Sveriges lantbruksuniversitet
Swedish University of Agricultural Sciences

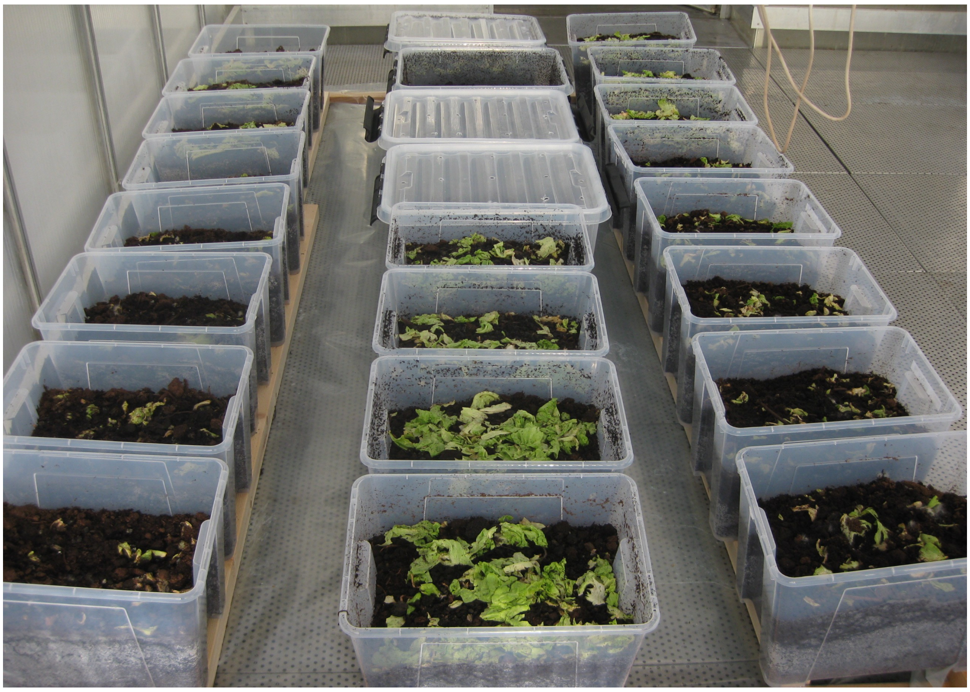
Fakulteten för landskapsarkitektur, trädgårds- och växtproduktionsvetenskap

Maskkompost med syftet att reducera mängden vegetabiliskt avfall - och en möjlighet att producera fiskfoder i akvaponiska system?

Engelsk titel:

Vermicompost for Reduction of Vegetable Waste – and a possible means to produce fish feed in aquaponic systems?

Lisa Sandell



Självständigt arbete • 15 hp
Alnarp 2014

Maskkompost med syftet att reducera mängden vegetabiliskt avfall - och en möjlighet att producera fiskfoder i akvaponiska system?

Vermicompost for Reduction of Vegetable Waste – and a possible means to produce fish feed in aquaponic systems?

Lisa Sandell

Handledare:	Sammar Khalil, SLU Institutionen för biosystem and teknologi
Examinator:	Georg Carlsson, SLU Institutionen för biosystem and teknologi

Omfattning: 15 hp
Nivå och fördjupning: G2E
Kurstitel: Kandidatarbete i biologi
Kurskod: EXO493

Examen: kandidatexamen i biologi
Ämne: Biologi (EXO493)
Utgivningsort: Alnarp
Utgivningsmånad och -år: oktober 2014
Omslagsbild: Lisa Sandell
Elektronisk publicering: http://stud.epsilon.slu.se

Nyckelord: aquaculture, earthworm protein, fish feed, recycling strategies**,** vermiculture

SLU, Sveriges lantbruksuniversitet
Fakulteten för landskapsarkitektur, trädgårds- och växtproduktionsvetenskap
Institutionen för biosystem och teknologi

Sammanfattning

Akvaponik är ett odlingssätt som kombinerar fisk- och/eller skaldjursodling med odling av växter i recirkulerande vatten. Det näringsrika vattnet från fisktanken utgör näringskälla för växterna. Bakterier omvandlar ammoniak från fisktanken till nitrat som tas upp av växterna. Växterna erhåller näring och fiskarna rent vatten.

Produktionen av fiskmjöl och fiskolja som används i konventionella fiskfoder förbrukar fossila bränslen och bidrar till stora koldioxidutsläpp.

För att åstadkomma en miljövänlig samt självförsörjande akvaponikanläggning kan fisk- och växtodlingen kompletteras med en maskkompost/maskodling.

Växtresterna från hydroponiken kan på så sätt återanvändas i komposten som föda åt kompostmaskarna. De proteinrika maskarna skördas, torkas och mals ned, och återförs sedan till akvakulturen i form av fiskfoder.

Syftet med studien var att undersöka hur vegetabiliska restprodukter påverkar maskkomposter vad gäller antal och vikt hos kompostmaskarna. Förändring i antal och vikt hos maskarna utvärderades efter skörd. Hypoteserna var att kompostmask kan odlas i vegetabiliska restprodukter samt att genom att tillföra gödsel till maskkomposterna är det möjligt att uppnå en näringshalt som är tillräcklig för att understödja en kontinuerlig skörd av maskar, med andra ord åstadkomma en maskodling.

Eisenia fetida och *Dendrobaena veneta* odlades i 20 L kompostlådor innehållande 70% torv uppblandat med 30% gödsel, kogödsel respektive hönsgödsel. Maskkomposterna tillfördes 35 g sallad (rester från en salladsodling) och 0.8 L vatten per vecka. Kompostlådorna placerades i en klimatkontrollerad växthuskammare med en konstant temperatur på 25.0°C.

Experimentet var två-faktoriellt med faktorerna maskart (två arter) och gödseltyp (två typer) vilket resulterade i fyra behandlingar. Fem replikat av varje behandling användes och experimentet pågick i fyra månader och utvärderades efter skörd.

Temperaturen i maskkomposterna låg konstant på 25.0°C under hela experimentet.

Värdet på pH varierade mellan 4.6-5.3 i maskkomposterna utan någon signifikant skillnad i pH-värde mellan de olika behandlingarna.

Fukthalten låg mellan 62-94 % i komposterna, också utan någon signifikant skillnad mellan de olika behandlingarna. Substratens C/N kvoter var relativt höga vid starten för experimentet, (C/N: 37-45), och sjönk sedan i samtliga behandlingar till C/N: 33-40.

Resultaten visade att antalet maskar ökat endast i *E. fetida* med hönsgödsel-behandlingen. Antalet maskar var signifikant högre i *E. fetida*-behandlingarna jämfört med *D. veneta*-behandlingarna men maskvikten hade sjunkit betydligt i samtliga behandlingar.

Ingen signifikant skillnad i antal maskar eller maskvikt indikerades mellan de två gödseltyperna.

Resultaten stöder inte hypoteserna men indikerar möjligen att valet av maskart kan ha större betydelse för utfallet vad gäller maskproduktionen än vad valet av gödseltyp i maskkomposterna har.

Abstract

Aquaponics is a food production system consisting of a consecutive cultivation of plants and aquatic animals, fish or shellfish, in recirculating water. The plants feed on the nutritious effluents from the fish tank and bacteria converts ammonia from the fish tank into nitrate that is absorbed by the plants. The plants receive nutrition and the fish gain purified water.

The environmental impact of the production of fish meal and fish oil used in conventional fish feed includes a large consumption of fossil fuels with subsequent carbon dioxide emission.

The aquaponic system can be made environmentally sustainable and self-supporting if supplemented with a vermicompost/vermiculture for the production of worm protein as a fish feed. Vegetable waste from the hydroponic part of the system can be reused as worm feed in the compost and the protein rich worms can be harvested, dried and grinded and brought back to the aquaculture as fish feed.

As a first step in this direction, I have investigated the effect of recycling of vegetable waste in vermicomposts on the growth and number of earthworms (this was evaluated after harvesting). The hypotheses were that earthworms can be cultivated in the vegetable waste and that by adding manure to the vermicompost it is possible to obtain a nutrient content adequate to support a continuous worm harvest, in other words create a vermiculture.

Eisenia fetida and *Dendrobaena veneta* were grown in 20 L bins containing peat mixed with either poultry or cattle manure. The proportions were 70% peat and 30 % manure. Discarded plant parts from lettuce cultivation was added to the vermicomposts (35 g per week) and water was supplied with 0.8 L per week. The bins were placed in a climate controlled greenhouse chamber with a constant temperature of 25.0°C.

The experiment was two factorial with the factors worm species (two types) and types of manure (two types) resulting in four treatments. Five replicates of each treatment were used.

The experiment lasted four months and was assessed once at the end of the experiment.

Temperatures within the composts were constant throughout the entire experiment (25.0°C).

Value of pH ranged between 4.6-5.3 in the composts, with no significant difference between treatments.

Moisture content varied between 62-94 % in the compost substrate, with no significant difference between treatments. C/N ratios in the substrates were relatively high at the start (C/N: 37-45) and dropped in all treatments to C/N: 33-40 during the experiment.

The results showed that the number of worms increased only in the *E. fetida* with poultry manure treatment. Number of worms were significantly higher in the *E. fetida* treatments compared to the *D. veneta* treatments but the worm weights had decreased substantially in all treatments.

No significant difference in number of worms or worm weight was found between the two types of manure.

The results did not support the hypotheses but may indicate that choice of earthworm species in the vermicomposts might have greater influence on the outcome in terms of worm production than the choice of manure added to the vermicomposts.

Table of contents

1. Introduction

1.1 Backround

Aquaponics is a food production system that combines the rearing of aquatic animals, aquaculture, with the cultivation of plants in water, hydroponics (Fig. 1).
The aquaculture part of the system consists of a tank hosting fish, crayfish or prawns. Vegetables, herbs and other edible plants can be grown in the hydroponic part (Hughey 2005). Nutrient rich water from the fish tank flows down to the hydroponic where it is purified by the plants and thereafter pumped back into the fish tank. The system is enriched with bacteria converting ammonia from the fish tank into the nitrate form (Fig. 1), preferred by the plants and less toxic to the aquatic organisms (Tyson et al. 2011; Al-Hafedh et al. 2008). Many plants are sensitive to ammonium and these levels are reduced as well (Hughey 2005).
This symbiotic relation between the three subsystems (fish, plants and bacteria) of an aquaponic, linked together by a natural biological nutrient cycle, fits the criteria of sustainable agriculture that makes efficient use of non renewable resources (Chan 1993).

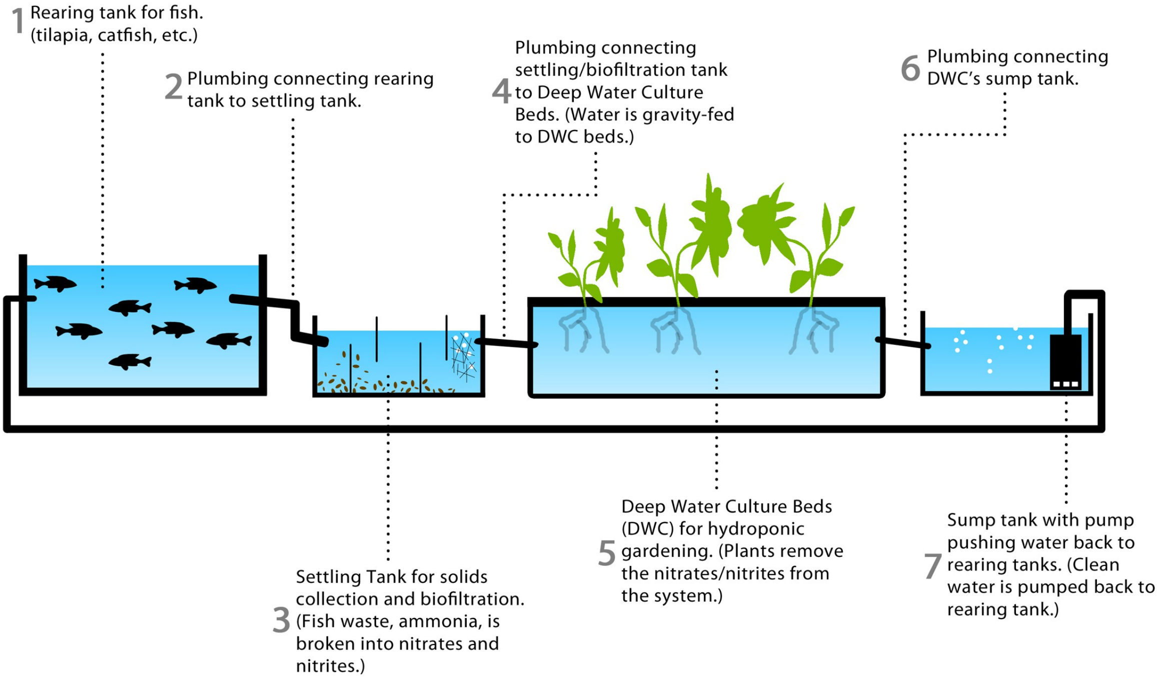


Figure 1. Aquaponic plant design, Trash Mountain Project. http://trashmountain.com.

The three main inputs to an aquaponic system are water, fish food and electricity to pump the water. A carefully constructed aquaponic system can function as an almost completely closed system, using mainly recirculated water and non-polluting electricity such as solar or wind energy (Al-Hafedh et al. 2008). Efficient recirculation and reuse of water is made possible by the plants, acting as a wetland in the way that they keep the water clean as they make use of the nutritious effluents, feces and undigested food, from the fish tank (Tyson et al. 2011). This results in less water usage and less waste discharge to the environment (Al-Hafedh et al. 2008).

Economic benefits of linking aquaculture with crop production includes shared costs for constructing and operating. Since the system simultaneously produces two important food items, fish and vegetables, it has the potential of an increased profit compared to traditional monocultures (Graber & Junge 2009).
The development of new agricultural systems and introduction of new crops is crucial to meet the demands of a growing human population, especially in areas with shortage of fresh water and lack of agricultural lands (Tyson et al. 2011; Chan 1993).

1.1.1 Fish feed in aquaponic systems

A controversial issue in fish farming is the reliance upon the environmentally unfriendly fish meal in the production of fish feed. Today 8 of the top 20 capture fish species are used primarily in the production of fish meal and fish oil for livestock and aquaculture feed and globally the aquaculture industry is the fastest growing consumer of fish meal (Tidwell 2012). Part of these fish meal species are not palatable to humans but many of them could be introduced as edible also for humans.

Marine fisheries are major consumers of fossil fuels and contribute to large carbon dioxide emissions as well as pollution of the seas (Tidwell 2012).
From a human health perspective, many environmental toxins accumulate higher up in the food chain with the result that fish feed made from fish meal will contain traces of these toxins, an issue that can be solved by replacing fish meal with a more sound protein source (Tidwell 2012).
It is difficult to predict all the environmental impacts from removing this many fish for the production of fish meal and fish oil and for this reason alone the precautionary principle should prevail.

Due to rising costs associated with manufacture and distribution the price of commercial fish feed containing a high proportion of fish product is rapidly increasing (Brett & Midmore 2008). Depletion of natural resources and stricter regulations regarding sustainable fisheries also contributes to the raising prices and since there is no reason why this trend would abate, profit margins of aquaponic and aquaculture businesses will gain from ridding themselves of the dependence on fish product feeds (Brett & Midmore 2008).

Substituting fish meal with a protein source further down on the food chain, like earthworms, would improve the sustainability of the aquaponic and there is also a large ecological advantage to be gained by transforming non-human food like protein rich worms, into human food in high demand such as fish (Muminovic 2010).
Other benefits from replacing fish meal with earthworm protein are fulfilling the goal of a circular flow of nutrients in the aquaponic, the possibility of reduced costs and also less need for transport since the worms can be cultivated on site with already available materials (Pantanella et al. 2011).
In order to create a natural closed system it is also necessary to reuse the solid waste products produced in the aquaponic, such as damaged plants and vegetables and find a way to bring these nutrients back to the system.

By cultivating compost worms in these waste products and thus creating a vermiculture as opposed to a common vermicompost, it may also be possible to obtain a sustainable worm harvest.
The harvested worms can be processed, dried and grinded and brought back to the fish tank as high quality fish food, substituting the outdated fish meal (Huu Yen Nhi et al. 2010).
Some farmed predatory fish species may advantageously be fed live worms but it would be a challenge to manage this in practice at a commercial aquaponic plant (Fadaee 2012).

The term vaquaponics (Brett & Midmore 2008) have been used to describe the integration of vermiculture, hydroponics and aquaculture utilising any kind of linkage, methodology or design (Fig. 2).
Optimizing the conditions in an integrated vermicompost for the production of worm protein as fish feed is possibly the most important step towards the development of sustainable and environmentally friendly recycling strategies in aquaponic systems and from an ecological point of view it is always desirable to meet any protein needs with a locally produced environmentally friendly protein product such as in this case earthworms produced at the same site where the intended consumers are located (Tidwell 2012).

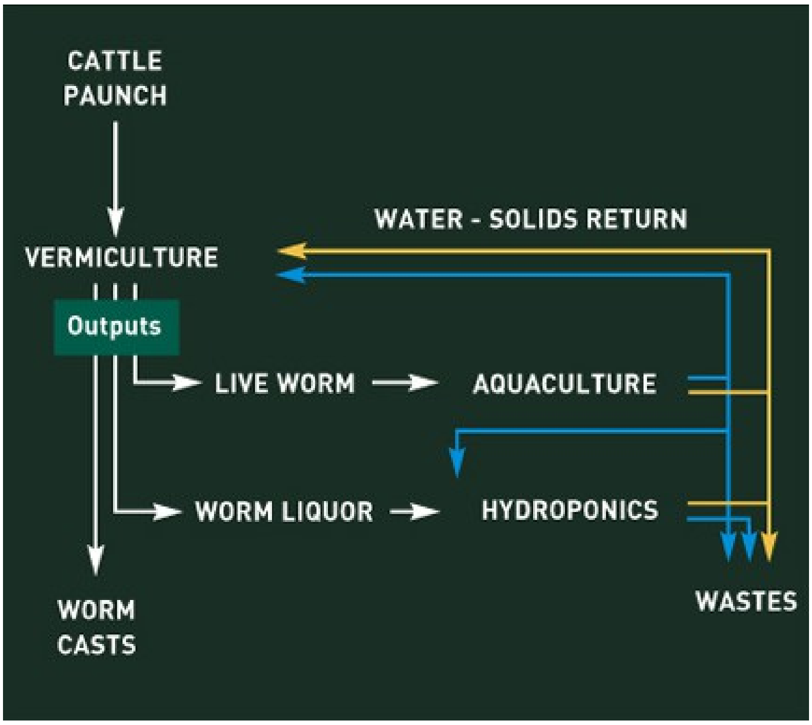


Figure 2. A diagrammatic representation of a vaquaponic system, Sustainable Aquaponics. http://www.hydroponics.com.au/. Primary nutrient inputs are fish feed and manure (or in this case cattle paunch) to the vermicultures. Liquid and solid waste products are reused in the system, whenever possible. The integrated vermiculture has the potential to constitute a dumping place for these waste products.

The pursuit of economically justifiable ecological improvements has accelerated the interest in using vermiculture in aquaponics as a technology for reusing the waste products from the hydroponic part in the production of worm protein as a fish feed.
As a first step in this direction I have in the current study investigated the effect of recycling of vegetable waste in vermicomposts on the growth and number of earthworms.

1.1.2 The fishes nutritional requirements and the nutritional value of earthworms

Providing the fish in a commercial aquaponic plant with the correct amount of fish feed is an important key in balancing the factors water volume, plant surface, grow media volume, vegetable biomass and fish biomass (Kattastrands kretsloppsodling 2013). One kg of fish feed produces about 20-40 kg of vegetable biomass. The daily ration of fish feed is about 2 % of the fish weight, depending on species (Kattastrands kretsloppsodling 2013).

Edible fish species suitable for aquaponics are the trout family, perch family, bass family and catfish (Aquaponics Sweden 2013). Crayfish and shrimp can also be bred, on their own or in combination with fish. Most frequently used fish species today is the tilapia and for this reason I will use tilapia as an example in comparing nutritional requirements of the fish with the nutritional value of the earthworm species *Eisenia fetida* and *Dendrobaena veneta.*

Optimum composition of a tilapia feed is 30 % protein, 30 % starch and maximum 6 % lipids (Storebakken 2014). Water content should be no more than 10 %. 1100 kg of feed is required for the production of 1000 kg of tilapia. If composed correctly no marine ingredients are required in the production of the feed (Storebakken 2014).
Based on dry weight *E. fetida* has a protein content of 59 % and a lipids content of 9 % (Fadaee 2012). *D. veneta* has a protein content of 45 % and a lipids content of 11 %, also based on dry weight. Provided that the earthworms have been dried without their gut contents (starved before

harvest), or that these have been ashed, the ash-free dry weight of an earthworm is about 40 % of its original fresh weight (Sinha & Valani 2011).
Using tilapia and the protein content of *E. fetida* as an example, how much worm protein is required for the production of 1000 kg of tilapia?
1100 kg of feed contains 330 kg of protein. This corresponds to 559 kg of dry weight or 1398 kg of fresh weight of *E. fetida* (Fadaee 2012). The lipids content of *E. fetida* is too high for tilapia (Fadaee 2012). In a commercial aquaponic plant maximum fish growth is required for profitability. This means assembling a feed that meets the exact nutritional requirements of the fish species used in the system.

Considering the above it is not realistic to think that earthworms can replace conventional fish feeds completely but the production of worm protein has great potential to replace current protein sources. Future research should preferably examine the possibilities to supplement the worm protein produced in vaquaponics. Is it possible to increase the protein content of the earthworms and to what degree can protein, lipid and starch content of the worms be manipulated? Worm protein from traditional vermiculture farms can also be used to satisfy the protein needs of a commercial aquaponic plant.

Earthworm protein is a complete protein containing all of the essential amino acids (Sinha & Valani 2011). Some of the amino acids occurs in higher amounts in earthworms than in fish meal (Table 1).

Table 1. Amino acids in vermimeal compared with fish meal (Sinha & Valani 2011):

Amino acids (g/100g protein)	Fish meal	Vermimeal
Arginine	3.9	6.1-6.5
Cysteine	0.8	1.6-1.8
Glutamic acids	8.4	13.8-14.2
Histidine	1.5	2.5-2.6
Isoleucine	3.6	4.5-4.6
Leucine	5.1	7.9
Lysine	6.4	7.1-7.5
Methionine	1.8	2.0-2.2
Pheylalanine	2.6	4.1
Threonine	2.8	4.8
Tyrosine	1.8	3.4
Valine	3.5	5

1.1.3 Vegetable waste in aquaponic systems

Plants are the primary crop in aquaponics, fish are secondary. Depending on conditions such as light and nutrient density there are few limitations as to what can be grown but proven more successful are leafy greens such as lettuce, basil, spinach, kale, cabbage and herbs (Aquaponics Sweden 2013).
An aquaponic system has a lower percentage of damaged plants and vegetables compared to conventional field cultivation. This means the majority of the production is of marketable quality. These numbers vary largely between commercial plants but around 10 to 30 % of the produced vegetable biomass is waste (Long 2012). This includes stalks, haulm and other non edible parts of fruiting plants. The C/N ratio of vegetable waste is about 25 (Vinje 2014), a value well suited for vermicomposting and vermicultures (Sinha & Valani 2011).

Using the presumably most frequently occuring vegetable crop lettuce as an example, an aquaponic plant with an annual fish production of 5400 kg (Nile tilapia) will produce around 90000 kg of lettuce annually (Nelson 2014). These numbers are an average of a wide range of results depending on factors such as light levels (natural seasonal light or year round optimum light levels) and how carefully the system is handled. This example will result in an annual waste production of 18000 kg (20 % of total production).

E. fetida with an average adult weight of 0.4 g consumes about 0.2 g daily, that is 73 g in a year. Thus 18000 kg of vegetable waste can sustain about 250000 *E. fetida* individuals annually. With an average weight of 0.3 g this corresponds to a worm weight of 75 kg, enough to produce 30 kg of vermimeal or 17.7 kg of worm protein. A worm protein weight of 17.7 kg is sufficient to produce fish feed corresponding to 53 kg of fish, in this case the species tilapia. The conclusion of the example is that the 5400 kg of fish results in 18000 kg of vegetable waste resulting in 53 kg of fish.
The result of this example establishes the fact that the vegetable waste produced in the system is far from enough to provide the system with the protein amount needed in the aquaculture section. This problem can be solved by adding manure, or any other suitable organic waste that is available, to the vermicultures.

1.1.4 Vermicomposting and vermiculture

The three main purposes for using earthworms in compost systems are waste management, production of vermicompost and production of worm biomass (Connely 2013).
Vermicomposting is a biotechnological process that utilizes naturally occurring organisms, earthworms, to improve waste disposal conditions as well as produce plant nutrition in terms of vermicompost (Nagavallemma et al. 2006).
It is an environmentally sound waste management practice with the added bonus of the production of vermicompost, a solid fertilizer, and vermi tea, a liquid fertilizer (Connely 2013).
In vermicomposting earthworms plays an important role as they recapture the nutrients in the waste products and thereby maintain the nutrient flows from one cropping system to another (Nagavallemma et al. 2006).

Earthworms are decomposers, transforming organic matter by mineralization of organically bound C and N into carbon dioxide and ammonium. Their decomposition of fibrous organic waste products increases the contact surface where molds and bacteria can access the material. If provided with an amount of feed appropriate for the species and the number of worms in the compost, the worms will transform 100 kg of waste into about 15-20 kg of humus. Evaporation of carbon dioxide and water are the main reasons for the reduced weight of the compost (Vincent 2012).
This humus, left after the worms have processed the material, is the vermicompost. It is a valuable growing medium that can help facilitate plant growth by improving the physical, chemical and biological properties of soil (Rupani et al. 2012). The vermicompost contains a wealth of worm manure, vermicast, which is rich in microbial activity and carries vital plant growth regulators (Nagavallemma et al. 2006). This vermicast has been found to contain as much as 20 times more beneficial microbes and bacteria compared to fertile soil (Sinha & Valani 2011).

Earthworm activities also results in a loosened and thereby ventilated compost structure, properties that enhances water retaining capacity, soil fertility and crop growth (Huu Yen Nhi et al. 2010). Awareness of the vermicompost as an organic soil amendment that possibly someday will replace chemical fertilizers is increasing with cultivation precursors such as aquaponic farmers (Singh et al. 2013).

It has been more than a hundred years ago since Sir Charles Darwin described in detail the many positive effects on soil and organic waste from earthworm activity (Darwin 1881) and today there is a global realization that the adoption of ecological and sustainable farming practices, such as allowing earthworms to process organic waste and reuse nutrients in waste products, is essential if we are to maintain both todays production volumes and environmental protection (Maize et al. 2010).

The demand for cultivated earthworms has risen as their potential has been proven also in fields other than the agricultural. Earthworms are rich in high quality protein which makes them suitable as animal feed and they can for example easily be incorporated to any large or small scale aquaponic system (Buch 1987). The possibility to raise them in simple rural conditions makes way for a better use of local resources such as organic waste and manures and creates an alternative source of income to farmers (Hussaini 2013).

Vermicomposting and vermiculture are similar processes, using earthworms to improve the properties of the compost system (Hussaini 2013). In the practice of vermicomposting, earthworms are used primarily for the production of vermicompost.
In a vermiculture however, all conditions are optimized in order to obtain a continuous harvest of worms. Population densities are lower in a vermiculture with the result that the organic waste will not be processed as quickly and efficiently but instead the reproduction rates are kept at its optimum at all time (Dominguez & Edwards 2011; Munroe 2009).

Compost worms can under optimum conditions be expected to double every 60 to 90 days. Optimum conditions includes continuous supply of nutritious food, a well aerated substrate with moisture content around 70-90%, stable temperatur in the range of 15-30°C and initial stocking densities appropriate for the species (Munroe 2009). Temperatures around 20-25°C are desirable in a vermiculture aiming for maximum worm production. Temperatures above 20°C stimulate reproduction but at 30°C the worms will begin to emigrate and degrees above 35°C are fatal (Dominguez & Edwards 2011).

The bedding substrate should have a low bulk density value in order to provide the worms with necessary air flow and also high enough absorbance capacity so that water is retained and the worms never risk to dry out. Optimal moisture content in a vermiculture is 70-90%, thus higher than the 45-60% prevailing in conventional composts. The worms breathe through their skin and substrates with moisture contents below 50% are hazardous to them.
Compost worms do not require a lot of oxygen but anaerobic conditions are fatal to them. A porous bedding substrate as well as the worms own activity help keep the system ventilated.
Optimizing the bedding substrate properties is an important key to a successful vermiculture (Munroe 2009).

Compost worms such as *E. fetida* are generally known to consume half their body weight per day, if offered suitable conditions. Vermiculture farmers commonly feed their worms manure, usually dairy and beef manure that are easily accessible. The high content of partially decomposed organic material allows the worms to consume it more rapidly than fresh foods. Manures are a natural food source but compost worms will eat basically anything organic, for example fresh food scraps, seaweed, corrugated cardboard, pre-composted fish and meat and biosolids (Munroe 2009).

Manures as well as many other feeds often contain more salts than the worms can tolerate. It is not a problem however as long as the salt contents are low (preferrably less than 0.5%) in the bedding substrate (Dominguez & Edwards 2011). Usually it does not take long for the salts to be leached out by precipation or watering and the worms can then begin consuming the feeds. Depending on how the livestock are kept the manures can sometimes contain a lot of urine that risk causing harmful levels of dangerous gases in the worm habitat. This problem can be resolved by watering the manure before using it in a vermiculture.
Other potentially toxic components in worm feeds are tannins from certain tree types, detergent cleansers, industrial chemicals and pesticides from different types of sewage sludge and de-worming medicine, often from horse manure. Greasy wastes that have not been pre-composted can also become harmful as toxic substances are released by microbes as they break down the oils.
Pre-composting reduces the risk of poisoning the worms but will also reduce the nutrient content so a shorter period of time is often the best compromise (Munroe 2009).

A search through the literature on the subject reveals differing opinions regarding optimum pH in vermicultures. Some researchers claim the worms prefer a pH of 7 or slightly higher (Munroe 2009), but other studies have shown that when given a choice in pH value, the worms move towards values around 5.0 (Dominguez & Edwards 2011). Low pH from an acidic bedding such as peat moss can however facilitate the development of mites and other pests.

Not all earthworm species are useful in vermiculture systems (Sinha & Valani 2011). Essential qualities are a wide tolerence of environmental conditions and fluctuations of these conditions. Rapid growth and reproductive rates combined with a relatively short life span are other desirable properties.
Species commonly used in vermicultures are *E. fetida, Eisenia andrei* and *Lumbricus rubellus*.

1.1.5 *Eisenia fetida* and *Dendrobaena veneta*

E. fetida and *D. veneta* are both epigeic earthworm species with a worldwide distribution. They feed on decaying organic matter and do not establish permanent burrows (Dominguez & Edwards 2004). They both belong to the family Lumbricidae and the genus *Eisenia.*

E. fetida colonizes organic substrates naturally, have short life cycles and a wide tolerance range regarding temperature and moisture, all of which are qualities desirable in vermicomposting and vermicultures (Sinha & Valani 2011). Their resilient body structure allows them to be readily handled and harvested.
E. fetida can survive in temperatures as low as 0°C (Table 2), but at single-digit temperatures they will be less active through not consuming as much food nor reproducing. A temperature of 15°C is required to keep the vermicompost efficient, but for a productive vermiculture with a harvest margin, it is necessary to keep temperatures at 20°C minimum. Maximum life expectancy is 4.5 to 5 years, an age uncommon under natural conditions (Dominguez & Edwards 2004).

D. veneta, also known as *Eisenia hortensis*, is larger than *E. fetida,* not as prolific as *E. fetida* but grow faster (Sinha & Valani 2011). Commercial vermiculture companies often breed *D. veneta* with the aim of using them in soil improvement projects or in protein production. It is a robust earthworm that can tolerate a wider moisture range than *E. fetida* but are somewhat more sensitive to high temperatures, preferring around 15-25°C (Table 2). The negative effects of high temperatures includes an increased chemical and microbial activity in the substrate, consuming the available oxygen (Dominguez & Edwards 2004).

Table 2. Comparison of some essential aspects of the biology of *E. fetida* and *D. veneta* (Dominguez & Edwards 2004).

Characteristics	*E. fetida*	*D. veneta*
Time to sexual maturity (days)	28-30	65
Incubation time (days)	18-26	42.1
Number of cocoons per day	0.35-0.5	0.28
Mean size of cocoons (mm)	4.85 x 2.82	3.14 x 1.93
Hatching viability (%)	73-80	20
Number of worms per cocoon	2.5-3.8	1.1
Self-fertilization	yes	no
Life cycle (days)	45-51	100-150
Limits and optimal Temp °C	25°C (0°C-35°C)	25°C (15°C-25°C)
Limits and optimal moisture	80%-85% (70%-90%)	75% (65%-85%)

1.2 Objective

The major aim of this study was to evaluate the possibility to replace the traditional fish feed used in aquaponic systems with an on site cultivated product and at the same time allow the vegetable waste products to be processed and reused within the aquaponic plant.
The detailed objective was to examine the possibility of using vegetable waste as feed in a vermicompost.

The overall question I was looking to answer was whether it is possible to establish an environmental sustainable form of aquaculture with a fully circular flow of nutrients?
This study intends to examine the first step in a sequence that hopefully ends with the possibility of using earthworms from aquaponic waste composts as fish feed in the aquaponic system in order to recapture the nutrients and obtain an ecologically sound aquaculture.
I also wanted to investigate whether there would be a difference in growth between two specific species of earthworms and if it would be possible to detect any differences in worm growth that could be linked to either of the two types of manure used in the vermicomposts.

1.3 Hypotheses

1. Earthworms can be cultivated in vegetable waste products.
2. By adding manure to the vermicompost it is possible to obtain a nutrient content adequate to support a continuous worm harvest, in other words create a vermiculture.

2. Material and Method

2.1 The vermicomposts

E. fetida and D. veneta were cultivated in top-fed 20 L plastic bins. Walls and floors had openings in order to allow aeration and for excess water to leak out. Lids with aeration holes were used to cover the bins in order to protect them from moisture loss and also to prevent the worms from escaping.
Each bin was filled to 2/3 of their volume, assuring there was room for the vegetable waste and also room to allow mixing of the top layer. This resulted in a total of 14 L out of which 10 L consisted of dark natural peat (Table 4), which constituted a 20 cm thick bedding layer.

The food source (Table 3) was composed by one part vegetable waste, rinsed iceberg lettuce (Table 7) and one part poultry manure (Table 6) or cattle manure (Table 5). Four L of pretreated garden trade cattle manure were added to the cattle manure bins. To the poultry manure bins 0.2 L of pretreated garden trade poultry manure was mixed in with four L of peat. The different amounts of manure was due to the much stronger concentration of nutrients in the poultry manure.
Initial stocking densities were 150 worms per 14 L, of either species, which corresponds to 10.7 worms per L. The bins were placed in a climate controlled phytotron chamber with natural daylight only. The temperature in the chamber was set to 25.0°C and the humidity in the chamber was set to 90 %.

Table 3. Compost bin set up

0.8 L water per week
35 g lettuce per week
30% manure
70% peat

After 3 weeks of frequent monitoring and adjustments the watering- and feeding schedule was set to 35 g of lettuce (iceberg) per week and 0.8 L of water per week (Table 3), in each compost bin. The lettuce was torn and supplied on one occation each week. The plan was to continuously adjust food supply as worm populations increased. Populations did not increase and therefor the watering- and feeding schedule was not altered during the experiment.

Room tempered water was supplied on three occasions every week with the total amount of 0.8 L.

Figure 3. Rows of compost bins in the phytotron chamber, photo by Lisa Sandell

2.2 Substrate, manures and vegetable waste

Since the early 2000s it is no longer permitted to sell non processed manure directly to consumers. For this reason I used heated and granulated manure that is also premixed with peat. The expected development was that the worms would gain increased access to the nutrients as the mineralization progressed.

Pore volym is about 95 % for peat and somewhat lower for the cattle and poultry manure (Bohlin 2014). Water retaining capacity (using a drain height of 10 cm) is about 8.5 dl per L for the peat and cattle manure and slightly lower for the poultry manure.

Data on the properties of the peat and manures are approximate as each package may have different origins in terms of litter, animal species and animal feed (Hasselfors Garden 2014).

The substrate and manures were measured and placed in the bins with the peat in the bottom and the manures above.

Table 4. Peat. 100 % natural dark peat (Hasselfors Garden 2014), a very nutrient poor substrate. Nutrients other than TN and K are in levels to low to detect (nutrients in mg per L):

Organic matter content	85.00%
C/N ratio	50
Grams dry matter per L	100
pH	4.4
N*	20
K	30

* total N

Table 5. Pretreated garden trade (Hasselfors Garden 2014) cattle manure (nutrients in mg per L):

Organic matter content	80.00%
C/N ratio	20
Grams dry matter per L	200
pH	6.5
N*	550
K	1500
P	350
Ca	250
Mg	240
Na	200
S	20
Fe	42
Mn	20

* total N

Table 6. Pretreated garden trade (Hasselfors Garden 2014) poultry manure (nutrients in mg per L):

Organic matter content	90.00%
C/N ratio	7
Grams dry matter per L	600
pH	7.5-8.0
N*	31850
K	18870
P	11210
Ca	19460

* total N

Table 7. Nutrient content (Livsmedelsverkets livsmedelsdatabas 2014-01-28) in iceberg lettuce (nutrients in mg per 35 g, the worms weekly ration).
* organic matter content is missing since a reliable value could not be found in the available litterature:

Organic matter content	*
C/N ratio (Vincent 2012)	17
pH	6
Protein	800
Carbohydrates	900
K	63
P	8
Ca	9
Mg	4
Na	1
Fe	0.12
Vitamin C	2
Vitamin E	0.3

2.3 Method

Twenty 1.5 cm holes were drilled in the floor of the plastic bins and ten 1 cm holes were drilled in the walls of the bins. Fifteen 1.5 cm holes were drilled in the lids. The bottom and part of the walls were covered with a dense plastic web with 0.08 cm openings in order to prevent the worms from escaping through these aeration holes. In the phytotron chamber the bins were placed on battens to allow for aeration and for the excess water to leak freely from the bottoms.

The lettuce was placed on top of the composts with only a very gentle mixing of the top layer. Perhaps the most problematic parameter to adjust was the moisture content. The manure layer was placed on top of the bedding layer, the peat, in order to allow for the worms to approach the food source at their own pace. This top layer was dried out faster in the poultry manure bins than in the cattle manure bins, probably due to the higher water retention capacity of the cattle manure. During the first three weeks of adjustments the bins received 0.15 L of water six times a week.

It was a challenge to compensate for the fact that the insolation was uneven in the chamber and due to lack of space in the chamber it was not possible to rotate the order of the bins. The problem was partially solved by taping up sun blocking foil on a part of the glass wall of the chamber.

The worms arrived in styrofoam packages from three different breeders.
They were counted by hand and weighed (± 0.1 g) on a portable weighing scale (Mettler PE 3600, Delta Range, Mettler Toledo) before they were placed in the composts. About 30 % of the worms arrived in sawdust and the rest of the worms arrived in organic waste. Each individual was carefully cleaned by hand. No water was used except for the water that was sprayed on the worms occasionally to prevent them from drying out during the process of removing all particles glued to their sticky bodies.
Judging by their size the worms were mainly juveniles. Number of worms that was placed in each compost bin was 150 individuals. The total worm weight in each *E. fetida* replicate was 30 g. Average weight of *E. fetida* was 0.2 g and average length of *E. fetida* was 0.22 cm.
The total worm weight in each *D. veneta* replicate was 90 g. Average weight of *D. veneta* was 0.6 g and average length of *D. veneta* was 0.43 cm.

After four months the worms were harvested and the experiment was evaluated.
Four days before harvest all residues of lettuce were removed and the worms were left to starve the remaining days. At harvest one liter at the time was placed on a plastic covered white table and the material was examined carefully by hand. The worms were once again meticulously cleaned from substrate particles and then counted and weighed on the weighing scale before being placed in plastic bags and put in a freezer. All visible individuals were counted, thus even millimeter-sized specimens. One liter of the substrate (measured with a standard liter bowl) of each of the 20 vermicomposts was placed in a plastic bag and sent to a laboratory (LMI Analyslab, Helsingborg) for analysis of nutrient content.

The C/N ratios from the start of the experiment were calculated using the data from the producer of the substrate and manures. The ratios after harvest were calculated using the data from LMI Analyslab.

2.4 Experimental set-up

The experiment was a two factorial experiment including as described in table 8 the factors 1: worm species, 2 types of earthworms, *Eisenia fetida* (E.f) and *Dendrobaena veneta* (D.v) and factor 2: type of manure, 2 types, poultry manure (P) and cattle manure (C).
All vermicomposts were added the same vegetable waste product which was rinsed iceberg lettuce.
Five replicates of each treatment were used resulting in a total of 20 vermicompost bins.
The duration of the experiment was four months and it was assessed once at the end of the experiment.

Table 8. The four different treatments used in the experiment. *E.fetida* (E.f) and *D.veneta* (D.v) combined with either poultry (P) or cattle (C) manure. Vegetable waste, lettuce, in all treatments. Five replicates of each treatment were used.

Treatments	E.f	D.v	P	C	Lettuce
1	X		X		X
2		X	X		X
3	X			X	X
4		X		X	X

The reason for the frequent monitoring of the parameters temperature, pH and moisture content was to establish an appropriate water supply, an adequate feeding schedule and also to adjust the temperature in the phytotron chamber according to the temperature generated by the activity from the worms and from the microbial community in the bins.
As there were little indication of worm activity during these first three weeks, the temperature in the chamber was slowly increased from an initial temperature of 10.0°C to a final temperature of 25.0°C, as an attempt to boost the worm activity. I started with a temperature of only 10.0°C in the phytotron chamber so that I would avoid chocking the worms who had been transported for three days in single digit temperatures.
This was also the case with the food supply, in order to promote the worm activity lettuce was supplied on two occasions every week during the first three weeks.
Indicating low worm activity was the lack of elevated temperature in the core of the vermicomposts where the temperatures were measured. Also there were few signs of mixing of the material or visible worm passages inside the transparent walls and lids of the bins.

2.5 Continuous analyses

Temperature, pH and moisture content were monitored twice a week during the experiment with the exception for the first three weeks after installing of the worms when these parameters were monitored and adjusted on a daily basis. Value of pH was measured with a portable pH

measurement instrument (pH/EC/TDS, Waterproof family, Hanna Instruments, Waterboys, Alvesta, Sweden) from three different locations within each vermicompost and a mean value (± 0.1) was calculated from these three values.
Temperature was measured with a portable temperature measurement instrument (Nordtech instrument AB, Göteborg, Sweden), also from three different locations within each vermicompost and a mean value with ± 1.0°C was then calculated from these three values.
Moisture content was measured with a portable moisture measurement instrument (Fieldscout TDR 300 device, Spectrum Technologies, Plainfield, Illinois, USA), again from three different locations within each vermicompost and a mean value with ± 1 % was then calculated from these three values.

2.6 Analyses

The following analyses were performed before start up and then again at harvest of the worms.
* Number of worms
* Worm weight
* C:N ratios in the substrate

2.7 Statistical analysis

One-way ANOVA and Tukey method in conjunction with the results of ANOVA. Sources of variation were worm weight versus treatment and worm count versus treatment.

3. Expected worm growth

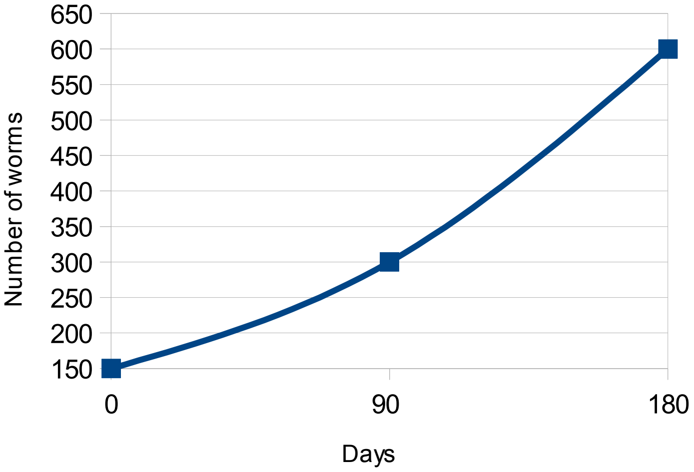


Figure 4. A conservative estimate of the expected growth in number of worms. The experiment lasted for 120 days, corresponding to a number of 390 worms in each replicate (Dominguez & Edwards 2011; Munroe 2009). An increase of 240 worms, from the initial starting number of 150 worms.

4. Results

The number of worms (Fig. 5) after harvest were significantly higher in treatments 1 and 3, that is the vermicomposts containing *E. fetida* treated with either poultry (treatment 1) or cattle (treatment 3) manure. In both *D. veneta* treatments the number of worms decreased, to 32 individuals in the poultry manure treatment (nr 2) and to 21 individuals in the cattle manure treatment (nr 4). Initially the number of worms in each replicate was 150 individuals.

Number of *E. fetida* was 132 individuals in the cattle manure treatment (nr 3) and 160 individuals in the poultry manure treatment, meaning that the number of worms actually only increased in one of the treatments, *E. fetida* treated with poultry manure (treatment 1).

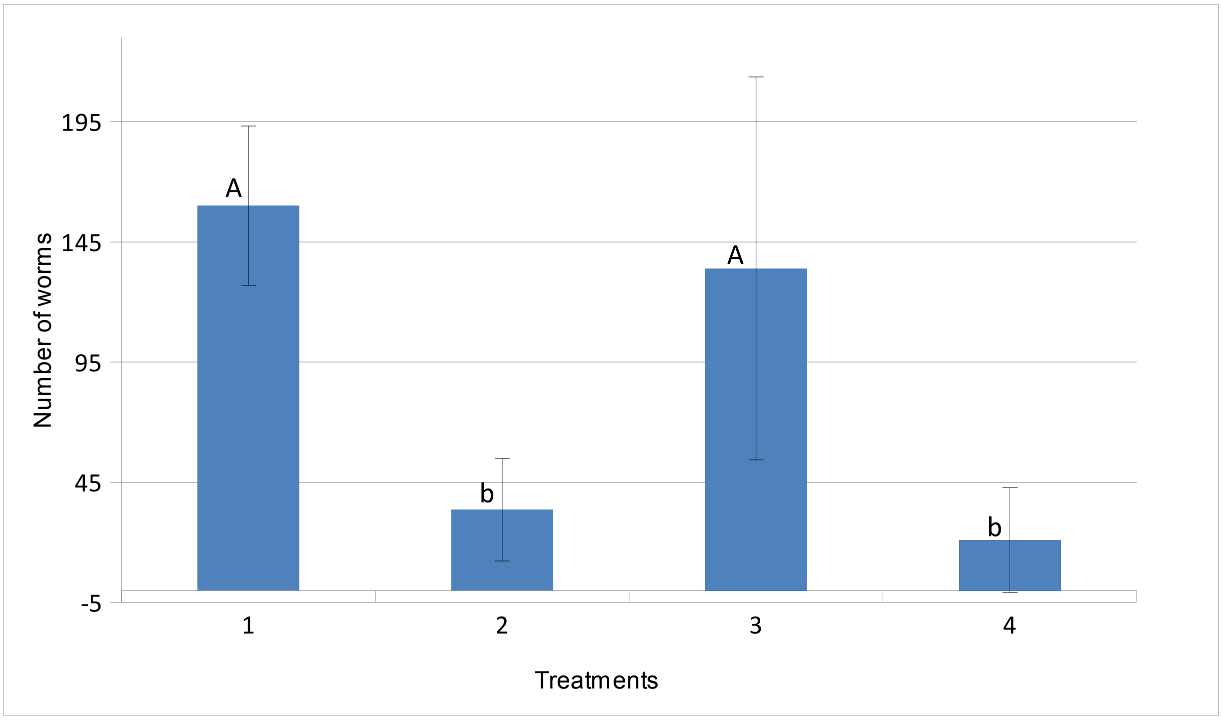


Figure 5. Counts of *E. fetida* cultivated in peat mixed with lettuce waste and poultry manure (treatment 1) or cattle manure (treatment 3) and of *D. veneta* cultivated in peat mixed with lettuce waste and poultry manure (treatment 2) or cattle manure (treatment 4). Analyses of variance were used. Treatment means of five replicates + SE were separated using Tukey's LSD, with P<0.05 considered significant, (P = <0.000). Means that do not share a letter are significantly different.
Standard error of mean (SE): treatment 1: 33.2, treatment 2: 21.4, treatment 3: 79.7, treatment 4: 21.9
The reason for not displaying the results as change of initial value (150) was to avoid downward staples.

At harvest the average weight of an *E. fetida* individual was 0.1 g, average length of an *E. fetida* individual was 0.16 cm and the total weight of *E. fetida* (the sum of ten replicates) was 169 g, corresponding to 360 g at the start.
Average weight of a *D. veneta* individual was 0.6 g, average length of a *D. veneta* individual was 0.40 cm and the total weight of *D. veneta* (the sum of ten replicates) was 185 g, corresponding to 1080 g at the start.

No significant differences between the treatments regarding worm weight (Fig. 6) could be found. At start up each *E. fetida* replicate contained a worm weight of 30 g and each *D. veneta* replicate contained a worm weight of 90 g. The worm weights decreased substantially in all treatments during the experiment.
After harvest the highest worm weight (20.3 g) was found in treatment 2, *D. veneta* with poultry manure. *D. veneta* with cattle manure contained a worm weight of 12.9 g.
E. fetida with poultry manure (treatment 1) weighed 16.0 g and *E. fetida* with cattle manure (treatment 3) weighed 13.2 g.

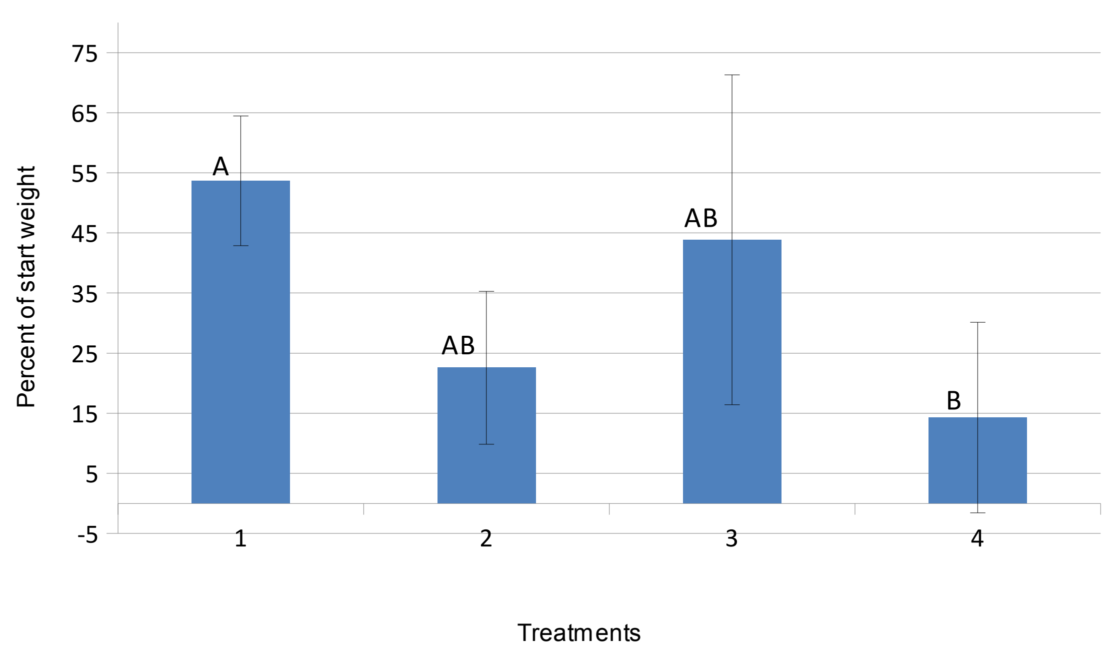


Figure 6. Weight of *E. fetida* cultivated in peat mixed with lettuce waste and poultry manure (treatment 1) or cattle manure (treatment 3) and of *D. veneta* cultivated in peat mixed with lettuce waste and poultry manure (treatment 2) or cattle manure (treatment 4). Considering the two species difference in starting weight, the weights are demonstrated as a mean percentage of initial weight of each replicate. Analyses of variance were used. Treatment means of five replicates + SE were separated using Tukey's LSD, with $P<0.05$ considered significant, ($P = 0.636$). Means that do not share a letter are significantly different.
Standard error of mean (SE): treatment 1: 10.8, treatment 2: 12.7, treatment 3: 27.4, treatment 4: 15.9

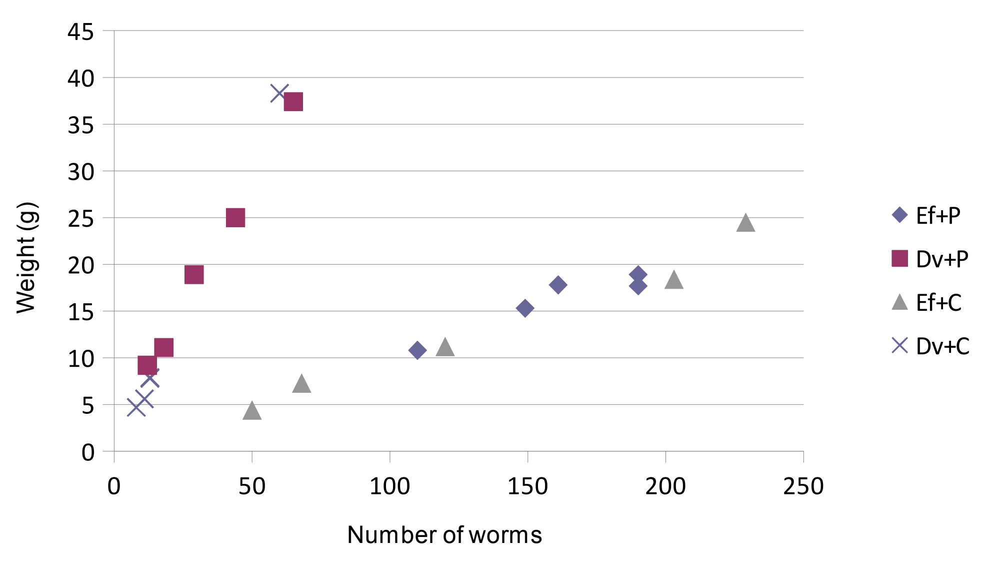


Figure 7. Weights and number of worms demonstrated together. Each one of the 20 replicates are shown in order to identify potential abnormal values.

Figure 7 displays the relation between worm weights and worm numbers in the different treatments. The *D. veneta* with cattle manure treatment appears to be the least successful of all treatments (Fig. 7) with one distinctive exception which drastically increases the mean value of the group.
The *D. veneta* with poultry manure replicates have relatively united values (Fig. 7) in terms of numbers of worms but this was not the case regarding the values of worm weights in the

treatment. Although the *E.fetida* with poultry manure treatment was more successful than the *E.fetida* with cattle manure treatment in general (Fig. 7), the *E.fetida* replicates with the highest number of worms are *E.fetida* with cattle manure replicates. Surprisingly, the *E.fetida* with cattle manure replicates also represents the *E.fetida* replicates with the lowest number of worms, rather much lower than the rest of the *E.fetida* values.

The most united values regarding both weight and numbers are found in the *E. fetida* with poultry manure treatment. This was also the most successful treatment in terms of growth of both weight and number of worms.
The wide distribution regarding both weight and numbers of both species makes it difficult to evaluate the results of the experiment.

Temperatures within the core of the vermicomposts were constant throughout the entire experiment, 25.0°C. That was the same temperature as the one prevailing in the climate controlled phytotron chamber which implies that there were not any form of measurable (by the heat generation) activity in progress within the vermicomposts.

Moisture content ranged between 62-94 % in the vermicompost substrate, with no significant difference between treatments. Out of a total of 126 moisture content measurements, 32 values have been removed from the results. These were extreme values of moisture contents between 100 and 180 %. These unrealistic values can only be explained by measurement and/or calibration errors.

Value of pH ranged between pH 4.6 and pH 5.3 in the vermicomposts, with no significant difference between treatments. Value of pH in the substrates was measured again at LMI Analyslab in Helsingborg four days after harvest (Table 9). These measurements showed values between pH 3.9 and pH 4.6 (mean values of each treatment).

Table 9. Mean values of pH and nutrient content (mg per L) in the four treatments after harvest, results from laboratory analysis (LMI Analyslab, Helsingborg):

	Treatment 1	Treatment 2	Treatment 3	Treatment 4
pH	4.6 (SE 0.3)	4.2 (SE 0.2)	4.2 (SE 0.3)	3.9 (SE 0.1)
N*	1.2 (SE 0.2)	1.4 (SE 0.4)	0.6 (SE 0.1)	0.8 (SE 0.1)
P	49 (SE 3.1)	51 (SE 9.0)	50 (SE 4.3)	49 (SE 2.3)
K	147 (SE 11.2)	129 (SE 16.9)	178 (SE 8.7)	167 (SE 19.0)
Mg	30 (SE 7.3)	41 (SE 6.4)	30 (SE 5.0)	55 (SE 4.8)
S	29 (SE 3.2)	39 (SE 4.1)	30 (SE 2.4)	36 (SE 1.9)
Ca	66 (SE 25.6)	117 (SE 22.8)	48 (SE 5.1)	122 (SE 4.4)
Na	63 (SE 10.9)	61 (SE 7.8)	55 (SE 1.9)	55 (SE 1.7)
Mn	0.7 (SE 0.1)	5 (SE 2.9)	0.8 (SE 0.1)	7.3 (SE 0.8)
Fe	116 (SE 4.0)	142 (SE 3.9)	98 (SE 3.2)	171 (SE 3.2)
Al	1.8 (SE 0.5)	0.9 (SE 0.2)	1.2 (SE 0.0)	1 (SE 0.2)

* total N

Most abundant after harvest were nutrients K and Fe (Table 9). Potassium levels were high also at the start of the experiment with 1500 mg/L in the cattle manure and as much as 18870 mg/L in the very concentrated poultry manure. The lettuce added contributed to the potassium levels with 63 mg each week. The peat did however dilute the potassium content with its low value of only 30 mg/L (each vermicompost contained 70 % peat).

Value of Fe are high in all treatments but the highest values are found in the *D. veneta* treatments.

Noteworthy is that the levels of nitrogen are very low in all treatments, ranging from 0.6 mg/L to 1.4 mg/L. Start values of nitrogen were 20 mg/L in the peat, 550 mg/L in the cattle manure and an entire 31850 mg/L in the much concentrated poultry manure.

The different treatments follow each other quite well in the nutrient spectrum. The major exception are the levels of calcium (Table 9) which are about twice as high in the *D. veneta* treatments compared to the *E. fetida* treatments. Value of calcium at the start of the experiment was highest in the poultry manure treatments (19460 mg/L in the poultry manure), treatments 1 and 2. The cattle manure contained 250 mg/L of calcium and the lettuce contributed with 9 mg per week.
Final values of phosphorus (Table 9) were remarkably low considering that the starting values were 11210 mg/L in the poultry manure and 350 mg/L in the cattle manure, plus the addition of another 8 mg per week from the lettuce.

C/N ratios in the substrates (Fig. 8) were relatively high at start up (C/N: 37-45) and decreased in all treatments during the experiment to C/N: 33-40.

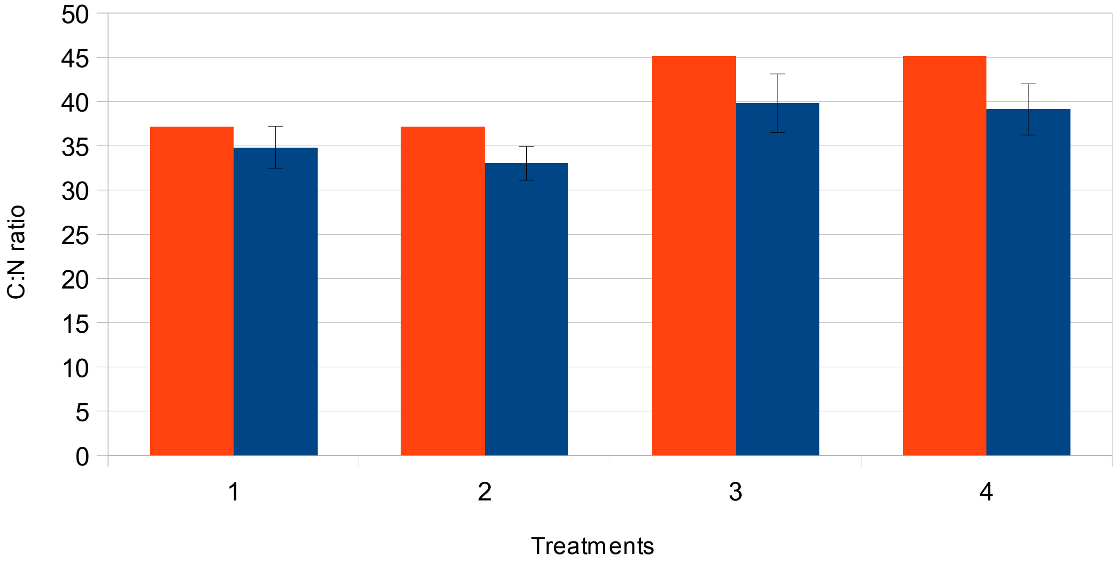


Figure 8. Mean value of C/N ratio of the substrate in each treatment at start up (left staple) and after harvest (right staple), (five replicates of each treatment). *E.fetida* combined with either poultry (treatment 1) or cattle manure (treatment 3) and *D.veneta* combined with either poultry (treatment 2) or cattle manure (treatment 4). There were no significant differences in C/N ratios between treatments (values after harvest) and no significant differences in the reduction of the ratios (p = 0.404). The percentage of the initial value of C/N ratios varied between 79 % and 103 % after harvest, in the 20 replicates.
Standard error of mean (of values after harvest): treatment 1: 2.4, treatment 2: 1.9, treatment 3: 3.3, treatment 4: 2.9

5. Discussion

Working with earthworms is always a much more complicated process than conventional composting.
The parameters moisture, pH, temperature, C/N ratio, oxygen supply and microbial activity all interact and influence the worm population in complex ways (Edwards 1998).
Species of earthworms used in vermicomposting are relatively tolerant of the varied environmental conditions in organic wastes. However, it has been demonstrated that earthworms have well-defined tolerance limits regarding especially the parameters pH and temperature with the result that wastes are processed much less efficiently outside their narrow range of favourable chemical and environmental conditions (Dominguez & Edwards 2004).
The earthworms major contribution to a compost is fragmenting the organic matter, but their

influence on their surroundings is far more extensive than that. As they move through the compost their excretions of ammonia and urea add to the nitrogen content in the substrate and their castings aswell as their mucus secretions provide nourishment for microbial populations (Edwards 1998; Edwards & Bohlen 1996).

5.1 The vermicompost

Provided favourable conditions, interactions between the organic matter, earthworms and other invertebrates and microorganisms will result in stabilization and homogenisation of the organic matter (Dominguez & Edwards 2004).
During harvest of the earthworms used in this experiment the vermicompost was studied visually and by hand and I came to the conclusion that the vermicompost showed no resemblance with a homogeneous vermicompost at its matured state.
This suggests low worm activity and low levels of degradation in all composts. Lettuce is an adequate food source for earthworms with its high water, nitrogen and potassium content.

However, the food source is not accessible to the worms until microorganisms such as bacteria, actinomycetes, molds and yeasts have had 5-6 days to decompose the material (Persson 1996). At harvest pieces of undegraded lettuce were found throughout the entire substrate in all compost bins indicating that the conditions required for the treatments to develop as a small scale ecosystems had not been met.
If the worm populations had increased as expected and the vermicomposts had reached a matured state an analysis of the vermicast would have been justified.

5.2 Worm growth

The results showed that the number of worms (Fig. 5) increased only in the *E. fetida* with poultry manure treatment. Number of worms were significantly higher in the *E. fetida* treatments compared to the *D. veneta* treatments but the worm weights (Fig. 6) had decreased substantially in all treatments. No significant difference in number of worms or worm weight was found between the two types of manure.

The results did not support the hypotheses but may indicate that choice of earthworm species in the vermicomposts might have greater influence on the outcome in terms of worm production than the choice of manure added to the vermicomposts. *D. veneta* decreased in both number (Fig. 5) and weight (Fig. 6) during the experiment. Even though *E. fetida* increased in number in the poultry manure treatment (Fig. 5), they actually decreased in weight (Fig. 6). All visible individuals were counted at harvest and the *E.fetida* composts with the higher worm count contained clusters of very tiny individuals drastically affecting the numbers.
Assessment of earthworm weight (Fig. 7) after harvest revealed that few individuals had reached for the species average adult size.

The hypothesis that earthworms can be cultivated in a vegetable waste compost was not supported by my results and despite the added manure the treatments never evolved into productive vermicultures supporting a continuous harvest of worms.

To be able to verify or reject the hypotheses the experimental treatments should always be compared against relevant control treatments. In the case of this study it means that there should be control treatments with manure but without vegetable waste as well as control treatments with vegetable waste but without manure. I did have four control composts that received no lettuce during the entire experiment. They contained each of the worm species combined with either type of manure. These four control treatments were measured and monitored in the exact same manner as the other treatments and the results were processed in the same way. The results from these control treatments showed no significant difference compared to the vegetable waste treatments. The conclusion to be drawn from this is that hypothesis number one could not be verified.

The lack of floor space in the phytotron chamber made it impossible to fit the four control treatments without manure needed in order to verify or reject the second hypothesis. In retrospect, I think it was a mistake that this problem was not remedied at start up.

Observations during the experiment and at harvest revealed that the vegetable waste was hardly decomposed at all. The worms were provided with a generous amount of feed as an attempt to promote worm activity and reproduction. When these actvities did not occur, the lettuce remained undegraded in the treatments and it is likely that the weekly ration of 35 g became to much for the worms. In a vermiculture incorporated to an aquaponic system a feeding schedule that amounts to one ration of vegetable waste every two weeks is more realistic.

E. fetida is the smaller species but also the more prolific (Table 2) of the two and they reach adult size sooner than *D. veneta.* Considering the negative outcome of this study it is obvious that the requirements for well functioning vermicultures had not been met. Since *E. fetida* appears to have coped with the inadequate conditions slightly better than *D. veneta* one might argue that *E. fetida* is the less sensitive of the two species, a fact that has already been established in earlier vermiculture research (Sinha & Valani 2011).

5.3 Nutrient levels and C/N ratios

Earthworms have major influences on nutrient cycling processes in composts as well as in many ecosystems (Edwards & Bohlen 1996). Many of these influences of earthworms on nutrient cycles and on the mineralization of organic matter are dependent on the interactions that takes place between earthworms and microorganisms.

Vermicompost has higher availability of nutrients N, P, K and Mg compared to conventional composts (Singh 2012). Processing compost through earthworms has been found to increase the NPK value by 3 to 4 times.

Essential for growth and activity of the decomposers are nutrients C and N. N is required for cell structure and C represents main energy source. Nutrients Cu, Ni, Mo, Fe, Zn and Na are also crucial for a prosperous micro society (Epstein 1997).

Vermicomposting generally retains more nitrogen than a regular compost and thus reduces the C/N ratio (Nagavallemma et al. 2006). C/N ratios are reduced in my vermicomposts as well (Fig. 8) but the difference is marginal and the continuous watering may very well be responsible for leaching out more of the nutrients compared to the amounts of nutrients added to the system as worm feed. I would have expected the weekly addition of lettuce with a C/N ratio of 17 to have a greater impact on the final C/N ratio values. There was a lot of undegraded lettuce in the treatments and this was all meticulously removed before harvest and therefor did not affect the final measurements.

Although reduced during the experiment, the C/N ratios (Fig. 8) were still above the equilibrium prevailing in a well balanced vermicompost and preferred by most decomposing microorganisms, C/N: 20-25 (Epstein 1997). Earthworms may also influence the C/N ratio by feeding selectively on the organic matter with the highest nitrogen content and thus increase the C/N ratio of the vermicompost. Considering the low activity and low survival rate (Fig. 7) of the earthworms in this study this was hardly a factor affecting the final C/N ratios.

My expectations were that the organic matter content would be gradually reduced, until reaching an equilibrium around C/N: 20 and that the majority of the carbon content in the organic matter would be released to the air as carbon dioxide through microbial cell respiration as they break down organic molecules to extract energy.

The high C/N ratios, the slow decomposition of the lettuce and the worm death are all indications of a non prosperous microbial community.

At start up the concentrations of total nitrogen was nearly three times as high in the poultry manure composts compared to the cattle manure composts. This difference was somewhat evened out during the experiment and after harvest the poultry manure composts had a concentration of nitrogen which was about twice the one found in the cattle manure composts.

Within the time frame of the experiment, it was not possible to investigate the reasons for the

unexpectedly low nitrogen concentrations at the end of the experiment.
Nitrogen dynamics in vermiculture systems possibly represents an exciting area for future research and further studies are needed in order to fully understand and optimize the conditions of such systems.

In a well-functioning vermicompost/vermiculture the concentration of phosphorus should be expected to increase as the organic matter decomposes, until a certain balance is reached (Pathade & Goel 2010). In my experiment the values of phosphorus after harvest (Table 9) were remarkably low, again indicating that the conditions required for the microorganisms to survive and function had not been met.

Concentrations of potassium, calcium and magnesium are often greater in vermicasts than in uningested compost (Edwards & Bohlen 1996). Again, due to the low activity and low survival rate of the worms in my vermicomposts it is difficult to draw any conclusions based on worm activity. Instead, the conclusions to be drawn from my experiment should be based on the absence of worm- and possibly also microbe activity and how this absence is demonstrated in the nutrient levels of the vermicomposts.
Potassium levels (Table 9) are high in my treatments but still lower than the levels at the start of the experiment and this is also the case with the calcium levels.

An interesting observation is how the three nutrients calcium, iron and manganese all follow the same pattern in that they are significantly higher in the *D. veneta* treatments compared to the *E. fetida* treatments. This could suggest that there was a higher percentage of vermicasts in the *D. veneta* treatments (Edwards & Bohlen 1996). Studies have shown that also micro nutrients such as iron and manganese occur more abundantly in vermicasts than in other compost materials (Dominguez & Edwards 2004).
The fact that *D. veneta* were, although fewer in number, much larger in average size than *E. fetida* provides a possible explanation for these differences in nutrient contents.
Fewer but larger worms produce more castings compared to numerous newly hatched worms aggregate vermicast production (Edwards & Bohlen 1996).
However, I have not been able to find any studies that compare the nutrient contents in the castings of the particular species at issue in this case, *E. fetida* and *D. veneta.*

The high levels of iron (Table 9) measured by LMI Analyslab after harvest are difficult to explain. The cattle manure contained 42 mg/L and the other substrates as well as the lettuce added to the vermicompost can hardly have contributed to these exceptional values.
The iron content in the treatments (Table 9) were about 3 to 4 times higher than what would be expected in a vermicompost,10-30 mg/L (Sinha & Valani 2011). While earthworms are quite tolerent to high iron levels, the microbial community is more sensitive and the biodiversity of microorganisms will be reduced if the iron content is too high (Sinha & Valani 2011). Growth and number of microorganisms was not examined in this study and it would be valuable to investigate also the state of the micro society in future studies on the topic.

It is possible that the experimental conditions did not favor the establishment of an active microbial community in the vermicomposts. Peat naturally contains very few microorganisms due to the very low nutrient content, and the cattle and poultry manure are both nearly sterile after being heated during the manufacturing process (Bohlin 2014). Sterilized water and carefully rinsed lettuce are not likely to have contributed with any microorganisms during the experiment.
Microorganisms might have been introduced into the vermicomposts via the skin and gut content of the worms, but this source was likely not of large importance due to the careful cleaning of the worms before the start of the experiment.
The undegraded lettuce is an indication that there was no or very low microbial activity. Without any degradation from the microbial community the lettuce has not been made accessible to the worms.
Consequently, there were perhaps never any reasonable conditions for the worms to survive and thrive.

5.4 Temperature and moisture contents

Worm activity and activity from the microbes were expected to cause an elevation in temperature in the core of the vermicomposts where temperature was measured but this sign of degradation activities did not occur at any time during the four months of the experiment.
The activity from worms and microbes was only evaluated by appearance, temperature and degradation of the vermicomposts.
The components pH, moisture, aeration, C/N ratio, organic matter content and microbe activity are all interdependent and any imbalance will soon cause a destructive chain reaction (Pathade & Goel 2010).

The negative effects are more noticeable in the vulnerable small scale system. Although in many aspects extremely tolerable, earthworms are sensitive to rapid changes in living conditions and in a small compost there is nowhere to escape. Balancing the various parameters in a small vermicompost requires meticulous surveillance of decisive parameters. The outcome of this study might have been different had I used larger composts with more buffer capacity regarding conditions harmful to both worms and microbes.

Moisture content ranged between 62 % and 94 % in the compost substrate, with no significant difference between treatments. Overwatering causes anaerobic conditions and the microbial community is replaced by sulphur bacteria (Singh 2012). At moisture contents above 60 % pore space starts to get blocked by water, restricting oxygen diffusion and thereby compromising the aerobic microbial community (Epstein 1997).

During harvest there was a distinct odor of sulphur in the bottom layer of several vermicomposts. The bottom of the bins were covered with a web to prevent the worms from escaping through the water holes. This web was clogged with dirt from the drainage, causing the water to stand still in the bottom layer. Due to measuring errors (the first four weeks I used a TDR 300 moisture meter whose needle did not reach the bottom layer) this problem was not discovered.
Inexperience from using the moisture meter might be one explanation for the large variation in measured moisture contents.

5.5 Values of pH

Earthworms in general are very sensitive to the concentration of hydrogen ions (pH) and to rapid changes in these concentrations with the result that the value of pH is often the factor that controls the numbers and distribution of earthworms in any particular substrate (Pathade & Goel 2010).
Except for the species *Dendrobaena octaedra,* which is a very acid-tolerant species, few earthworms will be found in substrates with a pH below pH 4.3 (Edwards & Bohlen 1996).
E. fetida has been reported to tolerate a pH range between pH 4.0 and pH 7.0, although they prefer and reproduce in the pH range from pH 7 to pH 8 (Edwards & Bohlen 1996).

Studies have shown that the vermicasts are often more pH neutral than the substrate in which the worms live. The worms are thus able to, to a certain extent, influence the pH value of their immediate environment. A probable explanation for this phenomenon is that the substrate is neutralized by excretion of ammonia and also secretions from the intestine.
Compost worms represents the more resistant earthworm species and can function in quite a wide pH range, from pH 5 to about pH 9. Some studies have even shown that when given a choice in pH value, the compost worms will move towards more acid pH values around pH 5.0 (Dominguez & Edwards 2011).
In the current study pH ranged between 4.6 and 5.3 in the vermicompost substrates.
Values of pH measured at LMI Analyslab in Helsingborg four days after harvest showed values between 3.9-4.6. It appears that pH has decreased after harvesting of the worms. The different values might also be due to different measuring methods.

Low pH from an acidic bedding substrate such as peat (pH 4.4) which was used in the treatments

of this study can facilitate the development of mites and other pests (Dominguez & Edwards 2011). The low values of pH measured in the vermicomposts of this experiment and/or a possible presence of pests is possibly one explanation for the deceased worms and the negative development of population growth. The possible presence of pests was not examined in this study. Earthworms will flee from values below pH 4.5 (Buch 1987), very close to my lowest measured values of pH 4.6.
Value of pH in the vermicomposts could have easily been adjusted upwards by adding calcium carbonate to the substrates.

5.6 Sources of error

The experiments began in January, the harsh winter of 2012 and the worms had to be transported across the country, 1500 km. It is reasonable to assume that the worms arrived attenuated by transport conditions and possible extreme shifts in temperatures. The breeders growing conditions regarding in particular substrate and fodder differed considerably from the conditions in my vermicomposts. It is possible that the worms had difficulties adjusting to their new environment and new feed.
With regard to their small size the worms used in this experiment at start up were mainly juveniles. Earthworms used in experimentation should be bred in an equivalent manner and be of an even size (Carlsson 2000). The worms used in this study were quite uneven in size.

Harvest and transport are both potential risk factors (Carlsson 2000) for the worms since they compose a stress for the worms that can lead to a fatal chock if the stress is repeated. For this reason worms should always be allowed a few days of rest between harvest and transport and this was not the case with the worms used in this study. In most cases earthworms can adjust to new environments very different from what they are used to as long as they are allowed enough time to acclimatize (Carlsson 2000). This means slowly exchanging the accustomed living substrate with the new substrate. Lack of experience and knowledge of vermicomposting is the reason why this was not done in this study. The issue of adjusting the worms to a new environment can be avoided by using worm eggs instead of worms and allowing them to hatch in the substrate of the experiment.

During the experiment I found occasional escapees, mostly *D. veneta.* They escaped through the aeration holes in the lids, well above the compost substrate. Lack of oxygen in the bottom layer can hardly explain this destructive behaviour. Search for food appears to be a more reasonable explanation, suggesting incorrect proportions in the composts with to much peat and not enough nutrients or that the worms were searching for their accustomed fodder. If the lights in the phytotron chamber would have been kept on at all time this might have prevented the worms from escaping.
The ventilation in the chamber was quite loud. In order to achieve a productive vermiculture it is important that the worms are left alone as much as possible and that their environment is kept calm and quiet.

In a study with several replicates it is important that the replicates are provided with equal conditions. The insolation in the chamber was quite uneven and the ventilation duct was more noticeable at one end of the room. Since rotating the compost bins was not an option the bins should have been placed randomly in the chamber and not in groups according to treatment as was the case. Again, lack of experience is the reason for this potential experimental error.

5.7 Future research

Many earthworm breeders testify that success follow after years of trial and error. Although the results of this study failed to live up to my expectations regarding worm production lots of previous research indicate that the vermicompost is superior to conventional compost in the following areas: level of beneficial microorganisms, ability to stimulate plant growth and supress plant disease, ability to repel pests and last but not least the level of plant-available nutrients (Munroe 2009; Dominguez & Edwards 2004).

Incorporating a vermicompost/vermiculture in an aquaponic system requires the creation of optimum conditions to meet the needs of all four involved organisms - fish, plants, bacteria and worms (fish feed). This challenge and also marketing the products (fish bred on worm protein and vermicompost as plant nutrition) properly, is subject of further refinement (Tyson et al. 2011).

Studies with frozen earthworms as fish feed, have shown poor growth rates in the fish stocks, due to the low palatability of the earthworms after being frozen. A higher survival rate with a fresh/dried earthworm diet presents a potential advantage in favour of on site vermicomposting (Huu Yen Nhi et al. 2010). Incorporating a vermiculture in an aquaponic system has the advantage of providing the fish with a freshly produced earthworm diet. A desirable follow-up of this study would be processing of the harvested worms on site.

Commercially, aquaponics is still in its infancy but the associated technology is rapidly evolving and the awareness of its many advantages is constantly growing (Al-Hafedh et al. 2008).
Further research concerning the fields production efficiency, conversion rates, harvesting rates and techniques, and in what ways earthworm biomass can be used as animal feed, would be beneficial also for the aquaculture department (Lofs-Holmin 1986).

6. Conclusions

* The results of my experiment do not support the hypothesis that earthworms can be cultivated in an aquaponic waste compost. Despite the added manure the treatments never evolved into productive vermicultures supporting a continuous harvest of worms.

* Further research is needed to answer the question whether integration of vermicultures in aquaponic systems is a possible means to produce fish feed in aquaponic systems.

* Balancing the various parameters in a small vermicompost requires experience and meticulous surveillance of decisive parameters. The outcome of this study might have been different had I used larger composts with more buffer capacity regarding conditions harmful to both worms and the microbial community.

7. Acknowledgements

This work has been financed by SLU - KON Vattenbruk which is greatly acknowledged.
I would also like to thank all enthusiastic aquaponic supporters whose fascinating home videos inspired this project.

8. References

Al-Hafedh, Y., Alam, A., Beltagi, M.S. (2008). *Food Production and Water Conservation in a Recirculating Aquaponic System in Saudi Arabia at Different Ratios of Fish Feed to Plants.* Journal of the world Aquaculture Society, vol. 39(4), pp. 510-519

Aquaponics Sweden (2013-02-09). *Aquaponics Sweden*. http://www.aquaponicssweden.com/ [2014-04-02]

Brett, R.R. & Midmore D.J. (2008). *Sustainable Aquaponics.* Practical Hydroponics and Greenhouses, vol. 103, pp. 87-90

Buch, W. (1987). *Daggmasken i trädgård och jordbruk*. 1. ed. Bokskogen, Göteborg

Carlsson, P. (2000). *Daggmask och daggmaskodling.* Swedish University of Agricultural Sciences. Deptartment of Biosystems and Technology (5 poängs examensarbete 2000:13)

Chan, G.L. (1993). *Aquaculture, Ecological Engineering: Lessons from China.* Ambio, vol. 22(7), pp. 491-494

Connely, D. (2013). *Vermiculture: Worm Farming as an Inexpensive and Green Way to Get Quality Compost.* Smashwords. Available: Scribd. [2014-04-05]

Darwin, C. (1881). *The Formation of Vegetable Mould Through the Action of Worms with Observations on their Habits.* 10. ed. Faber and Faber, London

Dominguez, J. & Edwards, C.A. (2004). *Vermicomposting organic wastes: A review.* Soil Zoology for Sustainable Development in the 21st Century. Report, Cairo

Dominguez, J. & Edwards, C.A. (2011). *Biology and Ecology of Earthworm Species Used for Vermicomposting.* Taylor & Francis Group LLC, Oxford

Edwards, C.A. (1998). *Earthworm Ecology*. CRC Press LLC, Boca Raton

Edwards, C.A. & Bohlen, P.J. (1996). *Biology and Ecology of Earthworms*. 3. ed. St Edmundsbury Press, Bury St Edmunds

Epstein, E. (1997). *The Science of Composting*. Technomic Publishing Company, Inc., Basel

Fadaee, R. (2012). *A review on earthworm Esienia fetida and its applications.* Annals of Biological Research, vol. 3 (5), pp. 2500-2506

Graber, A. & Junge, R. (2009). *Aquaponic Systems: Nutrient recycling from fish wastewater by vegetable production.* Desalination, vol. 246, pp. 147-156

Hasselfors Garden (2014-03-20). *Konsument/Produkter.* http://www.hasselforsgarden.se/konsument/produkter/torv/solmull-m.-goedsel-kalk [2014-04-01]

Hughey, T.W. (2005). *Barrel-ponics (a.k.a. Aquaponics in a barrel).* http://www.aces.edu/dept/fisheries/education/documents/barrel-ponics.pdf [2012-10-02]

Hussaini, A. (2013). *Vermiculture bio-technology: An effective tool for economic and environmental sustainability.* African Journal of Environmental Science and Technology, vol. 7(2), pp. 56-60

Huu Yen Nhi, N., Preston, T.R., Ogle, B., Lundh, T. (2010). *Effect of earthworms as replacement for trash fish and rice field prawns on growth and survival rate of marble goby* (*Oxyeleotris marmoratus*) *and Tra catfish* (*Pangasius hypophthalmus*). Livestock Research for Rural Development, vol. 22 (11), pp. 204-224

Kattastrands kretsloppsodling (2013-12-16). *Växter och fiskar i samma vatten.* http://www.rainbowfish.se/svenska/index.html [2014-04-02]

Livsmedelsverkets livsmedelsdatabas (2014-01-28). *Livsmedelsdatabasen - sök näringsinnehåll.* http://www7.slv.se/Naringssok/ [2014-04-05]

Lofs-Holmin, A. (1986). *Processing of municipal sludges through earthworms (Dendrobaena veneta).* Swedish Journal of Agricultural Research, vol. 16, pp. 67-71

Long, B (2012). *The EZ Guide to Aeroponics, Hydroponics and Aquaponics.* Smashwords. Available: Scribd. [2014-04-09]

Maize, P.V., Naikwade, S.T., Sankpal, J.B.B. (2010). *Management of waste by composting, vermicomposting and its use for improvement of growth, yield and quality of fodder.* International Journal of Research in IT, Management and Engineering, IJRIME , vol. 3(3), pp. 9-23

Mammoliti, C. (2014-01-05). *Trash Mountain Project.* http://trashmountain.com/ [2014-02-19]

Muminovic, M. (2010). *Domestic feed sources to farmed Arctic Charr (Salvelinus alpinus). An investigation of nutritional implications and impact on the ecological footprint.* Swedish University of Agricultural Sciences. Deptartment of Wildlife, Fish and Environmental Studies (MSc Thesis 2010: 21).

Munroe, G. (2009). *Manual of On-Farm Vermicomposting and Vermiculture*. Organic Agriculture Centre of Canada. http://oacc.info/docs/vermiculture_farmersmanual_gm.pdf [2012-09-14]

Nagavallemma, K.P., Wani, S.P., Stephane, L., Padmaja, V.V., Vineela, C., Babu Rao, M., Sahrawat, K.L. (2006). *Vermicomposting: Recycling Wastes into Valuable Organic Fertilizer*. Global Theme on Agrecosystems Report no.8, International Crops Research Institute for the Semi-Arid Tropics, India

Nelson, R. (2014-04-08). *Commercial systems*. http://aquaponics.com/page/commercial-systems [2014-04-06]

Pantanella, E., Danaher, J.J., Rakocy, J.E., Shultz, R.C., Bailey, D.S. (2011). *Alternative Media Types for Seedling Production of Lettuce and Basil.* Acta Horticulturae, vol. 891, pp. 257-263

Pathade, G.R. & Goel P.K. (2010). *Biotechnology in Environmental Management.* Global Media, Jaipur

Persson, K. (1996). *Att lyckas med komposten*. Utbildningsförlaget Brevskolan, Stockholm

Rupani, P.F., Embrandiri, A., Quaik, S., Ibrahim, H.M. (2012). *Sustainable Management of Palm Oil Mill Waste Using Vermicomposting Technology.* The Asian Conference on Sustainability, Energy & the Environment. Official Conference Report 2012, Osaka

Singh, K., Nath, G., Rai, R., Shukla, R.C. (2013). *Food Preference of Eisenia foetida among Different Combinations of Animal Dung and Agro/Kitchen Wastes.* Botany Research International, vol. 6 (1), pp. 23-26

Singh, R.P. (2012). *Organic fertilizers: types, production and environmental impact*. Nova Science Publishers, Inc., New York

Sinha, R.K. & Valani, D. (2011). *Engineering and Technology : Vermiculture Revolution: The Technological Revival of Charles Darwin's Unheralded Soldiers of Mankind.* Nova Science Publishers, Inc. Available: Ebrary [2014-04-05]

Tidwell, J.H. (2012). *Aquaculture Production Systems.* John Wiley & Sons, Inc., Oxford

Tyson, R., Treadwell, D., Simonne, E. (2011). *Opportunities and Challenges to Sustainability in Aquaponic Systems.* HortTechnology, vol. 21 (1), pp. 6-11

Vincent, W. (2012). *The Complete Guide to Working with Worms: Using the Gardener's Best Friend for Organic Gardening and Composting.* Atlantic Publishing. Available: Scribd. [2014-04-05]

Vinje, E. (2014-03-12). *Carbon-to-Nitrogen Ratios*. http://www.planetnatural.com/composting-101/c-n-ratio/ [2014-04-06]

Oral references:

Bohlin, Claes (2014), Horticulturalist at Hasselfors Garden, Örebro, Sweden

Storebakken, Trond (2014), Norwegian University of Life Sciences, Ås, Norway