Microalgae in aquaculture feeds for Nile tilapia, and the feasibility of urban aquaponics

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Summary

This thesis develops understanding of microalgae in Nile tilapia (*Oreochromis. niloticus)* feed, implications for aquaponics and how consumer perceptions influence feasibility of urban aquaponics. Aquaponics is the growing of crops, utilising nutrients from fish waste. Chapter one is a meta-analysis (36 papers) on the effects of microalgae inclusion on *O. niloticus*. This was done to determine if fish meal (FM) in aquaculture feeds can be replaced with microalgae, without being detrimental, to reduce pressure on wild fish stocks. An inclusion of microalgae, up to 30%, is beneficial to *O. niloticus* with no detrimental impacts. Effect sizes for specific growth rate (-0.15) and feed conversion ratio (-0.30) indicate microalgae can replace FM. Chapter two assesses effects of feed on water parameters (Ammonia etc) in *O. niloticus* systems, by systematic review. Optimal water parameters for *O. niloticus* are understood, but effects of feed type and quantity are poorly researched, which needs rectifying. Feed type has little importance, and feed quantity has overriding impact on water parameters. Chapter three investigates palatability of digestate cultured microalgae (DCM, Nannochloropsis & Scenedesmus), cultured on membrane microfiltered, food waste digestate on *O. niloticus*, assessing the possibility of DCM as an alternative to FM. Time to react, pellets eaten and ejected determined palatability. There were no statistically significant results ($P>0.05$), indicating that an alternative feed is as palatable as commercial feed. Chapter four examined feasibility of urban aquaponics via a consumer survey (254 participants), as future innovative farming will utilise space in urban areas (Biophilic living project). It created a baseline of perceptions in the UK, which could tailor systems/inform public on aquaponics based on current knowledge. 30% of participants had heard of aquaponics, but showed reservations to costs. Attitudes are neutral or in favour to aquaponics, but considerations are needed for location, product choice and system design.

Lay summary

This thesis is split into four parts relating to the feed of Nile tilapia and how it may be applied to a farming method called aquaponics. The first part investigated how the inclusion of microalgae can impact the growth and health of Nile tilapia. This was conducted to highlight the beneficial use of microalgae in the feed of Nile tilapia as opposed to fish meal. Results indicated that microalgae can replace fish meal in feed, without negative impacts to the fish. The second part looked at how type and quantities of feed influences water quality for Nile tilapia and how this may be incorporated into aquaponics. The amount of feed greatly impacts water quality; thus, quantities must be carefully controlled, meaning that aquaponics can balance feed quantity to the needs of the system. Even though the needs of Nile tilapia are well researched, there is little work on the effect of different feeds, which should be the focus of future research. The third part of this thesis looked at how attractive a feed formulated using a new method of culturing microalgae using food waste, was to Nile tilapia. The Nile tilapia were offered commercial feed as well as, the formulated feed and the attractiveness was determined based on individual fish reactions at Swansea university. This study found that Nile tilapia found the formulated diet just as attractive as the commercially available feed. Therefore, aquaculture feed can be made more sustainable and the use of this waste stream can have cascading positive impacts. The final section examined peoples baseline knowledge and perceptions of aquaponics to assess the feasibility of aquaponics within the UK and globally. Generally, opinions were neutral or positive, but there is a lack of understanding of aquaponics, hindering its uptake.

Declarations and statements

This work has not previously been accepted in substance for any degree and is not being concurrently submitted in candidature for any degree.

Signed ....[redacted]..... (candidate)

Date ....16/06/22...

STATEMENT 1

This thesis is the result of my own investigations, except where otherwise stated. Where correction services have been used, the extent and nature of the correction is clearly marked in a footnote(s).

Other sources are acknowledged by footnotes giving explicit references. A bibliography is appended.

Signed ....[redacted]........ (candidate)

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STATEMENT 2

I hereby give consent for my thesis, if accepted, to be available for photocopying and for inter-library loan, and for the title and summary to be made available to outside organisations.

Signed ....[redacted]..... (candidate)

Date ....16/06/22.......................................

Statement of expenditure

Student name: Samuel Files

Student number: 908455

Project title: Microalgae in aquaculture feeds for Nile tilapia, and the feasibility of urban aquaponics

Category	Item	Description	Cost IInc VAT)
Analysis	Pellet analysis	Sciantec Analytical Services to provide composition analysis of formulated feed,	£233.18
Equipment	Laptop	HP Probook 440 to be able to carry out data analysis and write up work. (To be returned upon submission	£718.80
Total			£951.98

The above expenditures were funded by the students KESS 2 Scholarship.

I hereby certify that the above information is true and correct to the best of my knowledge.

Signature (Student): [redacted]

Date: 16/06/2022

Signature (Supervisor): [redacted]

Date: 16/06/2022

Statement of contributions

SF – Samuel Files, MSc by Research student

CGL – Prof. Carlos Garcia de Leaniz, Academic supervisor

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Role	**Persons involved**
Conceptualization	CGL, SC, SF, CP, PH, RS
Data compiling	SF
Data analysis	SF, CGL
Funding acquisition	CGL, SC
Research	SF, CP, FF, AS
Methodology	SF, CP, PH, RS, FF, AS
Project Administration	CGL, SF
Supervision	CGL, SC
Writing – Draft	SF
Writing- review & editing	CGL, SF

Covid-19 statement

Covid-19 had a massive impact on this project. The initial plans were to carry out a meta-analysis of the use of microalgae in aquaponic systems and how it may be practically used in a system as an introduction. The main section of my thesis was going to focus on a purpose built aquaponic system in CSAR utilising the results from the meta-analysis. The diet was going to be formulated using digestate cultured microalgae and fed to Nile tilapia *O. niloticus* and fed long term to assess the effects on the growth of the Nile tilapia as well as the effects on lettuce within the system. However, as the lockdowns continued, I could not access CSAR and there were difficulties in acquiring Nile tilapia fry due to the pandemic even after the lockdowns lifted, this in conjunction with loss of close family members made my time in CSAR short. Therefore, the study was adapted, with a heavy focus on desk-based work, but still running an experiment using digestate focusing on the palatability to Nile tilapia, meaning a pilot study could still be run for my thesis. The meta-analyses were furthered with the systematic review on the effect of feed on water quality. To bring aquaponics further into the thesis, a survey was conducted as another chapter to analyse perceptions and link this to the feed aspect of aquaponics with making it more sustainable. Additionally, working from home has been difficult, transitioning from university facilities to confined, crowded house and Wi-Fi issues posed to be challenging.

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i. List of tables and figures

i.i Figures

i.i.i Tables

ii. Definitions of abbreviations

AIC – Akaike information criterion

CI – Confidence intervals

CP – Crude protein

CSAR – Centre for sustainable aquatic research

DCM – Digestate cultured microalgae

DO – Dissolved oxygen

DWC – Deep water culture

ED – Experimental design

FAO – Food and agriculture organisation

FCR – Feed conversion ratio

FM – Fish meal

GLM – Generalised linear model

HKSJ - Hartung-Knapp-Sidik-Jonkman

LN – Natural logarithm

NFT – Nutrient film technique

PEP - Peptidoglycan

RAS – Recirculating aquaculture system

SBM – Soy bean meal

SD – Standard deviation

SE – Standard error

SGR – Specific growth rate

SMD – Standardised mean difference

S_p – Pooled standard deviation

iii. Acknowledgements

I would like to thank all my friends and family for supporting me throughout my thesis, through the bad and the good they were there to support me and have been key to keeping me going through the toughest year.

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I would also like to thank Andrew Nixon and Marta Lopez from Powell Dobson and Hacer development for their funding and contribution to the project, and allowing me to be part of an exciting architectural and innovative project. Also, my thanks go to KESS2 East:

Mae Ysgoloriaethau Sgiliau'r Economi Wybodaeth (KESS 2 Dwyrain) yn fenter sgiliau lefel uwch ar gyfer Cymru gyfan, sy'n cael ei chefnogi gan Gronfa Gymdeithasol Ewrop drwy Lywodraeth Cymru ac yn cael ei harwain gan Fangor ar ran y sector addysg uwch yng Nghymru.

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iv. Justification and aims of thesis

Fish consumption globally has increased annually by 3.1% since 1961 and continues to grow, with farmed aquaculture increasing by 5.3% in the past three years (FAO., 2020). Nile tilapia (*Oreochromis niloticus*, Linnaeus 1758) are the third most produced finfish globally, behind two species of cyprinids and accounted for 8.3% of total finfish production in 2018 (FAO., 2020). With the current COVID-19 pandemic, aquaculture will suffer, as many countries are halting exports of seafood and incurring closures of aquaculture facilities (Gephart et al., 2020), yet this may be a chance for small scale, integrated aquaculture to take its place in providing food security for all. Nile tilapia are cultured to such an extent mainly due to their rapid growth and resulting productivity and their hardiness. They are able to tolerate and grow efficiently at higher stocking densities and are extremely tolerant of poor water quality in comparison to other species (Delong, Losordo, & Rakocy, 2009). Due to their general ease of culturing, they are often dubbed the "aquatic chicken" (Huecht, 2000) and can be raised and farmed by people with limited experience, making them ideal for developing countries with limited aquaculture knowledge and for large scale commercial farms.

While there is a trend for increased aquaculture production, which reduces stress to wild fisheries and allows developing countries a suitable form of protein (FAO., 2020), the sustainability of aquaculture, as well as the environmental impacts of the current practices caused by the industry are widely debated (Xuan & Sandorf, 2020). Again, this is where Nile tilapia come into their own, as they are omnivorous, leading to reduction in the amount of fishmeal-based diets and allowing for a more plant-based diet than other species (Love et al., 2014). The need for a more sustainable food source is extremely evident, with increasing world populations and finite natural resources, conventional farming practices need to be reconsidered. It has been found that the total tillable soil peaked in the year 2000, along with phosphate production, which is used as fertilizer (Sverdrup & Ragnarsdottir 2014), echoing the need for innovative farming methods, such as aquaponics.

iv.i What is aquaponics and its importance

Interest in aquaponics has increased in the past 20 years (Yep & Zheng, 2019). Although, its origins can be traced to at least 1,500 years ago in China with the cultivation of rice with catfish and cyprinids (Jones, 2002). Yet, a recent study found that 50% of people were unaware of what aquaponics is (Miličić, Thorarinsdottir, Dos Santos, & Hančič, 2017). Aquaponics is the combination of aquaculture and hydroponics into one closed-loop system and has been seen as a vital innovation to create more sustainable farming globally (Panades, 2015). Aquaponics has been defined in many ways, but it essentially is the growing of crops where >50% of the nutrients are supplied by fish waste (Palm et al., 2018). Whereas, hydroponics is defined as growing crops without any substrate, in a nutrient rich solution where the nutrients are added in liquid form (Jensen, 1997). Broadly speaking, aquaponics is the combination of rearing fish to produce waste into the system that by a process of nitrification is utilised by the plants as a fertiliser instead of using commercially produced nutrient additives, at an unsustainable level. Fish produce different type of waste in aquaculture systems; being urea, soluble waste, uneaten food and solid faecal waste. Urea is the principal component of fish waste used in aquaponics as it is easily available to the plants without further processing, with *O. niloticus* urea containing 33% of the available nitrogen and 17% of the available phosphates, uneaten feed accounts for 18% N and 18% P and solid waste accounts for 13% N and 37% P (Montanhini Neto & Ostrensky, 2015). However solids are now being utilised through the use of mineralisation to make the nutrients more available to the plants and leading to waste nutrient retention of up to 90% (Nicholsa & Savidov, 2012). The nutrient uptake efficiency of plants in aquaponics is greatly affected by flow rates in the hydroponic system, thus system design is key for nutrient availability for plants (Dediu, Cristea, & Xiaoshuan, 2014).

Aquaponics is deemed sustainable because it is a recirculating aquaculture system, that is used in a polyculture to grow plants. Of which, the benefits include; increased yield of fish and plants throughout a whole year in comparison to standard farming practices and outperforms hydroponics, reduces environmental impacts from waste water and commercial fertiliser (Delaide, Goddek, Gott, Soyeurt, & Jijakli, 2016). Aquaponics can be scaled to fit the space available and dramatically reduces the water usage in comparison to conventional aquaculture; up to 98% recycling of water to only require 320L per 1kg of fish (Al-Hafedh, Alam, & Beltagi, 2008; Goddek, Joyce, Kotzen), similar to that of Recirculating aquaculture systems (RAS). The concept behind aquaponics is shown in Figure i.i.

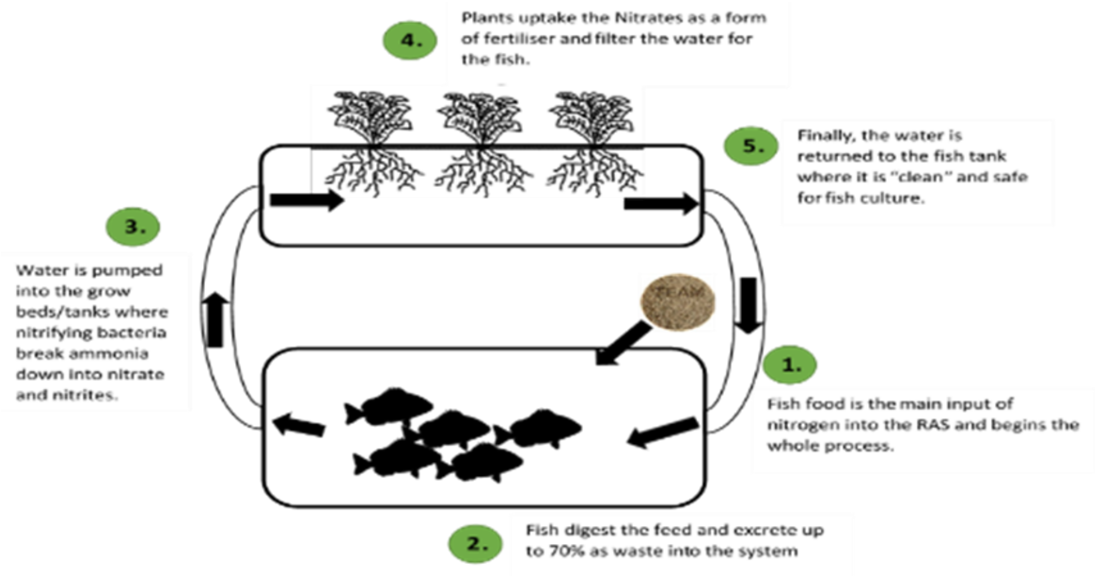


Figure i.i. Basic concept behind aquaponics in a RAS where water is recycled and both fish and crops are cultured together. (Wang, Olsen, Reitan, & Olsen, 2012)

iv. ii Urban aquaponics

Urban aquaponics it the use of old derelict space or new purpose built spaces, where aquaponics can be established within towns and cities in order to relive pressure of urbanisation (Where 50% of the world population lives in cities) on farmland and helps produce food in a more sustainable and efficient way for large urban areas (Goddek, Joyce, Kotzen, & Burnell Editors, 2019). As aquaponics requires less land, fertiliser, and produces less waste, it implies it is sustainable and subsequently aquaponics has successfully been scaled to even home-based systems as subsistence farming as well as in biophilic living projects utilising integrated greenhouses, showing the adaptability and potential for aquaponics in all urban settings (David et al., 2022). Local production of food utilising aquaponics with efficient building design can greatly reduce environmental impact of food production as can surpass the environmental sustainability of regular food production, as the increase demand on rural areas from expanding urban areas has lead to the transporting of food being a key contributor to greenhouse gas emissions and aquaponics farms can have more efficient energy consumption than aquaculture or soil-based farming. (David et al., 2022a; Körner et al., 2021). As it stands, the cost of urban aquaponics is high, and no cities have been thoroughly studied for its application, so it may not always be feasible. (Specht et al., 2014). However, with further education on how aquaponics is beneficial such as; job creation, food security, reducing food miles, using eco-conscious architecture, water catchment (Rizal et al., 2018) and filling the knowledge gap it can allow aquaponics to become socially accepted and therefore more feasible (Li et al., 2018).

iv.iii Biophilic living Swansea (Urban aquaponics in practice)

One such project that aims to be the forefront of urban aquaponics and provide a baseline stepping stone to urban aquaponics in the UK is the biophilic living project in Swansea, Wales. This project is the first of its kind in the UK, which is backed by the Welsh government and aims to tackle; energy consumption, food poverty and security, mental and physical wellbeing and bring communities together. The building project is a 12-story high rise, with a mix of residential, commercial and retail areas in the heart of Swansea city and will put a green infrastructure at the forefront of the city as a "new beacon" with an emphasis on the use of aquaponics to feed its residents and provide the nutrients for its greenhouses (Figure i.ii). Additionally to the use of aquaponics, the building will provide innovative technology to further the sustainability and feasibility of urban aquaponics systems such as; integrated solar panels into greenhouses, subsequent energy storage, Carbon dioxide capture and use in plant growth and water catchment devices to minimise the already lowered water use of aquaponics. The aquaponics system will be a vertical farm, where residents will be able to produce their own food to experience healthy food choices and be part of a new community centred on biophilic living. The aquaponics system will also function as an educational tool for both the public and prospective aquaponic farmers, therefore s pivotal in improving perceptions on aquaponics as well as its prospects for future development. This project has worked very closely with this thesis in the designing and functionality of their aquaponics system, as well as a keen interest in the use of microalgae as fish meal substitute and consumer perceptions of aquaponics within the UK, which was a driving force for the focus of this thesis. A conceptual design of the proposed aquaponics system in Swansea based on the needs of the building and functionality of the space is discussed next.

Figure i.ii Conceptual building design in Swansea, displaying the key biophilic aspects of living walls and the greenhouse systems. (https://www.biophilicliving.co.uk/about/)

iv.iv Conceptual system design

Due to the nature of a project being a community driven system, there is a clear need for a decoupled system. This is a contingency in case of any fertilizers or the like are added to the plants, to avoid contamination to the fish and potential death. Adding to this, there is a current trend in aquaponics to move towards a decoupled system, due to the trade-offs of having a single-loop system, whereby parameters are sub-optimal for both plants and fish (Table i.i). Single-loop aquaponics typically uses pH of around 7, leading to lower levels of nitrification and poorer water quality for fish and above the pH that is preferred by most plants. A decoupled system bypasses these problems and is often preferred as there is increased control of water parameters and results in better yields. The decoupled method is regarded as a more sustainable and efficient system, especially in a variable climate such as the UK (Goddek & Körner, 2019).

Table i.i. Water quality parameters that are optimal and tolerable for the culture of Nile tilapia in a RAS

Parameter	Unit	Optimal	Tolerance	References
Ammonia (NH_3)	Mg/L^{-1}	<0.1	<1	(Delong et al., 2009; El-Sayed, 2019; Makori, Abuom, Kapiyo, Anyona, & Dida, 2017; Sallenave, 2016; Setiadi, Widyastuti, & Prihadi, 2018)
Nitrite (NO_2)	Mg/L^{-1}	<0.3	5	
Nitrate (NO_3)	Mg/L^{-1}	<300	400	
Dissolved oxygen (DO	Mg/L^{-1}	5-6	>3, <9	
pH		6-9	5-10	(El-Sayed, 2019; Makori et al., 2017)
Temperature	°C	27-29	>25, <32	(Delong et al., 2009; Sallenave, 2016.; Setiadi et al., 2018)
Phosphorus (PO_4)	Mg/L^{-1}	0.05-1	<2	(Bhatnagar & Devi, 2013; Effendi, Widyatmoko, Utomo, & Pratiwi, 2020)

iv.v Sizing the system

Sizing of the system is mainly based on the size of the aquaculture component and planned stocking density. The general rule for stocking density in aquaponics is between 77-106 fish/m^3 marketable size tilapia (Rahmatullah, Das, & Rahmatullah, 2010; J. E. Rakocy, Bailey, Shultz, & Cole, 1997). However, at the highest density would yield 53kg/m^3, which is on the higher commercial end scale of aquaponics. For the purpose of this project, a lower initial stocking density to begin with to assess the needs and requirements of the system. Table i.ii shows the average feeding rates of Nile tilapia, which were used in calculating total feed and in sizing different aspects of the system.

Table i.ii. Average feeding rates of Nile tilapia at different sizes (FAO 2014)

Fish size (g)	% Biomass fed per day
0 – 20	20
20 – 40	7
40 – 100	5.5
100 – 200	4-2
200+	2-1.5

Based on the findings by (J. E. Rakocy et al., 1997), the optimum feeding rates to grow lettuce in aquaponics are 56-180g/m^2/day^{-1}. However, using aqua ecosystems prediction of 200m^2, it is not possible to for this system to support this much grow space. In reality with optimum feed ratios in the proposed system (Figure i.iii) it can support Ca 60m^2 of floating raft grow space (Bigelow Brook farm).

Leading from this, is the conceptual design of the system (Figure i.iii) & the conceptual footprint of the design (Figure i.iv). It should be noted that Ca 30% of space is typically reserved for walkways, which this design allows for slightly more, but pipework and spacing of the different aspects is unaccounted for. This design could also be changed with taller fish tanks to allow for higher stocking density, while the rest of the system should be sized to accommodate for an increased density.

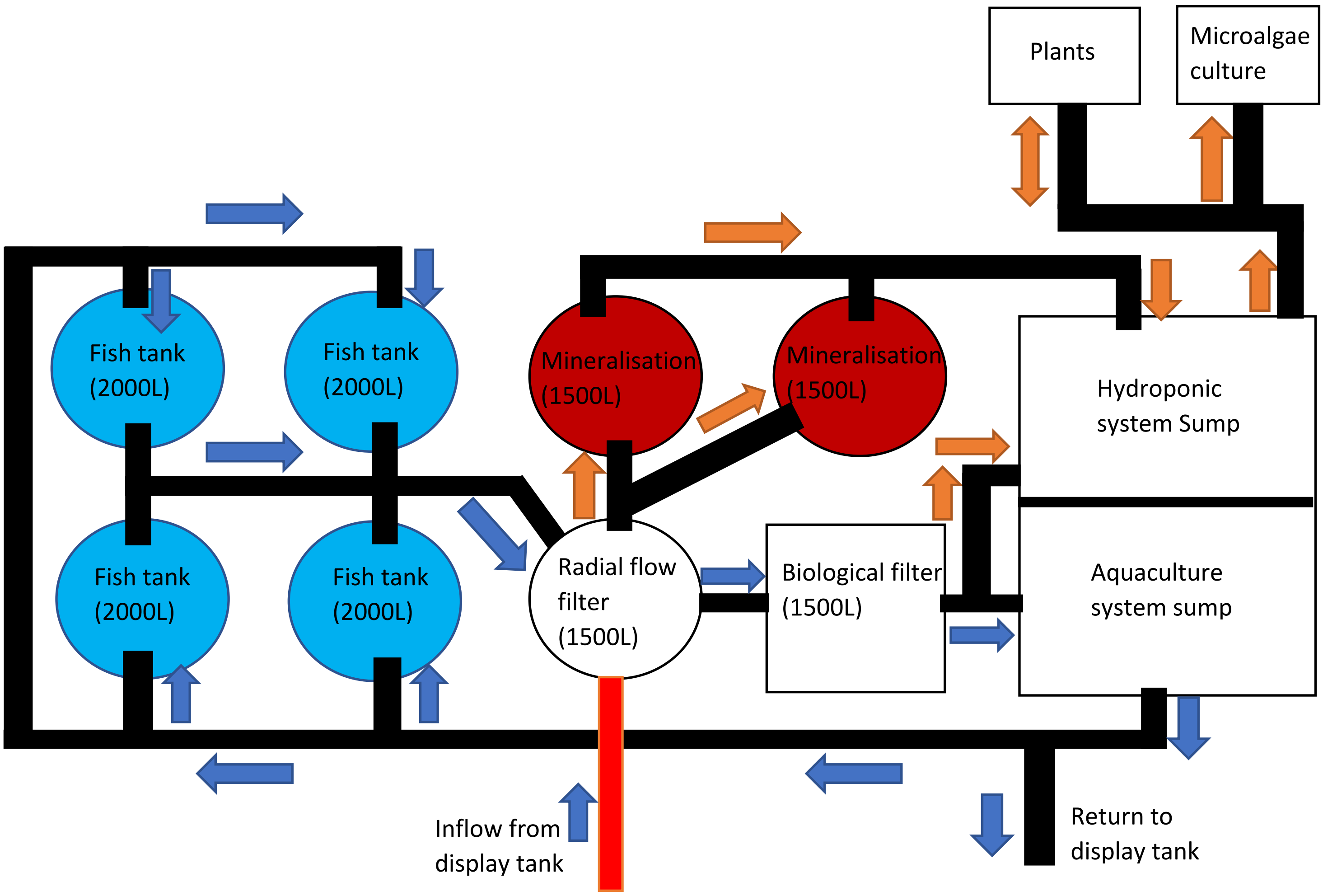


Figure i.iii. Conceptual diagram of the decoupled aquaponics system at Picton yard. Blue arrows indicate water movement within the RAS and orange indicate the flow to hydroponics component. Black/red rectangles are just visual to indicate piping and water flow.

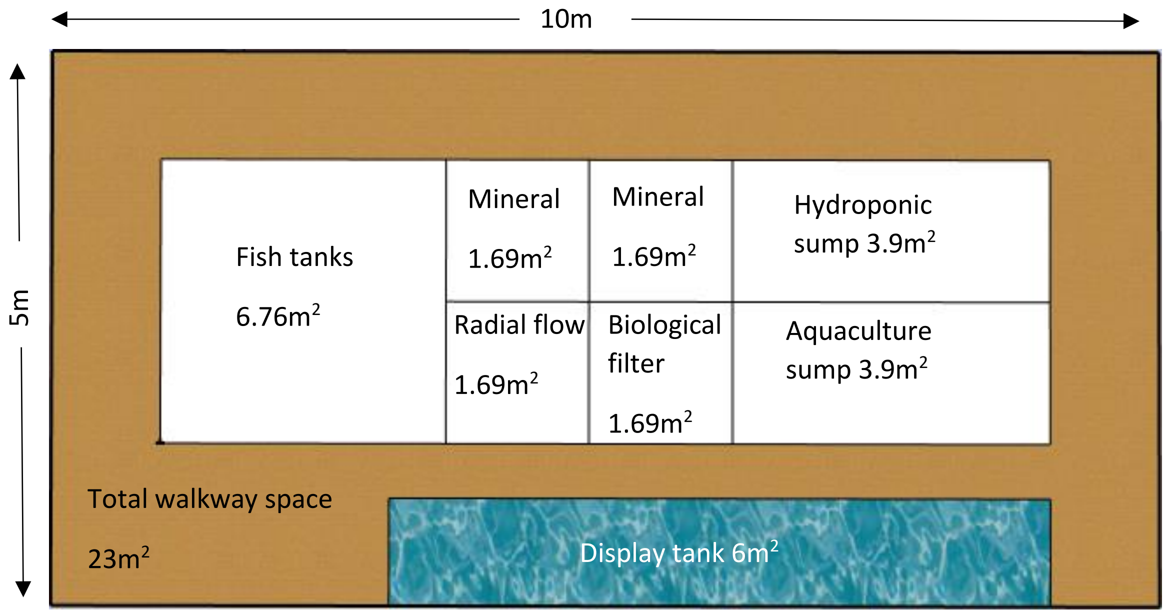


Figure i.iv. Conceptual foot print of system design working in a 50m^2 space, allowing for larger walkways, with the use for education and tours in mind to allow for larger groups and more space.

iv.vi Explanation of design

Using figure i.iii as a visual, the reasoning behind the different aspects of the system will be discussed.

Even though four fish tanks take up more room, it allows for adequate nutrient supply and uniform harvesting all year round. Nile tilapia are generally harvested at 24 weeks, so with four tanks the age and size of Nile tilapia are in 6 week increments, as proposed by (J. E. Rakocy et al., 1997). A consistent nutrient supply is kept as instead of only two tanks where 50% of the fish are harvested, hence 50% of the nutrient supply is removed, a maximum of 25% of the nutrients are reduced in a staggered approach and is more optimum for plant and fish growth (J. Rakocy, 2004). The radial flow filter is sized to account for the flow rate of one full circulation every 1hr 30mins from the fish tanks and display tanks using solid lifting outlets. Radial flow works by separating solids from the water column by slowing down the velocity of the water. A capacity of 1500L allows for an 8-minute retention time of water, which is adequate to remove majority of the solids in the system as a minimum of 2minutes is needed. The formula for sizing of radial flow filter is below.

$$\left(\frac{Retention\ time\ desired}{60}\right) \times Flow\ rate\ (LPH) = Size\ of\ filter\ needed$$

The flow from the radial flow filter is split to the mineralisation tanks and biofilter. Following the aquaculture system, water flows into the biological filter, where nitrifying bacteria converts ammonia (NH_3) to Nitrite (NO_2) and then Nitrates (NO_3), which can be utilised by plants. The sizing of biofilters is dependent on feeding rate and the TAN removal by different media types. The maximum feeding rate possible of the tanks, if they were all stocked at harvest weight (500g) would be up to 10kg of feed, which is what the filter will be sized to, allowing for contingency as most commercial media have a surface area of Ca. 200m^2 means that only a small filter would be needed (Losordo & Delong, 2018). The size of the filter also means that the aquaculture system can run independently all year round and means that while plants won’t necessarily grow all year in our climate, fish can still be grown (Goddek & Körner, 2019).

Water then travels to the aquaculture or hydroponic sump, dependant on the need of topping up the hydroponic sump etc. The sump must always be the lowest point in the system, so will need to be dug into the ground and theoretically this system allows for a one pump system to pump the water from the sump to the fish tanks. The general rule of thumb for sump size is to be at least 20% of the volume of the rest of the system and the bigger the better. This design has incorporated as large of sump as possible in the space which is well above the 20% rule. A larger sump is necessary as it ensures better water quality for longer and can allow for higher stocking densities.

In the hydroponics system, sludge (Solids) collected in the radial flow filter are transferred to mineralisation tanks, which are twice the volume of the radial flow filter to allow for continuous mineralisation. Strong aeration is required to promote heterotrophic bacteria to brake down solids and can lead up to 90% of the minerals in the sludge to be retained and used in the system (Delaide, Monsees, Gross, & Goddek, 2019). This is why mineralisation tanks are suggested in the system to reduce waste and be able to recirculate as much of the water as possible and make use of as much of the nutrients as possible. Mineralisation has further potential as methane is produced in the process, which has the potential to be utilised to run part of the system.

"Clean" water from the mineralisation tanks and biological filter then flows to a separate hydroponics sump of equal size to the aquaculture sump. From here, water is pumped to the plants and potentially a microalgae culture which will be discussed further. While a decoupled system is championed in this report, some forms of treatment including desalination and thorough filtering could allow for water to be returned to the aquaponic component (Goddek & Körner, 2019), but with a lack of space this may be unfeasible. Also, it may be an option to teach residents and people using the greenhouses of the risk of fertilisers to the system could lead to an understanding where by it is safe to return the water to the system.

Additionally, this system will need daily monitoring of pH, temperature and DO, with at least weekly tests of ammonia, nitrite, nitrate, phosphorus etc (J. Rakocy, 2004). By definition aquaponics need only supply >50% of nutrients from fish waste and will require further nutrient additions. Fish feed typically can supply 10/13 required nutrients by plants, but lacks in calcium, iron and potassium (Palm et al., 2018), which in a decoupled system can easily and safely be added to the hydroponics component. Also, many aquaponic farmers have advised the use of oversized piping, which results in less biofouling in the pipes and therefore less maintenance. Additional filters will also need to be incorporated such as UV filters to ensure good water quality and efficient running of the system. Finally, as water will heat up in the pipes and our cooler winters in the UK there is a need for a heating/cooling system to be in place to moderate any temperature fluctuations.

iv.vii Hydroponic component design

With a decoupled aquaculture system sized and designed, the subsequent hydroponic component can be sized/designed. The flow rate of hydroponics needs to be adjusted based on the type of system (Endut, Jusoh, Ali, Wan Nik, & Hassan, 2009), with floating rafts and gravel beds needing Ca. 500Lph/m^3 (Shete et al., 2016) and grow towers needing Ca 7Lph/Tower (Zipgrow). A decoupled system works best as plants require slower flow rates to fish, allowing for more efficient nutrient uptake in decoupled systems (Shete et al., 2016). As stated before, the system can comfortably support 60m^2 of grow space using media beds or floating raft systems. These two systems can be used to grow a variety of plants including tomatoes, chillies and plants that NFT cannot support, as well as both systems acting as additional biofilters that can remove up to 90.9 % of nitrates (Hussain et al., 2014), with grow beds out performing floating rafts and visually looking better.

However, NFT are Ca 20% less efficient at removing nutrients than the other two hydroponic systems. As a result, it can be argued that a mix of media beds (due to being aesthetically pleasing, potential for various crops and potential as biofilters) and NFT grow towers should be used to best utilise the supply of nutrients, such as the ones from Brightagrotech. 30m^2 of grow space can be used by media beds and 40m^2 for grow towers due to lower nutrient uptake. The 5ft grow towers have a growing area of 0.1524m^2, which will allow for a potential of 262 towers to be used in the system to grow leafy greens.

The microalgae component of the system is very promising. For this system the microalgae spirulina is suggested as they are generally easy to culture and don't require vast amounts of space or high levels of supplementation to be effective, with just 1% found to be beneficial to health and growth of Nile tilapia (B. Belal, 2012). The only thing they require is aeration and sunlight, so it would take up a small portion of greenhouse space. Most farms currently don't use spirulina due to its cost , but inclusion of the culture in an aquaponic system is very promising and could prove to be innovative (Rosas, Poersch, Romano, & Tesser, 2018). There are also recorded health benefits to humans with spirulina, which could be another possible route to take in the integrated culture. My research is planned to focus on the feasibility of culturing algae using Nile tilapia effluent and will commence once I have finalised methodology and can get back to labs to carry out the research.

iv.viii Summary of system design

- Decouple system as a contingency plan and to allow for optimum growth of plants and fish with the only trade off being higher water usage.

- An initial stocking density of 77fish/m^3 or lower is suggested to first develop the system and assess the demands and needs of the hydroponic component.

- Staggered stocking will work best to maintain constant nutrient supply and allow for ease in maintaining good water quality.

- Soybean meal & L-Lysine and microalgae are a good alternative to completely replace fish meal and will help to reduced costs and increase sustainability.

- The mineralisation of solids can help to reduce wastes and help minimise water usage.

- Water parameters need to be monitored accordingly to determine the efficiency of the system and support the welfare of the tilapia.

- A mix of media beds and grow towers will enable the growth of a wide variety of crops and enable the system to support up to 70m^2 grow space.

- The culture of microalgae in aquaponics is an innovative area and can be one step towards a more sustainable system.

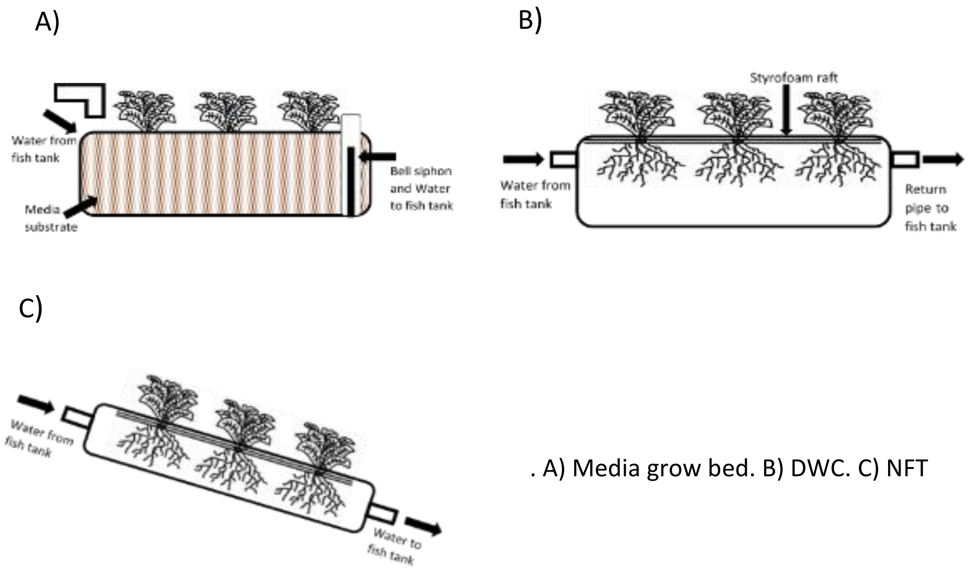
iv.ix Future of the system

As the system is unable to support the entire space that is available in the greenhouses, nutrients can be supplemented into the system at high levels to allow for more plants to be grown. However, as one of the first integrated aquaponics buildings, the use as an educational tool for future projects is huge and will help develop public understanding and opinions on aquaponics (Junge, Bulc, Anseeuw, Yavuzcan Yildiz, & Milliken, 2019). The prospect of using aquaponics as an educational tool has been needed for a long time (Miličić, Thorarinsdottir, Dos Santos, & Hančič, 2017), as most people are unfamiliar even with the term aquaponics, which brings in misconceptions of; system complication, ethics of rearing fish and the "ugly" aesthetics of aquaponics (Pollard, Ward, & Koth, 2017). However, integrating aquaponics into an urban setting is vital to teach people of the potential and practical aspects of aquaponics (dos Santos, 2016), which is very possible with this system, while maintaining a functioning system. This conceptual design attempts to reflect well on the possibility for the use of the system to grow fresh crops for residents of the building, while also having the potential to educate and inform people on the future prospects of sustainable farming.

iv.x. Types of aquaponic systems

There are three types of systems that are utilised in aquaponic systems (Figure i.ii). These include, media grow beds, deep water culture (DWC) (Also referred to as floating raft), and Nutrient film technique (NFT), with the addition of vertical towers. Most commonly used are Media beds, followed by DWC and then NFT in commercial systems (Maucieri et al., 2018). Media grow beds consist of raised beds, filled with a substrate. Typical substrate used is gravel based (containing no limestone) or hydroton clay balls, to provide surface area for nitrifying bacteria and as a way of roots to have anchorage. Media beds generally work on a constant flow or ebb and flood system and are the most commonly researched method of hydroponics within aquaponics, due to their success at nutrient removal due to high surface area on the substrate (Tyson, Treadwell, & Simonne, 2011). The DWC system, is almost exclusively used as a floating raft system, whereby, the water flows from the fish tanks to a larger raceway style of tanks that hold floating rafts with holes to allow plants to float in the water, with constant root contact to the water (Palm et al., 2018). NFT is the least reported, however, is very commonly used in commercial aquaponic set ups due to cheaper running cost and ease of maintenance.

NFT works on a principle of water constantly flowing through small pipes that plants sit in with a small amount of water touching the roots of the plants and has been adapted to use in vertical towers to efficiently use space (Allen Pattillo & Allen, 2017). NFT is viewed as the least effective at removal of nutrients, with reports showing it to remove up to 20% less nitrate when compared to the other two methods (W. A. Lennard & Leonard, 2006). Despite this, the system still functions as a sustainable method of aquaponics in the recycling of water to grow crops at an equal to or better rate than hydroponics (W. Lennard & Ward, 2019). and enables aquaponics to be adapted to very small-scale areas at a cheaper cost. Each system can have its advantages in its use dependant on the area available and the amount and types of plants that need to be grown, with leafy greens such as lettuce benefiting in all systems and fruiting crops including tomatoes being restricted to DWC or media beds (W. A. Lennard & Leonard, 2006; Maucieri et al., 2018).



. A) Media grow bed. B) DWC. C) NFT

Figure i.v. The three main hydroponic systems used in aquaponics.

Stocking density is the number of individuals kept in a system and it is one of the most commonly researched topics within aquaculture and is focused on in relation to aquaponics, due to the need of carefully balancing the fish to plant ratios (Robaina, Pirhonen, Mente, Sánchez, & Goosen, 2019). The key influencing factor in using Nile tilapia in RAS is that they perform best at higher stocking densities due to lower aggressive behaviour and an all-male cohort also allows for improved growth and again due to a high tolerance of poorer water quality (Suresh & Lin, 1992). Current studies have shown that tilapia and specifically Nile tilapia are used in approximately 69% of commercial aquaponic farms, showing their clear suitability to the system (Yep & Zheng, 2019). Adding to the complexity of stocking density, different types of systems (Described in iv.ii) can support a differing quantity of fish. This is due to floating raft and media bed systems having a higher capacity to uptake nutrients and therefore filter the water more efficiently, this allows for a higher stocking density than that of a nutrient film technique (NFT) system (Estrada-Perez et al., 2018). However, many papers have focused on ideal stocking densities for the best growth of Nile tilapia and thus general rules can be taken. Many papers focus on the growing of Nile tilapia fry, where the stocking density can be much greater of between 200-400 fish/m^3 (Babatunde, Ibrahim, Abdulkarim, Wagini, & Usman, 2019; Rayhan, Rahman, Hossain, Akter, & Akter, 2018; Yıldız & Bekcan, 2017), but these were only grown to small, non-market size or the fish welfare was seriously deteriorated by such high stocking density.

The typical system for commercial usage is the floating raft, whereby stocking densities can be achieved at up to 160fish/m^3 and grown to a harvestable size of 300-500g (Rakocy, Bailey, Shultz, & Cole, 1997), while maintaining fish welfare and optimum water quality. Additionally, at this high stocking density, it has been determined that Ca. 42 heads of lettuce/m^2 can be produced (Al-Hafedh, Alam, & Beltagi, 2008), which is of great value to commercial set ups. For smaller scale systems, it has been found that a lower stocking density is easier to manage for fish welfare and help control the water parameters best for fish and plants. This being a stocking density of between 90-106 fish/m^3 (553.96g±11.58g) ((Estrada-Perez et al., 2018; Rahmatullah et al., 2010), to allow for optimum growth of Nile tilapia to harvestable size, with a balancing of nutrients for the growth of plants in an easily maintainable system. Despite this, there are many other factors that influence the stocking density of fish including; feed ratio, plant ratio, water parameters, sex of the fish and many other influencing factors that can raise or lower potential stocking densities (Al-Hafedh et al., 2008; J. E. Rakocy et al., 1997; Rayhan et al., 2018), and they need to be calculated on a system-by-system basis.

iv.xi. Key issues in aquaponics

Aquaponics systems tend to sacrifice one part of the system in favour of another and there are plenty of papers on stocking density, photoperiods and plant yield. The only inputs to aquaponic systems are; feed, water and energy to run the system, and arguably the most crucial factor for a successful system is a balance between plant and fish growth is feed. However, it remains poorly studied in terms of sustainability and effects of different feeds on water parameters, which is why one focus of this thesis is the effects of feed on water parameters in *O.niloticus* systems. A number of studies have researched the ratio of feed to plant grow space and determined that between a feed amount of $56g/m^2$ – $180g/m^2$ is sufficient in a DWC system to enable to growth of 42 heads of lettuce/m^2 using Nile tilapia (Al-Hafedh et al., 2008; J. E. Rakocy et al., 1997), while maintaining a tolerable water quality. While these findings are useful for feed ratios, they do not focus on the very essence of aquaponics, being the nutrients produced by fish waste to be able to cultivate crops. More research needs to be conducted into how the nutrient removal can differ between crop type to maximise the efficiency of these systems and enable the welfare of the fish to be optimal, while being as sustainable as possible, this is why this thesis focuses on the use of microalgae as an alternative feed ingredient in order to provide evidence for beneficial use of microalgae. While the term microalgae normally refers to eukaryotic unicellular organisms, this thesis includes spirulina (Cyanobacteria) as microalgae for ease of nomenclature. Feed is the essential tool to aide in making aquaponics as sustainable and efficient as possible and it is key to make the feed optimal due to the rise in demand for aquaculture and subsequent rise in demand for feed (Robaina et al., 2019).

Commercial aquaponics is a rapidly increasing form of farming, and the formation of an aquaponics specific diet is almost non-existent. Commercial aquaculture feed is typically used to optimise fish growth, but overlooks nutrients output for crop growth (Junge, König, Villarroel, Komives, & Jijakli, 2017; Roosta & Hamidpour, 2013; Tyson et al., 2011). The need to make a more sustainable feed that replaces fish meal (FM) with a more sustainable source of protein such as; animal by-products and microalgae is pressing to reduce the environmental impact of feed (Gatlin et al., 2007; Hertrampf & Piedad-Pascual, 2000; van Huis & Oonincx, 2017).

A more sustainable feed can save money and is key to the feasibility of aquaponics, but the underpinning success of aquaponics is to be profitable (Greenfeld, Becker, McIlwain, Fotedar, & Bornman, 2019). Therefore, the emphasis in this thesis on the use of microalgae in a diet for *O. niloticus* is crucial not just for aquaculture, be also the feasibility of aquaponics in the future. However, many people have never heard of aquaponics (Greenfeld, Becker, Bornman, dos Santos, & Angel, 2020; Short, Yue, Anderson, Russell, & Phelps, 2017). The key issue in making a profit is consumer perceptions, as people favour aquaponics after being educated and are willing to spend more money (Greenfeld et al., 2019). Consumer perception remains extremely understudied and needs focus for aquaponics to be successful. (Miličić, Thorarinsdottir, Santos, & Hančič, 2017; Short et al., 2017; Suárez-Cáceres, Fernández-Cabanás, Lobillo-Eguíbar, & Pérez-Urrestarazu, 2021). As a result, despite being able to make a more sustainable feed to reduce impacts and potential costs, the consumer perception section of this thesis is fundamental for the successful application of aquaponics and is critical in forming a baseline for prospective aquaponic developments to understand how to best fit the system to local needs and desires.

The adaptation of feed for species like Nile tilapia can be easily formulated as they are omnivores that require between 20-56% crude protein (Tacón, Hasan, & Metian, 2011), opening opportunities for further waste removal into feeds and is a key reason as to why Nile tilapia were selected as a focus for this study. The supplementation of feed for tilapia is a saturated research topic, yet the applications for this supplementation has not been tested in an aquaponic system. The need for more research into feed type, public perceptions and nutrient release is key for the future of aquaponics and sustainable farming.

iv.xii. Aims and objectives

This thesis aims to help bridge the gap in knowledge within aquaponic and aquaculture systems, regarding how feed affects the growth of Nile tilapia and potential effects on water parameters, as well as assess the feasibility of aquaponics systems in the UK. The key objectives of this thesis are:

- Conduct a meta- analysis on the effect of feed supplementation with microalgae on the growth of Nile tilapia and review possible effects on body composition.

- Systematically review how the type and quantity of feed and stocking density can influence water parameters.

- Experimentally assess the palatability of a formulated feed, replacing fish meal with digestate cultured microalgae

- Assess public perceptions of aquaponics in the UK and worldwide, to ascertain how an urban system would be received.

1. A meta-analysis of microalgae supplementation on Nile tilapia growth

1.1 Introduction

One of the most costly and environmentally impactful aspects of aquaculture is the feed, with aquaculture using 70% of the global supply of FM, making aquaculture the most significant exploiter of the oceans (Sarker et al., 2018). Despite this, there is little research into how the use of supplementation of other ingredients can replace FM, which is vital to the sustainability of aquaponics/aquaculture and reducing environmental impacts. The transition away from FM has the potential for significant financial savings, making this a strikingly under-researched topic. As of July 2020 the cost of FM was £1122 per ton, while Soy bean meal (SBM) was a fraction at £280 per ton (Barrientos & Soria, 2020). Microalgae inclusion was analysed separately from other ingredients such as probiotics due to its potential for use in multi-trophic aquaponics systems, uses found with other farmed fish species and the implications of replacing FM. All aquaculture farms could benefit from further research into the effects of supplemented feed, to reduce costs and improve sustainability. Research will help to innovate the sector, by using multi-trophic aquaponics to reduce the damaging environmental footprint of aquaculture and potentially making aquaponics a more closed-loop system. There is a lack of studies that focus on the effects of supplementation in aquaponic systems, which is why this review only used papers using RAS or flow through aquaculture systems. Nevertheless, the need for a more sustainable substitute for FM is pressing. Nile tilapia are best suited for a FM free diet as they are omnivores and can require <5% FM in their diets, but currently most farms just use a commercial aquaculture feed that is high in FM inclusion (Byelashov & Griffin, 2014). Prior to the current trend with inclusion of microalgae in aquaculture feeds, microalgae was typically only used in salmonid farms to make them appear pinker and in shellfish farms for filter feeding bivalves and molluscs, which they still remain an integral part of the functioning of shellfish hatcheries (Hemaiswarya, Raja, Kumar, Ganesan, & Anbazhagan, 2011; Muller-Feuga, 2000). This diverse use of microalgae in aquaculture paved the way for the present and future integration into aquaculture feeds and use in waste treatment for large microalgae cultures.

Inclusion of 20% spirulina and the possibilities of SBM as an alternative to FM need further research to develop a sustainable and cost-effective technique to effectively replace FM on a large scale (Ungsethaphand et al., 2010). A shift from FM to plant-based protein sources can reduce demand on fish stocks, but then incurs an increase of pressure onto land resources and farming (Malcorps et al., 2019). This provides further evidence for the need of more sustainable and integrated systems that include the culture of microalgae with effluent and utilise plant waste as feed for Nile tilapia. Due to the increases prices of FM in the last 12 years as a result of demand, it is estimated that microalgae inclusion can replace up to 30% of world fish catches, reducing the demand for fisheries to produce fish meal (Beal et al., 2018), making integrated multi-trophic aquaponic systems key in a more sustainable future. Additionally, it has been found that microalgae inclusion can be feasible economically, with *Nannochloropsis oculata* being 3.5 times cheaper than fish meal (Sarker et al., 2020). Furthermore, the use of different cultivation technologies such as large scale, thin-layer cascade can reduce costs to €0.6/kg of microalgae biomass (Fernandez, Sevilla, & Grima, 2019), opposed to €1.122/kg of FM (Barrientos & Soria, 2020). However, as it currently stands the production costs of microalgae are much higher than that of FM and the use of new technologies and money saving methods such as using wastewater need to be widely implemented to make it feasible (Nagappan et al., 2021).

Currently, the use of microalgae in aquaculture is limited and not widely used in commercial settings due to the cost, lack of infrastructure and the use of digestate to culture microalgae is even more novel than microalgae cultured in traditional medium (Uggetti, Sialve, Latrille, & Steyer, 2014). While there are many papers that focus on the replacement of FM with microalgae, there is no clear baseline for the quantities that are best applicable. To date, no other meta-analysis has been conducted on different species of microalgae inclusion to compare the commonly used species, on the effects on growth of *O. niloticus*, but there has been wide focus on the use of microalgae in aquaculture feed. This chapter does not aim to record different microalgae species and their effects on Nile tilapia. Rather it aims to prove, using a meta-analysis, that inclusion of microalgae and subsequent removal of FM will be equal or better than commercial feeds for Nile tilapia growth. This chapter will also discuss the effects of microalgae inclusion on the body composition (crude protein content, lipid levels, moisture, omega-3 and ash content) of Nile tilapia to be able to discuss the implications of consuming tilapia fed on microalgae diets and establish if microalgae supplementation is beneficial to the fish and humans.

1.2 Methodology

1.2.1 Search method

This review was conducted by means of an extensive, repeatable literature review, based on peer-reviewed publications, followed by a meta-analysis. The review focused on how feed supplementation can influence the growth of Nile tilapia. The data bases were accessed on 13/06/2020 using the search term:

'Nile tilapia' OR '*Oreochromis niloticus'* AND 'Microalgae supplementation' AND 'recirculating aquaculture system'

The method of reviewing the literature is shown in Figure 1.1. The search yielded 2,010 results that included papers on Spirulina, Chlorella, Scenedesmus and Nannochloropsis. Despite that spirulina is a cyanobacteria, it is often referred to as a blue-green algae so papers using spirulina still appeared in the search. However, it is possible that some papers using spirulina did not appear due to the wording, which should be taken into account when analysing the data. The papers went through an initial screening, which 1,909 could be rejected as they were irrelevant from reading their title and abstract and any duplicates were excluded from the review. Each paper that passed the initial review was then recorded in a spreadsheet to allow for a more thorough second review to assess if it met the criteria for the analysis. A total of **36** papers (1998-2020) were included. The criteria for paper inclusion were; 1) The paper must have a control group, which was no supplementation. 2) There had to be one trial of only Nile tilapia. 3) Reported either feed conversion ratio (FCR) *or* specific growth rate (SGR) *or* data to calculate them. 4) Clear methodology that was standardised and comparable. 5) Provide stocking densities and type and amount of supplementation. 6) Report standard error/deviation *or* the data to be able to calculate. 7) The research was conducted in RAS systems. 8) Published in English. If the paper did not meet all of these criteria, then it was automatically rejected.

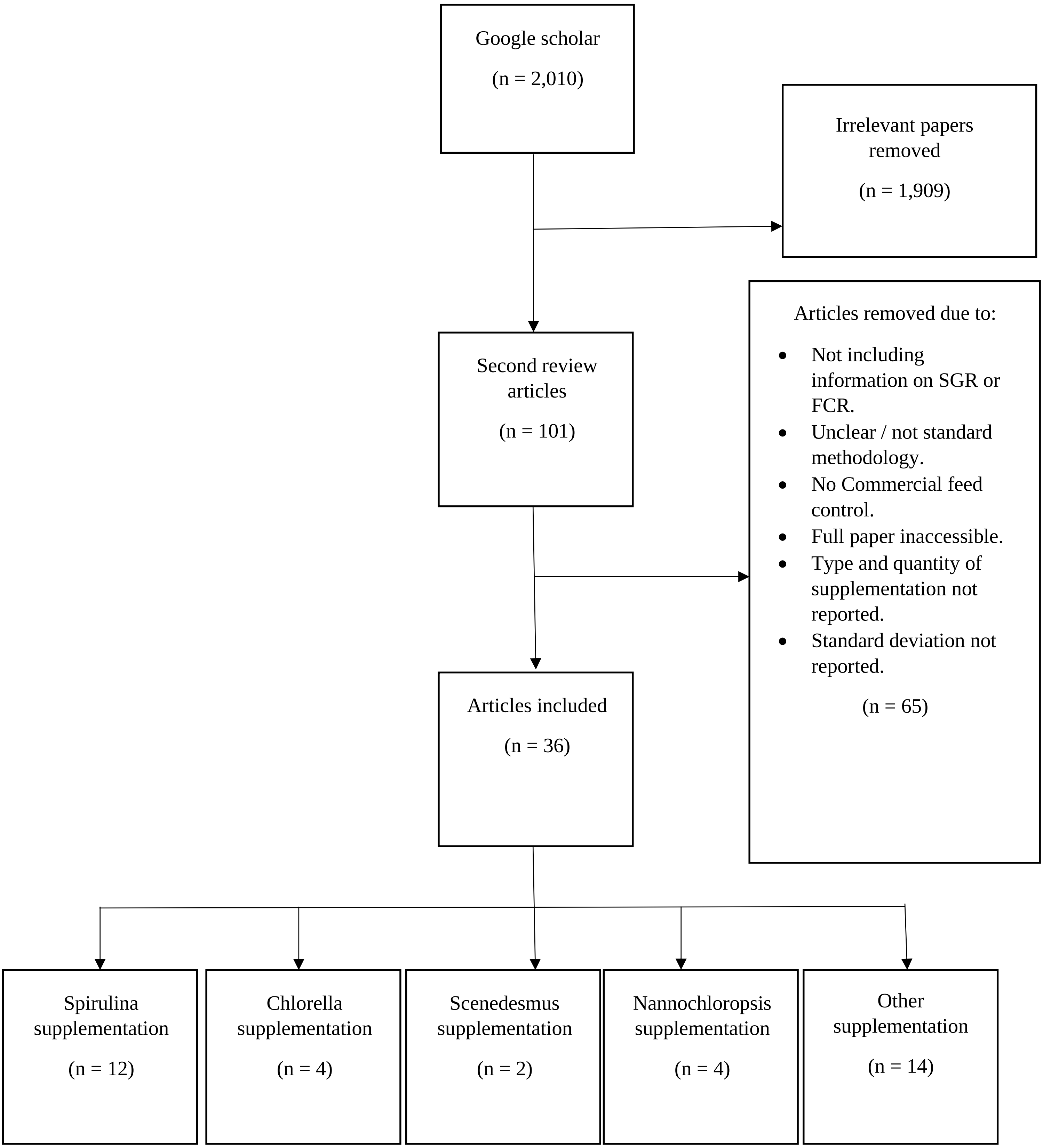


Figure 1.1. Flowchart visualising paper review and reasons for exclusion.

1.2.2 Data extraction and statistical analysis

Once the papers went through the second review, data on the growth of Nile tilapia was extracted and recorded in a spreadsheet, with the following information: 1) Authors, year of publication, journal published in, study duration, age of Nile tilapia, location of study and Title. 2) species of microalgae and percentage inclusion. 3) Crude protein percentage of feed. 4) Amount of feed given (% biomass). FCR and SGR were utilised to see determine the effects of the different supplementations and if any papers did not specifically report these parameters, they were calculated using the following formula.

$$FCR = \frac{Total\ weight\ of\ feed\ input\ (g)}{Average\ weight\ gain\ of\ fish\ (g)}$$

$$SGR = \frac{(lnFinal\ weight - \ln Initial\ weight)}{Days\ of\ study} \times 100$$

Where ln is the natural log of weight and the outcome is percentage growth per day.

As some of the papers only reported standard error, the data was standardised for the use of standard deviation. Standard deviation had to be calculated for some studies, which only reported standard error, with the equation below:

$$SD = SE \times \sqrt{N}$$

Where SE = Standard error and N = Sample size.

FCR and SGR were analysed separately in relation to the effect of supplementation. The Hartung-Knapp-Sidik-Jonkman (HKSJ) method in a random-effects model was used to estimate variance of the pooled effect sizes as it has been seen to provided better results than other methods and is easily utilised in R (Harrer, Cuiijpers, Furukawa, & Ebert, 2019). The analysis compared the FCR and SGR of *O. niloticus* when offered a feed with microalgae inclusion and a control feed. Thus, any differences seen in the growth or body composition in the analysis would be a result of the microalgae inclusion. Therefore, conclusions could be drawn on the effects of the addition of microalgae based on how the experimental diets compared to the control diets of each study.

1.2.3 Calculating effect size

Hedges' g* (Bias corrected, using pooled weighted standard deviations) was used to calculated effect sizes as it is best suited for smaller sample sizes (Hedges & Olkin, 1985), and was conducted for each experiment, with an average taken due to the controls being slightly different between experiments. The following formula were used for Hedges G* and for the pooled standard deviation:

$$G^* = \frac{\bar{x}_1 - \bar{x}_2}{S_p} \times J$$

$$S_p = \sqrt{\frac{(n_1 - 1)S_1^2 + (n_2 - 1)S_2^2}{(n_1 - 1) + (n_2 - 1)}}$$

Where $\bar{x}_1$, $\bar{x}_2$ and S_p are the means treatment (Supplemented feed), control (no supplementation), and Pooled and weighted standard deviation of both groups, respectively. n_1 *and* s_1 showing the number of observations in the supplemented feed and the standard deviation of the supplemented feed, respectively, and likewise for n_2 *and* S_2 with the control group. J is used for bias correction in smaller sample sizes and was calculated using this formula:

$$J = \frac{n - 3}{n - 2.25}\sqrt{\frac{n - 2}{n}}$$

Where n = the number of observations from the supplemented feed group + the number of observations from the control ($n_1 + n_2$).

When interpreting hedges g, 0.2 = small, 0.5 = medium and 0.8+ = large effect sizes. However, Cohen himself suggested that care should be taken when interpreting the results of the effect size as certain situations with a small effect size can still be significant, and it was created for use in social sciences (Cohen, 1977).

1.2.4 Guideline meta-analyses

The data extracted was used in a meta-analysis to quantify the effects of microalgae inclusion in feed, on the growth of Nile tilapia. This analysis was used to determine guidelines across different species of microalgae supplementation and quantity on supplementation, so that subsequent studies within this project can utilise this information to formulate an effective alternative feed for Nile tilapia. All the studies were included in the analysis, regardless of effect size, as even a small effect size demonstrates that a supplemented feed can be equal to that of a commercial feed. In studies which used a range of supplementation levels, the N from the control was distributed evenly to each level of supplementation

Forest plots were produced to visualise whether different types of supplementations influenced FCR or SGR with Nile tilapia. Further analysis was conducted on studies that used other supplementation separately to see how supplementation other than microalgae would influence growth of Nile tilapia, such as probiotics and *Ulva lactuca*, which both did not significantly affect SGR or FCR, but the probiotics improved gut health and immune response (Ramos et al., 2017). The meta-analyses were done to determine; between study heterogeneity to account for any outlying studies or anomalies that may cause heterogeneity, as well as testing for publication bias using funnel plots. Forest and funnel plots were created in R (Version 4.0.3, 2020) using the 'metafor', 'metacont' and 'tidyverse' packages.

1.2.5 Literature utilised

Papers in this analysis utilised four main species of microalgae, being Spirulina (Most common), Chlorella, Scenedesmus and Nannochloropsis. 55% of the studies used in this analysis were conducted in Egypt and they all had a wide range of microalgae inclusion rates (0.5%-100%) and differentiation in the feeding rates between satiation and 3% biomass per day, so certain studies offered more feed, influencing the potential for growth and any effects that a microalgae-based feed could have on Nile tilapia. A range of stocking densities and ages of Nile tilapia were used, from fry to juveniles, most commonly fingerlings as they show more significant results with growth parameters. SGR and FCR were compared in a meta-analysis to how they were influenced by different supplementation types in Nile tilapia feed. 22 papers were used in this meta-analysis. Information from the papers utilised is shown in Table 1.1.

1.3 Results

Table 1.1 A summary of all the papers utilised and their main findings on the use of supplemented feed. N/A indicates that the study did not report the parameters or meet the criteria of the study. Satiation indicates they were fed until they stopped eating.

Study	Microalgae species and inclusion level	Stocking density	Location	Duration (days)	Feed quantity (% biomass)	Age of tilapia	Effect on SGR and FCR	Effect on body composition
B. Belal, 2012	Spirulina (1%)	30	Egypt	90	4	Fingerling	Spirulina improved all growth parameters.	Spirulina increased protein content, but significantly decreased lipid levels and higher ash.
Amer 2016	Spirulina (0.5%, 1%, 1.5%)	45	Egypt	75	4	Fingerling	Generally improved growth.	Decrease of lipid content, but increased protein levels with supplementation.
Abdel Tawwab & Ahmad, 2009	Spirulina (1.25%, 2.5%, 5%, 10%)	60	Egypt	77	Starting at 10%, reducing to 5%	Fingerling	Up to 1% inclusion is positive.	1% inclusion levels improve lipid content, but no effect on ash or moisture.
El-Sheekh et al 2014	Spirulina (50%, 75%, 100%)	30	Egypt	65	12	Fry	Improved growth up to 75% inclusion.	Protein content increased with increased supplementation.
Hussein et al 2013	Spirulina (25%, 50%, 75%, 100%)	50	Egypt	77	10	Fry	100% supplementation can improve growth.	Increase protein levels, but decrease of lipid levels.

Table 1.1 Continued

Study	Microalgae species and inclusion level	Stocking density	Location	Duration (days)	Feed quantity (%	Age of tilapia	Effect on SGR and FCR	Effect on body composition
Olvera-Novoa et al 1998	Spirulina (20%, 40%, 60%, 80%, 100%)	45	Mexico	70	6	Fry	Up to 40% inclusion is positive. (20% optimal)	No clear negative impacts, up to 100% inclusion.
Sherif & Salama 2012	Spirulina (5%, 10%, 15%)	30	Egypt	90	3	Fingerling	Positive growth and higher survival rate with spirulina.	No significant difference.
Ungsethaphand et al 2010	Spirulina (5%, 10%, 20%)	50	Thailand	120	2	Fingerling	Growth not affected by any inclusion levels.	No significant difference.
M. Al-Zayat 2019	Spirulina (2.5%, 5%, 7.5%)	20	Egypt	60	Satiation	Fingerling	Significant improvement in growth.	Up to 7.5% inclusion has no adverse effects.
Badwy et al 2008	Chlorella & Scenedesmus (10%, 25%, 50%, 75%)	75	Egypt	90	3	Fingerling	Growth increased up to 50% inclusion.	Significantly higher dry matter and protein, but lower lipid levels.

Table 1.1 Continued

Study	Microalgae species and inclusion level	Stocking density	Location	Duration (days)	Feed quantity (% biomass)	Age of tilapia	Effect on SGR and FCR	Effect on body composition
Mahmoud et al 2020	Chlorella (5%, 15%)	72	Egypt	60	Satiation	Juvenile	5% inclusion improves growth and immune response.	N/A
Sarker et al 2018	Nannochloropsis (33%, 66%, 100%)	30	Illinois, USA	84	Satiation	Fingerling	Growth slightly lower but not significantly.	N/A
Gbadamosi & Lupatsch 2018	Nannochloropsis (82%)	45	UK	36	Satiation	Fingerling	Similar SGR, but significantly better FCR and PER with supplementation.	Increased protein levels and higher levels of dry matter with supplementation.
Ali, abo el makarem &	Spirulina & Nannochloropsis (3%, 5%, 7%)	45	Egypt	95	5	Fingerling	Both species enhance growth at low levels of inclusion.	Increase protein and dry matter, but decrease in ash content with supplementation.
El-Habashi et al 2019	Spirulina & Chlorella (15%)	30	Egypt	63	3	Juvenile	N/A	Decrease in moisture, but consortium is more beneficial.

Table 1.1 Continued

Study	Microalgae species and inclusion level	Stocking density	Location	Duration (days)	Feed quantity (% biomass)	Age of tilapia	Effect on SGR and FCR	Effect on body composition
Galal et al 2018	Chlorella (10%)	60	Egypt	60	3	Juvenile	Increase growth up to 10% inclusion.	N/A
Velasquez et al 2016	Spirulina (30%, 45%, 60%, 75%)	40	Philippines	60	Satiation	Juvenile	Improved growth with inclusion up to 30%	Significant differences in body composition, with increased Lipid (optimal at 45%), increased protein (30%) increased ash (lowest at 45%) and decreased moisture (lowest at 30%). Generally showing positive effects of supplementation up to 75%.
Teuling et al 2017	Spiruline, Nannochloropsis, Chlorella & Scenedesmus (30%)	45	Netherlands	36	Satiation	Juvenile	All four of the algae species showed increase of growth parameters, but Scenedesmus showed the least effect.	N/A

1.3.2 Specific growth rate

The effect size for changes in specific growth rate due to microalgae supplementation ranged from 20.58 to -51.27, shown in Figure 1.2. Generally, where there was an inclusion of over 50% microalgae, the SGR showed negative trends. Even in studies with large ranges of inclusion, when the percentage inclusion was higher than 30% there was decreased SGR than up to that level of inclusion (Figure 1.2). The Hartung-Knapp-Sidik-Jonkman (HKSJ) random effects model produced an overall effect size of -0.15 with a 95% confidence interval of -3.09 to 2.79. There was very high heterogeneity ($I^2 = 99\%$), thus showing the need for the random effects model and that variance may be greater than expected. This means that results taken from this study cannot, with absolute certainty be used to show the effect found in all situations. This heterogeneity allows for further speculation as to what can cause this difference and if the results are applicable. Additionally, further analysis was done utilising papers that used various types of other supplementations such as probiotics or Ulva meal to assess how various supplementations affect Nile tilapia growth.

To account for any publication bias, funnel plots were created to visualise if any studies were missing, by plotting the standard error (y-axis) against Hedges' g (X-axis) effect size. The more studies that lie within the funnel and show a symmetrical plot means there is less publication bias (Harrer et al., 2019). The funnel plots in Figures 1.3 shows that there is asymmetry to the studies for SGR, indicating publication bias just from visually assessing the plots, with the effect size being significant for many papers, to be expected in a smaller meta-analysis.

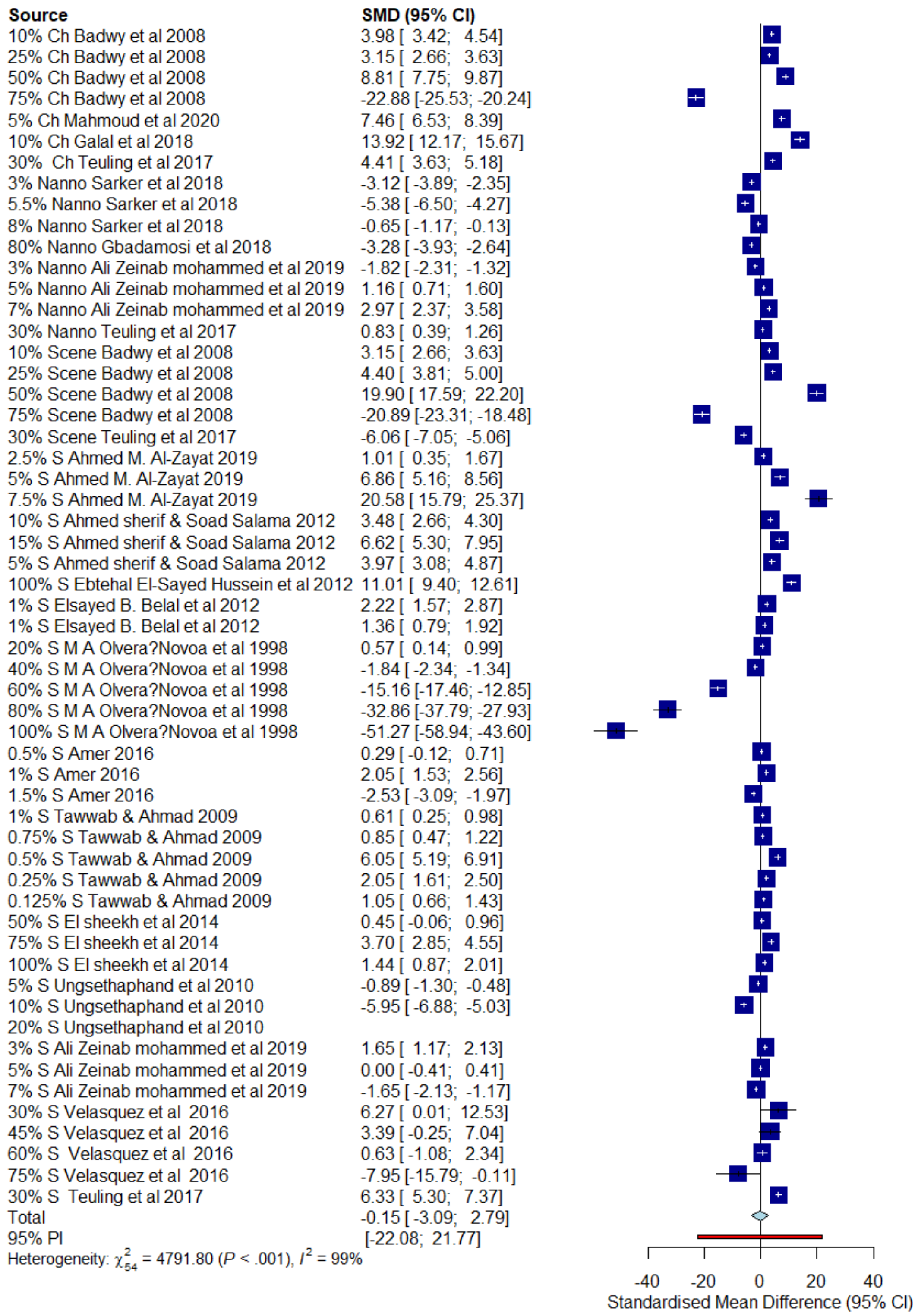


Figure 1.2. Forest plot on the effects of inclusion on Specific growth rate (SGR). Positive values indicate that inclusion improved SGR and negative show it is detrimental. The light blue diamond at the bottom of the forest plot shows the effect size and 95% CI of the HKSJ random effects model, accounting for variability in methods of diet formulation and ingredients. The line at 0 SMD is the line of no effect, where if the CI line overlaps, the inclusion was no different to that of the control. The size of the blue boxes shows the weighting of each study, the larger the box having more weight and the bars through each box indicates the 95% CI.

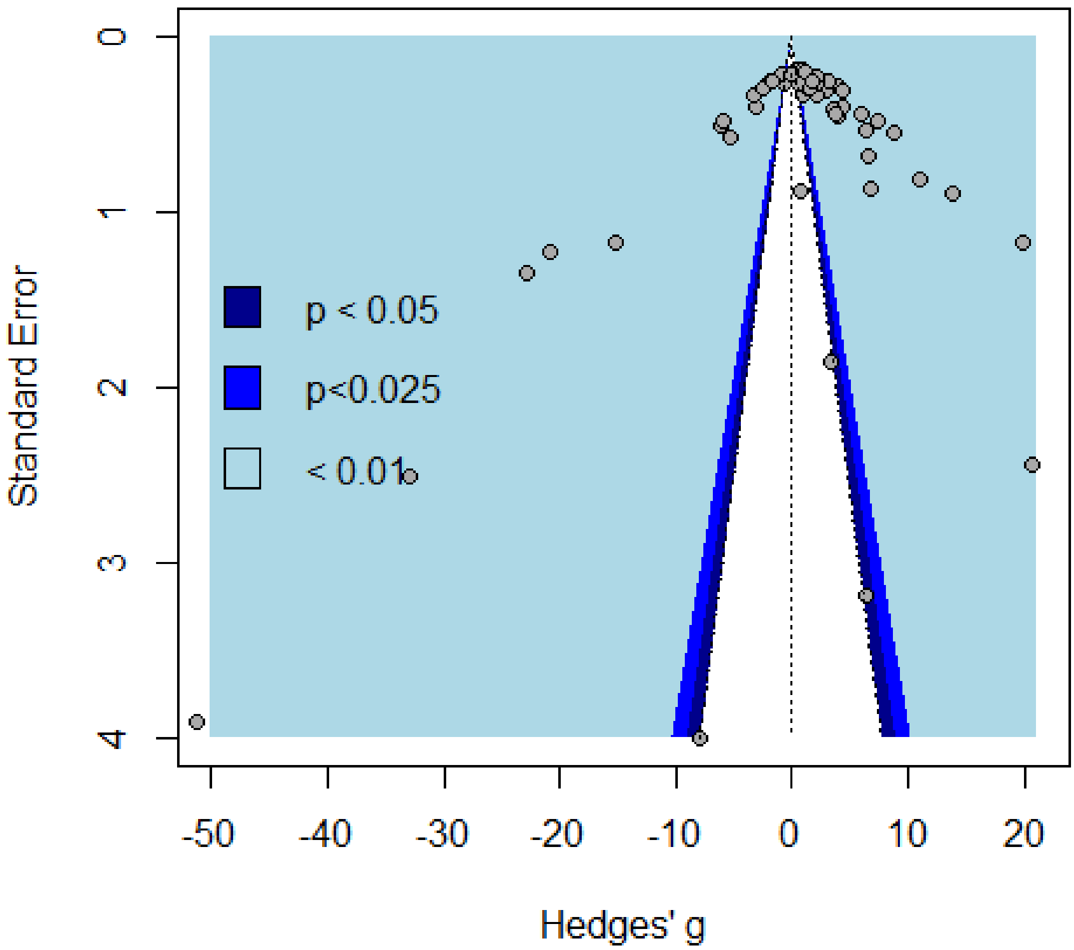


Figure 1.3. A funnel plot to demonstrate publication bias with the studies used in the SGR analysis. Each of the grey dots represents a study and the vertical dotted line indicates the results of the HKSJ random effects model (SMD = -0.15). If there was no publication bias, it would be expected that all the plots would lay symmetrically within the white funnel, asymmetry like in this funnel plot suggests publication bias.

1.3.3 Feed conversion ratio

It is important to note that with FCR, a higher level indicates that there is need for more feed to increase the weight of the Nile tilapia by 1kg. Thus, higher levels are negative, which needs to be taken into account when analysing the data. The effect size for changes in specific growth rate due to microalgae supplementation ranged from 28.75 to -16.57, show in Figure 1.4. Again, when the inclusion level of any microalgae surpasses 50%, the general trend is that FCR is negatively impacted, possibly to a greater extent than SGR. The range of inclusion percentages from the studies showed less of a variation in the results on FCR than SGR, with even smaller inclusions being equal to that of control feeds, yet up to 30% inclusion still appears to be beneficial for FCR (Figure 1.4). The Hartung-Knapp-Sidik-Jonkman (HKSJ) random effects model produced an overall effect size of -0.30 with a 95% confidence interval of -2.22 to 1.61. Once again, there was very high heterogeneity (I^2=99%), showing the need for the random effects model. This means that results taken from this meta-analysis cannot, with absolute certainty be used to show the effect found in all situations. There is evidence of publication bias within the FCR meta-analysis as the funnel plot was asymmetrical with more studies leaning to the left, indicating positive bias as FCR is inverse, shown in Figure 1.5.

Source	SMD (95% CI)
10% Ch Badwy et al 2008	-2.16 [-2.57; -1.76]
25% Ch Badwy et al 2008	-2.34 [-2.76; -1.92]
50% Ch Badwy et al 2008	-9.15 [-10.24; -8.05]
75% Ch Badwy et al 2008	9.29 [8.17; 10.40]
5% Ch Mahmoud et al 2020	-8.95 [-10.05; -7.85]
30% Ch Teuling et al 2017	-5.95 [-6.93; -4.97]
3% Nanno Sarker et al 2018	2.29 [1.63; 2.95]
5.5% Nanno Sarker et al 2018	3.42 [2.61; 4.23]
8% Nanno Sarker et al 2018	4.41 [3.45; 5.37]
80% Nanno Gbadamosi et al 2018	2.51 [1.95; 3.07]
3% Nanno Zeinab mohammed et al 2019	2.44 [1.89; 2.99]
5% Nanno Zeinab mohammed et al 2019	-0.38 [-0.80; 0.04]
7% Nanno Zeinab mohammed et al 2019	-0.76 [-1.19; -0.33]
30% Nanno Teuling et al 2017	0.99 [0.55; 1.43]
10% Scene Badwy et al 2008	-1.01 [-1.35; -0.67]
25% Scene Badwy et al 2008	-1.98 [-2.37; -1.59]
50% Scene Badwy et al 2008	-16.57 [-18.50; -14.65]
75% Scene Badwy et al 2008	1.33 [0.98; 1.69]
30% Scene Teuling et al 2017	14.87 [12.61; 17.13]
2.5% S Ahmed M. Al-Zayat 2019	0.09 [-0.53; 0.71]
5% S Ahmed M. Al-Zayat 2019	-0.84 [-1.49; -0.20]
7.5% S Ahmed M. Al-Zayat 2019	-0.42 [-1.05; 0.21]
10% S Ahmed sherif & Soad Salama 2012	-6.02 [-7.25; -4.80]
15% S Ahmed sherif & Soad Salama 2012	-3.18 [-3.96; -2.41]
5% S Ahmed sherif & Soad Salama 2012	-3.50 [-4.32; -2.68]
100% S Ebtehal El-Sayed Hussein et al 2012	-13.00 [-14.88; -11.12]
1% S Elsayed B. Belal et al 2012	-1.51 [-2.09; -0.93]
1% S Elsayed B. Belal et al 2012	-0.99 [-1.53; -0.45]
20% S M A Olvera?Novoa et al 1998	0.44 [0.02; 0.86]
40% S M A Olvera?Novoa et al 1998	2.75 [2.17; 3.34]
60% S M A Olvera?Novoa et al 1998	4.63 [3.82; 5.43]
80% S M A Olvera?Novoa et al 1998	9.69 [8.19; 11.20]
100% S M A Olvera?Novoa et al 1998	28.75 [24.44; 33.07]
0.5% S Amer 2016	-1.70 [-2.19; -1.22]
1% S Amer 2016	-8.56 [-9.90; -7.21]
1.5% S Amer 2016	1.35 [0.89; 1.81]
1% S Tawwab & Ahmad 2009	-1.20 [-1.59; -0.81]
0.75% S Tawwab & Ahmad 2009	-1.14 [-1.52; -0.75]
0.5% S Tawwab & Ahmad 2009	-2.16 [-2.62; -1.71]
0.25% S Tawwab & Ahmad 2009	-0.70 [-1.07; -0.33]
0.125% S Tawwab & Ahmad 2009	-0.79 [-1.16; -0.42]
50% S El sheekh et al 2014	-0.84 [-1.37; -0.31]
75% S El sheekh et al 2014	-6.73 [-8.07; -5.38]
100% S El sheekh et al 2014	-3.79 [-4.65; -2.92]
5% S Ungsethaphand et al 2010	0.35 [-0.05; 0.74]
10% S Ungsethaphand et al 2010	9.92 [8.47; 11.38]
20% S Ungsethaphand et al 2010	2.66 [2.12; 3.21]
3% S Zeinab mohammed et al 2019	-0.69 [-1.11; -0.26]
5% S Zeinab mohammed et al 2019	0.38 [-0.04; 0.80]
7% S Zeinab mohammed et al 2019	2.29 [1.75; 2.82]
30% S Teuling et al 2017	-10.91 [-12.59; -9.23]
Total	-0.30 [-2.22; 1.61]
95% PI	[-14.08; 13.47]

Heterogeneity: χ^2_{50} = 4216.31 (P < .001), I^2 = 99%

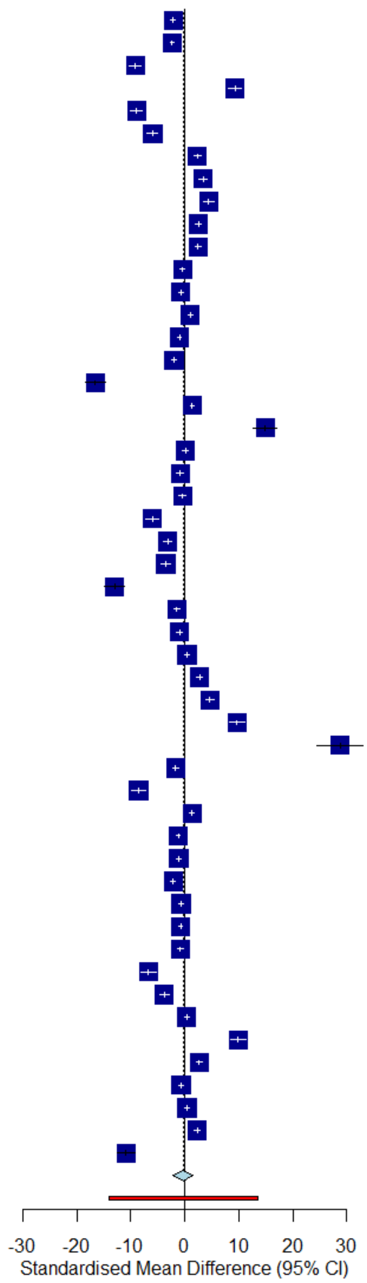


Figure 1.4. Forest plots of the effects of inclusion on Feed Conversion Ratio FCR. Positive values indicate the feed negatively impacted FCR. The light blue diamond at the bottom of the forest plot shows the effect size and 95% CI of the HKSJ random effects model. The line at 0 SMD is the line of no effect, where if the CI line overlaps, the inclusion was no different to that of the control. The size of the blue boxes shows the weighting of each study, the larger the box having more weight and the bars through each box indicates the 95% CI

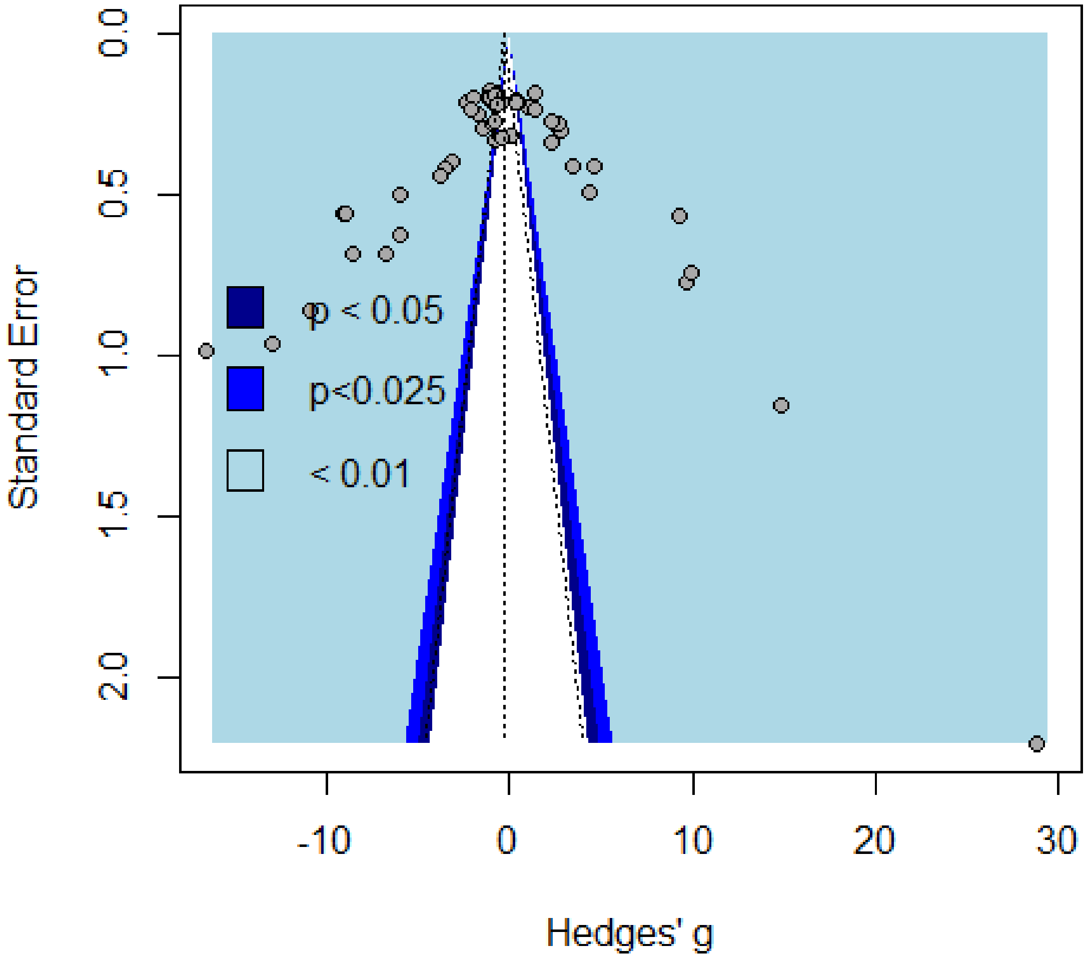


Figure 1.5. A Funnel plot to demonstrate publication bias with the studies used in the FCR analysis. Each of the grey dots represents a study and the vertical dotted line indicates the results of the HKSJ random effects model (SMD = -0.30). If there was no publication bias, it would be expected that all the plots would lay symmetrically within the white funnel, asymmetry like in this funnel plot suggests publication bias.

1.4 Discussion

This review explored the 4 main species that are utilised in alternative aquaculture feeds, which are Spirulina, Chlorella, Nannochloropsis and Scenedesmus and that there are significant differences of digestibility and effects of each species when used in feed (Hussein, Dabrowski, El-Saidy, & Lee, 2013). Despite chlorella being one of the most commercially important microalgae in aquaculture (Ahmad, Shariff, Md. Yusoff, Goh, & Banerjee, 2020) and having health benefits and high omega-3 content (Galal, Reda, & Abdel-Rahman Mohamed, 2018), there is a lack of research focusing on Nile tilapia, which is the case for most microalgae, apart from spirulina. Chlorella and spirulina are commonly utilised due to their high protein (Ca 70%) and the fact they have high digestibility coefficients, with chlorella being highest (Barone, Sonoda, Lorenz, & Cyrino, 2018; Olvera-Novoa, Dominguez-Cen, Olivera-Castillo, & Martinez-Palacios, 1998).

From this meta-analysis, the pooled data of 22 papers using microalgae supplementation demonstrates that there is a slight negative effect on SGR, but a slight positive effect on FCR (Figures 1.4 and 1.2). The findings from this meta-analysis can be used as a guideline to choosing the optimum species of microalgae to supplement Nile tilapia feed to obtain optimum growth and has implications for making aquaponics a more closed-loop, circular system as fish waste can be used to culture microalgae (Shanthi, Premalatha, & Anantharaman, 2021), thus aquaponics could produce some of its own feed. Determining the viability of microalgae supplementation on the growth of Nile tilapia requires; variations of supplementation levels, different species of microalgae, combinations of microalgae and comparisons to various commercial feeds etc. When the optimum species and level of inclusion are determined, this analysis can be used as a guideline to compare the success of the supplementation. Findings from this analysis show that FCR is more affected by supplementation than SGR.

While there is very limited data on the digestibility of different microalgae species, it is believed that species with peptidoglycan (PEP) cell walls are easier to digest than cellulose cell walls. Spirulina has PEP cell walls, linking to their digestibility and chlorella has a different type of cellulose, making them 'softer' (Teuling, Schrama, Gruppen, & Wierenga, 2017), hence the higher digestibility coefficient for chlorella or the process of formulating the feed could deteriorate the cell wall of microalgae, making it more digestible (Teuling et al., 2017) This being said, the apparent protein digestibility coefficient of chlorella, spirulina and Soy bean meal is comparable to that of fish meal, being 86.1%, 80%, 90% and 86% respectively (Hanley, 1987; Köprücü & Özdemir, 2005; Sarker et al., 2018; Sintayehu, Mathies, Meyer-Burgdorff, Rosenow, & Günther, 1996)**,** therefore with the further breaking down of microalgae, microalgae can equal the digestibility of fish meal. This could be a reason why FCR is improved by supplementation as it may make the feed more soluble and accessible to Nile tilapia, because it is closer related to their natural feed.

Results from this meta-analysis show that there is no real trend with high levels (30-100%) of different supplementations, supported by the lack in significance in the effect sizes for both SGR and FCR. High levels (30-100%) of inclusion can inhibit the lipid levels and not supply the correct amino-acids that FM is able to provide (Younis et al., 2018). However, this analysis does show very high heterogeneity (I^2 = 99%), which allows for speculation as to why this is. The most obvious cause of heterogeneity is that the type of supplementation varied across the studies, (Table 1.1). Duration of study and stocking density tended to be fairly uniform between the studies, so could be discounted as a cause for the differences observed, but feed quantity varies greatly and importantly as does age of Nile tilapia. The age of Nile tilapia has been group into broad categories (Table 1.1), to easily visualise differences and is crucial in understanding effects on growth, as both SGR and FCR decrease with age. Therefore, the differences in the results are a result of type of supplementation, feed quantity and experimental design (ED). ED can impact the water parameters, making the system have sub-optimal conditions for the culture of Nile tilapia, which may not have been recorded frequently or reported in the studies.

As a result of the heterogeneity, high levels of supplementation showed no trend or significance for SGR or FCR. Despite this, every single study reported that a lower inclusion rate of up to 20% in most cases was beneficial to SGR and FCR as well as having no impact on the body composition of the Nile tilapia and can also improve their immune response (Abdel-Tawwab & Ahmad, 2009). However, it was often reported that the feed conversion ratio (FCR) was increased with the inclusion of microalgae in the diet. Despite these findings from many papers, other research suggests that supplementation of 100% spirulina is possible to increase growth with no adverse effects (El-Sheekh et al., 2014; Hussein et al., 2013). This shows that the amount of FM used in diets can be dramatically reduced and that many more sustainable alternatives are available. In the case of increased crude protein content, there is a relationship between protein needs of Nile tilapia at certain ages. Nile tilapia fry require higher protein content (40%) compared to larger Nile tilapia (30%) (Al Hafedh, 1999). There are some studies that show little to no effect either way with the inclusion of supplementation, influencing the statistical significance of the results, but they are very useful results in the prospects of this study, as it shows that FM can be replaced, without any adverse effects on the growth of the Nile tilapia. On the other hand, some papers reported that microalgae lead to lower growth parameters, but were not significantly different to that of the control diets (Sarker et al., 2018).

While analysing the four species of microalgae together is useful to create a guideline on supplementation levels with microalgae, the effects of the different species are best analysed separately to assess their individual effects on growth. Overall, chlorella and Spirulina were the most beneficial to the growth of Nile tilapia. However, this does not mean Nannochloropsis or Scenedesmus should be discounted, as they are still beneficial to the growth of Nile tilapia and Nannochloropsis has the added benefit of high omega-3. Adding to this, these results should be taken into account with care as there were far more papers utilising spirulina than the other species, with most having two-four papers which would not be representative of how the species of microalgae can be used, and all papers indicated that a small inclusion of microalgae is beneficial regardless of the species used. Perhaps the most significant result reported from all the studies is that from (El-Sheekh, El-Shourbagy, Shalaby, & Hosny, 2014), where they completely replaced FM with an inclusion of 100% Spirulina in place of FM, with no adverse effect on SGR, FCR or body composition.

Even though the results from the forest plots indicate that there is no significance at all supplementation levels, when used at optimal supplementation there is clear evidence for suitable replacement of FM in aquaculture diets in Nile tilapia. This is of great importance globally, both economically so developing countries can grow their aquaculture industry and for the sustainability of aquaculture. Most importantly, the use of microalgae in an urban aquaponics setting has huge potential to improve food security in local areas (Li et al., 2019), as the use of microalgae will further reduce the need for outside sources for feed ingredients and create a more closed-loop system (Rizal et al., 2018). However, the current costs of large-scale microalgae culture limits its widespread usage, but when utilised at small scales in efficient systems, microalgae can become more economically feasible than FM (Ansari, Guldhe, Gupta, Rawat, & Bux, 2021; Sarker et al., 2020; Shah et al., 2018; Yarnold, Karan, Oey, & Hankamer, 2019).

It is important to note that the funnel plots for both SGR and FCR (Figures 1.3 and 1.5) show positive publication bias. As a result of this, it can be assumed that there are papers which showed negative effects or no effects on growth of Nile tilapia due to supplementation that were not published, or it may have been due to the criterion of inclusion causing this bias. This bias is a hindrance to the progression of aquaponics, in making it more sustainable and means good science with results that are not “desirable” are not shared and creates a loop of research into the effects of supplementation on Nile tilapia that is unnecessarily repeated. Nevertheless, because of this and the high heterogeneity, the results from this analysis should be interpreted with caution. While growth parameters show a clear positive trend in relation to supplementation (at the optimal levels). The body composition analyses indicate that supplementation is intrinsic and significant in altering the protein, lipid and ash content of Nile tilapia (Velasquez et al., 2016). However, a handful of studies suggest that a proximate analysis found no significant differences in body composition in relation to the amount of supplementation, even with levels of 100% inclusion having no effect (Olvera-Novoa et al., 1998; Sherif & Salama, 2012; Ungsethaphand et al., 2010).

The studies utilising spirulina were the only ones to report lipid content of the Nile tilapia carcasses. These studies showed that lipid content in spirulina supplementation is lower than that of the control and was significant (<0.05) (B. Belal, 2012). Yet it has been found that small inclusion levels can increase the lipid content slightly, without compromising other body composition parameters (Abdel-Tawwab & Ahmad, 2009). While this is clearly the case for spirulina, the other microalgae could have differing effects on the lipid content of Nile tilapia and could mean that body composition is not compromised for increased growth. In light of this, protein and dry matter were found to increase with increasing supplementation in almost every case. It was found that the ash content generally increased with increased supplementation and increased to a greater extent in spirulina diets in comparison to the other microalgae species. One notable result was from the study by (El-Habashi et al., 2019), whom found a significant decrease in moisture content and a significant increase in the protein content using Chlorella and consortium compared to the control and Spirulina diets.

1.5 Conclusion

In conclusion, the results from this meta-analysis indicate that a small inclusion of up to 30% microalgae can be beneficial to the growth parameters of Nile tilapia, without compromising the Nile tilapia health. Specifically, spirulina and chlorella are prime candidates due to their digestibility and wide applications already within aquaculture in comparison to Nannochloropsis and Scenedesmus. However, all four are still key in improving body composition, are beneficial to Nile tilapia growth and should still be considered for future use. The lack of studies on the effect of supplementation on the carcass composition hinders the ability to make definitive conclusions, but chlorella appears to perform better than spirulina in maintaining or improving body composition. These results are extremely useful and pertinent for this study as a replacement for FM is vital in the sustainability of aquaculture. Supplementation that yields the same growth and body composition of fish, without being detrimental and can lower the cost of feed are crucial to a more sustainable future. Future research will need to incorporate more algae species at all levels of inclusion and take measurements of growth and body composition to fully understand how microalgae can be utilised effectively.

2. The effects of aquaculture feed on water parameters

2.1 Introduction

Type of supplementation and different feeding regimes can greatly influence water parameters, thus can be damaging environmentally and can be key in formulating a feed specific to certain crops in aquaponics as different feeds release different nutrients (Goddek et al., 2019). The nutrient dynamics within aquaponics systems are the key to its success or downfall, as it requires a careful balance of nutrients to the plants, while maintaining good water quality for the fish (Seawright, Stickney, & Walker, 1998). It is widely accepted that aquaponic systems can provide most of the nutrients needed for optimal plant growth when the system is sized correctly, but will always need supplementation of certain nutrients such as iron, which is generally the most limiting factor (Goddek et al., 2019). Despite this, majority of papers that use aquaponics ignore the dynamics of water parameters in relation to feed and solely focus on the feed quantity (Delaide, Goddek, Gott, Soyeurt, & Jijakli, 2016; J. E. Rakocy et al., 1997; Yıldız & Bekcan, 2017). Furthermore, no studies have actually assessed the nutrient dynamics in relation to different feeds. The fact that no research has focused on this topic is staggering and means that they can lead to systems missing the very essence of aquaponics, being the interaction between nutrients produced and the uptake by crops.

It is essential that the nutrient dynamics in aquaponic systems are fully understood, as then they can be utilised in the most effective way possible, then tailored to particular crops, as aquaponics lends itself to crops with lower nutrient requirements such as lettuce (Nozzi, Graber, Schmautz, Mathis, & Junge, 2018). As no research has focused on the nutrients relating to type of feed, this study aimed to create a baseline of data that can help to design and formulate feeds for aquaponic systems in order to get the best possible outputs for different crop species. To do this, data was extracted from a number of studies that were both aquaponics and RAS systems, but no meta-analysis was run due to the studies all being very different as they did not focus on the nutrient dynamics.

2.2 Methodology

2.2.1 Literature search methodology

This systematic review was conducted by means of an extensive, repeatable literature review, based on peer-reviewed publications. The review focused on how feed type and quantity can influence water parameters in RAS. The data bases were accessed on 30/06/2020 using the search term:

‘Water quality ‘, ‘feed’ AND ‘Nile tilapia’ OR ‘*Oreochromis niloticus’* with variations using ‘aquaponics’, ‘effluent’ and ‘faeces’

The variations were used in attempts to search for papers focusing how feed can alter nutrient excretion by Nile tilapia and its potential for aquaponics. Majority of the papers that were found could be rejected immediately due to using other species or being clearly irrelevant to the research topic. It became apparent that no papers have focused on the nutrient excretion in relation to the feed input, so all used data had to be extracted from papers not necessarily focusing on the topic of the review. The criteria for inclusion of data from the reviewed papers were as follows; 1) The paper must have a control group, 2) There has to be at least one system comprised of solely of Nile tilapia, 3) The system must be a closed RAS, 4) Feed type and quantity must be given and 5) At least one water parameter is recorded throughout the study (Ammonia, Nitrite, Nitrate, Phosphorus, Dissolved oxygen (DO) or pH). 65 studies went through a second review and a total of 11 studies (1992-2020) were retained for use in the study and provided multiple sets of data through trials within the studies. Most studies were rejected due to the lack of studies reporting water parameters throughout the study and not meeting the full inclusion criteria, as well as, being outside the scope of this review.

2.2.2 Data extraction and statistical analysis

Once the papers went through the second review, data on water parameters was extracted and recorded in a spreadsheet, with the following information: 1) Authors, year of publication, journal published in, study duration, location of study and Title. 2) Crude protein percentage of feed. 3) Amount of feed given. 5) Water parameters recorded; Ammonia, Nitrite, Nitrate, Phosphorus, dissolved oxygen or pH, hence forth referred to as water parameters. 6) What crops were grown, if any. The feeding regime was used to determine how variations can influence the water parameters in RAS. This review used studies where the prime focus was not on nutrients from feed, so data had to be carefully extracted. Water quality parameters were obtained from each separate trial within each study, but not all papers reported all of the parameters so each were split into separate groups of Ammonia (NH3, if NH4 was reported, the results were not included) (11 trials), Nitrite (NO2) (15 trials), Nitrate (NO3) (13 trials), Phosphorus (PO4) (10 trials), DO (18 trials) and pH (18 trials). The data analysis for each water parameter was done separately to one another. Additionally, the crude protein (CP) percentage, quantity of feed (% biomass), stocking density, type of system, study length and location were recorded for comparisons to be made. Multiple Linear regression was used as a statistical tool to predict the relationship between the water parameter variables and feed type, quantity and stocking density. The formula used for the multiple linear regression is as follows:

$$Y = \beta_0 + \ \beta_1 X_1 + B_2 X_2 \ldots . + B_{10} X_{10}$$

X = Distinct predictor variables

β_0 = Value of Y when all of the independent variables (X1 through X_{10}) equal zero

β_1 = Change in Y in relation to a one unit change in X_1

The multiple linear regression was followed by a comparison model using the MuMIn package, using the ‘dredge’ to compare the models for each parameter with 3 predictors, 2 predictors and one predictor. The models were then assessed using Akaike information criterion (AIC) to determine which model best predicted each water parameter. All analyses and graphs were plotted in R version 3.6.3 (2020-02-29).

2.3 Results

Comparisons between the water parameters of; Ammonia, Nitrite, Nitrate, Phosphorus, DO and pH were made against feed quantity (% Biomass), feed type (% CP) and stocking density (kg/m^3). 11 papers were used in in this study, with inclusion of their separate trials to help infer conclusions as to why water parameters may differ in RAS. Information of the papers used is shown in Table 2.1. It should be noted that the system design of each study used varied, therefore the size and water treatment potential vary between studies which would influence the effects of feed on water parameters.

Table 2.1. list of research papers (1992-2020) included in the present systematic review, reporting also the initial stocking density, type of system, location/duration of study and additional information.

Author	Stocking density (kg/m^3)	System	Location	Duration (days)	Additional information
(Suresh & Lin, 1992)	15	RAS	Wädenswil, Switzerland	70	No plants, differing stocking densities
	3.75				
(Li et al., 2019)	0.027	Media bed	University of Stirling	130	60 celery and spinach/m^2 was grown in both systems
	0.027	NFT			
(Effendi et al., 2020)	1.4	NFT	Asian institute of technology Thailand	42	600g/m^2 of vevitier
	1.4				1800g/m^2 of vevitier
(Eissa, El-Lamie, Hassan, & El Sharksy, 2015)	2.4	DWC	University of Hawaii at Manoa	60	Green bell peppers
(Danaher, Shultz, Rakocy, & Bailey, 2013)	13.5	DWC	University of the Virgin Islands, St. Croix	55	Spinach grown at 3.3kg/m^2
(Knaus & Palm, 2017)	4.5	Media bed	Mecklenburg Western Pomerania	70	Lettuce, tomato & cucumber at 6/m^2
(Licamele, 2009)	2	DWC	University of Arizona	37	Differing stocking densities, and 32 lettuce plants/m^2
	8				
(Rayhan et al., 2018)	0.313	Media bed	Gazipur, Bangladesh	60	Differing stocking densities. Continuous flow instead of ebb and flood. Spinach plants at 12/m^2
	0.733				
	0.945				
(Kanial, 2006)	1.1	DWC	Sharkia, Egypt	180	10 bell pepper plants/m^2
	1.1				15 bell pepper plants/m^2
(Pinho, Molinari, de Mello, et al 2017)	4	DWC	Santa Catarina, Brazil	21	Use of biofloc technology and 20 lettuce plants/m^2
(Wahyunigsih, Effendi, & Wardiatno, 2015)	1	NFT	Bogor university, Indonesia	60	Lettuce plants at 5/m^2

Table 2.1 clearly visualises the differences between the studies utilised, thus the need to refrain from meta-analysis statistics and focus on systematic review, resulting from a lack of any papers focusing on the nutrients in relation to feed excretion. Therefore, there are too many differences between study designs, such as; using different system designs, stocking densities, species of plants and a large range in study duration. Correlations and relationships could be taken into account with caution of causation due to the differences in the studies, but it can set a good groundwork for future studies.

Multiple linear regression tests enabled the use of residual plots to help visualise correlations and the distribution and homogeneity of the data. Initial findings using trend lines from multiple linear regression plots and correlation statistic from RStudio indicate that the strongest correlations are between Ammonia (Figure 2.1) and Nitrate (Figure 2.3) against feed quantity, as well as; Dissolved oxygen against stocking density (Figure 2.5) and Ammonia and pH against feed type (Figure 2.6). It appears that most of the regressions show the expected trend in relation to the three independent variables in most cases

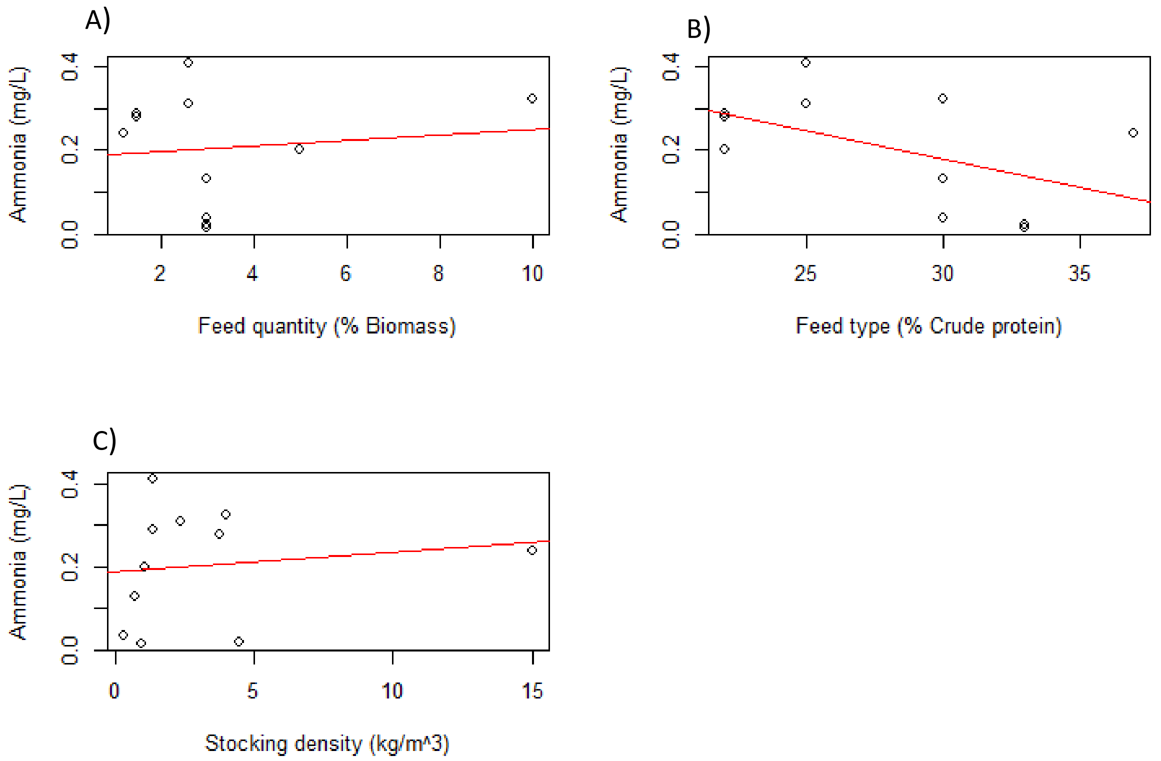


Figure 2.1 Quantity of Ammonia (NH_3, mg/l) in relation to; A) Feed quantity, B) Feed type and C) Stocking density

Figure 2.1 clearly shows that there is a more significant correlation to feed quantity than either feed type, with very little correlation to stocking density, which unexpectedly displayed a negative trend.

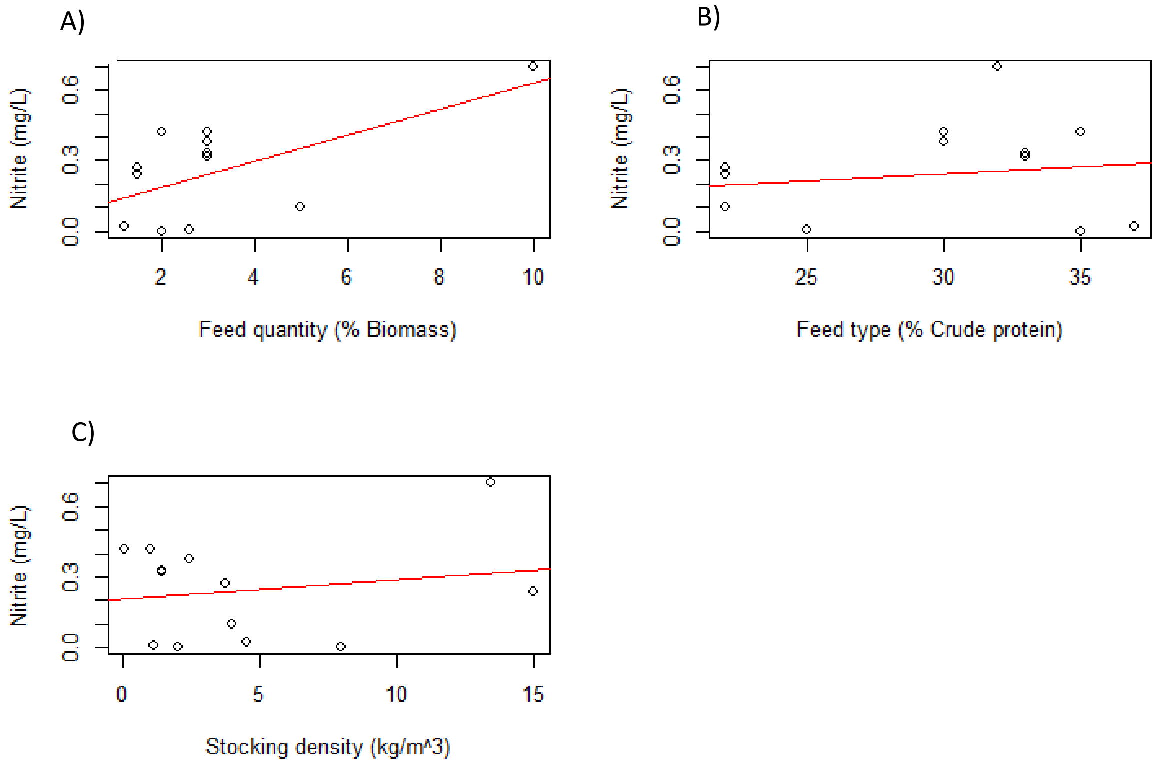


Figure 2.2. Quantity of Nitrite (NO2, mg/l) in relation to; A) Feed quantity, B) Feed type and C) Stocking density

Figure 2.2 reinforces the correlation between of poorer water quality and feed quantity, with a clear strong positive correlation and little correlation between feed type or stocking density.

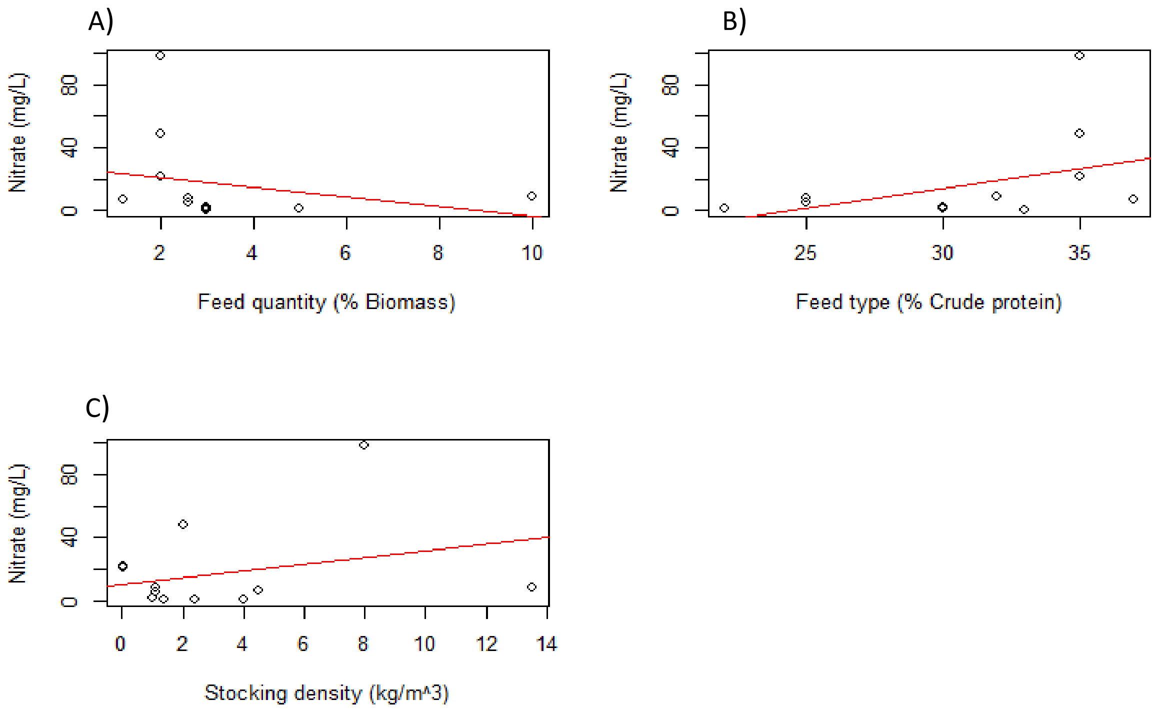


Figure 2.3 Quantity of Nitrate (N03, mg/l) in relation to; A) Feed quantity, B) Feed type and C) Stocking density

Figure 2.3 Displays an unexpectedly negative trend in Nitrates with feed quantity, but positive in relation to type and stocking density. This could be a result of the inclusion of aquaponic systems and just RAS systems skewing the amount of Nitrates through uptake by the plants.

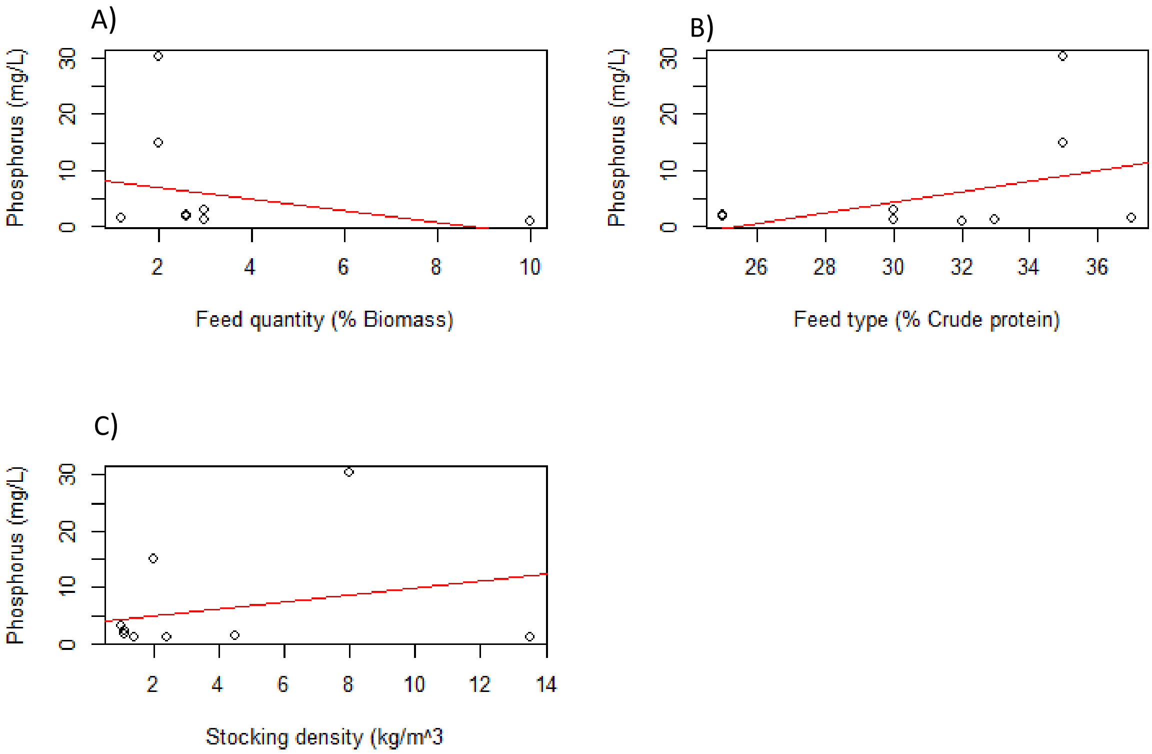


Figure 2.4. Quantity of Phosphorus (PO4, mg/l) in relation to; A) Feed quantity, B) Feed type and C) Stocking density

Figure 2.4 Shows similar results to the Nitrates found in each study, except this result can be expected, due to differing phosphorus content in the feeds and therefore feed type would affect the release of PO4 into the water.

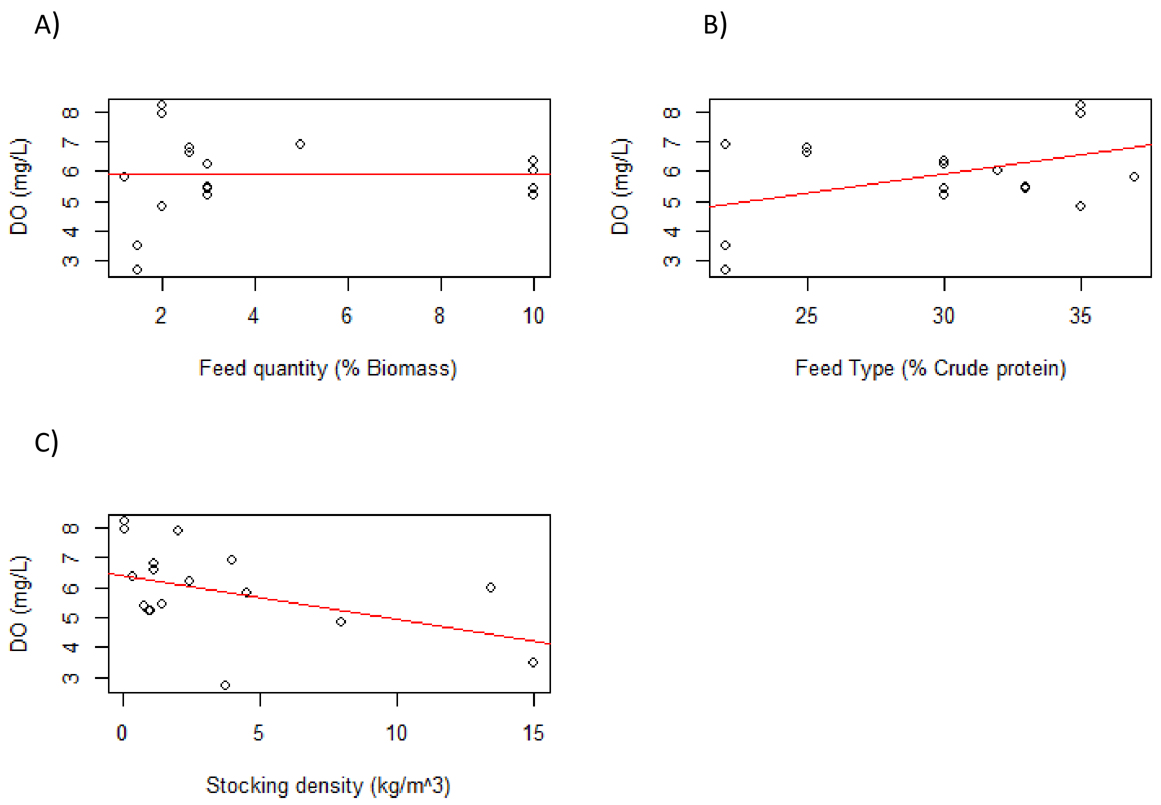


Figure 2.5. Quantity of Dissolved oxygen (DO, mg/l) in relation to; A) Feed quantity, B) Feed type and C) Stocking density

Figure 2.5 DO cannot be easily compared to the other parameters, as it is not directly affected by feed, but the most significant factor is stocking density. This is due to higher numbers of Nile tilapia in the systems utilising more oxygen in the water and therefore lowering DO levels

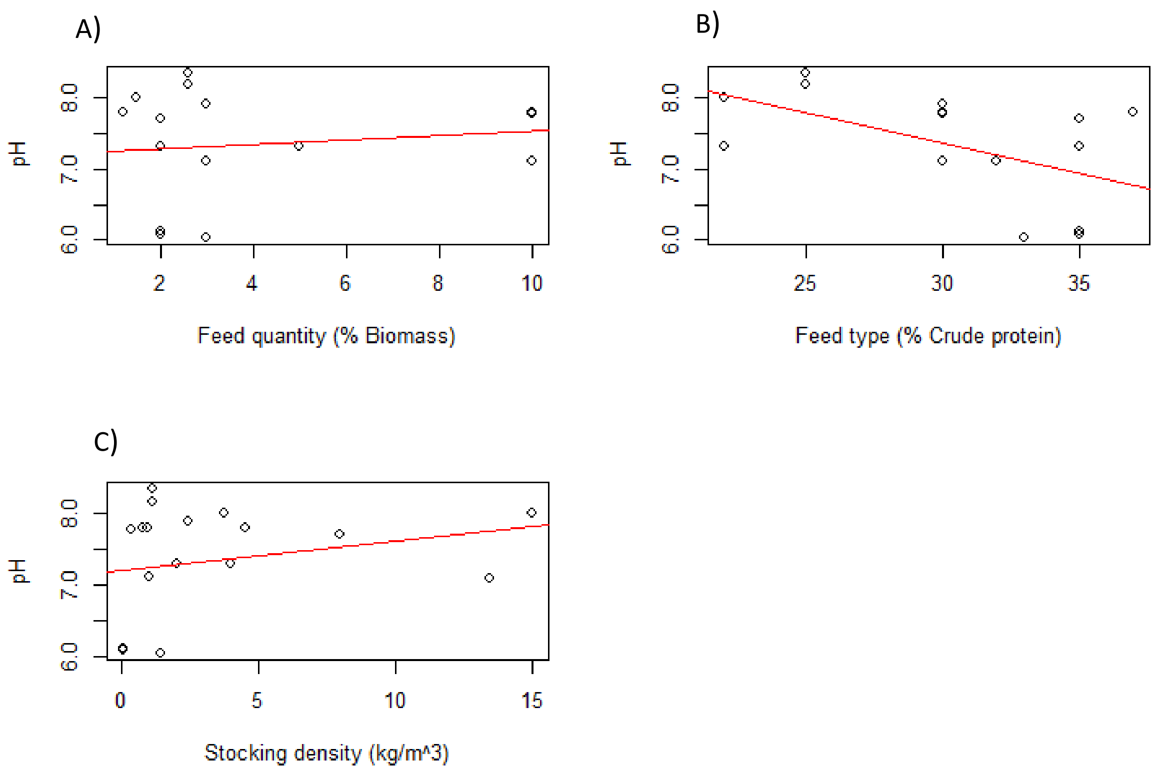


Figure 2.6. Quantity of pH in relation to; A) Feed quantity, B) Feed type and C) Stocking density

Figure 2.6 Shows interesting relationships with pH and the independent variables tested against it, possibly showing how the type of feed can affect digestibility to fish and as a result the ability for bacteria to break down the released nutrients. With increased nitrification the lower the pH, which could indicate a high nitrification at higher crude protein levels.

To estimate the relationship between the water parameters and feed quantity, feed type and stocking density shown in Figures 2.1 – 2.6, a multiple linear regression test was conducted for each water parameter to determine if there was any statistical significance ($P<0.05$) in the results, determining whether there was an effect of the three independent variables on the water parameters. This analysis allowed for the significance of the different independent variables to be assessed for each parameter, but it did not show which variable and model best explains the data. As a result, the dredge function (MuMIn package) was used was used to compare models and assess the weighting of each model using the Akaike information criterion (AIC) to identify the goodness of fit, as to which variables best predicted each water parameter. If the models had an AIC within 2 of the lowest AIC then they were considered as the best predictor model.

The most crucial finding was that ammonia was only significant when analysed against feed quantity (Figure 2.1). Allowing the null hypothesis to be rejected. Nitrate was also significant against feed quantity and stocking density, and pH against feed type. Unpredictably, DO was not significant ($P<0.05$) in relation to stocking density. Overall, the most influential aspect on nutrients in the water (excluding DO and pH) is feed quantity, playing a more significant role than feed type, yet feed type is still significant and will impact fish growth and health.

The goodness of fit test identified that feed quantity was the best predictor for ammonia and nitrite, while feed quantity and stocking density best predicted nitrate and phosphorus, stocking density for DO and feed type was the best predictor for pH. There is a clear relationship between ammonia, nitrite (which are toxic to fish) and nitrate and phosphorus with the feed quantity, shown in Figures 2.1 - 2.4 and from the results of the multiple linear regression and goodness of fit tests. There are other influencing factors to maintaining a good water quality, including type of feed and stocking density that are crucial to control DO and pH levels in the system.

After running the tests, the data was analysed to understand if there were similar trends at different levels (E.g., 10%feeding rate – 3% etc). The differences between 10% and lower feeding rates are the most significant than any other quantity comparison. Indicating that a higher feeding rate will result in higher ammonia levels, which is to be expected. Likewise, the same results were observed from Nitrite and Phosphorus against feed quantity. pH was significant against feed type and the largest difference lays between 33%-25% Crude protein and the larger differences were between the highest and lowest crude protein percentages. Additionally in studies that reported a higher stocking density there is a general trend of lower DO levels and vice versa.

2.4 Discussion

Results from this present study indicate that the main influential variable that will impact fish welfare appears to be feed quantity, as the more feed introduced into the system, the more toxic, ammonia and nitrites are produced (Figures 2.1 & 2.2). However, there are also other factors that influence fish welfare and the balance of aquaculture and aquaponic systems, including that of stocking density, which is most influential with DO in the water (Figure 2.5) and can lead to anoxia. Feed type is also crucial as it will influence the growth of the fish and importantly from an economic stand point, the quality of produce.

2.4.1. Ammonia, Nitrite and Nitrate

Ammonia nh3 is highly toxic to fish, but ammonium ion NH_4^+ is not as-toxic. Ammonia and nitrite were significantly affected by the quantity of feed offered to the Nile tilapia (Figures 2.1 & 2.2), with a significant increase in both with higher feeding rates. Similar results to this were found by (Al-Harbi, 2000) indicating the results are accurate. Ammonia enters aquaculture systems through the excretion of fish waste (Redner & Stickney, 1979) and is broken down by nitrification to nitrates ready for uptake by plants in aquaponic systems. Therefore, with a higher feeding rate, there is a larger amount of excretion from the fish stock that results in higher levels of ammonia, supporting the findings of this study and showing the results are reliable and are as to be expected. Ammonia and nitrite are extremely toxic to all fish at low levels, even though Nile tilapia can be more tolerant, they cannot withstand more than 0.1mg/L NH_3 (El-Shafai, El-Gohary, Nasr, Van Der Steen, & Gijzen, 2004).

The result of little significance or relationship to feed type (% Crude protein) is interesting as other research has found that with increased protein levels, there will be an increase of ammonia excretion (Brunty, Bucklin, Davis, Baird, & Nordstedt, 1997). However, in the case of increased feeding rates, a plausible conclusion would be that with an increase of quantity there is more protein available. Thus, the findings of (Brunty, Bucklin, Davis, Baird, & Nordstedt, 1997), support the results of this study with more protein influencing the water quality in aquaculture systems.

Another explanation for the findings of this study is the heterogeneity between the studies. The age of Nile tilapia changes how they utilise the protein in the feed in relation to amount of ammonia excreted (Begum, Chakraborty, Zaher, Abdul, & Gupta, 1994), as all the studies used varying ages and sizes of Nile tilapia at the beginning of the studies, it is possible that the results were found due to the differential in protein utilisation of the fish stock. Furthermore, ammonia, nitrites and nitrates are affected by the nitrogen cycle, meaning there are often fluctuations within systems. As a result, with most studies only reporting parameters once a week or as means over the whole study period, the true values cannot be observed over long study periods with few measurements. Therefore, the results from this study can help to determine general trends of water parameters and feed, but as they are not measured frequently enough, the true effect of feed quantity, type and stocking density is unknown. This highlights the need to conduct further research directly focusing on the impact of feed on water parameters.

2.4.2 Phosphorus

In this study, results showed that phosphorus was not significantly influenced by any variable, but the model that best fit was that of feed quantity and stocking density. Many papers recorded phosphorus levels over the optimum for Nile tilapia (0.05-1), but most papers report that there is no impact on Nile tilapia in relation to high exposure to phosphorus (Bhatnagar & Devi, 2013; Effendi, Widyatmoko, Utomo, & Pratiwi, 2020). While feed type had no significant effect on phosphorus, there was a positive trend, resulting from different levels of inclusion of phosphorus in the different feeds used in each study and higher protein content in the feed resulting in higher levels of phosphorus.

Phosphorus has little impact on the growth of Nile tilapia, as up to 65% of phosphorus in feed is available to Nile tilapia and they require very minimal inclusion in their diets (Watanabe, Takeuchi, Murakami, & Ogino, 1980). This would explain why an increase of feed quantity and stocking density best explain phosphorus, as the low requirement leads to high levels of excretion. Within the context of this study, it is a promising finding. In aquaponics systems, phosphorus is a key nutrient to maintain healthy growth of plants and is often limiting (Pantanella, Cardarelli, Colla, Rea, & Marcucci, 2012). Phosphorus is relatively an understudied water parameter, due to its limited effect on the growth and welfare of Nile tilapia, but with the increase of aquaculture and aquaponics (FAO., 2020), the need to understand nutrient dynamics in integrated systems is pressing to help innovate the aquaponics feed dilemma.

2.4.3 Dissolved oxygen

Higher stocking density reduced DO levels. Although Nile tilapia can tolerate low DO levels (Delong et al., 2009; El-Sayed, 2019; Makori, Abuom, Kapiyo, Anyona, & Dida, 2017; Sallenave, 2016; Setiadi, Widyastuti, & Prihadi, 2018), extremely levels could still have adverse effects chronically to the growth and development of the fish(Makori et al., 2017). Similarly, to the results found in this study, of relation to stocking density, other papers support these findings (Al-Harbi, 2000; R. Tyson, Simonne, White, & Lamb, 2004). An increase of feed leads to increased nitrification of ammonia due to higher excretion levels (Brunty et al., 1997), which is an oxidation process, reducing DO levels. Even though all studies used in this review maintained DO levels within the tolerable levels for Nile tilapia, DO can cause growth problems for Nile tilapia when exposed to low or high levels after long-term exposure. DO can also be affected by a number of different factors; including temperature, as with higher temperature there is less carrying capacity of DO (Abdel-Tawwab, Hagras, Elbaghdady, & Monier, 2014). Studies used had varying temperatures due to experimental design of being indoors or outdoors and being in different countries that vary in average temperatures, resulting in fluctuations in DO and levels of toxic ammonia. Additionally, the levels of aeration and flow rates varied between studied or were not reported, leading to various levels of DO even in systems that were very similar in all other aspects and could explain the results in this review (Al-Harbi, 2000)

Recent studies have suggested that Nile tilapia in aquaponic systems will perform best at recirculation rates of 200-400% per day based on optimum water parameters for *O. niloticus* **(Table i.i)** (Ngo Thuy Diem, Konnerup, & Brix, 2017), and would be similar to that required in RAS and flow through systems to supply optimum DO levels. Additionally, from reviewing the papers used, an optimum stocking density of Nile tilapia in aquaponics system was found to be 106 fish/m3 (Estrada-Perez et al., 2018; Rahmatullah et al., 2010; Rayhan et al., 2018), in order to maintain optimum water parameters and allow for efficient growth of fish and plants. DO is a widely researched topic in aquaculture and the requirements of Nile tilapia are well understood, as well as the methods to supply and maintain DO levels. The need for research into how DO can affect plant growth in aquaponics is important for further research to understand the dynamics of the inclusion of plants into the system on DO.

2.4.4 pH

pH was significant in relation to feed type, with increase protein decreasing pH (Figure 2.6). This is due to lower digestibility of feed at higher levels of protein, or showing higher levels of nitrification that results in lowering the pH. However, a more appropriate conclusion would be that due to most of the studies including an aquaponics component, the pH is a result of the system needs. Aquaponics has an optimum pH of 7 (R. Tyson et al., 2004), Yet the optimum pH for nitrification and most fish species is around 8 and show decreasing efficiency at a lower pH (Antoniou et al., 1990). As a result, the pH of a system is vital in maintaining optimum water quality, as lower pH will lead to slower nitrification and is very important in the understanding of the effects in aquaponic systems, especially the toxicity of ammonia (Kholdebarin & Oertli, 1977).

2.4.5 Implications for aquaponics

Understanding how the type and quantity of feed can influence an aquaponics system will allow for the maximum output to be achieved by altering the input of the feed. This control of water parameters such as ammonia and pH will mean that the fish species can have the best parameters for growth, while also controlling the nutrient flow into the hydroponics systems and therefore the available nutrients can be controlled via the feed. Adding to this, pH is a key talking point in aquaponics and can be influenced by the country and even local area that alters the pH of the local water source and in aquaponic systems by the plant species used. It has been found that tomatoes have a higher nitrogen use efficiency than pak-choi, therefore, tomatoes will lead to lower pH (Hu et al., 2015). Another positive use of lower pH in aquaponics is that there is higher availability of phosphorus for the growth of plants (Cerozi & Fitzsimmons, 2016). Therefore, pH is of upmost importance in aquaculture and aquaponics to maintain optimum water quality and can influence the entire system by affecting nitrification rates, which is why most commercial farms alter pH with buffers and use them to maintain optimum levels. Subsequently, the amount of feed waste can be reduced and ingredients or aspects of feed that have little impacts can be changed for other potentially more economical ingredients to have cascading economic impacts with aquaponics.

It should be noted that the systems each of the studies used in this review were all quite different in their hydroponic design and thus the specific conclusion of a certain feed for best results can’t be drawn, but a general consensus of the affect feed has on the system can be scaled and can be utilised in all tilapia systems. It is promising to see that work is being carried out in order to aide in the development of a feed that best suits an aquaponic system to supply the nutrients needed for the fish and plants to help create a precise feed working with nutrients in the feed and from the fish waste (Nelson & Shultz, 2018; Roy, Kajgrova, & Mraz, 2022). The production of a feed that is tailored to the system, thus being more economical and potentially sustainable for aquaponics can make it a more attractive business adventure and therefore has the possibility to lower prices of aquaponically grown crops and raise awareness of aquaponics. However, linking to this, a tailored feed is of little use if there is little uptake or poor public perception of aquaponics, and so its neds to be developed at the same time as educating and raising awareness. This is explored in chapter 4 in relation to public perception and subsequent willingness to pay, where feed is a key contributor to the overall running costs and prices, which many people have reservations to in aquaponics.

2.5 Conclusion

The results indicate that the dynamics of aquaculture systems are very complex interactions of many factors, and that no, one factor should be studied or used as a definitive conclusion of optimal system design. Ammonia and nitrites are arguably the most important parameter to maintain at optimum levels as they are directly toxic to fish stocks at very low exposure. Results from this review should be used with caution due to the variation of experimental design and many other factors shown in Table 2.1 that resulted in extreme heterogeneity between studies. However, the results of this study suggest that the best way to control water parameters is to control the feed quantity, which will influence the overall fish growth and potential for the number of fish that can be grown and in aquaponics, it has been found that between 56g/m2 – 180g/m2 is optimal for the growth of lettuce, while maintaining good water quality (Rakocy et al., 1997) so limiting the amount of feed directly influences the output of the whole system. The interactions between water parameters and Nile tilapia are fairly well understood in aquaculture systems. Yet, implications of feed quantity & type of feed remains understudied. The need for more research on feed to allow for a more sustainable, efficient and cost-effective system is of paramount importance.

3. A Pilot investigation on the palatability of microalgae cultured on digestate with Nile tilapia (*Oreochromis niloticus*)

3.1 Introduction

Having a feed that is best suited to an aquaponic/aquaculture system with optimal results for fish and nutrients for crops is a step in the right direction. However, as it currently stands, the use of commercial feed within aquaculture is unsustainable. The use of FM is decreasing with Nile tilapia and all farmed species, due mainly to cost (Tantikitti, 2014), but generally levels of fish meal in all feeds remains high and is not tailored to omnivorous species like *O.niloticus*. Feed is one of the most important factors for profitability and sustainability (Martins, Conceição, & Schrama, 2011). Aquaculture feed comprises of mostly FM and fish oils, as a result aquaculture is often run as "one fish in, one fish out". This experiment will assess the palatability and the efficacy of fish feed, which replace FM with microalgae. Palatability is accepted as the attractiveness and ingestion of feed (Glencross, Booth, & Allan, 2007). While research has begun to focus on alternatives to FM in diets of fish such as Nile tilapia (*Oreochromis niloticus*), little research has looked at the palatability or the effects of leeching from these formulated feeds. Any research prior on palatability has mostly studied the use of plant-based ingredients (e.g., soya meal) (Pereira-Da-Silva & Pezzato, 2000; Vinogradskaya & Kasumyan, 2019), rather than microalgae and showed that FM was more palatable. However, research on the use of microalgae has shown very promising results for Nile tilapia growth and body composition, thus should have good palatability (Badwy, Ibrahim, & Zeinhom, 2008; Sarker et al., 2018; Teuling, Wierenga, Agboola, Gruppen, & Schrama, 2019; Ungsethaphand, Peerapornpisal, Whangchai, & Sardsud, 2010). In particular, this experiment will observe how a microalgae based diet affects the palatability of the feed to Nile tilapia (Reaction of feed, rejection, behaviour etc), in individuals and in groups, whereas typically palatability studies focus on fish in groups and using on demand feeders (Jobling, Arnesen, Baardvik, Christiansen, & Jorgensen, 1995).

Microalgae has been used in supplementation of feed with Nile tilapia due to them being omnivores, but little work has been done on the use in aquaponics (Byelashov & Griffin, 2014). Microalgae use in aquaponic systems will benefit all aquaculture systems with omnivorous species, as it can supply the needed protein as well as other valuable nutrients which are currently missing in commercial feeds (Shah et al., 2018). Aquaponics can benefit even further from the use of alternative ingredients such as microalgae, due to the additional nutrients for the fish and subsequent nutrient release into the system from waste. Therefore, microalgae can help to supply the nutrients needed for crops, minimising costs of supplementation in the systems, whereas inorganic fertilizers continue to increase in price (Colt & Schuur, 2021; Nicholsa & Savidov, 2012)**.** Additionally to this, an aquaponics system is best suited to be able to accommodate a microalgae culturing system within it, to recycle the waste as a medium for culture, which can then be used in the fish feed, further making a more closed- loop system (Panades, 2015). The use of digestate has little to no research with applications in Nile tilapia feed (Uggetti et al., 2014) and the use of digestate in microalgae culture has only be proved on small scales (Fuentes-Grünewald et al., 2021). Digestate is a by-product produced from anaerobic digestion of food waste, producing massive amounts of nutrient-rich digestate, most of which cannot be used due to restrictions on fertiliser use and risk of nutrient run-off (Fernandes et al., 2020). Anaerobic digestion is an effective way of treating food waste and its key draw is that it can produce large amounts of energy, but it is a very costly process and the use of digestate in microalgae culture can improve the circular economy of anaerobic digestion (Chuka-ogwude, Ogbonna, & Moheimani, 2020).

It has been found that the use of digestate as a growing medium can successfully culture microalgae species such as Chlorella, Nannochloropsis and Scenedesmus (Fernandes et al., 2020). The DCM utilised in this study was cultured at Swansea university as part of the ALG-AD project and is further discussed in methodology. Additionally, Scenedesmus & nanochloropsis cultured on digestate has higher protein levels, omega-3, omega-9 and chl a than normal F2 cultured microalgae. Meaning that if this feed is found to be palatable by the Nile tilapia, it would make the body composition of Nile tilapia more beneficial to human consumption as well as using less FM. Therefore, the use of digestate cultured microalgae (DCM) will have cascading impacts for sustainability within aquaculture and can create a closed loop in waste treatment.

Due to the lack of focus on the use of DCM on Nile tilapia, with previous studies focusing on plant-based ingredient, this study aimed to determine the palatability of a formulated feed, replacing FM with DCM in a pilot study to assess if DCM can successfully be used in Nile tilapia feeds and subsequently aquaponics. To accomplish this, this study utilised DCM that was being grown as part of the ALG-AD project and incorporated it into a hand-made feed, where the palatability of the DCM could be investigated on Nile tilapia. Use of DCM would allow for aquaculture feed, aquaponics and anaerobic digestion to become more sustainable, profitable and closed loop systems. Therefore, the incorporation of DCM into aquaculture feed is of crucial importance, and this study aimed to bridge the gap in knowledge on if using digestate was feasible in Nile tilapia feed. Finally, the study aims to make recommendations on the use of DCM in aquaculture feeds and in aquaponics, established from the findings of this study.

3.2. Methodology

3.2.1 Fish and husbandry conditions

This research was carried out at the Centre for sustainable Aquatic Research (CSAR), at Swansea university, in march 2021. 300, mostly male Nile tilapia (*Oreochromis niloticus*) fry (1g) arrived in CSAR on 19/01/2021, were seperated into 25L opaque aquaria in a closed loop recirculating aquaculture system RAS. Nile tilapia were raised until the start of the experiment on 22/03/2021, of which, 104 of the Nile tilapias were used when they weighed (14.2 ± 4.78g). The Nile tilapia were obtained from Fish Gen LTD (Wales). The system was kept under a constant photoperiod (12 L:12 D), water temperature (26.73 ± 0.09 °C), pH (7.78 ± 0.02), Dissolved oxygen (DO) (Always between 5-8 mg/L), Alkalinity (35.7 mg/l $CaCO_3$) and Total ammonia (<0.02 mg/L), which were checked at least weekly (Daily for; temperature, DO and pH).

3.2.2 Experimental diet

Fish were fed at 5% total biomass, twice a day on a commercial pellet of 1.5mm (Alltech COPPENS 1.5mm) for the period they were in CSAR prior to the experiment starting. The experimental diet was formulated with DCM to fully substitute FM and natural/organic ingredients (Table 3.1), using the methods proposed by (Mcgoogan & Gatlin, 1997), whereby the dry ingredients are mixed first, followed by the wet ingredients (oils etc) and then processed through a meat grinder. The DCM of Nannochloropsis and Scenedesmus species were cultured in 800l photobioreactors, with a concentration of 2% membrane-filtered digestate at a temperature of 25°C an pH of 7.5. The cultures were harvested using membrane filtration (0.2 um pore size), then dewatered biomass was further centrifuged and then freeze-dried for 24 hours to obtain a fine powder.

The diet was then dried at 45°C in an oven for 2 hours and then ground into homogenous size using a blender into 1.5mm pellets and were then kept refrigerated for the duration of the experiment. At the end of the experiment, both the control diet and experimental diet were sent to Sciantec analytical for proximate analysis (Table 3.2). It should be noted that the control diet was a commercially available feed that was used due to constraints due to time and Covid-19 in the lab, which is why the feed has much higher protein levels and why only one level of DCM inclusion was utilised. Future studies should incorporate a range of inclusion levels and a control diet with equal levels of protein.

Table 3.1. Composition of experimental diet. Adapted from (El-Saidy & Gaber, 2002; El-Sheekh, El-Shourbagy, Shalaby, & Hosny, 2014)

Ingredients	(Percentage inclusion (%)
Soy bean meal	20
Cod liver oil	5
Wheat bran	8
Wheat	19
Wheat gluten	10
Maize	13
Nannochloropsis[1]	15
Scenedesmus[2]	5
Vitamin and mineral mix[3]	5

[1 & 2] Nannochloropsis and Scenedesmus cultured on digestate was harvested and the dried biomass was utilised in the feed

[3] Vitamin and mineral premix composition Per KG: Vitamins: Vitamin A 3a672a IU 3,036,000 – Vitamin D3 3a671 IU 66,000– Vitamin E 3a700 IU 142,000 – Choline chloride 3a890 mg 6,600 - Vitamin C 3a300 mg 275,000 – Niacinamide 3a315 mg 3,260 - Vitamin B2 mg 792 – Calcium D-Pantothenate 3a841 mg 717 - Vitamin B1 3a821 mg 660– Vitamin B6 3a831 mg 396 – Folic acid 3a316 mg 198 – Vitamin K3 3a711 mg 55 – Biotin 3a880 mg 6.6 – Vitamin B12 mg 1.6. Traces elements: Iron(II) sulphate monohydrate 3b103 (Iron) mg 3,300 - Zinc sulphate monohydrate 3b605 (Zinc) mg 3,300 - Manganous sulphate monohydrate 3b503 (Manganese) mg 1,925 - Copper(II) sulphate pentahydrate 3b405 (Copper) mg 825 - Coated granulated cobalt (II) carbonate 3b304 (Cobalt) mg 55 – Calcium iodate anhydrous 3b202 (Iodium) mg 55 – Sodium selenite 3b801 (Selenium) mg 5.5. Anti cacking agents: Silicic acid, precipitated and dried E551a mg 1,000

Table 3.2. Proximate analysis of the control and experimental diet, carried out at Sciantec analytical (UKAS accredited)

Test	Control diet (%)	Experimental diet (%)
Crude Protein (N X 6.25) (Kjeldahl)	56.3	31.2
Oil A (Ether Extract)	12.88	13.89
Total Oil (Oil B)	15.05	16.62
Crude Fibre	0.2	1.5
Moisture	6.3	13.8
Ash	11	4.5
FFA of extracted fat (as Oleic Acid)	6.1	23.8
Nitrogen-free extract	13.32	35.11

3.2.3 Experiment protocol and measurements

The Nile tilapia were seperated into separate aquaria at densities of 15 fish in the four experimental tanks. After this, the Nile tilapia were acclimated for five days to the feeding protocol. The feeding protocol throughout the five-day acclimation consisted of turning off the airflow to the tanks, siphoning the tanks, turning the tank inflow off and then lifting the divider and lowering it (Figure 3.1), then feeding and observing closely for one minute in order to make the Nile tilapia more comfortable with the dividers and being observed. After the acclimation period, the protocol then changed to lifting the dividers up until one Nile tilapia had swum through, then slowly lowering the divider to segregate one individual. They were then given five minutes to acclimate before being randomly offered 10 pellets of either the commercial feed or experimental diet.

The reaction time to feed, number of ejections (Each pellet ejected equalled one ejection), number of pellets eaten and any additional behavioural comments were recorded while stood behind a mesh barrier, as well as video recordings of some of the trials. The Nile tilapia were observed until they ate all the offered pellets or for five minutes. They were then allowed a further five-minute acclimation before being offered the diet they had previously not been given and the same observations were recorded. That individual was then removed from the experimental tanks to avoid double recording and were replaced to keep the stocking density at 15 fish per tank. This was repeated for a morning feed and afternoon feed, sampling eight individuals per day for 6 days, giving a sample number of 48 Nile tilapia.

Figure 3.1. Representation of the dividers used to separate the tilapia, whilst still retaining visual contact with the group to prevent stress and unnatural behaviour.

3.2.4 Statistical analysis

All statistical analyses were carried out using R version 4.0.3 (2020) and data was plotted using base R and the ggplot2 package and variability is reported as standard deviation, unless noted otherwise. The number of pellets eaten and the number of ejections were analysed using generalised linear models with Quasi-Poisson, with diet type, time of day and tank being used as predictor variables. The generalised linear model (GLM) was used test if the different diets, different times and tanks impacted palatability. Quasi-Poisson was used to account for over-dispersion, so the dispersion can be estimated from the data (Zeileis, Kleiber, & Jackman, 2008). The number of ejections was run using a zero inflated model, as it best fits data with excess of zeros (Lambert, 1992). Following the GLM with quasi-Poisson, a goodness of fit model was run using the 'dredge' function in the MuMIn package. The model that best predicted the dependent variable was identified using AIC, with any model within 2ΔAIC being considered. Also, a Scheirer-Ray-Hare test was run in Rstudio to test if there were any statistically significant differences in the means of the pellets eaten and ejected in relation to the two diets or time of feeding.

To analyse the time taken to start to feed, Survival analysis (A.K.A Time to event analysis) to determine the probability of Nile tilapia starting to feed as the five minutes of observations continued, using the 'survial' and 'survminer' packages (Emmert-Streib & Dehmer, 2019). In this analysis, the time corresponds to the time it took for the Nile tilapia to react to the feed in seconds from when it was dropped into the tank. The survival curve that this analysis produced is shown in Figure 3.6.

3.2.5 Ethics

Routine daily checks and observations were carried out and recorded to ensure that all of the Nile tilapia are healthy and behaving normally, with no signs of fin/skin damage, emaciation, exophthalmia or disease and specifically for Nile tilapia, eye or skin darkening as signs of stress.

As the experiment was heavily observation based it was easy to ensure that all of the Nile tilapia were healthy and behaving normally, no aggression, and no signs of stress. If the tilapia clearly rejected or were having adverse effects to their health to the feed and a significant number were not eating for more than two days, then the experiment would have been terminated and action taken in the interested of the fish's health (E.g. schedule 1), of which was not necessary. Ethical approval in appendices 7.12

3.3 Results

3.3.1 Pellets eaten

Each of the Nile tilapia in the study (48) were offered 10 pellets from each diet, which were offered in a random order. The mode number of pellets eaten for each diet was 10, but for certain individuals varied. Most noticeably there was more variation in the first two days of the experiment, notably more for the algae diet. The distributions of the number of pellets eaten in relation to diet and time are shown in Figures 3.2 & 3.3.

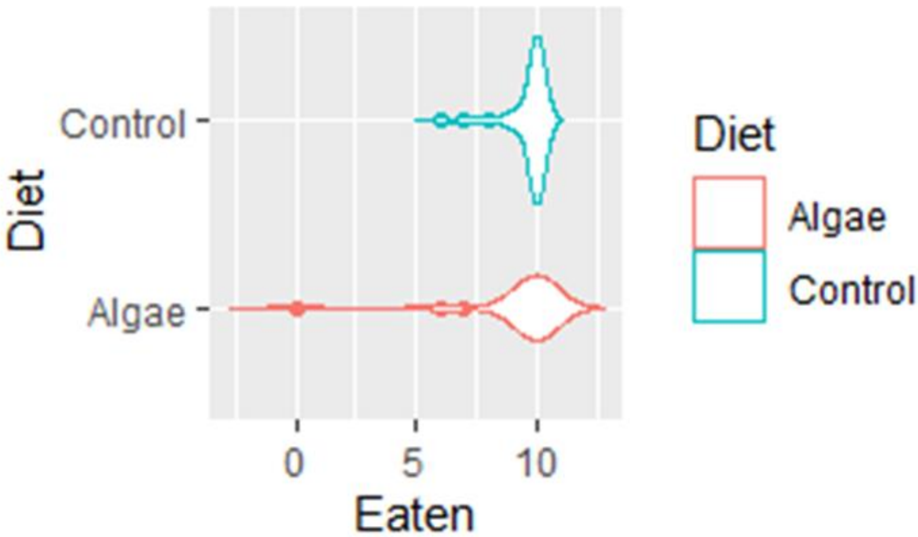


Figure 3.2. Distribution of the number of pellets eaten of the Algae diet (red) and control diet (blue) by Nile tilapia (*Oreochromis Niloticus*)

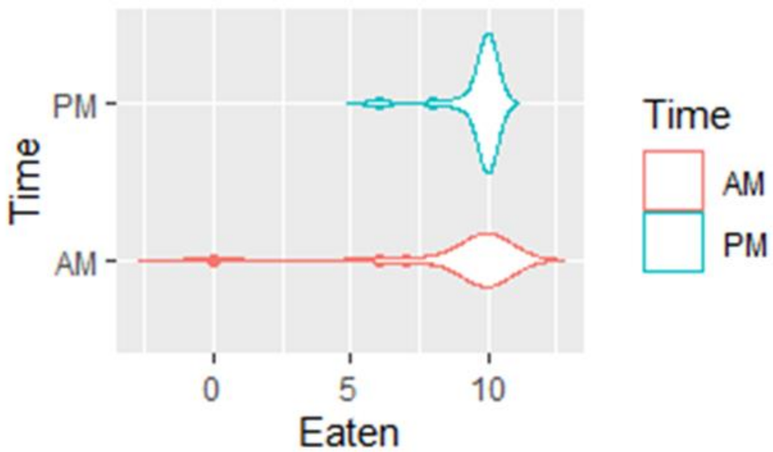


Figure 3.3. Distribution of the number of pellets eaten in relation to time fed. AM (red) and PM (blue) by Nile tilapia (*Oreochromis Niloticus*)

Figures 3.2 and 3.3 are violin plots that show the distribution of the number of pellets eaten in relation to diet and time they were fed. The Figures clearly show that both diets and feeding times follow very similar trends, with majority of the Nile tilapia eating all 10 pellets regardless of diet or time. Even though the pellets eaten show similar trends for diet and time, for the first two days the algae diet varied more in number eaten, which is evidenced in Figures 3.2 and 3.3. After the first two days, the Nile tilapia appeared to be more accustomed to the algae diet and readily ate the pellets, just as quickly as the control diet, while actively searching for more pellets.

The data was analysed using GLM on the number of eaten pellets and was then followed by dredging to find the model which best fit. The dredge produced three models within 2ΔAICs: (1) Time (AM/PM), AIC = 438.7, $\Delta = 0$. (2) Diet (Algae/control), AIC = 438.9, $\Delta = 0.2$. (3) Diet + Time, AIC = 440.3, $\Delta = 1.65$. Using Figures 3.2 and 3.3, results from the Scheirer-Ray-Hare test and the GLM, it was determined that model 3 best fit as a predictor, using both diet and time to determine the number of pellets eaten.

The Scheirer-Ray-Hare test indicated that there were no statistically significant differences ($P>0.05$) in the mean number of pellets eaten in relation to diet type or time of day, so a post-hoc test was not needed and null hypotheses could be accepted, based on no statistically significant differences in the mean number of pellets eaten in relation to diet or time. However, when the type of diet and time of day were analysed with an interaction factor, it showed the most significant result, supporting the GLM.

3.3.2 Ejections

Ejections were recorded as one ejection for each pellet ejected by the Nile tilapia. The same statistical analyses were run on the ejections as the number of pellets eaten. Likewise, to the number of pellets eaten, the number of ejections was slightly higher in the algae diet than that of the control diet, especially in the first two days. The Scheirer-Ray-Hare test revealed again that there were no statistically significant differences ($P>0.05$) in the means of ejected pellets in relation to diet type or time of day. But it is noted that time of day had the lowest P value in relation to number of pellets ejected. The results are supported by Figures 3.4 & 3.5, showing the distribution of the number of ejections, clearly showing very similar trends for diet type and time of day. There are one or two outliers present, which can be accounted for based on the size and behaviour of each Nile tilapia that was recorded.

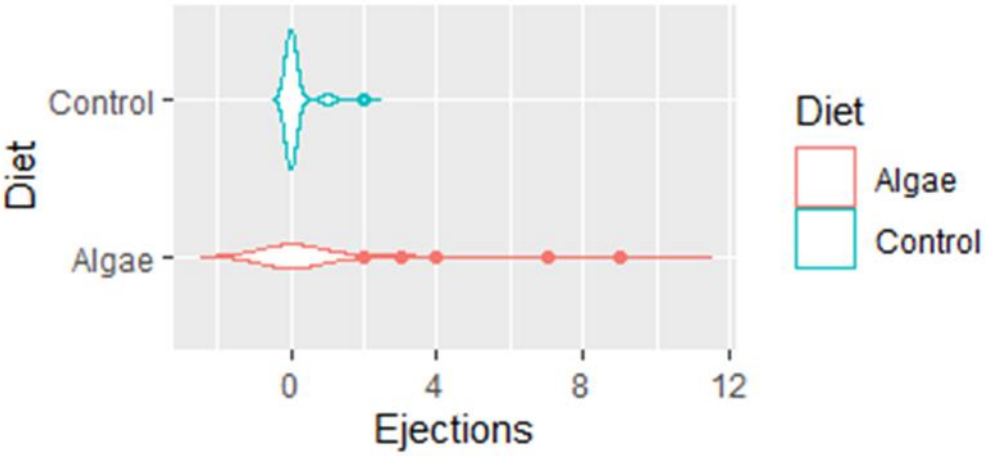


Figure 3.4. Distribution of the number of pellets ejected of the Algae diet (red) and control diet (blue) by Nile tilapia (*Oreochromis Niloticus*)

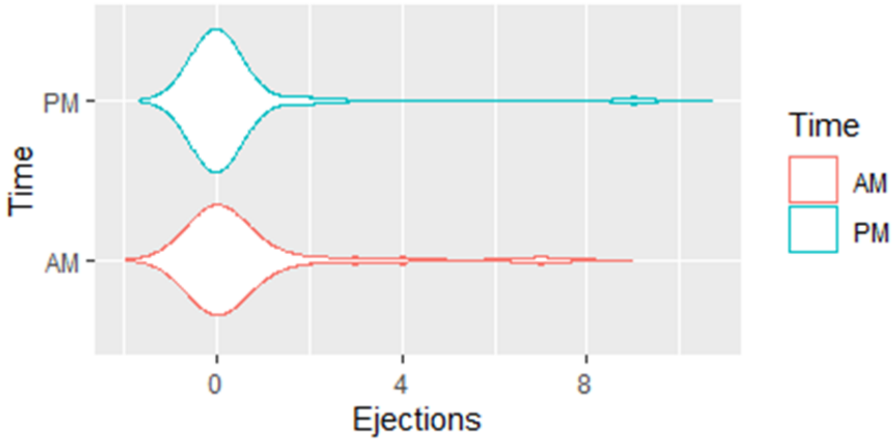


Figure 3.5. Distribution of the number of pellets ejected in relation to time fed. AM (red) and PM (blue) by Nile tilapia (*Oreochromis Niloticus*)

The GLM revealed that none of the predictor variables (Diet, Time and Tank) had a significant impact on the number of pellets that would be ejected, but it should be noted that the algae diet had the highest estimate of 0.67, as opposed to 0.3 for the control diet, meaning that it would be double as likely to be ejected than the control diet. Also, it is notable that time did have some impact with more pellets being ejected in the morning feeds, but not statistically significant. The tank that the fish were held in also had no significant impact on the number of ejections.

3.3.3 Survival analysis

The survival analysis assessed the time it took each Nile tilapia to start feeding in seconds. The survival curve is shown in Figure 3.6. Despite the curves showing that algae diet could take up to 300 seconds, this is down to one individual that did not feed. In fact, after 3 seconds of latency, 80% of the Nile tilapia had started feeding with the algae diet, but it was 6 seconds for the control diet, indicating it could be more palatable or noticeable to the tilapia.

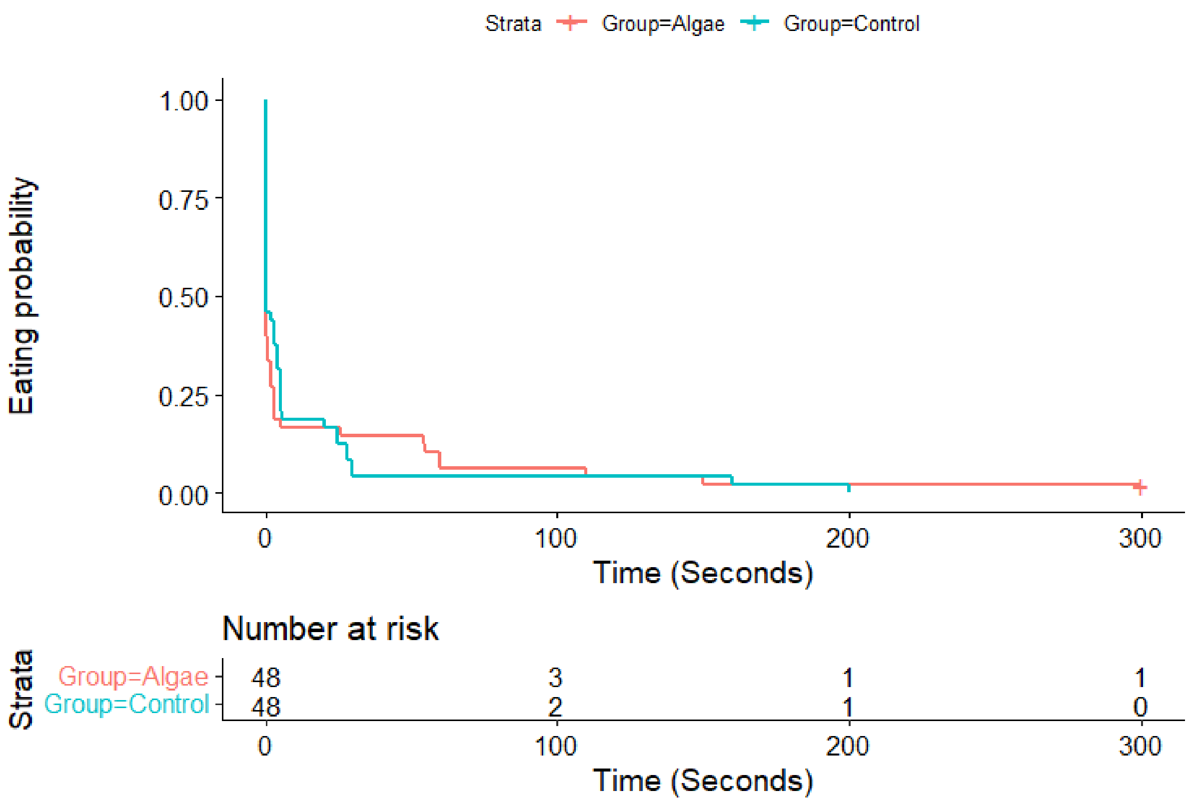


Figure 3.6. Plots of Kaplan-Meier estimates of Time to start eating of a group of Nile tilapias receiving two different diets.

Figure 3.6 shows that there is a clear trend for both diets of less likelihood of starting to eat as time goes on. This a positive result as it means that the Nile tilapia were readily eating both diets. Both diets had a very high percentage of Nile tilapia eating as soon as the feed hit the water, indicating high palatability and attractiveness of the algae diet.

3.3.4 Group observations

While no analysis was run on the group data, it is important to note on the behaviour of the groups when fed each diet. The tank nearest the door appeared to be the most timid and subsequently was the tank that had higher latency, possibly because it was closest to the door so was disturbed more, or the light conditions may have been different. The key observation worth noting is that there were little to no ejections when the algae diet was offered to the group and there were only a few left-over pellets on the first day of observation. This could be due to the social pressure of having to eat so the groups are less picky or else they could not eat, or due to the algae diet being attractive and palatable.

3.4 Discussion

This study is a pioneering move into the use of digestate integrated into an aquaculture feed and understanding the feasibility by proxy of palatability. The vast majority of studies on palatability, focus on fish in groups, using on demand feeders (Jobling et al., 1995), so individual behaviours and responses are seldom recorded. This pilot study aimed to establish how palatable DCM is to Nile tilapia (*Oreochromis niloticus*). This was achieved by formulating a feed and feeding pre-determined amounts to Nile tilapia and recording their behaviour and reactions to the feed. A GLM model on how Diet, Time and Tank influenced pellets eaten and pellets ejected showed that the diet and time of feeding best predicted the number of pellets eaten or ejected and the tank used did not significantly influence either. Using the Scheirer-Ray-Hare test, there was no significant difference in the means of pellets eaten or ejected, indicating that the palatability of the formulated feed is just as good as the commercial diet. This is a huge advancement into the use of DCM into the aquaculture industry and will lead to its incorporation in the food wate treatment industry as well as aquaculture.

Despite that statistically there were no significant differences between the diets, the initial increase in the first two days of latency, ejections and fewer pellets being eaten could indicate unpalatability. It is pertinent to explore why this initial apparent lack of palatability was observed, be it due to the taste, smell, feeding rhythm of the Nile tilapia or that the cohort was mostly male, causing differences in dominance and boldness of certain individuals (Schreck & Moffitt, 1987) Another possible explanation could be due to the method of separation of the individuals causing stress and rising cortisol levels, as some individuals have been found to acclimatise faster than others (Barreto & Volpato, 2011). In this pilot study, it was noted that some individuals appeared much more timid than others, but it would not likely be a stress response because of the separation, most likely it would be individual differences in boldness, thus their reaction to feed is more timid (Martins, Conceição, & Schrama, 2011). It has been found that there is low repeatability with studies in palatability experiments on individual Nile tilapia, which can explain why there is almost always variation in growth within cohorts (Martins et al., 2011). This supports the findings of this study, where it was noted that the only differences in relation to timidness were from smaller individuals, with variation in latency, ejections and pellets eaten in comparison to the other larger Nile tilapia, but they did not result in statistically significant differences.

Nile Tilapia follow a circadian rhythm of feeding, which could also explain why the palatability was similar for both diets. Nile tilapia can be flexible with their feeding habits, but they almost exclusively prefer to feed at night, where there is less risk involved for them to spend energy feeding and therefore can be more picky in what they will and will not eat (Fortes-Silva, Martínez, Villarroel, & Sánchez-Vázquez, 2010). As the study relied on individual observations, whilst being fed, they were fed in the mornings and afternoons in the CSAR building. Therefore, it was not carried out at their preferred time to feed. It could be argued that if the study took place at night, in line with their natural feeding rhythm, then the resulting palatability would have been even more compelling and that the formulated feed may have even been favoured or possibly the other way round, preferring the commercial diet.

Linking to this, Nile tilapia can successfully use on demand feeders and are often fed using them to improve FCR (Benhaïm et al., 2017; Sousa et al., 2012). The use of on demand feeders also leads to less cortisol, so less stress, improved feeding rate and palatability (Endo, Kumahara, Yoshida, & Tabata, 2002). This supports the findings of this study of variations in the apparent palatability of the formulated diet, as they may have had higher cortisol levels, as they tended to be smaller. Also, due to the fact they were only offered food twice a day rather than by on demand, so there was more competition due to feed not being readily available, thus the palatability may actually appear better than it would if they were fed by demand feeders or to satiation. This could explain the survival analysis and why they both show similar trends. Nevertheless, it is important to note that the group observations show even more promising results for the palatability of the formulated diet, but it must be interpreted with caution, without robust data or statistics. However, this study has shown that the use of DCM in aquaculture feed is possible and that Nile tilapia are prime candidates to use in future studies.

This study also found that the proximate analysis of the formulated feed (Table 3.2) was comparable to that of the control diet, so any differences seen in individual responses is less likely to be because of the composition of the feed. The protein levels in the formulated diet were much lower than the control, which is not an issue for *O. niloticus* as they are omnivores, with 30% protein showing no significant effects on body protein composition and if they are fed diets high in protein, it can decrease their body lipid composition (Al Hafedh, 1999). Furthermore, there were no mortalities throughout the study, suggesting the diet is digestible as well as palatable, with no obvious adverse effects on the tilapia’s health.

The results from this study, while not statistically significant, are extremely promising. The implications of using a DCM to reduce FM and plant meal products in aquaculture feed, of which, plant meal has a lower apparent palatability than microalgae and FM (Vinogradskaya & Kasumyan, 2019), are fundamental to the sustainability and advancement of aquaculture. Using a microalgae-based diet can be beneficial to SGR and FCR of Nile tilapia. Microalgae makes up the majority of the natural diet of Nile tilapia (Njiru, Okeyo-Owuor, Muchiri, & Cowx, 2004), thus microalgae will result in the best possible growth and natural behaviour in relation to feed. It is interesting to note that the digestibility coefficient of spirulina and chlorella is higher than that of Nannochloropsis and Scenedesmus, which were used in this study (Agboola, Teuling, Wierenga, Gruppen, & Schrama, 2019; Teuling, Wierenga, Agboola, Gruppen, & Schrama, 2019). Therefore, it could be argued that if other species of microalgae were utilised in experimental feed, the apparent palatability could be improved and their digestibility would be better for tilapia. As a result, other species and combinations should be trialled to fully understand which species best suits Nile tilapia. An enticing factor for the wider audience to incorporate digestate and microalgae is the potential profitability, thus monetary savings. Additionally by, utilising what is otherwise a waste stream can make steps towards being a more closed loop, as digestate is a by-product, so can only stand to make money (Fuentes-Grünewald et al., 2021). Also, feed constitutes up to 70% of maintenance in aquaculture systems (Castilho-Barros, Almeida, Henriques, & Seiffert, 2018), meaning if this could be up-scaled there would be no doubt for widescale uptake and is crucial for a more profitable system.

The implications these findings have for aquaponics are that they can create an even more closed-loop system with waste from aquaponics being used in anaerobic digestion, creating digestate that cultures microalgae, which subsequently is fed to the fish. This being said, it would be naïve to assume that systems can make a completely self-reliant system as it currently stands, however a small step towards sustainability will enable further work to develop new methodologies to help make the system as sustainable and efficient as it possibly can. Additionally, almost all aquaponic farms or systems only use commercial aquaculture diets, which is not always necessarily best for the species of fish or plants in the system (Robaina et al., 2019). The use of microalgae and a formulated feed means that it can supply other vital components such as omega-3 naturally. In addition to this the feed can be tailored to both the fish needs and customised to what nutrients is released into the system, so feed can be used to grow certain crops.

3.5 Conclusion

The results from the present study indicate that the palatability of feed, formulated using DCM is equal to that of commercially available feed, which is extremely promising for the food waste and aquaculture industries. Therefore, this study provides a benchmark of palatability for the inclusion of the DCM, whereby the use of DCM has no significant effects on the apparent palatability of the formulated feed. This study also highlighted the lack of studies on palatability within aquaculture, suggesting a need for further studies incorporating palatability as well as growth to understand feed dynamics more fully. Also, new feeds need to be fully understood to not been seen as novel in order to have a widescale uptake. Future studies should investigate into the effects on growth and body composition in *O. Niloticus.* Following this, supplementary research can follow on the applications of using DCM and different algae species into aquaponics and how it can create a more efficient, sustainable and profitable system.

4. The feasibility of aquaponics

4.1 Introduction

Optimising feed and making it more sustainable are important to the success of aquaponics, the fundamental underlying success is how it is perceived and subsequently adopted by the general public. For aquaponics to become mainstream and successful, it must return a profit, and it cannot do this without people buying into it, following cultural adjustment (Greenfeld, Becker, Bornman, dos Santos, et al., 2020). There has been an exponential increase in the number of papers focusing on aquaponics in the past 20years (Yep & Zheng, 2019), but there remains many concerns about the feasibility of aquaponics that are unexplored. There are however, some papers that focus on the cost (Of start-up and produce) being a common key concern (Short, Yue, Anderson, Russell, & Phelps, 2017) and how to mitigate these costs by means of alternate power, sustainable feed, water catchment etc (Greenfeld, Becker, McIlwain, Fotedar, & Bornman, 2019). It is remarkable that there are so few papers on consumer perception, as the economic feasibility of aquaponics is underpinned by the revenue it can create (Asciuto, Schimmenti, Cottone, & Borsellino, 2019).

Overall, the general consensus is that between 40-60% of people have at least heard the term aquaponics and generally people tend to be neutral or in favour of aquaponics (Miličić et al., 2017; Short et al., 2017; Suárez-Cáceres et al., 2021). This highlights the need for education on aquaponics being the key to its success, as currently there is higher consumer awareness for more sustainable and healthy alternatives (Miličić, Thorarinsdottir, Santos, et al., 2017). This general lack of knowledge is one of the biggest downfalls for aquaponics, as people fear what they do not understand and will not pay premium for something they do not know the benefits of, again lending to the need for education. Adding to this lack of knowledge, many new aquaponic systems fail to last more than a year because of the knowledge gap and the fact that up to 33% of new farmers have no previous experience in fish or crop culture, prior to starting an aquaponics farm (Greenfeld, Becker, Bornman, & Angel, 2020). This is also damaging to the progression of aquaponics as many people view it as a fad or proof that it doesn’t work, when in reality the system can be profitable when run well (Love et al., 2015).

One of the most significant hold ups for the wide-scale adoption of aquaponics is legislation, as it currently stands EU commission regulations No.889/2008 paragraph 4 and No.710/2009 paragraph 11, make it impossible for aquaponics to be classed as organic, as they require crops to be nourished by soil and RAS systems are prohibited to being classed as organic (Miličić, Thorarinsdottir, Santos, et al., 2017). This impacts both consumer perceptions, because without organic certification people tend to distrust the process of the farming and also means that the profitability is capped, due to the produce not being able to be sold at organic prices and there being no subsidies for being a non-organic systems (Cammies, Mytton, & Crichton, 2021). It is evident that for aquaponics to be successful, consumer perception needs to change alongside legislation to allow for organic certification, while minimising the costs of both start-up and maintenance to make it more widely available.

Extensive literature searches have shown that there is not a single study on public perceptions of aquaponics in the UK and only one paper had a small number of results from the UK (Miličić, Thorarinsdottir, Santos, et al., 2017). Therefore, this survey aimed to ascertain a baseline for the general knowledge of aquaponics in the UK and to compare this to the result of the world. In addition, this survey will aide in filling knowledge gaps and deepening understanding that people have about aquaponics and how these issues will impact the development of urban aquaponics and cultural acceptance.

4.2 Methodology

4.2.1 Survey outline and questions

This survey was conducted between 22/01/2021 – 01/03/2021 for participants from all around the world, using various online platforms (Email, social media, prolific, smart survey and survey circle) to distribute the survey to a wide range of people and avoid bias selection of the respondents of the survey. The survey consisted of 14 questions, of which included open, closed, slider, dichotomous (yes/no) and 7-point Likert type questions that could all be answered easily, to avoid survey fatigue and lack of personal questions to minimise false positives as people have anonymity so will be less likely to try to conform to what they think they need to (Miličić, Thorarinsdottir, Santos, et al., 2017). The survey questions are included in appendices 7.7. Broadly, the questions were split into;

1. Demographic information – age, location and highest level of education,

2. General knowledge of aquaponics – Terminology and basic knowledge.

3. Perceptions/reservations – Opinions, insight on costs and key reservations.

A total of 254 participants filled in the study, but 16 were invalidated due to not meeting the criteria, which were; 1) The participant must have taken at least 1minute 30 seconds to complete the survey (To ensure they had actually read the questions) and 2) They must have filled in all the demographic information. The first part of the survey asked participants demographic information and their general knowledge of aquaponics. After which they were given a very brief explanation of what aquaponics was, then various questions on public perceptions and reservations were after now having a basic understanding of what aquaponics was in order to gauge what key issues in aquaponics are. On top of this, a literature review was conducted on the perceptions and feasibility of aquaponics to assess how it can be mitigated to enable future projects, thus improving chances for education. Also, the literature review will enable a conceptual system design to be used by the industry partner to give a baseline system design. The conceptual design is included in the introduction.

4.2.2 Statistical analysis

Descriptive statistics will be used only with the dichotomous questions on the demographics of the participants and opinions on aquaponics, as well as for the responses to the open questions on reservations to aquaponics.

There is a great debate on the correct statistics to use when analysing Likert data, however many are not appropriate as there is no way of saying the interval between variables is even, but the Wilcoxon Mann-Whitney test best fits Likert data. In order to analyse the 7-point Likert data, it was transformed into numbered responses ranging from 1 – strongly disagree to 7 – Strongly agree. The mann Whitney null hypothesis is that the opinions on aquaponics from people who have heard of aquaponics is equal to that that had not. All statistical analyses were carried out using R version 4.0.3 (2020) and data was plotted using the ggplot2 package, variability is reported as standard deviation, unless stated otherwise.

4.2.3 Ethics

Prior to conducting this survey, ethic approval was sought and approved by Swansea university to be able to distribute and conduct the survey. As no personal data was to be obtained, ethical approval was given and is included in appendices 7.12.

4.3 Results

4.3.1 Demographics

In Total, 238 participants were included in this survey of various backgrounds, including; education level, range of ages from <18 to 65+ and location in the world Fig 4.1. The UK participants made up just over 50% of all participants and majority of participants were between the ages of 18 – 24 with undergraduate degrees Figures 4.2 and 4.3. This skew in the demographics needs to be taken into account when analysing the data and means conclusions made from these results may not be completely reliable and should be interpreted with the demographic in mind.

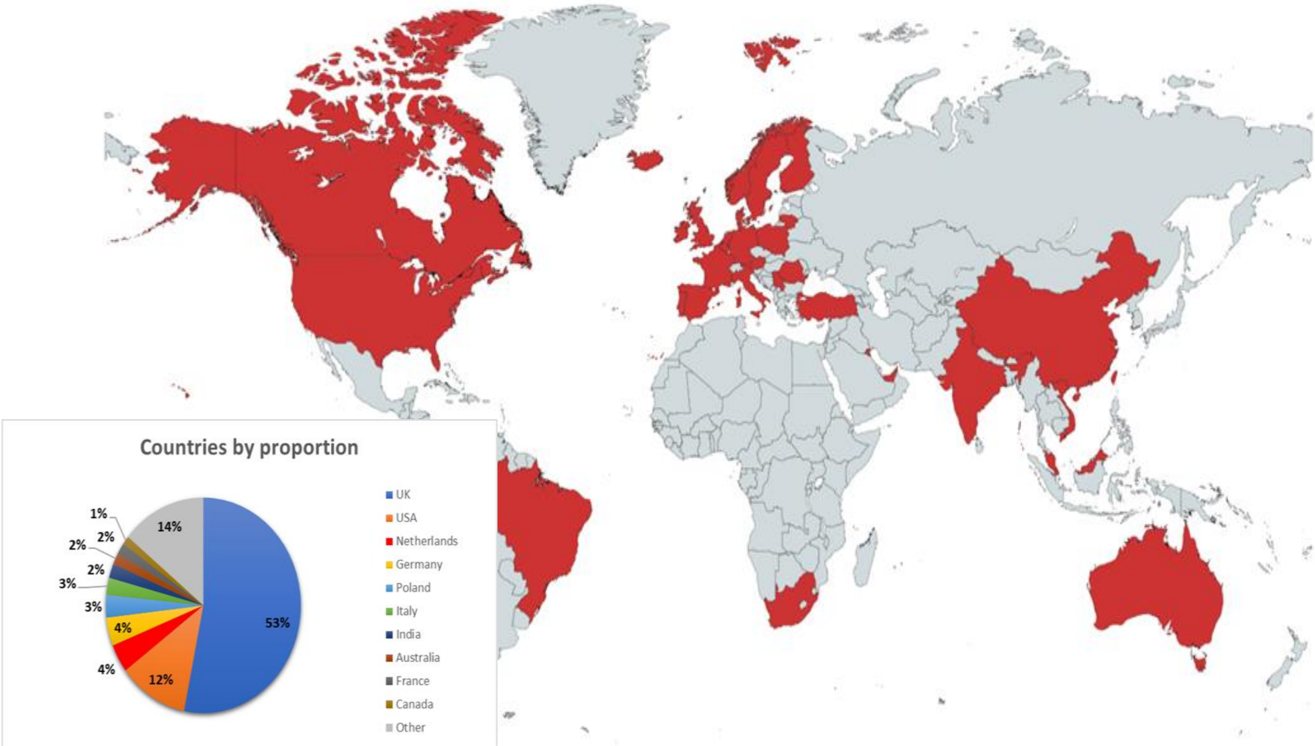


Figure 4.1. The distribution of participants based on country and the percentage each country made up of the total participants.

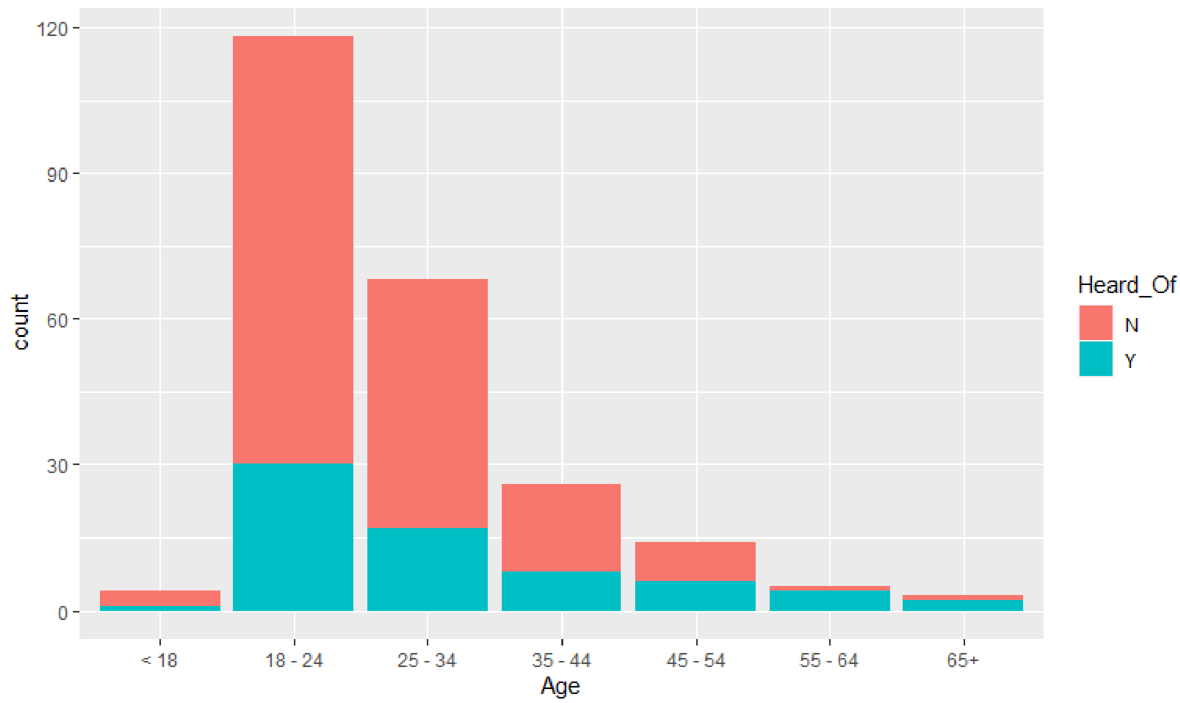


Figure 4.2. The distribution of participants based on age and if they had or had not heard of aquaponics.

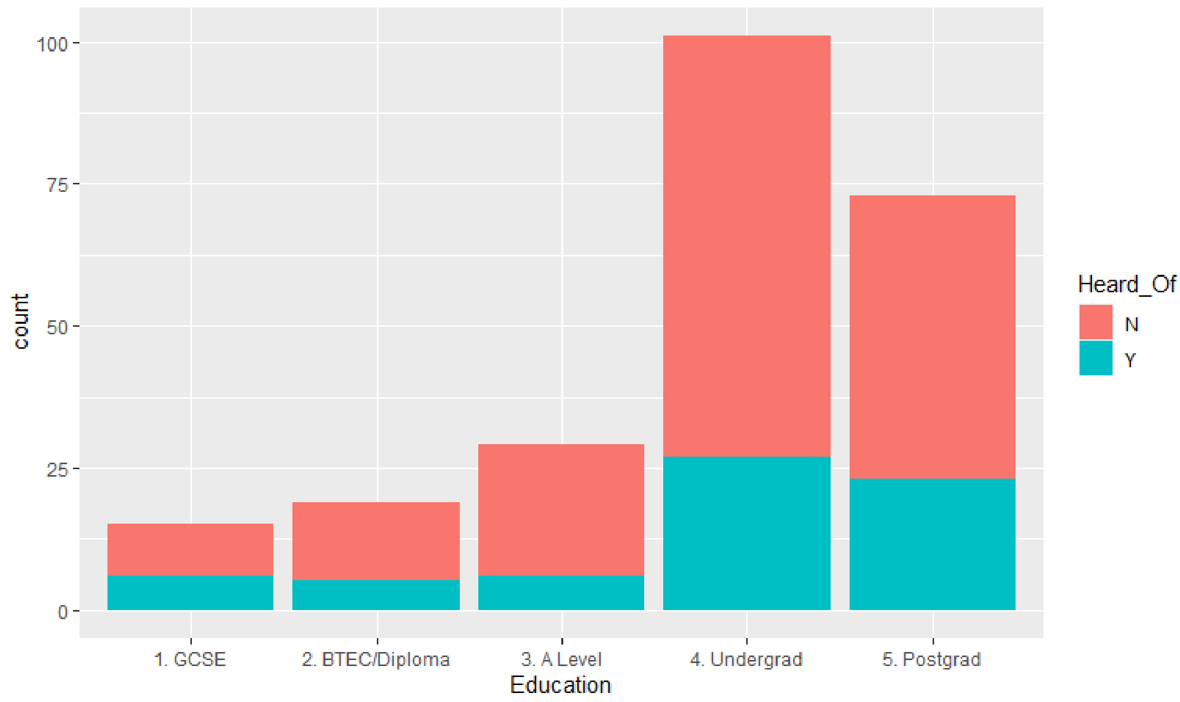


Figure 4.3. The distribution of participants based on educational level and if they had or had not heard of aquaponics.

4.3.2 Knowledge of aquaponics

Only 30% of all participants had heard of the term aquaponics (Table 4.1). Even though only 30% had heard of the term aquaponics, even fewer had competent knowledge of what it is. 43% of the people who had heard of aquaponics showed a working knowledge of what aquaponics is. Often, aquaponics was confused with hydroponics or people had just heard the term. Therefore, competent knowledge was based on if the participant managed to basically describe the concept of aquaponics with clear differentiation to hydroponics. The proportion of participants that had heard of aquaponics and had competent understanding in the UK and the rest of the world is shown in Table 4.1.

Table 4.1 Percentage of participants that had heard of aquaponics in the UK and the rest of the world and subsequently the percentage of which had a competent understanding.

Aquaponics	UK		World	
	Yes (%)	No (%)	Yes (%)	No (%)
Heard of aquaponics	30	70	29	71
Competent knowledge	43	57	42	58

4.3.3 Perceptions/reservations

An open question was used to ascertain what reservations people have towards aquaponics. There are clear trends with certain reservations for both the UK and the rest of the world (Figures 4.4 A and B).

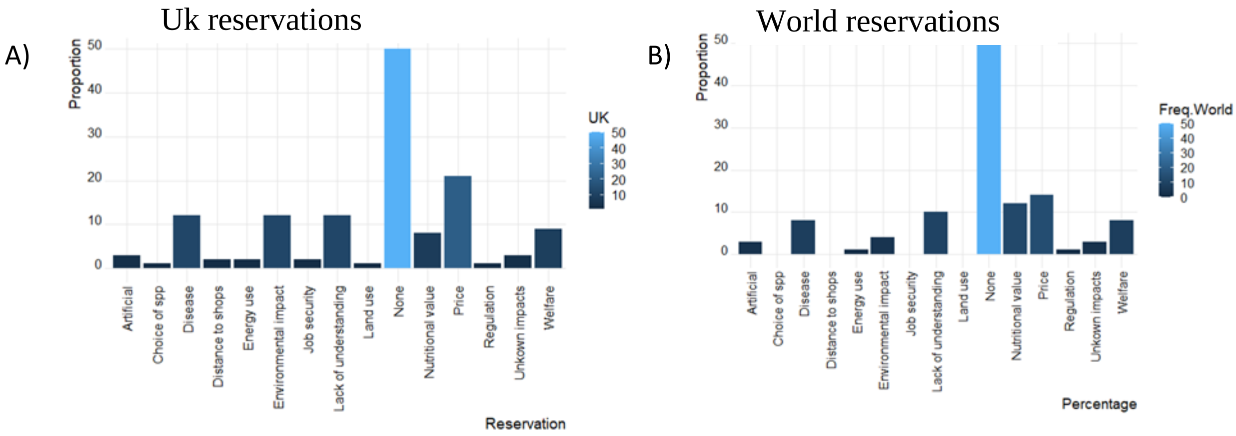


Figure 4.4 Reservations shown towards aquaponics by participants in the survey, as a percentage of responses. A) = Uk reservations. B) = World reservations

From the figures above, each reservation encapsulates the following; 1) Artificial = man-reared fish & lack of natural nutrition. 2) Choice of species = issue with species reared being undesirable. 3) Disease = Possible problems with spread of disease from the fish waste into the crops, as well as disease within the fish stock. 4) Distance to shops = food mileage, as it would defeat the purpose of the sustainable farm if it had to travel long distances. 5) Energy use = demand on the grid to run systems, power the pumps and lights etc. 6) Environmental impact = Discharge of nutrient rich water into the environment and how sustainable materials and build are. 7) Job security = Possibility of farmers losing jobs. 8) Lack of understanding = wanting to know more on aquaponics, or not fully understanding it. 9) Land use = using land that could alternatively be used for farming or residential buildings. 10) Nutritional value = Fish and crops being nutritionally poorer due to the method of farming. 11) Price = The price of the initial setup and maintenance costs of the system. 12) Regulation = As a lack of restrictions or legislation on this type of farming, who would regulate aquaponics and what restrictions would be in place. 13) Unknown impacts = as it is not mainstream or extremely common, what are the unknown impacts or long-term impacts. 14) Welfare = concerns over the welfare of the fish kept at high densities and keeping them in tanks rather than the wild. 15) None = there were no noted reservations.

Noticeably many participants had no concerns with the use of aquaponics. However, price is a major concern from all the participants, making up a larger percentage for both the UK and the world. The UK participants were more concerned about environmental impacts, but less so on nutritional value than the world, showing the differences based on culture and the need for perceptions to be changed. Generally, all the reservations showed a lack of education is the key issue for public perception of aquaponics. This is very pertinent for the industry partners for their development, as it shows the need for educating people on aquaponics and enables them to tackle these common perceptions prior to development. Additionally to this, the number of people who had no concerns with aquaponics, as well as the high percentage of people willing to pay organic prices (Table 4.2) are of vital importance to this industry partner as it shows there is a potential market for aquaponically grown crops. To further maximise the potential profits of aquaponic systems, it is clear that key emphasis needs to be drawn to what benefits aquaponics brings with less water use, potential for urban farms utilising dead space and food security, while other aspects such as high yields of fish may need to be de-emphasised due to bringing negative connotations of poor welfare and unethical practices.

Table 4.2 Percentage of participants that would choose aquaponics or wild raised fish and if they would pay organic prices for aquaponically grown crops, comparing the UK and the world.

Aquaponics	UK		World	
	Yes (%)	No (%)	Yes (%)	No (%)
Prefer Aquaponics	54	46	52	48
Pay organic prices	58	42	49	51

Despite the clear reservations that are seen towards aquaponics, Table 4.2 shows that majority of the people in the UK are more willing to buy aquaponics over wild and would pay organic prices, which are crucial for the success of aquaponic systems and means that legislative changes to make aquaponics organic would be supported by the majority of the population. The only difference being that the rest of the world showed more resentment towards paying organic prices than the UK.

4.3.4 Beliefs about aquaponics

Perceptions of aquaponics were assessed using 6 questions on a 1- 7 Likert scale type of question. Generally, people had a neutral or positive attitude towards aquaponics, but there was a varied range of responses to each question and interestingly varied between the UK and the rest of the world (Figures 4.5 and 4.6).

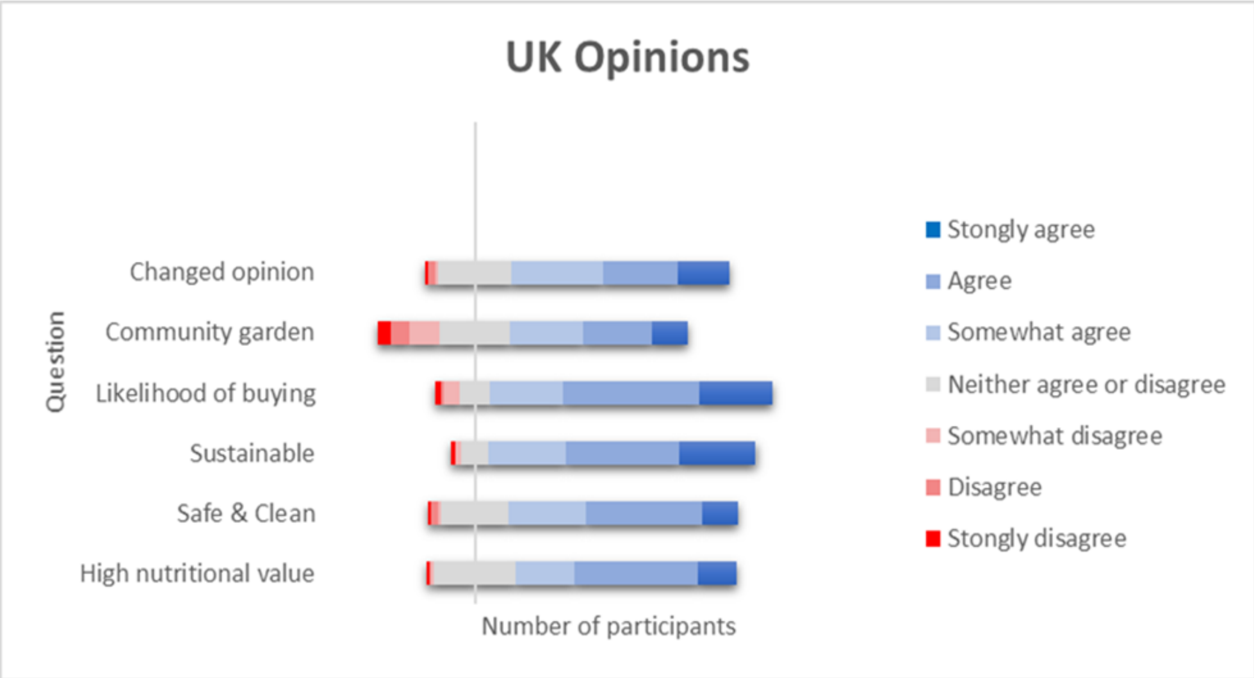


Figure 4.5. Shows the opinions of participants from the UK to six questions based on a 1 – 7 Likert scale.

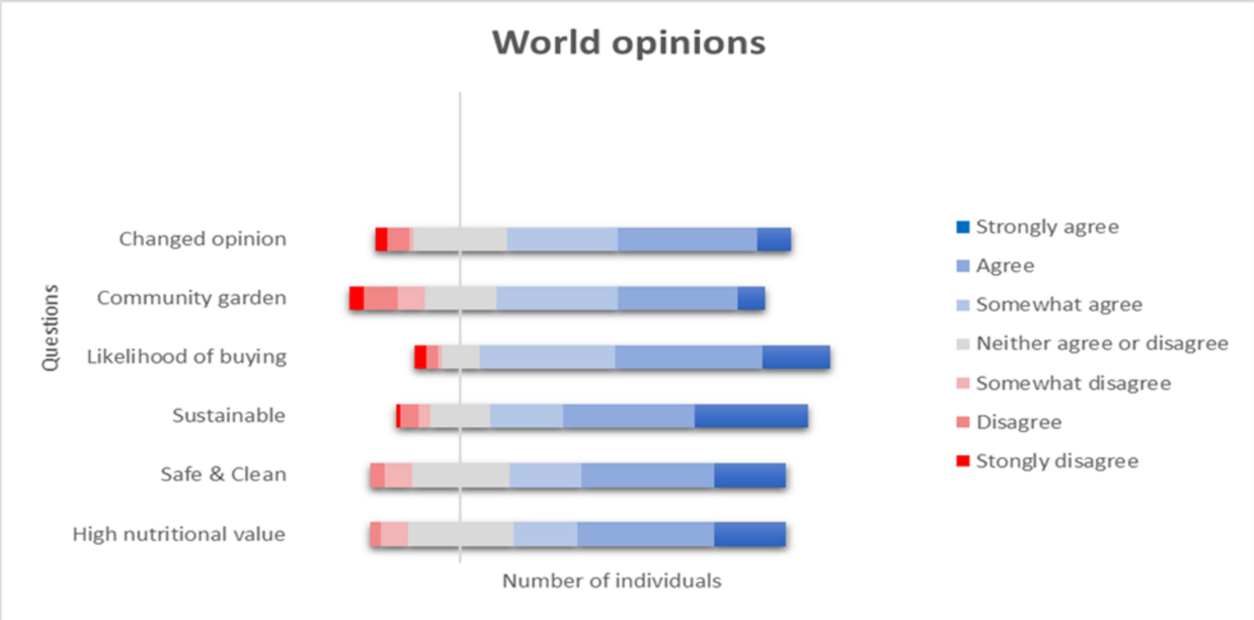


Figure 4.6. Shows the opinions of participants from the World excluding the UK to six questions based on a 1 – 7 Likert scale.

The data was analysed using Wilcoxon mann Whitney-U for the UK data, competent UK and the rest of the world. Results from the UK data is shown in Figure 4.7 A – F. The only significant result from the UK data was if people thought aquaponics was safe and clean, as the opinions were not statistically equal and people who had not heard of aquaponics had a much more varied response and typically more negative. While the other questions did not give statistically significant results, it is important to note that generally the responses were neutral or positive.

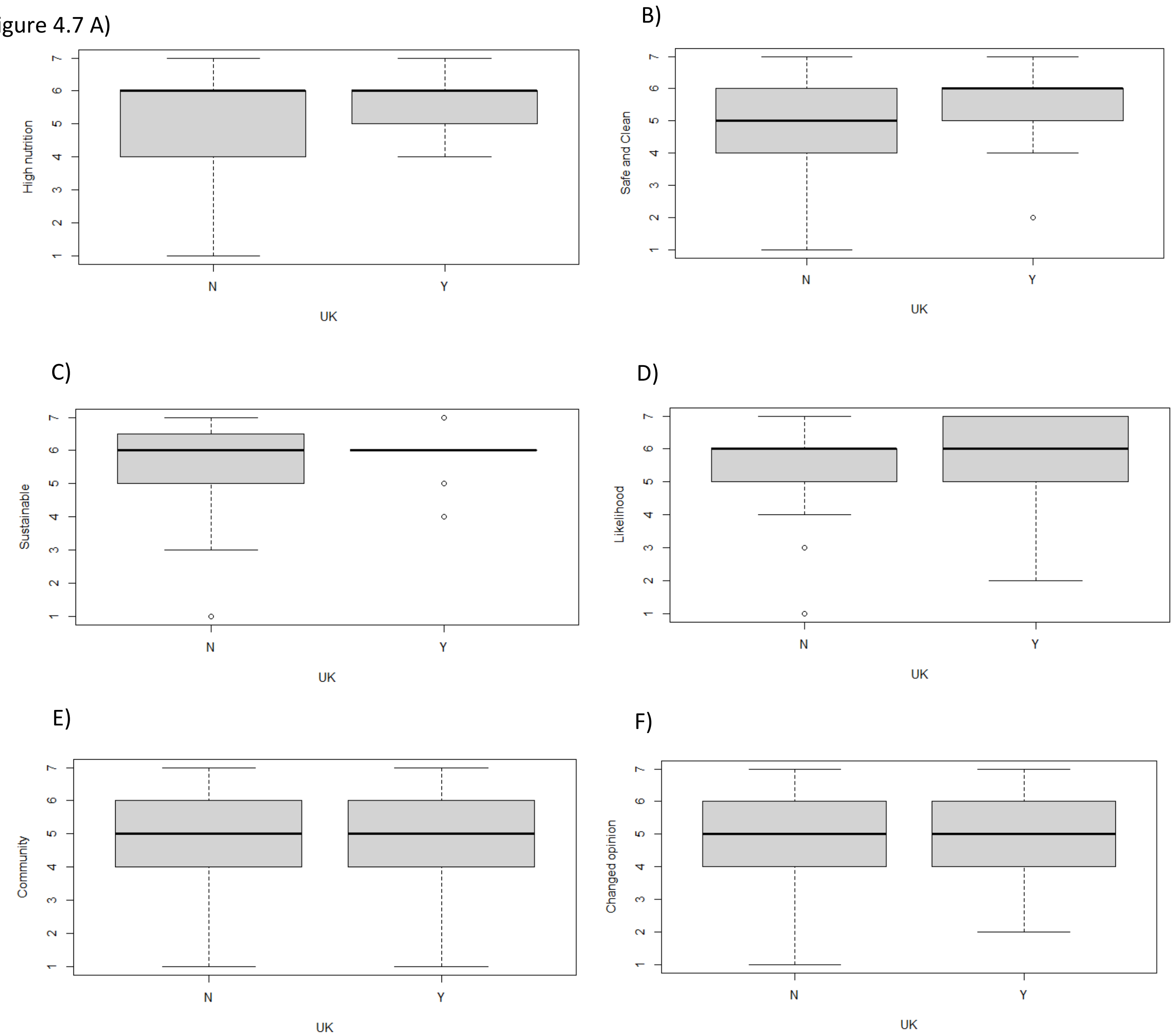


Figure 4.7A – 4.7F. Boxplots showing the distribution of answers from the UK data set. N refers to not having heard of aquaponics and Y have heard of it. The Y axis scale is on Likert data, with 1 denoting strongly disagree and 7 being strongly agree. The responses are from questions 6,7,11.12,13and 14 included in appendices 7.7.

who had competent knowledge and not competent knowledge of aquaponics. The responses from the 30% of the participants who had heard of aquaponics are shown in Figures 4.8 A – 4.8 F. The only significant result was in the community farm question where people who had competent knowledge of aquaponics were statistically significantly more likely to be willing to be part of and pay towards a community aquaponics farm Figure 4.8 E.

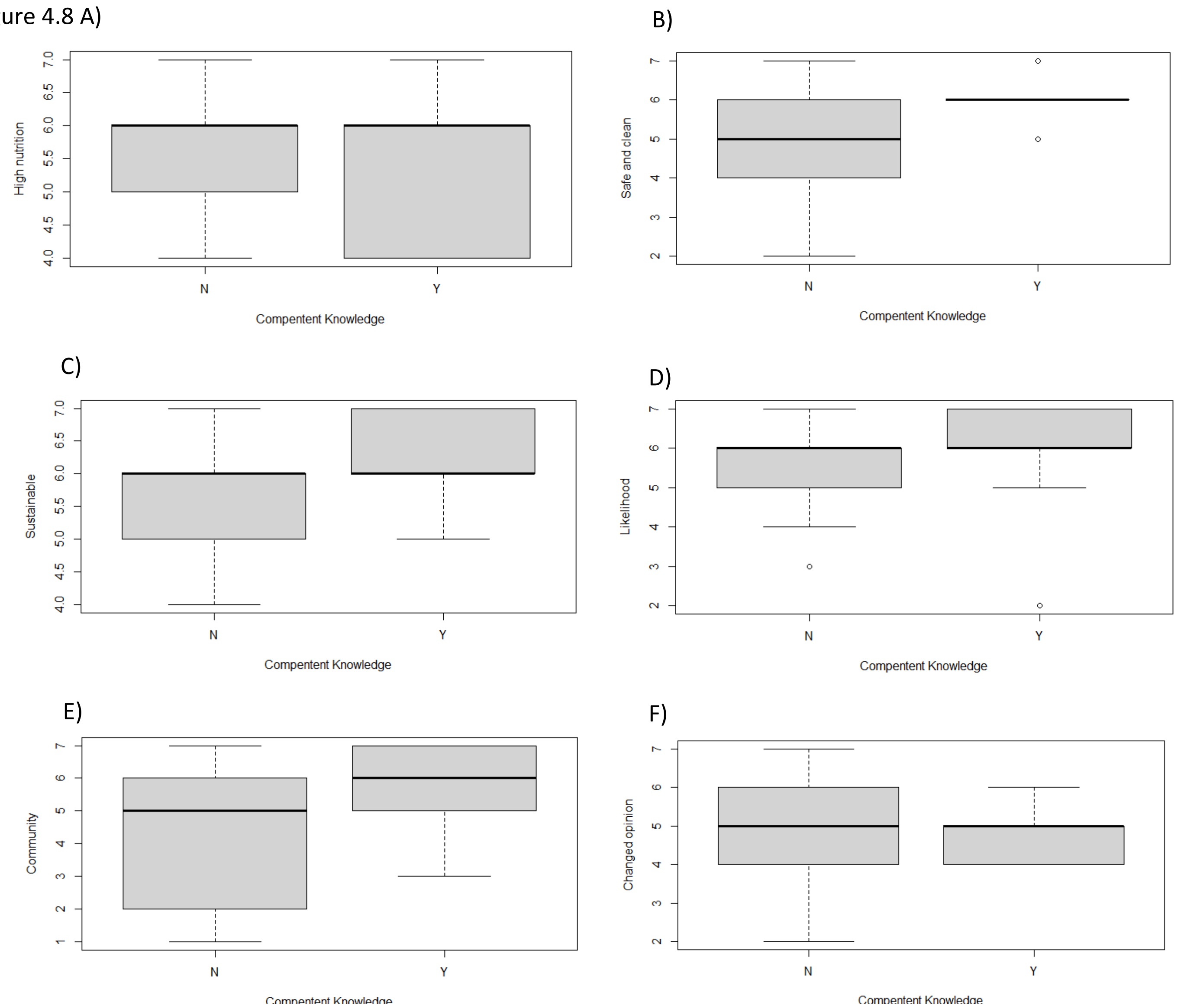


Figure 4.8 A – 4.8 F. Boxplots showing the distribution of answers from the UK competent data set. N refers to not having competent knowledge of aquaponics and Y have competent. The Y axis scale is on Likert data, with 1 denoting strongly disagree and 7 being strongly agree. The responses are from questions 6,7,11.12,13and 14 included in appendices 7.7.

For the rest of the world, it was found that the differences were significant for the nutritional value of aquaponics, the safety and cleanliness, and sustainability of aquaponics questions ($P<0.05$) Figures 4.9 A – 4.9 F visualise the data from the rest of the world that includes all the countries that is not the UK shown in Figure 4.1.

Figure 4.9 A)

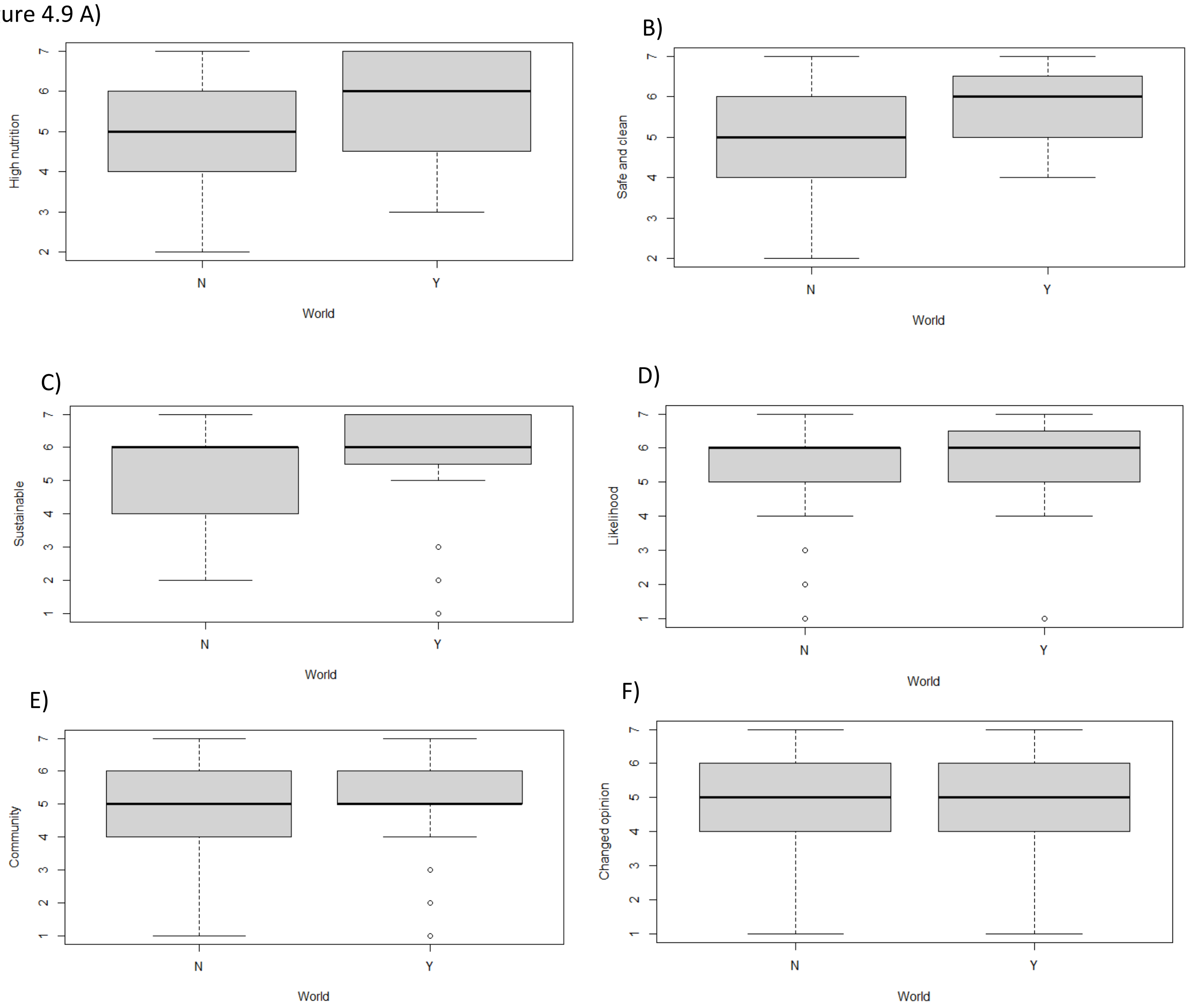


Figure 4.9 A – 4.9 F. Boxplots showing the distribution of answers from the World data set. N refers to not having heard of aquaponics and Y have heard of it. The Y axis scale is on Likert data, with 1 denoting strongly disagree and 7 being strongly agree. The responses are from questions 6,7,11.12,13and 14 included in appendices 7.7.

4.4 Discussion

For the aquaponics industry to thrive, its underlying success hinges on its perception by the general public. This survey found that only 30% of participants had heard of the term aquaponics, of which, only 43% of those had a basic competent understanding of what aquaponics is. Participants generally had a neutral or positive opinion of aquaponics, which typically improved with either having heard of aquaponics or a competent understanding. Just over 53% of participants were from the UK, which is useful for all current and prospective UK systems, as prior to this, there is little to no work on UK consumer perceptions (Miličić, Thorarinsdottir, Santos, et al., 2017). This enabled comparisons to be made between the UK and the rest of the world and a baseline to be created for future work in the UK. There are no clear trends with the demographics of aquaponics and their knowledge or opinions of aquaponics. The demographic data was not statistically analysed due to their being a disproportionately high number of participants that were between the ages of 18 – 24 and undergraduates (Figures 4.2 and 4.3)

Naturally, many people have reservations towards aquaponics and as evidenced in many previous studies, price is a major concern (Short et al., 2017). Many people are concerned over the initial investment costs and how much the produce will be, as generally people are not willing to pay premium prices for things they don’t fully understand (Suárez-Cáceres et al., 2021). However, interestingly, this study found that 58% of the respondents were willing to pay organic prices in the UK, and 49% from the rest of the world. This indicates that cultural differences and social awareness between countries on organic food plays a crucial role in the willingness to pay premium prices. These findings are an improvement on previous work that found 40% of participants were willing to pay organic prices (Miličić, Thorarinsdottir, Santos, et al., 2017), which is intriguing as fewer people had heard of aquaponics in this study, suggesting a positive cultural change.

It is important to note that many people had no reservations to aquaponics, which is incredibly promising for the future of aquaponics and its inclusion in urban farms. Pertinent reservations include; the nutritional value, disease, environmental impact and a lack of understanding. These reservations showed that the key issue for people's reservations to aquaponics is a lack of education, as many answers to some of the questions directly contradict the benefits and purpose of aquaponics. The subsequent issues this poses to aquaponics is that people don't understand it and fundamentally they are not actively going to pursue it through their own accord, evidenced by the reducing number of searches on google (Figure 4.10). The future uptake will be poor, unless strides are taken to educate and spread the knowledge and benefits of aquaponics, which can subsequently lead to improved economic feasibility of aquaponics.

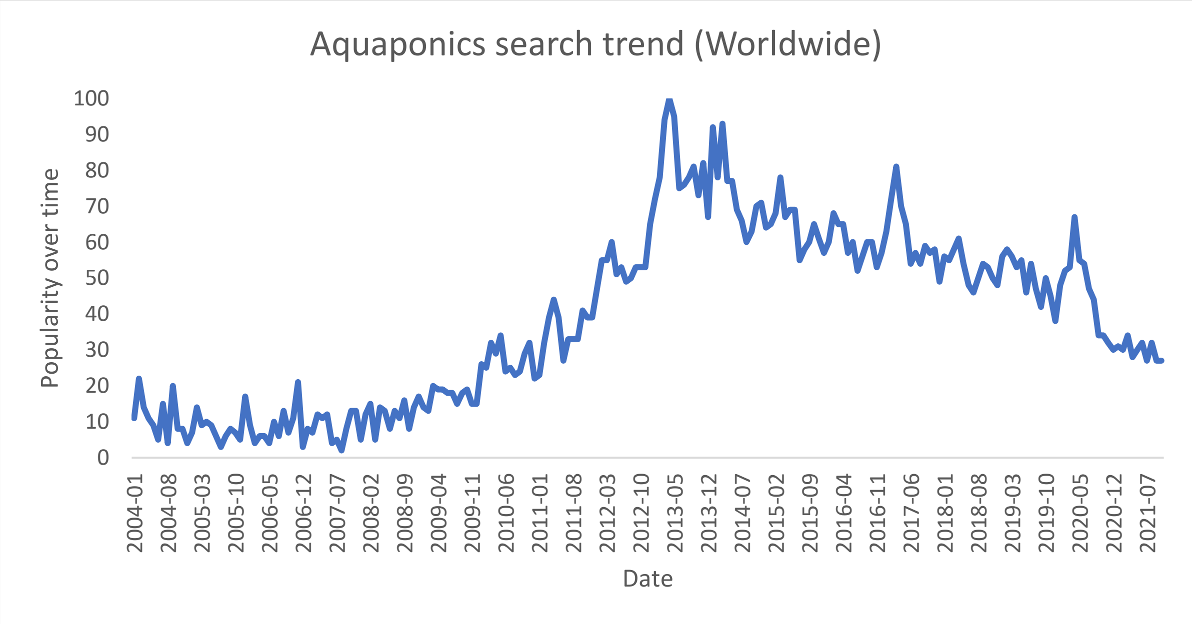


Figure 4.10 Data source: Google Trends of searches of the term aquaponics. 100 represents when the search term was most popular and 50 is half the searches etc. (https://www.google.com/trends). ("Aquaponics - Explore - Google Trends," 2021)

For aquaponics to be successful, it needs to be sold at premium prices and the produce selection must meet the needs and demands of the local area (Bosma et al., 2017). The main drawback for aquaponics to be economically feasible in the UK is that it cannot be sold at premium, organic prices. Unfortunately, the majority of economic reports on aquaponics are anecdotal due to their small scale, or being a research based system (Tokunaga, Tamaru, Ako, & Leung, 2015). However, it is known that feed can constitute up to 70% of total costs of a system (Castilho-Barros et al., 2018), labour up to 30% and water and electric can be 12% (Baganz, Baganz, Staaks, Monsees, & Kloas, 2020). Even a small scale system of 2,200l can have an initial investment of £2,200 and a monthly maintenance of £360 (Lobillo-Eguíbar, Fernández-Cabanás, Bermejo, & Pérez-Urrestarazu, 2020). The high cost of feed is why work with alternative ingredients, as seen in chapter 3 of this thesis and the inclusion of microalgae is key in mitigating costs, thus making aquaponics more economically feasible. Other ways of minimising costs is the use of community input to reduce labour costs, spreading awareness and using water catchment, as well as, renewable energy (Quagrainie, Flores, Kim, & McClain, 2018). While RAS/aquaponics systems typically use more electricity than pond/cage cultures (Badiola, Basurko, Piedrahita, Hundley, & Mendiola, 2018), meaning they do incur higher running costs, there is potential for money saving by using renewable energy. Most work however, focuses mainly on cost mitigation through feed as it is such a high cost and energy saving tends to be ignored due to being a much lower cost (Bergman et al., 2020). This being said, projects like biophilic living in Swansea, where the building will utilise solar power and new innovative technology to make an efficient system will allow for RAS/aquaponic systems to become more self-reliant and energy efficient. Also, it has been found that aquaponics systems even have the potential to be more energy efficient than RAS or soil-based farming (David et al., 2022), which is promising for prospective systems. As aquaponics cannot be classed as organic due to EU legislation (European commission, 2008), it cannot make as much of a profit as it needs to be economically feasible. This suggests the need for legislative and cultural change.

Despite this, the crucial underlying success of aquaponics lies with consumer perception and subsequent willingness to pay (Greenfeld et al., 2019; Turnsek, Joly, Thorarinsdottir, & Junge, 2020). It is recommended that future work be carried out on the economics of aquaponics, so that areas where costs can be mitigated and profits can be maximised, can work in conjunction with consumer perceptions in local areas to best fit the needs and demands of local communities.

The Likert analysis in the present study indicated that generally, public opinions are neutral or positive towards aquaponics, for the Uk and the rest of the world (Figures 4.5 and 4.6), which is comparable to results found in previous research (Miličić et al., 2017; Short et al., 2017). The question that received the most negative responses for both the UK and the world was "Would you be willing to pay towards and live in an urban building with a community aquaponics farm?" Which is pertinent information for Powell Dobson as their project will rely on people adopting the system in their community driven farms. The rest of the world had more negative responses than the UK, but still showed the same trends with the UK in regards to the rest of the questions. Few respondents strongly disagree with the community garden aspect of aquaponics, which is interesting as people are pro-innovative farming methods such as aquaponics, but they are worried that if they invest their time or money into something they don't fully understand or that is "novel" then they may have unforeseen consequences and fall out that they will have to deal with. Additionally, the question regarding paying towards and living with a community aquaponics farm yielded the most consistent responses regardless of any demographic or previous knowledge of aquaponics in comparison to the other questions and shows that the attitude towards being part of a community farm is the same across many demographics. The responses from the UK data are shown in Figure 4.7 A - 4.7 F, clearly shows that generally responses are neutral or positive and that when people had heard of aquaponics, they typically have a more positive opinion. The analysis of the UK Likert data revealed that there was only one statistically significant ($P<0.05$) result on the safety and cleanliness question of aquaponics, with people who had not heard of aquaponics having much higher variability and more likely to believe aquaponics is not a clean or safe method of farming.

Interestingly there was only one statistically significant result in the competent UK data for the community farm question. The results indicate that with competent knowledge of aquaponics, typically leads to a more positive opinion, other than for changing opinion of aquaponics as it is assumed they already had a good understanding of aquaponics, so a survey would not sway their opinions. It is important to note when people with competent knowledge of aquaponics were compared to people who were not, or had not even heard of it, the results were significant for all the questions in favour of a positive attitude to aquaponics, thus further showing the need for education and more widespread coverage of aquaponics, as understanding is key to its success and feasibility.

The rest of the world data revealed that the nutritional value, sustainability and the safety/cleanliness of aquaponically grown produce had statistically significant results ($P<0.05$) and they had a high variation in responses from people who had not heard of aquaponics, of which some responses that were very negative may have caused the statistically significant difference. The sustainability of aquaponics had variation in some responses of people that had heard of aquaponics, which could be explained by the fact that majority of the outlying negative responses were from people who did not have competent knowledge of aquaponics. Interestingly, the community farm prospect of aquaponics was not statistically significant, however it is important to note that bar a few responses that were negative, majority of people who had heard of aquaponics had positive opinions on the community farm. This heterogeneity in the responses for all the questions with the rest of the world data can be explained by the fact that perceptions are highly localised and will differ between towns let alone countries (Greenfeld et al., 2019), and the world data in this study, included perceptions from 33 different countries, which would explain the heterogeneity.

The results from this survey serve as a baseline guide for the UK perceptions of aquaponics, thus there is a better chance of aquaponics becoming sustainable and financially feasible by fully understanding the reservations and demands of local people. Also, the results highlight the need to move away from a "novel" idea to one of an innovative functioning method of farming, to be able to educate and physically shown people the huge benefits of aquaponics at work. Further work is needed to be conducted on the whole of the UK and at even more local scales, where new aquaponic systems are proposed to be built, to ascertain if the system would be successful and feasible based on the consumer willingness to cultural adjustment in farming methods and reservations of aquaponics (Greenfeld, Becker, Bornman, dos Santos, et al., 2020). The results have significant importance for producers or prospective farmers such as Powell Dobson in Swansea, as consumer perception is a key attributing factor to make aquaponics economically feasible (Short et al., 2017). It is recommended that further work is carried out in the UK to gain a better understanding of people's perceptions, as well as, committing resources and time into furthering the education of consumers and school pupils on aquaponics.

4.5 Conclusion

This study has provided a baseline of consumer perceptions to aquaponics in the UK. Generally, attitudes towards aquaponics are neutral or positive, but only 30% of people have heard of aquaponics, and there are clear reservations with aquaponics that hinder its progression and feasibility, which include; price, lack of understanding/knowledge and legislation. One of the most interesting results from this study was that, while people are willing to pay for aquaponics and think it is a positive method of farming, when it comes to cultural change, people are unwilling to be part of community farms or be directly involved, which should be explored further. Ways of reducing costs in aquaponic systems such as; labour costs, legislative changes and alternative feed ingredients need to be explored further to make aquaponics economically feasible. Crucially, the future of aquaponics is dictated by public awareness and perception. Therefore, future work should focus on education and integrating aquaponics into the mainstream, preventing aquaponics remaining stagnant as a novel farming method and spreading awareness of its significant benefits.

5 Thesis conclusion

Throughout this work, it has been argued that there is a need for a new feed that replaces FM, while still being beneficial to growth and that consumer perception is key to the feasibility of aquaponics. In particular, this thesis demonstrated; 1) Fish feed can be supplemented with up to 30% microalgae inclusion, without detriment to *O.niloticus* growth or body composition, 2) There is little impact of feed type on water parameters, but feed quantity is of great importance, thus aquaponics can base the system needs on quantity, 3) Microalgae cultured on digestate can successfully replace FM in the diet of *O.niloticus* and is just as palatable as commercial feed and 4) Generally people have neutral or positive perceptions of aquaponics, but only 30% of people have heard of the term and many people have reservations to costs and being part of urban farms. The results from this thesis help close the gap in aquaponics research on the use of "novel" ingredients to replace FM, creating a more closed-loop system and the huge gap in consumer perceptions, where no work has been conducted in the UK, creating a baseline for current and prospective aquaponic systems to utilise.

This thesis explored the use of four microalgae species (Chlorella, Spirulina, Nannochloropsis and Scenedesmus) as an alternative to FM and at what levels these microalgae were beneficial to *O. niloticus* growth, as the need for a sustainable alternative to FM is one of the most pressing concerns of today. As it stands, the use of microalgae is limited, due to costs, poor infrastructure and the use of microalgae being a novel ingredient that is not used on a wide scale. Critically, this thesis showed the need for further work on inclusion of microalgae in long term studies, so the use of microalgae can become widely utilised and make aquaculture more sustainable. However, the limitations from chapter 1 were that there are only a few papers reported on Nannochloropsis and Scenedesmus therefore conclusions on these species may not be as representative.

Additionally, this thesis assessed how feed influences water parameters, which many aquaponic and aquaculture papers ignore in relation to the feed, they tend to only focus on stocking density and the recirculation rate. It was found that generally, the key to nutrient dynamic in aquaculture systems is the feed quantity, but it is important to note that there is very little work on the nutrient dynamics relating to types of feed and future work on use of different ingredients needs to also focus on the effects on water parameters to better understand environmental impacts from discharge and to be able to tailor feed for use in aquaponics, hence why only a few papers were used, as they don't report water parameters, limiting the ability to make precise conclusions as there was high heterogeneity between the studies. Despite this, general conclusions could be drawn and crucially the findings relating to how different feeds can possibly influence pH is vital for aquaponics to be able to develop into an efficient functioning system.

Off the back of these reviews, this thesis conducted a pilot study on the palatability of a feed formulated using DCM, that replaced FM. Currently, aquaculture feeds are unsustainable, with many negative environmental impacts and no work has been done using DCM in *O. niloticus*. Key findings from this chapter were that there was no significant difference ($P>0.05$) in the number of pellets eaten or ejected. Therefore, this thesis provides a benchmark of palatability, where the feed that had microalgae and no FM was just as palatable as a commercially available feed. It is important to note that because of COVID-19, the feed formulated in this study did not match the commercial feed exactly in levels of proteins, therefore, the results should be interpreted with caution. It is however, still pertinent to say that such a difference in the formulation of the feed, would expect unpalatability as it is not what the tilapia were used to, but the palatability was the same as the commercial feed, supporting the findings of this study, despite the key differences in the feeds. This has huge implications for the aquaculture industry and the food waste industry, as a feed that can utilise waste from anaerobic digestion to formulate a feed in aquaculture can create new revenue streams and lead to a more sustainable and closed-loop system that can be utilised within aquaponics.

However, regardless of having a system or an aquaculture feed that is the most sustainable it possibly can be, if people will not buy it, it will not be feasible. The last chapter of this thesis bridged the vast gap in knowledge on consumer perceptions in the UK. The survey showed that there is a clear need for education to allow people to best understand aquaponics and make informed decisions. The key drawback for this particular survey was that it was solely online through various platforms, which may have inadvertently excluded some participants and made it more accessible to others and it also meant that it was impossible to be able to select a certain area for the surveys to take place, so no conclusions could be drawn to a local scale, such as the Swansea area. Additionally, highlighting the need to have local scale surveys to allow aquaponics farms to meet the needs of the local people. By doing so, aquaponics systems can be placed in areas that they are currently feasible, as opinions of aquaponics are highly localised.

This thesis was pioneering in multiple aspects relating to aquaculture and aquaponics. It is pertinent because the demand for fish has been increasing annually (FAO., 2020), thus the subsequent demand for feed increases, putting pressures on the industry to create sustainable alternatives. Additionally, Nile tilapia are the third most cultured species in the world, so any advances on the culture, feed or systems they are kept in can have massive positive impacts in sustainability and economically. Feed is of great importance, because it is the costliest and most environmentally damaging aspect of aquaculture (Sarker et al., 2018), the results from this thesis clearly show that alternatives to FM, especially soy bean and microalgae are excellent alternatives that will help reduce the costs of feed and lead to less environmental impact. Further to this, the successful inclusion of DCM in aquaculture feed will lead to greater positive environmental impacts, taking waste from an already established industry and utilising it to create a feed will mean that less of this waste becomes a pollutant and fits in well within the circular economy. The results also helped to create a baseline of palatability of DCM.

Additionally, prior to this thesis, there was no study on the perceptions to aquaponics in the UK. This lack of studies means that any systems in the UK are operating blind to what will make them feasible, which is having people knowing about aquaponics and its benefits. Therefore, this thesis created the first known baseline database of consumer perceptions within the UK. One example of where a baseline of consumer perceptions will benefit UK systems is the system being proposed by Powell Dobson in the heart of Swansea city, where it will be a community orientated farm and will require people to understand the system and be aware of how it works. Additionally, the local populations perception will be able to best dictate what the system will be used to grow and how it can best be used to educate and promote aquaponics. future work should focus on areas where systems are proposed, such as in Swansea to best understand the public's reaction to aquaponics and how to make it feasible in relation to the local perceptions.

The take home messages from this survey are that microalgae can successfully be utilised even at small quantities of up to 30% to replace FM in aquaculture feeds, without being detrimental to the growth or health of Nile tilapia. Also, feed quantity is the underlying factor that influences water parameters, thus water quality in aquaponics can be controlled using feed quantity as a fairly accurate measure. However, more work needs to be done in order to tailor feed to certain crops and better understand nutrient dynamics within systems. Furthermore, the palatability of a feed incorporating DCM has been proven to be equal to that of commercially available feed. Implying that the use of "novel" ingredients are suitable to replace FM completely and they should be more widely utilised to make aquaculture feed sustainable and better suited to the circular economy. Finally, consumer perception is crucial in making aquaponics feasible and generally people have a neutral or positive opinion. Despite this only 30% of people have heard of it and many people have reservations to the price of aquaponics and being part of a community farm, but there is a clear lack of understanding that will hinder the progression and adoption of aquaponics.

5.1 Future directions

This thesis leaves two key questions for the future; 1) Why are microalgae not commonly used in aquaculture feed with such clear benefits to the fish and costs? 2) Consumer perception is key, so why have there been no studies on public opinion in many countries? Future work should expand the use of DCM in feed in relation to long term effects on growth and body composition of many commercial fish species and there should be greater focus on consumer perceptions at global and local scales to best understand and educate people on aquaponics, with potential to ask follow up surveys for participants to see if the trends remain the same and if there are any further questions that would benefit local businesses.

Knowledge gaps and some limitations of this thesis have identified areas for future research to direct their focus. Work on utilising species such as spirulina and Chlorella in fish species like *O. niloticus* are saturated and work should focus on other species of microalgae. Future work also needs to incorporate different species of microalgae into feeds in combinations and following research into palatability, the effects on growth and body composition of Nile tilapia using a feed that is formulated with DCM needs focus, if the widescale uptake of DCM is to become a reality.

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7. Appendices

7.1 Chapter 1 raw data

#Key – Me = mean of treatment, Se = standard deviation of treatment, Mc = mean of control, Sc = standard deviation of control, Ne = sample size of treatment, Nc = sample size of control

7.1.1 Chapter 1 SGR raw data

Author	Microalgae	Me	Se	Mc	Sc	Ne	Nc
10% Ch Badwy et al 2008	Chlorella	1.6	0.01	1.56	0.01	75	75
25% Ch Badwy et al 2008	Chlorella	1.61	0.02	1.56	0.01	75	75
50% Ch Badwy et al 2008	Chlorella	1.7	0.02	1.56	0.01	75	75
75% Ch Badwy et al 2008	Chlorella	1.33	0.01	1.56	0.01	75	75
5% Ch Mahmoud et al 2020	Chlorella	1.62	0.02	1.47	0.02	72	72
10% Ch Galal et al 2018	Chlorella	0.42	0.01	0.28	0.01	60	70
30% Ch Teuling et al 2017	Chlorella	2.57	0.036	2.41	0.036	45	45
3% Nanno Sarker et al 2018	Nannochloropsis	3.18	0.04	3.38	0.08	30	30
5.5% Nanno Sarker et al 2018	Nannochloropsis	2.97	0.07	3.38	0.08	30	30
8% Nanno Sarker et al 2018	Nannochloropsis	3.05	0.7	3.38	0.08	30	30
80% Nanno Gbadamosi et al 2018	Nannochloropsis	3.19	0.04	3.34	0.05	45	45
3% Nanno Ali Zeinab mohammed et al 2019	Nannochloropsis	2.11	0.06	2.22	0.06	45	45
5% Nanno Ali Zeinab mohammed et al 2019	Nannochloropsis	2.29	0.06	2.22	0.06	45	45
7% Nanno Ali Zeinab mohammed et al 2019	Nannochloropsis	2.4	0.06	2.22	0.06	45	45
30% Nanno Teuling et al 2017	Nannochloropsis	2.44	0.036	2.41	0.036	45	45
10% Scene Badwy et al 2008	Scenedesmus	1.61	0.02	1.56	0.01	75	75
25% Scene Badwy et al 2008	Scenedesmus	1.63	0.02	1.56	0.01	75	75
50% Scene Badwy et al 2008	Scenedesmus	1.76	0.01	1.56	0.01	75	75
75% Scene Badwy et al 2008	Scenedesmus	1.35	0.01	1.56	0.01	75	75
30% Scene Teuling et al 2017	Scenedesmus	2.19	0.036	2.41	0.036	45	45
2.5% S Ahmed M. Al-Zayat 2019	Spirulina	1.08	0.04	1.05	0.01	20	20
5% S Ahmed M. Al-Zayat 2019	Spirulina	1.12	0.01	1.05	0.01	20	20
7.5% S Ahmed M. Al-Zayat 2019	Spirulina	1.26	0.01	1.05	0.01	20	20
10% S Ahmed sherif & Soad Salama 2012	Spirulina	0.7	0.02	0.61	0.03	30	30
15% S Ahmed sherif & Soad Salama 2012	Spirulina	0.76	0.01	0.61	0.03	30	30
5% S Ahmed sherif & Soad Salama 2012	Spirulina	0.7	0.01	0.61	0.03	30	30
100% S Ebtehal El-Sayed Hussein et al 2012	Spirulina	5.85	0.046188	5.04	0.092376	50	50
1% S Elsayed B. Belal et al 2012	Spirulina	2.05	0.08	1.87	0.08	30	30
1% S Elsayed B. Belal et al 2012	Spirulina	1.98	0.08	1.87	0.08	30	30
20% S M A Olvera Novoa et al 1998	Spirulina	4.76	0.07	4.72	0.07	45	45
40% S M A Olvera Novoa et al 1998	Spirulina	4.59	0.07	4.72	0.07	45	45

60% S M A Olvera Novoa et al 1998	Spirulina	3.65	0.07	4.72	0.07	45	45
80% S M A Olvera Novoa et al 1998	Spirulina	2.4	0.07	4.72	0.07	45	45
100% S M A Olvera Novoa et al 1998	Spirulina	1.1	0.07	4.72	0.07	45	45
0.5% S Amer 2016	Spirulina	13.49	0.79	13.32	0.21	45	45
1% S Amer 2016	Spirulina	14.22	0.58	13.32	0.21	45	45
1.5% S Amer 2016	Spirulina	12.39	0.47	13.32	0.21	45	45
1% S Tawwab & Ahmad 2009	Spirulina	2.44	0.05	2.41	0.047	60	60
0.75% S Tawwab & Ahmad 2009	Spirulina	2.45	0.047	2.41	0.047	60	60
0.5% S Tawwab & Ahmad 2009	Spirulina	2.62	0.013	2.41	0.047	60	60
0.25% S Tawwab & Ahmad 2009	Spirulina	2.49	0.028	2.41	0.047	60	60
0.125% S Tawwab & Ahmad 2009	Spirulina	2.45	0.026	2.41	0.047	60	60
50% S El sheekh et al 2014	Spirulina	4.69	0.161	4.59	0.266	30	30
75% S El sheekh et al 2014	Spirulina	5.3	0.033	4.59	0.266	30	30
100% S El sheekh et al 2014	Spirulina	4.9	0.141	4.59	0.266	30	30
5% S Ungsethaphand et al 2010	Spirulina	1.76	0.03	1.78	0.01	50	50
10% S Ungsethaphand et al 2010	Spirulina	1.72	0.01	1.78	0.01	50	50
20% S Ungsethaphand et al 2010	Spirulina	1.78	0	1.78	0.01	50	50
3% S Ali Zeinab mohammed et al 2019	Spirulina	2.32	0.06	2.22	0.06	45	45
5% S Ali Zeinab mohammed et al 2019	Spirulina	2.22	0.06	2.22	0.06	45	45
7% S Ali Zeinab mohammed et al 2019	Spirulina	2.12	0.06	2.22	0.06	45	45
30% S Velasquez et al 2016	Spirulina	4.63	0.06	4.16	0.06	3	3
45% S Velasquez et al 2016	Spirulina	4.46	0.08	4.16	0.06	3	3
60% S Velasquez et al 2016	Spirulina	4.23	0.11	4.16	0.06	3	3
75% S Velasquez et al 2016	Spirulina	3.4	0.09	4.16	0.06	3	3
30% S Teuling et al 2017	Spirulina	2.64	0.036	2.41	0.036	45	45

7.1.2 Chapter 1 FCR raw data

Author	Microalgae	Me	Se	Mc	Sc	Ne	Nc
10% Ch Badwy et al 2008	Chlorella	2.56	0.05	2.68	0.06	75	75
25% Ch Badwy et al 2008	Chlorella	2.56	0.04	2.68	0.06	75	75
50% Ch Badwy et al 2008	Chlorella	2.03	0.08	2.68	0.06	75	75
75% Ch Badwy et al 2008	Chlorella	3.24	0.06	2.68	0.06	75	75
5% Ch Mahmoud et al 2020	Chlorella	1.26	0.02	1.44	0.02	72	72
30% Ch Teuling et al 2017	Chlorella	0.89	0.01	0.95	0.01	45	45
3% Nanno Sarker et al 2018	Nannochloropsis	1.26	0.03	1.12	0.08	30	30
5.5% Nanno Sarker et al 2018	Nannochloropsis	1.58	0.17	1.12	0.08	30	30
8% Nanno Sarker et al 2018	Nannochloropsis	1.55	0.11	1.12	0.08	30	30
80% Nanno Gbadamosi et al 2018	Nannochloropsis	1.28	0.02	1.2	0.04	45	45
3% Nanno Zeinab mohammed et al 2019	Nannochloropsis	1.79	0.13	1.47	0.13	45	45
5% Nanno Zeinab mohammed et al 2019	Nannochloropsis	1.42	0.13	1.47	0.13	45	45
7% Nanno Zeinab mohammed et al 2019	Nannochloropsis	1.37	0.13	1.47	0.13	45	45
30% Nanno Teuling et al 2017	Nannochloropsis	0.96	0.01	0.95	0.01	45	45
10% Scene Badwy et al 2008	Scenedesmus	2.59	0.11	2.68	0.06	75	75
25% Scene Badwy et al 2008	Scenedesmus	2.57	0.05	2.68	0.06	75	75
50% Scene Badwy et al 2008	Scenedesmus	1.76	0.05	2.68	0.06	75	75
75% Scene Badwy et al 2008	Scenedesmus	2.74	0.02	2.68	0.06	75	75
30% Scene Teuling et al 2017	Spirulina	1.1	0.01	0.95	0.01	45	45
2.5% S Ahmed M. Al-Zayat 2019	Spirulina	1.78	0.08	1.77	0.13	20	20
5% S Ahmed M. Al-Zayat 2019	Spirulina	1.68	0.07	1.77	0.13	20	20
7.5% S Ahmed M. Al-Zayat 2019	Spirulina	1.73	0.02	1.77	0.13	20	20
10% S Ahmed sherif & Soad Salama 2012	Spirulina	1.22	0.02	1.66	0.1	30	30
15% S Ahmed sherif & Soad Salama 2012	Spirulina	1.15	0.2	1.66	0.1	30	30
5% S Ahmed sherif & Soad Salama 2012	Spirulina	1.39	0.04	1.66	0.1	30	30
100% S Ebtehal El-Sayed Hussein et al 2012	Spirulina	2.31	0.069282	4.39	0.21362	50	50
1% S Elsayed B. Belal et al 2012	Spirulina	1.27	0.15	1.5	0.15	30	30
1% S Elsayed B. Belal et al 2012	Spirulina	1.35	0.15	1.5	0.15	30	30
20% S M A Olvera?Novoa et al 1998	Spirulina	1.07	0.09	1.03	0.09	45	45
40% S M A Olvera?Novoa et al 1998	Spirulina	1.28	0.09	1.03	0.09	45	45
60% S M A Olvera?Novoa et al 1998	Spirulina	1.45	0.09	1.03	0.09	45	45
80% S M A Olvera?Novoa et al 1998	Spirulina	1.91	0.09	1.03	0.09	45	45
100% S M A Olvera?Novoa et al 1998	Spirulina	3.64	0.09	1.03	0.09	45	45
0.5% S Amer 2016	Spirulina	2.19	0.08	2.29	0.02	45	45
1% S Amer 2016	Spirulina	2.07	0.03	2.29	0.02	45	45
1.5% S Amer 2016	Spirulina	2.36	0.07	2.29	0.02	45	45
1% S Tawwab & Ahmad 2009	Spirulina	1.31	0.039	1.43	0.135	60	60
0.75% S Tawwab & Ahmad 2009	Spirulina	1.32	0.015	1.43	0.135	60	60
0.5% S Tawwab & Ahmad 2009	Spirulina	1.22	0.02	1.43	0.135	60	60
0.25% S Tawwab & Ahmad 2009	Spirulina	1.36	0.04	1.43	0.135	60	60
0.125% S Tawwab & Ahmad 2009	Spirulina	1.35	0.044	1.43	0.135	60	60
50% S El sheekh et al 2014	Spirulina	1.11	0.11	1.18	0.037	30	30
75% S El sheekh et al 2014	Spirulina	1	0.005	1.18	0.037	30	30

100% S El sheekh et al 2014	Spirulina	1.04	0.036	1.18	0.037	30	30
5% S Ungsethaphand et al 2010	Spirulina	1.78	0.08	1.76	0.01	50	50
10% S Ungsethaphand et al 2010	Spirulina	1.86	0.01	1.76	0.01	50	50
20% S Ungsethaphand et al 2010	Spirulina	1.82	0.03	1.76	0.01	50	50
3% S Zeinab mohammed et al 2019	Spirulina	1.38	0.13	1.47	0.13	45	45
5% S Zeinab mohammed et al 2019	Spirulina	1.52	0.13	1.47	0.13	45	45
7% S Zeinab mohammed et al 2019	Spirulina	1.77	0.13	1.47	0.13	45	45
30% S Teuling et al 2017	Spirulina	0.84	0.01	0.95	0.01	45	45

7.2 Chapter 1 R code

#Random-effects model pooling for meta-analysis

#Key – Me = mean of treatment, Se = standard deviation of treatment, Mc = mean of control, Sc = standard deviation of control, Ne = sample size of treatment, Nc = sample size of control

```
install.packages("metafor")

install.packages("meta")

install.packages("tidyverse")

library("metafor")

library("meta")

library("tidyverse")

#Specific growth rate analysis

SGRHigh <-read.csv(file.choose(), header = T)

View(SGRHigh)

str(SGRHigh)

names(SGRHigh)

SGRHigh.Pool <-metacont(Ne, Me, Se, Nc, Mc, Sc, data = SGRHigh, studlab = paste(Author),
comb.fixed = FALSE, comb.random = TRUE, method.tau = "SJ", hakn = TRUE, prediction = TRUE,
sm = "SMD")

SGRHigh.Pool

forest(SGRHigh.Pool, layout = "JAMA", text.predict = "95% PI", col.predict = "red",
colgap.forest.left = unit(15,"mm"))

install.packages("effsize")

library(effsize)

cohen.d(SGRHigh$Me, SGRHigh$Mc, na.rm=TRUE, pooled=TRUE, paired=TRUE, hedges=TRUE)

print(SGRHigh.Pool)
```

```
funnel(SGRHigh.Pool, xlab="Hedges' g", contour = c(.95,.975,.99),
col.contour=c("darkblue","blue","lightblue"))+ legend("left", c("p < 0.05", "p<0.025", "< 0.01"), bty
= "n",fill=c("darkblue","blue","lightblue"))

pdf(file = 'SGR.pdf')

SGRForest <- forest(SGRHigh.Pool, layout = "JAMA", text.predict = "95% PI", col.predict = "black",
colgap.forest.left = unit(15,"mm")) dev.off()

#Feed conversion ratio meta analysis

FCRHigh <-read.csv(file.choose(), header = T)

View(FCRHigh)

str(FCRHigh)

names(FCRHigh)

FCRHigh.Pool <-metacont(Ne, Me, Se, Nc, Mc, Sc, data = SGRHigh, studlab = paste(ï..Author),
comb.fixed = FALSE, comb.random = TRUE, method.tau = "SJ", hakn = TRUE, prediction = TRUE,
sm = "SMD")

FCRHigh.Pool

forest(FCRHigh.Pool, layout = "JAMA", text.predict = "95% PI", col.predict = "red",
colgap.forest.left = unit(15,"mm"))

install.packages("effsize")

library(effsize)

cohen.d(FCRHigh$Me, FCRHigh$Mc, na.rm=TRUE, pooled=TRUE, paired=TRUE, hedges=TRUE)

print(FCRHigh.Pool)

funnel(FCRHigh.Pool, xlab="Hedges' g", contour = c(.95,.975,.99),
col.contour=c("darkblue","blue","lightblue"))+ legend("left", c("p < 0.05", "p<0.025", "< 0.01"), bty
= "n",fill=c("darkblue","blue","lightblue"))

pdf(file = 'SGR.pdf')

FCRForest <- forest(FCRHigh.Pool, layout = "JAMA", text.predict = "95% PI", col.predict = "black",
colgap.forest.left = unit(15,"mm"))

dev.off()
```

7.3 Chapter 2 raw data

7.3.1 Chapter 2 Ammonia raw data

#Key – Me = mean of treatment, Se = standard deviation of treatment, Mc = mean of control, Sc = standard deviation of control, Ne = sample size of treatment, Nc = sample size of control, Feed type = Percentage crude protein, Feed percentage = percentage fed in relation to biomass.

Author	Feed type	Feed percentage	Me	Se	Mc	Sc	Ne	Nc	Stocking density (kg/m^3)
Knaus. U & H.W Palm 2017	37	1.2	0.24	0.2	0.17	0.14	20	20	15
Arul V.Suresh & C.Kwei Lin 1992 HSD	22	1.5	0.28	0.146969385	0.26	0.146969385	6	6	3.75
Arul V.Suresh & C.Kwei Lin 1992 LSD	22	1.5	0.29	0.171464282	0.26	0.146969385	6	6	1.4
Salah Kanial 2006 LP	25	2.6	0.41	0.108166538	0.75	0.108166538	13	13	1.4
Salah Kanial 2006 MP	25	2.6	0.31	0.108166538	0.75	0.108166538	13	13	2.4
Hefni Effendi et al 2020 LP	33	3	0.02	0.02	0.026	0.08	6	6	4.5
Hefni Effendi et al 2020 MP	33	3	0.015	0.005	0.026	0.08	6	6	0.945
Ismail Abd Elmonem Eissa et al 2020	30	3	0.036	0.010606602	0.068	0.038183766	18	18	0.313
Sri Wahyuningsih et al 2015	30	3	0.13	0.04	0.14	0.06	6	6	0.733
Sara Mello Pinho et al 2017	22	5	0.2	0.2	1.5	0.7	6	6	1.1
Md. Zahir Rayhan et al 2018 HSD	30	10	1.525	0.63	1.1	0.59	4	4	1.1
Md. Zahir Rayhan et al 2018 LSD	30	10	0.325	0.09	1.1	0.59	4	4	4
Md. Zahir Rayhan et al 2018 MSD	30	10	1.325	0.57	1.1	0.59	4	4	1

7.3.2 Chapter 2 Nitrite raw data

Author	Feed type	Feed percentage	Me	Se	Mc	Sc	Ne	Nc	Stocking density (kg/m^3
Arul V.Suresh & C.Kwei Lin 1992 HSD	22	1.5	0.24	0.073484692	0.24	0.073484692	6	6	15
Arul V.Suresh & C.Kwei Lin 1992 LSD	22	1.5	0.27	0.09797959	0.24	0.073484692	6	6	3.75
Chunjie Li et al 2019 Media bed	35	2	0.42	0.22	0.5	0.2	8	8	0.027
Chunjie Li et al 2019 NFT	35	2	0.42	0.17	0.5	0.2	8	8	0.027
Hefni Effendi et al 2020 LP	33	3	0.32	0.05	0.026	0.08	6	6	1.4
Hefni Effendi et al 2020 MP	33	3	0.33	0.03	0.026	0.08	6	6	1.4
Ismail Abd Elmonem Eissa et al 2020	30	3	0.377	0.49638896	0.936	0.576999133	18	18	2.4
Jason J. Danaher et al 2013	32	10	0.7	0.2	0.6	0.2	4	4	13.5
Knaus. U & H.W Palm 2017	37	1.2	0.02	0.02	0.03	0.05	20	20	4.5
Licamele, Jason David HSD	35	2	0	0	0	0	4	4	8
Licamele, Jason David LSD	35	2	0	0	0	0	4	4	2
Salah Kanial 2006 LP	25	2.6	0.006	0.007211103	0.014	0.072	13	13	1.1
Salah Kanial 2006 MP	25	2.6	0.006	0.007211103	0.014	0.072	13	13	1.1
Sara Mello Pinho et al 2017	22	5	0.1	0.4	0	0	6	6	4
Sri Wahyuningsih et al 2015	30	3	0.42	0.12	0.49	0.13	6	6	1

7.3.3 Chapter 2 Nitrate raw data

Author	Feed type	Feed percentage	Me	Se	Mc	Sc	Ne	Nc	Stocking density (kg/m^3
Sri Wahyuningsih et al 2015	30	3	2.1	0.27	2.24	0.44	6	6	1
Knaus. U & H.W Palm 2017	37	1.2	7.16	5.7	7.26	5.73	20	20	4.5
Salah Kanial 2006 LP	25	2.6	5.31	0.576888	17.28	0.576888	13	13	1.1
Salah Kanial 2006 MP	25	2.6	8.42	0.576888	17.28	0.576888	13	13	1.1
Sara Mello Pinho et al 2017	22	5	1.3	0.4	0.1	0.1	6	6	4
Licamele, Jason David HSD	35	2	98	47.392	73	27.952	4	4	8
Licamele, Jason David LSD	35	2	48.5	3.1092	73	27.952	4	4	2
Chunjie Li et al 2019 Media bed	35	2	21.24	3.18	24.85	3.81	8	8	0.027
Chunjie Li et al 2019 NFT	35	2	21.76	4.4	24.85	3.81	8	8	0.027
Hefni Effendi et al 2020 MP	33	3	0.72	0.03	0.79	0.05	6	6	1.4
Jason J. Danaher et al	32	10	9.1	2.8	10	3.6	4	4	13.5
Hefni Effendi et al 2020 LP	33	3	0.77	0.07	0.79	0.05	6	6	1.4
Ismail Abd Elmonem Eissa et al 2020	30	3	1.005	1.1243	0.476	0.725492	18	18	2.4

7.3.4 Chapter 2 Phosphorus raw data

Author	Feed type	Feed percentage	Me	Se	Mc	Sc	Ne	Nc	Stocking density (kg/m^3
Hefni Effendi et al 2020 LP	33	3	1.23	0.04	1.29	0.06	6	6	1.4
Hefni Effendi et al 2020 MP	33	3	1.19	0.04	1.29	0.06	6	6	1.4
Ismail Abd Elmonem Eissa et al 2020	30	3	1.27	0.296985	1.29	0.534573	18	18	2.4
Jason J. Danaher et al	32	10	1.1	1.4	0.7	0.6	4	4	13.5
Knaus. U & H.W Palm 2017	37	1.2	1.55	1.12	1.14	1.1	20	20	4.5
Licamele, Jason David HSD	35	2	30.25	12.764	18.25	3.304	4	4	8
Licamele, Jason David LSD	35	2	15	3.7417	18.25	3.304	4	4	2
Salah Kanial 2006 LP	25	2.6	1.8	0.793221	3.8	0.793221	13	13	1.1
Salah Kanial 2006 MP	25	2.6	2.3	0.793221	3.8	0.793221	13	13	1.1
Sri Wahyuningsih et al 2015	30	3	3.1	2.6	20.6	1.6	6	6	1

7.3.5 Chapter 2 DO raw data

Author	Feed type	Feed percentage	Me	Se	Mc	Sc	Ne	Nc	Stocking density (kg/m^3
Arul V.Suresh & C.Kwei Lin 1992 HSD	22	1.5	3.5	0.734847	3.6	0.734847	6	6	15
Arul V.Suresh & C.Kwei Lin 1992 LSD	22	1.5	2.7	0.489898	3.6	0.734847	6	6	3.75
Chunjie Li et al 2019 Media bed	35	2	7.93	1.92	7.17	0.96	8	8	0.027
Chunjie Li et al 2019 NFT	35	2	8.2	1.98	7.17	0.96	8	8	0.027
Hefni Effendi et al 2020 LP	33	3	5.42	0.69	5.24	0.63	6	6	1.4
Hefni Effendi et al 2020 MP	33	3	5.45	0.77	5.24	0.63	6	6	1.4
Ismail Abd Elmonem Eissa et al 2020	30	3	6.22	0.353553	6.12	0.212132	50	50	2.4
Jason J. Danaher et al	32	10	6	0.4	5.6	0.2	4	4	13.5
Knaus. U & H.W Palm 2017	37	1.2	5.8	0.8	4.7	1.2	70	70	4.5
Licamele, Jason David HSD	35	2	4.82	2.07	6.76	0.41	5	5	8
Licamele, Jason David LSD	35	2	7.91	0.36	6.76	0.41	5	5	2
Md. Zahir Rayhan et al 2018 HSD	30	10	5.2	0.42	6.2	0.42	4	4	0.945
Md. Zahir Rayhan et al 2018 LSD	30	10	6.35	0.43	6.2	0.42	4	4	0.313
Md. Zahir Rayhan et al 2018 MSD	30	10	5.4	0.29	6.2	0.42	4	4	0.733
Salah Kanial 2006 LP	25	2.6	6.8	2.817446	6.7	2.817446	180	180	1.1
Salah Kanial 2006 MP	25	2.6	6.6	2.817446	6.7	2.817446	180	180	1.1
Sara Mello Pinho et al 2017	22	5	6.9	0.7	7.3	0.3	21	21	4
Sri Wahyuningsih et al 2015	30	3	5.2	0.5	5.33	0.53	6	6	1

7.3.6 Chapter 2 pH raw data

Author	Feed type	Feed percentage	Me	Se	Mc	Sc	Ne	Nc	Stocking density (kg/m^3
Arul V.Suresh & C.Kwei Lin 1992 HSD	22	1.5	8	0.073485	8	0.073485	6	6	15
Arul V.Suresh & C.Kwei Lin 1992 LSD	22	1.5	8	0.04899	8	0.073485	6	6	3.75
Chunjie Li et al 2019 Media bed	35	2	6.08	0.85	6.55	0.78	8	8	0.027
Chunjie Li et al 2019 NFT	35	2	6.12	0.76	6.55	0.78	8	8	0.027
Hefni Effendi et al 2020 MP	33	3	6.04	0.23	6	0.28	6	6	1.4
Hefni Effendi et al 2020 LP	33	3	6.04	0.22	6	0.28	6	6	1.4
Ismail Abd Elmonem Eissa et al 2020	30	3	7.9	0.155563	7.69	0.148492	50	50	2.4
Jason J. Danaher et al	32	10	7.1	0.2	7	0.2	4	4	13.5
Knaus. U & H.W Palm 2017	37	1.2	7.8	0.2	7.7	0.1	70	70	4.5
Licamele, Jason David LSD	35	2	7.7	0.38	7.4	0.41	5	5	8
Licamele, Jason David HSD	35	2	7.3	0.32	7.4	0.41	5	5	2
Md. Zahir Rayhan et al 2018 LSD	30	10	7.8	0.54	7.7	0.56	4	4	0.945
Md. Zahir Rayhan et al 2018 MSD	30	10	7.77	0.58	7.7	0.56	4	4	0.313
Md. Zahir Rayhan et al 2018 HSD	30	10	7.79	0.56	7.7	0.56	4	4	0.733
Salah Kanial 2006 MP	25	2.6	8.34	0.67082	8.45	0.67082	180	180	1.1
Salah Kanial 2006 LP	25	2.6	8.17	0.67082	8.45	0.67082	180	180	1.1
Sara Mello Pinho et al 2017	22	5	7.3	0.1	7.3	0.1	21	21	4
Sri Wahyuningsih et al 2015	30	3	7.11	0.24	7.28	0.21	6	6	1

7.4 Chapter 2 R code

#Multiple linear regression for each water parameter (Dredging)

install.packages("MuMIn")

library("MuMIn")

library(ggplot2)

#First global model

Ammonia <- read.csv(file.choose(), header = T)

names(Ammonia)

ols <- lm(formula = Ammonia~Feedtype+Feedquantity+SD, data = Ammonia)

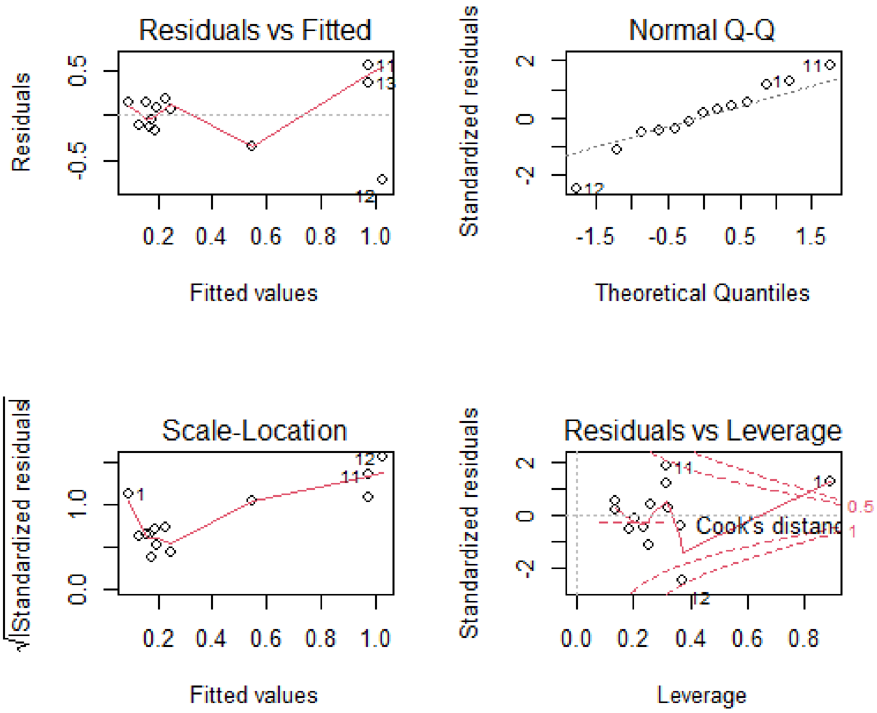
summary(ols)

```
Call:
lm(formula = Ammonia ~ Feedtype + Feedquantity + SD, data = Ammo
nia)

Residuals:
     Min       1Q   Median       3Q      Max 
-0.69946 -0.13496  0.06434  0.15044  0.54837 

Coefficients:
             Estimate Std. Error t value Pr(>|t|)   
(Intercept)   0.33952    0.68833   0.493  0.63365   
Feedtype     -0.01711    0.02687  -0.637  0.54006   
Feedquantity  0.11324    0.03420   3.311  0.00907 **
SD            0.01649    0.03402   0.485  0.63943   
---
Signif. codes:  
0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 0.3621 on 9 degrees of freedom
Multiple R-squared:  0.5669,	Adjusted R-squared:  0.4225 
F-statistic: 3.927 on 3 and 9 DF,  p-value: 0.04808
```

#Residual plots, 1st is linearity. 2nd is normality.

Par(mfrow = c(2,2)

plot(ols)



#Dredge to confirm variable and best model

options(na.action = "na.fail")

dredge(global.model = ols)

Model selection table

	(Intrc)	Fdqnt	Fdtyp	SD	df	logLik	AICc	delta	weight
2	-0.06149	0.1047			3	-3.152	15.0	0.00	0.767
4	0.20460	0.1067	-0.009673		4	-3.018	19.0	4.07	0.100
6	-0.07889	0.1060		0.004115	4	-3.137	19.3	4.30	0.089
1	0.39280				2	-8.289	21.8	6.81	0.025
8	0.33950	0.1132	-0.017110	0.016490	5	-2.850	24.3	9.30	0.007
5	0.45160			-0.020320	3	-8.108	24.9	9.91	0.005
3	0.36460		0.000993		3	-8.288	25.2	10.27	0.005
7	0.11710		0.012590	-0.028190	4	-8.028	29.1	14.09	0.001

Models ranked by AICc(x)

#Top model is best and holds most weight and lowest AIC so best fit. - Model with least predictors and lowest AIC is most significant

#Nitrite

Nitrite <- read.csv(file.choose(), header = T)

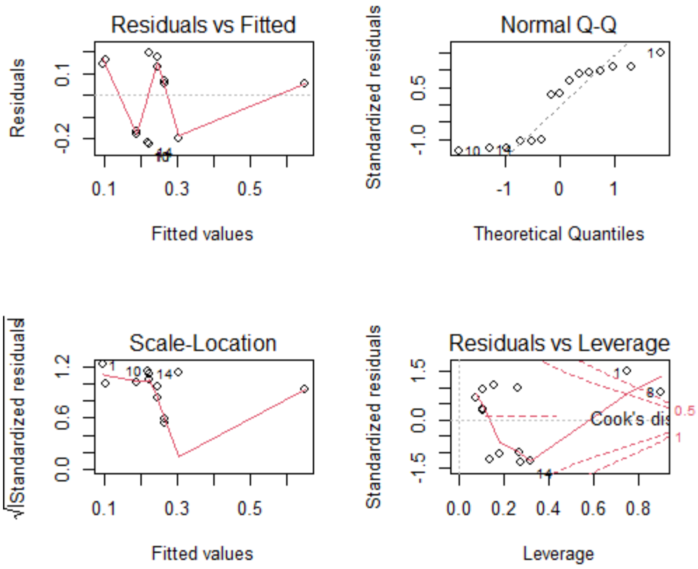
names(Nitrite)

ols1 <- lm(formula = Nitrite~Feedtype+Feedquantity+SD, data = Nitrite)

summary(ols1)

```
Call:
lm(formula = Nitrite ~ Feedtype + Feedquantity + SD, data = Nitrite)

Residuals:
     Min       1Q   Median       3Q      Max 
-0.22265 -0.18304  0.05387  0.15498  0.19588 

Coefficients:
               Estimate Std. Error t value Pr(>|t|)  
(Intercept)  -0.1283151  0.3153856  -0.407   0.6919  
Feedtype      0.0068306  0.0098417   0.694   0.5020  
Feedquantity  0.0566937  0.0264902   2.140   0.0556 .
SD           -0.0007445  0.0125439  -0.059   0.9537  
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 0.1945 on 11 degrees of freedom
Multiple R-squared:  0.3451,	Adjusted R-squared:  0.1664 
F-statistic: 1.932 on 3 and 11 DF,  p-value: 0.183
```

plot(ols1)



options(na.action = "na.fail")

dredge(global.model = ols1)

```
Model selection table
   (Intrc)   Fdqnt    Fdtyp          SD df logLik AICc delta weight
2  0.07811 0.05534                    3  5.245 -2.3  0.00  0.543
1  0.24190                            2  2.425  0.2  2.46  0.159
4 -0.13340 0.05605 0.006964           4  5.597  0.8  3.11  0.115
6  0.08173 0.05777          -0.0027350 4  5.278  1.4  3.75  0.083
5  0.20960                   0.0081960 3  2.671  2.8  5.15  0.041
3  0.05931         0.006074           3  2.607  3.0  5.28  0.039
8 -0.12830 0.05669 0.006831 -0.0007445 5  5.599  5.5  7.78  0.011
7 -0.04122         0.008065  0.0103100 4  2.988  6.0  8.33  0.008
Models ranked by AICc(x)
```

#Nitrate

Nitrate <- read.csv(file.choose(), header = T)

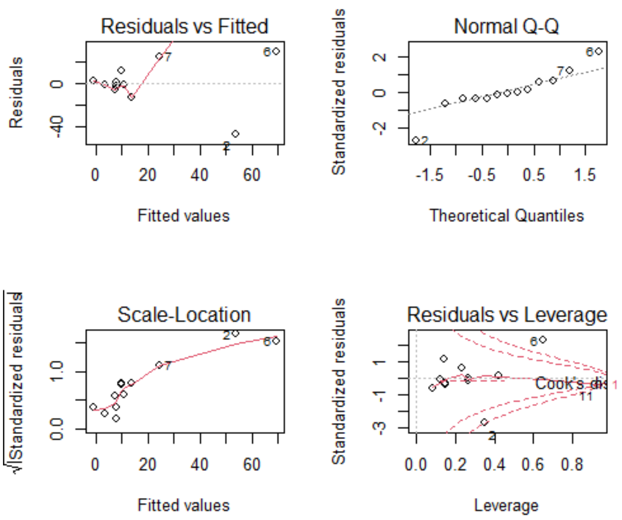
names(Nitrate)

ols2 <- lm(formula = Nitrate~Feedtype+Feedquantity+SD, data = Nitrate)

summary(ols2)

```
Call:
lm(formula = Nitrate ~ Feedtype + Feedquantity + SD, data = Nitrate)

Residuals:
    Min      1Q  Median      3Q     Max 
-46.447  -6.610  -1.459  11.211  28.804 

Coefficients:
             Estimate Std. Error t value Pr(>|t|)  
(Intercept)   24.4106    53.2933   0.458   0.6578  
Feedtype       0.2844     1.5419   0.184   0.8577  
Feedquantity -12.2688     4.7978  -2.557   0.0308 *
SD             7.4209     2.7324   2.716   0.0238 *
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 21.32 on 9 degrees of freedom
Multiple R-squared:  0.556,	Adjusted R-squared:  0.408 
F-statistic: 3.757 on 3 and 9 DF,  p-value: 0.05338
```

plot(ols2)



options(na.action = "na.fail")

dredge(global.model = ols2)

```
Model selection table
  (Intrc)   Fdqnt  Fdtyp    SD df  logLik  AICc delta weight
6   34.04 -12.720        7.655  4 -55.856 124.7  0.00  0.596
1   17.34                       2 -61.110 127.4  2.71  0.154
3  -59.69         2.4600        3 -59.878 128.4  3.71  0.093
5   10.84                2.088  3 -60.552 129.8  5.06  0.048
2   27.04  -3.047               3 -60.703 130.1  5.36  0.041
8   24.41 -12.270 0.2844 7.421  5 -55.832 130.2  5.52  0.038
7  -60.73         2.3140 1.805  4 -59.382 131.8  7.05  0.018
4  -46.75  -1.796 2.2300        4 -59.723 132.4  7.73  0.012
Models ranked by AICc(x)
```

#Phosphorus

Phosphorus <- read.csv(file.choose(), header = T)

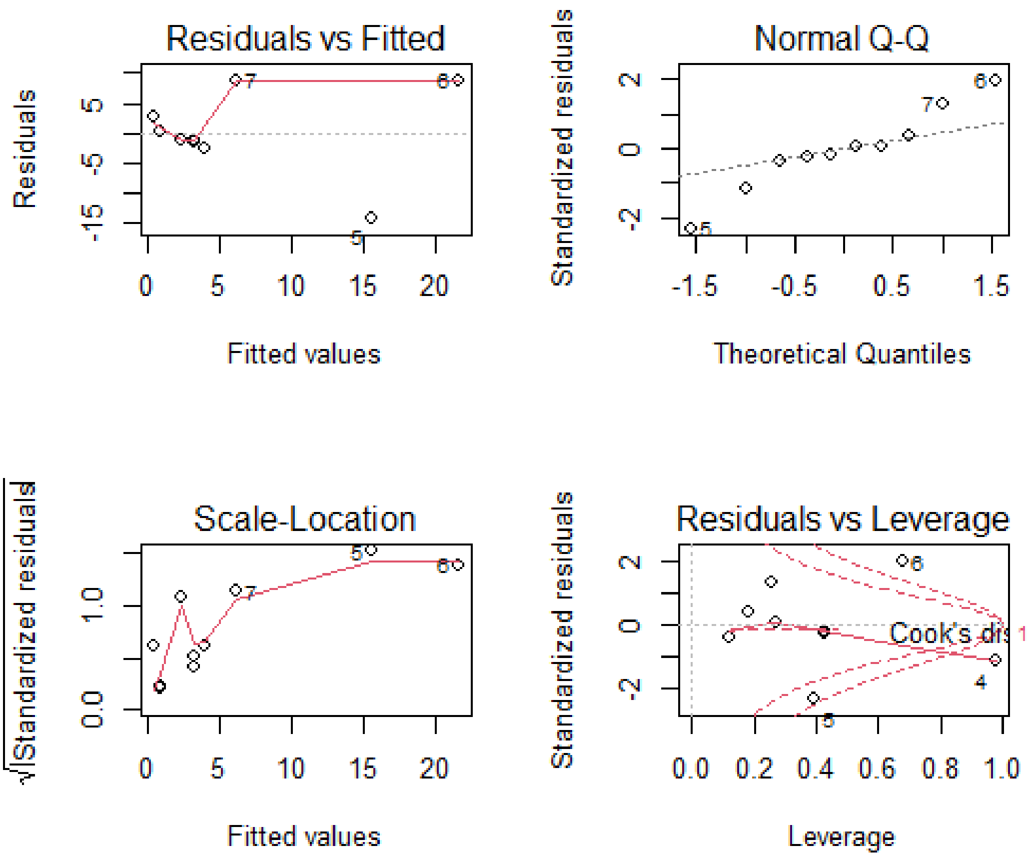
names(Phosphorus)

ols3 <- lm(formula = Phosphorous~Feedtype+Feedquantity+SD, data = Phosphorus)

summary(ols3)

```
Call:
lm(formula = Phosphorous ~ Feedtype + Feedquantity + SD, data = Phosph
orus)

Residuals:
     Min       1Q   Median       3Q      Max
-14.1147  -1.4558  -0.3605   2.0794   8.7185

Coefficients:
             Estimate Std. Error t value Pr(>|t|)
(Intercept)   16.1312    26.7901   0.602   0.5691
Feedtype      -0.1859     0.8182  -0.227   0.8278
Feedquantity  -4.2227     1.8939  -2.230   0.0673 .
SD             2.5507     1.1998   2.126   0.0776 .
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 7.853 on 6 degrees of freedom
Multiple R-squared:  0.5491,    Adjusted R-squared:  0.3237
F-statistic: 2.436 on 3 and 6 DF,  p-value: 0.1628
```

plot(ols3)



options(na.action = "na.fail")

dredge(global.model = ols3)

```
Model selection table
  (Intrc)   Fdqnt   Fdtyp     SD df  logLik AICc delta weight
1   5.879                         2 -36.227 78.2  0.00  0.513
6  10.120 -3.9880        2.3840  4 -32.288 80.6  2.41  0.154
3 -23.370          0.9285         3 -35.380 80.8  2.59  0.140
5   3.618                0.6213  3 -35.859 81.7  3.55  0.087
2   9.245 -1.0390                 3 -35.859 81.7  3.55  0.087
4 -18.860 -0.9056  0.8784         4 -35.052 86.1  7.94  0.010
7 -20.860          0.8087 0.3467  4 -35.262 86.5  8.36  0.008
8  16.130 -4.2230 -0.1859 2.5510  5 -32.245 89.5 11.32  0.002
Models ranked by AICc(x)
```

#DO

DO <- read.csv(file.choose(), header = T)

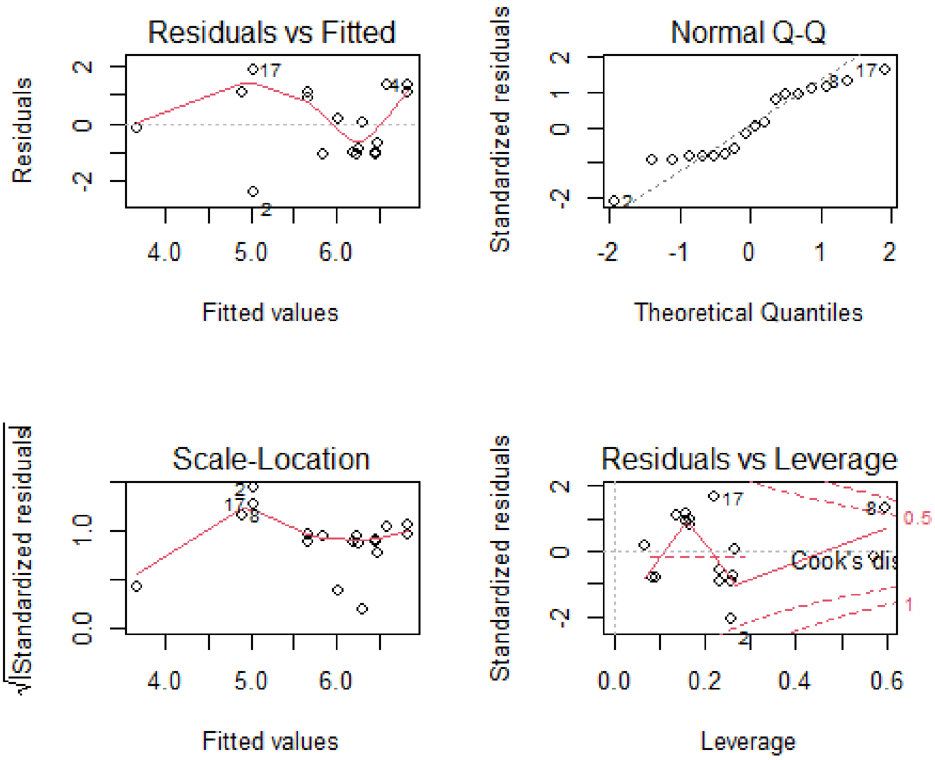
names(DO)

ols4 <- lm(formula = DO~Feedtype+Feedquantity+SD, data = DO)

summary(ols4)

```
Call:
lm(formula = DO ~ Feedtype + Feedquantity + SD, data = DO)

Residuals:
     Min       1Q   Median       3Q      Max
-2.33652 -0.99990 -0.05821  1.10816  1.88135

Coefficients:
              Estimate Std. Error t value Pr(>|t|)
(Intercept)   3.243262   2.107875   1.539    0.146
Feedtype      0.102078   0.065538   1.558    0.142
Feedquantity  0.003617   0.094647   0.038    0.970
SD           -0.122104   0.073048  -1.672    0.117

Residual standard error: 1.3 on 14 degrees of freedom
Multiple R-squared:  0.3217,    Adjusted R-squared:  0.1763
F-statistic: 2.213 on 3 and 14 DF,  p-value: 0.1319
```

plot(ols4)



options(na.action = "na.fail")

dredge(global.model = ols4)

```
Model selection table
  (Intrc)      Fdqnt  Fdtyp      SD df  logLik AICc delta weight
5   6.409                   -0.1465  3 -29.446 66.6  0.00  0.268
3   2.141            0.1254          3 -29.647 67.0  0.40  0.219
7   3.260            0.1020 -0.1221  4 -28.009 67.1  0.49  0.210
1   5.911                            2 -31.501 67.8  1.20  0.148
6   6.411 -0.0005708        -0.1465  4 -29.446 70.0  3.36  0.050
4   2.119  0.0046260 0.1255          4 -29.645 70.4  3.76  0.041
2   5.913 -0.0005116                 3 -31.501 70.7  4.11  0.034
8   3.243  0.0036170 0.1021 -0.1221  5 -28.008 71.0  4.41  0.030
Models ranked by AICc(x)
```

#pH

pH <- read.csv(file.choose(), header = T)

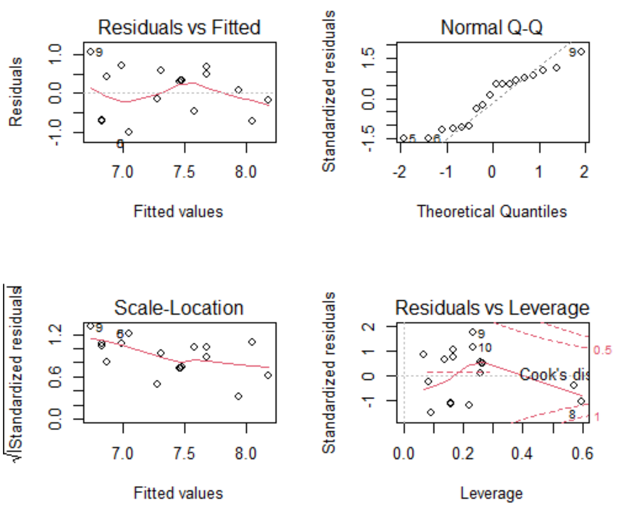
names(pH)

ols5 <- lm(formula = pH~Feedtype+Feedquantity+SD, data = pH)

summary(ols5)

```
Call:
lm(formula = pH ~ Feedtype + Feedquantity + SD, data = pH)

Residuals:
    Min      1Q  Median      3Q     Max 
-1.0014 -0.6453  0.1866  0.4835  1.0681 

Coefficients:
             Estimate Std. Error t value Pr(>|t|)    
(Intercept)   9.59544    1.15278   8.324  8.6e-07 ***
Feedtype     -0.08085    0.03584  -2.256   0.0406 *  
Feedquantity  0.02829    0.05176   0.546   0.5933    
SD            0.02091    0.03995   0.523   0.6089    
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 0.7112 on 14 degrees of freedom
Multiple R-squared:  0.3178,	Adjusted R-squared:  0.1716 
F-statistic: 2.174 on 3 and 14 DF,  p-value: 0.1366
```

plot(ols5)



options(na.action = "na.fail")

dredge(global.model = ols5)

```
Model selection table
  (Intrc)   Fdqnt    Fdtyp       SD df  logLik AICc delta weight
3   9.920          -0.08539          3 -17.503 42.7  0.00  0.549
4   9.788 0.02811 -0.08487          4 -17.319 45.7  2.99  0.123
7   9.730          -0.08141 0.02077  4 -17.335 45.7  3.03  0.121
1   7.353                            2 -20.587 46.0  3.25  0.108
5   7.217                   0.04019  3 -20.109 47.9  5.21  0.041
2   7.223 0.03159                    3 -20.422 48.6  5.84  0.030
8   9.595 0.02829 -0.08085 0.02091  5 -17.145 49.3  6.57  0.021
6   7.086 0.03160           0.04020  4 -19.935 50.9  8.23  0.009
Models ranked by AICc(x)
```

#Plotting graphs

plot(Ammonia$Ammonia~Ammonia$Feedquantity, xlab = "Feed quantity (% Biomass)", ylab = "Ammonia (mg/L)")

abline(lm(Ammonia$Ammonia~Ammonia$Feedquantity), col="red")

plot(Ammonia$Ammonia~Ammonia$Feedtype, xlab = "Feed type (% Crude protein)", ylab = "Ammonia (mg/L)")

abline(lm(Ammonia$Ammonia~Ammonia$Feedtype), col="red")

plot(Ammonia$Ammonia~Ammonia$SD, xlab = "Stocking density (kg/m^3)", ylab = "Ammonia (mg/L)")

abline(lm(Ammonia$Ammonia~Ammonia$SD), col="red")

plot(Nitrite$Nitrite~Nitrite$Feedquantity, xlab = "Feed quantity (% Biomass)", ylab = "Nitrite (mg/L)")

abline(lm(Nitrite$Nitrite~Nitrite$Feedquantity), col="red")

plot(Nitrite$Nitrite~Nitrite$Feedtype, xlab = "Feed type (% Crude protein)", ylab = "Nitrite (mg/L)")

abline(lm(Nitrite$Nitrite~Nitrite$Feedtype), col="red")

plot(Nitrite$Nitrite~Nitrite$SD, xlab = "Stocking density (kg/m^3)", ylab = "Nitrite (mg/L)")

abline(lm(Nitrite$Nitrite~Nitrite$SD), col="red")

```
plot(Nitrate$Nitrate~Nitrate$Feedquantity, xlab = "Feed quantity (% Biomass)", ylab = "Nitrate (mg/L)")

abline(lm(Nitrate$Nitrate~Nitrate$Feedquantity), col="red")

plot(Nitrate$Nitrate~Nitrate$Feedtype, xlab = "Feed type (% Crude protein)", ylab = "Nitrate (mg/L)")

abline(lm(Nitrate$Nitrate~Nitrate$Feedtype), col="red")

plot(Nitrate$Nitrate~Nitrate$SD, xlab = "Stocking density (kg/m^3)", ylab = "Nitrate (mg/L)")

abline(lm(Nitrate$Nitrate~Nitrate$SD), col="red")

plot(Phosphorus$Phosphorous~Phosphorus$Feedquantity, xlab = "Feed quantity (% Biomass)", ylab = "Phosphorus (mg/L)")

abline(lm(Phosphorus$Phosphorous~Phosphorus$Feedquantity), col="red")

plot(Phosphorus$Phosphorous~Phosphorus$Feedtype, xlab = "Feed type (% Crude protein)", ylab = "Phosphorus (mg/L)")

abline(lm(Phosphorus$Phosphorous~Phosphorus$Feedtype), col="red")

plot(Phosphorus$Phosphorous~Phosphorus$SD, xlab = "Stocking density (kg/m^3", ylab = "Phosphorus (mg/L)")

abline(lm(Phosphorus$Phosphorous~Phosphorus$SD), col="red")

plot(DO$DO~DO$Feedquantity, xlab = "Feed quantity (% Biomass)", ylab = "DO (mg/L)")

abline(lm(DO$DO~DO$Feedquantity), col="red")

plot(DO$DO~DO$Feedtype, xlab = "Feed Type (% Crude protein)", ylab = "DO (mg/L)")

abline(lm(DO$DO~DO$Feedtype), col="red")

plot(DO$DO~DO$SD, xlab = "Stocking density (kg/m^3)", ylab = "DO (mg/L)")

abline(lm(DO$DO~DO$SD), col="red")
```

```
plot(pH$pH~pH$Feedquantity, xlab = "Feed quantity (% Biomass)", ylab = "pH")

abline(lm(pH$pH~pH$Feedquantity), col="red")

plot(pH$pH~pH$Feedtype, xlab = "Feed type (% Crude protein)", ylab = "pH")

abline(lm(pH$pH~pH$Feedtype), col="red")

plot(pH$pH~pH$SD, xlab = "Stocking density (kg/m^3)", ylab = "pH")

abline(lm(pH$pH~pH$SD), col="red")
```

7.5 Chapter 3 raw data

#key. Diet – A = Experimental diet, C = control diet

Tank	Fish	Time	Diet	Latency (Reaction to feed) (Seconds)	Ejections	Eaten
7	1	AM	A	0	7	6
7	1	AM	C	0	0	7
8	2	AM	A	60	7	10
8	2	AM	C	0	0	10
9	3	AM	A	55	2	6
9	3	AM	C	0	0	10
10	4	AM	A	150	4	7
10	4	AM	C	160	0	10
7	5	PM	A	0	0	10
7	5	PM	C	0	0	10
8	6	PM	A	0	0	10
8	6	PM	C	0	0	10
9	7	PM	A	0	9	6
9	7	PM	C	0	0	10
10	8	PM	A	60	0	10
10	8	PM	C	200	2	10
7	9	AM	A	0	3	0
7	9	AM	C	30	0	10
8	10	AM	A	0	0	10
8	10	AM	C	0	0	10
9	11	AM	A	2	0	10
9	11	AM	C	28	0	10
10	12	AM	A	26	0	10
10	12	AM	C	30	1	10
7	13	PM	A	0	2	10
7	13	PM	C	4	0	10
8	14	PM	A	0	0	10
8	14	PM	C	0	0	10
9	15	PM	A	0	0	10
9	15	PM	C	0	0	10
10	16	PM	A	54	0	9
10	16	PM	C	28	0	6
7	17	AM	A	0	0	10
7	17	AM	C	5	0	10
8	18	AM	A	3	0	10
8	18	AM	C	0	0	10
9	19	AM	A	0	0	10
9	19	AM	C	0	0	10
10	20	AM	A	3	0	10
10	20	AM	C	3	0	8
7	21	PM	A	0	0	10
7	21	PM	C	0	0	10

8	22	PM	A	0	0	10
8	22	PM	C	0	0	10
9	23	PM	A	0	0	10
9	23	PM	C	0	0	9
10	24	PM	A	110	0	10
10	24	PM	C	25	0	10
7	25	AM	A	300	0	0
7	25	AM	C	0	0	10
8	26	AM	A	0	0	10
8	26	AM	C	25	1	9
9	27	AM	A	0	0	10
9	27	AM	C	3	0	10
10	28	AM	A	1	0	10
10	28	AM	C	5	0	9
7	29	PM	A	3	0	10
7	29	PM	C	4	0	10
8	30	PM	A	0	0	10
8	30	PM	C	5	0	10
9	31	PM	A	0	0	10
9	31	PM	C	0	0	10
10	32	PM	A	1	0	10
10	32	PM	C	5	0	8
7	33	AM	A	0	0	10
7	33	AM	C	0	0	10
8	34	AM	A	0	0	10
8	34	AM	C	0	0	10
9	35	AM	A	0	1	10
9	35	AM	C	0	0	10
10	36	AM	A	2	0	10
10	36	AM	C	4	0	10
7	37	PM	A	0	0	10
7	37	PM	C	0	0	10
8	38	PM	A	0	0	10
8	38	PM	C	0	0	10
9	39	PM	A	0	0	10
9	39	PM	C	0	0	10
10	40	PM	A	1	0	10
10	40	PM	C	2	0	10
7	41	AM	A	180	0	10
7	41	AM	C	6	0	10
8	42	AM	A	0	0	10
8	42	AM	C	3	1	10
9	43	AM	A	0	0	10
9	43	AM	C	0	0	10
10	44	AM	A	0	0	10
10	44	AM	C	20	0	10
7	45	PM	A	5	0	10
7	45	PM	C	0	0	9

8	46	PM	A	0	0	10
8	46	PM	C	0	0	10
9	47	PM	A	0	0	10
9	47	PM	C	0	0	10
10	48	PM	A	2	0	10
10	48	PM	C	5	0	10

7.6 Chapter 3 R code

```
#GLM for supplementation experiment

Feed <- read.csv(file.choose(), header = T)

summary(Feed)

View(Feed)

class(Feed$Diet)


install.packages("ggplot2")

install.packages("tidyverse")

install.packages("ggpubr")

install.packages("dplyr")

install.packages("ggridges")

install.packages("pscl")

install.packages("MuMIn")


library("MuMIn")

library(pscl)

library(ggplot2)

library(ggridges)

library(tidyverse)

library(ggpubr)

library(dplyr)

theme_set(theme_ridges())
```

```
ggplot(Feed, aes(x=Ejections, y=Time, color=Time)) + geom_violin()+
stat_summary(fun.y=mean, geom="point", size=2, color="red")+ geom_boxplot(width=0.1)
+ geom_violin(trim=FALSE)


ggplot(Feed, aes(x=Ejections, y=Diet, color=Diet)) + geom_violin()+
stat_summary(fun.y=mean, geom="point", size=2, color="red")+ geom_boxplot(width=0.1)
+ geom_violin(trim=FALSE)


ggplot(Feed, aes(x=Eaten, y=Time, color=Time)) +  geom_violin()+
stat_summary(fun.y=mean, geom="point", size=2, color="red")+ geom_boxplot(width=0.1)
+ geom_violin(trim=FALSE)


ggplot(Feed, aes(x=Eaten, y=Diet, color=Diet)) + geom_violin()+
stat_summary(fun.y=mean, geom="point", size=2, color="red")+ geom_boxplot(width=0.1)
+ geom_violin(trim=FALSE)

#Normaility

hist(Feed$Ejections)

mean(Feed$Ejections)

var(Feed$Ejections)



#overdispersion

hist(Feed$Eaten)

mean(Feed$Eaten)

var(Feed$Eaten)

#under dispersion

#Variaince does not equal the mean so use quasi-poisson so dispersion can be different
```

R0 <- glm(Eaten~Diet + Time + ï..Tank-1, family = quasipoisson, data = Feed)

summary(R0)

```
Call:
glm(formula = Eaten ~ Diet + Time + ï..Tank - 1, family = quasipoisso
n,
    data = Feed)

Deviance Residuals:
    Min       1Q   Median       3Q      Max
-4.1898   0.0279   0.1346   0.2403   0.4037

Coefficients:
            Estimate Std. Error t value Pr(>|t|)
DietAlgae    2.05817    0.14383  14.310   <2e-16 ***
DietControl  2.10438    0.14372  14.642   <2e-16 ***
TimePM       0.05502    0.03679   1.496    0.138
ï..Tank      0.01628    0.01645   0.990    0.325
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

(Dispersion parameter for quasipoisson family taken to be 0.3072897)
```

coef(R0)

exp(coef(R0))

options(na.action = "na.fail")

dredge(global.model = R0)

```
---
Model selection table
  Dit  ï..Tnk Tim df
1                  0
2   +              2
3     0.25570      1
4   + 0.01628      3
5               +  2
6   +           +  3
7     0.01628   +  3
8   + 0.01628   +  4
Models ranked by AICc(x)
```

R1 <- glm(Ejections~Diet +Time + ï..Tank-1, family = quasipoisson, data = Feed)

summary(R1)

```
Call:
glm(formula = Ejections ~ Diet + Time + ï..Tank - 1, family = quasipoi
sson,
    data = Feed)

Deviance Residuals:
    Min       1Q   Median       3Q      Max
-1.5289  -0.9989  -0.5441  -0.3775   6.0927

Coefficients:
            Estimate Std. Error t value Pr(>|t|)
DietAlgae     0.9994     2.4031   0.416    0.678
DietControl  -0.9465     2.5410  -0.373    0.710
TimePM       -0.7309     0.6732  -1.086    0.280
ï..Tank      -0.1205     0.2837  -0.425    0.672

(Dispersion parameter for quasipoisson family taken to be 3.976322)
```

coef(R1)

exp(coef(R1))

R1Zero <- zeroinfl(Ejections~Diet + Time + ï..Tank-1, data = Feed)

summary(R1Zero)

```
Call:
zeroinfl(formula = Ejections ~ Diet + Time + ï..Tank - 1,
    data = Feed)

Pearson residuals:
    Min      1Q  Median      3Q     Max
-0.5229 -0.3344 -0.2658 -0.2077  5.7784

Count model coefficients (poisson with log link):
            Estimate Std. Error z value Pr(>|z|)
DietAlgae    0.82311    1.33185   0.618    0.537
DietControl -1.43067    1.75390  -0.816    0.415
TimePM       0.41809    0.35660   1.172    0.241
ï..Tank      0.06236    0.15703   0.397    0.691

Zero-inflation model coefficients (binomial with logit link):
            Estimate Std. Error z value Pr(>|z|)
DietAlgae    0.66778    2.71987   0.246    0.806
DietControl  0.27725    3.09609   0.090    0.929
TimePM       1.46699    0.76140   1.927    0.054 .
ï..Tank      0.04265    0.31572   0.135    0.893
```

coef(R1Zero)

exp(coef(R1Zero))

options(na.action = "na.fail")

dredge(global.model = R1)

```
---
Model selection table
  Dit  ï..Tnk Tim df
1                  0
2   +              2
3     -0.1041      1
4   + -0.1205      3
5               +  2
6   +           +  3
7     -0.1205   +  3
8   + -0.1205   +  4
Models ranked by AICc(x)
```

AIC(R1, R1Zero)

```
       df      AIC
R1      4       NA
R1Zero  8 125.5086
```

#Scheirer-Ray-Hare test

Palatability <- read.csv(file.choose(), header = T)

View(Palatability)

names(Palatability)

No_of_eaten = c(0,0,6,6,7,10,10,10,10,10,10,10,10,10,10,10,10,10,10,10,10,10,10,7,8,9,9,10,10,10,10,10,10,10,10,10,10,10,10,10,10,10,10,10,10,10,10,6,9,10,10,10,10,10,10,10,10,10,10,10,10,10,10,10,10,10,10,10,10,10,10,6,8,9,9,10,10,10,10,10,10,10,10,10,10,10,10,10,10,10,10,10,10,10)

No_of_Ej = c(3,0,7,2,4,7,0,0,0,0,0,0,0,0,0,0,0,0,1,0,0,0,0,0,0,1,0,0,0,0,0,0,1,0,0,0,0,0,0,0,0,0,1,0,0,9,0,0,0,0,2,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,2,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0)

Diet = rep(c("Algae", "Control", "Algae", "Control"), each = 24)

Time = rep(c("AM", "PM"), each = 48)

#Variables to rank the data

Rank_eaten = rank(No_of_eaten)

Rank_Diet = rank(Diet)

Rank_Time = rank(Time)

Rank_EJ =rank(No_of_Ej)

#running analysis of variance

aov.eaten = aov(Rank_eaten ~ Rank_Diet * Rank_Time)

summary(aov.eaten)

```
                  Df Sum Sq Mean Sq F value Pr(>F)
Rank_Diet          1      1    1.04   0.003  0.954
Rank_Time          1    277  276.76   0.883  0.350
Rank_Diet:Rank_Time  1    298  297.51   0.949  0.333
Residuals         92  28848  313.57
```

aov.Ej = aov(Rank_EJ ~ Rank_Diet * Rank_Time)

summary(aov.Ej)

```
                  Df Sum Sq Mean Sq F value Pr(>F)
Rank_Diet          1    491   490.5   1.971 0.1638
Rank_Time          1    811   810.8   3.258 0.0744 .
Rank_Diet:Rank_Time  1    126   126.0   0.506 0.4785
Residuals         92  22900   248.9
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

#Extracting DF and sum of squares

DFEat = anova(aov.eaten)[, "Df"]

Sum_DfEat = sum(DFEat)

SSEat = anova(aov.eaten)[, "Sum Sq"]

Sum_SSEat = sum(SSEat)

DFEj = anova(aov.Ej)[, "Df"]

Sum_DfEj = sum(DFEj)

SSEj = anova(aov.Ej)[, "Sum Sq"]

Sum_SSEj = sum(SSEj)

#Calculating MS

MSEat = Sum_SSEat/Sum_DfEat

MSEj = Sum_SSEj/Sum_DfEj

#H value

H_Diet = SSEat[1]/MSEat

H_Time = SSEat[2]/MSEat

H_Interaction = SSEat[3]/MSEat

```
H_DietEJ = SSEj[1]/MSEj

H_TimeEJ = SSEj[2]/MSEj

H_InteractionEJ = SSEj[3]/MSEj

#Converting H value into P values

1-pchisq(H_Diet, DFEat[1])

1-pchisq(H_Time, DFEat[2])

1-pchisq(H_Interaction, DFEat[3])

1-pchisq(H_DietEJ, DFEj[1])

1-pchisq(H_TimeEJ, DFEj[2])

1-pchisq(H_InteractionEJ, DFEj[3])

# Time to event analysis - survival analysis

install.packages("survival")

install.packages("survminer")

install.packages("ggpubr")

install.packages("broom")

library(ggpubr)

library(survival)

library(survminer)

library(dplyr)

library(broom)

EatSur <- read.csv(file.choose(), header = T)

names(EatSur)
```

```
#define variables

Time <- EatSur$Reaction.to.feed

Event <- EatSur$Eaten

X <- cbind(EatSur$ï..Diet, EatSur$Time)

Group <- EatSur$ï..Diet

summary(Time)

summary(Event)

summary(X)

summary(Group)

kmsurvival <- survfit(Surv(Time,Event) ~ 1)

summary(kmsurvival)

plot(kmsurvival, xlab = "Time (Seconds)", ylab = "Survival probability")

Kmsurvival1 <- survfit(Surv(Time, Event) ~ Group)

summary(Kmsurvival1)
```

```
Call: survfit(formula = Surv(Time, Event) ~ Group)

                Group=Algae
 time n.risk n.event survival std.err lower 95% CI upper 95% CI
    0     48      29   0.3958  0.0706       0.2791        0.561
    1     19       3   0.3333  0.0680       0.2234        0.497
    2     16       3   0.2708  0.0641       0.1703        0.431
    3     13       4   0.1875  0.0563       0.1041        0.338
    5      9       1   0.1667  0.0538       0.0885        0.314
   26      8       1   0.1458  0.0509       0.0735        0.289
   54      7       1   0.1250  0.0477       0.0591        0.264
   55      6       1   0.1042  0.0441       0.0454        0.239
   60      5       2   0.0625  0.0349       0.0209        0.187
  110      3       1   0.0417  0.0288       0.0107        0.162
  150      2       1   0.0208  0.0206       0.0030        0.145


                Group=Control
 time n.risk n.event survival std.err lower 95% CI upper 95% CI
    0     48      26   0.4583  0.0719       0.3370        0.623
    2     22       1   0.4375  0.0716       0.3174        0.603
    3     21       3   0.3750  0.0699       0.2603        0.540
    4     18       3   0.3125  0.0669       0.2054        0.475
    5     15       5   0.2083  0.0586       0.1200        0.362
    6     10       1   0.1875  0.0563       0.1041        0.338
   20      9       1   0.1667  0.0538       0.0885        0.314
   25      8       2   0.1250  0.0477       0.0591        0.264
   28      6       2   0.0833  0.0399       0.0326        0.213
   30      4       2   0.0417  0.0288       0.0107        0.162
  160      2       1   0.0208  0.0206       0.0030        0.145
  200      1       1   0.0000     NaN           NA           NA
```

```
ggsurvplot(Kmsurvival1, data = EatSur, risk.table = TRUE, xlab = "Time (Seconds)", ylab = "Eating probability" )
```

7.7 Chapter 4 Survey questions.

Q1 *

How old are you

- <18
- 18 - 24
- 25 - 34
- 35 - 44
- 45 - 54
- 55 - 64
- 65+

Q2 *

Where are you from?

Q3 *

What is your highest level level of education?

- GCSE
- A Level
- BTEC/Diploma
- Undergraduate degree
- Postgraduate degree

Q4

Have you Heard of the term aquaponics before?

- Yes
- No

Q5

If you have heard of aquaponics, please briefly describe what you know.

Q6-7

Your aquaponic beliefs

		Strongly agree	Agree	Somewhat agree	Neither agree nor disagree	Somewhat disagree	Disagree	Strongly disagree
Q6	Aquaponically grown crops and fish have the high nutritional value	☐	☐	☐	☐	☐	☐	☐
Q7	Aquaponics is a safe and clean method of farming	☐	☐	☐	☐	☐	☐	☐

Aquaponics is the combination of fish farming and hydroponics (Crop farming) in a single system to farm crops and fish together in an intensive method. The nutrient rich waste from the fish system is converted by nirtifying bacteria to nitrates and acts as a fertilizer to promote plant growth. After the plants filter the water, it is then returned to the fish system in a recirulating closed-loop. Please answer the following on your opinions of aquaponics.

Q8

Would you prefer to buy aquaponically grown fish/crops or wild caught/traditionally grown produce?

- ○ Aquaponics
- ○ Wild/Traditional

Q9

Would you be willing to pay organic prices for aquaponically grown crops?

- ○ Yes
- ○ No

Q10

What, if any are your reservations/worries with aquaponically grown produce?

Q11

Aquaponics has three main disadvantages;

1.Initial investment cost.

2. Negative public perceptions.

3.Energy use

However, key advantages of aquaponics are;

1. Significant reduction of water! It uses 1/10th of the water used in traditional farming.

2. Reduces chemical/pesticide use (None)

3. Can be used in urban cities and areas, reducing food insecurity and carbon footprint by having local produce

4. Land use is approximately 20% of traditional farming.

5.Food can be grown year round at up to 10 times the speed of normal farming.

Knowing these key advantages and disadvantages of aquaponics, how likely are you to look for and buy aquaponically grown produce?

- ○ Extremely likely
- ○ Moderately likely
- ○ Slightly likely
- ○ Neither likely nor unlikely
- ○ Slightly unlikely
- ○ Moderately unlikely
- ○ Extremely unlikely

Q12 - 14

		Strongly agree	Agree	Somewhat agree	Neither agree nor disagree	Somewhat disagree	Disagree	Strongly disagree
Q12	Would you be willing to pay toward and live in an urban building with a communitiy aquaponics farm	☐	☐	☐	☐	☐	☐	☐
Q13	Aquaponics is a sustainable and environmentally conscious method of farming	☐	☐	☐	☐	☐	☐	☐
Q14	Completing this survey has changed my opinions/feelings towards aquaponics	☐	☐	☐	☐	☐	☐	☐

7.8 Chapter 4 raw data

#key Y = Yes, N = No, N/A = not applicable, 1 = Strongly disagree – 7 = strongly agree.

Each of the responses is results taken from the survey questions in appendices 7.7.

#	Age	Location	Education	Heard_Of	Competent	High_Nutrition	Safe_Clean	Aquaponics_Wild	Organic	Reservations	Likelihood	Community	Sustainable	Changed
1	18 - 24	UK	Postgrad	Y	Y	N/A	N/A	Wild	N	Nutritional value	5	N/A	N/A	N/A
2	18 - 24	UK	Undergrad	Y	Y	N/A	N/A	Wild	Y	Price	7	N/A	N/A	N/A
3	18 - 24	UK	Undergrad	Y	Y	N/A	N/A	Wild	Y	Job security	6	N/A	N/A	N/A
4	18 - 24	UK	Undergrad	N	N/A	N/A	N/A	Aquaponics	Y	Environmental impact	6	N/A	N/A	N/A
5	65+	UK	Postgrad	Y	N	N/A	N/A	Wild	N	Disease	5	N/A	N/A	N/A
6	18 - 24	UK	Undergrad	Y	N	N/A	N/A	Wild	N	Price	6	N/A	N/A	N/A
7	55 - 64	UK	GCSE	Y	N	N/A	N/A	Aquaponics	Y	Disease	6	N/A	N/A	N/A
8	18 - 24	UK	Postgrad	Y	N	N/A	N/A	Aquaponics	N	None	7	N/A	N/A	N/A
9	18 - 24	UK	Postgrad	N	N/A	N/A	N/A	Aquaponics	Y	None	6	N/A	N/A	N/A
10	18 - 24	UK	Undergrad	N	N/A	N/A	N/A	Aquaponics	Y	Disease	7	N/A	N/A	N/A
10										Environmental impact				
10										Welfare				
11	25 - 34	UK	Postgrad	Y	Y	6	6	Aquaponics	N	Price	2	5	6	5
11										Environmental impact				
12	55 - 64	UK	GCSE	Y	N	5	4	Wild	N	Price	3	1	5	2
12										Nutritional value				
13	18 - 24	UK	Postgrad	N	N/A	6	6	Wild	Y	None	6	4	6	6
14	18 - 24	Netherlands	Undergrad	Y	Y	7	5	Aquaponics	Y	Welfare	7	7	6	1
15	25 - 34	UK	Undergrad	Y	N	6	7	Aquaponics	Y	Disease	6	2	6	6
16	25 - 34	UK	BTEC/Diploma	Y	N	6	4	Wild	N	Price	4	4	5	4
18	18 - 24	UK	Undergrad	N	N/A	6	6	Aquaponics	N	Disease	6	5	6	5
19	18 - 24	UK	Postgrad	N	N/A	6	6	Aquaponics	Y	None	7	6	6	6
20	18 - 24	UK	Postgrad	Y	Y	6	6	Aquaponics	Y	None	6	7	6	5
21	18 - 24	UK	Postgrad	N	N/A	6	6	Aquaponics	Y	Price	3	6	7	5
22	18 - 24	UK	Undergrad	Y	N	6	7	Aquaponics	Y	Job security	7	7	7	5
23	25 - 34	UK	Undergrad	N	N/A	7	7	Aquaponics	N	Price	7	7	7	7
24	18 - 24	UK	Undergrad	N	N/A	6	5	Aquaponics	Y	Disease	7	6	7	7
25	18 - 24	UK	Postgrad	N	N/A	6	5	Wild	Y	Environmental impact	6	5	5	5

25										Welfare				
26	18 - 24	Hong Kong	Postgrad	N	N/A	5	4	Aquaponics	Y	Unkown impacts	6	6	6	6
27	18 - 24	UK	Postgrad	Y	Y	4	6	Aquaponics	Y	Unkown impacts	7	7	6	4
28	18 - 24	UK	A Level	N	N/A	5	6	Aquaponics	Y	None	6	6	6	6
29	18 - 24	UK	Undergrad	Y	N	7	6	Aquaponics	Y	None	7	5	7	5
30	18 - 24	UK	Postgrad	Y	N	4	5	Wild	N	Welfare	6	5	4	4
31	25 - 34	France	Undergrad	Y	N	4	4	Aquaponics	Y	Welfare	5	5	5	5
31		France								Environmental impact				
32	18 - 24	UK	Undergrad	N	N/A	4	5	Aquaponics	N	None	6	6	6	6
33	35 - 44	UK	Undergrad	Y	N	6	6	Aquaponics	Y	Energy use	6	6	6	5
34	18 - 24	UK	Postgrad	N	N/A	4	5	Aquaponics	Y	None	7	4	6	5
35	18 - 24	UK	Undergrad	N	N/A	6	6	Aquaponics	Y	None	7	7	7	7
36	25 - 34	Netherlands	BTEC/Diploma	N	N/A	6	4	Wild	N	None	6	1	6	4
37	25 - 34	UK	BTEC/Diploma	N	N/A	6	5	Wild	Y	Lack of understanding	6	4	5	4
38	35 - 44	UK	BTEC/Diploma	N	N/A	4	4	Wild	N	None	5	5	4	4
39	45 - 54	UK	Postgrad	Y	N	5	4	Aquaponics	Y	Environmental impact	7	6	5	5
40	45 - 54	UK		Y	N	4	5	Wild	Y	Lack of understanding	6	4	5	6
41	18 - 24	UK	Postgrad	Y	N	5	4	Wild	N	Price	5	2	5	7
42	55 - 64	UK	Postgrad	Y	N	6	6	Aquaponics	Y	None	6	1	5	5
43	18 - 24	UK	Undergrad	N	N/A	7	7	Aquaponics	Y	Price	5	2	7	2
44	25 - 34	UK	GCSE	N	N/A	7	6	Aquaponics	Y	Price	6	6	6	N/A
45	18 - 24	UK	Postgrad	N	N/A	4	4	Wild	N	None	6	4	5	5
46	45 - 54	UK	GCSE	Y	N	4	5	Wild	N	Lack of understanding	4	1	4	4
47	25 - 34	UK	BTEC/Diploma	N	N/A	5	5	Wild	N	Lack of understanding	6	4	5	4
48	45 - 54	UK	Undergrad	N	N/A	4	4	Wild	N	None	4	4	4	4
49	65+	UK	GCSE	Y	Y	4	5	Wild	N	None	5	6	5	4
50	18 - 24	UK	Undergrad	N	N/A	6	6	Aquaponics	Y	None	6	4	6	4
51	18 - 24	UK	Undergrad	N	N/A	6	6	Wild	N	None	6	5	6	6
52	35 - 44	UK	GCSE	N	N/A	1	1	Wild	N	Welfare	1	1	1	1
53	18 - 24	UK	BTEC/Diploma	N	N/A	4	4	Wild	Y	Disease	5	7	5	7
54	35 - 44	UK	Undergrad	Y	N	5	2	Wild	N	Unkown impacts	5	4	4	4
55	25 - 34	Austria	Undergrad	N	N/A	6	6	Aquaponics	Y	Welfare	5	5	6	4
56	25 - 34	Malaysia	Postgrad	N	N/A	6	7	Wild	N	Price	5	5	6	5

56		Malaysia								Nutritional value				
57	35 - 44	France	Undergrad	N	N/A	7	7	Aquaponics	Y	Welfare	5	2	4	4
58	18 - 24	Netherlands	Undergrad	N	N/A	5	3	Aquaponics	Y	Lack of understanding	2	2	4	5
59	35 - 44	USA	BTEC/Diploma	Y	N	7	7	Wild	N	Price	4	5	5	5
60	18 -24	USA	Undergrad	N	N/A	7	5	Wild	Y	Lack of understanding	5	5	5	5
61	45 - 54	UK	Postgrad	N	N/A	4	4	Wild	N	Lack of understanding	4	4	4	4
62	18 - 24	Italy	A Level	N	N/A	6	6	Aquaponics	Y	None	6	5	6	6
63	18 - 24	India	Postgrad	Y	N	6	6	Aquaponics	N	None	6	6	6	6
64	< 18	UK	GCSE	N	N/A	6	6	Wild	N	None	3	6	7	4
65	25 - 34	Turkey	BTEC/Diploma	N	N/A	4	5	Wild	N	Disease	5	4	6	5
66	25 - 34	Poland	Undergrad	N	N/A	5	6	Aquaponics	Y	Nutritional value	6	6	7	6
67	18 - 24	UK	Undergrad	N	N/A	5	4	Aquaponics	Y	Disease	4	3	6	6
68	18 - 24	UK	A Level	N	N/A	5	6	Aquaponics	Y	None	6	6	6	6
69	25 - 34	USA	Postgrad	N	N/A	4	4	Wild	N	Lack of understanding	4	6	4	4
70	45 - 54	UK	GCSE	N	N/A	7	7	Aquaponics	Y	None	7	6	6	6
71	25 - 34	India	Postgrad	N	N/A	6	6	Aquaponics	N	None	6	6	6	6
72	18 - 24	UK	Postgrad	N	N/A	6	6	Wild	Y	Welfare	4	3	6	4
73	25 - 34	UK	Postgrad	N	N/A	6	6	Aquaponics	Y	None	6	6	6	4
74	35 - 44	France	Postgrad	Y	N	6	6	Aquaponics	Y	Energy use	7	6	7	7
75	65++C215BB2:B236	UK	GCSE	N	N/A	6	6	Wild	Y	None	7	4	6	6
76	25 - 34	Singapore	Undergrad	Y	Y	6	6	Wild	N	Nutritional value	6	6	6	6
77	18 - 24	UK	Undergrad	N	N/A	4	5	Aquaponics	Y	None	5	4	6	5
78	35 - 44	UK	Postgrad	N	N/A	6	6	Wild	N	None	6	3	4	5
79	18 - 24	UK	Undergrad	N	N/A	5	5	Wild	Y	Lack of understanding	5	2	5	5
80	18 - 24	Netherlands	Postgrad	N	N/A	6	7	Aquaponics	N	None	6	5	6	6
81	25 - 34	UK	A Level	N	N/A	6	2	Wild	N	Disease	6	6	6	6
82	18 - 24	Finland	Undergrad	N	N/A	7	7	Wild	Y	Environmental impact	5	3	6	6
83	18 - 24	USA	A Level	Y	Y	4	6	Aquaponics	N	None	6	5	7	4
84	18 - 24	USA	Undergrad	N	N/A	5	4	Wild	N	Artificial	5	2	4	5
85	18 - 24	USA	Undergrad	Y	N	4	4	Wild	N	Lack of understanding	4	4	4	4
86	25 - 34	Germany	Undergrad	N	N/A	6	3	Wild	N	None	1	2	4	2

87	25 - 34	Denmark	Postgrad	N	N/A	2	5	Wild	N	Environmental impact	6	6	6	5
88	45 - 54	Portugal	Postgrad	N	N/A	4	4	Aquaponics	N	None	5	4	5	5
89	25 - 34	UK	Undergrad	N	N/A	5	5	Aquaponics	Y	Price	6	5	5	5
90	18 - 24	UK	Postgrad	N	N/A	4	2	Wild	N	Environmental impact	5	5	4	5
91	25 - 34	Netherlands	Postgrad	N	N/A	6	4	Aquaponics	N	Disease	6	5	6	7
92	18 - 24	UK	A Level	N	N/A	4	4	Aquaponics	Y	None	5	4	4	3
93	18 - 24	UK	A Level	N	N/A	5	5	Aquaponics	N	None	5	4	5	4
94	55 - 64	Italy	Undergrad	N	N/A	2	4	Wild	Y	Artificial	5	1	5	4
95	45 - 54	UK	Postgrad	N	N/A	4	5	Aquaponics	Y	Lack of understanding	6	5	5	5
96	< 18	UK	A Level	N	N/A	6	5	Wild	Y	None	7	4	7	7
97	18 - 24	USA	A Level	N	N/A	4	4	Wild	N	None	5	4	5	5
98	18 - 24	Netherlands	BTEC/Diploma	N	N/A	3	3	Wild	N	Lack of understanding	5	5	5	5
99	18 - 24	UK	A Level	N	N/A	5	4	Wild	N	None	3	3	5	4
100	18 - 24	UK	Undergrad	N	N/A	4	4	Wild	N	Price	4	4	4	4
100										Nutritional value				
101	18 - 24	UK	A Level	N	N/A	2	5	Wild	N	None	6	3	5	6
102	18 - 24	UK	Undergrad	N	N/A	6	5	Aquaponics	N	Price	6	4	7	7
102										Disease				
103	18 - 24	Germany	A Level	N	N/A	7	7	Aquaponics	Y	None	5	5	6	7
104	25 - 34	UK	Undergrad	Y	Y	4	7	Wild	Y	Regulation	6	6	7	4
105	18 - 24	USA	GCSE	N	N/A	5	6	Aquaponics	Y	None	6	5	7	6
106	18 - 24	UK	Undergrad	N	N/A	7	4	Aquaponics	Y	Welfare	1	2	7	6
107	25 - 34	Norway	Undergrad	N	N/A	4	4	Wild	N	None	6	4	4	4
108	18 - 24	UK	Postgrad	Y	N	5	6	Aquaponics	Y	None	7	6	6	6
109	18 - 24	Singapore	A Level	N	N/A	5	5	Wild	N	None	6	6	6	6
110	25 - 34	UK	Postgrad	Y	Y	6	7	Aquaponics	Y	Distance to shops	7	7	7	6
111	18 - 24	UK	Undergrad	N	N/A	4	4	Aquaponics	Y	None	6	5	6	6
112	35 - 44	UK	Undergrad	N	N/A	6	6	Aquaponics	Y	None	7	1	7	5
113	18 - 24	USA	GCSE	Y	N	3	6	Wild	N	Price	4	3	7	6
114	< 18	USA	GCSE	N	N/A	5	5	Wild	N	None	5	3	6	6
115	25 - 34	UK	Undergrad	N	N/A	5	6	Aquaponics	Y	None	6	5	5	6
116	18 - 24	USA	Undergrad	N	N/A	4	5	Aquaponics	Y	None	6	6	6	6
117	18 - 24	UK	Undergrad	N	N/A	6	7	Aquaponics	Y	Disease	7	6	7	7
118	25 - 34	Romania	Postgrad	N	N/A	6	6	Wild	N	None	6	6	7	6

119	18 - 24	Poland	Undergrad	N	N/A	3	3	Wild	N	Nutritional value	6	5	5	6
120	18 - 24	UK	Undergrad	N	N/A	6	6	Aquaponics	Y	Environmental impact	6	7	6	6
121	18 - 24	UK	A Level	N	N/A	6	6	Aquaponics	Y	None	6	5	7	6
122	25 - 34	Netherlands	Undergrad	N	N/A	6	3	Wild	N	Price	6	5	5	6
123	25 - 34	Poland	Postgrad	N	N/A	2	2	Wild	Y	None	6	4	3	2
124	25 - 34	Lithuania	A Level	N	N/A	6	6	Aquaponics	N	Nutritional value	7	5	7	7
125	25 - 34	Serbia	Postgrad	Y	N	6	6	Wild	N	Nutritional value	6	5	6	4
125		Serbia								Price				
126	18 - 24	Canada	Undergrad	N	N/A	5	4	Wild	N	Disease	5	5	5	6
127	35 - 44	India	Postgrad	Y	Y	6	7	Aquaponics	N	Nutritional value	7	6	7	4
128	45 - 54	USA	Undergrad	Y	N	7	7	Wild	y	None	7	6	7	4
129	18 - 24	Germany	BTEC/Diploma	N	N/A	6	2	Wild	N	None	6	2	6	5
130	18 - 24	UK	A Level	N	N/A	7	7	Aquaponics	Y	None	7	6	7	7
131	18 - 24	UK	A Level	Y	Y	4	6	Aquaponics	N	Price	6	3	6	6
132	< 18	USA	GCSE	Y	N	6	6	Aquaponics	Y	None	6	6	7	2
133	18 - 24	UK	Undergrad	N	N/A	4	5	Aquaponics	N	Price	5	3	5	5
134	25 - 34	Spain	BTEC/Diploma	N	N/A	6	6	Wild	Y	None	4	4	4	4
135	25 - 34	USA	Undergrad	Y	N	6	6	Aquaponics	Y	None	6	6	6	5
136	25 - 34	Romania	Postgrad	N	N/A	4	4	Aquaponics	N	None	6	4	4	4
137	25 - 34	USA	Postgrad	N	N/A	5	5	Wild	N	Nutritional value	5	6	6	5
138	25 - 34	Germany	Undergrad	Y	Y	6	6	Aquaponics	Y	Welfare	7	5	6	5
139	18 - 24	UK	A Level	N	N/A	7	5	Aquaponics	N	Nutritional value	5	6	7	5
139										Environmental impact				
140	45 - 54	UK	Postgrad	N	N/A	7	6	Aquaponics	Y	Nutritional value	7	5	6	7
141	18 - 24	UK	A Level	Y	Y	7	7	Aquaponics	Y	None	6	5	6	5
142	18 - 24	Ireland	Undergrad	N	N/A	4	4	Aquaponics	N	None	4	4	4	4
143	18 - 24	UK	Undergrad	Y	Y	6	6	Wild	Y	Distance to shops	7	6	7	6
144	35 - 44	USA	Undergrad	Y	N	6	5	Wild	Y	Lack of understanding	6	7	6	6
145	18 - 24	UK	Undergrad	N	N/A	5	6	Aquaponics	N	Price	5	5	6	5
146	18 - 24	UK	Undergrad	N	N/A	4	4	Wild	Y	None	5	4	4	4
147	18 - 24	Sweden	A Level	Y	Y	7	7	Aquaponics	Y	Welfare	6	6	7	4
148	25 - 34	Spain	Postgrad	N	N/A	4	4	Aquaponics	Y	Lack of understanding	6	4	4	4
149	18 - 24	Germany	A Level	N	N/A	4	5	Aquaponics	Y	None	5	4	5	5

150	35 - 44	UK	Postgrad	N	N/A	4	4	Wild	N	Artificial	4	4	4	5
151	25 - 34	Kuwait	Undergrad	N	N/A	5	4	Wild	N	None	1	6	6	2
152	18 - 24	Germany	Undergrad	N	N/A	4	2	Wild	N	None	2	2	6	2
153	18 - 24	UK	Undergrad	N	N/A	4	4	Wild	Y	None	7	5	5	7
154	25 - 34	Australia	Postgrad	Y	Y	4	4	Wild	N	Welfare	1	1	1	1
155	18 - 24	UAE	Undergrad	Y	N	7	6	Wild	N	Disease	5	6	7	6
156	18 - 24	Ireland	Postgrad	N	N/A	7	5	Aquaponics	Y	Price	6	7	7	7
157	18 - 24	Netherlands	A Level	N	N/A	3	3	Wild	Y	None	6	5	5	5
158	35 - 44	Africa	BTEC/Diploma	Y	N	5	4	Aquaponics	N	None	5	5	4	5
159	18 - 24	Poland	Undergrad	N	N/A	6	6	Aquaponics	Y	None	5	5	5	6
160	18 - 24	USA	Undergrad	N	N/A	4	4	Aquaponics	N	Disease	5	6	6	6
161	18 - 24	Poland	Undergrad	N	N/A	5	4	Wild	Y	None	5	5	4	5
162	25 - 34	UK	Postgrad	N	N/A	5	5	Wild	N	Lack of understanding	7	7	7	7
163	18 - 24	UK	Undergrad	N	N/A	6	6	Aquaponics	Y	None	6	4	6	6
164	35 - 44	Australia	Undergrad	N	N/A	6	6	Aquaponics	N	Price	7	4	6	6
165	18 - 24	Netherlands	A Level	Y	Y	7	7	Aquaponics	Y	Price	7	6	7	4
166	25 - 34	Canada	Undergrad	Y	N	4	6	Wild	Y	None	5	2	6	6
167	25 - 34	UK	Undergrad	N	N/A	6	6	Wild	Y	Nutritional value	6	6	6	7
167										Welfare				
168	18 - 24	UK	Undergrad	N	N/A	5	5	Wild	N	Unkown impacts	6	5	5	6
169	35 - 44	Australia	Postgrad	N	N/A	4	4	Aquaponics	Y	None	5	6	5	5
170	25 - 34	UK	Undergrad	N	N/A	4	4	Wild	N	None	3	3	6	4
171	18 - 24	UK	Undergrad	N	N/A	5	4	Wild	N	None	4	4	4	4
172	18 - 24	UK	Undergrad	N	N/A	4	4	Aquaponics	Y	None	6	5	5	6
173	25 - 34	Netherlands	Postgrad	N	N/A	4	4	Wild	N	None	4	4	4	4
174	18 - 24	USA	Undergrad	Y	N	7	7	Aquaponics	N	None	5	4	7	5
175	18 - 24	USA	BTEC/Diploma	N	N/A	4	4	Wild	N	Nutritional value	5	5	6	5
176	25 - 34	UK	Postgrad	Y	Y	6	6	Wild	Y	None	7	6	6	4
177	18 - 24	UK	Postgrad	Y	Y	5	6	Aquaponics	Y	Environmental impact	7	7	7	5
178	35 - 44	UK	BTEC/Diploma	N	N/A	4	4	Wild	N	Welfare	4	3	5	4
179	25 - 34	UK	Undergrad	Y	Y	7	6	Aquaponics	Y	Energy use	7	6	6	4
179										Choice of spp				
180	45 - 54	Iceland	A Level	N	N/A	6	6	Wild	N	Lack of understanding	6	6	5	6
181	25 - 34	UK	Undergrad	N	N/A	6	6	Wild	N	None	5	5	5	5

182	35 - 44	USA	BTEC/Diploma	Y	Y	7	7	Aquaponics	Y	Unkown impacts	7	6	7	4
183	25 - 34	Germany	Postgrad	N	N/A	6	6	Wild	Y	None	6	6	6	6
184	25 - 34	Brazil	Postgrad	N	N/A	5	4	Aquaponics	Y	None	6	6	5	4
185	25 - 34	UK	Postgrad	N	N/A	6	6	Aquaponics	Y	Disease	6	7	7	7
186	25 - 34	UK	Undergrad	N	N/A	6	6	Aquaponics	Y	Price	5	5	7	7
186										Environmental impact				
187	25 - 34	UK	Undergrad	N	N/A	6	5	Aquaponics	N	None	6	6	5	5
188	55 - 64	UK	Postgrad	Y	N	7	6	Aquaponics	Y	Land use	6	5	5	6
189	18 - 24	UK	Postgrad	N	N/A	5	5	Wild	Y	Price	5	3	5	5
189										Environmental impact				
189										Nutritional value				
190	45 - 54	UK	Postgrad	N	N/A	4	4	Wild	Y	Price	6	4	6	5
191	25 - 34	USA	Undergrad	N	N/A	7	7	Aquaponics	Y	None	5	1	7	5
192	25 - 34	Ireland	Postgrad	N	N/A	7	7	Aquaponics	Y	None	7	7	7	7
193	25 - 34	Germany	BTEC/Diploma	N	N/A	7	5	Aquaponics	Y	Artificial	7	7	7	6
194	18 - 24	UK	A Level	N	N/A	6	6	Aquaponics	N	Artificial	5	5	6	5
195	18 - 24	UK	A Level	N	N/A	5	5	Wild	Y	None	6	5	6	7
196	18 - 24	Italy	Undergrad	Y	N	5	6	Aquaponics	Y	Nutritional value	5	6	3	6
196		Italy								Environmental impact				
197	35 - 44	Vietnam	Postgrad	N	N/A	7	7	Aquaponics	Y	Disease	7	7	7	7
198	18 - 24	Poland	Undergrad	N	N/A	6	6	Wild	Y	Disease	4	2	5	6
199	35 - 44	UK	Postgrad	N	N/A	4	5	Aquaponics	N	None	6	6	7	7
200	25 - 34	Turkey	Postgrad	N	N/A	6	6	Aquaponics	Y	Regulation	7	6	6	7
201	35 - 44	Finland	Postgrad	N	N/A	4	3	Wild	Y	None	6	4	6	5
202	25 - 34	Italy	Postgrad	Y	Y	6	6	Aquaponics	Y	Price	6	2	6	2
203	25 - 34	UK	Postgrad	N	N/A	3	3	Wild	N	Lack of understanding	3	2	4	2
204	18 - 24	India	Undergrad	Y	N	7	7	Aquaponics	Y	None	7	6	6	6
205	18 - 24	UAE	Undergrad	Y	Y	3	5	Aquaponics	Y	Unkown impacts	5	3	5	5
206	18 - 24	UK	Undergrad	N	N/A	5	4	Wild	Y	Environmental impact	6	4	6	6
207	25 - 34	Germany	Postgrad	N	N/A	6	6	Aquaponics	Y	Welfare	6	6	5	4
208	18 - 24	USA	Undergrad	N	N/A	4	5	Aquaponics	N	None	2	4	6	4
209	25 - 34	Belgium	BTEC/Diploma	N	N/A	3	5	Wild	Y	Lack of understanding	5	5	5	6

210	45 - 54	Canada	Postgrad	Y	Y	6	6	Wild	N	Price	5	5	6	5
211	18 - 24	UK	Undergrad	Y	N	6	5	Aquaponics	Y	Artificial	5	5	6	6
212	25 - 34	UK	Postgrad	Y	N	6	7	Wild	N	Price	6	7	7	7
213	18 - 24	UK	A Level	Y	Y	6	5	Aquaponics	Y	None	5	5	3	5
214	18 - 24	Poland	Undergrad	N	N/A	5	5	Aquaponics	Y	None	6	4	6	6
215	25 - 34	UK	Undergrad	N	N/A	4	5	Aquaponics	N	Lack of understanding	6	2	5	5
216	18 - 24	UK	Undergrad	N	N/A	6	6	Wild	N	Price	4	3	6	4
216										Nutritional value				
217	18 - 24	Italy	Undergrad	N	N/A	6	6	Aquaponics	Y	None	5	5	6	6
218	35 - 44	USA	Undergrad	N	N/A	4	6	Wild	N	Nutritional value	3	5	6	5
219	25 - 34	USA	Undergrad	N	N/A	4	7	Wild	N	Price	7	3	7	1
220	18 - 24	USA	Undergrad	N	N/A	4	6	Aquaponics	Y	None	7	5	6	4
221	45 - 54	Australia	BTEC/Diploma	Y	Y	5	5	Wild	N	None	5	5	6	4
222	18 - 24	UK	A Level	N	N/A	7	7	Aquaponics	N	None	5	4	5	5
223	18 - 24	USA	BTEC/Diploma	N	N/A	6	6	Aquaponics	Y	Price	5	5	6	6
224	25 - 34	USA	Undergrad	Y	N	4	5	Wild	N	Lack of understanding	5	5	6	5
225	35 - 44	UK	GCSE	N	N/A	7	7	Wild	N	Lack of understanding	6	7	7	7
226	35 - 44	China	Postgrad	N	N/A	3	2	Aquaponics	Y	None	6	6	6	6
227	18 - 24	Poland	GCSE	N	N/A	5	5	Aquaponics	N	Price	5	4	5	6
228	25 - 34	Taiwan	Postgrad	N	N/A	6	6	Wild	N	Price	4	6	7	5
229	18 - 24	Italy	Postgrad	N	N/A	4	4	Wild	N	Nutritional value	6	3	3	3
230	35 - 44	UK	Undergrad	N	N/A	6	6	Wild	N	Lack of understanding	5	4	4	4
231	25 - 34	India	Postgrad	N	N/A	7	7	Aquaponics	N	None	7	5	7	6
232	18 - 24	UK	Undergrad	N	N/A	6	6	Aquaponics	Y	None	5	6	7	5
233	35 - 44	China	Undergrad	N	N/A	4	7	Aquaponics	Y	Disease	7	7	7	7
234	35 - 44	France	Postgrad	N	N/A	6	4	Wild	N	None	4	4	4	4
235	18 - 24	Germany	Undergrad	N	N/A	6	6	Wild	N	None	6	3	6	6
236	18 - 24	UK	A Level	N	N/A	6	6	Aquaponics	Y	Disease	7	5	7	7
237	25 - 34	Germany	Postgrad	N	N/A	6	6	Aquaponics	Y	None	6	5	5	6
238	25 - 34	Germany	Undergrad	N	N/A	4	5	Wild	N	None	6	5	4	4

7.9 Chapter 4 R code

```
#Wilcox Mann whitney U test for Likert data

UKlikert <- read.csv(file.choose(), header = T)
View(UKlikert)
names(UKlikert)

boxplot(UKlikert$High_Nutrition ~ UKlikert$Heard_Of, xlab = "UK", ylab = "High
nutrition")
wilcox.test(UKlikert$High_Nutrition ~ UKlikert$Heard_Of, mu=0, alt="two.sided",
conf.int=T, conf.level=0.95, paried=F, exact=F, correct=T)

boxplot(UKlikert$Safe_Clean ~ UKlikert$Heard_Of, xlab = "UK", ylab = "Safe and Clean")
wilcox.test(UKlikert$Safe_Clean ~ UKlikert$Heard_Of, mu=0, alt="two.sided", conf.int=T,
conf.level=0.95, paried=F, exact=F, correct=T)

boxplot(UKlikert$Sustainable ~ UKlikert$Heard_Of, xlab = "UK", ylab = "Sustainable")
wilcox.test(UKlikert$Sustainable ~ UKlikert$Heard_Of, mu=0, alt="two.sided", conf.int=T,
conf.level=0.95, paried=F, exact=F, correct=T)

boxplot(UKlikert$Likelihood ~ UKlikert$Heard_Of, xlab = "UK", ylab = "Likelihood")
wilcox.test(UKlikert$Likelihood ~ UKlikert$Heard_Of, mu=0, alt="two.sided", conf.int=T,
conf.level=0.95, paried=F, exact=F, correct=T)
```

```
boxplot(UKlikert$Community ~ UKlikert$Heard_Of, xlab = "UK", ylab = "Community")

wilcox.test(UKlikert$Community ~ UKlikert$Heard_Of, mu=0, alt="two.sided", conf.int=T, conf.level=0.95, paried=F, exact=F, correct=T)


boxplot(UKlikert$Changed ~ UKlikert$Heard_Of, xlab = "UK", ylab = "Changed opinion")

wilcox.test(UKlikert$Changed ~ UKlikert$Heard_Of, mu=0, alt="two.sided", conf.int=T, conf.level=0.95, paried=F, exact=F, correct=T)


#Analysis of world data

Worldlikert <- read.csv(file.choose(), header = T)

View(Worldlikert)

names(Worldlikert)


boxplot(Worldlikert$High_Nutrition ~ Worldlikert$Heard_Of, xlab = "World", ylab = "High nutrition")

wilcox.test(Worldlikert$High_Nutrition ~ Worldlikert$Heard_Of, mu=0, alt="two.sided", conf.int=T, conf.level=0.95, paried=F, exact=F, correct=T)


boxplot(Worldlikert$Safe_Clean ~ Worldlikert$Heard_Of, xlab = "World", ylab = "Safe and clean")

wilcox.test(Worldlikert$Safe_Clean ~ Worldlikert$Heard_Of, mu=0, alt="two.sided", conf.int=T, conf.level=0.95, paried=F, exact=F, correct=T)


boxplot(Worldlikert$Sustainable ~ Worldlikert$Heard_Of, xlab = "World", ylab = "Sustainable")

wilcox.test(Worldlikert$Sustainable ~ Worldlikert$Heard_Of, mu=0, alt="two.sided", conf.int=T, conf.level=0.95, paried=F, exact=F, correct=T)
```

```
boxplot(Worldlikert$Likelihood ~ Worldlikert$Heard_Of, xlab = "World", ylab = "Likelihood")

wilcox.test(Worldlikert$Likelihood ~ Worldlikert$Heard_Of, mu=0, alt="two.sided", conf.int=T, conf.level=0.95, paried=F, exact=F, correct=T)


boxplot(Worldlikert$Community ~ Worldlikert$Heard_Of, xlab = "World", ylab = "Community")

wilcox.test(Worldlikert$Community ~ Worldlikert$Heard_Of, mu=0, alt="two.sided", conf.int=T, conf.level=0.95, paried=F, exact=F, correct=T)


boxplot(Worldlikert$Changed ~ Worldlikert$Heard_Of, xlab = "World", ylab = "Changed opinion")

wilcox.test(Worldlikert$Changed ~ Worldlikert$Heard_Of, mu=0, alt="two.sided", conf.int=T, conf.level=0.95, paried=F, exact=F, correct=T)


#analysis of opinions with competent knowledge in the UK

UKcomp <- read.csv(file.choose(), header = T)

View(UKcomp)

names(UKcomp)


boxplot(UKcomp$High_Nutrition ~ UKcomp$Competent, xlab = "Compentent Knowledge", ylab = "High nutrition")

wilcox.test(UKcomp$High_Nutrition ~ UKcomp$Competent, mu=0, alt="two.sided", conf.int=T, conf.level=0.95, paried=F, exact=F, correct=T)
```

```
boxplot(UKcomp$Safe_Clean ~ UKcomp$Competent, xlab = "Compentent Knowledge",
ylab = "Safe and clean")

wilcox.test(UKcomp$Safe_Clean ~ UKcomp$Competent, mu=0, alt="two.sided",
conf.int=T, conf.level=0.95, paried=F, exact=F, correct=T)


boxplot(UKcomp$Sustainable ~ UKcomp$Competent, xlab = "Compentent Knowledge",
ylab = "Sustainable")

wilcox.test(UKcomp$Sustainable ~ UKcomp$Competent, mu=0, alt="two.sided",
conf.int=T, conf.level=0.95, paried=F, exact=F, correct=T)


boxplot(UKcomp$Likelihood ~ UKcomp$Competent, xlab = "Compentent Knowledge",
ylab = "Likelihood")

wilcox.test(UKcomp$Likelihood ~ UKcomp$Competent, mu=0, alt="two.sided", conf.int=T,
conf.level=0.95, paried=F, exact=F, correct=T)


boxplot(UKcomp$Community ~ UKcomp$Competent, xlab = "Compentent Knowledge",
ylab = "Community")

wilcox.test(UKcomp$Community ~ UKcomp$Competent, mu=0, alt="two.sided",
conf.int=T, conf.level=0.95, paried=F, exact=F, correct=T)


boxplot(UKcomp$Changed ~ UKcomp$Competent, xlab = "Compentent Knowledge", ylab =
"Changed opinion")

wilcox.test(UKcomp$Changed ~ UKcomp$Competent, mu=0, alt="two.sided", conf.int=T,
conf.level=0.95, paried=F, exact=F, correct=T)
```

```
#Making graphs to display dichotomous and demographic information

library(ggplot2)

RawSurvey <- read.csv(file.choose(), header = T)

View(RawSurvey)

names(RawSurvey)

attach(RawSurvey)

ggplot(data= RawSurvey, aes(x = Education, fill = Heard_Of)) +geom_bar()

ggplot(data= RawSurvey, aes(x = Age, fill = Heard_Of)) +geom_bar()+ theme(axis.text.x = element_text(angle = 90, vjust = 0.5, hjust=1))

Exploring relationships between demographics and opinions

ggplot(data= RawSurvey, aes(x = Age, fill = Aquaponics_Wild)) +geom_bar()+ theme(axis.text.x = element_text(angle = 90, vjust = 0.5, hjust=1))

ggplot(data= RawSurvey, aes(x = Age, fill = Organic)) +geom_bar()+ theme(axis.text.x = element_text(angle = 90, vjust = 0.5, hjust=1))

ggplot(data= RawSurvey, aes(x = Education, fill = Aquaponics_Wild)) +geom_bar()+ theme(axis.text.x = element_text(angle = 90, vjust = 0.5, hjust=1))

ggplot(data= RawSurvey, aes(x = Education, fill = Organic)) +geom_bar()+ theme(axis.text.x = element_text(angle = 90, vjust = 0.5, hjust=1))

UKRes <-read.csv(file.choose(), header = T)

names(UKRes)

ggplot(data= UKRes, aes(x = Catagories.uk, fill = UK)) +geom_bar(stat="identity") +labs(x="Percentage", y="Proportion")
```

7.10 Risk assessments

COS Risk Assessment for Teaching, Administration and Research Activities

Swansea University; College of Science

Name Sam Files **Signature** [redacted] **date 17/03/2021**

Supervisor* Carlos Garcia De Leaniz **Signature** [redacted] **date 17/03/2021**

Activity title: MRes KESS 2 research: Palatability of digestate cultured microalgae................
Base location (room no.) CSAR C2

(* the supervisor for all HEFCW funded academic and non-academic staff is the HOS)

School Activity Serial # (enter Employee No. or STUREC No....
Start date of activity (cannot predate signature dates) 17/03/2021 ....
End date of activity (or 'on going') 31/03/2021- 07/04/2021...

Level of worker (delete as applicable) ....

~~UG~~, **PG**, ~~research assistant~~, ~~technician~~, ~~administration~~, ~~academic staff~~, ~~other (state)~~

Approval obtained for Gene Manipulation Safety Assessment by UWS ? Yes/not applicable
Licence(s) obtained under "Animals (Scientific Procedures) Act (1986)" ? Yes/not applicable
Approval obtained for use of radioisotopes by COS ? Yes/not applicable

Record of specialist training undertaken

Course	date

COS Protocol Risk Assessment Form

(Expand or contract fields, or append additional sheets as required; insert NA if not applicable)

Protocol #	**Title: MRes KESS 2 research** Use of C2 to assess palatability of feed formulated using digestate microalgae
Associated Protocols ***#..........................***	**Description:** Preparing fish feed using microalgae cultured on digestate. Daily experiment, using a divider to separate one tilapia and offer the digestate diet or commercial diet to assess palatability of the feed and then removing that individual. Carrying out routine daily duties to feed and care for the fish and collecting samples when required.

Location: C1, Centre for Sustainable Aquaculture Research (CSAR), Llyr Building

Identify here risks and control measures for work in this environment:

Slips, trips and falls:

· Suitable footwear to be worn in this area, floors to be dried after water spillages.

· PPE including latex gloves and laboratory coats must be worn at all times.

· In case of injury: use SafeZone app., inform Security and/or go to Hospital if needed.

· Risk should be minimized by using the appropriate tools.

Chemicals:

· This lab holds a number of chemicals.

· MSDS must be obtained prior to use of any chemicals and appropriate PPE worn.

· Risk assessments for the use of specific chemicals need to be completed prior to use.

Use of machines

· I will be using a new pellet machine and blender to break up the feed.

· Due care will be taken and gloves warn to ensure a minimal risk of cutting or injuring fingers

· In case of injury: use SafeZone app., inform Security and/or go to Hospital if needed.

Use of Oven

· The oven will be used to dry the formulated feed.

· Thermal protective gloves will be used we moving anything in or out of the oven.

· In case of burns apply cold water and use SafeZone app., inform Security and/or go to Hospital if needed.

COVID-related:

· I will most likely be the only person working in C2 apart from technical support on occasion.

· New working practices have been implemented in order to keep users and technical staff safe (refer to Visitor info CSAR_CGR.doc).

· If any other person to be in the same lab (max. 3 working at the same time), keep 2m social distancing or wear face masks if social distancing not possible.

· Wear gloves at all times and regular washing of hands (for 20 seconds).

· Disinfection with own alcohol-based gel before and after entering/exiting the rooms (especially when touching door handles or high-touch points).

· Cleaning and disinfection of the workspace before and after use with 70% Ethanol and 20% bleach. Use gloves for disinfectants as they will be in a common area.

· A give- way system is in place and effective communication with technical staff to ensure no clashes in room/equipment use and enough distance is kept at entering/exiting the facility.

· If working during the weekends, ensure regular communication with staff (time of arrival and departure will be confirmed on a daily basis).

· This is in addition to compliance with wider University policies detailed in the generic risk assessment document when inside and outside the CSAR.

Chemicals	Quantity	Hazards	Category (A,B,C,D)*	Exp.Score
-	-	-	-	-

Hazard Category (known or potential)
A (e.g. carcinogen/teratogen/mutagen)
B (e.g. v.toxic/toxic/explosive/pyrophoric)
C (e.g. harmful/irritant/corrosive/high flammable/oxidising)
D (e.g. non classified)

Exposure Potential Circle the **highest Exposure Score** above. Use this to calculate the exposure potential for the entire protocol (see handbook). Indicate this value below.

Low Medium High

Primary containment (of product) sealed flask/bottle/glass/plastic/other (state) :- N/A

Storage conditions and maximum duration :- N/A

Secondary containment (of protocol) open bench/fume hood/special (state) :- N/A

Disposal e.g. autoclaving of biohazard, UWS chemical disposal

Identify other control measures (circle or delete) - ~~latex/nitrile/heavy gloves; screens; full face mask; dust mask;~~ protective shoes; ~~spillage tray; ear defenders~~; other: lab coat, latex gloves, face mask (if 2m social distance cannot be kept)

Justification and controls for any work outside normal hours

Working out of hours should be avoided where possible. If it is absolutely necessary to be working in this lab outside normal working hours, this should be done in pairs. If working alone then signing in and out using the CSAR in/out board located in the changing rooms and log book (Wallace building) is imperative.

Emergency procedures (e.g. spillage clearance; communication methods)

Fire extinguisher available on site. Fire service to be called in case of fire. Wallace building alarm to be sounded for evacuation. COVID directional signs will not be followed in this case and congregation to be avoided outside if evacuation occurs.

Supervision/training for worker (circle)

~~None required~~ **Already trained** ~~Training required~~ ~~Supervised always~~

Declaration I declare that I have assessed the hazards and risks associated with my work and will take appropriate measures to decrease these risks, as far as possible eliminating them, and will monitor the effectiveness of these risk control measures.

Name & signature of worker *Sam Files* [redacted]

[redacted]

Name & counter-signature of supervisor *Carlos Garcia De Leaniz* [redacted] ***Date.*** *17/03/2021*

Date of first reassessment Frequency of reassessments

COS Protocol Risk Assessment Form

(Expand or contract fields, or append additional sheets as required; insert NA if not applicable)

Protocol #	**Title: Using power tools**
Associated Protocols **#..........................**	**Description: The use of power tools including drill and jigsaw in and around the CSAR facilities**

Location: Across all CSAR facilities

circle which COS Local Rules apply –

Boat . Field Genetic-Manipulation Laboratory Office/Facility Radioisotope

Identify here risks and control measures for work in this ~~environment,~~ additional to Local Rules

The use of power tools in CSAR pose a number of risks:

The use of electrical tools near water and in damp environments leads to a risk of electrocution – Where possible battery powered tools should be used, failing that low voltage tools should be used. Use of 240V tools should be restricted to dry facilities i.e., tool shed.

These types of tools have a risk of injury from sharp blades and drill bits, these can be dangerous if misused or if they shatter – Operator to wear appropriate Personal Protective Equipment including eye protection and heavy gloves.

Use of power tools can produce dust and/or excessive noise – Operator to wear dust mask and ear defenders if operating power tools in confined or poorly ventilated areas.

Chemicals	Quantity	Hazards	Category (A,B,C,D)*	Exp. Score

Hazard Category (known or potential)

A (e.g. carcinogen/teratogen/mutagen)
B (e.g. v.toxic/toxic/explosive/pyrophoric)
C (e.g. harmful/irritant/corrosive/high flammable/oxidising)
D (e.g. non classified)

Exposure Potential Circle the **highest Exposure Score** above. Use this to calculate the exposure potential for the entire protocol (see handbook). Indicate this value below.

Low **Medium** **High**

Primary containment (of product) sealed flask/bottle/glass/plastic/other (state) :-

Storage conditions and maximum duration :-

Secondary containment (of protocol) open bench/fume hood/special (state) :-

Disposal e.g. autoclaving of biohazard, UWS chemical disposal

Identify other control measures (circle or delete) ~~latex/nitrile/~~heavy gloves~~; screens; full face mask;~~ dust mask; protective shoes~~; spillage tray; ear-defenders; other (state)~~

Justification and controls for any work outside normal hours. Work using power tools should be avoided outside normal working hours.

Emergency procedures (e.g. spillage clearance; communication methods) The nearest emergency telephone, first aider and first aid box should be identified.

Supervision/training for worker (circle)

None required ~~Already trained~~ ~~Training required~~ ~~Supervised always~~

Declaration I declare that I have assessed the hazards and risks associated with my work and will take appropriate measures to decrease these risks, as far as possible eliminating them, and will monitor the effectiveness of these risk control measures.

Name & signature of worker [redacted] Sam Files

Name & counter-signature of supervisor [redacted] *Date* 16.03.21

Date of first reassessment requency of reassessments

7.12 Ethical approval.

Student Details

Name:	Samuel Files
Student Number:	[redacted]
Level:	7
Course:	Research Study
Project Supervisor:	Prof. Carlos Garcia De Leaniz
Last Updated Date:	14 Apr 2021, 5:16 p.m.
Last Reviewed Date:	1 Mar 2021, 10:23 a.m.
Reviewed by:	Rebecca Stringwell

Projects Ethics Assessment Status

Project Title	Status	Approval Number
Can microalgae cultured on digestate be more beneficial to tilapia growth?	Completed	SU-Ethics-Student-140421/3620
Peoples perceptions of aquaponics	Completed	SU-Ethics-Student-140421/3630
Can microalgae supplementation of tilapia feed affect palatability	Completed	SU-Ethics-Student-140421/3789

Additional forms to be submitted as part of this assessment and their status(only Project Supervisor can submit additional forms to relevant Committee):

- AWERB Review Form(STU_BIOL_157593_190221104250_3) - Approved Proposal :AWERB Group DECISION Details
- AWERB Review Form(STU_BIOL_157593_190121100210_1) - Approved Proposal :AWERB Group DECISION Details