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Fatty acid composition of black soldier fly larvae - impact of the rearing substrate

Fettsyrasammansättningen i amerikansk vapenfluga – påverkan från födosubstratet

Nils Ewald

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Abstract

With an increasing world population and meat consumption the black soldier fly (BSF; *Hermetia illucens*) shows potential as feed for animals, while recycling nutrients from food waste. To produce larvae of high quality as animal feed, further understanding is needed of how the substrate affects the nutritional composition of the larvae. In this project the aim was to investigate how the chemical composition of the substrate affects the one of the larvae, with focus on fatty acids. The chemical composition of BSF larvae (BSFL) reared on six different substrates was investigated: 1) retaken bread, 2) rainbow trout, 3) food waste, 4) fresh mussels, 5) ensiled mussels and 6) rancid mussels. Significant differences were recorded in proximate and fatty acid composition between larvae reared on different substrates, especially in the crude fat and ash content. Linear regression analysis indicated mainly the carbohydrate, crude protein and ash content of the substrate affected the proximate composition of the larvae. The proportion of saturated fatty acids (SFA), especially lauric acid, increased in the larvae with an increased larval weight, while mono- and polyunsaturated fatty acids decreased. The main factor for finding omega-3 fatty acids in the larvae was the concentrations of these fatty acids in the substrate. The analysis of malondialdehyde concentration in the substrates did not produce reliable results for the samples analysed. While the high SFA content in the larvae could be problematic in aquaculture, the use of substrates such as mussels and fish could improve the quality of the BSFL as a feed alternative.

Keywords: Black soldier fly, *Hermetia illucens*, fatty acids, omega-3 fatty acids, malondialdehyde, blue mussels, waste management

Sammanfattning

I takt med att jordens befolkning ökar och äter allt mer animaliska livsmedel, kan den amerikanska vapenflugan (BSF; *Hermetia illucens*) komma att bli viktig då den potentiellt kan användas som djurfoder och samtidigt tar vara på livsmedelsavfall. För att kunna producera larver av hög kvalitet behövs dock en vidare förståelse för hur olika avfallsprodukter påverkar den slutgiltiga näringssammansättningen i larverna. Syftet med detta projekt var att undersöka hur näringssammansättningen i olika avfallsprodukter påverkade sammansättningen i BSF-larver, med fokus på fettsyrasammansättningen. Näringssammansättningen analyserades i larver som fötts upp på sex olika substrat: 1) återtaget bröd, 2) regnbågslax, 3) livsmedelsavfall, 4) färska musslor, 5) ensilerade musslor och 6) härskna musslor. Signifikanta skillnader hittades i näringssammansättningen mellan de olika larverna, speciellt i fett- och askhalten. Regressionsanalys visade på att främst halten kolhydrater, protein och aska i substraten påverkade larvernas näringssammansättning. Mängden mättade fettsyror, främst laurinsyra, ökade i takt med att larverna blev större, medan mängden enkel- och fleromättade fettsyror minskade. Den viktigaste faktorn för att det skulle finnas omega-3 fettsyror i larverna var att dessa fettsyror också återfanns i substratet. Analys av koncentrationen malondialdehyd i proverna gav inga tillförlitliga resultat. Medan den höga andelen mättade fettsyror i larverna kan vara ett problem i fiskodling, visar resultaten från denna studie också att användningen av substrat såsom fisk och musslor kan öka kvalitén i BSF-larverna som fiskfoder.

Nyckelord: Amerikansk vapenfluga, *Hermetia illucens*, fettsyror, omega-3 fettsyror, malondialdehyd, blåmusslor, avfallshantering

There are only three subjects: love, death and flies. Ever since man was invented, this emotion, this fear and the presence of these insects have been his constant companions. Other people can take care of the first two subjects. Me, I just concern myself with flies – a much greater theme than men, though maybe not greater than women.

Augusto Moterroso

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Abbreviations

ALA	α-linolenic acid[1]
ANOVA	Analysis of variance
BCR	Waste-to-biomass conversion ratio
BSF	Black soldier fly
BSFL	Black soldier fly larvae
DHA	Docosahexaenoic acid[1]
EPA	Eicosapentaenoic acid[1]
GC	Gas chromatography
MDA	Malondialdehyde
MUFA	Mono-unsaturated fatty acids
PUFA	Poly-unsaturated fatty acids
SFA	Saturated fatty acids
SLU	Swedish University of Agricultural Sciences
TBA	Thiobarbituric acid

[1]Abbreviations for all 21 fatty acids analysed in this study are found in Appendix 2.

1 Introduction

1.1 Global food challenges

From the world population in 2017 of 7.6 billion inhabitants, the United Nations (2017) predicts that the world population will increase to 9.8 billion in 2050, and to 11.1 billion in 2100. With an additional 2 billion inhabitants until 2050, the food production will need to increase, in a world where, as of 2016, 794 million people were still undernourished (FAO, 2017). At the same time, an increased production of bio-based fuels has set aside more agricultural land for production of non-food biomass, while growing incomes in many countries has resulted in a higher consumption of meat, which requires more land for production (FAO, 2017).

Another global concern are the great amounts of food wasted globally. It has been estimated that one third of all the food produced in the world is thrown away as waste (FAO, 2017). While poor infrastructure and harvesting systems are main causes of losses in low-, and middle income countries, great amounts of food are thrown away by the consumers in high-income countries (Parfitt *et al.*, 2010). Only within the European union, a total of 94 million tonnes of animal- and vegetable waste was created in 2016 (Eurostat, 2018). According to the food waste hierarchy, as presented by FAO (2013), prevention and reduction are the most effective ways of dealing with food waste, in terms of environment, social and economic factors. The second best option in the hierarchy, is to reuse the food waste, followed by recycling, and landfill at the bottom of the hierarchy. Therefore it should be considered to use the food waste (not fit for human consumption) as feed for animals (reuse), before sending it for digestion to biogas (recycling) or incineration (FAO, 2013). While the pig has historically been an animal fed large amounts of food waste (FAO, 2013), a new group of omnivorous animals has gotten increasing attention lately: insects.

1.2 Potential of the Black soldier fly

Insects can feed on various waste streams, and in comparison with conventional livestock, they have been found to emit less ammonia and greenhouse gases, and have a higher feed conversion efficiency (van Huis & Tomberlin, 2017). Additionally, in comparison with meat products, various insect species has been shown to have a beneficial nutritional composition (Payne *et al.*, 2016). For these reasons, insects can be considered an alternative feed source for animals (van Huis & Tomberlin, 2017). Out of the more than one million insects species found worldwide (Resh & Cardé, 2009) a few species are considered for rearing on organic waste streams. The yellow mealworm (*Tenebrio molitor*) and various crickets have potential as human food. For animal feed though, the black soldier fly (BSF; *Hermetia illucens*) is one of the most promising species, due to the ability to efficiently convert various organic waste streams into biomass (van Huis & Tomberlin, 2017). The BSF, and especially the larvae thereof, has been described by Tomberlin and Cammack (2017) as "voracious, generalist feeders, able to consume a wide variety of materials". Through this ability, the BSF larvae (BSFL) has been suggested for a wide variety of applications such as; manure management systems in animal production (Sheppard *et al.*, 2002), brewery waste management (Chia *et al.*, 2018) and management of the hazardous waste from the Ugandan gin brewing (Dobermann *et al.*, 2019). Due to the high protein and fat content, Wang and Shelomi (2017) suggested the use the BSFL could be used as a human food, but mainly saw the potential use as a feed for animals. Therefore, BSFL composting systems can be used to convert various waste streams into larval biomass, with the potential use as a feed product for animals.

Even though the BSF in many aspects is distinctly different from conventional agricultural animals, it is still considered as a production animal in the legislation of the European Union (Čičková *et al.*, 2015). The EG regulation 1069/2009, which severely limits the use of animal by-products and catering waste for feeding to animals, therefore also applies to insect. Recently progress was made, with the approval of EC regulation 2017/893. This regulation allows the use of processed proteins from seven insect species (including the BSF) reared on plant derived substrates, to be used as feed in aquaculture (Meneguz *et al.*, 2018b). In the United States and Canada, similar legislation is found; as of 2017, the BSF was allowed – as the only insect species - as animal feed for broiler poultry (only Canada) and salmonid fish (Tomberlin & Cammack, 2017). Even though the BSFL has been proposed as feed for various different animals (Tomberlin *et al.*, 2015), it is therefore mainly within aquaculture that it can be legally used, as of today.

1.3 Black soldier fly in aquaculture

While the world population has been increasing since the 1960s with a rate of 1.6% per year, the meat consumption has been increasing with 2.8% per year. The consumption of fish though, has been increasing with 3.2% per year over the same period (FAO, 2018). Fish was earlier mainly provided by captures of wild fish, but the production of fish from aquaculture has been steadily rising since the end of the 20th century, and now provide as much fish as the one caught in the wild (FAO, 2018). However, also aquaculture is dependent on the capture of wild fish, since the feed used in aquaculture is partly based on fish meal (Vidaković, 2015). While the inclusion levels of fish meal in the feed has decreased in the last decades, the overall growth of aquaculture creates a great demand for fish meal and wild fish stocks nevertheless (Vidaković, 2015). Also, a high percentage of the feed is now based on plant based substitutes such as soy and sunflower meal, which could have instead be used for human consumption (Vidaković, 2015). The use of BSFL fed on food waste could therefore be a way of reducing the pressure upon wild fish stocks, while at the same time reducing the use of plant based substitutes which could have been used for human consumption.

Earlier studies (Lock *et al.*, 2016; Kroeckel *et al.*, 2012; St-Hilaire *et al.*, 2007b) has found that it is possible to partially replace fish meal with meal from BSFL for various fish species, such as Atlantic salmon (*Salmo salar*), Turbot (*Psetta maxima*) and Rainbow trout (*Oncorhynchus mykis*). Kroeckel *et al.* (2012) and St-Hilaire *et al.* (2007b) found that it was possible to include up to 25% of BSFL meal in the feed, and Lock *et al.* (2016) found that inclusion levels up to 50% was possible without negatively affecting the fish growth. However, St-Hilaire *et al.* (2007b) also found that, independent of inclusion level, the lipid content and content of α-linolenic acid (ALA; C18:3), eicosapentaenoic acid (EPA; C20:5) and docosahexaenoic acid (DHA; C22:6) in the fish fed with BSFL meal was significantly lower. Since ω-3 fatty acids are essential for fish (Vidaković, 2015), and EPA and DHA are associated with a reduced risk for cardiovascular disease for humans (WHO, 2003), the lower concentration of these fatty acids could be a problem. On the other hand, St-Hilaire *et al.* (2007a) found that BSFL reared on cow manure and fish offal contained considerable amounts of ALA, EPA and DHA. This indicate that it is possible to produce BSFL with a more interesting fatty acid profile through alteration of the substrate. More recent studies (Meneguz *et al.*, 2018b; Spranghers *et al.*, 2017) has also concluded that the rearing substrate impacts the fatty acid composition BSFL, but there is still little knowledge regarding the exact mechanisms. To be able to produce BSFL with high nutritional quality, a further understanding is needed for how different waste products affect the final fatty acid composition of the larvae.

1.4 Aim of the project

This study was part of a larger project with the aim of producing BSFL to be used as feed in aquaculture. The specific objective was to investigate the impact of the rearing substrate on the chemical composition of the BSFL with focus on the fatty acid composition. To examine how the lipid oxidation status of the substrate affected the larvae, the concentration of malondialdehyde (MDA) was also analysed.

2 Background

2.1 The Black Soldier Fly

The BSF is thought to originate from the Americas, but is today spread all over globe in tropical and subtropical areas (Rozkošný, 1997). The BSF has four distinct stages: egg, larva, pupa and fly (Tomberlin & Cammack, 2017), of which the larval stage consist of six instars (May, 1961). The larvae grows from <1 mm in length upon hatching, up to 3.5 cm and a weight of up to 300 mg under optimal conditions (Tomberlin & Cammack, 2017). During the larval stage the larvae consume large quantities of food until it reaches the sixth instar, known as the prepupal stage. The prepupa leaves the feeding substrate in search for a dry and dark place where it after approximately a week in optimal conditions turns into a pupa (Holmes *et al.*, 2013). After two weeks, an adult BSF emerge from the pupa. The adult BSF is black and has the appearance of a wasp, ranging between 1.0-2.5 cm in length (Tomberlin & Cammack, 2017). While water is necessary, Tomberlin and Sheppard (2002) found that the adult BSF is not dependent on the supply of food. Since the adult BSF does not consume any food, it is solely dependent on the energy stored as a larva, which is thought to explain the high fat content of the BSFL (Tomberlin & Sheppard, 2002). The adult flies mate and after a couple of days the females lay eggs, which eventually give rise to a new generation of larvae (Tomberlin & Sheppard, 2002)

From the mid-20th century, the BSF has been suggested for a variety of applications such as a natural control-method for prevention of house flies (Bradley & Sheppard, 1984; Furman *et al.*, 1959), as feed to swine (Newton *et al.*, 1977) and as a way of adding value to manure management (Sheppard *et al.*, 1994). However, until Sheppard *et al.* (2002) was able to breed BSF under controlled conditions in the end of the 20th century, most studies had been reliant on flies caught in the wild. Nowa-days, most studies investigates domesticated BSF colonies in controlled conditions.

2.2 BSF composting

In earlier studies (e.g. Sheppard *et al.*, 2002), BSFL was mainly reared on manure from various animals. Lately though, a wide variety of substrates has been tried out for the BSFL; examples include restaurant waste, fish offal, cow manure, biogas digestate, brewery by-products, sewer sludge and human faeces (Lalander *et al.*, 2019; Meneguz *et al.*, 2018b; Spranghers *et al.*, 2017; St-Hilaire *et al.*, 2007a). There is a consensus in the literature, that the growth and feed conversion of the BSFL, as well as nutritional composition, are affected by the substrate that the larvae are reared on. For example, in the study by Lalander *et al.* (2019) BSFL reared on abattoir waste took 12 days to reach the prepupal stage, while it took up to 40 days when the larvae were reared on digested sewage sludge. Also, in the same study, the prepupae reached a weight of 250 mg when the larvae were reared on abattoir waste, while it was as low as 70 mg when reared on digested sewage sludge.

In the study by Lalander *et al.* (2019) it was observed that the amount of volatile solids and protein of the substrate had a large impact on the size and development time of the larvae. The impact of the protein content of the substrate has also been investigated in other studies. Pimentel *et al.* (2017) observed morphological changes in the fat body of the BSFL, as well as starvation response in the gene expression, when the larvae were reared on substrates poor in nitrogen. While the protein and volatile solids content in the substrate appears as important for the larval development, the BSFL has been observed to withstand wide variations in substrate pH. In the study by Meneguz *et al.* (2018a) no significant differences were found in final larval weight, mortality or development time between larvae reared on substrates with pH-values between 4.0-9.5. Additionally, during the trial, the pH-value changed to 9, independent of the initial pH. It also seems like the BSFL are able to reduce pathogens in the rearing substrate. In a study by Lalander *et al.* (2015), a 7 log reduction of *Salmonella* spp. was observed during the BSF composting trial.

In addition to the substrate quality, factors such as temperature and relative humidity also affects the development of the larvae (Tomberlin & Cammack, 2017). BSF mating and oviposition has been observed at temperatures of 24-40°C and at relative humidity between 30-90% (Sheppard *et al.*, 2002). The temperature usually used for the fly larvae composting step is 27-29°C at a relative humidity of 60-70% (e.g. Meneguz *et al.*, 2018b; Spranghers *et al.*, 2017). Another factor which has been observed to affect the larval development is the feeding system. Meneguz *et al.* (2018a) found that when larvae were given the substrate in one batch, the prepupae developed faster, but when given the same amount of substrate spread over the whole feeding period, the larvae grew bigger.

2.3 Nutritional Composition

Earlier studies investigating BSFL reared on different waste streams, has reported the solid fraction of the larvae to contain 31-53% protein, 26-41% fat and 3-20% ash (Table 1). Chitin, a chemical compound found in many insects, has also been found in amounts of 1-7% in the larvae. Meneguz *et al.* (2018b) found that considerable amounts of the larvae consisted of various fibres. Differences in the nutritional composition between and within studies can depend on different reasons. Spranghers *et al.* (2017) found that mainly the fat and ash content in the larvae were affected by using different substrates. In a comparison between larvae of different age, Liu *et al.* (2017) also found significant differences especially in the fat and ash content of the larvae. While the protein content of the larvae varied between 31-53% in the study by Meneguz *et al.* (2018b), the results by Spranghers *et al.* (2017) were similar to the 39-44% reported by Lalander *et al.* (2019). In contrast to the fat and ash content, earlier results therefore indicate that the protein content of the larvae is less prone to variations between substrates and larvae of different age. Lalander *et al.* (2019) found significant differences in the amino acid profiles in larvae reared on different substrates, but the variations in concentrations were within ±20% for the majority of the 21 amino acids analysed.

Table 1. *Summary of lowest (Lo) and highest (Hi) value reported in the nutritional composition of BSFL in two earlier studies. Total solids are presented as percentage of wet weight, remaining components are presented as percentage of total solids.*

	Meneguz *et al.* (2018b)		**(Spranghers *et al.*, 2017)**	
	Lo	**Hi**	**Lo**	**Hi**
Total solids	22.0	29.1	38.1	41.0
Crude Protein	30.8	53.0	39.9	42.1
Crude Fat	26.3	40.7	33.6	38.6
Ash	7.3	14.6	2.7	19.7
Chitin	1.4	6.2	5.6	6.7
Neutral detergent fibre	8.7	19.8	-	-
Acid detergent fibre	6.5	11.3	-	-
Acid detergent lignin	0.8	4.5	-	-

In a comparison between 32 different insects, Stanley-Samuelson and Dadd (1983) found that palmitic (C16:0), palmitoleic (C16:1), stearic (C18:0), oleic (C18:1), linoleic (C18:2) and ALA (C18:3) generally accounted for approximately 98% of the total fatty acids in the insects. While the same fatty acids have also been found in BSFL, earlier studies (Meneguz *et al.*, 2018b; Spranghers *et al.*, 2017; St-Hilaire *et al.*, 2007a) has reported that the fatty acid found in the largest proportion in BSFL is lauric acid (C12:0) (Appendix 1: Table 10). These studies found in general, that,

the majority of the fatty acids found in the BSFL is constituted of saturated fatty acids (SFA). However, significantly different fatty acid compositions have been reported for the BSFL, both within and between studies. St-Hilaire *et al.* (2007a) found that it was possible to introduce ω-3 fatty acids in the BSFL fat, by rearing the larvae on cow manure mixed with fish offal. Also, the age of the larvae appears to affect the fatty acid composition, which was found by Liu *et al.* (2017). These earlier studies indicates that the substrate affects the fatty acid composition of BSFL.

3 Materials and methods

3.1 Materials

3.1.1 Feeding trials

The trials were carried out in the research facilities at Swedish University of Agricultural Sciences (SLU) in Uppsala, Sweden. A continuous culture of BSF including the full life cycle from egg to adult fly, is maintained in these facilities, from which the larvae for the experiment was provided.

Blue mussels (*Mytilus edulis*) were harvested at Baltic Sea Sankt Anna mussel farm (St. Anna Musselodling, Vattenbruk centrum Öst) in June 2018, transported and stored alive at 4°C until treatment. Homogenized household food waste was received from Eskilstuna Strängnäs Energi och Miljö AB waste treatment facility (Eskilstuna, Sweden). Rainbow trout (*Onchorhynchus mykiss*) was received from the Department of Animal Nutrition and Management at SLU (Uppsala, Sweden). Wheat bran was received from Lantmännen Foder (Uppsala, Sweden). Reclaimed bread was received from the bread company Fazer (Uppsala, Sweden).

3.1.2 Chemicals

For lipid extraction, chloroform (VWR, CAS No. 67-66-3) was mixed with methanol (Merck Millipore, CAS No. 67-56-1) volumetrically to 2:1 ratio. Sodium chloride (Merck Millipore, CAS No. 7647-14-5) was diluted in deionized water to the concentrations 0.9% and 20% weight by volume. Sodium hydroxide (Merck Millipore, CAS No. 1310-73-2) was diluted to the molar concentration 0.01 M in anhydrous methanol (Merck Millipore, CAS No. 67-56-1). Also used for methylation was 20% boron triflouride-methanol complex in methanol (VWR, CAS No. 373-

57-9) and hexane (Fisher Chemicals, CAS No. 110-54-3). Methanol 15-mehylheptanedecanoate (Larodan, Sweden) was used as internal standard. For identification of peaks in the chromatograms, fatty acid methyl ester corresponding to 21 different fatty acids were used (Appendix 2: Table 11). Methyl laureate (Larodan, Sweden) was delivered as a single unit, while the fatty acid methyl esters for to the remaining 20 fatty acids (C14:0 to C24:1) were found in varying concentrations in the standard mix GLC68D (Nu-Check-Prep INC, Minnesota).

For lipid oxidation analysis Thiobarbituric Acid (TBA) and 4.17 M Malondialdehyde (MDA) standard from the "Lipid Peroxidation (MDA) Assay Kit" MAK085 (Sigma-Aldrich, Missouri) was used. TBA was diluted in glacial acetic acid (Merck Millipore, CAS No. 61-19-7) and ultrapure water according to the instructions of the kit to a final acetic acid concentration of 30%. Perchloric acid (Merck Millipore, CAS No. 7601-90-3) was diluted in ultrapure water to the molar concentration 2.0 M. Butylated Hydroxytoluene (BHT; MP Biomedicals, CAS No. 128-37-0) was diluted to the concentration 1.0% weight to volume in 99.9% ethanol (Merck Millipore, CAS No. 64-17-5).

3.2 Experimental Setup

The study consisted of in total six different BSF rearing trials, where young larvae were set to rear on six different substrates for two weeks (*Figure 1).*

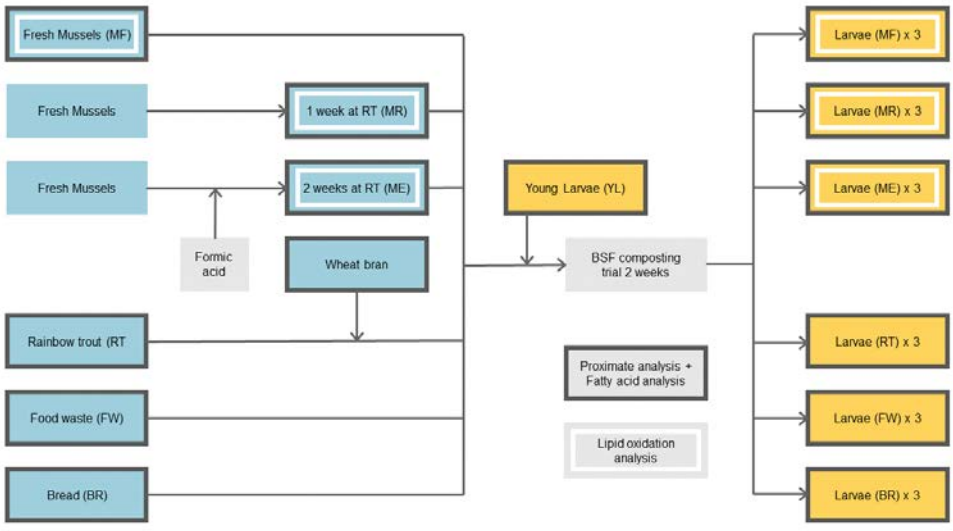

Figure 1. Schematic illustration of the experimental setup of the study. Blue blocks represent substrates and yellow blocks BSFL. Proximate and fatty acid analysis was carried out on samples with dark frames. Lipid oxidation was analysed in samples with an additional white frame.

3.2.1 Substrate preparation

Fresh mussels were crushed on arrival to become more available to the larvae. One part of the fresh crushed mussels (MF) was stored at -20°C between feedings. The rancid mussel substrate (MR) consisted of fresh crushed mussels stored at room temperature (30°C) for 1 w before the start of the feeding trial to simulate worst case scenario storage conditions. In order to simulate a simplified handling of the mussels to the treatment, the fresh crushed mussels (ME) were also ensiled in 3% formic acid for 2 w at room temperature. This was done in order to preserve the nutritional composition of the mussels. The Rainbow Trout and wheat bran substrate (RT) was based on one whole Rainbow Trout which was homogenized and mixed with wheat bran to a weight-ratio of 5:1 between fish and wheat bran. The bread substrate (BR) consisted of in total eight kinds of breads that were coarsely mixed. The bread mix was stored at room temperature between feedings. The food waste substrate (FW) was received as a slurry, and was not further processed. Between feedings the food waste was stored at -20°C.

BSF hatchlings were reared on chicken feed to 5 days age at 28°C before being transferred to the rearing substrate. Each trial was carried out in triplicate and lasted for 2 w from the point where the young larvae were introduced to the rearing substrate. The trials varied in size in terms of larval density and the amount of feed added per larvae (Table 2). After the 2 w trials the larvae were separated from the substrates by sieving, washed briefly and dried with towel paper. At the end of the experiment the weight of 50 larvae was recorded, as well as the total weight of surviving larvae, to be able to calculate the final larval weight, survival rate and waste-to-biomass conversion ratio.

Table 2. *Starting amount of larvae, larval density, total amount of feed added over the (1-3) feedings and the corresponding amount of volatile solids (VS) in the substrate added* per larvae.

Trial	Substrate	Larvae at start	Larval density (larvae/cm^2)	Total feed added (kg)	VS/larva (mg)	Number of feedings
MF	Fresh mussels	800	0.3	20	1500	3
MR	Rancid mussels	800	0.3	20	1100	3
ME	Ensiled mussels	800	0.3	20	1700	3
RT	Rainbow trout and wheat bran	1300	6.3	0.9	230	1
FW	Food waste	700	3.4	0.8	170	3
BR	Reclaimed bread	15000	6.3	6.0	250	3

3.2.2 Sampling

Fatty acid composition and proximate composition - including total solids, crude protein, crude fat and ash – was analysed in all larvae (including 5-d old larvae) and substrates (Figure 1). Fatty acid composition analysis was carried out in duplicate for substrates, and triplicate for the larvae (one analysis for each of the three trial replicates). Proximate composition analysis was carried out in singlets for the substrates, and triplicate for the larvae. Also, for mussel-substrates and larvae reared on mussels, the concentration of MDA was analysed in duplicate, to determine the degree of lipid oxidation in these samples.

3.3 Fatty acid analysis

Fatty acids were extracted using a modified version of the method described by Folch *et al.* (1957). Enough sample to extract 50 mg of lipids (10 g for mussels, 2 g for remaining samples) was weighed on an analytical scale. For every gram of sample, 20 ml of chloroform:methanol 2:1 (v/v) was added. The solution was homogenised with an Ultra-Turrax T25 homogeniser (Janke and Kunkel, Germany) for 3x30 s and cooled on ice in between. The homogenate was filtered using a Buchner funnel, and rinsed using an additional 5 ml of chloroform: methanol 2:1 (v/v) per gram of original sample. The filtrate was transferred to a separation funnel. A solution of 0.9% NaCl was added to the volume giving the ratio 8:4:3 between chloroform, methanol and water. After separation of phases, the lower phase was emptied into a round bottom flask and remaining chloroform and methanol was evaporated in a rotary evaporator (Büchi Labortechnik, Switzerland). The extract was diluted in 2 ml chloroform and stored in -80°C until further analysis.

The chloroform was evaporated using nitrogen gas in a sample concentrator coupled to a heating block (Techne, United Kingdom). From the remaining lipids, 5 mg was weighed, to which 60 µl of internal standard (methyl 15-methylheptadecanoate) and 2 ml 0.01 M NaOH in water-free methanol was added. The sample was vortexed followed by heating at 60°C for 10 min. Further, 3 ml 20% BF_3-methanol complex was added, the sample was vortexed, followed by heating at 60°C for an additional 10 min. After cooling to room temperature, 2 ml 20% NaCl solution and 2 ml hexane was added. The sample was vortexed and then centrifuged at 480 xg (Hermle Labortechnik, Germany) for 5 min. The upper phase was transferred to a GC-vial, and evaporated in a sample collector using N_2-gas. Before injection into the gas chromatograph (GC), 300 µl hexane was added. With each GC-run, a standard solution was also injected, consisting of 100 µl GLC68D standard and 50 µl internal standard (methyl 15-methylheptadecanoate) which were diluted in 150 µl hexane.

Hexane extracts (1µl) were injected (split ratio 1:10) by an Agilent 7683 auto sampler (Agilent, California) onto a Agilent 6890 system with a flame ionization detector attached (Agilent, California). Hydrogen was used as carrier gas at a constant flow of 1 ml/min and separation was conducted on a SGE BPX70 capillary column (50m x 0.22 mm x 0.25 µm; SGE/Trajan, Australia). The oven was maintained at 158°C for 5 min, ramped up to 220°C at 2°C/min and held for 8 min. The temperature of the FID was 250°C with flow rates of hydrogen, oxygen and N_2 (make up gas) at 40, 400 and 50 ml/min. Each sample was injected twice.

3.4 Lipid oxidation analysis

The concentration of MDA was determined in the mussel substrates and larvae reared on mussels. A modified version of the method included in the "Lipid Peroxidation (MDA) Assay Kit" MAK085 (Sigma-Aldrich, Missouri) was followed. Before analysis, the shells in the mussel-substrates were removed. To 1 g of sample the following was added: 2.7 ml ultrapure water, 300 µl 1% BHT in ethanol and 3 ml 2 N perchloric acid. The sample was homogenized for 2x30 s on ice using an Ultra-Turrax T25 homogenizer (Janke and Kunkel, Germany). The sample was centrifuged at 13,000 xg (Thermo Scientific, Massachusetts) for 10 min. From the centrifuged sample, 200 µl of supernatant was transferred and mixed with 600 µl TBA in 30% acetic acid. Blanks and five standards containing 4-20 nmol MDA in 200 µl ultrapure water (20-100 nmol/ml) were prepared in duplicate. Samples, blanks and standards were incubated at 95°C in a water bath for 60 min, followed by cooling on ice for 10 min. Samples were analysed alongside blanks and standards at 532 nm using an Infinite M1000 microplate reader (Tecan, Switzerland).

3.5 Proximate analysis

Proximate analysis was carried out by the staff at the Department of Animal Nutrition and Management at SLU (Uppsala, Sweden). All samples were pre-dried in a freeze-drier before further analysis. Pre-dried samples were dried at 103°C for 16 h to determine the total solids content, followed by drying at 550°C for 3 h to determine the ash content. Total nitrogen was measured using the Kjeldahl method in accordance to NMKL (1976). To estimate the protein content, the conversion factor 6.25 was used. Determination of crude fat content was carried out by hydrolysis in hydrochloric acid followed by extraction in light petroleum as described by the European Commission (1998). Due to high content of calcium in mussel shells, it was not possible to hydrolyse the mussel-substrates prior to lipid extraction.

3.6 Calculations and statistical analysis

3.6.1 Calculations – Larval growth

The survival ratio (Survival %) of the larvae was calculated as:

$$Survival\ \% = \ 100\ \times \frac{Larvae_{Out}}{Larvae\ _{In}} \quad (1.)$$

where $Larvae_{In}$ and $Larvae_{Out}$ were the total amount of larvae put on the substrate in the beginning of the feeding trial (In) and the amount of surviving larvae in the end of the trial (Out).

The waste-to-biomass conversion ratio (BCR_{TS}) percentage was calculated on total solids in the substrate and larvae as:

$$BCR_{TS}\ \% = \ 100\ \times \frac{TS_{Larvae-out}}{TS_{Sub-in}} \quad (2.)$$

where $TS_{Sub\text{-}in}$ and $TS_{Larvae\text{-}out}$ were the total solids (in g) of the total substrate given to the larvae throughout the trial (Sub-in), and the total solids of all surviving larvae in the end of the trial (Larvae-out).

3.6.2 Calculations – Fatty acid analysis

Using the peak areas in the standard chromatogram, the response factor for each fatty acid methyl ester (RF_{FAME}) was calculated as:

$$RF_{FAME} = \frac{PA_{FAME}/m_{FAME}}{PA_{IS}/m_{IS}} \quad (3.)$$

where PA_{FAME} and PA_{IS} were the peak areas in the standard chromatogram, and m_{FAME} and m_{IS} the masses added to the standard solution of a specific fatty acid methyl ester and the internal standard (IS).

Using the retention times of the peaks corresponding to each fatty acid in the standard chromatogram, the peaks in the sample chromatograms were identified. The corresponding mass of each fatty acid (m_{FA}) was calculated as:

$$m_{FA} = \frac{PA_{FAME}}{\frac{RF_{FAME} \times PA_{IS}}{m_{IS}} \times 1.048} \quad (4.)$$

where PA_{FAME} and PA_{IS} were the peak areas in the sample chromatogram, RF_{FAME} the response factor calculated for a specific fatty acid methyl ester and m_{IS} the mass added of internal standard (IS) to the sample. The average weight ratio between fatty acid methyl esters and free fatty acids is 1.048, which was used to convert the weight from fatty acid methyl ester to free fatty acid.

As reported by Khan et al. (2006), blue mussels might contain considerable amount of fatty acids not included as fatty standards in this study. Since these fatty acids may have a considerable impact on the total fatty acid profile in some samples, the percentage of unidentified fatty acids was also estimated. It was assumed that the samples injected in the GC was pure from non-fatty acid compounds. Peaks found in all chromatograms with similar retention time and peak area were assumed to be contaminants, but all other peaks which did not correlate to any of the 21 fatty acids standards (Appendix 2: Table 11) of this study, were considered as unidentified fatty acids. Using Equation 4 and an average of the 21 RF_{FAME}-values for the fatty acid standards the percentage of these unidentified fatty acids was estimated. By comparing the retention times of the peaks in different sample chromatograms, it was possible to identify whether unidentified fatty acids were occurring in more than one sample.

The percent of each identified and unidentified fatty (FA %) out of the total amount of fatty acids was calculated as:

$$FA\ \% = \frac{m_{FA}}{\sum\left(m_{C12:0} + m_{C14:0} + \ldots + m_{C24:1} + m_{unidentified}\right)} \quad (5.)$$

where m_{FA}, $m_{C12:0}$, $m_{C14:0}$ up to $m_{C24:1}$ and $m_{unidentified}$ were the mass calculated for each identified and unidentified fatty acid.

Unidentified fatty acids with a concentration lower than 0.5% were excluded, since they were not considered relevant. However, for identified fatty acids, concentrations down to 0.1% were included, since these were seen as more relevant, and could be distinguished from contaminations with a higher certainty. Therefore, fatty acid concentrations presented as 0.0 in the results, could also indicate that the concentration is <0.1% for identified, or <0.5% for unidentified fatty acids.

For the comparison between different larvae, it was assumed that the contribution of other lipid components to the crude fat component of the larvae was negligible. Absolute amounts of fatty acids (FA_{Abs}) were therefore calculated as:

$$FA_{Abs} = FA\% \times CF \times TS \times m_{Larvae} \quad (6.)$$

where FA%, CF, TS and m_{larvae} were the percentage of a specific fatty acid, the crude fat content, the total solids content and the final larval weight

3.6.3 Calculations – proximate analysis

It was assumed that the remaining mass of total solids in larvae and substrates not being protein, fat or ash, was carbohydrates. The percentage of carbohydrates (Cbh %) was calculated as:

$$Cbh\,\% = 1 - (CP + CF + Ash) \quad (7.)$$

where CP, CF and ash were the percentage of crude protein, crude fat and ash on total solids basis in the specific sample.

Since the BSFL were not assumed to consume the mussel shells, only the proximate composition of the mussel meat was considered. To be able to calculate the proximate composition of the mussel meat it was assumed that the solid fraction of the mussel meat in this study contained 10% ash, and that the mussel shell was constituted of 100% ash. These assumption were based on the ash value (9%) reported by Swedish national food agency (2011) for blue mussels, and the amount of calcium carbonate (>95%) found by in mussel shells by Hamester *et al.* (2012). This implicate that all fat, protein and carbohydrates found in the analysis, originates from the mussel meat, which made it possible to estimate the proximate composition of the mussel meat (Appendix 3: Table 12).

3.6.4 Statistical analysis

Minitab (Minitab Inc., Pennsylvania) was used for one-way analysis of variance (ANOVA) with a 95% confidence interval to identify statistically significant differences between the proximate compositions, fatty acid profiles and MDA concentrations of the larvae and substrates. A Tukey post-hoc with 95% confidence interval was performed on statistically significant different values. Linear regression models were set up with different combinations of proximate composition and fatty acid profiles of the substrates and larvae, as well as the larval weight. Microsoft Excel 2013 (Microsoft, Washington) was used for creating graphical representations of the data.

4 Results

4.1 Larval growth

At the end of the two week composting trials, differences were observed in final larvae weight, survival rate and waste-to-biomass conversion ratio between the different rearing substrates (Table 3). Larvae raised on the ensiled mussels grew poorly and reached the lowest larval weight (30 mg/larva) while larvae raised on the fresh mussels grew largest (230 mg/larva). However, also the larvae reared on food waste reached relatively high weight (190 mg/larva). Larvae raised on the ensiled mussels and rainbow trout had a very low survival rate (10 and 20%). The highest waste-to-biomass conversion ratio was reached in the larvae reared on food waste (40%), while the ratios of larvae reared on ensiled and rancid mussels as well as rainbow trout were very low (0, 1 and 2%).

Table 3. *Weights per larva, survival rate and waste to biomass conversion ratio counted by total solids (BCR_{TS}) for the six rearing trials.*

	BR		**RT**		**FW**		**ME**		**MF**		**MR**		**YL**	
	Avg	**SD**	**Avg**	**SD**	**Avg**	**SD**	**Avg**	**SD**	**Avg**	**SD**	**Avg**	**SD**	**Avg**	**SD**
Weight per larva (mg)	137.4	6.6	88.5	17.6	190.5	19.2	25.0[1]	-	234.6	14.8	106.0	29.1	1.5	0.2
Survival (%)	69.8	9.8	18.4	2.6	89.1	6.0	11.0	4.5	89.3	6.8	55.1	11.2		
BCR_{TS} (%)	13.6	1.5	2.3	0.1	37.2	2.8	<0.1[2]	0.0	11.6[2]	0.5	1.2[2]	0.0		

Abbreviations: Average (Avg.), standard deviation (SD), bread (BR), rainbow trout (RT), food waste (FW), ensiled mussels (ME), fresh mussels (MF), rancid mussels (MR) and young larvae (YL).
[1] An estimate based on measurement of <10 larvae
[2] The total solids corresponding to the mussel shells has been excluded from the calculation.

4.2 Proximate composition

The total solids content of the larvae varied slightly between different rearing substrates, but the most considerable differences were observed in the composition of the solid fraction (Table 4). The crude protein (40-50%) and crude fat (10-60%) were the largest constituents in the majority of the larvae raised on different substrates. The exception was the larvae reared on ensiled mussels, which had a considerably lower crude fat (10%) and higher ash content (30%). However, the proportion of carbohydrates and crude protein could not be accurately estimated in these larvae, as there was too little material to perform a crude protein analysis. Comparing the larvae before and after the trials, the crude fat content was significantly higher in all larvae trials, except those reared on ensiled mussels. The carbohydrates content was in general low after the rearing trials, and negative values were estimated for the larvae reared on bread and rainbow trout. The young larvae had a considerably higher amount of estimated carbohydrates, than the larvae after the two week rearing trials.

Table 4. *Proximate composition of the larvae (L) and substrate (S) as well as the young larvae (YL). The total solids (TS) are presented as percentage of the wet weight, while crude protein (CP), crude fat (CF), ash and carbohydrates (Cbh) are presented as as percentage of total solids. The letters represents significant differences row-wise with a 95% confidence-level.*

		BR		**RT**		**FW**		**ME**		**MF**		**MR**		**YL**	
		Avg %	**SD**	**Avg %**	**SD**	**Avg %**	**SD**	**Avg %**	**SD**	**Avg %**	**SD**	**Avg %**	**SD**	**Avg %**	**SD**
TS	L	35.5^{a}	1.1	27.0^{b}	2.1	$33.0^{a,b}$	1.3	$27.3^{a,b}$	-	$31.3^{a,b}$	0.8	27.5^{b}	0.4	$32.7^{a,b}$	5.0
	S	62.8	-	37.7	-	16.7	-	9.3	-	8.0	-	6.5	-	-	-
CP	L	$39.2^{b,c}$	2.6	52.6^{a}	2.2	36.6^{c}	0.3	-[1]	-	44.6^{b}	1.4	42.3^{b}	0,4	44.7^{b}	3.4
	S	13.5	-	41.8	-	20.5	-	59.6	-	60.1	-	79.6	-	-	-
CF	L	57.8^{a}	1.5	46.7^{b}	1.5	40.7^{c}	2.3	11.2^{e}	-	33.1^{d}	1.2	29.7^{d}	0.3	9.7^{e}	3.8
	S	5.3	-	22.5	-	20.7	-	3.7	-	4.8	-	9.7	-	-	-
Ash	L	3.9^{d}	0.3	5.7^{d}	0.3	16.3^{c}	1.8	33.0^{a}	-	$18.7^{b,c}$	1.4	22.6^{b}	1.2	15.9^{c}	3.1
	S	2.6	-	7.9	-	10.4	-	10.0	-	10.0	-	10.0	-	-	-
Cbh	L	$-0.9^{b,c}$	2.4	-5.0^{c}	2.6	6.4^{b}	3.2	-[1]	-	3.5^{b}	0.9	5.4^{b}	0.7	29.6^{a}	5.2
	S	78.6	-	27.8	-	48.4	-	26.7	-	25.0	-	0.8	-	-	-

Abbreviations: Average (Avg), standard deviation (SD), bread (BR), rainbow trout (RT), food waste (FW), ensiled mussels (ME), fresh mussels (MF) and rancid mussels (MR).

[1]Due to a low growth, the amount of ME-larvae produced were not enough for determination of crude protein content, and carbohydrates could therefore not be estimated.

4.3 Fatty acid profile

4.3.1 Identified fatty acids

In total 17 different fatty acids were identified in the larvae, in significantly different concentrations (Table 5, Figure 2). The content of saturated fatty acids (SFA) constituted 40-80%, mono-unsaturated fatty acids (MUFA) 20-30% and poly-unsaturated fatty acids (PUFA) 10-20% of the fatty acids in the larvae. The concentration of ω-3 PUFAs ranged between 2-6% in most larvae, but larvae reared on ensiled mussels contained a considerably higher concentration (15%).Ten different fatty acids (C12:0, C14:0, C14:1, C16:0, C16:1, C18:0, C18:1 Δ9, C18:1 Δ11, C18:2 and C18:3) were identified in all larvae. The largest constituent in most larvae, except young larvae and those reared on ensiled mussels, was lauric acid (C12:0). This fatty acid was only found in one substrate, food waste. Seven fatty acids (C20:0, C20:1, C20:2, C20:4, C20:5, C22:6 and C24:0) were only found in varying amounts in some of the larvae, and only in the larvae reared on a substrate containing the same fatty acids. EPA (C20:5) and DHA (C22:6) was only found in the larvae reared on rainbow trout and mussels, substrates which all contained considerable amounts of these fatty acids (2-12% EPA and 5-22% DHA) The remaining four fatty acids analysed (C20:3, C22:0, C22:1 and C24:1) were not found in any of the larvae.

Table 5. Fatty acid composition of the larvae (L) of each substrate (S). The results are presented in percentage of the total fatty acids (identified + unidentified). Values that do not share the same letter row-wise are significantly different with a 95% confidence level. Concentrations in substrates marked with + are significantly different from the concentration in the larvae fed on the same substrate with a 95% confidence level.

		BM		FB		FW		ME		MF		MR		YL	
		Avg	**SD**	**Avg**	**SD**	**Avg**	**SD**	**Avg**	**SD**	**Avg**	**SD**	**Avg**	**SD**	**Avg**	**SD**
C12:0	L	52.6^{a}	3.4	28.8^{d}	2.1	$40.4^{b,c}$	5.2	12.7^{e}	3.7	$50.8^{a,b}$	1.9	$30.1^{c,d}$	7.3	7.9^{e}	1.9
	S	0.0^{+}	0.0	0.0^{+}	0.0	1.3^{+}	0.0	0.0^{+}	0.0	0.0^{+}	0.0	0.0^{+}	0.0		
C14:0	L	9.1^{a}	0.9	5.8^{c}	0.3	$6.5^{b,c}$	0.3	5.2^{c}	0.4	7.4^{b}	0.4	8.9^{a}	0.1	2.3^{d}	0.3
	S	0.0^{+}	0.0	1.8^{+}	0.0	3.2^{+}	0.1	5.9	0.4	5.7^{+}	0.2	7.2	0.1		
C14:1	L	0.2^{c}	0.0	0.2^{c}	0.0	0.2^{c}	0.0	0.3^{b}	0.0	$0.2^{b,c}$	0.0	0.5^{a}	0.0	0.0^{d}	0.0
	S	0.0^{+}	0.0	0.0^{+}	0.0	0.2	0.0	0.0^{+}	0.0	0.0^{+}	0.0	0.0^{+}	0.0		
C16:0	L	12.5^{c}	0.7	12.1^{c}	0.4	15.9^{b}	1.0	19.8^{a}	0.4	11.2^{c}	1.2	$17.7^{a,c}$	1.8	$19.0^{a,b}$	1.8
	S	7.8^{+}	0.1	11.4	0.0	22.5^{+}	0.6	15.6^{+}	0.2	14.1	0.2	20.8	0.6		
C16:1	L	2.8^{e}	0.2	4.6^{d}	0.5	2.6^{e}	0.3	12.7^{a}	0.2	6.5^{c}	0.7	8.7^{b}	1.0	0.8^{f}	0.1
	S	0.3^{+}	0.0	2.6^{+}	0.0	1.6	0.1	5.7^{+}	0.0	6.1	0.8	4.9^{+}	0.2		
C18:0	L	1.5^{d}	0.3	$2.1^{c,d}$	0.2	$2.0^{c,d}$	0.6	3.6^{b}	0.2	1.6^{d}	0.3	$2.9^{b,c}$	0.5	6.8^{a}	0.6
	S	2.3	0.1	2.5	0.0	7.8^{+}	0.4	2.3	0.1	2.5	0.2	5.0^{+}	0.4		
C18:1 Δ9	L	12.1^{c}	1.2	24.9^{a}	0.6	19.1^{b}	2.1	13.0^{c}	0.4	10.0^{c}	2.3	11.8^{c}	1.8	26.9^{a}	3.5
	S	43.2^{+}	2.3	38.6^{+}	0.2	38.1^{+}	0.5	3.7^{+}	0.2	6.8	0.8	3.9^{+}	0.1		

		BM		FB		FW		ME		MF		MR		YL	
		Avg	**SD**	**Avg**	**SD**	**Avg**	**SD**	**Avg**	**SD**	**Avg**	**SD**	**Avg**	**SD**	**Avg**	**SD**
C18:1 Δ11	L	0.2^{c}	0.1	1.1^{b}	0.1	0.4^{c}	0.1	2.1^{a}	0.1	1.3^{b}	0.3	1.5^{b}	0.2	1.1^{b}	0.1
	S	2.5^{+}	0.1	3.0^{+}	0.0	2.0^{+}	0.0	2.0	0.0	2.1^{+}	0.1	2.9^{+}	0.1		
C18:2	L	7.4^{d}	0.2	12.0^{b}	0.3	9.6^{c}	0.8	4.0^{e}	0.3	2.4^{e}	0.3	3.8^{e}	0.6	30.6^{a}	1.3
	S	34.7^{+}	2.3	17.6^{+}	0.0	18.1^{+}	0.6	2.7	0.2	3.6	0.1	2.5	0.2		
C18:3	L	$1.6^{b,c}$	0.1	3.3^{a}	0.1	1.8^{b}	0.3	3.3^{a}	0.5	$1.2^{b,c}$	0.2	0.9^{c}	0.1	3.6^{a}	0.2
	S	7.1^{+}	0.0	4.3^{+}	0.0	3.1^{+}	0.1	4.0	0.5	4.4^{+}	0.1	1.7	0.3		
C20:0	L	0.0^{b}	0.0	0.0^{b}	0.0	0.0^{b}	0.1	0.0^{b}	0.0	0.0^{b}	0.0	0.1^{b}	0.1	0.2^{a}	0.0
	S	0.5^{+}	0.0	0.3^{+}	0.0	0.6^{+}	0.0	0.0	0.0	0.0	0.0	0.0	0.0		
C20:1	L	0.0^{d}	0.0	$0.5^{b,c}$	0.0	0.0^{d}	0.0	1.2^{a}	0.2	0.7^{b}	0.2	1.3^{a}	0.1	$0.2^{c,d}$	0.0
	S	0.9^{+}	0.1	3.2^{+}	0.0	0.7^{+}	0.0	3.8^{+}	0.3	3.7^{+}	0.0	6.1^{+}	0.3		
C20:2	L	0.0^{c}	0.0	0.1^{b}	0.0	0.0^{c}	0.0	0.3^{a}	0.0	0.2^{b}	0.0	0.3^{a}	0.1	0.0^{c}	0.0
	S	0.0	0.0	0.7^{+}	0.0	0.2^{+}	0.0	1.1^{+}	0.0	1.0^{+}	0.1	1.9^{+}	0.1		
C20:3	L	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	S	0.0	0.0	0.2^{+}	0.0	0.0	0.0	0.0	0.0	0.4^{+}	0.0	0.4^{+}	0.0		
C20:4	L	0.0^{c}	0.0	$0.1^{b,c}$	0.0	0.2^{b}	0.0	1.1^{a}	0.1	0.2^{b}	0.0	$0.1^{b,c}$	0.0	0.0^{c}	0.0
	S	0.0	0.0	0.3	0.0	0.2	0.0	2.3^{+}	0.2	1.7^{+}	0.1	0.3^{+}	0.1		
C20:5	L	0.0^{c}	0.0	1.7^{b}	0.1	0.5^{c}	0.0	7.4^{a}	0.9	1.9^{b}	0.1	1.7^{b}	0.1	0.0^{c}	0.0
	S	0.0	0.0	1.7	0.0	0.2	0.0	11.6^{+}	0.6	9.6^{+}	0.2	2.6	0.5		
C22:0	L	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	S	0.5^{+}	0.0	0.3^{+}	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		
C22:1	L	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	S	0.0	0.0	2.2^{+}	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		
C22:6	L	0.0^{b}	0.0	0.7^{b}	0.0	0.0^{b}	0.0	4.1^{a}	0.8	0.5^{b}	0.0	0.3^{b}	0.0	0.0^{b}	0.0
	S	0.0	0.0	6.2^{+}	0.1	0.3	0.0	21.6^{+}	0.2	18.7^{+}	0.9	5.1^{+}	1.3		
C24:0	L	0.0^{b}	0.0	0.0^{b}	0.0	0.0^{b}	0.0	0.1^{a}	0.1	0.0^{b}	0.0	0.0^{b}	0.0	0.0^{b}	0.0
	S	0.2^{+}	0.0	0.2^{+}	0.0	0.0	0.0	0.7^{+}	0.0	0.6^{+}	0.0	0.0	0.0		
C24:1	L	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	S	0.0	0.0	0.4^{+}	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.6^{+}	0.1		
UI	L	0.0^{d}	0.0	1.8^{c}	0.1	$0.8^{c,d}$	0.2	9.1^{a}	0.7	4.1^{b}	0.6	9.3^{a}	1.1	$0.6^{c,d}$	0.2
	S	0.0	0.0	2.7	0.0	0.0	0.0	17.1^{+}	0.3	19.1^{+}	0.9	34.1^{+}	1.3		
SFA	L	75.7^{a}	1.6	48.8^{d}	1.5	$64.8^{b,c}$	3.9	$41.4^{d,e}$	3.4	$71.0^{a,b}$	1.5	59.7^{c}	4.9	36.2^{e}	4.7
	S	11.3^{+}	0.2	16.4^{+}	0.1	35.4^{+}	1.1	24.5^{+}	0.6	22.9^{+}	0.6	32.9^{+}	0.9		
MUFA	L	15.2^{d}	1.4	31.4^{a}	1.2	22.3^{c}	2.5	$29.2^{a,b}$	0.2	$18.7^{c,d}$	1.4	$23.8^{b,c}$	3.1	$29.0^{a,b}$	3.4
	S	46.9^{+}	2.5	49.9^{+}	0.2	42.6^{+}	0.4	15.2^{+}	0.4	18.7	0.1	18.3	0.2		
PUFA	L	$9.1^{c,d}$	0.4	18.0^{b}	0.4	12.0^{c}	1.2	20.4^{b}	2.6	6.2^{d}	0.3	7.1^{d}	0.8	34.2^{a}	1.5
	S	41.8^{+}	2.3	31.1^{+}	0.1	22.0^{+}	0.8	43.2^{+}	0.2	39.3^{+}	1.6	14.7^{+}	2.4		
ω-3 PUFA	L	1.6^{c}	0.1	5.7^{b}	0.2	2.3^{c}	0.4	14.9^{a}	2.1	$3.5^{b,c}$	0.3	2.9^{c}	0.2	$3.6^{b,c}$	0.2
	S	7.1^{+}	0.0	12.4^{+}	0.1	3.5	0.1	37.1^{+}	0.1	33.0^{+}	1.3	9.9^{+}	2.2		

Abbreviations: Average (Avg), standard deviation (SD), bread (BR), rainbow trout (RT), food waste (FW), ensiled mussels (ME), fresh mussels (MF), rancid mussels (MR), young larvae (YL) and unidentified fatty acids (UI).

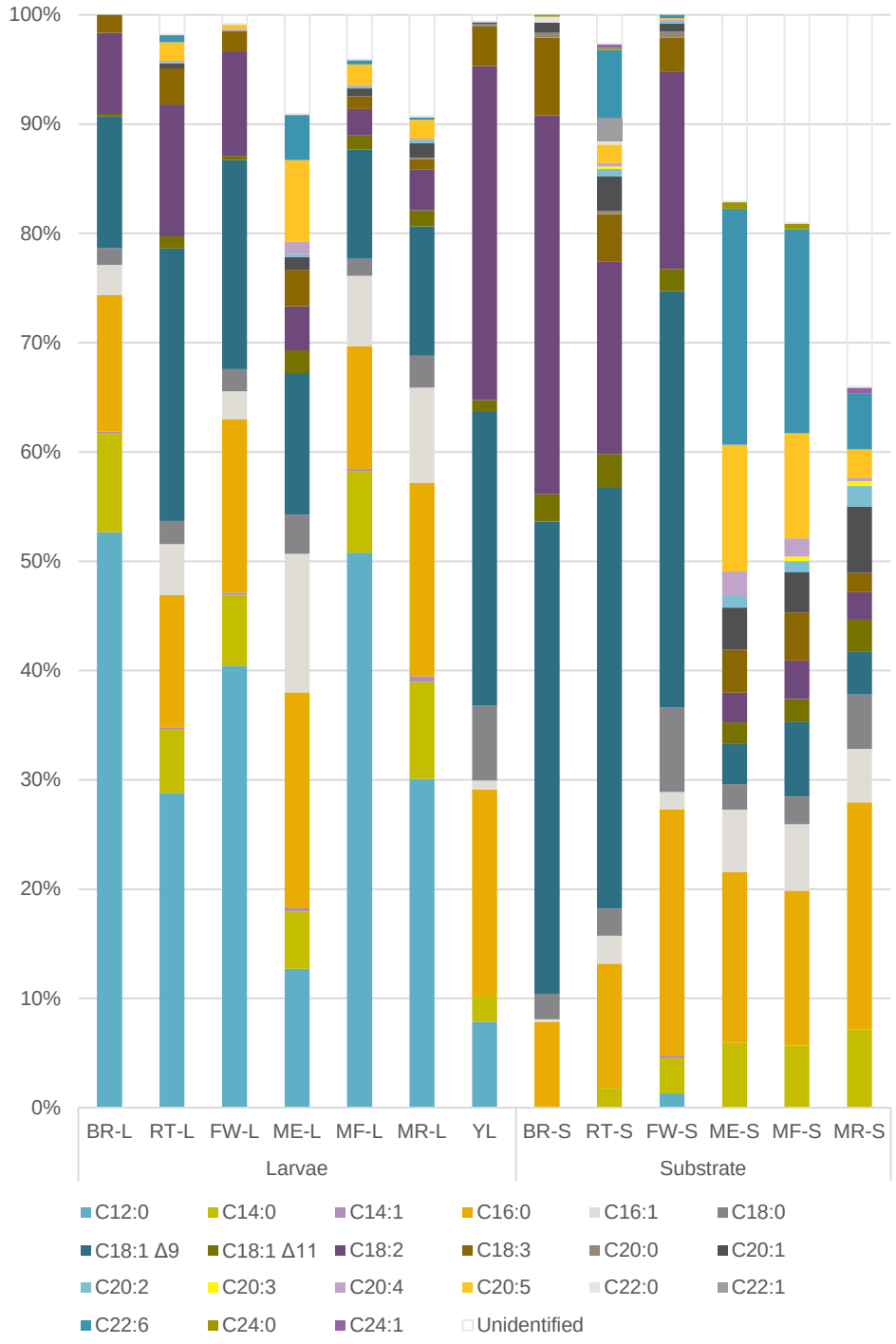

Figure 2. The fatty acid composition of the larvae (L) of each substrate (S) presented as percentage of total fatty acids (identified + unidentified fatty acids). Abbreviations: bread (BR), rainbow trout (RT), food waste (FW), ensiled mussels (ME), fresh mussels (MF), rancid mussels (MR) and young larvae (YL).

Large differences were found between the larvae reared on different substrates, when comparing the absolute amounts of total SFA, MUFA, PUFA and ω-3 PUFA (Figure *3*). The larvae reared on bread contained the highest amount of total fatty acids (28mg/larva), but amounts found in the larvae reared on food waste and fresh mussels (25 mg/larva) was just slightly lower. Larvae reared on fresh mussels contained the highest absolute amounts of ω-3 PUFA (0.9 mg/larvae), while the amounts in found in larvae reared on bread, rainbow trout and food waste were comparable (0.5-0.6 mg/larva) despite different larval weight and crude fat content.

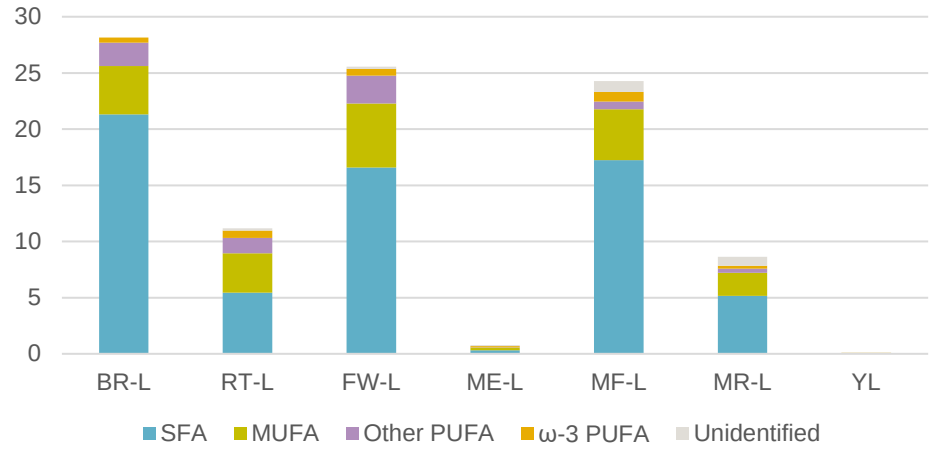

Figure 3. Absolute amounts of fatty acids in the larvae in mg/larva. Other PUFA and ω-3 PUFA adds up to the total amount of PUFA in the larvae. Abbreviations: bread (BR), rainbow trout (RT), food waste (FW), ensiled mussels (ME), fresh mussels (MF), rancid mussels (MR) and young larvae (YL).

4.3.2 Unidentified Fatty Acids

Compounds, assumed to be unidentified fatty acids, were found in varying proportions in the larvae and substrates (Figure 4). These fatty acids represented up to 30% of the total fatty acids, which was observed in the rancid mussels. In total 28 different peaks, assumed to correspond to unidentified fatty acids, with unique retention times, were distinguished in the larval and substrate samples (Appendix 4: Table *13*). Of these 28 unidentified fatty acids, 21 were found in the rancid mussels (Appendix 5: Table 14). One peak with a particular retention time, assumed to correspond to a particular fatty acid (denoted X5), was found in all larvae, except those reared on bread.

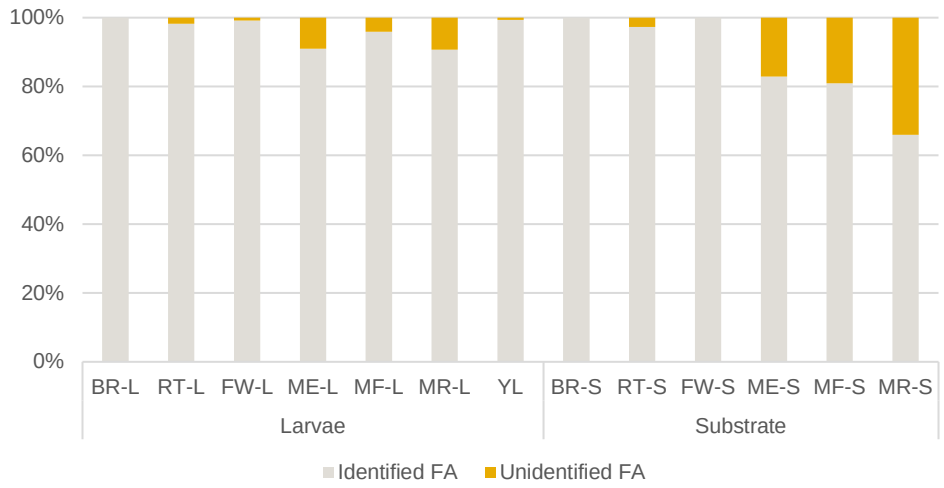

Figure 4. The percentage of unidentified fatty acids estimated in the larvae (L) and substrates (S). Abbreviations: bread (BR), rainbow trout (RT), food waste (FW), ensiled mussels (ME), fresh mussels (MF), rancid mussels (MR) and young larvae (YL).

4.3.3 Fatty acids in mussels

In general terms, more similarities were found in the fatty acid composition between the ensiled and fresh mussels, than towards the rancid mussels (Table 6). Out of the 16 identified fatty acids found in the mussels, only the proportion of palmitoleic acid (C16:1) was found significantly indifferent between the three treatments. The proportions of nine fatty acids (C14:0, C16:0, C18:0, C18:1 Δ11, C18:3, C20:1, C20:2, C22:6 and C24:1) were not significantly different between the ensiled and fresh mussels, but significantly different in comparison with the rancid mussels. The rancid mussels contained almost double the amount (35%) of unidentified fatty acids as was found in the ensiled (15%) and fresh mussels (20%). Comparing the proportions of SFA- and PUFA, the rancid mussels were significantly different from the other treatments, whereas no significant differences were found in the case of MUFA. The amount of ω-3 PUFA was significantly lower in the rancid mussels, while the ensiled mussels contained a significantly higher amount compared to the fresh mussels.

Table 6. *Fatty acid composition of the mussels of different treatment. The results are presented in percentage of the total fatty acids (identified + unidentified). Values that do not share the same letter row-wise are significantly different with a 95% confidence level.*

	ME		**MF**		**MR**	
	Avg	**SD**	**Avg**	**SD**	**Avg**	**SD**
C12:0	0.0	0.0	0.0	0.0	0.0	0.0
C14:0	5.9[b]	0.4	5.7[b]	0.2	7.2[a]	0.1
C14:1	0.0	0.0	0.0	0.0	0.0	0.0
C16:0	15.6[b]	0.2	14.1[b]	0.2	20.8[a]	0.6
C16:1	5.7	0.0	6.1	0.8	4.9	0.2
C18:0	2.3[b]	0.1	2.5[b]	0.2	5.0[a]	0.4
C18:1Δ9	3.7[b]	0.2	6.8[a]	0.8	3.9[b]	0.1
C18:1 Δ11	2.0[b]	0.0	2.1[b]	0.1	2.9[a]	0.1
C18:2	2.7[b]	0.2	3.6[a]	0.1	2.5[b]	0.2
C18:3	4.0[a]	0.5	4.4[a]	0.1	1.7[b]	0.3
C20:0	0.0	0.0	0.0	0.0	0.0	0.0
C20:1	3.8[b]	0.3	3.7[b]	0.0	6.1[a]	0.3
C20:2	1.1[b]	0.0	1.0[b]	0.1	1.9[a]	0.1
C20:3	0.0[b]	0.0	0.4[a]	0.0	0.4[a]	0.0
C20:4	2.3[a]	0.2	1.7[b]	0.1	0.3[c]	0.1
C20:5	11.6[a]	0.6	9.6[b]	0.2	2.6[c]	0.5
C22:0	0.0	0.0	0.0	0.0	0.0	0.0
C22:1	0.0	0.0	0.0	0.0	0.0	0.0
C22:6	21.6[a]	0.2	18.7[a]	0.9	5.1[b]	1.3
C24:0	0.7[a]	0.0	0.6[b]	0.0	0.0[c]	0.0
C24:1	0.0[b]	0.0	0.0[b]	0.0	0.6[a]	0.1
UI	17.1[b]	0.3	19.1[b]	0.9	34.1[a]	1.3
SFA	24.5[b]	0.6	22.9[b]	0.6	32.9[a]	0.9
MUFA	15.2[b]	0.4	18.7[a]	0.1	18.3[a]	0.2
PUFA	43.2[a]	0.2	39.3[a]	1.6	14.7[b]	2.4
ω-3 PUFA	37.1[a]	0.1	33.0[b]	1.3	9.0[c]	2.2

Abbreviations: Average (Avg), standard deviation (SD), ensiled mussels (ME), fresh mussels (MF), rancid mussels (MR) and unidentified fatty acids (UI).

4.4 Lipid oxidation

The concentration of MDA was found significantly different between the three mussel treatments (Table 7). The highest concentration of MDA was found in the fresh mussels and the lowest in the rancid mussels. The same pattern was not observed in the larvae, where the larvae reared on fresh mussels had a significantly lower MDA-concentration in comparison with those reared on ensiled mussels. It was not possible to distinguish the larvae reared on rancid mussels from the larvae reared on fresh and ensiled mussels.

Table 7. *The contents of MDA in the Larvae (L) and Substrates (S) for each mussel treatment. The results are presented as mg MDA per kg of tissue. Values that do not share the same letter row-wise are significantly different with a 95%* confidence level.

		ME		**MF**		**MR**	
		Avg.	**SD**	**Avg.**	**SD**	**Avg.**	**SD**
MDA (mg/kg)	L	4.7^{a}	-	2.1^{b}	0.4	$3.1^{a,b}$	0.4
	S	13.3^{b}	0.6	22.4^{a}	0.6	5.4^{c}	0.1

Abbreviations: Average (Avg), standard deviation (SD), ensiled mussels (ME), fresh mussels (MF) and rancid mussels (MR).

4.5 Linear regression models

4.5.1 Fatty acid models

Two parameters were found to correlate to the concentration of various fatty acids found in the larvae: the concentration of the same fatty acid in the substrate, and the larval weight. Three linear regression models were set up as:

$$FA_{Lar} = a + b \times FA_{Sub} \quad \text{(Model 1)}$$

$$FA_{Lar} = a + c \times Ww_{Lar} \quad \text{(Model 2)}$$

$$FA_{Lar} = a + b \times FA_{Sub} + c \times Ww_{Lar} \quad \text{(Model 3)}$$

Where, FA_{Lar} and FA_{Sub} were the concentration of a specific fatty acid (in %) in the larvae and substrate, Ww_{Lar} was the final wet weight (in mg) of the larvae, and *a*, *b* and *c* were coefficients.

From these three models, one or more significant ($P<0.05$) models were found for 17 of the fatty acids found in the larvae. Significant models were also found for the total unidentified fatty acids, SFA, MUFA, PUFA and ω-3 PUFA (Table 8). For

most fatty acids Model 3 was the statistically most significant with the lowest probability value (P) and highest coefficient of determination (R^2) in comparison with Model 1 and Model 2. This pattern was seen for example for palmitic acid (C16:0; Figure 5). However, for many of the fatty acids the R^2-value for Model 1 and Model 2 were comparable to that of Model 3, indicating that it was mainly one of the two parameters (larval weight or fatty acid concentration in the substrate) which had the largest impact.

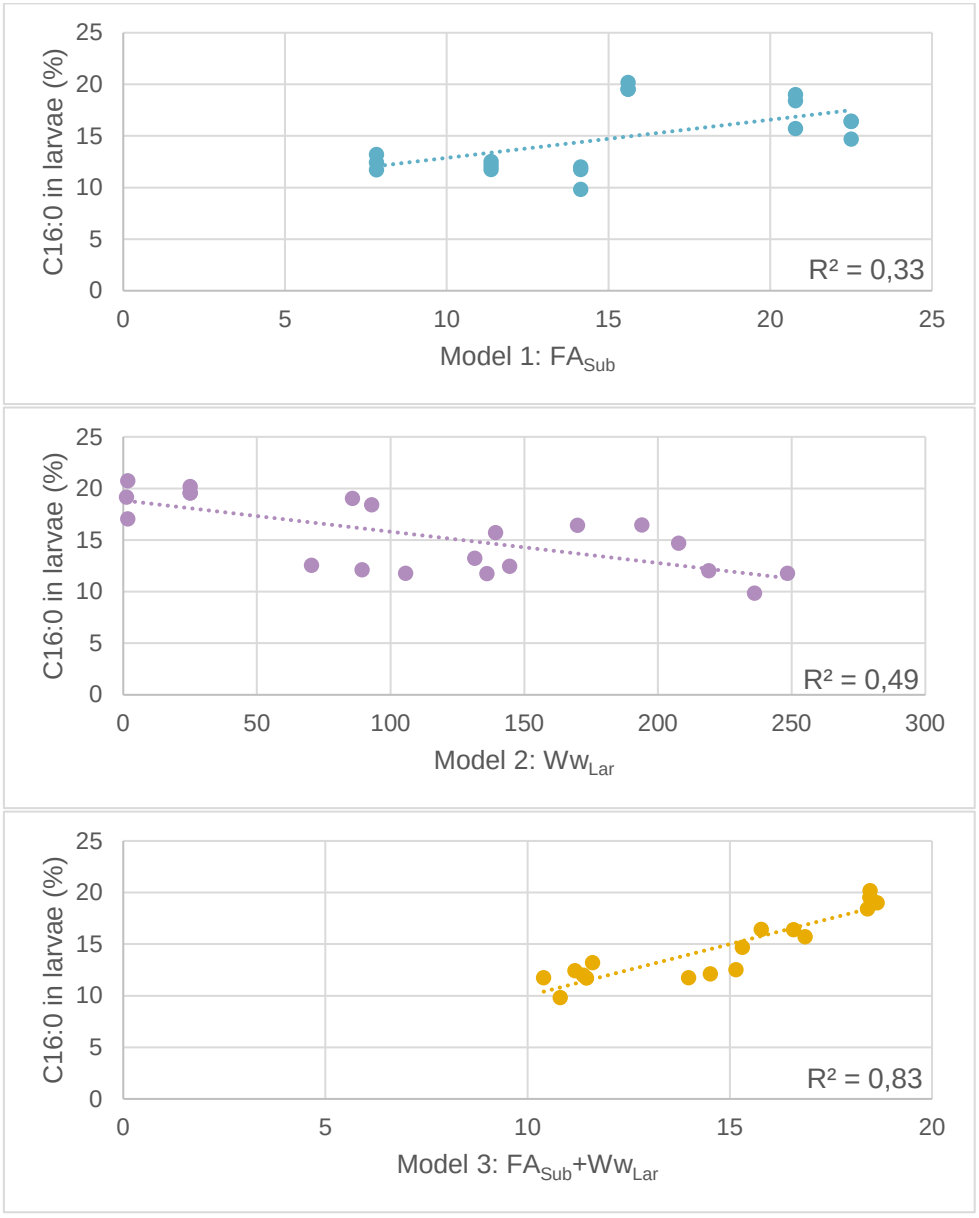

Figure 5. Graphical illustrations of the three fatty acid models applied for palmitic acid (C16:0). Model 1, 2 and 3 (x-axis) were plotted towards the percentage of palmitic acid in the larvae (y-axis).

Table 8. *Percentage of variation (R^2) for the three models applied to each identified and unidentified (UI) fatty acid found in the larvae. The model marked with a bold R^2-value was the statistically most significant for the specific fatty acid. The P-values and coefficients (a, b and c) are presented for the statistically most significant model.*

	Model 1: FA_{Sub}	**Model 2: Ww_{Lar}**	**Model 3: FA_{Sub}+Ww_{Lar}**	**Model parameters of most significant model**			
	R^2	**R^2**	**R^2**	**P**	**a**	**b**	**c**
C12:0	0.020	**0.810**	0.763	>0.001	10.84	-	0.188
C14:0	0.007	**0.391**	0.128	0.002	4.49	-	0.018
C14:1	0.089	0.027	**0.163**	0.263	0.34	-0.22	-0.002
C16:0	0.330	0.494	**0.830**	>0.001	12.66	0.43	-0.033
C16:1	0.657	0.023	**0.944**	>0.001	5.55	1.24	-0.028
C18:0	0.000	0.615	**0.685**	>0.001	3.15	0.12	-0.010
C18:1 Δ9	0.355	0.246	**0.458**	0.010	14.02	0.20	-0.025
C18:1 Δ11	0.010	0.198	**0.367**	0.032	2.84	-0.42	-0.006
C18:2	0.398	0.322	**0.435**	0.014	5.24	0.19	-0.010
C18:3	0.024	**0.591**	0.499	>0.001	3.43	-	-0.010
C20:0	0.019	**0.281**	0.029	0.013	0.12	-	-0.001
C20:1	0.870	0.044	**0.899**	>0.001	0.03	0.25	-0.001
C20:2	0.831	0.022	**0.901**	>0.001	0.07	0.17	-0.001
C20:4	0.632	0.117	**0.854**	>0.001	0.38	0.32	-0.003
C20:5	0.662	0.106	**0.930**	>0.001	2.97	0.39	-0.019
C22:6	0.543	0.173	**0.849**	>0.001	1.54	0.11	-0.012
C24:0	0.302	0.128	**0.500**	0.006	0.04	0.09	-0.001
UI	0.776	0.041	**0.925**	>0.001	3.90	0.25	-0.021
SFA	0.018	**0.758**	0.725	>0.001	39.22	-	0.157
MUFA	0.004	**0.517**	0.483	>0.001	30.18	-	-0.053
PUFA	0.086	**0.651**	0.582	>0.001	25.87	-	-0.095
n-3 PUFA	0.511	0.258	**0.869**	>0.001	6.70	0.22	-0.040

The coefficient of determination (R^2) for Model 1, indicates that the concentration of the selected fatty acid in the substrate, was the main predictor (R^2>0.5) for six fatty acids (C16:1, C20:1, C20:2, C20:4, C20:5 and C20:6), as well as for unidentified fatty acids and the total concentration of ω-3 PUFA. The same coefficient for Model 2 on the other hand, indicates that larval weight is the main determinant (R^2>0.5) for the concentration of three fatty acids (C12:0, C18:0 and C18:3), but also for the total concentration of SFA, MUFA and PUFA. For three of the other fatty acids (C16:0, C18:1Δ9 and C18:2) the coefficients for Model 1 and Model 2 were similar, indicating that the concentration of these fatty acids in the substrate, and the larval weight had similar importance for the concentration in the larvae.

The model coefficients (b and c) are rates describing whether the correlation towards a certain parameter is negative or positive. For the fatty acid concentration in the substrate, the coefficient (b) indicating that higher a concentration of a fatty acid in the substrate gave a higher concentration of the same fatty acid. The exception was for two fatty acids (C14:1 and C18:1 Δ11) where the coefficient (b) was negative. In contrast, the larval weight had a negative coefficient (*c*), indicating an inverse correlation, for most fatty acids. In the case of larval weight, the exceptions were lauric (C12:0) and myristic acid (C14:0) as well as for total SFA, which all increased with an increased larval weight.

4.5.2 Impact of proximate composition

Models of relationships between all possible combinations (n=36) of total solids, crude protein, crude fat, ash and carbohydrates in the substrate and larvae, as well as the larval weight were set up as:

$$P_{Lar} = m + k \times P_{Sub} \qquad \text{(Model 4)}$$

where P_{Lar} and P_{Sub} were specific parameters (total solids, crude protein, ash, carbohydrates or larval weight) in the larvae (Lar) and substrate (Sub) in percentage or mg, and *m*, and *k* were coefficients.

From this, eight significant models (P<0.05) were found with coefficients of determination (R^2) values above 0.5 (Table 9). Two of these models explain the proportion of total solids in the larvae, four models explained the proportion of crude fat and two the proportion of ash in the larvae. However, none of these significant models were found explaining the larval weight or the proportion of crude protein or carbohydrates in the larvae.

Table 9. *Significant (P<0.05) linear regression models with varying combinations of proximate composition in the substrate as predictor (P_{Sub}), and proximate composition in the larvae (P_{Lar}) as response. The P- and R^2-values and coefficients (m and k) are presented for each model.*

	Model Parameters				Model Strength	
Model	**P_{Lar}**	**P_{Sub}**	**m**	**k**	**R^2**	**P**
a	Total Solids	Crude Protein	0.351	-0.102	0.520	0.002
b	Total Solids	Carbohydrates	0.267	0.111	0.685	<0.001
b	Crude fat	Total Solids	0.268	0.510	0.773	<0.001
d	Crude fat	Crude Protein	0.569	-0.388	0.584	0.001
e	Crude fat	Ash	0.678	-3.389	0.630	<0.001
f	Crude fat	Carbohydrates	0.269	0.362	0.561	0.001
g	Ash	Total Solids	0.237	-0.356	0.763	<0.001
h	Ash	Ash	-0.041	2.266	0.570	0.001

5 Discussion

5.1 Larval development

In this study, large variations were observed in the larval weight, survival rate and the BCR of larvae reared on different substrates (Table 3). The larvae reared on ensiled mussels were distinguished by a considerably lower growth, survival and BCR compared to the larvae reared on other substrates. In comparison, larvae reared on bread, rainbow trout and rancid mussels in this study were of similar weight of larvae reared on sewage sludge (70-150 mg/larvae) in the study by Lalander *et al.* (2019). The authors of that study concluded that sewage sludge was less suitable as a substrate for BSFL. However, it should be noted that the larvae reared on sewage sludge took 15-40 days to reach prepupal stage. Since the trials in this study was only carried out for a total of two weeks, it is possible that the larvae reared on bread, rainbow trout and ensiled mussels would have gained more weight if given more time. However, larvae reared on rainbow trout and ensiled and rancid mussels, were also distinguished by low survival rates (11-55%) and low BCR (0-2%). This indicates that these three substrates might not be suitable for the growth of BSFL.

The BCR for larvae reared on bread and fresh mussels, were similar to those reported for larvae reared on abattoir waste (15%) in the study by Lalander *et al.* (2019). In that study abattoir waste was considered a suitable substrate for BSFL. The BCR reported for food waste (14%) in the same study though, was considerably lower than the BCR observed for larvae reared on food waste (37%) in this study. In that study the process was less optimised, which is a likely reason for the difference in BCR in comparison between the studies. For example the larval density, feed per larvae and feeding frequency was different in the two studies.
While the larval weight, survival ratio and BCR by themselves does not necessarily tell anything about the nutritional quality of the larvae, they can give valuable information about the efficiency of the BSF composting system. A high BCR value

indicates that a higher degree of the substrate is converted into larval biomass, which potentially can be used as an animal feed. If the survival ratio and larval weights are higher, it implies that less BSF hatchlings has to be produced to keep the system running. Also, larger larvae are easier to separate from the remaining substrate. With these parameters in mind, the results of this study indicates that fresh mussels and food waste, but also bread, are potential substrates in an efficient BSFL waste-management system where waste is efficiently converted to larval biomass.

5.2 Proximate composition

In terms of proximate composition, similarities can be found between the larvae reared on different substrates in this study (Table 4) and results earlier reported by Meneguz et al. (2018b) and Spranghers et al. (2017) (Table 1), especially in terms of total solids and crude protein content. However, the high degree of variation observed in crude fat (11-58%) and ash content (4-33%) in this study, does not compare to the lower variations reported in the earlier studies. One reason for this, could be the differences in larval weight in this study, which indicates that the larvae were in different stages of development. As reported by Liu *et al.* (2017), differences in proximate composition can be observed between larvae of different age and larval stage, especially in the ash and crude fat content. Since the larvae reared on ensiled mussels had a very low weight in comparison with those reared on other substrates, this is likely to be one reason contributing to the considerably lower crude fat (11%) and higher ash (33%) values observed in those larvae.

The lowest content of crude fat in this study was measured in the smallest larvae, the ones on ensiled mussels. No significant correlation ($P>0.05$) was found though, between larval weight and crude fat content in the larvae. However, four other significant models were found though describing a positive correlation between crude fat in the larvae and total solids and carbohydrates in the substrates, and a negative correlation to the protein and ash content (Table 9). The positive correlation between crude fat and carbohydrates, is in line with the study by Spranghers *et al.* (2017), where it was theorized that more energy dense substrates (high non-fibre carbohydrates and fat) resulted in a higher synthesis of fatty acids, mainly lauric acid (C12:0), in the larvae. Also, Li *et al.* (2015) reported that the addition of glucose to the substrate, increased the amount of lipids found in the BSFL. Further, Pimentel *et al.* (2017) observed a gene expression contributing to increased lipid accumulation in the fat body of BSFL, when reared on substrate poor in protein, which is in line with the negative correlation between protein and crude fat found in this study.

Significant models (P<0.5) was also found describing a correlation between the ash content in the larvae and the total solids and ash content of the substrate (Table 9). This is in line with Spranghers *et al.* (2017), who also found a correlation (R^2 = 0.954, P = 0.023) between ash content in the substrate and the larvae. The correlation between ash content in substrate and larvae found in this study (R^2 = 0.570, P = 0.001) explained less of the variation compared to the mentioned study. It is possible that a stronger correlation would have been found if the proximate composition of the mussel meat would have been analysed, instead of estimated though calculations. The highest concentration of ash in this study were observed in young larvae, and larvae reared on mussels (Table 4). Being both small, and reared on mussels, this could explain why the highest proportion of ash (33%) was observed in the larvae reared on ensiled mussels

Except for the young larvae, the estimated amounts of carbohydrates were below 10% in all larvae (Table 4). To the knowledge of author, no earlier study has estimated the amount of carbohydrates in BSFL in the same way as in this study, therefore no comparable numbers can be found. However, Meneguz *et al.* (2018b) reported considerable amounts of neutral and acid detergent fibres (6-20%), acid detergent lignin (1-4%) as well as chitin (1-6%) in the BSFL. It is possible that the value presented as carbohydrates in this study, may represent these fibrous compounds. In larvae reared on bread and rainbow trout, negative carbohydrate values were estimated. This could be explained by low accuracy in the proximate analysis, but it could also likely be because of the conversion factor used to estimate the amount of protein. Since protein in average contain 16% nitrogen, the conversion factor 6.25 is commonly used to estimate of the protein content based on the total nitrogen content in a sample (Coultate, 2016). It has recently been found though, that when analysing whole BSFL the conversion factor 4.76 would be more suitable, because of the considerable amounts of non-protein nitrogen, mainly from chitin (Janssen *et al.*, 2017). If the conversion factor 4.76 is used, the estimated amount of crude protein will be 24% less than when using 6.25 as factor. In the case of this study, this would result in positive estimations of the “carbohydrate” content in the larvae reared on bread and rainbow trout. For future studies the conversion factor 6.25 might be useful for various substrates, but the conversion factor of 4.76 may be more appropriate for the BSFL. However, it should be kept in mind that the estimated carbohydrates fraction in that case may include a wide variety of fibrous compounds such as chitin, as reported by Meneguz *et al.* (2018b).

5.3 Fatty acid composition

Significant differences were found in the fatty acid profiles of the larvae in this study (Table 5). In most larvae the fatty acid profile followed a similar pattern; lauric acid (C12:0) was by far the largest constituent, followed by palmitic (C16:0) and oleic acid (C18:1 Δ9). Similar patterns can be found in the fatty acid profiles (Appendix 1: Table 10) for larvae reared on various substrates as reported in earlier studies (Meneguz *et al.*, 2018b; Spranghers *et al.*, 2017; St-Hilaire *et al.*, 2007a). Also the total proportions of SFA, MUFA and PUFA observed in this study, compares to those reported in the earlier studies: SFA was the main component followed by MUFA; PUFA was found in the lowest proportions.

A high percentage of lauric acid (C12:0) was found in all larvae, but it was only present in one substrate, food waste. This strongly indicates that lauric acid can be synthesised by the larvae, a hypothesis which has earlier been suggested by Spranghers *et al.* (2017). In this study, a significant positive correlation was found between larval weight and the content of lauric acid in the larvae (R^2=0.810, P<0.001). This suggests that more lauric acid was accumulated as the larvae grew, which was also observed by Liu *et al.* (2017) in larvae of different age. This could explain why the lauric acid content was lowest in the young larvae and those reared on ensiled mussels, which both had low weights. However, the larvae reared on bread contained the highest percentage (53%) and absolute amount (14 mg/larva) of lauric acid, but were not the larvae that grew largest. As mentioned in section 5.2, factors such as carbohydrate and protein content of the substrate seems to contribute to the crude fat content of the BSFL, and Spranghers *et al.* (2017) suggested that energy dense substrates give rise to larvae with higher crude fat and lauric acid content. The high amount of carbohydrates estimated in bread (79%) could therefore explain the high amounts of lauric acid in larvae reared on this substrate.

Besides lauric acid (C12:0), nine other fatty acids (C14:0, C14:1, C16:0, C16:1, C18:0, C18:1 Δ9, C18:1 Δ11, C18:2 and C18:3) were found in all larvae, irrespective of rearing substrate (Table 5). Myristoleic acid (C14:1) was found in all larvae, but not in all substrates, which might indicate that this fatty acid, like lauric acid, can be synthesised by the larvae. However, unlike lauric acid, no significant model (P<0.05) was found indicating a correlation with myrsitic acid. The eight other above mentioned fatty acids, were found in both larvae and substrates. From this information, it is therefore not possible to draw any conclusions whether the fatty acids origin from the substrate, or were synthesised in the larvae.

Seven fatty acids compromising 20 or more carbons (C20:0, C20:1, C20:2, C20:4, C20:5, C22:6 and C24:0) were found in various amounts in some, but not all, larvae. These fatty acids were only found in larvae reared on substrates containing the specific fatty acid (Table 5). This suggests that fatty acids longer than 18 carbons need to be found in the substrate, to be incorporated in the fat of the larvae. St-Hilaire *et al.* (2007a) had similar results. While very low concentrations (<0.1%) of C20:4, C20:5, C22:5 and C22:6 were detected in larvae reared on cow manure in that study, considerably higher concentrations (0.1-2.2%) were found in larvae reared on fish offal. Humans and many other animals have the ability of enzymatic elongation and desaturation of fatty acids such as linoleic (C18:2) and ALA (C18:3) to arachidonic acid (C20:4), EPA (C20:5) and DHA (C22:6) (Becker, 2013). The results of this study, and those of St-Hilaire *et al.* (2007a), indicates that BSFL does not possess the same enzymatic abilities, at least not to an extent giving proportions of ≥0.1%. This was clearly observed in the larvae reared on bread which contained linoleic acid (7%) and ALA (2%), but no fatty acids exceeding 18 carbons in length.

Peaks, assumed to correspond to unidentified fatty acids, were found in the chromatograms of many samples in this study. The unidentified peaks were mainly found in mussels and larvae reared on mussels. As seen in the study on blue mussels from Newfoundland by Khan *et al.* (2006), considerable amounts of fatty acids not included in the standards of this study, can be found in mussels. This indicates that at least some of the unidentified peaks in this study, most likely corresponds to various fatty acids which has not been possible to identify. However, it cannot be confirmed whether it was valid to assume that all unidentified fatty acids corresponded to fatty acids. In the study by Spranghers *et al.* (2017), BSFL which had been reared on biogas digestate contained 6.5% branched fatty acids. This indicates that fatty acids with less common conformations can be incorporated in BSFL, which seems to have happened also in the larvae of this study. One particular peak, assumed to be that of an unidentified fatty acid (denoted X5) was found in all larvae except those reared on bread (Appendix 5: Table 14). This assumed fatty acid (X5) was not found in any substrate except the rancid mussels. It is therefore possible that this is another fatty acid, like C12:0 and C14:1, which can be synthesised by the larvae. Since the retention time of the peak was slightly shorter than the one of C16:1 (Appendix 4: Table *13*), it is possible that the fatty acid is another monounsaturated 16-carbon fatty acid, in which the double bond has a different location in the carbon chain.

5.4 Factors affecting fatty acid composition

The results from the linear regression models (Table 8) gives further understanding for which parameters that might affect the fatty acid composition in the larvae. A strong correlation between the fatty acid concentration in the substrate and in the larvae (Model 1), indicates that the concentration in the larvae is reliant on the concentration in the substrate. R^2-values exceeding 0.5 were mainly found for fatty acids compromising 20 or more carbons, which further demonstrate the importance of the fatty acid composition of the substrate, to incorporate these fatty acids in the larvae. The concentration of a particular fatty acid in the larvae, was found positively correlated (positive coefficient *b*) to the concentration in the substrate, for all but two fatty acids. This could explain why four fatty acids (C20:3, C22:0, C22:1 and C24:1) were not found in any of the larvae. These fatty acids were found in low concentrations (0.2-2.2%) in the substrates, which appears to have been too low to be incorporated in detectable (≥0.1%) amounts in the larvae.

As discussed regarding lauric acid (C12:0), a strong correlation to the larval weight (Model 2) could indicate that the synthesis of the fatty acid within the larvae, is important for the final concentration. The concentration of lauric acid and myristic acid (C14:0) were found positively correlated (positive coefficient *c*) to larval weight in this study. However, for the major part of the fatty acids investigated, a negative correlation (negative coefficient *c*) was found to the larval weight. A similar pattern was observed in the results presented by Liu *et al.* (2017) where the proportion of C12:0 and C14:0 increased as the larvae grew older, while the proportion fatty acids such as C16:0, C18:0, C18:1 Δ9 and C18:2, decreased. While Model 2 was found significant for many fatty acids, only the models for C12:0, C18:0 and C18:3 had R^2-values above 0.5, which suggests that these three fatty acids are synthesised by the larvae, in increasing or decreasing amounts over time.

Since lauric acid (C12:0) was the most prevalent fatty acid in most larvae, and it was positively correlated to the larval weight, this is likely the main reason for the positive correlation found between total SFA and larval weight (Model 2: R^2=0.758). This correlation agree with the results earlier reported by Liu *et al.* (2017), where SFA constituted 40% of the total fatty acids in six day old larvae, to increase to 90% in the prepupae. Since the adult BSF do not feed, it needs to store a lot of energy during the larval stage (Tomberlin & Sheppard, 2002). The large amount of SFA stored in the larvae is likely an energy reserve for the adult fly. The reason for storing SFA instead of unsaturated fatty acids, could be because of the additional enzymatic processing that is required to degrade unsaturated fatty acids (Berg *et al.*, 2012). While palmitic acid (C16:0) is the end product of the fatty acid

synthesis in many organisms (Berg *et al.*, 2012), the same does not seem to be the case in BSFL. It is possible that the lower melting temperature of lauric acid in comparison with longer fatty acids (Coultate, 2016), in combination with the temperate climate the BSF is found in (Rozkošný, 1997), makes lauric acid the preferable fatty acid for storing energy. However, it is currently only possible to speculate about the reason for the BSFL storing especially lauric acid in such high amounts.

When taking in account both the larval weight, and fatty acid concentration in the substrate (Model 3), the R^2-value increased for 13 fatty acids (Table 8). The higher R^2-value indicates that, even though the larval weight or fatty acid concentration in the substrate was the main determinant, combining both parameters gives a higher degree of explanation for the variations in the larval fatty acid concentration. While SFA, MUFA and PUFA were mainly correlated to larval weight, the concentration of ω-3 PUFA in the larvae, was found mainly correlated to the concentration of the same fatty acids in the substrate. However, the higher R^2-value found for Model 3 ($R^2 = 0.859$) compared to Model 1 ($R^2 = 0.511$), indicates that the larval weight also has an impact on the concentration. If BSFL rich in ω-3 PUFA are desired, it seems like the most important factor is the concentration of these fatty acids in the substrate, but with an increased larval growth, the relative amount decrease. It is likely that a high degree of synthesis of especially lauric acid (C12:0), but also myristic acid (C14:0), during the growth of the larvae, makes the relative amounts (in percentage) of all other fatty acids decrease during the growth.

5.5 Lipid Oxidation

Comparing the fatty acid profiles of the fresh, ensiled and rancid mussels, the concentrations of various fatty acids in the ensiled and fresh mussels were more similar, than in comparison with the rancid mussels (Table 6). The similarities in fatty acid concentrations, but also in proximate composition, between ensiled and fresh mussels indicates that formic acid was able to preserve the mussels The percentage of SFA was found higher in rancid mussels, and the amount of PUFA lower, while the MUFA was indifferent. Because free radicals are more likely to attack carbons close to double bonds, MUFA in general and PUFA in particular are more prone to oxidative rancidity (Coultate, 2016). Most likely, the absolute amounts of MUFA and PUFA decreased due to oxidative rancidity in the rancid mussels, while the amounts of SFA was more or less unchanged. In relative amounts it therefore appeared as if the SFA increased. The oxidative rancidity process, initiated by a free radical, sets of a chain reaction initially giving rise to increasing amounts of hydroperoxides.

These hydroperoxides eventually breaks down giving rise to various aldehydes, alcohols and ketones (Coultate, 2016). It is possible that some of the unidentified fatty acids found in the rancid mussels could be hydroperoxides or other breakdown products from this process.

One specific product of oxidative rancidity, is MDA (Coultate, 2016). Therefore it would have been expected that the rancid mussels contained high concentrations of this compound. However, the results from the lipid oxidation analysis shows the complete opposite. The highest concentration of MDA was found in the fresh mussels (22 mg MDA/kg) and the lowest concentration was found in the rancid mussels (5 mg MDA/kg). While these results are contradictory, similar results has been reported earlier by Khan *et al.* (2006). In that study the changes in MDA content was analysed in blue mussels from Newfoundland stored on ice for 14 days. While the MDA concentration in the mussels increased until day 10, the concentration at day 14 was significantly lower. According to Shahidi and Spurvey (1996), the concentration of MDA measured after more than 10 days of storage can be misleading as indicator of oxidation. It therefore seems, like the MDA molecule is degraded at a longer storage times. Since the ensiled mussels still contained 13 mg MDA/kg after two weeks of storage, it is possible that the formic acid partly inhibited the degradation of the MDA molecule in these mussels.

In comparison with the MDA concentrations in the mussels, the larvae does not follow the same pattern. However, conclusions drawn from this would be very uncertain; both because the uncertain long term storage effects on MDA, but also because of the uncertainties of the used method. While analysis of MDA with the use of TBA is a commonly used method, various sources of error are known. There is a risk of TBA reacting with other substances, so called TBA reactive substances (TBARS), and the risk of inducing further oxidation in the sample by the high temperature required in the method (Barriuso *et al.*, 2013).

5.6 Implications for aquaculture

Partial substitution of fish meal with BSFL meal has earlier been shown to be a possibility for various fish species (Lock *et al.*, 2016; Kroeckel *et al.*, 2012; St-Hilaire *et al.*, 2007b). One problem pointed out by St-Hilaire *et al.* (2007b) was the fatty acid composition of the larvae in comparison with the fish meal, which resulted in lower amounts of ω-3 PUFA recorded in fish fed with BSFL meal. In this study it was found that, as reported earlier by Meneguz *et al.* (2018b), Spranghers *et al.* (2017) and St-Hilaire *et al.* (2007a) that SFA, mainly lauric acid (C12:0), constitute

the major part of the fat fraction in BSFL. In line with the results of St-Hilaire *et al.* (2007a), this study also indicates the possibility to modify the fatty acid composition of the BSFL, and especially the introduction of ω-3 PUFA, by using substrates of a certain fatty acid composition.

By analysing the results from 16 different feeding trials, Hua and Bureau (2009) observed lower apparent digestibility coefficient for lipids in Rainbow trout and Atlantic salmon when the amount of SFA exceeded 23% of the total fatty acids. Therefore the high amounts of SFA found in the larvae of this study (40-75%) could become a problem if the larvae are to be used as feed in aquaculture, especially the larvae reared on bread, which contained the highest amounts of SFA. However, it should also be pointed out that the exact composition of SFA could be important. For example, Lock *et al.* (2016) found a considerably higher digestibility of lauric acid (C12:0) than for C14:0, C16:0 and C18:0 when BSFL were fed to turbot. Since lauric acid constitutes such a large portion of the BSFL fat, the high percentage of SFA should not necessarily be concluded as something solely negative.

In absolute amounts, the larvae reared on fresh mussels contained the highest amounts of ω-3 PUFA. Indicated by the high BCR observed for food waste in this study (37%) in comparison with the one reported for food waste (14%) by Lalander *et al.* (2019), there might also be room for optimisation of the process. In this study, the feeding dose of fresh mussels was 1500 mg volatile solids per larva, which is almost nine times the amount given to the larvae reared on food waste (170 mg volatile solids per larva). It should however not be completely ruled out to ensile the mussels as well, as this would result in the possibility to store the mussels at room temperature. Even though BSFL have been shown to grow at pH as low as 4.0 without negative impact on growth (Meneguz *et al.*, 2018a), the low growth of larvae reared on ensiled mussels strongly indicates that addition of formic acid had an inhibiting effect on the larval growth. However, when it comes to the use of formic acid in silage for pigs and ruminants, the EFSA FEEDAP Panel (2014) recommend that 10 g formic acid per kg of feed is enough to preserve the feed. It should therefore be considered to use lower concentrations (than 3%) of formic acid to investigate the possibility to preserve the mussels, without inhibiting the growth of the larvae.

Also the larvae reared on rainbow trout also contained considerable amounts of ω-3 PUFA, but only a low percentage of larvae survived and the larvae reached a low weight. A possible explanation for this is that high levels of ammonia were measured during the trial when larvae were reared on rainbow trout. The atmospheric levels of ammonia during the trial was 480 ppm, and the substrate reached levels of 15g ammonia/kg. Another reason for the low growth of these larvae could

be the high amount of oil observed during the trial, which could have covered the larvae, making them unable to breathe. For both the ensiled mussels and rainbow trout, it could be an idea to co-compost these substrates with other substrates, as demonstrated in the study by Lalander *et al.* (2019). In that study it was found that larvae took almost 30 days to reach the prepupal stage when reared on fruit and vegetables. However, when the fruit and vegetables was mixed with abattoir waste in the same study, it only took 12 days for the larvae to reach prepupal stage. It is possible that co-composting substrates such as mussels or fish with a substrate such as food waste could be a way of reaching a higher larval growth, while at the same time increasing the amount of ω-3 fatty acids in the substrate.

5.7 Further studies

In this study, all larvae were reared on the substrates for two weeks. As a result of the different substrates used, the growth, survival and BCR varied between larvae reared on different substrates. This made it possible to compare the fatty acid composition of the larvae at different stages, but it also became more complicated to distinguish whether the variations in fatty acid compositions were because of the fatty acid composition of the substrate, or because of the different stage of development of the larvae. However, it should be noted that the larval weight has been shown to correlate to certain parameters in chemical composition of the substrate. Lalander *et al.* (2019) found that the main contributor to the larval weight, was the volatile solids feeding dose of the substrate.

To draw further conclusions about the mechanics and factors affecting the fatty acid composition of the BSFL, it would be recommended to analyse the fatty acid composition of larvae at different age, in similarity with what was done in the study by Liu *et al.* (2017). However, in addition to that, it would be recommended to compare larvae reared on different substrates, preferably such as food waste or abattoir and food waste where BSFL has earlier been found to grow well (Lalander *et al.*, 2019). It could also be possible to spike substrates with fatty acids at different levels. This could make it possible to identify the effects of different factors, such as larval growth and the fatty acid composition of the substrate as well as the substrate matrix. By calculation of the weight of each fatty acid throughout the development of the larvae, it could be possible to distinguish whether the BSFL accumulates fatty acids from the substrate at a certain stage.

Finally, the results of this study, in combination with what has earlier been reported by Khan *et al.* (2006), indicates that the analysis of MDA concentration is not suited for analysis of mussels stored for extended periods of time. Since the substrates used for BSFL in some cases could have been stored a long time, it is possible that the same problem would occur also when analysing other substrates. If any conclusions should be drawn regarding the impact of lipid oxidation in the substrate on the larvae, it would be recommended to consider another method. Luckily, MDA is not the only compound possible to analyse as indicator of lipid oxidation. As concluded by Barriuso *et al.* (2013), a wide variety of methods can be considered for the analysis of lipid oxidation status (in foods), and in some case it can be more convenient to combine different methods.

Conclusion

In this study the impact of six different substrates on the nutritional composition of BSFL was investigated. The larval growth was better on fresh mussels, food waste and bread, than on rancid mussels, rainbow trout and ensiled mussels. Especially the crude fat and ash content varied between larvae reared on different substrates, which seems to have been mainly affected by the carbohydrate, crude protein and ash content of the substrate. With increased weight the larvae accumulated more SFA, while the proportion of MUFA and PUFA decreased, which could become a problem if BSFL are to be used as feed in aquaculture. It was possible, to incorporate ω-3 PUFA in the larvae if these fatty acids were present in the substrate. Therefore, the use of substrates such as mussels and fish could improve the quality of the BSFL as a feed within aquaculture. The analysis of MDA in the substrates did not produce reliable results for the samples analysed, and it would be recommended to use other methods for future analysis of lipid oxidation status.

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Appendix 1 – Earlier reported fatty acid contents

Table 10. *Summary of earlier reported fatty acid contents in BSF larvae and prepupae, presented as percentage of total fatty acids. The lowest (Lo) and highest (Hi) value of each fatty acid reported in each of the studies are presented. The 21 fatty acids written in* ***bold*** *were included in this study. The delta (Δ) sign specifies the conformation of the double bond, and the location counted from the carboxyl-carbon. Fatty acids marked with a dash (-) were not reported in the earlier studies.*

		(Meneguz *et al.*, 2018b)- Larvae		**(St-Hilaire *et al.*, 2007a) - Prepupae**		**(Spranghers *et al.*, 2017) - Prepupae**	
	Δ/ c	**Lo**	**Hi**	**Lo**	**Hi**	**Lo**	**Hi**
C10:0	-	-	-	-	-	1.2	2.0
C12:0	-	32.4	57.4	19.0	52.5	43.7	60.9
C14:0	-	6.6	10.4	3.5	8.5	6.9	9.5
C14:1	9	-	-	-	-	-	-
C16:0	-	13.1	20.4	13.7	21.3	9.7	10.3
Iso- and ante-iso[1]	-	-	-	-	-	0.1	6.5
C16:1	9	2.9	6.1	-	-	2.0	7.6
C18:0	-	1.8	2.8	1.6	8.0	1.0	1.8
C18:1	9	8.5	12.5	15.1	39.0	5.7	8.0
C18:1	11	0.3	0.6	-	-	0.1	2.3
C18:2	9,12	4.1	23.5	3.9	5.5	4.5	11.6
C18:3	9,12,15	0.4	2.5	0.2	1.1	0.7	1.4
C18:4	?	-	-	-	-	0.1	0.9
C20:0	-	-	-	-	-	-	-
C20:1	11	-	-	-	-	-	-
C20:2	11,14	-	-	-	-	-	-
C20:3	11,14,17	-	-	-	-	-	-
C20:4	5,8,11,14	-	-	0.0	0.2	-	-
C20:5	5,8,1,14,17	-	-	0.0	2.2	0.0	0.2
C22:0	-	-	-	-	-	-	-
C22:1	13	-	-	-	-	-	-
C22:5	7, 10,13,16,19	-	-	0.0	0.7	-	-

		(Meneguz *et al.*, 2018b)- Larvae		(St-Hilaire *et al.*, 2007a) - Prepupae		(Spranghers *et al.*, 2017) - Prepupae	
	Δ/ c	**Lo**	**Hi**	**Lo**	**Hi**	**Lo**	**Hi**
C22:6	4,7,10,13,16,19	-	-	0.0	2.1	0.01	0.02
C24:0	-	-	-	-	-	-	-
C24:1	15	-	-	-	-	-	-
SFA	-	63.0	81.9	-	-	64.8	82.8
MUFA	-	12.7	19.0	-	-	9.5	19.1
PUFA	-	4.8	26.0	-	-		
ω-3 PUFA	-	-	-	0.3[2]	5.1[2]	0.9	2.3

[1]Branched fatty acids

[2]Includes C18:3, C20:5 and C22:6

Appendix 2 - Fatty acid standards

Table 11. *Fatty acids corresponding to the 21 fatty acid methyl esters used as standards in this study. The delta (Δ) sign specifies the conformation of the double bond, and the location counted from the carboxyl-carbon.*

Abbreviation	**Full name**	**Δ**
C12:0	Lauric acid	-
C14:0	Myristic acid	-
C14:1	Myristoleic acid	cis - 9
C16:0	Palmitic acid	-
C16:1	Palmitoleic acid	cis - 9
C18:0	Stearic acid	-
C18:1 Δ9	Oleic acid	cis - 9
C18:1 Δ11	Vaccenic acid	trans - 11
C18:2	Linoleic acid (ω-6)	cis – 9,12
C18:3	α-Linolenic acid (ω-3)	cis – 9,12,15
C20:0	Arachidic acid	-
C20:1	Eicosenoic acid	cis-11
C20:2	Eicosadienoic acid (ω-6)	cis-11,14
C20:3	Eicosatrienoic acid (ω-3)	cis-11,14,17
C20:4	Arachidonic acid (ω-6)	cis-5,8,11,14
C20:5	Eicosapentaenoic acid (EPA) (ω-3)	cis-5,8,11,14,17
C22:0	Behenic acid	-
C22:1	Erucic acid	cis-13
C22:6	Docosahexaenoic acid (DHA) (ω-3)	cis-4,7,10,13,16,19
C24:0	Lignoleric acid	-
C24:1	Nervonic acid	cis-15

Appendix 3 - Estimation of mussel meat proximate composition

The results from proximate analysis of whole (including both shells and meat) fresh, ensiled and rancid mussels (Table 12) were used to estimate the proximate composition of the mussel meat in each substrate, using the following equations (8-17).

Table 12. *Proximate composition of fresh, ensiled and rancid mussels, where values measured in the proximate analysis (whole - including both mussel shell and meat) were used to estimate the composition of the mussel meat. Total solids are presented on wet weigh basis, and the other components are presented on total solids basis.*

	ME		**MF**		**MR**	
	Whole (%)	Meat (%)	Whole (%)	Meat (%)	Whole (%)	Meat (%)
Total Solids (TS_{PX})	26.9	9.3	23.0	8.0	28.1	6.5
Crude protein (CP_{PX})	16.6	59.6	17.4	60.1	14.2	79.6
Crude fat (CF_{PX})	1.0	3.7	1.4	4.8	1.7	9.7
Ash (Ash_{PX})	74.9	10.0	74.0	10.0	84.0	10.0
Carbohydrates (Cbh_{PX})	7.4	26.7	7.2	25.0	0.1	0.8

Abbreviations: Ensiled mussels (ME), fresh mussels (MF), rancid mussels (MR).

The weight of the total solids ($m_{TS,PX}$) in the whole mussels was calculated as:

$$m_{TS,PX} = TS_{PX} \times x \quad (8.)$$

where TS_{PX} was the measured total solids content (in Table 12) in the whole mussels and the variable "x" was an assumed wet weight of the whole mussels.

The weight of the crude protein ($m_{CP,PX}$), crude fat ($m_{CF,PX}$), ash ($m_{Ash,PX}$) and carbohydrates ($m_{Cbh,PX}$) in the whole mussels was calculated as:

$$m_{CP,PX} = m_{TS,PX} \times CP_{PX} \quad (9.)$$

where $m_{TS,PX}$ was the weight of the total solids in the whole mussels and CP_{PX} was the measured crude protein content (in Table 12) in the whole mussels.

Mussel shells consists to more than 95% of calcium carbonate (Hamester *et al.*, 2012). For the possibility of estimating the chemical composition of the mussel meat, all protein, fat and carbohydrates found in the whole mussels were therefore assumed to origin from the mussel meat. The weight of the crude protein ($m_{CP,PX}$),

crude fat ($m_{CF,PX}$), and carbohydrates ($m_{Cbh,PX}$)in the mussel meat were set to equal the weights in the whole mussels ($m_{CP,PX}$; $m_{CF,PX}$ and $m_{Cbh,PX}$) as:

$$m_{CP,meat} = m_{CP,PX} \quad (10.)$$

The weight of the total solids ($m_{TS,meat}$) in the mussels meat was calculated as:

$$m_{TS,meat} = \frac{m_{CP,meat} + m_{CF,meat} + m_{Cbh,meat}}{1 - a} \quad (11.)$$

where $m_{CP,meat}$, $m_{CF,meat}$ and $m_{Cbh,meat}$ were the weights of the crude protein (CP), crude fat (CF) and carbohydrates (Cbh) in the mussel meat. The constant *a* was given the value 0.1 since the it was assumed that the ash content in the mussel meat was 10%, based on the ash content reported in blue mussels by the Swedish national food agency (2011).

The crude protein (CP_{meat}), crude fat (CF_{meat}), and carbohydrate (Cbh_{meat}) content in the mussel meat was calculated as:

$$CP_{meat} = \frac{m_{CP,meat}}{m_{TS,meat}} \quad (12.)$$

where $m_{CP,meat}$ and $m_{TS,meat}$ were the weights of the crude protein and total solids in the mussel meat.

The weight of the ash ($m_{Ash,meat}$) in the mussel meat was calculated as:

$$m_{Ash,meat} = a \times m_{TS,meat} \quad (13.)$$

where $m_{TS,meat}$ was the weight of the total solids in the mussel meat. The constant *a* was given the value 0.1 since it was assumed that the ash content in the mussel meat was 10%, based on the ash content reported in blue mussels by the Swedish national food agency (2011).

The weight of the ash ($m_{Ash,shell}$) in the mussel shell was calculated as:

$$m_{Ash,shell} = m_{Ash,PX} - m_{Ash,meat} \quad (14.)$$

where $m_{Ash,PX}$ and $m_{Ash,meat}$ were the weight of the ash in the whole mussels and the mussel meat.

The wet weight of the mussel shell ($m_{ww,shell}$) was calculated as:

$$m_{ww,shell} = \frac{b \times m_{Ash,shell}}{c} \quad (15.)$$

where $m_{Ash,shell}$ was the weight of the ash in the mussel shell. The constants "b" and "c" were given the value 1.0, since the mussel shell was assumed to consist of 100% ash and 100% total solids (source).

The wet weight of the mussel meat ($m_{ww,meat}$) was calculated as:

$$m_{ww,meat} = x - m_{ww,shell} \quad (16.)$$

where $m_{ww,shell}$ was the wet weight of the mussel shell and the variable "x" was an assumed weight of the whole mussels.

The total solids content (TS_{meat}) in the mussel meat was calculated as:

$$TS_{meat} = \frac{m_{TS,meat}}{m_{ww,meat}} \quad (17.)$$

where $m_{TS,shell}$ and $m_{ww,meat}$ were the total solids and wet weight of the mussel meat.

Appendix 4 – Unidentified fatty acids (retention times)

Table 13. *Retention times for peaks in the chromatograms, which were assumed to correspond to unidentified fatty acids (denoted X1-X28). Average retention times for the 21 fatty acid standards and the internal standard (IS;methyl 15-methylheptadecanoate) are also included for comparison. The delta (Δ) sign designate the location of the double bond(s) counted from the carboxyl-carbon.*

Retention time	Fatty acid	Δ	Retention time	Fatty acid	Δ
2.350	X1		15.141	C20:0	-
3.021	C12:0	-	15.500	X15	
4.340	C14:0	-	15.750	X16	
4.901	C14:1	9	15.813	X17	
5.380	X2		16.119	C20:1	11
6.869	C16:0	-	16.220	X18	
7.107	X3		16.534	X19	
7.144	X4		16.865	X20	
7.331	X5		17.351	X21	
7.534	C16:1	9	17.622	C20:2	11,14
7.612	X6		17.942	X22	
7.944	X7		19.347	C20:4	5,8,11,14
8.891	X8		19.602	C20:3	11,14,17
9.237	X9		20.248	C22:0	-
9.976	IS	-	20.634	X23	
10.387	X10		21.276	C22:1	13
10.588	C18:0	-	21.486	C20:5	5,8,11,14,17
10.895	X11		21.869	X24	
11.383	C18:1	9	22.058	X25	
11.534	C18:1	11	23.459	X26	
12.044	X12		23.603	X27	
12.594	X13		25.377	C24:0	-
12.697	C18:2	9,12	26.364	C24:1	15
14.128	X14		26.851	X28	
14.507	C18:3	9,12,15	27.594	C22:6	4,7,10,13,16,19

Appendix 5 - Unidentified fatty acids (estimated concentrations)

Table 14. *Estimated weight percentage for the chromatogram peaks, supposed to correspond to unidentified fatty acids, found in the larvae (L) and substrates (S) for each treatment. The results are presented in percentage of the total fatty acids (identified + unidentified).*

		BR		RT		FW		ME		MF		MR		YL	
		Avg (%)	SD	Avg (%)	SD	Avg (%)	SD	Avg (%)	SD	Avg (%)	SD	Avg (%)	SD	Avg (%)	SD
X1	L	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.7	0.1	0.0	0.0	0.0	0.0
	S	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		
X2	L	0.0	0.0	0.0	0.0	0.0	0.0	0.9	0.1	0.5	0.1	0.8	0.0	0.0	0.0
	S	0.0	0.0	0.0	0.0	0.0	0.0	0.7	0.1	0.0	0.0	1.0	0.1		
X3	L	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.6	0.1	0.0	0.0
	S	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.8	0.0	0.0	0.0		
X4	L	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.6	0.1	0.0	0.0
	S	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.4	0.3		
X5	L	0.0	0.0	1.2	0.0	0.8	0.2	1.5	0.0	0.8	0.1	1.4	0.2	0.6	0.2
	S	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.4	0.1		
X6	L	0.0	0.0	0.0	0.0	0.0	0.0	0.9	0.1	0.5	0.1	0.9	0.1	0.0	0.0
	S	0.0	0.0	0.0	0.0	0.0	0.0	1.0	0.0	0.0	0.0	1.7	0.1		
X7	L	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.9	0.1	0.0	0.0
	S	0.0	0.0	0.0	0.0	0.0	0.0	0.9	0.0	0.8	0.0	1.4	0.1		
X8	L	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	S	0.0	0.0	0.0	0.0	0.0	0.0	2.4	0.1	4.6	0.3	1.4	0.3		
X9	L	0.0	0.0	0.0	0.0	0.0	0.0	1.3	0.1	0.0	0.0	0.7	0.2	0.0	0.0
	S	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		
X10	L	0.0	0.0	0.0	0.0	0.0	0.0	0.7	0.1	0.0	0.0	0.0	0.0	0.0	0.0
	S	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		
X11	L	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	S	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.1	0.1		
X12	L	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.1	0.2	0.0	0.0
	S	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.2	0.4		
X13	L	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	S	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.1	0.4		
X14	L	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	S	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.7	0.0	0.0	0.0		

		BR		RT		FW		ME		MF		MR		YL	
		Avg (%)	SD	Avg (%)	SD	Avg (%)	SD	Avg (%)	SD	Avg (%)	SD	Avg (%)	SD	Avg (%)	SD
X15	L	0.0	0.0	0.6	0.0	0.0	0.0	2.0	0.3	1.1	0.2	0.8	0.1	0.0	0.0
	S	0.0	0.0	0.8	0.0	0.0	0.0	2.8	0.2	3.1	0.2	1.1	0.5		
X16	L	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	S	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.7	0.1	0.0	0.0		
X17	L	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	S	0.0	0.0	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		
X18	L	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	S	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.7	0.2		
X19	L	0.0	0.0	0.0	0.0	0.0	0.0	1.7	0.2	0.0	0.0	0.0	0.0	0.0	0.0
	S	0.0	0.0	0.0	0.0	0.0	0.0	3.7	0.2	3.6	0.4	1.9	0.1		
X20	L	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	S	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.9	0.2		
X21	L	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	S	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.9	0.2		
X22	L	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	S	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.1	0.3		
X23	L	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.3	0.6	0.2	0.0	0.0
	S	0.0	0.0	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.7	0.0		
X24	L	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	S	0.0	0.0	0.0	0.0	0.0	0.0	0.9	0.2	1.0	0.0	1.2	0.0		
X25	L	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	S	0.0	0.0	0.0	0.0	0.0	0.0	2.2	0.1	1.9	0.1	2.5	0.1		
X26	L	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	S	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.8	0.3	1.0	0.2		
X27	L	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	S	0.0	0.0	0.0	0.0	0.0	0.0	1.2	0.1	0.0	0.0	1.1	0.0		
X28	L	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	S	0.0	0.0	0.6	0.0	0.0	0.0	1.2	0.0	1.0	0.0	3.3	0.2		
Tot	L	0.0	0.0	1.8	0.1	0.8	0.2	9.1	0.7	4.1	0.6	9.3	1.1	0.6	0.2
	S	0.0	0.0	2.7	0.0	0.0	0.0	17.1	0.3	19.1	0.9	34.1	1.3		

Abbreviations: Average (Avg), standard deviation (SD), bread (BR), rainbow trout (RT), food waste (FW), ensiled mussels (ME), fresh mussels (MF), rancid mussels (MR), and young larvae (YL).

Appendix 6 – Popular science summary

The black soldier fly larvae – soon a little bit healthier

The world population and meat consumption is increasing, and at the same time we waste huge amounts of food. This is an equation that won't work in a world with finite resources. But a small insect might be able to help us, and soon it might become a little bit healthier as well.

Out of the more than one million insect species found worldwide there is one especially voracious species. The larvae of the black soldier fly (*Hermetia Illucens*) is able to consume a wide variety of foods. And what the black soldier fly larvae call food, you would rather not eat. Since the potential of this omnivorous larva was discovered researchers all over the world has tried feeding it with a wide variety of products. Human faeces, abattoir waste, fish offal, used brewery grains, sewage sludge, cow manure and food waste are just some examples. When the larva is finished eating, it contains a high portion of fat and proteins. The nutritional contents make the larvae of the black soldier fly a potential feed for animals.

Fish is one group of animals that has been found possible to feed with the

The black soldier fly larvae is happily eating your food waste (Photo: Nils Ewald).

Black soldier fly larvae. Since aquaculture is reliant on the supply of fish meal, substitution of the fish meal with meal from black soldier fly larvae might become a way to lower the pressure on the overfished seas. Usually though, fish contains a lot of omega-3 fatty acids; fatty acids which has been found to reduce the risk of cardiovascular disease for humans. One problem that arise when feeding fish with black soldier fly larvae though, is that the amount of omega-3 fatty acids decrease in the fish. For consumers expecting to find these healthy fatty acids in the fish, this might therefore be a problem.

In a recent study by Nils Ewald, a Master's student at the Swedish Uni-

versity of Agricultural Sciences, it was found that the main component of the black soldier fly larval fat, was saturated fatty acids. While omega-3 fatty acids are associated with health benefits, saturated fatty acids are in general regarded as less healthy. In the study, carried out during the fall of 2018, Black soldier fly larvae were fed with different feed materials; bread, which was retaken from the store, food waste, fish waste, and mussels from the Baltic Sea. What became apparent was that the larvae accumulated more saturated fatty acids the more they grew. But another pattern also became clear: the fatty acid in the larvae feed affected the fatty acids found in the fat of the larvae. Therefore, larvae which were fed with mussels and fish, also contained a lot of omega-3 fatty acids.

If the larvae with higher amounts of omega-3 fatty acids would be fed to fish it might become possible to substitute the fish meal in the production, without compromising the healthy omega-3 fatty acids. It should be noted though, that the aim of this study was not to use fish, to feed to larvae, and then feed again to fish. The study was set up mainly to get more insight in which fatty acids are created in the black soldier fly larvae, and how it is affected by what the larvae eat. However, it might be a good idea to use mussels. Another current problem is the eutrophication of the Baltic Sea. By catching blue mussels in the Baltic Sea it might become possible use these nutrients, and then convert it into larval meal, that can be used as feed for fish or other animals.

Many people might become disgusted by the thought of entomophagy, to eat insects. But what about eating fish, pork or chicken meat, which has been fed with insects? Without you noticing, the meat in the store might not be fed with soy meal from Brazil, or fish meal from the overfished seas, but larvae that has gobbled on your food waste, and tied together the loose ends between the fork and the field.