ORNAMENTAL FISH AS COMPLEMENTARY SPECIES IN AQUAPONICS

WASIIM MOHAMMAD KADER BATHIA

ORNAMENTAL FISH AS COMPLEMENTARY SPECIES IN AQUAPONICS

by

© Wasiim Mohammad Kader Bathia

A thesis submitted to the School of Graduate Studies in partial fulfilment of the requirements for the degree of Master of Science in Aquaculture

Faculty of Science
Memorial University of Newfoundland

June 2012

St. John's Newfoundland

Abstract

Running costs of aquaponic systems are much higher in temperate regions due to supplementary greenhouse heating and lighting. Finding routes to maximize revenues would be critical for good business operations. Ornamental fish production is a highly lucrative sector whereby profitability (profit/m^3 culture water) is very high. The aim of this study was to investigate the possibility of using two ornamental fish species, namely platy (*Xiphophorus maculatus*) and goldfish (*Carassius auratus*), as sole major nutrient sources for three species of plants in greenhouse aquaponic culture. No significant differences ($p > 0.05$) were observed regarding fish growth parameters (sex-wise) for platys across treatments. Productivity of basil with goldfish was higher as compared to platys ($p < 0.05$) as the goldfish treatment had a higher nutrient input and build-up. Being a low nutrient-requirement and temperature-tolerant plant, watercress showed better growth than basil and spinach. It was concluded that small ornamental fish alone would not be suitable for commercial plant production. They can, however, be a very good value added element in aquaponic systems in temperate regions, and goldfish (a medium-sized ornamental fish) has excellent potential as a sole species in aquaponics.

Acknowledgements

To begin with, I would like to express my utmost gratitude to my principal supervisor, Dr. Laura C. Halfyard, for her precious guidance and her everlasting support, not only throughout the whole project but since the beginning of my M.Sc. program. This pilot project would not have been a reality without her help and perseverance. I have also deeply appreciated the contribution of my co-supervisors: Mr. Cyr Couturier and Dr. C.C. Parrish, for always providing me with constructive critiques and advice.

I would also like to thank the personnel of the Centre for Aquaculture and Seafood Development (Fisheries and Marine Institute of Memorial University of Newfoundland). Mention is made here of Heather Manuel, Marsha Clarke, Brian Gillett, Tracy Granter, Jason Nichols, Wade Murphy, Mark Santos and Stephen King. In addition, I would like to specially thank Chris Dawe for the invaluable help he provided me in the design, construction and setting-up of the mini-aquaponic systems at Lester Farms Inc. I would also like to thank Ryan Pretty for his help with regard to laboratory work I performed at the Fisheries and Marine Institute. The School of Fisheries/Centre for Aquaculture and Seafood development also paid for and supplied me with some materials for the setting up of the project.

I would like to thank Mr. Brian Burke, from Burke Consulting Inc., for reviewing the economic analyses and projections I have performed at the end of this study.

In addition, the financial assistance which I have received from Memorial University, namely, the School of Graduate Studies Fellowship support and the Graduate Officer support (from Mr. Cyr Couturier), has been of great help during the course of my study. Besides this, I am grateful to Mr. Danny Boyce (Ocean Sciences Centre, Memorial

University of Newfoundland), who hired me to work part-time at the Dr. Joe Brown Aquatic Research Building since May 2011. The financial assistance which I received through him made my stay in Canada much easier.

I would also like to express heartfelt thanks to the whole Lester Family: Jim, Michelle, Samantha, John and Caleb. Jim and Michelle, owners of Lester Farms Inc. (St. John's, NL), willingly provided us with their greenhouse facility for conducting the experimentations and they were ever-present with assistance in different situations. A special mention goes to Samantha who fed the fish on some weekends.

All the more, I am grateful to my parents and my brother for their continuous moral and financial support. It is thanks to the hard-earned savings of my parents that I am in Canada and I am a Masters candidate at Memorial University of Newfoundland today.

Finally, I would like to heartily thank my fiancé, Tasneem, for always being here for me, believing in me and encouraging me. I would not have been able to go through these two years alone without her moral support. I would also like to thank her for having proofread my thesis.

Table of Contents

List of Tables

List of Figures

Page

List of Appendices

Page

List of Abbreviations and Symbols

ANCOVA	Analysis of covariance
ANOVA	Analysis of variance
cm	Centimetre (s)
d.p.	Decimal places
°C	Degree Celsius
et al.	and others
FCR	Feed conversion ratio
g	Gram (s)
gph	Gallons per hour
h	Hour (s)
kg	Kilogram (s)
L	Litres
M	Molar
m^3	Metre cube
mg	Milligram (s)
mL	Millilitre (s)
mm	Millimetre (s)
nm	Nanometre
®	Registered trademark
TAN	Total ammonia-nitrogen
UIA	Un-ionized ammonia-nitrogen

µL	Microlitre (s)
µS	Microsiemens
UVI	University of Virgin Islands
vs	Against
w/v	Weight by volume

CHAPTER 1: INTRODUCTION AND REVIEW OF LITERATURE

1.1 Introduction

Aquaponics is the combined culture of fish and soil-less plants in recirculating aquaculture systems. The technique falls under the category of *integrated production systems*, whereby the by-products or effluents from the fish unit are fed into the hydroponic unit in a totally closed system set-up. This method allows for the production of other cash crops at practically no additional running costs (as the plants receive most of their nutrients from the fish), increasing profitability. Dissolved nutrients, in an aquaponic system, are generated by daily application of fish feed, direct excretion by the fish and the microbial breakdown of fish wastes (Diver, 2006; Rakocy et al., 2006). In tropical areas, open field cultivation is possible as the climate is suitable for year-round production. However, the issue is totally different in temperate regions. Here, production should be carried out under greenhouse facilities, where investments and running costs are much higher. For production to be possible, greenhouse heating and lighting are compulsory during certain periods of the year, due to low temperatures and lack of sunlight. Rakocy et al. (2006) indicated that aquaponic production in temperate climates might not be profitable due to the increased costs mentioned above. Nonetheless, successful growth trials under commercial conditions have been performed in Canada at the Crop Diversification Centre South (Brooks, Alberta) by Dr. N. Savidov (Savidov, 2004; Savidov, 2005). All the more, there are commercial aquaponic systems already in existence for over a decade in Canada in Alberta, Quebec and British Columbia. Good

planning and management would be key factors in making aquaponic production viable in temperate regions.

One way to maximize profit (per square metre of greenhouse space per year) is to produce culinary herbs, as these fetch higher market prices per kg of produce, as compared to fruiting crops (Savidov, 2004; Savidov, 2005; Rakocy et al., 2006). Additional routes to maximize profitability would be of definite help to recover the initial high investments and subsequent running costs in temperate climates. One such possibility would be the production of ornamental fish, as a complementary species to the main food fish being reared, in the same aquaponic system. The ornamental fish sector is a highly lucrative field and ornamental fish keeping is rapidly growing in popularity worldwide. The profitability (profit/m^3 culture water) of ornamental fish production is very high when compared to food fish. When considering the integration of ornamental fish into aquaponics, Rakocy et al. (2006) noted that any species of fish, including large ornamentals such as koi carps and medium-sized ornamental fish such as goldfish, can be used as the fish species in an aquaponic system. However, no mention has been made in the literature about using small ornamentals as alternatives. Such species are even more profitable than bigger ones and they potentially represent greater value for production in aquaponics.

The main aim of this pilot study was to investigate the potential of using a small ornamental fish such as the platy (*Xiphophorus maculatus*) and a medium-sized species such as the goldfish (*Carassius auratus*), as the main species in aquaponics for the production of specific crops. The hypothesis under investigation was that small and

medium-sized ornamental fish can be used as sole major nutrient sources in aquaponic culture.

1.1.1 Objectives

- To downscale and adapt the *Rakocy Model* to a small-scale local Newfoundland greenhouse production.
- To compare major nutrient profiles and productivity of basil, spinach and watercress under two species of ornamental fish culture.
- To assess the possibility of using ornamental fish as a value added factor to aquaponic production in temperate climates.

1.2 Review of literature

1.2.1 Aquaponic systems

Aquaponics combines the culture of fish and plants in the same system. This is possible as the effluent water from the fish tanks is used to directly grow plants in another coupled unit known as the hydroponic sub-system, and culture water is constantly recirculated across the whole system (Savidov, 2004; Savidov, 2005; Diver, 2006; Rakocy et al., 2006). The nutrient solution in an aquaponic system supplies 10 of 13 required plant nutrients in adequate amounts. Out of these 10, nitrogen (N), magnesium (Mg), phosphorous (P) and sulphur (S) represent macronutrients while chlorine (Cl), manganese (Mn), boron (B), zinc (Zn), copper (Cu) and molybdenum (Mo) are micronutrient supplied by the culture water. The three nutrients needing supplementation are calcium (Ca), potassium (K) and iron (Fe). Fish wastes continuously generate nutrients which prevent nutrient depletion. Plants, for their part, take up nutrients from the culture solution to prevent toxic nutrient accumulation (Rakocy et al., 2004a).

Before the 1980s, the concept of aquaponic production faced many unsuccessful attempts due to the complex nature of the technology involved. Aquaponics requires effective management of the production and marketing of two different agricultural products (fish and plants). Fortunately, innovations since then have helped to turn this concept into a viable system of food production. Even more, today, it is regarded as a working model of sustainable food production (Diver, 2006). This concept has become so popular that in the USA, hundreds of school districts have included aquaponics as a science learning tool in their curriculum (Rakocy et al., 2006).

1.2.2 System design: The *Rakocy Model*

The *Rakocy Model* of aquaponic production has been developed by Dr. James E. Rakocy and associates at the University of the Virgin Islands (UVI). The system is geared towards commercial production whereby red and Nile tilapia (*Oreochromis spp.*) are raised in tanks and the effluent water is fed to a raft hydroponic sub-system (Diver, 2006; Rakocy et al., 2006). Rakocy et al. (2006) reported that the essential elements of the system are: fish-rearing tanks, a settleable and suspended solids removal component, an integrated biofilter, a hydroponic component and a sump. Figure 1 gives the optimum arrangement of these components, as described by Rakocy et al. (2006), in the aquaponic system. Effluent water from the fish tanks passes first through the solids removal component where the organic matter load in the water is removed as settleable and suspended solids. The water then undergoes biofiltration where ammonia is converted in a two-step process to nitrate by bacteria. Water is fed to the plants in the hydroponic sub-system where nutrient uptake occurs and it then exits to a sump. The water is then pumped back to the fish rearing tanks and the process is repeated continuously.

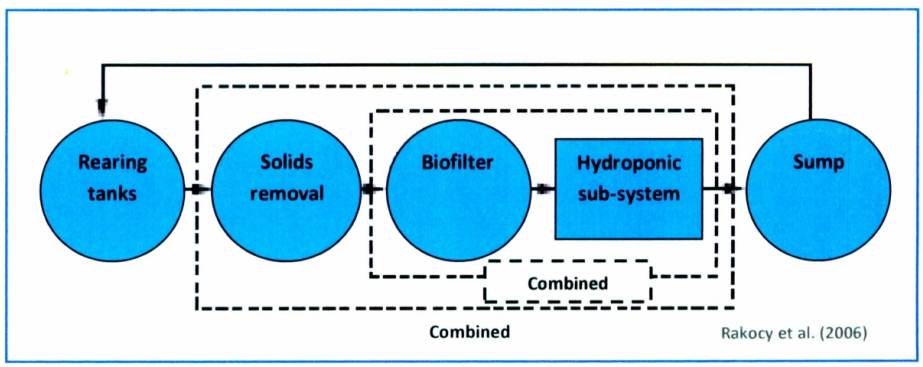

Figure 1. Optimum arrangement of different components in the *Rakocy system*. The arrows show the direction of water flow between the components, represented in blue.

Raft hydroponics is used for the culture of plants in the hydroponic sub-system. This technique makes use of floating sheets of polystyrene and net pots for plant support, and plants float in about 30 cm of effluent solution. If the plant area is sufficiently large, the raft system can provide adequate biofiltration for the efficient breakdown of ammonia and hence eliminating the expense of a separate biofilter. Another independent design combines solids removal with biofiltration and plant culture by using hydroponic support media such as pea gravel or coarse sand. However, this method is subject to clogging and higher maintenance is required. Figure 2 gives a layout of the actual system at the UVI.

Rakocy et al. (2006) described the solids removal component as a critical segment of the system. They further added that wastes such as fecal fish matter, uneaten feed and other particulate matter such as excess organisms (e.g. bacteria, fungi and algae) which grow in the system should be removed from the effluent water before it is fed to the hydroponic sub-system. If accumulation of these occurs, carbon dioxide and ammonia will be generated as they decay. In addition, the deposition of suspended solids on plant roots may create anaerobic regions, preventing nutrient uptake by active transport (a process requiring oxygen). Anaerobic decomposition can also result in the production of methane and hydrogen sulphide which are toxic to fish and plants. Microorganisms in the water column, however, represent a crucial part of the biofiltration capacity of an aquaponic system.

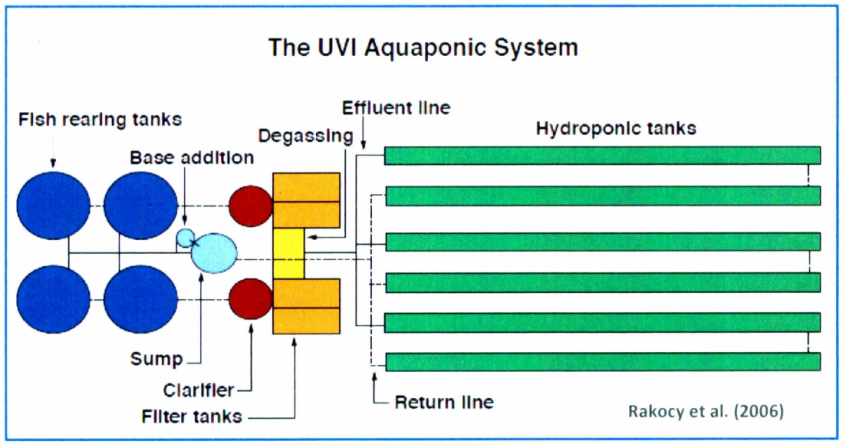

Figure 2. Layout of the UVI aquaponic system.

The *Rakocy Model* has been successfully adapted to Canadian growing conditions under greenhouse facilities by Dr. Nick Savidov at the Crop Diversification Center South in Brooks, Alberta. Sixty crops and varieties have been tested and the technology has been described as having commercial potential for Alberta, a cold, temperate climate location (Savidov 2004; Savidov 2005). The system is practically the same representation as the one at the UVI with some modifications regarding system set-up and running. At UVI, the system runs in open-field, while in Alberta the system is under heated greenhouses. Other modifications are that in Alberta pure oxygen is used rather than aeration, a lower water pH of 6.0 is maintained for plant grow-out, plant beds are used rather than channels and computerized system control has been applied. In the present study, general concepts of the Rakocy system have been applied but components have been downscaled into mini-aquaponic systems (Savidov, 2004; Savidov, 2005).

1.2.3 Component ratios of the model

As a general guide for raft aquaponics, Rakocy et al. (2006) recommends a ratio ranging from 60 to 100 g of fish feed/m^2 of plant growing area/day. Under this feeding ratio, plants such as basil, lettuce and several others have been successfully produced with tilapia in the UVI system. Another important factor to consider is the microbial activity in an aquaponic system. A maximum ratio of 180 g of fish feed/m^2 of plant growing area/day has been recommended by the authors, representing the capacity of the system to treat wastes without the use of a separate biofilter. Hence, a balance between nutrient generation by the addition of feed and efficient microbial activity over a definite area should be achieved when designing an aquaponics system using raft culture. Microorganisms are not only involved in the process of nitrification. They also help as follows: in the breakdown of organic wastes, in the ammonification process, they stimulate nutrient uptake by plant roots and they help in the competitive inhibition of pathogenic organisms. They are fundamental in the successful running of an aquaponic system. Examples of such microorganisms are bacteria, protozoa, *Archaea* and algae (Leininger et al., 2006; Lehtovirta-Morleyae et al., 2011; Rakocy et al., 2006; Savidov, 2004; Savidov, 2005).

1.2.4 Fish species

Tilapia is the most common fish cultured in commercial aquaponic systems (Diver, 2006; Rakocy et al., 2006) but most freshwater fish which tolerate crowding will do well, including ornamental fish. Rakocy et al. (2006) reported that channel catfish, largemouth

bass, crappies, rainbow trout, pacu, common carp, koi carp, goldfish, Asian sea bass and Murray cod have been used in aquaponics. Hybrid striped bass, however, performs poorly because it cannot tolerate the high potassium level in the system as this essential plant nutrient is often added to enhance plant growth.

DeLong et al. (2009) stated that tilapia possess a myriad of attractive characteristics which make them well suited for tank culture. They can adapt to crowding and handling conditions required in a tank-based culture system. They possess a heavy slime coat protecting them from abrasion and bacterial infections, which would be of definite concern in many other species. The authors also added that they show good growth even at the high densities in the confinement of tanks. Production levels range from 60 to 120 kg/m^3 or more (FAO, 2006). Besides, tilapia is tolerant to fluctuating pH, temperature, oxygen and dissolved solids in culture water (Parker, 2000; Diver, 2006; FAO, 2006; Popma and Masser, 2006; DeLong et al., 2009).

Regarding goldfish, no scientific evidence exists with respect to the use of this species to sustain commercial aquaponic systems. Many people, however, use them in their backyard mini-aquaponic systems, which are widely broadcasted over the internet.

1.2.5 The ornamental fish sector

Ancient Egyptians are believed to be the first real aquarium keepers and fish were kept in their homes to impress friends. High-ranked Roman officials then also adopted this practice. Fish keeping began spreading from Rome to the Far East where oriental fish keepers became very attracted to the common goldfish and they started to

selectively breed them. Public aquaria began to appear in the 1800s but it started gaining much popularity only in the 1900s. Today, aquarium keeping is hugely popular where people can choose from a myriad of fish species and state of the art equipment (Hargrove and Hargrove, 2006).

Although exact figures regarding the world ornamental fish trade do not exist (Livengood and Chapman, 2011), the wholesale ornamental trade market was valued at nearly US$1 billion in 2005, while the retail trade amounted to US$3 billion annually. However, when non-exported products, wages, retail sales and associated materials within the entire industry are taken into consideration, the sector has been estimated to be worth around US$15 billion. Moreover, the scope of the ornamental fish business and its related impact on human and aquatic communities are unappreciated and inaccurately understood (FAO, 2005a). As compared to aquaculture food production, the aquaculture of ornamental fishes does not require large space and returns are much higher per unit of biomass produced (Moothy and Bhikajee, 2000).

Since 1985, the value of exports in ornamental fish has increased at an average growth rate of 14% per annum (FAO, 2005b). On a worldwide basis, Singapore is ranked first in ornamental fish exportation and the country occupied 24 to 26% of the world ornamental fish export trade during 1996-2000 (Lewbart and Harms, 2008). The countries which follow are China/Hong Kong with 10.6%, Indonesia with 7.1% and Malaysia with 6.3% of the total world ornamental fish exports. Small ornamentals are at the top of the world demand list with Tetras (Family: Characidae) coming in first place followed by

guppies (*Poecilia reticulata*), with regard to leading exported species. Platys are also among the most important species of interest (Ling and Lim, 2006).

In the United States, it is estimated that around 7.2 million households have an aquarium at home and in the European Union, the figure is estimated at 3.2 million. The global demand for ornamental fishes is constantly increasing and aquarium fish keeping is one of the most popular hobbies worldwide (Ghosh et al., 2003; Livengood and Chapman, 2011).

1.2.5.1 Platys

Platys (*Xiphophorus maculatus*, Günther 1866) are benthopelagic small freshwater fish typically found in the tropics where water temperature ranges optimally from 18°C to 25°C (Riehl and Baensch, 1991). The maximum length (tail length) of males is 4.0 cm (Keith et al., 2006) while females reach 6.0 cm (Dennis and Hugg, 1996). Platys are live bearers and in the wild, adults can be found in warm springs, canals and ditches, exhibiting slow water movements with weedy banks. They can also inhabit creeks and swamps (Allen et al., 2002). Platys exhibit several colour variations making them popular aquarium fishes where they reach sexual maturity after three to four months and they would readily reproduce (Riehl and Baensch, 1991).

Platys are among the most beautifully coloured freshwater fish. They can be found with different fin shapes and in nearly all colours imaginable. These fish have been developed extensively via commercial breeding for the ornamental fish market. Some of the well-known varieties are the Mickey Mouse, the Marigold, the Calico, the Painted, the

Wag Tail and the Sunburst platy, amongst others (Hargrove and Hargrove, 2006). Hargrove and Hargrove (2006), who are aquarium-fish keeping experts, also added in their book that platys are their favourite species of fish because of 'their beauty, cheery round body shape and amazing colours'.

1.2.5.2 Goldfish

Goldfish (*Carassius auratus auratus*, Linnaeus 1758) are benthopelagic freshwater fish which can be found in Central Asia, China and Japan; however, they have been introduced throughout the world (Kailola et al., 1993). Goldfish can inhabit tropical as well as cold water systems. The maximum reported tail length of a goldfish is 32.0 cm (Kottelat and Freyhof, 2007) and they can live for very long, making them very good companions. The longest living goldfish has been reported to be 41 years old (Bobick and Peffer, 1993). Goldfish introduced in the wild commonly inhabit rivers, lakes, ponds and ditches (Man and Hodgkiss, 1981; Etnier and Starnes, 1993) in either stagnant or slow-flowing water (Billard, 1997). While wild forms are generally dull-coloured, the cultured ones show a myriad of colourations such as scarlet, red-pink, silver, brown, white, black and a combination of all of these (Trautman, 1957).

Hargrove and Hargrove (2006) described that goldfish are one of the oldest aquarium fish known. They were specifically bred from carp 'for their beauty' and no natural known varieties are known. They further added that all goldfish varieties are grouped into the same species, the *Carassius auratus*. However, four main types exist,

namely the Egg, the Wen, the Dragon-eye and the Common goldfish. The latter resembles the carp but exhibits more colour variations.

1.2.6 Crop selection, yield and productivity

1.2.6.1 Crop selection

The goal when selecting crops for an aquaponic system is to culture those that will generate the highest productivity (per unit area per unit time) and bring the highest value on the market. The best choices are culinary herbs such as basil, cilantro, chives, parsley, portulaca and mint. These have low to medium nutrient requirements and adapt well to the type of culture system used in aquaponics. In addition, they command higher market value and hence generate more income as compared to fruiting vegetables such as tomatoes, cucumbers, eggplant and okra (Savidov, 2004; Savidov, 2005; Diver, 2006; Rakocy et al., 2006). Rakocy et al. (2006) stated that the gross income from culinary herbs grown in aquaponics is 20 times higher than when growing fruiting crops. The authors presented a comparison made in the UVI commercial-scale system whereby on an annual basis, basil yielded 11,000 pounds for a gross income of US$110,000 as compared to okra which yielded only 6,400 pounds for just US$6,400. They further compared the break-even and sale prices of two commodities. The break-even price for lettuce was US$6.15/case for a selling price of US$20.00/case while the break-even price for basil was US$0.75/pound for a selling price of US$10.00/pound. These figures give a clear indication of the comparative advantage of culturing herbs with respect to fruiting

vegetables. Basil and cilantro bring the highest income on the market among all the herbs (Rakocy et al., 2006).

1.2.6.2 Yield and productivity

In a study carried out by Rakocy et al. in 2004, the production of tilapia and basil in two different cropping systems, namely batch and staggered production, in aquaponics were compared. Further comparison was made with basil production in open fields. Plants were grown at a density of 8.0/m^2 and final results yielded 2.0, 1.8 and 0.6 kg/m^2 for batch, staggered and field, respectively. Annual yields were 25.0, 23.4 and 7.8 kg/m^2 respectively. These were lower compared to hydroponically grown basil at a yield of 56.2 kg/m^2/yr at a plant density of 25 per m^2 (Bradley and Marulanda, 2001). However, when adapting the UVI system in Alberta, Savidov (2005) produced basil at around 31 plants/m^2 and his results yielded a production of 42.0 kg/m^2/yr in a staggered cropping system. Both Rakocy et al. (2004a) and Savidov (2005) reported that batch production is not sustainable in the UVI system at the current fish output as there is high nutrient depletion during the maximum growth stage of the plants, leading to some acute deficiency symptoms. Rakocy et al. (2004a) found that due to these deficiencies, the majority of the produce was unmarketable at harvest. They further concluded by saying that 'basil production is sustainable in an aquaponic system with a feed input ratio of 99.6 g/day/m^2 and a staggered cropping system'. They also added that further research could determine the feasibility of batch production of basil at a higher feeding ratio.

Comparing batch and staggered production, in the former, all plants are planted and harvested at the same time (all in, all out), while with staggered production plants of different stages are planted simultaneously and partial harvests are carried out (Rakocy et al., 2006). Rakocy et al. (2004a) reported that in their system at the UVI, basil seedlings, for example, were planted on one fourth of the total growing area every week until filling the entire system. According to Rakocy et al. (2006), batch production causes a high nutrient pressure on the system during the maximum growth phase of the plants and hence a higher fish feeding ratio is required to sustain adequate nutrient output from the fish component. Staggered production, however, allows for a balance in nutrient uptake thus not requiring higher feed input. They further added that leafy green vegetables, herbs and other crops with short production cycles are very well adapted to continuous staggered production.

In the present study, all plants were planted and harvested at the same time because the plant growing area was relatively very small (0.8 m^2/system). In addition, for statistical replication purposes, staggered plant culture would not have been adequate under the conditions of the present study.

CHAPTER 2: MATERIALS AND METHODS

2.1 Preliminaries

2.1.1 Mini-aquaponic systems

Experimentations were conducted under a greenhouse facility at Lester Farms Inc. (St. John's, NL), which specializes in flower and vegetable production. The technology could help expand the visibility of this family-owned business, as the owners intend to invest into a commercial aquaponic system with tilapia in the near future.

Eight mini-aquaponic systems were built specifically for the purpose of this pilot study, with general technical specifications as described by Rakocy et al. (2006). The main components of each system were fish tanks, a solids removal unit, plant growing beds with floating food-grade polystyrene sheets and a sump with bio-spheres for additional biofiltration. Aeration was provided in the fish tanks via an air blower, as an additional source of oxygen.

In each system, water moved from four 40 L fiberglass fish tanks to a common filter where the effluent fish water was sprayed via a spray bar into a filter bag (10 μm). Each fish tank had its individual water inlet and outlet, and common water from the four tanks joined before moving into the filter. Out from the filter, cleaner water was distributed to four plant tanks (arranged in line) via four individual inlet valves. Water then moved out from the plant tanks through individual outlets and common water then flowed to the sump. The whole flow from the fish tanks to the sump was by gravity. Water from the sump was then pumped back to the fish tanks by a *Laguna® PT-8125* (750 gph) water pump. Flow rates across all the tank inlets were maintained at 3 L/min.

In addition, water in each system was heated at the sump by a *Finnex*® 800W titanium high-output heater and temperature was set to 25°C. Each system had a total water volume of 450 L. Figure 3 shows the arrangement of the different components in the aquaponic systems.

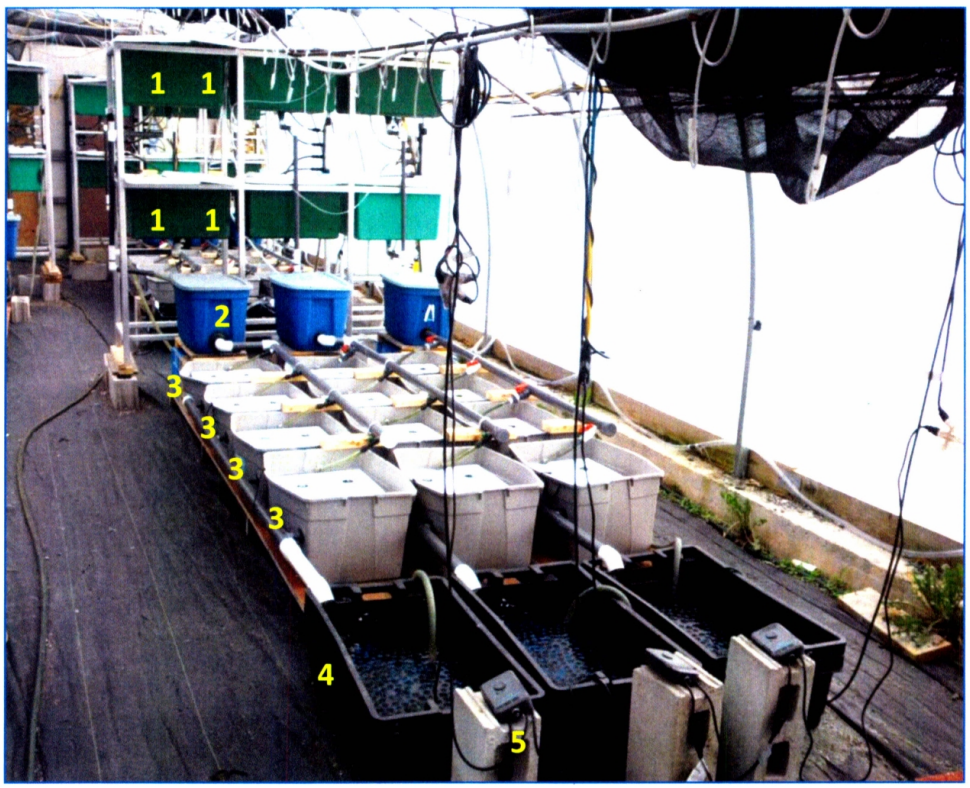

Figure 3. Arrangement of main components in the mini-aquaponic systems (1: Fish tanks; 2: Solids removal unit; 3: Plant growing tanks; 4: Sump; 5: Temperature controller). All the labels show components in system 1 (1.0 platy/L-plant 1).

2.1.2 Fish

Seven hundred goldfish (variety: common) and 1,400 platys (variety: red wag tail) were imported via local pet stores for this study. The goldfish came from Ontario while the platys from Singapore. The fish were acclimated in separate holding tanks, at

the greenhouse facility, upon their arrival (under Memorial University of Newfoundland Animal Care protocol number: 11-24-LH).

It should be noted here that the goldfish suffered high stress at the end of the shipping process. They were lethargic, were swimming at the surface and they had very minimal feeding activity. The main reason was that the fish were counted again at the local pet store upon their arrival and no oxygen was used when they were again bagged (a situation beyond our control). About 500 died in the two-week period following this. As a result of this event, the initial planned treatments were modified and hence only one system subsequently accommodated goldfish.

The platys, however, which came from a different supplier, were in good health and only two mortalities resulted from the shipping process.

2.1.3 Plants

Basil (*Ocimum basilicum*) and spinach (*Spinacia oleracea*) seeds were sown in rockwool media and seedlings were transplanted to the floating polystyrene in the mini-aquaponic systems, once roots emerged across the rockwool. Watercress (*Nasturtium officinale*), however, was vegetatively propagated from cuttings. Watercress shoots of 10 cm in length were cut from parent plants at the nodes, disinfected with iodine, washed and placed directly into net pots (used for plant support) in holes drilled through the polystyrene sheets.

Two plant cycles were undertaken. The first plant cycle was run with basil and spinach. As the experimentation started in the early fall season, temperatures inside the

greenhouse were too cold for good basil growth by the end of the cycle (air temperatures reached freezing at night). In addition, seeds set for the second cycle showed low germination rates and poor seedling growth even under 24-hour supplementary light and heat. The decision was thus taken to switch from basil to watercress in respective treatments. Watercress is a more cold-tolerant and it is a low-nutrient requirement plant (McHugh et al., 1987; McHugh and Constantinides, 2004; Savidov, 2004). Spinach is also very tolerant to cold temperatures with its peak growing season being the fall season (Guy et al., 1985; Guy and Haskell, 1987; Kaye et al., 1998). The second plant cycle was thus run with watercress and spinach.

2.2 Fish culture and sampling

2.2.1 Fish culture

After the acclimation period, fish were randomly distributed across the eight mini-aquaponic systems. Seven systems received platys while the last one accommodated goldfish. Growth of two plants was investigated under three platy densities namely 0.75 fish/L, 1.0 fish/L and 1.25 fish/L, in a total of six treatments. A seventh system was set up as the control with no plants and the highest density of platys. The final treatment received goldfish at 0.625 fish/L (half highest platy density) but with twice the feeding regime, as an investigation of a higher feed input on the nutrient profiles. For the platys, each tank was stocked with half males and half females. Basil was grown in the goldfish treatment in the first cycle while watercress was grown in the second cycle. Figure 4 shows some fish tanks within three of the treatments.

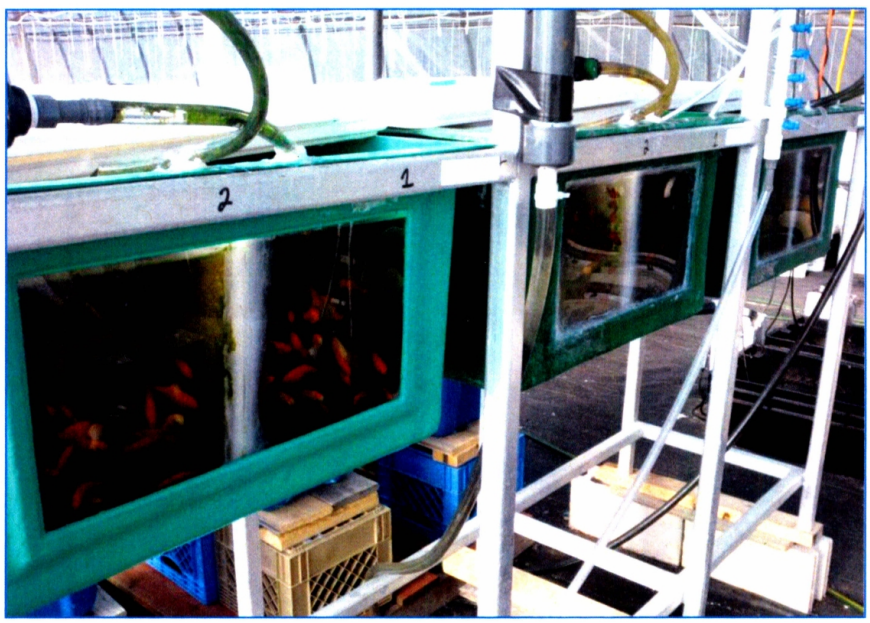

Figure 4. Arrangement of fish tanks in three different systems in the greenhouse. Each tank was split into half making two individual compartments. The bottom tanks of three systems are shown in this picture. The system on the extreme left held goldfish while the other two contained platys.

A special feeding regime (Table 1) was devised for this study. Fish were fed twice a day with *Blackwater Gold-N® professional* (professional koi and goldfish feed) crushed pellets throughout the study. This feed contains a minimum of 40% crude protein, minimum 10% crude fat and maximum 4% fiber. The fish were under study for 11 weeks.

Table 1. Feeding regime devised for this aquaponics study with platys and goldfish.

Weeks	Platys feeding/10 fish/day (g)	Goldfish feeding/5 fish/day (g)
1 - 2	0.2	0.4
3 - 6	0.3	0.6
7 - 11	0.4	0.8

2.2.2 Fish sampling

Ten male and 10 female platys were randomly sampled every two weeks for length and weight assessment. For goldfish, no visual difference could be made between males and females. Hence, 10 fish were sampled from each tank irrespective of sex.

Length was measured, as standard length, with a digital pair of *Mitutoyo®* vernier calipers (accuracy: 0.01 mm) as shown in Figure 5. The wet weight of each individual fish was also recorded, following length measurement, with an *A&D® ER-120A* electronic balance (accuracy: four d.p.). Each individual fish was placed onto dry absorbent paper before being weighed (Figure 6).

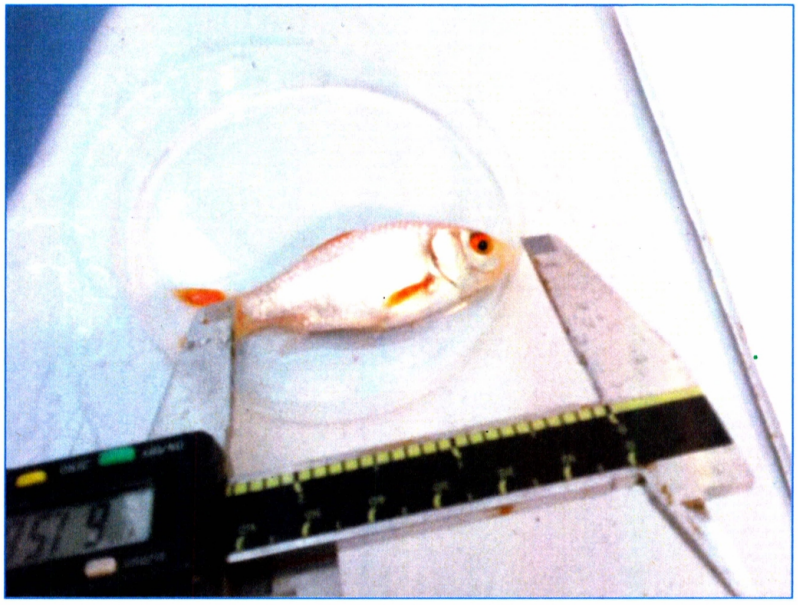

Figure 5. Standard length measurement on a goldfish with a pair of vernier calipers. A plastic petri, with a thin film of water, was used to hold the fish for a few seconds while measurements were taken. The small fish stopped moving after a few seconds after being transferred to the petri with the thin water film acting as a sticking medium.

Figure 6. Weight measurement on a platy with a four d.p. electronic balance.

2.3 Plant culture and sampling

2.3.1 Plant culture

As mention earlier, the first plant cycle was undertaken with spinach and basil, while in the second cycle, spinach and watercress were employed for growth trials. Four plants were grown in each plant tank making a density of 20 plants/m^2 (16 plants/treatment). Each plant tank was 54 cm (L) x 47 cm (W) with a water height of 23 cm for a volume of 58.4 L. Floating polystyrene sheets with net pots placed in drilled holes, were used to hold the plants (Figure 7). The net pots were just touching the water surface (Rakocy et al., 2006) and the roots grew into the flowing water. Plants were grown under ambient light conditions and water temperature of 25°C.

The first plant cycle lasted five weeks while the second cycle was four weeks. Plants (first cycle) were introduced in the systems two weeks after fish were introduced.

Figure 7. Plant beds with watercress. The grey pipes running parallel to the plant tanks are the water inputs supplying each tank. Each input line had four valves, each supplying one individual plant tank. Three systems are shown in the above picture. The one on the right was using goldfish while the other two, used platys.

Iron-EDTA was added every two weeks at a concentration of 2.0 mg/L. pH was adjusted towards 7.0 with phosphoric acid and potassium hydroxide, so as to achieve a balance between maximum plant nutrient uptake and effective biofiltration (Rakocy et al., 2006). The same amounts were added each time to all eight systems. In addition, 1.2 kg of crushed coral was laid at the bottom of every solids removal tank, as a source of calcium for the plants.

2.3.2 Plant sampling

All 16 plants from each system were assessed at the end of each plant cycle for shoot length (main stem), root length (main root) and plant weight. A metre ruler was

used for measuring the shoots and roots while the *A&D® ER-120A* electronic balance (accuracy: four d.p.) was used for plant weight measurement.

Table 2 gives a summary of the complete layout of the experiment.

Table 2. Experimental design used across the eight mini-aquaponic systems. Each treatment has been replicated four times in both plant cycles. There were hence a total number of 32 fish tanks and 32 plant tanks in both plant cycles.

Plant Cycle	Treatments x Replicates			Abbreviation
1st	Basil	0.75 platy/L	x 4	D1P1
		1.0 platy/L		D2P1
		1.25 platy/L		D3P1
		goldfish		Goldfish
	Spinach	0.75 platy/L	x 4	D1P2
		1.0 platy/L		D2P2
		1.25 platy/L		D3P2
	No plants	1.25 platy/L	x 4	Platy control
2nd	Watercress	0.75 platy/L	x 4	D1P1
		1.0 platy/L		D2P1
		1.25 platy/L		D3P1
		goldfish		Goldfish
	Spinach	0.75 platy/L	x 4	D1P2
		1.0 platy/L		D2P2
		1.25 platy/L		D3P2
	No plants	1.25 platy/L	x 4	Platy control

2.4 Water quality assessment

Several water quality parameters such as dissolved oxygen, temperature, pH, electrical conductivity, total ammonia-nitrogen, un-ionized ammonia-nitrogen, nitrate-nitrogen, phosphate and potassium were periodically assessed throughout the study.

2.4.1 Dissolved oxygen

Dissolved oxygen data were collected from each fish and plant tank, across all treatments every week, with an *Oxyguard® beta* dissolved oxygen meter. The meter was always re-calibrated before use. Data were collected both as percentage saturation oxygen and mg/L oxygen. Spot checks were, however, carried out on a daily basis to ensure that the dissolved oxygen levels were in the desired range.

2.4.2 Temperature

Temperature (°C) data were collected on a weekly basis from all the fish and plant tanks using the *Oxyguard® beta* dissolved oxygen meter.

2.4.3 pH

Weekly pH data were collected from all the fish tanks with a *Sper scientific® 850050* pH pen. The pen was re-calibrated every two weeks.

2.4.4 Electrical conductivity

Electrical conductivity was assessed weekly from each plant tank using a *YSI® Y30* conductivity meter. Data were measured in microsiemens (µS).

2.4.5 Un-ionized ammonia-nitrogen

Total ammonia-nitrogen was assessed using a *LaMotte Smart® 2* colorimeter. Water samples were collected from each fish tank and analyzed immediately at the farm site, as per instructions provided by the manufacturer.

Thereafter, the un-ionized ammonia-nitrogen level (NH_3-N) was calculated from the values of total ammonia-nitrogen (NH_3-N + NH_4^+-N). The proportion of un-ionized ammonia-nitrogen in solution is dependent on the water temperature and pH (Emerson et al., 1975; Boyd, 1982).

Finally, theoretical values for total ammonia-nitrogen production were calculated from the formula in Appendix 1, and compared with actual values obtained in the eight systems.

2.4.6 Nitrate-nitrogen

Nitrate-nitrogen concentration was assessed frequently using a rapid spectrophotometric method designed by Velghe and Claeys (1985). Water samples were collected from all plant tanks across all treatments. The samples were cooled and immediately transported to the Fisheries and Marine Institute of Memorial University of Newfoundland for analysis.

For calibration, a stock solution of 1000 mg/L nitrate was prepared by dissolving 1.6306 g anhydrous potassium nitrate in one litre of distilled water. Standards of known nitrate concentrations, namely 5 mg/L, 10 mg/L, 15 mg/L, 20 mg/L, 25 mg/L, 30 mg/L and 35 mg/L were then prepared from the stock solution. Two mL of the standards and

sample water from the eight systems were transferred in separate test tubes and 100 µL 5% (w/v) resorcinol solution was added to each. All test tubes were shaken slightly and 2.6 mL of 98% concentrated sulphuric acid (analytical grade) was immediately added to each. The colour was left to develop until the test tubes cooled down to room temperature. Absorbance of the standards and samples were read using a *Jenway® 6405* spectrophotometer at 360 nm and the nitrate concentration from each sample was obtained by comparing with the absorbance of the standards.

Samples were diluted with de-ionized water when the concentration was expected to reach over 35 mg/L nitrate.

2.4.7 Potassium

Potassium concentration (mg/L) was assessed frequently by the *LaMotte Smart® 2* colorimeter. Samples were taken from all plant tanks across all treatments and analyzed immediately at the farm. The tests were performed as per the manufacturer's guide.

2.4.8 Phosphate

Phosphate concentration (mg/L) was assessed on the same day as potassium by the *LaMotte Smart® 2* colorimeter. Samples were also taken from all plant tanks across all treatments and analyzed immediately at the farm. The tests were performed as per the manufacturer's guide.

2.5 FCR

At the end of the study, the feed conversion ratio (FCR) was calculated for the fish in each of the eight systems. The following formula was used:

$$FCR = \frac{\text{Total feed fed (g)/system}}{\text{[Final total fish weight (g) – Initial total fish weight (g)]/system}}$$

2.6 Economic assessment

An economic assessment was performed at the end of the study, to compare the hypothetical gross return from ornamental fish versus a food fish, like tilapia. The Rakocy system of aquaponic production was used as a model for the calculations.

2.7 Data treatment and statistical analyses

All statistical analyses were performed on raw data using the statistical package *Minitab® 15*. The tolerance for type 1 error was set at 5%. All charts have been generated by *Microsoft® Excel 2007*, from the raw data.

ANCOVAs have been used to compare between treatments over a time scale for fish growth and water quality parameters. Where statistical assumptions were not met, one-way and two-way ANOVAs have been applied. ANCOVAs, rather than the Von Bertalanffy Growth model, were used to assess fish growth parameters as the fish under study were still in a linear growth phase during the whole of the experiment. For the three plant parameters: plant weight, root length and shoot length, one-way ANOVAs have been used to compare between specific treatments. In addition, three-way ANOVAs were

used to compare dissolved oxygen levels between fish and plant tanks across treatments. For planned comparisons, the error rate was adjusted by dividing the 5% by the number of tests performed. Four planned comparisons were performed for several parameters, to better analyze the raw data. An error rate of 1.25% (5%/4) was used everywhere for all planned comparisons, unless otherwise specified.

To start with, goldfish was compared to all other platy treatments. Then, differences were tested among the platy treatments when the goldfish treatment was excluded from the analysis. Following this, the control platy treatment was tested against the other platy treatments. Finally, treatments under one plant were compared to those under the second plant for differences (goldfish treatment excluded).

For every statistical test performed, all statistical assumptions have been verified. These are: (1) normality of distribution of residuals, (2) randomization and independent sampling and (3) homogeneity or equality of variance.

CHAPTER 3: RESULTS

3.1 Standard fish length

3.1.1 Growth of female platys over time

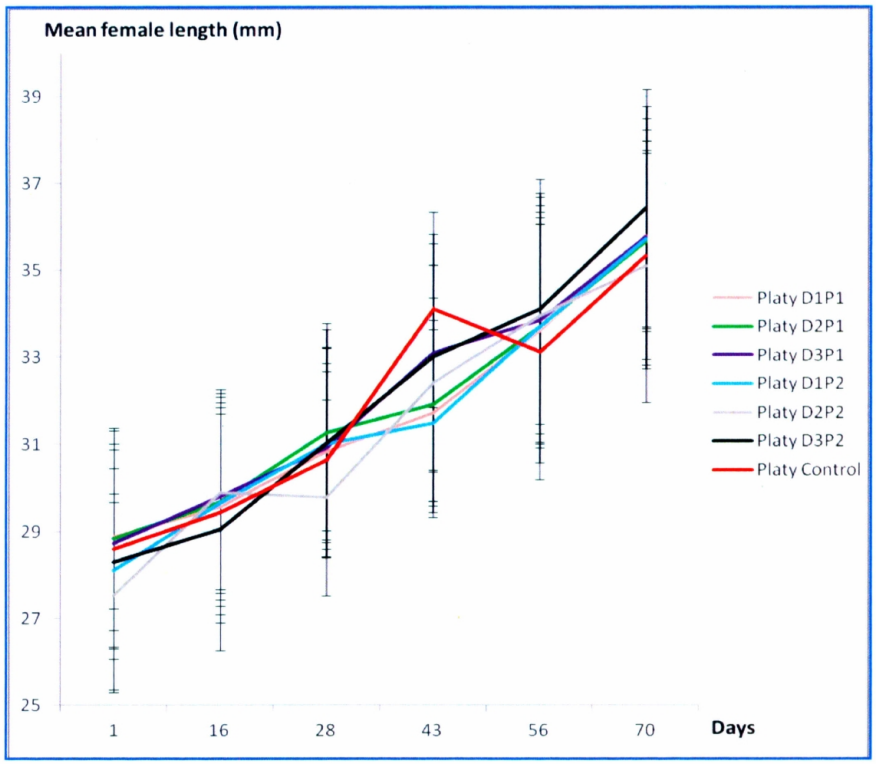

Figure 8. Standard length of female platys over time in all treatments with mean ± standard deviation. n= 40 per measurement time per treatment. D1P1= 0.75 platy/L-plant 1, D2P1= 1.0 platy/L-plant 1, D3P1= 1.25 platy/L-plant 1, D1P2= 0.75 platy/L-plant 2, D2P2= 1.0 platy/L-plant 2, D3P2= 1.25 platy/L-plant 2, Platy Control= 1.25 platy/L-no plants.

No difference (p= 0.154) was noted in the mean standard length of female platys over time, across all the treatments (Figure 8). The fish showed linear growth at this stage which made analysis using an ANCOVA possible. In addition, no difference was observed between standard length of females among treatments (p= 0.314) in the first sampling. All statistical assumptions were met.

3.1.2 Growth of male platys over time

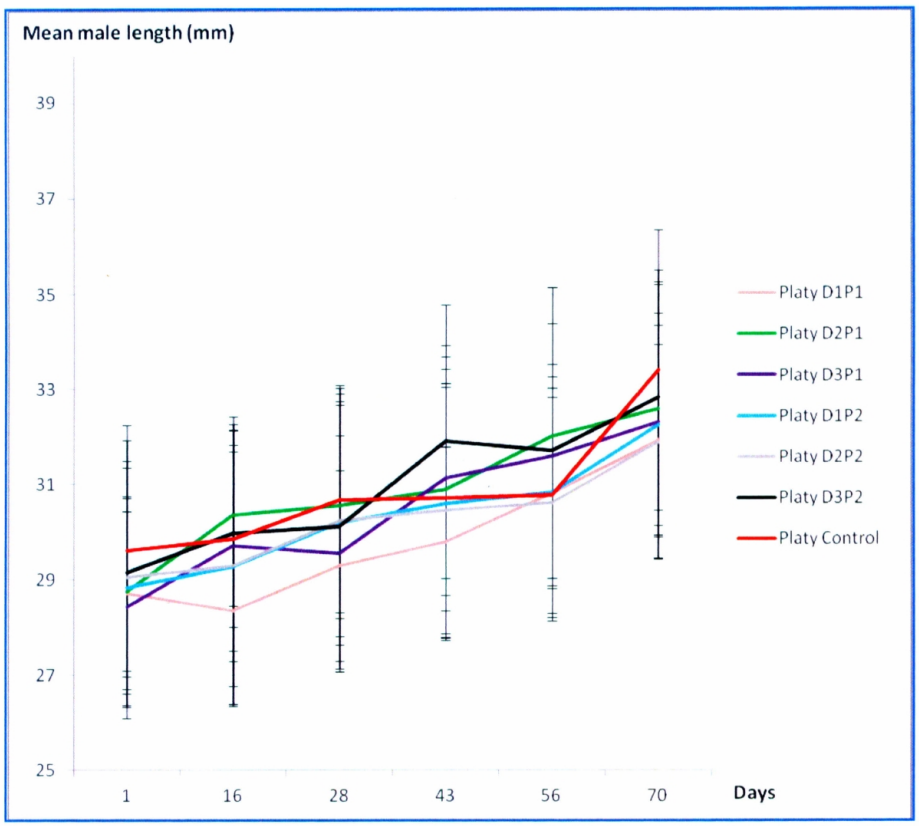

Figure 9. Standard length of male platys over time in all treatments with mean ± standard deviation. n= 40 per measurement time per treatment. Designations explained in legend to Fig. 8.

There was no difference in the growth of the male platys ($p= 0.604$) with respect to their standard length over time, among treatments (Figure 9). The analysis using an ANCOVA was possible, since the male platys have also displayed a linear growth. All statistical assumptions were met.

In addition, there were no differences in the mean standard length of males in the first sampling across treatments ($p= 0.582$).

3.1.3 Growth of male *vs* female platys over time

Figure 10. Standard length of male platys compared to females across all treatments over time with mean ± standard deviation (orange for males; black for females). n= 40 per measurement time per treatment, for both males and females.

Female platys showed better growth (p= 0.000) over time (Figure 10). At the start of the study, males were bigger than females across all treatments (p= 0.010). Mean male standard length was 28.9 ±2.4 mm while females were 28.4 ±2.3 mm. However, at the end, females grew bigger than males (p= 0.000) with a mean standard length of 35.7 ±2.6 mm. Males were 32.3 ±2.8 mm in mean standard length overall.

3.1.4 Growth of goldfish over time

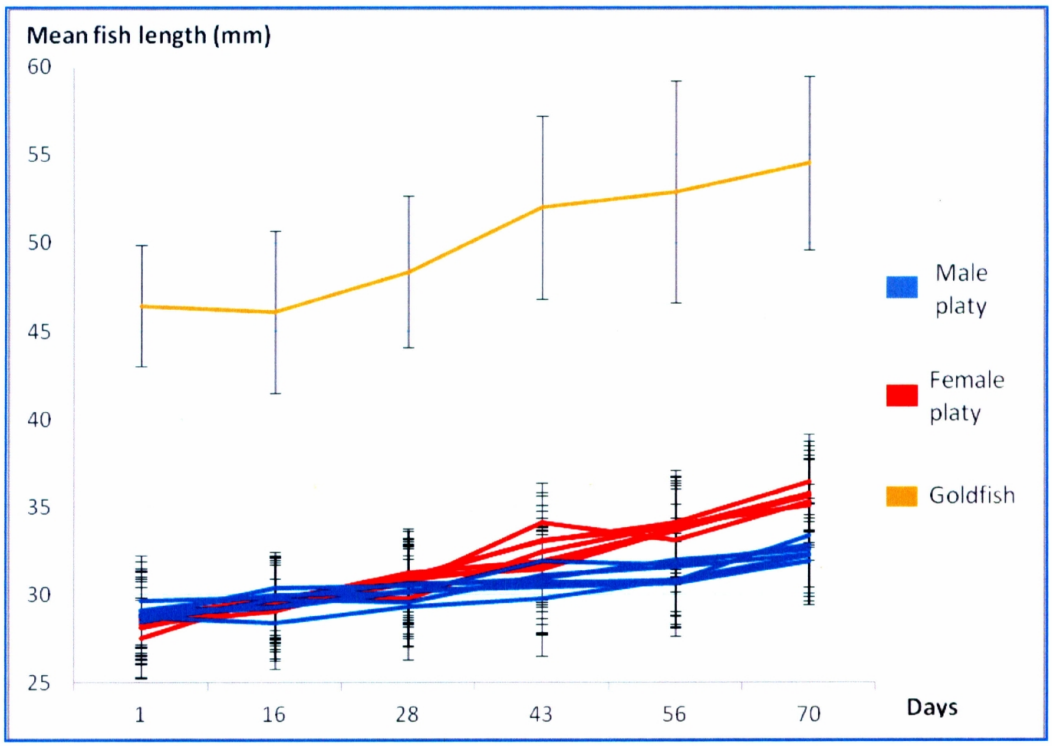

Figure 11. Goldfish standard length over time compared to platys with mean ± standard deviation. Goldfish n= 40 per measurement time; platys n= 40 per measurement time per treatment for both males and females.

Figure 11 gives a visual comparison of the mean standard length of goldfish with respect to male and female platys over the 11-week study period. The goldfish were 46.4 ±3.4 mm and 54.6 ±4.9 mm, respectively, at the start and end of the study.

3.2 Fish weight

3.2.1 Fish loading in the systems

Table 3. Initial and final fish loading in the eight systems including initial and final average body weight in the systems.

Treatments	Fish density (Fish/L)	Initial total fish loading/system (g/L)	Final total fish loading/system (g/L)	Initial average body weight (g)	Final average body weight (g)
D1P1	0.75	0.55	0.95	0.74	1.29
D2P1	1.0	0.72	1.31	0.72	1.34
D3P1	1.25	0.90	1.69	0.72	1.37
D1P2	0.75	0.55	0.99	0.73	1.33
D2P2	1.0	0.73	1.23	0.73	1.29
D3P2	1.25	0.90	1.67	0.72	1.38
Platy control	1.25	0.94	1.72	0.75	1.42
Goldfish	0.625	2.27	3.85	3.63	6.16

Table 3 gives a comparison of fish loading in the eight mini-aquaponic systems at the various fish densities (fish/L) used. The fish loading are much less as recommended in a typical aquaponic system (Rakocy et al., 2006). This fact is more pronounced in the platys because of the small sizes of small ornamental fish. Goldfish, which is a medium-sized ornamental fish, has a higher loading even at half of the highest platy density.

3.2.2 Growth of female platys over time

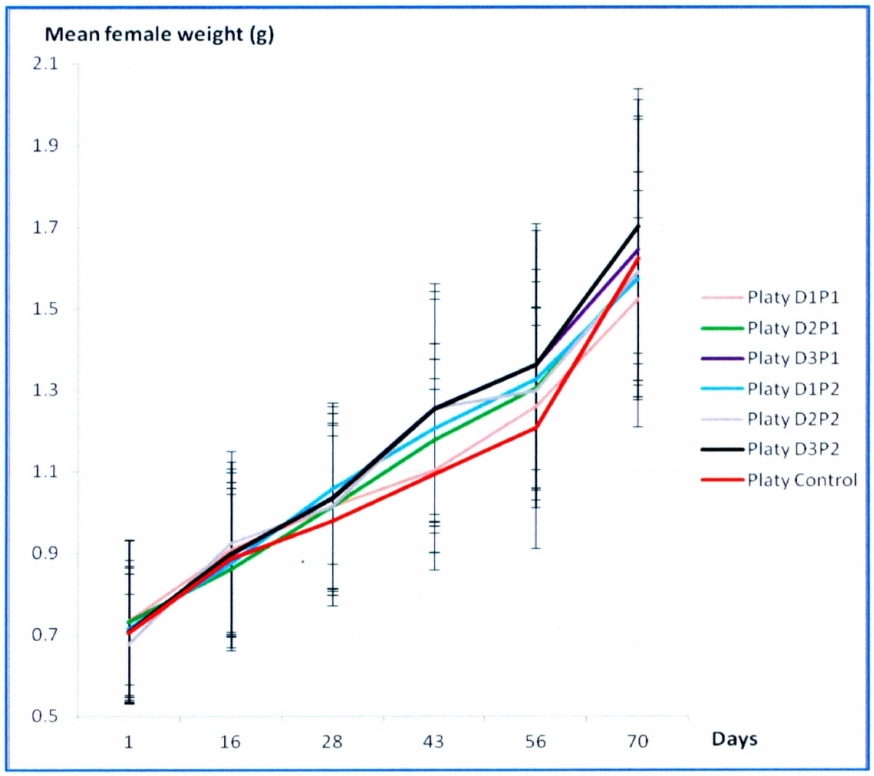

Figure 12. Weight of female platys over time in all treatments with mean ± standard deviation. n= 40 per measurement time per treatment. Designations explained in legend to Fig. 8.

A difference in mean fish weight over time was observed between the treatments ($p= 0.010$) over time when an ANCOVA was performed (Figure 12). When the D3P2 treatment (1.25 platy/L-spinach) was excluded, no significant difference was observed ($p= 0.055$; generated by performing 2,000 ANCOVA randomizations).

The weight gains of the fish were still in the linear phase making the use of an ANCOVA possible. All statistical assumptions were met.

3.2.3 Growth of male platys over time

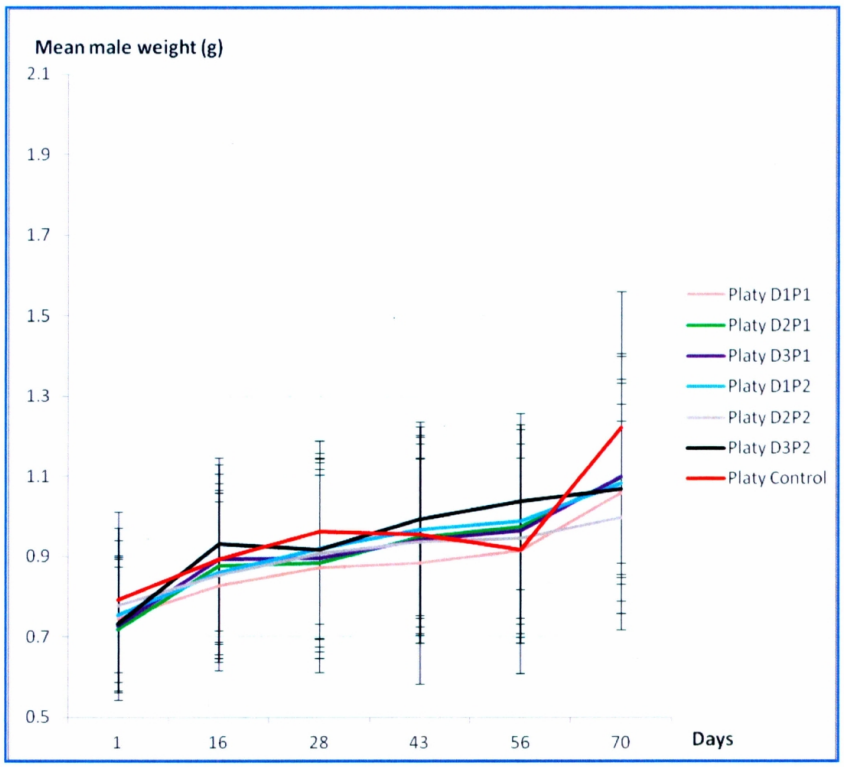

Figure 13. Weight of male platys over time in all treatments with mean ± standard deviation. n= 40 per measurement time per treatment. Designations explained in legend to Fig. 8.

No significant difference (p= 0.809) was observed among treatments in the growth rate of the male platys with respect to their weight as shown in Figure 13. Linear analysis was performed using an ANCOVA and all statistical assumptions were met.

3.2.4 Growth of male *vs* female platys over time

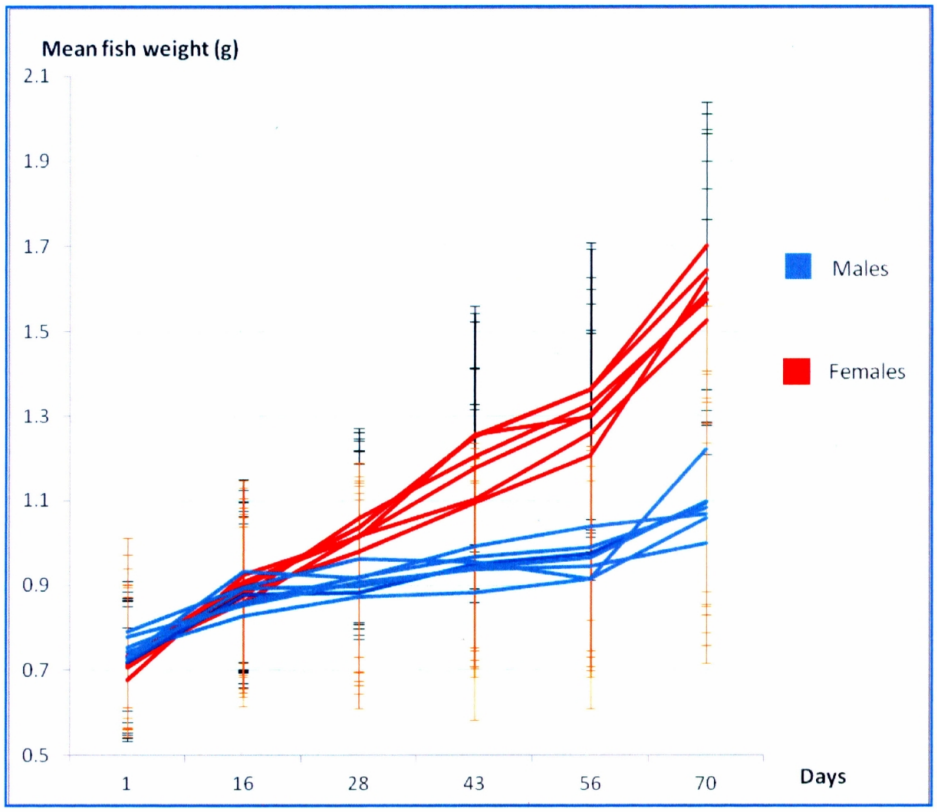

Figure 14. Weight of male platys compared to females across all treatments over time with mean ± standard deviation (orange for males; black for females). n= 40 per measurement time per treatment, for both males and females.

Female platys showed better growth over time (p= 0.000) with respect to weight as illustrated in Figure 14. At the start of the study, the mean body weight of males (irrespective of treatments) was higher (p= 0.003). Males were 0.75 ±0.17 g while females were 0.71 ±0.15 g. However, mean female body weights were bigger than males (p= 0.000) at the end. Females were 1.60 ±0.32 g while males were 1.07 ±0.28 g.

3.2.5 Growth of goldfish over time

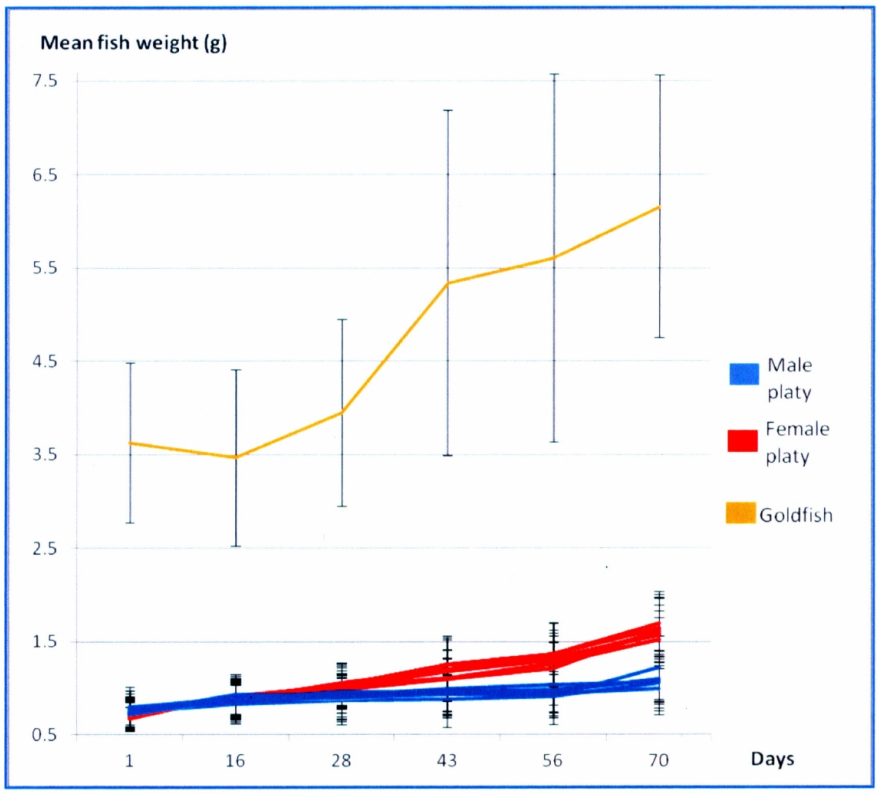

Figure 15. Goldfish weight over time compared to platys with mean ± standard deviation. Goldfish n= 40 per measurement time; platys n= 40 per measurement time per treatment for both males and females.

Figure 15 gives a visual account of the mean weight of goldfish compared to male and female platys over the 11-week study period. Initial and final weights of goldfish were, respectively, 3.63 ±0.85 g and 6.16 ±1.40 g.

3.3 FCR

Table 4 gives an account of total fish weight gain and FCR for all treatments. High FCR values have been obtained in each treatment relative to what is expected in aquaculture production.

Table 4. Weight gain and FCR for all treatments. The values for platys include combined data for both males and females, as each tank had half of each sex. The total feed fed per system is the cumulative total amount of feed fed during the course of the whole study. Designations explained in legend to Fig. 8.

Treatments	Initial total fish weight/system (g)	Final total fish weight/system (g)	Total weight gain/system (g)	Total feed fed/system (g)	FCR
D1P1	88.7	151.8	63.1	302.4	4.79
D2P1	116.0	209.1	93.1	403.2	4.33
D3P1	143.5	269.9	126.4	504.0	3.99
D1P2	88.1	158.4	70.3	302.4	4.30
D2P2	116.3	196.8	80.5	403.2	5.01
D3P2	144.1	267.7	123.6	504.0	4.08
Platy control	149.8	275.3	125.5	504.0	4.02
Goldfish	363.1	615.9	252.8	1008.0	3.99

3.4 Fish feeding compared to recommended

Table 5 describes the amount of feed applied to each system on a daily basis compared to what Rakocy et al. (2006) recommends for good plant production. It is notable that the amount of feed needed for good plant production is far less in the present study, than what is recommended.

Table 5. Total amount of feed fed per system per day compared to the recommended value for good plant production. The recommended value is with respect to the plant growing area per system, which was 0.8 m^2.

Weeks	Treatments	Amount of feed fed/system/day (g)	Recommended feeding/day (g)
1 - 2	0.75 platy/L	2.4	≥ 80
	1.0 platy/L	3.2	
	1.25 platy/L	4.0	
	goldfish	8.0	
3 - 6	0.75 platy/L	3.6	
	1.0 platy/L	4.8	
	1.25 platy/L	6.0	
	goldfish	12.0	
7 - 11	0.75 platy/L	4.8	
	1.0 platy/L	6.4	
	1.25 platy/L	8.0	
	goldfish	16.0	

3.5 Plant weight

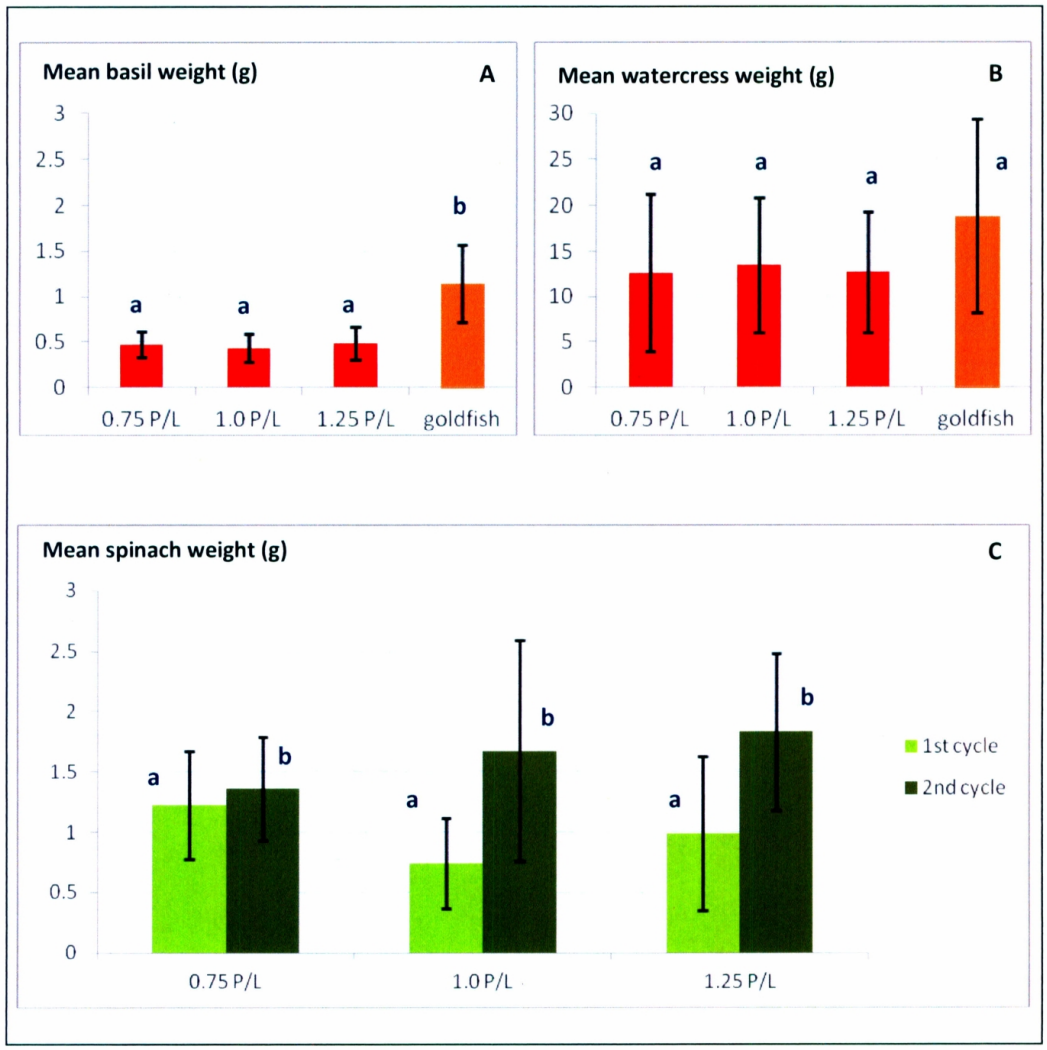

Figure 16. Mean plant weight for basil treatments at the end of the first plant cycle (A), mean plant weight for watercress treatments at the end of the second plant cycle (B) and mean plant weight for spinach at the end of the first and 2nd plant cycles (C) ± standard deviation. n for A= 12-16; n for B= 16; n for C= 11-16. 0.75 P/L= 0.75 platy/L, 1.0 P/L= 1.0 platy/L, 1.25 P/L= 1.25 platy/L, goldfish= 0.62 goldfish/L.

3.5.1 Basil weight at the end of the first plant cycle

A difference among the four treatments containing basil (p= 0.000) was observed in the first plant cycle (Figure 16 A). Following this, planned comparisons were set up to identify the differences. It was found that the mean plant weight in the goldfish treatment was higher than for platys (p= 0.000). Mean plant weight for basil with goldfish was 1.14 ±0.43 g while mean plant weight in the platy treatments was 0.47 ±0.16 g. Besides this, there was no difference in mean plant weight among the platy treatments (p= 0.863).

3.5.2 Watercress weight at the end of the second plant cycle

No difference in mean plant weights was observed among the four treatments with watercress (p= 0.130) in the second cycle. Mean plant weights for 0.75 platy/L, 1.0 platy/L, 1.25 platy/L treatments and goldfish were 12.6 ± 8.6 g, 13.5 ±7.4 g, 12.7 ±6.7 g and 18.8 ±10.6 g, respectively, as illustrated in Figure 16 B.

3.5.3 Spinach weight in both plant cycles

In the first spinach cycle, no difference in mean plant weights was observed among the three treatments (p= 0.071; generated by performing 1,500 ANOVA randomizations). Mean plant weights for 0.75 platy/L, 1.0 platy/L and 1.25 platy/L treatments were 1.22 ±0.45 g, 0.74 ±0.37 g and 0.99 ±0.64 g, respectively. The 1.0 platy/L-spinach data were corrected for one outlier, by performing the Grubb's test for the first plant cycle (p= 0.000). At the end of the second cycle, there was still no significant difference (p= 0.163) in mean plant weight for the treatments under spinach. Mean plant weights for 0.75

platy/L, 1.0 platy/L and 1.25 platy/L treatments were 1.36 ±0.43 g, 1.67 ±0.92 g and 1.83 ±0.65 g, respectively. However, spinach plants in the second cycle were bigger (p= 0.000) compared to the first cycle (Figure 16 C).

3.6 Root length

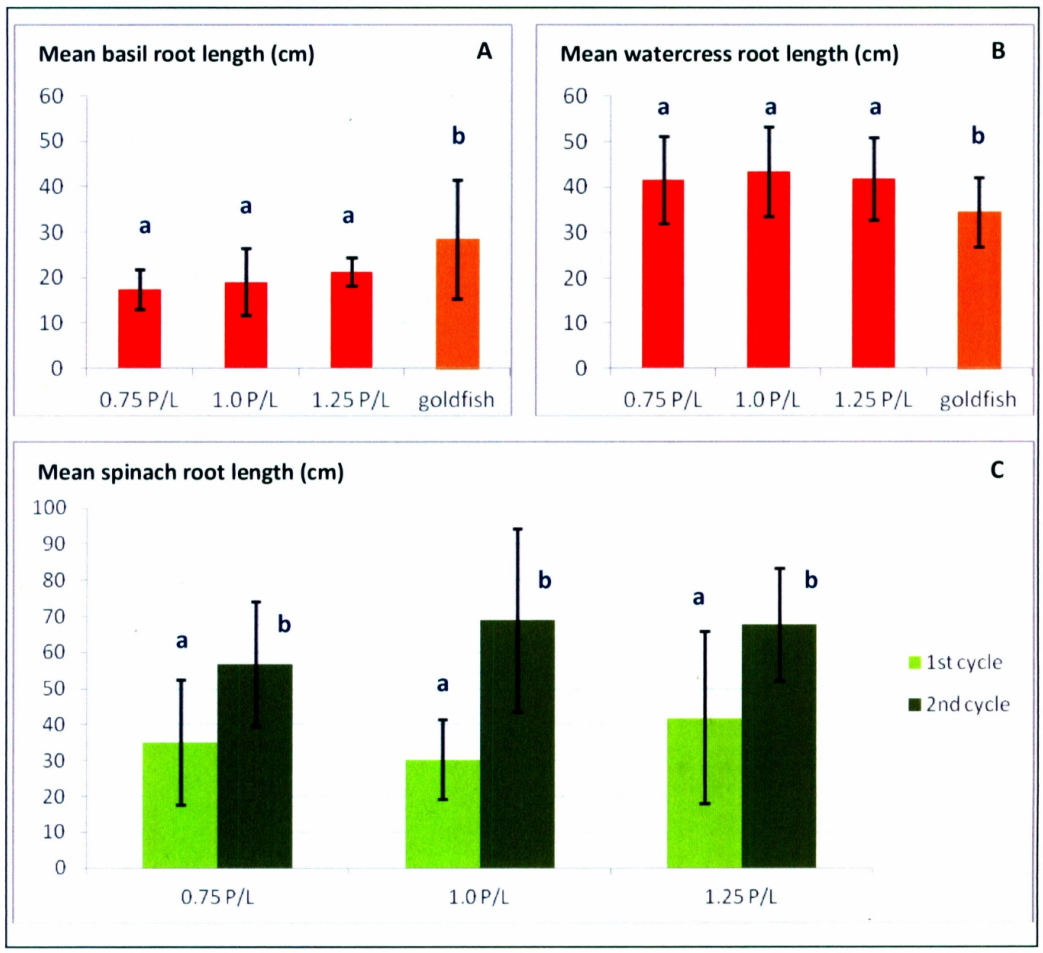

Figure 17. Mean root length for the basil treatments at the end of the first plant cycle (A), mean root length for watercress treatments at the end of the second plant cycle (B) and mean root length for spinach at the end of the first and 2nd plant cycles (C) with ± standard deviations. n for A= 12-16; n for B= 16; n for C= 9-16. Designations explained in legend to Fig. 16.

3.6.1 Root length of basil at the end of the first plant cycle

When an ANOVA was performed, a difference in the length of roots ($p= 0.002$) was observed among the basil treatments. But when goldfish was excluded in a planned comparison, there was no longer a difference ($p= 0.402$) between the basil treatments with platys (Figure 17 A), meaning that the root length of basil plants in goldfish was longer ($p= 0.000$). Basil with goldfish had a root length of 28.5 ±13.0 cm while the root of basil plants with the platys treatments were 19.3 ±5.2 cm.

3.6.2 Root length of watercress at the end of the second plant cycle

At the end of the second cycle, a difference ($p= 0.035$) in mean root length was observed among the four treatments for watercress (Figure 17 B). When goldfish was excluded in a planned comparison, no difference ($p= 0.822$) among the platy treatments was observed, meaning that the difference lay with goldfish. Root length of watercress plants with goldfish was hence found to be shorter ($p= 0.004$) compared to spinach plants in the three other watercress treatments with platys. Root length of watercress plants with goldfish was 34.5 ±7.7 cm compared to 42.3 ±9.4 cm for the platys.

3.6.3 Root length of spinach at the end of both plant cycles

At the end of the first plant cycle, no difference ($p= 0.362$) in the root length of spinach plants was observed. Mean root lengths for the 0.75 fish/L, 1.0 fish/L and 1.25 fish/L platy treatments were 35 ±17 cm, 30 ±11 cm and 42 ±24 cm, respectively. After the second plant cycle, still no difference ($p= 0.179$) was noted in the root length of basil plants in the three platy treatments. Mean root lengths for 0.75 fish/L, 1.0 fish/L and 1.25

fish/L platy treatments were 57 ±17 cm, 69 ±25 cm and 68 ±16 cm, respectively. However, the root length for spinach plants in the second plant cycle was longer (p= 0.000) compared to the first, as illustrated in Figure 17 C.

3.7 Shoot length

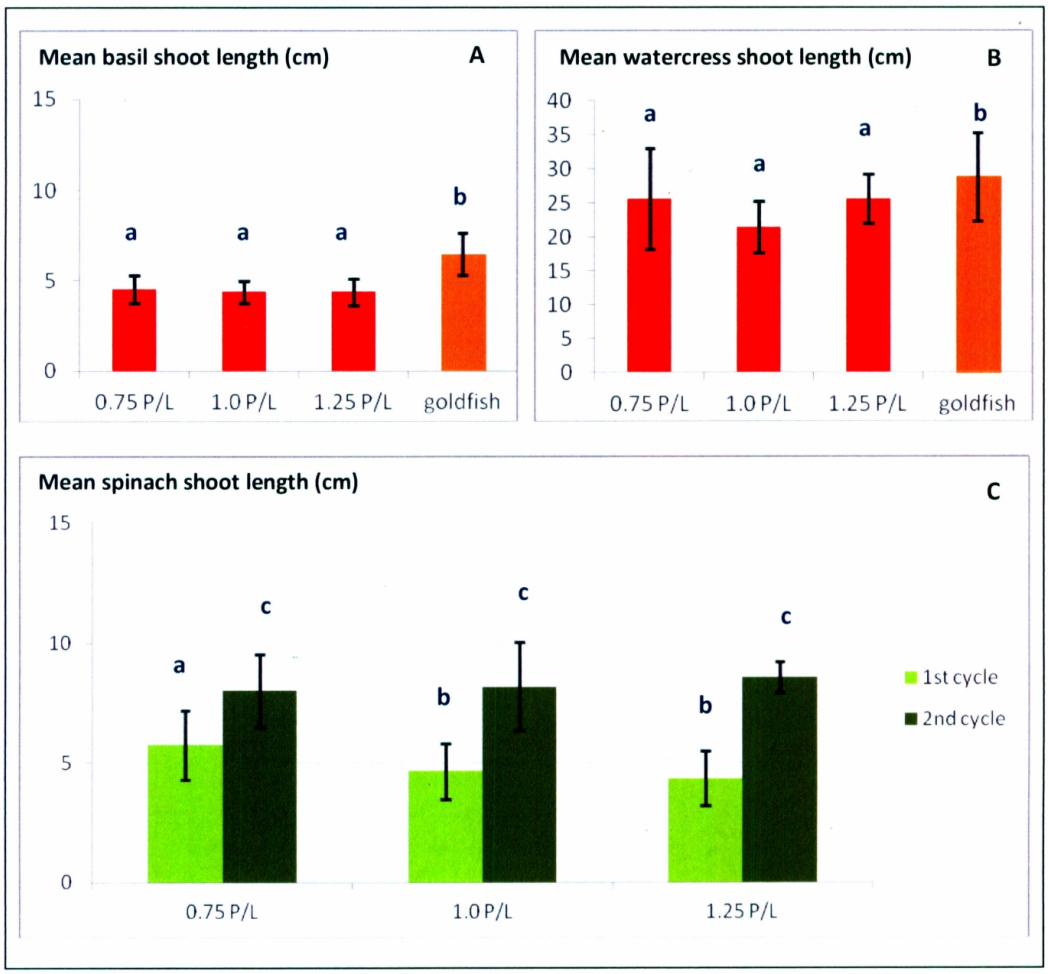

Figure 18. Mean shoot length for the basil treatments at the end of the first plant cycle (A), mean shoot length for watercress treatments at the end of the second plant cycle (B) and mean shoot length for spinach at the end of the first and 2nd plant cycles (C) ± standard deviation. n for A= 12-16; n for B= 16; n for C= 11-16. Designations explained in legend to Fig. 16.

3.7.1 Shoot length of basil at the end of the first plant cycle

A difference (p= 0.000) in shoot length was observed among the treatments with basil (Figure 18 A). However, when the goldfish treatment was excluded in a planned comparison, no difference (p= 0.862) was observed among the treatments with platys. Mean shoot length in the goldfish treatment was thus higher (p= 0.000) compared to the the platy treatment with basil. Mean shoot length in the goldfish treatment was 6.5 ±1.2 cm compared to 4.5 ±0.7 cm in the platys treatment.

3.7.2 Shoot length of watercress at the end of the second plant cycle

A difference (p= 0.006) was observed among the treatments with watercress at the end of the second plant cycle (Figure 18 B). But when the goldfish treatment was excluded in a planned comparison, no difference (p= 0.063) was observed among the treatments under platys. Mean shoot length in the goldfish treatment was thus higher (p= 0.006) with a length of 28.9 ±6.5 cm. Mean shoot length in the platy treatments was 24.3 ±5.5 cm.

3.7.3 Shoot length of spinach at the end of both plant cycles

A difference (p= 0.015) in shoot length among the spinach treatments was observed at the end of the first cycle. When the 0.75 platy/L treatment was excluded, no difference (p= 0.567) between the other two treatments was found. The 0.75 platy/L-spinach treatment was higher with a mean shoot length of 5.8 ±1.5 cm. The 1.0 platy/L and 1.25 platy/L-spinach treatments had mean shoot lengths of 4.6 ±1.2 cm and 4.4 ±1.1 cm, respectively.

No significant difference (p= 0.527) was observed in mean shoot length in the treatments with spinach at the end of the second plant cycle. Mean shoot lengths for the 0.75 fish/L, 1.0 fish/L and 1.25 fish/L platy treatments were 8.0 ±1.5 cm, 8.2 ±1.8 cm and 8.6 ±0.6 cm, respectively. When comparing between the two cycles, shoot lengths in the second cycle were longer (p= 0.000).

3.8 Water quality parameters

3.8.1 Water temperature in fish tanks

An ANCOVA did not fit the model and hence an ANOVA was performed. A difference (p= 0.000) was observed in water temperature of the fish tanks over time in all the treatments (Figure 19 A). Hence, separate ANOVAs were performed at specific time points which were judged to be critical. Temperature was assessed at four time points among the treatments. These were: at the start of the study, at the end of the first plant cycle, at the start of the second plant cycle and at the end of the study.

A significant difference (p= 0.000) in temperature was observed among all treatments at days 14, 49, 57 and 76. In the planned comparisons, water temperature in the goldfish treatment was higher (p= 0.000) compared to the platy treatments at days 14, 49 and 57. At day 76, no difference (p= 0.137) was noted.

When all the platy treatments were compared, a difference (p= 0.000) was found to be present at each of the four sampling points. Water temperature in the control treatment was higher than the other platy treatments at days 14 and 76 ($p \leq 0.001$). It was, however, lower (p= 0.000) at days 49 and 57.

When treatments under specific plants were compared, the treatments under basil showed a higher (p= 0.000) water temperature compared to those under spinach at day 14. But at day 49, no difference (p= 0.084) was noted. In the second plant cycle, at day 57, water temperature in the spinach treatments was higher (p= 0.000) compared to those under watercress. But at day 76, the inverse was observed where the water temperature in the watercress treatments was higher (p= 0.010) compared to those under watercress.

3.8.2 Dissolved oxygen in fish tanks

No significant difference (p= 0.194) in dissolved oxygen (% saturation) was observed among the eight treatments over time (Figure 19 B). An ANCOVA was performed and it fitted the model for the analysis. Dissolved oxygen (mg/L) also showed no difference (p= 0.504) over time among all treatments when an ANCOVA was performed. The trend is illustrated in Figure 19 C.

3.8.3 Water temperature in plant tanks

An ANCOVA did not fit the model here and hence an ANOVA was performed over time to test for significant differences in temperature among the treatments in the plant tanks. A difference (p= 0.000) was found to be present. Hence, differences were analysed at the four specific time points. A difference (p= 0.000) in water temperature was observed at each of days 14, 49, 57 and 76. Figure 20 A illustrates how water temperature varied over time in the plant tanks.

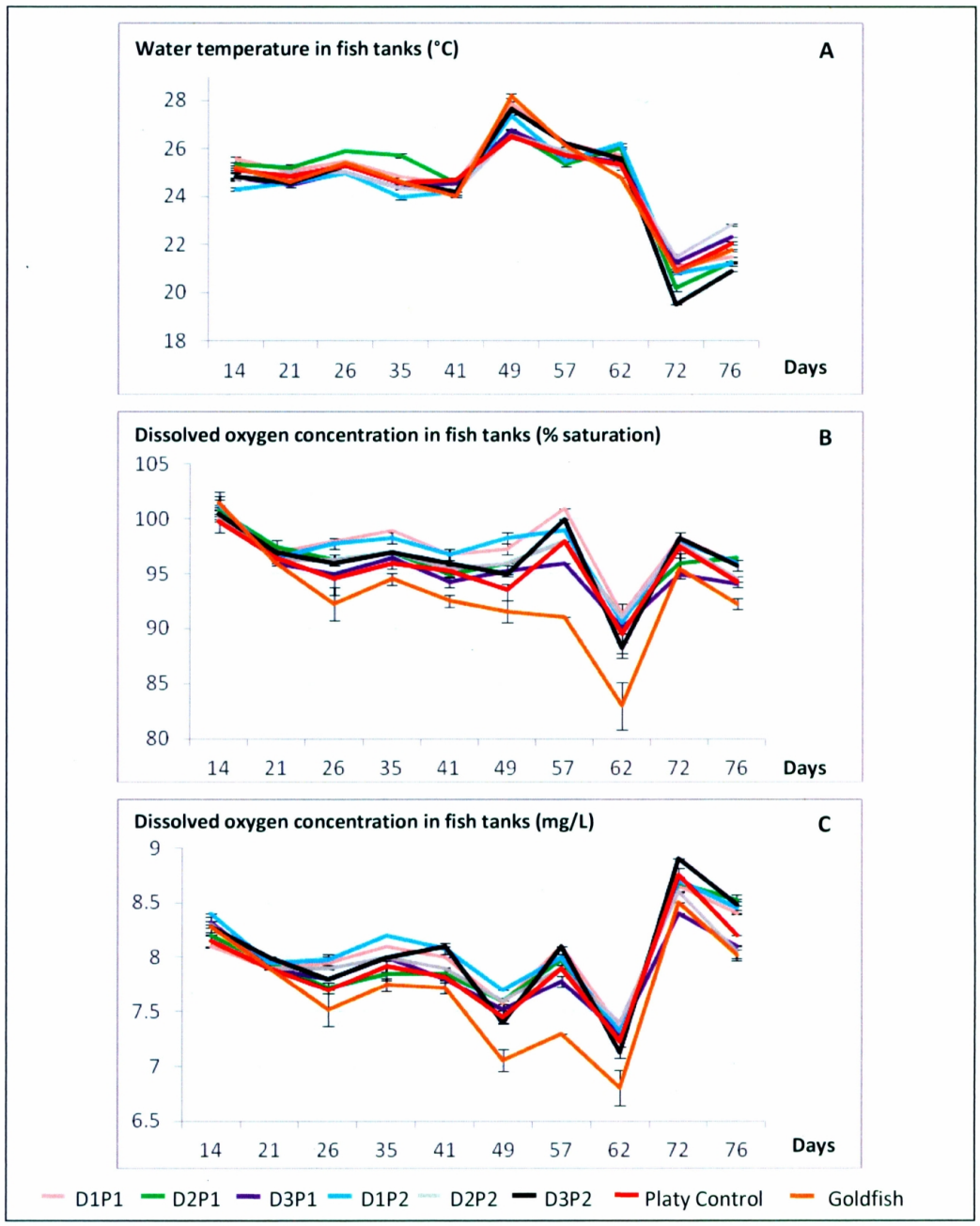

Figure 19. Water temperature (A), Dissolved oxygen - % saturation (B) and Dissolved oxygen - mg/L (C) in fish tanks over time with mean ± standard deviation. n for A, B and C= 4 per measurement time per treatment. Designations explained in legend to Fig. 8.

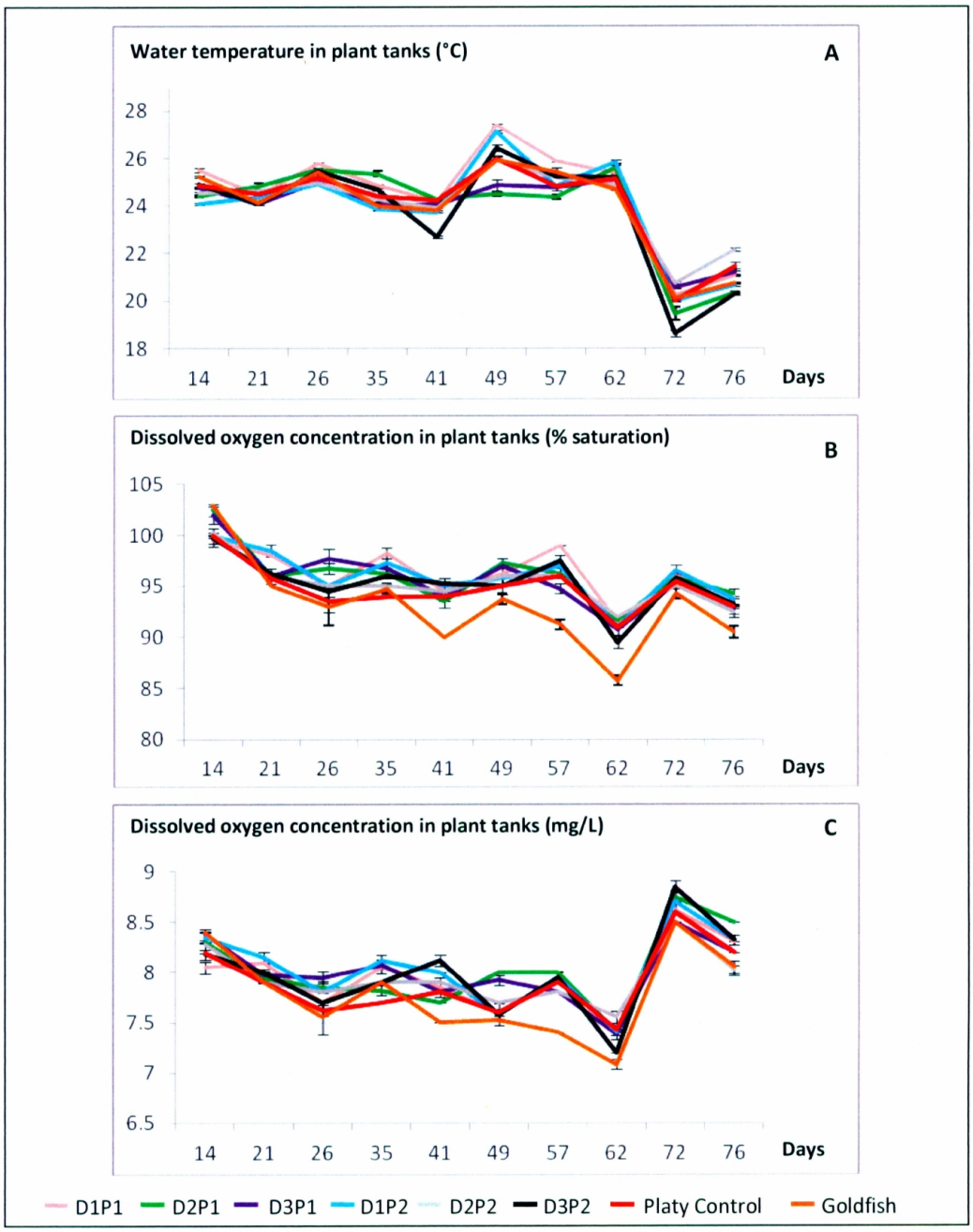

Figure 20. Water temperature (A), Dissolved oxygen - % saturation (B) and Dissolved oxygen - mg/L (C) in plant tanks over time with mean ± standard deviation. n for each of A, B and C= 4 per measurement time per treatment. Designations explained in legend to Fig. 8.

In the planned comparisons, the water temperature was noted to be higher ($p= 0.000$) in the goldfish treatment compared to the platys at each of days 14 and 57. However, at day 76, the inverse was observed where temperature in the goldfish treatment was lower ($p= 0.000$) compared to those under platys. At day 49, no difference was present ($p= 0.242$).

Comparing all the platy treatments, a difference ($p= 0.000$) was observed throughout each of the four specific time points. No difference was observed between the control treatment and other platy treatments at days 14 and 49 ($p\geq 0.361$). However, the control treatment had a higher ($p= 0.000$) water temperature at each of days 57 and 76.

When treatments under specific plants were compared, a non-homogeneous trend was again observed. Water temperature in the basil treatments was higher ($p= 0.000$) compared to spinach treatments at day 14. However, the inverse was noted at day 49 where the treatments under spinach was higher ($p= 0.000$). No difference between the treatments under watercress and the treatments under spinach was observed at day 57. However, at day 76, water temperature in watercress tanks was lower ($p= 0.000$) compared to that in spinach.

Furthermore, in three-way ANOVAs, differences in dissolved oxygen levels were investigated between fish and plant tanks. It was found that the fish tanks had a higher ($p= 0.000$) % saturation of dissolved oxygen level compared to the plant tanks but oxygen levels (mg/L) were higher ($p= 0.006$) in the plant tanks compared to the fish tanks. This is because the water temperature got cooler ($p= 0.000$) in the plant tanks when moving from the fish tanks due to low temperatures inside the greenhouse.

3.8.4 Dissolved oxygen in plant tanks

A significant difference ($p= 0.045$, generated by performing 3,000 ANCOVA randomizations) was observed in dissolved oxygen (% saturation) level over time among all treatments. However, when the goldfish treatment was excluded from the analysis, no difference ($p= 0.402$) was observed. Dissolved oxygen level was hence lower in the goldfish treatment throughout the study (Figure 20 B). Looking at the dissolved oxygen level (mg/L), no difference ($p= 0.475$) was observed among the treatments over time when an ANCOVA was again performed. Dissolved oxygen (mg/L) change over the time of the study is illustrated in Figure 20 C.

3.8.5 pH

Raw data were fixed into classes to carry out an ANOVA as an ANCOVA did not fit the model here. A difference ($p= 0.000$) in pH was observed among all treatments over the time of the study, even when the goldfish treatment was excluded from the analysis ($p= 0.000$). Hence, the data were again assessed at the four critical time points. An illustration of how pH changed with time is shown in Figure 21 A.

Differences ($p\leq 0.002$ at days 13, 50, 57 and 76) were observed among all treatments throughout the study. When performing planned comparisons, it was found that pH in the goldfish treatment was higher ($p= 0.004$) than the platy treatments only at the first sampling point. Otherwise, it was lower than the other platy treatments at days 50, 57 and 76 ($p\leq 0.001$).

Regarding the platy treatments, a difference was observed among all of them at days 13, 57 and 76 ($p\leq 0.009$). At day 50, no difference ($p= 0.245$) was noted. When the

control treatment was compared to the other platy treatments, no difference was found to be present at days 13 and 50. However, the control treatment had a lower pH at days 57 and 76 ($p= 0.000$).

Furthermore, in the first plant cycle, no difference was present between the basil and spinach treatments at days 13 and 50 ($p\geq 0.134$). In the second plant cycle, no difference in pH was noted at day 57 ($p= 0.057$) but at day 76, the pH in the spinach treatments was lower ($p= 0.000$) compared to those with watercress.

3.8.6 Ammonia concentration

3.8.6.1 Total ammonia-nitrogen

An ANOVA was performed to test for differences among the treatments over time, as an ANCOVA did not fit the model. A difference ($p= 0.000$) was observed in the total ammonia-nitrogen concentration with time, even when goldfish was excluded from the analysis ($p= 0.000$). Hence, four planned comparisons were set up to further analyze the raw data.

Total ammonia-nitrogen concentration in the goldfish treatment was higher ($p= 0.000$ at days 13, 48, 54, 76) compared to the platy treatments, throughout the study. In addition, there was a difference ($p\leq 0.002$ at days 13, 48, 54 and 76) among all the platy treatments throughout the study. The control was higher ($p= 0.000$) than the other platy treatments at days 13 and 54 but no differences ($p\geq 0.054$ at days 48 and 76) were observed at the other two time points. Comparing treatments for plants 1 and 2, at the first sampling point, no difference ($p= 0.082$) was observed between the basil and spinach treatments. The same occurred on day 76 where no difference ($p= 0.122$) was observed

between the watercress and spinach treatments. However, total ammonia-nitrogen was higher in the spinach treatments at days 48 and 54 ($p \leq 0.004$) compared to the basil and watercress treatments, respectively. An illustration of how total ammonia-nitrogen (TAN) concentration varied throughout the study is given in Figure 21 B.

3.8.6.2 Un-ionized ammonia-nitrogen

An ANOVA was performed to analyse the generated data for un-ionized ammonia-nitrogen (from total ammonia-nitrogen as a factor of pH and temperature). An ANCOVA did not fit the model. A difference ($p= 0.000$) was observed among all treatments, even when goldfish was excluded from the analysis ($p= 0.000$).

At day 13, a difference ($p= 0.000$) was present among all treatments. In the planned comparisons, un-ionized ammonia concentration in the goldfish treatment was higher ($p= 0.000$) compared to all the platys. Besides, a difference was observed ($p= 0.000$) among all the platy treatments and it was found that the control treatment was higher ($p= 0.000$) compared to the other platy treatments. However, no difference ($p= 0.866$) was present between the platy treatments with basil compared to those with spinach.

At day 48, a difference was noted among all treatments. In a planned comparison (error rate 2.5%) the goldfish treatment was higher ($p= 0.016$) compared to the other platy treatments. However, no difference was observed among the platy treatments ($p= 0.152$). At day 54, no differences ($p= 0.281$) were present among the treatments.

At the final sampling, a difference ($p= 0.000$) was present among all treatments. In the planned comparison, un-ionized ammonia-nitrogen concentration in the goldfish treatment was lower ($p= 0.000$) compared to the other platy treatments. Besides this, there

was a difference (p= 0.008) among all platy treatments and it was found that the control treatment had a lower (p= 0.001) un-ionized ammonia concentration compared to the other platy treatments. Further, there was no difference (p= 0.455) in un-ionized ammonia-nitrogen concentration between the treatments under watercress and those with spinach. Figure 21 C illustrates how un-ionized ammonia-nitrogen (UIA) varied with time in all the treatments.

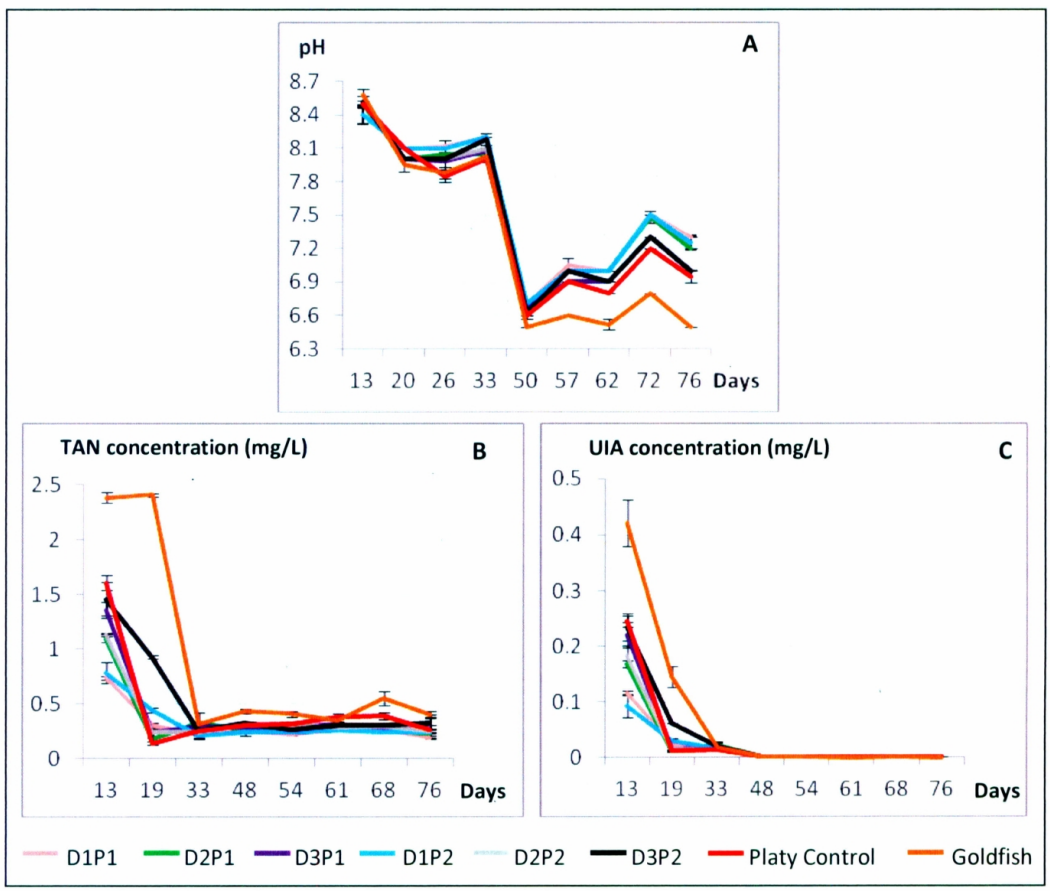

Figure 21. pH (A), Total ammonia-nitrogen concentration (B) and Un-ionized ammonia-nitrogen concentration (C) over time with mean ± standard deviation. n=4 per measurement time per treatment for all the parameters. Designations explained in legend to Fig. 8.

3.8.7 Electrical conductivity

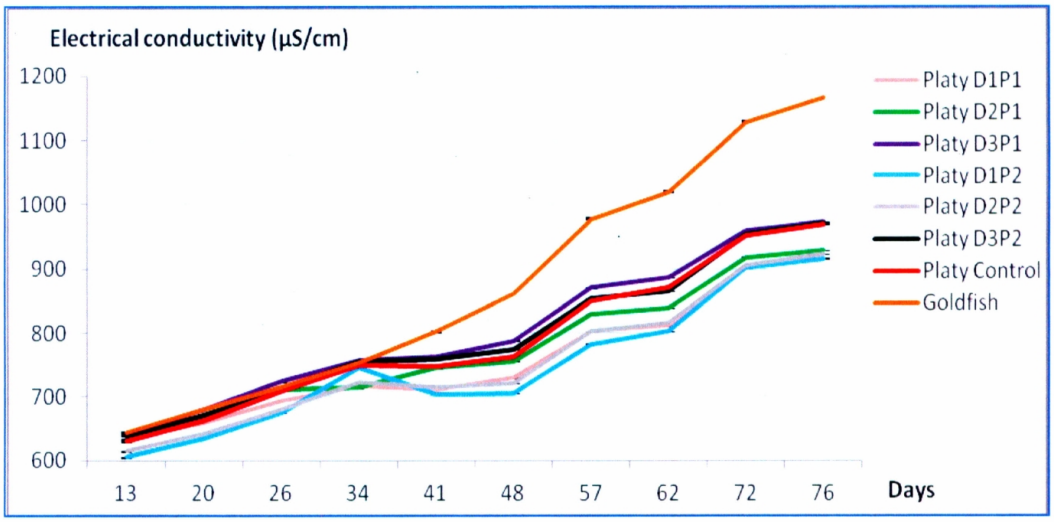

Figure 22. Electrical conductivity over time in all the treatments with mean ± standard deviation. n= 4 per measurement time per treatment. Designations explained in legend to Fig. 8.

A difference (p= 0.000) in electrical conductivity over time was observed among all treatments when an ANOVA was performed (an ANCOVA did not fit the model). Even when the goldfish treatment was excluded from the analysis, a difference (p= 0.000) among the platy treatments was still observed. Following this, assessment of electrical conductivity at the four critical time points was made. Differences (p= 0.000) were observed among the treatments at each of days 13, 48, 57 and 76. Figure 22 illustrates how electrical conductivity changed over time in all the treatments.

In the planned comparisons, it was found that electrical conductivity in the goldfish treatment was higher (p= 0.000) compared to the other platy treatments at each of days 13, 48, 57 and 76.

In addition, differences (p= 0.000) were observed among all the platy treatments at each of the four time points. Apart from day 13 where there was no difference (p= 0.015) between the control treatment and the other platy treatments, on days 48, 57 and 76, the control treatment had a higher (p= 0.000) electrical conductivity compared to the other platy treatments.

Moreover, it was noted in the first plant cycle that the electrical conductivity was higher (p= 0.000) in the basil treatments compared to those under spinach at each of days 13 and 48. In the second plant cycle, electrical conductivity in the watercress treatments was also higher (p= 0.000) than in the treatments under spinach at each of days 57 and 76.

Values for electrical conductivity at the end of the first and second plant cycles are illustrated in Table 6.

Table 6. Final electrical conductivity at plant harvest, at the end of the first and second plant cycles in all treatments.

Plant Cycle	Treatments		Mean electrical conductivity (µS/cm)
1st	Basil	0.75 platy/L	729.5
		1.0 platy/L	755.8
		1.25 platy/L	788.2
		goldfish	863.0
	Spinach	0.75 platy/L	706.0
		1.0 platy/L	720.5
		1.25 platy/L	774.8
	Control	1.25 platy/L	763.5
2nd	Watercress	0.75 platy/L	915.0
		1.0 platy/L	928.8
		1.25 platy/L	973.0
		goldfish	1165.8
	Spinach	0.75 platy/L	916.2
		1.0 platy/L	923.5
		1.25 platy/L	972.0
	Control	1.25 platy/L	969.8

3.8.8 Nitrate-nitrogen

An ANCOVA did not fit the model and hence an ANOVA was used to test for differences among the treatments over time. A significant difference ($p= 0.000$) was observed in nitrate-nitrogen concentration among all treatments, even when the goldfish treatment was excluded from the model ($p= 0.000$). Besides, a difference ($p= 0.000$) among all treatments was observed at each of days 13, 47, 54 and 75. Figure 23 A illustrates how nitrate concentration varied in all the treatments over time.

In the planned comparisons, no difference ($p= 0.839$) in the nitrate concentration was observed between the goldfish treatment and the other treatments at day 13. However, for the other three specific sampling points which followed, the nitrate concentration in the goldfish treatment was higher ($p= 0.000$) compared to the other treatments.

In addition, differences ($p= 0.000$) among the platy treatments were noted at each of days 13, 47, 54 and 75. At each of the sampling points, nitrate concentration in the control treatment was higher ($p= 0.000$) compared to the other platy treatments.

Finally, when comparing between specific plants, no definite trend was observed. In the first plant cycle, the nitrate concentration in the basil treatment was higher ($p= 0.008$) compared to those under spinach at day 13. But at day 47, the nitrate concentration in the spinach treatments was higher ($p= 0.001$). After the start of the second plant cycle (day 54), no difference ($p= 0.041$) was observed between the treatments under watercress and those under spinach. But at day 75, the nitrate concentration in the watercress treatments was lower ($p= 0.000$) than that in the spinach treatments.

3.8.9 Phosphate

Phosphate concentration in all the treatments was also analysed into two phases, namely before acid addition (day 15 to day 34) and after acid addition (day 49 to day 76). Acid was added at day 34 to lower the pH.

Before acid was added, a significant difference ($p= 0.000$) was observed among the treatments when an ANCOVA was performed. Hence, further analyses were performed at day 15 and day 34 to have a better understanding of the data. At day 15, no difference ($p= 0.141$) in phosphate concentration was observed among the treatments. A difference ($p= 0.000$) was, however, present at day 34. In the planned comparisons, phosphate concentration in the goldfish treatment was found to be higher ($p= 0.000$) than in the platy treatments. Besides this, there was a difference ($p= 0.001$) among all the platy treatments. There was, however, no difference ($p= 0.124$) between the control treatment and the other platy treatments. Moreover, no difference ($p= 0.407$) was present between the treatments under basil and spinach.

After acid was added, a difference ($p= 0.000$) in phosphate concentration was observed among all treatments when an ANOVA was performed over time (an ANCOVA did not fit the model).

At day 49, a difference ($p= 0.000$) in phosphate concentration was present among all treatments. In the four planned comparisons, there was no difference ($p= 0.037$) in the concentration of phosphate between the goldfish and platy treatments. There was, however, a difference ($p= 0.000$) among all the platy treatments. Phosphate concentration in the control treatment was higher ($p= 0.009$) than the other platy treatments but there was no difference ($p= 0.511$) between the basil and the spinach treatments.

At the end of the study, a difference ($p= 0.048$, generated by performing 3000 ANOVA randomizations) in phosphate concentration was still present among all treatments. In the planned comparisons (error rate= 2.5%), it was found that the goldfish treatment had a higher phosphate level ($p= 0.011$) compared to the platy treatments and no difference was present among the platy treatments ($p\geq 0.207$).

Phosphate concentration over time for all treatments is illustrated in Figure 23 B.

3.8.10 Potassium

Potassium concentration data was analysed in two phases, namely, a period before base addition (day 15 to day 49) and a period after the start of base addition (day 49 to day 76). Potassium hydroxide was added at day 50 but for statistical analysis purposes, the second phase was started at the fourth sampling point (day 49) to assess the trend.

Before the base was added, a difference ($p= 0.000$) in the level of potassium was noted among the treatments, when an ANOVA was performed over time. In the planned comparisons, potassium concentration in the goldfish treatment was found to be higher ($p= 0.000$) at each of days 15 and 49.

In addition, a difference among all the platy treatments was present at both day 15 ($p= 0.0117$) and day 49 ($p= 0.000$). There was no difference in potassium concentration at days 15 and 49 ($p\geq 0.086$).

However, when comparing between treatments under the two specific plants, at day 49, the potassium concentration in the basil treatments was found to be higher ($p= 0.000$) than in spinach treatments. At day 15, no difference ($p= 0.708$) was present.

Figure 23. Nitrate-nitrogen (A), Phosphate (B) and Potassium (C) concentrations in all treatments over time with mean ± standard deviation. n= 4 per measurement time per treatment for all the parameters. Designations explained in legend to Fig. 8.

After the base was added, no significant difference ($p= 0.795$) in potassium concentration was observed over time in the treatments. An ANCOVA did not fit the model and hence an ANOVA over time was performed here. Figure 23 C illustrates how potassium concentration changed over time in all the treatments.

3.9 Economic advantage of ornamental fish rearing

Tables 7, 8 and 9 compare the return of ornamental fish in an aquaponic system relative to a food fish such as tilapia. The *Rakocy Model* of aquaponic production has been used here as a reference for the calculations.

Tables 7 and 8 compare the wholesale and retail returns per year, respectively, between tilapia, goldfish and platys if the fish were to be produced in one tank in an aquaponic system. Table 9 compares the return per year between goldfish and tilapia if both species were used as sole species in all four tanks of 7.8 m^3 each, in the Rakocy aquaponic system.

Table 7. Hypothetical gross economic returns per tank between tilapia, goldfish and platys, in the UVI aquaponic system. *The selling price for tilapia (whole, fresh) is retail price, as projected by Lester Farms Inc. Prices for goldfish and platys are expected wholesale selling price based on current market prices. The unit selling price for tilapia is per pound of fish while for goldfish and platys, it refers to unit price per fish.

Wholesale economic returns by species per tank					
Species	**Stocking density (fish/m^3)**	**Number of cycles/year**	**Harvest/ tank/cycle (lbs or fish)**	**Unit selling price ($)**	**Return/tank/year ($)**
Tilapia	77	2	1,056 lbs	6.00*	12,672
Common goldfish	3,125	2	24,375 fish	0.50	24,375
Exotic goldfish	3,125	2	24,375 fish	5.00	243,750
Platys	2,000	3	15,600 fish	0.60	28,080

Table 8. Hypothetical gross economic returns per tank between tilapia, goldfish and platys, in the UVI aquaponic system. All unit selling prices are retail prices. The unit selling price for tilapia (whole, fresh) is per pound of fish while for goldfish and platys, it refers to unit price per fish.

Retail economic returns by species per tank					
Species	**Stocking density (fish/m^3)**	**Number of cycles/year**	**Harvest/ tank/cycle (lbs or fish)**	**Unit selling price ($)**	**Return/tank /year ($)**
Tilapia	77	2	1,056 lbs	6.00	12,672
Common goldfish	3,125	2	24,375 fish	1.75	85,312
Platys	2,000	3	15,600 fish	2.00	93,600

Table 9. Hypothetical gross economic returns between tilapia and goldfish as sole species in the UVI aquaponic system. *The price for tilapia (whole, fresh) is retail price per pound of fish as projected by Lester Farms Inc. ^The price for goldfish is wholesale price per unit fish based on current market prices.

Economic returns by species, when used alone in aquaponics					
Species	**Stocking density (fish/m^3)**	**Number of cycles/year**	**Total harvest/ cycle (lbs or fish)**	**Unit selling price ($)**	**Total returns/ year ($)**
Tilapia	77	2	4,224 lbs	6.00*	50,688
Common goldfish	3,125	2	97,500 fish	0.50^	97,500

Note: All prices indicated in the Tables are in Canadian dollars.

CHAPTER 4: DISCUSSION

4.1 Fish growth

Regarding weight gain, females in the 1.25 platy/L-spinach treatment (D3P2) showed slightly better growth (weight gain) than the other treatments, although no difference ($p= 0.818$) in weight among all females was present at the start of the study. Being closer to the greenhouse plastic covering, the fish tanks in this treatment compared to the tanks in the other treatments, might have been exposed to more sunlight since more algal growth was observed on surfaces. As *Xiphophorus* sp. are omnivorous (Tamaru et al., 1996; Froese and Pauly, 2011), the female platys in this treatment have probably supplemented their diet more with proliferating algae than female platys in other treatments. As females characteristically show better growth than males after the juvenile stage (Kallman and Borkoski, 1977; Froese and Pauly, 2011), this small diet supplementation might have been more effective in the females. Males in the same 1.25 platy/L-spinach treatment showed similar growth to males in other treatments. The above theory is further supported when looking at different water quality parameters, where the 1.25 platy/L-spinach treatment does not seem to differ from the other treatments.

It should be noted that male platys were bigger than females at the start of the study. But with time, females grew bigger and better than males. Kallman and Borkoski (1977) pointed out in their paper that regardless of genotype, female and immature male platys have the same growth rate. As the male grows, the growth rate declines acutely under the influence of androgenic hormones, hence explaining why the graphs crossed each other in Figures 10 and 14.

Typical stocking densities for *Xiphophorus* sp. in commercial intensive recirculating tank production is between 1.0 and 2.0 fish/L, where maximum survival rate is achieved as well as optimum water quality (Tamaru et al., 1996). For the purpose of this study and due to budget constraints, three culture densities of platys were chosen, namely 0.75 fish/L, 1.0 fish/L and 1.25 fish/L. The maximum stocking density in this study falls well under the maximum acceptable 2.0 fish/L for commercial production. The main concern when considering stocking densities is water quality. In aquaponic systems, water treatment is enhanced by the important role played by plants in the uptake of nutrients and possible harmful components such as un-ionized ammonia, directly via the root systems (Savidov, 2004; Savidov, 2005; Rakocy et al., 2006). This presents an advantage where no water exchange is needed compared to recirculating aquaculture systems. A grow-out density higher than 2.0 fish/L could be well achieved for platys in aquaponics. An interesting fact to note is that the fish in the control platy system (highest fish density and no plants) also showed similar growth patterns compared to the other treatments. Growth rates of fish were not suppressed, confirming the fact that the highest density used in this study was far lower than the maximum tolerable by the systems.

Regarding commercially available feeds for ornamentals, there is a lack of information for consumers. On the majority of feed bags, guidelines for feeding are just given as: 'feed two to three times daily the amount of feed your fish will consume in five minutes', which is unusable in the setting up of scientific feeding charts for growth trials. Concerning the *Blackwater Gold-N® professional* feed used in this study, no feeding instruction was present either on the feed bag or on the manufacturer's website. This is

why a special feeding regime was devised for this study. Based on fish feeding behavior and belly size after feeding, feed amounts were adjusted to finally give the feeding chart described in Table 1. The fish grew well under this regime over the 70 days of the growth study. The female platys had more than doubled in weight at the end, when compared to their initial weights. Overall, no apparent health problems were noted and the fish showed brilliant colouration throughout. This could be attributed to the fact that the feed used is one containing colour-enhancing ingredients and probiotics which increase growth, luster and boost the immune system of the fish (as described by the manufacturer). All the more, the platys were reproducing and readily producing fry in the fish tanks, suggesting that the feed and culture conditions were adequate for the species.

Uncharacteristically high values of feed conversion ratio (FCR) have been obtained for each of the systems (Table 4). This might be explained by the fact that some feed was lost from the tank outflows whenever the fish were fed. The design of the fish tanks did not allow the use of centre drains. Thus, overflow pipes were used, which caused some of the floating feed to be lost during feeding. While the platys seemed to be at the maximum amount of feed they could absorb per day, the goldfish, however, could have taken in more feed. But the amount was not increased because the goldfish treatment was fed exactly twice the daily ration as compared to the highest platy density, and this practice was maintained throughout the study. Judging by the feeding behaviour of the goldfish, they could easily have consumed all the feed delivered (actual ingestion plus feed lost through the tank overflows). Moreover, the lost feed did not deplete dissolved oxygen levels (Section 4.3) and might possibly have enhanced the nutrient loads in all systems, as

uneaten feed also contributes to nutrient generation for the plants in an aquaponic system (Rakocy et al., 2006). Furthermore, as growth rates did not differ among the platy treatments, it might be expected that the fish had practically the same FCR and feed efficiency in all the platy systems.

The above explanations are further confirmed when looking at theoretical values for the production of total ammonia-nitrogen in each system (Appendix 1). Apart from the first total-ammonia nitrogen sampling, all other sampling points showed far lower values in the systems when compared to the theoretical values. The first possible reason is supported by the fact that not all feed supplied into the system were consumed by the fish, as already indicated by the FCR (because not all feed fed were digested). Secondly, nitrification by colonizing nitrifying bacteria coupled with direct root uptake contributed to keeping the levels of total-ammonia nitrogen at lower than expected in aquaculture systems (Savidov, 2004). In the goldfish treatment for example, the treatment efficiency of total-ammonia nitrogen by the system was calculated to be 92.4% (at the end of the study).

4.2 Plant yield and productivity

Basil, which is a medium-nutrient requirement plant needing nutrient solution concentrations of 1000 to 2000 µS/cm (Savidov, 2004; Savidov 2005), showed better growth in the goldfish treatment compared to the other three platy densities ($p = 0.000$; for plant weight, root length and shoot length). In addition, the majority of the water quality parameters were higher in the goldfish treatment when compared to the other platy

treatments (pH was, however, lower). All these differences can be attributed to the fact that the goldfish treatment, having a higher fish biomass, received a higher feeding regime compared to the platy treatments. The goldfish treatment had half the density of fish compared to the highest density of platys but it received twice the feeding rate. Rakocy et al. (2006) described that the amount of feed input in an aquaponics system is what governs the nutrient load in the system. A higher feeding rate allows for better plant productivity.

Although the basil in the goldfish treatment showed better growth, productivity was far lower than values achieved at the Crop Diversification Centre South in Alberta. Highest basil productivity in the current study was 0.24 $kg/m^2/yr$ (goldfish treatment) while in Alberta, basil productivity reached 42.0 $kg/m^2/yr$. This can be attributed to three main reasons, namely: very low levels of nutrients as production was only at the start, nutrient levels were low because there was lower input from the ornamental fish used in this study compared to a bigger species like tilapia, and temperatures were too low for basil as early November was reached when the first plant cycle was over.

Regarding spinach, no differences were observed in plant weight ($p \geq 0.071$) and root length ($p \geq 0.179$) among the three platy densities in the two cycles. However, the shoot length in the 0.75 platy/L treatment was longer ($p = 0.015$) compared to the other two densities. With no differences in mean plant weight and root length, the longer shoot length in the 0.75 platy/L-spinach treatment could be attributed to the fact that the plants in this treatment produced an adaptive response to the very low nutrient levels at this point in time by elongating their shoots. The electrical conductivity of 706 µS/cm at the

end of the first plant cycle, was the lowest recorded in this treatment as compared to the other treatments. After having reached their maximum root extension capacity, the spinach plants in this treatment have probably started to elongate their stems in searching to capture more energy for growth.

Moreover, all three parameters showed significant increases in the second plant cycle compared to the first ($p= 0.000$ for each of plant weight, root length and shoot length). Overall, spinach productivity remained low with the highest productivity being only 0.48 $kg/m^2/yr$ in the 1.25 platy/L treatment compared to around 18.0 $kg/m^2/yr$ in Alberta. Low nutrient levels, lack of sunshine and cool temperatures inside the greenhouse were the three main reasons for this poor spinach productivity.

Electrical conductivity is a direct measure of nutrient solution concentration used in aquaponic systems (Rakocy et al., 2004b; Savidov, 2004; Savidov, 2005; Rakocy et al., 2006). For both basil and spinach, recommended levels of nutrients range between 1000 to 2000 µS/cm (Savidov, 2004). This was never achieved in the platy treatments, not even at the end.

The electrical conductivity values were, however, adequate for the production of watercress, as indicated by Savidov (2004). Watercress is a low-nutrient requirement plant needing a nutrient solution concentration having an electrical conductivity ranging from 200 to 1000 µS/cm. Besides, it is cold temperature tolerant (McHugh et al., 1987; McHugh and Constantinides, 2004). As a result, even if the goldfish treatment had bigger watercress plants, no significant difference ($p= 0.130$) was observed in mean plant weight among the treatments (Figure 16 B). The shoot length was, however, longer ($p= 0.006$)

and it was very interesting to note that the roots were shorter ($p= 0.035$) in watercress plants with the goldfish treatment. As noted in Table 6, the electrical conductivity in the goldfish treatment was over that recommended for watercress production. The plants could have adapted themselves to shorten their roots as there was no need to over-extend them since nutrients were readily available for growth (Lopez-Bucio et al., 2003; Gojon et al., 2009).

The productivity of watercress in the goldfish treatment was 4.9 $kg/m^2/yr$ while in the platy treatment having the highest yield (0.75 fish/L), the productivity of watercress was 3.5 $kg/m^2/yr$. In Alberta, productivity of watercress reached around 9.0 $kg/m^2/yr$. The lower productivity of watercress obtained in this study may be attributed to the lack of sunlight and too low temperatures at this time of the year. During culture periods where the plants could benefit from a few days of continuous sunlight, a fast change in the growth could be visually noted. Had artificial lighting and supplementary heating been used, the production of watercress in this study could have been matched with that in Alberta. In addition, plant culture density used in this study was lower (20 plants/m^2 *vs* 31 plants/m^2 in Alberta). In a nut shell, the nutrient content of all the treatments with watercress was adequate for watercress production and the watercress was overall growing very well with lush green leaves (Appendix 2), suggesting no nutrient limitation.

The levels of nutrients in the treatments were thus adequate for the production of low-nutrient requirement plants. The production of a medium-requirement plant could not be sustained as spinach did not grow well even in the second cycle. Apart from too cold temperatures, the electrical conductivity data at the end of the study (76 days) shows

clearly that nutrient contents were too low for the production of medium-nutrient requirement plants. The ratio of fish water volume to plant water volume in the present study was 1: 1.4 while Rakocy et al. (2006) had a ratio of 1: 2.2. Hence, a more concentrated nutrient solution should have been expected in this study, which was not the case due to a lower feeding rate. In the Alberta system, good plant production was achieved after two months of the system running (Savidov, 2004) which was not achieved after a two-month period in the present study. Hence, small ornamentals like platys could possibly only sustain the production of low-nutrient requirement plants. The same results would be expected for other small species of common ornamental fish such as guppies (*Poecilia reticulata*), mollies (*Poecilia latipinna*), swordtails (*Xiphophorus hellerii*) and others.

However, at the end of the second plant cycle, the electrical conductivity in the goldfish treatment was still rising at a good rate compared to the nearly stabilized platy treatments (Figure 22). Further study is needed to assess how medium-requirement plants would perform under the culture of a medium-size ornamental fish such as the goldfish, for a longer period of time with supplementary lighting and greenhouse heating, in the same set-up (same system design and fish density).

4.3 Water quality parameters

Total ammonia-nitrogen levels in the goldfish treatment remained higher ($p= 0.000$) than the other platy treatments throughout the study. The higher feed input into this system could explain this. In addition, the goldfish treatment showed a good production

of nitrate-nitrogen and at the end, values were still rising sharply compared to other treatments which were levelling off at that time (Figure 23 A). Had the study been continued, desired concentrations of around 250 mg/L (Savidov, 2004), could have been reached. However, the platy treatments would have never attained required concentrations for good plant production, emphasizing on the fact that small ornamental fish are not suitable for commercial aquaponics production of medium-nutrient requirement plants.

After oxygen, ammonia-nitrogen is the second most critical factor which can affect fish negatively. When present in small amounts, ammonia causes stress which can lead to the damage of gills and other body tissues. If duration persists, fish become more susceptible to bacterial infections, show poor growth and a decreased tolerance to routine handling (Francis-Floyd et al., 2009). At the end of the study, un-ionized ammonia-nitrogen levels were far lower in all systems compared to the maximum of 0.05 mg/L recommended in aquaculture systems (Timmons et al., 2002; Francis-Floyd et al., 2009). This corroborates the findings of Savidov (2004) and supports the statement that aquaponics 'provides one of the best water quality controls in the aquaculture industry'. Apart from the first sampling period, un-ionized ammonia-nitrogen levels remained well under the maximum lethal limits in all treatments once effective nitrification had been established, between day 19 and 33 (Figure 21 C). At the start, the levels were high because higher pHs were present. A higher pH favours a higher percentage of un-ionized ammonia out of total ammonia (un-ionized + ionized) in culture water (Emerson et al., 1975; Boyd, 1982; Durborow et al., 1997; Parker, 2000; Canadian Environment Protection Act 1999, 2001; Hargreaves and Tucker, 2004; Francis-Floyd et al., 2009). For

every unit increase in pH, the amount of un-ionized ammonia increases 10 fold (Durborow *et al.*, 1997).

Regarding phosphate levels at the end of the study, they were not far from the 60 mg/L desired concentration (Savidov, 2004) in all treatments. Statistical analyses showed no difference among the platy treatments ($p \geq 0.207$) but levels in the goldfish treatment were, however, higher ($p = 0.011$). Phosphoric acid was added to lower the pH in all the treatments as from day 34 because the pH resisted change and remained high. The same was observed in Alberta where preliminary analysis showed a high pH of 8.6 in the aquaponic system (Savidov, 2004). The gradual addition of the same amounts of acid to all systems directly inputted phosphate which explains the sudden rise in the phosphate level seen in Figure 23 B after day 34. Following acid addition, the systems then reacted normally where pH decreased with time due to biofiltration activity, fish growth and respiration, and plant root respiration (Buttner et al., 1993; Lewbart and Harms, 2008; Tucker and D'Abramo, 2008). As a pH of 7.0 is desired in aquaponic systems for optimum balance between nutrient uptake and efficient biofiltration (Savidov, 2004; Savidov, 2005; Rakocy et al., 2006), potassium hydroxide solution was then added as from day 50, to increase the pH. This is why the potassium levels rose sharply as from day 50. The concentrations in each system remained, however, very low compared to the potassium level achieved in the Alberta system (Savidov, 2004). Potassium reached a concentration of 440 mg/L in Alberta while in the present study, the maximum level achieved at day 76 was 72.5 ±5.0 mg/L in the 1.25 platy/L-spinach treatment. Fish feed are very limiting in potassium (Savidov, 2004; Savidov, 2005; Rakocy et al., 2006).

Besides, the feeding regime in this study was very low. This has two implications: first, less potassium is available via the feed and secondly, the pH did not drop naturally enough to add sufficient amounts of potassium hydroxide as a good supplementation. Small fish add less pressure to water quality regarding water acidification as feed application, fish respiration and nitrifying activity are lower when compared to a big species like tilapia. This is confirmed when looking at the pH profile for the goldfish treatment. The lower pH in the goldfish treatment (Figure 21 A) suggests possibly more fish respiration taking place as well as more nitrifying activity under a higher fish biomass. In addition, more total ammonia-nitrogen was present in the system, which was subsequently converted to nitrate and hence liberated more H^+ ions, compared to the other platy treatments.

Regarding dissolved oxygen levels, they remained above the acceptable levels of 80% saturation and 6 to 7 mg/L (Rakocy et al., 2006), throughout the study, as shown in Figures 19 and 20. Hence, plant roots were not stressed as there was no oxygen limitation in the treatments.

Finally, looking at the water temperature in both fish and plant tanks, it was noticed that it varied with time, although the temperature in all the systems was set to 25°C. On days where the sun was shining, temperatures inside the greenhouse increased dramatically which heated the water above the set-point. A difference was noted among all treatments as described in Sections 3.8.1 and 3.8.3. This may be due to different positioning of the systems inside the greenhouse whereby one system was getting more sunlight than the other. Moreover, at the end of the study, there was considerable loss of

heat from the systems as indicated by the low temperatures in Figures 19 and 20. Air temperatures went below freezing during night time and although very powerful heaters were used, heat loss from the systems could not be prevented and water temperatures went down substantially.

4.4 Feeding regime

As the ornamental fish used in this study were much smaller compared to a common species for aquaponics such as tilapia, the feeding regime adopted was also comparatively much lower. In the *Rakocy Model*, it has been described that what drives good production of plants in an aquaponic system is the amount of feed being inputted on a daily basis. To be able to sustain good and healthy plant productivity, a feeding ratio of 60 to 100 g of fish feed to one m^2 of plant growing area has been recommended by Rakocy et al. (2006). However, only staggered production is sustainable at this feeding ratio because batch production has too big a nutrient pressure on a system, resulting in unmarketable produce (Rakocy et al., 2004a). It has thus been suggested that a higher feeding regime could be applied to have better production (Rakocy et al., 2004a; Rakocy et al. 2004b; Rakocy et al., 2006).

In the present study, the feed input in the systems was far lower than recommended for good plant production as indicated in Table 5. If we scale up the proper density of platys to achieve a feeding rate of 80 g of feed applied, we would end up with a stocking density of 12.5 fish/L, which is unfeasible. The maximum recommended density for *Xiphophorus* sp. is 2.0 fish/L (although a higher density could be achieved in aquaponics,

as indicated in Section 4.1). Tamaru et al. (1996) showed that as fish culture density increases, fish survival starts to decline sharply. Further, if the Rakocy system was stocked with only platys, a total of 62,400 could be cultured at 2.0 fish/L in four fish tanks of 7800 L each. These fish would be fed 2,496 g of fish feed at the end of the 11 week period hence being able to sustain only 24.96 m^2 of plant growing area. This is far less than the total plant growing area of 214 m^2, which tilapia is able to sustain in the same fish tank volume in the Rakocy system (Rakocy et al., 2006). Besides, the feeding rate here is an over-estimate as it is based on the feeding regime for platys in this study. Hence, with the feeding rate being adjusted, less plant growing area would be possible. Platys alone would thus not be able to sustain good plant production when used as a sole species in aquaponics, even under optimum greenhouse environmental conditions.

When scaling up the density of goldfish, it reaches a density of 3.12 fish/L. By considering goldfish biomass, this will result in a density of 19.4 kg/m^3 (for the mean final goldfish weight of 6.2 g). In a study performed in 1999, Feldlite and Milstein recommended a stocking density of 2222 goldfish fry/m^3 in earthen pond production, where limited water treatment would be expected. Fry reached 3 g at marketable size. With adequate water treatment as in aquaponic systems, the goldfish density of 3.12 fish/L could possibly be applied. Rakocy et al. (2006) described a harvest weight of 61.5 kg/m^3 for tilapia. The system could thus easily tolerate goldfish at the 19.4 kg/m^3 stocking density. Besides this, goldfish are very hardy species (Hargrove and Hargrove, 2006).

Considering the Rakocy system, the four tanks would accommodate a total of 97,500 goldfish. This biomass represents a total feeding regime of 15.6 kg of fish

feed/day (or 2.6% bodyweight) and a potential plant growing area of 156 m^2. As mentioned in Section 4.1, the goldfish in this study could have taken in more feed and hence the total feeding regime mentioned above would be more or less a good estimate. Goldfish alone could hypothetically be used as a sole species in a commercial aquaponics system. Further studies should be carried out to test the productivity of different plants at different goldfish densities/feeding rations under optimal greenhouse environmental conditions, and also how the goldfish will perform at high stocking densities.

4.5 Economic advantage of ornamental fish rearing

As indicated earlier, a platy density of 2.0 fish/L and goldfish at 3.12 fish/L are densities which could be applied in the grow-out of these ornamentals in an aquaponic system. Based on these, comparative economic analyses have been performed.

Comparing platys, goldfish and tilapia, the much higher potential value of the ornamental fish at sale clearly comes forward. Ornamental fish bring much higher revenues per m^3 of culture water, even at very low wholesale prices, as compared to the retail price of tilapia (Table 7). If retail price to retail price was compared, the figures illustrated in Table 8 could be achieved. The common goldfish and platys would then bring, respectively, 6.7 times and nearly 7.4 times more revenues than tilapia. A possibility exists to use one tank in an aquaponic system for the production of ornamental fish and the other tanks would still be under tilapia. The same fish feed could be used and partial harvest could be carried out throughout a year across the different fish cycles.

As the cost of greenhouse heating and supplementary lighting in temperate regions can be a challenge (Rakocy et al., 2006), the production of ornamental fish in additional smaller tank units coupled to the main system, can also be used as a means to generate more income. Otherwise, another approach (Table 9) could be adopted whereby goldfish could hypothetically be used as the sole fish species in an aquaponic system. As discussed earlier, the feeding rate for an ornamental species like goldfish would be suitable to sustain commercial aquaponic plant production.

Compared to tilapia, common goldfish could be 1.9 times more profitable when cultured in the same system. This would not only support the high costs of greenhouse heating and lighting in temperate regions, but would also decrease the dependency on plant production to generate high income as is presently the case in aquaponics (Rakocy et al., 2006). The pay-back period with respect to the high initial construction costs would be shorter and the whole business would be much more profitable.

However, when opting for such high production of ornamentals, a farmer would have to go for only wholesale of the fish to be able to sell such a big stock. Proper management would be required. In order to generate the highest profit and to have a constant supply of fish fry, investment into breeding programs for goldfish would be preferable rather than just specializing in fish grow-out. Besides, a larger number of smaller fish tanks could be used in the aquaponic system making multiple rearing units. Each tank would have fish of a different age as used in the Rakocy system for tilapia production. In addition, different species of goldfish could also be cultured in the same

system. There is a big variation in goldfish price and exotic species could bring as much as $5.00 per unit at wholesale (Watson et al., 2004).

As the growing ornamental fish market generally demands a constant supply of fish, very precise management would be needed to make this theory a reality. Research should be carried out to test the production of goldfish in commercial aquaponic systems where all aspects of production should be looked at, starting from breeding strategies, fish grow-out/management to secure market identification and development of risk-proof marketing strategies. A farmer could always revert back to tilapia or partially produce tilapia at any point in time if he or she wishes to, while still keeping the system running. In addition, other medium-sized ornamentals such as the goldfish or larger ornamental fish could be experimented in an aquaponic system.

The production of goldfish would be more easily achieved by a goldfish farmer than for an existing aquaponics farmer (producing a food fish species). The goldfish farmer would already have all the breeding strategies and set-ups in place. The farmer could just use a commercial aquaponic system for the grow-out of the fish. This would thus be adding a much higher value to the business as very high profitability can be achieved regarding culinary herbs culture in aquaponics (Savidov, 2004; Savidov, 2005; Diver, 2006; Rakocy et al., 2006). Additional advantages and benefits would be: far more superior water treatment, decrease in water usage and decrease in effluent discharge to the environment.

However, in temperate climates, the partial culture of ornamental fish in an aquaponic system could be worth the investment, as a means to cover the high running

costs involved. All the more, the production of species like exotic goldfish varieties could not only be highly profitable but it could also add potentially unparalleled value to an aquaponic system (Table 7).

4.6 Conclusions

1. Ornamental fish could be used as complementary species in an aquaponic system to support the high costs of greenhouse heating and lighting in temperate regions.
2. Small species of ornamental fish are suitable for the production of low-nutrient requirement plants alone. They would not be able to sustain commercial plant production in aquaponics, as they generate low levels of nutrients.
3. Goldfish, which is a medium-sized ornamental fish, showed good potential to be used as a sole species for commercial plant production in an aquaponic system, as they have a higher feeding regime due to a bigger biomass.
4. The production of goldfish could potentially bring 4.6 times more gross income when compared to tilapia.
5. Greenhouse heating and supplementary lighting would be key factors for production of aquaponic crops in Newfoundland.

4.7 Recommendations

1. Experimentations should be carried out to test for the feasibility of culturing goldfish and other medium-sized ornamental fish species as sole species in aquaponic systems, at high densities and at different feeding rates.
2. Studies on proper breeding strategies to constantly supply aquaponic production with fish fry, fish grow-out/management strategies and secure market identification and development of risk-proof marketing strategies should be performed as fundamental backbones to future studies.

References

Allen, G.R., Midgley, S.H. and Allen, M. (2002). *Field guide to the freshwater fishes of Australia*. Australia: Western Australian Museum.

Billard, R. (1997). *Les poissons d'eau douce des rivières de France, Identification, inventaire et répartition des 83 espèces*. France: Lausanne, Delachaux & Niestlé.

Bobick, J.E. and Peffer, M. (1993). Science and technology desk reference. Gale Research Inc.

Boyd, C.E. (1982). Water Quality Management for Pond Fish Culture. Netherlands: Elsevier Science Publishers.

Boyd, C.E. (1982). *Water Quality Management for Pond Fish Culture*. Netherlands: Elsevier Science Publishers B.V.

Bradley, P. and Marulanda, C. (2001). Simplified hydroponics to reduce global hunger. *Acta Horticulturae*, *554*, 289-296.

Buttner, J.K., Soderberg, R. W. and Terlizzi, D.E. (1993). An Introduction to Water Chemistry in Freshwater Aquaculture. *Northeastern Regional Aquaculture Center, University of Massachusetts Factsheet, 170*, 4 pp.

Delong, D.P., Losordo, T.M. and Rakocy, J.E. (2009). Tank culture of Tilapia. *Southern Regional Aquaculture Center*, *282*, 8 pp.

Dennis, O. and Hugg, S. (1996). *Freshwater and estuarine fishes of North America, MAPFISH georeferenced mapping database*. USA: Life Science Software.

Diver, S. (2006). Aquaponics- Integration of hydroponics with aquaculture. *ATTRA-National Sustainable Agriculture Information Service*, *IP 163*, 28 pp.

Durborow, R.M., Crosby, D.M. and Brunson, M.W. (1997). Ammonia in Fish Ponds. *Southern Regional Aquaculture Center* Factsheet, *463*, 2 pp.

Emerson, K., Russo, R.C., Lund, R.E. and Thurston, R.V. (1975). Aqueous Ammonia Equilibrium Calculations: Effects of pH and Temperature. *Journal of the Fisheries Research Board of Canada*, *32*, 2379-2383.

Emerson, K., Russo, R.C., Lund, R.E. and Thurston, R.V. (1975). Aqueous Ammonia Equilibrium Calculations: Effects of pH and Temperature. *Journal of the Fisheries Research Board of Canada* **32**, 2379-2383.

Environment Canada and Health Canada (2001). Canadian Environment Protection Act 1999, Priority Substances List Assessment Report: Ammonia in the Aquatic Environment. Canada: Minister of Public Works and Government Services. 103 pp.

Etnier, D.A. and Starnes, W.C. (1993). *The fishes of Tennessee*. Knoxville, Tennessee: The University of Tennessee Press.

FAO. (2005a). International trade in aquaculture products. In *FAO Fisheries and Aquaculture Department*. Retrieved from. http://www.fao.org/fishery/topic/14884/en

FAO. (2005b). Ornamental fish. In *FAO Fisheries and Aquaculture Department*. Retrieved from. http://www.fao.org/fishery/topic/13611/en

FAO. (2006). Cultured aquatic species information programme- Oreochromis niloticus. In FAO Fisheries and Aquaculture Department. Retrieved from. http://www.fao.org/fishery/culturedspecies/Oreochromis_niloticus/en

FDEP (2001). Chemical laboratory methods manual: Calculation of un-ionized ammonia in fresh water. Tallahassee: Florida Department of Environmental Protection.

Feldlite, M. and Milstein, A. (1999). Effect of Density on Survival and Growth of Cyprinid fish fry. *Aquaculture International*, *7*, 399-411.

Francis-Floyd, R., Watson, C., Petty, D. and Pouder, D.B. (2009). Ammonia in Aquatic Systems. *Department of Fisheries and Aquatic Sciences, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida, FA-16*, 5 pp.

Froese, R. and Pauly, D. (2011). *Xiphophorus maculatus*, southern platy. In FishBase. Retrieved from. http://www.fishbase.org/summary/Xiphophorus-maculatus.html

Ghosh, A., Mahapatra, B.K. and Datta, N.C. (2003). Ornamental Fish Farming – Successful Small Scale Aqua business in India. *Aquaculture Asia*, *8* (3), 14-16.

Gojon, A., Nacry, P. and Davidian, J-C. (2009). *Current Opinion in Plant Biology*, *12* (3), 328-338.

Guy, L.C. and Haskell, D. (1987). Induction of Freezing Tolerance in Spinach is associated with the Synthesis of Cold Acclimation Induced Proteins. *Plant Physiology*, *84*, 872-878.

Guy, L.C. Niemi, K.J. and Brambl, R. (1985). Altered Gene Expression during Cold Acclimation of Spinach. *Proceedings of the National Academy of Sciences, 82*, 3673-3677.

Hargreaves, J.A. and Tucker, C.S. (2004). Managing Ammonia in Fish Ponds. *Southern Regional Aquaculture Center Factsheet, 4603*, 8 pp.

Hargrove, M. and Hargrove, M. (2006). *Freshwater aquariums for dummies (2nd ed.)*. Hoboken, New Jersey: Wiley Publishing, Inc.

Kailola, P.J., Williams, M.J., Stewart, P.C., Reichelt, R.E., McNee, A. and Grieve, C. (1993). *Australian fisheries resources*. Canberra, Australia: Bureau of Resource Sciences.

Kallman, K.D. and Borkoski, V. (1977). A sex-linked gene controlling the onset of sexual maturity in female and male platyfish (*Xiphophorus maculatus*), fecundity in females and adult size in males. New York: New York Zoological Society.

Kaye, C., Neven, L., Hofig, A., Li, Q-B., Haskell, D. and Guy, C. (1998). Characterization of a Gene for Spinach CAP160 and Expression of Two Spinach Cold-Acclimation Proteins in Tobacco. *Plant Physiology, 116*, 1367-1377.

Keith, P., Marquet, G., Valade, P., Bosc, P. and Vigneux, E. (2006). *Atlas des poissons et des crustacés d'eau douce des Comores, Mascareignes et Seychelles, Patrimoines naturels* (pp. 65-250). Paris: Muséum national d'Histoire naturelle.

Kottelat, M. and Freyhof, J. (2007). *Handbook of European freshwater fishes*. Cornol, Switzerland: Publications Kottelat.

Lehtovirta-Morley, L.E., Stoecker, K., Vilcinskas, A., Prosser, J.I. and Nicol, G. (2011). Cultivation of an obligate acidophilic ammonia oxidizer from a nitrifying acid soil. *Proceedings of the National Academy of Sciences*, *108*, 15892-15897.

Leininger, S., Urich, T., Schloter, M., Schwark, L., Qi, J., Nicol, G.W.,... Schleper, C. (2006). Archaea predominate among ammonia-oxidizing prokaryotes in soils. *Nature*, *442*, 806-809.

Lewbart, G.A. and Harms, C.A. (2008). Freshwater Ornamental Fish. *Zoological Medicine Teaching Rounds*, *817*, 19 pp.

Lewbart, G.A. and Harms, C.A. (2008). Freshwater Ornamental Fish. *Zoological Medicine Teaching Rounds,* 817, 19 pp.

Ling, K.H. and Lim, L.Y. (2006). The Status of Ornamental Fish Industry in Singapore. *Singapore Journal of Primary Industries*, *32*, 59-69.

Livengood, E.J. and Chapman, F.A. (2011). The Ornamental Fish Trade: An Introduction with Perspectives for Responsible Aquarium Fish Ownership. *University of Florida IFAS Extension, FA 124*, 8 pp.

Lopez-Bucio, J., Cruz-Ramirez, A. and Herrera-Estrella, L. (2003). The role of nutrient availability in regulating root architecture. *Current Opinion in Plant Biology*, *6*, 280-287.

Man, S.H. and Hodgkiss, I.J. (1981). *Hong Kong freshwater fishes*. Hong Kong: Urban Council, Wishing Printing Company.

McHugh, J.J. and Constantinides, L.N. (2004). Pest Management Strategic Plan for Watercress Production in Hawaii. Honolulu, Hawaii: University of Hawaii at Manoa.

McHugh, J.J., Fukuda, S.K. and Takeda, K.Y. (1987). Hawaii Watercress Production. *University of Hawaii Research Extension Series, 088*, 12 pp.

Moothy, V. and Bhikajee, M. (2000). Development of an ornamental fish breeding unit for the export market at Rose Belle Sugar Estate: Final Report. *Private Sector Collaborative Research Scheme of the Mauritius Research Council*, 55 pp.

Parker, R.O. (2000). *Aquaculture Science (2nd ed.)*. Clifton Park, New York: Cengage Learning.

Popma, T. and Masser, M. (1999). Tilapia- Life history and biology. *Southern Regional Aquaculture Center, 283*, 4 pp.

Rakocy, J.E., Bailey, D.S., Shultz, R.C. and Thoman, E.C. (2004b). Update on tilapia and vegetable production in the UVI aquaponic system. Virgin Islands, USA: Agricultural Experimentation Station, University of Virgin Islands.

Rakocy, J.E., Masser, M.P. and Losordo, T.M. (2006). Recirculating aquaculture tank production systems: Aquaponics- Integrating fish and plant culture. *Southern Regional Aquaculture Center, 454*, 16 pp.

Rakocy, J.E., Shultz, R.C., Bailey, D.S. and Thoman, E.C. (2004a). Aquaponic production of Tilapia and Basil: comparing a batch and staggered cropping system. *Acta Horticulturae, 648*, 63-69.

Riehl, R. and Baensch, H.A. (1991). *Aquarien Atlas*. Germany: Mergus, Verlag für Natur- und Heimtierkunde.

Savidov, N. (2004). Evaluation and development of aquaponics production and product market capabilities in Alberta. Ids *Initiatives Fund Final Report, 679056201*, 190 pp.

Savidov, N. (2005). Evaluation and development of aquaponics production and product market capabilities in Alberta- Phase II. *New Initiatives Fund Final Report, 2004-67905621*, 57 pp.

Tamaru, C.S., Cole, B., Bailey, R. and Brown, C. (1996). A manual for the commercial production of the Swordtail, *Xiphophorus helleri*. Honolulu: USDA Center for Tropical and Subtropical Aquaculture, University of Hawaii Sea Grant Extension Service.

Timmons, M.B., Ebeling, J.M., Wheaton, F.W., Summerfelt, S.T. and Vinci, B.J. (2002). *Recirculating aquaculture systems* (*2nd ed.*). United States: Northeastern Regional Aquaculture Center.

Trautman, M.B. (1957). *The fishes of Ohio*. Ohio: Ohio State University Press.

Tucker, C.S. and D'Abramo, L.R. (2008). Managing High pH in Freshwater Ponds. *Southern Regional Aquaculture Center Factsheet, 4604*. 5 pp.

Velghe, N. and Claeys, A. (1985). Rapid spectrophotometric determination of nitrate in mineral waters with resorcinol. *Analyst, 110*, 313-314.

Watson, C.A., Hill, J.E., and Pouder, D.B. (2004). Species profile: Koi and Goldfish. *Southern Regional Aquaculture Center, 7201*, 6 pp.

Appendix 1

$$\text{Production}_{(TAN)} = \frac{F \times PC \times 0.092 \times 10^6}{t \times 450}$$

F= total amount of feed input/day (kg)
PC= Protein content of feed (decimal)
0.092= constant
10^6= conversion factor from kg/L to mg/L
t= time between 2 feedings (day)
450= Total water in each system (L)

(Timmons et al., 2002)

Table i. Total ammonia-nitrogen (TAN) sampling 1 of culture water at day 13.

Sampling	Treatment	Actual TAN (mg/L)	Theoretical TAN (mg/L)
1st	D1P1	0.74	0.78
	D2P1	1.10	1.05
	D3P1	1.36	1.31
	D1P2	0.78	0.78
	D2P2	1.13	1.05
	D3P2	1.46	1.31
	Platy Control	1.60	1.31
	Goldfish	2.38	2.24

Table ii. Total ammonia-nitrogen (TAN) sampling 2 of culture water at day 19.

Sampling	Treatment	Actual TAN (mg/L)	Theoretical TAN (mg/L)
2nd	D1P1	0.31	1.18
	D2P1	0.18	1.57
	D3P1	0.25	1.96
	D1P2	0.45	1.18
	D2P2	0.24	1.57
	D3P2	0.93	1.96
	Platy Control	0.14	1.96
	Goldfish	2.40	3.92

Table iii. Total ammonia-nitrogen (TAN) sampling 3 of culture water at day 33.

Sampling	Treatment	Actual TAN (mg/L)	Theoretical TAN (mg/L)
3rd	D1P1	0.22	1.18
	D2P1	0.33	1.57
	D3P1	0.30	1.96
	D1P2	0.21	1.18
	D2P2	0.24	1.57
	D3P2	0.26	1.96
	Platy Control	0.26	1.96
	Goldfish	0.32	3.92

Table iv. Total ammonia-nitrogen (TAN) sampling 4 of culture water at day 48.

Sampling	Treatment	Actual TAN (mg/L)	Theoretical TAN (mg/L)
4th	D1P1	0.26	1.57
	D2P1	0.27	2.09
	D3P1	0.28	2.62
	D1P2	0.26	1.57
	D2P2	0.32	2.09
	D3P2	0.33	2.62
	Platy Control	0.30	2.62
	Goldfish	0.44	5.23

Table v. Total ammonia-nitrogen (TAN) sampling 5 of culture water at day 54.

Sampling	Treatment	Actual TAN (mg/L)	Theoretical TAN (mg/L)
5th	D1P1	0.22	1.57
	D2P1	0.24	2.09
	D3P1	0.28	2.62
	D1P2	0.24	1.57
	D2P2	0.30	2.09
	D3P2	0.27	2.62
	Platy Control	0.32	2.62
	Goldfish	0.41	5.23

Table vi. Total ammonia-nitrogen (TAN) sampling 6 of culture water at day 61.

Sampling	Treatment	Actual TAN (mg/L)	Theoretical TAN (mg/L)
6th	D1P1	0.26	1.57
	D2P1	0.26	2.09
	D3P1	0.32	2.62
	D1P2	0.26	1.57
	D2P2	0.29	2.09
	D3P2	0.31	2.62
	Platy Control	0.38	2.62
	Goldfish	0.35	5.23

Table vii. Total ammonia-nitrogen (TAN) sampling 7 of culture water at day 68.

Sampling	Treatment	Actual TAN (mg/L)	Theoretical TAN (mg/L)
7th	D1P1	0.26	1.57
	D2P1	0.26	2.09
	D3P1	0.28	2.62
	D1P2	0.24	1.57
	D2P2	0.30	2.09
	D3P2	0.31	2.62
	Platy Control	0.39	2.62
	Goldfish	0.55	5.23

Table viii. Total ammonia-nitrogen (TAN) sampling 8 of culture water at day 76.

Sampling	Treatment	Actual TAN (mg/L)	Theoretical TAN (mg/L)
8th	D1P1	0.19	1.57
	D2P1	0.24	2.09
	D3P1	0.32	2.62
	D1P2	0.24	1.57
	D2P2	0.26	2.09
	D3P2	0.33	2.62
	Platy Control	0.26	2.62
	Goldfish	0.40	5.23

Appendix 2

Figure i. Watercress from one of the tanks in the goldfish treatment, near the end of the study.