

with that in RO or TW treatments. Despite this difference, and similar to our study, they did not observe differences between lettuce sap or tissue content in 'Tropicana' leaf lettuce. The S accumulation in hydroponic lettuce did not appear to be affected by excessive S or the salinity of the nutrient solution.

The Ca and Mg levels were higher in the recirculating TW nutrient solution compared with that in RO and WW throughout the experiment, but they were not significantly higher at 17 and 24 DAT (Table 4; Fig. 3C, 3D). In the TPSC water treatment system that generates the RO and WW, municipal water passes through a water softener in which Ca and Mg ions in the municipal water are exchanged with Na from a NaCl solution to reduce the hardness of the water (i.e., Ca and Mg contents) in the RO (Fig. 1). Therefore, the water that flows from the softener to the RO components of the system have lower Ca and Mg and higher Na and Cl levels. Because WW is composed of RO system reject water, it also has lower Ca and Mg than that in TW.

The declines in Ca and Mg levels in solution from 3 to 10 DAT (Fig. 3C, 3D) can be attributed to the replenishment of the nutrient reservoirs with the treatment water sources without fertilizer at 7 DAT (Fig. 2). The increasing levels of Ca and Mg in the recirculating nutrient solutions of all three treatments from 10 to 24 DAT (Fig. 3C, 3D) suggested that the supply exceeded plant uptake. Tissue concentrations of Ca and Mg were low compared with the standards (Table 5); Ca was at least 1% lower than optimal and Mg was on the low end of the sufficiency range (Mills and Jones 1996). This may be partially explained by antagonization of Ca and Mg uptake by the higher levels of Na and EC, in general, in WW. The plant tissue results supported this possibility because Ca was 0.25%

Table 4. Nutrient content of nutrient solution reservoirs (n = 3) from weekly samples collected 3, 10, 17, and 24 d after transplant (DAT) in a nutrient film technique hydroponics system used to produce 'Casey' butterhead lettuce grown with reverse osmosis (RO), municipal [wap water (TW)], and reject water from RO system [wastewater (WW)] water sources.

		Nutrient (ppm)											
DAT	Treatment	N	P	K	Ca	Mg	S	Fe	Mn	Zn	Cu	Cl	Na
3	RO[i]	205.2	50.3	219.6	132.8 b[iii]	62.6 b	81.6 c	3.05 a	0.70	0.42 a	0.30	6.90 c	4.91 c
	TW	184.5	45.7	216.6	157.3 a	74.5 a	128.1 b	2.56 b	0.61	0.36 b	0.27	63.5 b	55.9 b
	WW	197.0	47.0	226.2	131.3 b	63.9 b	185.7 a	2.66 ab	0.57	0.38 ab	0.30	129.7 a	218.7 a
	P value[ii]	NS	NS	NS	**	**	***	*	NS	*	NS	***	***
10	RO	141.1	39.3	162.0	119.2 b	56.6 b	74.0 c	2.75	0.59	0.36	0.27 a	4.12 c	4.64 c
	TW	171.2	36.9	166.5	149.2 a	70.8 a	131.9 b	2.44	0.51	0.33	0.22 b	69.2 b	62.5 b
	WW	143.6	35.3	161.6	117.0 b	57.9 b	214.4 a	2.71	0.52	0.29	0.25 ab	143.7 a	268.9 a
	P value	NS	NS	NS	**	**	***	NS	NS	NS	*	***	***
17	RO	89.6	41.5	73.9	153.0 ab	74.2	145.5 b	3.20	0.42	0.37	0.37	9.87 c	5.82 c
	TW	114.4	36.8	124.0	181.8 a	88.3	184.7 b	2.93	0.42	0.32	0.27	75.0 b	73.2 b
	WW	68.4	24.0	88.0	149.4 b	73.8	354.0 a	3.34	0.53	0.30	0.35	170.3 a	360.0 a
	P value	NS	NS	NS	*	NS	***	NS	NS	NS	NS	***	***
24	RO	0.01	37.6	0.01	188.0	89.8	213.2 b	3.43	0.29	0.35	0.49	8.36 c	1.11 c
	TW	9.54	23.4	39.5	210.7	104.9	255.8 b	3.46	0.47	0.29	0.34	84.0 b	84.8 b
	WW	7.34	30.2	10.8	173.9	85.4	467.0 a	3.17	0.35	0.24	0.40	166.6 a	423.0 a
	P value	NS	NS	NS	NS	NS	**	NS	NS	NS	NS	***	***

[i] n = 3 samples per treatment of the reported average.

[ii] Analyzed using a one-way analysis of variance.

[iii] Letters indicate the mean difference according to Tukey's honest significant difference multiple comparison procedure (a = 0.05).

Ca = calcium; Cl = chloride; Cu = copper; Fe = iron; K = potassium; Mg = magnesium; Mn = manganese; N = nitrogen; Na = sodium; NS = not significant; P = phosphorus; Zn = zinc.

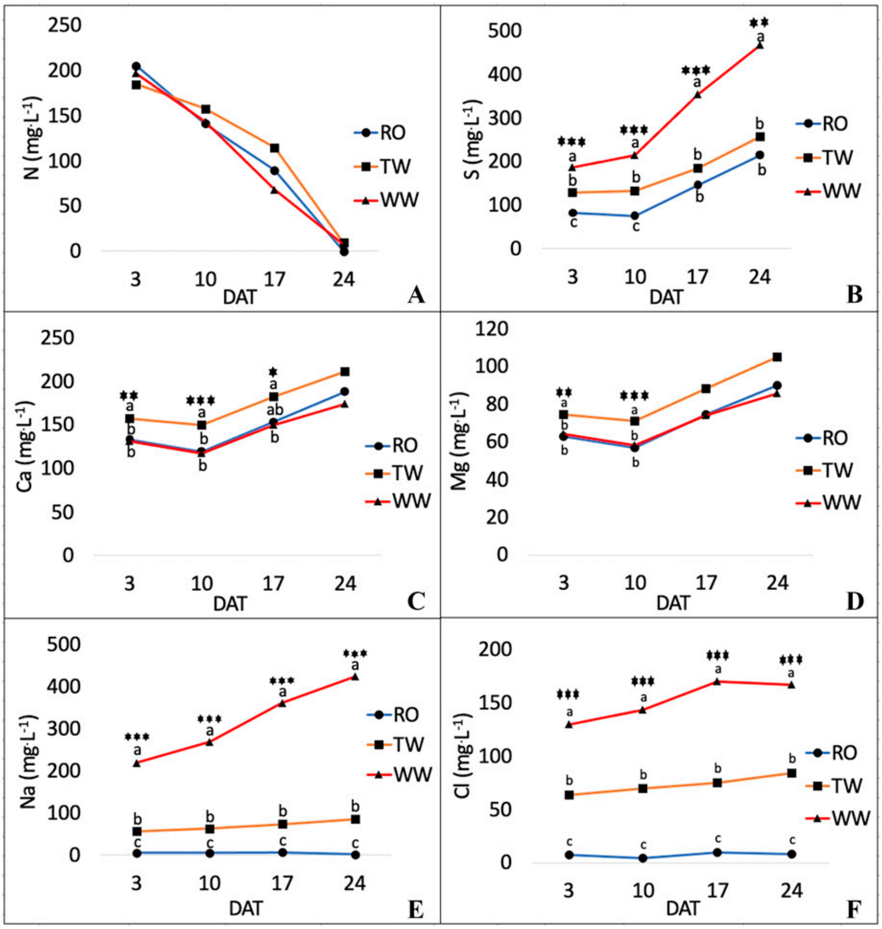

Fig. 3. Nutrient contents of nitrogen (**A**), sulfur (**B**), calcium (**C**), magnesium (**D**), sodium (**E**), and chloride (**F**) from weekly water collections for 4 weeks of lettuce grown in three water sources: reverse osmosis (RO), municipal (tap) water (TW), and RO concentrate backflush [wastewater (WW)]. Letters indicate the mean difference (n = 3) according to Tukey's honest significant difference multiple comparison procedure on a given date (a = 0.05).

and Mg was approximately 0.1% lower at mid-harvest in plants grown with WW compared with those grown with RO or TW (Table 5), and Na in WW nutrient solution exceeded that of RO and TW by 160 $mg{\cdot}L^{-1}$ or more (Table 4). Grattan and Grieve (1998) noted that Na can impact Ca mobility and distribution in plants. By end-harvest, differences in tissue Ca and Mg between the three treatments were less significant despite high levels of Na in recirculating solution of WW. Niu et al. (2018) reported that tissue Ca and Mg levels of 'Tropicana' leaf lettuce were within the same range as that of our study and did not find a difference between their RO, TW, or TW plus NaCl treatments.

Different levels of both Na and Cl were found between all three of the water sources during each week of plant growth in recirculating culture (Table 4; Fig. 2E, 2F). Based on the water softening process described for the TPSC water treatment system, it is not surprising that Na and Cl levels were negligible in RO water and much higher in WW compared with the nutrient solutions in TW during the entire month of production (Fig. 2E, 2F). The Na level of the WW was approximately 220 $mg{\cdot}L^{-1}$ at 3 DAT and accumulated to approximately 420 $mg{\cdot}L^{-1}$ by 24 DAT, which was a 48% increase over the production cycle. The Na level of TW was approximately 5 $mg{\cdot}L^{-1}$ at 3 DAT and stayed at approximately that level for the duration of the production cycle. The Cl levels in the TW and WW nutrient solutions increased 24% and 22%, respectively, from 3 to 24 DAT. Niu et al. (2018) also observed increasing Na and Cl in their tap water treatment over 20 d of production in recirculating culture.

Tissue Na and Cl levels were different among water treatments at both mid-harvest and end-harvest (Table 5), with plants grown with WW accumulating 1.8% Na and 2.9% Cl after 1 month of production. In contrast, plants grown in TW accumulated 1.0% Na and 1.5% Cl. The higher levels of these elements in the water source translated to increased plant uptake and accumulation in plant tissue. Similar results were reported by Niu et al. (2018).

Levels of the heavy metals As, Pb, Cr, and Cd were not different in plant tissue between water sources, except for Pb at end-harvest (Table 6), which was similar in TW and WW but reduced in RO compared to

Table 5. Tissue nutrient analyses of 'Casey' butterhead lettuce shoots grown with reverse osmosis (RO), municipal water [tap water (TW)], and reject water from RO system [wastewater (WW)] water sources (n = 3) at mid-harvest (week 2) and end-harvest (week 4) in a nutrient film technique hydroponics system.

Harvest[i]	Treatment	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	SO_4 S (%)	Fe ppm	Mn ppm	Zn ppm	Cu ppm	Cl (%)	Na (%)	Al ppm
Mid	RO	5.61	0.93	7.18	1.08 a[iii]	0.44 ab	0.29	119.7[iv]	142.6	47.6 b	9.23	0.58 c	0.04 c	24.7
	TW	5.79	1.01	7.82	1.11 a	0.45 a	0.30	145.4	129.5	64.4 ab	9.37	1.03 b	0.24 b	31.7
	WW	5.58	0.92	7.52	0.85 b	0.36 b	0.30	128.6	129.7	67.3 a	10.0	1.50 a	0.97 a	23.0
	P value[ii]	NS	NS	NS	**	*	NS	NS	NS	*	NS	***	***	NS
End	RO	4.89	1.04	7.05	1.28	0.54 a	0.29	154.3	160.7	69.9	9.47 b	0.34 c	0.07 c	53.3
	TW	5.03	0.96	7.98	1.23	0.49 ab	0.30	189.6	186.5	68.1	9.60 b	1.80 b	0.33 b	52.7
	WW	4.78	0.96	7.33	1.03	0.43 b	0.31	177.9	175.8	84.9	13.0 a	2.86 a	1.76 a	40.0
	P value	NS	NS	NS	NS	*	NS	NS	NS	NS	**	***	***	NS
Standard range[v]		4.0–5.5	0.5–1.0	6.0–9.0	2.0–3.5	0.5–2.0	0.2–0.5	50–150	15–250	25–250	6–25	ND[vi]	ND	ND

[i] Mid-harvest comprised three samples per experimental unit, and end-harvest comprised four samples per experimental unit.
[ii] Analyzed using a one-way analysis of variance.
[iii] Letters indicate the mean difference according to Tukey's honest significant difference multiple comparison procedure (a = 0.05).
[iv] Indicates a missing data point for one sample of the reported average.
[v] Tissue concentrations reported for *Lactuca sativa* var. *capitata* (Boston or butterhead lettuce) in Mills and Jones (1996).
[vi] No data reported.
Al = aluminum; Ca = calcium; Cl = chloride; Cu = copper; Fe = iron; K = potassium; Mg = magnesium; Mn = manganese; N = nitrogen; Na = sodium; NS = not significant; P = phosphorus; S = sulfur; Zn = zinc.

Table 6. Nutrient analyses of heavy metals in 'Casey' butterhead lettuce shoots grown with reverse osmosis (RO), municipal [tap water (TW)], and reject water from RO system (WW) water sources (n = 3) at mid-harvest (week 2) and end-harvest (week 4) in a nutrient film technique hydroponics system.

Harvest[i]	Treatment	As ($\mu g \cdot g^{-1}$)	Pb ($\mu g \cdot g^{-1}$)	Cr ($\mu g \cdot g^{-1}$)	Cd ($\mu g \cdot g^{-1}$)
Mid	RO	0.73	0.66	1.30	<0.1
	TW	0.86	<0.1	1.66	<0.1
	WW	<0.1	0.86	<0.1	<0.1
	P value[ii]	NS	NS	NS	NS
End	RO	2.33	<0.1 b	2.37	<0.1
	TW	1.60	2.37 a	0.86	<0.1
	WW	0.89	0.89 ab	4.06	<0.1
	P value	NS	*	NS	NS
USDA-FAS limit[iii]		0.5	0.3	0.5	0.2

[i] Mid-harvest comprised three samples per experimental unit, and end-harvest comprised four samples per experimental unit.
[ii] Analyzed by a one-way analysis of variance.
[iii] US Department of Agriculture Foreign Agricultural Service (2023).
As = arsenic; Cd = cadmium; Cr = chromium; NS = not significant; Pb = lead.

TW. The levels in plant tissue were compared with the maximum acceptable levels in food (Table 6) (US Department of Agriculture Foreign Agricultural Service 2023). The As, Pb, and Cr levels in tissue exceeded safe limits, with a few exceptions. The Cd levels, however, were below the maximum acceptable limit. Despite the water analyses of all three sources indicating less than detectable levels (<0.01 $mg \cdot L^{-1}$) of these four heavy metals, tissue analyses suggested that they accumulated in the plant tissue over the production cycle.

Nutrient budgets. Nutrient budgets allowed for comparisons between treatments regarding where and how much N, S, Ca, Mg, Na, and Cl accumulated during the 4 weeks of hydroponic production. The amount of each nutrient that was estimated to remain in the system was based on analyses of the nutrient reservoir solution at harvest. Differences in the total amount of N added to the system were minimal between water sources, and this translated to no differences between treatments for any budget segment (budget not shown). With RO, a total of 4088 mg N was added to the system, 70% was recovered in shoot tissue, and 8% remained in reservoirs at harvest. With TW, a total of 4106 mg N was added to the system, 56% was recovered in shoot tissue, and 23% remained in reservoirs at harvest. With WW, 4107 mg N (the same as that in TW) was added to the system, 70% was recovered in shoot tissue, and 18% remained in reservoirs at harvest. At the end of the study, the lettuce shoots contained 334, 270, and 291 mg N per head when produced with RO, TW, and WW, respectively. The high salt content of the WW treatment did not dramatically influence N uptake into shoot tissue.

The nutrient budget for S was different between the treatments for budget segments of pH adjustment and both the calculated and estimated S that remained in reservoirs (Table 7). The WW had the highest S in each instance. This result was partly attributable to the greater amounts of sulfuric acid required to manage the pH of WW; however, it was primarily attributable to the water source, which contained more than twice the amount of S compared to that in TW and more than 50-times the amount of S compared to that in RO. Despite this, S in plant tissue was not different between water sources. With RO, a total of 2741 mg S was added to the system, 6% was recovered in shoot tissue, and 83% remained in reservoirs at harvest. With TW, a total of 3759 mg S was added to the system, 3.6% of the total was recovered in the shoot tissue, and 86% remained in the reservoirs at harvest. With WW, a total of 4972 mg S was added to the system, 3.3% was recovered in shoot tissue, and 84% remained in the reservoirs at harvest. Therefore, although the total amount of S varied between the water sources, the amount of applied S that remained in the nutrient reservoirs at harvest was between 83% to 86% for all treatments. At the end of the study, the lettuce shoots contained 20, 16, and 19 mg S per head when produced with RO, TW, and WW, respectively. As with N, the high salt content of the WW treatment did not influence S uptake into shoot tissue.

The nutrient budget for Ca was different among the treatments for budget segments of water samples and calculated Ca that remained in reservoirs (Table 8). In each

Table 7. Sulfur budget of 'Casey' butterhead lettuce grown in nutrient film technique hydroponics system with three water sources of reverse osmosis (RO), municipal water [tap water (TW)], and reject water from RO system (WW) for 4 weeks.

	Sulfur				
Nutrient source	RO[i]	TW	WW	Significance[ii]	Budget calculations
Full fertilizer recipe (mg)	1450	1450	1450	NA	Application (1×), g * $\mu g \cdot g^{-1}$ S fertilizer analysis
Two-thirds fertilizer recipe (mg)	1087	1087	1087	NA	Sum of total applications (3×), g applied * $\mu g \cdot g^{-1}$ S fertilizer analysis
pH Adjust (mg)	201.6 c[iii]	333.1 b	491.0 a	***	NA
Water added (mg)	2.55	888.8	1944	NA[iv]	Sum of additions (4×), L added * $\mu g \cdot L^{-1}$ S water source analysis
Shoot tissue (mg)	171.0	136.8	163.4	NS	Mid-harvest [(avg (n = 3) DW g * $\mu g \cdot g^{-1}$ S tissue analysis) * 3 plants] + End-harvest [(avg (n = 4) DW g * $\mu g \cdot g^{-1}$ S tissue analysis) * 8 plants]
Root tissue (mg)[v]	41.5	33.3	39.7	NS	Mid-harvest [(avg (n = 3) DW g * $\mu g \cdot g^{-1}$ S tissue analysis) * 3 plants] + End-harvest [(avg (n = 4) DW g * $\mu g \cdot g^{-1}$ S tissue analysis) * 8 plants]
Water samples (mg)	257.1 b	350.2 b	610.6 a	***	Sum of water samples (4×), 0.5 L * $\mu g \cdot L^{-1}$ S water reservoir analysis
Calculated remaining (mg)	2272 c	3239 b	4158 a	***	Total inputs (fertilizer, pH adjust, water added) − total removed (shoot tissue, root tissue, water samples)
Estimated remaining (mg)	2192 b	3570 ab	5725 a	*	Estimated ending reservoir amount in L * $\mu g \cdot L^{-1}$ S week 4 water reservoir analysis

[i] Reported values are the average of n = 3 repetitions of each treatment.
[ii] Analyzed using a one-way analysis of variance.
[iii] Letters indicate the mean difference according to Tukey's honest significant difference multiple comparison procedure (a = 0.05).
[iv] Statistics were not possible because n = 1.
[v] Root dry weight estimation determined by multiplying shoot dry weight at mid-crop or end-crop by the respective root-to-shoot ratio (mid-harvest ratio: 0.25538; end-harvest ratio: 0.242236) determined from the data of Both et al. (1999).
DW = dry weight; NA = not applicable; NS = not significant; S = sulfur.

Table 8. Calcium budget of 'Casey' butterhead lettuce grown in nutrient film technique hydroponics system with three water sources of reverse osmosis (RO), municipal water [tap water (TW)], and reject water from RO system (WW) for 4 weeks.

	Calcium				
Nutrient source	RO[i]	TW	WW	Significance[ii]	Budget calculations
Full fertilizer recipe (mg)	2123	2123	2123	NA	Application (1×), g * μg·g^{-1} Ca fertilizer analysis
Two-thirds fertilizer recipe (mg)	1591	1591	1591	NA	Sum of total applications (3×), g applied * μg·g^{-1} Ca fertilizer analysis
pH Adjust (mg)	NA	NA	NA	NA	NA
Water added (mg)	2.98	1045	36.0	NA[iii]	Sum of additions (4×), L added * μg·L^{-1} Ca water source analysis
Shoot tissue (mg)	732.8	561.4	537.6	NS	Mid. harvest [(avg (n = 3) DW g * μg·g^{-1} Ca tissue analysis) * 3 plants] + End harvest [(avg (n = 4) DW g * μg·g^{-1} Ca tissue analysis) * 8 plants]
Root tissue (mg)[iv]	178.0	136.4	130.7	NS	Mid-harvest [(avg (n = 3) DW g * μg·g^{-1} Ca tissue analysis) * 3 plants] + End-harvest [(avg (n = 4) DW g * μg·g^{-1} Ca tissue analysis) * 8 plants]
Water samples (mg)	296.5 b[v]	349.5 a	285.8 b	*	Sum of water samples (4×), 0.5 L * μg·L^{-1} Ca water reservoir analysis
Calculated remaining (mg)	2510 b	3712 a	2796 b	***	Total inputs (Fertilizer, pH adjust, water added) − total removed (shoot tissue, root tissue, water samples)
Estimated remaining (mg)	1951	2946	2118	NS	Estimated ending reservoir amount in L * μg·L^{-1} Ca week 4 water reservoir analysis

[i] Reported values are the average of n = 3 repetitions of each treatment.
[ii] Analyzed using a one-way analysis of variance.
[iii] Statistics were not possible because n = 1.
[iv] Root dry weight estimation determined by multiplying shoot dry weight at mid-crop or end-crop by the respective root-to-shoot ratio (mid-harvest ratio: 0.25538; end-harvest ratio: 0.242236) determined from the data of Both et al. (1999).
[v] Letters indicate the mean difference according to Tukey's honest significant difference multiple comparison procedure (a = 0.05).
Ca = calcium; DW = dry weight; NA = not applicable; NS = not significant.

instance, Ca in TW was greater than that in RO or WW. These results are explained by the increased levels of Ca in the TW water source. With RO, a total of 3717 mg Ca was added to the system, 20% was recovered in the shoot tissue, and 68% remained in the reservoirs at harvest. With TW, a total of 4759 mg Ca was added to the system, 12% was recovered in shoot tissue, and 78% remained in the reservoirs at harvest. With WW, a total of 3750 mg Ca was added to the system, 14% was recovered in the shoot tissue, and 75% remained in the reservoirs at harvest. The percent of Ca taken into shoot tissue was greatest in RO (20%) and similar in TW (12%) and WW (14%). At the end of the study, the lettuce shoots contained 87, 67, and 63 mg Ca per head when produced with RO, TW, and WW, respectively. As a benchmark, a 12.7-cm (5-inch), 163-g head of butterhead lettuce would contain 57 mg Ca (US Department of Agriculture 2019), which is comparable to that of lettuce produced in this study.

Similar to Ca, the nutrient budget for Mg was different between the treatments for budget segments of water samples and calculated Mg that remained in reservoirs (budget not

Table 9. Sodium budget of 'Casey' butterhead lettuce grown in nutrient film technique hydroponics system with three water sources of reverse osmosis (RO), municipal water [tap water (TW)], and reject water from RO system (WW) for 4 weeks.

	Sodium				
Nutrient source	RO[i]	TW	WW	Significance[ii]	Budget calculations
Full fertilizer recipe (mg)	55.5	55.5	55.5	NA	Application (1×), g * μg·g^{-1} Na fertilizer analysis
Two-thirds fertilizer recipe (mg)	41.6	41.6	41.6	NA	Sum of total applications (3×), g applied * μg·g^{-1} Na fertilizer analysis
pH Adjust (mg)	NA	NA	NA	NA	NA
Water added (mg)	5.10	1671	6890	NA[iii]	Sum of additions (4×), L added * μg·L^{-1} Na water source analysis
Shoot tissue (mg)	41.7 b[iv]	152.8 b	905.0 a	***	Mid-harvest [(avg (n = 3) DW g * μg·g^{-1} Na tissue analysis) * 3 plants] + End-harvest [(avg (n = 4) DW g * μg·g^{-1} Na tissue analysis) * 8 plants]
Root tissue (mg)[v]	10.1 b	37.1 b	219.7 a	***	Mid-harvest [(avg (n = 3) DW g * μg·g^{-1} Na tissue analysis) * 3 plants] + End-harvest [(avg (n = 4) DW g * μg·g^{-1} Na tissue analysis) * 8 plants]
Water samples (mg)	8.24 c	138.2 b	635.3 a	***	Sum of water samples (4×), 0.5 L * μg·L^{-1} Na water reservoir analysis
Calculated remaining (mg)	42.1 c	1440 b	5227 a	***	Total inputs (fertilizer, pH adjust, water added) − total removed (shoot tissue, root tissue, water samples)
Estimated remaining (mg)	12.8 b	1185	5151 a	**	Estimated ending reservoir amount in L * μg·L^{-1} Na week 4 water reservoir analysis

[i] Reported values are the average of n = 3 repetitions of each treatment.
[ii] Analyzed using a one-way analysis of variance.
[iii] Statistics were not possible because n = 1.
[iv] Letters indicate mean difference according to Tukey's honest significant difference multiple comparison procedure (a = 0.05).
[v] Root DW estimation determined by multiplying shoot dry weight at mid-crop or end-crop by the respective root-to-shoot ratio (mid-harvest ratio: 0.25538; end-harvest ratio: 0.242236) determined from data by Both et al. 1999.
DW = dry weight; Na = sodium; NA = not applicable; NS = not significant.

Table 10. Chloride budget of 'Casey' butterhead lettuce grown in nutrient film technique hydroponics system with three water sources of reverse osmosis (RO), municipal water [tap water (TW)], and reject water from RO system (WW) for 4 weeks.

	Chloride				
Nutrient source	RO[i]	TW	WW	Significance[ii]	Budget calculations
Full fertilizer recipe (mg)	62.4	62.4	62.4	NA	Application (1×), g * μg·g^{-1} Cl fertilizer analysis
Two-thirds fertilizer recipe (mg)	46.8	46.8	46.8	NA	Sum of total applications (3×), g applied * μg·g^{-1} Cl fertilizer analysis
pH Adjust (mg)	NA	NA	NA	NA	NA
Water added (mg)	290.3	1904	3607	NA[iii]	Sum of additions (4×), L added * μg·L^{-1} Cl water source analysis
Shoot tissue (mg)	204.6 c[iv]	803.1 b	1471 a	**	Mid-harvest [(avg (n = 3) DW g * μg·g^{-1} Cl tissue analysis) * 3 plants] + End-harvest [(avg (n = 4) DW g * μg·g^{-1} Cl tissue analysis) * 8 plants]
Root tissue (mg)[v]	49.8 c	194.9 b	357.1 a	**	Mid-harvest [(avg (n = 3) DW g * μg·g^{-1} Cl tissue analysis) * 3 plants] + End-harvest [(avg (n = 4) DW g * μg·g^{-1} Cl tissue analysis) * 8 plants]
Water samples (mg)	14.6 c	145.9 b	305.1 a	***	Sum of water samples (4×), 0.5 L * μg·L^{-1} Cl water reservoir analysis
Calculated remaining (mg)	130.4 c	869.3 b	1583 a	**	Total inputs (fertilizer, pH adjust, water added) − total removed (shoot tissue, root tissue, water samples)
Estimated remaining (mg)	89.2 c	1173 b	2006 a	**	Estimated ending reservoir amount in L * μg·L^{-1} Cl week 4 water reservoir analysis

[i] Reported values are the average of n = 3 repetitions of each treatment.
[ii] Analyzed using a one-way analysis of variance.
[iii] Statistics were not possible because n = 1.
[iv] Letters indicate mean difference with Tukey's honest significant difference multiple comparison procedure (a = 0.05).
[v] Root dry weight estimation determined by multiplying shoot dry weight at mid-crop or end-crop by the respective root-to-shoot ratio (mid-harvest ratio: 0.25538; end-harvest ratio: 0.242236) determined from the data of Both et al. (1999).
Cl = chloride; DW = dry weight; NA = not applicable; NS = not significant.

shown). In each instance, Mg in TW was greater than in RO or WW because of the increased levels of Mg in the TW water source. With RO, a total of 1505 mg of Mg was added to the system, 20% was recovered in the shoot tissue, and 65% remained in the reservoirs at harvest. With TW, a total of 2091 mg Mg was added to the system, 11% was recovered in the shoot tissue, and 78% remained in the reservoirs at harvest. With WW, 1523 mg Mg was added to the system, 15% was recovered in the shoot tissue, and 72% remained in the reservoirs at harvest. At the end of the study, lettuce shoots contained 37, 27, and 27 mg Mg per head when produced with RO, TW, and WW, respectively. As a benchmark, a 12.7-cm (5-inch), 163-g head of butterhead lettuce would contain 21 mg Mg (US Department of Agriculture 2019), which is comparable to that of lettuce produced in this study.

The nutrient budget for Na was different between the treatments for all budget segments possible. Notably, shoot tissue and Na that remained in reservoirs was highest when produced with WW (Table 9). Totals of 102, 1768, and 6987 mg Na were added to the system for RO, TW, and WW, respectively. For WW, 13% of Na was recovered in shoot tissue and 75% remained in the reservoirs at harvest. At the end of the study, lettuce shoots contained 5, 18, and 108 mg Na per head when produced with RO, TW, and WW, respectively. As a benchmark, a 12.7-cm (5-inch), 163-g head of butterhead lettuce would contain 8 mg Na (US Department of Agriculture 2019), which is comparable to that of lettuce produced with RO and TW in this study; however, the Na content of lettuce produced with WW was 13.5-times greater than the benchmark. This difference may result in taste and health ramifications that require further study; however, Ünlükara et al. (2008) did not find that increased salinity influences lettuce taste.

The nutrient budget for Cl was different between the treatments for all budget segments. As with Na, shoot tissue and Cl that remained in reservoirs were notably higher when produced with WW (Table 10). Totals of 400, 2013, and 3716 mg Cl were added to the system for RO, TW, and WW, respectively. For both TW and WW, 40% of Cl was recovered in shoot tissue and 43% remained in the reservoirs at harvest. The average Cl values (mg) per head of lettuce were 23, 97, and 177 for RO, TW, and WW, respectively. Compared with Na, treatments with TW and WW resulted in more comparable Cl distribution based on both the amount in shoot tissue and the amount that remained in reservoirs at harvest; however, it was greater in both instances when WW was the water source.

Conclusion

The Cl, and especially Na, from WW accumulated in the nutrient solution reservoirs. Although lettuce yield was not inhibited during production over 4 weeks in this study, in a continuing cropping cycle of sequential lettuce crops, the nutrient solution reservoirs would have to be amended to reduce the salt content by, for example, back-mixing with RO or low EC municipal water. Growing lettuce with higher EC than that recommended for Na and Cl may have antagonized the uptake of some nutrients, such as Ca and Mg, and resulted in higher levels of Na and Cl in lettuce shoot tissue (Table 5). Solution analyses did not detect levels of heavy metals in any water source; however, shoot tissue analyses suggested that the accumulations of As, Pb, and Cr exceeded US Department of Agriculture Foreign Agricultural Service (2023) limits for RO and TW, as well as WW; therefore, further investigations are required. Ultimately, this study indicated that high-quality lettuce can be successfully produced with higher salt levels than those currently recommended, and that reject water from RO systems can be successfully managed to grow high-quality hydroponic lettuce. Further investigations may be necessary to evaluate lettuce for taste and health ramifications because of the higher tissue salt levels. Future research could include hydroponic crop production with feedwater of lesser quality to continue developing more sustainable water use strategies.

References Cited

Adhikari ND, Simko I, Mou B. 2019. Phenomic and physiological analysis of salinity effects on lettuce. Sensors. 19(21):4814. https://doi.org/10.3390/s19214814.

Al-Maskri A, Al-Kharusi L, Al-Miqbali H, Mumtaz Khan M. 2010. Effects of salinity stress on growth of lettuce (*Lactuca sativa*) under closed-recycle nutrient film technique. Int J Agric Biol. 12:377–380.

Both AJ, Albright LD, Scholl SS, Langhans RW. 1999. Maintaining constant root environments in floating hydroponics to study root – shoot relationships. Acta Hortic. 507:215–222. https://doi.org/10.17660/ActaHortic.1999.507.25.

Brechner M, Both AJ, Staff CEA. 2013. Hydroponic lettuce handbook. Cornell University CEA Program, Ithaca, NY, USA. https://cpb-us-e1.wpmucdn.com/blogs.cornell.edu/dist/8/8824/

files/2019/06/Cornell-CEA-Lettuce-Handbook-.pdf.
Bugbee B. 2004. Nutrient management in recirculating hydroponic culture. Acta Hortic. 648: 99–112. https://doi.org/10.17660/ActaHortic.2004.648.12.
Centers for Disease Control and Prevention. 2023. A guide to drinking water treatment technologies for household use. https://www.cdc.gov/healthywater/drinking/home-water-treatment/household_water_treatment.html.
Connecticut Department of Public Health. 2009. Reverse osmosis treatment of private drinking water systems. https://portal.ct.gov/-/media/Departments-and Agencies/DPH/dph/environmental_health/pdf/21ReverseOsmosisTreatmentofPDWSpdf.pdf.
Culligan. 2016. The Culligan® E2 series reverse osmosis system. https://products.reynoldsculligan.com/Asset/34924-E2-Series-RO_05-09-16.pdf.
Culligan. 2021. What is reverse osmosis and how does it work? https://www.culligan.com/blog/what-is-reverse-osmosis.
Culligan. 2023a. Carbon filter: Non-backwash: Culligan Igh, MN. Culligan of IGH 5.0. https://www.culligan4water.com/water-treatment-solutions/carbon-filter/.
Culligan. 2023b. What are water softeners? The complete guide. https://www.culligan.com/blog/everything-you-need-to-know-about-water-softeners.
Dvorak BI, Skipton SO. 2013. Drinking water treatment: Activated carbon filtration. NebGuide. G1489. https://extensionpublications.unl.edu/assets/html/g1489/build/g1489.htm.
Dubbeling M, van Veenhuizen R, Halliday J. 2019. Urban agriculture as a climate change and disaster risk reduction strategy. Field actions science reports. The Journal of Field Actions. Special Issue 20:32–39. https://journals.openedition.org/factsreports/5650.
Environmental Protection Agency. 2009. National primary drinking water regulations. EPA 816-F-09-004.
Environmental Protection Agency. 2023. Overview of drinking water treatment technologies. https://www.epa.gov/sdwa/overview-drinking-water-treatment-technologies.
Gieseking JE, Snider HJ, Getz CA. 1935. Destruction of organic matter in plant material by the use of nitric and perchloric acids. Ind Eng Chem Anal Ed. 7(3):185–186. https://doi.org/10.1021/ac50095a021.
Grattan SR, Grieve CM. 1998. Salinity-mineral nutrient relations in horticultural crops. Sci Hortic. 78(1–4):127–157. https://doi.org/10.1016/S0304-4238(98)00192-7.
Kansas Department of Health and Environment 2024. City of Manhattan, consumer confidence report - 2024. https://www.kdhe.ks.gov.
Kim HJ, Fonseca J, Choi JH, Kubota C, Kwon D. 2008. Salt in irrigation water affects the nutritional and visual properties of romaine lettuce (*Lactuca sativa* L.). J Agric Food Chem. 56(10): 3772–3776. https://doi.org/10.1021/jf0733719.
Langenhoven P. 2016. Opportunities in hydroponics. Purdue University Extension, Lafayette, IN, USA. https://ag.purdue.edu/department/hla/extension/_docs/langenhoven_hydroponics_ivgs2016b.pdf.
Mattson NS, Peters C. 2014. A recipe for hydroponic success. Inside Grower. January 2014:16–19.
Mattson NS, van Iersel MW. 2011. Application of the "4R" nutrient stewardship concept to horticultural crops: applying nutrients at the "right time." HortTechnology. 21(6):667–673. https://doi.org/10.21273/HORTTECH.21.6.667.
Mehran A, Agha Kouchak A, Nakhjiri N, Stewardson MJ, Peel MC, Phillips TJ, Wada Y, Ravalico JK. 2017. Compounding impacts of human-induced water stress and climate change on water availability. Sci Rep. 7(1):6282. https://doi.org/10.1038/s41598-017-06765-0.
Minnesota Department of Health Public Water Supply Unit. 1999. Madison leads the way first reverse-osmosis system for municipal water supply in Minnesota. https://www.health.state.mn.us/communities/environment/water/waterline/featurestories/madison.html.
Mills HA, Jones JB. 1996. Plant analysis handbook II: A practical sampling preparation, analysis and interpretation guide. MicroMacro Publishing, Inc., Athens, GA, USA.
Murray J, Basset G, Sandoya G. 2021. Nutritional benefits of lettuce consumed at recommended portion sizes. EDIS. 2021(3). https://doi.org/10.32473/edis-hs1416-2021.
Niu G, Sun Y, Masabni JG. 2018. Impact of low and moderate salinity water on plant performance of leafy vegetables in a recirculating NFT system. Horticulturae. 4(1):6. https://doi.org/10.3390/horticulturae4010006.
Os EA, Blok C, Voogt W, Waked L. 2016. Water quality and salinity aspects in hydroponic cultivation. https://edepot.wur.nl/403810.
Pennisi G, Orsini F, Blasioli S, Cellini A, Crepaldi A, Braschi I, Spinelli F, Nicola S, Fernandez J, Stanghellini C, Gianquinto G, Marcelis LFM. 2019. Resource use efficiency of indoor lettuce (*Lactuca sativa* L.) cultivation as affected by red:blue ratio provided by LED lighting. Sci Rep. 9(1):14127. https://doi.org/10.1038/s41598-019-50783-z.
Puretec 2021. DI-tank user guide. https://puretecwater.com/storage/app/media/downloads/deionized-water/di-tank-user-guide.pdf.
Sharma N, Acharya S, Kumar K, Singh N, Chaurasia OP. 2018. Hydroponics as an advanced technique for vegetable production: An overview. J Soil Water Conser. 17(4):364–371. https://doi.org/10.5958/2455-7145.2018.00056.5.
Sheikh BA. 2006. Hydroponics: Key to sustain agriculture in water stressed and urban environment. Pak J Agri Agril Engg Vet Sc. 22(2). https://citeseerx.ist.psu.edu/document?repid=rep1&type=pdf&doi=5109e0d8e536202a64d4cbb0da563e65f083c732.
Shrestha A, Dunn B. 2017. Hydroponics. Oklahoma State Extension, Stillwater, OK, USA. HLA-6442. https://extension.okstate.edu/fact-sheets/hydroponics.html.
Thimijan RW, Heins RD. 1983. Photometric, radiometric, and quantum light units of measure: A review of procedures for interconversion. HortScience. 18(6):818–822. https://doi.org/10.21273/HORTSCI.18.6.818.
Ünlükara A, Cemek B, Karaman S, Erşahin S. 2008. Response of lettuce (*Lactuca sativa* var. *crispa*) to salinity of irrigation water. N Z J Crop Hortic Sci. 36(4):265–273. https://doi.org/10.1080/01140670809510243.
US Department of Agriculture. 2019. Lettuce, butterhead (includes boston and bibb types), raw. FoodData Central. FDC ID: 168429. https://fdc.nal.usda.gov/fdc-app.html#/food-details/168429/nutrients.
US Department of Agriculture Foreign Agricultural Service. 2023. China releases the standard for maximum levels of contaminants in foods. https://fas.usda.gov/data/china-china-releases-standard-maximum-levels-contaminants-foods-0.
Vetrano F, Moncada A, Miceli A. 2020. Use of gibberellic acid to increase the salt tolerance of leaf lettuce and rocket grown in a floating system. Agronomy. 10(4):505. https://doi.org/10.3390/agronomy10040505.
Williams KA, DeWolf OJ. 2022. Wastewater from a commercial water purification system results in hydroponic lettuce and basil growth comparable to reverse osmosis and municipal water. HortScience. 57(9):S199.
Yaqub M, Nguyen MN, Lee W. 2022. Treating reverse osmosis concentrate to address scaling and fouling problems in zero-liquid discharge systems: A scientometric review of global trends. Sci Total Environ. 844:157081. https://doi.org/10.1016/j.scitotenv.2022.157081.