Deliverable 20.1
Analysis of access provided by WU aquaculture experimental facilities: types and users

Version 2

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Executive Summary

Objectives

WU offers two infrastructures, the metabolic research unit (WU-MRU) and the independent RAS (WU-RAS).
The WU-MRU offers a research environment for studies on nutrient and energy balances and metabolism in fish (both over a production cycle and for within-day variations). The research questions in the metabolic research unit relate to how animal factors (genetics, phenotypic differences, and health status), nutritional factors and environmental factors (temperature, oxygen concentration, carbon dioxide concentration, stocking density, sex ratio and housing conditions) affect responses of animals.
The WU-RAS is used to measure how nutritional, animal, environmental and management factors (including RAS configuration) affect water quality, water treatment unit performance, waste production and waste discharge (system level, WU-RAS).

Main Results

Three TNA projects were completed (two for the WU-MRU and one for the WU-RAS), one visit/experiment is currently running (WU-RAS), and two more TNA projects are scheduled (approved) on short notice (WU-MRU). So far, pike perch, Nile tilapia, and rainbow trout have been used; the current TNA is with Atlantic salmon, and the remaining two experiments will be with seabass and rainbow trout. The guest researcher was trained/supported in the design of the experiment (writing/assisting in the writing of the protocols required for approval and use during the experiment) and obtained while visiting some hands-on experiments.

Overview completed TNA's:
PID: 23110 - Adjustment of trace mineral nutrition of trout grown in RAS
8 units of access; one person trained: Claudia Figueiredo Silva

PID: 24853 - Bioavailability of novel protein sources for pikeperch (*Sander lucioperca*) juveniles
8 units of access; one person trained: Hung Quang Tran

PID:32385 - Hempseed as an alternative protein source for Nile tilapia (*Oreochromis niloticus)*
8 units of access; two persons trained: Stuart McMillan, Monica Betancor

Pending/scheduled TNA's:
PID: 34992 - Effect of coagulation-flotation on phosphate removal, water quality, biofilter efficiency, and fish performance in recirculating aquaculture systems (RAS)
8 units of access;

PID: 35091 – Exploring the effects of increasing levels of insect chitin on the digestibility of diets for European seabass and chitin metabolism
8 units of access;

PID: 34788 – The effect of Milk Fat Globule Membrane in diets of Rainbow Trout (Oncorhynchus mykiss) on productive parameters, digestibility, fat deposition and liver health.
8 units of access;

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Authors/Teams involved:

For WU, scientific supervision was done by Marit Nederlof, Fotini Kokou, Johan Schrama and Roel Maas. Additional supporting staff to facilitate the stay of the TNA guests was involved, as was staff at the animal facilities.

Wageningen University (NL): Marit Nederlof, Fotini Kokou, Johan Schrama, Roel Maas
Zinpro Corporation (USA): Claudia Figueiredo Silva
Institute of Aquaculture and Protection of Waters (IAPW) (CZ): Hung Quang Tran
The University of Stirling (UK): Stuart McMillan, Monica Betancor
Norwegian University of Life Sciences (NO): Kiran Vettathkuzhuppully Subash, Vasco Mota
CIIMAR - Interdisciplinary Centre of Marine and Environmental Research PT): Rafaela Silva Costa, Luísa Maria Pinheiro Valente
Nukamel N.V. (BE): Evi Croes, Hans Boon

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1. Overview of TNA users projects realized in WU

1.1.1. Installations

WU-MRU

The WU-MRU consists of twelve metabolic chambers (200L each) linked to a recirculation system with a total water volume of ± 7m3. The recirculation system is equipped with an independent water quality (pH, salinity, temperature) measurement and control system. The metabolic unit is placed in a room with adjustable photoperiod. It has on-line measurement of actual and cumulative water flow per metabolic chamber, oxygen, temperature, pH, conductivity (µS), salinity, CO2 production/consumption, TAN, urea, NO2-N, NO3-N, dissolved protein, and PO4-P in the rearing water, using an auto-analyzer (Type San autoanalyzer adapted with flow through cuvettes, Skalar, Breda, The Netherlands). The twelve metabolic chambers can be equipped with a mobile feeding registration system. Mobile faecal collection units (12 sedimentation funnels) can be used to study the digestibility of feed nutrients. Mobile webcams (N=16) and imaging analysis software are available to record and analyse behavioural data. The metabolic research unit is equipped with a data acquisition system in which all data can be stored and made available in excel spreadsheets for later analysis. The unit can be connected to two identical RAS differing in water quality (pH, salinity, water exchange rate, nitrate level) to study the effects of these factors on the response of fish.


Picture WU-MRU

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WU-RAS

Fish Production Systems research is performed in 6 replicated RAS. An auto analyser is available for online measurements of TAN, urea, NO_2-N, NO_3-N, dissolved protein, CO_2 and PO_4-P in the rearing water of the RAS. Fish species used in research include: rainbow trout, Atlantic salmon, Nile tilapia, European eel, African catfish, Inbred and outbred lines of common carp, seabass, turbot, sole and Yellowtail Kingfish. The WU-RAS: (1) can be used with a drum filter or settling unit; (2) can be extended with a single sludge denitrification reactor (4 reactors available); (3) can be used to test different biofilters (trickling vs. moving bed, different biofilter media, etc.) (4) enables researchers to perform experiments for both freshwater and marine fish (salinity can be varied from 0 to 35ppt) and; (5) for cool and warm water fish (water temperature can be controlled between 12 and 32 °C).

Picture WU-RAS

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1.1.2. User projects

Min. quantity of access units to be provided according ther DoA: 48
Total number of access units (sum of access units in the table): 48

Installation number	Installation code	Project title	Project acronym	Description about the experiment	Coordinator	Already used installation (Yes/No)	Nature of the access unit*	Number of used access units during the project	(Potential) paper	How many people was trained by this procedu ?
1	WU-RAS	Adjustment of trace mineral nutrition of trout grown in RAS	TRACEMINERAL-RAS	Feeding trial with trout to compare mineral sources. Mineral availability and accumulation in water were examined.	Claudia Figueiredo Silva	Yes	All systems/week	8	Yes, results look promising and have been presented already at international conference.	1
2	WU-MRU	Bioavailability of novel protein sources for pikeperch (*Sander lucioperca*) juveniles	AlProSan	Feeding trial with pike perch to evaluate protein sources. Measuring oxygen consumption, ingredient digestibility, and fecal characteristics	Hung Quang Tran	Yes	Whole system/week	8	Yes, TNA user is planning to write a paper.	1
3	WU-MRU	Hempseed as an alternative protein source for Nile tilapia (*Oreochromis niloticus)*	OreoHEMP	Feeding trial with Nile tilapia to assess hempseed as a protein source. Measuring oxygen consumption and assessing ingredient digestibility. ONGOING	Stuart McMillan	Yes	Whole system/week	8	Yes, expriment was succesfull, all data analyses almost complete.	2

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4	WU-RAS	Effect of coagulation-flotation on phosphate removal, water quality, biofilter efficiency, and fish performance in recirculating aquaculture systems (RAS)	CLEAN-RAS	Testing the effect of a protein skimmer and coagulant on water quality, including ortho-P removal and COD.	Kiran Vettathkuzhuppully Subash	No	All systems/week	8	Yes, as part of the PhD of the TNA user. Experiment still ongoing.	2
5	WU-MRU	Exploring the effects of increasing levels of insect chitin on the digestibility of diets for European seabass and chitin metabolism	ChiBass	SCHEDULED	Rafaela Silva Costa	No	Whole system/week	8	Yes, as part of the PhD of the TNA user. Experiment is scheduled.	1 or 2
6	WU-MRU	The effect of Milk Fat Globule Membrane in diets of Rainbow Trout (Oncorhynchus mykiss) on productive parameters, digestibility, fat deposition and liver health.	MFGMAQUA1	SCHEDULED	Hans Boon	No	Whole system/week	8	Yes, that is the intention of the user. Experiment is scheduled	1 or 2

** Access units describe how accesses are calculated, typically 1 day x 1 pot, 1 season x 1 microplot, etc …*

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Table: overview TNA users

Researcher (TNA user)	Employing organisation/Home institution (TNA user affiliation)			User-project acronym (TNA project)			
Name	Name	Legal Status	Country		Installation Short Name	Units of access	Date of access
Amelia Claudia Silva	Zinpro Corporation	PRV	United States of America	TRACEMINERAL-RAS	WU-RAS	8	16/05/2023 - 10/07/2023
Hung Quang Tran	Institute of Aquaculture and Protection of Waters (IAPW)	UNI	Czech Republic	AIProSan	WU-MRU	8	12/09/2023 - 07/11/2023
Stuart McMillan	The University of Stirling	UNI	United Kingdom	OreoHEMP	WU-MRU	8	26/09/2024 - 21/10/2024
Kiran Vettathkuzhuppully Subash	Norwegian University of Life Sciences	UNI	Norway	CLEAN-RAS	WU-RAS	8	17/03/2025 - 12/03/2025
Rafaela Silva Costa	CIIMAR-Interdisciplinary Centre of Marine and Environmental Research	RES	Portugal	ChiBass	WU-MRU	8	21/07/2025 - 15/09/2025
Evi Croes	Nukamel N.V.	SME	Belgium	MFGMAQUA1	WU-MRU	8	05/05/2025 - 30/06/2025

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2. TNA projects

2.1.1. TNA projects description

PID: 23110 - Adjustment of trace mineral nutrition of trout grown in RAS
Claudia Figueiredo Silva
Acronym: TRACEMINERAL-RAS
Trace minerals (TM), such as zinc (Zn), play an important role in fish nutrition by performing a variety of functions in the body and for growth. TM are required in small quantities and are involved in a variety of biochemical processes such as cellular metabolism, skeletal structure formation, and acid-base equilibrium regulation. While being important for the fish, TM levels in the water have a negative impact on the natural environment. Adjustments to dietary aquafeed diets with better bioavailability of TM for the fish should contribute in lowering TM supplementation requirements and, as a result, mineral accumulation in recirculating aquaculture systems (RAS) and effluent. It is unclear whether increased dietary TM availability (for example, Zn) increases mineral retention and thus the effect of water mineral levels (due to reduced excretion). The current project investigates two factors that can affect the TM availability/uptake in the fish and the water/effluent level of TM.
30 trout (per tank (each replicated RAS has one fish tank)) were randomly counted, batch weighed and stocked. Fish were fed by hand twice daily and Faecal matter was collected. The day before the experiment ended, three fish from each tank were collected (after the last feeding moment). Blood was drawn from those three fish, and the fish were killed and tissue samples (e.g. gills and liver) and digesta from the gut (for mineral availability) were collected from these fish to evaluate potential trace mineral (TM) toxicity/oxidative stress (e.g. TM content, enzyme activity (catalase) ATPase activity). The remaining fish were caught, sedated, and batch weighted on the final day of the experiment. Following that, 10 fish per tank were killed in order to determine body composition. The experiment and samplings/analyses went well. All analyses are performed and the results have been presented at EAS 2024 Copenhagen. The results will be published in an open-access peer reviewed journal.

PID: 24853 - Bioavailability of novel protein sources for pikeperch (*Sander lucioperca*) juveniles
Hung Quang Tran
Acronym: AlProSan
Pikeperch (*Sander lucioperca*) is an emerging species in aquaculture, valued for its high meat quality and increasing market demand. In its natural habitat, this carnivorous species primarily consume crustaceans, insects, and fish. Under commercial farming conditions, pikeperch are typically fed formulated diets with high protein levels ranging from 43% to 50%. These diets often require substantial inclusion of fishmeal sourced from marine environments – a practice that raises concerns regarding long-term sustainability due to declining fish stocks and the ecological impact on marine ecosystems. In this context, alternative protein sources such as insect meal derived from yellow mealworm (*Tenebrio molitor*) and krill meal (*Euphausia superba*) have gained attention as promising ingredients for aquafeeds.
Two ingredients, including defatted yellow mealworm (*T. molitor*) (TM), and krill meal (*E. superba*) (KM) were evaluated for nutrient digestibility. A control diet (CON) was formulated, containing 50% crude protein, 15% lipid, and 22 MJ/kg with fishmeal as a dominant protein source, in order to meet the nutrient requirements of pikeperch. The other two test diets contained 80% of CON, diluted by 20% with the corresponding test ingredients (TM and KM), abbreviated diets as TMD and KMD, respectively. Fish were fed with the respective diets at 2% body weight for a duration of 8 weeks. During the last four weeks of the experiment, nutrient digestibility (dry matter, ash, crude protein, fat, carbohydrate, energy, phosphorus and calcium), waste production, and faeces recovery of the eight diets was determined. Moreover, oxygen consumption and ammonia production were determined from week 3 to week 7. Faeces particle size (quality) was determined from week 6 to week 8; faeces

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recovery was determined during week 8 after a 72-hour faeces collection. All data analyses are complete, the results will be presented at EAS 2025, and a manuscript is being prepared.

PID:32385 - Hempseed as an alternative protein source for Nile tilapia (*Oreochromis niloticus)*
Stuart McMillan, Monica Betancor
Acronym: OreoHEMP
The rapid growth of aquaculture must be made in a sustainable manner and the use of responsibly sourced raw materials is a priority. Therefore, the search continues for more environmentally friendly terrestrial plant alternatives, compared to traditional feed constituents' fishmeal and fish oils, and current alternatives such as soy. Hempseed from industrial hemp (*Cannabis sativa*) is one promising alternative as the crop is grown within Europe and is a secondary product because the crops primary use is to produce materials for construction and energy using the plants stalks. As a by-product this makes hempseed a promising low carbon footprint resource.
The aim of this research was to evaluate industrial hempseed meal (HM) as a sustainable alternative protein source for Nile tilapia (*Oreochromis niloticus*). This was accomplished by evaluating nutrient digestibility, growth, short chain fatty acids (SCFA) in the faeces, fish behavior, and metabolic measurements after juvenile fish (~50 g) are fed one of four diets. A commercial formulation was used to create a control diet containing approximately 30-35% protein and 10% lipid. To create the three experimental diets, 30% of the control diet was replaced with either soy bean meal (SBM), single cell protein (SCP), or hempseed meal (HM). Three hundred and sixty (360) Nile tilapia juveniles (approximately 40-50 g/fish) were randomly assigned to one of twelve metabolic chambers (30 fish/tank) and fed one of the diets during the experiment. The experiment was overall a success, with no major issues. The growth, nutrient digestibility of the diet and its ingredients, and the effect of the diet's behaviour are all evaluated. All lab analyses have been completed, and data analyses are currently being finalized. The plan is to publish the findings in a peer-reviewed journal. The experiment and sampling/analysis went well. All analyses were completed, and the results were presented at EAS 2024 Copenhagen. The findings will be published in an open-access, peer-reviewed journal. The results are described in greater detail in 2.1.2.

The other (three) projects (PID: 34992, PID: 35091 and PID: 34788) are ongoing/scheduled.

2.1.2. Selection of One exemplary project

PID: 23110 - Adjustment of trace mineral nutrition of trout grown in RAS
The study investigated two factors that can affect the trace mineral (TM) availability/uptake by the fish and the water/effluent level of TM: composition of the TM premix (inorganic vs TM-AA complexes) and the water refreshment level (RAS vs. flow through system), resulting in four different treatments. With this purpose, two diets were formulated to be equal in their nutrient composition with the exception of the type and level of TM used. One of the diets was supplemented with inorganic forms of TM (TM-I; sulphates of Fe, Zn, Cu, Mn and Se) and the other with TM-AA complexes (TM-AA; Fe-, Zn-, Cu-, Mn- and Se-AA complexes). In the TM-AA diet, TMs were supplemented at half the level of the inorganic. The two diets were fed for 8 weeks to ±78 g initial body weight rainbow trout reared in two different systems differing in water refreshment rate, high (HWR; 1.5 times the system volume) and low water refreshment (LWR; ±311 L / kg feed). At the end of the feeding period, the effect of the diet, rearing system and their interaction were evaluated in terms of growth performance, fish composition, TM retention and losses, plasma and hepatic TM content, and activity of hepatic enzymes playing a key role in the antioxidant defence (e.g. SOD, GPx, CAT). Rainbow trout grew better (SGR %/d) in the LWR (1.98) system compared with the HWR (1.92), regardless of the diet fed. Replacement of inorganic TM with TM-AA complexes by the half has significantly improved digestibility of P, Zn, Mn and Se. Moreover, and regardless of the system type, the supplementation with TM-AA complexes reduced significantly Fe, Cu, Mn and Se total losses while maintaining TM body retentions similar. The analysis of hepatic TM content revealed a reduction on Fe and Se contents with TM-AA vs TM-I and in

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the Se content of trout raised at HWR vs LWR system. However, neither the diet nor the system type affected the activity of enzymes playing a key role on antioxidant defense system. Plasma analysis showed higher levels of Mn and lower levels of Zn in trout fed TM-AA vs TM-I diet, regardless of the system type. In summary, the findings prove that replacement of inorganic with TM-AA complexes can offer an interesting strategy to reduce TM losses into the environment, while respecting EU legislation for upper limits of TM and still maintaining fish performance and health.

3. Reflection on results of the TNA programme

The TNA programme proved to be an effective way to initiate new collaborations. This TNA may result in new projects and collaboration with the visitors. Furthermore, it is beneficial to train people from various institutes/companies and share knowledge about research methods. Especially for some of the PhDs who visited, it is a nice way to boost their PhD project, as adequate funding for such an experiment is not always available. Overall, the programme was beneficial and fruitful. The online platform (ARIA) could have been more user-friendly.

4. References

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Work Participants				

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