Insects reared on seaweed as novel feed ingredients for Atlantic salmon (*Salmo salar*)

Investigating the transfer of essential nutrients and undesirable substances along the seaweed-insect-fish food chain

Irene Biancarosa

Thesis for the degree of Philosophiae Doctor (PhD)
University of Bergen, Norway
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Scientific environment

This PhD project started in March 2015 as a collaboration between the Department of Biology at the University of Bergen (UiB) and the research group Requirement and Welfare at the National Institute of Nutrition and Seafood Research (NIFES), which was merged with the Institute of Marine Research in Bergen (IMR) on January 1st 2018.

The work for this doctoral thesis was performed under the supervision of Prof. Rune Waagbø (IMR, UiB), Dr. Erik-Jan Lock (IMR) and Dr. Heidi Amlund (Technical University of Denmark, National Food Institute, Lyngby, Denmark).

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Abstract

Traditionally, major sources of protein and lipid in aquaculture fish feeds have been fish meal and fish oil. However, fish stocks used for fish meal and fish oil production are fully exploited, therefore prices of these ingredients continue to increase. In recent years, substantial progress has been made by the research community and feed producers to test novel sources of protein and lipid to replace marine feed ingredients in aquaculture. Insects have been identified as feed ingredients of great potential for farmed fish. In particular, being high in energy and protein content, they seem a good source of ingredients in compound feeds for Atlantic salmon (*Salmo salar*). However, insects reared on terrestrial feedstuff are not a source of the essential marine omega-3 fatty acids, which Atlantic salmon has a dietary requirement for. The *AquaFly* project aimed to develop novel insect feed ingredients for Atlantic salmon, contributing essential nutrients to produce robust and healthy fish. To achieve this, tailoring of the nutrient composition of the insect feed ingredients towards fish nutrient requirements was investigated through the use of seaweed as feeding substrate for the insects.

Seaweeds are known to contain marine omega-3 fatty acids and essential minerals (like iodine) which are generally absent in terrestrial feedstuff for insects. At the same time, seaweeds can contain undesirable substances, especially heavy metals and arsenic, which could be transferred to the insects, therefore enter the food production chain. The focus of this PhD project, as part of *AquaFly*, was to evaluate the suitability and safety of the seaweed-insect-fish food production chain, by studying the transfer of both nutrients and undesirable substances along the food chain.

Several species of seaweeds from Norwegian waters were screened for their chemical profile. The seaweed species studied contained both nutrients and undesirable substances (heavy metals and arsenic); the concentrations thereof were highly dependent on species and taxonomic group. Based on the data obtained, the brown alga *Ascophyllum nodosum* was chosen for insect rearing. This seaweed species showed the highest lipid content and the lowest concentrations of undesirable substances among the species investigated. In the insect feeding trial, larvae of the black soldier fly (*Hermetia*

illucens) were fed plant-based media enriched with *A. nodosum* in increasing percentages (from 0 to 100 % seaweed inclusion). The larvae could grow on such media, up to 100 % seaweed. However, the best growth performance, nutrient utilization and retention were seen up to 50 % seaweed inclusion in the media. Transfer of heavy metals and arsenic from seaweed to larvae occurred, with higher concentrations of these undesirable substances in larvae fed higher inclusion of seaweed in the media. Based on the results, large batches of insect larvae fed 50 % seaweed in the media were grown to produce insect meal and insect lipid for the fish trials.

Two fish feeding trials were conducted with i) freshwater-phase juvenile fish and ii) seawater-phase post-smolt fish. In the freshwater trial, fish were fed six diets: a control diet with protein and lipid from traditional ingredients (fish meal, fish oil, plant-based protein and vegetable oil) and five experimental diets where insect meal replaced 85 % of the dietary protein and /or all the vegetable oil was replaced by insect lipid. In this trial, the inclusion of insect feed ingredients in the fish diet did not affect the overall growth parameters, nutrient digestibility and whole fish nutrient composition. In the seawater trial, insect meal was used as replacement for fish meal in the feed (33, 66, 100 % replacements). This did not impact growth performances of the fish, nutrient utilization or fillet composition. In both fish trials, low levels of heavy metals were found in feed and fish. In the seawater trial, transfer of arsenic from feed to fish occurred; however, levels of arsenic in the fillet decreased in fish fed increasing insect meal in the diet.

Based on the results of this PhD work, it can be concluded that the seaweed-insect-fish food chain holds great benefits for farming Atlantic salmon by producing nutritious feeds and healthy, robust fish. Moreover, it is an overall safe approach, in terms of both feed and food safety.

Abbreviations

AB	Arenobetaine
ARA	Arachidonic acid
BSF	Black soldier fly
DGI	Daily growth index
DHA	Docosahexaenoic acid (22:6n-3)
DMA	Dimethylarsinate
DW	Dry weight
EAA	Essential amino acid
EPA	Eicosapentaenoic acid (20:5n-3)
FA	Fatty acid
FCR	Feed conversion ratio
FM	Fish meal
FO	Fish oil
HSI	Hepatic somatic index
LC-PUFA	Long-chained polyunsaturated fatty acid
LOQ	Limit of quantification
IM	Insect meal
IL	Insect lipid
ML	Maximum level
N-Prot	Nitrogen-to-protein
NPN	Non-protein nitrogen
PAP	Processed animal protein
PUFA	Polyunsaturated fatty acid
SGR	Specific growth rate
SPC	Soy protein concentrate
VO	Vegetable oil
WW	Wet weight

List of Publications

Paper I

Biancarosa I., Espe M., Bruckner C.G., Heesch S., Liland N.S, Waagbø R., Torstensen B.E. and Lock E-J. (2017). "Amino acid composition, protein content, and nitrogen-to-protein conversion factors of 21 seaweed species from Norwegian waters". Journal of Applied Phycology 29(2): 1001-1009.

Paper II

Biancarosa I., Belghit I., Bruckner C.G., Liland N.S., Waagbø R., Amlund H., Heesch S. and Lock E-J. (2018). "Chemical characterization of 21 species of marine macroalgae common in Norwegian waters: benefits of and limitations to their potential use in food and feed". Journal of the Science of Food and Agriculture 98(5): 2035-2042.

Paper III

Liland N. S., **Biancarosa I**., Araujo P., Biemans D., Bruckner C.G., Waagbø R., Torstensen B.E. and Lock E-J. (2017). "Modulation of nutrient composition of black soldier fly (*Hermetia illucens*) larvae by feeding seaweed-enriched media". PLoS One 12(8): e0183188.

Paper IV

Biancarosa I., Liland N.S., Biemans D., Araujo P., Bruckner C.G., Waagbø R., Torstensen B.E., Lock E-J. and Amlund H. (2018). "Uptake of heavy metals and arsenic in black soldier fly (*Hermetia illucens*) larvae grown on seaweed-enriched media". Journal of the Science of Food and Agriculture 98(6): 2176-2183.

Paper V

Belghit I., Liland N.S., Waagbø R., **Biancarosa I**., Pelusio N., Li Y., Krogdahl Å. and Lock E.-J. (2018). "Potential of insect-based diets for Atlantic salmon (S*almo salar*)". Aquaculture 491: 72-81.

Paper VI

Belghit I., Liland N.S., Gjesdal P., **Biancarosa I.**, Menchetti E., Li Y., Waagbø R., Krogdahl Å. and Lock E.-J. (2019). “Black soldier fly larvae meal can replace fish meal in diets of sea-water phase Atlantic salmon (*Salmo salar*)”. Aquaculture 503: 609-619.

Paper VII

Biancarosa I., Sele V., Belghit I., Ørnsrud R., Lock E-J. And Amlund H. (2019). “Replacing fish meal with insect meal in the diet of Atlantic salmon (*Salmo salar*) does not impact the amount of contaminants in the feed and it lowers accumulation of arsenic in the fillet”. Food Additives and Contaminants: Part A 36(8): 1191-1205.

Contents

1. Introduction

The aquaculture industry has been the fastest growing food sector in the world during the last three decades, with an annual growth rate of 5.8 % in the period 2001-2016 (FAO 2018) (**Figure 1**). Considering the increase in the world population and the demand for food, aquaculture will likely continue to expand in the near future, and it is expected to contribute up to 60 % of the global fish consumption by 2030 (FAO 2018). In Norway, aquaculture has been mainly focused on farming Atlantic salmon (*Salmo salar*). In the last 20 years, the Norwegian salmon industry has increased its output considerably, making Norway the second-largest exporter of fish and fish products worldwide, behind China (FAO 2018). The Norwegian government aims for five million tons of aquaculture production by 2050, which is a five-fold growth of the current production (Hersoug 2015). An intensification of the aquaculture sector will require new strategies to maintain a sustainable development. A sustainable development "conserves land, water, plant and animal genetic resources, and it is environmentally non-degrading, technologically appropriate, economically viable and socially acceptable" (FAO 2018).

In Atlantic salmon aquaculture, the availability of sustainable feed ingredients is a major obstacle. Carnivorous fish like salmonids are typically fed high-energy diets, containing large amounts of protein and lipid (~40 and ~30 % of the diet, respectively), and low level of carbohydrates (NRC 2011). Traditionally, major sources of protein and lipid in salmon feeds have been fish meal (FM) and fish oil (FO) obtained from wild-caught fish. These marine feed ingredients are considered the most nutritious and digestible feed ingredients for farmed Atlantic salmon, due to high-quality protein and lipids. They contain a well-balanced profile of essential amino acids (EEA) as well as beneficial omega-3 long-chain polyunsaturated fatty acids (n-3 LC-PUFAs) which Atlantic salmon has a dietary requirement for (Roselund et al. 2016). However, fish stocks targeted for FM and FO production are fully exploited, and, consequently, prices of these feed ingredients continue to increase (FAO 2018) (**Figure 1** and **Figure 2**). Therefore, in the last two decades, research has focused on finding novel sources of protein and lipid to replace marine feed ingredients in aquaculture.

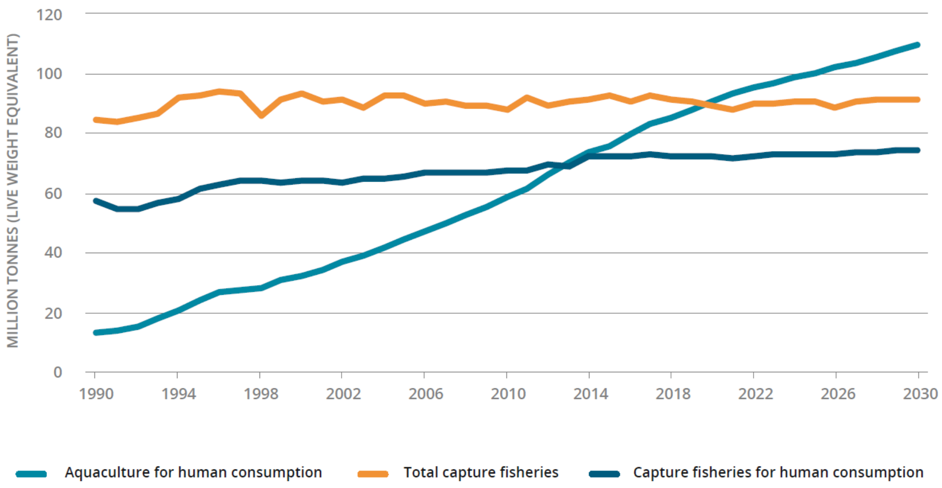


Figure 1. Development of world capture fisheries and aquaculture production (expressed as million tonnes), 1990-2030 © FAO (FAO 2018). Orange line: total capture fisheries; dark blue line: aquaculture for human consumption; light blue line: capture fisheries for human consumption.

So far, plant-based feed ingredients like soy-based proteins have been the main replacement of FM and FO in aquafeeds for salmonids, but also other farmed species. This is mainly because of lower prices for the raw materials compared to marine feed ingredients, and an all year-round availability (**Figure 2**). However, using plant-based feed ingredients for fish nutrition has led to adverse effects on fish health, due to nutrient deficiencies or imbalances, and occurrence of anti-nutrients and toxic substances in plant-based ingredients (Francis et al. 2001; Waagbø et al. 2013). Additionally, the replacement of marine ingredients with plant-based ingredients shifted resource demand from oceans to lands, adding pressure to the terrestrial environment and food production systems for human consumption. Today, research is therefore focused on finding novel sources of feed ingredients to replace both marine and plant ingredients, among which single cell proteins, microalgae and insects, have received the highest attention.

Figure 2. Fish meal and soybean meal prices (expressed as USD/tonne) trend in Germany and the Netherlands (above), fish oil and soybean oil prices trend in the Netherlands (below) 1983-2017 © FAO (FAO 2018). Orange line: soybean oil; blue line: fish oil.

The interest in using insects in animal nutrition has increased tremendously in the last decade. Insects are natural components of the diet of many animal species, therefore the inclusion of insect ingredients in feed formulas seems self-evident. They are typically protein-rich and have a well-balanced profile of amino acids, which makes insect meal (IM) highly digestible for animals (Gasco et al. 2019; Sogari et al. 2019). The nutritional composition of insects strongly reflects the composition of the insect feeding media (Meneguz et al. 2018; Pinotti et al. 2019). This is a major advantage that allows insect producers to tailor the nutrient profile of the IM towards a desired profile, through the insect feeding media.

Insect larvae can convert low quality carbohydrate-rich organic materials (such as food waste) into high quality feed ingredients to be used in feed formulas (Fowles and Nansen 2020). In Europe, 88 million tons of food is wasted per year and this is expected to increase (Stenmark et al. 2016). Rearing insects on waste materials could contribute to reduced waste while responding to the demand for feed ingredients in animal food production chains. This fits well into the Circular Economy principle which is the ability to maintain values of products and systems through their use and reuse (Kirchherr et al. 2017; Rizos et al. 2017). Preliminary studies have also shown that insects have a lower environmental footprint than other livestock animals, however more data is needed (Oonincx and de Boer 2012; Mertenat et al. 2019).

The insect industry in Europe has mainly focused on the production of insect protein destined to pet food. However, research on the use of insect feed ingredients for farmed animals has developed rapidly in the last five years, leading to the authorization of insect processed animal protein (PAP) in feed for farmed fish in 2017 (Commission Regulation (EU) 2017/893) (EU 2017). However, this regards only a few insect species: black soldier fly (BSF) (*Hermetia illucens*); common housefly (*Musca domestica*), yellow mealworm (*Tenebrio molitor*), lesser mealworm (*Alphitobious diaperinus*), house cricket (*Acheta domesticus*), banded cricket (*Gryllodes sigillatus*) and field cricket (*Gryllus assimilis).* The production of insect protein in Europe is expected to rise up to 75 % by 2025 (**Figure 3**). Of this production, more than 50 % has been used for aquaculture since authorization in the EU (IPIFF 2018). The use of insect PAP in feed for other animals (e.g. poultry, swine) is expected to be also authorized in the near future.

In the European Union (EU), insects are considered full-fledged “farmed animals”-i.e. animals that are kept for the production of food, feed or other derived products (Regulation (EC) No 1069/2009, Commission Regulation (EU) 2017/893) (EU 2009; EU 2017). Like other farmed animals, insects can only be fed authorized substrates like “feed grade materials” (e.g. plant-based materials, vegetable and fruit residues, wheat bran, grass and brewery by-products, hay, among others) (Regulation (EC) No 178/2002, Regulation (EC) No 767/2009 and Commission Regulation (EC) No

68/2013) (EU 2002a; EU 2009; EU 2013). However, insect producers have also shown interest in using other media for insect rearing.

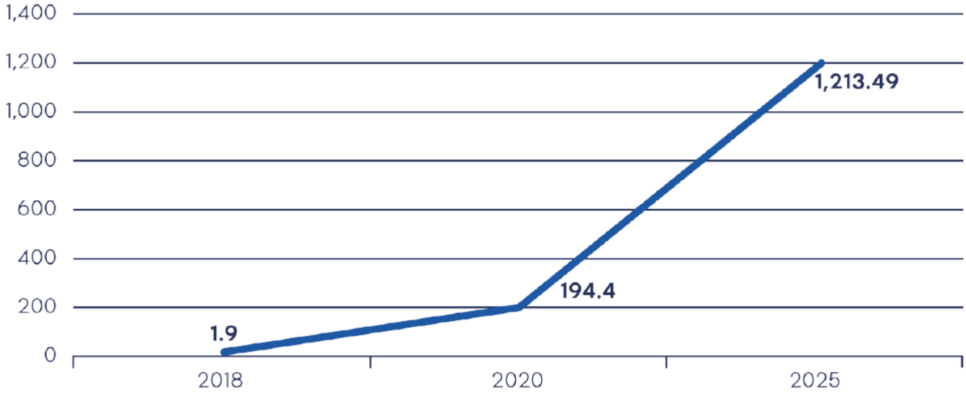


Figure 3. Estimated volumes of production of insect protein until 2025 in Europe (in thousands of tonnes) (IPIFF 2018).

In the last decade, seaweeds have been studied as potential fish feed ingredients in aquaculture, due to their richness in both macro- and micronutrients (Satoh et al. 1987; Wassef et al. 2013; Norambuena et al. 2015). Low inclusion of seaweed meal in fish feed (<10 % of the diet) seemed to have positive effects, while high seaweed inclusion levels could be detrimental for fish (Soler-Vila et al. 2009; Wassef et al. 2013; Norambuena et al. 2015). As such, the use of seaweed in aquaculture is most likely limited to the inclusion of seaweed-based additives, rather than as a realistic replacement of marine resources (i.e. FM and FO) in aquafeeds. In Norway, large volumes of seaweed naturally grow along the Norwegian coastline, of which only 1 % is harvested every year, mainly for the industrial production of thickeners (e.g. agar and alginates) (Olafsen et al. 2012; Skjermo et al. 2014). As such, seaweed represents an under-exploited marine resource for the Norwegian economy and the interest in exploiting this resource for other applications has increased.

In this PhD work, seaweed was used as feeding substrate for rearing BSF larvae. BSF is considered one of the most suitable species for feeding salmonids as the larvae of this fly are protein- and lipid-rich and have an EAA profile similar to FM (Barroso et al. 2014; Henry et al. 2015; Lock et al. 2018). However, BSF larvae are naturally absent in n-3 LC PUFAs, like most terrestrial insect species (Henry et al. 2015). By feeding seaweed to BSF larvae, we aimed to tailor the nutritional composition of the insect larvae towards a "marine profile" which better suits the nutritional requirements of Atlantic salmon. The suitability of seaweed as rearing substrate for insect larvae was not known in the literature. Growth and performance of BSF larvae reared on seaweed-enriched media were investigated, while assessing the potential transfer of nutrients from seaweed to larvae. The BSF larvae fed seaweed were therefore utilized to develop IM and insect lipid (IL), which were later included in the diet of farmed Atlantic salmon. We investigated nutrient digestibility of the insect-based diets as well as growth and health parameters of the fish.

A major aim in food production chains is to produce safe food for consumers which means to ensure food safety. When food comes from farmed animals, it is also necessary to provide safe feed for the animals, that is to guarantee feed safety. Novel feed ingredients may bring undesirable substances to aquafeeds. Feed and food safety regulations are therefore in place to ensure that feed and food stuff do not represent a danger to human health, animal health or the environment. The EU legislation has set maximum levels (MLs) for undesirable substances in feed and food stuff (EC Directive 2002/32 and amendments; Commission Regulation (EC) No 1881/2006 and amendments) (EU 2002; EU 2006). This covers a wide range of compounds such as heavy metals, arsenic, polychlorinated biphenyls, dioxins, pesticides, plant and fungal toxins.

This PhD project evaluated availability, access and utilization of nutrients as well as challenges with undesirable substances along the seaweed-insect-fish food production chain (**Figure 4**). My focus was put on heavy metals (cadmium, lead and mercury) and the metalloid arsenic, as they are major potential chemical hazards associated with seaweed (Almela et al. 2006; Chakraborty et al. 2014). Therefore they were expected to enter the food chain when seaweed was fed to insect larvae. I evaluated the potential transfer of these undesirable substances from seaweed to insects to feed to fish. In this PhD thesis, I discuss the overall suitability and safety of the seaweed-insect-fish food production chain in Atlantic salmon farming.

2. Objectives of the PhD work

To achieve the aims of this PhD work, I followed three main objectives:

1. To survey the chemical composition of seaweed species from Norwegian waters.
2. To evaluate the suitability of seaweed biomass as feeding substrate for insect larvae production, with focus on the potential transfer of heavy metals and arsenic from seaweed to insects.
3. To study the suitability of insect feed ingredients in the diet of farmed Atlantic salmon juveniles and post-smolt, with focus on the potential transfer of heavy metals and arsenic from feed to fish.

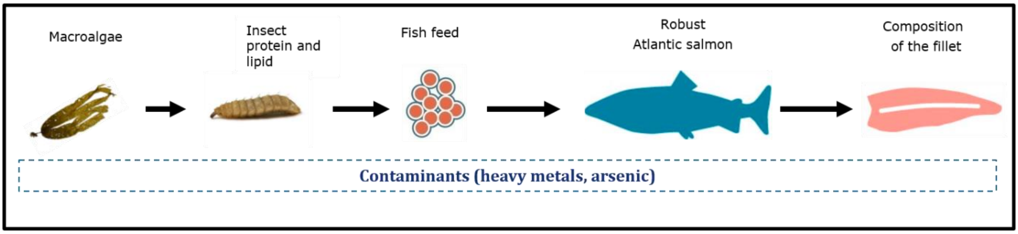


Figure 4. The seaweed-insect-fish food production chain.

3. Background

3.1 Insects for Atlantic salmon nutrition. For wild Atlantic salmon, insects comprise more than 50 % of the diet in freshwater (Gabler and Amundsen 1999); as such, the inclusion of insect feed ingredients in the diet of salmon appears appropriate. When choosing which insect species to incorporate into a fish diet, it is pivotal to determine the nutritional composition of the insect, as it should meet the nutrient requirements of the fish. Carnivorous fish like salmonids require between 40 and 55 % protein, and EAA for optimal growth (NRC 2011). The protein content of most insect species range from 50 to 82 % on dry weight (DW) basis (Barroso et al. 2014), which is comparable to the average protein content of FM (70 %) and higher than that of soybean meal (45 %) (NRC 2011). Moreover, while plant-based feed ingredients have some EAA imbalances and deficiencies, the EAA profile of most insects meets the EAA requirements of most fish species and, in some cases, even exceed these requirements (Henry et al. 2015).

Besides protein and amino acids, fish require lipids as a source of energy. Commercial feeds for Atlantic salmon typically contain 20 % and 30 % of lipids in freshwater and seawater diets, respectively. Dietary lipids above these levels can lead to reduction of fish growth and fat deposition (New and Wijkstroem 2002). The lipid content of insects ranges from 6 to 38 % (DW basis) and it is higher than lipid levels typically found in FM (8.2 %) and soybean meal (3 %) (St-Hilaire et al. 2007; Barroso et al. 2014). Besides energy, lipids provide essential fatty acids (FAs) such as the n-3 LC-PUFAs eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Most insects species lack n-3 LC-PUFAs (Barroso et al. 2014), which could hamper the use of insect feed ingredients in the diet of Atlantic salmon. However, the FA profile of insects is highly affected by the FA profile of their feeding substrate, and there are evidences that feeding insects on substrates rich in n-3 LC-PUFAs (e.g. fish offal) enriches the larvae with such nutrients (Sealey et al. 2011; St-Hilaire et al. 2007a). Sealey et al. (2011) highlighted the importance of the insect rearing substrate on the quality of the IM in a trial with rainbow trout (*Onchorhynchus mykiss*). Fish fed IM produced from BSF larvae reared on subsrates enriched with fish offal, performed better (in terms of fish growth) than fish fed IM produced from BSF larvae fed substrates without the fish offal enrichment (Sealey et al. 2011).

Besides macronutrients, insects are a good source of other essential nutrients such as minerals (i.e. potassium, calcium, iron, magnesium, zinc, selenium) (Finke 2002; Banjo et al. 2006; Schabel 2010; Rumpold and Schluter 2013). However, calcium and phopshorus levels in insects are usually lower than the ones found in FM (Makkar et al. 2014).

3.2 The black soldier fly. The BSF belongs to the order of *Diptera* (the true flies) and originates from the American continent, although its diffusion is nowadays ubiquitous in tropical and temperate regions. The larvae of BSF typically contain high amounts of both protein (~40 %) and lipids (~35 %) (of DW) (Barroso et al. 2014). BSF has a relatively short lifecycle including four stages: eggs (4 days), larvae (18 days), pre-pupae (14 days) and finally adults (9 days) (Sheppard et al. 2002). BSF larvae are scavengers and can grow on a wide range of organic substrates, while adults do not feed and rely on the fat storage from the larval stage (Diclaro and Kufman 2009). BSF larvae are able to withstand extreme environmental conditions such as drought, dehydration, shortage of food and oxygen deficiency, and this robust nature makes rearing activities quite simple (Diener et al. 2011). Rearing of BSF has been done since the 1990s, mainly for biodegradation of organic waste by the larvae (Diener et al. 2009; Čičková et al. 2015; Fowles and Nansen 2020). To date, BSF larvae are also reared for feed purposes and biodiesel production (Diener et al. 2009; Čičková et al. 2015; Fowles and Nansen 2020).

Studies investigating the effects of IM for salmonids are only a few, as reviewed by Lock et al. (2018). St-Hilaire et al. (2007) investigated the use of a full fat meal from BSF pre-pupae as substitution of FM and FO in the diet of rainbow trout. In this study, the inclusion of 15 % of BSF meal in the diet of rainbow trout (corresponding to 25 % and 36 % substitution of FM and FO, respectively) was possible without significant impacts on growth performances. Renna et al. (2017) showed that a partially defatted IM from BSF larvae can be included in the diet of rainbow trout up to 40 % (50 % of

FM substitution), without impairing growth performance of the fish. Lock et al. (2016) showed a full (100 %) replacement of FM with IM from BSF larvae in the diet of Atlantic salmon, finding no adverse effects on fish performance parameters (growth, condition factor, hepatic somatic index (HSI), visceral somatic index (VSI)). Similarly, the partial substitution of FM with BSF meal has been successfully performed in other fish species such as European seabass (*Dicentrarchus labrax*) (Magalhães et al. 2017), Yellow catfish (*Pelteobagrus fulvidraco*) fry (Xiao et al. 2018), Jian carp (*Cyprinus carpio*) (Li et al. 2017; Zhou et al. 2018), and Nile tilapia (Devic et al. 2018). Recently, meagre (*Argyrosomus regius*) juveniles fed diets including 10 %, 20 %, and 30 % of BSF meal, replacing 17 %, 35 %, and 52 % of FM, showed no major adverse effects on growth, feed utilization, whole-body composition and FA profile (Guerreiro et al. 2020). Moreover, results of a trial performed feeding Siberian sturgeons (*Acipenser baerii*) with BSF diets, showed that dietary inclusion of BSF meal up to 18.5 % (25 % of FM replacement) does not impair growth performance, condition factor, biometric and morphometric indices, and whole body proximate composition of the fish (Caimi et al. 2020).

Figure 5. Black soldier fly larvae (Photo: Irene Biancarosa).

3.3 Seaweeds. Seaweeds, also known as marine macroalgae, are autotrophic (plant-like) organisms distributed worldwide. They are commonly divided into three taxonomic groups, on the basis of thallus colour: brown algae (*Phaeophyceae, Heterokontophyta*), green algae (*Chlorophyta*), and red algae (*Rhodophyta*). To date, about 10 000 seaweed species have been described, of which 6500 are red, 2000 are brown and 1500 are green algae (http://www.algaebase.org/). For centuries, seaweeds have been part of the traditional food culture in Asian countries, while their use in Europe has been limited to the extraction of bioactive compounds such as alginates, fucoidans and pigments.

Seaweeds are known to be rich in high-quality nutrients: they generally contain all the EEA and up to ~30 % of the algal DW can be protein, although high variations are found between species and due to the method used for protein determination (Gupta and Abu-Ghannam 2011; Skjermo et al. 2014; Mæhre et al. 2014). The most recommended approach for protein determination in seaweed is by analyzing each species for its content of amino acids, and then estimating protein contents as the sum of proteomic amino acids (or amino acid residues) after hydrolysis (the so-called "true protein" method) (Aitken et al. 1991; Lourenço et al. 2002; Diniz et al. 2011; Shuuluka et al. 2013). This method, although widely recommended, requires sophisticated and expensive equipment and specialized laboratories. As such, most of the existing studies on seaweed protein have been based on the crude protein method (Kjeldahl 1883). This is the elemental analysis of nitrogen followed by an indirect estimation of protein contents using a nitrogen-to-protein (N-Prot) conversion factor. Traditionally, a standard N-Prot conversion factor of 6.25 is used for protein determination in biological matrixes. However, this would not be ideal for seaweed (Angell et al. 2016). The use of 6.25 is built on the assumption that protein is the only source of nitrogen in the analyzed tissue and the amount of non-protein nitrogen (NPN) is negligible. Seaweeds, however, contain a considerable amount of NPN in chlorophyll, nucleic acids, free amino acids and inorganic nitrogen, among other components (Lourenço et al. 1998; Belghit et al. 2017). As such, using the crude protein method with the standard N-Prot factor of 6.25 can overestimate the actual protein content of seaweed samples (Angell et al. 2016). Species-specific N-Prot conversion factors should be calculated (and applied) when using the crude protein method for protein quantification in seaweed (Angell et al. 2016).

Despite being typically low in lipids, some red algae species, e.g. *Porphyra* species, are found to be rich in n-3 LC-PUFAs (Mæhre et al. 2014). Also, total mineral content in seaweed is generally high, up to 55 % of the DW in some species (Holdt and Kraan 2011). In particular, calcium, phosphorus, zinc, selenium, are abundant (Mæhre et al. 2014). Marine macroalgae typically contain high concentrations of iodine (up to 4.5 % of DW in some brown algae species from the order of *Laminariales*) (Gall et al. 2004; Holdt and Kraan 2011). Iodine is an essential trace element for both human and animal nutrition, as it regulates the metabolisms through the synthesis of the thyroid hormones. Dietary iodine deficiencies are linked to severe health disorders in both humans and animals (Penglase et al. 2013; Leung et al. 2014).

Due to their valuable nutrient profile, seaweeds have a great potential for feed applications. Commercial use of seaweed destined for animal feed began in Europe in the early 1930 with the harvest of the brown alga *A. nodosum* (rockweed) which was and still is very abundant, and easy to collect and process (Evans and Critchley 2014). *A. nodosum* is the most used seaweed species for animal feed applications, with an annual world harvest of about 100 000 tons wet biomass (Evans and Critchley 2014). The inclusion of seaweed meal in animal nutrition has been reported to be beneficial for both marine and terrestrial organisms (Evans and Critchley 2014). Small amounts (2.5-10 % of the diet) of seaweed meal in fish feeds improved growth performance, feed utilization and fillet nutrient composition in red sea bream (*Pargus major*), gilthead seabream (*Sparus aurata*), European seabass and rainbow trout (Wassef et al. 2005; Valente et al. 2006; Soler-Vila et al. 2009; Evans and Critchley 2014). Also, adding 2.5 and 5 % of seaweed meal in the diet of Atlantic salmon did not impact growth and feed efficiency, while it increased total fatty acid concentrations as well as n-3 LC-PUFAs levels in the fish fillet (Norambuena et al. 2015; Wilke et al. 2015). On the other hand, high inclusion levels of seaweed meal in feeds (>10-15 % of the diet) have been reported to be detrimental for fish, causing negative effects on fish growth and nutrient utilization, especially in marine carnivorous fish species like salmonids (Nakagawa et al. 1987; Nakagawa et al. 1993; Diler et al. 2007; Azaza et al 2008). This is believed to be due to the presence, in seaweeds, of complex carbohydrates (e.g. alginates, fucoidans, laminarin) and substances of anti-nutritional properties (e.g. lectins, tannins, phytic acid, proteinase and amylase inhibitors). These compounds can interfere with nutrient utilization and digestion, thus affecting fish growth (Jiao et al. 2011; Evans and Critchley 2014; Makkar et al. 2015; Melanson and MacKinnon 2015). These compounds can comprise up to 80 % of the algal DW, although this is highly dependent on species and season of harvest (Wijesekara and Karunarathna 2017).

3.4 Status of seaweed research in Norway. Enormous volumes of seaweed grow along the Norwegian coastline, which is among the world's longest (over 100 000 km) and most productive. Harvesting of seaweed in Norway has been a common practice for more than five decades, especially of brown algae species such as *Laminaria hyperborea* (commonly known as "forest kelp") and *A. nodosum* (FAO 2016). These species can be harvested from the intertidal zone or more often collected on the beach, after storms. However, to date, only a small portion of the total seaweed biomass is harvested, making seaweeds an under-exploited marine resource (Skjermo et al. 2014). For example, c.a. 50 million tons of *L. hyperborea* grow in Norwegian coastal waters every year, but only 150 000 tons (less than 1 %) are harvested (Skjermo et al. 2014; Steen 2016).

Besides harvesting, there has been a growing interest in cultivating seaweed in Norway in the last two decades. This activity, however, is still mostly on a research scale. Seaweed cultivation in Norway has been focusing on brown algae species (kelps species), particularly *Saccharina latissima* (sugar kelp) and *Alaria esculenta* (winged kelp). Cultivation of these species at sea has increased by four times between 2014 and 2016 (8, 24 and 36 cultivation sites at sea in 2014, 2015 and 2016, respectively) (Directorate of Fisheries 2016a and 2016b). A few seaweed cultivation sites in Norway are located in the vicinity of Atlantic salmon farms, the so-called integrated multitrophic aquaculture (IMTA) systems. Seaweeds in such systems utilize excess nutrients produced by fish farming wastes, reducing the environmental impact of intensive aquaculture while increasing the yield of valuable biomass produced (Directorate of Fisheries 2016a and 2016b).

To date, the use of seaweed biomass in Norway has been limited to the extraction of bioactive compounds such as alginates destined to e.g. food additives, health products and cosmetics (Stevant et al. 2017). However, other applications such as human food, animal feed products and fertilizers are believed likely to play an increasing role in the Norwegian bio-economy in the foreseeable future (Skjermo et al. 2014). This has led to an increasing number of studies on seaweed from Norway in the last few years, although knowledge in this field remains rather scant. A few studies have described the chemical profile of seaweed species common in Norwegian waters (Jensen et al. 1985; Rødde et al. 2004; Mæhre et al. 2014; Duinker et al. 2016). These studies found that seaweed species from Norway contain valuable nutrients (e.g. EEA, iodine, n-3 LC-PUFAs), which makes them suitable resources for food and feed purposes. More data is however needed on species-specific and geographical/seasonal variations on seaweed chemical composition (Skjermo et al. 2014).

3.5 Heavy metals. The term “heavy metal” is commonly applied to non-essential elements such as cadmium, lead and mercury (Duffus 2002). They have no biological functions in living organisms, and they are usually toxic. Heavy metals occur in nature due to both natural and anthropogenic releases, have long half-lives in the environment and can easily bioaccumulate in living tissues, leading to adverse effects on health. FM is the major source of heavy metals in fish feeds, but alternative feed ingredients (e.g. vegetable protein and oils, krill meal), may also contribute to the levels of metals found in aquafeeds (Amlund et al. 2012; Berntssen et al. 2010).

Heavy metals are major potential chemical hazards associated with seaweeds, as they can easily accumulate in the algal tissue from the surrounding water (van Netten et al. 2000; Almela et al. 2002; Roberts et al. 2008). Concentrations of heavy metals in seaweed, however, are highly dependent on species, season of harvest, and location (Almela et al. 2002; Villares et al. 2002; Chakraborty et al. 2014).

As underlined by the European Food Safety Authority (EFSA), heavy metals are also associated with farmed insects, in particular cadmium (EFSA 2015). However, studies on metal contamination in insects and insect derived products are few (Charlton et al. 2015; Diener et al. 2015; Purschke et al. 2017; Camenzuli et al. 2018). These studies have shown that metals are naturally present in insects but can also be transferred to insects from the rearing substrates (Vijver et al. 2003; Diener et al. 2015).

Cadmium. Cadmium is classified as carcinogen and an endocrine disruptor in humans (IARC 1993). It can cross biological membranes through metal transporters (e.g. calcium channels) and can efficiently bind to metal-binding proteins (e.g. metallothioneins), therefore it readily accumulates in cells (Ray 1984). Although the toxicity mechanisms are still poorly understood, it is believed that the generation of oxidative stress is the primary cause of cell damage due to cadmium exposure (Liu et al. 2009). Moreover, by mimicking other metals that are essential to biological functions, cadmium can cause adverse effects to several tissues and organs (EFSA 2009a). Human exposure to cadmium is mainly from food consumption (90 %), with cereals and vegetables being the major sources of this heavy metal (EFSA 2009a). Generally, meat and fish contain lower concentrations of cadmium than plant-based food products, although animal offal (e.g. kidney and liver) can contain high levels (UNEP 2008).

Cadmium occurs in nature in its inorganic form by e.g. weathering of rocks and volcanic activities as well as industrial manufacturing of various products (e.g. batteries) and zinc mining (Pinot et al. 2000). It is present in the marine environment with average concentrations ranging from less than 5 ng/L to 40 ng/L in unpolluted surface waters (Ray 1984). Higher levels of cadmium (80-250 ng/L) have been reported in Norwegian costal zones (EFSA 2009a). Marine organisms tend to accumulate cadmium from the surrounding water or through dietary ingestion of food and detrital particles. Concentrations of cadmium in marine samples are usually lower than in terrestrial samples, however one exception are crustaceans, in particular the brown crab (*Cancer pagurus*), where cadmium levels can be very high (Järup et al. 2009; Wiech 2018).

FM is the major source of cadmium in aquafeeds, while plant-based ingredients are usually lower in cadmium than marine ingredients (**Table 1**) (Amlund et al. 2012). Commercial feeds for Atlantic salmon in Norway have been reported to contain relatively low concentrations of cadmium in the last few years (< 0.5 mg/kg sample) (**Table 1**). Research has shown that transfer of cadmium from feed to fish is generally low (2-6 %). In Atlantic salmon, this metal mainly accumulates in the intestine, liver and kidney, while very little cadmium is stored in the muscle tissue of the fish (Amlund et al. 2012).

*Lead***.** Lead is classified as carcinogen by the International Agency of Cancer (Anttila et al. 2006). A common toxic effect of lead is oxidative stress which can therefore cause DNA, proteins and cell membranes damage (Gidlow 2015). In living organisms, lead poisoning affects many systems in the body: the cardiovascular, renal, endocrine, gastrointestinal, immune and reproductive systems, among others (Anttila et al. 2006; ATSDR 2019). Human exposure to lead can occur through consumption of food, drinking water, inhalation of dust and air (EFSA 2010). In the marine environment, lead is present with average concentrations ranging from 0.002 to 0.05 µg/L in unpolluted seawater (ILA 2019). Aquatic organisms take up lead from both the surrounding water and the diet, but the effects of dietary exposure to lead are relatively minor (ILA 2019).

In fish feeds, lead is mainly associated with feed additives, rather than feed ingredients (Adamse et al. 2017; Sele et al. 2018). Mean concentrations of lead in mineral premixes for Atlantic salmon feed were 0.82, 0.73 and 2.5 mg/kg in 2014, 2015 and 2017, respectively (Sanden et al. 2015 and 2016; Sele et al. 2018). In contrast, low levels of lead (<0.1 mg/kg) have been found in FM and plant-based ingredients in the last years (**Table 1**). Since mineral premixes are usually added in small volumes to the diets, low levels of this metal (<0.1 mg/kg) have been found in commercial feeds for Atlantic salmon (**Table 1**). Lead has little capacity to accumulate in fish muscle and this metal in Atlantic salmon fillets is found in low concentrations (<0.03 mg/kg wet weight, WW) in the muscle meat of fish (Berntssen et al. 2010; Hannisdal et al. 2016; Nøstbakken et al. 2015).

*Mercury***.** Mercury is a widespread pollutant which is released in the environment from both natural and anthropogenic sources. The toxicity of mercury is highly variable, depending on the chemical form of mercury, the route of exposure and metal concentrations, among other factors (Bernhoft 2012). Common adverse effects of mercury poisoning in humans are genesis of oxidative stress, protein damage and neurodegenerative diseases (Branco et al. 2017). In the aquatic environment, mercury exists in three main chemical forms: elemental mercury, inorganic mercury and organic mercury (mainly methylmercury, MeHg) (EFSA 2012). Among these species, MeHg rises the highest concerns, due to its potent toxicity for living organisms, as it affects growth, development and reproduction of both humans and animals (EFSA 2012). MeHg easily accumulates in aquatic food chains at low trophic levels and it is also biomagnified up through the food web (Wiener et al. 2003). Fish consumption is by far the biggest source of exposure to this heavy metal, making population with high intakes of fish and seafood vulnerable to its poisoning (Rice et al. 2014).

In fish feeds, the main source of mercury is FM, as this metal is strongly bound to protein fractions (Sissener et al. 2010). The average concentration of total mercury in FMs between 2003 and 2008 was 0.10 mg/kg, with relatively higher levels of mercury in FMs from North Atlantic catches (0.14 mg/kg), compared to FMs from the South America (0.06 mg/kg) (Sissener et al. 2010). Sanden and colleagues reported a similar average concentration of mercury (0.14 mg/kg) in FM samples in 2014 (Sanden et al. 2015). Average mercury concentrations in feeds for Atlantic salmon have been reported to be generally low (< 0.05 mg/kg) in the last years (**Table 1**). In feeds for Atlantic salmon, mercury levels were found to correlate significantly with the percentage inclusion of FM in feeds (Sissener et al. 2010). Due to a lower use of FM in commercial feeds in the last years, a clear decline of total mercury concentrations in Norwegian farmed Atlantic salmon has been observed (Nøstbakken et al. 2015). In the last years, also low levels of mercury (< 0.05 mg/kg WW) in muscle meat of farmed Atlantic salmon has been reported (Nøstbakken et al. 2015; Hannisdal et al. 2016 and 2017).

	Fish meal		**Plant protein**		**Complete feed**	
	2008	**2014**	**2010-2013**	**2014-2017**	**2010-2013**	**2014-2017**
Cd	0.7	0.52	0.03	0.04	0.3	0.2
Pb	0.12	0.15	0.04	0.03	0.07	0.05
Hg	0.12	0.14	<0.004	<0.004	0.03	0.03
As	6.1	7.3	0.03	0.03	2.6	2.6

Table **1. Mean concentrations (mg/kg sample) of heavy metals and arsenic in fish meal, plant protein and complete Atlantic salmon feed in 2008-2017. Data obtained from the Norwegian surveillance program, available online (www.hi.no).**

3.6 Arsenic. Arsenic is classified as metalloid and it is released into the environment by both anthropogenic and natural sources. Natural releases of arsenic include weathering of rocks and volcanic activities, while mining, fossil fuel combustion and the use of arsenic-containing pesticides are the major anthropogenic sources (Mandal et al. 2002). Arsenic is present in both terrestrial and marine environments, however, its concentrations in marine samples are higher than in terrestrial samples (Francesconi and Kuehnelt 2002).

In seawater, concentrations of total arsenic are generally low and uniform, between 0.5 and 2 µg/L (Andreae et al. 1979; Cullen et al. 1989). Marine organisms accumulate arsenic from the seawater or through the diet (Edmonds et al. 1998; Rahman et al. 2012). Fish, crustaceans and algae have between 1 and 100 mg/kg arsenic WW, although high variations of arsenic levels are found between species and within the same species collected in different locations (Cullen et al. 1989; Francesconi and Kuehnelt 2002). Terrestrial samples usually contain less than 0.02 mg/kg arsenic (WW) (Kuehnelt et al. 2003). In the marine environment, several chemical forms or arsenic species exist. In seawater, arsenic occurs mainly as inorganic arsenic, i.e. sum of arsenite and arsenate (Sloth et al. 2005; Julshamn et al. 2012). Despite being the major form in seawater, inorganic arsenic only contributes to less than 1 % of total arsenic concentrations in marine organisms (Sloth et al. 2005; Julshman et al. 2012). From the seawater, inorganic arsenic is mainly taken up by phytoplankton and marine bacteria, and further converted into organic arsenic species by methylation processes (Sanders 1980).

Arsenic is commonly known to be toxic to humans, as it promotes cancer in several tissues and organs such as skin, liver, lungs, among others (Tapio and Grosche 2006). However, toxicity of arsenic highly depends on the chemical form of this element as well as its route of exposure (Shiomi 1994). In general, inorganic arsenic is considered to be the most toxic form, while organic forms of arsenic are of a lower toxicity (EFSA 2009b; Lorenzana et al. 2009). The toxicity of inorganic arsenic is believed to be due to chemical similarities between arsenic and phosphorus, also belonging to the group 15 in the periodic table. Phosphate is therefore substituted by arsenate in compounds such as the ATP, leading to inactivation of enzymes involved in cellular energy pathways and DNA replication and repair (Ratnaike et al. 2003). Humans are mainly exposed to organic forms of arsenic, mostly arsenobetaine (AB), through consumption of seafood (EFSA 2009b). AB is considered non-mutagenic, non-cytotoxic and non-immunotoxic for mammalian cells (Borak and Hosgood 2007), and it is rapidly excreted in the urine of seafood consumers (EFSA 2009b).

Arsenic is an element of concern for feed and food safety. Being naturally present in pelagic fish, it is transferred to marine feed ingredients, i.e. FM and FO, which are the major sources of arsenic in aquafeeds (Berntssen et al. 2010). Based on the results obtained from the National surveillance programs in Norway in the last years, arsenic in FM was found to be 6-8 mg/kg on average (Sloth et al. 2005; Sissener et al. 2013; Sele et al. 2018) (**Table 1**). FOs were also found to contain relatively high levels of total arsenic, ranging from 4.6 to 15 mg/kg (Sloth et al. 2005; Espe et al. 2010; Sissener et al. 2013; Sele et al. 2018). However, the replacement of FM with plant-based ingredients in the last years caused a reduction in total arsenic levels in feed (Berntssen et al. 2010). Plant-based ingredients are indeed known to contain relatively low concentrations of arsenic, in the range of 0.02-0.08 mg/kg (Berntssen et al. 2010; Espe et al. 2010; Sissener et al. 2013; Sanden et al. 2015) (**Table 1**).

4. Research questions

Are seaweed species from Norwegian waters nutritious and safe?

Hypothesis:

- Norwegian seaweeds contain valuable nutrients and low levels of undesirable substances (**Paper I**, **Paper II**)

Is seaweed from Norwegian waters a suitable and safe rearing substrate for insect larvae?

Hypotheses:

- Feeding seaweed to insect larvae promotes growth of the larvae and enriches the nutritional profile of the larvae with marine nutrients (**Paper III**)
- Undesirable substances are transferred from seaweed to insect larvae (**Paper IV**)

Are insect feed ingredients from larvae fed seaweed-enriched media suitable and safe for farmed Atlantic salmon?

Hypotheses:

- Feeding Atlantic salmon a diet containing insect feed ingredients from larvae fed seaweed-enriched media contributes to the production of healthy fish (**Paper V**, **Paper VI**)
- Insect-based diets contain low levels of undesirable substances, ensuring a safe end-product for the consumer (**Paper VII**)

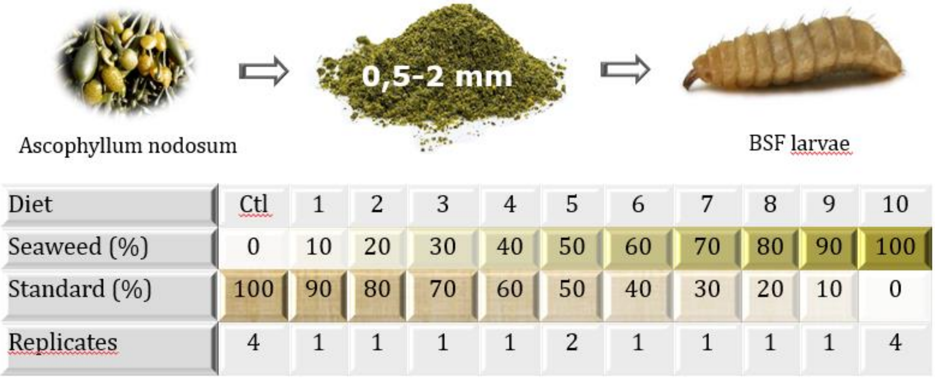
5. Methodological approach

This project was designed to develop insect feed ingredients for Atlantic salmon juveniles and post-smolt, aiming to contribute with essential nutrients to produce robust and healthy fish. To achieve this, tailoring of the insect nutritional composition towards the fish nutrient requirements was investigated through the use of seaweed as rearing substrates for the insects.

Samples of over 20 seaweed species were collected from Norwegian waters: each sample consisted of pooled material of at least five individuals per species, identified at first by morphology. Sub-samples of each species were sent to collaborators (University of Ireland, Galway) to verify the initial morphological identifications using genetic tools (**Paper I**). The seaweed samples were therefore analyzed for their content of macro- and micronutrients, as well as undesirable substances (i.e. heavy metals and arsenic) (**Paper II** and **Paper II**). The protein contents of the seaweeds were estimated using two methods: crude protein (nitrogen x 6.25) and true protein (sum of proteomic amino acids); and species-specific nitrogen-to-protein conversion factors were calculated for each species (**Paper I**). From the data acquired, the seaweed species with the most promising nutritional profile and the lowest heavy metal concentrations was chosen for insect rearing.

The insect feeding trial was run at the facility of Protix Biosystem (The Netherlands). The aim of this trial was to investigate the suitability of the seaweed biomass in rearing BSF larvae. The control diet for the larvae consisted of a plant-based medium (processed wheat) used as reference material for larval growth at Protix. The trial followed a regression design where the control medium was gradually replaced by seaweed biomass (*A. nodosum,* rockweed), in steps of 10 %, leading to 11 diets and 18 experimental groups. This design (**Figure 6**), built on different replicates per seaweed inclusion level, was chosen over the ideal regression design of six inclusion levels in triplicates, as the uncertainty associated with the proposed design was lower.

The seaweed biomass was prepared by grinding fresh whole thalli of *A. nodosum* in a blender. This step was quite challenging, as the seaweed biomass became sticky once ground. The particle size of such seaweed biomass was therefore not homogeneous, and its size was approximately 500-2000 µm. BSF larvae were fed the experimental diets for eight days. During the feeding trial, we measured larval weight daily, to investigate growth of the larvae. At the end of the trial, we took samples of insect larvae, feeding media, and residue, to investigate the transfer of nutrients from seaweed to larvae and nutrient retention/utilization (**Paper III**). I focused on the uptake of heavy metals and arsenic from seaweed to larvae (**Paper IV**). At sampling, larvae grown on high inclusion of seaweed in the feeding media (> 70 %) were smaller and more difficult to separate from the media than the ones grown on lower inclusion of seaweed. This may have affected some of the results, as some residues of the media might have been analyzed together with the insects.



Diet	Ctl	1	2	3	4	5	6	7	8	9	10
Seaweed (%)	0	10	20	30	40	50	60	70	80	90	100
Standard (%)	100	90	80	70	60	50	40	30	20	10	0
Replicates	4	1	1	1	1	2	1	1	1	1	4

Figure 6. Schematic illustration of the regression design for BSF larvae feeding trial.

Large batches of BSF larvae fed seaweed-enriched media (50 % seaweed) were grown at Protix to produce insect feed ingredients (IM and IL) for the fish trials. The seaweed biomass (*A. nodosum*) used for insect production was this time purchased from a company (Ocean Harvest, Ireland) as dry seaweed powder. IM and IL from BSF larvae fed seaweed were produced by Protix. Later, two fish feeding trials were conducted with i) fresh water-phase Atlantic salmon (juveniles) and ii) seawater-phase Atlantic salmon (post-smolt).

In the freshwater trial, Atlantic salmon juveniles were fed diets containing insect feed ingredients for 8 weeks. The control diet contained protein from FM and soy protein concentrate (SPC) (50:50), and lipids from FO and vegetable oil (VO) (33:66). Five experimental diets were formulated, where 85 % of the dietary protein was replaced by IM and/or all the VO was replaced by IL from BSF larvae grown on i) seaweed-enriched media (IL1) or ii) plant-based media without the seaweed enrichment (IL2). This trial followed a factorial 2 x 3 way-ANOVA experimental design with lipids (VO, IL1 and IL2) and protein (FM+SPC or IM) as varying factors. With this design, we could investigate the effect of dietary protein sources and/or dietary lipid sources on our target parameters, and the interaction between main effects of the two varying factors. Fish were grown from 50 g to 150 g. At the end of the trial, samples of feed, feces and fish were taken for analysis of macro- and micronutrients. Growth performance, body composition, feed utilization and nutrient digestibility were investigated. Levels of heavy metals and arsenic in feed and fish were also investigated (**Paper V**).

In the second fish trial, seawater-phase Atlantic salmon (post-smolt) were fed diets where FM was replaced by 33 %, 66 % and 100 % IM from BSF larvae fed seaweed-enriched media, until market-size (~4 kg). This trial was conducted to test growth potential, nutrient utilization, fish health parameters and also fish fillet sensory parameters. At the end of the trial, samples of feed, feces and fish were taken for analysis of macro- and micronutrients. Growth performance, body composition, feed utilization and nutrient digestibility were investigated (**Paper VI**). This trial followed a regression design, to test the effect of increasing dietary replacement of FM with IM on growth potential, nutrient utilization, fish health parameters and also fish fillet sensory parameters. Within this trial, my major focus was the transfer of heavy metals and arsenic from feed to fish and speciation analysis of arsenic in IM, feed and fish (**Paper VII**).

6. Summary of the results

Paper I - "Amino acid composition, protein content, and nitrogen-to-protein conversion factors of 21 seaweed species from Norwegian waters"

and

Paper II – "Chemical characterization of 21 species of marine macroalgae common in Norwegian waters: benefits of and limitations to their potential use in food and feed"

Findings:

- Twenty-one seaweed species were identified: seven red algae, three green algae and 11 brown algae species
- Brown algae species had lower protein contents than green and red algae species, but the highest lipid contents
- Crude protein analysis resulted in an overestimation (18-44 %) of the protein content compared to the true protein analysis
- Species-specific N-Prot conversion factors ranged from 3.5 to 5.1
- The species were high in iron, calcium and iodine
- The heavy metals cadmium, lead and mercury were found in all species at concentrations below the EU MLs
- Arsenic concentrations were in gradation in relation to the taxonomic group (brown algae > red algae > green algae)
- Inorganic arsenic comprised < 7 % of total arsenic in the species studied, excepted for one brown algae species (*H. siliquosa*, sea oak)
- Most of the seaweed species contained arsenic at concentrations below the EU ML

Conclusions:

- The results confirm that seaweeds from Norwegian waters contain nutrients which are relevant for both human and animal nutrition, the concentrations thereof are highly dependent on species
- Some of the brown algae in the present study (e.g. *L. digitata, S. latissima and A. esculenta*) contained high levels of arsenic and iodine, which could hamper their utilization for food and feed purposes

Paper III - "Modulation of nutrient composition of black soldier fly (*Hermetia illucens*) larvae by feeding seaweed-enriched media"

and

Paper IV – "Uptake of heavy metals and arsenic in black soldier fly (*Hermetia illucens*) larvae grown on seaweed-enriched media"

Findings:

- Of the larvae added to each crate, the majority (> 95 %) survived through the whole trial when grown on media with ≤ 80% seaweed inclusion
- The larvae grown on seaweed-enriched media were smaller than the control larvae, decreasing in size with increasing seaweed inclusion
- Total protein of dry larvae did not vary between groups (~30 % of dry matter)
- The amino acid profile slightly changed between groups, with glutamic acid increasing in the larvae when more seaweed was added to the media
- Total lipid content of the larvae was reduced in the larvae fed increasing amounts of seaweed in the media
- The larvae lipid profile changed into a higher percentage of mono- and polyunsaturated FAs (% of total lipids) and lower levels of saturated lipids (% of total lipids) with increasing amounts of dietary seaweed
- The larvae accumulated marine nutrients from the feeding media, in particular EPA, vitamin E and iodine

- The EPA levels in the larvae significantly ($p<0.05$) increased with increasing seaweed inclusion in the media and reached up to 1 % of total lipids in the larvae fed 100 % seaweed
- The concentrations of heavy metals and arsenic in the larvae increased when more seaweed was added to the media
- Arsenic and cadmium concentrations in the larvae exceeded the EU MLs for these elements in feed ingredients, when larvae where fed ≥ 20 % and 70 % seaweed in the media, respectively
- Mercury and lead levels in the larvae were always below MLs for these elements in feed and feed ingredients

Conclusions:

- *A. nodosum* is a suitable rearing substrate for BSF larvae up to 50 % inclusion level in the feeding media
- Including seaweed in the feeding media for the larvae improve their nutrient profile by enriching the larvae with marine nutrients
- Larvae accumulate heavy metals and arsenic from the seaweed in the media
- Feeding high percentages of seaweed in the insect media is not recommended as the concentrations of arsenic and cadmium in the larvae could exceed MLs set for these elements in feed ingredients
- The high concentrations of arsenic and cadmium in the larvae should be taken into account if production of BSF is upscaled for food and feed purposes

Paper V - "Potential of insect-based diets for Atlantic salmon (*Salmo salar*)"

Findings:

- Feed intake and feed conversion ratio (FCR) were similar between the feeding groups
- The fish grew up to three-fold of the initial body weight in all groups
- Replacing FM and SPC with IM in the diet significantly reduced the digestibility of protein, lipids and amino acids, though all diets remained highly digestible

- Fish fed diets with IL1 had slightly lower specific growth rate (SGR) and daily growth index (DGI) compared with fish fed VO and IL2
- There were no significant effects on growth due to changing protein sources in the diet
- The use of IM-based diets significantly increased both HSI and VSI of the fish
- Whole-body protein, lipid, amino acid and mineral contents were comparable between groups
- Concentrations of heavy metals and arsenic in the diets did not exceed any of the EU MLs for these elements set for fish feed
- Diets containing insect feed ingredients showed lower concentrations of arsenic and mercury compared to the control, which was reflected in the fish (lower levels of arsenic and mercury in the fish fed insect feed ingredients than control fish)
- Concentrations of cadmium were higher in the insect-based diets than the control diet, but this was not reflected in the fish

Conclusions:

- Insect feed ingredients from BSF larvae are overall good sources of high-quality nutrients for Atlantic salmon juveniles
- The inclusion of insect feed ingredients in the diets did not pose any challenges in terms of feed safety

Paper VI – "Black soldier fly larvae meal can replace fish meal in diets of sea-water phase Atlantic salmon (*Salmo salar*)"

and

Paper VII – "Replacing fish meal with insect meal in the diet of Atlantic salmon (*Salmo salar*) does not impact the amount of contaminants in the feed and it lowers accumulation of arsenic in the fillet"

Findings:

- Feed intake, DGI and FCR were not affected by dietary substitution of FM with IM
- Replacing FM with IM in the diet did not affect the digestibility of macronutrients (i.e. protein, lipids and amino acids)
- The FA composition of the whole fish reflected the FA composition of the diets
- Sensory testing of the fillet revealed minor differences between dietary groups
- Similar concentrations of heavy metals and arsenic were found in the diets, at levels below current EU MLs for these substances in complete feed
- There was little/no transfer of heavy metals from feed to fish
- The level of total arsenic in the IM was above the EU ML set for feed ingredients
- Arsenic was transferred from feed to fish fillet, however arsenic levels in the fillet decreased with higher inclusion of IM in the diets
- In the diets, higher inclusion of IM led to lower levels of AB and increased levels of inorganic arsenic, dimethylarsinate (DMA) and unidentified species
- In the fillet samples, AB was the major arsenic species detected in all groups

Conclusions:

- Total replacement of FM with IM in the diet of seawater-phase Atlantic salmon is possible with no adverse effects on growth performance, feed utilization, nutrient digestibility, health parameters and nutritional quality of the fish fillet
- IM from BSF larvae fed seaweed-enriched media is overall a safe source of protein for seawater-phase Atlantic salmon, with low concentrations of undesirable substances in the feed
- Feeding seawater-phase Atlantic salmon a diet containing IM ensure a safe product for the consumer, with respect to the concentrations of undesirable substances in the fillet

7. Results and Discussion

7.1 Seaweed screening. The data from the seaweed screening showed a great variation in nutritional composition between the three taxonomic groups (red, green and brown algae) but also between different species of the same group (**Paper I** and **II**). It is well established in literature that the nutritional profile of seaweeds highly varies between species and taxonomic groups, but also seasonal and geographical variations contribute to this variation (Aitken et al. 1991; Fleurence et al. 1999; Rødde et al. 2004; Khairy and Elshafay 2013). Despite this, our findings are mostly in accordance with previous reports on the same species from Norwegian waters (Mahre et al. 2014; Angell et al. 2016) and other species worldwide (Ramos et al. 2000; Wong and Cheung 2000; Lourenço et al. 2002; Diniz et al. 2011).

Seaweeds are often described as good protein sources (Holdt and Karaan 2011). Protein is a key factor when assessing the nutritional value of food/feed products, as it is an essential macronutrient for normal growth in animals and humans. Whether seaweeds are used as feed or food, accurate knowledge of protein quantity is important. In this PhD work, protein contents of the seaweed samples were estimated via both crude (nitrogen x 6.25) and true protein methods (sum of proteomic amino acids) (**Paper I**). Crude protein values were found to be 18-44 % higher than true protein values, in agreement with previous studies assessing protein content in seaweed using both methods (Aitken et al. 1991; Lourenço et al. 2002; Diniz et al. 2011; Shuuluka et al. 2013). In materials with high NPN such as seaweed, specific N-Prot conversion factors (other than 6.25) are recommended for a more accurate estimation of protein when total nitrogen is a proxy for quantification, as in the crude protein method (Sosulski and Imafidon 1990; Salo-väänänen and Koivistoinen 1996; Sriperm et al. 2011; Diniz et al. 2011; Shuuluka et al. 2013).

In this PhD work, I established species-specific N-Prot conversion factors for the seaweed species collected, which ranged from 3.53 to 5.13 (**Paper I**). These findings are in accordance with existing literature on N-Prot conversion factors for seaweeds from tropical and temperate regions (Aitken et al. 1991; Lourenço et al. 2002; Diniz et al. 2011; Shuuluka et al. 2013). To the best of my knowledge, no data on N-Prot conversion factors for seaweeds from Norway are available beyond this work. The N-Prot conversion factors calculated in **Paper I** could therefore be applied retrospectively to previous protein data from the same species collected in Norwegian waters. Environmental factors can influence the nitrogen content of seaweeds, resulting in high individual variations of N-Prot conversion factors between different samples of the same species (Aitken et al. 1991; Angell et al. 2016). I therefore suggest that sample-specific N-Prot conversion factors are calculated when possible, for a more accurate protein estimation in seaweeds. When calculation of specific N-Prot factors is not available, Angell et al. (2016) suggested a universal seaweed N-Prot factor of 5, to be used for protein estimation. Regardless of the method used for protein quantification, the highest protein concentrations belonged to red alga species (8-21 % of the algal DW) while brown algae species had the lowest (3-10 % of the algal DW). Green algae species contained moderate concentrations of protein. This pattern fits well with the literature on seaweed species worldwide (Angell et al. 2016).

The protein quality of a food/feed source is (in part) defined by its relative concentrations of amino acids. Of the 20 amino acids, ten are known to be essential for both animal and human nutrition: arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine (NRC 2011). Seaweeds have higher concentrations of EAAs than protein from most terrestrial plants (Holdt and Kraan 2011). Relevant for our purposes, the seaweed species in our study contained all the EAAs, in suffieint amounts to cover the dietary EAA requirements of Atlantic salmon (NRC 2011) (**Paper I**).

In carnivorous fish like salmonids, EPA and DHA are required in the diet to maintain optimal growth, health and welfare. This requirement has been estimated in Atlantic salmon at 0.5 and 1 % of the diet for EPA+DHA in freshwater and seawater growth, respectively (NRC 2011; Rosenlund et al. 2016). EPA and DHA are commonly known as beneficial FAs for both human and animal nutrition. These nutrients play essential roles in cell membranes and signaling (eicosanoids), brain and eye development, the immune system and the inflammatory response (Hodge et al. 2005; Siriwardhana et al.

2012; Swanson et al. 2012). In our study, DHA was not present in any of the species collected, while EPA comprised more than a third of total FAs in red algae species, in line with previous findings (Van Ginneken et al. 2011; Mahre et al. 2014) (**Paper II**). However, the absolute concentrations of this nutrient in red algae were low (up to 2.7 mg/g DW), due to the low total lipid contents in these species (up to 3 % DW). As such, red algae cannot be considered good dietary sources of EPA as stand-alone dietary products for animals or humans, while they may be used as feed or food supplements. On the other hand, brown algae species had lower relative percentages of EPA of total FAs, but higher absolute concentrations of EPA than red algae (per algal DW), due to higher total lipid concentrations (up to 10 % DW) (**Figure 7**).

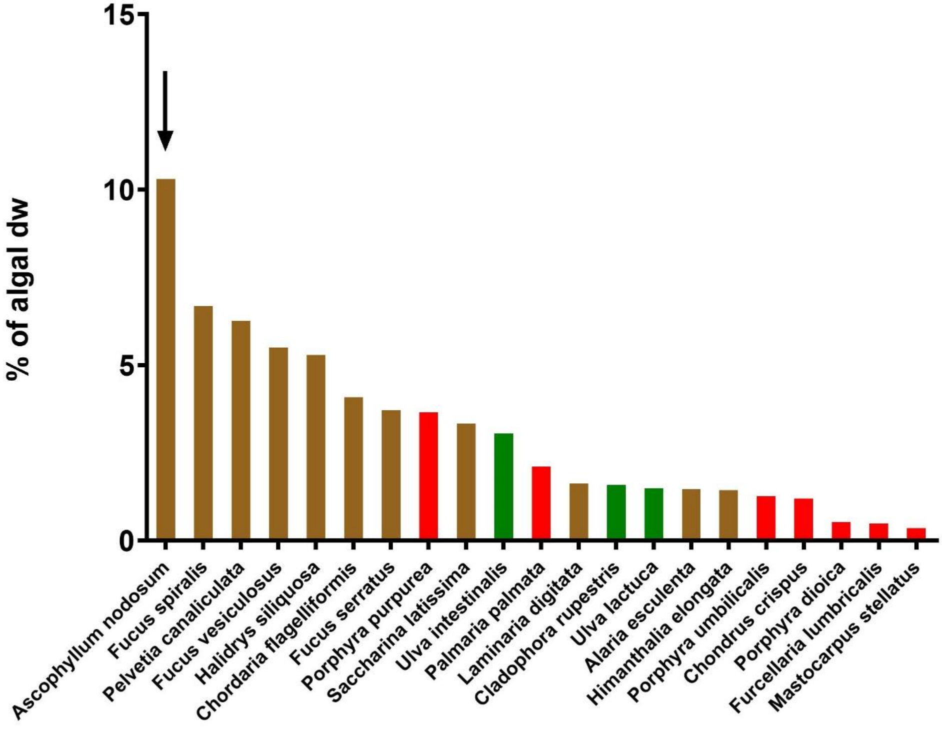


Figure 7. Lipid concentrations of brown, red and green algae species (percentage of algal DW). The arrow indicates the seaweed species chosen as substrate for insect rearing.

Screening the nutritional profile of several seaweed species allowed us to identify the brown algae *A. nodosum* as the most suitable species for insect rearing. This species had among the lowest protein content (3 % of algal DW) compared to that of some red algae species such as *Porphyra* species (**Paper I**). However, red algae species are generally small in size and lack the potential for providing large biomasses through harvesting and cultivation in the sea. On the contrary, natural and easily available stocks of brown algae are enormous in Norway. The biomass of *A. nodosum* is estimated around 1.8 mill tons along the intertidal zone of the Norwegian coast and until recently, only 2 % of this biomass is harvested every year (Skjermo et al. 2014; Steen 2016). As such, this species represents a great and under-exploited biomass resource.

A. nodosum had the highest lipid (10.3 % of algal DW) and PUFAs amounts (~7 mg/g of algal DW), among all the species analyzed (**Figure 7**). Also, it was rich in essential micronutrients such as iodine and calcium, and finally, it showed the lowest heavy metals and arsenic concentrations among the brown algae species (**Paper II**). By choosing *A. nodoum* as substrate for insect larvae rearing, we aimed to i) exploit an under-used feed resource for insect production, ii) enrich the larvae of valuable "marine" nutrients contained in the seaweed, iii) tailor the IM from these larvae towards the nutrient requirement of Atlantic salmon.

Figure 8. *A. nodosum* selected for insect production (Photo by: Kjersti Sjøtun).

7.2 Insect production. All the tested substrates allowed for BSF larval growth and development, although differences in growth were observed between feeding groups (**Paper III**). The media did not have a large effect on the survival of the larvae until seaweed were included at 80 % or more. At the end of the trial, ≥ 95 % of the larvae survived in most feeding groups. This is in accordance with similar studies on BSF larval growth on carbohydrate-rich substrates (Oonincx et al. 2015; Chia et al. 2018; Meneguz et al. 2018; Bava et al. 2019). However, in larvae fed over 50 % seaweed in the media, we found a large decline in most growth parameters (e.g. mean size of the larvae and total larvae yield). I suggest that the protein content of the feeding media determined the growth of the larvae, therefore media containing high seaweed inclusions had too little protein to sustain good growth of the larvae.

Previous studies have also shown that protein is a key nutrient for larval development in insects. Cammack and Tomberlin investigated how varying the protein:carbohydrate ratio in the feeding medium impacts the life-history growth of BSF larvae (Cammack and Tomberlin 2017). Larvae fed low protein-high carbohydrate (7-35 % of the diet) media developed slower than those feeding on high protein-low carbohydrate (35-7 % of the diet) media. In the same study, larvae reared on protein-carbohydrate balanced diets (21-21 % of the diet) had the fastest growth and the greatest survival rate. Oonincx et al. (2015) tested diets with 1) high protein-high fat (22-10 % of the diet); 2) high protein-low fat (23-1 % of the diet); 3) low protein-high fat (13-15 % of the diet); and 4) low protein-low fat (15-2 % of the diet) for BSF larval rearing. Larvae developed fastest on the high protein-high fat diet while slowest on low protein diets, independent on the fat content.

Within the *AquaFly* project, I also conducted a pilot study to explore the potential of the sugar kelp (*S. latissima*) as feed additive in the diet of BSF larvae (Biancarosa et al., unpublished results). Specifically, the aim was to assess whether the seaweed biomass could be used as replacement for the carbohydrate and/or protein component of the larval diet, and how this would have affected growth and nutritional composition of the larvae. To achieve this aim, different percentages of powdered sugar kelp (from 1 to 13 %) were mixed with conventional feeding materials used for BSF larvae growth at the insect producer (Protix), while the control diet had no seaweed. Given a fixed level of seaweed, the diets with more protein than carbohydrates resulted in better growth of the larvae. The best growth was observed in larvae fed media enriched with 11 % of seaweed, 50 % protein and 31 % carbohydrates in the diet. This pilot study confirmed the hypothesis that protein is a key factor for insect larval growth.

In the insect trial, the reduction of most growth parameters in the larvae fed high dietary seaweed in the media (more than 50 %), compared to control larvae, could also be linked to a significant decrease of total feed intake in these larvae (**Paper III**). I think that the particle size of the seaweed biomass (500-2000 µm) was difficult to process for BSF larvae, leading to a prolongation of the developmental time in larvae fed high seaweed in the media, compared to the control. I propose that a further reduction of the seaweed particle size could increase feed intake, therefore improve growth of the larvae.

The larvae fed the control media consisted of 37 % dry matter, of which 29 % was protein, 33 % lipid and 5 % ash (**Paper III**). These values are within the range of those reported in literature for BSF larvae reared on organic plant-based media (Diener et al. 2009; Oonincx et al. 2015; Spranghers et al. 2017; Meneguz et al. 2018). In our study, the proximate composition of the larvae was highly affected by the composition of the feeding media. This is in accordance with several studies investigating the role of different rearing substrates on the nutrient composition of BSF larvae (Diener et al. 2009; Nash and Chapman 2014; Oonincx et al. 2015; Tschirner and Simon 2015; Cammack and Tomberlin 2017; Spranghers et al. 2017; Meneguz et al. 2018; Bava et al. 2019). The true protein contents of the larvae were similar between feeding groups, ranging from 25 to 29 % of DW (**Paper III**). Previous studies have reported higher protein concentrations in BSF larvae (37-56 % DW) (Bava et al. 2019; Čičková et al. 2015; Diener et al. 2009; Oonincx et al. 2015; Finke 2013; Tschirner and Simon 2015; Spranghers et al. 2017). These values were obtained by using the crude protein method with the standard N-Prot conversion factor of 6.25. As such, I suggest that these authors have likely overestimated the protein content of the BSF larvae. Insects contain high concentrations of NPN in compounds like chitin, nucleic acids, phospholipids and ammonia (Janssen et al. 2017), therefore using 6.25 as N-Prot factor is not appropriate.

In **Paper III**, quite high percentages of NPN of total nitrogen (19-30 %) were found in BSF larvae. It has been suggested that a N-Prot conversion factor of 4.67 would be more accurate than 6.25 and should be used when choosing the crude protein method in insects (Janssen et al. 2017). However, I suggest that the estimation of true protein through the amino acid analysis is always to be preferred.

The amino acid profile of BSF larvae (**Paper III)** showed good correlation with literature data (Henry et al. 2015). Feeding larvae with seaweed in the media did not affect the composition of the amino acids in the larvae, as only small differences between groups were observed. This is consistent with previous studies reporting small-to-no difference in amino acid contents of BSF larvae due to changes in their feeding media (Tschirner and Simon 2015; Spranghers et al. 2017). Some amino acids in BSF meal have often been described as limiting (e.g. cysteine, methionine and threonine) (Henry et al. 2015). In the current study, the larvae contained low levels of methionine (up to 1.8 % of crude protein) compared to e.g. FM (3.1 % of crude protein) (NRC 2011) (**Paper III**), confirming previous findings. On the contrary, levels of threonine in the larvae was not limiting in our study. Cysteine is destroyed during the acid hydrolysis in the method and, therefore it was not estimated. Overall, the EAA profile and levels of the BSF larvae found (**Paper III**) were relevant for Atlantic salmon feed, in the light of the EAA requirements of this fish species (NRC 2011).

While no big changes were observed in protein and amino acid composition of the larvae, the larval quality and quantity of the lipids were affected by the feeding media (**Paper III**), confirming previous studies (St.Hilaire et al. 2007a; Spranghers et al. 2017; Meneguz et al. 2018). The largest lipid fraction in the larvae (up to 68 % of total lipids) was saturated lipids, of which half was the medium-chained saturated fatty acid 12:0 (lauric acid), in line with previous findings (Oonincx et al. 2015; Ushakova et al. 2016; Spranghers et al. 2017). In animal and humans, lauric acid has antibacterial and antiviral properties (Liu 2015). Moreover, lauric acid is a fast substrate for energy and insect-derived lauric acid has been shown to promote less lipid storage in the liver of fish fed insect feed ingredients (Belghit et al. 2019).

The fatty acid productive values (FAPVs) reflects the retention efficiency of a specific fatty acid and/or groups of FAs. Values below 1 reflect a net loss of a FA during the trial, while values above 1 indicate a net production of a FA. Based on FAPVs, there was an overall net production of FAs in larvae fed up to 50 % seaweed in the media (**Paper III**). However, larvae fed higher proportions of seaweed biomass in the media had significantly lower FAs levels than the control group. This reduction was dramatic in larvae fed the sole seaweed medium which had five times fewer total FAs than the control group. I hypothesize that the carbohydrates present in the seaweed biomass were not accessible for the larvae, therefore not sufficient as source of energy for larvae fed high amounts of seaweed in the media. As such, lipids were likely used as substrates for energy in these larvae. Seaweeds, especially brown algae species, can contain up to 40 % of complex carbohydrates on a DW basis such as alginate, fucose-containing sulfated polysaccharides and laminarin (Hashim and Chu 2004; Jiao et al. 2011; Evans and Critchley 2014). These compounds are poorly digestible for some animals, e.g. ruminants, pigs, poultry and fish (Nakagawa et al. 1987; Soler-Vila et al. 2009; Wassef et al. 2013; Makkar et al. 2015; Norambuena et al. 2015) and also possibly for BSF larvae. As the feed intake of the larvae fed high amounts of seaweed in the media was low, I also suggest that these larvae experienced a period of hunger which enhanced the oxidation of lipids for energy (**Paper III**).

Although larvae fed higher amounts of seaweed in the media produced and contained less lipids, their lipids had a higher percentage of both n-6 and n-3 LC-PUFAs such as arachidonic acid (ARA) and EPA. The concentrations of EPA in the larvae reached up to 1 % of total FAs (**Paper III**). EPA is naturally not present in terrestrial insect like BSF (Henry et al. 2015), and it was introduced in the larvae from the seaweed in the media. Previous studies have reported that BSF larvae fed media enriched with marine substrates (fish offal), can retain EPA up to 2.3 % of total FAs (St-Hilaire et al. 2007a). Interestingly, the retention of this fatty acid in the larvae was highest in the larvae fed 50 % seaweed in the media (18 % of total added EPA was retained in these larvae).

Accumulation of marine LC-PUFAs in insect larvae fed marine substrates has been reported also for the superworm (*Zophobas morio*) and the yellow mealworm

(Biancarosa et al. 2017). In a pilot study, larvae of these species were first reared on a standard plant-based medium and transferred, one day before harvesting, to media containing biomass from FM, fish offal, seaweed or tunicates. Larvae fed such media had a higher percentage of LC-PUFAs (e.g. ARA, EPA and DHA) compared to larvae fed the control medium. This confirms the plasticity of the lipid make-up of insect larvae, which could help to tailor the insect feed ingredients into nutrient profiles for specific feed and food purposes.

Feeding seaweed to the larvae introduced iodine into the larvae. Concentrations of iodine in the larvae fed the control medium were below the limit of quantification (LOQ). Iodine concentrations increased enormously with higher inclusion of seaweed in the media (more than ten times higher iodine in larvae fed the 100 % seaweed medium (700 mg/kg DW), compared to larvae fed 10 % seaweed in the media (61 mg/kg DW)) (**Paper III**). Data from the Norwegian surveillance programme on commercial fish feeds showed relatively low concentrations (0.2-5 mg/kg) of iodine in feeds for Atlantic salmon in the last years (Sanden et al. 2015 and 2016). These concentrations were close to the requirement of salmonids for dietary iodine (1.1 mg/kg) (NRC 2011) and often below. I suggest that using insects fed seaweed for the production of feed ingredients in fish feed, could improve iodine supply for fish. By using seaweed in the media, the larvae were also enriched with calcium, iron, magnesium and potassium, making the larvae an overall good source of minerals.

Seaweeds, and especially brown algae species, are known to be a good source of vitamin E (Holdt and Kraan 2011). This was reflected in the larvae that had higher vitamin E levels when grown on media containing higher levels of seaweed. Larvae fed the sole seaweed medium had six times higher levels of vitamin E than control larvae. Vitamin E is known for its antioxidant properties as it prevents the formation of reactive oxygen species (ROS), protecting cell membranes through the same property. As such, the transfer of vitamin E from seaweed to larvae improves the quality of the larvae, when they are intended for feed purposes.

In this study, the performance and chemical composition of BSF larvae largely depended on the composition of the feeding substrate. This suggests that insects, like farmed animals, have nutritional requirements which have to be met for optimal growth. However, very little is known regarding the requirements of BSF larvae, so further research on this topic is needed. From the results of **Paper III**, it can be concluded that BSF larvae can successfully be reared on media containing up to 50 % of the brown seaweed *A. nodosum*. The larvae fed seaweed-enriched media had a more “marine” profile than the control larvae, and thereby better suited the purpose of using IM and IL from these larvae as fish feed ingredients. However, adding seaweed beyond this level in the insect media was not ideal, as it led to significant reductions in growth and survival of the larvae as well as poorer nutrient accumulation.

7.3 Atlantic salmon production. Using insect feed ingredients in the diet of freshwater-phase Atlantic salmon did not affect its palatability, as no significant effects on voluntary feed intake were observed between feeding groups (**Paper V**). This is in accordance with previous studies where FM was replaced by IM in the diet of salmonids (St-Hilaire et al. 2007; Sealey et al. 2011; Lock et al. 2016; Renna et al. 2017). All diets allowed for optimal fish growth and development, as minor changes in growth parameters were observed between groups. This is again in line with previous reports where IM from BSF larvae was used as replacement for FM in fish diets, at inclusion levels similar to our study (St-Hilaire et al. 2007; Sealey et al. 2011; Karapanagiotidis et al. 2014; Lock et al. 2016; Li et al. 2017; Magalhães et al. 2017; Renna et al. 2017).

Interestingly, fish fed diets with IL from BSF larvae reared on seaweed-enriched media (IL2) performed equally good as the control group, and better than fish fed diets with IL from BSF larvae fed the standard plant-based medium (IL1). Specifically, lower SGR and daily growth rate were found in fish fed IL1 than fish fed VO and/or IL2 in the diet (**Paper V**). Diets containing IL2 had higher concentrations of ARA and EPA than the other dietary groups. I suggest that it is likely that BSF larvae reared on seaweed-enriched media accumulated these LC-PUFAs from the seaweed, as seen earlier (**Paper III**). This improved the overall quality of the IL, which resulted beneficial for the fish growth. Unfortunately, neither data on the nutritional composition of the BSF larvae nor the IL used in this trial were available. A positive effect on growth has been previously reported in rainbow trout fed diets containing BSF prepupae reared on cow manure enriched with fish offal (EBSF), compared to fish fed diets containing prepupae reared on the sole cow manure (BSF) (Sealey et al. 2011).

The inclusion of insect feed ingredients partly reduced the digestibility of macronutrients (lipid, protein and ash). However, the digestibility values were in general high and comparable to previous studies (Lock et al. 2016; Renna et al. 2017). Moreover, the whole-body proximate composition of the fish was not affected by the use of insect feed ingredients in the diet, indicating that IM and IL can be efficiently utilized as protein and lipid sources by Atlantic salmon juveniles (**Paper V**).

In the seawater trial, Atlantic salmon post- smolt were fed diets where FM was replaced by IM at increasing levels, until total replacement. The IM was produced by Protix from BSF larvae fed 60 % *A. nodosum* in the media. A partial or total substitution of FM with IM did not affect feed intake, growth performance or nutrient utilization of the fish (**Paper VI**). This is in line with other studies testing IM in the diet of salmonids (Lock et al. 2016; Renna et al. 2017; Dumas et al. 2018). No significant effects on macronutrient (protein, lipid, ash) digestibility were observed by replacing FM with IM; digestibility values were also in line with literature data on fish fed dietary insect feed ingredients (Magalhães et al. 2017; Dumas et al. 2018; Belghit et al. 2018).

The whole-body composition of the fish was partly affected by the diet. In particular, we found a slight decrease (~8 %) of total FAs content of the fish when replacing all the FM with IM in the diet (**Paper VI**). This might be due to an increased amount of lauric acid in diets containing more IM, which could have reduced lipid storage in fish. Lauric acid is the dominant fatty acid of BSF larvae (Oonincx et al. 2015), and it is easily oxidized for energy (Nordrum et al. 2003; Smith et al. 2005; Belghit et al. 2019). Despite a slight decrease of total FAs in fish with increasing IM in the diet, the fish whole-body composition of EPA and DHA increased. This could be due to extra FO added to the diet containing IM, in order to guarantee the required levels of dietary omega-3 for fish health and welfare.

An aim of the seawater trial was to test sensory parameters of the fish fed IM in the diet. Changing dietary feed ingredients can affect color, flavor and aroma of the fish fillet (Olsson et al. 2003; Turchini et al. 2009; Borgogno et al. 2017). Our results showed only marginal changes in sensory parameters of the fish, that was an increased rancid odor in baked fillets of salmon fed increasing IM inclusion in the diet **(Paper VI**). An increase in saturated FAs of the fish, like in the current trial, renders fish flesh more susceptible to oxidation during thermal treatment, which can cause rancidity (Medina et al. 1998). Borgogno et al. (2017) found significant changes in the flavor, color and texture characteristics of the fillet of rainbow trout fed IM from BSF larvae, in replacement of FM. Other feeding trials, however, did not find any sensory differences in the fillet of fish fed with insect-based diets (Sealey et al. 2011; Lock et al. 2016; Stadtlander et al. 2017). Gasco et al. (2019) suggested that the training of sensory panelists may significantly affect their capability of perceiving sensory differences (Gasco et al. 2019).

7.4 Feed and Food Safety aspects of the seaweed-insect-fish food chain. The safety of the seaweed-insect-fish food production chain was evaluated in terms of the transfer of heavy metals and arsenic along the food chain. These undesirable substances are often associated with seaweed (Almela et al. 2006; Chakraborty et al. 2014), therefore they were expected to enter the food chain when using seaweed biomass in the feeding media of insect larvae. Also, heavy metals and arsenic can be naturally found in insects and their products, which enhances the need for safety evaluations when insect feed ingredients are included in animal feeds (EFSA 2015).

Heavy metals. The heavy metals cadmium, lead and mercury were all found in *A. nodosum*, during the first seaweed screening (**Paper II**). Average concentrations of three samples (each of pooled algal material of several individuals) were: 0.23 ± 0.08, 0.08 ± 0.03, 0.02 ± 0.0 mg/kg (DW) for cadmium, lead and mercury, respectively. Seaweeds take up heavy metals from the surrounding waters, thus levels of these undesirable substances in a seaweed species highly depend on both seasonal and geographical variations (Villares et al. 2002). However, concentrations found in **Paper II** are overall in agreement with previous reports on *A. nodosum*, collected in Norway but also worldwide (Sharp 1995; Morrison et al. 2008; Duinker et al. 2016). Insects can be fed materials of vegetable origin (some exceptions are milk, eggs, honey, among others), according to the current regulatory framework in the EU (Commission Regulation (EU) 2017/893) (EU 2017). In this context, feeding seaweed to insect larvae is allowed by legislation, although it has been done only on a research scale. However, levels of undesirable substances in the seaweed-enriched media should be within threshold levels (MLs) set by the EU in the feed legislation (EU 2002) (**Table II**).

The control plant-based medium for BSF larvae contained relatively low concentrations of cadmium and lead (< 0.1 mg/kg DW). The concentrations of these metals in the mixed media slightly increased when more seaweed was included (**Paper IV**). The pure seaweed medium (100% seaweed) had three times higher levels of cadmium and lead than the control medium. However, the concentrations of cadmium and lead in the feeding media of the larvae can be considered relatively low and they were well below the EU MLs set at 0.5 and 5 mg/kg (based on 12 % moisture content) for cadmium and lead in complete animal feed, respectively (EU 2002). Mercury was not detected in the control medium for the larvae (below LOQ), while it was introduced in the medium by adding seaweed (**Paper IV**). This was expected, as this metal is typically associated with substrates of marine origin. The 100% seaweed medium had 10 times higher mercury than the medium containing 10 % seaweed; however, concentrations of this metal in the media remained relatively low and well below the EU ML. In light of the current feed legislation, rearing insect larvae with media enriched with the brown alga *A. nodosum* is therefore safe with respect to the presence of heavy metals in the feeding media for insects.

A major consideration for the evaluation of insects as safe feed ingredients is the need to quantify their levels of metals. However, studies on this topic are rather scant (Vijver et al. 2003; Diener et al. 2015; EFSA 2015; van der Fels-Klerx et al. 2016). BSF larvae accumulated heavy metals when these were present in the feeding media, and a direct positive correlation existed between dietary and larval metal concentrations (**Figure 9**) (**Paper IV**). I suggest that metal accumulation in BSF larvae was not strongly regulated, as previously observed in many studies with both insects in the wild and farmed (Vijver et al. 2003; Diener et al. 2015; van der Fels-Klerx et al. 2016). Cadmium was readily accumulated by the larvae of all groups and retained up to 93 % in larvae fed ≤ 50 % seaweed in the media. Previous studies have also reported the strong capability of BSF larvae to accumulate cadmium from substrates contaminated with this metal (Diener et al. 2015; van der Fels-Klerx et al. 2016; Gao et al. 2017). This has also been shown in other Dipteran species (Wu et al. 2006). Accumulation of cadmium in insects is believed to happen due to the ability of this metal to i) pass through calcium channels on cell membranes, but also ii) bind to metallothioneins which increases storage capacity of this metal in insect bodies (Braeckman et al. 1999; Craig et al. 1999). BSF have an exceptionally high content of calcium compared to other Dipteran species (Finke 2013; Barroso et al. 2014; Makkar et al. 2014), which can partly explain why the larvae of this species easily accumulated cadmium.

Accumulation of cadmium in the larvae reached a *plateau* level in larvae fed ≥ 50 % seaweed in the media. I suggest that this was due to reduced growth, survival and poor nutrient utilization in these larvae, which affected cadmium accumulation. Previous studies have shown that BSF larvae are able to accumulate much higher concentrations of cadmium than in our study, in a linear fashion (Diener et al. 2015; van der Fels-Klerx et al. 2016; Gao et al. 2017). This difference might be due to higher exposure levels of cadmium used in the feeding media for the larvae compared to our study, but also exposure time and different developmental larval stages used. Moreover, spiked cadmium in the feeding media, as in previous studies, might have been more bioavailable than the cadmium contained in seaweed-enriched media as in the present study. Seaweeds, especially brown algae species, contain complex polysaccharides which can bind metals (Hashim and Chu 2004; Jiao et al. 2011) and this could have lowered the bioavailability of cadmium for the larvae fed high amounts of seaweed in the media.

The current EU feed legislation set MLs for cadmium at 1 and 2 mg/kg (based on 12 % moisture content) in complete fish feed and feed ingredients, respectively (EU 2002). The larvae in the present study exceeded the MLs for cadmium in fish feed when reared on ≥ 50 % seaweed in the media (1.4 mg/kg in larvae fed 50 % seaweed, based on 12

% moisture content) (**Paper IV**). This is not concerning as interest in the use of insects in fish feed mainly regards the inclusion of insect-derived ingredients (IM, IL), rather than the whole larvae as the sole component of the fish diet. Cadmium concentrations exceeded the EU MLs for cadmium in feed ingredients only in larvae fed ≥ 70 % seaweed in the media. This is again not concerning, as inclusion of seaweed at high percentages in the insect feeding media is most likely not relevant for the industrial production of BSF larvae and their products due to poor growth and survival of the larvae.

Larvae fed the control medium did not contain mercury (<LOQ). This supports previous studies reporting low concentrations of this metal in terrestrial insects, both wild and farmed (Charlton et al. 2015; EFSA 2015). However, this metal was introduced in the larvae when they were fed seaweed-enriched media (**Paper IV**). In a pilot study, rearing insect larvae on substrates containing marine materials (seaweed, tunicates, FM) resulted in the uptake of mercury also in mealworms and superworms (Biancarosa et al., unpublished results). Concentrations of mercury in larvae fed 50 % and 100 % seaweed were three and five times higher than the control larvae, respectively (**Paper IV**). Similar results were observed regarding accumulation of lead in the larvae. However, levels of both mercury and lead were overall low and only slightly higher than in the feeding media, which suggests poor accumulation of these metals in the larvae. This supports previous findings on BSF larvae (Diener et al. 2015; van der Fels-Klerx et al. 2016). Concentrations of both lead and mercury in the larvae did not exceed any of the current EU MLs for these heavy metals in both feed ingredients and complete feed (**Paper IV**) (**Table 2**); this raises no concerns in terms of feed safety, regarding these undesirable substances.

In the freshwater trial, IM-based diets had slightly higher concentrations of cadmium (0.4 mg/kg feed) than diets without IM (0.3 mg/kg feed) (**Paper V**). This was also observed for levels of lead which showed a two-fold increase in IM-based diets (0.14 mg/kg feed) compared to diets without IM (0.07 mg/kg feed). These findings were expected, as both cadmium and lead have been associated with farmed insects and especially BSF larvae (Charlton et al. 2015; Diener et al. 2015; EFSA 2015; van der

Fels-Klerx et al. 2016). On the contrary, concentrations of mercury in the diets decreased by using IM (0.01 mg/kg feed), compared to diets without IM (0.03 mg/kg feed). This was again expected, as this metal is usually associated with marine ingredients, especially FM (Sissener et al. 2010).

	Feed ingredients	**Animal feed**	**Fish feed**
Cd	2	0.5	**1**
Pb	10	5	**5**
Hg	0.1	0.1	**0.2**
As	2 (40)	2	**10**

Table 2. EU MLs (mg/kg) in feed ingredients, complete feed for farmed animals and complete feed for fish, based on 12 % moisture content, according to the Directive 2002/32 EC (and amendments) on undesirable substances in animal feed. Value in parenthesis refers to the ML for arsenic in "seaweed meal and and feed ingredients derived from seaweed".

Similar findings were observed in the seawater trial (**Paper VI**). The concentrations of cadmium, lead and mercury were low in the IM (0.98, 0.36, 0.016 mg/kg for cadmium, lead and mercury, respectively). These levels were very well below the MLs set for these undesirable substances in feed ingredients (EU 2002). As in the freshwater trial, when IM replaced FM in the diet, cadmium and lead concentrations increased while mercury levels decreased (**Paper VI**). A previous study by Lock et al. (2016) found 12 times higher concentrations of lead in Atlantic salmon diets, when FM was fully replaced by IM from BSF larvae. However, the concentration of cadmium in the diets did not change between dietary groups (~0.2 mg/kg feed) (Lock et al. 2016). In Lock et al. (2016), dietary concentrations of mercury were below LOQ, as seen in the current study.

The concentrations of cadmium, lead and mercury in the diets, in both the freshwater and seawater trials, were comparable to concentrations typically found in commercial fish feed for Atlantic salmon in the last years (**Table I**), and below the EU MLs for fish feed (EU 2002) in all dietary groups (**Paper V** and **VII**). In whole fish from the freshwater trial, cadmium was found at 0.01 mg/kg (DW) in all dietary groups, while lead was not quantified (< LOQ) (**Paper V**). In the fish fillet samples from the seawater trial, both cadmium and lead were not detected (< LOQ) (**Paper VII**). This suggests that fish do not accumulate these undesirable substances in the muscle tissue, as previously reported (Handy 1992; Atobatele and Olutona 2015). Mercury levels were low in whole fish fed diets without IM (0.02 mg/kg (DW) and even lower (0.01 mg/kg (DW) in fish fed IM in the diets (**Paper VII**). This is likely due to low concentrations of this metal in the fish diet and the IM. The levels of cadmium, lead and mercury in the fish were all well below the MLs set for these undesirable substances in muscle meat of fish (**Paper V** and **VII**) (Commission Regulation (EC) No 1881/2006) (EU 2006).

Based on the results, I suggest that the inclusion of insect feed ingredients from BSF larvae fed seaweed in the media, in the diet of both freshwater- and seawater-phase Atlantic salmon, is not a concern in terms of feed and food safety. This regards the presence and transfer of the heavy metals cadmium, lead and mercury through the seaweed-insect-fish food chain.

Arsenic. In the first seaweed screening, we found 23 and 30 mg/kg (DW) of total arsenic in two different samples of *A. nodosum* (**Paper II**). Inorganic arsenic comprised less than 1 % of total arsenic found in this species. These concentrations are similar to previous reports on total arsenic and inorganic arsenic levels in *A. nodosum* (Morrison et al. 2012; Taylor and Jackson 2016; Ronan et al. 2017). The EU has set a ML for arsenic at 40 mg/kg in “seaweed meal and feed ingredients derived from seaweed” (based on 12 % moisture content). This maximum level is set for total arsenic, but authorities can request documentation showing that concentrations of inorganic arsenic in feed ingredients are below 2 mg/kg (12 % moisture content). When a factor of 12 % moisture was applied to results in the current study, the concentrations of arsenic (total and inorganic arsenic) in the samples from *A. nodosum* were all below the MLs set by the EU (**Paper II**).

Mixing seaweed biomass of *A. nodosum* with the standard plant-based medium for BSF larvae, increased the concentrations of arsenic in the media for the larvae (**Paper IV**). Total arsenic was present at low levels (0.08 mg/kg DW) in the control medium, reaching concentrations of 17 mg/kg and 36 mg/kg (DW) at 50 % and 100 % seaweed in the media, respectively. Overall, the media enriched with seaweed (10 to 100%) had values of total arsenic below the ML of 40 mg/kg for arsenic in seaweed meal. However, these values were above the ML for arsenic in feed ingredients (2 mg/kg) (**Table II)**. This may rise some concerns regarding the inclusion of seaweed in the feeding media for insects, depending on which threshold level is considered. On the other hand, the concentrations of inorganic arsenic, lowest in the control medium (0.05 mg/kg DW), increased only slightly in the seaweed-enriched media (0.08 and 0.09 mg/kg at 50 and 100 % seaweed in the media, respectively) and they were all below the ML.

Feeding BSF larvae with seaweed-enriched media led to accumulation of arsenic in the larvae, as a linear function of arsenic concentrations in the media (**Figure 9**) (**Paper IV**). When a factor of 12 % moisture was applied in the current study, levels of total arsenic found in BSF larvae were above the MLs for arsenic in feed materials (2 mg/kg), when larvae were fed ≥ 20 % seaweed in the media. This should be taken into account whether larvae fed seaweed are intended for feed purposes. On the other hand, levels of inorganic arsenic found in BSF larvae were overall low, and well below the ML for inorganic arsenic set by the EU (EU 2002).

In the freshwater trial, levels of total arsenic in the diets containing insect ingredients were more than two times lower than diets without insect ingredients (**Paper V**). Previous studies have shown that arsenic is typically associated with marine ingredients, especially FM and FO (Sloth et al. 2005; Berntssen et al. 2010). I suggest that IM and IL in the current study contained lower concentrations of arsenic than marine ingredients in the diets; however, data on arsenic levels in each feed ingredient were unfortunately not available to support this hypothesis.

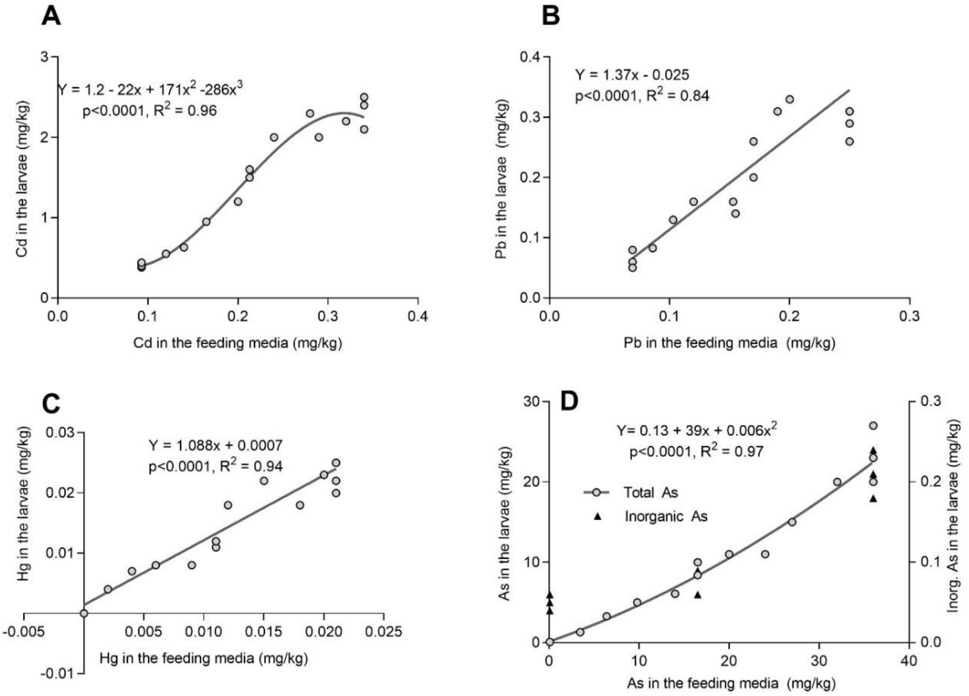


Figure 9. Accumulation of heavy metals and arsenic in BSF larvae fed seaweed-enriched media. Concentrations (mg kg−1 DW) of cadmium (A), lead (B), mercury (C) and arsenic (D) in feeding media (0-100% of a plant-based feeding medium replaced with seaweed and in BSF larvae fed these media for 8 days. Regression lines are indicated as continuous lines.

These findings are similar to Lock et al. (2016) who reported lower concentrations of arsenic in fish diets containing IM, compared to control diets without IM. Overall, concentrations of total arsenic in the diets were comparable to concentrations typically found in commercial fish feed for Atlantic salmon in the last years (**Table I**). Moreover, they were lower than MLs set for arsenic in fish feed (EU 2002) (**Paper V**). As such, the inclusion of insect feed ingredients in the diets of freshwater-phase fish, did not rise safety issues. However, levels of inorganic arsenic in the diets were not quantified. The reduction of arsenic concentrations found in the diets were reflected in the whole fish composition. Total arsenic levels were reduced by 85 % in fish fed IM as protein source, compared to fish fed diets without IM inclusion (**Paper V**).

In the seawater trial, levels of arsenic in the IM were relatively high (4.7 mg/kg DW) and above the ML for total arsenic in feed ingredients (EU 2002) (**Paper VII**). This may limit the use of IM from BSF larvae fed seaweed (*A. nodosum*) as fish feed ingredient. However, inorganic arsenic accounted for only 6 % of total arsenic in IM and it was lower than the MLs set by the EU in feed ingredients. Despite replacing FM with IM, the concentrations of total arsenic in the diets were similar among groups (**Paper VII**). This is different from what was observed in the freshwater trial. This could be due to adding FO to the diets containing IM in the seawater trial, to ensure the required dietary levels of n-3 FA for fish growth and welfare. FO is known for containing high concentrations of arsenic (Sloth et al. 2005; Sele et al. 2014; Sissener et al. 2013). Overall, the concentrations of total arsenic in the diets were all below the EU ML of 10 mg/kg (12 % moisture content) arsenic in complete fish feed (EU 2002). Moreover, the concentrations of inorganic arsenic in the four diets were also below the ML. This reduces the safety issues of using IM from BSF larvae fed seaweed (*A. nodosum*) in fish diets, regarding arsenic contamination.

One of the most interesting results of this work is that, despite similar concentrations of total arsenic in the diets, the fish fillet showed a significant decrease of total arsenic concentrations when fish were fed increasing IM in the diet, compared to the control diet (**Paper VII**). I hypothesize that the arsenic in the IM was less bioavailable for the fish than the arsenic contained in the other feed ingredients. To test this hypothesis, I conducted speciation analyses on the IM, the diets and the fish fillet from the seawater trial, as discussed below.

Arsenic speciation. Seaweed absorbs inorganic arsenic from the surrounding water through phosphate uptake mechanisms, due to similarities in the chemical structures between arsenic and phosphorus (Klumpp 1980; Francesconi 2010). Once absorbed into the seaweed, inorganic arsenic is further converted into organic species through methylation and alkylation processes (Klumpp 1980; Edmonds and Francesconi 2003). The main end products of the arsenic metabolism found in seaweeds are complex carbohydrates, the so-called arsenosugars (Edmonds et al.1998; Edmonds and Francesconi 2003; Feldmann and Krupp 2011). Other arsenic species have been found in seaweeds: these are lipid-soluble species, the arsenolipids, but also DMA and inorganic arsenic (Edmonds et al. 1998; Rose et al. 2007).

The metabolism of arsenic by BSF larvae is currently not known. When BSF are fed media enriched with seaweeds at increasing inclusion levels, the percentage of inorganic arsenic (relative to total arsenic) in the larvae did not vary between dietary groups, remaining below 1 % (**Paper IV**). Based on this data, I suggest that BSF larvae do not convert organic forms of arsenic into inorganic arsenic species.

Speciation analysis of organic arsenic (water-soluble) species in the IM used for the seawater trial showed that DMA was the major arsenic species, accounting for 18 % of total arsenic (**Paper VII**). DMA has been described as a metabolic product of arsenosugars (Edmonds and Francesconi 1987; Feldmann and Krupp 2011). AB only accounted for 1.2 % of total arsenic in IM. A major fraction of arsenic in IM was unidentified arsenic species. I speculate that these species could be other metabolites of arsenosugars produced by BSF larvae fed the seaweed-enriched media, e.g. monomethylarsonic acid (MA), arsenocholine, dimethylarsinoylacetate, dimethylarsinoylethanol and their thio-analogues (Edmonds and Francesconi 1987; Feldmann and Krupp 2011). A fraction (13 %) of total arsenic in the IM was not extracted, and it could be arsenolipids, as the method used for arsenic speciation only extracted water soluble arsenic species.

The concentrations of total arsenic in the IM were much lower than total arsenic levels found in BSF larvae fed the same amount of seaweed in the media (60 %) in the first insect trial (see **Paper IV**). I therefore suggest that processing of insect larvae into IM (e.g. defatting of IM, exoskeleton removal) might have affected the content of arsenic in the insect end product. However, levels of total arsenic in the larvae used for the production of the IM were unfortunately not quantified, therefore this remains a speculation.

The digestibility of total arsenic decreased by replacing FM with IM in the seawater trial (**Paper VII**). This supports the idea that IM contained arsenic species which were less bioavailable than the ones in other feed ingredients in the fish diet (e.g. FM).

Speciation analyses of the diets showed that the concentrations of DMA, inorganic arsenic and unidentified arsenic species increased in the diets when IM replaced FM, while AB decreased (**Paper VII**). This was expected as AB is the major form of arsenic contained in FM (Sloth et al. 2003). In the fish fillet, AB was the major arsenic species in all dietary groups. It is well known that AB is the major arsenical compound in fish (Shiomi et al. 1995; Nam et al. 2010; Özcan et al. 2016). Interestingly, AB accounted for a higher percentage of total arsenic in fish fed the control group without IM, compared to fish fed IM-containing diets. Based on the results from arsenic speciation, I suggest that, although total arsenic levels in the diets were approximately similar, the type of arsenic species present in the diets affected the accumulation of arsenic in the fish.

8. Conclusions

Seaweed species from Norwegian waters contained essential macro- and micronutrients as well as undesirable substances (heavy metals and arsenic); the concentrations thereof were highly dependent on species and taxonomic group. Levels of heavy metals and arsenic found were mostly lower than MLs set by the feed legislation in the EU.

Seaweed (*A. nodosum*) was a suitable feeding substrate for rearing BSF larvae up to 50 % seaweed biomass of total feeding medium. Moreover, the inclusion of seaweed in the medium for BSF larvae introduced valuable nutrients into the larvae such as EPA, iodine and vitamin E, generally associated with the marine environment. At the same time, transfer of heavy metals and arsenic from seaweed to larvae occurred. The concentrations of these undesirable substances in the larvae, mostly followed the concentrations of the media in a linear trend. Levels of cadmium and arsenic in the larvae were above the EU MLs for feed ingredients, when larvae were fed more than 70 and 20 % of seaweed in the media, respectively.

Feeding diets containing insect feed ingredients (from BSF larvae fed seaweed in the media), to freshwater-phase and seawater-phase Atlantic salmon, did not affect growth parameters, digestibility of the nutrients, whole-body composition of the fish or sensory parameters of the fish fillet. In the seawater trial, both the IM and the IM-based diets contained heavy metals; however, levels of these undesirable substances were all below the EU MLs. Fish fed IM-based diets did not accumulate these undesirable substances in the fillet. In the same trial, concentrations of total arsenic in the IM were above the EU MLs for feed materials, while concentrations of arsenic in IM-based diets were all below the EU MLs. Transfer of arsenic from feed-to-fillet occurred. However, there was a clear reduction of total arsenic concentrations in the fillet of fish fed higher IM in the diet.

Overall, the seaweed-insect-fish food chain holds a great potential for farming Atlantic salmon, by producing nutritious feeds, and healthy and robust fish. Moreover, this approach rises only minor concerns in terms of feed and food safety.

9. Future perspectives

This PhD work demonstrated that feed ingredients from insects reared on seaweed are suitable and safe for farmed Atlantic salmon. To the best of my knowledge, no other studies have tested seaweed biomass as rearing substrate for insects destined to animal feed. In this project, seaweed biomass of the brown alga *A. nodosum* (rockweed) was harvested from the environment, where it represents a massive and under-exploited resource in Norway. However, beside harvesting, cultivating seaweeds in Norway is a growing sector. Seaweed cultivation in Norway has been focusing on *S. latissima* (sugar kelp) and *A. esculenta* (winged kelp), therefore testing these species for rearing of insects is suggested.

A few seaweed cultivation sites in Norway are located in IMTA systems, where seaweeds in such systems utilize excess nutrients produced by fish farming wastes. Further research could therefore focus on insect production using seaweed biomass from side streams of seaweed cultivation in IMTA systems. This approach would fit into the principle of Circular Economy, which has taken hold in most countries to fight waste production in the last few years. Also, it would add a greater value to insect farming, in terms of sustainability.

The results of this PhD work have shown that the main driver of nutrient and contaminant composition in insects and their products is the feeding medium of the larvae. Feeding insects on biomass from *A. nodosum* introduced “marine” nutrients into the insects, and this partly tailored the nutritional quality of the insect raw materials towards the requirement of Atlantic salmon. However, levels of these nutrients in the insect ingredients were not sufficient to fully meet these nutritional requirements. The nutrient composition of marine macroalgae varies widely, based on environmental, seasonal and/or specific properties such as genetics. As such, further research should focus on feeding insect larvae with different marine macroalgae species, where the nutrient composition varies, in order to test if the nutritional profile of the insect raw materials could be improved to a greater extent. Other marine organic materials (e.g. blue mussels) could be considered as insect feeding substrates, for the same purpose.

In this PhD work, speciation analysis of arsenic showed that most of the arsenic species present in the IM were unknown, therefore further research is necessary to evaluate the potential presence of toxic arsenic species in insect and their products. Gaining knowledge in this topic will help to better understand which insect fraction the arsenic is bound to, then to improve processing of the insects and their products, to finally reduce arsenic contamination in insect feed ingredients.

The use of antibiotics in animal farming has led to antibiotic resistance and reduction of the effectiveness of medical treatments, with negative effects on both animals and humans. Finding new alternative sources of antimicrobial compounds is therefore necessary. Seaweeds are rich sources of structurally diverse bioactive components with valuable pharmaceutical and biomedical potentials. To my knowledge, transfer of these compounds from seaweed to insects has not been elucidated. Insects also produce antimicrobial compounds as defense to their natural environment, highly contaminated by pathogens. The research community should further explore the potentials of using bioactive compounds from insects reared on seaweed as alternatives to antibiotics in feeds.

Source of data

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