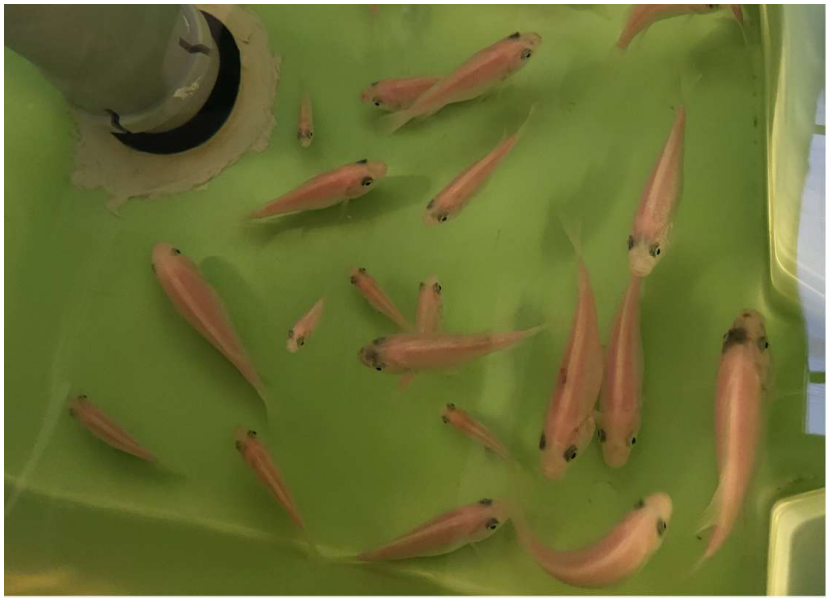
Sustainable aquaculture development in Sweden:

An assessment of novel feed in aquaponic cultivation of Nile tilapia



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Degree project for Master of Science (120 ECTS) with a major in sustainable production and utilization of marine bio resources
MABI20 Master project in Sustainable production and utilization of marine bio resources 60 ECTS
Second cycle

Semester/year: Autumn/spring 2024/2025

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Abstract

As the global population continues to rise, the demand for resilient and resource efficient food production is becoming increasingly urgent. Aquaculture is the fastest growing food sector, although transitioning to low-impact practices requires innovative strategies to reduce dependence on conventional methods. One major bottleneck in aquaculture is the reliance on conventional feed ingredients such as fishmeal and soy protein. Another challenge is nutrient loss from these systems, which not only contributes to environmental pollution but also represents a waste of valuable resources. To make aquaculture truly sustainable, a shift toward circular, sustainable systems is important. This study compared a regionally available alternative feed, based on mussel meal and pea protein concentrate, to a conventional feed containing fishmeal and soy protein in aquafeeds for Nile tilapia (*Oreochromis niloticus*) reared in coupled aquaponic systems. An experiment was set up to compare fish performance, water quality, plant growth, and microbial activity between trials of conventional diets and alternative diets. Fish reared in aquaponic systems exhibited high growth and feed efficiency when fed the alternative diet (FCR: 1.07), performing slightly below the commercial control feed and the fish in recirculating aquaculture systems (RAS), although still within acceptable limits (FCR: 0.90 - 1.80). Once nutrients began to accumulate during the stabilization phase, the aquaponic systems maintained stable water quality and supported Tatsoi (*Brassica rapa*) growth that was comparable to, or even greater than that of the hydroponic control systems, throughout the feeding trials. Microbial assessments and cortisol analyses indicated that aquaponic systems supported fish welfare comparable to that of RAS. When fish were fed the alternative feed, microbial loads were generally lower in both rearing systems compared to those fed the conventional diet. Overall, the findings support the potential of blue mussel and pea protein as sustainable feed components in integrated aquaponic production, contributing to nutrient circularity and reducing dependence on limited marine stocks and imported resources.

Contents

1. Introduction

1.1 Background

The global population is projected to reach 9.7 billion by 2050, an increase of approximately 2 billion people over the next 30 years (United Nations, 2019). With this population growth, and increased stresses on resources such as water, land, and nutrients, the demand for sustainable food production becomes increasingly critical (Klinger & Naylor, 2012; Searchinger et al., 2019).

Aquaculture is the fastest growing food sector (D'Abramo, 2024; Troell et al., 2014), and in 2022 the farming of aquatic animals; 94 million tons surpassed the wild caught fisheries; 91 million tons for the first time (FAO, 2024). Aquatic food systems contribute to the global food security and economic development (FAO, 2024). They can serve as a more sustainable source of food compared to many terrestrial agricultural systems, as numerous farmed fish species exhibit inherently higher production efficiency due to their physiological characteristics (D'Abramo, 2024; Duarte et al., 2009). Compared to terrestrial animals, aquatic species typically have lower feed conversion ratios (FCR), requiring less feed to gain body weight (D'Abramo, 2024). In general, fish have an FCR of 1.0 - 2.0 (A. Tacon & Metian, 2008), chicken 1.7 - 2.0 (Zuidhof et al., 2014), pigs 2.70 - 5.00 (MacLeod et al., 2013), and cattle 6 - 10 (Shike, 2013) (**Fig. 1**).

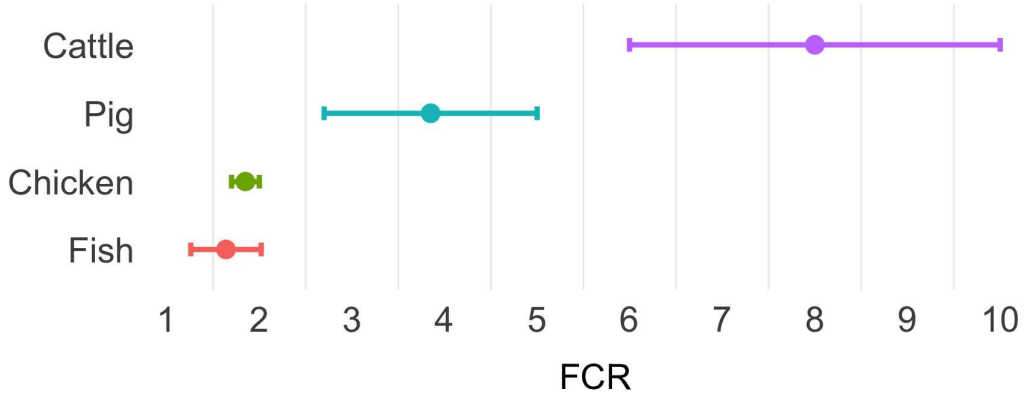


Figure 1. Feed conversion ratio (FCR) for common livestock, with dots representing mean values and bars indicating the range. The figure is adapted from data gathered by Fry et al., 2018.

Despite aquaculture being inherently efficient in production, it comes with some associated challenges (D'Abramo, 2024). Most intensive net-cage farming for fed finfish occurs in public waters, including coastal areas, lakes, and rivers, with minimal environmental control (Tacon, 2023). These systems are prone to disease outbreaks, local pollution, and fish escapes (Tacon, 2023). As a result, there has been a significant shift away from open-cage farming towards land-based, indoor recirculat-

ing aquaculture systems (RAS), which offer a fully controlled farming environment (Mota et al., 2022).

However, RAS faces challenges such as high energy consumption and operational costs due to water treatment processes, compared to conventional aquaculture (Ahmed & Turchini, 2021), and large amounts of nitrogen (N) and phosphorus (P) are released and wasted from the system (Ende et al., 2024). An advancing farming technology that could have the potential to address these issues is aquaponics (Ahmed & Turchini, 2021; Goddek et al., 2019; Klinger & Naylor, 2012), a combination of aquaculture and hydroponics in a recirculating system (Diver, 2006). Aquaponic systems offer several benefits, including nutrient recovery, optimal use of water resources, and increased profitability through the simultaneous production of two valuable yields (FAO, 2022; Wongkiew et al., 2017).

Among the fish commonly raised in aquaponic systems is Nile tilapia (*Oreochromis niloticus*) (Sommerville et al., 2014) (**Fig. 2a**). According to recent data from FAO (2024), Nile tilapia is one of the top ten farmed aquatic species worldwide (5.3 million tonnes in 2022). It has proven suitable for aquaponic farming due to its hardiness and adaptability (Sommerville et al., 2014). They have high resistance to several pathogens and can withstand large fluctuations in water quality conditions, such as pH-levels between 6 - 9, ammonium (NH_4^+) < 2 mg L^{-1}, nitrite (NO_2^-) < 1 mg L^{-1}, and nitrate (NO_3^-) < 400 mg L^{-1} (Mengistu et al., 2020; Sommerville et al., 2014).

Leafy greens such as Tatsoi (*Brassica rapa*) (**Fig. 2b**), lettuce and spinach, are ideal candidates for aquaponic and hydroponic systems due to their fast growth rates, compact root structures, low nutrient requirements, and adaptability to varying environmental conditions (GoGreenAquaponics, 2025).

(a)

(b)

Figure 2. (a) Nile tilapia (*Oreochromis niloticus*), (Gårdsfisk, 2023), and (b) Tatsoi (*Brassica rapa*), (Maria Berger, 2024).

By integrating fish farming with plant cultivation, aquaponics creates a closed-loop system allowing for the harvest of both crops and fish, all without the use of soil (Racoky et al., 2006). Waste from the fish becomes a nutrient source for the plants (Diver, 2006; Eck, Körner, & Jijakli, 2019). Fish excretes NH_4^+ as a waste product,

and through the process of nitrification, it is converted into NO_2^- and subsequently into NO_3^- by nitrifying bacteria (Van Rijn, 2013). If not properly managed, these N compounds can accumulate to toxic concentrations, posing serious risks to aquatic animal health (Kim et al., 2019). Chronic exposure to elevated NH_4^+ levels has been linked to altered metabolic activity and neurotoxicity in fish (Rodrigues et al., 2014; Sinha et al., 2012). Elevated concentrations of NO_2^- and NO_3^- can impair oxygen transport by oxidizing hemoglobin to methemoglobin, which cannot bind oxygen efficiently (Roques et al., 2015; Van Bussel et al., 2012). NO_3^- is generally less toxic to most species than NO_2^- (Sommerville et al., 2014), and it is the preferred N form for plant uptake, playing a vital role in supporting plant growth (Marschner, 2011).

Aquaponics relies on maintaining a balanced ecosystem of fish, plants, and bacteria. Each group has specific water quality requirements, and although their tolerance ranges overlap, compromises are necessary—meaning not all organisms can operate at their optimum conditions (Sommerville et al., 2014). **Table 1** shows approximate acceptable ranges for temperature, pH, nitrogen compounds, and dissolved oxygen (DO).

Table 1. Optimal water quality parameters for aquaponic systems as a compromise between plants, fish, and bacteria. The table is adapted from Sommerville et al., 2014.

Temp (°C)	**pH**	**NH_4^+ (mg L^{-1})**	**NO_2^- (mg L^{-1})**	**NO_3^- (mg L^{-1})**	**DO (mg L^{-1})**
18–30	6–7	< 1	< 1	5–150	> 5

A typical aquaponic system includes a fish tank, a biofilter for nitrification, and other processes that detoxify NH_4^+ and compounds excreted by the fish, a sump for removing solids, and a grow bed for hydroponic cultivation (Racoky et al., 2006). The three most commonly used types of grow beds in aquaponics are the nutrient film technique (NFT), floating raft (deep water culture), and media-filled (ebb- and flow) (**Fig. 3**) (Delaide et al., 2017; Engle, 2015; Wongkiew et al., 2017). The NFT consists of channels that support plants, with a thin film of nutrient solution flowing through them, allowing for efficient water use and minimal water loss from evaporation (Racoky et al., 2006). It is suitable for herbs and leafy greens but not larger vegetables since the roots can clog the system (Engle, 2015).

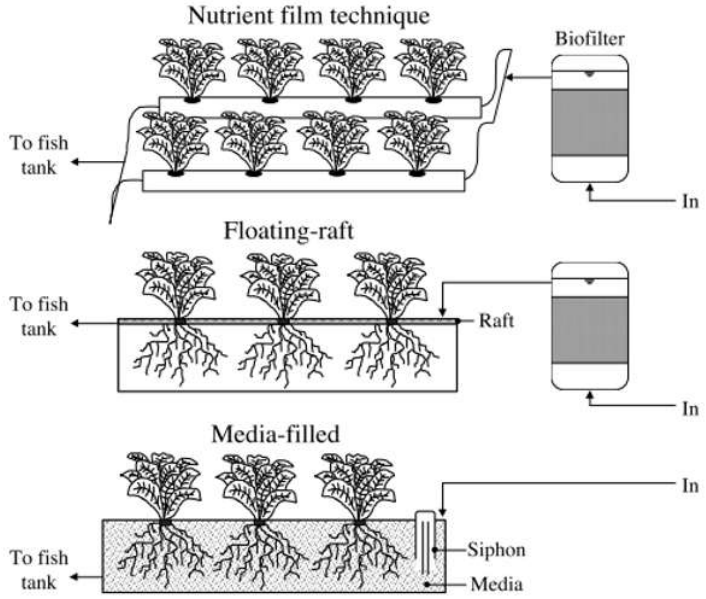


Figure 3. Aquaponic systems based on types of grow bed (Wongkiew et al., 2017).

Most of the nutrients required by the plants in the aquaponic system are retrieved from fish and feed waste. Nevertheless, plants require certain macronutrients that are commonly deficient in aquaponic systems: potassium (K), phosphorus (P), iron (Fe), manganese (Mn), and sulfur (S) (Robaina et al., 2019). Especially Fe is essential for vital processes in plants and is commonly added as a supplement (Kasozi et al., 2019). One way to achieve a water composition that meets hydroculture needs is by optimizing the fish feed composition (Goddek et al., 2015).

Traditionally, fish feed for intensive systems such as RAS and aquaponics has relied heavily on fish meal and fish oil derived from wild-caught fish (Aas et al., 2022). This practice has raised significant sustainability concerns due to overfishing and the depletion of marine resources (FAO, 2020). To reduce dependence on marine ingredients, alternative feeds have been developed using plant-based proteins and oils, such as soy, corn, and wheat (Aas et al., 2022; Jannathulla et al., 2019; Little et al., 2016). However, these alternatives present their own challenges (Zlaugotne et al., 2022). They often require large amounts of arable land and freshwater, which raises concerns about their environmental impact and competition with human food crops (Zlaugotne et al., 2022).

To address these challenges, researchers are increasingly exploring sustainable protein alternatives that support both environmental sustainability and optimal fish health and growth (Agboola et al., 2021; Warwas, 2023). Several studies have investigated the full or partial replacement of fish meal with alternative protein sources, such as insect meal, yeast, fish processing side streams, seaweed fly larvae and mussel meal (Alfiko et al., 2022; Biancarosa, 2020; Rasidi, 2022; Vidakovic et al., 2016; Warwas et al., 2023, 2024). For carnivorous species like Atlantic salmon (*Salmo salar*), reducing fish meal and fish oil in feed formulations can significantly im-

pact growth and performance (Kousoulaki et al., 2022). In contrast, tilapia are an omnivorous species capable of efficiently digesting plant-based diets. (FAO, 2006). Increasing plant-based ingredients while reducing fish meal and fish oil in tilapia diets may be a more sustainable approach, as it minimizes environmental impact without compromising digestibility (Magbanua & Ragaza, 2024).

Among plant protein sources, field peas have been identified as a valuable alternative for aquafeeds, showing promising results in species such as Atlantic salmon (Aslaksen et al., 2007; Øverland et al., 2009) and Rainbow trout (*Oncorhynchus mykiss*) (Thiessen et al., 2003). Incorporating the legume into fish diets can contribute to reducing the environmental footprint of aquaculture (Wilfart et al., 2023). As a locally produced crop in Sweden, field peas represent a more sustainable and regionally adapted protein source and help reduce dependence on soy-based ingredients, which are often imported and associated with environmental concerns (WWF, 2014). Field peas also require fewer inputs compared to other legumes, making them environmentally efficient, and there is a long-standing tradition of using peas in Swedish animal feed formulations (SLU, 2023). In 2023, 54,900 tonnes of peas were produced in Sweden (Jordbruksverket, 2024).

To not fully loose the marine components in alternative aquafeeds, cultivated blue mussels (*Mytilus edulis*) are of significant interest as a high-quality ingredient (Albrektsen et al., 2022; Rasidi, 2022). They contain high protein levels (50–70 % dry weight) and lipids (5–16 % dry weight) (Jusadi et al., 2020) and offer a favorable amino acid and fatty acid profile comparable to fishmeal (Jusadi et al., 2020; Langeland et al., 2016). Additionally, they are rich in n-3 Polyunsaturated Fatty Acids (PUFAs), making them a valuable dietary component (Albrektsen et al., 2022). Beyond their nutritional benefits, blue mussels provide important ecosystem services by acting as filter feeders that help mitigate eutrophication through N removal from the water column (Lindahl et al., 2005). While blue mussel meal has been studied as a feed ingredient for various fish species in aquaculture (Berge & Austreng, 1989; Jaeger et al., 2024; Langeland et al., 2016; Vidakovic et al., 2016), its application in Nile tilapia diets within an aquaponic system remains largely unexplored.

1.2 Aim and Objectives

This study aims to evaluate the integration of regionally available, alternative protein sources—blue mussel meal and pea protein concentrate—into aquafeeds for Nile tilapia within a coupled aquaponic system. The objective is to assess the effects of this novel feed formulation on fish growth performance, water quality parameters, and plant nutrient dynamics, using Tatsoi as a model crop.

By comparing a control diet based on conventional protein sources (fishmeal and soy protein concentrate) with a diet incorporating blue mussel meal and pea concentrate, this work contributes to evaluating the viability of alternative feed ingredients in recirculating aquaculture systems. Given the favorable environmental profiles of both mussels and field peas—including high nutrient density, low input requirements, and reduced dependency on imported feedstuffs—their inclusion may improve the sus-

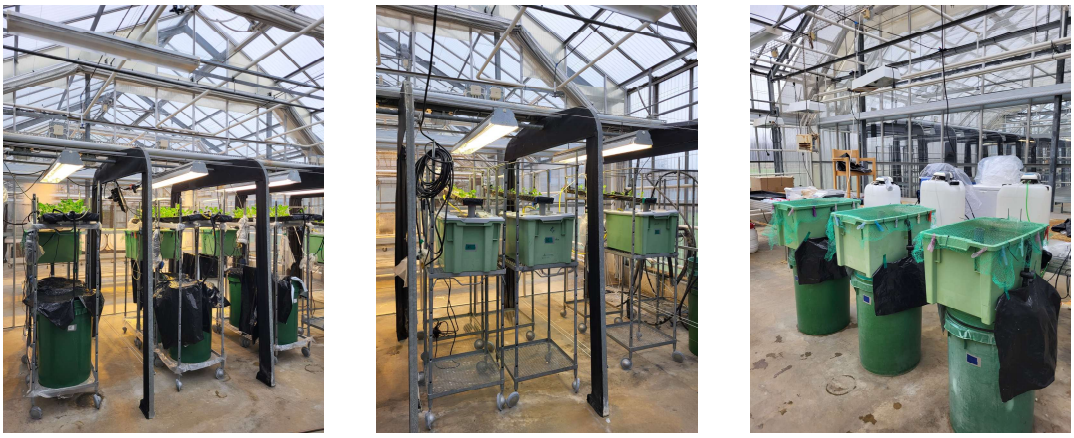
tainability, nutrient circularity, and resource efficiency of aquaponic food production systems.

2. Methods

2.1 Fish husbandry and experimental setup

Two hundred nursery-stage (post-larval) Nile tilapia with an initial weight of 0.15 ± 0.05 g (mean ± SD) were obtained from Gårdsfisk (Kristianstad, Sweden) and introduced into experimental systems located in the greenhouse at the Department of Biosystems and Technology, SLU Alnarp, Sweden. The experiment involved aquaponic systems (A), hydroponic control systems (H), and RAS (R) controls in triplicate (**Fig. 4**).

System compartments had the following volumes: fish tanks and hydroponic reservoirs – 50 L; biofilters – 70 L, containing 10 % nitrifying bio media; and sump tanks – 80 L. The hydroponic gutters measured 155 × 13 × 5 cm and accommodated ten plants each. In both A and R, temperature was maintained using aquarium heaters placed in the fish tanks and biofilters. These compartments were also continuously aerated with air stone diffusers. H were likewise aerated with air stones. Lighting was applied above all A and H systems in a 14:10 (light:dark) photoperiod at an intensity of 200 µmol m^{-2} s^{-1}. Environmental conditions in the greenhouse were monitored and maintained at 70 % relative humidity and an air temperature of 24 °C.



(a) **(b)** **(c)**

Figure 4. Experimental setup of (a) aquaponic systems, (b) hydroponic controls, and (c) RAS controls in triplicate in the greenhouse chamber.

Aquaponic system

Tatsoi was cultivated in a coupled aquaponic system using nutrient film technique (NFT) (**Fig. 5a**) at a temperature of 25.00 ± 2.15 °C (mean over the whole experimental period ± SD). Each system comprised a fish tank, with the water being

directed into a sump tank, followed by a biofilter tank. From the biofilter, water was pumped to three hydroponic gutters, where it returned to the fish tank by gravity.

Hydroponic system

The hydroponic control units consisted of a nutrient solution reservoir containing Hoagland's solution (Hoagland & Arnon, 1950), see **Table S1** in the supplementary material for compound composition. The solution was pumped to a single hydroponic gutter, and the system operated at a temperature of 20.90 ± 1.56 °C (**Fig. 5b**).

RAS

The RAS control units were operated at a temperature of 25.10 ± 1.34 °C throughout the experimental period. The water was pumped from the biofilter to the fish tank, where it returned to the biofilter by gravity (**Fig. 5c**).

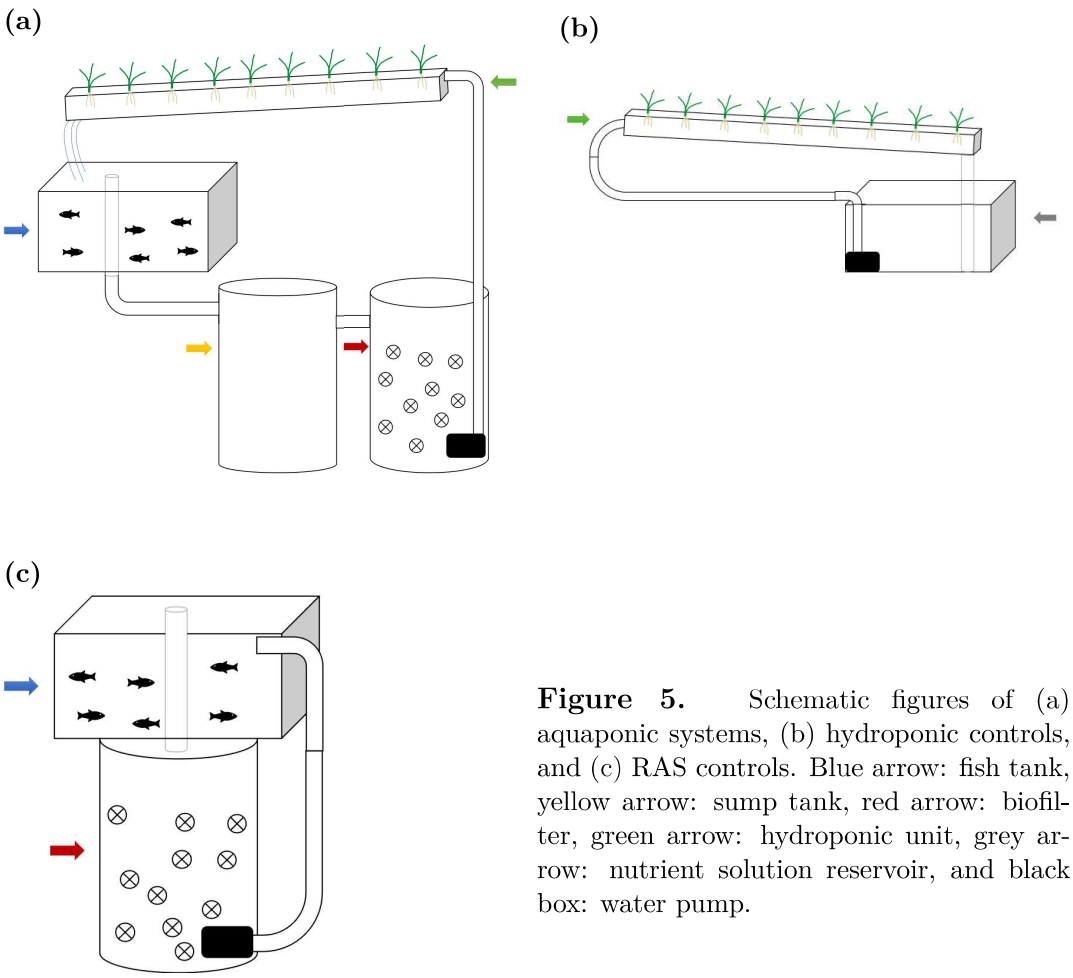


Figure 5. Schematic figures of (a) aquaponic systems, (b) hydroponic controls, and (c) RAS controls. Blue arrow: fish tank, yellow arrow: sump tank, red arrow: biofilter, green arrow: hydroponic unit, grey arrow: nutrient solution reservoir, and black box: water pump.

2.2 Experimental design

The experiment was conducted in three phases: Phase 1 – System Stabilization, Phase 2 – Feeding trial with fishmeal and soy protein (Control feed), and Phase 3 – Feeding trial with alternative feed (ECO feed).

A transition period of four days between the feeds was applied to ensure proper adaptation from the old- to the new feed:

1st day: 75 % old feed & 25 % new feed, 2nd day: 50 % old feed & 50 % new feed, 3rd day: 25 % old feed & 75 % new feed, 4th day: 100 % new feed.

According to the manufacturer's recommendations (Cultiwool, 2025), Tatsoi seeds were germinated in rock wool cubes 14-19 days before being transplanted into the systems.

System stabilization

The first ten weeks of the experiment served as a stabilization period, during which the A and R systems were conditioned to establish stable water quality and a functional microbial community suitable for rearing nursery-stage Nile tilapia.

To promote initial biofiltration, both system types were initiated prior to fish introduction using circulating water obtained from Groburket (Alnarp, Sweden), which provided a source of active microbial inoculum. NO_2^- accumulation, as seen in **Fig. S2**, during this phase was mitigated by regular water exchanges and the addition of biologically active filter media.

Tatsoi seedlings were introduced after fish stocking. A total of 20 seedlings were randomly distributed across the three A systems, while 10 seedlings were placed in each H system. To address observed micronutrient deficiencies in the aquaponic systems, they were supplemented with 1.72 g of Fe as YaraTera REXOLIN® D12 (Yara International ASA, Oslo, Norway) and 24 g of $MgSO_4$ as Epsom salt (BitterMag, Helsingborg, Sweden), applied two and six weeks after transplantation.

Throughout the stabilization phase, fish were fed Nutrasprint starter feed for trout (Skretting, Stavanger, Norway), the same feed used at the Gårdsfisk facility. It is a common practice for Gårdsfisk to raise tilapia on salmonid feed the ifrst part of their lives.

Fish were redistributed to balance the weight in each tank following the first stabilization period. While fish remained within their respective system types (A or R), the biomass was homogenized, and individuals below 1.00 g and over 14.00 g were removed from the experiment. The systems were re-stocked with 15 fish each with the following weights: A-systems: 7.80 ± 3.49, R1: 7.20 ± 4.00, R2 & R3: 7.40 ± 4.32.

Feeding trials

The experimental feeds used during the feeding trials were custom-formulated by the Swedish University of Agricultural Sciences (SLU) in Uppsala. Initially extruded as 2 mm floating pellets, the feeds were further ground into granules ranging from 0.3

to 2 mm in diameter to accommodate the size of the fish. This grinding process resulted in primarily slow-sinking pellets.

Each feeding trial lasted four weeks. The first trial employed a control feed designed to reflect a conventional commercial aquafeed, with fish meal and soy protein concentrate as the main protein sources (**Table 2**). The second trial utilized an ECO feed incorporating blue mussel meal and pea protein concentrate as alternative, regionally sourced ingredients.

Into A systems, 15 Tatsoi seedlings were randomly distributed across the three gutters, while 9 seedlings were placed in each H system.

Feeding protocol

Fish were hand-fed twice daily on weekdays (at 09:30 and 15:00), once on Saturdays (at 09:30), and on Sundays the fish were not fed. Uneaten feed was collected from each tank 30 minutes after feeding using a gentle siphoning technique. The recovered feed was dried at room temperature and weighed to calculate daily feed waste.

If feed waste was below 5 % for three consecutive days, the feed ratio was increased by 10 %. Conversely, if waste exceeded 10 %, the feeding amount was reduced to the previous level, following the approach described by Warwas et al., 2023.

2.3 Fish growth and biometrics

Initial, intermediate, and final biometric measurements were conducted across all three experimental phases, a total of nine measurement points during the study. Prior to each sampling event, fish were fasted for 24 hours. For sedation, 2 L of water from each system were transferred into a bucket with 50 mg L^{-1} Tricaine Methanesulfonate (MS-222, Finquel®, Argent Chemical Laboratories, Redmond, Washington, USA), buffered with 100 mg L^{-1} calcium carbonate ($CaCO_3$) to maintain a pH of 6.8–7.0.

During the stabilization period, 10 fish per system (A and R) were randomly selected using a net and placed in the anesthetic solution. During the feeding trials, all fish in each system (15 individuals) were handled using the same procedure. Once visibly sedated, fish were gently placed on a scale for weighing, and then transferred to a recovery bucket with continuous aeration until all fish had been measured and fully recovered.

At the final biometry of the ECO feed trial, which marked the end of the experimental period, a different protocol was applied for blood sampling and dissection. After a 24-hour fasting period, fish were anesthetized with 12 mg L^{-1} metomidate hydrochloride (Aquacalm, Syndel, Canada) dissolved in water for deep sedation. Eight of the representative fish from each system were selected and placed in the solution. Once deeply anesthetized, fish were weighed, measured, and photographed.

Table 2. Ingredient composition and nutritional values of the Control and ECO feed. The table is adapted after recipe from Aleksandar Vidacovic (SLU, Uppsala).

Feed Ingredient (%)	**Control**	**ECO**
Fish meal	9.60	–
Maize meal	11.04	5.00
Wheat gluten	10.00	10.00
Wheat meal	18.25	18.00
Pot starch	3.00	1.00
Fish oil	–	–
Rapeseed oil	3.00	1.73
Wheat bran	–	9.11
Poultry meal	17.00	17.00
Guar gum Suncol 205	2.00	2.00
Pea concentrate (AMN-30835 IAAFD)	–	15.50
Soy protein concentrate	23.00	–
Baltic mussel meal	–	17.90
Choline chloride	0.01	0.01
Vitamin mineral premix	1.00	1.00
Lysine sulfate	0.30	–
DL-methionine	0.30	0.25
Monocalcium phosphate	1.50	1.50
Total	100.00	100.00
Nutritional Composition		
Crude Protein (%)	42.66	42.83
Digestible Protein (%)	38.39	38.11
Gross Energy (MJ kg_{-1})	18.13	18.59
Digestible Energy (MJ kg_{-1})	15.32	15.19
Crude Fat (%)	7.62	7.61
Lysine (g kg_{-1})	25.79	28.43
Methionine (g kg_{-1})	10.15	10.42
Phosphorus (%)	1.38	1.26
DP:DE	2.51	2.51
Digestible Phosphorus (%)	0.97	0.89
Fe (mg kg_{-1})	76.84	72.71
Mg (%)	0.13	0.14

Blood samples were collected via caudal vessel puncture using a 23-gauge-needle and a 1 mL heparinized syringe to prevent coagulation. Samples were transferred into 0.5 mL Eppendorf tubes and centrifuged at 10,000 rpm for 5 minutes. The resulting plasma was aliquoted into new tubes and frozen on dry ice before storage at –80°C for later analysis.

Following blood sampling, fish were dissected to collect and weigh the liver, and

their sex was recorded. The remaining fish were sedated using the MS-222 protocol as previously described, then weighed, photographed, and euthanized to conclude the trial.

2.4 Growth parameters

The following growth performance indices were calculated after the feeding trial; averaged per experimental tank, experimental type of system and per fish where applicable. Using the initial weight (IW), final weight (FW), duration of the trial in days (d), feed consumption (FC), number of fish in the system (n), weight gain (WG), liver weight (LW), number of fish at the start (FI), and at the end (FF) of the feeding trial:

$$\text{WG (Weight gain, g)} = \text{FW} - \text{IW}$$

$$\text{SGR (Specific growth rate, \%W d}^{-1}) = \left(\frac{\ln(\text{FW}) - \ln(\text{IW})}{\text{d}}\right) \times 100$$

$$\text{FCR (Feed conversion ratio)} = \frac{\text{FC}}{\text{WG}}$$

$$\text{HSI (Hepatosomatic Index, \%)} = \left(\frac{\text{LW}}{\text{FW}}\right) \times 100$$

$$\text{Survival (\%)} = \left(\frac{\text{FF}}{\text{FI}}\right) \times 100$$

2.5 Cortisol analysis

Radio immuno assay (RIA) was used to determine cortisol concentrations in the blood plasma of fish as described by (Young, 1986) using a cortisol antibody validated by (Sundh et al., 2011). Shortly, each sample, run in duplicate, was placed in a test tube containing a known amount of radioactively labeled cortisol, ^{3}H (tracer). An antibody specific to cortisol was then added. The labeled and unlabeled cortisol competed for binding to the antibodies. As the cortisol concentration in the sample increases, less of the labeled cortisol binds to the antibody — therefore, a sample with high cortisol levels will result in lower measured radioactivity.

After incubation, the bound and free hormones were separated, and the radioactivity of the bound fraction was measured using a beta counter. A standard curve, based on eleven known cortisol concentrations made out of hydrocortisone, ranging from 0.5 to 512 ng mL^{-1} (prepared by serial dilution and run in duplicate), was used to calculate the cortisol concentration in each sample. A reference plasma sample containing a known concentration of cortisol was included in the assay to verify its accuracy and reliability.

2.6 Plant growth

Plant growth was monitored weekly throughout all experimental phases. During the stabilization phase, 10 plants from each A system and 5 from each H system were randomly selected for measurement. In the feeding trials, all plants were measured—15 in A systems and 9 in H systems. Plant height (cm) was recorded using a ruler.

2.7 Water quality

Temperature (°C), pH, electrical conductivity (EC), and total dissolved solids (TDS) were measured daily in the fish tanks of both A and R, as well as in the hydroponic water reservoirs in H, using a multiparameter probe (Combo, Hanna Instruments, Woonsocket, RI, USA). In addition, water quality was monitored twice weekly using commercial photochemical test kits (Hach Lange GmbH, Düsseldorf, Germany). NH_4^+, NO_2^-, and NO_3^- concentrations were measured with test kits LCK303, LCK341, and LCK339, respectively, using a DR3900 spectrophotometer (Hach Lange GmbH, Düsseldorf, Germany). Water consumption was measured, and the tanks were filled up accordingly with freshwater weekly.

Additionally, water samples from the fish tanks, biofilters, and hydroponic units were analyzed by a commercial lab for nutrient analysis (LMI, Helsingborg, Sweden) for micro- and macronutrients in the first five weeks and at the end of the stabilization phase, as well as initial and intermediate for Control feed and intermediate and final for ECO feed.

2.8 Microbial content

A microbiological assessment was conducted through viable counting of both water and root samples. Microbial populations were enumerated using selective culture media, each targeting specific groups of microorganisms. All media were prepared using 1000 mL of distilled water:

(I) Tryptic Soy Agar (TSA) for the enumeration of general bacteria (80 g TSA; DIFCO 0369-17-6).

(II) Malt Extract Agar (MA) for the enumeration of general fungi (half-strength – 10 g MA DIFCO 0186-17-7 and 20 g agar).

(III) King's B Agar (KB) supports the growth of *Pseudomonas* spp. and other fluorescent bacteria (1.5 g K_2HPO_4, 1.5 g $MgSO_4 \cdot 7H_2O$, 15 mL glycerol, 20 g proteose peptone (no. 3 Difco), and 15 g agar).

(IV) Violet Red Bile Dextrose (VRBD) applied only to water samples from the fish tanks for the enumeration of *Enterobacteriaceae* spp. (39.5 g Crystal-violet neutral-red bile dextrose agar).

Water samples were collected from the fish tank, biofilter, and hydroponic water

unit at the end of each experimental phase, in connection with the plant harvest. Additionally, root samples were collected by harvesting 10 grams from 3 randomly selected plants from each A and H system. The samples were placed into vials containing system-specific water.

To prepare the root samples for microbial analysis, 50 mL of a detergent solution [0.1 % (w v^{-1}) peptone in 0.2 % (w v^{-1}) sodium hexametaphosphate] was added to each vial (Khalil & Alsanius, 2009). The vials were then shaken at 200 rpm for 20 minutes at room temperature to dislodge bacteria from the root surfaces into the surrounding water.

Microbial populations were quantified using serial dilution (**Fig. 6**). Five sterile test tubes were each filled with 4.5 mL of sterile 0.9 % NaCl solution. A 0.5 mL aliquot of the original sample was added to the first tube and thoroughly mixed. Serial 0.5 mL transfers were then made from tube to tube, with mixing between each step to ensure homogeneity.

Aliquots from each dilution were placed onto the culture media. For each dilution, two replicates were prepared for all water and root samples with spread techniques. For the stabilization phase, 1 mL of the sample was spread across the agar surface using sterile glass beads. For the feeding trials, plates were divided into two sections for replication: five 20 µL drops from the appropriate dilution were placed on each half of the plate.

All plates were incubated at 25°C for 24–36 hours. Following incubation, colony-forming units (CFUs) were counted. Only plates with fewer than 300 colonies were considered for enumeration to ensure reliability and minimize error due to overlapping colonies. The $\log_{10}(\text{CFU mL}^{-1})$ and $\log_{10}(\text{CFU g}^{-1})$ were calculated on a system type basis as follows:

$$\log_{10}(\text{CFU mL}^{-1}) \;\&\; \log_{10}(\text{CFU g}^{-1}) = \log_{10}\left(\frac{\text{Number of colonies} \times \text{Dilution factor}}{\text{Volume plated (mL)}}\right)$$

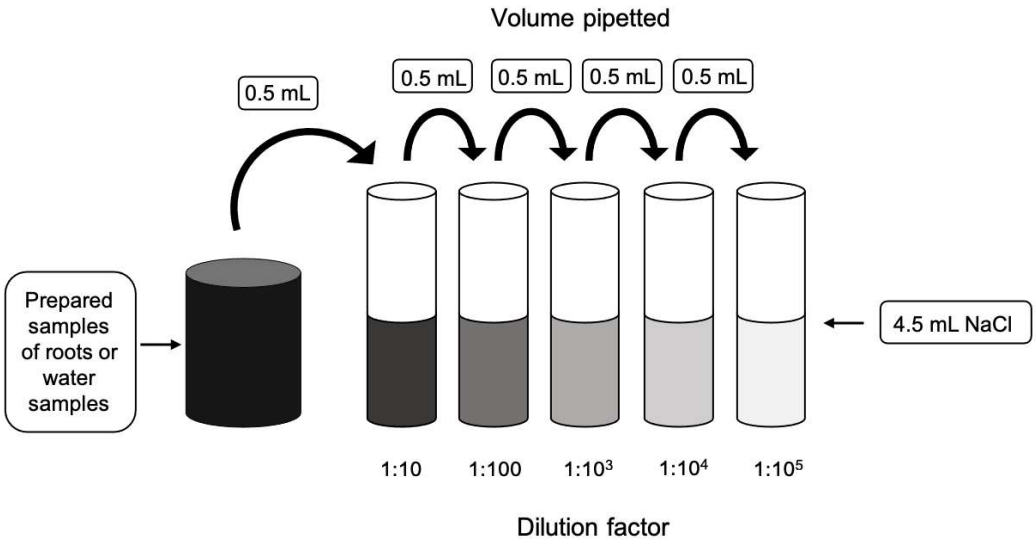


Figure 6. Schematic figure of the serial dilution procedure.

2.9 Statistical analysis

Statistical analyses were conducted using the Student's t-test for independent samples to compare mean differences between treatment groups. Welch's correction was applied in cases of unequal variances. A 95 % confidence interval was used for all comparisons, and significance was set at $p < 0.05$. Analyses were performed using R version 4.4.1.

3. Results

3.1 Growth and biometrics

3.1.1 Fish

Growth

Nile tilapia had an initial weight of 0.15 ± 0.05 g (**Fig. 7**). During the stabilization period, fish from A grew significantly more than fish from R, 9.18 ± 6.62 g and 6.53 ± 5.63 g respectively (T-test, p-value=0.04). The total initial biomass was higher in A than in R at the beginning of the control feed trial: 117 ± 0.00 and 110 ± 1.73 respectively (T-test, p-value = 0.020) (**Table 3**). Fish in the R systems showed slightly higher specific growth rates (SGR) than A in the control feed trial: 3.56 ± 0.06 % day^{-1} and 3.49 ± 0.26 % day^{-1} respectively, and in the ECO feed trial significantly higher: 2.35 ± 0.16 % day^{-1} and 1.87 ± 0.17 % day^{-1} respectively. The FCR was comparable between A and R in the control feed trial (0.62 ± 0.08 and 0.64 ± 0.03 respectively), whereas in the ECO feed trial the FCR was higher in A than in R (1.07 ± 0.14 and 0.83 ± 0.05 respectively).

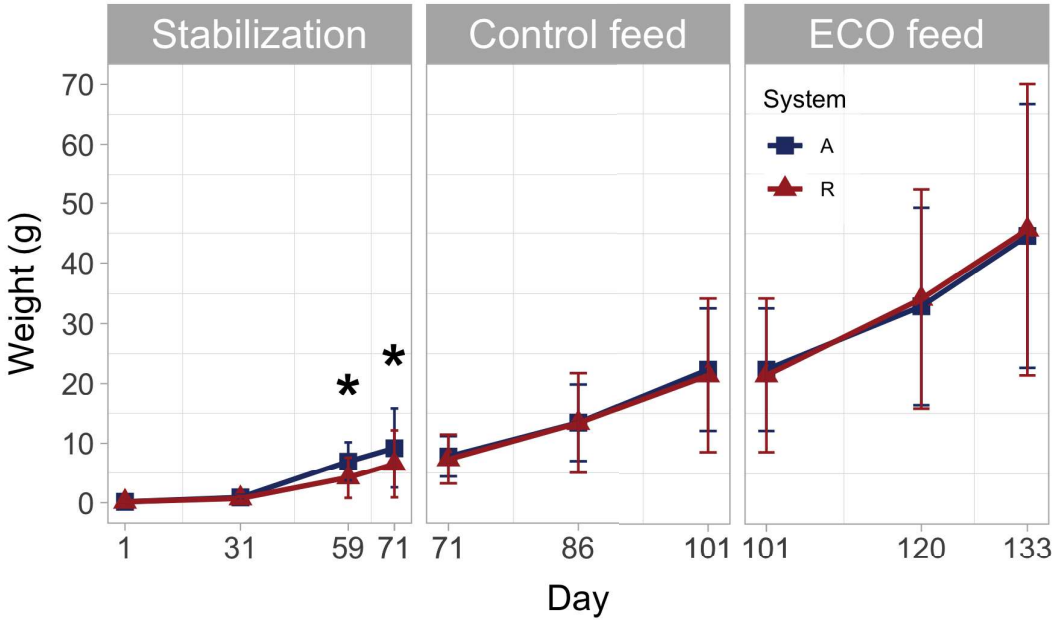


Figure 7. Average body weight (g) of Nile tilapia in aquaponic systems (A), and RAS (R) throughout the experimental period. The experiment was divided into three phases: Stabilization (71 days), Control feed (31 days), and ECO feed (33 days). Data are presented as mean ± SD (n = 3).
* Statistically significant at $p < 0.05$ from T-test.

Plasma

The average cortisol concentrations in A and R systems were 28.70 ± 30.50 ng mL^{-1} and 30.40 ± 24.80 ng mL^{-1}, respectively, with no significant differences observed between the system types. However, a distinct tank effect was evident, as cortisol levels varied considerably among replicates. Notably, A3 exhibited significantly lower ($p < 0.05$) cortisol concentrations (4.60 ± 4.80 ng mL^{-1}) compared to both A1 (43.50 ± 23.80 ng mL^{-1}) and A2 (38.00 ± 38.10 ng mL^{-1}). A3 also differed significantly from R1 (43.70 ± 24.70 ng mL^{-1}) and R3 (27.30 ± 25.30 ng mL^{-1}) (**Table 4**).

Table 3. Growth performance, feed utilization, and survival of Nile tilapia in aquaponic systems and RAS during the feeding trials. Values are presented as mean ± SD. P-values are according to analysis with T-test.

Control feed **Parameter**	**Aquaponic**	**RAS**	**p-value**
Initial individual weight (g)[1]	7.80 ± 3.41	7.33 ± 4.12	0.560
Final individual weight (g)[1]	22.30 ± 10.21	21.34 ± 12.82	0.696
Individual weight gain (g)[2]	14.50 ± 1.75	14.01 ± 0.52	0.684
Initial total weight (g)[2]	117.00 ± 0.00	110.00 ± 1.73	0.020*
Final total weight (g)[2]	334.48 ± 26.38	320.14 ± 9.31	0.452
Total weight gain (g)[2]	217.48 ± 26.38	210.14 ± 7.81	0.683
Specific growth rate (% day^{-1})[2]	3.49 ± 0.26	3.56 ± 0.06	0.706
Feed intake (g tank^{-1})[2]	134.39 ± 1.69	133.37 ± 1.05	0.434
Feed conversion ratio (FCR)[2]	0.62 ± 0.08	0.64 ± 0.03	0.836
Survival (%)[2]	100.00 ± 0.00	100.00 ± 0.00	1.00
ECO feed			
Initial individual weight (g)[1]	22.30 ± 10.21	21.34 ± 12.82	0.696
Final individual weight (g)[1]	44.62 ± 22.02	45.67 ± 24.33	0.834
Individual weight gain (g)[2]	22.26 ± 1.03	24.33 ± 1.50	0.131
Initial total weight (g)[2]	334.48 ± 26.38	320.14 ± 9.31	0.452
Final total weight (g)[2]	609.84 ± 55.54	678.47 ± 16.61	0.157
Total weight gain (g)[2]	275.35 ± 37.20	358.34 ± 24.93	0.039*
Specific growth rate (% day^{-1})[2]	1.87 ± 0.17	2.35 ± 0.16	0.025*
Feed intake (g tank^{-1})[2]	291.89 ± 5.13	296.30 ± 2.98	0.283
Feed conversion ratio (FCR)[2]	1.07 ± 0.14	0.83 ± 0.05	0.082
Survival (%)[2]	96.67 ± 3.34	100.00 ± 0.00	0.226

[1] The average values are calculated for n = 45 for A and R with Control feed, and n = 43.5 for A (adjustment due to mortality), and n = 45 for R with ECO feed.
[2] The average values are calculated for n = 3 (system replicates).
* Statistically significant at $p < 0.05$ from T-test.

Table 4. Cortisol concentration (mean ± SD) in Nile tilapia and sample size (n) per system replicate and system type.

System	n	Cortisol (ng mL $^{-1}$)
A1	8	43.55 ± 23.76 ^{a}
A2	8	37.98 ± 38.14 ^{a}
A3	8	4.58 ± 4.76 ^{b}
R1	7	43.66 ± 24.66 ^{a}
R2	8	21.98 ± 22.33 ^{ab}
R3	8	27.33 ± 25.27 ^{a}
A	24	28.70 ± 30.50 ^{a}
R	23	30.44 ± 24.76 ^{a}

Letters indicate significant differences ($p < 0.05$) between replicates.

3.1.2 Plants

Distinct growth patterns were observed between A and H systems across all experimental phases (**Fig. 8**). During the Stabilization phase, plants were generally taller in H than in A, with significant differences from day 12 to 33 (**Table 5**). However, from day 40 onward, plant height in H systems began to decline, whereas plants in A systems continued to grow, ultimately surpassing H in average height.

In the Control feed trial, plant height was consistently greater in A systems, with significant differences observed from day 14 through day 24. During the ECO feed trial, highly significant differences were evident throughout the entire period (day 11 to day 28), with A systems demonstrating markedly greater growth performance.

By the end of the feeding trials, average plant height in A systems reached 14.00 ± 2.76 cm with the Control feed (day 24) and 14.31 ± 2.45 cm with the ECO feed (day 28). In contrast, H systems reached lower final heights of 12.04 ± 2.11 cm and 5.48 ± 1.44 cm, respectively. Compared to a similar time point during the Stabilization phase (day 26), A systems exhibited lower growth (7.95 ± 2.86 cm), while H systems showed higher growth (13.39 ± 3.16 cm).

As shown in **Fig. 8**, plant growth during the feeding trials was more consistent and exhibited less variability compared to the Stabilization phase. For a detailed view of individual system replicates, see **Fig. S1** in the supplementary material.

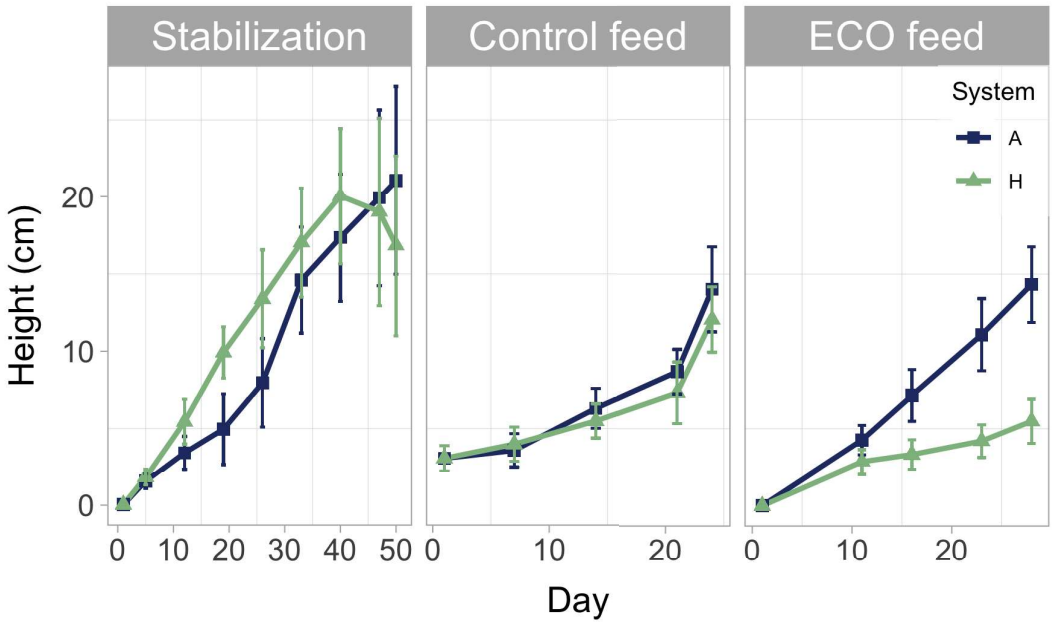


Figure 8. Average plant height (cm) of Tatsoi in aquaponic (A) and hydroponic (H) systems during the Stabilization period (50 days), Control feed trial (24 days), and ECO feed trial (28 days). Each system type included three replicates (n = 3), based on the following number of plants: Stabilization — A: n = 10, H: n = 5; Control and ECO feed trials — A: n = 15, H: n = 9. Data are presented as mean ± SD.

3.2 Water parameters

Water temperature (**Fig. 9a**) remained relatively stable in both A and R systems throughout all phases, although greater fluctuations were observed during the stabilization period. H systems consistently maintained lower temperatures than both A and R systems across the entire experimental period. pH levels (**Fig. 9b**) showed the greatest variability during the stabilization phase, followed by a gradual decline during the feeding trials in all systems, with H reaching the lowest values. Electrical conductivity (**Fig. 9c**) and total dissolved solids (**Fig. 9d**) displayed similar patterns, with higher levels in H systems across all phases. Meanwhile, A and R systems maintained lower, comparable levels that gradually increased over time.

3.3 N - Analysis

Ammonium

NH_4^+ concentrations fluctuated throughout the experiment (**Fig. 10a**). During the Stabilization period, NH_4^+ levels remained low in both system types, with A systems showing slightly lower concentrations but occasional high outliers, while R systems exhibited greater overall variability. In the Control feed trial, A systems experienced

higher NH_4^+ levels, while R remained at lower concentrations. In the ECO feed trial, A systems displayed moderate variability, though still less than R systems. Median values were similar, although R systems exhibited more extreme values and greater variability.

Nitrite

Overall, NO_2^- levels remained low throughout the experimental period (**Fig. 10b**). During the Stabilization period and Control feed trial, accumulation was minimal with limited variability, aside from a few outliers. In contrast, the ECO feed trial showed greater variation, particularly in A systems.

Nitrate

NO_3^- concentrations increased steadily across all phases in both A and R systems (**Fig. 10c**). During the Stabilization period, levels remained low in both systems, though R exhibited greater variability. Throughout the feeding trials, NO_3^- concentrations kept on accumulating, with R systems consistently showing significantly higher median values than A.

Table 5. Plant height (cm) and T-test results, comparing aquaponic and hydroponic systems across the experimental periods. Values represent mean ± SD.

Stabilization			
Day	**Aquaponic**	**Hydroponic**	**p -value**
1	0.04 ± 0.01	0.04 ± 0.01	1.000
5	1.56 ± 0.48	1.83 ± 0.52	0.104
12	3.42 ± 1.07	5.44 ± 1.45	< 0.001
19	4.96 ± 2.30	9.92 ± 1.65	< 0.001
26	7.95 ± 2.86	13.39 ± 3.16	< 0.001
33	14.60 ± 3.43	17.04 ± 3.55	0.037
40	17.35 ± 4.13	20.04 ± 4.40	0.059
47	19.94 ± 5.70	19.03 ± 6.08	0.631
50	21.07 ± 6.11	16.83 ± 5.84	0.008
Control feed			
1	3.07 ± 0.81	3.07 ± 0.81	1.000
7	3.58 ± 1.08	3.98 ± 1.12	0.143
14	6.31 ± 1.28	5.49 ± 1.11	0.006
21	8.66 ± 1.45	7.31 ± 1.98	0.003
24	14.00 ± 2.76	12.04 ± 2.11	0.005
ECO feed			
1	0.04 ± 0.01	0.04 ± 0.01	1.000
11	4.26 ± 0.96	2.86 ± 0.78	< 0.001
16	7.14 ± 1.66	3.32 ± 0.96	< 0.001
23	11.06 ± 2.33	4.20 ± 1.06	< 0.001
28	14.31 ± 2.45	5.48 ± 1.44	< 0.001

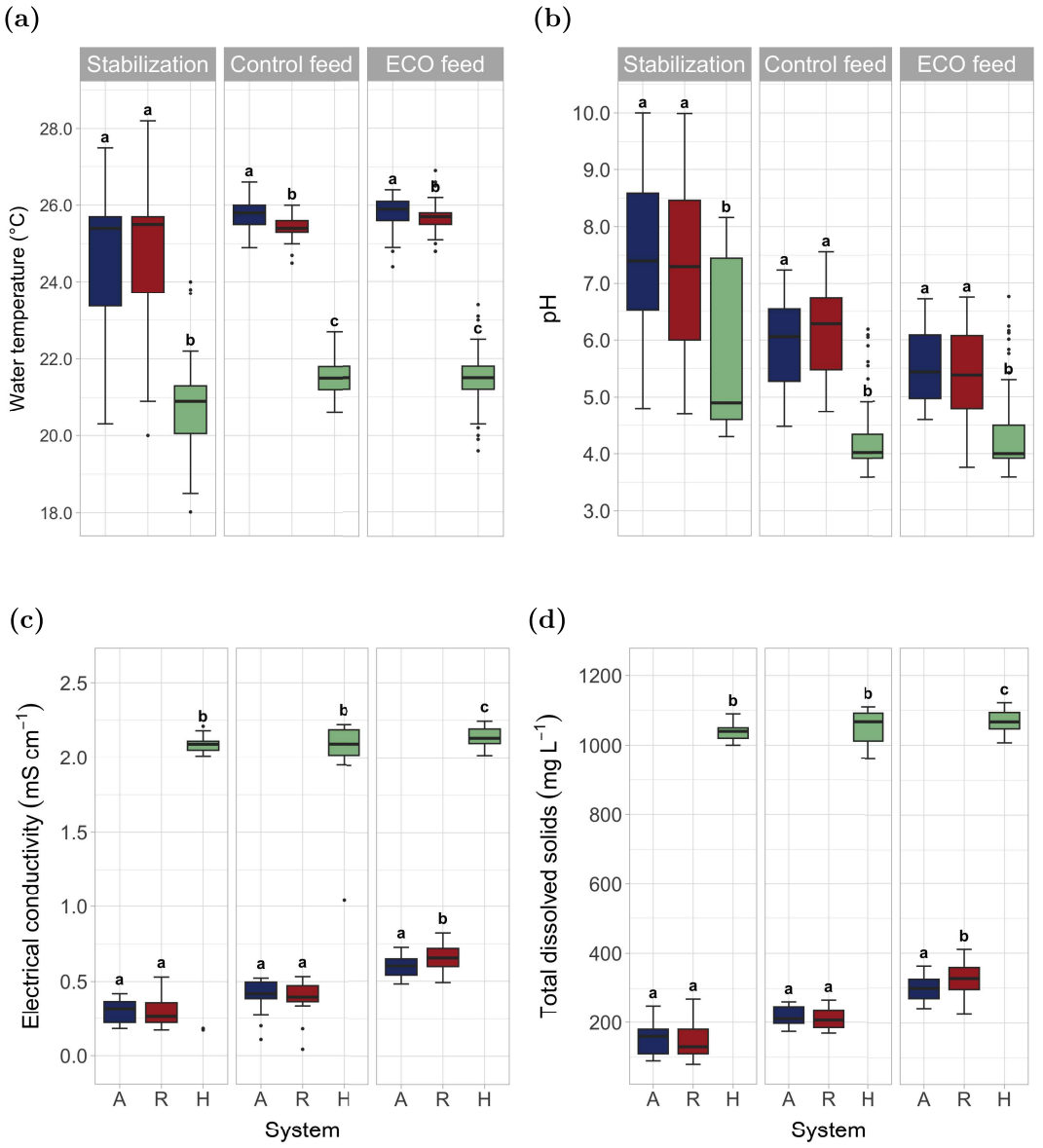


Figure 9. Boxplot overview of the measured fish tank water parameter variables: (a) water temperature, (b) pH, (c) electrical conductivity and (d) total dissolved solids. Measurements were taken daily during the Stabilization period (71 days), Control feed trial(31 days), and ECO feed trial (33 days) in the aquaponic- (A), RAS (R) and hydroponic systems (H), n=3. Points beyond the whiskers represent outliers. Letters indicate significance groups from T-test, $p < 0.05$.

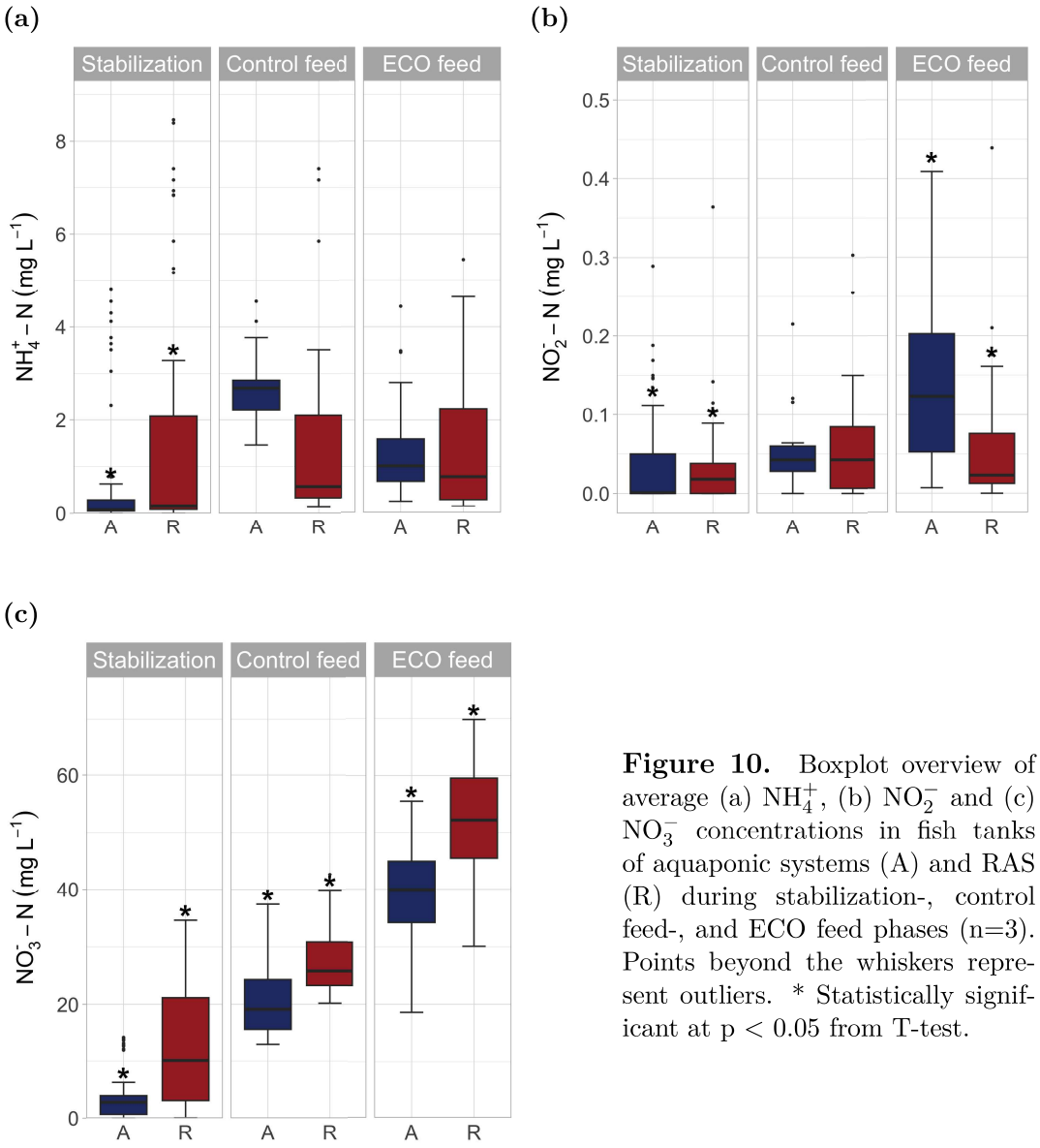


Figure 10. Boxplot overview of average (a) NH_4^+, (b) NO_2^- and (c) NO_3^- concentrations in fish tanks of aquaponic systems (A) and RAS (R) during stabilization-, control feed-, and ECO feed phases (n=3). Points beyond the whiskers represent outliers. * Statistically significant at $p < 0.05$ from T-test.

3.4 Microbial content

Microbial abundance showed distinct patterns across sample types, system types, and experimental phases (**Fig. 11**). Fish tank water samples (**Fig. 11a**) supported microbial growth on all four media. Notably, growth on King's B agar (KB), which targets *Pseudomonas* spp. and other fluorescent bacteria, was minimal and detected only in a single R system during the control feed trial.

During the stabilization phase, fungal counts on MA medium were generally higher in R systems compared to A systems. Bacterial counts on TSA medium were unde-

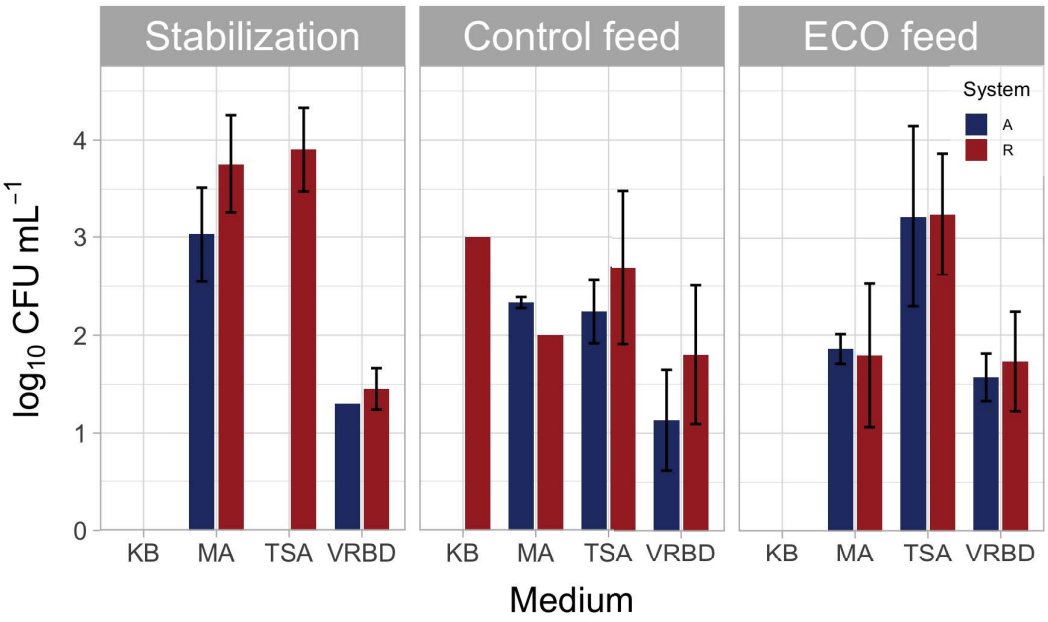
tectable in A systems, whereas they dominated the microbial profiles in R systems. As the systems matured, bacterial counts increased while fungal counts declined. This pattern was consistent across both system types. *Enterobacteriaceae* spp. (VRBD) were initially low but gradually increased over the course of the experiment, with consistently higher counts in R systems compared to A systems.

In biofilter samples (**Fig. 11b**), fungal growth was detected exclusively in R systems during the stabilization phase. During the control feed trial, both fungal and bacterial counts were slightly higher in A systems. However, this trend reversed in the ECO feed trial, with R systems exhibiting higher overall microbial loads. As the experiment progressed, a general decline in microbial abundance was observed in both system types, with bacterial populations continuing to dominate over fungi.

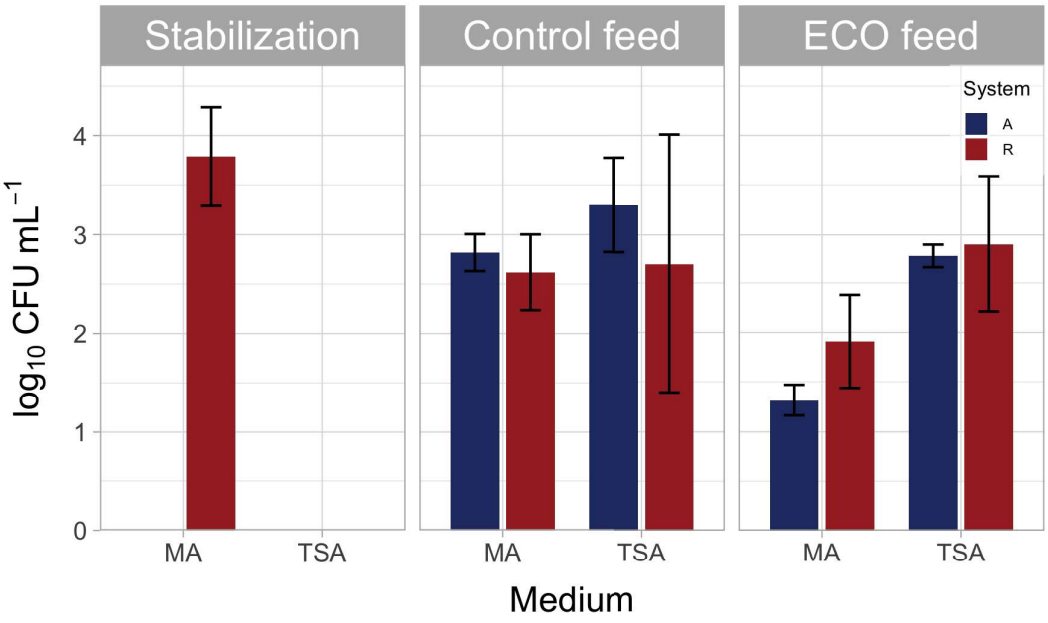
In the hydroponic units (**Fig. 11c**), only fungal growth was observed during the early stabilization period, with H systems showing higher counts than A systems. By the control feed trial, bacterial counts surpassed fungal counts in A systems, while microbial abundance in H systems declined to similar levels. In the ECO feed trial, bacterial growth was observed exclusively in A systems and clearly dominated over fungi, which remained low and comparable in both system types.

Root samples (**Fig. 11d**) were initially dominated by bacterial populations during the stabilization phase, with significantly higher counts in A systems than in H systems. Throughout the feeding trials, all microbial groups — including fungi, bacteria, and *Pseudomonas* spp.— were detected in both systems, with bacterial counts consistently dominating. A systems maintained slightly higher microbial loads than H systems. However, microbial abundance declined over time in both A and H systems.

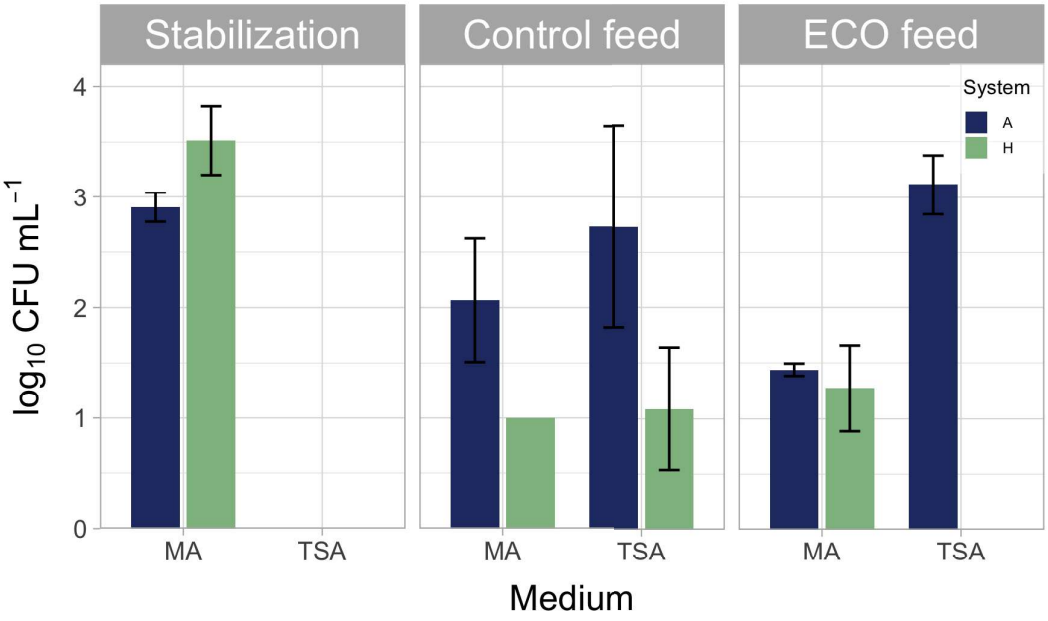
(a) Fish tank



(b) Biofilter



(c) Hydroponic unit



(d) Root samples

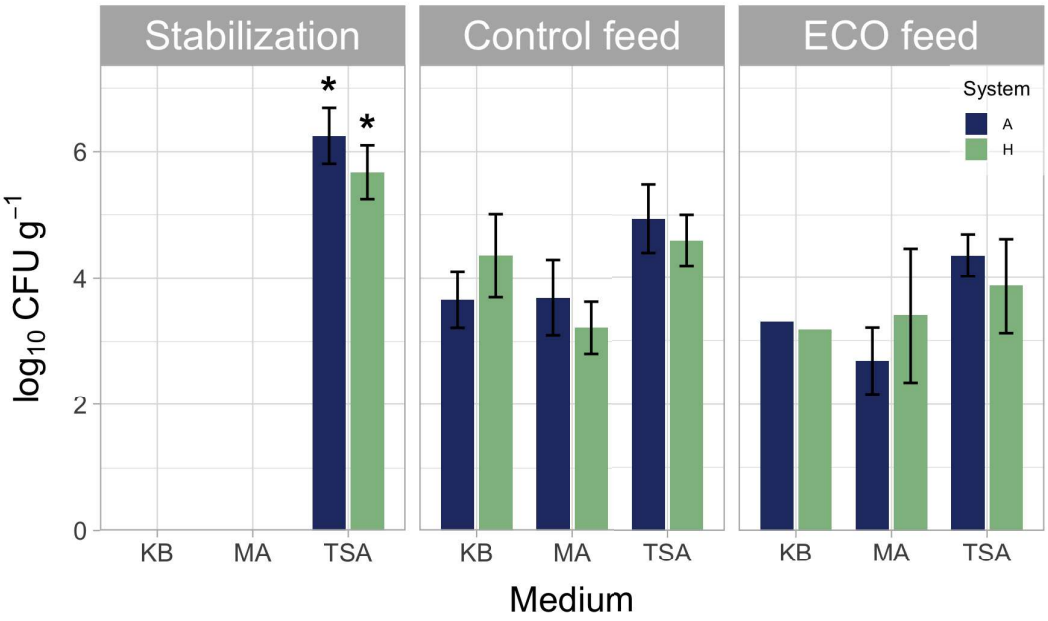


Figure 11. Microbial content is presented as the average $\log_{10}$(CFU mL^{-1}) of water samples from (a) fish tanks, (b) biofilters in aquaponic systems (A) and RAS (R), (c) hydroponic units in (A) and hydroponic (H) systems, and as average $\log_{10}$(CFU g^{-1}) from (d) root samples in (A) and (H) at the end of each phase. Enumeration was performed on: tryptic soy agar (TSA) for general bacterial flora, half-strength malt extract agar (MA) for general fungal flora, King's B agar (KB) for fluorescent *Pseudomonas* spp., and violet red bile dextrose agar (VRBD) for *Enterobacteriaceae* spp. The glass bead method was employed during the stabilization phase, while the drops technique was used during the feeding trials. Data are presented as mean ± SD, with n = 3.
* Statistically significant at $p < 0.05$ from T-test.

4. Discussion

This study explored the use of blue mussel meal and pea protein as sustainable alternatives to conventional fish feed ingredients in aquaponic systems with Nile tilapia and Tatsoi as model organisms. The findings contribute to a growing body of research supporting an undiscovered species-specific evaluation of this alternative feed up to this point.

Growth performance and feed utilization were similar between A and R systems during the control feed trial. However, in the ECO feed trial, fish reared in R systems showed better growth and feed efficiency compared to those in A systems. SGR values were high and FCR values were low in both systems under the control feed. In contrast, during the ECO feed trial, SGR declined—more noticeably in A systems—while FCR increased, again with a greater rise in A systems than in R systems.

These changes can be attributed to the natural progression of fish size and life stage. During the Control feed trial, fish were smaller, exhibiting higher metabolic efficiency and faster growth rates, resulting in lower FCR values. As the fish grew larger in the ECO feed trial, growth rates naturally declined and maintenance energy demands increased, contributing to lower SGR and higher FCR (Wainaina et al., 2023).

Furthermore, FCR has been reported to vary widely, not only between different studies, but also among the life stages of tilapia (larvae, fingerlings, and adults), and depending on whether the fish are reared in RAS or aquaponic systems (Rodríguez-Hernández et al., 2025). In aquaponic systems, an average FCR of 1.23 was calculated for fingerling-sized fish (10–20 g), compared to a slightly higher FCR of 1.41 in RAS (Rodríguez-Hernández et al., 2025). A recent comparison study showed an FCR of 1.13 in aquaponic systems and 1.27 in RAS for juvenile fish (Kiu et al., 2024).

Reported FCRs in aquaponic systems vary between 0.72 for fry (Amin et al., 2020), 1.38 and 1.47 for fingerling (Estim et al., 2019; Félix-Cuencas et al., 2021), and 1.14 – 1.27 depending on stocking density (Tawaha et al., 2020), up to 1.60 and 1.62 for juvenile fish (Effendi et al., 2017; Félix-Cuencas et al., 2021).

In RAS, FCRs for tilapia fry have been reported as low as 0.65 (Amin et al., 2020), with variations from 1.27 - 1.78 depending on stocking density for fingerlings (Gullian-Klanian & Arámburu-Adame, 2013), and 0.90 - 1.06 with increasing inclusion of pea concentrate as protein source (Schulz et al., 2007).

Moreover, productivity in the farming of Nile tilapia is influenced by several environmental and management factors, including temperature, pH, dissolved oxygen, feeding rate, and stocking density (Mengistu et al., 2020; Thoa et al., 2016). Optimal growth and feed efficiency in tilapia are achieved at the upper end of their preferred temperature range (27–32°C) (Mengistu et al., 2020). Water temperature remained stable at approximately 25 °C in both A and R systems throughout both feeding trials, which is rather low for optimal growth, although it is a compromise for the wellbeing of the plants as well (Sommerville et al., 2014).

While tilapia are capable of surviving and thriving across a broad pH spectrum, the best growth performance and FCR are observed within a pH range of 6–9 (Mengistu et al., 2020; Popma & Masser, 1999). During the Control feed trial, pH levels averaged around 6, whereas during the ECO feed trial, pH declined to values closer to 5–5.5 in both system types. Nile tilapia prefer slightly higher pH levels for optimal physiological performance, which may help explain the observed decrease in SGR and increase in FCR between the Control and ECO feed trials. Nonetheless, the growth performance and feed efficiency results from this study align with values reported in the available literature, suggesting that the experimental trials were successfully conducted and that both system types provided suitable rearing conditions for Nile tilapia.

The dietary shift to an alternative protein source in the ECO feed trial may have further influenced nutrient digestibility and feed utilization efficiency. Previous findings for inclusion of mussel meal in aquafeeds show that the responses vary considerably among species, underscoring the importance of species-specific evaluations.

In species such as Atlantic salmon, Rainbow trout, Turbot (*Scophthalmus* spp.), and Japanese flounder (*Paralichtys olivaceus*), diets containing blue mussel meal at inclusion levels between 10–25 % have proven effective in maintaining growth performance, feed acceptance, and nutritional quality (Azad et al., 2023; Berge & Austreng, 1989; Kikuchi & Sakaguchi, 1997; Nagel et al., 2014; Weiß & Buck, 2017).

Pea protein has likewise been studied as a fishmeal replacement. In Nile tilapia, up to 30 % pea protein was included without compromising growth performance (Schulz et al., 2007). Similar success has been reported with 20 % inclusion in Rainbow trout, Atlantic salmon, and gilthead sea bream (*Sparus aurata*) (Øverland et al., 2009; Pereira & Oliva-Teles, 2002; Thiessen et al., 2003), and with sea bass (*Dicentrarchus labrax*) tolerating replacements of up to 60 % (Tibaldi et al., 2005).

A combined diet containing 17.90 % blue mussel meal and 15.50 % pea protein concentrate in this study, resulted in less favorable growth performance and feed utilization compared to a conventional fishmeal- and soy protein-based diet. Nevertheless, the outcomes remain within the range of values reported in the existing literature, suggesting that these alternative ingredients hold potential as sustainable feed components for use in RAS and aquaponic systems.

Plasma cortisol levels serve as a key indicator of stress in fish, as cortisol is a primary hormonal response to both acute and chronic stressors (Balm et al., 1989). The mean plasma cortisol concentrations for Nile tilapia at the end of the ECO feed trial were 28.70 ng mL^{-1} in A and 30.40 ng mL^{-1} in R, which is in accordance with levels described in other studies. Basal plasma cortisol levels in tilapia have ranged from 16.43 to 39.22 ng mL^{-1} (Volpato & Barreto, 2001), 27 to 39 ng mL^{-1} (Roza e Silva et al., 2020), and 10 to 30 ng mL^{-1} (Correa et al., 2003).

However, substantial variation in cortisol levels among replicates indicated a tank effect. In particular, fish in tank A3 exhibited significantly lower cortisol concentrations compared to both A1, A2, R1, and R3. The most common triggers of acute stress responses in fish include handling and sudden environmental changes. In contrast, chronic stress is typically caused by factors such as overcrowding, repeated disturbances, and prolonged deterioration of water quality (Barcellos et al., 1999). Such tank-specific differences could be attributed to localized factors such as differences in tank microenvironments, social interactions, stocking densities, or other environmental stressors not captured at the system-wide level (Barcellos et al., 1999). In this study, it seems that the effectiveness in the sampling procedure was a possible cause for the varying plasma cortisol levels, showing acute stressors rather than chronic. Since the fish were to small to draw blood from up until the end of the ECO feed trial, there is no evidence of the latter.

Overall cortisol concentrations in both A and R systems were moderate and did not differ significantly between system types, suggesting that the rearing environment in both systems was generally conducive to maintaining fish welfare. Nonetheless, the observed intra-system variation highlights the importance of considering individual tank conditions when evaluating fish welfare in aquaponic and RAS setups.

There was a clear difference in NO_3^- accumulation between the fish rearing system types, with significantly higher levels observed in R due to the absence of plants

to absorb excess nutrients (Diver, 2006; Endut et al., 2011). In aquaponic systems, plant uptake contributes to the regulation of NO_3^- concentrations, supporting a more balanced and sustainable nutrient environment (Estim et al., 2019). In contrast, NO_3^- accumulation in RAS can reach potentially harmful levels, posing a risk to fish health, feed intake and growth (Mota et al., 2015). Although, in the scope of this study, no such tendencies were observed.

During the Stabilization phase, H systems initially outperformed A systems in plant growth. However, the latter gradually caught up as NO_3^- levels accumulated toward the end of the phase. Pantanella et al., 2012 found no difference in crop yield between hydroponic and aquaponic systems when NO_3^- concentrations exceeded 1.4 mmol L^{-1}, a finding supported by Graber and Junge, 2009, who observed similar results across three different vegetable species.

In the present study, comparable plant yields and quality were observed in both systems during the Control feed trial. However, during the ECO feed trial, significant differences emerged, with H systems showing notably poorer performance. This decline was likely linked to issues with the Hoagland solution; following its renewal after ten days, plant growth improved, indicating previous limitations in nutrient availability. Nutrient management in hydroponic systems can be challenging, as imbalances may arise if the chemical composition is not carefully monitored and maintained (Bunyuth & Mardy, 2024). Since the nutrient solution in the H systems was replaced at the start of each phase, these systems may have been more prone to instability. In contrast, the A systems maintained a more stable nutrient environment, gradually building up nutrient concentrations over time.

The comparable or even improved plant growth observed in A systems during the ECO feed trial suggests that the inclusion of blue mussel meal and pea protein in fish diets may have supported nutrient release conducive to plant uptake. These findings provide promising evidence for the use of this alternative, more sustainable aquafeed in integrated aquaponic systems.

The stabilization period of the experiment is critical for establishing biofiltration and the associated microbial communities (Sommerville et al., 2014). Trends in the study in availability and stability of NH_4^+, NO_2^-, and NO_3^-, suggest that it took approximately two months for the systems to reach a stable state. This period allowed for the establishment of sufficient microbial activity, which in turn improved nutrient processing and availability. Previous studies have shown that variations in organic matter loading can significantly alter microbial community composition in commercial RAS (Dahle et al., 2023), indicating that both time and nutrient input play important roles in shaping system microbiota.

Microbial abundance varied across sample types, system types, and experimental phases, with bacterial populations generally dominating over fungi. Fungal growth was more sporadic and showed a tendency to decline over time. By the end of the ECO feed trial, microbial counts in fish tank and biofilter samples were comparable between A and R systems, with bacterial populations continuing to dominate. Similar bacterial communities have been observed in both aquaponic and aquaculture systems, suggesting a shared core microbiota across system types (Eck, Sare, et al.,

2019; Schmautz et al., 2017).

In H units, early fungal dominance shifted to bacterial dominance in A systems, while microbial abundance in H systems decreased progressively. Root-associated microbial communities were initially more abundant in A systems and remained slightly higher throughout the experiment, though overall abundance declined over time in both A and H systems. *Pseudomonas fluorescent* were detected in moderate numbers in root samples during both the Control and ECO feed trials, but were otherwise only found in a single R system fish tank during the Control feed trial. This distribution likely reflects the ecological role of *Pseudomonas fluorescent*, being a rhizobacteria naturally associated with the root zone, where they are also known to produce metabolites that inhibit plant pathogens (Hultberg et al., 2008; Thomas et al., 2024).

There was a presence of pathogens *Enterobacteriaceae* spp. in both A and R systems, which have previously been reported in recirculating water systems (Khalil et al., 2021). The recirculating nature of RAS and aquaponic systems can promote pathogen persistence and transmission, as shared water flow facilitates system-wide dissemination (Kasozi et al., 2021; Lee & Lee, 2015). These findings highlight the importance of continuous microbial monitoring and the implementation of biosecurity measures in closed-loop systems.

The inclusion of blue mussel meal and pea protein in the ECO feed did not result in pronounced system-wide effects when compared to the control feed. This aligns with findings by Schmidt et al., 2016, who reported that replacing fishmeal with plant-based protein had no significant impact on the microbiome when rearing Atlantic salmon in freshwater, and the effect of dietary ratios did not affect the microbial abundance in a RAS with Rainbow trout (Huang et al., 2024).

However, the stable or even enhanced plant growth observed in A systems during the ECO feed trial suggests that nutrients derived from the alternative feed were effectively utilized by the plants, thereby supporting overall system balance. These results underscore the interconnected nature of aquaponic systems, where feed composition not only influences fish performance but also affects water chemistry, microbial interactions, and nutrient availability for plants.

Taken together, the findings of this study demonstrate that aquaponic systems can support both fish welfare and plant productivity when alternative protein sources such as blue mussel meal and pea protein are used in the feed. While growth performance and feed efficiency were slightly reduced in A systems compared to R, particularly during the ECO feed trial, plant growth in A systems remained stable and, in some cases, superior to that in hydroponic units. This suggests a trade-off between fish and plant performance that may be managed through careful system design and nutrient balancing. Furthermore, microbial community dynamics and stress indicators such as plasma cortisol supported the conclusion that both system types provided generally suitable rearing conditions. Nonetheless, working with small fish presents several limitations, particularly in terms of handling sensitivity and growth variation. Variability among replicates and compartments highlights the importance of system-specific monitoring, particularly during transitional phases.

Future studies should explore long-term effects of alternative feeds, the development of beneficial microbial communities, and the optimization of environmental parameters for integrated production.

5. Conclusions

This study evaluated the effects of alternative aquafeeds containing blue mussel meal and pea protein on the performance of Nile tilapia and Tatsoi in integrated aquaponic systems. While R systems supported slightly better fish growth under ECO feed conditions, A systems maintained stable plant production and microbial function. Overall, both system types showed performance values within the range of existing literature, confirming the feasibility of sustainable feed strategies in coupled aquaponic production. These findings contribute to the development of circular aquaculture solutions and support further investigation into environmentally friendly feed alternatives.

Acknowledgements

First and foremost, I would like to thank my supervisors, Samar Khalil and Jonathan Roques. Samar – thank you for the warm welcome at SLU in Alnarp and for your invaluable guidance throughout the experimental work. Jonathan – thank you for your insightful input on all things fish-related and for your constructive feedback along the way.

Thank you to Niklas Hjelm and Niclas Mörck at Grobruket for your practical support and help with the system setups, and big thanks to Aleksandar Vidakovic at SLU Uppsala for formulating the experimental feeds.

A heartfelt thanks to everyone at SLU Alnarp for making me feel welcome during my time there, and to everyone in the master's room in Bodø for the good company during long days of writing.

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A. Supplementary material

Table S1. Composition of Hoagland hydroponic nutrient solution.

Compound Name	Formula	mg L^{-1}
Ammonium nitrate	NH_4NO_3	30
Ammonium sulfate	$(NH_4)_2SO_4$	87
Boric acid	H_3BO_3	2.80
Calcium nitrate	$Ca(NO_3)_2 \cdot 4H_2O$	910
Copper sulfate heptahydrate	$CuSO_4 \cdot 7H_2O$	0.09
Ferrous sulfate heptahydrate	$FeSO_4 \cdot 7H_2O$	13
Magnesium nitrate	$Mg(NO_3)_2 \cdot 6H_2O$	34
Magnesium sulfate	$MgSO_4 \cdot 7H_2O$	320
Manganous chloride	$MnCl_2 \cdot 4H_2O$	1.53
Monopotassium phosphate	KH_2PO_4	67
Phosphoric acid	H_3PO_4	50
Potassium nitrate	KNO_3	556
Sodium molybdate dihydrate	$Na_2MoO_4 \cdot 2H_2O$	0.025
Zinc sulfate heptahydrate	$ZnSO_4 \cdot 7H_2O$	0.23

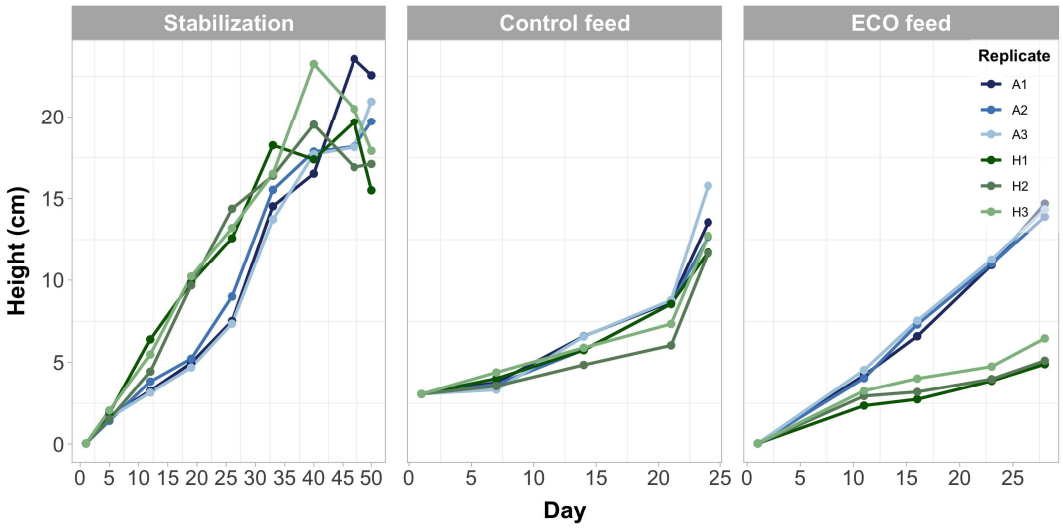
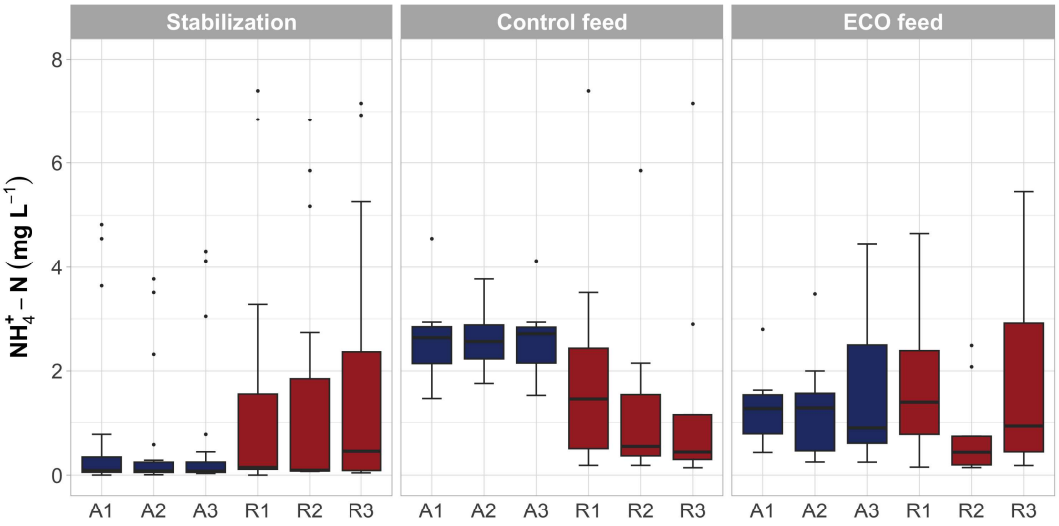
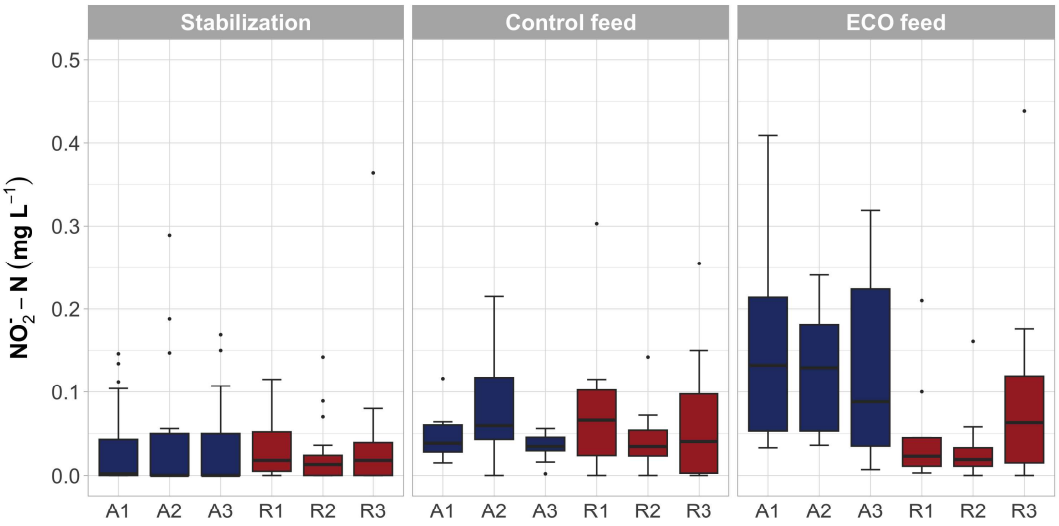


Figure S1. Line plot showing the average plant height (cm) in aquaponic (A) and hydroponic (H) system replicates across the experimental period. During the stabilization phase, n = 10 for A and n = 5 for H; during the feeding trials, n = 15 for A and n = 9 for H.

(a)


Stabilization
Control feed
ECO feed
$NH_4^+ - N$ (mg L^{-1})
0
2
4
6
8
A1 A2 A3 R1 R2 R3

(b)


Stabilization
Control feed
ECO feed
$NO_2^- - N$ (mg L^{-1})
0.0
0.1
0.2
0.3
0.4
0.5
A1 A2 A3 R1 R2 R3

(c)

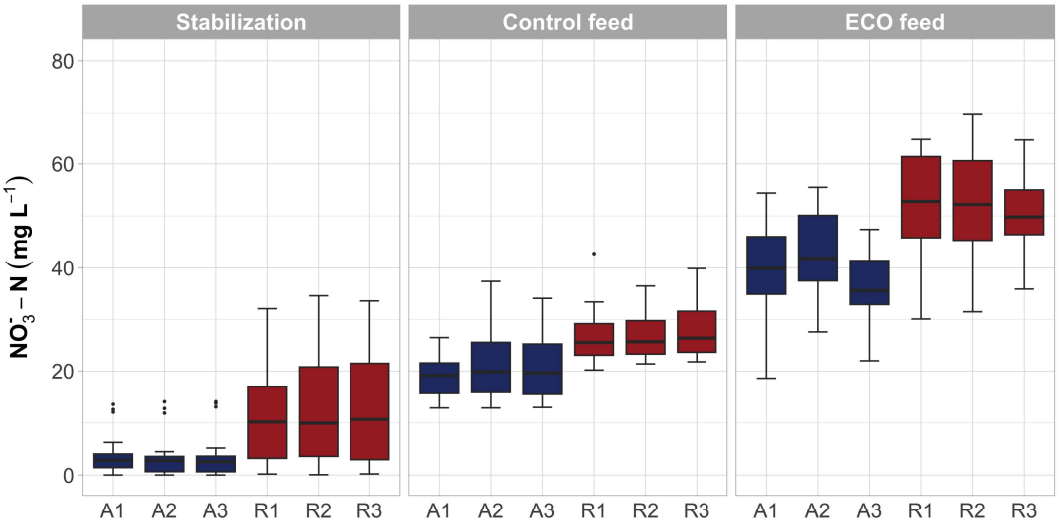


Figure S2. Boxplot overview of (a) ammonium, (b) nitrite and (c) nitrate in fish tanks of replicates of aquaponic systems (A) and RAS (R) during the Stabilization, Control feed, and ECO feed phases. Points beyond the whiskers represent outliers

B. Popular science summary

Feeding a growing global population in an environmentally responsible way is one of the greatest challenges of our time. As the number of people on Earth rises, so does the demand for food. Aquaculture, the farming of aquatic animals, has become the fastest growing food sector in the world. However, while it offers a promising alternative to overfishing and agricultural methods, aquaculture still faces sustainability challenges.

One of the biggest issues in aquaculture is the feed. Traditionally, fish feed has relied heavily on fishmeal made from wild caught fish and soy protein, which is often imported and linked to deforestation and high carbon emissions. These ingredients are expensive, environmentally damaging, and raise concerns about long-term availability. Another key challenge in aquaculture is the loss of nutrients from the system. When not reused, it is a huge waste of valuable resources which can also lead to pollution of the environment. To make aquaculture truly sustainable, a shift toward circular, sustainable systems is important.

This study explored the use of two regionally available and more sustainable protein sources in Sweden: blue mussel meal and pea protein concentrate, as substitutes for traditional feed ingredients. The research focused on Nile tilapia, a commonly farmed freshwater fish, raised in aquaponic systems. Aquaponics combines fish farming with plant cultivation in a resource-efficient, closed-loop system, where fish waste fertilizes plants.

Experimental trials were set up to compare a conventional feed to the alternative feed. The fish were raised in two types of systems: traditional recirculating aquaculture systems (RAS) and aquaponic systems. In addition to fish growth and feed efficiency, the study evaluated water quality, Tatsoi plant growth, and microbial growth, factors that are critical to the health and balance of the system.

The results were promising. In the alternative feed trial, the fish in aquaponic systems showed high growth and feed efficiency, only slightly below the results from the conventional feeding trial. Fish reared in RAS had marginally better growth, but the difference was small and remained within acceptable limits. Aquaponic systems maintained stable water quality and supported healthy, sometimes superior, plant growth throughout the trial.

Microbial analysis and cortisol levels (an indicator of stress) showed that fish welfare in aquaponic systems was comparable to that in RAS, suggesting that the integrated design did not harm animal health. In fact, aquaponics offers added benefits, such as improved resource use, nutrient recycling, and the ability to produce both fish and vegetables in one system.

Overall, the study supports blue mussel and pea protein as sustainable alternatives to fishmeal and soy in aquafeeds. When combined with circular systems such as aquaponics, these ingredients can reduce dependence on imported and marine resources, helping to make aquaculture more resilient, eco-friendly, and regionally adapted.