Processing and storage of mussels: mussels to feed through fly larvae

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EU STRATEGY FOR THE BALTIC SEA REGION FLAGSHIP

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About

Baltic Blue Growth is a three-year project financed by the European Regional Development Fund. The objective of the project is to remove nutrients from the Baltic Sea by farming and harvesting blue mussels. The farmed mussels will be used for the production of mussel meal, to be used in the feed industry. 18 partners from 7 countries are participating, with representatives from regional and national authorities, research institutions and private companies. The project is coordinated by Region Östergötland (Sweden) and has a total budget of 4,7 M€.

Partners

- *Region Östergötland (SE)*
- *County Administrative Board of Kalmar County (SE)*
- *East regional Aquaculture Centre VCO (SE)*
- *Kalmar municipality (SE)*
- *Kurzeme Planning Region (LV)*
- *Latvian Institute of Aquatic Ecology (LV)*
- *Maritime Institute in Gdańsk (PL)*
- *Ministry of Energy, Agriculture, Environment, Nature and Digitalization of Schleswig-Holstein (DE)*
- *Municipality of Borgholm (DK)*
- *SUBMARINER Network for Blue Growth EEIG (DE)*
- *Swedish University of Agricultural Sciences (SE)*
- *County Administrative Board of Östergötland (SE)*
- *University of Tartu Tartu (EE)*
- *Coastal Research and Management (DE)*
- *Orbicon Ltd. (DK)*
- *Musholm Inc (DK)*
- *Coastal Union Germany EUCC (DE)*
- *RISE Research institutes of Sweden (SE)*

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1 Introduction

The process line developed in the project is shown in Figure 1, which illustrates the different steps from harvest of Baltic Sea blue mussels to fish feed through fly larvae composting. This report describes the process line from Pre-process treatment (No 3.) to Post-process treatment (No 7.).

The most crucial part of the process line is the fly larvae compost, as this involves living organisms that must be treated in certain ways to survive. SLU has long experiences of this and has operated fly larvae composting with municipal source separated organic waste since 2011. Several tests with blue mussels have been done in this project, where the experiences from the use of organic waste is used as a reference. Other parts of the process line - ensilage (optional); crushing of mussels before feeding them to the fly larvae; separation of fly larvae, shells and droppings and finally drying of fly larvae – are more straight forward and dependent on the technical equipment that are selected. Thus, other techniques, than those selected in this project, could be used depending on e.g. experiences, availability, local circumstances and economy.

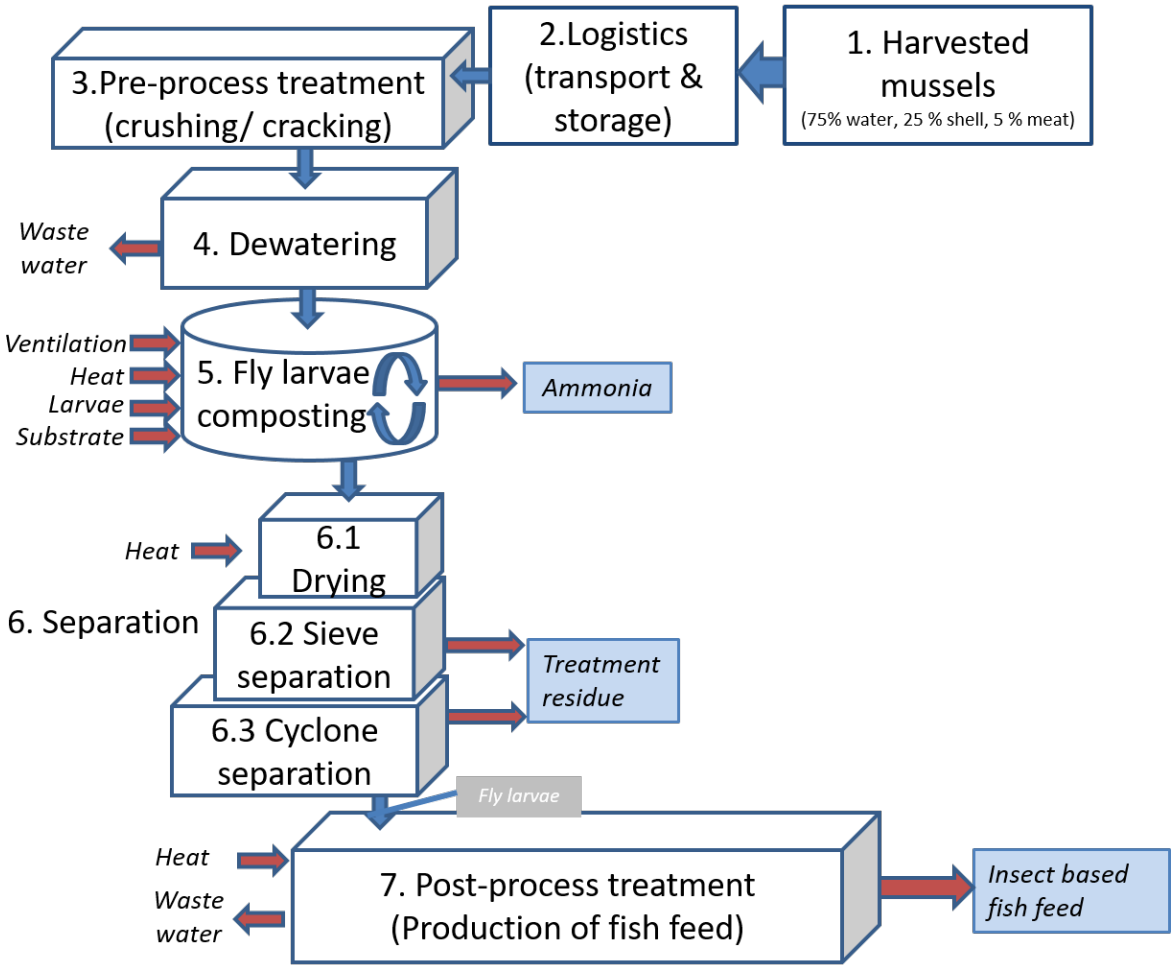


Figure 1. Process line flowchart

2 Aim

The aim of this report is to describe the different process steps in a production line where fly larvae from black soldier fly (*Hermetia illucens*) is used to compost/convert harvested blue mussels (*Mytilus edulis*) to a fish feed. The description should make it possible for a commercial actor to develop a process line. Economical aspects of post-harvest use of mussels are described and discussed in another project report by PP07 Zaiga Ozolina (cost and benefit analysis of mussel farming in the Baltic Sea region).

3 Impact of harvest methods on mussel quality

The impact on different harvest methods used before the mussels reach the site for fly larvae composting have not been tested, since the crushing/grinding is more impactful than the consequence of any harvest method.

In this project we have tested both frozen and fresh mussels. However, to freeze mussels will add an additional cost both for the freezing and for the transport of frozen mussels. Frozen mussels also need to be thawed before using them as a feed in the larvae compost.

4 Storage and transport of mussels - effect on quality

Several storage durations of freshly harvested mussels have been tested in order to identify optimal storage and transport times and temperatures in relation to quality e.g. fatty acid profiles and lipid oxidation. The mussels were harvested from a farm in St. Anna archipelago in May 2018. They were placed immediately on ice in containers with lids and transported by truck to Uppsala, transport took about 4 hours. Upon arrival mussels were kept in a cold room (5 °C) over night and on the following day (day 1) divided into 3 treatments: on ice (cold room), 5°C (cold room) and freezing at -18 °C - in order to evaluate oxidation levels depending on storage conditions. Control sample was taken in the morning on day 1 and prepared for the analysis. Samples were then taken from each treatment after 24 and 96 hours giving a total number of seven samples. All samples were tested for oxidation levels by analysis of 2-thiobarbituric acid (TBA) values and expressed indirectly as levels of oxidative stress marker, malondialdehyde (MDA). Changes in fatty acid profiles were also analysed. More detailed description on the analytic methods used are available in the appendix.

4.1 Oxidation of blue mussels at different conditions

The results for MDA levels (Figure 2) showed only minor difference in concentration between treatments when the mussels were stored for 24 hours. However, the 96-hour samples showed increasing MDA levels in mussels stored at -18 °C and decreasing in mussels stored on ice and at 5°C. The observed decrease has been reported earlier in similar studies by Khan et al. (2006) and (Shahidi & Spurvey, 1996) who observed decreasing values in tissues of mussels and mackerel after first 96 hours. These studies observed a rapid increase of MDA only after initial 120 hours of storage at 5°C. Therefore, it is quite possible that the period of more intense oxidative changes in the tissue of mussels likely appears after the 96-hours.

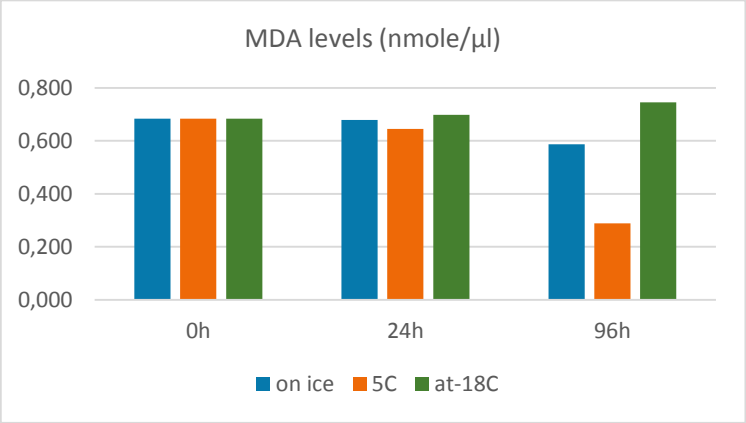
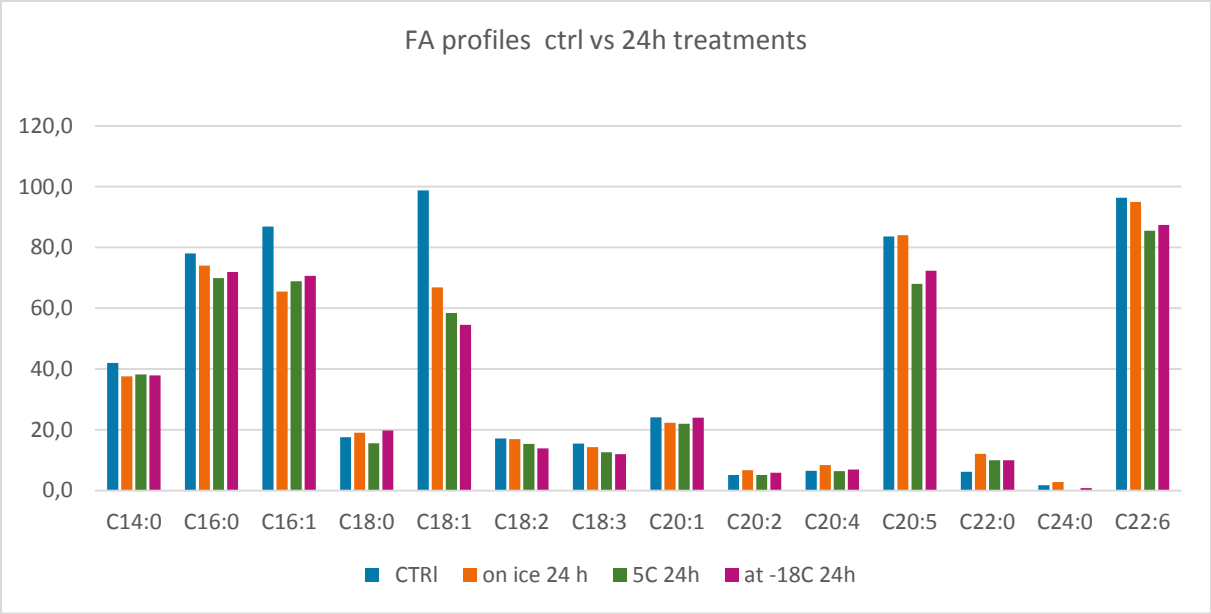


Figure 2. Malondialdehyde concentration in samples from 3 treatments stored for 0, 24 and after 96 hours.

4.2 Changes in fatty acid composition

In conjunction with MDA analysis, attention was directed towards establishing fatty acid levels during storage. We followed 15 different fatty acids belonging to saturated (SFA), monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA). Omega-3 fatty acids were of particular interest due to their value as nutritional compounds and sensitivity to oxidation. Eicosapentaenoic acid or EPA (C20:5) and docosahexaenoic acid or DHA (C22:6) are usually found only in marine environments. The levels of EPA and DHA in Baltic blue mussels after 24 hours of storage at 5°C and -18°C dropped somewhat similarly (39.5-43.2% drop for EPA) while the levels in mussels stored on ice were unchanged (table 3- appendix and figure 3). At 96-hour sampling, mussels stored on ice lost approximately 50% of EPA and 25% DHA while the levels were no different from the other two treatments.

This indicates that in order to preserve the EPA and DHA concentration for the first 24- 36 hours, mussels should be kept on ice rather than stored in dry conditions at 5°C or frozen at -18°C. The overall fatty acids composition seems to deteriorate after 96 hours independent of storage conditions.



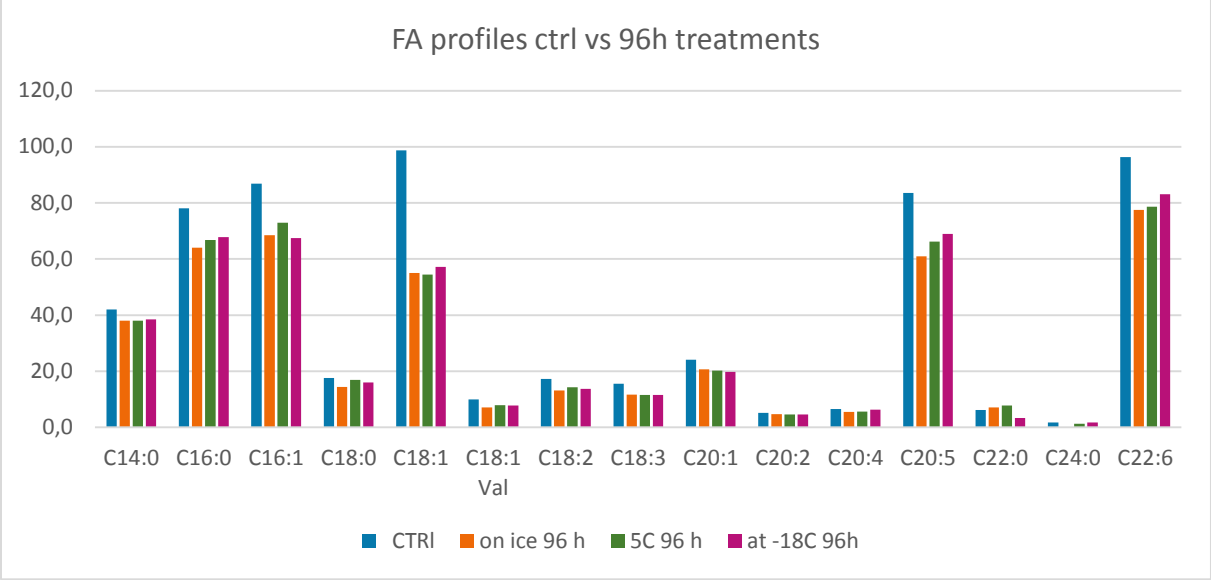


Figure 3. Comparative changes in individual fatty acid levels after 24 and 96 hours of storage. The values are expressed as mg/g of extracted fat.

5 Mechanical pre-treatment of mussels

When a blue mussel is harvested the shell is closed in order to prevent the mussel to dry out and die. Thus, the mussels need to be crushed or grinded before use in order to expose the soft tissue and uses a feed for fly larvae composting. If the mussels arrive already cracked or whole, should only have a minor effect on the suitability of the substrate going into the fly larvae production. The difference should only be noticeable when using a grinding disc with very large sized holes or no grinding disc at all. Results from testing at RISE suggest that the tested grinding process is suitable for a wide range of mussels and is not limited to mussels of certain standard or quality.

Process line – mussels to feed through fly larvae

After the fly larvae composting there must be a separation between fly larvae, shells and droppings from the larvae. The technique used for separation and the performance of it is very much dependent on the particle size of the mussel shells, thus how crushing/grinding is done must consider the ability and technique used during separation. Rather large particle size of shells is preferable, which often means that mussels should be crushed/grinded course. We used a 55 kW meat grinder of model Palmia (Figure 4).

Figure 4: Palmia 200 55 kW industrial meat grinder

The grinder operates by having a transport screw that funnels the material to the first of two sets of knives in the series (see Figure 5). Each set of knives is accompanied by a grinding disc with holes of a certain size (Figure 5).

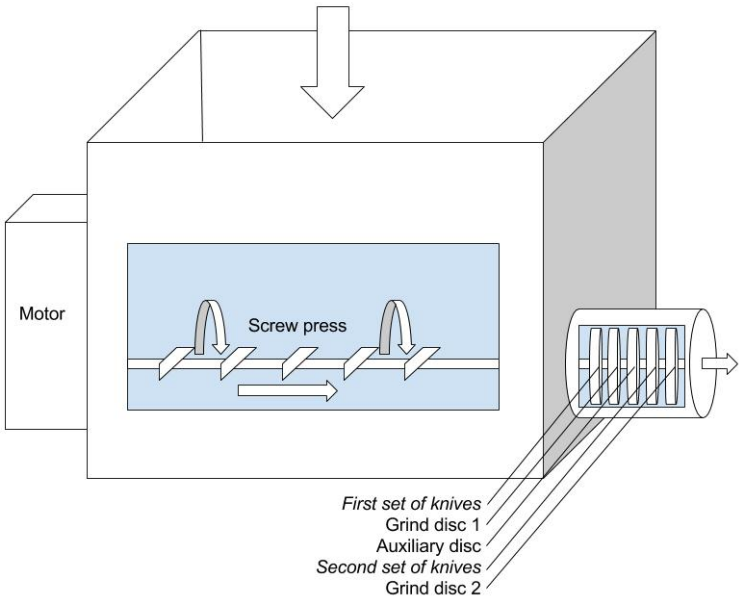


Figure 5: Schematic of the Palmia 200 used for the test

Figure 5 is showing the process with five discs/knives, but the machine can be run with as few as one knife and a disc, which was attempted in our tests.

Figure 6: Grinding disc with 10 mm holes (left) and one set of the knives in the grinder (right)

A fraction size for the shells of less than 4 mm seems to be optimal, since the full-grown larvae are too large to fall through a 4 mm sieve, when larvae and shells should be separated later. Having a shell fraction size of 4 mm or less would allow the separation to be simpler. To reach this or smaller size, the material was ground two times.

Detailed information on the grinding of mussels could be found in the project report "Post-harvest process report: Crushing and grinding" written by Oscar Lagnelöv, dated 14 December 2017.

6 Fly larval composting Baltic blue mussels

Black soldier fly larvae (BSFL) composting was evaluated as a biological harvesting method for the mussel meat. At the first part of the project, different methods to fly larvae compost mussels were evaluated, investigating the impact of different process parameters such as mussel crushing strategy on the efficiency of the process. Once the process parameters had been verified, larvae were reared on mussels. The larvae were then used to produce fish feed that later was tested in fish trial to evaluate the aquaculture feed potential of the mussel reared larvae. In conjunction to the feed trial, a thorough investigation of the fatty acid profile of the mussel reared larvae was undertaken. More information on fly larvae composting can be found in Lalander et al. (2019); Lalander et al. (2018); Lalander et al. (2015). Results from the fish feed trails as well as fatty acid profiles of mussel reared larvae will be included in separate project reports.

6.1 Efficiency of composting Baltic blue mussels

As described above (4) Mechanical pre-treatment of mussels) it was found that a light crushing of mussels, using only knifes and no grinding disc, was the most efficient method (Table 1.) for pre-process treatment before the BSFL composting (step no 3 in Figure 1). The mussel-to-biomass conversion ratio (BCR) describes how much of the total mussel mass that has been converted into larval biomass. The wet weight conversion includes both shells and water, while the conversion on a volatile solids (VS) basis only includes the organic component. The wet weight BCR for mussels was around 6%, which is lower than what has been found for food waste (20%) (Lalander et al., 2018). The conversion on VS basis was considerably higher (20-25%), which us closer to the value found for food waste (30-35%). The reduction on VS basis was 35-55%, which is lower than what has been

found for food waste (>70%) (Lalander et al., 2018). The survival of the larvae was similar to what has been found for food waste in all treatments.

Table 1. The efficiencies of fly larvae composting in terms of crushed dewatered mussels-to-biomass conversion ratio (BCR) on basis of wet weight and volatile solids (VS), volatile solids reduction and survival rate when treating larvae differently pre-treated Baltic blue mussels.

Treatment	TS (%)	VS (% of TS)	BCR (% wet weight)	BCR (% VS)	VS reduction (%)	Survival rate (%)
Crushed mussels (knifes only)	34.9	21.9	7.3	25.6	54.7	100
Mussels crushed with 20 mm discs	35.7	23.1	6.0	19.6	46.7	100
Mussels crushed with 10 mm discs	30.5	21.7	6.0	24.0	34.9	90

6.2 Process parameters for composting Baltic blue mussels

Based on the results from the mussel composting studies a protocol for composting mussel was set up. EU standard size 40 x 60 cm^2 polypropylene crates with a height of 17 cm (Figure 7a. b) were used for the treatment. The number of young larvae added per crate was lowered compared to other waste substrates commonly used (e.g. food waste), from 10.000 – 15.000 to 5.000 larvae per crate. as the amount of nutrients available to the larvae was considerably smaller in mussels. In general, unprocessed Baltic blue mussels comprised 75% water. 20% shell and 5% meat. The crushed dewatered mussels had a TS of 30-35% and a VS of around 22% on a TS basis and the larvae could only degrade the meat fraction. The amount of mussels added to each crate is adjusted by the height of the total amount mussels added that cannot exceed 5 cm in height as the larvae run the risk of suffocating in greater material heights (Dortmans et al., 2017). The total addable amount of mussels per crate was 15 kg on a wet weight basis (TS 35%; VS 22% on a TS basis). The larvae feeding dose, i.e. how much VS that that is proportion per larva, is 0.2 g VS/larva. With 15 kg being treated per crate, the total number of larvae added was 5.000 young larvae per crate. The young larvae (1-week-old) were delivered from SLU's black soldier fly colony.

At the start of the treatment. 5 kg of fresh crushed mussels and 5.000 (1-week-old) BSFL where added to the treatment crate. At day 3 and 7, additional 5 kg of mussels where added to the crate. to sum up to a total addition of 15 kg of mussels per treatment tray. On day 10 the treatment was terminated and the larvae where separated from the treatment residue (Section 7) and the larvae were killed by placement in -20 °C. On average. 1 kg of larvae where generated per crate in the process (BCR 6.4% on a wet weight basis).

The crates where stacked in order to make the treatment more area efficient (Figure 7c). In order for the material to dry out, heavy ventilation is required (0.2 m^3/h. kg mussels).

a) b) c)

Figure 7. Pictures of a) the crate used for treatment with the crushed mussels added. b) the crate with mussels and the added larvae. c) the stacked treatment crates.

6.3 Suggestion to a mobile medium-scale treatment facility

For the treatment to be mobile, the treatment is to take place in a container into which the highest number of stacks possible are fitted (Figure 8), following the semi-centralized modular waste treatment described in (Diener et al., 2015).

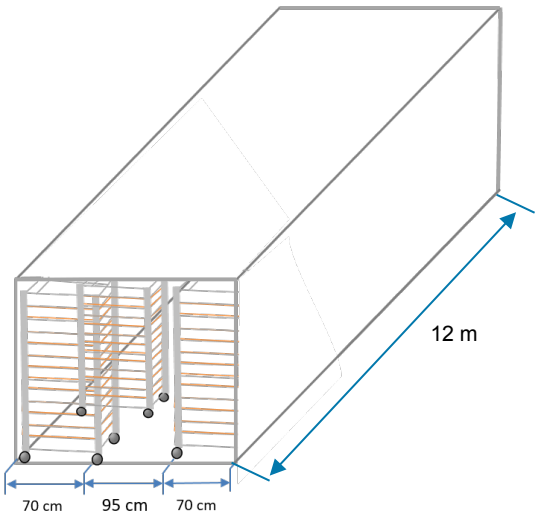


Figure 8. A schematic illustration of the treatment container filled with treatment stacks.

The total number of stacks estimated to fit into the container is 528 and thus it will be possible to treat 790 kg of mussels per working day. Every working day, five racks with fresh mussels are added to the container. and five racks are removed for sieving. The young larvae are produced at a centralised BSF colony facility and the 1-week-old BSFL are taken to the mobile treatment container for the treatment. The total ventilation requirement for the container would be 119 m^3/h. During work in the container. additional ventilation could be necessary due to the formation of ammonia and carbon dioxide from the BSF.

7 Separation between fly larvae. shells and larvae droppings

To be able to do the separation between fly larvae. shells and larvae droppings the mixture must be rather dry to avoid clogging in the separation equipment. The following process steps were found to work out well:

1. Light drying with a fan to get the material dry enough for sifting and cyclone separation, but not dry out the larvae.

2. Shaking (high power, low frequency movement) sifting separation with mesh sizes of 8 mm, 4 mm and possibly a 1.5-2 mm sieve, regardless of if circular or square meshes are used.

3. Cyclone separation of the 4mm fraction with roughly 6 m/s airflow capacity (optimized for a 125 mm airflow inlet nozzle).

The end results will be one fraction of fly larvae, which is the wanted fraction, and one fraction with a mixture of shells and droppings from the larvae (manure). The later fraction could be used as a soil fertilizer, containing calcium (lime effect) and plant nutrients (N, P and K). Detailed information on the separation between fly larvae, shells and larvae dropping could be found in the project report "Post-harvest process report: Separation" written by Oscar Lagnelöv, dated 22 December 2017.

The larvae fraction must be dried further in order to allow it to be stored.

8 Producing mussel and insect-based fish feed

Following the separation, and in order to prepare the material for feed production larvae fraction was transported to SLU (Department for Animal Nutrition and Management) where it was subjected to additional air-drying at 40 °C for 12 hours to constant weight. Larvae were later analysed for proximate composition and dry matter was determined to be 93.7 %.

Larvae were then milled into a meal using a high impact small hammer mill (Novital Davide, Novital S.r.l., Lonate Pozzolo, Italy) to a moderately uniform particle size of ≤1 mm. Due to high fat content larvae were subjected to partial freezing at -18C prior to grinding (2 hours). This prevented the fat from accumulating on the walls of the grinding chamber and clogging the screen, which was a common problem when larvae were milled at room temperature.

The mussels was acquired from a project partner Borgholm municipality and the mussel meal was produced by the research institute Nofima in 2018.

One feed recipe including larvae meal and one including mussel meal were formulated to be used in a growth/digestibility study on rainbow trout. Recipes were formulated in accordance with nutrient requirements of the rainbow trout (NRC, 2011) and are described in Table 2 below. The growth/digestibility test was conducted at the aquatic facility of Department of Animal Nutrition and Management at SLU. The design and the results of this test will be a subject of a separate project report (report on fish and poultry trials-food and feed safety aspects).

Table 2. Feed formulation and the proximate nutritional composition of Larvae and Mussel diets

	Diet	
Ingredients (Dry matter basis in g kg^{-1})	**Larvae diet**	**Mussel diet**
BSF larvae meal	200	-
Mussel meal	-	165
Pea-protein concentrate	425	395
Wheat meal	135	140
Cellulose	25	30
Rapeseed oil	110	130
Fish oil	20	55
Gelatin	60	60
Titanium dioxide	5	5
Mineral-vitamin premix	20	20
Proximate analysis (as is basis)		
Dry matter	942	942
Crude protein[3]	476	479
Crude lipid	212	210
Neutral detergent fibre	66	46
Ash	55	36
Gross energy (MJ kg^{-1})	23.3	23.2

In order to prepare the feed, all dry ingredients were weighed and mixed into a feed mix according to recipe (table 2) using a paddle mixer. Gelatine previously dissolved in hot water was added as a binder to each 500 grams feed batch and the mixing was performed in a bench-top mixer. The wet feed mash was pressed through a single-screw meat grinder with a 3 mm die/orifice (Nima maskin-teknik AB, Örebro, Sweden, Figure 10) and air-dried for 12 hr at 50 °C.

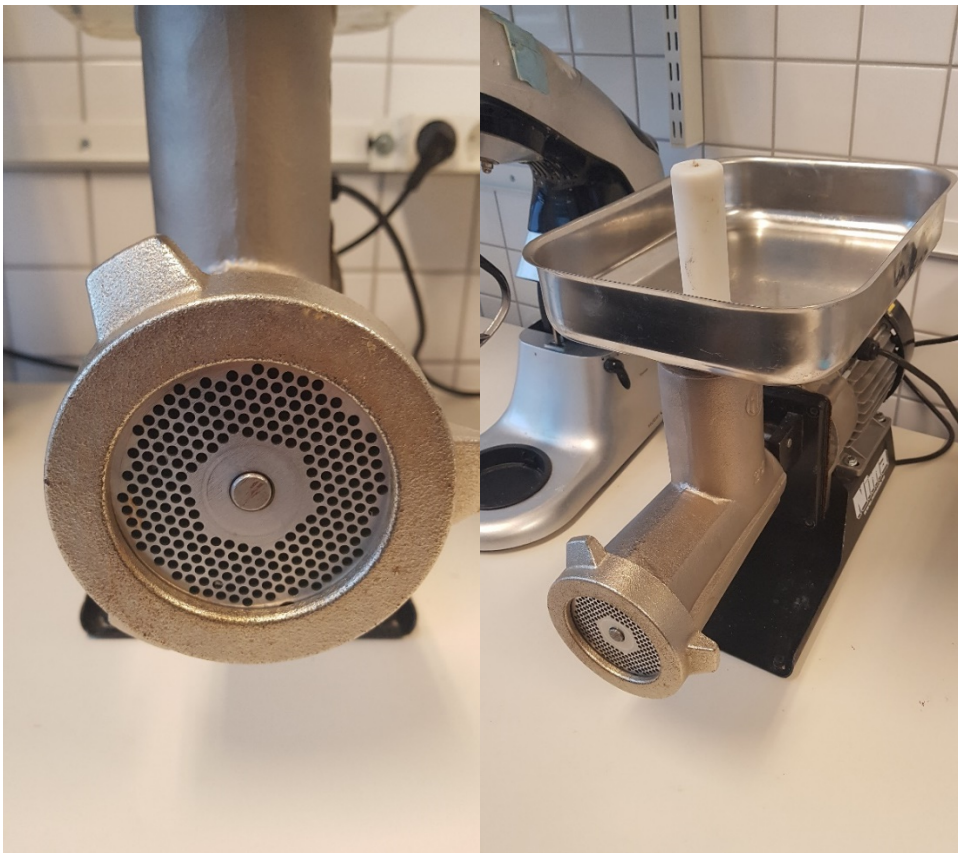


Figure 9. A single screw 'Nima' meat grinder used for feed production, visible 3 mm orifice. .

Pellets were cut to 3-5 mm length using a blender, manually sieved to remove pellets smaller than 3 mm and stored at 4 °C until distribution. Feed batches were prepared at the SLU Feed Science Laboratory (Uppsala, Sweden). The two types of feed are shown in Figure 10.

In an industrial setup, a hygienisation step should be considered to counteract possible contamination of the mussels and larvae. This could be achieved through short heat exposure of the material in a pre-processing step. Alternatively, a common feed processing step in the aqua-feed industry is high-temperature extrusion, which by itself can act as a hygienisation step.

Figure 10. Pelleted mussel meal diet (left) and BSF larvae diet (right)

9 Marketable products

In the treatment, two products are generated: fly larvae, that can be refined into fish feed; and treatment residue that can be used as an organic fertilizer. Furthermore, ammonia is emitted in the fly larvae composting process and have to be captured so that it is not released into the atmosphere where it can contribute to acidification and eutrophication of water bodies. Equipment and costs for ammonia recovery has not been investigated in this project.

9.1 Insect based fish feed

9.1.1 Nutritional composition of larvae reared on Baltic blue mussel

BSF larvae generally comprise 35 – 40% dry matter, of which the protein fraction is 37-48% and the fat 20-40%, depending on the substrate they have been reared on and the larval stage (Wang & Shelomi, 2017). The protein content in larvae reared on fresh mussels (45% of dry matter) were in line with what has been observed for larvae reared on other substrates. BSF larvae reared on fresh mussels contained mainly saturated fat (74 mg/g fat), but also a small proportion of Omega-3 fatty acids (4 mg/g fat). Therefore, feeding black soldier fly larvae with fresh mussels could be a way of improving the nutritional quality of the larvae as an animal feed. However, the mussels should not be stored for long periods upon harvest if not frozen. During the storage for one week at room temperature, the Omega-3 fatty acids in the mussels were degraded to a large extent and larvae reared on these mussels grew poorly in comparison with those reared on fresh mussels. In a separate test, focusing on comparing various short-term storage conditions of blue mussels, it was found that no differences in FA profiles were present after 96 hour storage at +4°C, on ice and at -18°C. A more in-depth description of the nutritional composition of BSF larvae, with special focus on fatty acids, is given in Nils Ewald's master thesis entitled *Fatty acid composition of black soldier fly larvae – impact of rearing substrate*. The thesis is planned to be complied into a scientific article during winter/spring 2019.

The proximate nutritional composition of produced feed is given in Table 2. Both diets had similar proximate composition resulting in identical dry matter content of 94.2% and a high crude protein content of ~48%. Crude lipid levels were also rather similar while the higher content of neutral detergent fiber in larvae meal diet is probably a result of high chitin content. Chitin is a polymer largely responsible for the structure of the larval cuticle.

9.2 Treatment residue

The treatment residue comprises of mussel shells and larvae faeces/droppings. Whole mussel have been suggested as fertiliser and assumed to have three agronomic values: content of plant available nitrogen. phosphorous and liming effect (Spångberg et al., 2013). In the BSF treatment residue, the shell fraction will mainly have a liming effect. The larvae feaces component when looking at BSF composted faeces and food waste, on the other hand, has been demonstrated to have fertilising effects comparable to chemical fertiliser, when growing Swiss chard (Chirere, 2016).

10 Conclusions

Using fly larvae composting as a method to harvest the mussel meat from the mussels was demonstrated to be possible. It was furthermore shown that it was possible to produce a larvae meal of the mussel reared BSF larvae. The mussel reared larvae were shown to contain Omega-3 fatty acids, which larvae reared on substrates not containing Omega-3 fatty acids do not (Ewald, 2019).In terms of transport and storage conditions, the result show that storage on ice for a period of up to 96 hours does not affect the quality of mussels in terms of fatty acid composition.

11 Further reading

Information on economic aspects of using Baltic blue mussels to produce fish feed through a processing step of BSF larvae composting can be found in a separate project report ‘Cost and benefit analysis of mussel farming in the Baltic Sea region’. For further reading on nutritional quality of BSF larvae and Baltic blue mussel meal in diets for rainbow trout please see the ‘Report on fish and poultry trials- food and feed safety aspects’.

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13 Appendix – method for fatty acid analysis and lipid oxidation

Extraction of fatty acids was performed using a modified version of the method described by Folch et al. (1957). Sample was taken in order to extract 50 mg of lipids (10 g for mussels, 2 g for remaining samples) and weighed on an analytical scale. For every gram of sample, 40 ml of hloroform:methanol 2:1 (v/v) was added. The solution was homogenized 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 in2 ml chloroform and stored in -80°C until methylation.

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% BF3-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 hexanewas added. The sample was vortexed and then centrifuged for 5 min at 480 xg (Hermle Labortechnik, Germany). The upper phase was transferred to a GC-vial, and evaporated in a sample collector using N2-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 N2 (make up gas) at 40, 400 and 50 ml/min. Each sample was injected twice and run for 35 min.

Using the peak areas in the standard chromatogram, the retention factor for each fatty acid methyl ester (RFFAME) was calculated as:

$$RF_{FAME} = \frac{PA_{FAME}/m_{FAME}}{PA_{IS}/m_{IS}}$$

where PAFAME and PAIS were the peak areas in the standard chromatogram, and mFAME and mIS 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 (mFA) was calculated as:

$$m_{FA} = \frac{PA_{FAME}}{\frac{RF_{FAME} \times PA_{IS}}{m_{IS}}} \times 1.048$$

where PAFAME and PAIS were the peak areas in the sample chromatogram, RFFAME the response factor calculated for a specific fatty acid methyl ester and mIS 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 calculate are more accurate estimate of the fatty acid weight.

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).

14 Appendix- fatty acid composition

Table 3. Fatty acid composition of mussels in 3 different treatments stored for 0, 24 and 96 hours. The values are expressed as mg/g of extracted fat.

Fatty acid	0h	ice 24 h	5C 24h	-18C 24h	Ice 96 h	5C 96 h	-18C 96h
C14:0	42.0	37.5	38.2	37.9	38.0	38.0	38.4
C16:0	78.1	74.0	70.0	71.9	64.0	66.8	67.8
C16:1	86.9	65.5	68.9	70.6	68.4	72.9	67.5
C18:0	17.6	19.0	15.6	19.8	14.4	16.9	16.0
C18:1	98.8	66.8	58.4	54.5	55.0	54.5	57.2
C18:1 Val	10.0	9.2	8.1	8.5	7.1	7.8	7.7
C18:2	17.2	17.0	15.4	13.9	13.1	14.3	13.6
C18:3	15.5	14.3	12.6	12.0	11.7	11.5	11.5
C20:1	24.1	22.3	22.0	24.0	20.6	20.2	19.8
C20:2	5.2	6.7	5.1	5.9	4.7	4.6	4.6
C20:4	6.5	8.4	6.4	6.9	5.4	5.6	6.3
C20:5	83.6	84.0	68.0	72.4	60.9	66.2	69.0
C22:0	6.2	12.1	10.0	10.0	7.0	7.7	3.3
C24:0	1.7	2.8	0.0	0.8	0.0	1.2	1.7
C22:6	96.3	94.9	85.5	87.4	77.5	78.7	83.1
SFA	145.60	145.39	133.71	140.42	123.42	130.65	127.15
MUFA	219.67	163.91	157.41	157.65	151.22	155.42	152.24
PUFA	224.23	225.31	193.01	198.48	173.31	180.84	188.10