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**Evaluating the acute and chronic toxicity of potassium carbonate and the interactive effects
between sodium and potassium on rainbow trout (*Oncorhynchus mykiss*)**

By

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BS in Marine, Freshwater, & Estuarine Biology, University of New Hampshire, 2017

THESIS

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Abstract

Capture-and-reuse of recirculating aquaculture system (RAS) effluents as hydroponic fertilizer can mitigate the high costs of waste treatment and disposal. However, RAS operators must address limitations in macro- (K, Ca) and micro-nutrients (Fe, Zn, Mo, Cu) and the phytotoxic sodium concentrations (>50 mg/L Na) present in solution. One option is to replace sodium-based pH buffering salts such as sodium bicarbonate with potassium-based derivatives such as potassium carbonate or bicarbonate. However, findings in previous studies demonstrate adverse sublethal impacts on fish health due to elevated potassium concentrations. Lethal effects of potassium salts (KCl, KHCO_3 , and K_2SO_4) have been demonstrated in fathead minnows (*Pimephales promelas*) and bluegills (*Lepomis macrochirus*). Research findings indicate that potassium toxicity in fishes may be mitigated by concentrations of other cations such as sodium, magnesium, and calcium. However, published research on potassium toxicity and interactive effects between sodium and potassium on fish health is limited. The objectives of this research were to determine the acute toxicity of potassium carbonate (K_2CO_3), the interactive acute toxic effects between two salts (K_2CO_3 and NaCl), and the chronic toxicity of K_2CO_3 on growth performance of rainbow trout (*Oncorhynchus mykiss*) cultured in RAS.

The acute toxicity of potassium carbonate (K_2CO_3) was determined using a median lethal concentration (LC_{50}) study. Five concentrations (707, 1502, 2298, 3093, and 3888 mg/L K_2CO_3) were tested with ten replicates per treatment. Percent mortality was recorded over a 96-hour period and LC_{50} concentrations were determined at 24, 48, 72, and 96 hours using Probit regression. Two rounds of preliminary mortality studies with three replicates per treatment were used to determine

the interaction between sodium and potassium. The first round of studies tested the effect of K: Na ratio and the second round tested the effects of a balanced (1:1) K: Na ratio using the same potassium concentrations (from K_2CO_3) used in Experiment 1. Percent mortalities were recorded over a 96-hour period and the results of round two were compared to Experiment 1. A grow-out study was conducted to determine the effects of increased potassium concentrations on the growth performance of rainbow trout. Growth data were collected over 79 days in a replicated study where potassium carbonate was used as the pH buffering salt. Feed conversion ratios (FCRs) and specific growth rates (SGRs) were calculated after the study was concluded. The growth data obtained in this study were compared to growth data obtained in Spring 2018.

Median lethal concentrations (LC_{50}) of 3470, 3016, 2831, and 2672 mg/L K_2CO_3 were obtained at 24, 28, 72, and 96-hours, respectively. However, interactive effects between sodium and potassium on acute toxicity were not observed. In the grow-out study, the SGR was significantly higher ($p=0.0093$) in the potassium treatment (SGR- 1.32 ± 0.02 %/day) than in the control (SGR- 0.87 ± 0.06 %/day) and the FCR was significantly lower ($p=0.013$) in the potassium treatment (FCR- 1.18 ± 0.04) than in the control (FCR- 2.96 ± 0.22).

The results of this research displayed low acute toxicity to elevated potassium concentrations in rainbow trout. Growth performance was also greater in the potassium treatment than in the control. Furthermore, no interaction between sodium and potassium was demonstrated. This potentially indicates that the replacement of potassium carbonate as the sole buffering salt will not negatively impact fish health. However, there are sources of error including the use of different cohorts and the fluctuations in culture water potassium concentrations. Therefore, another grow-out study should be run using the same cohort of fish reared at the same time under different conditions to obtain a more valid understanding of the effects of elevated potassium on fish health.

Chapter 1

Grand Introduction

1. Sustainability in Aquaculture:

Global aquaculture production has increased greatly over the last 40 years (FAO, 2018). Aquaculture represents the fastest growing food production industry despite a significant decrease in the annual growth rates in recent years (5.8% between 2001-16) compared to years past (10.8% and 9.5% in the 1980's and 1990's, respectively). In 2016, aquaculture contributed nearly 110.2 million tons of marketable seafood (including aquatic plants), accounting for 46.8% of global capture fisheries and aquaculture production (FAO, 2018). The four most commonly utilized production methods are: ponds, net-pens, raceways, and recirculating aquaculture systems (RAS) (Swann, 1992; Timmons et al., 2018). Aquaculture production in ponds, net-pens, and raceways faces a number of issues including high land and water requirements, disease pressure, predation, escapees, natural disasters, and large environmental footprint due to improper waste management strategies. These can result in large yield losses and produce a commodity that is neither economically nor environmentally sustainable (Hall et al., 2011; Timmons et al., 2018). Indoor RAS offers a greater degree of control to growers and reduces the water to land use ratio while increasing the production intensity (kg/ha/y) significantly. It is also more environmentally sustainable as waste is captured and treated prior to discharge. However, further commercial expansion of RAS is greatly limited by high operation costs due to the intensive treatment required to meet regulatory discharge standards set by the Environmental Protection Agency (EPA) (EPA, 2004; Mugg et al., 2007; Klinger and Naylor, 2012).

Waste management presents a considerable challenge to growers operating an aquaculture facility (Mugg et al., 2007). Solid waste must be removed immediately from culture water in order to mitigate proliferation of heterotrophic bacteria, accumulation of nitrogenous waste products, and maintain fish health and biofilter efficiency in a recirculating system (Masser et al., 1992; Davidson et al., 2011a, 2011b; Thorarinsdottir et al., 2015). Removal of suspended and settleable solids is an intensive process, requires high capital investment, and incurs high maintenance and operating costs. Additionally, aquaculture effluent must undergo expensive treatment processes in order to meet the regulatory standards for discharge of aquaculture effluents set by the EPA (EPA, 2004; Klinger and Naylor, 2012). Treated RAS effluents are often saturated with high concentrations of phosphorus and nitrogen and may contribute to eutrophication in aquatic environments if not properly managed (Mugg et al., 2007; Martins et al., 2010). Development of a capture-and-reuse model for aquaculture similar to other animal agriculture production systems can mitigate environmental impact and costs of waste treatment and disposal (NRCS, 2013). The large concentrations of aqueous nutrients present in RAS effluents makes it more suitable for integration with hydroponic systems, a soilless growing technique that relies solely on nutrients dissolved in solution (Rakocy et al., 2006; Graber and Junge, 2009; Rakocy, 2012; Goddek et al., 2015). Adopting a capture-and-reuse model for RAS requires significant changes to water quality parameters, which may affect finfish production and health.

2. Integrating RAS Waste Capture-and-Reuse strategy with Hydroponic Production

Adoption of capture-and-reuse in aquaculture waste has increased in recent years due to its ability to reduce effluent discharge into the environment and monetize the waste treatment process (Martins et al., 2010; Klinger and Naylor, 2012). Captured solid waste from RAS can be applied directly as fertilizer in land-based agriculture or composted and utilized as a soil amendment (van

Rijn, 1996; Summerfelt and Vinci, 2008; Guerdat et al., 2013; NRCS, 2013). However, aquaculture sludge must undergo expensive thickening processes beforehand to remove excess water and minimize costs of storage and transport and prevent nutrient loss into groundwater (van Rijn, 1996; Cripps and Bergheim, 2000; Summerfelt and Vinci, 2008; Sharrer et al., 2009; Guerdat et al., 2013). Following sludge thickening processes, the total solids concentration comprises only 5-15% of RAS effluent stream (Cripps and Bergheim, 2000; Summerfelt and Vinci, 2008; Sharrer et al., 2009). The remaining RAS effluent is concentrated with aqueous nutrients making it more suitable to use as fertilizer in hydroponic production, which relies mostly on nutrients dissolved in solution (Rakocy et al., 2006; Graber and Junge, 2009; Rakocy, 2012; Goddek et al., 2015)..

Aquaponics is an integrated multitrophic aquaculture (IMTA) practice that utilizes the nutrients dissolved in RAS culture water as fertilizer in hydroponic cropping systems (Rakocy et al., 2006; Graber and Junge, 2009; van Rijn, 2013; Goddek et al., 2015). RAS effluents contain sufficient concentrations of a number of macro-nutrients (plant-essential nutrients required in greater mass, proportionally) to support plant growth including nitrogen (N), phosphorus (P), and sulfur (S) (Rakocy et al., 2006; Rakocy, 2012; Delaide et al., 2016). The use of RAS effluents as hydroponic fertilizer results in greater quantities of water being recirculated, thus reducing the costs of waste treatment and environmental discharge (Rakocy et al., 2006; Somerville et al., 2014; Goddek et al., 2015; Delaide et al., 2016). However, the nutrient profile of RAS effluents doesn't provide the complete complement of nutrients essential for optimal plant growth.

RAS effluents lack sufficient concentrations of some macro-nutrients such as potassium (K) and calcium (Ca), as well as a number of micro-nutrients (plant-essential nutrients required in lower mass quantities, proportionally) (e.g. iron (Fe), copper (Cu), zinc (Zn), molybdenum (Mo)) essential to support hydroponic production (Seawright et al., 1998; Rakocy et al., 2006; Anderson,

2016; Delaide et al., 2016). Sodium concentrations in RAS effluent often exceed concentrations considered to be phytotoxic (>50 mg/L) due to use of sodium-based buffering salts such as sodium bicarbonate and sodium hydroxide, osmoregulatory activity of the fish, and the high concentrations found in many commercial fish feeds (Seawright et al., 1998; Guerdat et al., 2013; Delaide et al., 2016; Anderson et al., 2017). Sodium concentrations typically vary based on water exchange rates, nutrient characterization of make-up water, and feed rates and can range from 4 mg/L to >700 mg/L in rainbow trout RAS (Davidson et al., 2009, 2011a, 2011b). At the University of New Hampshire's Agricultural Experiment research program (Durham, NH, USA), the water quality and nutrient profiles of the replicated research rainbow trout (*Oncorhynchus mykiss*) RAS are regularly monitored in the culture water and effluent streams, and sodium concentrations typically range between 50 and 150 mg/L (*Unpublished operational data*). Sodium disrupts plant growth by competitively inhibiting the uptake of essential nutrients such as K, Ca, and Fe and results in issues such as chlorosis and disrupted growth rates (Ball et al., 1987a; Resh, 1995; Tavakkoli et al., 2010a, 2010b). Disrupted growth rates and chlorosis can affect the marketability of the product and decrease the profit margin (Tavakkoli et al., 2010a, 2010b). One option for RAS producers to decrease the high sodium concentrations in RAS effluents and make them more suitable for use as hydroponic fertilizer has been to replace the sodium-based pH buffering salts such as sodium bicarbonate with potassium or calcium derivatives. Replacement with calcium-based buffering salts is complicated due to its low solubility in the presence of high organic carbon concentrations, which is present in high concentrations in RAS effluents (Roosta, 2014). Potassium-based buffering salts such as potassium hydroxide (KOH) and potassium bicarbonate (KHCO₃) can buffer pH and account for insufficient concentrations in RAS effluent (Seawright et al., 1998; Goddek et al., 2015). However, even though these negative impacts of elevated concentrations of

potassium on fish health have been identified, more research is need to clarify the toxic effects of increased potassium concentrations on fish in RAS (Trama, 1954; Mount et al., 1997; Davidson et al., 2011b; Borvinskaya et al., 2016).

3. Sodium vs Potassium as a Potential Limitation to Integration

Replacing of sodium-based buffering salts with conjugate potassium-based salts is done in order to reduce the phytotoxic effects of sodium in plants and to account for limited potassium concentrations in RAS effluent (Seawright et al., 1998; Goddek et al., 2015). Potassium is important in photosynthesis, enzyme function in glycolysis, water uptake, sugar production and transportation, and fruit ripening (Resh, 1995; Seawright et al., 1998; Cakmak, 2005; Somerville et al., 2014; Thorarinsdottir et al., 2015). However, it is often found in limited concentrations in fish feeds since it is not essential to support fish growth (Seawright et al., 1998; Goddek et al., 2015). Limited concentrations of potassium in RAS effluents and culture water would allow it to get competitively inhibited by sodium, thus disrupting important physiological functions in plants, thereby causing chlorosis, and reducing plant growth and fruit yield (Al-Hafedh et al., 2008; Graber and Junge, 2009; Somerville et al., 2014). Supplementation of potassium into an aquaponic system accounts for this limitation and mitigates the competitive effects of sodium (Tavakkoli et al., 2010a, 2010b; Roosta and Hamidpour, 2011). The costs of replacing sodium-based buffering salts with potassium-based derivates and the impacts on fish production need to be further assessed.

Excessively high potassium concentrations in RAS can have detrimental impacts on fish health (Mount et al., 1997; Rakocy et al., 2006; Rakocy, 2012; Borvinskaya et al., 2016). Excessive potassium concentrations affect the ionic balance of the RAS culture water, which disrupts the electrochemical gradient of K^+ across fish cellular membranes. This affects many physiological

functions that are dependent on intracellular and extracellular sodium and potassium concentrations including osmoregulation, acid-base balance, muscle-contraction, and nerve function (Evans, 2008; Opoku-Okrah et al., 2015; Borvinskaya et al., 2016). Sublethal effects associated with elevated potassium concentrations include stunted growth rate, erratic swimming behavior, and gill irritation (Davidson et al., 2011b; Borvinskaya et al., 2016). Davidson et al (2011b) provided anecdotal evidence from Mac Laberge in Aquaponiques (Quebec, CA) noting gill irritation at concentrations of 110-120 mg/L when potassium was supplemented in the culture water. Elevated concentrations of potassium salts have caused lethal effects in multiple fish species including hybrid striped bass (female white bass *Morone chrysops* x male striped bass *M. saxatilis*), rainbow trout (*Oncorhynchus mykiss*) (see Experiment #1, Chapter 2), bluegill (*Lepomis macrochirus*) and fathead minnows (*Pimephales promelas*) (Trama, 1954; Mount et al., 1997; Rakocy et al., 2006; Davidson et al., 2011b; Borvinskaya et al., 2016). Survival of bluegill and fathead minnows decreased significantly in water treated with potassium salts (KNO_3 , KCl , K_2SO_4) compared to the conjugate calcium ($Ca(NO_3)_2$, $CaCl_2$, $CaSO_4$) and sodium salts ($NaNO_3$, $NaCl$, Na_2SO_4), suggesting the toxicity was due to K rather than to the ions themselves (Trama, 1954; Mount et al., 1997). Both Trama (1954) and Mount et al. (1997) identified the following order of cation toxicity: $K > Ca > Na$. The toxic effects of elevated potassium concentrations need to be further researched in order to develop a more sustainable capture-and-reuse model for integrated aquaculture and hydroponic cropping systems.

The interaction between sodium and potassium in the culture water is an important consideration as aquaculture systems integrate with hydroponic production systems. The presence of sodium, magnesium, and calcium salts can reduce some of the sublethal and lethal impacts of potassium salts (Mount et al., 1997; Borvinskaya et al., 2016). Acute toxicity of potassium salts

(KCl, K₂SO₄, and KHCO₃) in fathead minnows decreased in LC₅₀ assays testing two salts where equal volumes of sodium and potassium salts were used (Mount et al., 1997). Furthermore, the growth performance of whitefish (*Coregonus lavaretus*) decreased significantly in culture water with a 5:1 K:Na balance compared to a 1:1 K:Na balance, even with similar potassium concentrations (171.6 mg/L and 117.6 mg/L K respectively). There was a corresponding increase in liver malondialdehyde (MDA), an indicator of oxidative stress in whitefish reared in the culture water with the 5:1 K:Na molar ratio (Borvinskaya et al., 2016). Reduction in growth performance is a tertiary response in many fish species to environmental stressors (Jobling, 1995). Nutrient analysis of the culture water at the UNH Aquaponic Research Greenhouses at Kingman Farm (Madbury, NH) demonstrated a 4.8:1 molar ratio between potassium and sodium concentrations (*Unpublished operational data*). However, no apparent adverse effects were observed in the growth performance of tilapia (*Oreochromis* spp.). Since there is a lack of published research of the effects of the K:Na molar ratio on fish health, more research is needed in order to assess the interactive effects such that a sustainable balance can be reached to optimize production of both plant and fish systems.

4. Sustainable Rainbow Trout Aquaculture:

Rainbow trout are a widely cultured species in aquaculture and continue to see a growth in popularity in markets all over the world since 1950. Salmon and trout have been the largest fish commodity by value since 2013, providing 18.1% of the trade and sale of global aquaculture and capture fisheries products (FAO, 2018). Global aquaculture production of rainbow trout has increased exponentially since the 1950s and approximately 800 kilotons (kt) are currently produced annually (Hinshaw et al., 2004; Cowx, 2005; FAO, 2018). Trout production in the

United States experienced an expansion in the 1870s-80s and has since remained relatively consistent despite the global exponential increase (Hinshaw et al., 2004). There are currently approximately 300 farms in operation in the United States producing close to 49 million pounds (22.2 kt) of market size trout (400-650 g/fish) annually (Fornshell, 2002; Hinshaw et al., 2004; Perdue and Hamer, 2019).

Although rainbow trout are cultured in ponds, net-pends, and recirculating aquaculture systems, nearly 90% of rainbow trout aquaculture in the United States is conducted in concrete raceways (Fornshell, 2002; Hinshaw et al., 2004). Due to high volumes of wastewater discharge associated with trout production in raceways and limited availability of suitable sites, further expansion of trout farms using raceway systems is limited despite the high demand for market size farmed trout. In order to meet consumer demand and more effectively compete for limited ground water resources, trout farming needs to adapt more intensive production techniques such as recirculating aquaculture systems (RAS) (Fornshell, 2002; Hinshaw et al., 2004). Rainbow trout RAS can be successfully integrated with hydroponic cropping systems growing leafy greens since trout effluents contain most nutrients required for optimal plant growth at adequate concentrations and water temperatures are at an ideal range of 15-19°C (Adler et al., 2000; Thorarinsdottir et al., 2015). Since potassium concentrations are greatly limited in aquaculture effluents, potassium needs to be supplemented into the culture water in order to support growth of other hydroponic crops such as tomatoes (Roosta and Hamidpour, 2011; Goddek et al., 2015). Further research is needed in order to understand the impacts of supplementing potassium at higher concentrations on the health of rainbow trout in order to optimize fish and plant production.

5. Conclusions and Research Needs

As aquaculture practices continue to expand, development of an effective capture-and-reuse model for aquaculture is essential to mitigate costs of conventional waste treatment, monetize the nutrient stream in the effluent, and reduce overall environmental impact (Martins et al., 2010; Klinger and Naylor, 2012). Aquaponic effluents are more ideal as fertilizer in hydroponic cropping systems than land-based agriculture due to the high volume of water and have been shown to support plant growth in multiple applications (Seawright et al., 1998; Cripps and Bergheim, 2000; Rakocy et al., 2006; Rakocy, 2012; Guerdat et al., 2013; Anderson, 2016). Aquaculture effluents often contain optimum concentrations of multiple plant-essential macronutrients including nitrogen (N), phosphorus (P), and sulfur (S). However, sodium is often present at above phytotoxic concentrations (>50 mg/L Na) and it limits the effectiveness of RAS effluents as hydroponic fertilizer (Seawright et al., 1998; Guerdat et al., 2013; Delaide et al., 2016; Anderson et al., 2017). One current approach taken to mitigate the negative effects of sodium is replacement of sodium-based buffering salts (i.e. sodium bicarbonate and sodium hydroxide) with the potassium-based derivatives (Seawright et al., 1998; Goddek et al., 2015). However, since elevated potassium concentrations are detrimental to fish health and can be problematic to long-term production, research is needed to establish optimal operating parameters for recirculating aquaculture systems (Mount et al., 1997; Davidson et al., 2011b; Borvinskaya et al., 2016).

Research is needed which addresses the inherent differences between hydroponic and RAS systems such that the two processes may be successfully integrated to further support the development of an economically sustainable integrated farming model for RAS. Evaluating the acute and chronic toxicity of potassium in fish is an important first step to understanding the effect of increased potassium in integrated aquaculture and hydroponic cropping systems. Once the acute threshold of potassium carbonate is determined, a toxicity trial using two salts (sodium chloride

and potassium carbonate) can be performed to assess the sodium-potassium interaction. A long-term growth study can then be performed to assess the effects of elevated potassium concentrations on the growth performance of rainbow trout. The goals of this research project are to assess (1) the acute toxicity of potassium carbonate (K_2CO_3) on rainbow trout (*Oncorhynchus mykiss*), (2) the acute toxicity using two salts potassium carbonate (K_2CO_3) and sodium chloride (NaCl) to understand the interactive effects between potassium sodium, and (3) the impact of replacing sodium bicarbonate with potassium carbonate on fish growth performance. It is hypothesized that the result from this study will demonstrate a corresponding increase in mortality with respect to the concentration of potassium carbonate, and a decrease in mortality with respect to increased sodium concentrations. Replacement with potassium carbonate as a buffering source should result in decreased growth rate and survivorship among fish in potassium treated culture water.

Chapter 2

Evaluating the acute toxic effects of potassium carbonate (K₂CO₃) on rainbow trout (*Oncorhynchus mykiss*) cultured in recirculating aquaculture systems (RAS)

1. Introduction

Commercial expansion of recirculating aquaculture systems (RAS) is limited by current waste treatment processes. Waste management poses one of the largest economic challenges to operating an aquaculture facility as operators are forced to internalize the costs. The RAS culture water is first treated to rapidly remove suspended and settleable solids, then treated using biological filtration to renovate water quality and enable reuse within the facility (Masser et al., 1992; Losordo et al., 1999; Badiola et al., 2012). RAS water treatment systems require special equipment which have high start-up, maintenance, and operating costs. In the United States, RAS effluent requires intensive treatment on-site to meet regulatory discharge standards set by the Environmental Protection Agency (EPA) (EPA, 2004; Klinger and Naylor, 2012). Treated RAS effluents may still contain large masses of phosphorus and nitrogen, both of which can contribute to eutrophication in aquatic environments (Mugg et al., 2007; Martins et al., 2010). Development of a capture-and-reuse waste nutrient model similar to other animal agriculture production systems can mitigate environmental impacts and costs of waste treatment and disposal (NRSC AWM, 2013).

The capture-and-reuse of aquaculture effluents has expanded in recent years due to the ability to reduce effluent discharge into the environment and monetize the waste treatment process by providing an additional marketable commodity (Martins et al., 2010; Klinger and Naylor, 2012). Solid waste from RAS can be applied as a land-based fertilizer or composted

and utilized as a soil amendment (van Rijn, 1996; Summerfelt and Vinci, 2008; Guerdat et al., 2013). However, aquaculture sludge must undergo expensive thickening processes beforehand to remove excess water and minimize costs of storage and transport and prevent nutrient loss to groundwater (van Rijn, 1996; Cripps and Bergheim, 2000; Summerfelt and Vinci, 2008; Sharrer et al., 2009; Guerdat et al., 2013). Following sludge thickening processes, the total solids concentration comprises only 5-15% of the effluent stream (Cripps and Bergheim, 2000; Summerfelt and Vinci, 2008; Sharrer et al., 2009). The remaining RAS effluent is concentrated with aqueous waste nutrients making it more suitable for use as fertilizer in hydroponic systems. Hydroponic production is a soilless growing technique that relies solely on nutrients dissolved in solution (Graber and Junge, 2009; Goddek et al., 2015). Hydroponic cropping systems offer a more suitable application of RAS effluents than field application for the production of agricultural crops. The wasted nutrients from RAS can support hydroponic production in many different settings (Adler et al., 2000; Harmon, 2005; Rakocy et al., 2006; Rakocy, 2012; Turcios and Papenbrock, 2014)

RAS effluents provide a number of plant-essential macro-nutrients including nitrogen (N), phosphorus (P), and sulfur (S) which are readily available at sufficient concentrations for plant growth (Seawright et al., 1998; Rakocy et al., 2006; Guerdat et al., 2013; Delaide et al., 2016). However, several other macro-nutrients such as potassium (K) and calcium (Ca), as well as some plant-essential micro-nutrients (e.g. Fe, Cu, Zn, Mo) are present at insufficient quantities to support hydroponic production (Seawright et al., 1998; Rakocy et al., 2006; Anderson, 2016; Delaide et al., 2016). Sodium (Na) concentrations in RAS effluents typically exceed concentrations considered to be phytotoxic (50 mg/L) due to the use of sodium-based buffering salts such as sodium bicarbonate (NaHCO_3) and sodium hydroxide (NaOH) and through the

osmoregulatory activity of the fish (Seawright et al., 1998; Guerdat et al., 2013; Delaide et al., 2016; Anderson et al., 2017). The water quality and nutrient profiles of the replicated research RAS as part of the Agricultural Engineering research program at the University of New Hampshire (Durham, NH, USA) are monitored regularly in the culture water and effluent streams, and sodium concentrations typically range between 50 and 150 mg/L (*Unpublished operational data*). Sodium disrupts plant growth by competitively inhibiting the uptake of essential nutrients such as K, Ca, and Fe (Ball et al., 1987b; Resh, 1995; Tavakkoli et al., 2010a, 2010b). One option for RAS producers to mitigate the potential problems with sodium in RAS effluents is replacing the sodium pH-buffering salts with potassium derivatives such as potassium carbonate (K_2CO_3) or potassium bicarbonate ($KHCO_3$) (Seawright et al., 1998; Goddek et al., 2015; Thórarinsdóttir et al., 2015). However, negative impacts on fish health resulting from elevated potassium concentrations have been documented in other studies (Trama, 1954; Mount et al., 1997; Davidson et al., 2011b; Borvinskaya et al., 2016).

Excessively high potassium concentrations disrupt various physiological functions of fish including osmoregulatory function, acid-base balance, muscle-contraction, and nerve function by disrupting the intra/extracellular electrochemical gradient of K^+ (Evans, 2008; Opoku-Okrah et al., 2015; Borvinskaya et al., 2016). Sublethal impacts of excess potassium concentrations may include stunted growth, erratic swimming behavior, and gill irritation (Davidson et al., 2011b; Borvinskaya et al., 2016). Survival of common bluegill (*Lepomis macrochirus*) and fathead minnows (*Pimephales promelas*) was reduced in water treated with potassium salts (KNO_3 , KCl , K_2SO_4) compared to the conjugate calcium ($Ca(NO_3)_2$, $CaCl_2$, $CaSO_4$) and sodium salts ($NaNO_3$, $NaCl$, Na_2SO_4), suggesting that the toxicity was due to K rather than the anions themselves (Trama, 1954; Mount et al., 1997). Although some research has been conducted to quantify the

negative impacts of elevated potassium concentrations on certain fish species, there isn't enough research to fully understand the potential operational issues that may arise in commercial RAS when it is coupled with hydroponic cropping systems.

Determining the acute toxicity of potassium carbonate (K_2CO_3) will establish sensitivity of rainbow trout to high potassium concentrations and is the first step to developing operating parameters for integrated aquaculture systems. Rainbow trout is a widely cultured species in aquaculture and is being evaluated for commercial production in aquaponic systems, particularly in Nordic countries such as Denmark and Norway (Adler et al., 2000; Rakocy et al., 2006; Jokumsen and Svendsen, 2010; Thórarinsdóttir et al., 2015; Yep and Zheng, 2019). Furthermore, this research directly addresses a gap in research on the acute toxicity of potassium in rainbow trout and provides a framework for further research on sublethal effects. Therefore, the objective of this research was to assess the acute toxic effects of potassium carbonate (K_2CO_3) in rainbow trout through a median lethal concentration (LC_{50}) trial.

2. Methods/Materials:

This research was conducted at the University of New Hampshire Anadromous Fish and Invertebrate Research (AFAIR) lab at (Durham, NH, USA) using a system of individual tanks constructed as a rack system for LC_{50} assays. Details about the rack system and study organism are summarized in the sections below.

2.1. Study Organism:

Rainbow trout averaging 14.9 g/fish were received in September 2019 (Danaher Farms, Shrewsbury, VT, USA). Upon arrival, the fish were stocked in a 3500-L quarantine recirculating aquaculture system and reared for 4 weeks. The fish were fed a commercially available starter

feed (Skretting Nutra Fry 1.8 mm; a 50% crude protein, 20% lipid, sinking feed). The quarantine RAS temperature was maintained at a temperature range of 13-15 °C.

Water quality parameters were monitored and assessed daily. Dissolved oxygen (mg/L), temperature (°C), and electrical conductivity (mS/cm) were monitored daily (YSI Pro Optical Dissolved Oxygen meter, Xylem Inc., OH, USA). Dissolved oxygen (DO) was maintained between 80-95% saturation via direct injection into a down-flow, oxygen saturating column constructed of PVC pipe. Additional water quality parameters monitored included pH, alkalinity, nitrate-nitrogen (NO₃-N), nitrite-nitrogen (NO₂-N), and total ammoniacal nitrogen (TAN). The pH and alkalinity of the culture water were assessed daily with a bench-top pH meter (Accumet AB250, Fisher Scientific, NH, USA). Alkalinity (mg/L as CaCO₃) was measured by titration to pH endpoint (APHA, 2012, Method: 2320). All nitrogenous species (NO₃-N, NO₂-N and TAN) were measured regularly (four times per week) using a HACH DR3900 spectrophotometer following EPA approved HACH methods for water quality analysis (HACH Methods 8039, 8507, and 8038 respectively; HACH Company, CO, USA).

2.2. Range-finding Studies

Preliminary range-finding studies were conducted prior to the LC₅₀ assay to determine an optimum range of potassium carbonate concentrations for analysis similar to Mount et al. (1997). Each range-finding study tested five different potassium carbonate concentrations, each with three replicates. The optimum test range was defined as total mortality in the highest concentration, no mortality in the lowest concentration, and varying degrees of mortality in all other doses over the 96-hour period similar to previous studies (Mount et al., 1997; Hamlin, 2006).

2.3. *LC*₅₀ Assay

A single 96-hour semi-static *LC*₅₀ assay was conducted using individual rainbow trout, all from the same cohort, weighing an average of 30.4 g (\pm 4.0 SD) per fish. Five potassium treatments (3888.3, 3093.0, 2297.6, 1502.3, and 707.0 mg/L as K₂CO₃) and a dilution water control were tested utilizing 10 replicates per treatment for a total of 60 fish. Each treatment was prepared by diluting a stock solution (10,000 mg/L as K₂CO₃ stock solution or 5658.24 mg/L as K) into a dilution medium consisting of a 50:50 mix between reverse osmosis (RO) and well water. Well water was first analyzed to provide a comprehensive nutrient profile prior to treatment solution mixing (Table 2-1). Mixing of well water was performed to reduce any interactive effects between sodium and potassium which could potentially affect the results (Mount et al., 1997; Borvinskaya et al., 2016). The potassium stock solution used in this study was mixed using food grade potassium carbonate (> 99.0% assay) and RO water. HCl was used to adjust each treatment solution pH between 7.5-8.5. The control treatment solution was prepared by diluting the well water (28 mg Na/L) with RO water at a 50:50 mix ratio to achieve the same sodium concentration of 14 mg Na/L as the potassium treatment solutions.

Each fish was housed in individual 5L transparent high-density polyethylene (HPDE) Sterlite containers filled with 4 L of treatment solution. A lid was placed over each container to prevent escape. The Sterlite containers were placed on a 1.8 x 1.9 m² metal rack, with 12 containers per each of the five shelving units. Each shelving unit housed all 10 replicates of an entire treatment level plus two control replicates. Treatments were not randomized across the shelving units. Aeration was provided to individual containers through forced air injection from an air pump using small airstone diffusers. DO and temp were measured daily, while pH, NH₃,

and NO₂ were measured at the beginning of the study. Water quality parameters (DO, pH, temp, NH₃, and NO₂) are summarized in Table 2-2.

Feed was withheld from fish for 24 hours prior to starting the LC₅₀ assay in order to prevent buildup of feces and nitrogenous waste products (Environmental Protection Series, 1990). Fish were monitored regularly every 3-4 hours from 08:00-20:00 each day of the 4-day assay for mortality or change in physical condition. Changes in physical condition of the fish were recorded, including loss of equilibrium, erratic swimming, surface breathing, and loss of color (Environmental Protection Series, 1990). Total mortality for each treatment was determined at the end of every 24-hour period through the course of this study. Half of the water in each of the containers was exchanged daily (50% exchanged every 24 hours) to mitigate confounding effects in mortality due to excess accumulation of nitrogenous waste products such as TAN and NO₂-N (Hamlin, 2006). At the conclusion of the 96-hour assay, all fish were euthanized by cervical dislocation per the University of New Hampshire (UNH) International Animal Care and Use Committee (IACUC) Protocol #181205.

TABLE 2-1 Well water nutrient constituent characterization

Nutrient	Concentration (mg/L)
K	8.08
Ca	22.03
Mg	7.34
Na	28.35
P	0.29
S	6.24
Cl	20.72
NH₄-N	0
NO₃-N	0
ALK	107.47
pH	7.96
EC (mS/cm)	0.26

Urea	0.64
Fe	0
Mn	0.06
B	0
Cu	0
Zn	0
Mo	0.02
Al	0.14

TABLE 2-2 Characterization of water quality parameters for LC₅₀ treatments

Treatment #	K₂CO₃ (mg/L)	pH	NH₃-N (mg/L)	NO₂-N (mg/L)	DO (mg/L O₂)	Temp (°C)
Control	0	8.07	0.05	0.001	7.71 ± 0.13	15.17 ± 0.05
1	707.0	8.26	0.04	0.001	7.56 ± 0.17	14.93 ± 0.05
2	1502.3	8.44	0.04	0.002	7.79 ± 0.21	14.94 ± 0.05
3	2297.6	8.36	0.05	0.001	7.53 ± 0.18	14.54 ± 0.05
4	3093	8.56	0.03	0.002	7.35 ± 0.13	14.60 ± 0.00
5	3888.3	8.68	0.02	0.001	7.32 ± 0.12	14.89 ± 0.03

2.4. Statistical Analysis

Mortality data were compiled for each 24-hour period during the 96-hour study. A Probit analysis was conducted using JMP® Pro 14.0.0 (SAS Institute Inc., Cary, NC) to determine median lethal concentrations (LC₅₀) and 95% confidence intervals. LC₅₀ values were determined for 24, 48, 72, and 96-hours.

3. Results:

Complete mortality was observed in the highest treatment level (3888.3 mg/L K₂CO₃) within 72 hours of starting the study. No mortality was observed in the control or in the lowest treatment level (707.0 mg/L K₂CO₃) during the 96-hour study period (Figure 2.1). Moribund fish exhibited outward symptoms of toxicity including erratic swim behavior, loss of equilibrium, increased surface breathing, periods of rapid gilling, and sluggishness. Mortality was observed

typically within 12 hours after the initial onset of physical symptoms.

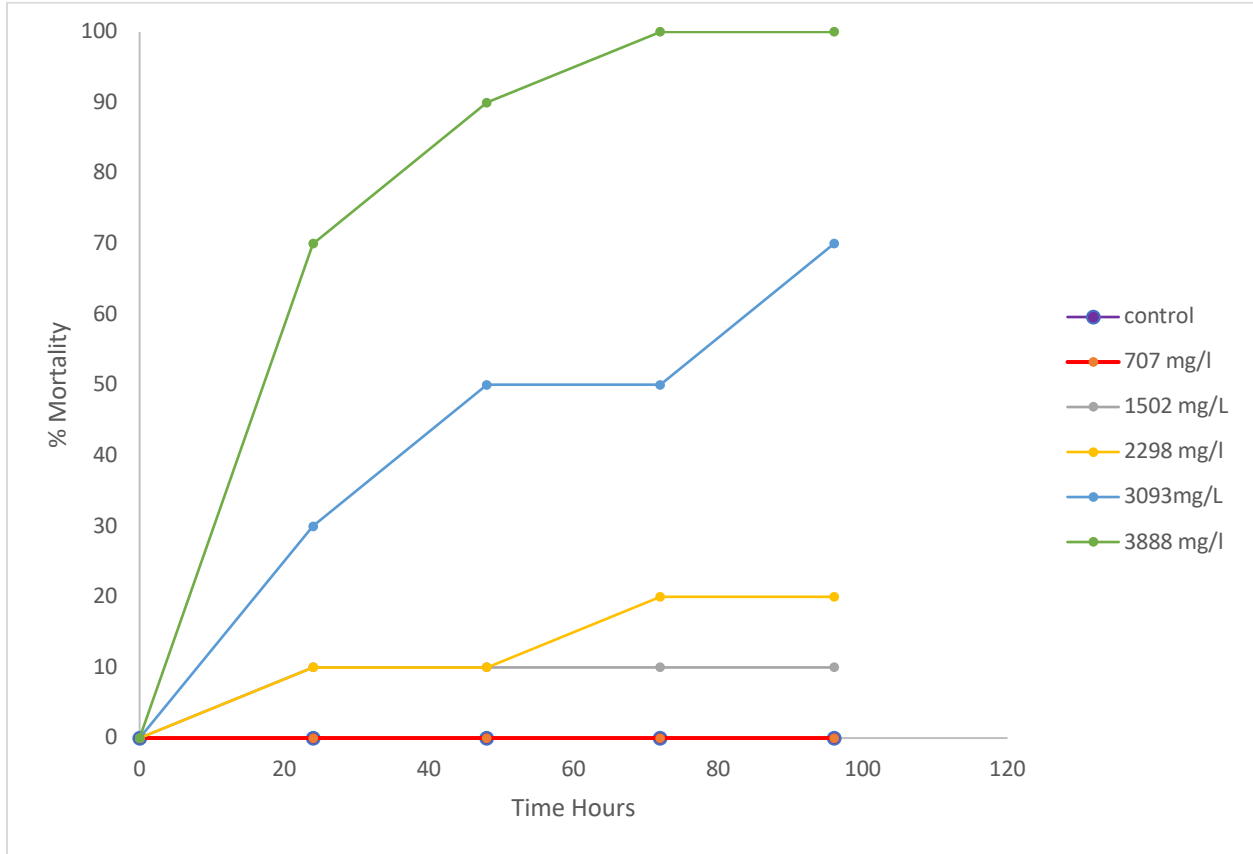


FIGURE 2.1 Percent mortalities at each K treatment (in mg/L) over the 96-hour study period. No mortality was observed in the control and lowest K_2CO_3 treatment (707 mg/L) during of the study period.

Median lethal concentrations (LC_{50}) and 95% confidence intervals of K_2CO_3 for rainbow trout at 24, 48, 72, and 96-hrs were determined to be 3470.1, 3016.3, 2830.6, and 2672.1 mg/L K_2CO_3 using the Probit model for regression (Table 2-3; Figure 2.2).

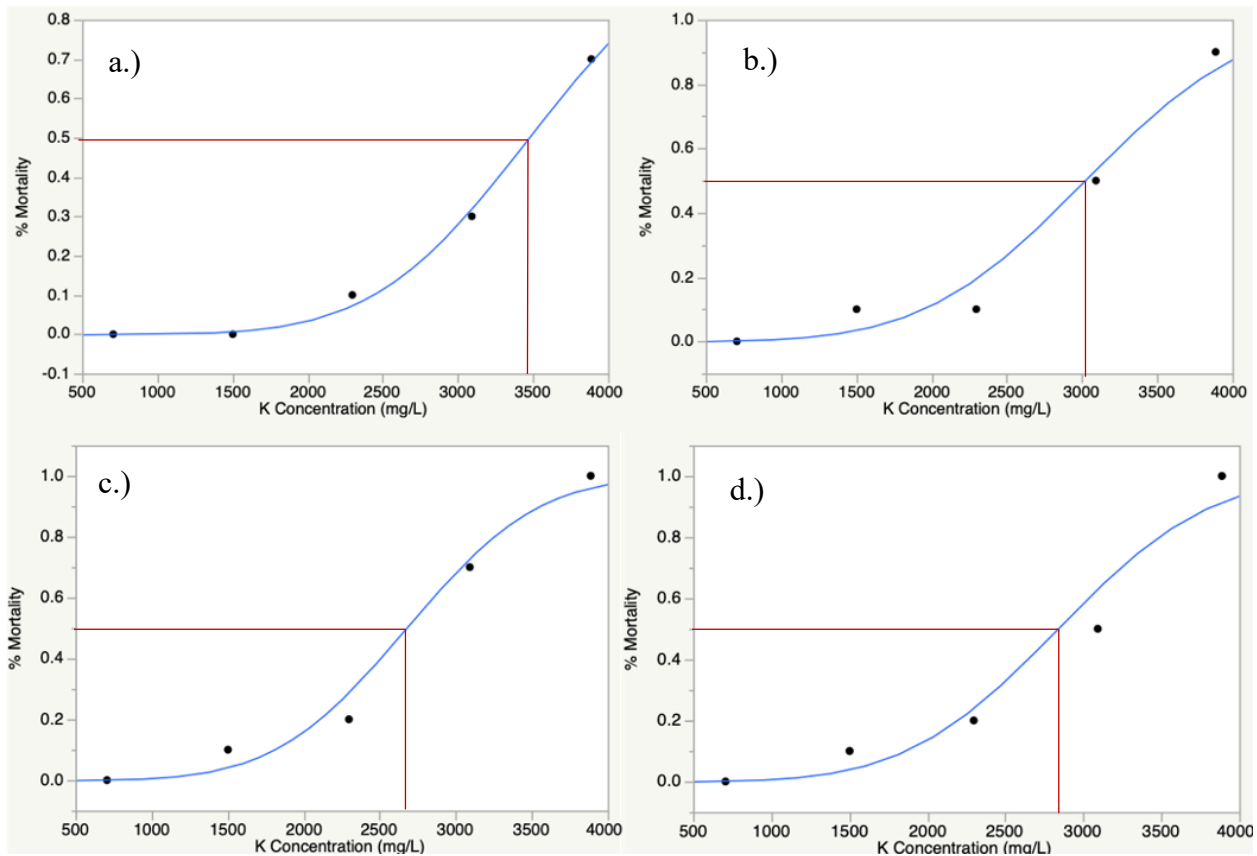


FIGURE 2.2 Probit regression curves fit to mortality across all K₂CO₃ treatments in rainbow trout (*Oncorhynchus mykiss*) at 24, 48, 72, and 96-hours (clockwise from the top left). Red lines are used to depict the position of median lethal concentration (mg/L K₂CO₃)

TABLE 2-3: Median lethal concentrations and 95% confidence intervals of K₂CO₃ at 24, 48, 72, and 96 hours

	Calculated LC₅₀ Values and 95% confidence intervals (mg/L K₂CO₃)
24-hrs	3470.1 (2062.3-4877.8)
48-hrs	3016.3 (1732.5-4300.0)
72-hrs	2830.6 (1657.7-4002.5)

96-hrs	2672.1 (1588.4.7-3755.9)
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4. Discussion:

This study demonstrated the acute toxic effects of potassium carbonate on rainbow trout over a 96-hour period with lethal concentrations ranging from 2672 - 3470 mg/L K_2CO_3 . The median lethal concentrations obtained in this assay are considerably higher than values previously reported in other studies (See Table 4). The discrepancy between the results of this research and previous studies likely is due to younger fishes being tested in these earlier assays. This study utilized fish that weighed approximately 30 g each while the other studies used fish ranging in size between 0.8-1.2 g/fish (Waller et al., 1993; Mount et al., 1997; EPA, 2002). Smaller, younger fish are far considered to be far more susceptible to most aquatic toxicants than adult fish (Weber, 1993).

Variation in toxic responses to potassium salts among fish species ranges widely (Table 2-4). Rainbow trout 96-hr LC_{50} concentrations for $KHCO_3$ and KCl (1200 and 1610 mg/L respectively) were double the LC_{50} concentrations for fathead minnows (*Pimephales promelas*) (510 mg/L and 880 mg/L) (Waller et al., 1993; Mount et al., 1997; PMRA, 2016). Bluegill (*Lepomis macrochirus*) exhibited greater tolerance to potassium in a 96-hour LC_{50} trial, reported as 2010 mg/L KCl (Trama, 1954). However, sensitivity to potassium-based salts can vary greatly when different salts are used (Wallen et al., 1957; Mount et al., 1997). Anionic constituents play an important role in determining the relative toxicity of potassium salts (Mount et al., 1997). Based on a review of the literature, potassium bicarbonate is more toxic than potassium sulfide and chloride, and potassium permanganate is more toxic than either salt (Trama, 1954; Mount et al., 1997; Kori-Siakpere, 2008).

TABLE 2-4. Median lethal concentrations for potassium-based salts in representative studies.

Species	K Salt	Concentration (mg/L)	Study Duration	Reference
Fathead minnow (<i>Pimephales promelas</i>)	KHCO ₃	510	96-hr	Mount et al., 1997
Fathead minnow (<i>Pimephales promelas</i>)	KCl	880	96-hr	Mount et al., 1997
Fathead minnow (<i>Pimephales promelas</i>)	K ₂ SO ₄	680	96-hr	Mount et al., 1997
Common bluegill (<i>Lepomis macrochirus</i>)	KCl	2010	96-hr	Trama, 1954
Rainbow trout (<i>Oncorhynchus mykiss</i>)	KHCO ₃	1200	96-hr	PMRA, 2016
Rainbow trout (<i>Oncorhynchus mykiss</i>)	KCl	1610	48-hr	Waller et al., 1993
Mosquitofish (<i>Gambusia affinis</i>)	KCl	920	96-hr	Wallen et al., 1957
Mosquitofish (<i>Gambusia affinis</i>)	KMnO ₄	12	96-hr	Wallen et al., 1957
African Catfish (<i>Clarias gariepinus</i>)	KMnO ₄	3.02	96-hr	Kori-Siakpere, 2008)

The presence of sodium, magnesium, and calcium have been shown to ameliorate toxic effects of potassium (Mount et al., 1997; Borvinskaya et al., 2016). Acute toxicity of potassium salts (KCl, K₂SO₄, and KHCO₃) in fathead minnows decreased in LC₅₀ studies when equal volumes of stock solutions of potassium and sodium salts were used (Mount et al., 1997). Furthermore, the growth performance of whitefish (*Coregonus lavaretus*) decreased considerably at a higher potassium: sodium ratio (5:1) than a balanced ratio (1:1) (Borvinskaya et al., 2016).

The potassium concentrations tested in this assay do not represent realistic rearing parameters in either RAS or aquaponic production. At optimal rearing conditions for rainbow trout grown in RAS, potassium concentrations in the culture water typically will not exceed 20-25 mg/L when the water exchange rate is at the minimum recommended 0.26% total flow rate (Davidson et al., 2009; Davidson et al., 2014). The concentrations seen in aquaponic systems are usually higher due to the lower exchange rates and the use of potassium-based buffering salts (Goddek et al., 2015). At the UNH Aquaponic Research Greenhouses at Kingman Farm, the maximum observed potassium concentrations observed typically average 250 mg/L K when culture system water is exchanged daily at approximately 1.5% of the total system volume and K_2CO_3 is used as the sole pH buffering mineral. Potassium concentrations of 171.6 mg/L significantly decreased the growth performance of whitefish over a 20-day study period without any notable decrease in feed consumption (Borvinskyaya et al., 2016). Furthermore, Davidson et al. (2011b) provided anecdotal evidence from Marc Laberge in Aquaponiques (Quebec, CA) noting gill irritation at concentrations as low as 110-130 mg/L K. However, the high concentrations of K_2CO_3 required to produce lethal effects in this assay may imply a need to further investigate whether these negative effects occur solely as a result of high potassium concentrations or whether there is an interactive effect between potassium and other ionic constituents and nutrients.

The sodium-potassium interaction is an important consideration as aquaculture systems integrate with hydroponic production systems. Potassium is essential for photosynthesis, glucose transportation, water uptake, synthesis of protein, carbohydrates, and starch, and enzyme activation (Cakmak, 2005; Thórarinsdóttir et al., 2015). Its limitation from hydroponic fertilizer solutions causes issues such as chlorosis, increased disease susceptibility, and reductions in fruit

development and yield (Cakmak, 2005; Roosta and Hamidpour, 2011; Thórarinsdóttir et al., 2015). Sodium concentrations are phytotoxic above 50 mg/L Na and can have detrimental implications on plant growth by limiting uptake of important nutrients including potassium (Delaide, et al., 2016). Although this deleterious effect can be accounted for by supplementing additional potassium into the culture water (e.g. potassium carbonate), the effects of increased potassium on fish health must be considered over a long-term production scenario (Tavakkoli et al., 2010; Davidson et al., 2011).

In conclusion, this study suggests that potassium carbonate (K_2CO_3) has a low degree of acute toxicity to rainbow trout, which is promising for the proposed use of potassium to replace sodium-based pH buffering salts. Further research is needed to assess the long-term effects of elevated potassium concentrations and the interactive effects of sodium and potassium on fish health. Establishment of safe operating parameters for potassium concentrations is important to the development of an economically sustainable model for the capture-and-reuse of aquaculture wastes in hydroponic cropping systems.

Chapter 3

Evaluating the interactive effects between potassium and sodium on rainbow trout (*Oncorhynchus mykiss*) mortality through preliminary mortality studies

1. Introduction:

Development of an effective waste nutrient capture-and-reuse model is essential to mitigating environmental impacts and costs associated with treatment and disposal of aquaculture effluents (EPA, 2004; Martins et al., 2010; Klinger and Naylor, 2012). Solid waste from recirculating aquaculture systems (RAS) can be directly utilized as a land-based fertilizer or composted to be made into a soil amendment (Guerdat et al., 2013; Timmons et al., 2018). However, aquaculture sludge comprises only 5-15% solid waste by mass, and is usually concentrated by expensive thickening processes before final disposal (Cripps and Bergheim, 2000; Summerfelt and Vinci, 2008; Sharrer et al., 2009; Guerdat et al., 2013). Capture-and-reuse of effluents from recirculating aquaculture systems (RAS) presents a unique challenge due to the high water content and low nutrient mass relative to other animal agricultural waste streams, which makes direct land application infeasible due to the hydraulic limitation of the soil (Salazar and Saldana, 2007; Tidwell, 2012; NRCS, 2013). Hydroponic cropping systems offer a more suitable application of RAS effluents than field application since they directly utilize these aqueous nutrients (Graber and Junge, 2009; Goddek et al., 2015). The wasted, aqueous nutrients from RAS can support hydroponic production in many different settings since most, if not all plant-essential macro-nutrients (plant-essential nutrients required in greater mass, proportionally) including nitrogen (N), phosphorus (P), and sulfur (S) are available at sufficient concentrations for crop production (Seawright et al., 1998; Adler et al., 2000b; Harmon, 2005; Rakocy et al., 2006; Rakocy, 2012; Turcios and Papenbrock, 2014; Anderson et al., 2017).

However, some macro-nutrients such as potassium (K) and calcium (Ca) and micro-nutrients (plant-essential nutrients required in lower mass quantities, proportionally) (e.g. iron (Fe), copper (Cu), zinc (Zn), molybdenum (Mo)) are not present at sufficient concentrations to support plant growth (Rakocy et al., 2006; Delaide et al., 2016; Timmons et al., 2018). Sodium (Na) concentrations in RAS effluents also typically exceed concentrations considered to be phytotoxic levels (50 mg/L) due to the use of sodium-based buffering salts such as sodium bicarbonate (NaHCO_3) and sodium hydroxide (NaOH), high concentrations present in commercial feeds, and the osmoregulatory activities of the fish (Seawright et al., 1998; Guerdat et al., 2013; Delaide et al., 2016; Anderson et al., 2017). Excessive sodium concentrations are disruptive to plant growth and competitively inhibit the uptake of essential nutrients such as K, Ca, and Fe, leading to issues such as chlorosis and reduced growth rates (Ball et al., 1987a; Resh, 1995; Seawright et al., 1998; Tavakkoli et al., 2010b, 2010a). In order to make RAS effluents suitable for hydroponic cropping production, one option for RAS producers to mitigate issues with excess sodium is to completely replace sodium pH-buffering salts with potassium derivatives such as potassium carbonate (K_2CO_3) or potassium bicarbonate (KHCO_3) (Seawright et al., 1998; Goddek et al., 2015; Thórarinsdóttir et al., 2015). However, there are known negative impacts on fish health resulting from elevated potassium concentrations, although recent research has shown that the toxicity of potassium may not be as significant as previously believed (Mount et al., 1997; Rakocy et al., 2006; Rakocy, 2012; Borvinskaya et al., 2016; Experiment #1, Chapter 2).

Excessive potassium concentrations are disruptive to fish physiology and affect osmoregulatory function, acid-base balance, muscle-contraction, and nerve function (Evans, 2008; Opoku-Okrah et al., 2015; Borvinskaya et al., 2016). Sublethal effects of excess potassium concentrations may include stunted growth rate, erratic swimming behavior, and gill irritation (Davidson et al., 2011b; Borvinskaya et al., 2016). Lethal effects have also been observed at higher

concentrations in a number of species including hybrid striped bass (female white bass *Morone chrysops* x male striped bass *M. saxatilis*), rainbow trout (*Oncorhynchus mykiss*), bluegill (*Lepomis macrochirus*) and fathead minnows (*Pimephales promelas*) (Trama, 1954; Mount et al., 1997; Rakocy et al., 2006; Davidson et al., 2011b; Rakocy, 2012; Borvinskaya et al., 2016).

Potential issues with fish health resulting from excessive potassium concentrations can be mitigated by instituting a partial replacement regime that utilizes both sodium and potassium pH-buffering salts in a balanced ratio. There may be a mitigating effect of sodium upon the toxic effects of potassium (Mount et al., 1997; Borvinskaya et al., 2016). The growth performance of whitefish (*Coregonus lavaretus*) was significantly lower when cultured at a K:Na molar ratio of 5:1 as opposed to a 1:1 K:Na molar ratio even with similar potassium concentrations (171.6 mg/L K and 117.6 mg/L K respectively) (Borvinskaya et al., 2016). The acute toxic effect of potassium salts (KHCO₃, K₂SO₄, and KCl) in fathead minnows was reduced in two-salt LC50 assays using sodium salts (NaHCO₃, Na₂SO₄, and NaCl) (Mount et al., 1997). Partial replacement can mitigate the costs associated with complete replacement of sodium-based pH-buffering salts with potassium based derivatives. However, in an integrated production scenario it may be difficult to justify maintaining sodium and potassium at an even balance for the sake of plant health due to the competitive effects of sodium on uptake of potassium, calcium, and iron (Resh, 1995; Tavakkoli et al., 2010a, 2010b).

Further research is needed to quantify an appropriate nutrient balance whereby fish and plant production are both optimized. In order to develop an economical model for integrated RAS and hydroponic cropping systems, operating parameters must first be optimized. This study was conducted in order as a follow up to the previous K₂CO₃ toxicity experiment (see Experiment #1, Chapter 2) to assess the mitigating effects of sodium on acute toxicity of potassium (K) on rainbow trout (*Oncorhynchus mykiss*) through an LC₅₀ trial. Demonstrating this mitigating effect can

provide a framework for conducting future research on long-term impacts of elevated potassium concentrations and the K:Na interaction on fish and plant production.

2. Methods/Materials:

This research was conducted at the University of New Hampshire Anadromous Fish and Invertebrate Research (AFAIR) lab at (Durham, NH, USA) using a system of individual tanks constructed as a rack system for LC50 assays. See Experiment #1, Chapter 2 for a full description of the rack LC50 evaluation tank system used in this study.

Preliminary range-finding studies were conducted prior to a complete LC50 assay to determine the ideal testing range of K and Na for optimal research results. The first round of studies tested five ratios of potassium and sodium with three replicates per treatment using fixed K and varying Na concentrations to achieve different molar ratios of the two ions in solution. The second round of studies evaluated a fixed K:Na molar ratio of 1:1 by varying the concentrations of K and Na.

2.1. Study Organism:

Rainbow trout (*Oncorhynchus mykiss*) were received at an average weight of 14.9 g/fish in September 2019 (Danaher Fishery, Shrewsbury, VT, USA). Upon arrival, they were stocked in a 3500-L quarantine RAS and reared for 6 weeks. The fish were fed a commercially available starter feed (Skretting Nutra Fry 1.8 mm; a 50% crude protein, 20% lipid, sinking feed). The quarantine RAS temperatures were maintained at a temperature range of 13-15 °C.

Water quality parameters in the quarantine system were monitored and assessed daily. Dissolved oxygen (mg/l), temperature (°C), and electrical conductivity (mS/cm) were monitored daily using a handheld meter (YSI Pro Optical Dissolved Oxygen meter, Xylem Inc., OH, USA).

Water quality parameters including pH, alkalinity, Nitrate-nitrogen ($\text{NO}_3\text{-N}$), nitrite-nitrogen ($\text{NO}_2\text{-N}$), and total ammoniacal nitrogen (TAN) were also measured regularly. The pH and alkalinity of the culture water were assessed daily with a bench-top pH meter (Accumet AB250, Fisher Scientific, NH, USA). Alkalinity (mg/L as CaCO_3) was measured by titration to pH endpoint (APHA, 2012, Method: 2320). All nitrogenous species ($\text{NO}_3\text{-N}$, $\text{NO}_2\text{-N}$, and TAN) were measured regularly using a HACH DR3900 spectrophotometer following US EPA approved HACH methods for water quality analysis (HACH Methods: $\text{NO}_3\text{-N}$; 8507 $\text{NO}_2\text{-N}$; 8038 TAN, HACH Company, CO, USA).

2.2. Mortality Studies Testing Different Potassium-Sodium Ratios

Two trials were conducted during the first round of preliminary studies. The potassium concentrations were held constant at 1750 mg/L and 2200 mg/L for each trial, respectively, while sodium concentrations were varied to achieve desired K: Na ratios. A kill control was used to establish baseline performance with K concentrations established at 1750 and 2200 mg/L K for the two assays to confirm the lethal effects of the increased potassium concentrations. For the purpose of this research, the ideal testing range was defined as total mortality in the highest ratio, low mortality in the lowest ratio, and varying degrees of mortality in all other doses over the 96-hour period (Mount et al., 1997; Hamlin, 2006).

Desired K:Na ratios were developed for each treatment by mixing stock solutions prepared from K_2CO_3 (10,000 mg/L K_2CO_3 or 5658.24 mg/L K) and NaCl (80,000 NaCl or 31,471 mg/l Na) dissolved in a dilution water solution comprised of a 50:50 mix of reverse osmosis (RO) and well water. Well water was first analyzed to provide a comprehensive nutrient profile prior to treatment solution mixing. Mixing of well water into the test solutions was included in the analysis to reduce additional interactive effects between the sodium and potassium present in the well water,

which could potentially affect the results (Mount et al., 1997; Borvinskaya et al., 2016). The K and Na concentrations present in the well water were 8 mg/L and 28 mg/L, respectively. The potassium and sodium stock solutions used in this study were mixed using food grade potassium carbonate (>99.0% assay) and sodium chloride (>99.0 % assay), respectively and RO water. HCl was used to buffer pH between 7.5-8.5. The control condition was prepared by diluting the well water (28 mg/L Na) with RO water at a 50:50 mix to achieve a sodium concentration of 14 mg/L. This control was distinct from the kill control as its purpose was to establish that the 50:50 well water and RO dilution solution would not result in lethal effects.

Using the same LC₅₀ evaluation system as described in Experiment #1, Chapter 2, each fish was housed in individual 5 L transparent high-density polyethylene (HDPE) containers filled with 4 L of treatment solution. A lid was placed over each container to prevent escape. The containers were placed on a 1.8 x 1.9 m² metal rack, with 12 containers per each of the five shelving units. Each shelving unit housed all 10 replicates of an entire treatment level plus two control tanks. Aeration was provided to individual tanks through forced air injection from an air pump using small airstone diffusers.

Feed was withheld from fish for 24 hours prior to starting the mortality assays to prevent accumulation of feces and nitrogenous waste products in the testing culture vessels (Environmental Protection Series, 1990). Fish were monitored regularly every 3-4 hours from 08:00-20:00 for mortality or change in physical condition. Changes in physical condition of the fish were recorded including loss of equilibrium, erratic swimming, surface breathing, and loss of color (Environmental Protection Series, 1990). Total mortality for each treatment was determined at the end of each 24-hour period. Water exchanges were conducted daily, replacing 50% of the volume in the containers to mitigate confounding effects in mortality due to excess accumulation of nitrogenous waste products such as ammonia and nitrite (Hamlin, 2006). At the conclusion of each

96-hour assay, all fish were humanely euthanized by cervical dislocation per the University of New Hampshire (UNH) International Animal Care and Use Committee (IACUC) Protocol #181205.

2.3. Mortality Studies Testing a Balanced Potassium-Sodium Molar Ratio

A second round of preliminary studies was conducted to test different potassium and sodium concentrations at a balanced (1:1) K: Na molar ratio. Desired K: Na concentrations were developed by mixing stock solutions prepared from K_2CO_3 (10,000 mg/L K_2CO_3 or 5658.24 mg/L K) and NaCl (80,000 NaCl or 31,471 mg/l Na) dissolved in a dilution solution comprised of a 50:50 mix of reverse osmosis (RO) and well water. The treatment concentrations used in this study were as follows: 400:234, 850:498, 1300:761, 1750:1025, and 2200:1288 mg/L K: mg/L Na. The same study design and data collection methods from the first round of preliminary studies were used for this portion of the study also.

2.4. Statistical Analysis

Percent mortalities for each treatment were compared for each study and were graphically presented using Microsoft Excel. The mortality data from the second round of preliminary studies (testing a 1:1 K: Na balance) was compared to the findings of Experiment #1, Chapter 2.

3. Results:

3.1. Mortality Studies Testing Different Potassium-Sodium Ratios

In the first study, mortality was observed in all treatments over 96-hours with 33, 100, 100, 100, 67, 100, and 33%, mortality for 3:1, 5:1, 10:1, 25:1, 40:1, 55:1, and 70:1 K: Na, respectively (Figure 3.1). The kill control did not demonstrate complete mortality within 96-hours. In the second study, the percent mortalities after 96-hours mortality was 100, 66.67, 0, 100, 100, and 100% for 0.5:1, 1:1, 5:1, 10:1; 25:1, and 40:1 K: Na respectively (Figure 3.2). Again, the kill control did not demonstrate complete mortality within 96-hours.

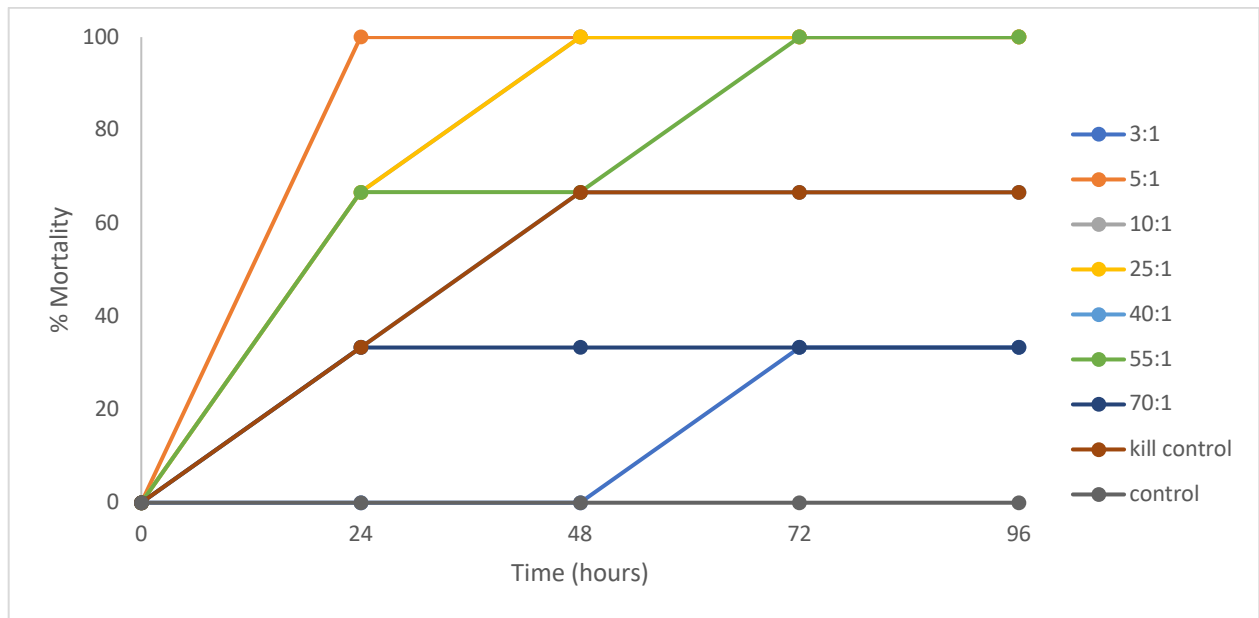


FIGURE 3.1 Percent mortality for Rainbow Trout for each K:Na treatment over a 96-hour period where K concentration remained constant at 2200 mg/L K

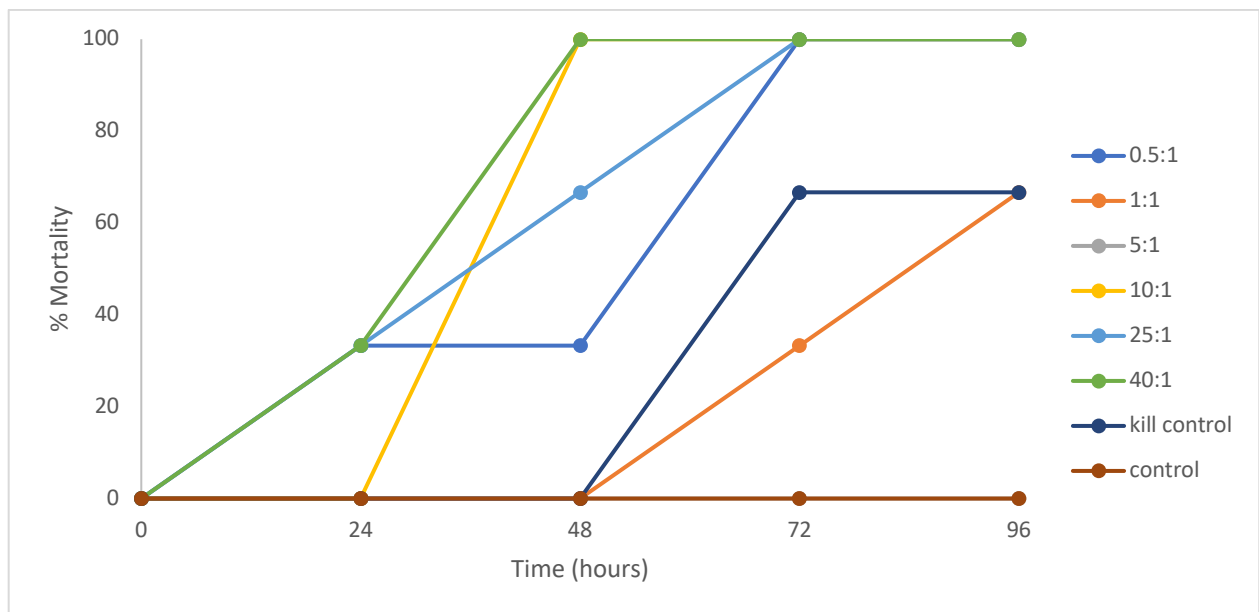


FIGURE 3.2 Percent mortality for Rainbow Trout for each K: Na treatment over a 96-hour period, where the K concentration remained constant at 1750 mg/L K

3.2. Mortality Studies Testing Balanced 1:1 Sodium and Potassium Ratios

In the study testing balanced 1:1 K:Na ratios, the percent mortalities were 0, 33, 67, 33, and 100% for 400:234, 850:498, 1300:761, 1750:1025, and 2200:1288 mg/L K: Na respectively (Figure 3.3). For comparison, the percent mortalities from Experiment #1, Chapter 2 were 0, 10, 20, 70, and 100%, respectively (Figure 3.3).

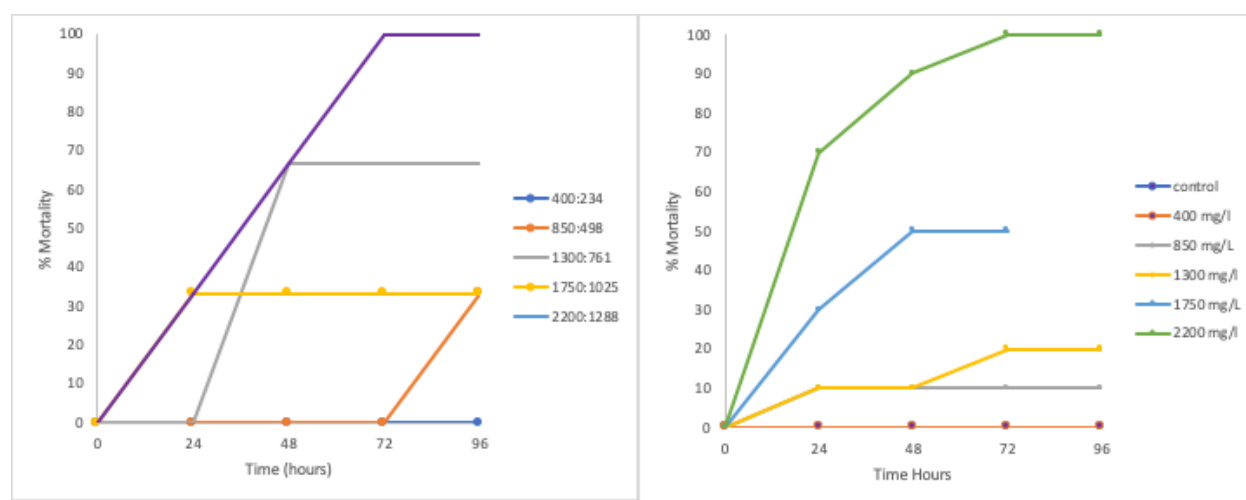


FIGURE 3.3 Rainbow trout mortality in ranging study conducted with 1:1 K:Na (left) compared to the LC₅₀ toxicity results of Experiment #1, Chapter 2 (right)

4. Discussion:

No clear interactive effects between sodium and potassium concentrations in rainbow trout were demonstrated in any of the three trials. There was pattern observed between K: Na ratios and mortality to demonstrate any mitigating effects of sodium. In the case of the constant K trials, for both 1750 and 2200 mg/L K, mortality increased as a function of the ionic content, potentially indicating ionic stress-induced toxicity. Additionally, the mortality in the 1:1 K:Na ratios increased as a function of K concentration similarly to Experiment #1, Chapter 2 indicating no effect of sodium mitigation on K toxicity.

The results from this study do not support the findings of Mount et al. (1997) who demonstrated

the mitigating effects of sodium salts (NaCl, NaHCO₃, Na₂SO₄) on the acute toxicity of potassium salts (KCl, KHCO₃, K₂SO₄) when their respective concentrations were maintained at balanced ratios. However, Mount et al. (1997) prepared the balanced two-salt treatment solutions by mixing equal volumes of the respective stock solutions rather than using molar equivalents, which was the method used in this study. Based on the results of this study and the lack of prior relevant published literature, it is apparent that the effect of the cation ratio of potassium and sodium salts on acute toxicity in fish is not yet fully understood.

Sublethal effects associated with the K:Na balance have been observed, however. The growth performance of whitefish (*Coregonus lavaretus*) decreased considerably at a higher potassium:sodium ratio (5:1) compared to a balanced ratio (1:1) even with similar potassium concentrations (171.6 and 117.6 mg/L K respectively) (Borvinskaya et al., 2016). There was no difference in feed consumption between the 1:1 and 5:1 K:Na treatments despite the significant difference in growth performance indicating a tertiary stress response to the ionic characterization of the culture water (Jobling, 1995; Borvinskaya et al., 2016). Since this grow-out study was conducted over a 21-day period, however, it only provides limited scope of the sublethal effects associated with increased potassium concentrations. There is limited research beyond Borvinskyaya et al. (2016) that explores the effect of the K:Na balance on fish growth performance. Future studies should assess the interactive effects between sodium and potassium on fish growth performance over a longer timeframe of 10-18 months, akin to commercial production (Fornshell, 2002; Cowx, 2005; Davidson et al., 2011b, 2014).

The interaction between sodium and potassium is an important consideration in integrating aquaculture with hydroponic production (Rakocy et al., 2006; Graber and Junge, 2009; Rakocy, 2012; Goddek et al., 2015). RAS effluents contain low concentrations of potassium and high concentrations of sodium (Seawright et al., 1998; Graber and Junge, 2009; Sharrer et al., 2009).

Potassium is involved in photosynthesis, enzyme function in glycolysis, water uptake, sugar production and transportation, and fruit ripening (Resh, 1995; Seawright et al., 1998; Cakmak, 2005; Somerville et al., 2014; Thórarinsdóttir et al., 2015). However, excessively high sodium concentrations (>50 mg/L Na) in RAS competitively inhibit the uptake of potassium and other important macro- and micro- nutrients resulting in issues such as chlorosis, lower growth rates, and fruit and flower development (Al-Hafedh et al., 2008; Graber and Junge, 2009; Somerville et al., 2014; Thórarinsdóttir et al., 2015). Therefore, more research is needed in order to develop an operating regime for sodium and potassium concentrations whereby RAS effluents can be effectively captured and reused as hydroponic fertilizer without negatively affecting fish and plant production systems.

5. Conclusions:

This research did not clearly demonstrate any interactive effects between sodium and potassium on acute toxicity in rainbow trout. However, the K:Na balance is an important consideration in capturing and reusing RAS effluents for hydroponic cropping. More research is needed in order to understand the sublethal effects of the K:Na molar ratio on fish health. Future research should evaluate the effects of various K:Na molar ratios on growth performance of rainbow trout.

Assessing the chronic effects of elevated potassium concentrations and various K:Na ratios is important in assessing potential fish health issues that may occur during long-term production. Reduced growth performance as a result of high potassium concentrations and K:Na ratios can indicate stress response in fish due to adverse rearing conditions. Determining the effect of K:Na ratio is important in order to optimize fish and plant production systems in coupled operations.

Chapter 4

Determining the effects of elevated potassium carbonate (K₂CO₃) concentrations on the growth performance of rainbow trout (*Oncorhynchus mykiss*) grown in recirculating aquaculture systems (RAS)

1. Introduction:

Evaluating the chronic toxicity of elevated potassium concentrations on fish health is an important consideration when developing a capture-and-reuse model for RAS effluents to be used as fertilizer in hydroponic cropping systems. The captured waste nutrients from RAS effluents have been reused as fertilizer for hydroponic production across many different settings (Adler et al., 2000b; Harmon, 2005; Rakocy et al., 2006; Rakocy, 2012; Turcios and Papenbrock, 2014). RAS culture water provides several plant-essential macro-nutrients (required at higher rates) and micro-nutrients (required at lower rates) at sufficient concentrations to support plant growth (Seawright et al., 1998; Rakocy et al., 2006; Guerdat et al., 2013; Delaide et al., 2016).

Several macro-nutrients including nitrogen (N), phosphorus (P), and sulfur (S) are present at sufficient concentrations to support plant growth (Seawright et al., 1998; Adler et al., 2000b; Harmon, 2005; Rakocy et al., 2006; Rakocy, 2012; Turcios and Papenbrock, 2014; Anderson et al., 2017). However, other important macro-nutrients (potassium (K) and calcium (Ca)) and multiple micro-nutrients (e.g. zinc (Zn), molybdenum (Mo), copper (Cu), iron (Fe)) are not present at sufficient concentrations to maintain plant growth (Seawright et al., 1998; Rakocy et al., 2006; Anderson, 2016; Delaide et al., 2016). Furthermore, sodium (Na) concentrations in RAS effluent often exceed the phytotoxic threshold (>50 mg/L Na) due to in use of sodium bicarbonate (NaHCO₃) and sodium hydroxide (NaOH) as buffering salts, osmoregulatory activity of fish, and the high concentrations found in commercial feed

(Seawright et al., 1998; Guerdat et al., 2013; Delaide et al., 2016; Anderson et al., 2017). Sodium concentrations in rainbow trout RAS vary based on water exchange rates, nutrient characterization of make-up water, and feed rates and can range from 4 mg/L to >700 mg/L Na (Davidson et al., 2009, 2011a, 2011b).

Excessive sodium concentrations are disruptive to plant growth and competitively inhibit the uptake of essential nutrients such as K, Ca, and Fe, leading to issues such as chlorosis (Ball et al., 1987b; Resh, 1995; Tavakkoli et al., 2010a, 2010b). In order to reuse RAS effluents as hydroponic fertilizer, issues with excess sodium concentrations need to be resolved. One approach taken by RAS operators to mitigate issues with excess sodium concentrations in RAS effluents and make it more suitable for use as fertilizer by plant growers is to replace sodium pH-buffering salts with potassium derivatives such as potassium carbonate (K_2CO_3) or potassium bicarbonate ($KHCO_3$) (Seawright et al., 1998; Goddek et al., 2015; Thórarinsdóttir et al., 2015). However, negative impacts on fish health resulting from elevated potassium concentrations have been documented in other studies (Trama, 1954; Mount et al., 1997; Davidson et al., 2011b; Borvinskaya et al., 2016).

Excessive potassium concentrations can negatively affect fish health by disrupting the electrochemical gradient of K^+ in the environment resulting in disrupted osmoregulation, acid-base balance, muscle-contraction, and nerve function in fishes (Evans, 2008; Opoku-Okrah et al., 2015; Borvinskaya et al., 2016; see Experiment #1, Chapter 2). The growth performance of whitefish (*Coregonus lavaretus*) was significantly reduced in the presence of excessively high potassium concentrations and low sodium concentrations over a 21-day period (Borvinskaya et al., 2016). Sublethal effects in rainbow trout (*Oncorhynchus mykiss*) associated with increased potassium concentrations include increased erratic swimming behavior (side-swimming and

upside-down swimming) and gill irritation (Davidson et al., 2011b). However, Davidson et al. (2011b) was not able to definitively isolate potassium as the sole cause of these sublethal effects due to excessive concentrations of other nutrients present in the culture water. Elevated concentrations of several different potassium-based salts (e.g. K_2CO_3 , $KHCO_3$, KCl , and K_2SO_4) have caused lethal effects in a number of species including hybrid striped bass (female white bass *Morone chrysops* x male striped bass *M. saxatilis*), rainbow trout (*Oncorhynchus mykiss*), bluegill (*Lepomis macrochirus*) and fathead minnows (*Pimephales promelas*) (Trama, 1954; Mount et al., 1997; Rakocy et al., 2006; Davidson et al., 2011b; Borvinskaya et al., 2016; see Experiment #1, Chapter 2). In order to understand the effects of altering the RAS water quality conditions to suit the nutrient profile requirements of hydroponic cropping systems as a means for monetizing the RAS effluent treatment process, more research should be conducted to assess the chronic, long term effects of elevated potassium concentrations on different economically important aquaculture species.

The objective of this research was to assess the chronic effects of increased potassium (K) concentrations on the growth performance of rainbow trout (*Oncorhynchus mykiss*) in RAS by using potassium carbonate (K_2CO_3) as the sole pH buffering salt in replacement for the typical use of sodium bicarbonate ($NaHCO_3$). A reduction in growth performance could indicate a tertiary stress response since fish rapidly deplete energy reserves to adapt to chronic exposure to toxicants (Jobling, 1995). The results of this study could further support the findings of Borvinskyaya et al. (2016) by demonstrating sublethal effects of elevated potassium concentrations over a longer timeframe (>60 days). Assessing the chronic toxicity of potassium can provide a more detailed account of its long-term impacts on fish production in RAS and provide framework to balance potassium concentrations in culture water to suit both plant and fish needs.

2. Methods

This research was conducted at the University of New Hampshire Anadromous Fish and Invertebrate Research (AFAIR) lab (Durham, NH). The AFAIR lab was a recirculating aquaculture research facility which housed three pilot-scale replicated RAS (4.65 m³ each) and two separate quarantine RAS. The replicated RAS were comprised of two 1300 L circular tanks (1.8 m diameter), a standpipe well, pumping sump, water circulation pump, moving bed bioreactor (MBBR) using 0.7 m³ Kaldness K1 biocarrier media, and a rotary drum screen filter fitted with 40 µm screens (Hydrotech 501, Vellinge, Sweden). Water temperatures were maintained between 14-16°C using a water chiller with drop-in coil (CY-6 Cyclone chiller, Aqua Logic, Inc., CA, USA). Dissolved oxygen (DO) was maintained using forced air injection from a regenerative air blower (Sweetwater, S45, Pentair AES, FL, USA). Animal feed-grade sodium bicarbonate (NaHCO₃) (Agway, Dover, NH) was used as the pH buffering salt in the baseline, control grow-out studies and food-grade potassium carbonate (K₂CO₃) (99.9% assay; Morewine, CA) was used in the potassium treatment study.

2.1. Original Sodium Baseline Growth Study

Rainbow trout (*Oncorhynchus mykiss*) were acquired from Danaher Fishery (Shrewsbury, VT, USA) in October 2017 at 35.2 g/fish. Upon arrival, they were stocked in a 3500-L quarantine RAS system and reared for 8 weeks before transfer into the triplicate RAS at an average weight of 107 g/fish (91 fish/tank). The trout were then grown under intensive production conditions (stocking densities of approximately 40-60 kg/m³) until July 2018, when they were harvested at 671.3 g/fish (70-72 fish/tank). These growth data were collected by Alexander Sitek for his master's thesis research and used as the control for this current study. All rainbow trout used in the research conducted at UNH were supplied by the same source.

2.2. Potassium Treatment Growth Study

Rainbow trout were acquired from Danaher Fishery (Shrewsbury, VT, USA) in May 2019 at an average weight of 3.12 g/fish. Upon arrival, they were stocked in a 3500-L quarantine RAS system and reared until October 2019, when they were transferred into the triplicate RAS at 236.2 g/fish (83-84 fish/tank). The trout were then grown under intensive production conditions until January 2020, when they were harvested at 681.7 g/fish (77-84 fish/tank). Potassium carbonate was used as the sole pH buffering salt in the triplicate RAS, and dosing was contingent upon water quality analyses (see section 2.4).

2.3. Replicated Sodium Baseline

In order to obtain a more reliable baseline for comparison, another grow-out study was conducted in Spring 2020. Rainbow trout acquired from Danaher Fishery (Shrewsbury, VT, USA) in September 2019 at 14.9 g/fish were transferred from the quarantine to the triplicate RAS in January 2020 at an average weight of 245.5 g/fish (84 fish/tank). The trout were grown under intensive production conditions; however, the study was prematurely ended in March 2020 due to growing concerns from COVID-19. The final harvest weight of this cohort was 385.7 g/fish (78-83 fish/tank).

2.4. Water Quality

Dissolved oxygen (mg/L) and temperature (°C) were monitored daily using a YSI Pro Optical Dissolved Oxygen meter (Xylem Inc., OH, USA). Water usage (L) was measured daily using an inline water meter (Flexible Axis Water Meter, Master Meter North American, Mansfield, TX). Additional water quality parameters monitored included pH, alkalinity (mg/L as CaCO₃), nitrate-nitrogen (NO₃-N), nitrite-nitrogen (NO₂-N), and total ammoniacal nitrogen (TAN). The pH and alkalinity were assessed daily with a bench-top pH meter (Accumet AB250, Fisher Scientific,

NH, USA). Alkalinity was measured by titrating to pH endpoint of 4.8 (APHA, 2012). Concentrations (mg/L) of all nitrogenous species (NO₃-N, NO₂-N and TAN) were measured regularly (four times per week) using a HACH DR3900 spectrophotometer (HACH Methods: 8039, 8507, and 8038 respectively; HACH Company, CO, USA). Water samples were sent to JR Peters Laboratory (Allentown, PA) biweekly for additional characterization of dissolved nutrients (including: K, Ca, Mg, Na, P, S, Cl, NH₄-N, NO₃-N, urea, Fe, Mn, B, Cu, Zn, Mo, Al) and to determine changes in sodium and potassium concentrations over time.

2.5. Feed

Fish were fed a standard commercial diet (Bio-Oregon, Bio Trout, ME, USA) in two different pellet sizes (4.0- and 6.0-mm) based on fish growth and development. The 4.0-mm (45% crude protein; 24% crude fat) contained a higher protein content than the 6.0-mm feed (43% crude protein; 24% crude fat) pellet. Fish were transitioned from the 4.0-mm to the 6.0-mm pellets once they reached an average weight of 400 g/fish. Feed rates were fed on a % total biomass basis as recommended by feed manufacturer guidelines. However, they were regularly adjusted based on observations of growth performance and accumulation of uneaten feed in the standpipe wells (Davidson, et al. 2014). Fish were fed hourly over a 24-hour period using automatic vibratory feeders (Pentair Vibratory Feeders, Pentair Aquatic Eco-Systems, Apopka, FL, USA). Feed samples were sent to JR Peters (Allentown, PA) for nutrient analysis. Constant overhead lighting was maintained for the study period.

2.6. Sampling Protocol:

Fish weights were sampled once per week throughout the course of this study. Random samples comprised of a minimum of 10% of tank inventory were weighed (kg) to obtain representative samples of growth. Average weight per fish (g/fish) and system biomass (kg) were

calculated. Feed conversion ratios (FCR; Equation 1) and specific growth rate (SGR; Equation 2) were calculated at the end of the study to determine weight gain in biomass per feed added (Davidson et al., 2014; Borvinskaya et al., 2016; Muchlisin et al., 2017). The growth performance data from this study was compared to growth data obtained from the baseline growth performance study performed in Spring 2018 and an additional baseline study performed in Spring 2020.

(Equation 1)

$$FCR = \frac{Feed\ fed\ (g)}{Weight\ gain\ (g)}$$

Here, FCR is reported as total mass feed fed per total mass of fish weight gain (g feed / g weight gain). Feed fed is the total amount of feed administered for the analysis period (g), and weight gain is the total fish biomass accumulated for the analysis period (g).

(Equation 2)

$$SGR = 100 \times (LN(average\ end\ weight\ (g)) - LN(average\ start\ weight\ (g))) \times \frac{1}{day}$$

Here, SGR is reported as a percent change in average weight per day from the beginning of the study to its conclusion.

2.7. Statistical Analysis:

All statistical analyses were conducted using JMP® Pro 14.0.0 (SAS Institute Inc., Cary, NC). A One-way ANOVA followed by a Tukey HSD test ($\alpha = 0.05$) was used to compare means of water quality parameters between the spring 2018 baseline, spring 2020 baseline, and potassium treatment and individual replicates. Mean FCR and SGR were compared between the potassium treatment and spring 2018 baseline using a T-Test assuming unequal variances ($\alpha = 0.05$). Due

to the lack of sufficient data, mean FCR and SGR were not compared from the spring 2020 baseline.

3. Results:

3.1. Water Quality

Average DO (mg/L), pH, alkalinity (mg/L as CaCO₃), NH₃-N (mg/L), NO₂-N (mg/L), and water exchange rate (%) were significantly different between the 2018 control and the potassium treatment (Table 4-1). Between the 2020 control and potassium treatment, only average DO (mg/L) and temperature (°C) were significantly different (Table 4-1). Potassium concentrations in the treatment culture water fluctuated throughout the course of the study. While potassium concentrations increased throughout the course of the study, there was a sharp drop from day 43 to day 57, before a sharp increase by day 59 (Figure 4.1).

TABLE 4-1 Mean water quality parameters in the potassium-treatment and 2018 and 2020 controls. Significant differences in means determined using Tukey HSD test ($\alpha = 0.05$). Means with the same letters are not significantly different.

Treatment	Control (2018)	Control (2020)	Potassium
Average DO (mg/L)	7.8 ± 0.05 (A)	8.6 ± 0.07 (B)	8.04 ± 0.06 (C)
Average Temp (°C)	15.64 ± 0.08 (A)	15.31 ± 0.11 (B)	15.7 ± 0.09 (A)
pH	7.75 ± 0.02 (A)	7.34 ± 0.03 (B)	7.41 ± 0.03 (B)
Alkalinity (mg/L as CaCO ₃)	75.59 ± 1.24 (B)	126.37 ± 1.67 (A)	127.50 ± 1.40 (A)
NH ₃ -N (mg/L)	0.31 ± 0.01 (A)	0.28 ± 0.01 (AB)	0.25 ± 0.01 (B)
NO ₂ -N (mg/L)	0.048 ± 0.003 (A)	0.019 ± 0.004 (B)	0.029 ± 0.003 (B)

Average Daily Water Usage (L)	1501.20 ± 91.48 ^(A)	719.15 ± 122.90 ^(B)	433.34 ± 103.33 ^(B)
Average Water Exchange Rate (%)	0.54 ± 0.04 ^(A)	0.26 ± 0.05 ^(B)	0.31 ± 0.042 ^(B)

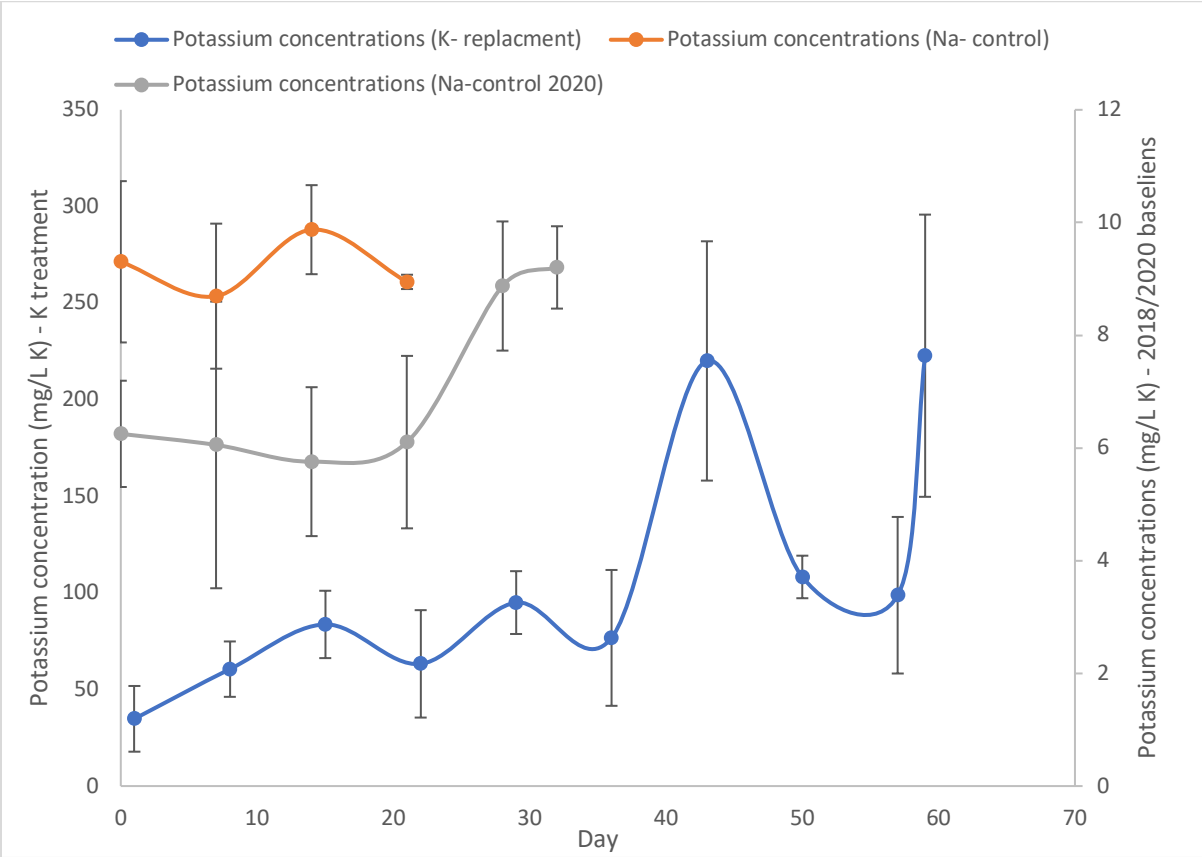


FIGURE 4.1 Mean culture water potassium (K) concentrations (mg/L) (\pm standard deviation) for the length of each study period.

3.2. Growth Performance

The SGR was significantly higher ($p=0.0093$) in the potassium treatment (1.32 ± 0.02 %/day) than in the control (0.87 ± 0.06 %/day) (Table 4-2, Figure 4.2). The FCR was significantly higher ($p=0.013$) in the control (2.96 ± 0.22) than in the potassium treatment (1.18 ± 0.04) (Table 4-2, Figure 4.3).

TABLE 4-2 Comparison between the average (mean \pm se) specific growth rate (SGR) and feed conversion ratio (FCR) between the potassium replacement treatment and control RAS. p-values >0.05 denote significant differences

	Control	Potassium	P-values ($p < 0.05$)
Specific growth rate (SGR) (%/day)	0.87 \pm 0.06	1.32 \pm 0.02	0.0093
Feed conversion ratio (FCR)	2.96 \pm 0.22	1.18 \pm 0.04	0.013

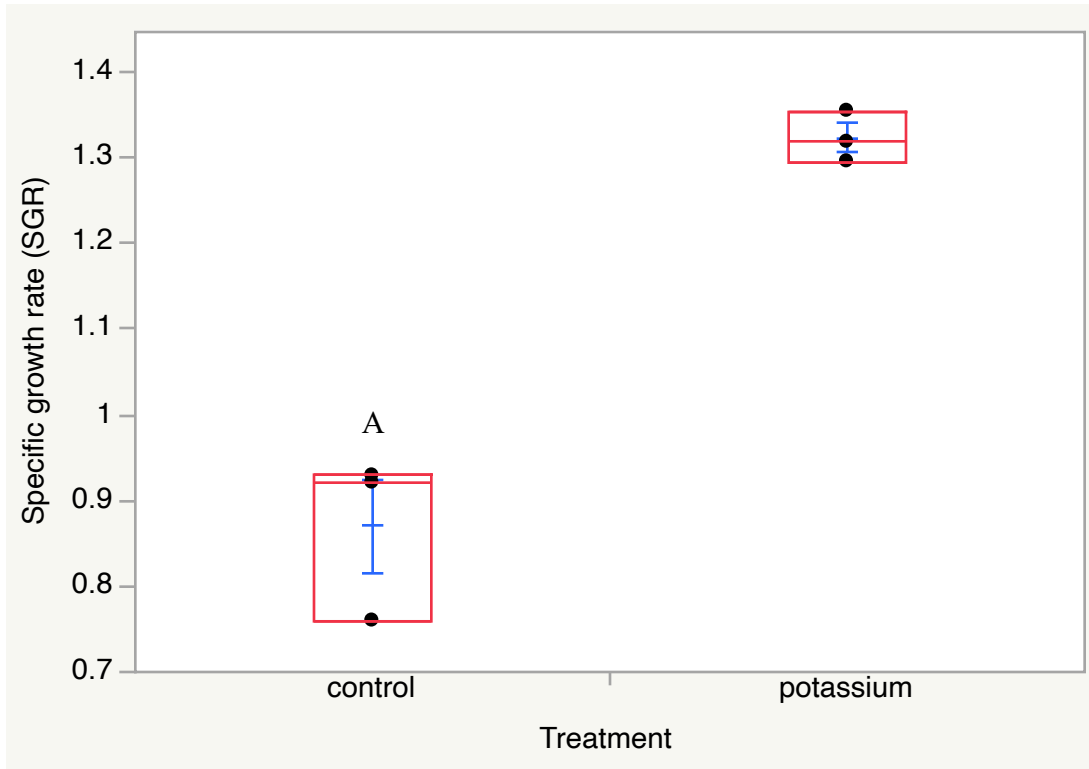


FIGURE 4.2 Differences in specific growth rate (SGR) of rainbow trout between the potassium treatment and 2018 baseline RAS. Means (\pm standard deviation) are depicted by the blue lines. individual data points are depicted by the red brackets.

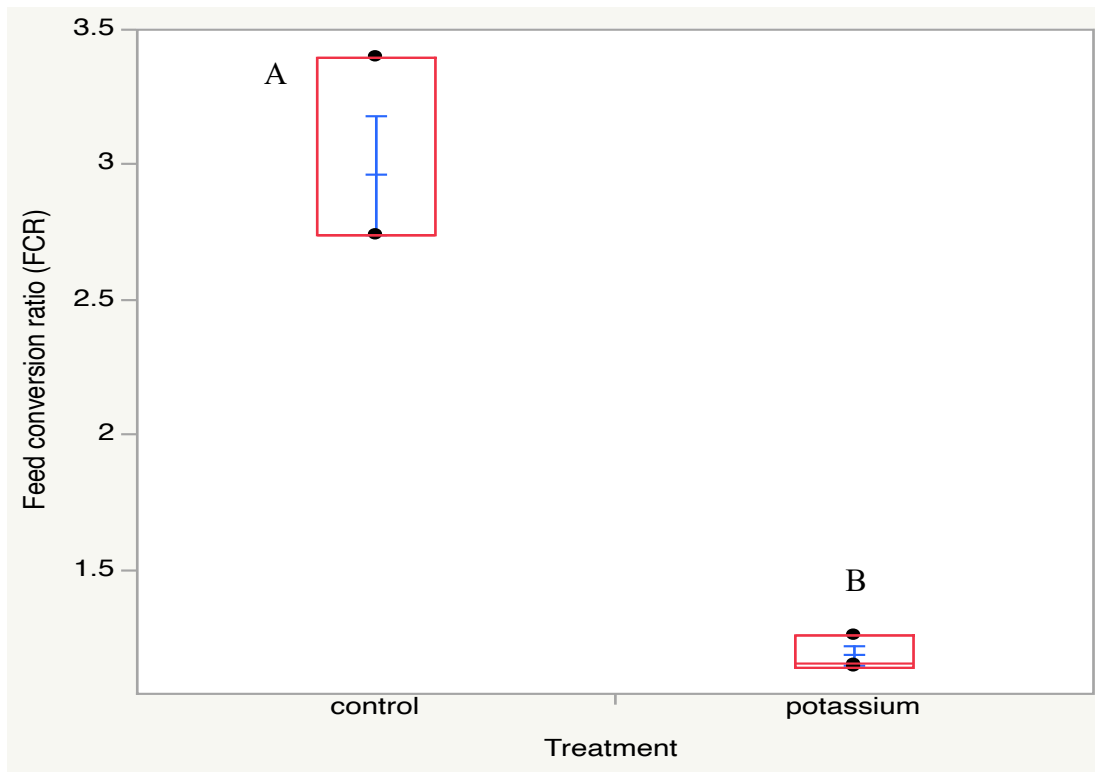


FIGURE 4.3 Differences in feed conversion ratio (FCR) of rainbow trout between the potassium treatment and 2018 baseline RAS. Means (\pm standard deviation) are depicted by the blue lines. Individual data points are depicted by the red brackets.

4. Discussion:

Increased growth performance was observed in the potassium treatment relative to the control. Specific growth rate (SGR) was significantly higher in the potassium treatment than in the control. Feed conversion ratio (FCR) was significantly lower in the potassium treatment than in the control. These results indicate higher growth rates and more efficient feed utilization in the potassium treatment compared to the control. The findings of this study directly contradict the findings of Borvinskyaya et al. (2016) who demonstrated a significant reduction in growth performance in whitefish cultured in the presence of higher potassium relative to sodium concentrations despite no apparent decrease in feeding activity.

Findings from previous research have identified sublethal impacts including gill irritation and reduced growth performance occurring at potassium concentrations ranging from 100-171.6

mg/L K (Davidson et al., 2011b; Borvinskaya et al., 2016). Nutrient analysis of the culture water at the UNH Aquaponics Research Greenhouses at Kingman Farm demonstrate potassium concentrations typically range between 200 and 250 mg/L K when potassium carbonate is supplemented as the sole pH buffering salt with daily total system volume exchange rates of approximately 1.5% (*Unpublished, operational data*). Lethal effects of elevated potassium concentrations have been documented in a number of species including hybrid striped bass (female white bass x male striped bass), rainbow trout, bluegill, and fathead minnows (Trama, 1954; Mount et al., 1997; Rakocy et al., 2006; Davidson et al., 2011b; Borvinskaya et al., 2016). Chronic exposure to toxicants could potentially reduce survivability of fish (Davidson et al., 2011b, 2014). Therefore, the long-term impacts of these higher concentrations on fish health should be reevaluated before integrating aquaculture with hydroponic production.

This study did not utilize the same cohort of fish grown under different water quality conditions and instead, used separate cohorts of fish for each of the grow-out studies. As such, there were significant differences among several water quality parameters including DO (mg/L O₂), pH, alkalinity (mg/L CaCO₃), NH₃-N (mg/L), NO₂-N (mg/L), and average daily water usage (L). Other deviations in operation included frequency of fish weight and culture water nutrient characterization sampling periods. While weight was sampled weekly and culture water nutrient characterized biweekly in the potassium treatment study and the 2020 control, samples from the 2018 control were taken less frequently. The use of two different cohorts of rainbow trout added additional variability since there were differences in fish age and life stage of fish. Younger life stages of fish are considered to be far more susceptible to most aquatic toxicants than adults (Weber, 1993; Davidson et al., 2014). Although potassium concentrations increased in the treatment culture water due to the use of potassium carbonate as the buffering salt, there were large

fluctuations in concentrations throughout the study. These fluctuations were potentially due to the timing of sampling relative to potassium carbonate addition and the location from which samples were collected. Samples were collected in the pumping sump as the source was assumed to represent the system nutrient concentrations. However, water is automatically replenished in the pumping sump when the water levels drop too low. As such, the replenishment of fresh well water may have been occurring without notice during sampling, potentially diluting the samples inadvertently. The use of peristaltic pumps should be considered in future studies to maintain target potassium concentrations in the culture water (Davidson et al., 2014). Other factors such as blood chemistry, liver MDA (malondialdehyde) concentration, muscle and liver GSH (glutathione) concentrations, histopathology, and swim behavior should be evaluated in addition to growth performance in order to better understand the stress response of rainbow trout to elevated potassium concentrations better (Davidson et al., 2014; Borvinskaya et al., 2016).

5. Conclusions:

Evaluating the long-term impacts of elevated potassium concentrations on fish health is important to developing an economical capture-and-reuse model for aquaculture effluents. Potassium supports photosynthesis, glucose transportation, water uptake, synthesis of protein, carbohydrates, and starch, and enzyme activation in plants (Cakmak, 2005; Thórarinsdóttir et al., 2015). Its limitation from hydroponic fertilizer solutions causes issues such as chlorosis, increased disease susceptibility, and reductions in fruit development and yield (Cakmak, 2005; Roosta and Hamidpour, 2011; Thórarinsdóttir et al., 2015). The results of this study demonstrated improved growth performance in rainbow trout cultured in the potassium treatment compared to the control. However, due to discrepancies in the study design and other contradictory studies, more research is needed in order to establish the chronic effects of elevated potassium concentrations (Davidson et al., 2011b; Borvinskaya et al., 2016).

Chapter 5

Grand Conclusion:

The goals of this study were to determine the acute and chronic toxicity of potassium carbonate (K_2CO_3) and the interactive effects between sodium and potassium on rainbow trout (*Oncorhynchus mykiss*) grown in recirculating aquaculture systems (RAS). The acute toxicity of potassium carbonate was determined, however, interactive effects between sodium and potassium on percent mortality were not determined in mortality studies using two salts (K_2CO_3 and NaCl). Furthermore, the growth rates of rainbow trout were significantly higher in the presence of elevated potassium carbonate concentrations when compared to the sodium control. Based on the results of this study, elevated potassium concentrations are not as toxic as originally hypothesized and may not detrimentally affect fish health if supplied as potassium carbonate for the purpose of pH buffering by RAS operators. However, there are a number of shortcomings and weaknesses within the study design that likely affected the results.

The lethal concentrations of K_2CO_3 obtained in this research were higher than has been found for potassium salts (KCl, K_2SO_4 , $KHCO_3$) for rainbow trout, fathead minnow, and common bluegill in previous studies (Trama, 1954; Waller et al., 1993; Mount et al., 1997; PMRA, 2016). However, it is important to note that the fish in this study were older than the fish used in previous studies (Waller et al., 1993; Mount et al., 1997; EPA, 2002). Generally, younger fish in juvenile and fry stages are considered far more susceptible to most aquatic toxicants than adult fish (Weber, 1993). However, Hamlin (2006) demonstrated the opposite effect with respect to nitrate toxicity in Siberian sturgeon. Future studies should assess the toxicity of potassium carbonate over multiple life stages in order to understand the potential impacts on fish health through a typical RAS production cycle. Although the median lethal concentrations attained in this study are considerably

higher than those from previous studies evaluating toxicity of potassium salts (KCl and KHCO₃) on rainbow trout, it is currently unknown whether this trend of increased tolerance continues into more mature life stages.

No interactive effects were demonstrated between sodium and potassium on acute toxicity in mortality trials involving two salts (K₂CO₃ and NaCl). There was no consistent relationship between K:Na ratio and percent mortality and no notable changes in percent mortality when results of 1:1 K:Na study were compared to the results of Exp 1 (see Experiment #1, Chapter 2). The mitigating effects of sodium on the toxicities of potassium salts have been documented in other studies (Mount et al., 1997; Borvinskaya et al., 2016). Future studies should be conducted to further assess the interactive effects of the K:Na molar ratio by testing a greater variety of ratios over a longer time frame than was tested in this study to understand the effects of sustained exposure to high potassium concentrations in culture water.

Growth performance was significantly higher in the potassium treatment than in the baseline study conducted in 2018. However, there were significant differences in water quality parameters (NO₂-N, NH₃-N, pH, alkalinity, water usage, etc.), feed rates, and frequency of nutrient constituent characterization and fish weight sampling frequency between the potassium treatment and 2018 baseline, which likely affected the validity of the results. Potassium concentrations fluctuated throughout the course of the potassium treatment study. Additionally, different cohorts of fish were used for each individual study, which may account for discrepancies in the respective growth rates due to the different ages of the fish (Davidson et al., 2014). Future studies should use same cohort of fish run under different conditions, test a range of potassium concentrations (with peristaltic pumps to maintain consistent concentrations throughout the study), and look at other factors of chronic stress such as blood chemistry, liver MDA (malondialdehyde) concentration,

muscle and liver GSH (glutathione) concentrations, histopathology, and swim behavior in order to further understand the stress response (Davidson et al., 2011b, 2014; Borvinskaya et al., 2016).

This research demonstrated the acute toxicity of K_2CO_3 on rainbow trout, however it did not demonstrate sublethal effects of elevated potassium concentrations or interactive effects between sodium and potassium due to limitations in the study design. Therefore, more research is needed in order to readdress these questions before guidelines for culture water potassium and sodium concentrations can be established for RAS operators looking to integrate with hydroponic cropping systems. Balancing potassium and sodium concentrations in the RAS culture water is important for optimizing plant and fish production in integrated aquaculture and hydroponic cropping systems (Seawright et al., 1998; Cakmak, 2005; Goddek et al., 2015; Thórarinsdóttir et al., 2015; Delaide et al., 2016; Anderson et al., 2017). The broader implications of this research are that by identifying limited conditions for rainbow trout RAS, the reuse of the aquaculture effluent stream is enabled as a naturally derived nutrient source for crop fertilization. Integration of rainbow trout RAS with the hydroponic industry ultimately provides an economic benefit to RAS operators as it enables the monetization of the wastewater treatment process.

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