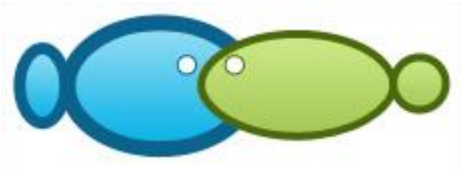

# A comparison of the effect of starting nitrate concentration on fish growth, plant production and water quality within a research-scale, recirculating aquaponics system

[1]Wilson A. Lennard

[1] School of Science, Technology, Engineering and Mathematics, University of South Australia, Mawson Lakes Campus, Mawson Lakes, South Australia, Australia 5095. Corresponding author: W. A. Lennard, willennard@gmail.com

**Abstract**. Murray cod, *Maccullochella peelii peelii*, and Green Oak lettuce, *Lactuca sativa*, were used to determine the effects of three different starting nitrate concentrations, in a research-scale aquaponic system using gravel bed hydroponic components: control (initial nitrate = 0 mg $L^{-1}$), medium nutrient (initial nitrate = 25 mg $L^{-1}$) and high nutrient (initial nitrate = 50 mg $L^{-1}$), where plant nutrients were supplied from fish wastes while the plants removed nutrients from the water before it was returned to the fish tanks (25.9 g of fish feed $m^{-2}$ of plant growing area $day^{-1}$). Murray cod had FCR's and biomass gains that were statistically identical in all systems (mean SGR = 1.16% $replicate^{-1}$ $day^{-1}$; mean FCR = 0.84). Lettuce, in terms of biomass gain and yield, within both the medium and high nutrient treatments (medium yield of 4.28 kg $m^{-2}$ and high yield of 4.41 kg $m^{-2}$) had superior results when compared to the control (yield of 3.47 kg $m^{-2}$). In terms of nitrate removal (nitrate accumulation: control = 5.60 mg $L^{-1}$; medium nutrient = - 0.50 mg $L^{-1}$; high nutrient = - 1.57 mg $L^{-1}$) and phosphate removal (phosphate accumulation: control = 0.92 mg $L^{-1}$; medium nutrient = - 5.53 mg $L^{-1}$; high nutrient = - 9.04 mg $L^{-1}$), conductivity accumulation (control = 237 µS $cm^{-1}$; medium nutrient = 186 µS $cm^{-1}$; high nutrient = 189 µS $cm^{-1}$), pH, buffer additions (control = 0.7 g $day^{-1}$; medium nutrient = 0.9 g $day^{-1}$; high nutrient = 0.9 g $day^{-1}$) and water use (control = 1.15 L $day^{-1}$; medium nutrient = 0.98 L $day^{-1}$; high nutrient = 1.04 L $day^{-1}$), no statistical difference was observed between any treatments. Overall, results demonstrate that starting nitrate concentrations (with nutrients derived from fish wastes) between 25 mg $L^{-1}$ and 50 mg $L^{-1}$ provided improvements in the research-scale aquaponic system for plant yield. This study demonstrates nutrient levels should be allowed to accumulate in coupled aquaponic systems by feeding fish for a few weeks before plants are introduced.
**Key Words**: aquaculture, hydroponic, Murray cod, lettuce.

**Introduction**. One of the goals of integrating hydroponically grown plants with recirculating fish culture (RAS) is the removal of nutrient wastes from the water, to control nutrient accumulation and therefore, lower the reliance of RAS on water exchanges (Rakocy & Hargreaves 1993; Rakocy et al 2006; Moya et al 2016; Endut et al 2009; Calone et al 2019; Palm et al 2019). The plants integrated are also of commercial importance and therefore, their requirements must be met, since they contribute substantially to the economic success of the aquaponic enterprise (Rakocy & Hargreaves 1993; Love et al 2015; Saha et al 2016; Lennard 2017). Fish are constantly producing nutrient wastes in aquaponic systems and therefore, nutrients are constantly becoming available for plant use (Rakocy & Hargreaves 1993; Lennard 2013). If the balance between fish waste production and plant nutrient use is optimal within fully recirculating (coupled) aquaponic systems, the net nutrients available in the recirculating waters should stay relatively constant and close to the initial concentration of nutrients in the system before plants were introduced (Lennard 2013; Lennard 2017). Because of this, the question of what is the optimal, basal net nutrient concentration for the plants in the aquaponic system arises? It is recommended by Lennard (2005), Rakocy et al (2006) and Lennard (2017), that when aquaponic systems are commissioned for operation, the fish should be introduced first and fed for a few weeks before any plants are introduced.

This is done to allow time for all of the nutrients available in the fish feed, which are ultimately used for plant growth, to accumulate to the minimum levels required for optimal plant growth (Lennard 2017). On the other hand, if nutrients are allowed to accumulate for too long, the total nutrient concentrations may exceed a maximum safe level for plants and can become toxic and decrease production (Rakocy et al 2006).

Hydroponic producers and researchers have optimised hydroponic systems, including the configuration of the nutrient solution, so they achieve the highest yields possible (Jensen & Collins 1985; Graves 1993; Resh 2013). Many nutrient solutions are now directly tailored to the needs of the particular plant variety being grown (Mason 1990; Bugbee 2004; Resh 2013). In the case of lettuce varieties (*L. sativa*), the configuration of the nutrient solution used in hydroponic culture is well known and many producers use nutrient solutions with standard nutrient profiles (Morgan 1999; Resh 2013). Analyses of standard hydroponic lettuce nutrient solutions shows that nitrogen is usually present in solution at concentrations between 100 – 200 mg $L^{-1}$, phosphorous is present at concentrations between 15 – 90 mg $L^{-1}$ and the electrical conductivity (EC) is often no higher than 2,500 µS $cm^{-1}$ (Morgan 1999; Resh 2013).

Many hydroponic nutrient solutions are designed and configured so that adequate nutrients are available for several weeks of culturing (Cooper 1996; Resh 2013). Once hydroponic nutrient solutions are depleted of nutrients, they are generally discarded and replaced by a new batch of fresh solution or the existing solution is replenished so that the nutrient mixture is again, optimised (Jensen & Collins 1985; Morgan 1999; Bugbee 2004; Resh 2013). This means hydroponic nutrient solutions are made with ion concentrations at the upper limit of the plant's requirement, so the nutrient solution may be used for as long as possible and still maintain plant-available nutrient concentrations and mixtures (Resh 2013). Aquaponic systems are different from standard hydroponic systems, because in aquaponics the fish are constantly renewing the nutrients in solution via the metabolism of fish feed and the release of fish waste (Rakocy & Hargreaves 1993; Lennard 2013; Lennard 2017). Because the fish are constantly renewing the nutrients for plant growth, it would be expected that the basal levels of nutrients within an aquaponic system could be lower than the initial concentrations typically used in hydroponic nutrient solutions. In addition, the relative amounts of each nutrient within an aquaponic system are dependent on the nutrient configuration of the fish feed being added, which may produce a very different nutrient profile outcome to a standard hydroponic nutrient solution (Rakocy et al 2006; Maucieri et al 2019). Whatever the nutrient mix within an aquaponic system, the upper limit of electrical conductivity for the particular plant being grown must be adhered to, as this limit is set so the plants aren't stressed by the osmotic potential of the recirculating water, which may ultimately lead to lowered growth rates and yields (Rakocy & Hargreaves 1993; Resh 2013; Lennard 2017).

The fish species used in the current experiment was the Australian, freshwater native Murray cod, *Maccullochella peelii peelii* (Mitchell, 1838), and the hydroponic vegetable was lettuce, *Luctuca sativa* (Green Oak variety) (Linnaeus 1753). The three systems were compared for fish growth, plant growth, nutrient concentrations and other parameters in the recirculating water.

This experiment was devised to test and compare three different starting (initial) nitrate concentrations (as indicators of overall initial nutrient level) in a research-scale aquaponic system. This was done to answer a number of questions with regard to the performance of the hydroponic component of the research-scale aquaponic system:

1. Are Murray cod growth rates and feed conversion ratios (FCRs) in the research-scale, aquaponic system, using three different initial nitrate concentrations, comparable to (better than, equal to or worse than) each other?

2. Are lettuce growth rates and yields in the research-scale, aquaponic system, using three different initial nitrate concentrations, comparable to (better than, equal to or worse than) each other?

3. Is nutrient (nitrate and phosphate) accumulation in the research-scale, aquaponic system, using three different initial nitrate concentrations, comparable to (better than, equal to or worse than) each other?

4. Does the use of a particular initial nitrate concentration in the research-scale, aquaponic system, confer any advantage, with respect to the other parameters measured?

**Material and Method**

***Fish origin and holding***. Murray cod were obtained from Australian Aquaculture Products Pty. Ltd., Melbourne, Victoria, Australia. Fish were held indoors at the Royal Melbourne Institute of Technology University Aquaculture Annex and ranged in size from 120 g to 220 g. All fish were kept in 1,000 L, cylindrical tanks receiving flow-through water at a flow rate of 3,000 L $day^{-1}$, until required for experimentation. Size distributions of the Murray Cod were similar among replicates and treatments (mean = 195 ± 20 g). Water was of domestic origin (Melbourne tap water – catchment rainwater sourced; specific conductance = 100 µS $cm^{-1}$; Total alkalinity as $CaCO_3$ = 10 mg $L^{-1}$), carbon-filtered and heated to approximately 22 $^oC$.

***Experimental recirculating aquaponic system***. The experimental set-up was located, and all research was performed, within the Aquatic Culturing Laboratory Annex, Building 223, RMIT Bundoora Campus.

The experimental, recirculating aquaponic system array consisted of 9 individual, identical aquaponic units, allowing replication of experimental treatments. Each aquaponic unit consisted of a fish holding tank, an associated biofilter and a hydroponic component (Figure 1, Figure 2).

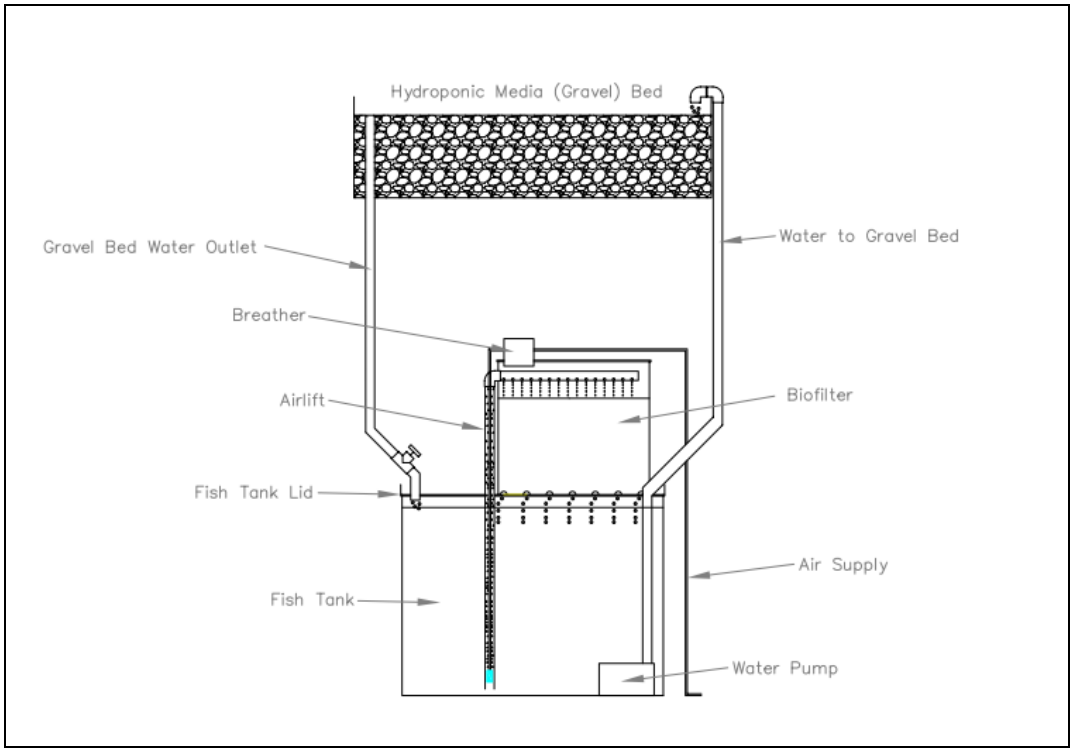


Figure 1. Schematic representation of a single, recirculating aquaponic test unit.

The fish tank of each aquaponic unit was a round 100 L, opaque, white plastic tank (570 mm diameter x 460 mm deep). As well as fish, the tank contained an airlift pipe (to the biofilter), a submersible water pump (to the hydroponic component) and a 100 W thermostatically controlled electrical resistance aquarium heater. A plastic "core flute" (3

mm) lid covered the fish tank to lower evaporation and to stop fish from jumping from the tank.

Each fish tank had an associated, 20L biofilter (360 mm L x 330 mm W x 290 mm D), made from a plastic storage box. This biofilter sat above the fish tank and was of a wet/dry trickling design. Water entered the biofilter by way of a 20mm airlift pipe (at an average of 250 L $h^{-1}$), running from the base of the fish tank and into the top of the biofilter. A 6 mm plastic hose delivered air to the airlift pipe via an air stone. Water from the airlift entered the top of the biofilter via a "spray bar", trickled across the biological filter medium (polystyrene "bean bag" beads @ approx. 300 $m^2$ $m^{-3}$: area $volume^{-1}$) and out through a series of 6 x 10 mm holes (drilled in the bottom, front area of the biofilter) and back into the fish tank. The biofilter had a plastic "core flute" (3 mm) lid to lower evaporation. This lid contained a breathing hole in one corner made from a short length of 65 mm PVC pipe to allow gas exchange within the biofilter.

Each tank and biofilter unit had an associated hydroponic plant growth component which contained standard, washed aquarium gravel (6 mm average particle size) to a depth of approximately 200 mm. This component was rectangular in shape (780 mm L x 670 mm W x 220 mm D) and was placed above the fish tank/biofilter unit on a separate shelving system. A submersible water pump (Rio 1700, 1200 L $h^{-1}$ @ 1.2 m head) located within the fish tank continuously delivered water to the hydroponic component via a 19 mm pipe. Water from the hydroponic component was returned to the fish tank via a 20 mm drainpipe, situated at the opposite end of the hydroponic bed from the water inlet.


Figure 2. Images of the research-scale, recirculating aquaponic system; top-left: view of a fish tank and biofilter of one unit; top-right: view of the grow beds from above; bottom-left: view of the grow beds, lights and fan; bottom-right: view of the individual aquaponic units with fish tanks below and hydroponic beds above.

A continuous flow of water through the hydroponic gravel bed was used because previous experiments (Lennard 2005) demonstrated that this was the most efficient method for the research-scale aquaponic system for the experimental duration applied.

Water for all experimental tanks was supplied from the aquaculture laboratory water supply system. Air for all biofilters and associated airlifts was supplied by a centralised, pressurised air supply that delivered air to the entire aquaculture lab.

Lighting (for plant growth in the hydroponic sub-systems) consisted of six x 400 W metal halide lamps. Lights were situated above the hydroponic beds at a height of 700 mm above the gravel surface, with one lighting unit located at the interface between two hydroponic sub-systems (i.e. one lighting unit supplied the required light for two hydroponic beds). Lights (i.e. day-length) were controlled by a digitally timed electrical switch.

***Experimental methodology***. The experiment was designed to compare the effects of three starting nitrate concentrations in the water of the aquaponic unit(s). The hydroponic component of each aquaponic unit for all treatments and the control used a pumping rate of 540 L $h^{-1}$ (9 L $min^{-1}$) through the gravel bed. One control and two separate treatments, each with three replicates, were tested:

1. Control: fish in tank, plants in the hydroponic bed (gravel), constant flood regime, starting nitrate concentration of 0 mg $L^{-1}$ - this was a control regime to compare fish growth, nitrate and phosphate accumulation and plant growth with the two test treatments, based on the starting nitrate concentration used in previous experiments (Lennard 2005).

2. Medium nutrient: fish in tank, plants in the hydroponic bed (gravel), constant flood regime, starting nitrate concentration of 25 mg $L^{-1}$ - this was a test regime to compare fish growth, plant growth, nutrient use and other parameters to the Control and the other starting nitrate test treatment.

3. High nutrient: fish in tank, plants in the hydroponic bed (gravel), constant flood regime, starting nitrate concentration of 50 mg $L^{-1}$ - this was a test regime to compare fish growth, plant growth, nutrient use and other parameters to the Control and the other starting nitrate test treatment.

The lighting regime for plant growth was 10 hrs on : 14 hrs off, with lights coming on at approximately 08:30 am (AEST) and going off at 18:30 pm (AEST).

All aquaponic units had operated with fish and plants present for several months prior to experimentation, to establish stable biofiltration treatment capacity (confirmed by daily ammonia determinations – data not presented here). At the initiation of the experiment, all aquaponic unit replicates were proportionally filled with fresh water to 100 L (i.e. new, freshwater mixed with existing aquaponic water) to establish the required initial nutrient concentrations of the test treatments (based on nitrate concentration; control – 0 mg $L^{-1}$; medium nutrient – 25 mg $L^{-1}$; high nutrient – 50 mg $L^{-1}$) and initial actual nitrate and phosphate concentrations were recorded. Fish weights were determined for each aquaponic unit replicate and adjusted to a treatment biomass of approximately 1,000 g (individual tank fish biomass was recorded).

Fish were fed at a percentage of the total initial fish biomass per day (for six of seven days per week) with a 9 mm, sinking pellet (43% protein) (Skretting Classic SS, Skretting Pty. Ltd. Hobart, Tasmania, Australia) (Table 1). Feeding rates were set at 1.0% of fish biomass (per day) for the first 5 days, then adjusted to 1.5% for the remaining 15 days of the experiment. Weights of fish feed fed to each aquaponic unit daily were recorded.

Twenty lettuce seedlings (*L. sativa* Green Oak variety) were planted using an evenly distributed planting scheme into each of the replicate hydroponic beds. The individual initial weight (biomass) of these twenty seedlings was recorded (weight with attached soil plug). Because seedlings had attached plugs of soil, initial leaf weight was estimated by recording the weights of fifteen additional seedlings from the same batch as those used for the experiment with and without attached soil plugs. These weights were

used to establish a mean ratio of leaf-only to leaf + plug weight. This ratio was then used to estimate the initial leaf-only weight of the tested seedlings (Lennard 2005).

Six of seven days a week (at the same time every day – 9:30 am, AEST; Monday to Saturday, inclusive), the amount of fish feed fed (g), air temperature (°C), water replaced per tank (L) (to adjust for evapotranspiration – see below), the amount of buffer added (to adjust pH to between 6.80 and 7.00; added directly to the fish rearing compartment) (g), pH, temperature of the tank water (°C), conductivity (µS $cm^{-1}$) and dissolved oxygen (mg $L^{-1}$) were recorded. Twice a week, tanks were sampled for ammonia (mg $L^{-1}$) and nitrite (mg $L^{-1}$), whilst once a week, tanks were sampled for nitrate (mg $L^{-1}$) and phosphate (mg $L^{-1}$). All samples for analysis were taken from the fish-rearing compartment of the aquaponic unit(s).

The amount of feed fed, and the buffer added per tank (to maintain pH) were measured using a top loading balance (A&D HL-200). The amount of water replaced per tank was determined by re-filling the tank to a pre-measured 100 L mark with a measuring cylinder. Temperature (tank water), pH, conductivity and dissolved oxygen were determined using a TPS 90-FL multi-parameter meter and associated probes. Ammonia, nitrite and nitrate were determined using a Hanna, C203 Multiparameter ion-specific meter (H025463) and Hanna Ammonia LR reagent (HI 93700-01), Hanna Nitrite LR reagent (HI 93707-01) and Hanna Nitrate HR reagent (HI 93728-01), respectively. Phosphate was determined using a Merck Spectroquant, colour reagent test (code: 1.1482.0001)**,** read against a standard curve using a spectrophotometer at 400 nm (Varian Cary 50 Bio UV-Vis).

The entire experiment ran for 21 days in 2005 from water flushing/proportional mixing and planting to harvest. At the end of the experiment, fish biomass was determined by wet weight (A&D HL-200) and plant (leaf only) biomass was determined by wet weight (A&D HL-200). Gains in both fish biomass and plant biomass per unit (or hydroponic component) were determined by the difference between initial and final wet biomasses recorded. Fish biomass was determined on a per-tank basis, whilst plant biomass (leaf only) was determined on an individual, per plant basis.

Comparisons between treatments and controls at the end of the experimental period for fish biomass, fish feed conversion ratio (FCR = feed fed {g}/fish weight gain {g}), fish specific growth rate (SGR = [(ln final wt. – ln initial wt.)/(time (days)) x 100)] and nitrate and phosphate concentrations were analysed using Mann-Whitney, two independent population, non-parametric analysis. For lettuce growth and yield parameters, the replicates within a treatment (n=3) were initially analysed for homogeneity (using individual plant weight gains; (n=20)) and when confirmed, replicate individual plant weight gains were pooled (n=60) for overall treatment comparisons.

***Statistical analysis***. Comparisons for all other parameters were analysed using ANOVA and Least Significant Difference (LSD) post-hoc analysis. Homogeneity of variance was tested using Levenes test (Zar 1984). In the cases of parameters concerned with the lettuce, high sample numbers were always available, and these cases always tested positively for normality and homogeneity of variances (Levenes test). In the cases for water quality parameters, data sets were compared for the length of the experiment, therefore the statistical analysis only tested for differences across the entire experimental period. All ANOVA results were also analysed (as a secondary "check" procedure) using post-hoc analysis based on the assumption of non-equal variances (Dunnett's C test) (Zar 1984), to make sure that homogeneity of variance was not a factor that may have affected outcomes, as well as a test to make sure the results from the initial analyses were correct. All statistical analyses were performed using SPSS (Version 10.0) software.

## Results

***Fish***. Survival of Murray cod in all replicates was 100% for the 21-day trial. Fish biomass gain (wet weight gain/treatment replicate) averaged 295.0 g, 273.3 g and 243.3 g for control, medium nutrient and high nutrient treatments respectively, whilst specific growth rate (SGR) averaged 1.25%, 1.20% and 1.04% for control, medium nutrient and high

nutrient treatments respectively (Table 1). Feed conversion ratios (FCR) were 0.78, 0.80 and 0.95 for control, medium nutrient and high nutrient treatments respectively (Table 1). No significant differences (Mann-Whitney: P>0.05, n=3) were detected between any treatments in terms of any fish growth parameters.

Table 1

Mean wet-weight gain, specific growth rate (SGR), feed conversion ratio (FCR) and feed consumption for Murray Cod for control, medium nutrient and high nutrient treatments over the 21-day trial

| *Treatment* | *Wet-Weight gain[1] (g Replicate$^{-1}$)* | *SGR[1] (% Replicate$^{-1}$ Day$^{-1}$)* | *FCR[1]* | *Feed Fed (g Replicate$^{-1}$)* |
|---|---|---|---|---|
| Control | 295.0 ± 15.0 [a] | 1.25 ± 0.03 [a] | 0.78 ± 0.04 [a] | 230.0 |
| Medium | 273.3 ± 8.8 [a] | 1.20 ± 0.01 [a] | 0.80 ± 0.03 [a] | 230.0 |
| High | 243.3 ± 3.3 [a] | 1.04 ± 0.01 [a] | 0.95 ± 0.01 [a] | 230.0 |

Note: [1]Values are means ± S.E
a & b: values showing the same letter are not significantly different (P>0.05, n=3) (Mann-Whitney)
SGR specific growth rate (% day$^{-1}$): [(ln final wt. – ln initial wt.)/(time (days))]x100
FCR feed conversion ratio: feed fed/(wet weight gain)

***Lettuce***. Lettuce production values (g treatment replicate$^{-1}$) for control, medium nutrient and high nutrient treatments are presented in Table 2. There was a significant difference (ANOVA: $F_{x,y}$=14.435, P=0.000) between the control (control lower) and the other two test treatments in terms of both biomass gain and yield (Table 2).

Table 2

Mean biomass gain and mean yield (g plant$^{-1}$ & kg m$^{-2}$) for lettuce plants for control, medium nutrient and high nutrient treatments over the 21-day trial.

| *Treatment* | *Biomass Gain [1] (g Replicate$^{-1}$)* | *Yield (g plant$^{-1}$) [1]* | *Yield (kg m$^{-2}$) [1]* |
|---|---|---|---|
| Control | 1814.0 ± 25.4 [a] | 90.70 ± 5.69 [a] | 3.47 ± 0.22 [a] |
| Medium | 2239.2 ± 11.7 [b] | 111.96 ± 2.61 [b] | 4.28 ± 0.10 [b] |
| High | 2306.2 ± 43.3 [b] | 115.31 ± 9.69 [b] | 4.41 ± 0.37 [b] |

Note: [1]Values are means ± S.E.
a & b: values showing the same letter are not significantly different (P>0.05, n=60) (ANOVA)

No significant difference (ANOVA: $F_{x,y}$=3.313, P=0.444) was detected between the medium nutrient treatment and the high nutrient treatment. Yields averaged 3.47 kg m$^{-2}$, 4.28 kg m$^{-2}$ and 4.41 kg m$^{-2}$ for the control, medium nutrient and high nutrient treatments respectively (Table 2). Statistical differences followed the same associations as for lettuce production values. No qualitative indications of sub-optimal plant growth (tip burn, bolting or wilting) were noted in any control or experimental treatments.

***Metabolites, nitrates, and phosphates***. Ammonia and nitrite were recorded daily to ascertain biological filter conversion efficiency. All replicates in all treatments showed undetectable ammonia concentrations (<0.25 mg L$^{-1}$), over the duration of the experiment (data not presented). Nitrite levels remained at zero (<0.05 mg L$^{-1}$) for all replicates in all treatments for the duration of the experiment (data not presented).

Final phosphate accumulations (the difference between initial and final concentrations) averaged 0.92 mg L$^{-1}$, -5.53 mg L$^{-1}$ and -9.04 mg L$^{-1}$ for control, medium nutrient and high nutrient treatments respectively (Table 3). Negative phosphate accumulations represent the fact that plants stripped more phosphate from the water than was produced by the fish. No significant differences (Mann-Whitney: P>0.05, n=3) were detected between any of the control or test treatments for final phosphate accumulation (Table 3).

Final nitrate accumulations averaged 5.60 mg $L^{-1}$, -0.50 mg $L^{-1}$ and -1.57 mg $L^{-1}$ for control, medium nutrient and high nutrient treatments respectively (Table 3). No significant differences (Mann-Whitney: P>0.05, n=3) were detected between any of the control or test treatments with respect to final nitrate accumulation (Table 3).

Table 3

Mean net (final-initial) phosphate and nitrate accumulations, for control, medium nutrient and high nutrient treatments over the 21-day trial

| *Treatment* | *Phosphate (mg $L^{-1}$)* [1] | *Nitrate (mg $L^{-1}$)* [1] |
|---|---|---|
| Control | 0.92 ± 0.54 [a] | 5.60 ± 0.57 [a] |
| Medium | -5.53 ± 0.66 [a] | -0.50 ± 1.51 [a] |
| High | -9.04 ± 1.59 [a] | -1.57 ± 1.60 [a] |

Note: [1]Values are means ± S.E.
a & b: values showing the same letter are not significantly different (P>0.05, n=3) (Mann-Whitney)

***Physical/chemical parameters***. Air temperature was measured daily and remained steady at 24°C. Tank water temperatures averaged 21.9°C, 22.0°C for control, medium nutrient and high nutrient treatments respectively (data not shown).

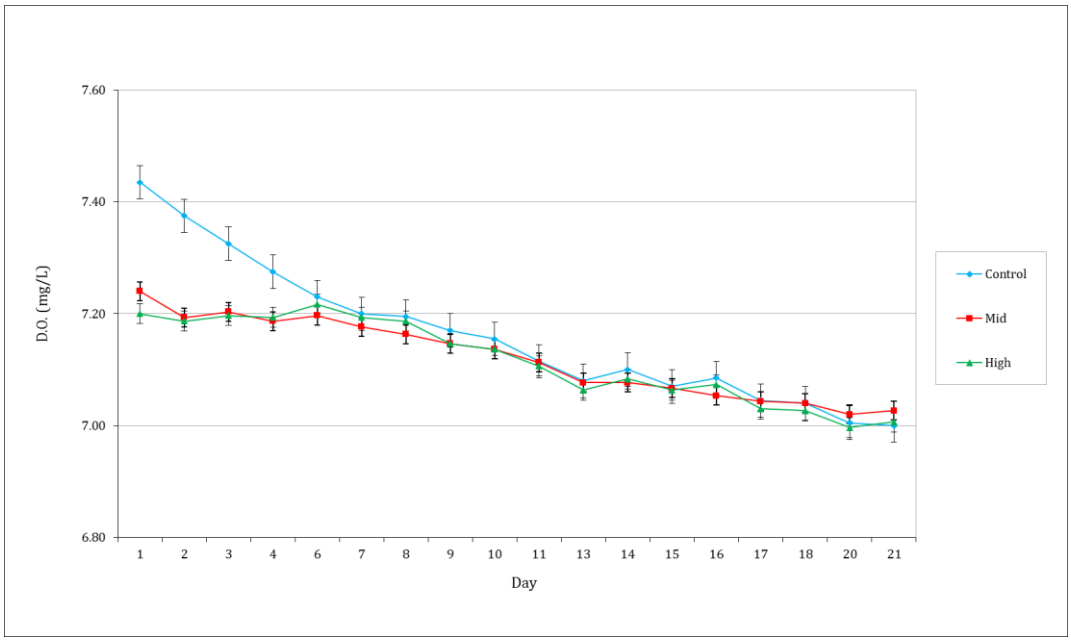


Figure 3. Mean daily dissolved oxygen concentrations for control, medium nutrient and high nutrient treatments. Error bars represent standard errors.

Mean daily dissolved oxygen (D.O.) concentrations differed significantly between the control and the two test treatments (ANOVA: $F_{x,y}$=3.075, P=0.049), but no significant difference (ANOVA: $F_{x,y}$=0.0031, P=0.864) was detected between the two test treatments. This is most likely due to the fact that the control replicates exhibited higher D.O. (ANOVA: $F_{x,y}$=-2.0567, P=0.632) concentrations in the first 5 days of the experiment (Fig. 3), because tanks were completely flushed and new water was added at the beginning of the experiment. D.O. concentrations dropped over the length of the experiment in all replicates but remained above 7.00 mg $L^{-1}$ for all treatments (Figure 3).

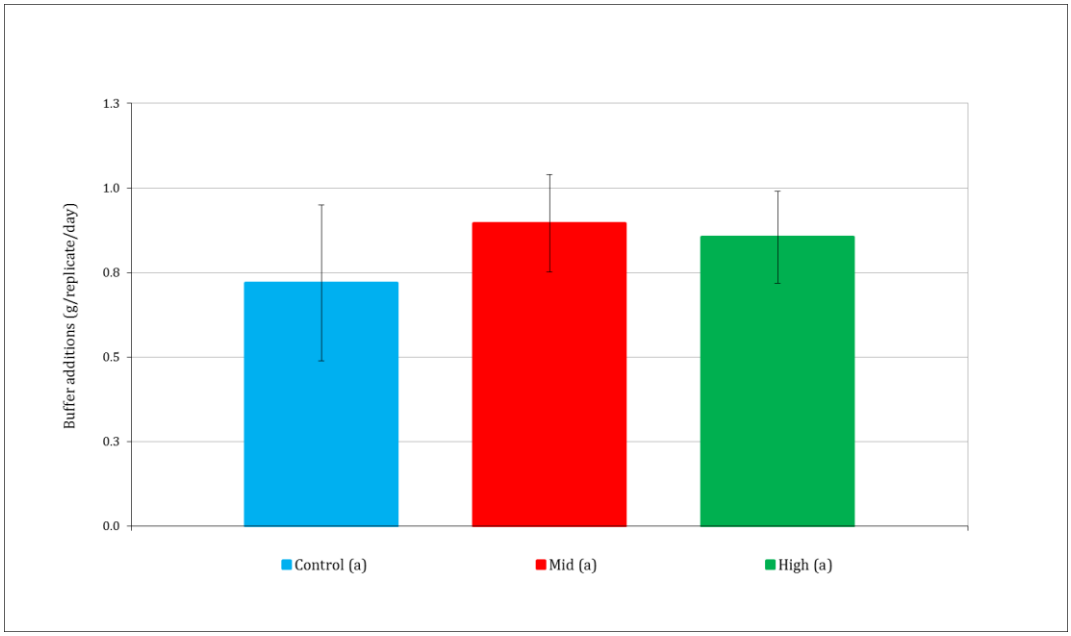


Figure 4. Mean daily buffer additions (per treatment replicate) for control, medium nutrient and high nutrient treatments. a & b: Treatments showing the same letter are not significantly different (P>0.05, n=51). Error bars represent standard errors.

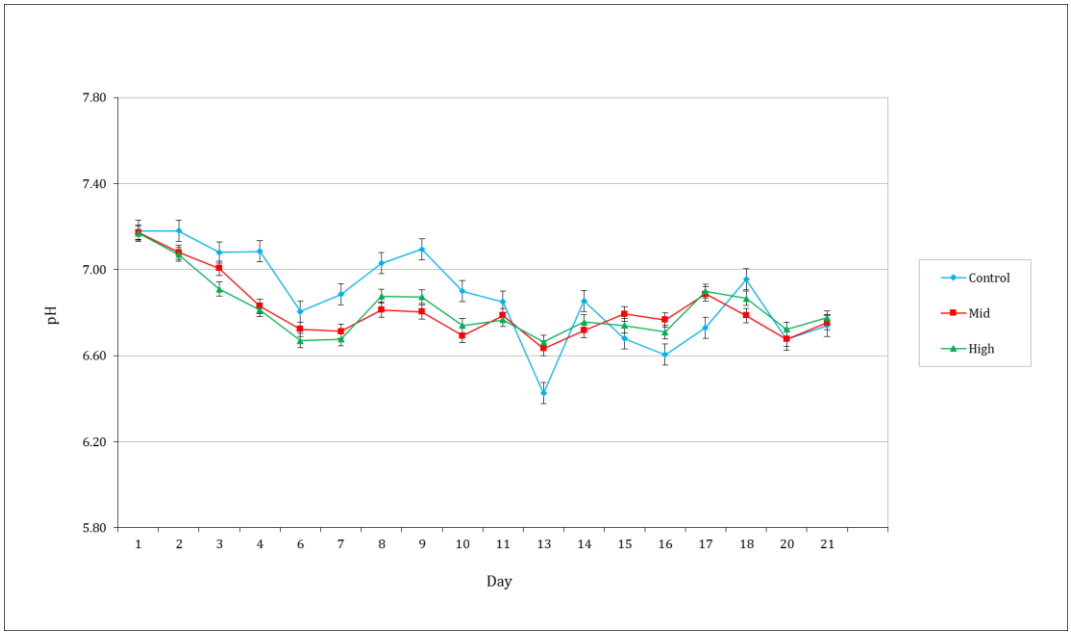


Figure 5. Mean daily pH readings for control, medium nutrient and high nutrient treatments. Error bars represent standard errors.

Buffer additions (Figure 4) and pH (Figure 5) are integrally linked in aquatic systems. Buffer additions averaged 0.7, 0.9 and 0.9 g treatment replicate$^{-1}$ day$^{-1}$ for control, medium nutrient and high nutrient treatments respectively. No significant differences (ANOVA: $F_{x,y}$=0.2870, P=0.751) were detected between any control or test treatment for buffer additions.

Mean daily pH readings are represented in Figure 5 pH levels averaged 6.88, 6.81 and 6.82 for control, medium nutrient and high nutrient treatments respectively. No significant differences (ANOVA: $F_{x,y}$=1.559, $P$=0.214) were detected between any control or test treatment (Figure 5).

Mean daily conductivity readings are represented in Figure 6. Conductivity in all control and test treatments followed a very similar slope (Figure 6), although, differed

markedly between each other because of the effect of the different starting nitrate and phosphate concentrations.

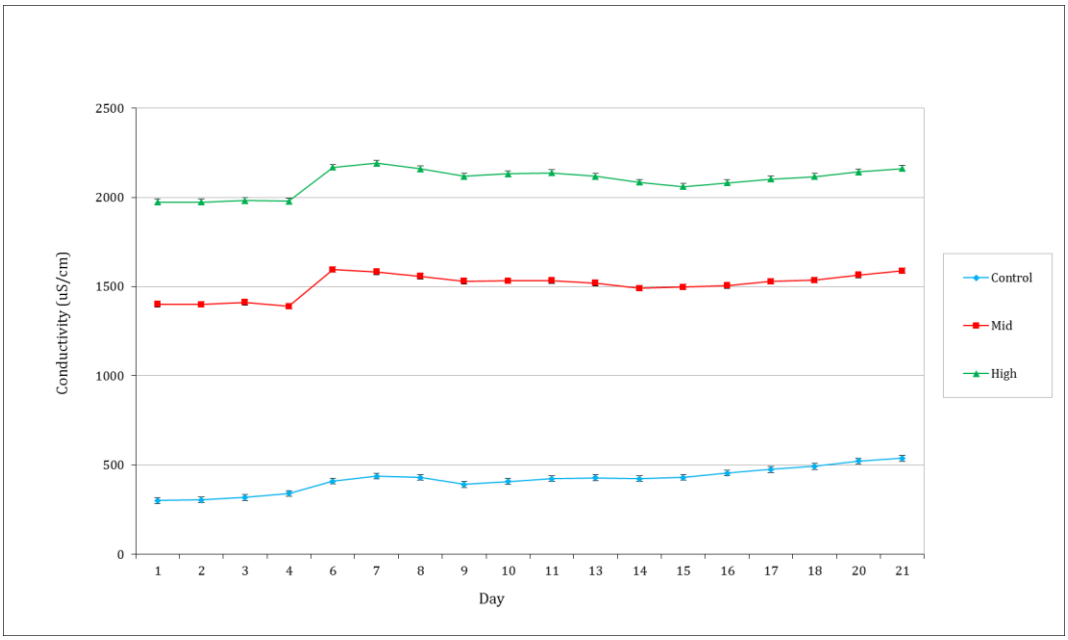


Figure 6. Mean daily conductivity for control, medium nutrient and high nutrient treatments. Error bars represent standard errors.

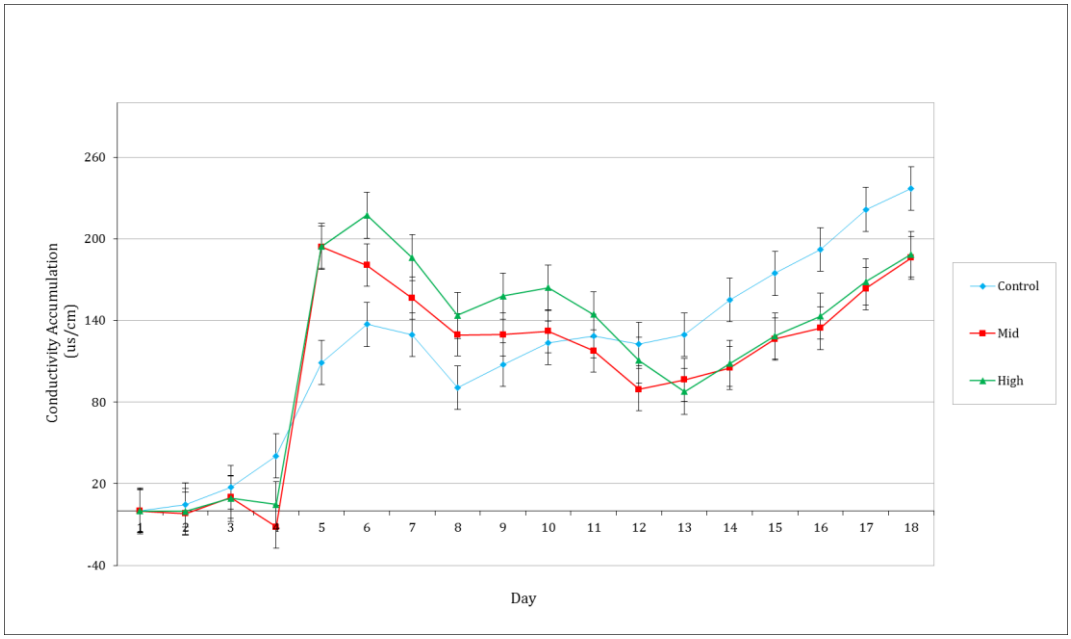


Figure 7. Mean daily conductivity accumulation for control, medium nutrient and high nutrient treatments. Error bars represent standard errors.

Because of this difference in initial conductivities, conductivity accumulation was also calculated (Figure 7). No significant differences (ANOVA: $F_{x,y}=0.1700$, $P=0.844$) were detected between any control or test treatments in terms of conductivity accumulation. Conductivity did not accumulate beyond 240 µS $cm^{-1}$ for any treatment (control or test) (Figure 7).

Water was replaced daily to compensate for evapotranspiration (Figure 8). Overall, daily water replacement averaged 1.15 L $replicate^{-1}$, 0.98 L $replicate^{-1}$ and 1.04 L $replicate^{-1}$ for the control, medium nutrient and high nutrient treatments respectively. No significant differences (ANOVA: $F_{x,y}=1.2680$, $P=0.288$) were detected between any control or test treatments with respect to water consumption (Figure 8).

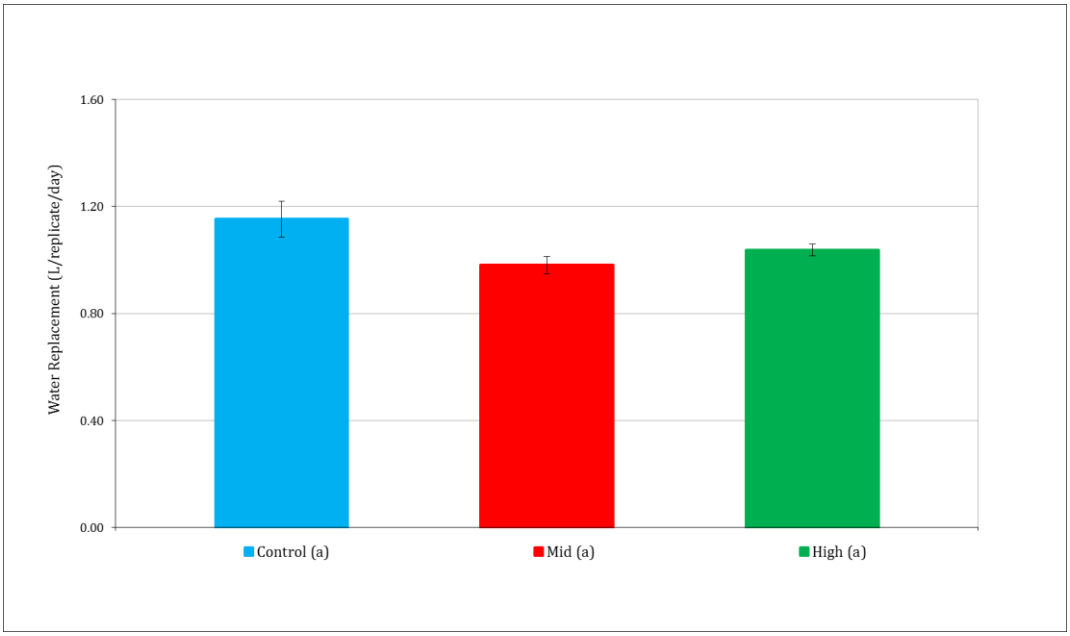

Figure 8. Mean daily water replacement (per treatment replicate) for control, medium nutrient and high nutrient treatments. a & b: Treatments showing the same letter are not significantly different (P>0.05, n=54). Error bars represent standard errors.

**Discussion**. Past research has suggested that aquaponic systems need to be started as fish-only systems for a few weeks before plants are introduced (Lennard 2005; Rakocy et al 2006). This is because the feeding of the fish, without plants present, allows the accumulation of nutrients to levels that are more conducive to optimal plant growth (Lennard 2005; Rakocy et al 2006; Lennard 2017). This initial nutrient concentration can then be maintained by balancing the waste the fish produce with that which the plants use, so zero net nutrient accumulation occurs after the plants are introduced (Lennard 2005; Lennard 2013; Lennard 2017). In the current study, it was decided to accumulate nutrients (as indicated by nitrate concentration) using normal fish metabolism (as opposed to increasing nitrate concentrations alone by adding a nitrate salt). Nutrient accumulation via fish metabolism was considered more appropriate than adding a nitrate salt, because it meant a full complement of nutrients arising from the fish wastes were available to the plants (as in a normal aquaponic situation) (Lennard 2005). However, it must also be noted that the differences in nutrient concentrations within the test treatments (compared to the control) may have had an effect on other parameters within the aquaponic treatments (most notably dissolved oxygen – see below).

Fish mortality in all treatments was zero. Ingram (2002) obtained less than 5% mortality for Murray cod exceeding 50 g in weight in culture trials and therefore, the mortality in the present study is what should be expected for Murray cod of the size tested (250 – 400 g) in standard recirculating aquaculture. In terms of feed conversion efficiency, Ingram (2002) (feed containing 43% protein) obtained a mean FCR for Murray cod over 150 g in weight of 1.2, therefore the FCR values obtained in the present study (Table 1) are comparable with research results using industry-standard, recirculating culture methods. Whether the basal concentration of nutrients (measured as nitrate) in the system affects fish growth is also an important question. In the present study, no significant differences in any fish growth parameter (biomass gain, SGR or FCR) were detected between any treatments or controls (Table 1), therefore, suggesting none of the nutrient starting concentrations tested had a deleterious effect on fish growth or survival. This is also what should be expected for nitrate concentrations in the range tested, as nitrate is considered to be relatively non-toxic to most fish species at relatively high concentrations (over 300 mg $L^{-1}$) (Masser et al 1999; Timmons et al 2002).

Whilst plants were added to the system to remove nutrient, and therefore, improve water quality for the fish, it was plant growth, health and yield that were

considered the best indicators of the optimal starting nutrient concentration. Lettuce production as wet, leaf weight gain or yield (weight gain per unit area) within the three treatments followed the relationship high = medium > control (Table 2). Therefore, the starting nitrate concentration (and hence, the starting concentration of all of the nutrients with origins from fish wastes) of the system did affect the efficiency of the aquaponic system when measured in terms of plant production, although no difference was detected between the two starting nitrate concentrations tested. This suggests that allowing some nutrient to accumulate via fish metabolism before adding plants to the research-scale, aquaponic system assisted plant production.

It is unknown whether the highest starting nitrate concentration tested could be considered the upper threshold for nitrate concentration in the research-scale aquaponic system. Upper thresholds to nutrient concentrations for lettuce are generally set using electrical conductivity as the indicator (Mason 1990; Morgan 1999; Bugbee 2004; Resh 2013). Hydroponic nutrient solutions are recommended to be below 2,500 μS $cm^{-1}$ for lettuce (Morgan 1999; Resh 2013). In the present experiment, the highest starting nitrate concentration (high treatment replicates) was set at 50 mg $L^{-1}$, based on recommendations by Morgan (1999) for lettuce. Starting conductivity in this treatment averaged approximately 2,000 μS $cm^{-1}$ (Figure 6), suggesting that the accumulation of fish wastes to approximately 50 mg $L^{-1}$ of nitrate before the start of the experiment, may be an approximate upper limit, as it caused conductivity to rise to a level that is considered close to maximal for lettuce (Morgan 1999; Resh 2013). In addition, in previous experiments, hydroponic nutrient solutions have been used as comparative controls to ascertain the performance of nutrients derived from fish wastes in the same research-scale, aquaponic system (Lennard & Leonard 2004; Lennard 2005; Lennard & Leonard 2006; Lennard 2020). To achieve electrical conductivity within the maximum recommended for lettuce, hydroponic nutrient solutions were diluted to half strength in these experiments (Lennard 2005). On analysis, the nitrate concentration of these half-strength solutions was determined to be approximately 52 mg $L^{-1}$ (Lennard 2005), which is comparable to the highest nitrate concentration in the present study, therefore suggesting that approximately 50 mg $L^{-1}$ of nitrate, taken in the context of overall nutrient accumulation (measured as approximately 2,000 μS $cm^{-1}$) within the research-scale, aquaponic system, may well have been an upper limit for nutrient accumulation. It must be noted, however that hydroponic nutrient solution is a complete nutrient mix (Resh 2013). Therefore, although initial nitrate and overall conductivity levels were similar in the high nutrient test treatment, these may not represent as complete a mixture in aquaponic systems as hydroponic nutrient solutions do in hydroponic systems (Rakocy et al 2006; Maucieri et al 2019).

Lettuce yields in the present study were equal to, or better than, the studies of Burgoon and Baum (1984), Seawright et al (1998), Lennard and Leonard (2004), Lennard and Leonard (2006), Geisenhoff et al (2016), Johnson et al (2017), Jordan et al (2018), Maucieri et al (2018) and Lennard (2020); suggesting the nutrient availability in the two test treatments was adequate for lettuce production.

Net nutrient accumulation within the aquaponic system is a further indicator of efficient plant growth and production, as well as being an indicator of the balance between fish biomass (based on ultimate fish feeding rate) and plant numbers (Lennard 2005). It can be seen from the results of net nitrate accumulation (final - initial nitrate concentrations), both test treatments finished with negative nitrate accumulations, whilst the control finished with a positive nitrate accumulation (Table 3). This suggests the plants in both test treatments used more nitrate than was produced by the fish as waste across the experimental period (21 days), whereas the plants in the control used less nitrate than the fish produced as waste. With all other test parameters equal between the control and test treatments, it may be concluded that the increased initial nitrate concentrations of the two test treatments aided plant metabolism and resulted in greater plant growth and yield (Table 2) and increased nitrate removal (Table 3), when compared with the control (initial nitrate concentration of 0.0 mg $L^{-1}$). Given the negative accumulation of nitrate in the test treatments, more fish or fewer plants may be required for long-term commercial production system nutrient balance.

Net accumulation of nitrate within all treatments (Table 3) ranged from – 1.57 ± 1.60 mg $L^{-1}$ (high treatment) to 5.60 ± 0.57 mg $L^{-1}$ (control). This accumulation is lower than previous research results using the same research-scale, aquaponic system with a similar constant flow regime through the gravel plant-growing bed, of 11.80 ± 1.78 mg $L^{-1}$ over the same time period (Lennard & Leonard 2004), similar to a further study using the same system, where nitrate accumulated to 4.63 ± 2.85 mg $L^{-1}$ (Lennard & Leonard 2006) and lower than all treatments in another study using the same system (nitrate accumulation range of 7.80 ± 2.20 mg $L^{-1}$ to 13.77 ± 2.23 mg $L^{-1}$) (Lennard 2020). Delaide et al (2017) achieved nitrate accumulations of 58 mg $L^{-1}$ in their small-scale, deep-water culture aquaponic system growing lettuce and basil. Hasan et al (2017) achieved average nitrate accumulations of approximately 40 mg $L^{-1}$ after three weeks growing Sangkuriang catfish (*Clarias gariepinus*) and Nile tilapia (*Oreochromis niloticus*) with water spinach (*Ipomoea aquatica*) and lettuce (*L. sativa*). Dediu et al (2012) observed nitrate accumulations of 34.52 ± 6.26 mg $L^{-1}$ and 32.25 ± 7.06 mg $L^{-1}$ in an aquaponic system applying high and low hydraulic retention times growing bester sturgeon (*Huso huso* x *Acipenser ruthenus*) and lettuce (*L. sativa*). Therefore, nitrate accumulations in the current study were lower than other studies and may suggest that the current study employed a fish waste to plant nutrient use ratio of better balance than that of these other studies.

Phosphate accumulation (Table 3) is also an indicator of efficient plant metabolism and overall system optimisation (Lennard 2005). As was the case for nitrate accumulation, phosphate accumulations in both test treatments were negative at the end of the experiment, and control phosphate accumulations were positive (Table 3). Again, as for nitrate, this suggests that the plants in both test treatments used more phosphate than the fish produced as waste over the length of the experiment. Again, this suggests that the higher initial phosphate concentrations within the two test treatments aided plant metabolism and led to higher plant growth and yields within these two test treatments, when compared with controls (Table 2). As for nitrate accumulation, more fish or fewer plants may be advisable in a long-term, commercial, rotational lettuce harvest situation to balance nutrients.

Net accumulation of phosphate ranged from -9.04 ± 1.59 (high treatment) to 0.92 ± 0.54 mg $L^{-1}$ (control). This is lower than previous research results using the same aquaponic research system with a similar constant flow regime through the gravel plant-growing bed, of 3.87 ± 0.71 mg $L^{-1}$ over the same time period (Lennard & Leonard 2004), lower than a further study with the same system, where phosphate accumulated to 3.42 ± 0.11 mg $L^{-1}$ (Lennard & Leonard 2006) and lower than all treatments in another study using the same system (phosphate accumulation range of 2.60 ± 0.11 mg $L^{-1}$ to 3.92 ± 0.33 mg $L^{-1}$) (Lennard 2020). Makhdom et al (2017) observed phosphate accumulations in an aquaponic system growing pearl gourami (*Trichopodus leerii*) and cherry tomato (*Solanum lycopersicum var. cerasiforme*) at the highest planting density, of approximately 15 mg $L^{-1}$ after 30 days. Liang and Chien (2013) achieved phosphate accumulation of 38.1 mg $L^{-1}$ (lowest concentration achieved) when growing red tilapia (*Oreochromis* sp.) and water spinach (*Ipomoea aquatica*) in an aquaponic system testing fish feeding frequencies and plant exposure photoperiods. Therefore, phosphate accumulations in the current study were lower than those of other studies and may suggest that the current study employed a fish waste to plant nutrient use ratio of better balance than that of these other studies.

Dissolved oxygen concentrations are presented in Figure 3. A significant difference was detected between the control and both test treatments. As can be seen, control D.O. concentrations were significantly higher in the first five days of the experiment, but from day 5 onwards were similar to both test treatments. This is most likely because control replicates were completely flushed (at time zero) and refilled with new water to obtain initial nitrate concentrations as close as possible to zero. All treatments were started several weeks earlier with only fish in them, so as to accumulate nutrient levels to obtain the required initial nitrate concentrations within the particular treatment (0, 25 or 50 mg $L^{-1}$). Test treatments were not completely flushed with new water, with less new water added (than controls) to achieve the required starting nitrate concentrations (25 or 50

mg $L^{-1}$) via mixing new fresh water with existing aquaponic water. Therefore, test treatments contained levels of organic matter, arising from fish wastes (dissolved and non-dissolved) which may have contributed to greater initial oxygen use within them, when compared to the control replicates. Despite the initial differences in D.O between the control and both test treatments, the initial higher nitrate concentrations (and associated organic matter) in the test treatments still had little effect on the ability of the system water to maintain dissolved oxygen concentrations in the range required. D.O., in all treatments (control and test), was maintained at levels clearly above those stated as the minimum requirement for lettuce (2.1 mg $L^{-1}$ – Goto et al 1987), warm water fish (5.0 mg $L^{-1}$ – Masser *et al.* 1999) and nitrifying bacteria (2.0 mg $L^{-1}$ – Alleman & Preston 2002). It is therefore likely, that the oxygen levels in all treatments had no negative impact on lettuce growth.

Buffer addition and pH are integrally linked in aquaponic systems. No significant differences were detected between any control or test treatments in terms of both buffer use and pH maintenance (Figure 4 & Figure 5). Therefore, it can be concluded that the different initial nitrate levels of the two test treatment systems had no effect on the overall ability of the test systems to maintain pH or to consume buffer, when compared to the control system. Lennard (2005) demonstrated that the inclusion of plants in the research-scale, recirculating aquaponic system led to an outcome whereby buffer additions to control pH may be lowered (when compared to fish-only controls). When nitrate and phosphate ions are assimilated by plants, negative ions ($OH^-$, $HCO_3^-$) are released in order to maintain homeostatic, cellular pH levels within the roots (Imsande & Touraine 1994, Lennard 2017, Maucieri et al 2019) and the release of these negative ions lowers the requirement for external buffer additions (Lennard 2017). Lennard & Leonard (2004) achieved buffer additions of 0.8 ± 0.14 g $day^{-1}$ in the same research-scale, aquaponic system when comparing a constant flow regime to a reciprocating flow regime, Lennard and Leonard (2006) achieved a range of buffer additions from 0.9 ± 0.21 to 2.1 ± 0.20 g $day^{-1}$ in the same system for several different hydroponic component types (gravel, nutrient film technique and deep flow culture) and Lennard (2020) achieved buffer additions in the range of 1.3 ± 0.16 g $day^{-1}$ to 1.9 ± 0.19 g $day^{-1}$ for several different buffer species in the same research-scale, aquaponic system.

It is evident from the conductivity curves of the present experiment that the control and both test systems started at very different conductivities (Figure 6). As stated earlier, this is because nitrate (and all other nutrients arising from fish metabolism) accumulation to higher initial levels was required in the two test treatments, which thus, contributed to higher conductivities in test treatments. Because of the difference between all treatments in terms of initial conductivity, net conductivity accumulation (final – initial conductivity) over the course of the experiment was used to assess for differences in conductivities between control and test treatments (Figure 7). Comparisons between all control and test treatments in terms of conductivity accumulation returned no significant differences between any treatment (Figure 7). Therefore, a difference in the initial nitrate concentration (and overall nutrient concentration) of the particular test system had no effect on the accumulation of ions (conductivity) within the research-scale, aquaponic system replicates and therefore, it is considered that, as long as the initial conductivity level is below the maximum allowed for the plant species being grown, initial nitrate levels can be set as high as required.

In terms of water use, all control and test treatments used statistically similar amounts (Figure 8). Accepting that evaporation should be similar across all treatments, this suggests that transpiration in all treatments was statistically similar. Therefore, plants transpired and used similar amounts of water across all treatments, suggesting that the initial nutrient concentrations had little effect on the amount of water used by the respective systems.

**Conclusions**. It is evident that increased initial nutrient concentrations (measured as nitrate in this study) within the research-scale, aquaponic systems, aided plant metabolism and improved the overall efficiency of the aquaponic systems in terms of both plant yield and nutrient use by the plants (measured as net nitrate and phosphate

accumulation). In terms of all of the other parameters tested, it appears that the initial nutrient level has had little effect. This study, therefore, agrees with past research suggestions that nutrient levels should be allowed to accumulate in aquaponic systems, to a level below the toxic limit for plant growth, by feeding fish for a few weeks before plants are introduced to the system.

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Authors:
Wilson Andrew Lennard, School of Science, Technology, Engineering and Mathematics, University of South Australia, Mawson Lakes Campus, Mawson Lakes, South Australia, Australia 5095, e-mail: willennard@gmail.com

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