There are no longer plenty more fish in the sea

The potential of wrack macro-invertebrates as an alternative source of the marine origin omega-3 fatty acids: EPA and DHA

Submitted for the degree of Doctor of Philosophy

by

Finlay Bryson Richardson

May 2023

University of Stirling

And

(The) Institute of Marine Research

Statement of Originality

I hereby confirm that this thesis is an original piece of work conducted independently by the undersigned and was done so with the ethical approval of the Animal Welfare and Ethical Review Body. All research material has been duly acknowledged and cited. Any mistakes or errors are mine alone.

Finlay Bryson Richardson,

12th May 2023

Acknowledgements

I have been very fortunate throughout this PhD to have worked on a project which I have found extremely interesting and enjoyable, albeit for slightly too long. To have called the beautiful beaches of East Lothian my office, to have travelled to conferences and most of all, to have met some wonderful people and friends to whom I will be forever grateful to. None of this would have been possible without them.

First and foremost, I would like to express my deepest gratitude to my supervisor Dr. Andre Gilburn for both giving me this opportunity and guiding me through it. Thank you for keeping me on track when I threatened to bite off more insect protein than I could chew by continuously trying to expand the scope of the project with every thought and idea I had. Similarly, I am extremely grateful for the advice, time and knowledge of my supervisors Dr. Robin Ørnsrud and Dr. Ikram Belghit at The Institute of Marine Research, Bergen. The work in this thesis has only been possible thanks to them and their expertise have been invaluable.

This PhD is not my achievement alone, but also belongs to my family. Thanks to my parents for their selfless support and guidance throughout my life, I have no doubt the upbringing and values you bestowed upon me led me here. Thank you Mam, for the sacrifices you have made for all of us and for making sure fun and happiness are deeply rooted in our values. Thank you Dad, for always supporting our family and for showing me that there are many measures of success and which of those I value highest. If I am half as successful as you, I will have done very well. Thank you both for patiently encouraging my curiosity from the moment I started talking and showing me the value of the natural world even before then. I expect this all started with the pet stick insects I had whose babies escaped all over the house, I am endlessly grateful. Thank you for letting me use your house as a base when I was on fieldwork and turn your shed into a lab. 20 years on from the stick insects, and it was seaweed flies all over the house, I'd like to say that will be it now, but I can't make any promises. I love you both and am so lucky to have such supportive parents. Thank you to my sisters Molly and Erin for spending hours sifting through rotting seaweed looking for maggots in the freezing cold with me. Thank you Erin for the moral support and thank you Molly for the sense of sanity you provide amongst those three.

Similarly, I am infinitely grateful to my Grandad for his continuous guiding support. Thank you for our honest conversations, for helping me push myself to achieve more and for being a voice of

reason when it was most needed. Moreover, thank you to you and Gran for the financial support you have given me, I would never have gotten this far without it.

I would also like to express my deepest gratitude to Dr. Gareth Padfield, for his time and patience in his medical care of my heart, thank you for taking on that responsibility. I don't think I would have finished this PhD without it. Similarly, I am extremely grateful to Dr. Catherine Labinjoh for her holistic care and positive outlook when I was at my lowest and needed it most. A heartfelt thank you to you both and all the cardiology team at Forth Valley Royal Hospital.

A huge thank you to my office mates Dr. Jess Burrows and Dr. Kelsey Wilson for keeping me sane and motivated throughout this project. I am fortunate we landed in an office together, I'm not sure I would have been able to see this to the end without you both.

I am also very grateful to my high school geography teachers Miss Miller and Mr Lanyon who introduced me to the world of geographical and environmental sciences and whose infectious enthusiasm set me on this path which I have enjoyed so much. I remember drawing my first podzol and knowing I would love a career in this subject.

Lastly, and most importantly, the biggest thank you goes to my fiancé Brooke. Continuing this PhD wasn't always the easiest decision, but your unwavering support in every aspect has allowed me to finish it. I truly would not have been able to complete this without you and I am forever grateful. Thank you for sticking by me through thick and thin, this achievement is both of ours. I look forward to the rest of our lives together and where it will take us.

"All we have to decide is what to do with the time that is given us."

J.R.R.Tolkien

Abstract

Increasing population and affluence puts expanding pressure on global food security. Sustainably meeting future food demand requires novel and innovative food production techniques and technologies that are more efficient than those used today. Furthermore, the disconnect between the diets of people today and the diets for which we were naturally selected has numerous negative health effects. As such, future food must also be of high quality. This is particularly true of our omega-3 consumption relative to our omega-6. However, over recent years, rather than improving, the quantity of omega-3 in farmed fish produce, which is the most significant source of dietary omega-3 globally, has decreased. This is a result of overfishing reducing fish oil supply, creating a lower omega-3 content in the food of these farmed fish. Thus, finding an alternative omega-3 source that is more environmentally sustainable than fish oil is a top priority. This alternative must also be economically attractive and come with social benefits as the comprehensive adoption of any alternative relies on it improving the sustainability of all three of these pillars of society. Insects show much promise as a sustainable source of protein in food and feed and preliminary studies have identified wrack macro-invertebrates as having similar potential as a fish oil replacement. This research assesses the potential of this alternative by investigating the ease with which they could be cultured and their nutritional composition before crudely estimating their production cost and market value following this. The results suggest the biggest barrier to the use of wrack macro-invertebrates as a fish oil replacement is the inherent value of the seaweed substrate which prevents this from being economically viable. Furthermore, even if it were, higher returns of omega-3 would be obtained from the use of the seaweed as a feed ingredient directly.

Table of contents

Table of Figures

Table of Tables

Abbreviations

ω-3 - Omega 3

ω-6 - Omega 6

ALA - α-linolenic acid

BSFL - Black soldier fly larvae

CEF - Controlled environment facility

CI - Confidence interval

CO_2 - Carbon dioxide

DHA - Docosahexaenoic acid

DOM - Dissolved organic matter

DPA - Docosapentaenoic acid

dw - dry weight

EFSA - European food safety authority

EPA - Eicosapentaenoic acid

EU - European union

FA - Fatty acid

FAO - Food and Agriculture Organisation

FM - Fishmeal

GASB - Great Atlantic Sargassum belt

GC - Gas chromatography

GHG - Green house gas

HPLC - High performance liquid chromatography

ICP-MS - Inductively coupled plasma-mass spectrometry

IM - Insect meal

LOQ - Limit of quantification

MS - Mass spectrometry

MCT – Modern coexistence theory

MUFA - Mono-unsaturated fatty acid

NHS - National Health Service

O_2 - Oxygen

OM - Organic matter

PAP - Processed animal protein

PCA - Principal component analysis

PPH - Preference performance hypothesis

PUFA - Poly-unsaturated fatty acid

SD - Standard deviation

SFA - Saturated fatty acid

SLM - Spectral library matching

TFA - Total fatty acid

UN - United Nations

UPLC - Ultra performance liquid chromatography

USDA - United States Department of Agriculture

WFP - World Food programme

WHO - World Health Organisation

ww - wet weight

Chapter 1

AN INTRODUCTION

1.1 Prelude

Over the course of this research project, global societies, economies and environments have undergone an historic shift. The unprecedented worldwide pandemic realigned the values of many, the forced break from economic focused goals made people realise how far removed from the natural world they have become and how important that natural world is (Office for National Statistics 2021). Just having a garden, never mind living close to woodland, upland, or the coast, was looked on with envy. Countries faced large supply shocks as global supply chains came under pressure, leading to panic buying as people feared they would run out of food. Then, as many of the worlds' global economies began to reopen and industrial demand for fossil fuels returned, hostilities in Ukraine escalated. Given that in 2020 the EU imported 39%, 23% and 46% of gas, oil and coal respectively from Russia (European Commission 2020), this led to an energy supply shock. This disruption to global energy markets put pressure on governments as they experienced high and volatile prices of fossil fuels (European Commission 2020). Lastly, both the COVID-19 pandemic and the conflict in Ukraine placed pressure on food supplies, and the global price of food rose sharply making food security a top concern of many governments (Jargin 2022, Rizal and Nordin 2022).The World Food Programme (WFP) estimated that 345 million people faced food insecurity in 2022 across the 82 countries they operate in, 47 million of which were directly linked to the knock on effects of the conflict in Ukraine (Food Security Information Network 2022, World Food Programme 2022). As a result of these events, and their cumulative effects, global markets and economies entered a sharp economic downturn.

Hardship often precedes improvement, and these recent events have also had some positive effects. Improvements of mRNA vaccine technology to combat COVID-19 have demonstrated promise against cancer (Chakraborty *et al* 2021, Ladak *et al* 2022). The halt of global economies saw a sharp decrease in green house gas (GHG) emissions which had numerous benefits, among which was the strikingly visible improvement in air quality in some of the world's major cities such as Dehli, demonstrating the circular benefits of sustainable development (Kotnala *et al* 2020). Furthermore, increased connectedness to, and value of nature made people more climate conscious (Haasova *et al* 2020). The pandemic not only compelled individuals to adopt a healthier lifestyle (Public Health

England 2021), but highlighted the inequalities in physical activity opportunities both within and between communities, emphasising the need for healthier, fairer and more sustainable urban development (McDougall *et al* 2021). The energy crisis has acted as a catalyst in progress towards, and investment in, renewable energy as it can provide an independent supply making countries less reliant on imports (EMBER 2022 UN 2022). Lastly, the shocks to the supply of food have shown that the importance of food security cannot be understated and is perhaps only surpassed by climate change. It has served as a pertinent example of the benefits of local food production and importance of food security.

It is deeply regrettable that it has taken a global pandemic and a large-scale international conflict to make world leaders and governments begin to take some of the most pressing challenges of this generation seriously. However, at least it seems there is potential for some good to come from these difficult years. Finally, governments and authorities are beginning to understand:

Sustainability is Holistic

The word 'sustainability' is used frequently but means something different from person to person and sector to sector and many forms of sustainability exist. In truth, the word 'sustainability' simply means to be maintained at a rate or level. To be sustained. Each year it becomes more evident that humankind's presence cannot be sustained. Many of our individual measures of progress and success in life are unsustainable. Most individuals aspire to owning a big house, a nice car and good food for them, their children and pets. Regardless of whether this can be achieved within their societal structure, it cannot be achieved en-mass because the planets' natural limits cannot be surpassed. As a result, we must either change our society and its values to include the natural world and its limits or, have those natural limits change it for us.

The three pillars of sustainability (Figure 1) are a familiar depiction of the holism of sustainability (Purvis *et al* 2019). Here, sustainability refers to the indefinite persistence of humankind, and the figure visualises our reliance on the environment, economy and society. If one is neglected it will become increasingly unable to support sustainability. Much like a pillar weathering and not being maintained would eventually lack the structural integrity to hold

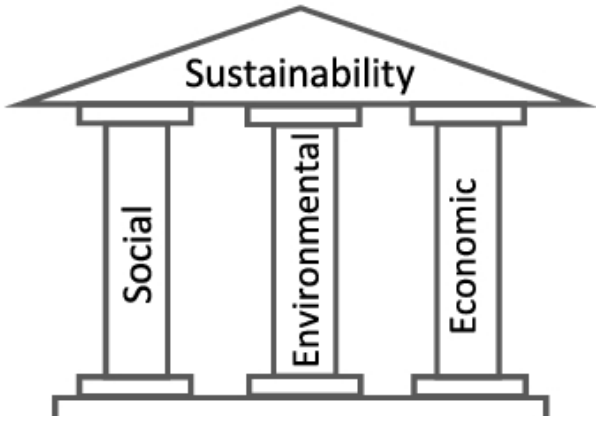


Figure 1. The three pillars of sustainability. The common depiction of the need to balance all three sectors to maintain sustainability. Figure created using GIMP 2.10.28.

the part of the roof it supports, leading to a collapse.

For decades, people, groups and organisations have been trying to make clear the ramifications of disregarding environmental sustainability. Not because people feel unbearable pain and empathy every time a piece of ice melts or a tree is cut down. Of course, this is facetious, and the effects of climate change on individual organisms and their species can be heart-breaking and the loss or change of the ecosystem service they provide can be catastrophic. But it is unrealistic to expect a picture of a starving and displaced polar bear to encourage governments, corporations and individuals to prioritise the environment at an economic and/or social cost. Instead, evidencing the social and economic costs of not prioritising the environment and offering solutions that come with economic and social benefits is likely to be more motivating. Given the holistic nature of sustainability this is not as unachievable as it may first seem.

With the backdrop of recent global events highlighting the need and benefits of prioritising, or at least not neglecting, the environment's role in humankinds' sustainability, much was anticipated from the 27th meeting of the Conference of Parties (COP-27). Investing in renewable energy and local food production reduces reliance on imports making economies more robust in dealing with disturbances such as we have recently seen. At the same time, an increase in renewable energy and local food production would reduce GHG emissions. Which in turn would benefit the environment, economy and society. For example; the WHO estimate 23% of global yearly deaths could be avoided with a healthier environment (WHO 2017). This increase in global health and quality of life would make a happier, healthier and larger workforce, in turn, facilitating increased economic growth. Assuming this growth is somewhat evenly distributed, this creates a happier society. Despite the clear circular benefits of prioritising the environment in this way, many are disappointed with the outcomes of COP27 (Siva 2022). There was no commitment to working towards a removal of fossil fuels, only a pledge to reduce them which have been pledged in previous COP meetings . Arguably the most significant outcome was the agreement of 'rich' countries to create a fund for loss and damage caused by climate change to 'developing' countries. However, there are no specifics surrounding this agreement, such as what a 'rich' or 'developing' country is, and none are expected until COP-28 by which time any number of circumstances and factors could have changed (UNFCCC 2022). Nevertheless, this may be an important milestone as it could represent countries beginning to take some responsibility for climate change.

1.2 Population growth and food security

In the last century, the global population has grown at an unprecedented rate (UN Department of Economic and Social Affairs 2021). Yet, our population is still growing faster than anticipated, a modelled prediction from 2011 suggested the global population would reach 8 billion between 2024 and 2033 (Schervbov *et al* 2011). However, on the 15th November 2022, the UN estimated we reached a global population of 8 billion people (UN Department of Economic and Social Affairs 2021). Figures as high as this often lack true appreciation. To give the number of people that now exist on earth some perspective, it would take 253.5 years to count to 8 billion, 1 second at a time. Whilst the rate of growth is expected to slow, the population is predicted to reach 10 billion around 2058 (UN Department of Economic and Social Affairs 2021). Not only is the population growing, but it is also developing, becoming more affluent and living longer. As such, the amount of food that needs produced is increasing by more than just that to keep up with population growth. In 2011, Tilman *et al* (2011) predicted by 2050 global crop demand would increase by 100-110% despite the population only predicting to increase by around 33% (2.3 billion). As already evidenced, the population has been growing faster than previously expected and this model was based on the work by Godfray *et al* (2010), which predicted a plateau in the global population at roughly 9 billion around the middle of the century. However, the UN now project the population to exceed 9 billion around 2037 and 10 billion around 2058 (UN Department of Economic and Social Affairs 2021). The UN's Food and Agriculture Organization (FAO) predict that the increase in agriculture production will be significantly less than the expected demand (FAO 2014). Similarly, Ray *et al* (2013) suggest there will be a shortage of 67% maize, 42% rice, 38% wheat and 55% soybean by 2050. On top of this, the increase in demand will have to be met without an increase in the size of the planet. Therefore, our current food production will have to undergo a period of rapid innovation and increased sustainability to prevent a global food security crisis. Consequently, food security must be a global priority (Godfray *et al* 2010, Tomberlin *et al* 2015).

As discussed, the 3 pillars of (humankind's) sustainability (Figure 1), visualise the importance of maintaining all 3 pillars equally. It could be argued that because the pillar of environmental sustainability has been neglected over recent decades, it now deserves priority. But, due to the nature of many of our societies, most innovations to improve environmental sustainability will not be embraced if they come at a social or economic cost (Hartmann and Siegrist 2017). The current energy crisis shows that the alignment of environmental, social and economic goals can occur, as all three sectors are now calling for a large-scale increase in renewable energy. This makes a case for these 3 pillars to be thought of as more interconnected and possibly self-governing through an

inverse correlation. Perhaps visualizing the importance of these 3 aspects as pillars, creates a misleading perception of them as static and independent. This idea is visualised in Figure 2.

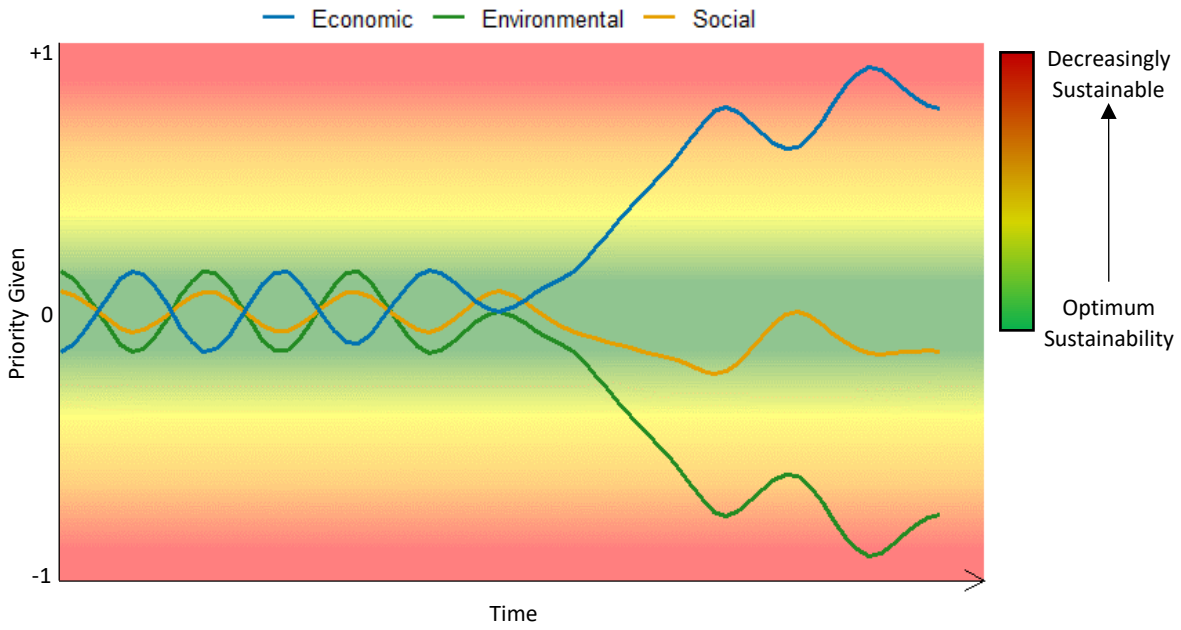


Figure 2. Conceptualisation of treating the 3 aspects of human sustainability as dynamic and interconnected rather than static and independent.

If this were the case, the current generational priority of the environment may not be as revolutionary as it seems. It could, instead, be environmental sustainability beginning to retrace towards the mean of overall sustainability. Whilst it would be ideal if motivations for an increase in environmental sustainability were inherently independent, it is important to be aware that this is just that, idealistic. In reality, people from different backgrounds and upbringings have different values, priorities and ambitions. This is why innovations must benefit the environment, society and the economy to be comprehensively adopted. Consequently, although an immediate and systematic shift to innately environmentally sustainable practices is desirable to many, a more realistic and therefore achievable ambition is finding ways for social and economic outputs to be improved in ways which also increase environmental sustainability. Rather than trying to force society and economies to change their outputs for the benefit of the environment. This, combined with increasing environmental education and awareness, could lead to a more gradual, but all-inclusive, sustainable sustainability.

1.3 Food vs Feed

As highlighted, the global output of food will have to increase over the coming decades to meet increasing demand and affluence. This presents an opportunity to improve a social and economic

output in a way that also improves environmental sustainability. Again, convincing all, or the majority, of the population to eat locally, sustainably produced food and waste little to none of it, is likely the fastest way to significantly increase food security. Yet people and businesses are unlikely to accept the economic and social costs this would come with (Hartmann and Siegrist 2017). It could be hypothesised this is because previous, and to some extent current, generations have developed their values in a society that prioritised social and economic sustainability over environmental. Thus, expecting them to make a generational shift in their values is unrealistic. As such, a shift to environmentally sustainable food production will have to come at a generational pace. The consumption of and demand for animal products is increasing (Cole *et al* 2018). Given that each step up a food chain (trophic level) sees approximately 90% of the initial energy produced, lost (Saito *et al* 2001) the more animal products, specifically meat, a person's diet contains, the less environmentally sustainable it is (Peters *et al* 2016). This is due to the fundamentals of carrying capacity, the number of individuals that can be maintained from a given set of resources. The populations of wild animals are governed by carrying capacity and species that are carnivores have smaller populations than herbivores (Rockwood 2015). The classic example of this is a food chain of grass, rabbits, and foxes (Figure 3). Due to the energy loss at each increase in trophic level, populations are more efficient and therefore numerous the lower the trophic level is. Specifically, at each level increase around 10% of the energy-yielding carbon compounds produced are incorporated into biomass at the next trophic level.

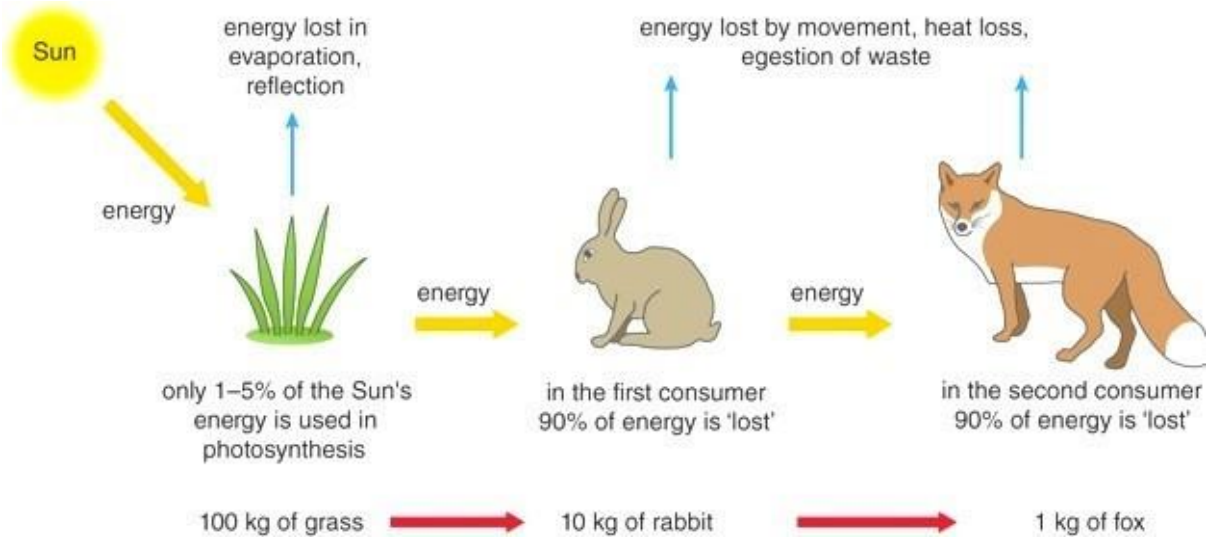


Figure 3. The common depiction of a simple food chain highlighting the loss of energy at each increase in trophic level. Figure from https://tinyurl.com/rtnueyp2

If this were applied to humans in an oversimplified hypothetical example, it becomes clear that more people could be sustainably (consistently and repeatably) fed from a given area of land by consuming food from the 1st trophic level rather than the 2nd (Figure 4).

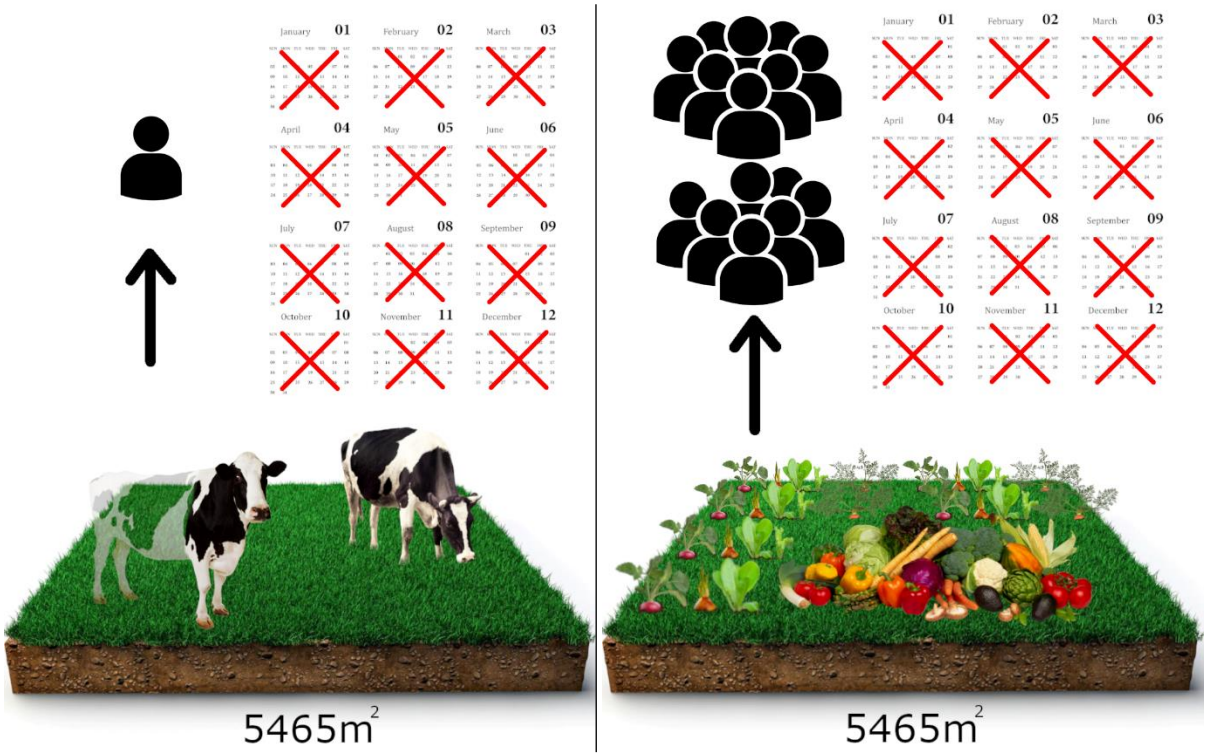


*Figure 4. Left; To meet the recommended daily calorie intake, averaged between men and women, of 2250cal (NHS 2022) for 1 year, by only consuming beef, would require 1.35 steer/bullocks. This is based on data from the US Department of Agriculture (USDA) which states a 1000lb steer will yeild 430lbs of retail cuts (*Dept. of Agriculture, food, & forestry, 2019*) and the average caloric value of all beef cuts is 262cal per 3oz (United States Department of Agriculture 2011). Using the agricultural rule of thumb which is 1 cow per acre, 5465m^2 would be needed to maintain 1.35 steer yielding enough calories to sustain 1 person for 1 year. Right; Comparatively, Jeavons (2001) found 371m^2 were required to grow enough vegetable matter to meet the calorific requirements of one person for one year. This is based on growing vegetables with a higher calorific value, using a realistic crop yield and includes space to grow crops to be used as fertiliser (Jeavons 2001). From these data, in the same space required to sustain 1 person on beef for a year, 14.7 people could have their calorie requirements met for 1 year. Figure created using GIMP 2.10.28.*

This is a rough, oversimplified example, and in reality, there are many factors that determine the sustainability of human food consumption. Water requirements, transport and the need for variation in the diet are just a few. Nevertheless, it emphasises the significance food vs feed competition has on global food security. An estimated three quarters of the world's agricultural area is devoted to producing livestock either directly or indirectly (Foley *et al* 2011). Of global crop production, Cassidy *et al* (2013) estimated 36% of calories and 53% of protein produced are used in feed for livestock. In addition, the production of feed has a high-water demand and livestock often

occupy arable land on which crops for direct human consumption could be grown (Manceron *et al* 2014, Mungkung *et al* 2014). In certain developed countries, food consumption trends are moving in the direction of less meat (Stoll-Kleemann and Schmidt 2017). However, in developing countries where, in general, population growth rates are higher, demand for animal products in the diet is expected to increase (FAO 2012, Gerbens-Leenes *et al* 2010). Given the predicted increase in demand and the negative affect food vs feed competition has on food security, reducing the competition between food and feed is a social and economic goal that could be met, or improved, in a way which also increases environmental sustainability.

1.4 The Blue Revolution

As mentioned, space represents one of the major challenges to meeting the predicted global demand for food. Rather than simply not increasing, the amount of arable land is expected to decrease. As population increases, global cropland per capita decreases with each new person requiring additional infrastructure (FAO 2020b). Additionally, the observed anthropogenic climate change and associated global warming is expected to further reduce the amount of land available for crop production. Predicted sea level rise would reduce the amount of arable land directly as well as indirectly through soil salinization and increased competition with human infrastructure (Pandey *et al* 2023, van Scheltinga *et al* 2023). What's more, the frequency and severity of natural disasters are expected to increase further exacerbating the problem (van Scheltinga *et al* 2023).

In the last decade, exciting advancements and innovations in agriculture have been made, such as vertical farming and the use of stem cell culture technology (Eibl *et al* 2018, Sandison *et al* 2022). However, it is expected that much of the increased demand for food will be met by food grown in the ocean in the so called 'Blue Revolution' (Costello *et al* 2020, FAO 2020a, Free *et al* 2022, Little *et al* 2016). This is largely because it has the space to do so. Currently, despite occupying around 71% of the earth's surface (United States Geological Survey 2019), only around 17% of global animal protein consumed is from seafood (FAO 2020a). Furthermore, aquaculture is the fastest growing food producing sector (Garlock *et al* 2020). Between 1950 and

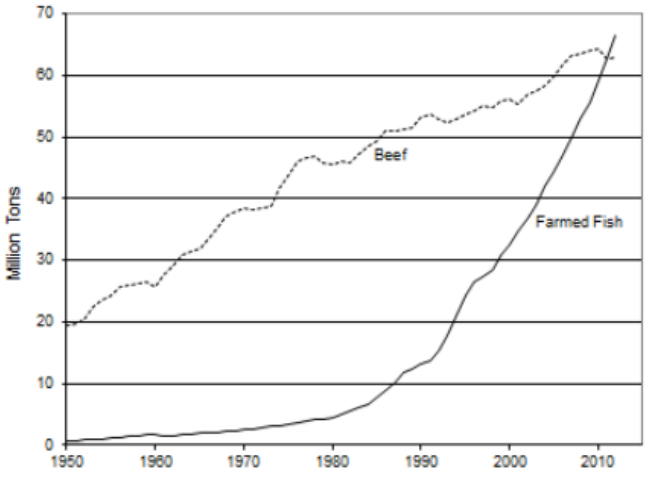


Figure 5. The amount of beef and farmed fish produced between 1950 and 2012 in million tons. Figure from Larsen and Roney (2013).

2006 fish farming grew on average, by 8.5% each year (Tacon and Metain 2008). This growth has occurred to such an extent that in 2011, farmed fish production (66million tons) exceeded beef production (63 million tons) (Larsen and Roney 2013) (Figure 5).

The demand for fish has recently increased partially due to an awareness of the health benefits over red meat, which is associated with a higher risk of heart disease, as well as an economic incentive as the rising cost of grain and soy has increased the price of grain fed meat (Larsen and Roney 2013). Fish consumption is set to keep rising and this demand will be met through farmed fish rather than wild caught (FAO 2020a). Whilst removing this demand would have a far greater environmental impact, it is unrealistic. As such, it is essential that the social and economic goal of increasing aquacultural output is done so in a way which also increases its environmental sustainability.

1.5 Fishmeal

As discussed, food vs feed competition represents the biggest restricting factor to the sustainability of many food production systems. This is the case to a larger extent in the farming of fish. Figure 4 shows how more people can be sustained by consuming food from the 1st trophic level as opposed to the 2nd. Figure 6 applies this same concept to aquaculture showing that eating certain fish species can be as high up as the 5th trophic level where 99.99% of the initial energy produced is lost.

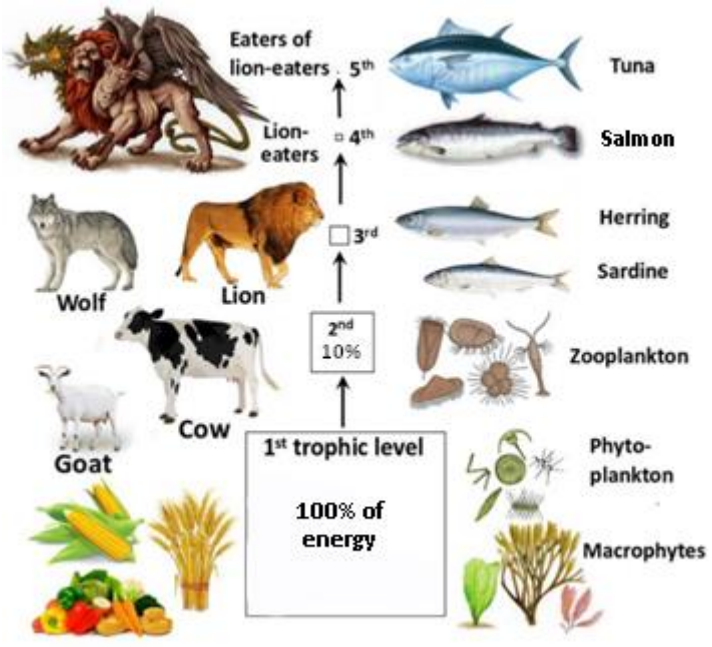


Figure 6. Right, the trophic levels of a marine food chain highlighting the consumption of Salmon or Tuna as consuming the 4th or 5th trophic order respectively. Left, what such trophic levels would look like in a terrestrial food chain. Figure modified from Kolding et al 2016.

The existence of such high trophic levels in the marine example and the seemingly absurd terrestrial comparisons suggests the food chains may behave in a different manner between the environments,

however, as can be seen in Figure 7, fish from capture fisheries is making up a continuously smaller percentage of global fish production (FAO 2020a) to which the food chain in Figure 6 is most applicable.

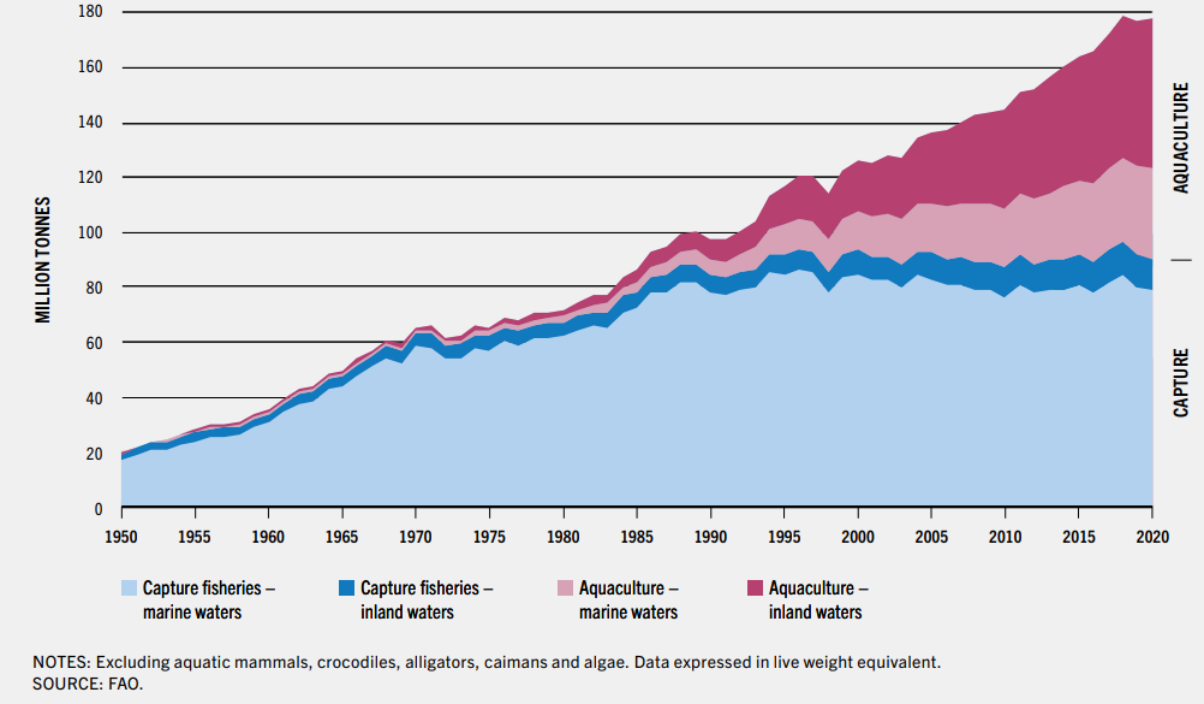


Figure 7. World capture fisheries and aquaculture production. From the FAO's most recent report on the state of world fisheries and aquaculture. Figure from FAO 2020a.

Nonetheless, figure 6 highlights an area with clear room for innovation and improvement towards increased efficiency and sustainability, capturing more of the available energy through the trophic levels consumed by people. Therefore, it comes as no surprise that the FAOs most recent "The State of World Fisheries and Aquaculture" report states that:

> *"Focus priority areas for innovative aquaculture practices are aquafeeds and feeding" (FAO 2020a, pg. 21)*

And;

> *" the future sustainability of the fed aquaculture sector nevertheless remains intimately dependent on the sourcing of new and nutritionally balanced feed components" (FAO 2020a, pg. 121)*

To support growth, feed needs a relatively high protein content, which must be palatable, digestible and have a suitable profile of amino-acids (Sánchez-Muros *et al* 2014). Protein that comes from animals is known to have a higher digestibility and better balance of amino acids than that which is plant based (Webster *et al* 1992, Refstie *et al* 1998). Consequently, fishmeal (FM) has been a key

ingredient in feeds (Barrows *et al* 2008). FM is a protein-rich flour made by milling and drying fish or fish parts. It is mostly produced directly from wild caught fish and, as a valuable resource with a limited legal framework and further limited enforcement of this, it has been overexploited (FAO 2020a, Tomberlin *et al* 2015). This has occurred to such an extent that FM production is decreasing and fell from 30.2 million tons in 1994 to 16.3 in 2012 (Tomberlin *et al* 2015). As FM becomes less readily available it is subject to higher prices (Van Huis *et al* 2015). The price rose from US$60/ton in 2005 to US$2000/ton in 2010 (Mungkung *et al* 2014). These higher prices can, in turn, encourage overfishing exacerbating the problem. The number of fish caught globally is going to decline further, thus increasing the price of fishmeal and further decreasing its availability (Lock *et al* 2015).

Whilst the effects of overexploiting fisheries are less visible (out of sight, out of mind), fisheries are requiring a much larger investment of time and money to achieve the same levels of catch and are catching smaller fish (Larsen and Roney 2013). Additionally, most fisheries that specifically catch fish to be used in the production of FM are classed as either recovering from overexploitation, or are over exploited (Alder *et al* 2008, Larsen and Roney 2013). Of all aspects of livestock production, fish farming is the most reliant on FM. In 2006, it used 68.2% of the total FM produced globally (Tacon and Metain 2008) and 73% in 2010 (Tomberlin *et al* 2015). This trend is confirmed to have continued by the FAO (2020a) as can be seen in Figure 8.

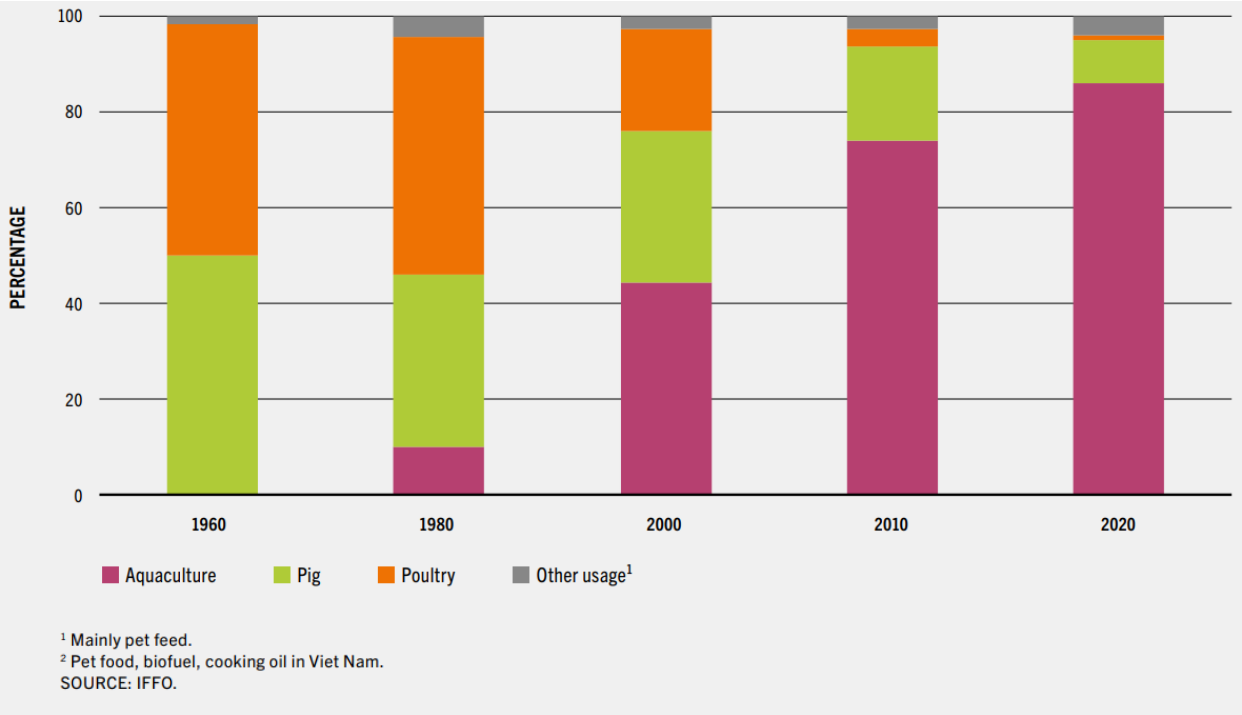


Figure 8. The global utilisation of fishmeal. Figure from FAO (2020a).

FM is steadily being replaced by vegetable-based proteins, however, as discussed, significant crop shortages are predicted in the future (Lock *et al* 2015, Tacon and Metain 2008). Consequently, a more sustainable protein source, with less food vs feed competition is required to meet future demand for animal feed.

1.6 Insect Meal

Insect meal (IM) has the potential to meet the demand for animal feed protein (Nijdam *et al* 2012, Rumpold and Schlüter 2013, Van der Spiegel *et al* 2013, Van Huis *et al* 2013, Makkar *et al* 2014, Mungkung *et al* 2014, Lock *et al* 2015, Payne *et al* 2016, Van Huis 2017). Insects contain more species than any other animal class and make up between 70-95% of species globally (Chapman 2009). Many of these have short life cycles, large population densities and high rates of reproduction (Yen 2015). This means a relatively large amount of protein can be generated quickly and from a comparatively small space. Farming insects requires less land area for production (Oonincx and De Boer 2012) and emits lower levels of GHGs than current methods of protein production (Oonincx *et al* 2010).

The protein from insects is, in general, both high in quantity and quality (de Guevara *et al* 1995, Ramos-Elorduy *et al* 1997). Mungkung et al (2014) collated information on the nutritional composition of a variety of insects and highlight their abundance of essential amino acids. In a similar collation, Makkar et al (2014) reported a crude protein content between 42% and 63% across a range of insect species. Furthermore, insects can often be raised on products that ordinarily have both an economic and environmental cost and can convert these into a valuable protein source (Mungkung et al 2014). Additionally, insects have a high feed conversion efficiency as they are ectotherms so do not spend energy maintaining body temperature (Nijdam *et al* 2012, Van Huis *et al* 2015).

Insects are already used in pet food (REGULATION (EC) No 1069/2009, Schabel 2010) and there is a long history of insect consumption in Asia (Bukkens 1997, Yen 2015). In China, insects have been eaten for at least 3,200 years as well as farmed and used as feed (Yi *et al* 2010). In South Africa, a caterpillar known as the mopane worm (*Gonimbrasia belina*) is more valuable than beef. During the harvest period of mopane worms, beef sales experience a significant fall. These mopane worms are so valuable that they are overharvested and in some areas are regarded as locally extinct. Similarly in Africa, the caterpillar *Cirina forda* is considered a delicacy and sold based on weight, the price of which is twice that of beef. (Womeni *et al* 2009). Two thousand different species of insect are already consumed globally (Van Huis *et al* 2015, Yen 2015) and are an integral part in the diets of

approximately two billion people (Makkar *et al* 2014). As the trophic levels of consumption have shown, the direct consumption of insect protein by humans would be more sustainable than using it to feed an animal which people then eat.

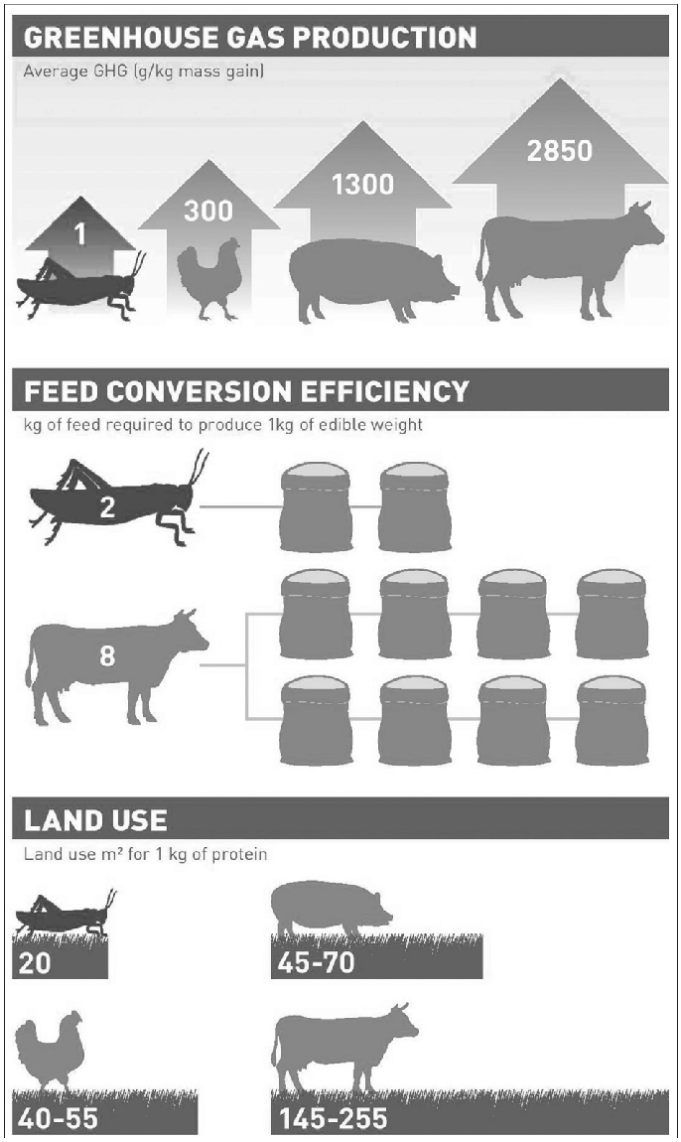


Figure 9 A comparison of the sustainability of protein production based on the type of animal being farmed. Figure from Giovanni 2015.

The staggering extent of this can be seen in Figure 9. The potential of insect farming to increase the sustainability of food production becomes even greater when it is considered that the land used by insects (Figure 9) does not need to be arable so further reduces food vs feed competition. But as discussed, expecting entire populations and societies to make such sudden changes at a social and/or economic cost is unrealistic. Instead, introducing farmed insects to the human food chain through animal feed could be a way to facilitate that shift more gradually and sustainably.

Given that insects are naturally in many fish species' diets (St-Hilaire *et al* 2007) the use of IM as a replacement for FM seems very logical. If you were asked to draw a cartoon person fishing, chances are there is a worm on their hook as bait, not a fish. In addition to increasing global food security and environmental sustainability, the development of a product that can replace FM could represent a significant economic opportunity. This use of IM in fish feed does already take place with various species of fish and insect and varying rates of inclusion. Examples of this are collated in Table 1. These studies highlight that the palatability of fish diets rich in IM rather than FM are good and can feasibly replace at least a proportion of the FM being used in aquaculture feed. Additionally, many of these studies also show that the inclusion of IM in the diet of fish does not alter the texture, aroma or acceptance

by the customer (Makkar *et al* 2014). This evidences the potential surrounding the replacement of FM with the more sustainable and cost effective IM.

Table 1. Collated information on the highest % of FM which could be replaced by relative insect meal before detrimental effects were observed with regards to the fish species being raised. These % are the amount of FM replaced in the diet of the fish an

Insect Species	Fish Species	Amount successfully replaced	Reference
Black Solider Fly	Atlantic salmon	100%	Lock *et al* (2014)
Black Solider Fly	Blue Tialpia	100%	Makkar *et al* (2014)
Black Solider Fly	Immature Channel Catfish	10%	Bondari and Sheppard (1987)
Black Solider Fly	Yellow Catfish	25%	Zhang *et al* (2014)
Black Solider Fly	Rainbow Trout	25%	St-Hilaire *et al* (2007)
Black Solider Fly	Rainbow Trout	50%	Sealey *et al* (2011)
Black Solider Fly	immature Turbot	33%	Kroeckel *et al* (2012)
Mealworm	African Catfish	40%	Ng *et al* (2001)
Mealworm	Gilthead Seabream	25%	Piccolo *et al* (2014)
Mealworm	Rainbow Trout	50%	Gasco *et al* (2014a)
Mealworm	European Seabass	25%	Gasco *et al* (2014b)
Silkworm	Carp	100%	Rahman *et al* (1996)
Silkworm	Silver Barb	38%	Mahata *et al* (1994)
Silkworm	Mahseer	50%	Shyama and Keshavanath (1993)

1.7 Fish oil

Fishmeal is not only sought after for its value as a complete (containing all essential amino-acids) protein source, but fish oils is also produced by the fish from which FM is made. Fish oil is the most significant source of the marine origin omega-3 fatty acids: eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Liland *et al* 2017). FM and the amino acids it contains are steadily being replaced with alternative sources, yet, these sources are mostly vegetable based protein (Lock et al 2016, Tacon and Metain 2008). The discussed competition for cropland means also feeding farmed fish with terrestrial grown feed and intensifying food vs feed competition is not a sustainable solution. But IM shows promise in addressing this (Ortuño *et al* 2021). However, vegetable oil cannot replace fish oil as it lacks the polyunsaturated fatty acids (PUFAs) which fish oil is rich in. These

PUFAs, specifically EPA and DHA are required in the diets of fish and are essential to the appeal of the final product to the consumer (Lock et al 2016). These omega-3 fatty acids can: reduce the risk of chronic pathologies and cardiovascular disease, are essential to brain and retina structure, promote cardiovascular health and have anti-inflammatory properties (Cazan 2009, Kris-Etherton *et al* 2002, Lauritzen *et al* 2001, McNamara and Carlson 2005, Simopoulos 1999a, Wall *et al* 2010). They can also boost brain function and development, ease rheumatoid arthritis, protect against the development of dementia and decrease depression (Ruxton *et al* 2004). As such, it is vital that any alteration in the feed of these fish does not significantly reduce their PUFA content. Nevertheless, of the studies in Table 1 only two; St-Hilaire *et al* 2007 and Liland *et al* 2017, account for this.

St-Hilaire *et al* (2007) supplemented Black Soldier Fly larvae (BSFL)'s diets with trout offal. This increased the EPA and DHA content of the larvae to ~0.7g/100g. However, it seems logical that this trout offal would have had a higher total EPA and DHA content than that of the final larvae. Yet, this does provide clear evidence that the nutritional composition of black soldier fly larvae can be manipulated through their substrate. Liland *et al* (2017) found that enriching the substrate BSFL were reared on with the brown macroalgae *Ascophyllum nododsum*, enriched the EPA concentration in the larvae from <LOQ to 0.14g/100g. This was done by enriching 50% of the growing media with algae and worked out as a recuperation rate of 18% of added EPA. However, it was suggested that most of the excess energy within the BSFL in this trial was stored as monosaturated and saturated fats with the PUFAs being used in biological function (Liland *et al* 2017). BSFL present an alternative protein source to replace fishmeal, and this switch is already taking place in the industry (FAO 2020a). However, further investigation into their potential as a fish oil replacement is required.

1.8 Wrack Flies

Some invertebrates have evolved to thrive on seaweed that washes ashore (wrack) and thus have the potential to provide a feed ingredient rich in the marine PUFAs. A single investigation of the nutritional composition of two species of wrack fly larvae, *Coelopa frigida* and *Coelopa pilipes*, by Biancarosa *et al* (2018), identified their potential as a feed ingredient reporting an EPA content of ~1.0g/100g. However, this study makes clear that some enhancement and improvement would be required before their nutritional composition represents a potentially viable feedstuff. There are several variables to be explored which may increase the marine PUFA content in the larvae of wrack flies and other wrack invertebrates.

The two species of wrack fly used by Biancarosa *et al* (2018) were *Coelopa frigida* and *Coelopa pilipes*. These two species are very closely taxonomically related. A review of the literature has

revealed at least 72 species of fly globally which are documented to breed in these detached macroalgae deposits (not including those from the *thoraochaetaeridea* or *tethina* genera as these are a very small species) coming from 7 different families and 21 genera (Mathis 2011, Mathis and Mcalpine 2011, Egglishaw 1958, Stenton-Dozey and Griffiths 1980, Mcalpine 1991). These are discussed further in Chapter 2. Given the taxonomic range these flies present, it is probable that different species will accumulate fatty acids, amino acids, nutrients and contaminants differently. Investigating a broader range of species would facilitate a better understanding of those most efficient at converting algae into protein and crucially, the marine origin PUFAs. Thus, further assessing their potential as an alternative to fish oil.

Similarly, different species of algae have different nutritional compositions (Biancarosa *et al* 2016,2017, Belghit *et al* 2017). Investigation of different seaweed species as the larval substrate could also alter their nutritional composition. Moreover, it is documented (Biancarosa *et al* 2017) that the chemical composition of algae differs depending on season and its growing stage. Manipulation could provide further opportunity to improve the nutrition available to the larvae and potentially their nutritional composition. In both wrack fly (Biancarosa *et al* 2018) and black soldier fly (Liland *et al* 2017) studies, larvae were not given time to clear their gut before being frozen for analysis. This is practised in other studies (Charlton *et al* 2015) and ensures the reported nutrient composition of the larvae is not influenced by any substrate still present in the gut of the larvae. However, leaving them too long may result in oxidation of the desired nutritional content. Thus, among other aspects, the potential of wrack flies and other wrack invertebrates to provide an alternative PUFA source will be investigated through these and other variables.

1.9 Research Objectives

As highlighted both by this chapter, and so clearly by the FAO in their most recent report on "The State of World Fisheries and Aquaculture" (FAO 2020a), to promote the sustainable growth of the aquaculture industry in response to its forecast increase in demand, innovations are required in aquafeed. This research project aims to assess what potential certain invertebrates, which feed on detached macroalgae, have to be one such innovative feedstuff. Throughout this thesis, such detached macroalgae will be referred to as wrack. However, it is worth noting, some sources may use the term 'wrack' to describe species of algae from the genus *Fucus*. This potential will be assessed through the following structure and research questions:

Chapter 2 introduces the principles of fatty acids, particularly those considered essential, before discussing wrack deposits and the macro-invertebrates found in them.

Chapter 3 reviews the ecology of wrack deposits and presents an alternative theory on the succession of their colonisation. Previous rearing of wrack macro-invertebrates in the literature are then discussed before exploring collected data to assess the following research questions:

- Which study species can successfully be cultured in a laboratory environment?
- To what extent do the species of algae making up the substrate, larval density and cage status influence this success?

Chapter 4 explores the use of ovipositional preferences of wrack flies as an indicator of optimal larval substrate and niche boundaries. In doing do, the following research questions will be investigated:

1. Does the substrate type influence the number of eggs laid on that substrate by any of the fly species?
2. Does the substrate type influence the success of offspring development of any of the fly species?

Chapter 5 aims to quantify the nutritional composition of eight wrack invertebrates and from this assess the following research questions:

1. Does the EPA or DHA content differ between the study species or within species between substrates?
2. Is the accumulation of contaminants different between the study species or within species between substrates?
3. Does the method of larval processing affect their EPA, DHA and/or contaminant composition?
4. Is the EPA, DHA and/or contaminant composition of a 2nd generation of larvae different from that of the 1st reared on the same substrate?

Chapter 6 investigates the use of proteomics as a regulatory tool in the field of insects as food and feed by asking the following research question:

- Can proteomics be used as a taxonomically accurate identifier of closely related wrack fly species?

Chapter 7 provides a discussion on the findings of the previous chapters and from these and assesses what potential the wrack invertebrates studied have to provide an alternative source of the marine origin PUFAs EPA and DHA. Currently, the most promising innovations in this industry are the culturing of microalgae, the main global producers of EPA and DHA, and the genetic modification of

food grade organisms to also produce these fatty acids (Gladyshev *et al* 2013). The potential of wrack macro-invertebrates will be discussed in comparison to these methods.

Chapter 2

Fatty Acids, Algae and Wrack

2.1 Fatty Acid Structure

To assess the potential of any fish oil replacement and the PUFAs, specifically EPA and DHA, it provides, it is important to understand these PUFAs. Fatty acids (FAs) are free individual molecules consisting of a carboxylic acid and an attached chain of carbon atoms with bound hydrogen atoms. These individual molecules are used as components in lipids, which are a group of hydrophobic (insoluble in water) compounds that include fats, oils and waxes. Fatty acids play a crucial role in all life as the membranes of cells, and of structures within a cell (organelles) are made from two layers of phospholipids. Additionally, they are essential to many biological functions such as the communication within and between cells, immune function and energy regulation (Nagy and Tiuca 2017). FAs are split into three groups; saturated, mono-unsaturated and poly-unsaturated (Figure 10). They can have between 2-28 carbon atoms and always contain an even number of them. The end carbon atom, furthest from the carboxylic acid, is known as the omega (ω) carbon. Saturated fatty acids (SFA) are those in which all carbon bonds are saturated (filled) with hydrogen atoms as can be seen in Figure 10A. Due to the structure of SFA, all

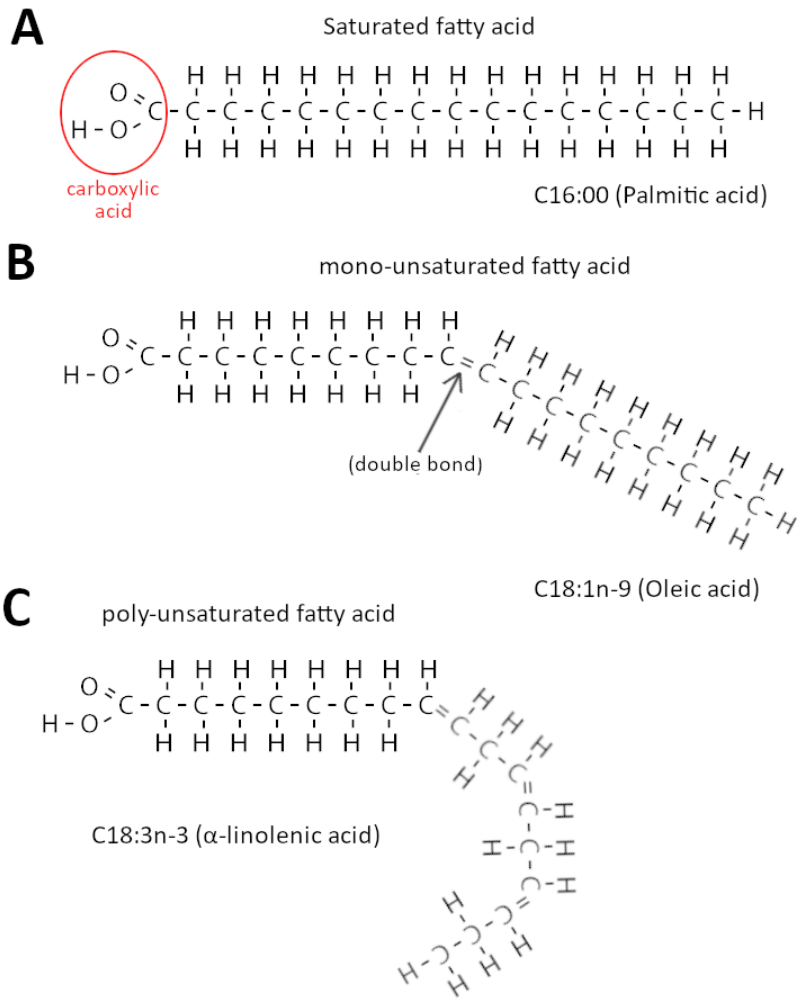


Figure 10. The structural differences between the 3 groups of fatty acid. Figure created using GIMP 2.10.28.

bonds are single and thus equally strong making them straight and pack together tightly. As a result, saturated fats are generally solid at room temperature. FAs are classified by the number of carbon atoms they have, as well as the number and position of double bonds when present. SFA contain no double bonds so are simply named by the number of carbon atoms they have, Figure 10A shows a saturated FA with 16 carbon atoms so is named C16:00, more commonly the C at the start of fatty acid names is omitted (16:00). Some FAs have a 'common' name and C16:00 is also known as palmitic acid from the French word palmier meaning palm tree and is the main component of palm oil.

Conversely, unsaturated FAs are those in which all carbon bonds do not have a bound hydrogen. Where a hydrogen atom does not fill the carbon bond, the available bond binds to the adjacent carbon atom creating a double bond (Figure 10B). Thus, the number of double bonds represents the number of unsaturated carbon atoms. Unsaturated fatty acids are further split by a prefix derived from ancient Greek: mono, from *mónos* meaning alone/only/single. Or poly, from *polús* meaning many. Mono-unsaturated fatty acids (MUFAs) like that in Figure 10B have only one double bond hence the prefix mono and poly-unsaturated fatty acids (PUFAs) have more than one. Unsaturated FAs are classified by both the number of carbon atoms they have and the position of the first double bond relative to the omega carbon (the end). Figure 10B shows a fatty acid with 18 carbons (C18), one double bond (1) and counting back from the omega (ω) carbon the double bond is first encountered on the 9^{th} carbon atom (omega minus 9; ω-9). Hence, this fatty acid is classified C18:1 ω-9. However, an n is more commonly used to denote the distance from the omega carbon, C18:1n-9. The double bond is stronger than a single bond, and this strength bends the rest of the carbon chain (Figure 10B). This shape means these fatty acids cannot be packed as tightly as saturated FAs and so are most often liquid at room temperature. PUFAs are classified in the same way, but the number of double bonds will always be greater than 1. Figure 10C shows a PUFA with 18 carbons and 3 double bonds, the first of which is three carbons away from the ω carbon so is C18:3n-3. If more than one double bond is present, they will occur three carbons from the previous double bond. Like MUFAs, the double bonds in PUFAs also bend the carbon chain. As there are more than one of these bends, their shapes are more complex and thus can be compacted the least. The difference in shapes within and between these groups allow fatty acids to perform different biological functions. In the case of figure 10C all the double bonds bend the chain in the same direction creating an inverted C shape, this is not necessarily the case in C18:3n-3 and is for illustrative purposes only, the double bonds can bend the chain in either direction depending on which side of the chain they occur. There is a fourth group called trans-fatty acids however, these

are not common in nature and so are not discussed here. So called “omega-3’s” are well known for their health benefits and it can be a common misconception to assume this is because they have three “omega pieces”. As demonstrated, they are named thus because their first double bond is three carbons from the ω carbon, something all fatty acids have. Figure 10B shows an omega-9 FA. Omega -3 (ω-3) and omega-6 (ω-6) FAs are always PUFAs.

It is important to note that fat and fatty acids are not synonymous. Fatty acids are free individual molecules, but they rarely occur as such. Most often they are found as part of a more complex lipid (Nagy and Tiuca 2017). Triglycerides, better known as fat, are one such lipid and are used as an energy store as lipids produce more energy than that of the same weight of other carbon compounds, carbohydrates or proteins (Gladyshev *et al* 2013). These triglycerides (fat molecules) are comprised of a glycerol molecule, often called the head, with three FAs attached, referred to as the tails. They can be imagined as a jellyfish like structure with three tentacles.

2.2 Fatty Acid Synthesis

Along with other complex organisms including fish and mammals, humans can synthesise all the fatty acids required for normal bodily function, apart from ω-3 and ω-6 FAs (Castro *et al* 2011, Gladyshev *et al* 2013, Öz *et al* 2022, Rustan and Drevon 2001). As the body cannot synthesise these FAs they are known as essential meaning they must be present in the diet. These two groups (ω-3 and ω-6) are vital for several mechanisms within the body such as gene interactions and membrane fluidity (Öz *et al* 2022). For example, 22:6n-3, also known as docosahexaenoic acid (DHA), as can be seen from the name, is an omega-3 with 22 carbon atoms and 6 double bonds. This makes it a relatively complex FA and, as such, when incorporated into a cell membrane it creates a more open/loose structure. This openness, among other things, facilitates faster signalling between cells. Around 60% of the human brain is comprised of fatty acids and owing to this faster signalling around 50% of these FAs are DHA (Kaur *et al* 2014). Hence, DHA is vital to normal brain function, health and development (Öz *et al* 2022, Rustan and Drevon 2001, Weiser *et al* 2016). DHA is also vital to visual health and development; Birch *et al* (2010) found that the visual sharpness of infants given DHA in their food from birth to 12 months old was significantly better than that of the control group after the 12 months (Birch *et al* 2010). Additionally, EPA and DHA are documented to: decrease the risk of heart disease and sudden cardiac death, be structurally and functionally important to the nervous system, promote healthy skin, reduce insulin resistance decreasing the risk of type 2 diabetes, supress the development of some cancers, reduce asthma risk and protect against depression or rather, their absence facilitates depression (Öz *et al* 2022). Given these functions ω-3 FAs perform in

the body are so crucial to survival, it is surprising, and even incredible, that we, along with most other organisms, have evolved in such a way that we are unable to produce them.

Whilst all ω-3 and ω-6 FAs cannot be synthesised *de novo* (from new), due to their physiological importance only the following are broadly considered essential, from the ω-3s: α-linolenic acid (ALA) C18:3n-3, eicosapentaenoic acid (EPA) C20:5n-3 and docosahexaenoic acid (DHA) 22:6n-3. From the ω-6s: linoleic acid (LA) C18:2n-6, gamma-linolenic acid (GLA) C18:3n-6 and arachidonic acid (AA) C20:4n-6 (Kaur *et al* 2014). Additionally, the ω-3 FA docosapentaenoic acid (DPA) C22:5n-3, has recently been the subject of interest and is increasingly regarded as one of the essential FAs (Calder 2021, Drouin *et al* 2019).

The ω-6 FAs are readily abundant as they can be synthesised by most plants (Gladyshev *et al* 2013, Meyer *et al* 2003, Simopoulos 2001) and thus are regularly consumed through cereals and cereal products, vegetable products, nuts and seeds, meat which has been reared on cereals and most of all, through vegetable fats and oils (Meyer *et al* 2003). The ω-3 ALA can also be synthesised by most plants and is rich in some vegetable sources such as green leafy vegetables, flaxseed, pumpkin seeds, walnuts, legumes and rapeseed (Öz *et al* 2022). There is a common misconception that only the ω-3 ALA is essential and that all other ω-3s can be produced from this. Whilst it is well documented that the body can produce the ω-3s EPA and DHA from ALA (Gladyshev *et al* 2013, Öz *et al* 2022, Stark *et al* 2008), it is equally well documented that the conversion efficiency of this is poor and cannot provide sufficient amounts to meet our requirements (Gladyshev *et al* 2013, Öz *et al* 2022, Wall *et al* 2010, Ruiz-Lopez *et* al 2012). Therefore, it is crucial that all essential FAs, particularly the ω-3s, EPA and DHA, are present in people's diets (Coletta *et al* 2010). This is especially the case in pregnant women as many studies have shown the importance of ω-3s on the development of fetal brain and retina, cognitive function and ability of the infant(s) once born and in preventing post-partum depression (Coletta *et al* 2010, Helland *et al* 2003, Judge *et al* 2007).

De novo synthesis of EPA and DHA is a much rarer physiological trait. They can only be synthesized by algae and some protists, fungi, mosses and bacteria (Gladyshev *et al* 2013, Leblond *et al* 2003, Pernet *et al* 2003, Sayanova and Napier 2004, Taipale *et al* 2020, Uttaro 2006). Examples of these and their contents were collated from the literature by Gladyshev *et al* (2013) (Table 2). As can be seen from this table, microalgae, in particular, diatoms, cryptophytes and dinophytes synthesise considerable amounts of EPA and DHA. Globally, algae synthesise more than half of all EPA and DHA and are the main source of these PUFAs (Gladyshev *et al* 2013, Guschina and Harwood 2009).

Of terrestrial plants, only soil algae and some lichens are known to produce EPA and DHA (Gladyshev *et al* 2013). Certain symbiotic arbuscular mycorrhizal fungi as well as some saprotrophic fungi can also synthesize EPA and/or DHA (Table 2). However, these fungi, soil algae and lichen species are not very abundant in comparison to algae (Gladyshev *et al* 2013, Rillig 2004). Interestingly, Sempedro *et al* (2006) found that compared to the soil they were in, earthworms (*Lumbricus terrestris*) had high concentrations of EPA and DHA, and it originated in their gut suggesting some gut microorganisms can synthesise these PUFAs (Sampedro *et al* 2006). Similarly, the nematode *C. elegans* appears to be able to synthesize EPA de novo (Tanaka *et al* 1996).

Table 2. Adapted from Gladyshev et al 2013. EPA (20:5n-3) and DHA (22:6n-3) contents in organisms capable of their synthesis. Values give in mg per g of carbon in their biomass

Taxon	20:5n-3	22:6n-3	Sum
	Microalgae		
Eustigmatophyceae			
Nannochloropsis limnetica	100.9	0.0	100.9
Cryptophyceae			
Cryptomonas sp.	29.9	5.3	35.2
Rhodomonas lacustris	26.2	7.6	33.8
Rhodomonas lacustris	44.5	3.0	47.5
Rhodomonas salina	22.6	17.9	40.5
Rhodomonas salina	20.7	13.3	34.0
Rhodomonas sp.	5.8	2.9	8.7
Bacillariophyceae (Diatomea)			
Chaetoceros calcitrans	20.6	2.1	22.7
Cyclotella meneghiniana	40.8	11.4	52.2
Thalassiosira oceanica	45.9	7.5	53.4
Thalassiosira weissflogii	18.6	3.2	21.8
Dinophyceae (Peridinea)			
Gyrodinium dominans	11.1	38.4	49.5
Oxyrrhis marina	4.0	47.6	51.6
Prorocentrum dentatum	0.5	6.2	6.7
Peridiniopsis borgei	8.3	11.7	20.0
Prymnesiophyceae			
Isochrysis galbana	3.7	22.1	25.8
Isochrysis galbana	8.1	27.0	35.1
Phaeocystis globosa	1.7	4.9	6.6
Prasinophyceae			
Tetraselmis suecica	22.0	0.0	22.0
	Macroalgae		
Pheophyceae			
Laminaria saccharina	1.7	0.0	1.7
Laminaria digitata	1.5	0.0	1.5
Fucus vesiculosus	1.5	0.0	1.5
Undaria pinnatifida	4.5	0.0	4.5
Halidrys siliquosa	0.5	0.0	0.5
Rhodophyceae			
Porphyra umbilicalis	26.1	0.0	26.1
Chondrus crispus	1.0	0.0	1.0
Palmaria palmata	13.4	0.1	13.6
Gracilaria verrucosa	5.2	0.0	5.2
	Water moss		
Bryophyta			
Fontinalis antipyretica	10.1	0.4	10.5
	Arbuscular mycorrhizal fungi		
Glomeromycota			
Glomus spp.	6.0	0.0	6.0
Glomus irregulare	6.6	0.0	6.6
	Saprotrophic fungi		
Zygomycota			
Mortierella alpina	8.1	0.0	8.1
	Thraustochytrids		
Labyrinthulomycetes			
Aurantiochytrium sp.	5.4	86.4	91.7
Schizochytrium sp.	7.8	30.0	37.8
Thraustochytrium sp.	14.7	56.6	71.3
	Bacteria		
Gamma Proteobacteria			
Shewanella putrefaciens	2.4	0.0	2.4
Shewanella gelidimarina	11.0	0.0	11.0

Whilst there are some terrestrial sources of EPA and DHA, their contribution to global production is considered negligible (Gladyshev *et al* 2009). Given the physiological importance of these PUFAs, how then do terrestrial animals obtain them? Following synthesis, mostly by aquatic algae, EPA and DHA pass up through various food chains to terrestrial omnivorous and carnivorous consumers (Gladyshev *et al* 2013). The global export of these PUFAs from aquatic to terrestrial ecosystems via the main vectors was estimated by Gladyshev *et al* (2013). They estimated riparian predators export 2 million kg/y, shore deposits of carrion and seaweed export 24 million kg/y, the emergence of amphibiotic (aquatic larval stage) insects export 240 million kg/y and water birds export 432 million kg/y (Gladyshev *et al* 2013). The total natural export is estimated as 0.2% of all EPA and DHA production (Gladyshev *et al* 2013). They also estimated the global anthropogenic

export by generalising an average quantity of 2mg/g wet weight across total world catch and concluded a value of 180 million kg/y. Whilst this is an underestimate, as it only accounts for declared commercial export, it still suggests both amphibiotic insects and water birds export more EPA and DHA from aquatic to terrestrial ecosystems than do humans. This highlights just how important these food chains are for the global health of all terrestrial omnivores and carnivores, not just humans.

As discussed in Chapter 1, at each increase in trophic level, only around 10% of energy (carbon) is passed on. The food chains which export EPA and DHA to terrestrial ecosystems are relatively long and with an expected loss of 90% of these PUFAs at each trophic level increase, it seems improbable that sufficient quantities would reach terrestrial consumers. However, Gladyshev *et al* (2013) found that the transfer efficiency of the essential PUFAs was 3x higher than that of non-essential fats, including non-essential PUFAs. This again shows their importance as they are naturally conserved and not oxidised. Gladyshev *et al* (2011) found that this transfer efficiency was reduced between trophic levels in a river ecosystem as a result of pollution by copper (Cu), lead (Pb), and cadmium (Cd).

The importance of the essential FAs EPA and DHA is something evidenced in nature; birds have been documented preferentially feeding on amphibiotic insects as opposed to terrestrial insects and they have up to 35 times more ω-3 PUFA (Shipley *et al* 2022). Many studies, such as that by dos Santos Aguilar (2021), conclude a high ALA content fulfils all ω-3 PUFA requirements. As previously discussed, this is not the case and is evidenced by Twining *et al* (2016). In a full factorial design, Twining *et al* (2016) manipulated the quality and quantity of food in diets of tree swallow chicks (*Tachycineta bicolor).* These diets were either; high in EPA and DHA or, low in EPA and DHA but high in ALA; each of these treatments was then also split into either high or low quantity. They found chicks on both high and low quantity high EPA and DHA diets grew faster, were in better condition, had greater immunocompetence and lower basal metabolic rates compared to chicks on both high and low quantity ALA rich diets. Their findings suggested that tree swallows time their breeding with the emergence of aquatic insects high in EPA and DHA (Twining *et al* 2016), and this was later confirmed as a driver for breeding success (Twining *et al* 2018). Evidently, these ω-3s are of vital importance for the healthy development and maintenance of most animals even if the exact mechanisms of this importance are not fully understood (Gladyshev *et al* 2013). The scarcity of the ω-3s is what makes them of greater importance than the essential ω-6 FAs; the ω-6s are so abundant that our overconsumption of them goes full circle and is regarded as excessive (Candela *et al* 2011, Simopoulos 2002).

2.3 Too much of a good thing

Both ω-3 and ω-6 are necessary for healthy bodily function however, the ratio of ω-3: ω-6 is equally important (Candela *et al* 2011, Simopoulos 2002). Crawford *et al* (1999) evidence a strong association between the evolution of cognitive ability in *Homo* species (sp) with dietary changes that utilised more food from aquatic food chains. The evolution of the species *Homo erectus* around 2 million years ago marked a significant increase in the cognitive ability of species from the *Homo* genus (Crawford *et al* 1999, Wood 1996). In the evolution of closely related Hominid species as body size increased, relative brain size decreased. For example, chimpanzee's (*Pan troglodytes*) brains make up around 0.5% of their body weight (Crawford *et al* 1999). Conversely, as species from the genus *Homo* evolved, relative brain size increased to the roughly 2% we *Homo sapiens* have today (Crawford *et al* 1999). Crawford *et al* (1999) present compelling evidence, supported by the fossil record, that this increase in brain size was facilitated by diets rich in ω-3 PUFAs. This suggests our brains evolved to be dependent on dietary ω-3 FAs. This is supported by the evidence already discussed that our brains are some 30% DHA and that infants whose diets were supplemented with DHA showed increased cognitive ability. Our inability to convert the more common ALA to sufficient quantities of EPA and DHA attests to the diet dependence of this further. This would also explain why terrestrial herbivores can acquire sufficient EPA and DHA as they will have a lower cerebral demand, thus can meet their requirements from ALA conversion alone. What's more, in humans 50–70% of oral doses of ALA are oxidised (used for energy) within 24 hours, in comparison, DHA is selectively incorporated into cell membranes, and only 15% is oxidised (Broadhurst *et al* 2002, Plourde and Cunnane 2007). This shows a physiological awareness of the importance of these dietary FAs.

However, when this evolution took place the diets of *Homo sapiens* were very different from that of their descendants today. As such, our nutritional intake today, does not match that which our genetic make-up was naturally selected and is programmed for (Candela *et al* 2011, Konner and Eaton 2010, Simopoulos 1999b, 2002). This dietary change, to which we are not genetically suited, promotes a range of chronic diseases and illnesses such as atherosclerosis (narrowing of the arteries), hypertension, heart disease, obesity, food intolerances, diabetes and some cancers (Eaton and Konner 1985, Konner and Eaton 2010, Simopoulos 2002).

The earliest discovered *Homo sapien* remains are 30-40'000 years old. Since then, despite some significant cultural and technological advancements, our genetic make-up has changed little (Crawford *et al* 1999, Eaton and Konner 1985). As evidenced by Zuk (2013), it has undoubtedly changed more than "common knowledge" would have many people believe, but is still relatively

unchanged when compared with the rapid exposure to selection pressures *Homones sapientes* are enabling. One such advancement was the development of agriculture around 10'000 years ago which we are still not genetically equipped for today (Eaton and Konner 1985, Innes and Calder 2018, Shetty *et al* 2023). For example, the development of agriculture and domestication of ruminants meant milk became readily available. However, as in most mammals, the DNA of humans was programmed to "switch off" the enzyme lactase following the weaning phase (Gerbault *et al* 2011). Lactase is responsible for breaking down the main carbohydrate present in milk, lactose. As such, adult humans were unable to digest this readily available milk. Instead, the processing and fermenting of milk into products such as cheese and yoghurt greatly reduced the lactose content making it more digestible. However this came at a time, energy and resource cost (Salque *et al* 2013). In response to this cultural evolution and introduced selection pressure, an evolutionary adaptation took place. A mutation to DNA meant the enzyme lactase was produced into adulthood, this trait is known as lactose persistence (LP). Individuals with this trait had a competitive advantage over those without it as they now had access to high quality nutrition relatively easily compared to the complicated processes of creating milk products (Salque *et al* 2013). As a result, these individuals were more likely to survive bad harvests and had increased calcium and vitamin D in their diets giving positive selective pressure (Leonardi *et al* 2012). At the same time, those who were not lactose persistent (lactose intolerant) would have been subject to symptoms of lactose intolerance if they ingested too much lactose, potentially exerting a negative selective pressure. The result of this competitive advantage was a clear example of natural selection and the relatively rapid spreading of this evolutionary adaptation in one of humans' most recent genetic mutations (Eaton and Konner 1985, Evershed 2022, Salque *et al* 2013). Despite the speed this adaptation spread at, helped by convergent evolution (Leonardi *et al* 2012), only around a third of humans globally are lactose persistent today (Itan *et al* 2010).

Around 10'000 years on from the domestication of the first animals, the majority of the adult human population are still unable to digest milk. This clearly evidences the imbalance between our genetic makeup and our lifestyles which has occurred because we, as a species, are evolving culturally and technologically, much faster than we can genetically evolve, at least naturally. Furthermore, the rate of our technological advancement is exponential and this imbalance has become especially pronounced in the last ~200 years. The industrial revolution has facilitated cultural evolution at an unprecedented pace. Given that we are not even genetically equipped to deal with the lifestyle changes brought about by the agricultural revolution some 10'000 years ago, it should come as no surprise that our lifestyles today are often having fatal consequences. It is very conceivable that this

exponential change to our lives, to which we are not genetically equipped, may result in selection pressures to which we cannot genetically adapt in time. Essentially, we could ultimately be working towards the creation of a niche in which we cannot survive. On the other hand, this technology is also likely to facilitate our survival through innovations such as gene modification and medical advancements. This raises a fascinating question which is well outside the scope of this thesis, but is too thought provoking not to include:

> Is the medical treatment of negative selective pressures exacerbating negative selective pressures?

Nonetheless, until such innovations entirely realign our diets and our genetic makeup, they remain at an imbalance. Mass food production systems facilitated by the industrial revolution have set our diets the furthest from that of which we genetically evolved than they have ever been (Eaton and Konnor 1985). This separation is evident in the ratio of ω-3: ω-6. Our ancestors' diets, which, as discussed, may have facilitated the evolution of our cognitive ability, were comprised of an approximate ω-3: ω-6 ratio of 1:1-2, so for every unit of ω-3, 1-2 units of ω-6 were consumed (Candela *et al* 2011, Simopoulos 2002). Due to the scarcity of ω-3 and prevalence of ω-6, particularly in cooking oils, in our diets today, a ratio of 1:25-50 is common (Abedi and Sahari 2014, Doughman *et al* 2007, Shetty *et al* 2023). Whilst there are clear negative effects of this ratio being too high (the reverse of that which increased ω-3 improves), the mechanisms of these effects are less clear (Abedi and Sahari 2014, Doughman *et al* 2007, Sargent *et al* 1999, Simopoulos 2002). It seems they are a result of both ω-3 and ω-6 competing as they can provide opposing metabolic functions (Sargent *et al* 1999, Shetty *et al* 2023, Simopoulos 2002). For example, ω-6 facilitates inflammation, which is a necessary bodily function, while ω-3 inhibits it (Bernaerts *et al* 2019, Innes and Calder 2018, Shetty *et al* 2023). Owing to the importance of this ratio, it is vital that ω-3 and ω-6 intakes are not considered individually, but relative to each other.

2.4 The Wonders of Algae

The essential ω-3 and ω-6 fatty acids, and the ratio between them, are fundamental to the survival of all vertebrate species as well as many invertebrates (Castro *et al* 2012, Twining *et al* 2018). With more than half of all global EPA and DHA being produced by algae (Gladyshev *et al* 2013), it is no stretch of the imagination to say they are central to the global ecosystem. However, algae are far more central than this alone. For centuries algae have been used for food, medicine and in agriculture. More recently they have also become valuable in the industrial production of many products such as plastics, chemicals, lubricants, biofuels, food additives and cosmetics (Ahmad *et al* 2022, Chapman 2013, Goswami *et al* 2015, Kandale *et al* 2011, Pooja 2014, Wang *et al* 2015).

Furthermore, algae have, and continue to fuel much of our societal and technological advancement as major oil and gas deposits are largely comprised of marine algal deposits from the Cretaceous period (Chapman 2013). In addition, recent innovations show there are still undiscovered benefits of algae: Koch *et al* (2020) evidences the potential of cyanobacteria for a sustainable, carbon neutral, industrial production of the bio-plastic poly-hydroxy-butyrate. Some species of red algae are anti-methanogenic and thus can inhibit methane production in ruminants when present during digestion (Abbott *et al* 2020). Roque *et al* (2021) found supplementing the diet of beef steers with the red algae *Asparagopsis taxiformis* at an inclusion rate of 0.5% reduced methane production by 68%. Similarly, Ford *et al* (2020) evidence the potential seaweed inclusion in livestock diets has in limiting antibiotic resistance as the polyphenols from seaweed species can offer a natural source of antimicrobials. Lastly, Beal *et al* (2018) and Froehlich *et al* (2019) highlight the potential seaweed farming has as a tool for offsetting climate change through bioenergy carbon capture and storage without competing with agriculture.

Yet, all of this only scratches the surface of how fundamental algae are to the world we live in today. 4.5 billion years ago the earth formed but no life was present. Around a billion years later, as the earth cooled and stabilised simple, single cell organisms in the form of bacteria and archaea emerged. These types of simple, single cell organisms are known as prokaryotes. As these evolved to fill different niches, some 3 billion years ago the first photosynthesising organisms appeared in the form of cyanobacteria. Through their photosynthesis, cynobacteria began to change the chemical composition of the earth. Oxygen (O_2) is a bi-product of photosynthesis and thus these organisms slowly began to oxygenate the earth (Chapman 2013, Kump 2008). Furthermore, this introduction of O_2 also created ozone whereby the ultraviolet (UV) radiation from the sun split these O_2 molecules into individual O molecules which then bound to O_2 to create O_3 (ozone). Ozone then formed a layer around earth which absorbs and reflects the most potent of the suns UV radiation, UV-C rays. Until this point, organisms were confined to living in water as the UVC radiation, if on land would have caused mutations which most often resulted in the death of the organism. The development of this ozone created a more habitable terrestrial environment.

Another 0.5 billion years of evolution in this chemically changing environment and some of these simple, single cell prokaryotes evolved into eukaryotes. Eukaryotes are more complex organisms which can be multicellular, the crucial difference between prokaryotes and eukaryotes are that the more complex eukaryotic cells contain a nucleus and the organelles within the cell have their own membrane. All organisms apart from bacteria and archaea are eukaryotes. It is hypothesised that these first evolved as a result of endosymbiosis where a simple prokaryote which had developed a

nucleus engulfed another organism in a primitive form of eating. However, the engulfed organism did not break down and instead a symbiotic (mutually beneficial) relationship formed whereby the ingested cell benefited the cell it was in, likely through the production of energy, which in turn gave it protection from heterotrophs. This theory was first proposed by Mereschkowsky (1905).

Today, algae produce around half of all oxygen and they do so with impressive efficiency as the total global biomass of algae is approximately one tenth of all other plants (Chapman 2013). Similarly, Phytoplankton account for <1 % of the photosynthetic biomass on Earth but are responsible for nearly 50 % of global net primary production (Field et al 1998). Furthermore, all land plants which we rely on for food and materials evolved from algae (Chapman 2013). So, algae are central to the evolution and continuation of almost all life on Earth, but what are algae?

2.5 The *Colocasia* in the room

At this point, it seems appropriate to address the elephant ear plant (*Colocasia*) in the room. The definition of a plant can be a source of surprising controversy and some people would protest (vigorously) that plants are only terrestrial organisms with a vascular system and roots (Chapman 2013). What then of multicellular aquatic 'organisms' which have roots, photosynthesising leaves and a stem to transport nutrients between the two, such as *Hygrophila corymbose*? Okay, then perhaps aquatic species can be included in the definition. Perfect, but does this mean mosses are not plants? Mosses do not have a vascular structure (Charron and Quatrano 2009) and while mosses have rhizoids which provide the same anchoring function as roots (Jones and Dolan 2012) they do not take up nutrients as a root is defined to. If anything, the rhizoids of mosses are more akin to the holdfasts of what are commonly referred to as seaweeds, so if mosses are plants seaweeds must be too. But hold on a second, now we're really crossing a line, seaweeds are algae and algae are definitely not plants. The truth of that statement now depends on both the definition of a plant and of an alga. This debate can (and does) rage all the way back to those first photosynthesising organisms, cyanobacteria, and even beyond to if archaebacteria technically photosynthesised (Chapman 2013). Different definitions likely suit different needs and specificities. As this thesis is not on the evolution of plants (as interesting as it is) broader definitions will make for easier reading. Therefore, here, plants are defined as any organism which produces its own energy through oxygenic photosynthesis and algae are defined as any aquatic plant which does not have roots. Thus, for the purposes of this thesis cyanobacteria are included as algae.

Using these definitions, some of the first eukaryotes were algae, a photosynthesising cyanobacteria within another cell. These individual organisms, along with other microscopic algae, cannot be seen

with the naked eye and as such are known as microalgae. Under the right conditions however, microalgae can form blooms which are a collection of microalgae so numerous they can be seen without a microscope. Such blooms can be toxic. Microalgae are also known as phytoplankton, phyto meaning of a plant and plankton meaning an aquatic organism which cannot move against the current. Organisms which cannot move against the current but are not plants (eukaryotic photosynthesisers) are known as zooplankton. The definition for zooplankton can also be debated but broadly speaking represents all aquatic organisms that are consumers (heterotrophs) rather than producers (autotrophs) that cannot move against the current. Under this definition they range from single cell organisms, through some crustaceans and insect larval stages to jellyfish. However, zooplankton is also often used to define only those which are microscopic.

A particularly interesting group of these early microscopic organisms are called dinoflagellates which can be classed as both phyto and zoo plankton as they can both photosynthesise and consume depending on their conditions. Some dinoflagellates are also bioluminescent. For the purposes of this discussion, they will be classed as microalgae as they are photosynthesising organisms. Microalgae are an old and diverse group of organisms, but commonly the four most abundant groups are discussed, dinoflagellates being the first (Patras *et al* 2019, Garcia *et al* 2017). These can be very abundant achieving high densities (Graham and Wilcox 2000) and also have a symbiotic relationship with corals. Coral bleaching is a result of dinoflagellates leaving their hosts due to unfavourable conditions such as pollution or temperature change (Chapman 2013). The next group are called diatoms and are unique in that they make their cell wall out of silica so quite literally live in glass houses. These silica coatings make them very slow to decompose and so form deposits on lake and ocean floors (Chapman 2013). Over time these deposits fossilised into a sedimentary rock known as diatomite which is an industrially valuable resource and is used in the production of a wide range of products (Reka *et al* 2014). Similarly, Coccolithophores are a group of microalgae which coat themselves in calcium carbonate platelets. This makes them a significant carbon sink as they both consume carbon during photosynthesis and deposit it onto their exterior. Thus, as they die and sink to the bottom it becomes trapped in sediment layers. These can also fossilise into a sedimentary rock and create chalk deposits such as the white cliffs of dover. The last, and most abundant microalgae are the already discussed cyanobacteria, some species of which are among the 0.01% never to have gone extinct (Chapman 2013, Remize *et al* 2021).

From these microalgae, macroalgae evolved. The definitions of all algae and where one becomes the next are messy and often overlap. Generally, the term macroalgae is used synonymously with the word seaweed to describe any visible (macroscopic) algal organism. This does not include blooms of

microalgae as it is not the individual organisms which can be seen. Confusingly, this means seaweed also describes freshwater macroalgae and thus is not confined to the sea. Macroalgae are further split into three groups: Phaeophyta (brown algae), Rhodophyta (red algae) and Chlorophyta (green algae). These groupings are largely based on the different photosynthetic pigments each type have, which give them their colouration. However, there are a few structural markers for the groups as well. Brown algae contain the following photosynthetic pigments chlorophylls *a*, c_1, and c_2, β-carotene, diatoxanthin and fucoxanthin. The xanthophyll fucoxanthin mask the other pigments giving the brown-olive green colour (Anderson 2004, Graham *et al* 2009, Wehr 2015). All but 1% of brown algae are marine (Wehr 2015). Red algae contain chlorophyll *a* and phycoerythrin, the latter of which gives the red colour. Red algae are mostly marine but not uncommon in freshwater (Dodds 2002, Sheath and Vis 2015). Lastly, green algae contain chlorophyll *a* and *b* and are more commonly found in freshwater than the ocean (Bunker *et al* 2017, Leliaert *et al* 2012).

Whilst these groupings are usually used to split macroalgae, they can also add confusion to the distinctions between microalgae. Green algae is routinely used to specify any algae containing chlorophyll *a* and *b* as the dominant pigments. A prime example are species from the genus *Volvox* which are well studied as they mark a crucial stepping-stone in the evolution of multicellular organisms; these are consistently referred to as green algae despite being microscopic (Umen 2020). In reality, green algae seems most frequently used to refer to any algae which is not a red or brown seaweed/macroalgae. But even this leaves room for some confusion as brown algae can also refer to the microalgae diatoms as they too contain the pigments diatoxanthin, fucoxanthin and β-carotene which give them a brown colour (Kuczynska *et al* 2015).

In summary, the same organism can be classified in several ways creating controversy, confusion and debate. For example, the genus *Sargassum* are marine algae, which in recent years have received much attention as a result of the great Atlantic *Sargassum* belt (GASB). In the 15th century, Christopher Columbus reported these large mats of floating seaweed and they are such a phenomenon that an area of the Atlantic Ocean is called the Sargassum Sea. These mats create essential habitat which are often teaming with marine wildlife and are described as 'upside down coral reefs' (Butler *et* al 1983). Prior to 2011, these mats or belts of *Sargassum* were not a permanent feature, but since then have been. This has grown to form an enormous belt which in 2018 was 8850km long, weighed more than 20 million tons, and, at times, extended from West Africa to the Gulf of Mexico (Wang *et al* 2019). As a result, coastal areas around the south-east of America and the Caribbean have been periodically plagued by enormous *Sargassum* deposits which begin to decompose and adversely affect the local population and environment (Wang *et al* 2019).

This *Sargassum* could be described in any number of ways: some would call it a plant (Chapman 2013) while others would not (Moroney *et al* 2001). The etymology of phytoplankton suggests it would describe *Sargassum* perfectly but few would agree it is a phytoplankton (Winder and Sommer 2012). Instead, the term seaweed appears to fit *Sargassum* but seaweeds are often regarded as being fixed to the substrate and coastal (Wienck and Bischof 2012) and as seaweed and macroalgae are so often used interchangeably calling it either of these leaves room for confusion. It also appears to fit the classification brown algae, but again this typically only defines fixed seaweeds. Lastly, like most other plants, *Sargassum* go through the alteration of generations and so it is only during the sporophytic stage of its life that the organism is actually visible and the other stages are microscopic (Hurd *et al* 2014). Fortunately, this thesis only deals with a few species of algae directly, all of which are brown, macroscopic, coastal and fixed to the substrate so can comfortably be referred to as either macroalgae, seaweed or brown algae.

2.6 Wrack and Coastal Ecosystems

The seaweed species used in this project fall under two orders within the class phaeophyceae (brown algae); Laminariales and Fucales. Seaweeds from the order Laminariales are commonly called kelp (though some will use kelp and brown algae synonymously (Ye *et al* 2015)) and are characterised by flat bladed fronds similar to leaves, a strong but flexible stipe similar to a tree trunk and a holdfast similar to the roots of a tree. These can be seen in Figure 11. The comparison to trees extends beyond the physical structure of kelp as they can form giant kelp forests which are the home of diverse ecosystems and are central to the marine food web (Schiel and Foster 2015). Kelp forests are among the most productive ecosystems on earth (Kain 1979, Steneck et al 1982, Colombini and Chelazzi 2003), and much of Scotland's coasts contain large kelp forests (Walker 1954). They are the most complex and largest brown algae and considered one of the most important owing to their high number of species, biomass and ecological and economic significance (Baweja *et al* 2016). Recently, Eger *et al* (2023) estimated the global value of three ecosystem services: fishery production, nutrient cycling and carbon removal, provided by the six seaweed genera which make up the majority of kelp forests. These kelp forests were estimated to have a value of between $465 and $562 billion/year worldwide (Eger et al 2023). Within this research project, the following kelp species were used as substrates in laboratory experiments: *Laminaria digitata*, *Alaria esculenta* and *Saccharina latissima*. *L.*digitata, commonly called oar weed, is broadly distributed along the northern European coast, with the southern limit clearly defined on the Atlantic coast of Southern Brittany (Oppliger *et al* 2014). They appear similar to the *L.hyperborea* (commonly called forest kelp) in Figure 11, but reach a maximum height of around 1.5m. A third

species also has a very similar appearance to these two; *L.ochroleuca* (commonly called golden kelp). Juvenile organisms of each species can be hard to tell apart, however the stipe of *L.digitata* is oval in a cross section where the other two are round, this is particularly pronounced at the top of the stipe and is diagnostic of the species. Additionally, the stipe of *L.digitata* is more flexible and when exposed by the tide lies almost flat, whereas forest kelp and golden kelp, which can also survive in the intertidal zone, are firmer and thus remain more upright. *L.digitata* along with *L.hyperborea* and *L.ochroleuca* are very abundant and contribute significantly to the primary production and habitat creation of the nearshore environments, particularly around the coasts of Britain and the North Sea (Bunker *et al* 2017). These species are often farmed and wild harvested, and whilst edible, they are more often used commercially for their high alginate and iodine content (MacArtain *et al* 2007).

Figure 11. A Norwegian kelp forest comprised mainly of Laminaria hyperborea.The stipes of which are covered in epiphytes. From: https://www.hi.no/en/hi/news/2018/october/chronicle-part-of-our-ocean-is-dying Photo: Erling Svensen / Havforskningsinstituttet

Alaria esculenta, commonly called dabberlocks, has a spearhead shaped delicate frond with a distinctive midrib (large strengthened vein like that of a leaf). *Saccharina latissima,* commonly called sugar kelp, also has a spearhead shaped frond but lacks a midrib and is uneven and ungulated at its edges. Both species also reach a maximum height of around 1.5m, can survive in the intertidal zone and are common throughout the nearshore of Britain and the North Sea, though prefer more sheltered areas than those from the genus *Laminaria* (Bunker *et al* 2017). Both species are edible and are commonly farmed for this purpose (Stévant *et al* 2017, Afonso *et al* 2021). The other group of species used in this project were from the genus *Fucus*. Like the Laminariales are referred to as kelp, species from the Fucales order are sometimes called wrack. However, this is less common as

wrack can also be used to describe shore deposits of detached seaweed as it is in this thesis. Initially, only the species *Fucus serratus* (Figure 12) was used, however, once this and similar species became fragmented on the shore, it can be difficult to tell them apart. So whilst the majority was *F.serratus,* it is likely some were also from the species *F.spiralis*, *F.guiryi* and *F.vesiculosus*. As such, throughout this thesis, this substrate is referred to as *Fucus* species (sp). Species from the genus *Fucus* tend to dominate intertidal zones, where some Laminariales can tolerate emersion but are more abundant below the low tide mark, *Fucus* species are most abundant in the intertidal zone. They are also much smaller growing up to 60cm depending on the species, have a darker olive-green colour and can appear closer to black than brown especially when out of the water (Bunker *et al* 2017).

Figure 12. Intertidal F.serratus. Photo by Ryan Hodnett (image license CC-by-SA *https://commons.wikimedia.org/w/index.php?curid=118294987)*

As well as primary production, macroalgae provide shelter and habitat and thus are vital to marine ecosystems. Seaweeds are consumed by a diverse assemblage of herbivores that includes fishes, urchins, gastropods, crabs and numerous smaller herbivores such as amphipods and isopods (Duffy and Hay 1990). Sea urchins frequently graze on the holdfasts (of kelp in particular), which anchor the seaweed in place. In a balanced successive ecosystem, this poses little threat to its stability. However, if the grazing of such seaweed herbivores, especially sea urchins, is allowed to intensify. As result of anthropogenic removal or displacement of their predators, they can disrupt the equilibrium and turn kelp forests into areas known as sea urchin barrens (Filbee-Dexter and Scheibling 2014, Norderhaug and Christie 2013). Herbivorous grazing of the holdfasts of seaweed is one way in which their detachment from the substrate can occur. Many species such as *L.digitata* are perennial but shed their fronds in late winter, thus seaweed is also detached as part of the plants life cycle through

senescence and shedding (Bunker *et al* 2017, Krumhansl and Scheibling 2011, 2012). Lastly, erosion, particularly during storms and rough seas, can detach significant amounts of seaweed from their substrate (Walker and Richardson 1955, Filbee-Dexter and Scheibling 2012). The majority of this detached macroalgae is washed ashore (Colombini and Chelazzi 2003) with some remaining in the water column (Rothäusler *et al* 2012) and little sinking to the sea floor (Wilding 2006). Therefore, in winter, through a combination of rougher seas and shedding, detached seaweed is generally more abundant.

Detached seaweed that is washed ashore collects in the intertidal and supralittoral zones and forms deposits known as wrack. One such deposit can be seen in Figure 13. Wrack plays a central role in shore ecosystems as they have very little primary production as a result of the highly mobile sediment and dynamic and harsh conditions (Brown and McLachlen 1990, Colombini and Chelazzi 2003, Gilburn 2012). Therefore, the ecosystem is almost entirely dependent on allocthonous (introduced from elsewhere) material as primary production (Colombini and Chelazzi, 2003, Ruiz-Delgado *et al* 2016). This is most often wrack and provides a food source for many secondary consumers, particularly Dipteran (fly) larvae and Amphipods (Orr 2013). However, wrack not only provides a food source for those organisms feeding on it directly, but is central to the ecosystem of the shore as a whole. The wrack fauna support a wider range of organisms and are an essential food source for shorebirds, even more so during their pre-migratory fattening period and during migratory stop-offs (Dugan et al 2003, Dierschke 1998, Gilburn 2012). Orr (2013) found wrack macroinvertebrate biomass to increase 3.7 fold during the pre-migration fattening period of waders, and this increase was principally Dipteran larvae. Compared to other wrack fauna,

Figure 13. A large, and relatively fresh, deposit of wrack consisting primarily of Laminaria species. This photo was taken around high tide on 16th January 2020 making it a winter deposit.

Dipteran larvae are present in larger densities and have a greater calorific value (Fuller et al 2013, Summers *et al* 1990). During this period these birds' dietary needs increase and migration only occurs if they have adequate energy stores, which can be as much as a 40% increase in mass (Metcalfe and Furness 1984). Dipteran larvae are a crucial food source for birds (Backlund 1945, Egglishaw 1958, Gilburn 1992), migratory birds in particular (Orr 2013). Once cast ashore, wrack undergoes fragmentation, decomposition and remineralisation and as it breaks down much of the organic matter (OM) is lost through leaching which leads to high levels of dissolved organic matter (DOM) in the beach sediments and nearshore waters (Colombini and Chelazzi 2003, Griffiths *et al* 1983). OM, derived from wrack deposits washed back to sea, supports the growth of marine primary production, amphipods, isopods and suspension feeders like many shellfish (Baweja *et al* 2016, Crawley *et al* 2009, Dugan *et al* 2011, Orr 2013). The amount of dissolved nitrogen in the nearshore/surf zone has been shown to have a positive correlation with the wrack deposits on the adjacent beach (Dugan *et al* 2011, Kirkman and Kendrick 1997). Some of this OM also accumulates in the shore sediment where it facilitates primary production and is fed on by amphipods and polychaetes (Bolam *et al* 2000). Orr *et al* (2014) found that macroalgae derived OM concentration was three times higher than that of Chlorophyll-a in the nearshore suggesting it plays a more important role in the nearshore food web than that of microalgae. Similarly, kelp detritus has been documented as being more dominant than phytoplankton in the diet of some primary consumers (Dunton and Schell 1987, Sturaro *et al* 2010, Duggins *et al* 1989, Kaehler *et al* 2006).

Beaches that accumulate wrack are rich in biological activity and are essential in the processing and remineralisation of detached macroalgae and associated organic matter. This is true to such an extent that models by Orr (2013) found the total system throughput for the intertidal zones of beaches rich in wrack deposits are comparable to that of coral reefs (Heymans *et al* 2012, Orr 2013). When comparing eight beaches, half with a high amount of wrack and half a low, Orr (2013) found a 42 fold increase in the biomass and an 87 fold in the abundance of benthic infauna at low water. This is a remarkable increase and shows how vital wrack deposits and their remineralisation are to the nearshore ecosystem. The findings from one of these 'high wrack' beaches can be seen in Figure 14 which also visualises a typical food web in shores which accumulate wrack.

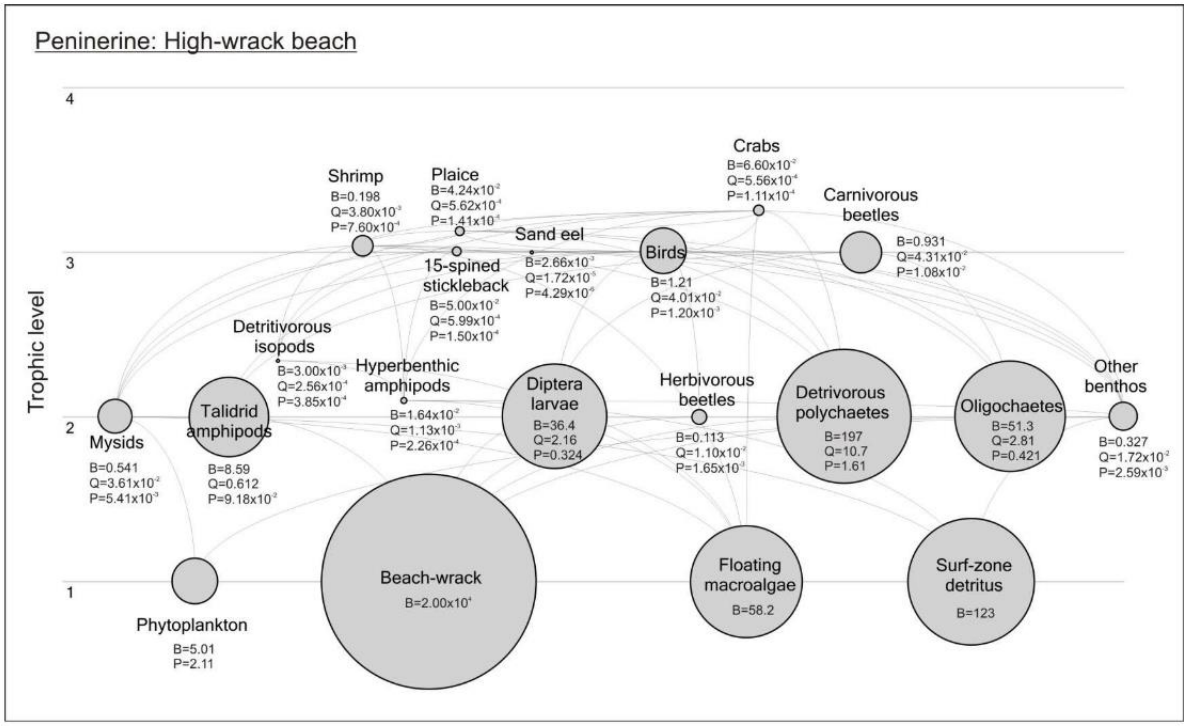


Figure 14. A flow diagram from Orr (2013) depicting the trophic links on a beach which accumulates a large amount of wrack. The size of each circle is proportionate to the biomass it represents. B: Biomass (g.m-2), P: Production (g.m-2.day-1), Q: Consumption (g.m-2.day-1). Figure from Orr (2013).

As evidenced, shores rich in wrack deposits support far greater faunal biomass, abundance and diversity than those which do not, but this can be further evidenced by the absence of wrack. Beaches that are groomed (have their wrack removed to increase aesthetics) have been found to support an average of four times fewer taxa than those which are not (Gilburn 2012). Similarly, removal of wrack has been associated with depleted abundance and species richness of macro-invertebrates and thus a decline in top predators such as shorebirds (Dugan *et al* 2003) and fish (Lavery *et al* 1999). Beaches free of wrack also have a lower proportion of organic matter, bacteria and meiofauna in the sediments (McLachlan 1985, Gheskiere *et al* 2006, Malm *et al* 2004). This results in a significant decline in the amount of nutrients leached into sediment pore water and the adjacent water column (Malm *et al* 2004, Dugan *et al* 2011). Thus, beach grooming has also been shown to reduce the survival and reproduction rates of dune plants which are essential in stabilising beach sands and facilitating succession (Dugan and Hubbard 2010). The importance of wrack in the stabilisation of beaches is evidenced in Uist's worst erosion period in history, which has been closely linked to the 'kelp boom' between 1770-1815 (Angus and Elliott 1992), when large-scale harvesting of wrack took place for the production of alkaline ash (Gray 1951, Orr 2013).

The type of wrack deposits found on a shore will be dependent on the quantity and type(s) of seaweed present in the sea of that shore's 'catchment'. However, unlike terrestrial catchments

which are dictated by topography, these 'wrack catchments' are dictated by the physical and chemical properties of the sea, the local current(s) and most significantly, the prevailing wind direction and strength. This makes wrack catchments dynamic. The type of wrack deposit is also dependant on the shape of the coastline itself. The steeper the shore, the less wrack will accumulate as the waves by which it is transported lack the energy to force detached seaweed up the slope. If a coast is more open the seas will be rougher giving them a higher likelihood of detaching seaweed. If the coast is straight, wrack will be evenly dispersed along the shore, in which case large wrack banks will only form when there is a high abundance of incident material. Most often, large wrack banks form when other factors force a congregation of this incident material, this can be anthropogenic like skerries which direct the waves or natural, like curved coasts and bays where wrack is deposited to specific areas of the coast. As such, the shape of the coastline and direction of dominant winds dictates the deposition of wrack (Baring *et al* 2014). As well as being the driving force in the formation of wrack beds, tidal action can also be responsible for their breakdown. Thus, even very large wrack banks can be rather ephemeral. The apparent stability of wrack beds generally occurs as a result of an equilibrium between the two. Being deposited by the tide, wrack deposits are linked to lunar cycles and are sometimes described as having a 'lifespan' of 28 days existing between full moons and their spring tides (Backlund 1945, Colombini and Chelazzi 2003, Dobson 1974, Leggett 1993). This can be interrupted by high neap tides and storms. However, the deposits can remain for much longer than this and Dobson (1974) recorded a maximum of 82 days. There can also be periods when no wrack is deposited (Dobson 1973).

Backlund (1945) uses the term wrack bed to refer to any wrack deposit and classifies them as one of three types:

1. Wrack strings, which are never more than 15cm wide or deep. They are usually dry unless they consist of green algae, which retains moisture while decaying.

2. Wrack flakes which have no limit to their width but are never more than 5cm deep, they are also relatively dry.

3. Wrack banks which are anything more than 15cm deep and wide. They consist of 3 distinct layers the bottom of which is always wet and decomposition is anaerobic. The middle layer is where wrack fauna are found, and the mechanical action of these quickly turn the wrack into more of a humus/frass. The upper layer is the exposed surface and contains the least moisture but is generally not dry to the touch.

These wrack deposits are rarely so clearly defined with different types merging into each other or large deposits that would be classed as wrack banks being relatively dry. Furthermore, these characterisations were made based purely on the above ground wrack, yet often another several cm of wrack can be found below the substrate surface. Nevertheless, these classifications give a good base description of the physical differences between different types of wrack deposits.

The rate at which wrack decomposes is predominantly dependent on its composition and quantity as this dictates the microclimate of the wrack deposit and which organisms will colonise it (Colombini and Chelazzi 2003, Backlund 1945, Smith and Foreman 1984). The large wrack banks are where decomposition occurs fastest and the most biological activity can be found. Biological activity is fastest in the middle layer between the dry upper layer and the anaerobic lower layer. In the upper and lower layers, individual pieces of seaweed can be recognised though those in the bottom layer are covered in a thick biofilm which is often white in appearance. In the middle layer however, mechanical activity of the macrofauna quickly turn the wrack into a more homogenous structure not dissimilar to a wet humic soil layer or a loose peat. The bottom layer is tightly packed and what little pore space is present is mostly full of water released during the breakdown of the layer above making it anaerobic. In contrast, the middle layer is less densely packed and drains into the layer below this and the mechanical activity of the fauna keep it aerated. The upper layer serves as a buffer between the middle layer and the exterior environment and prevents desiccation of the wrack beneath.

The result in the middle layer is an aerated and moist environment made almost entirely of organic matter which has a high surface area and thus is ideal for bacterial growth. Yet this is not a one-sided relationship where the macrofauna create the perfect environment for the microfauna, instead it is mutualistic. In turn, these bacteria can raise the temperature in the middle layer to one considerably above that of the exterior environment with Backlund (1945) observing a maximum temperature of 40°C when investigating the wrack fauna of Sweden and Finland. Leggett (1993) observed a maximum of 55°C in a deposit in the North-East of England. As a general rule, insects have a fixed number of generations each year and life cycle stages are season specific. However, the microbial activity in the middle layers of wrack banks, combined with the poor heat conduction of organic matter (Egglishaw 1945), creates a temperature and micro-climate independent of that in which it is situated. This is the case to such an extent that the outer layer of wrack banks can be frozen while the middle layer can remain above 30°C (Egglishaw1945). This can facilitate the constant presence of insects in all life cycle stages at any time of the year. This higher temperature also allows the

ectothermic ('cold-blooded') invertebrates to develop faster and reach a life cycle stage in which flooding or removal of the wrack deposit by the next spring tide would not be fatal.

This scenario describes a wrack deposit in which decomposition occurs fastest, however, to achieve this depends on the size and composition of the deposit. While decomposition occurs fastest in a relative sense, a deposit has to be large to facilitate this state and thus can be present for a long time. In comparison, the smaller wrack strings and flakes decompose slower, and, unless physically removed, individual deposits can last months, although this is less common (Backlund 1945). These smaller deposits tend to breakdown either physically or biologically. Where the physical structure of the deposit, influenced by its size and seaweed composition, creates a high surface area to volume ratio the wrack is very dry and less supportive of biological activity. Such deposits breakdown physically as they are dry and brittle, so wind and sand abrasion reduce the deposit down into smaller fragments which are more easily transported by the wind and sea. Alternatively, when these smaller deposits have a lower surface area to volume ratio, they can form a moisture retaining layer between the surface of the wrack and the substrate. This is much thinner and drier than the middle layer of a wrack bank but does create a micro-climate in which biological activity and decomposition can occur but does so at a temperature in equilibrium to that of its environment. Lastly, as the substrate of shores is often highly mobile, smaller wrack deposits can easily become buried. Such burying facilitates moisture retention and thus biological activity.

In addition to the size of the wrack deposit, the type of seaweed(s) it is comprised of also affects its decomposition. Around the coasts of Scotland, Northern England and Scandinavia wrack deposits are almost entirely comprised of seaweeds from the genus *Fucus* and/or *Laminaria* (Backlund 1945, Leggett 1993). *Fucus* sp have a branched structure which make them pack together more loosely than *Laminaria* sp, they also have a lower moisture content. This lends *Laminaria* species to the type of breakdown described in the larger wrack banks and *Fucus* sp to that described in the smaller wrack deposits.

Wrack fauna play a fundamental role in the decomposition of beached detached macroalgae (wrack). These organisms largely fall into the following groups: microorganisms (mainly bacteria), Oligochaetes (worms), Amphipoda (often referred to as sand hoppers), Diptera (flys) and Coleoptera (beetles). Occasionally isopods and predatory spiders are also present. This thesis focuses on the macro herbivorous species. Within the literature, the following Diptera are consistently recorded as breeding in wrack deposits in the UK, Norway and Sweden: *Coelopa frigida, Coelopa pilipes, Orygma luctuosum, Heterochelia buccata, Helcomyza ustulata, Fucellia fucorum, Fucellia maritima, Fucellia tergina, Malacomyia sciomyzina* and *Thoracochaeta* species, along with Amphipoda from the

Talitridae family (Backlund 1945, Egglishaw 1958, 1965, Gilburn 2012, Orr 2013). These can all be thought of as 'true wrack species' as wrack is necessary to their life cycle. In addition to these, several species, for which wrack is not considered necessary to their life cycle, are also documented but appear more sparingly and erratically. These can be thought of as 'false wrack species' and are; *Hydrophorus oceanus* (a predatory fly from the family Dolichopodidae)*, Hydrotaea irritans* (house fly species), *Hylemya* species (root-maggot flies)*, Chersodromia hirta* (dance fly)*, Sciaridae* species (fungus gnats)*, Fannia canicularis* (Lesser house fly)*, Scathophaga stercoraria* and *Scathophaga litorea* (dung flies), *Psychodidae* species (drain flies), *Tabanus bromius* (Horse fly), *Tipula* species (crane flies), *Tinea pallescentella* (Large pale clothes moth) and *Eristalinus aeneus* (hoverfly) (Backlund 1945, Egglishaw 1958, Orr 2013).

Globally however, a review of the literature has revealed some 70+ fly species which can all be classed as 'true wrack species'. These have been compiled into a phylogenetic tree which can be accessed from Figure 15. Of these 70+ species *Paractora dreuxi-mirabilis,* in particular, deserves a mention. Firstly, because the findings of Crafford (1971) and of Crafford and Scholtz (1987) suggest the larvae of this flightless Antarctic wrack fly can consume and process vast quantities of wrack relatively quickly. And secondly, because in the 1950s, it featured on a stamp in the oversees French territory the French Southern and Antarctic Lands (Figure 15).

Figure 15. Left. A QR code leading to a phylogeny tree of global true wrack flies. This can also be accessed from https://bit.ly/2UGL7nM Right. A stamp from the French Southern and Antarctic Territories featuring the wrack fly Paractora dreuxi-mirabilis

Of the UK true wrack flies, all aforementioned species were encountered within this project apart from *Fucellia fucorum* and *Fucellia tergina*. Species from the genus *Thoracochaeta* are considerably smaller than the others with adult flies reaching a maximum length of no more than 4mm. This small size makes them an impractical potential feed ingredient and so were not included in this study. As they were not encountered, neither were *Fucellia fucorum* or *Fucellia tergina*, all others were.

2.7 Wrack Fauna

This section (2.7) provides a brief introduction to these common wrack species, their ecology is discussed in Chapter 3.

Coelopid

Coelopid is a genus of flies, and along with others in the family Coelopidae, are known as seaweed flies or kelp flies as they are the most common in wrack deposits globally (Dobson 1976). In Britain, only the species *C.frigida* and *C.pilipes* are present and can be seen in Figure 16. Their literal translations from Latin are *Coelopa frigida* – Cold coelopa and *Coelopa pilipes*- Hair(y) coelopa.

Figure 16. Left. Dorsal view of a male Coelopa frigida. Photo kindly provided by Brandon Woo Copyright © 2015 (https://bugguide.net/node/view/1176743). Right. Dorsal view of a male Coelopa pilipes image taken using GXCAM-U3-5.

C.frigida is a bristly dorsoventrally flattened black/brown fly and varies the most in size and appearance within *Coelopid*. Males range from 3 -9mm from between antennae to the tip of the abdomen, females 3-7mm. Longer flies have proportionally longer bristles which can make them appear very different. The males are more bristly than the females. *C.pilipes* is similar but in the place of bristles has more hair like structures particularly on the males which makes them easy to identify. However, the females in each species are similar but can be told apart by the more prominent bristle on the tibia of *C.frigida*. Both males and females of *C.pilipes* are darker and this is particularly pronounced on the legs as those of *C.frigida* are a light brown colour. Females from both species can also be differentiated by the ventral appearance of the abdomen with *C.frigida* presenting a browner shiny abdomen and *C.pilipes* presenting an almost black matte abdomen. *C.pilipes* is less variable in size measuring between 4.5-7mm and is generally slightly smaller than *C.frigida* though not always (Egglishaw 1958).

Orygma luctuosum

Orygma luctuosum is a black fly, which is more dorsoventrally flattened than *C.pilipes* and *C.frigida*. It is relatively large and the size fairly constant with males and females ranging between 6-10mm. They are also noticeably wider than all other true wrack flies making them fairly distinctive. *O.luctuosum* have few hairs/bristles, the most prominent being few long hairs on their metathoracic spiracles. *O.luctosum* are quite different in their evolution and taxonomy from the *Coelopids* as they are in the family Sepsidae commonly referred to as dung flies. However, where Sepsidae are generally described as small slender and ant-like, *O.luctosum* are large and broad making them fairly unique both within wrack deposits and within the family Sepsidae. In a key to the Families of British Diptera (Ball 2015), Sepsidae are described as often having a black spot at the wing tips and a basally constricted abdomen. As can be seen in Figure 17, *O.luctuosum* have neither.

Figure 17. Top. Dorsal views of a male O.luctuosum. Bottom left. Ventral view of male a O.luctuosum. Bottom right. Dorsal view of male (left) and female (right) O.luctosum, thelighter colour of the females legs makes them easily seperable. Images taken using GXCAM-U3-5.

Fucellia

In Britain, there are three species of the genus *Fucellia* all of which are documented to be present in wrack deposits: *F.fucorum*, *F.maritima* and *F.tergina*. *Fucellia* are in the family Anthomyiidae most species from which are commonly called root-maggot flies as their larvae develop in the roots and stems of plants. However, there are some, such as *Fucellia,* whose larvae develop in decaying plant matter. Many flies from this family are occasional visitors of flowers, those from the genus *Fucellia* included. *Fucellia fucorum* can be easily distinguished by its wholly black leg. In comparison *F.maritima* and *F.tergina* have light brown tibias. Discerning *F.maritima* from *F.tergina* requires much closer inspection of the individual. The presence of a single or no hairs between the two rows of presutural acrostical bristles is diagnostic of *F.tergina*. The presence of several hairs is diagnostic of *F.maritima* (Figure 18). Additionally, *F.maritima* have a wider gena than that of *F.tergina*. (Ackland *et al* 2017).

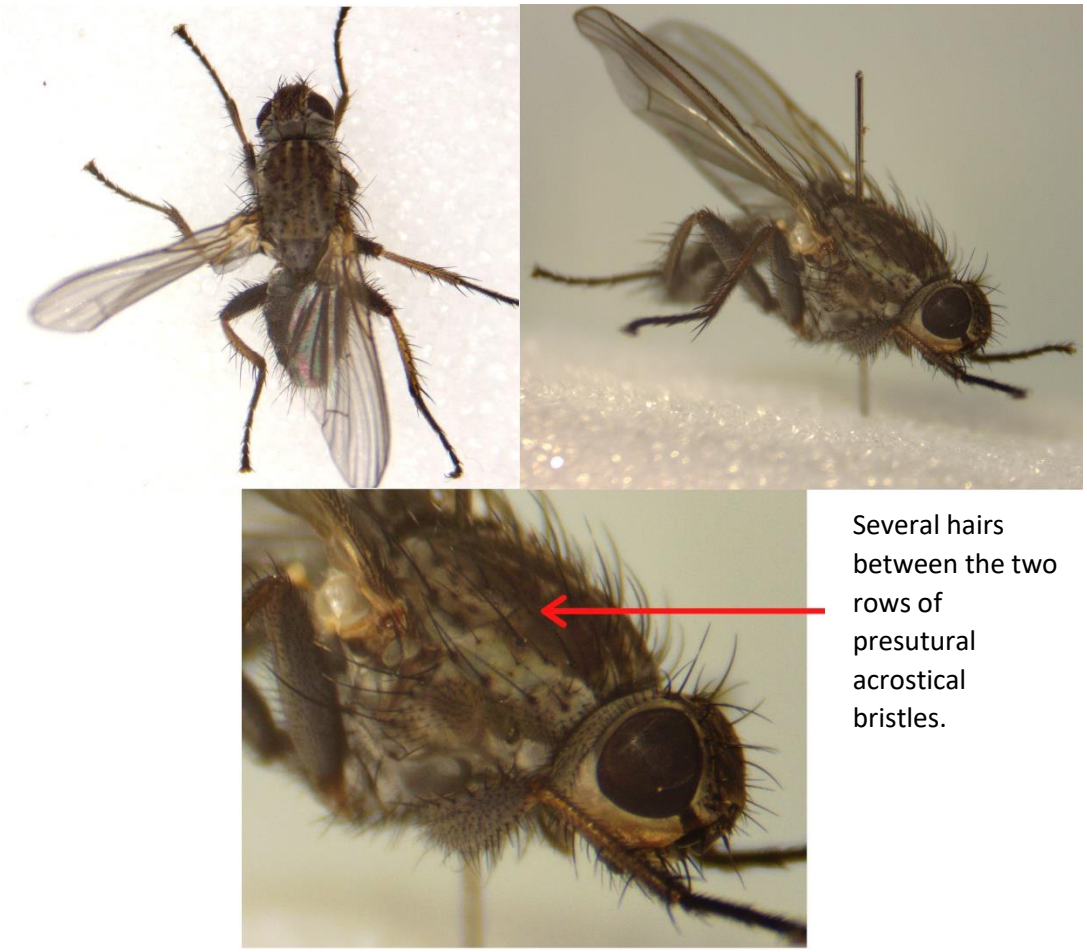


Figure 18. Top left. Dorsal view of a male Fucellia maritima. Top right. Dorsal-Lateral view of a male Fucellia maritima. Bottom. Close up Dorsal-Lateral view of the thorax where the several diagnostic hairs can be made out between the two rows of presutural acrostical bristles. Images taken using GXCAM-U3-5.

Helcomyza ustulata

Helcomyza ustulata is the only species from the family Helcomyzidae present in Britain, it was previously considered a subfamily of Dryomyzidae (Chandler 1998). *H.*ustulata is a large and long winged light grey fly which can attain a wing span of over 20mm, making it one of the largest British Acalypterates. The light grey colour with a brown dusting and large size makes these adults readily distinguishable (Figure 19).

Figure 19. A male Helcomyza ustulata viewed dorsally (left) and laterally (right). Images taken using GXCAM-U3-5.

Heterocheila buccata

Similar to *H.ustulata, Heterocheila buccata* is the only fly from the family Heterocheilidae and was also previously placed in the family Dryomizidae (Chandler 1998). *H.buccata* are a light brown colour, almost appearing orange at times, with a grey thorax. They are relatively small ranging between 4.5-6mm. *H.buccata* (Figure 20) and the following species *Malacomyia sciomyzina* (Figure20) can be difficult to tell apart in the field.

Figure 20. A male Heterocheila buccata viewed dorsally (left) and laterally (right). Photos kindly provided by Steven Falk Copyright © 2016 (https://www.flickr.com/photos/63075200@N07/albums/72157695010328734/).

Malacomyia sciomyzina

Malacomyia sciomyzina is the only species in the genus *Malacomyia* and is currently situated in the family Coelopidae making it a close relative of *C.frigida* and *C.pilipes*. However, it has been the subject of debate and has previously also been placed in the family Helcomyzidae along with *H.buccata* to which it appears very similar and in the family Dryomizidae before that (Chandler 1998). Like *H.buccata, M.sciomyzina* is a light brown/yellow fly with a grey thorax. It is similar in size to *H.buccata* but averages slightly smaller. The two can most easily be told apart by the mouthpieces as that of *M.sciomyzina* protrudes forwards (Figure 21) where that of *H.buccata* is retreating (Figure20).

Figure 21. Left. A dorsally viewed M.sciomyzina. Photo by Maloclm Storey (Image license: CC-BY-NC, https://species.nbnatlas.org/species/NBNSYS0000134862) *Right. A laterally viewed M.sciomyzina focusing on the head and thorax highlighting the difference in mouth piece from H.buccata.*

Amphipoda

Amphipoda are a diverse order of the class Crustacea with at least 7900 known species (Glazier 2014). While they are often described as aquatic organisms (Beiras 2018), there are a few species which are entirely terrestrial though require very moist environments (Glazier 2014, Lincoln 1979, Morritt and Stevenson 1993). Amphipods are omnivorous most commonly feeding on live or decaying plant matter through filter feeding or direct grazing. However, they can also be predatory and even cannibalistic in some species so do not neatly fit the functional feeding groups (Glazier 2014). Amphipods tend to reach a maximum of 2-3cm when fully grown (Lincoln 1979), recently however, some in excess of 30cm have been recorded in the deep sea (Jamieson *et al* 2014). They are often the most abundant macro-invertebrates in an ecosystem and play a major role in detritus processing, nutrient cycling and trophic transfer. Most Amphipod species are marine and, as shown

in Figure 14, play an essential role in the breakdown and remineralisation of wrack and suspended macroalgae (Orr 2013). Those most commonly found in the supralittoral and intertidal zones are from the families Talitridae or Gammaridae (Backlund 1945). However, some species from Talitridae are entirely terrestrial within the shore ecosystem whereas those from Gammaridae are not found out of water (Backlund 1945, Lincoln 1979) examples of species from each family can be seen in Figure 22.

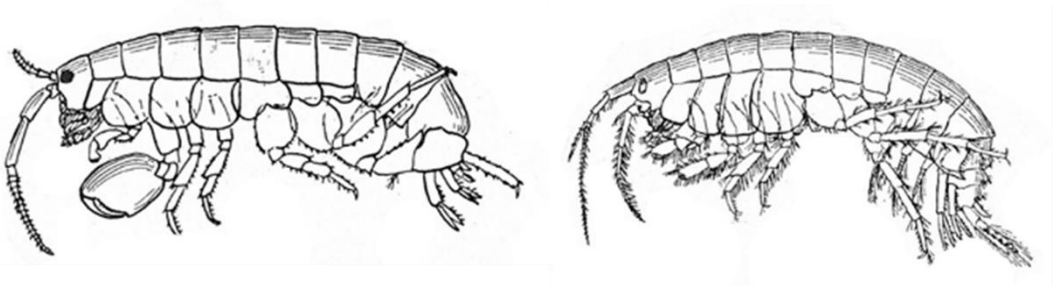


Figure 22.Left. Example of a Talitrid Amphipod (Orchestia gammarellus). Right. Example of a Gammerid Amphipod (Gammerus locusta). Drawings from Stephensen (1925)

Isopoda

Isopoda are another order in the class Crustacea and are taxonomically similar to the Amphipoda. But where the Amphipoda are characterized by being laterally compressed, the Isopoda are dorsoventrally compressed. Terrestrial Isopoda fall into the sub-order Oniscoidea and are commonly referred to as woodlice and are likely the most familiar of the Isopoda. However, they are one of the most morphologically diverse Crustacea orders and the majority are marine. Like the Amphipoda they can be scavengers, predators and herbivores but many are also parasitic, especially on fish (Brusca *et al* 2007). In Britain, several species of isopod are present in the intertidal zone alone, and range considerably in their habitat and appearance. All of which are either detritivores or herbivores feeding predominantly on seaweed in one form or another (Campbell 1994). The most common, distinctive and easily recognisable is the species *Ligia oceanica* as it most resembles a terrestrial 'woodlouse' Figure 23. They are usually found in between and under rocks where small fragments of detached seaweed and resuspended wrack collect.

Figure 23. The intertidal Isopoda Ligia oceanica. Photo By Gilles San Martin (image license: CC BY-SA, https://commons.wikimedia.org/w/index.php?curid=17576709)

The interactions between these species and the roles they play in the breakdown of wrack are discussed in Chapter 3. From the base knowledge of fatty acids, algae, wrack and wrack macro-invertebrates introduced in this Chapter, this thesis now aims to assess the potential of wrack macro-invertebrates as an alternative source of the marine origin omega-3 fatty acids: EPA and DHA as outlined in Chapter 1.

Chapter 3

THE WRACK ECOSYSTEM AND CULTURING OF ITS FAUNA

As global population and affluence rise, the demand for food is predicted to increase, and much of this demand is expected to be met by aquaculture (FAO 2020a, Free *et al* 2022). As highlighted by the UN's FAO (2020a), innovative feed and feed ingredients are fundamental to this being done sustainably. However, when changing these aquafeeds, the importance of the essential omega-3 fatty acids, to both the appeal and nutritional benefit of the final product as well as the health of the animal being reared, must not be forgotten (Lock *et al* 2016). Globally, algae are responsible for the majority of essential ω-3 FA production (Gladyshev *et al* 2013). As organisms that can achieve high population densities that feed on the initial source of essential ω-3 FAs, wrack invertebrates from the 2nd trophic level are suggested as one such sustainable innovative feed ingredient. The first barrier to any wrack invertebrate establishing potential as a novel feedstuff is how easily they can be reared and ultimately mass produced. Attempts to rear these organisms requires an understanding of their ecology. This chapter will discuss the current state of knowledge on the ecology of wrack beds and the succession of their colonisation before proposing an alternative theory of colonisation based on observations throughout this project. Then, previous culturing of wrack invertebrates from the literature will be discussed before presenting data on successful and unsuccessful cultures during this research.

3.1 The distribution of wrack invertebrates

Fruit flies (*Drosophila* sp) have many traits and characteristics that have long made them the subject of choice for genetic research. Similarly, particularly in the 1980s and 1990s, the seaweed fly *C.frigida* found it also gained the attention of geneticists. This was mainly owing to its ease of culture, polymorphic chromosome inversion system and ecological traits. As such, some of the ecological information on *C.frigida*, and to a lesser extent *C.pilipes*, came from such genetic studies. The rest of the ecological understanding of wrack beds comes from various studies carried out specifically on this subject, most of which occurred between 1945 and 1999. These studies largely focus on *C.frigida* and, to a lesser extent *C.pilipes*, and many come to conflicting conclusions. However, they all agree that *C.frigida* are competitively superior to *C.pilipes* (Butlin 1983, Dobson 1974, Egglishaw 1958, Leggett 1993).

The organisms found in wrack beds lack cognitive reasoning and decision making. As such, they respond to differences and changes, which can be both physical or chemical and can occur internally or externally. Their response is most often genetic, but in some cases can be an inherited/learned behavioural trait. This raises an interesting theoretical null hypothesis which many people may assume not to be true without the data to support this. That null hypothesis is as follows:

> *H_0:When placed in a truly homogenous environment, organisms which are genetically identical and lack cognitive reasoning and decision making will behave identically.*

This is a theoretical hypothesis and is not tested in this thesis, but was contemplated during this project. Whether it is responded to identically or not, identifying what changes or differs in an ecosystem can help understand the niches different species occupy and their distributions. In investigating said distributions both within and between wrack beds, the factor which the literature disagrees on most is the importance of seaweed species on the ecology of wrack deposits.

Role of seaweed species in wrack invertebrate distribution

Wrack deposits in Northern Britain are almost entirely comprised of macroalgae species from the genus *Fucus* and/or the genus *Laminaria.* While other species are present on occasion, they are never in quantities large enough to be the dominant constituent (Edward and Gilburn 2013). Therefore, whether the wrack deposit is comprised of *Fucus*, *Laminaria,* or a mixture of the two is a visually apparent difference between wrack beds and has received the most investigation as a driver in their ecology. However, the results have been somewhat conflicting with Hodge and Arthur (1997) stating:

> *"In earlier studies, there has been some confusion regarding whether seaweed flies require the presence of laminarian or fucoid seaweeds or both in cultures to induce egg laying and enhance larval survival"*

According to some, *C.frigida* can only be found and/or reared on *Laminaria* (Leggett 1993,Rowell 1969). While others state *C.frigida* can be reared on both, but *Laminaria* is easier and more successful (Biancarosa *et al* 2018, Dunn *et al* 2002, Edward and Gilburn 2013, Thompson 1951). Yet, Dobson (1974) concludes *C.frigida* cannot grow on *Fucus* alone but only on *Laminaria* or on mixes of the two, which led to the prediction that areas with a high amount of *Laminaria* would favour C.*frigida*. Similarly, Burnet and Thompson (1960) suggest the needs of the larvae can only be met with a mixture of both *Laminaria* and *Fucus.* Using stable isotope analysis, Edward *et al* (2008) found the δ13C in *C.frigida* reared on *Laminaria* only and on a mixture of *Laminaria* and *Fucus* comparable

suggesting a preferential consumption of the *Laminaria* (Edward *et al* 2008). *Coleopa frigida* females are more inclined to oviposit (Phillips *et al* 1995) and males more inclined to mate (Edward and Gilburn 2007) on *Laminaria* than *Fucus*. Edward and Gilburn (2013) show that *C.frigida* reared on *Laminaria* have an increased fitness over those reared on *Fucus* with a larger body size and increased larvae to adult viability. Yet, this comes at the cost of increased development time. This increased fitness meant each *Laminaria* cage yielded more and larger flies than any of the *Fucus* cages. However, each *Fucus* cage developed faster than any *Laminaria*. *C.pilipes* is equally contested in the literature with suggestions that they can only be reared on *Fucus* (Colombini and Chelazzi 2003, Leggett 1993), only on *Laminaria* (Thompson1951) or only on a mix of both (Egglishaw 1968, Dobson 1974). While others report them being reared on either *Laminaria* or *Fucus* (Biancarosa *et al* 2018, Dunn *et al* 2002). Butlin (1983) suggests the presence of *C.pilipes* correlates with the presence of *Fucus* and Phillips *et al* (1995) note *C.Pilipes* were not present in the north-west of Britain and in the majority of these locations deposits were *Laminaria* rather than *Fucus*. Whilst this clearly evidences conflict in the literature with regards to the influence of seaweed species on *Coelopa* success and distribution, the 'average consensus' leans towards *C.frigida* favouring *Laminaria* and *C.pilipes Fucus.* Regarding the other wrack invertebrates introduced in chapter 2, only *Fucellia*, as a genus, and their correlation with seaweed species is discussed in the literature and they are recorded as being indifferent between *Laminaria* and *Fucus* by Egglishaw (1958).

Role of time in wrack invertebrate distribution

Time can influence the ecology of wrack beds in two primary ways. Firstly, through the differences in development time between species and secondly, through the changing of physical and environmental conditions with time. Holding all other variables constant, the egg to adult development time of *C.frigida* is consistently reported as being quicker than that of *C.pilipes* which in turn is quicker than *O.luctuosum* (Dobson 1973, Egglishaw 1958, Leggett 1993, Thompson 1951). Owing to their longer development time, Dobson (1973) predicted that areas with greater intervals between wrack deposition would favour *C.pilipes*. However, intervals between wrack deposition are largely irrelevant if wrack remains present in between. Additionally, relying on their ideal timing to locally exclude *C.frigida* would require a remarkable consistency of these depositions, and as discussed wrack catchments are highly dynamic. It therefore seems unlikely that nature has selected for such a strategy.

Perhaps a more plausible theory of the role of development time is suggested by Leggett (1993). They suggest having staggered development times would reduce interspecific competition as the fastest species (*C.frigida*) would have a relatively 'peaceful' period in the beginning, then as the next

fastest (*C.pilipes*) begin to develop competition between them increases at which point *C.frigida* are ready to pupate and thus stop competing with *C.pilipes*. In turn, *C.pilipes* then experience their own 'peaceful' period before the cycle repeats with *O.luctuosum*. This idea is visualised by Leggett (1993) in Figure 24 (left). Another proposed theory by Leggett (1993) is that of temporal differentiation of colonisation whereby *C.frigida* are drawn to the fresher wrack deposits, the chemical signals of decomposition or increased temperature created by *C.frigida* then attracts *C.pilipes.* Thus, the development of each species is staggered to reduce interspecific competition. This idea is visualised by Leggett (1993) in Figure 24 (right).

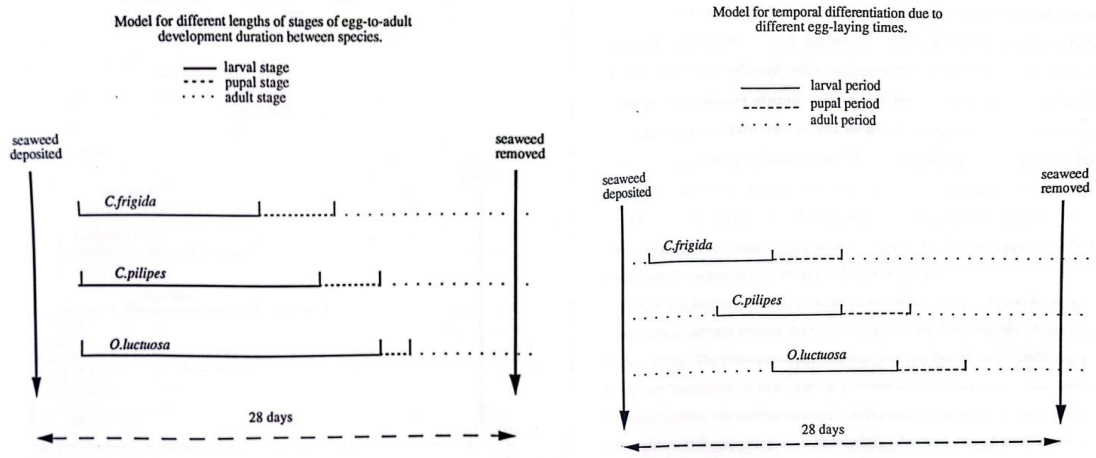


Figure 24. Left. A visual representation of the theory of differential development time facilitating coexistence of species within wrack deposits. Right. A visual representation of the theory of differential colonisation facilitating coexistence of species within wrack deposits. Figures from Leggett (1993).

These are interesting and plausible theories, however, as mentioned by Leggett (1993) both theories, or a combination of the two, assume that once adults emerge they survive without reproducing until then next full moon spring tide deposit and there is no evidence for this specifically. In addition to this, there is a more glaring error of which Leggett (1993) is not the only one to make as it is also made by Dobson (1974) among others. That is the frequency at which spring tides occur. Whilst the height of tides can vary as a result of numerous factors, the most pronounced is a result of the neap-spring tidal cycles driven by phase changes of the moon. Spring tides occur when the Sun, Earth and Moon are in alignment, an event which is observed as either a full or a new Moon. Thus spring tides occur twice evry lunar cycle (Kvale 2006), not once as suggested in Figure 24. However, the occurrence of two spring tides per lunar cycle lends itself to the theory of differential development time proposed by Leggett (1993) as it better suits the lifespan of the adults

and does not require some inhibition to their willingness to mate until a specific wrack deposit occurs.

In addition to the temporal effects of wrack organisms' biology on the distribution of wrack species, temporal environmental affects play a role. The biggest temporal change to local environments is that brought about by the seasons. Although, the effects of this are also conflicting in the literature as there is no consensus on whether the distributions of *C.frigida* and *C.pilipes* changes seasonally. Dobson (1974) and Butlin (1983) found no correlation between the time of year and which of the two *Coelopid* was most common, though Butlin (1983) does note cases of very rapid changes in the proportions of each species. However, Egglishaw (1965) states that *C.frigida* can only be found from October to January and *C.pilipes* from January to March. Similarly, Colombini and Chelazzi (2003) state that *C.frigida* prefer colder conditions than *C.pilipes* and that their distribution extends further North.

Of the other species discussed in Chapter 2, Egglishaw (1958) recorded adults from the *Fucellia* genus from March until October/November and stated that larvae which pupated in October did not eclose until March. Of *Helcomyza ustulata,* Egglishaw (1958) found pupation to last around 4 weeks during the warmer months (March – September), but after September, pupation lasted until the following March. Adults were only present in the field during the months of June and September and as larvae in July, August, September and early October (Egglishaw 1958).

Though this is inconclusive and sparing evidence, which highlights the need for a thorough investigation into the temporal distribution of wrack flies, it does show that some species likely follow the more common life cycle stages of insects previously discussed. Where life cycle stages are season specific and, in temperate environments, pupation generally occurs during the colder winter months (Denlinger 2009).

Role of temperature in wrack invertebrate distribution

This seasonal change is not the only hypothesised way in which temperature affects ecology within wrack beds. Phillips *et al* (1995) undertook an experiment in which larvae of both *Coelopa* species were reared in containers of minced *Fucus* substrate which had an increasing temperature gradient from one end to the other. They conclude that both species freely coexist, despite competing, because *C.frigida* aggregate in the colder parts of the wrack, whereas *C.pilipes* do so in the warmer. This distribution on the local scale also matches the broader geographical distribution of the species with *C.frigida* occurring further north than *C.pilipes* (Colombini and Chelazzi 2003). Phillips *et al* (1995) found this aggregation to be driven by larval preference rather than oviposition preference or

increased survival. Additionally, they report that the decrease in *C.frigida* body size that could be attributed to the presence of *C.pilipes* was greater with increasing temperature. Conversely, the decrease in *C.pilipes* size attributed to competition was greater with decreasing temperature and did not exist in the high temperature areas. This suggests the species possess different thermal niches and as they move out of these, the affect(s) of competition increase. This is supported by an observed difference in the temperature of wrack beds through their cross section displayed in Figure 25 from Phillips *et al* (1995).

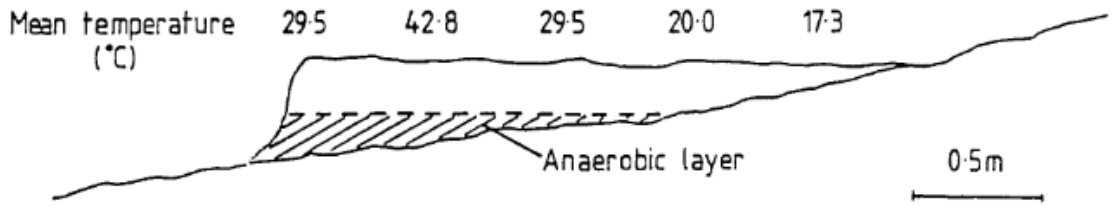


Figure 25. A diagram created by Phillips et al *(1995) showing a cross section through a typical wrack bed. Mean temperature values at points across the wrack bed are generated from eleven sample points all at an approximate depth of 10cm. Figure from Phillips* et al *(1995).*

This figure, nor its discussion in the paper, give a representation of the variation observed in these temperatures. Nonetheless, they are in alignment with similar figures reported, such as those by Egglishaw (1958), making the proposed theory of different thermal niches facilitating co-existence between *Coelopa* possible. However contrary to this, Burnet (1961) showed that *C.frigida* chooses warmer egg-laying sites over colder, and that many more eggs are laid in warm than in cold conditions. Furthermore, if this theory does explain a significant amount of the distribution of *C.frigida* and *C.pilipes* within a wrack bed then an aggregation of *C.pilipes* would be expected in the warmer area(s) of the deposit, along with either an aggregation or even distribution of *C.frigida* in the cooler area(s).

Such a distribution is not documented in the literature, yet the reverse is. Both Burnet (1961) and Butlin (1983), when analysing cored wrack bed samples, found that distribution of *C.pilipes* larvae was no different from random but the distribution of *C.frigida* was aggregated. This same pattern of *C.frigida* aggregation and a more constant, or faintly aggregated, distribution of *C.pilipes* is reported several times in the literature. These studies attribute this aggregation to the limited mobility of the larvae and the laying of eggs in batches of up to 70 eggs by *C.*frigida and singularly or in batches up to 10 by *C.*pilipes (Backlund 1945, Egglishaw 1958, Butlin and Day 1984). The source of these conflicting results could be evidenced by a publication, which came the year before Phillips *et al*

(1995), Phillips and Arthur (1994). In the aforementioned literature which report an aggregation of *C.frigida* larvae and no or little aggregation of *C.pilipes,* larval identification of the species took place by allowing the larvae to develop and identifying the adult stage of the life cycle. However, Phillips and Arthur (1994) report a study where samples of wrack bed were taken, transported to the lab and frozen. Once frozen a section was cut free and the proportions of macro-invertebrates counted. In this they found over 7500 3rd instar *C.pilipes* larvae and 2874 3rd instar *C.frigida* larvae. However, Backlund (1945) and Egglishaw (1958) state that the larvae of *C.frigida* and *C.pilipes* could not be told apart and this is something no other publication has evidenced since. Phillips and Arthur (1994) also do not explain how the larvae were told apart and thus their findings on the distributions between *C.frigida* and *C.pilipes* come with some scepticism. Phillips *et al* (1995) do not specify how species are identified so perhaps the same larval identification was used as in Phillips and Arthur (1994). If so, the observed preference for the warmer parts of the substrate by *C.pilipes* and relatively cooler parts for *C.frigida* may be inaccurate. This could explain the inconsistency with the rest of the literature. Perhaps their larval identification technique did work but identified larvae as the opposite *Coelopa* from that which they were. This would explain the observed aggregation of *C.frigida* as well as the findings of Phillips and Arthur (1994) where *C.pilipes* were over twice as numerous as *C.frigida* in samples. Nonetheless, Phillips and Arthur (1994) did find that *Coelopa* larvae, Amphipods and Oligochaetes had differing centres of distribution within wrack beds. They conclude that *Coelopa* are wrack specialists and are adapted to the hot and deep areas within wrack beds. The Amphipods and Oligochaetes are adapted to ambient temperature and are more widely and evenly distributed along the shore.

Role of physical adaptations in wrack invertebrate distribution

Thanks to the works of Darwin (1859), survival of the fittest is a phrase and concept many, both in and out of natural sciences, are familiar with. It suggests organisms best physically adjusted to their environment are the most successful in surviving and thus reproducing (Darwin 1859). Physical conditions differ within and between wrack beds, thus physical differences between wrack invertebrates may explain some of the variation in their distribution and coexistence. In particular, that of Dipteran larvae as they appear to coexist despite being ecologically similar (Backlund 1945, Egglishaw 1958, Leggett 1993). Only the works of Backlund (1945) and Egglishaw (1958) have investigated this. The physical adaptations between the Diptera present in wrack beds are split into the following groups: egg adaptations, feeding adaptations, movement adaptations, respiratory adaptations and pupal adaptations.

Figure 26. An egg of the species O.luctuosum highlighting the presence of two filaments at the base. Image taken using GXCAM-U3-5.

Of the egg adaptations, only *O.luctuosum* have any noticeable structural differences which come in the form of two long filaments which extend from the base of the egg (Figure 26). These filaments have a respiratory function and help keep the eggs from sinking allowing them to be laid in soft substrate (Egglishaw 1958). Such filaments are present in the eggs of other Diptera like *Drosophila* and Syrphidae so could be a remnant of the evolution of *O.luctuosum* rather than an adaptation, especially given their taxonomy as 'dung flies' which often breed in soft material. In comparison, eggs of the other wrack Diptera are similar (Figure 27).

Figure 27. From top to bottom, left to right eggs of the wrack fly species C.frigida, H.buccata, F.maritima and M.sciomyzina. Images taken using GXCAM-U3-5

Concerning feeding adaptations, Egglishaw (1958) found that the mouth hooks of *C.frigida* and *C.pilipes* are bifurcated, each composed of 2 downward curving projections which are rounded at the edges (Figure 28). Mouth hooks are also known as mandibular brushes (McAlpine *et* al 1981). What Egglishaw refers to as the teeth, better described a plate or potentially the labrum, is broad, flattened and extends from the surface of the head lobe. It is hardened as it is made from sclerotin (similar to keratin) and thus referred to as sclerotised. When they feed, the head is raised from the surface and extended forwards before being lowered onto the surface and is then retracted again.

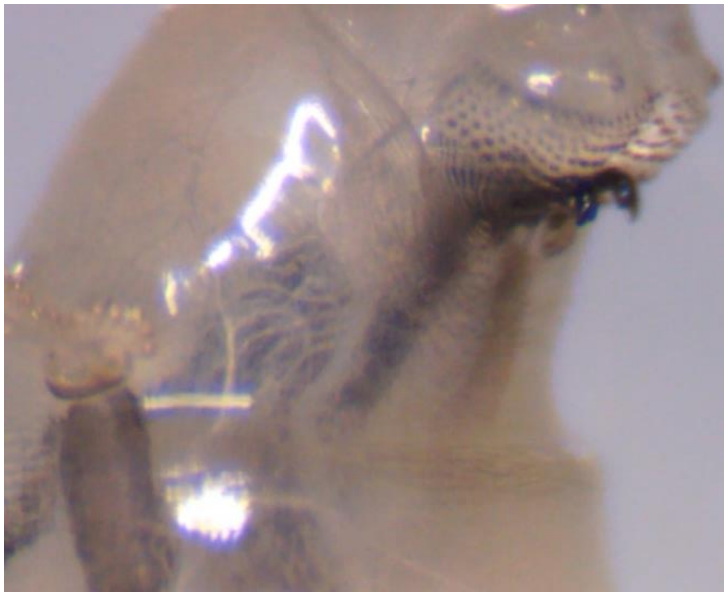


Figure 28. A dorsal-lateral view of the mouth hooks of C.frigida larvae. Image taken using GXCAM-U3-5

As this occurs, the mouth hooks and sclerotised plate pull food into the mouth. As their mouth hooks are rounded and their 'tooth' broad and flat, these larvae are best suited to ingesting soft material which needs scrapped rather than bitten (Egglishaw 1958).

In comparison, larvae from *O.luctuosum* have mouth hooks, which are unbranched and pointier than those of the *Coelopa* larvae (Egglishaw 1958), the morphology of their 'tooth' is not mentioned. Lastly, the mouth hooks of *F.maritima*, *H.ustulata* and *H.buccata* are described as being similar to each other and all being pointed with sharp edges (Figure 29). Their sclerotized plate is narrower and firmer than those of Coelopa larvae and only project slightly from the head lobes (Egglishaw 1958). As a result Egglishaw (1958) concludes that the *Coelopa* species are more suited to consuming softer wrack, *F.maritima*, *H.ustulata* and *H.buccata* to fairly hard wrack and *O.luctuosum* between the two groups.

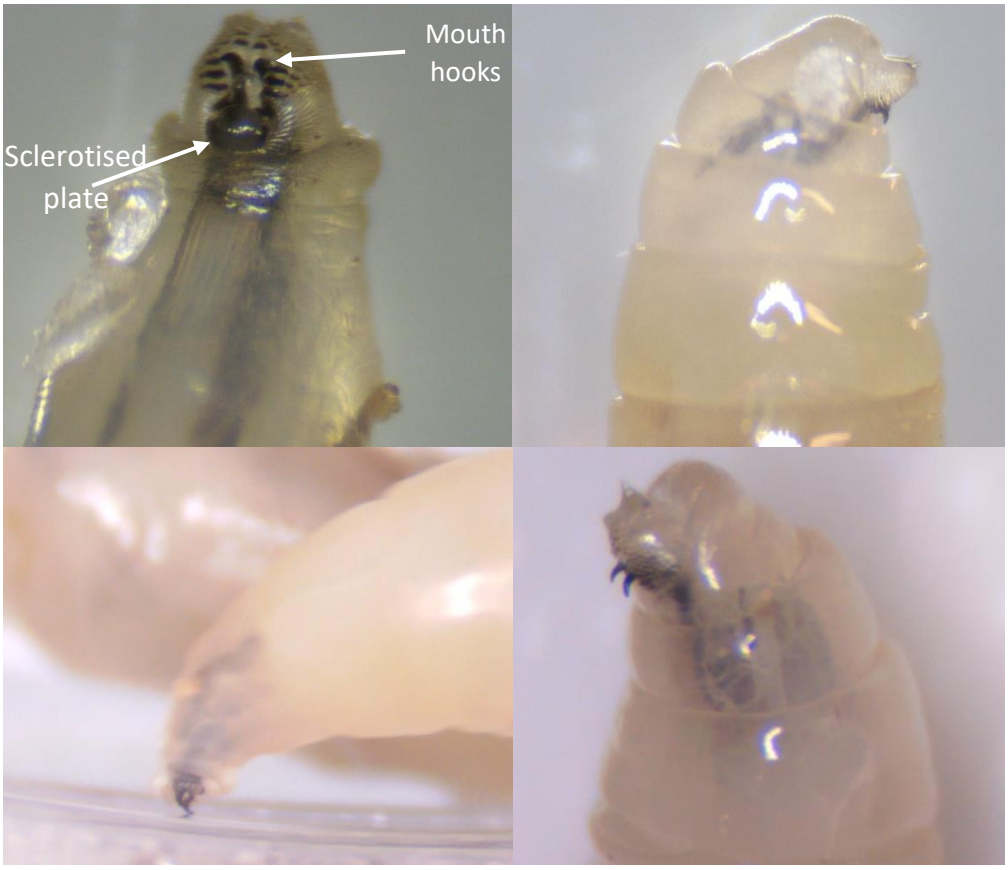


Figure 29. Top left. A ventral view of the mouth parts of O.luctuosum. Top right. A lateral view of the mouth hooks of F.maritima. Bottom right. A lateral view of the mouth hooks of H.ustulata. Bottom right. A ventral lateral view of the mouth hooks of H.buccata. Images take using GXCAM-U3-5.

Larval adaptations for movement in the wrack come in the form of black sclerotised spines on transverse rows on the ventral surface. These spines act as a gripping surface, so more and bigger spines suggest larvae have been naturally selected to move through wetter substrate (Egglishaw 1958). Egglishaw (1958) states that the *Coelopa* larvae have more of these spines than the other species discussed. They also state that *O.luctuosum* have a similar number of spines to the *Coelopa*, but the largest of which are smaller than the largest of the *Coelopa* species and that the larvae of all three species are "about the same size" (Egglishaw 1958). Of the other species, *F.maritima* and *H.buccata* are reported as having rows of very small white spines and *H.ustulata* has none at all. These ventral spines in *C.frigida* and *O.luctuosum* can be seen in Figure 30, where the relative size of each species can also be compared.

Figure 30. A lateral view of an O.luctuosum larvae (left) and a dorsal and ventral-lateral view of C.frigida larvae (right) highlighting the presence of the ventral spines in each species and their sizes relative to each other. Both images were taken at the same magnification and distance from the camera thus sizes are realtive. Image taken using GXCAM-U3-5

Regarding respiratory adaptations, Egglishaw (1958) notes the presence of hairs on the posterior respiratory spiracles of some of the Dipteran larvae. Like the gripping ventral spines, these hairs are only reported as being present on the *Coelopa* species and *O.luctuosum.* Egglishaw (1958) suggests they serve three functions. Firstly, these hairs are naturally splayed which provide resistance against the larvae becoming fully submerged in the wrack and loosing contact with the air. Second and thirdly, when the larvae are fully submerged, these hairs are forced backwards and form a cone around the spiracle which both prevents it from becoming blocked and traps a bubble of air in the cone allowing respiration to continue for a short time. Egglishaw (1958) suggests this is further evidence that these species are better physically adapted to the wetter and more liquid wrack substrates. The posterior spiracles of *C.frigida* and *O.luctuosum* can be seen in Figure 31.

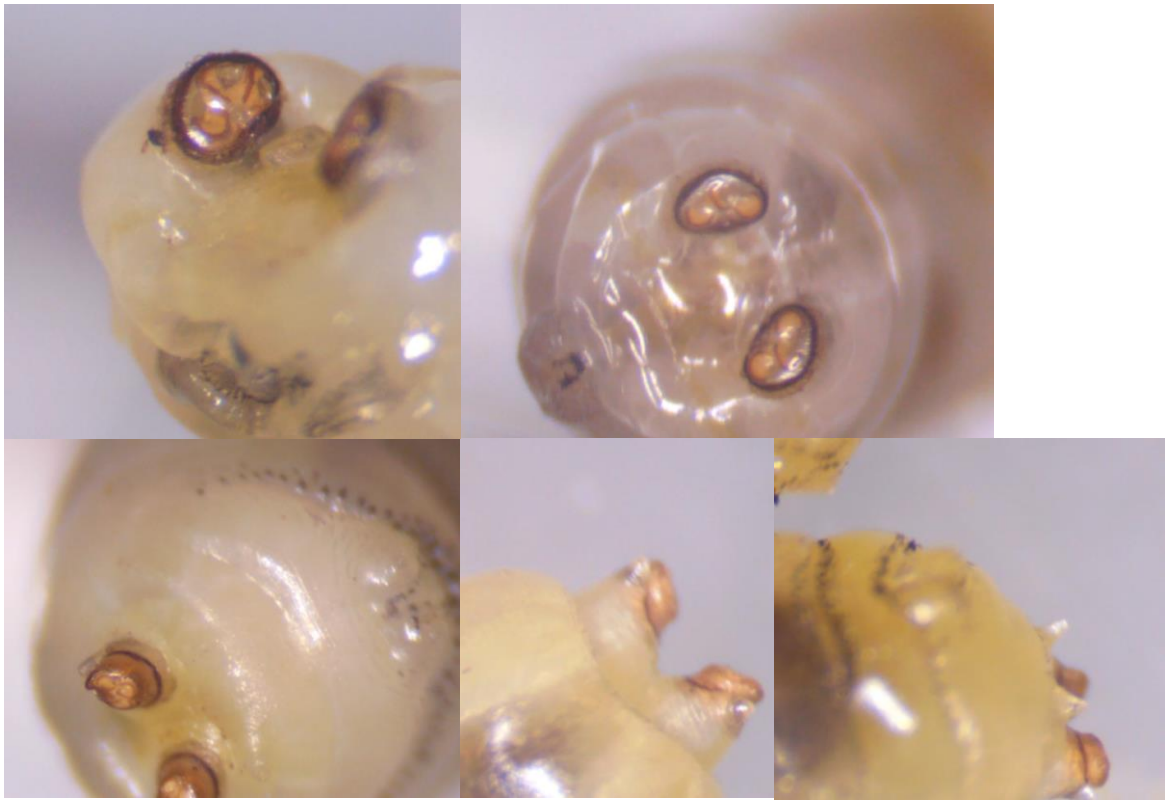


Figure 31. The posterior spircales of C.frigida (top) and O.luctuosum (bottom). Images taken using GXCAM-U3-5.

Lastly, Egglishaw (1958) suggests that the location in which larvae pupate is indicative of the type of wrack deposit they are best suited to inhabit. They report larvae of *C.frigida* and *C.pilipes* pupate in the upper layers of wrack deposits, or among the sand/shingle on the landward side of the deposit. Comparatively, larvae of *F.maritima*, *H.ustulata* and *H.buccata* pupate in the lower layers of the deposit or in the sand/shingle directly below it. They state *O.luctuosum* is the only species to pupate exclusively in the wrack and covers it's pupae in a white, calcium rich, secretion when it does so. Once again, it is suggested that this evidences an adaptation of the *Coelopa* species to the wetter deposits as they actively seek somewhere drier to pupate to prevent drowning. Conversely, *F.maritima*, *H.ustulata* and *H.buccata* seek somewhere wetter to pupate to prevent desiccation making them more adapted to relatively dry wrack deposits.

In their works Egglishaw (1958) found the distribution of *C.frigida* and *C.pilipes* larvae to be mostly confined to the large and wet wrack banks, while that of *F.maritima*, *H.ustulata* and *H.buccata* was exclusively in smaller and much drier wrack deposits, larvae of *O.luctuosum* were generally found in wrack of an intermediate moisture content. Egglishaw then inspects the structural differences between the larval species in an attempt to explain this observed distribution and this classically 'Darwinian' explanation is largely agreeable. However, there are a few irregularities in some of the

descriptions which support this theory. Firstly, the mouth hooks of *C,frigida* were not ibserved to be bifurcated or as rounded as Egglishaw (1958) describes them, as can be seen in Figure 28. Secondly, no hairs on the posterior spiracles of *O.luctuosum* or *Coelopa* larvae could be seen as Egglishaw (1958) describes, as can be seen in Figure 31. Lastly, the ventral rows of spines present on *O.luctuosum* larvae are much more pronounced and larger than those of *Coelopa* larvae, so much so they have reliably been used to identify *O.luctuosum* larvae from *Coelopa* during this project, and the larvae of *O.luctuosum* are also noticeably larger than the *Coelopa* larvae unlike Egglishaw (1958) describes (Figure 30). Of course, there have been many advancements since 1958, and the work of Egglishaw (1958) was the first thorough investigation into the fauna of British wrack beds. The observed distributions of the larval species by Egglishaw are largely consistent with those in this research however, it seems slightly less of this distribution can be attributed to the physical adaptations than Egglishaw (1958) suggests.

Amphipod distribution

The wrack fly species and their distributions are discussed relative to each other both here, and in the literature, because they appear to occupy very similar ecological niches (Backlund 1945, Butlin and Day 1984, Egglishaw 1958, Leggett 1993). This makes them of particular interest as Gause's (1934) competitive exclusion theory states no two species that compete for the same limiting resource can coexist indefinitely. As expected however, given their different taxonomy and evolution, Amphipods occupy a different niche from that of wrack fly larvae. Though some Amphipod species do also appear to occupy very similar ecological niches as each other. However, Amphipods were a late addition to this project and as they are often difficult to both catch and identify to a species level, only the family Talitridae and Gammaridae are used in this research. Similarly, studies on shore ecology in the literature often group Amphipods. Phillips and Arthur (1994) discuss them as one ecological group. Orr (2013) splits them into Talitrid Amphipods, those coming from the family Talitridae, which are semi-aquatic, and hyperbenthic Amphipods. While others only discuss those from a genus such as *Orchestia* (Talitridae) (Backlund 1945) or the more common and easily identifiable species such as *T.saltator* and *Orchestia gammarelus* (MacMillan and Quijón 2012, Moore and Francis 1986 , Morritt and Stevenson 1993, Ruiz-Delgado *et al* 2015, 2016).

Amphipods play a central role in the food web as an intermediate between the washed-up wrack and higher trophic levels (Griffiths et al. 1983, Brown and McLachlan 2002, Dugan et al. 2003). Talitrid Amphipods inhabit the area between the fore-dunes and the strandline, generally buried during the day and emerging at night to avoid desiccation and reduce visual predation (Orr 2013, Scapini *et al* 1992; Ruiz-Delgado *et al* 2016). Species from the genus *Gammerus* dominate detached

macroalgae and resuspended wrack in the nearshore (Backlund 1945). Where wrack deposits are present on the upper-shore, the density of Talitrid amphipods can be vast. (MacMillan and Quijón 2012; Ruiz-Delgado *et al* 2015, 2016). Amphipods have at least 2 broods a year (Persson 1999), and breeding is triggered by photoperiod and/or temperature: *O.gammarellus* breeds April-September and the onset is driven by temperature once max air temp reaches 10°C (Morritt and Stevenson 1993). In *T.saltator,* photoperiod is thought to initiate and end breeding (Williams 1985).

Stable isotope analysis suggests Amphipods feed directly on wrack and derived OM (Crawley and Hyndes 2007, Sturaro et al 2010). Although, Persson (1999) suggest they feed on the microorganisms present on the detatched algae/wrack. There appears to be more indication of them feeding on the algae directly. *Orchestia gammarelus* can produce the enzyme cellulose thus can decompose the cell walls of algae (Moore and Francis 1985) and Backlund (1945) report that *Orchestia platensis* survived for 7 months on sterile seaweed. Additionally, many sources agree that Amphipods are the first macrofauna to colonise wrack in the intertidal zone (Crafford and Scholtz 1987, Egglishaw 1958, Morritt and Stevenson 1993, Orr 2013).

3.2 Succession of wrack and its colonisation

Deposits that can be described as discrete and ephemeral (temporary) are often referenced as experiencing a clear sequence of invasion through their decomposition (Hodge and Arthur 1997). Crucial to this is the arrival of species at different times. The most intense competition will take place between species which are most ecologically similar, and in being ecologically similar these species are also likely to colonise at the same time (Hodge and Arthur 1997). Wrack beds have received less investigation than other discrete ephemeral resources like fallen fruit, dung and carcasses (Hodge and Arthur 1997, Shorrocks *et al* 1979). The discussed distribution of wrack invertebrates is also influenced by the succession of wrack colonisation and vice versa.

Following detachment from the substrate, algae becomes suspended in the water column and the majority of it works its way to the nearshore (Colombini and Chelazzi 2003). As it is transported, microorganisms, both those that were present when the algae were attached, as well as newcomers, colonise it and begin its decomposition (Orr 2013). While suspended in the nearshore, this algae and its particulate matter is fed on by suspension feeders, and crustaceans such as Amphipods and Isopods, which are in turn eaten by fish (Orr 2013). As a result of the nutrition and shelter detached algae provide, it plays an important role in the survival of juvenile fish in particular, and the abundance of juvenile fish has been shown to be positively correlated with the volume of drifting macroalgae (Crawley *et al* 2006). Prior to being deposited above the high tide line, detached

macroalgae is again colonised by Amphipods, though of different species, in the intertidal zones where they graze on the tough epidermis of the detached seaweed making it easier for successive decomposition to occur (Crafford and Scholtz 1987). However, Backlund (1945) state that nematodes are the first to colonise detached intertidal algae and perform a similar function.

Upon being deposited on or above the high tide line by the spring tide this detached seaweed forms a wrack bed and this moist, sheltered habitat is rapidly colonised by scavengers and detritivores, namely Talitrid Amphipods, Diptera and less often, isopods (Backlund 1945, Colombini and Chelazzi 2003, Egglishaw 1958, Orr 2013). However, at this point Diptera are present on the wrack as adults rather than larvae and do not feed on it or contribute to its decomposition, therefore it could be argued they have not yet colonised the wrack. Indeed, some of the wrack flies, *F.maritima* in particular, are generally present on the stranded seaweed in the intertidal zone. Dobson (1974) and Egglishaw (1958) note wrack flies as laying eggs 1-2 days after the wrack is deposited at which point they can be classed as having colonised the deposit. This delay may have been naturally selected for as the period between deposition of the wrack and oviposition of the Diptera eggs would allow the organisms that have already colonised the wrack to manipulate the deposit into one more suited to the Dipteran larvae. Additionally, since there is confusion on whether the Dipteran larvae feed on the wrack directly, or on the microorganisms that consume the wrack, this delay would allow these microbial populations to increase ensuring there is sufficient food for the larvae if indeed they do consume the microorganisms.

The evidence in the literature does suggest the larvae are dependent on the microorganisms as Backlund (1945) attempted to rear *Coelopa* larvae on sterile seaweed, but was unsuccessful. However, attempts to rear them on bacteria and fungi cultured from wrack were also unsuccessful suggesting this dependence is facilitative. Of course, there are several potential drivers for these results, but they do suggest *Coelopa* larvae require the wrack to be somewhat decomposed to make nutrition available to them, whether this is through consumption of the microfauna directly or the availability of nutrition following breakdown by organisms which colonise the wrack first is unknown.

As found by Egglishaw (1958), the microclimate and habitat formed by a wrack bed dictates which Diptera species will colonise it. Wetter deposits were found to contain *Coelopa* and some *O.luctuosum* larvae and the drier banks *F.maritima*, *H.ustulata* and *H.buccata*. Which species are present is also dependent on the time of year and although the literature is conflicting it appears that *Coelopa* and *O.*luctuosum larvae can be present all year while the others are only present seasonally (Butlin 1983, Egglishaw 1958,1965).

As succession continues, the wrack can also be colonised by terrestrial macro-invertebrates such as predatory beetles, mites, spiders and parasitic Hymenoptera (Deidun et al 2009, Colombini et al 2009). These fauna feed on the macro-invertebrates already inhabiting the wrack (Egglishaw, 1958). The larvae are also a high energy food source to many shorebird species and are prevalent in their diets (Dierschke 1998, Goss-Custard *et al* 1977, Mendonca *et al* 2007, Prys-Jones *et al* 1992, Robinson 2005, Summers *et al* 1990). As more OM is released from the wrack through its breakdown it either accumulates in the substrate where Amphipods and Polychaetes feed on it (Raffaelli 2000, Rossi and Underwood 2002, Bolam *et al* 2000) or, is transported back to the nearshore environment where it can play three times as big a role in 'primary production' than that of chlorophyll-a (Orr *et al* 2014).

Once Diptera larvae are developed, they pupate either in the wrack on in the substrate around it and, following eclosion after pupal development, these adults then colonise the next wrack deposit. Although, Crafford and Scholtz (1987), when investigating the Antarctic wrack fly *Paractora dreuxi-mirabilis,* found the fragmentation of wrack leads to a reservoir of wrack detritus below the surface of the substrate where larvae can be sustained even if the deposit is swept away by a storm. They argue that the colonisation of new wrack deposits comes as much from adults that have developed in this reservoir as from existing deposits. They also suggest that the presence of fresh wrack stimulates eclosion. Compared to Dipteran larvae, Amphipods are highly mobile and are adept at swimming and burrowing, so submersion poses little threat to them. However, Dipteran larvae and pupae still present in the wrack, if and when the sea reaches the deposit, can be swept away if the tide has the strength to disassemble part or all of the wrack bed. While Dobson (1974) found *C.frigida* larvae could survive up to six days suspended in aerated seawater and often observed larvae that had been removed from the wrack washed back onto shore, this usually proves fatal. When suspended in the water, these larvae are eaten by fish and birds (Orr 2013), and it was noted during this research that, in areas with high amounts of wrack, at high tide the waterline often had shorebirds, particularly waders, along its length allowing the tidal action to bring displaced wrack invertebrates to their feet.

This description of the succession and colonisation of wrack beds from the literature is one in which studies are largely in agreement. There are of course differences between studies, but the literature broadly agrees.

3.3 Observed succession and wrack fauna distribution

For a 2 year period during this research project, field days were spent collecting samples, generally this took place at least once a month. During this time several observations on the ecology of wrack beds were made. The study sites are displayed in Figure 32. Note this figure extends over two pages.

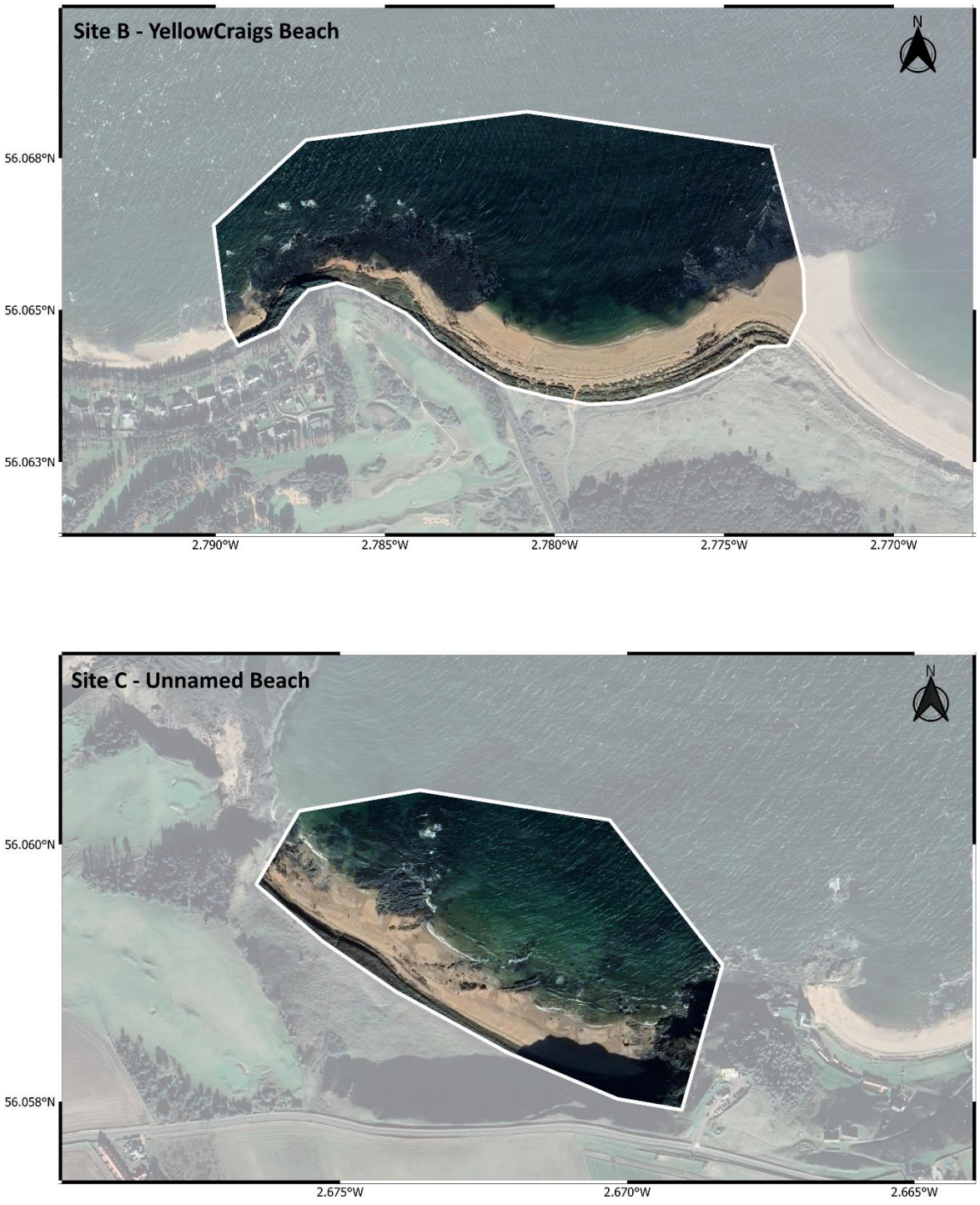


Figure 32. The location of the study sites of this research on a national (Scotland), regional (East Lothian) and local (site A,B,C) scale. Map derived from QGIS Desktop 3.30.1. Base maps: © MapTiler https://www.maptiler.com/copyright/

As described by Backlund (1945) and Egglishaw (1958), the colonisation of wrack partially depends on the physical habitat that wrack deposit creates, and this observation is reinforced by others (Butlin 1983, Colombini and Chelazzi 2003, Leggett 1993). Similarly, as discussed in chapter 2, the type of seaweed a deposit is comprised of influences how it decomposes. Furthermore, the seaweed species the wrack deposit is comprised of has received much attention regarding its influence on which invertebrate species will colonise the wrack bed (Butlin 1983, Colombini and Chelazzi 2003, Dobson 1973, Hodge and Arthur 1997, Leggett 1993). However, in the literature, there does not appear to be a discussion that brings all of these observations together and asks why wrack deposits tend to be comprised of either *Fucus* or *Laminaria*?

The discussion on seaweed species, and the nature of decomposition that they facilitate, in Chapter 2 concludes that *Laminaria* facilitates the wet decomposition typical of wrack banks, and *Fucus* facilitates the drier decomposition typical of the smaller wrack deposits. However, this also depends on size, and a larger deposit is also required to facilitate this 'wetter' decomposition, which creates its own microclimate. Interestingly, these two factors nearly always align. While some *Fucus* can become incorporated into wrack banks, they are usually dominated by *Laminaria* and conversely, while *Laminaria* can form the smaller deposits, these tend to be comprised solely of *Fucus*. Although buried, small deposits of *Laminaria* are not infrequent. There is a case for each type of decomposition preserving itself where faster decomposition releases more moisture facilitating more decomposition, however this doesn't explain why it tends to only be *Laminaria* which form large deposits.

Here, it is hypothesised that this is a result of the ease of which each seaweed type can be transported. Indeed, the only time large fucus deposits were observed during this research project were a result of aeolian transport to a natural collection point such as a large rocky outcrop or curve in the shoreline. Perhaps then, *Fucus* are lighter and/or have greater buoyancy allowing the tide to deposit them at its highest point. In doing so, they are exposed to the wind for ~12 hours until the next high tide in which time evaporation reduces their water content such that they can be transported by the wind more easily. Though if some moisture can be retained at the bottom of the deposit, this will help fix it to the substrate and facilitate biological activity which will release more moisture further anchoring it. On the other hand, it is hypothesised *Laminaria* require more energy to be transported and thus remain in the intertidal zone. The shape and slope of the shore and the wind direction will slowly work these individual pieces of detached *Laminaria* in the same direction until they too gather in a 'collection feature'. Their deposition then occurs on mass when wave(s) with more energy deposit these all at once. Additionally, amphipods and isopods feeding on this

detached seaweed, which remain in the intertidal zone, will gradually reduce the particle size also aiding deposition.

This theory of *Laminaria* aggregating intertidally as a result of the morphodynamics of the coastline until a wave(s) with sufficient energy can transport it to the high tide line is further supported by an alternative interpretation of the role spring tides play. As discussed, several studies suggest wrack is largely deposited and removed by spring tides which occur once a month, thus giving a standard lifecycle to a wrack bed of around 28 days though they can be present longer (Dobson 1974, Leggett 1993). Therefore, previous discussions, give a rather discrete impression of a wrack bed's presence in the sense that they appear the morning after a full moon and then disappear 28 days later, the morning before the next full moon. However, spring tides occur twice per lunar cycle (Figure 33) and during this research, their influence was observed as being of a more gradual and continuous nature.

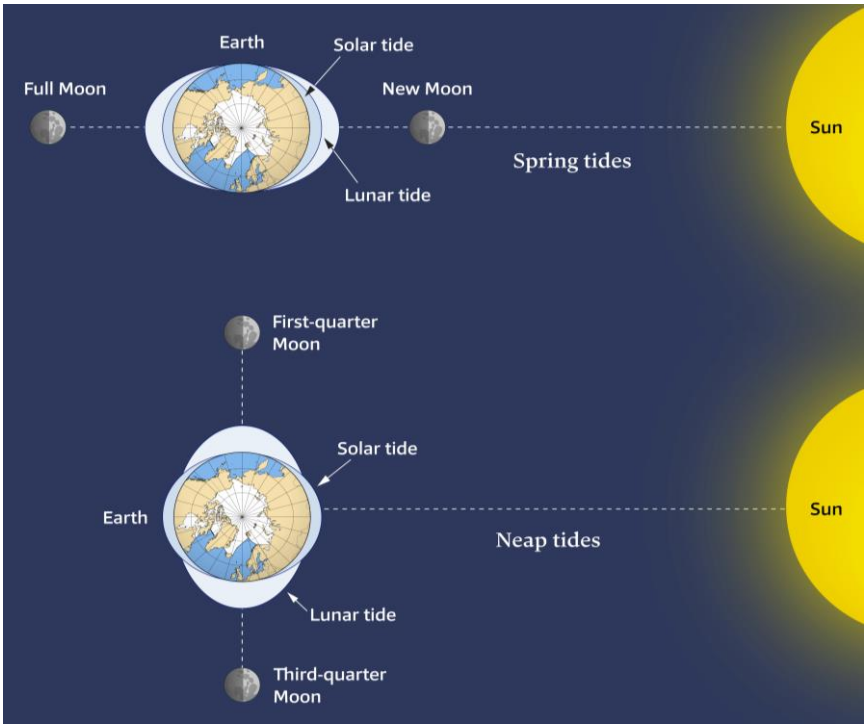


Figure 33. Earth – Moon – Sun positions and corresponding tides. Image adapted from https://www.sailingissues.com/navcourse6.html

When the gravitational pull of the Sun and Moon are at 90° to each other they are as opposing as can be and this opposition means the lowest high tides of the lunar cycle occur known as neap tides (Figure 33). This is the case when the moon reaches a quarter phase which is seen from Earth as a half Moon. During the ~7 days following a quarter phase, the position of the Moon relative to the Sun and Earth moves at an even rate and at the end of these ~7 days the position of the Moon

relative to the Earth is in line with the sun, this is viewed as either a full or new Moon. At this point the gravitational pull of the Sun and Moon are in the same direction and thus compound. This creates the highest high tides of the lunar cycle, the spring tides. During the intermediate tide between the neap and the spring tides, the height of the high tide increases linearly as the Moon's position progresses to the spring tide position (Kvale 2006). It is therefore hypothesised that *Laminaria* which aggregates intertidally gradually progresses up the shore during these 7 days. However, depending on the size of the aggregation, this alone may not be enough to deposit the mass of seaweed at the high tide line.

This hypothesis of wrack deposition does theoretically explain why wrack deposits tend to occur as either *Laminaria* or *Fucus* rather than an even mixture of the two. It also potentially explains another observation on the distribution of wrack. It has been observed during this research that further landward from large *Laminaria* wrack banks, often at the vegetation line, small deposits of highly decomposed *Fucus* can be found, which are wetter, more decomposed and more biologically active than *Fucus* deposits not behind *Laminaria* deposits. In these, *C.pilipes* adults and larvae as well as *O.luctuosum* larvae can often be found, they also contain a higher proportion of wood and inorganic material. It is hypothesised that as the tide height increases between neap and spring tides the denser *Laminaria* is transported further up the shore, but not to the tide's highest point. Thus, the *Laminaria* is deposited around the height of the intermediate tide's maximum height. As a result, the large *Laminaria* beds that exist there are at least partially submerged before, during and after the spring high tide. At this time, the less dense *Fucus* and anything else which is buoyant is floated out of the wrack bed and to the spring high tide line further landward of the wrack bed. This would result in a deposit of *Fucus* which has partially decomposed along with wood, inorganic matter and the slower developing *C.pilipes* and *O.luctuosum* larvae, occurring landward of the wrack bank and differing from other *Fucus* deposits.

Of course, it is likely that all the factors discussed, as well as some which are unconsidered, contribute in varying amounts to the deposition of wrack, it's succession and it's colonisation. However, this hypothesis theoretically explains the observed distribution of seaweed species within wrack which in turn influences the manor of decomposition and the invertebrate species which can thrive there reported in the literature. This theory of wrack and wrack fauna distribution is visualised and evidenced in Figure 34 (N.B. this figure extends over two pages).

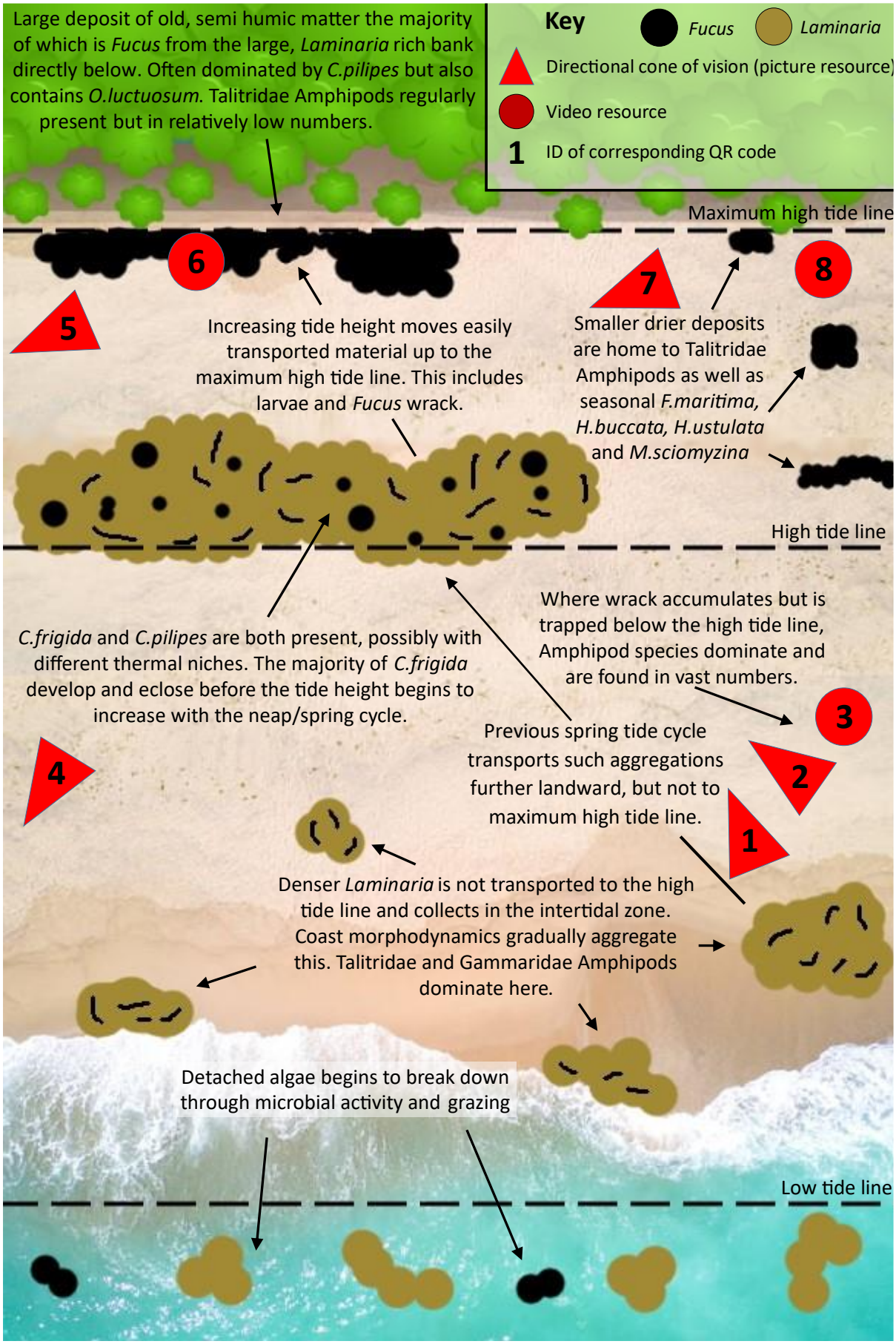

Large deposit of old, semi humic matter the majority of which is *Fucus* from the large, *Laminaria* rich bank directly below. Often dominated by *C.pilipes* but also contains *O.luctuosum*. Talitridae Amphipods regularly present but in relatively low numbers.
Key
Fucus
Laminaria
Directional cone of vision (picture resource)
Video resource
1 ID of corresponding QR code
Maximum high tide line
6
5
7
8
Increasing tide height moves easily transported material up to the maximum high tide line. This includes larvae and *Fucus* wrack.
Smaller drier deposits are home to Talitridae Amphipods as well as seasonal *F.maritima, H.buccata, H.ustulata* and *M.sciomyzina*
High tide line
C.frigida and *C.pilipes* are both present, possibly with different thermal niches. The majority of *C.frigida* develop and eclose before the tide height begins to increase with the neap/spring cycle.
Where wrack accumulates but is trapped below the high tide line, Amphipod species dominate and are found in vast numbers.
3
2
1
4
Previous spring tide cycle transports such aggregations further landward, but not to maximum high tide line.
Denser *Laminaria* is not transported to the high tide line and collects in the intertidal zone. Coast morphodynamics gradually aggregate this. Talitridae and Gammaridae Amphipods dominate here.
Detached algae begins to break down through microbial activity and grazing
Low tide line

Figure 34. A visual representation of the proposed theory of wrack deposition and colonisation. This figure presents interactive evidence for the theory in the form of photos and videos accessed via the QR codes. Red triangles represent a photograph with the cone of vision in the photograph mirrored by the triangle. Red circles denote video resources representing their relative location. The numbers identify which QR code matches which resource. Picture resources contain text explaining them. That of video resources is found here: 3. An example of the high densities of Amphipods present in seaweed deposits trapped below the high tide line. 6. An example of the high densities of C.pilipes adults observed in Fucus deposits landward of large Laminaria deposits. The cold weather prevents them from flying upon being disturbed. 8. An example of the high densities of Talitrid Amphipods often present under the common small Fucus deposits. Figure created in GIMP 2.10.28.

It has also been observed during this research that the stipes of *Laminaria* can provide their own habitat due to their structure. Moving from the outside to the centre, this structure, seen in Figure 35, can be broadly split into a tough tightly packed outer layer, the epidermis, a less densely packed and thus softer cortex and a loose and very soft centre, the medulla.

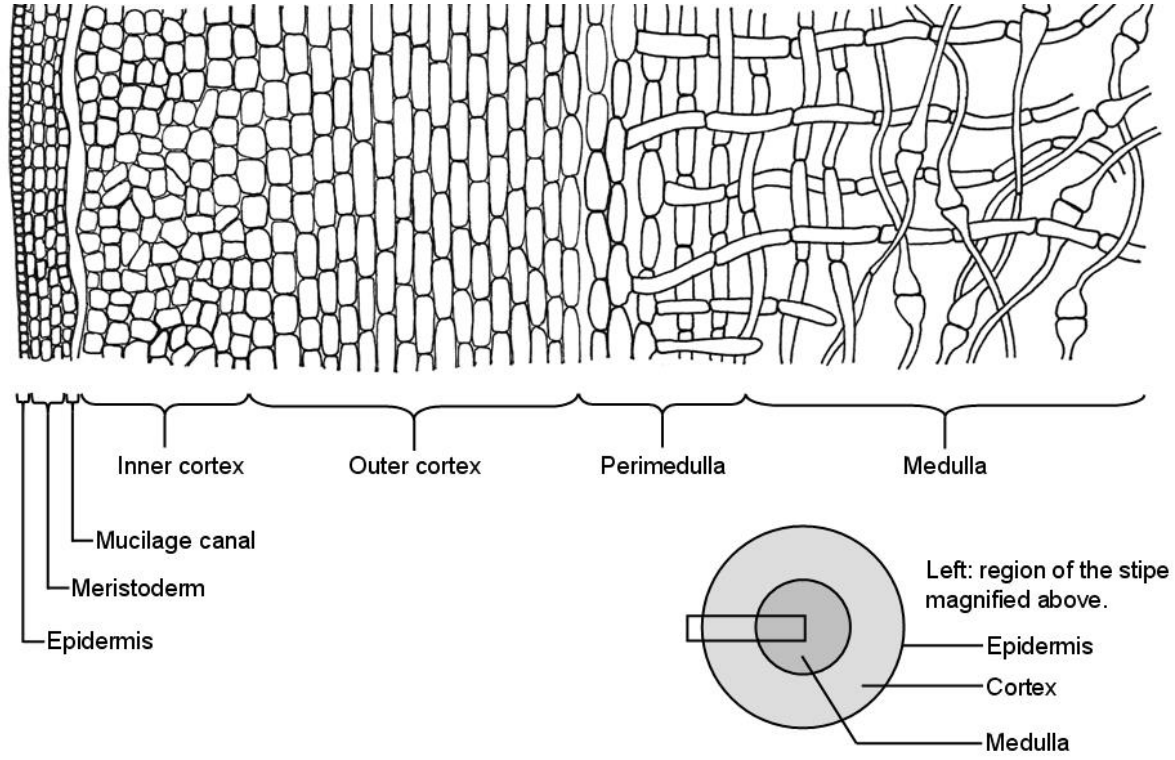


Figure 35. The structure of the stipes of brown seaweeds as viewed from a dorsal cross section. Figure from https://cronodon.com/BioTech/Seaweeds.html

The medulla can be easily burrowed into by 1st instar larvae, which, as they feed and grow, work their way deeper into the medulla and the cortex. As the larvae develop, they are protected from predation, desiccation and tidal displacement and are also insulated. Thus, these stipes may create a habitat in which larvae have a higher rate of survival and those which require a more moist substrate are not entirely dependent on the presence of large wrack banks to create this. However, it was not only the *Coelopa* species and *O.luctuosum* that were found thriving in these stipes. Individual pieces of stipe, particularly those buried, were also found to contain the larvae of *F.maritima*, *H.ustulata* and/or *H.buccata* though these were never found with *Coelopa* species. Concerning the *Coelopa* species, there was an observed increased likelihood of *C.pilipes* to be found in the stipes rather than *C.frigida*. However, no data to quantify this were collected, and it is observational only. Figure 36 shows one such observed aggregation of *Coelopa* larvae in a *Laminaria digitata* stipe.

Figure 36. A Laminaria digitata stipe found at site 2 in November 2019. Splitting the stipe open revealed a high density of 3rd instar Coelopa larvae. Note the medulla of the stipe is still intact below the larvae but is missing where they are present and above.

Another observation on the distribution of wrack fauna made during this research is that of the temporal distribution of species. Over a 2 year period, shores along the East Lothian coast were frequently visited and notes on species present were taken. *Coelopa frigida* and *Coelopa pilipes* could be found all year round. They were sometimes absent on a local scale i.e on individual shores, particularly during the summer months, but were consistently present regionally. *Orygma luctuosum* were also present all year round, although they are fairly elusive as adults, preferring to scurry deeper into wrack than fly and occur in lower density than the *Coelopid* species making them easy to miss in the field. However, their larvae are very distinct. During the warmer months, approximately April through September, *Fucellia maritima* adults replaced the *Coelopa* species as the most abundant on a given shore, and this is likely linked to reduced wrack deposits generally occurring during this time creating less of the large wrack banks *Coelopa* thrive in. *Helcomyza ustulata* was very rarely encountered and was only done so from June until August/September. No more than 5 adults were captured at once even when spending hours searching for them specifically. *Malacomyia sciomyzina* were also rarely encountered and were done so in May, June and July. *Heterochelia buccata* were commonly encountered during September, October and November.

Lastly, *F.maritima* adults were often observed aggregating around faecal matter or other decomposing organic matter deposited onto the shore, most frequently crabs and jellyfish. Therefore, the company of a spaniel, or any other *Canis lupus familiaris,* can facilitate in the baited capture of ample *F.maritima* adults as was the case in this research. Although, said companion was equally as effective in disturbing and scattering Diptera.

3.4 Culturing wrack invertebrates

3.4.1 Introduction

Understanding the ecology of species and ecosystems is essential for us and the natural world we are part of. The more they are understood, the better they can be reactively protected from anthropogenic disturbances, or better still, proactively protected. In turn, this also protects the dependence of people on nature that is vital for numerous aspects of our lives such as food and material production, maintaining clean air and water and sustaining biodiversity. The understanding of the ecology of wrack beds and their organisms has been used for several functions such as: quantifying the effect of harvesting beach cast seaweed for biofuel (Orr 2013), protecting coastal and nearshore ecosystems by evidencing the negative impact of beach grooming on them (Gilburn 2012), protecting against coastal erosion by demonstrating the importance of wrack in the stabilisation of the backshore and dunes and the succession of plants there (Dugan and Hubbard 2010) and advancing the field of genetics through the culturing of wrack flies and their use in experiments (Butlin 1983, Leggett 1993, Thompson 1951).

To meet some of the demand for ω-3 PUFAs, any alternative will have to be industrially scalable, thus being cultured is a prerequisite of any wrack fauna's potential as an alternative ω-3 source. *C.frigida* are regarded as the most reliably cultured wrack fly and thus have received the most experimental attention and culturing (Butlin 1983, Dobson 1974, Egglishaw 1958, Leggett 1993, Thompson 1951). *C.pilipes* and *O.luctuosum* have been cultured with less success (Butlin 1983, Dobson 1974, Egglishaw 1958, Leggett 1993). Only Egglishaw (1958) report attempting to culture *F.maritima*, *H.buccata* and *H.ustulata,* the latter of which was unsuccessful.

Egglishaw (1958), similar to Thompson (1951), cultured all wrack flies on split *Laminaria* stipes in 1-2lb Kilner jars with a lid of perforated cardboard. Wet sand or cellulose wool were placed in the bottom to prevent the *Laminaria* drying out. If larvae were not present, the *Laminaria* most often became mouldy or covered in a thick opaque biofilm. When larvae were present, they quickly broke the stipe down into a semifluid of which some was removed if it became too deep and larvae began to drown. In these conditions, Egglishaw (1958) found *C.frigida* "eventually laid eggs"(pg50) in one,

two or three batches each containing 60-80 eggs. Once the larvae developed, they pupated in clusters mostly in the upper drier parts of the jar. At 24°C, Thompson (1951) found an egg to adult development time of 11-12 days under these conditions. Egglishaw (1958) report *C.pilipes* as "very reluctant to lay eggs in the described conditions" (pg50) and out of 53 jars five batches of eggs were laid, however the resulting larvae thrived in the same fashion as *C.frigida*. In response Egglishaw (1958) altered the moisture content in the jar, the state of decomposition of the wrack provided and the species of the provided wrack, none of these were found to improve egg laying of *C.pilipes*. At room temperature, the development of *C.pilipes* took 17-25 days.

Egglishaw (1958) observed *O.luctuosum* embedding their eggs in wrack tissue. To provide a suitable substrate for this, nematodes found in wrack beds were added to the cultures. These nematodes reproduced quickly and softened the stipes. In these conditions, adults of *O.luctuosum* readily mated and up to 180 eggs were laid by each female. Eggs were laid in the tissue of the stipe with the filaments in the air or on the stipe surface. The development time was found to vary greatly, in summer at room temperature, the 1st and 2nd instars lasted two -three days, however, some larvae pupated after ten days while others were still 3rd instar larvae six weeks later. In winter, the 1st and 2nd instars were 6-8 days each and the 3rd lasted up to 12 weeks. Pupation occurred in the wool at the bottom of the jars. In summer, this lasted 8-13 days. However, those which pupated in November did not emerge as adults until the end of the following March.

Similarly, Egglishaw (1958) found *H.buccata* 1st and 2nd instars lasted 3-5 days each and the 3rd varied considerably. Generally, the 3rd instar larvae were active for 4 weeks before becoming lethargic under the wool or sand; after 5-6 weeks of this lethargy, they pupated. The pupal stage lasted approximately eight weeks. Unless the jars were actively kept dry through the removal of moisture with a pipette, a very low egg to adult viability was found. Of 189 eggs laid in unmaintained jars, only nine adults emerged. Lastly, of *F.maritima,* Egglishaw (1958) found mating occurred readily and eggs were laid on *Fucus* and *Laminaria* in all states of decomposition but larvae died if they didn't have access to partially decomposed wrack. Females also laid eggs in jars with no seaweed on any moist surface. At room temperature, the 1st and 2nd instars lasted 4-6 days and the 3rd 8-10 days. When attempting to culture *H.ustulata* mating was evident but females died within a week without laying eggs (Egglishaw 1958).

From these methods established by Thompson (1951) and Egglishaw (1958), the culturing of the *Coelopa* species was adopted in the genetic field, and methods progressed through research such as Dobson (1974) and Butlin (1983) to those used by Leggett (1993) who investigated the competitive

interactions between *C.frigida, C.pilipes* and *O.luctuosum*. During this research adults were kept in 1/3 pint bottles with a piece of cellulose soaked in 0.5% mannitol solution which provided a food source and kept the humidity high. *C.frigida* were stored at 4°C and C.*pilipes* at 10°C as *C.pilipes* died after a few days at lower temperature. Adults could be kept like this for up to two months with no apparent detrimental effects. *O.luctuosum* could only be maintained like this for up to four days. Each species was cultured separately in 30x20x20cm glass tanks containing predominantly *Fucus* species of seaweed harvested live from the intertidal zone. This was collected around every three weeks and was stored at 4°C. Cultures were setup in this way once a month for *Coelopa* species which had 30 mating pairs of adults added, *O.luctuosum* were setup every two months with 20 pairs of adults. The *C.frigida* cages produced up to 2000 adults, the other two were less productive, though *O.luctuosum* broke down a greater volume of seaweed.

When acquiring eggs for experiments, great difficulty was had with C.*pilipes* and more adults and temperature increases were used in response to this with no effect. Given Dobson's (1974) observation that *C.pilipes* require a mixture of *Fucus* and *Laminaria* some *Laminaria* was added, despite it not being present nearby and so rarely being used, this still did not work. Seaweed at different levels of decomposition was used, and the presence of larvae, *E-coli* and yeast was also experimented with to no avail. Finally, mincing the seaweed in a meat mincer before adding it to the tank resulted in a small number of eggs being acquired.

More recently, the *Coelopa* species are reported as being cultured in studies that investigate sexual conflict (Blyth and Gilburn 2011, Dunn *et al* 2005, Edward and Gilburn 2013, Gilburn *et al* 2009, Meader and Gilburn 2008) among others, and most recently, the chromosome inversion system has been the subject of genetic investigation once again (Berdan *et al* 2021). These all give relatively brief overviews of the culturing methods which mostly add adult flies to boxes stored at 24°C, on a 12:12 light/dark cycle containing an excess of minced *Fucus* sp substrate, though some also use minced *Laminaria*. The study by Biancarosa *et al* (2018), which can be thought of as a precursor to this research, give a slightly more detailed description of the rearing methods. The same methods as described above were used but it is specified that the seaweed was stored at -20 °C before use and 2kg of either minced *Fucus serratus* or *Laminaria digitata* were added to 10L plastic containers along with 25 mating pairs of *C.frigida* or *C.pilipes*.

In addition to wrack fly larvae, Amphipod species that feed on wrack are considered as an alternative ω-3 PUFA source here. This consideration was a late addition to this project and thus they are not cultured in it. However, their ability to be cultured is evidenced in the literature (Baeza-

Rojano 2013) and in industry as Amphipod species are a popular live food in the Aquarium hobby. This chapter now presents the methods and results of attempts to culture the wrack fly species present in South-Eastern Scotland.

3.4.2 Methods

Colony setup

Throughout this research, the culturing of wrack flies took place in a controlled environment facility (CEF) at 24°C on an 8:16hr light:dark cycle and at a relative humidity of 60%. Six different substrates were used: the frond of *Laminaria digitata*, the stipe of *Laminaria digitata*, *Fucus* species, *Alaria esculenta*, *Saccharina latissima* and farmed *Laminaria digitata.* Three of these represent the same species (*Laminaria digitata*) thus this variable is referred to as substrate rather than substrate species or seaweed species. Of these six substrates, the latter three were sourced from seaweed farms. Each of *Alaria esculenta*, *Saccharina latissima* and *Laminaria digitata* were obtained from both Islander Kelp LTD. Rathlin Island, Northern Ireland and The Scottish Association for Marine Science (SAMS) Oban, Scotland. When not specified as farmed, *Laminaria digitata* along with the *Fucus* substrate were collected from the shores highlighted in Figure 32. These wild samples consisted of detached algae taken from the backshore or intertidal zone, only fresh seaweed was taken. Where necessary, these collections were separated into frond and stipe and stored along with the farmed seaweeds at -20°C. To be used in cultures, portions of these substrates were broken off with a hammer and placed in water for around 10 minutes to soften before being minced in a meat mincer.

Cultures were either created with adults from existing lab colonies or samples from the field. In either case, the container holding the flies was placed in a dark room which contained a light box, a pump and a lidless plastic box. The box was placed on its side with the base against the face of the light box and fabric mesh attached to one side of the lid opening so it draped to create a screen. The screen was lifted and the container holding the flies opened. As most flies are positively phototactic they move to the light. Once the container was empty, the screen was released and flies could be pooted into sample pots with the aid of the pump. Following this, pressurised carbon dioxide (CO_2) was fed into the sample pot sedating the flies, which were then transferred to a porous plate to which the CO_2 was then connected. This kept flies sedated while they were identified, sexed, counted and transferred to sample pots or cultures. Flies were sedated for no more than 2 minutes and only sedated once. Thus, it was important not to overfill each sample pot at the light box. Flies not used in cultures were kept at room temperature and had access to a small petri dish containing coarse grained pearl sugar and a cuvette on its side filled with water and capped with cotton wool.

Ease of culture

The aim of this analysis is to assess the ease at which each wrack fly species could be cultured on each substrate. In attempting to successfully culture wrack flies on different substrates, two variables were experimented with: the density of individuals relative to the substrate and whether the cage was open or closed, referred to as cage status. Density was manipulated through the number of mating pairs added to substrate and expressed as mating pairs per 100g of substrate. Cage status had two potential configurations. Closed, where the plastic container housing the culture (referred to as the cage) had a lid, of which part had been replaced by either fabric mesh or blue roll to allow gas exchange. Or open, where the plastic container had no lid and was placed inside a mesh cage. The open and closed configurations can be seen in Figure 37.

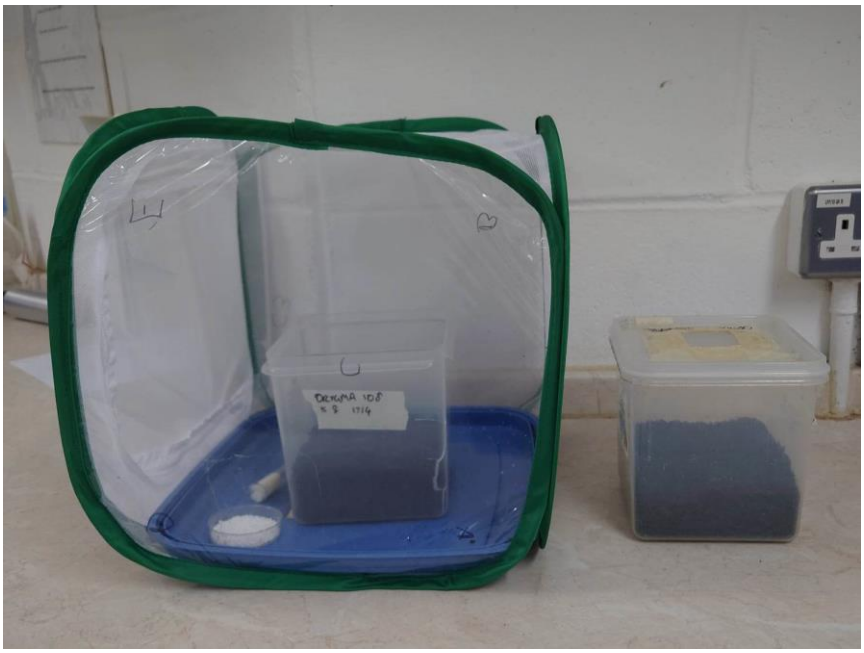


Figure 37. Left. The setup of an open cage configuration. Right. The setup of a closed cage configuration.

Data were gathered on successful and unsuccessful cultures. A successful culture was considered one from which more adults eclosed than initially populated the culture. These data are inherently unbalanced, rather than being from a balanced experimental design, they represent reactive changes to unsuccessful cultures throughout this research. For example, if species x could be reliably cultured on substrate y in a closed cage, the only time this needed altered was in experiments investigating the affect(s) of substrate type. The resulting data and they were analysed is examined in the results section. All statistical analysis were performed in R programming version 4.2.2 (R Core Team 2022).

Optimum larval density

Alongside this analysis of ease of culture, an experiment was setup which aimed to replicate the findings of the optimum larval density in *C.frigida* by Leggett (1993). Leggett (1993) modelled a relationship between larval density and survival in *C.frigida* and *C.pilipes.* These results have advised the optimum densities used for both species during this research thus it seems wise to corroborate them. To do so, 200ml square plastic tubs (n=153) were filled with 50g of minced *Laminaria digitata* frond. To these, *C.frigida* eggs were added, the number of eggs added was used to manipulate the density. After 48hrs, the piece of substrate eggs were placed on was removed and the number of unhatched eggs counted. From this, density expressed as larvae per gram of substrate (larvae/g) was calculated. Density ranged from 0.1 - 2 larvae/g. Eggs were collected from *C.frigida* cultures using a moistened paintbrush under a magnifying ring lamp. The number of flies which eclosed from each replicate was recorded.

3.4.3 Results

The following Diptera were obtained from wild samples: *C.pilipes, C.frigida, O.luctuosum, F.maritima*, *H.buccata, M.scioymzina* and *H.ustulata*. Of these, *M.scioymzina* and *H.ustulata* could not be cultured. Few *H.ustulata* individuals were sampled and only three mating pairs ever obtained. From these, no eggs were laid in an open cage containing *Fucus*, *Laminaria* and a mixture of the two, the adults died after three days. *M.scioymzina* were more abundant when present, though no more than 10 mating pairs were ever sampled at once. Under the same conditions as *H.ustulata,* they also laid no eggs. From August to October, *H.buccata* were very abundant and ample mating pairs could be obtained. Culturing of these appeared successful at the start and, in an open cage with *Fucus* substrate, eggs were laid and 3rd instar larvae developed. However, many of these did not pupate and appeared happier to die than do so. Of those that did, no flies eclosed after three months at which point the culture was terminated. While *O.luctuosum* were present year round, their abundance was consistently low and few mating pairs were obtained at any one time. Adults tended to die in the lab, but when they stayed alive, mating was evident. Despite frequent mating, eggs were not often laid but when they were, quickly developed into 3rd instar larvae. Similarly to *H.buccata* some did not pupate but rather than this leading to a high mortality, larvae seemed capable of staying thus as long as adequate nutrition was present. Of those which did pupate, very few ever eclosed. These were left for up to 5 months before being terminated. The remaining *C.frigida*, *C.pilipes* and *F.maritima* could be reliably cultured allowing colonies to be setup providing adults for experiments.

Additionally, the observed attraction of *F.maritima* to faecal matter, and the taxonomy of *O.luctuosum* placing it in the family Sepsidae, often referred to as dung flies, prompted attempts to rear them on cattle (*bos taurus*) dung. Both species laid eggs in the dung, though *O.luctuosum* did so more readily. However, no *F.maritima* larvae developed beyond the 1st instar. Some *O.luctuosum* developed into 3rd instar larvae but a higher mortality rate was observed than on algae and no pupation occurred.

Ease of Culture of C.frigida

The probability of a *C.frigida* culture being successful, depending on the substrate the culture contained, was modelled using a binomial logistic regression (Appendix 3.1). From this, the mean probability and 95% confidence interval (CI) for each substrate was estimated. Each estimated probability was compared pairwise using a tukey multiple comparison test with the 'emmeans' package (Lenth 2023). These pairwise comparisons and the estimated probabilities are visualised in Figure 38. The likelihood of the differences in the estimated probabilities of success between each pairwise comparison having occurred by chance if there is truly no difference, are represented as p-values in Table 3. *C.frigida* reared on the frond of *Laminaria digitata* is estimated to have a higher probability of being successful than when reared on *Fucus* sp (p=0.008), *A.esculenta* (p<0.001), *S.latissima* (p<0.001) or farmed *L.digitata* (p<0.001). The probabilities of all other pairwise comparisons of *C.frigida* being successful are estimated not to differ between substrates at a significance level of 0.05.

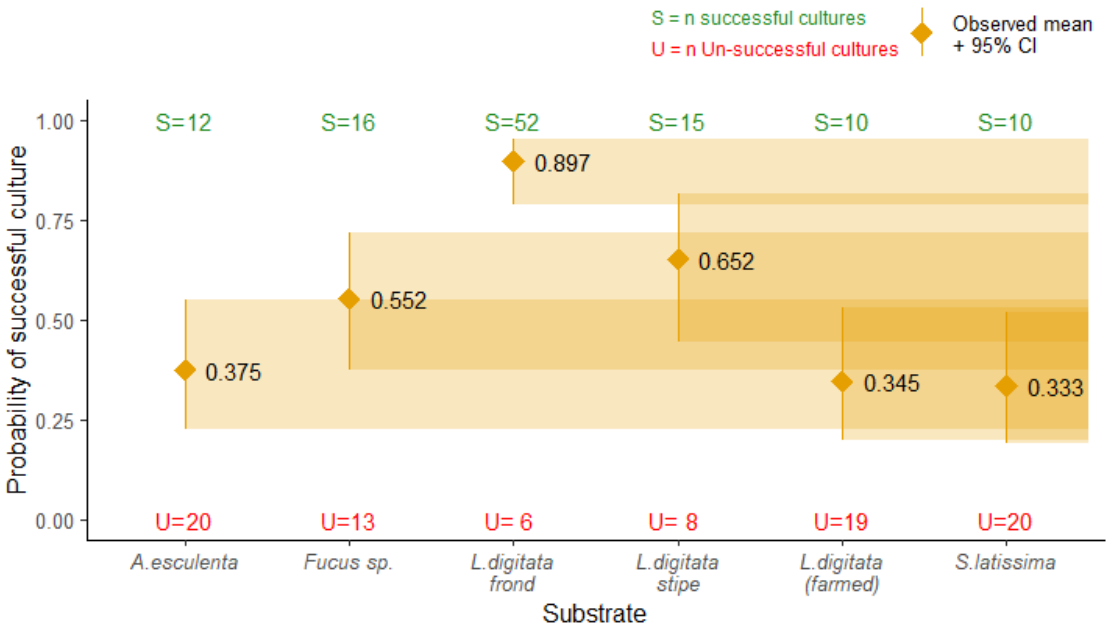


Figure 38. The estimated probability of the success of a C.frigida culture depending on the type of substrate that culture is comprised of. The 95% confidence intervals for each estimated mean probability have been projected right to make for easy

pairwise comparison. Note, the range in which there is a 95% likelihood the mean probability of L.digitata frond lies in, goes beyond 1.

Table 3. The p-values of each pairwise comparison from figure 38. Values in green denote those which are below the significance level of 0.05.

	S.latissima	***L.digitata (farmed)***	***L.digitata stipe***	***L.digitata frond***	***Fucus sp.***
L.digitata (farmed)	>0.999				
L.digitata stipe	0.210	0.254			
L.digitata frond	<0.001	<0.001	0.127		
Fucus sp.	0.549	0.617	0.978	0.008	
A.esculenta	>0.999	>0.999	0.343	<0.001	0.742

When *C.frigida* was unsuccessfully reared on a substrate, density and/or cage status were changed in an attempt to successfully culture *C.frigida* on that substrate. Density was increased and/or cage status changed from the default closed, to open. Because these were changed in response to substrate, density and cage status were correlated with both each other and substrate. Thus, if *C.frigida* had a high probability of being successful on a given substrate, there were little or no cultures with higher density and/or an open cage on that substrate. Therefore, some of the variation in both density and cage status can be explained by substrate making density and cage status a predictor of substrate. All 3 variables were significant in the initial multiple regression, but as these three were all correlated, and substrate was the variable of most interest, a simple regression using only substrate was performed. This correlation between substrate and density is visualised in Figure 39 and between substrate and cage status in Figure 40.

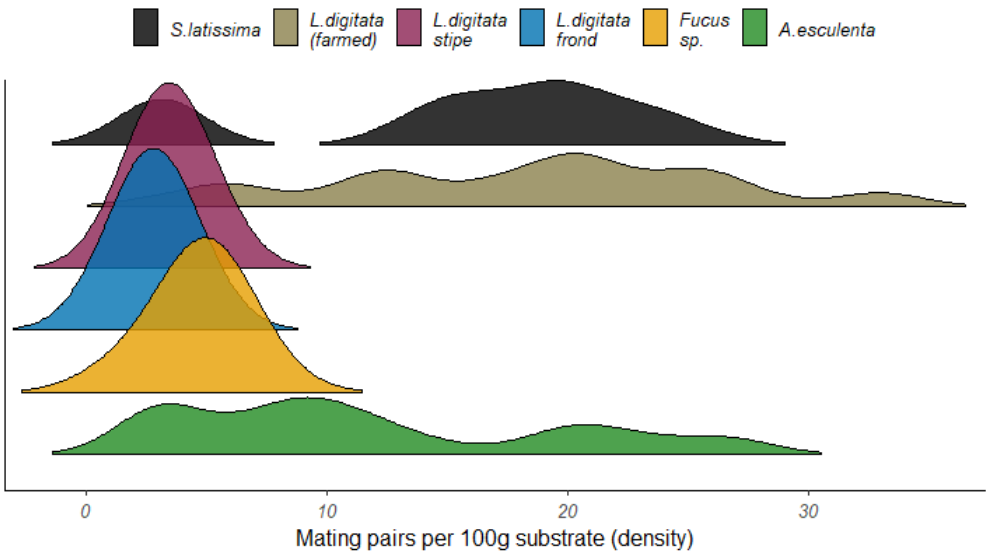


Figure 39. The distributions of the densities of C.frigida in cultures of each substrate type. Density was increased in response to unsuccessful cultures thus increasing density represent difficulty rearing C.frigida. Knowing the density can help predict the substrate.

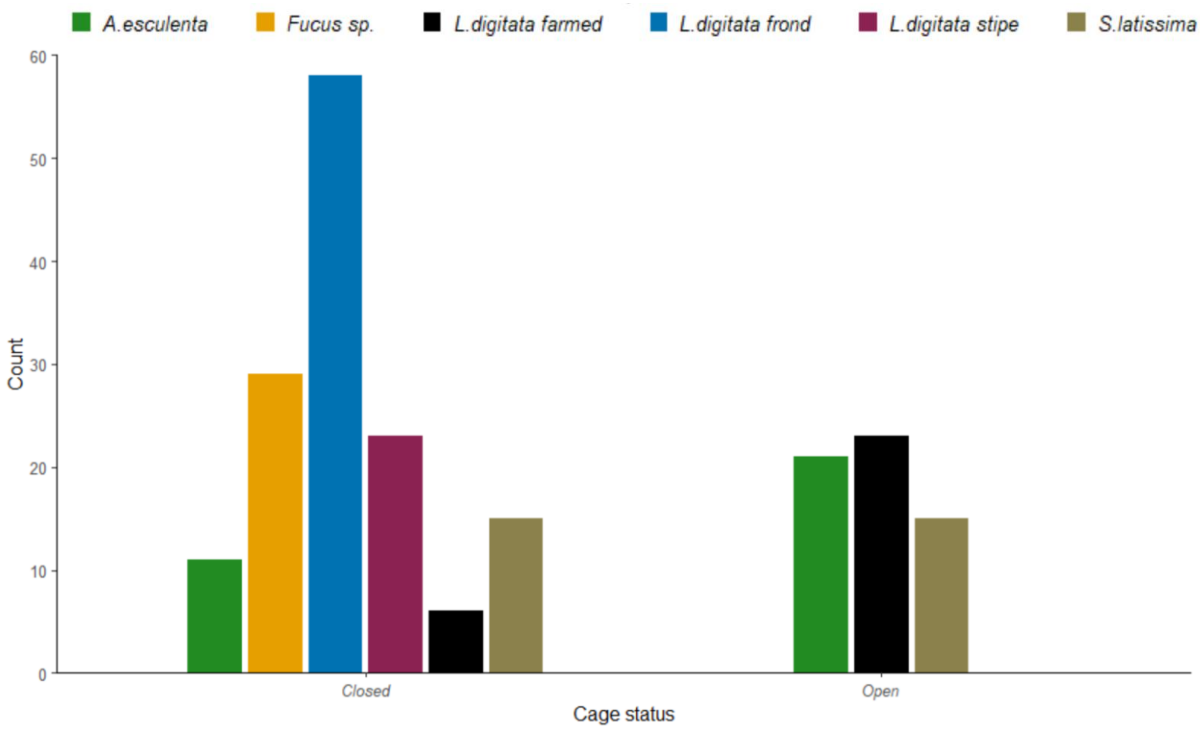


Figure 40. The counts of cage statuses of C.frigida in cultures of each substrate type. Cage status was changed to open in response to unsuccessful cultures thus open cages represent difficulty rearing C.frigida. Knowing the cage status can help predict the substrate.

Ease of Culture of C.pilipes

The probability of a *C.pilipes* culture being successful on a given substrate was modelled using a logistic binomial regression (Appendix 3.2). From this, the mean probability and 95% CI for each substrate was estimated. Each estimated probability was compared pairwise using a tukey multiple

comparison test with the emmeans package (Lenth 2023). These pairwise comparisons and the estimated probabilities are visualised in Figure 41. The p-values of each pairwise comparison are presented in Table 4. *C.pilipes* reared on the stipe of *Laminaria digitata* were estimated to have a higher probability of being successful than when reared on *L.digitata* frond (p<0.001), *A.esculenta* (p<0.001), *S.latissima* (p<0.001) or *Fucus* sp (p=0.016). *C.pilipes* reared on *Fucus* sp were estimated to have a higher probability of being successful than when reared on *A.esculenta* (p<0.008) or *S.latissima* (p<0.021). *C.pilipes* was not successfully reared on farmed *L.digitata* thus the probability of its success could not be estimated. The probabilities of all other pairwise comparisons of *C.pilipes* being successful is estimated not to differ between substrates at a significance level of 0.05.

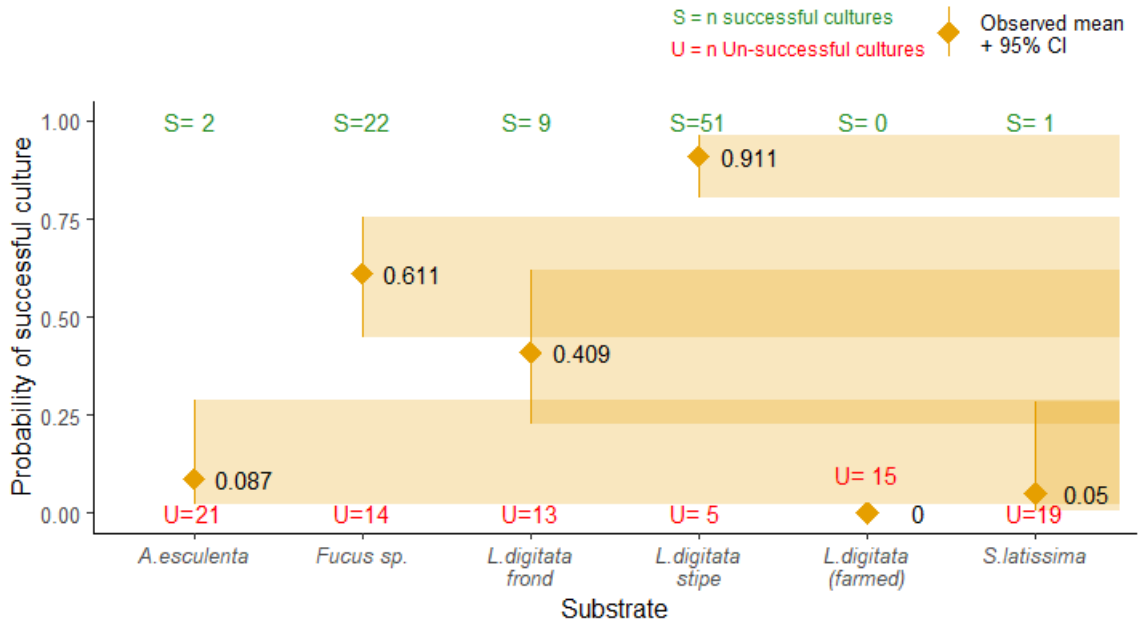


Figure 41. The estimated probability of the success of a C.pilipes culture depending on the type of substrate that culture is comprised of. The 95% confidence intervals for each estimated mean probability have been projected right to make for easy pairwise comparison. Note, the range in which there is a 95% likelihood the mean probability of A.esculenta, S.latissima and L.digitata stipe lies in, goes beyond 0,0 and 1 respectively.

Table 4. The p-values of each pairwise comparison from figure 41. Values in green denote those which are below the significance level of 0.05.

	S.latissima	L.digitata (farmed)	L.digitata stipe	L.digitata frond	Fucus sp.
L.digitata (farmed)	>0.999				
L.digitata stipe	<0.001	>0.999			
L.digitata frond	0.189	>0.999	<0.001		
Fucus sp.	0.021	>0.999	0.016	0.674	
A.esculenta	0.997	>0.999	<0.001	0.189	0.008

In the same way as in *C.frigida* density and cage status were changed in response to unsuccessful cultures and thus correlate with substrate. However, unlike in *C.frigida* cage status was not significant in predicting the variation in successful and unsuccessful cultures in the multiple regression, density and substrate were. Again, as these were both correlated a simple regression using substrate as the predictor variable was used. This correlation between substrate and density is visualised in Figure 42.

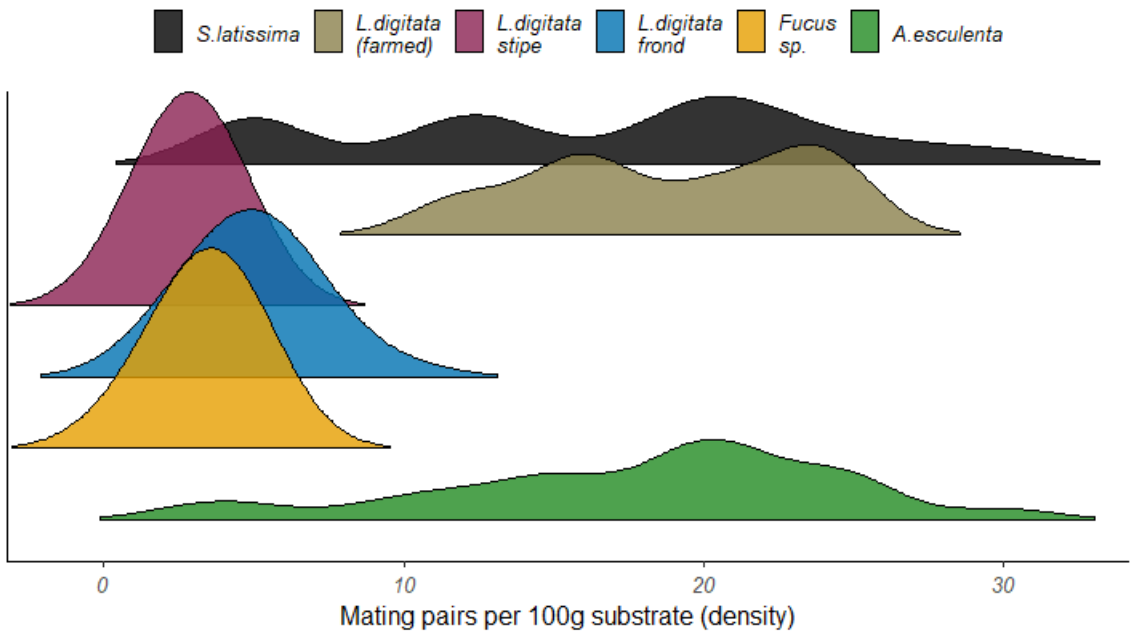


Figure 42. The distributions of the densities of C.pilipes in cultures of each substrate type. Density was increased in response to unsuccessful cultures thus increasing density represent difficulty rearing C.pilipes. Knowing the density can help predict the substrate.

Ease of Culture of F.maritima

In *F.maritima,* the initial multiple regression showed substrate and cage status to explain a significant amount of the variation in the probability of a culture being successful. Crucially on this occasion, these two variables explained different amounts of the variation as they were not correlated. This can be seen in Figure 43 as knowing the cage status does not help predict the substrate type.

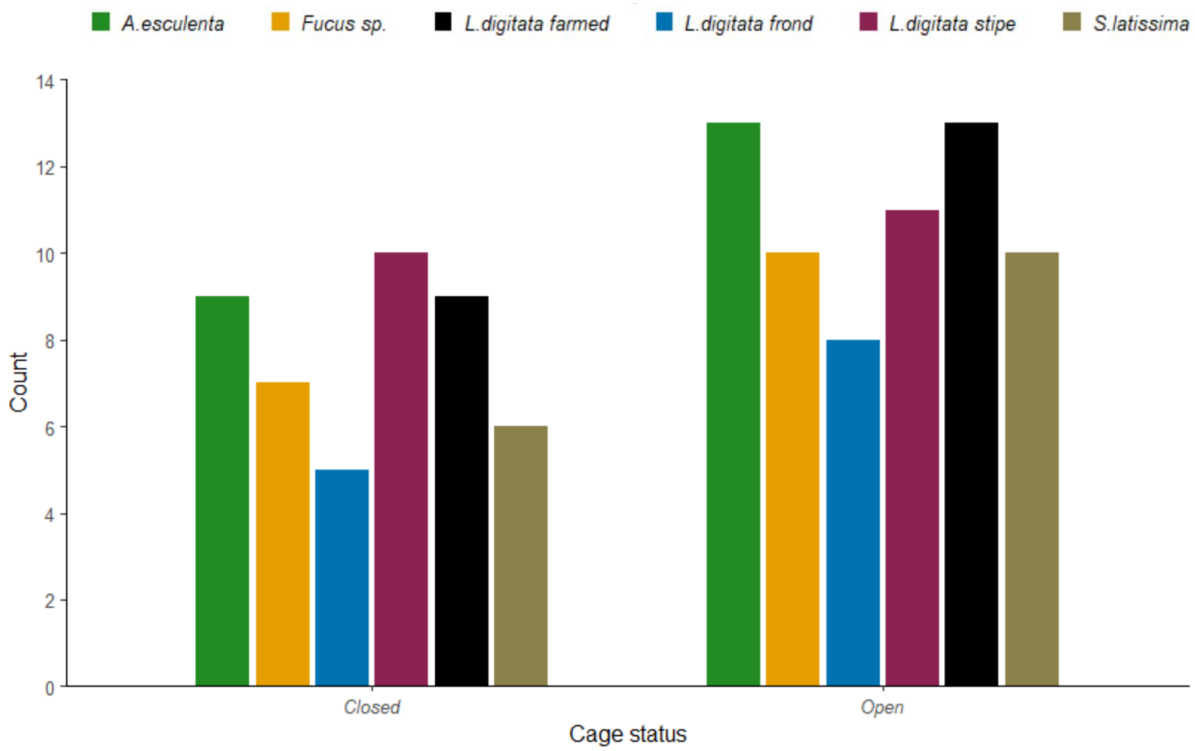


Figure 43. The counts of cage statuses of F.maritima in cultures of each substrate type.

Density was also not correlated but was insignificant. No significant interaction between substrate and cage status was found. As such, the probability of a *F.maritima* culture being successful on a given substrate or cage status was modelled using a multiple binomial lofistic regression (Appendix 3.3). From this, the mean probability and 95% CI for each substrate was estimated. Each estimated probability was compared pairwise using a tukey multiple comparison test with the emmeans package (Lenth 2023). These pairwise comparisons and the estimated probabilities of substrate are visualised in Figure 44. The p-values of each pairwise comparison of substrate are presented in Table 5. *F.maritima* reared on the frond of *Laminaria digitata* is estimated to have a higher probability of being successful than when reared on *S.latissima* ($p<0.046$). *F.maritima* reared on the stipe of *Laminaria digitata* is estimated to have a lower probability of being successful than when reared on *L.digitata* frond ($p<0.003$), *A.esculenta* ($p<0.028$) or *Fucus* sp ($p<0.018$). The probabilities of all other

pairwise comparisons of *F.maritima* being successful is estimated not to differ between substrates at a significance level of 0.05.

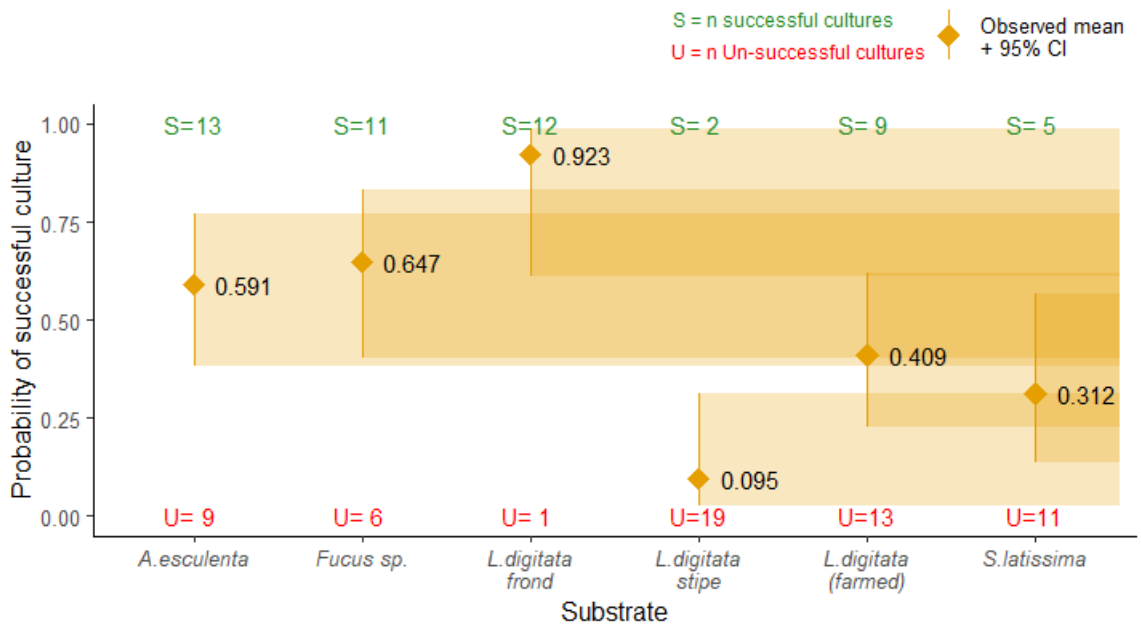


Figure 44. The estimated probability of the success of a F.maritima culture depending on the type of substrate that culture is comprised of. The 95% confidence intervals for each estimated mean probability have been projected right to make for easy pairwise comparison. Note, the range in which there is a 95% likelihood the mean probability of L.digitata frond and stipe lies in, goes beyond 1 and 0 respectively.

Table 5. The p-values of each pairwise comparison from figure 44. Values in green denote those which are below the significance level of 0.05.

	S.latissima	***L.digitata (farmed)***	***L.digitata stipe***	***L.digitata frond***	***Fucus sp.***
L.digitata (farmed)	0.985				
L.digitata stipe	0.654	0.255			
L.digitata frond	0.046	0.101	0.003		
Fucus sp.	0.354	0.661	0.018	0.573	
A.esculenta	0.490	0.820	0.028	0.398	>0.999

Additionally, *F.maritima* reared in an open cage is estimated to have a higher probability of being successful than in a closed cage ($p<0.029$) (Figure 45).

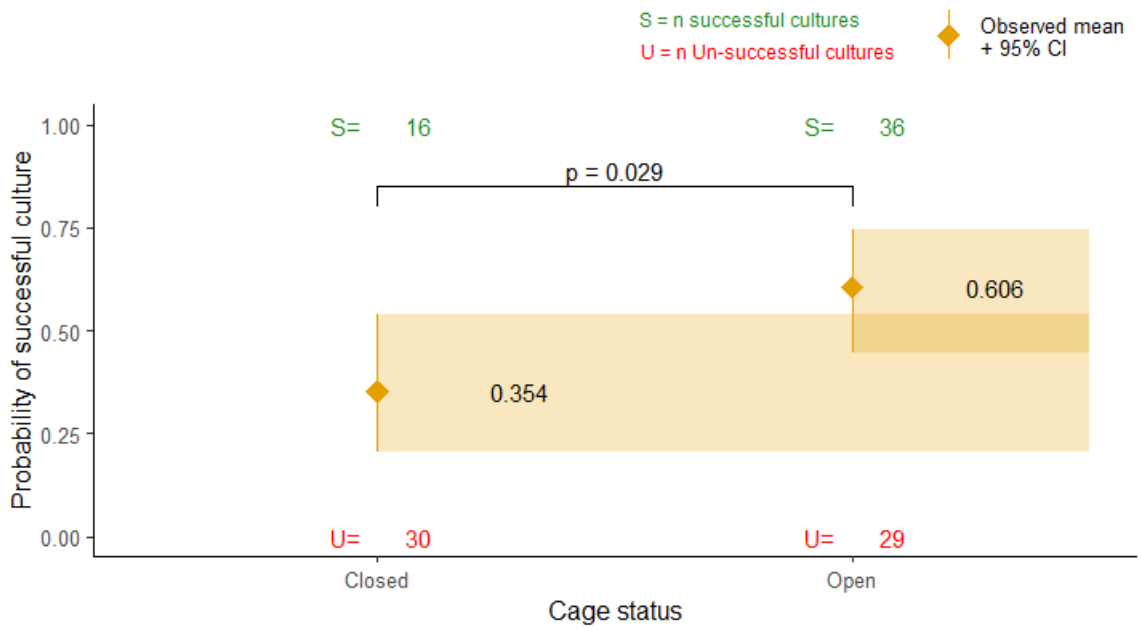


Figure 45. The estimated probability of the success of F.maritima being reared in an open or closed cage. The 95% confidence intervals for each estimated mean probability have been projected right to make for easy comparison.

Optimum larval density of C.frigida

The data gathered to assess the relationship between density and survival were count data, the number of adults that eclosed at a given density. As the number of hatched eggs was known, these counts can be considered as successes out of n (number of hatched eggs) attempts. Thus, these data could be represented as a ratio, probability or percentage. The aim of this experiment was to identify the density at which survival was most likely, therefore, identifying the peak of the relationship was most valuable. As such, the relationship between density and survival was modelled using a 3rd order polynomial regression (Appendix 3.4). A 3rd order polynomial was selected as the 3rd order explained a significant amount of variation unexplained by the 2nd order and the standard linear regression (1st order). A 4th order was insignificant in the model (Appendix 3.5). Using this model to predict values outwith the observed data would result in impossible values outside the boundaries of the data being obtained. However, for modelling the relationship in the observed data, the polynomial regression describes it well without overfitting. This modelled relationship and the observed data are described in Figure 46.

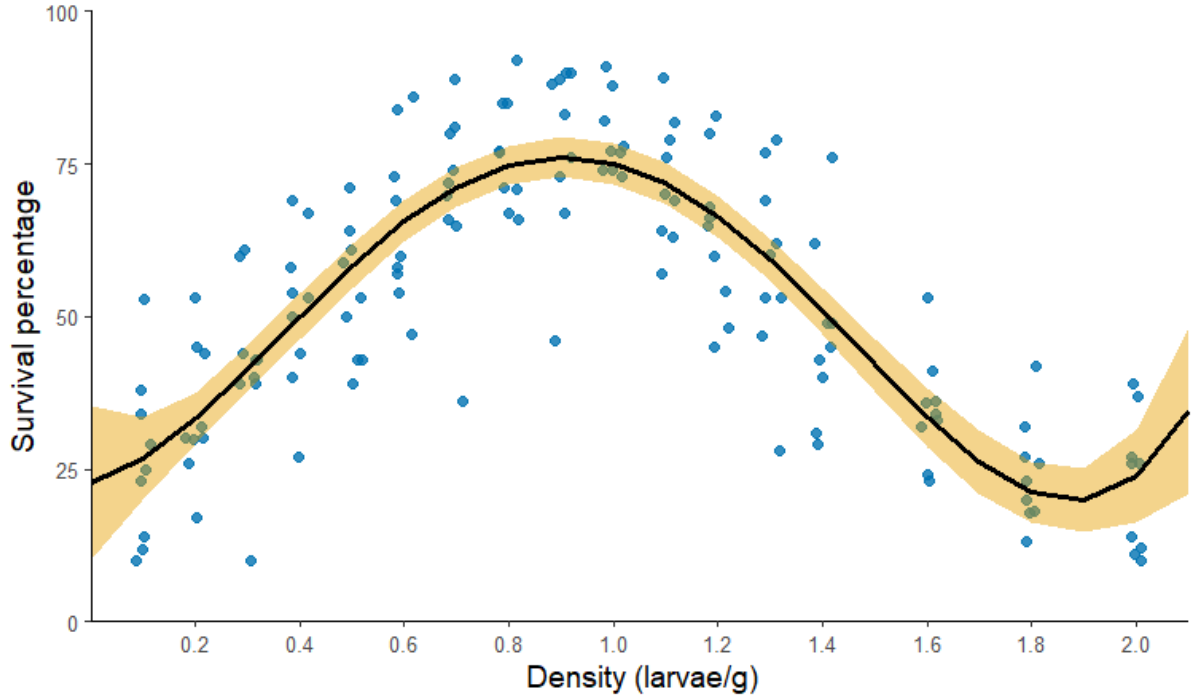


Figure 46. The relationship between the density of C.frigida larvae in a culture, relative to the amount of substrate, and the percentage of those larvae which developed into adulthood as described by a 3rd order polynomial regression. The yellow band represents the 95% CI of the mean. The plotted points show observed data values and are jittered horizontally.

3.4.4 Discussion

Attempts were made to culture seven species of wrack fly. Of these, only three can be considered wholly successful in that attempts to rear them resulted in more adults being obtained from the culture than initially populated it. These were *C.frigida, C.pilipes,* and *F.maritima*. In being successfully cultured, these 3 species have the capacity to be farmed/mass produced evidencing their potential as an ω-3 PUFA source to replace some of the demand for fish oil. However, especially given these results are not the product of a balanced experiment, this does not mean the other four species do not share this capacity. While this could be the case, it could also be that these were not successfully cultured as a result of variables unaccounted for in the conditions. Two such variables are as follows. Firstly, the different spatiotemporal distributions of each species resulted in a varying number of individuals from each species being collected both at any one time and throughout the research. Species of which more mating pairs could be collected and returned to the lab were at an advantage to those of which less could be obtained. More mating pairs facilitated a greater likelihood of a lab colony being established from which variables affecting culture success could be investigated. Secondly, as found in this study, and by Egglishaw (1958), *O.luctuosum* and *H.buccata* demonstrate a reluctance to pupate under the conditions provided. While this may simply represent increased difficulty in rearing and less potential to be farmed, it seems more likely that an

environmental trigger not present in the conditions of this study prompts changes between life cycle stages.

In response to environmental conditions, many insect species possess the adaptive, genetically regulated, trait of diapause (Dhillon *et al* 2022). Similar to hibernation in higher animals, diapause is a mechanism by which some insects and related arthropod species can weather harsh or adverse conditions by halting growth and development. While this most often occurs to avoid winter in the temperate regions, it can also be used to avoid extreme heat and aridity in tropical climates (Denlinger 2009). *O.luctuosum, F.maritima*, *H.buccata, M.scioymzina* and *H.ustulata* are all evidenced to undergo a period of diapause (Egglishaw 1958). The temporal distribution of these species observed support this in all but *O.luctuosum,* which were observed year round. Crafford and Scholtz (1987) when investigating the ecology of the Antarctic wrack fly *Paractora dreuxi-mirabilis*, suggest pupation in them is, to some extent, triggered by the presence of fresh wrack. This example evidences the possibility that manipulation of environmental conditions in the laboratory setting may facilitate improved culture success for certain species. Thus these species may also represent an alternative ω-3 PUFA source, but that is not evidenced here.

The observed mean likelihood, under the conditions described above, of a *C.frigida* culture being successful when reared on the frond of *L.digitata* was found to be 89.7%. This high probability of success evidences the potential of *C.frigida* to be farmed in an effort to provide an alternative ω-3 PUFA source on an industrial scale. As does that of *C.pilipes* on the stipe of *L.digitata* being 91.1% and that of *F.maritima* on the frond of *L.digitata* being 92.3%. Additionally, these results have ecological implications, which add information to the discussion of wrack fly species distribution within the shore ecosystem.

The significantly higher probability of a *C.frigida* culture being successful on the frond of *L.digitata* than on *Fucus* sp adds evidence to the consensus in the literature that *C.frigida* prefer/are better suited to growing on *Laminaria* than on *Fucus* (Biancarosa *et al* 2018, Dunn *et al* 2002, Edward and Gilburn 2013, Leggett 1993, Thompson 1951, Rowell 1969). This preference for *Laminaria* also aligns with the proposed theory of wrack aggregation and with the observations of Egglishaw (1958) on the physical adaptations of *Coelopa* larvae. Said theory proposes that detached *Laminaria* are prone to forming large wrack deposits owing to the increased energy required to transport the denser seaweed. In being both large and comprised of *Laminaria,* these deposits are predisposed to a 'wet' form of decomposition (Backlund 1945, Egglishaw 1958) and as discussed, the physical adaptations of *Coelopa* larvae make them well suited to this type of decomposition (Egglishaw 1958). However, the higher probability of a *C.pilipes* culture being successful on the stipe of *L.digitata* than on any

other substrate also suggests they prefer/are better suited to *Laminaria*, something less of the literature supports with Butlin 1983, Colombini and Chelazzi 2003, Leggett 1993 and Phillips *et al* 1995 all suggesting a preference for *Fucus* over *Laminaria*.

These results in combination with the proposed theory and findings from other studies previously discussed suggest *Coelopa* species prefer and are better suited to wrack comprised of *Laminaria* than *Fucus*. However, it is important to note that there is no evidence this preference is necessarily for the substrate itself and may instead be a preference for the environment that substrate creates. This environment may promote oviposition and/or larval development resulting in a greater probability of a successful culture. This distinction would be relatively easy to make in an experiment where the micro-climate of each substrate is manipulated along with the substrate type.

It may be the case that the distribution of wrack fly species within an ecosystem depends more on the micro-habitat of a substrate than on the species of substrate. These results also support this theory. Under the conditions of this experiment, differences in micro-habitat only arose as a result of different substrates and/or cage statuses. In the CEF, humidity was high, which kept substrates relatively moist thus suiting the morphology of *Coelopa* species best. The only way the moisture content of substrates could be reduced was by placing them in the open cage configuration allowing evaporation to occur. As the *Coelopa* species appear to prefer 'wetter' substrates, this was rarely done, hence the effects of substrate and cage status were correlated. Alternatively *F.maritima* both in the literature, and the observations of this research, appear to inhabit a niche of drier substrate. This is evidenced by open cages being estimated to have a higher probability of success than closed cages, in addition to the estimated effects of substrate. Furthermore, the effect of substrate on the success of *F.maritima* cultures seems less than that of cage status as the mean probability across all substrates was, remarkably, exactly 0.5, although a measure of effect size is required to confirm this. This requirement of a drier substrate is further evidenced by *F.maritima*'s success being less likely on *L.digitata* stipe than on frond, *A.esculenta* or *Fucus* sp as the substrate comprised of the minced stipe was undoubtedly the wettest. The reverse of this may also be expected to hold true under this hypothesis whereby, the driest substrate (*Fucus* sp) has the highest estimated probability of success, but this was not observed. However, when in open cages, the *Fucus* sp substrate could become exceptionally dry, especially if larval activity was not present to mechanically break the substrate down thus releasing water and lowering the surface area to volume ratio reducing evaporation.

The last ecological implication discussed is that of *C.frigida*'s highest estimated probability of success being on the frond of *L.digitata* while *C.pilipes* was on the stipe. How the two ecologically and taxonomically similar *Coelopa* species appear to coexist within a niche is an unanswered question

which disagrees with Gause (1934)'s competitive exclusion theory. This question and the competitive exclusion theory are further discussed in Chapter 4. As discussed in section 3.1, several studies have attempted to describe the mechanism(s) of this coexistence with differing optimal thermal ranges having the most merit. These results suggest that *C.pilipes* prefer/are more suited to *L.digitata* stipe than frond and *C.frigida* frond than stipe. This could be a result of either the direct effect of the different substrates or, the differing micro-climates they create. However, due to the unbalanced nature of this study, results of one species are not directly comparable to another so caution must be taken when interpreting results between species.

Furthermore, the conditions under which these results were obtained did not involve intraspecific competition and/or facilitation, something which would occur in the natural ecosystem. Similarly, throughout this research, the development of cultures was treated as linear with discrete generations and life cycle stages. However, naturally they are not like this. Sampling on any specific day did not give *C.frigida* as either larvae, adults or pupae depending on where they are in this "linear development". Instead, they were found as all three at once. Both intra and interspecific competition and facilitation in *C.frigida*, *C.pilipes* and *O.luctuosum* were demonstrated by Leggett (1993). As the generations and life cycle stages are naturally not discrete, but were in this study, this interspecific facilitation was absent. This likely contributed to a decreased probability of success in the results, as every culture represented the initial colonisation of the resource. These conditions were constant, so the results are comparable to each other, but less so to the natural ecosystem. Unsuccessful cultures were most often deemed so as a result of microbial activity making the culture uninhabitable, this problem was likely exacerbated because each culture represented an initial colonisation.

These results suggest cultures across all three species are less likely to be successful when reared on substrates from farmed seaweeds than wild harvested. This may also be a result of microbial activity as the farmed substrates were much quicker to become overrun by microbial populations than those not farmed, under the same conditions. This is likely a result of these two groups of substrates, farmed and non-farmed, coming from different habitats and environments. The farmed species of substrate were grown offshore and frozen soon after harvest. Comparatively, the non-farmed substrates have gone through the natural succession leading up to a wrack deposition which wrack flies have evolved to thrive on. The implications of this in regard to the industrial scale production of wrack fauna as an ω-3 PUFA replacement are discussed in Chapter 7.

Lastly, the optimum density of *C.frigida* larvae per gram of substrate is estimated to lie between 0.7 and 1.1 larvae/g with the peak of the estimated mean lying at 0.9 larvae/g (Figure 46). These results

are in agreement with those obtained by Leggett (1993) who, in a comparable experiment, found survival to peak around 0.8 larvae/g. The methods used in this study followed those of Leggett (1993) except, where all replicates were performed with 50g of substrate in this study, those of Leggett (1993) were not always, "although the majority of cultures used 50g of seaweed" (Leggett 1993, pg 61). This observed pattern of increasing survival with density leading to a peak and then decreasing represents the initial intraspecific facilitation increasing survival through the mechanical breakdown of the substrate increasing surface area and releasing moisture which in turn promotes microbial growth. As discussed, the larvae may or may not feed on the microbes directly, but either as a food source or facilitative decomposers this benefits the larvae. However, at densities beyond the peak, the negative effects of the intraspecific competition outweigh the positives of facilitation. As highlighted by Butlin *et al* (1994) this is typical of scramble competition which is common in Diptera among other insects.

These results, among other ecological implications discussed, suggest *C.frigida*, *C.pilipes* and *F.maritima* show potential as an alternative ω-3 PUFA source as far as their ability to be cultured and thus produced industrially is concerned. The highest mean probability of a culture being successful across all substrates for each species type was estimated as 89.7% , 91.1% and 92.3% respectively. These probabilities were all observed on substrates deriving from wild harvested detached *L.digitata*. While these results suggest a reduced capacity of the other wrack fly species to be cultured under the conditions of this study, they do not indicate a lack of potential to be industrially produced under any other conditions.

Chapter 4

OVIPOSITIONAL PREFERENCE IN WRACK FLIES AS AN INDICATOR OF OPTIMAL LARVAL SUBSTRATE AND NICHE BOUNDARIES

4.1 Introduction

The majority of wrack deposits in Northern Britain fall into one of two groups: Those which are large and comprised predominantly of *Laminaria* sp or those which are small and comprised primarily of *Fucus* sp (Egglishaw 1958, Orr 2013). The following Diptera are commonly found in the smaller deposits, though their abundances differ temporally: *F.maritima*, *H.ustulata*, *H.buccata* and *M.sciomyzina*. *O.luctuosum* can also be present in these smaller deposits but not exclusively. Conversely, *C.frigida* and *C.pilipes* are commonly found in the larger wrack banks and can be present in any life cycle stage all year round. The larger wrack beds are of a size that facilitates their own micro-climate and have been recorded at a temperature of 55°C (Leggett 1993). As such, when other species enter a period of diapause to avoid the harsh conditions of winter, those which inhabit the larger wrack beds do not. *O.luctuosum* can also be found in these larger deposits, but their temporal distribution is less clear, as is that of the much smaller *Thoracochaeta* sp. Many other macro-invertebrate species can also be present in both types of wrack deposits, of which Amphipod and Nematode species are the most abundant (Orr 2013). The two *Coelopa* species (*C.frigida* and *C.pilipes*) are very similar in their taxonomy, distribution, life cycle and colonisation of wrack beds. This similarity has often prompted the question: what mechanism(s) enables the coexistence of *Coelopa* species which both appear to occupy the relatively homogenous middle layer of large wrack beds (Butlin and Day 1984, Edwards *et al* 2008, Egglishaw 1958, Leggett 1993, Phillips *et al* 1995)?

The ability of more than one species to coexist within a habitat and exploit the same resource without a dominant species competitively excluding the other/rest has been the subject of much investigation (Hubbell 2001, Pinsky 2019, Rosindell *et al* 2011, Scheffer and Van Nes 2006). Since Gause's (1934) competitive exclusion theory, stating no two species which compete for the same limiting resource can coexist indefinitely, there have been many advancements on this principle. The theory of limiting similarity (limsim) by Abrams (1983) (Figure 47, 1.), neutral theory (Hubbell 2001) (Figure 47, 3.), the theory of aggregation such as that driven by oviposition (Atkinson and Shorrocks 1984) Figure 47, 4.) and the impact of temporal variation/differentiation (Chesson 2000) (Figure 47,5). Furthermore, Arthur (1986) showed that seemingly homogenous lab environments can be

complex and have scope for niche differentiation. These mechanisms (Figure 47) represent some of the processes by which coexistence between *C.frigida* and *C.pilipes* may occur.

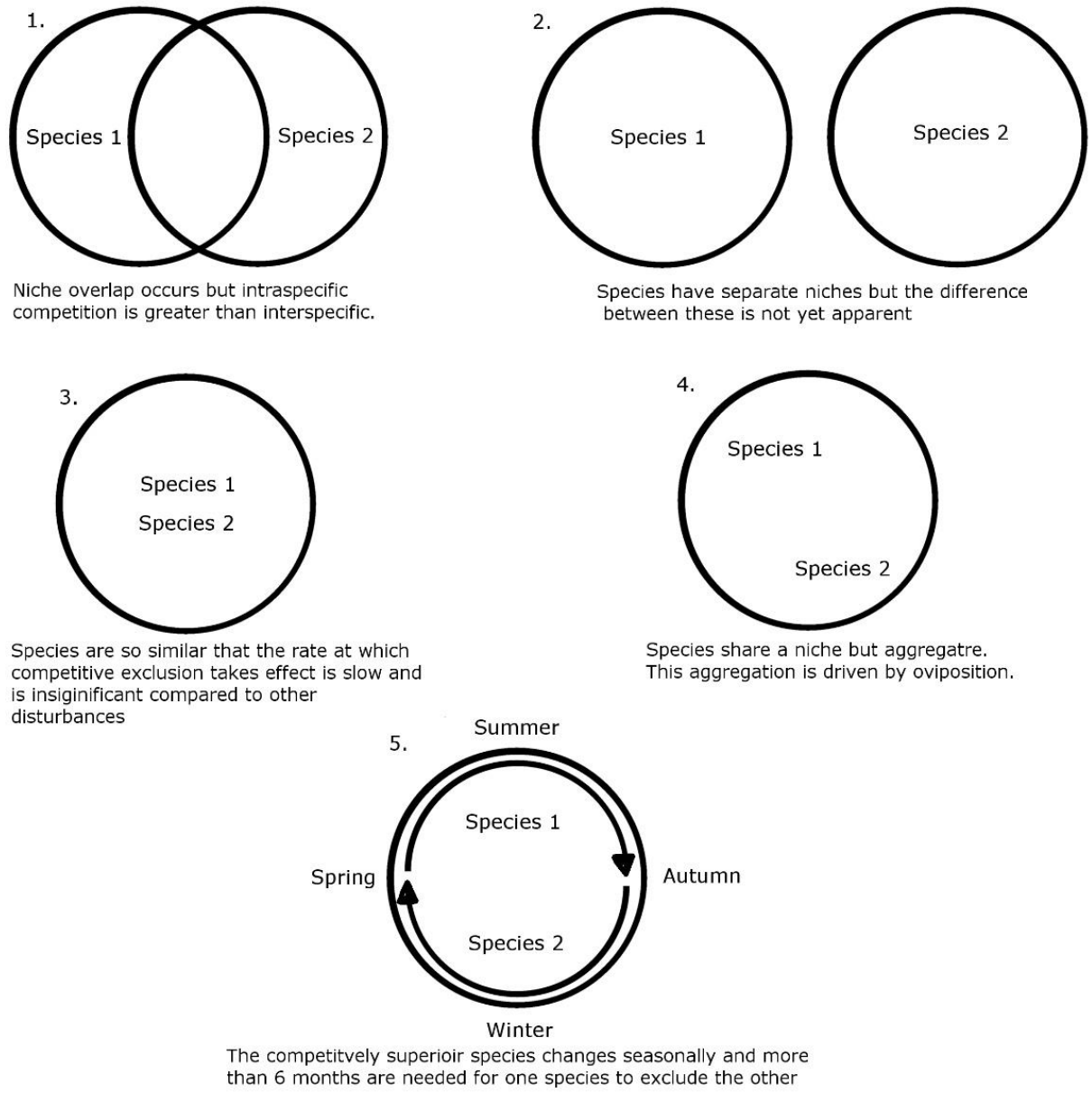


Figure 47. Mechanisms by which multiple species can coexist while occupying the same niche in an ecosystem. Figure created using GIMP 2.10.28.

In addition to these mechanisms which can facilitate coexistence within Gause's (1934) competitive exclusion theory, advancements have been made in the key understanding of community ecology. Chief among these is what is often referred to as 'Modern Coexistence Theory' developed by Chesson and colleagues and evolved throughout Chesson and Warner (1981), Chesson and Huntly(1997), Chesson and Kuang(2008) and Chesson (1990, 1994, 2000a,2000b, 2003, 2011,2013,2018). This theory of modern coexistence prompts a revision of niche concepts and

empirically evidences how competitor coexistence can occur as a result of a spatiotemporal fluctuations maintaining biodiversity among a balance between other equalising and stabilising mechanisms (Barabás *et al* 2013, Chesson 2000b, 2003). The diversity and makeup of co-occurring species can undergo significant fluctuations over time and space. A central focus in MCT is to comprehend the mechanisms responsible for creating and upholding this variability (Chase 2003, Chesson 2000a,2000b, HilleRisLambers *et al* 2012, Mittelbach and Schemske 2015). As populations expand, the interactions and dynamics within and between species play a crucial role in determining which specific combinations of species coexist (HilleRisLambers *et al* 2012, Spaak and Schreiber 2023).

Modern coexistence theory (MCT) attempts to unify our understanding of the different devices of coexistence by disentangling stabilizing and equalizing mechanisms (Chesson, 2000a,2000b). Equalising mechanisms are processes that diminish the competitive advantage of dominant species, allowing less competitive species to persist (Chesson, 2003). These mechanisms can include: disturbance, predation and density dependence where some populations experience reduced reproductive success or increased mortality as their density increases. This can create a feedback loop that prevents any one species from dominating an ecosystem. Stabilizing mechanisms work to reduce competition between species by promoting niche differentiation, allowing species to coexist (Chesson, 2003). Such as spatial and/or temporal resource partitioning. Allesina and Levine (2011) argue that when species are limited by multiple factors, the coexistence of many species is the most probable outcome and that habitat heterogeneity interacts with network structure to favour diversity.

Prior to the development of MCT, several investigations into the ecological relationship between *C.frigida* and *C.pilipes* have taken place in attempts to better understand and explain their coexistence. As discussed in Chapter 3, consumption of different seaweed species, different temporal distributions, differing thermal preferences and differing physical adaptations have been investigated. The results of investigations into different seaweed species and different temporal distributions are contested (Colombini and Chelazzi 2003, Dobson 1974, Egglishaw 1958, Leggett 1993). The comparison and interpretation of different physical adaptations between the larvae of wrack flies by Egglishaw (1958) appears useful in accounting for some of the variation in overall wrack fly distribution. However, the physical adaptations of *C.frigida* and *C.pilipes* are reported as almost identical (Egglishaw 1958), providing evidence they should not be able to coexist rather than the contrary. Of the mechanisms discussed in Chapter 3, differing thermal requirements/preferences show the most potential in accounting for some of the coexistence.

Phillips *et al* (1995) reared both *Coelopa* sp on a *Fucus* substrate that contained a temperature gradient from 25°C – 45°C. *C.pilipes* were found to aggregate in the warmer areas where as *C.frigida* did so in the colder. Neither species oviposited in the hotter half of the substrate. Nor did differential survival create this aggregation, making it driven by larval preference. *C.frigida* showed a greater decrease in body size at the higher temperatures and *C.pilipes* at the lower suggesting competition is fiercer for the respective species at these temperatures. This suggests the species possess different thermal niches and that these match the broader geographical distribution of the species with C.*frigida* occurring further north in colder climates than does C.*pilipes* (Phillips *et al* 1995). However, this finding by Phillips *et al* (1995) is in opposition to the in situ sampling, which finds *C.frigida* tend to aggregate and *C.pilipes* do not (Burnet 1961, Butlin 1983, Colombini and Chelazzi 2003). Although, this opposition could be accounted for by the method of identification used by Phillips *et al* (1995), as this method is not provided and no other publication reports being able to differentiate larvae from either *Coelopa* sp.

As defined by MCT, to evaluate which mechanism(s) may facilitate the coexistence of the *Coelopa* sp, the nature of their interaction must be understood. In a series of experiments, Leggett (1993) investigated the interactions within and between *C.frigida, C.pilipes* and *O.luctuosum*. All of these experiments took place on a substrate comprised of minced *F.serratus*. To assess the intraspecific interactions the affect(s) of larval density on survival, development time and wing length were explored as a proxy of size. The nature of interaction(s) was found to be density dependent. In *C.*frigida, at low densities larvae facilitated each other, but at approximate densities of 0.8 larvae per gram of substrate (l/g) and 1.5l/g this facilitation peaked and negative effects on survival and size were observed respectively. Leggett (1993) found obtaining eggs of *C.pilipes* very difficult thus experiments were done in small batches and high density pots were only setup rarely when enough eggs were obtained. At low density, survival was low but this rose to a peak faster than in *C.frigida* and peaked at a higher density of around 1.5l/gw after which it rapidly decreased. The steepness in the phases either side of the peak were greater in *C.pilipes* suggesting they are more sensitive to changes in density. *C.pilipes* size did not increase with density at any point but decreased and female variance was greater than male which is the reverse of that observed in *C.frigida*. On average, *C.pilipes* were larger than *C.frigida* and reached their threshold size at a lower density. However, the differences in experimental setup resulting from the difficulty obtaining *C.pilipes* eggs makes the results between species less comparable.

In assessing interspecific interactions between *C.frigida* and *C.pilipes,* densities of both species were manipulated in cultures (n=135) at varying proportions (Leggett 1993). *C.pilipes* was found to

facilitate *C.frigida* as increasing density of the former increased survival of the latter. However, the effect(s) of increasing *C.pilipes* density on *C.frigida* size is not reported, so whilst survival might increase, individuals may be smaller owing to a competitive interaction. A competitive effect of *C.pilipes* on *C.frigida* was evidenced by increasing heterozygote frequency in *C.frigida* with increasing *C.pilipes* density. The heterozygotes of *C.frigida* are more fit than the homozygotes. The effect of *C.frigida* on *C.pilipes* was solely competitive with survival and size of *C.pilipes* decreasing with *C.frigida* density. In this experiment, Leggett (1993) reports *C.frigida* are competitively superior. The results of intraspecific interaction in *O.luctuosum* were found only to be competitive. Interspecific interactions between *C.frigida* and *O.luctuosum* were facilitative for *C.frigida* and competitive for *O.luctuosum*. *O.luctuosum* survival decreased with increasing *C.frigida* density and *C.frigida* survival increased and development time decreased with *O.luctuosum* density. Between *O.luctuosum* and *C.pilipes* increasing density of *C.pilipes* decreased *O.luctuosum* survival, and *O.luctuosum* density had no effect on *C.pilipes*.

The asymmetrical nature of the interaction between *C.frigida* and *C.pilipes* reported by Leggett (1993) is supported by Hodge and Arthur (1997). They found the presence of *C.frigida* increased the development time of *C.pilipes* by two days and that the development of *C.frigida* was not affected by the presence of *C.pilipes*. Hodge and Arthur (1997) suggest this facilitation of *C.frigida* is either a result of a swarm effect where the presence of *C.pilipes* induces *C.frigida* to lay more eggs, or *C.pilipes* modifying the resource for *C.frigida* in an unknown way.

The faster life cycle and greater number of eggs laid by *C.frigida* compared to *C.pilipes* makes the former regarded as the competitively superior of the two (Butlin 1983, Dobson 1974, Egglishaw 1958, Leggett 1993). These studies show that, at least under the conditions analysed, *C.frigida* is competitively superior and is helped by the presence of the other two species. As such, the main interactive force acting negatively on *C.frigida* is intraspecific competition. Therefore, increasing *C.frigida* density above 0.8l/g reduces *C.frigida* survival which in turn would reduce their competitive effect on *C.pilipes*. However, as the competitive effect of *C.frigida* reduces *C.pilipes* survival, over time the former could be expected to outcompete the latter into extinction.

Species will only become extinct from competition if it is a major cause of their mortality and interspecific competition is greater than intraspecific. The fitness of larvae is determined by several biotic and abiotic factors and the importance of a single factor may depend on the impact of any, or all, of the other factors making them context dependant (Rudolf and Rodel 2005). For example, the availability and amount of seaweed as well as predation and competition with other species may

result in interactions between the two *Coelopa* being of little consequence to their ecology and survival. *C.frigida* and *C.pilipes* are frequently found together and can be maintained under the same lab conditions. No environmental variable previously investigated correlates with their distribution, and they appear to occupy the same niche. This study proposes the use of the preference performance hypothesis as an indicator of niche boundaries and is tested on the wrack fly species *C.frigida*, *C.pilipes* and *F.maritima*.

The preference-performance hypothesis (PPH) suggests the fitness of females' offspring, which receive no parental care and are limited in their movement, is strongly influenced by the oviposition site she chooses (Jaenike 1978, Thompson 1988). This applies to almost all insects, and the female's site selection will determine much of the resource quantity and quality, competition and predation her offspring are exposed to. Therefore, a strong selective pressure is expected on females to oviposit most frequently in sites where offspring survival and performance would be highest (Altesor and González 2018, Craig *et al* 2016, Lambret *et al* 2018, Potter *et al* 2012, Rieger *et al* 2004, Rudolf and Rodel 2005). However, this has been shown not always to be the case as the choice of oviposition site may be influenced by more than offspring performance (Clark *et al* 2011, Potter *et al* 2011), such as maximising maternal survival or indirect oviposition site choice via mating (Refsnider and Janzen 2011). Additionally, there are multiple ways to measure offspring performance including embryonic survival, development speed and egg to adult viability, and it is unlikely these can all be maximised based solely on the oviposition site. Thus, larval preference may account for some of their performance as is the case in the Mediterranean fruit fly (*Ceratitis capitata*). *C.capitata* larvae develop in fallen oranges and the performance of larvae can vary within a single orange, yet females show no preference for the more nutritious regions as their site of oviposition. Instead, upon hatching, larvae migrate to the most valuable areas (Fernandes-da-Silva and Zucoloto 1993). Therefore, the site a female chooses to oviposit does not always have to be that of which larval performance would be highest. In the case of *C.capitata* it could be argued that the ability of larvae to identify areas of increased nutritional value makes embryonic survival of more importance in oviposition site selection.

Whether a female selects the site where larvae performance is highest to oviposit or not, she must oviposit most frequently within her species' niche. Natural selection requires this. As a result, the PPH could be used to characterise the niches of species that occupy separate niches that are not yet apparent, making the species appear to coexist. Under this theory, when given a choice of conditions in which to oviposit, the more oviposition differs from random, the closer to the fundamental niche of the species that set of conditions would be. Additionally, larval success in non-choice performance

assays can be used to identify fundamental niche boundaries. Therefore, by assessing the ovipositional preferences of *C.frigida*, *C.pilipes* and *F.maritima* and their larval success in each of the oviposition choices presented, the following research questions will be investigated:

1. Does the substrate type influence the number of eggs laid on that substrate by any of the fly species?
2. Does the substrate type influence the success of offspring development of any of the fly species?

Assessing this will then allow discussion of the following questions:

1. Does the preference-performance hypothesis apply to any of the study species?
2. Do the oviposition preferences or offspring performances of the study species demonstrate that they differ in their niches?
3. Does niche separation facilitate the coexistence of the study species?

4.2 Methods

Ovipositional preferences

Experimental design

To assess the ovipositional preferences of each of *C.frigida*, *C.pilipes* and *F.maritima,* females were given a choice of five substrates on which to lay their eggs. Flies used in this experiment were obtained by collecting wild samples of adults from the study sites presented in Figure 32; these were then bred and their offspring used in the experiments. Thus, individuals used were the first generation to eclose in the lab. This approach was used instead of taking individuals from the established lab colonies to reduce any selective pressures laboratory rearing may have and to reduce any displayed preferences being a result of previous laboratory experience and exposure. Three replicate cages for each fly species were setup and consisted of a $30cm^3$ mesh cage with a 30x30cm plastic lid on the bottom to give the floor some rigidity. On top of which, five clear, lidless $7cm^3$ plastic containers were placed in a quincunx pattern, each containing 50g of a different substrate. The substrates used were *A.esculenta*, *Fucus* sp, *L.digitata* frond, *L.digitata* stipe and *S.latissima*. Each position on the quincunx was assigned a letter ID from A-E and the positional ID of each substrate was different across all three replicates. The position of each substrate was rotated daily from A through E and then back to A etc. This allowed any environmental changes within the cage to be somewhat accounted for. A small petri dish containing coarse grained pearl sugar and a test tube filled with water and capped with cotton wool were also placed in each cage. 12 mating pairs, giving a total of 24 adults, were added to each of the three replicate cages across all three fly species and placed in the CEF at 24°C on an 8:16hr light:dark cycle and at a relative humidity of 60%. Each

morning for 14 days, substrates were removed from the cages and replaced with new replicates in their next quincunx position. Prior to the experiment, an excess of each substrate was minced and stored separately in large plastic containers at -20°C. The morning substrate was required some was broken off with a hammer and broken into its minced particle size. 50g was then added to the experiment tubs. Once removed from the cage substrates were scoured for eggs using a magnifying ring lamp, tweezers and a paintbrush and the number of eggs found was recorded. This experiment was run separately for each species one after the other with approximately one week in-between.

Statistical analysis

Statistical analysis was performed using R version 4.2.2 (R Core Team 2022) and the number of eggs laid on each substrate was modelled using a zero-inflated negative binomial mixed effects model with cage ID treated as a random effect. Days where no eggs were laid within a cage were removed from the data. This removed some of the background noise created from the many variables which can affect if oviposition occurs. Variables such as sexual maturity or mating success which this experiment was not investigating. A Poisson model was also considered however, a negative binomial was used instead as it allowed for greater deviance from the assumption of the variance equalling the mean, making a false positive less likely to be observed. The generalised linear mixed-effect model with a binomial distribution was used to estimate the mean number of eggs laid per substrate. Each of the three cages within a species were treated as having a random intercept. Models were created using the 'NBZIMM' package (Yi 2020). The estimated mean number of eggs laid on each substrate was then compared pairwise within species using a tukey multiple comparison test with the 'emmeans' package (Lenth 2023).

Similarly, to investigate any influence of time (in days) on the number of eggs laid on each substrate, a linear mixed-effects model was fitted to the data. A random intercept was included for each replicated cage to account for potential variation among cages. The model was also fitted using the 'lme4' package (Bates *et al* 2015). To assess whether the effect of time varied across substrates, an interaction term between time and substrate was included in the model.

Offspring success

Experimental design

To evaluate offspring success, three replicates containing 50 eggs from of each of *C.frigida*, *C.pilipes* and *F.maritima* were setup on each of the five substrates. The number of adults that eclosed and when they did so was recorded. Thus, larval success was measured by egg to adult viability and development time. Larval success experiments were setup from that species' ovipositional

preference experiment. Whereby, when sufficient eggs were laid on the same day, after being counted these were manipulated such that each of the 50g pots of the five substrates contained 50 eggs. This was done with as minimal movement of the eggs as possible. Each substrate had three replicates giving a total of 150 eggs per substrate per species. These pots were then sealed with parafilm with small holes poked through to allow gas exchange before being placed in the CEF under the same conditions as above. Where sufficient eggs were not obtained on the same day, a separate egg laying culture was setup and eggs from this were used instead. This was only the case with *C.pilipes*.

Statistical analysis

Statistical analysis was performed using R programming version 4.2.2 (R Core Team 2022). A survival-time analysis was used to assess any influence of substrate type on development time and egg to adult viability. Survival-time analysis, sometimes called failure-time analysis, is often used to study times to events and rates at which events occur (Scheiner and Gurevitch 2001). To assess the effect of substrate, the Kaplan-Meier method with a log-rank test was used (Kaplan and Meier 1958). The proportion of flies which emerged from each substrate on each day was calculated, and Kaplan-Meier survival curves fitted for each species/substrate combination. This was done using the 'survival' package (Therneau and Grambsch 2000). Differences in survival curves among substrates were tested for using a log-rank test and were compared pairwise within species. The false discovery rate was controlled for using the Benjamini–Hochberg procedure (Benjamini and Hochberg 1995). Any effects were considered significant at a p-value <0.05. This approach allowed comparisons between substrates that accounted for both speed of development and success of development in the same model. Prior to this statistical method, a generalized linear mixed-effects model with a negative binomial distribution and a logistic regression were considered, however, these could not account for the day on which eclosion occurred and the magnitude of the eclosion at the same time.

4.3 Results

Ovipositional preferences

Of the wrack fly species investigated, *C.pilipes* laid no eggs in any of the three replicate cages across the 14 days the experiment ran. Eggs were laid in all replicates of *C.frigida* and *F.maritima.* The relative quincunx position of substrates was found to have no effect on the number of eggs laid.

From the negative binomial model (appendix 4.1), the substrate on which *C.frigida* females chose to lay their eggs was estimated to be significantly different across six of the pairwise comparisons. However, five of these six had marginal effect sizes of which *S.latissima* had the highest estimated

mean. Conversely, a more pronounced effect was estimated in females' choice not to lay eggs in the *Fucus* sp substrate. These estimates and their relative effect sizes are visualised in Figure 48. The p-values from each pairwise comparison are reported in Table 6.

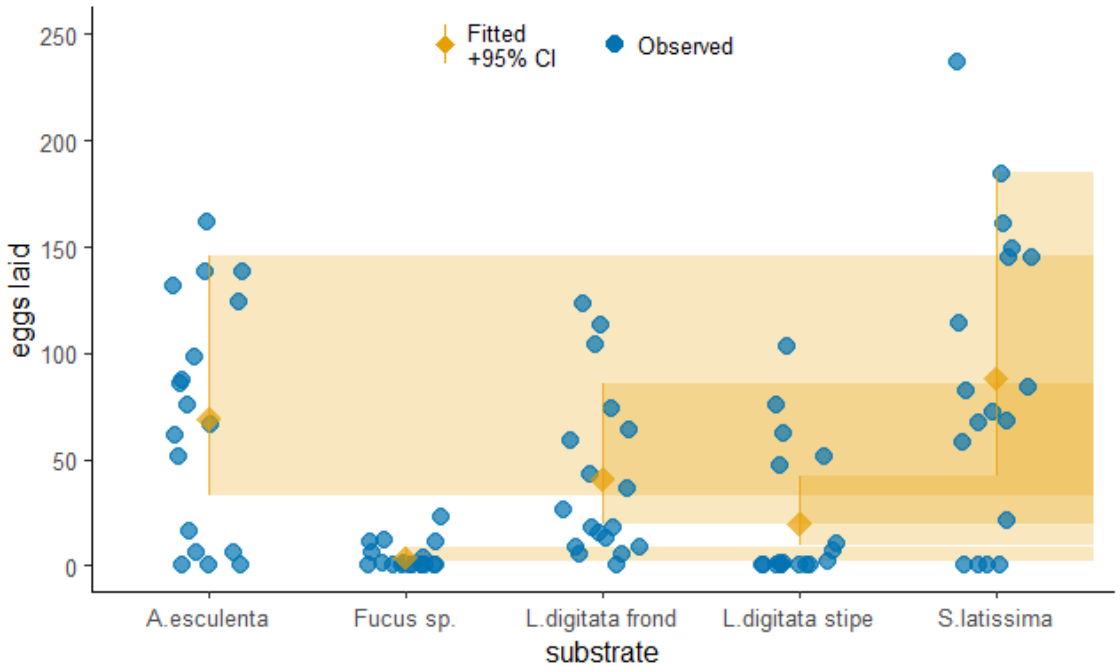


Figure 48. The number of eggs laid by C.frigida females per substrate. Data represent values across three replicate cages and a time period of 14 days. The mean number of eggs laid and the 95% CI for this mean are represented by yellow data and the estimated CI has been projected right to allow easy pairwise comparison. Blue data points represent observed values during the experiment.

Table 6. The p-values of each pairwise comparison of estimated eggs laid by C.frigida per substrate. Values in green denote those which are below the significance level of 0.05.

	S.latissima	***L.digitata stipe***	***L.digitata frond***	***Fucus sp.***
L.digitata stipe	0.046			
L.digitata frond	0.603	0.677		
Fucus sp.	<0.001	0.023	<0.001	
A.esculenta	0.992	0.142	0.861	<0.001

The mean number of eggs laid by *C.frigida* on each substrate over time, along with 95% confidence intervals, were estimated and these results are presented in Figure 49. As reported, substrate had a

significant effect on the number of eggs laid; naturally this was also found in this model (χ^2= 36.662 , df = 4, p < 0.001). Additionally, the day was found to significantly affect the number of eggs laid (χ^2= 69.451, df = 13, p < 0.001). The interaction between substrate and day was insignificant in explaining some of the observed variance in egg laying (χ^2= 73.697, df = 52, p =0.083). This can be seen in Figure 49 as the number of predicted eggs laid can vary notably between substrates, particularly *Fucus* sp, but all eggs are laid at similar times across the substrates.

These results suggest, under the conditions of the experiment, substrate plays a marginally significant role in a *C.frigida* female's oviposition site selection and that this is influenced more by where not to oviposit than where to oviposit.

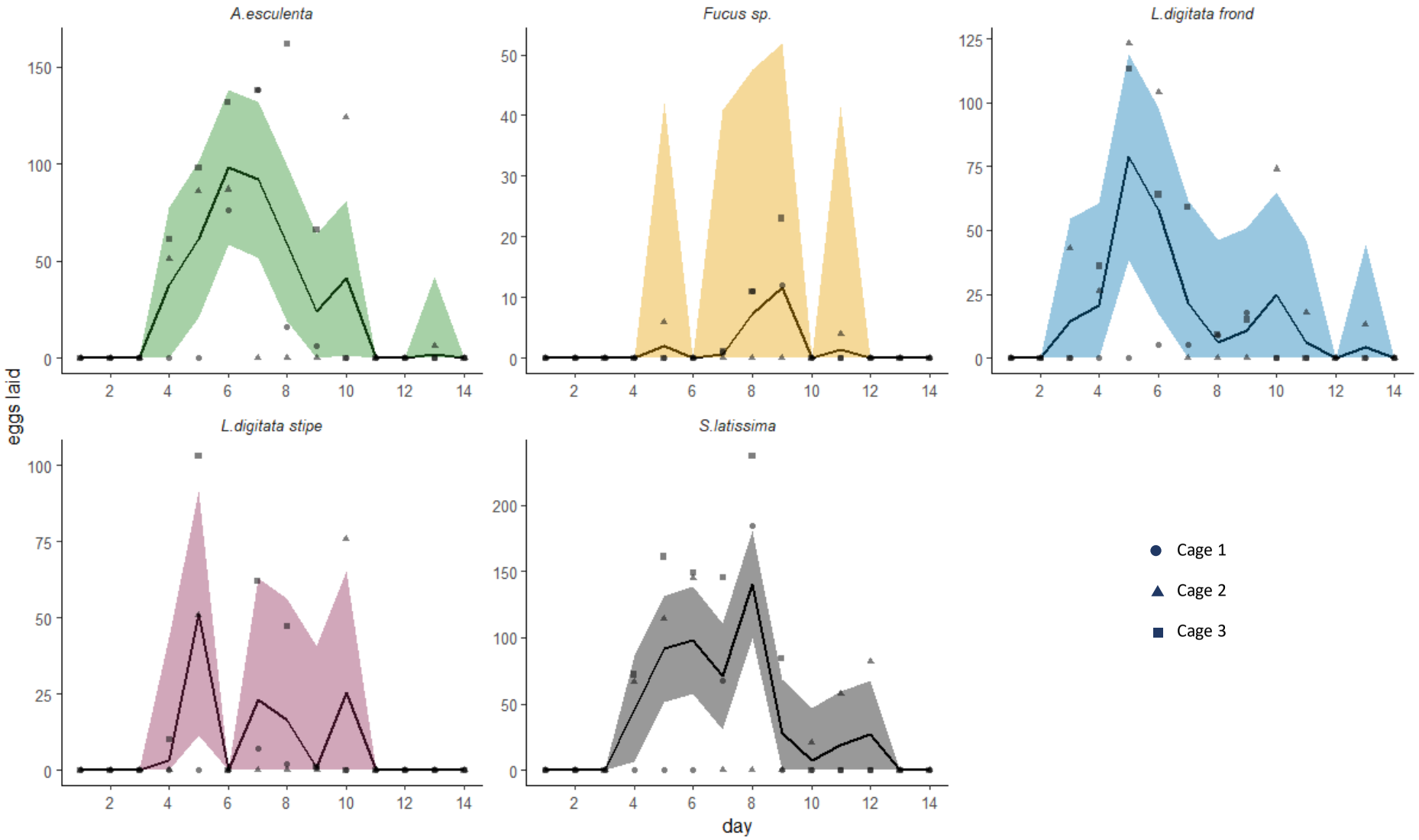


Figure 49. The estimated mean and 95% CI of the number of eggs laid by C.frigida on each substrate as time progressed. The black lines represent the estimated means and the shaded area around these represent the 95% CI. Observed values from each of the three replicates are plotted by shape. Note, y-axis scales vary between substrate plots.

From the negative binomial model (appendix 4.2), the substrate on which *F.maritima* females chose to lay their eggs was estimated not to be significantly different across any of the pairwise comparisons. However, a slight preference for *S.latissima* is suggested. These estimates and their relative effect sizes are visualised in Figure 50. The p-values from each pairwise comparison are reported in Table 7.

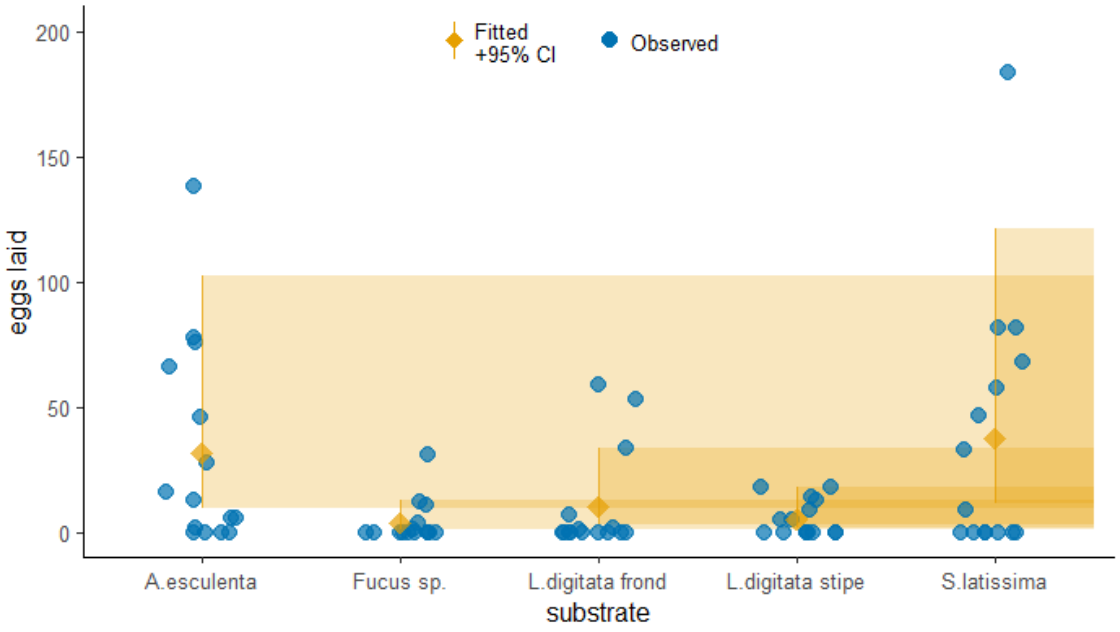


Figure 50. The number of eggs laid by F.maritima females per substrate. Data represent values across three replicate cages and a time period of 14 days. The mean number of eggs laid and the 95% CI for this mean are represented by yellow data and the estimated CI has been projected right to allow easy pairwise comparison. Blue data points represent observed values during the experiment.

Table 7. The p-values of each pairwise comparison of estimated eggs laid by F.maritima per substrate. Values in green denote those which are below the significance level of 0.05.

	S.latissima	***L.digitata stipe***	***L.digitata frond***	***Fucus sp.***
L.digitata stipe	0.159			
L.digitata frond	0.557	0.944		
Fucus sp.	0.065	0.996	0.790	
A.esculenta	>0.999	0.239	0.686	0.106

The mean number of eggs laid by *F.maritima* on each substrate over time, along with 95% confidence intervals, were estimated and these results are presented in Figure 51. Again, substrate was naturally also significant in this model (χ^2=23.859, df = 4, p = < 0.001). In *F.maritima* both day (χ^2= 68.564, df = 13, p < 0.001) and the interaction between substrate and day (χ^2= 119.968, df = 52, p < 0.001) were each found to explain a significant amount of the variation in observed oviposition. However, the high χ^2 of 119.968 relative to the 52 degrees of freedom suggests the model does not fit the data particularly well. This is evident in Figure 51 where the differences in timing between substrates are relatively small. The most noticeable differences are a ~1 day delay in the onset of oviposition in *L.digitata* frond and an earlier peak in *Fucus* sp, although the peak of 30 eggs is relatively low.

These results suggest, under the conditions of the experiment, substrate plays a marginally significant role in a *F.maritima* female's oviposition site selection.

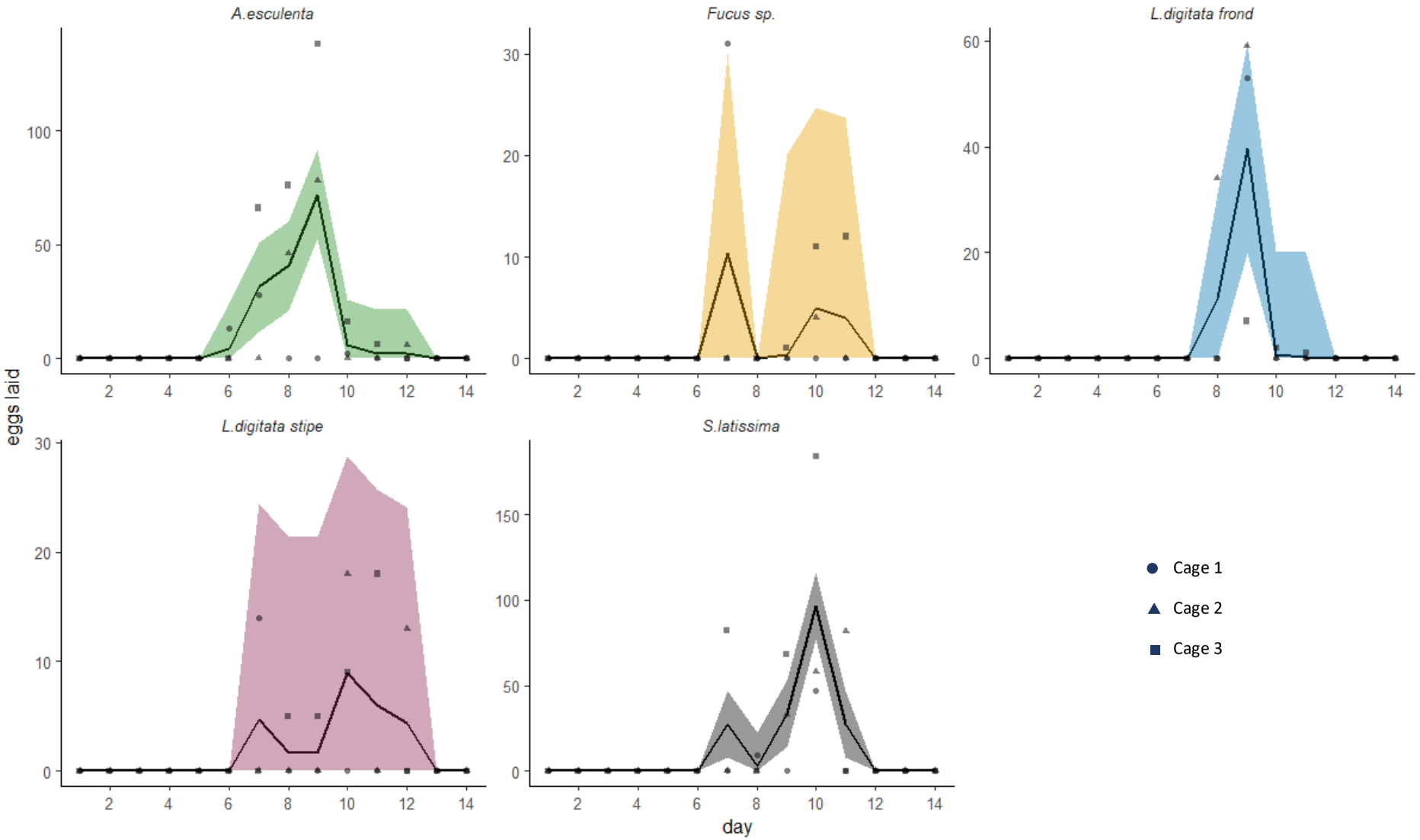
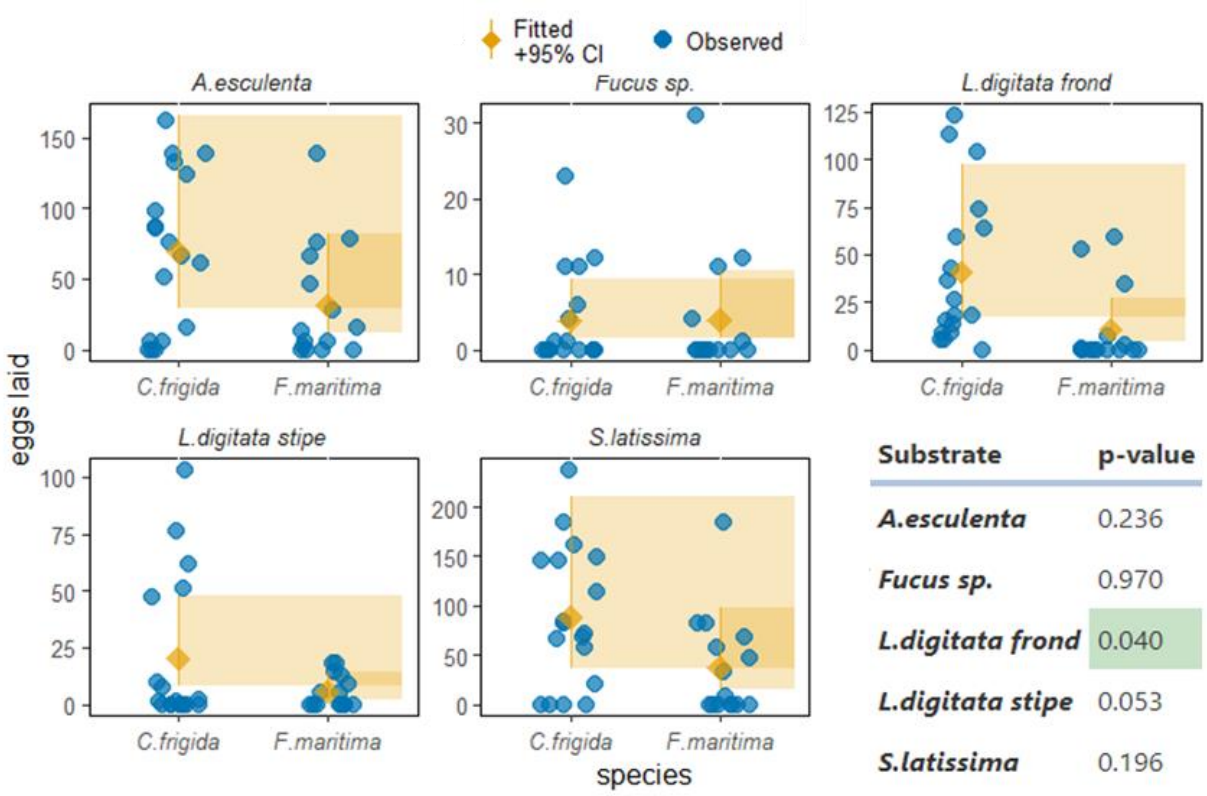


Figure 51. The estimated mean and 95% CI of the number of eggs laid by F.maritima on each substrate as time progressed. The black lines represent the estimated means and the shaded area around these represent the 95% CI. Observed values from each of the three replicates are plotted by shape. Note, y-axis scales vary between substrate plots.

Lastly, the estimated number of eggs laid by both *C.frigida* and *F.maritima* across each substrate were compared. The estimated eggs laid were compared across each substrate with a t-test via the package 'emmeans' (Lenth 2023). The only substrate on which the number of eggs laid was estimated to be different between *C.frigida* and *F.maritima* was *L.digitata* frond. However, the effect size of this was also very marginal as can be seen in the visualisation of these results in Figure 52. Thus, under the conditions of the experiment, these results suggest the number of eggs laid by each fly species is more likely to be the same for matching substrates than different.



Substrate	p-value
A.esculenta	0.236
Fucus sp.	0.970
L.digitata frond	0.040
L.digitata stipe	0.053
S.latissima	0.196

Figure 52. The number of eggs laid by C.frigida and F.maritima facetted by substrate. The mean number of eggs laid and the 95% CI for this mean are represented by yellow data and the estimated CI has been projected right to allow easy pairwise comparison. Blue data points represent observed values during the experiment. Resulting p-values from t-tests between fly species for each substrate are displayed in the table, the value in green denotes this p-value is below the significance level of 0.05.

Offspring success

The estimated survival curves for *C.frigida* larvae on each substrate were compared using a log-rank test and were significantly different between the substrate groups (χ^2= 874, df = 4, p < 0.001) (appendix 4.3). The survival curves of each substrate were compared pairwise and a higher proportion of eggs reached adulthood when reared on the substrate *L.digitata* frond than any other. They also reached adulthood faster than those on any other substrate. The p-values of each pairwise

comparison are reported in Table 8. The only comparisons in which a difference was not found was between *Fucus* sp and *L.digitata* stipe and between *A.esculenta* and *S.latissima*. There was no difference in survival on the latter two substrates as 100% of eggs did not reach adulthood on either. The total number of eggs that reached adulthood in each replicate are reported in Table 9. The survival curves for each substrate are visualised in Figure 53. These results suggest that the development of *C.frigida* larvae differs significantly depending on the substrate on which it is reared under the conditions of this experiment.

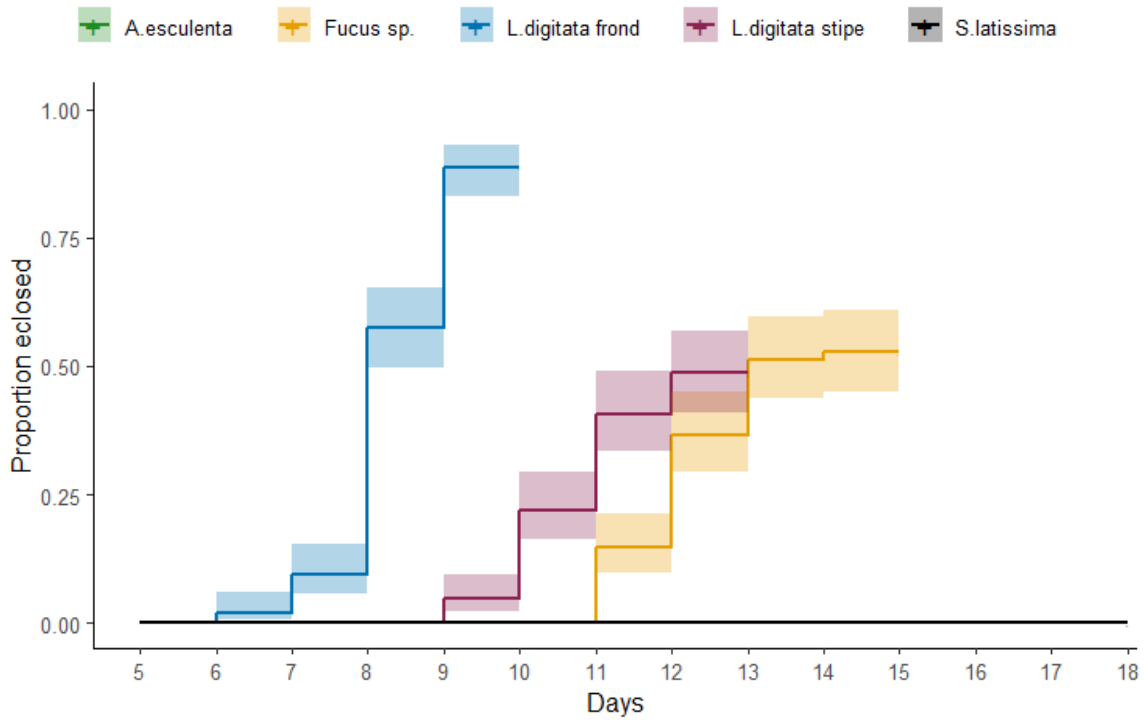


Figure 53. The survival rates of C.frigida eggs to adulthood for each substrate. The x-axis represents the development time in days and begins on day 5, the day before the first eclosion took place. The y-axis represents the proportion of eggs that survived to eclosion. Shaded areas either side of the line represent the 95% CI.

Table 8. The p-values of each pairwise comparison of C.frigida survival curves per substrate. Values in green denote those which are below the significance level of 0.05.

	A.esculenta	***Fucus sp.***	***L.digitata frond***	***L.digitata stipe***
Fucus sp.	<0.001			
L.digitata frond	<0.001	<0.001		
L.digitata stipe	<0.001	0.186	<0.001	
S.latissima	>0.999	<0.001	<0.001	<0.001

Table 9. The total number of C.frigida eggs which reached, and did not reach, adulthood per substrate for each replicate.

	Reached adulthood			Did not reach adulthood		
	rep 1	rep 2	rep 3	rep 1	rep 2	rep 3
L.digitata frond	46	44	43	4	6	7
L.digitata stipe	36	13	24	14	37	26
A.esculenta	0	0	0	50	50	50
S.latissima	0	0	0	50	50	50
Fucus sp	23	19	37	27	31	13

Whilst no *C.pilipes* eggs were laid in the ovipositional preference experiment, their larval success could still be comparatively estimated using eggs obtained outwith the experiment. The estimated survival curves for *C.pilipes* larvae on each substrate were compared using a log-rank test and were also significantly different between the substrate groups (χ^2= 664, df = 4, $p < 0.001$) (appendix 4.4). The survival curves of each substrate were compared pairwise and a higher proportion of eggs reached adulthood when reared on the substrate *L.digitata* stipe than any other. They also reached adulthood faster than those on any other substrate. This was followed by *L.digitata* frond and then *Fucus* sp. As with *C.*frigida, 100% of eggs did not reach adulthood on *A.esculenta* and *S.latissima*. Thus, these two substrates were the only pairwise comparison in which larval success was not estimated to differ (Table 10). The total number of eggs which reached adulthood in each replicate are reported in Table 11. The survival curves for each substrate are visualised in Figure 54. These results suggest that the development of *C.pilipes* larvae differs significantly depending on the substrate on which it is reared under the conditions of this experiment.

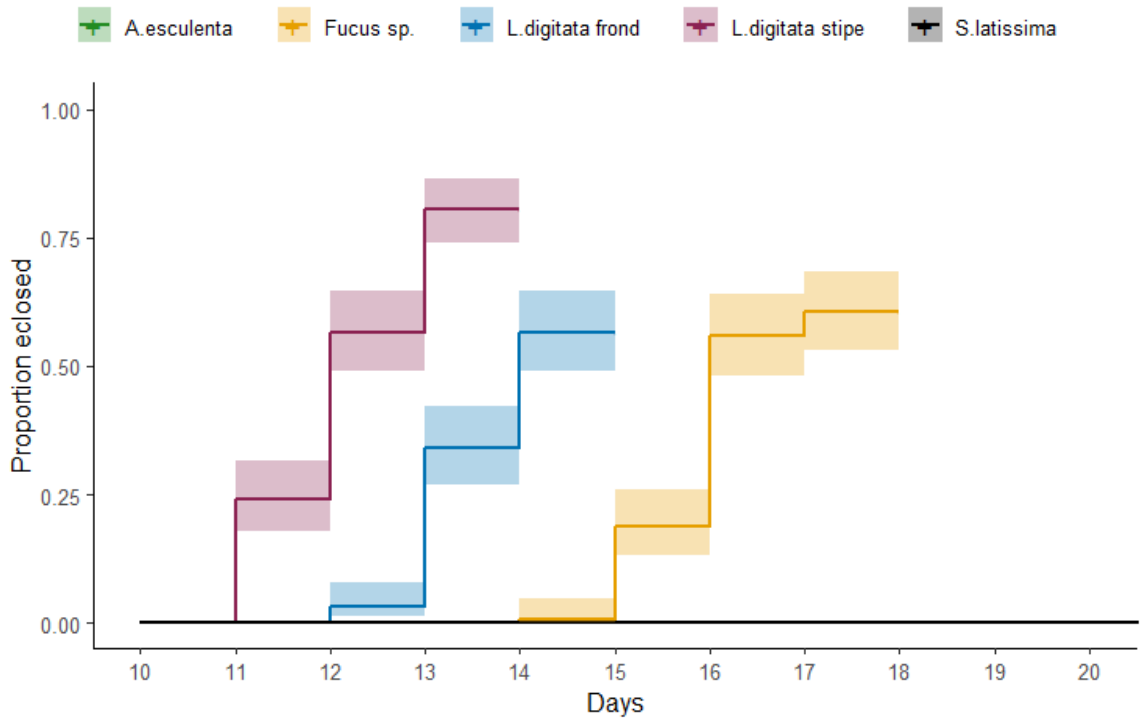


Figure 54. The survival rates of C.pilipes eggs to adulthood for each substrate. The x-axis represents the development time in days and begins on day 10, the day before the first eclosion took place. The y-axis represents the proportion of eggs that survived to eclosion. Shaded areas either side of the line represent the 95% CI.

Table 10. The p-values of each pairwise comparison of C.pilipes survival curves per substrate. Values in green denote those which are below the significance level of 0.05.

	A.esculenta	***Fucus sp.***	***L.digitata frond***	***L.digitata stipe***
Fucus sp.	<0.001			
L.digitata frond	<0.001	<0.001		
L.digitata stipe	<0.001	<0.001	<0.001	
S.latissima	>0.999	<0.001	<0.001	<0.001

Table 11. The total number of C.pilipes eggs which did and did not reach adulthood per substrate for each replicate.

	Reached adulthood			Did not reach adulthood		
	rep 1	rep 2	rep 3	rep 1	rep 2	rep 3
L.digitata frond	27	34	24	23	16	26
L.digitata stipe	43	41	37	7	9	13
A.esculenta	0	0	0	50	50	50
S.latissima	0	0	0	50	50	50
Fucus sp	38	28	25	12	22	25

The estimated survival curves for *F.maritima* larvae reared on each substrate were compared using a log-rank test and were significantly different between the substrate groups (χ^2= 419, df = 4, p < 0.001) (appendix 4.5). The survival curves of each substrate were compared pairwise and a higher proportion of eggs reached adulthood when reared on either the substrate *L.digitata* frond or *A.esculenta* than any of the other three. No difference between these two was found. However, those reared on *L.digitata* frond did develop faster than those on *A.esculenta*. Although, in contrast to the *Coelopa* species, substrate(s) on which the most *F.maritima* eggs reached adulthood was not also the substrate on which development occurred fastest. *F.maritima* eggs reached adulthood significantly faster on *L.digitata* stipe than on any other substrate. The p-values of each pairwise comparison are reported in Table 12. The only comparisons in which a difference was not found was between *Fucus* sp and *S.latissima* on which larval success was poorest. The total number of eggs which reached adulthood in each replicate are reported in Table 13. The survival curves for each substrate are visualised in Figure 55. These results suggest that, under the conditions of this experiment, the development of *F.maritima* larvae differs significantly depending on the substrate on which it is reared.

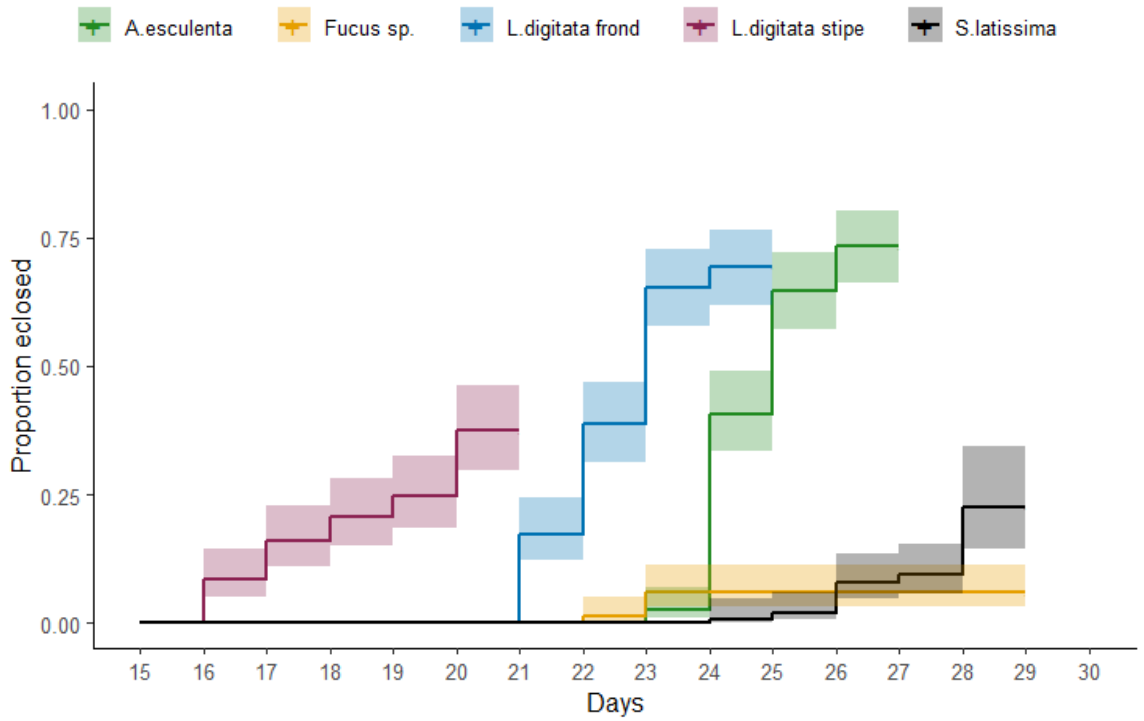


Figure 55. The survival rates of F.maritima eggs to adulthood for each substrate. The x-axis represents the development time in days and begins on day 10, the day before the first eclosion took place. The y-axis represents the proportion of eggs that survived to eclosion. Shaded areas either side of the line represent the 95% CI.

Table 12. The p-values of each pairwise comparison of F.maritima survival curves per substrate. Values in green denote those which are below the significance level of 0.05.

	A.esculenta	***Fucus sp.***	***L.digitata frond***	***L.digitata stipe***
Fucus sp.	<0.001			
L.digitata frond	<0.001	<0.001		
L.digitata stipe	<0.001	<0.001	<0.001	
S.latissima	<0.001	0.263	<0.001	<0.001

Table 13. The total number of F.maritima eggs which did and did not reach adulthood per substrate for each replicate.

	Reached adulthood			Did not reach adulthood		
	rep 1	rep 2	rep 3	rep 1	rep 2	rep 3
L.digitata frond	40	29	35	10	21	15
L.digitata stipe	9	26	17	41	24	33
A.esculenta	39	33	38	11	17	12
S.latissima	7	9	5	43	41	45
Fucus sp	0	6	3	50	44	47

Overall, these estimated effects of substrate on larval success suggest the larvae of all three wrack fly species perform best on different substrates than each other. Additionally, the results also suggest that on each species' "best" substrate, *C.frigida* develop quicker and with a higher egg to adult viability than *C.pilipes* or *F.maritima*.

4.4 Discussion

This study aimed to investigate the following research questions:

1. Does the substrate type influence the number of eggs laid by any of the fly species?
2. Does the substrate type influence the success of offspring development of any of the fly species?

The presented results show, in the case of *C.frigida* and *F.maritima,* that the substrate type significantly influenced how many eggs were laid on that substrate and the probability of observing this if the null hypothesis was true was <0.1%. Thus, it can be stated that, under the conditions of this experiment, substrate type does influence how many eggs will be laid on a substrate by *C.frigida* or *F.maritima. C.pilipes* laid no eggs throughout the experiment so the influence of substrate type on their oviposition was not assessed. Across all three species, it can be stated that substrate type does influence the success of offspring development ($p<0.001$).

These questions were assessed in the context of the PPH and its potential use in niche characterisation. The results suggest the PPH does not apply to any of the studied wrack fly species as the substrate females chose to lay their eggs in was not the substrate on which larvae performed best. There are many ways larval success can be measured, and it could be that development time and egg to adult viability used in this experiment, do not provide a complete measure of success, thus accounting for the disconnect between ovipositional preference and offspring success.

However, ovipositional preference and larval success were not simply un-aligned, they were contradictory. *C.frigida* laid more eggs on *S.latissima* than any other substrate and this difference was significant in all but one pairwise comparison, that with *A.esculenta*. Conversely, their larvae were equally least successful on these two substrates as no eggs developed into adulthood on them. Similarly, *F.maritima* laid significantly more eggs on *S.latissima* than any other substrate, yet, larvae performed joint poorly on this and *Fucus* sp. While an incomplete assessment of larval success could explain some discrepancy between it and ovipositional preference. It is unlikely a measure of success as fundamental as egg to adult viability would be in complete opposition with ovipositional preference if the PPH applied to these species. Therefore, it is more likely that of the substrate choices given, and the range of variables such as nutrition, moisture content and microbial population they represent, females did not choose where to oviposit based on larval success.

The selection of ovipositional site is highly complex and many factors and variables must be considered. For example, ovipositioning in a habitat where conspecific (same species) offspring are already present will increase intraspecific competition, decreasing survival. However, the avoidance of such sites can lead to the use of lower quality oviposition habitats and could have a greater detrimental effect. In this sense, the presence of conspecific offspring may be a cue for high quality habitat (Rudolf and Rodel 2005, Wilton 1968). Therefore, females may be choosing among conflicting forms of quality that may vary spatially and temporally (Potter *et al* 2012). As a result, determining that host species defines where a female will lay her eggs most frequently, based on a lab experiment where this is the only variable, can be irrelevant in the field. As such, this relatively simple experiment should be validated through those which are more complex, and preferably done in the field, before any robust conclusions on how the PPH applies to these wrack flies are drawn.

Nevertheless, as eggs were laid by *F.maritima* and *C.frigida*, and many of those eggs likely would have reached adulthood, it can be argued that the conditions presented in the experiment were within the fundamental niche of each species. Conversely, the laying of 0 eggs by *C.pilipes* under the same conditions could be interpreted as evidence these are not within this species' niche. However, if this were the case, one might expect larval performance to also be poor. Furthermore, *C.pilipes* can be reared relatively well in some of these conditions when presented individually as shown in Chapter 3. This highlights a limitation of this experiment in the proximity of the substrate choices to each other. *C.pilipes* can successfully and reliably be reared on *L.digitata* stipe but not on *A.esculenta* or *S.latissima* (Chapter 3). Therefore, the presence of 0 eggs across all three replicates may be a result of the variable(s) represented in *A.esculenta* and *S.latissima,* which makes culture on them unsuccessful, inhibiting *C.pilipes* oviposition across the cage. This is feasible as the

substrates were all in close proximity within the 30cm^3 cage. This may also be responsible for some of the small effect size in the other species.

The results on larval success are more suggestive of species occupying different niches, or at least having different optimal conditions. Each of the three study species performed best on different substrates. Each substrate differs by several variables such as moisture content and likely differs by several more unknown. Thus, differential optimum performance on different substrates suggests each species are adapted to different conditions. *C.frigida* developed fastest and with the highest egg to adult viability on *L.digitata* frond, *C.pilipes* did so on *L.digitata* stipe. This is a particularly interesting finding as it evidences a realistic mechanism that could facilitate some of the coexistence between these *Coelopa* species. This is supported by the findings of Edwards *et al* (2008) who, through stable isotope analysis, found even in a laboratory monoculture, *C.frigida* and *C.pilipes* metabolised algal material differently. They suggest this is indirect evidence of adaptations to avoid intraspecific competition. It seems unlikely the coexistence of these species is a result of *C.pilipes* carrying out their life cycles in the stipe of *L.digitata* and *C.frigida* in the frond. However, these results, supported by those of Edward *et al* (2008), do suggest these species digest wrack differentially and some of this is represented in the difference between *L.digitata* stipe and frond.

Although these results may advance our understanding on the coexistence of *C.frigida* and *C.pilipes,* it is important to remember that such findings based on niche separation and/or intraspecific competition do not exist in isolation in the field as they can in the lab. Therefore, if niche separation and or interspecific competition are not the most significant factor in controlling natural coexistence, the results are of little consequence (Rudolf and Rodel 2005). Thus, there is a need for a holistic study into the coexistence of these species, and this is especially true given the nature of the resource (wrack beds). A key aspect of Gause (1934)'s competitive exclusion theory is that the same limiting resource cannot be shared indefinitely. The ephemeral nature of wrack beds relates to the indefinite aspect and the duration a given deposit exists is best measured in weeks. As such, a deposit does not exist for a long, or short, enough period of time for one species to entirely outcompete the other. Although, over time the less dominant of the two could be expected to reduce into extinction. Additionally, wrack beds are often large relative to the biomass of adults which colonise them, particularly the *Laminaria* banks *Coelopa* are most often found in, and have the capacity to be indefinitely replenished by further tidal deposition. Thus, they are not always a limited resource. Moreover, the discrepancies in the literature regarding the temporal distribution of *C.frigida* and *C.pilipes* (Colombini and Chelazzi 2003, Dobson 1974, Egglishaw 1958, Leggett 1993) could be evidence of the dominant species changing seasonally further reducing one species'

capacity to competitively exclude the other as shown in Figure 47,5. Lastly, the coexistence of the *Coelopa* species in wrack beds could also be a result of species occupying a realised niche in the presence of the other species, something Phillips *et al* (1995)'s findings could indicate is temperature driven.

The results presented in this study build on the previous findings of Chapter 3 which evidence the potential of *C.frigida*, *C.pilipes* and *F.maritima* to be farmed in the production of an alternative ω-3 PUFA source. Here it is evidenced *C.frigida* have more potential to be produced on an industrial scale, owing to their fastest development time and highest egg to adult viability when reared on *L.digitata* frond, compared to the other two species on their respective best performing substrates.

Chapter 5

THE NUTRITIONAL COMPOSITION OF EIGHT WRACK INVERTEBRATES

5.1 Introduction

Understanding the ecology of wrack invertebrates and using this to establish reliable methods of rearing them is the first step to assessing their feasibility as an alternative ω-3 source. This is the case for two reasons. Firstly, to meet at least some of the demand for ω -3 rich products currently met by fish oil, such species would need to be produced on an industrial scale. Naturally, this requires them to be reared with relative ease and efficiency. Secondly, to begin to assess the nutritional composition of these species and if/how it can be manipulated requires their rearing in experimental analysis. To this end, the nutritional composition of eight of the wrack invertebrates, introduced in Chapter 2, were analysed, and some initial experiments into what may account for any variability in this composition undertaken. In addition to these wrack invertebrates, one sample of *Daphnia* species, as an ecologically different invertebrate, which feeds on live micro-algae, was analysed for comparison.

Currently, there is one study that reports the nutritional composition of *C.frigida* and *C.pilipes* (Biancarosa *et al* 2017). To the best of current knowledge, that of *F.maritima*, *O.luctuosum* and *L.oceanica* have not been reported, this study addresses that knowledge gap. Comparatively, studies into the nutritional composition of Amphipod species are available (Kolanowski *et al* 2007, Lahdes *et al* 2010), though none which report specifically on intertidal Talitridae could be found. In addition to assessing the potential of these species as an alternative PUFA source, data on their nutritional composition will also help quantify their role in their ecosystems.

Biancarosa *et al* (2017) report the following findings on the composition of *C.frigida* and *C.pilipes* larvae as well as the *F.serratus* and *L.digitata* they were reared on. Regarding the two algal substrate species, they found both contained levels of the essential amino acids histidine, methionine and phenylalanine below the limit of quantification (<LOQ). Among other differences, *L.digitata* had significantly more iron, copper, arsenic and mercury. It also contained more lead, but this difference was not significant. Conversely, *F.serratus* had significantly more cadmium. The levels of cadmium, mercury and lead were relatively low in both species. Biancarosa *et al* (2017) highlight the arsenic and cadmium levels in the larvae as a cause for concern and suggest the use of substrate management to limit this. The *L.digitata* and *F.serratus* analysed contained 1.1mg/g and 8.0mg/g of

EPA respectively and DHA was <LOQ for both species. Concerning the EPA in larval samples, *C.frigida* was estimated to contain 4.8mg/g and 8.4mg/g when reared on *L.digitata* and *F.serratus* respectively. That of *C.pilipes* was 4.4mg/g and 4.8mg/g. These values were reported as % of total fatty acid by the authors and have been converted from these published data. The species and the species substrate interaction were estimated to be non-significant in the quantity of EPA along with most other FAs, minerals and amino acids. This is surprising given the ~57% increase in the EPA of *C.frigida* between *L.digitata* and *F.serratus.*

Assessing the fatty acid composition of five freshwater Gammarid Amphipod species Kolanowski *et al* (2007) report a range of EPA, DHA and DPA values between 3.7-12.8mg/g, 1.1-2.7mg/g and 0.3-1.3mg/g respectively. These values were also converted from % of total Fa to mg/g. Ladhes *et al* (2010) report the fatty acid composition of marine Amphipod species identified to the *Gammerus* genus, these are also presented in % of total fatty acids. Unfortunately, the amount of fatty acid is not reported making the data meaningless outside any comparisons within the study. However, they do evidence a significant difference in the composition depending on the time of year, a change they hypothesise is temperature driven.

The amino acid compositions of insects are known to be relatively consistent compared to that of certain fatty acids and minerals, this is a result of their efficiency at conserving and recycling amino acids and difference in which amino acids they can synthesise *de novo* (Behmer and Nes 2003, Slansky, 1993, Wilson and Lee 200). As such, several studies investigating the effects of diet on nutritional composition of insects, such as that on black soldier fly larvae (*Hermetia illucens*) by Spranghers (2017), find little variability in amino acid composition between treatments. Therefore, to reduce the time and financial costs of analysis in this study, fewer amino acid samples were analysed than fatty acid or mineral. Similarly, only one sample of each substrate type underwent analysis as Biancarosa *et al* (2018) report the chemical composition of 21 species of seaweed wild harvested from the coast of Norway. If a relationship between the invertebrates and the substrate they are reared on was established, their predicted nutritional composition based on that of a given substrate could be modelled.

The method(s) used in the processing of samples can influence their nutritional profile (Ojha *et al* 2021). For example, the drying method used prior to the nutritional analysis of seaweed has been shown to affect the results (Chan *et al* 1997) and the choice of solvent extraction technique has been shown to affect the fatty acid composition of *Pistacia vera* (pistachio) oil (Abdolshahi *et al* 2013). Additionally, some studies investigating the nutritional value of insects leave individuals for 24 hours with no food in order to clear their gut prior to analysis (Charlton *et al* 2015), while others do not

(Biancarosa *et al* 2017). Therefore, quantifying the effects of gut clearing would prove useful in establishing a standard practice for the processing if insect foodstuffs.

Lastly, as discussed in Chapter 3, at low densities, the larvae of *C.frigida* appear to facilitate each other but between 0.7 and 1.1 larvae per gram of substrate this relationship seems to turn competitive. The default in interactions like this is often to assume this competition is for food/nutrition, but this may not be the case. If it were for nutrition, it could be hypothesised that a second generation reared on the same substrate, would be both less successful and of poorer nutritional quality. If this is not the case, the use of insects as an alternative food source may be more sustainable than many estimates suggest as the substrate could be reused.

This study aims to quantify the nutritional composition of eight wrack invertebrates and investigate the following research questions:

1. Does the EPA or DHA content differ between the study species or within species between substrates?

2. Is the accumulation of contaminants different between the study species or within species between substrates?

3. Does the method of larval processing affect their EPA, DHA and/or contaminant composition?

4. Is the EPA, DHA and/or contaminant composition of a 2nd generation of larvae different from that of the 1st reared on the same substrate?

5.2 Methods

Sampling

To assess these questions, the following samples were collected to have their mineral/chemical, amino acid and fatty acid contents analysed, here referred to as nutritional composition. The six substrates: *Laminaria digitata* frond, *Laminaria digitata* stipe, *Fucus* species, *Alaria esculenta*, *Saccharina latissima* and farmed *Laminaria digitata*, the collection of which is described in Chapter 3, were used in the rearing of laboratory samples. One sample of each substrate (n=6) was analysed as the nutritional content of seaweed species are well documented (Belghit *et al* 2018) thus the purpose of their analysis was largely to confirm the nutritional composition of the substrates used were in line with the literature. Although, making a distinction between the frond and stipe of *L.digitata*, as done here, is not reported anywhere else. In addition to which, nine species of invertebrate were sampled (n=103). These invertebrates can be split into two groups the first of

which make up the bulk of the samples and can be thought of as the 'experiment samples'. Wild samples of Talitridae (n=17), *O.luctuosum* larvae (n=10), *F.maritima* larvae (n=3) and *Coelopa* species larvae (n=15) were collected over a 2 year period as described in Chapter 2. *Coelopa* species refers to larvae of *C.frigida* and/or *C.pilipes* as this was the lowest level larvae could be identified to. In addition to the wild harvested larval samples were those reared in the laboratory and consisted of *C.frigida* (n=25), *C.pilipes* (n=12), *F.maritima* (n=12) and *O.luctuosum* (n=5). These invertebrate and algal samples each had a unique sample ID and were represented by the variable 'species' (though not every algal sample represented a distinct species such as *L.digitata* frond). From these samples, an estimate of the effect of species on nutritional composition could be made.

Samples from the laboratory were reared according to the same culturing methods described in Chapter 3. Under ideal rearing, approximately 300g of substrate was added to a 10x10x15cm plastic tub along with adult flies and was closed with a lid fashioned to allow gas exchange. The number of adults differed depending on fly species and was calculated based on the number of eggs females from each species were expected to produce in attempts to achieve a rough larval density of 1 larvae per gram of substrate. This was 4-5 mating pairs in *C.frigida*, 8-10 in *C.pilipes* and 12-15 in *F.maritima*. However, as discussed in Chapter 3, when difficulty was had in rearing a certain species substrate combination, this adult density was increased in attempts to improve success rate. Furthermore, as also discussed in Chapter 3, *F.maritima* were more successful in 'open' cages and thus were only reared such in this experiment.

To improve the accuracy of the results, each laboratory reared sample was comprised of at least three biological replicates. Creating samples from three biological replicates improved the accuracy of the results without tripling the costs that would be associated with analysing each biological replicate separately. The more difficulty was had in rearing each species on a given substrate the more biological replicates were needed to achieve the required sample amount of 2g dw.

After being harvested, larvae were rinsed to remove any substrate present on them and patted dry with blue roll. They were than placed on a piece of blue roll for ~10 minutes to allow any additional external moisture to evaporate or soak into the blue roll. Following this, larvae were euthanised by storing them at -20°C for at least 24 hours. Lastly, these samples were transferred to a -80°C freezer for 24hours before being freeze dried in a vacuum (lyophilised) ready for analysis. Samples were stored at -80°C prior to freeze drying to ensure sublimation (transition from solid to vapor without passing through the liquid state), rather than melting, occurred.

To assess any effect of invertebrate substrate on nutritional composition, the rearing of *C.frigida*, *C.pilipes* and *F.maritima* was attempted on each of the six substrates. However, due to the unanticipated difficulty in rearing certain species substrate combinations, this was not wholly successful. Three samples of each species substrate combination were ideally collected but particular difficulty was had with the farmed species of substrate, which was not easily replaced like that of the wild harvested substrates. As such, the farmed substrates quickly ran out before enough samples from which to model the effects of each could be obtained. Upon analysing the nutritional composition of the few samples successfully reared on farmed substrates, their effects on nutritional content did not appear to differ hugely. This, combined with the observed similarity in their difficulty of culturing, led to the decision to group all farmed substrate into one substrate level: 'farmed sp' allowing the samples to be included in modelling.

Lastly, samples to explore the effect(s) of both larval processing method and larval generation from substrate on the nutritional composition were gathered using *C.frigida* larvae reared on *L.digitata* frond. This combination was selected as it was the easiest, fastest and most reliable. The effect of two different processing methods were compared to the standard method described above. The first of which was leaving the larvae for six hours between removal from the substrate and freezing thus creating larval samples with a cleared gut. The second of which was oven drying the larval samples instead of freeze drying them. Whereby, larvae were transferred from the -20°C freezer to a drying oven preheated to 50°C where they were left for 24-36 hours and subsequently stored for analysis. Samples to assess the effect of larval generation from substrate were obtained by rearing *C.frigida* larvae on *L.digitata* frond at an approximate density of 1l/g as described above. A sample of larvae was collected from each replicate, and the rest were allowed to develop into adults. Once no more larvae were present, the pupae and any dead larvae were removed and a second batch of adults added to the culture. Thus, the larvae of these adults represented the 2nd generation to be reared on that culture's substrate. As above, each of these treatments (gut cleared, oven dried and 2nd generation) had three samples (n=3), and each of these samples was comprised of three biological replicates.

In addition to these 'experimental samples', some 'comparison samples' were collected. These consisted of one sample (n=1) of four additional invertebrate species, which feed on algae/wrack. The first of these were Gammarid Amphipods, Nematode species present in the wrack and *L.oceanica* (sea slater) these three samples were wild harvested, processed as described above and also contained three biological replicates per sample. The last of these samples were *Daphnia* species bought already dried from Chubby Pet Products LTD. The purpose of these was to gain a

deeper understanding of how nutritional composition may differ between invertebrate species which feed on wrack/algae.

Nutritional composition analysis

The mineral/chemical, amino acid and fatty acid compositions of each sample were quantified in technical duplicates using inductively coupled plasma-mass spectrometry (ICP-MS), ultra-performance liquid chromatography (UPLC) and gas-liquid chromatography (GC) respectively. Technical duplicates were used to ensure the reliability and accuracy of the data by assessing any measurement errors or variability in the analysis process. All analysis was performed at havforskningsinstituttet, Bergen, Norway.

Mineral/chemical

The mineral/chemical compositions were analysed using ICP-MS based on the method described by Julshamn *et al* (2001). First, wet digestion of the samples was carried out to break down the organic and inorganic components within them, making it easier to measure the element concentrations. Approximately 0.2 g of dry sample, were digested in 69% nitric acid (2 ml) and 30% hydrogen peroxide (0.5 ml) using a microwave digestion system (UltraWAVE). The use of a microwave digestion system allowed for rapid and efficient digestion of the samples. The solutions were then diluted to 25 ml with deionized water to help ensure they were within the working range of the ICP-MS instrument. The composition of samples were then quantified by ICP-MS (iCapQ ICPMS; Thermo Fisher Scientific) equipped with an autosampler (FAST SC-4Q DX; Elemental Scientific), which allowed for efficient and precise sample introduction minimizing potential errors. Finally, data were processed using the Qtegra ICPMS software ensuring accurate quantification and interpretation of the results.

Amino acid

The analysis of total amino acids was performed using UPLC and was based on that by Biancarosa *et al* (2017). Prior to analysis, wet, powdered samples were hydrolysed, as the amino acids need to be in a free, unbound form to be detected by the instrument. Hydrolysis was achieved by heating the samples in 6M hydrochloric acid at 110 °C for 22 hours. This breaks down the peptide bonds between the amino acids reducing the protein into its constituent amino acids. To ensure the accuracy of the results, an internal standard (3.125 mM Norvaline) was added prior to hydrolysis. In addition, an antioxidant agent (0.1 M dithiothreitol) was added to protect methionine from degradation during acid hydrolysis. The sample tubes were also topped up with nitrogen gas to further prevent oxidation by displacing O_2 gas present. Cysteine and tryptophan were destroyed during acid hydrolysis and therefore were not reported in the results. After hydrolysis, the samples

were cooled to room temperature and centrifuged in a vacuum until dry. The residue was then diluted in deionized water and filtered through a syringe-driven filter. A derivatisation agent (AccQ.Tag™) was added to each sample prior to instrumental analysis to improve detection sensitivity. The amino acids were then separated by UPLC using a column with a flow rate of 0.7 ml/min, and the results were integrated by Empower 3.

Fatty acid

The various fatty acid compositions were identified and quantified using GC coupled with a flame ionization detector based on that described by Torstensen *et al* (2004). Lipids were isolated from the other components of the sample by powdering and wetting them before homogenising in chloroform and methanol in a respective ratio of 2:1, v:v. The extracted lipids were then analysed using an 'Autosystem XL GC with Autosampler' (Perkin Elmer) into which pre-column Silica tubing with an internal diameter (i.d) of 0.53 mm and a CP-sil-88™ column, 50m long with a 0.32mm i.d, were installed. Helium was used as the carrier gas at 1.5ml/min, and hydrogen was used as the detector gas at 45ml/min. To be effectively analysed by GC, the fatty acids were converted into a more volatile form through methyl esterification. This is done via the reaction of the fatty acids with methanol in the presence of an acid catalyst, which results in the formation of methyl esters. These methyl esters are more stable and have lower boiling points compared to the parent fatty acids, making it easier to pass them through the GC machine. The peaks corresponding to each fatty acid were identified using Chromeleon® version 6.8 (Dionex) and individual methyl esters were identified by comparison to known standards and published values (Ackman 1980). The quantification of fatty acids was done using 19:0 methyl ester as an internal standard which serves as a reference to correct for variability in extraction and analysis.

Statistical analysis

Mineral/chemical analysis

Not all of the response variables measured were minerals; some such as cadmium and arsenic are chemical elements, however, here, the term mineral is used to describe all the mineral and chemical elements assessed. The effects of each variable on mineral content were assessed on each mineral individually. This was done using a multiple linear regression, an interaction term between species and substrate was included to assess the effect of substrate within species. Pairwise comparisons were conducted using a tukey multiple comparison test with the 'emmeans' package (Lenth 2023).

Amino acid analysis

Amino acid compositions are reported as less variable in similar species than that of mineral and fatty acid composition as well as being less influenced by substrate (Spranghers *et al* 2017). In light of this and given the financial and time costs of the analysis, fewer samples had their amino acid compositions quantified. Only enough samples to loosely assess (n=3) the composition of the species, substrates and any species substrate interaction were analysed. Therefore, the aim of the amino acid analysis was to report the values in each species and substrate and assess if a similar reduced variability in amino acid content is likely. Similar to the mineral analysis, this was done using a simple linear regression with an interaction term between species and substrate. Pairwise comparisons were conducted using a tukey multiple comparison test with the 'emmeans' package (Lenth 2023).

Fatty acid analysis

The main aim of this study was to investigate the effects of species and substrate on the content of fatty acids in invertebrate samples. To achieve this, a linear mixed-effects model was fitted to the data using the 'lme4' package (Bates *et al* 2015). Rather than assess the effects on each fatty acid separately, the data were converted to a long format with levels of the variable 'FA' being each of the fatty acids. Therefore, the model included species, substrate, FA and their interaction as fixed effects, as well as a random effect of cage ID. This random effect allowed any variation between samples within variables to be quantified. The significance of the fixed effects was assessed using an anova and post-hoc pairwise comparisons of effects were conducted using a tukey multiple comparison test with the 'emmeans' package (Lenth 2023).

5.3 Results

Mineral/chemical composition

Only the findings with the most implication to the research questions of this chapter are presented here followed by a table of mean observed mineral values, +/- 1 standard deviation, for each invertebrate species (Table 24) and each substrate type (Table 25). Full results for each mineral can be found in Appendix 5.1.

The species of invertebrate had a significant effect on the content of every mineral. Though this ranged from a slight effect and poor overall fit such as in iron (Fe) ($F(5, 85) = 2.9608$, $p= 0.016$) to a pronounced effect and high proportion of variance explained such as in calcium(Ca) ($F(5, 85) = 80.677$, $p < 0.001$). A significant amount of the observed variation in mercury (Hg)(Figure 56), lead(Pb)(Figure 57), cadmium(Cd)(Figure 58),copper(Cu)(Figure 59) and arsenic(As)(Figure 60) was

explained by species. The estimated concentrations (+95%CI) of each species were compared pairwise to quantify the differences between them. These findings are visualised in each minerals' respective graph. These graphs are followed by a table of p-values of each pairwise comparison evidencing which are estimated to be significantly different.

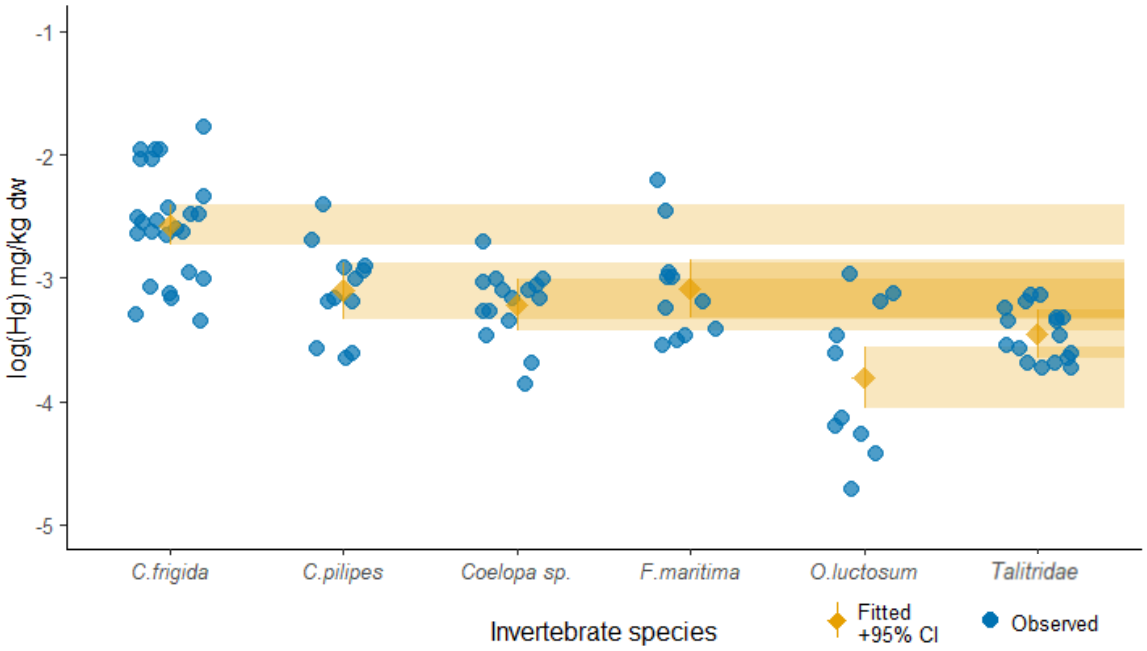


Figure 56. The estimated mean and 95%CI mercury content of each species. Estimates modelled from observed data (blue circles). The 95%CIs have been projected right to allow easy pairwise comparison. A log transformation was applied to the response variable Hg in order to normalise its distribution.

Table 14. P-values of each pairwise comparison of species' estimated mercury content, visualised in Figure 56.

	Talitridae	*O.luctosum*	*F.maritima*	*Coelopa sp.*	*C.pilipes*
O.luctosum	0.240				
F.maritima	0.179	0.001			
Coelopa sp.	0.551	0.006	0.964		
C.pilipes	0.194	0.001	>0.999	0.977	
C.frigida	<0.001	<0.001	0.007	<0.001	0.004

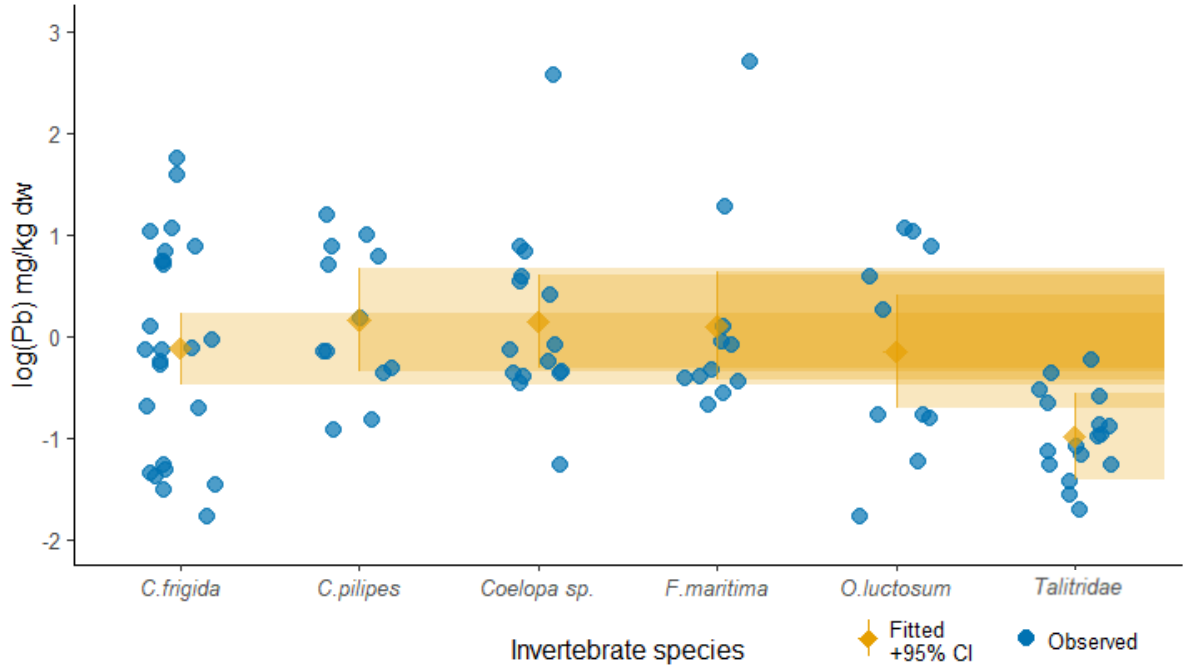


Figure 57. The estimated mean and 95%CI lead content of each species. Estimates modelled from observed data (blue circles). The 95%CIs have been projected right to allow easy pairwise comparison. A log transformation was applied to the response variable Pb in order to normalise its distribution.

Table 15. P-values of each pairwise comparison of species' estimated lead content, visualised in Figure 57.

	Talitridae	*O.luctosum*	*F.maritima*	*Coelopa sp.*	*C.pilipes*
O.luctosum	0.185				
F.maritima	0.025	0.985			
Coelopa sp.	0.007	0.963	>0.999		
C.pilipes	0.011	0.957	>0.999	>0.999	
C.frigida	0.030	>0.999	0.982	0.944	0.939

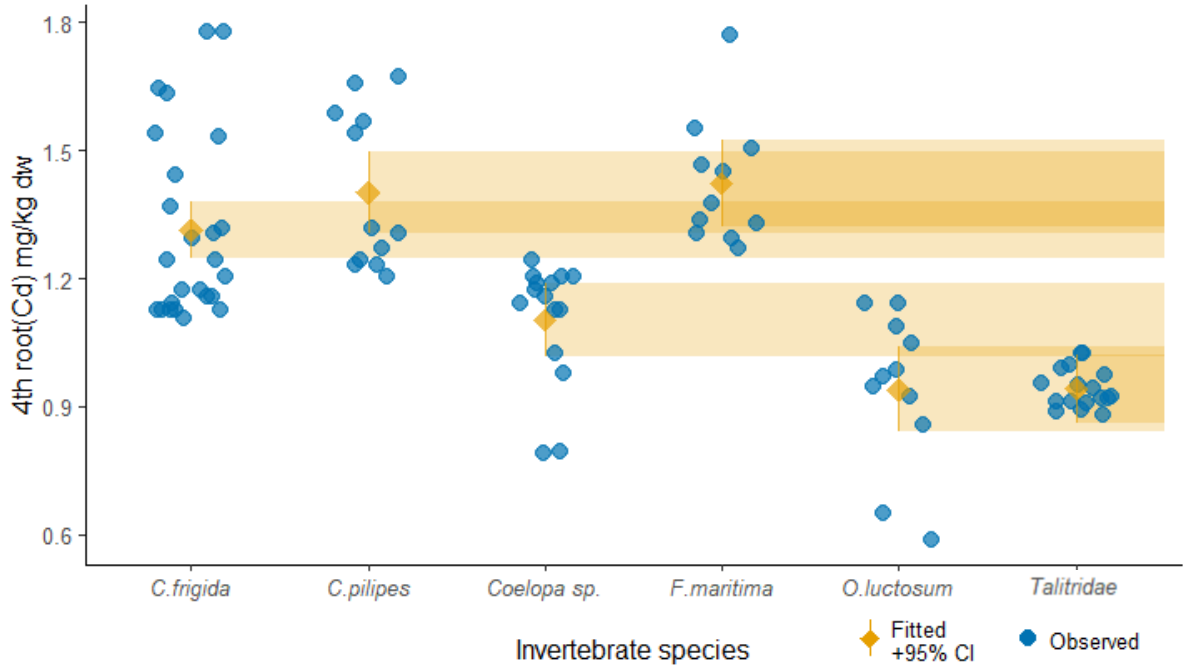


Figure 58. The estimated mean and 95%CI cadmium content of each species. Estimates modelled from observed data (blue circles). The 95%CIs have been projected right to allow easy pairwise comparison. A 4th root transformation was applied to the response variable Cd in order to normalise its distribution.

Table 16. P-values of each pairwise comparison of species' estimated cadmium content, visualised in Figure 58.

	Talitridae	*O.luctosum*	*F.maritima*	*Coelopa sp.*	*C.pilipes*
O.luctosum	>0.999				
F.maritima	<0.001	<0.001			
Coelopa sp.	0.079	0.148	<0.001		
C.pilipes	<0.001	<0.001	>0.999	<0.001	
C.frigida	<0.001	<0.001	0.475	0.003	0.673

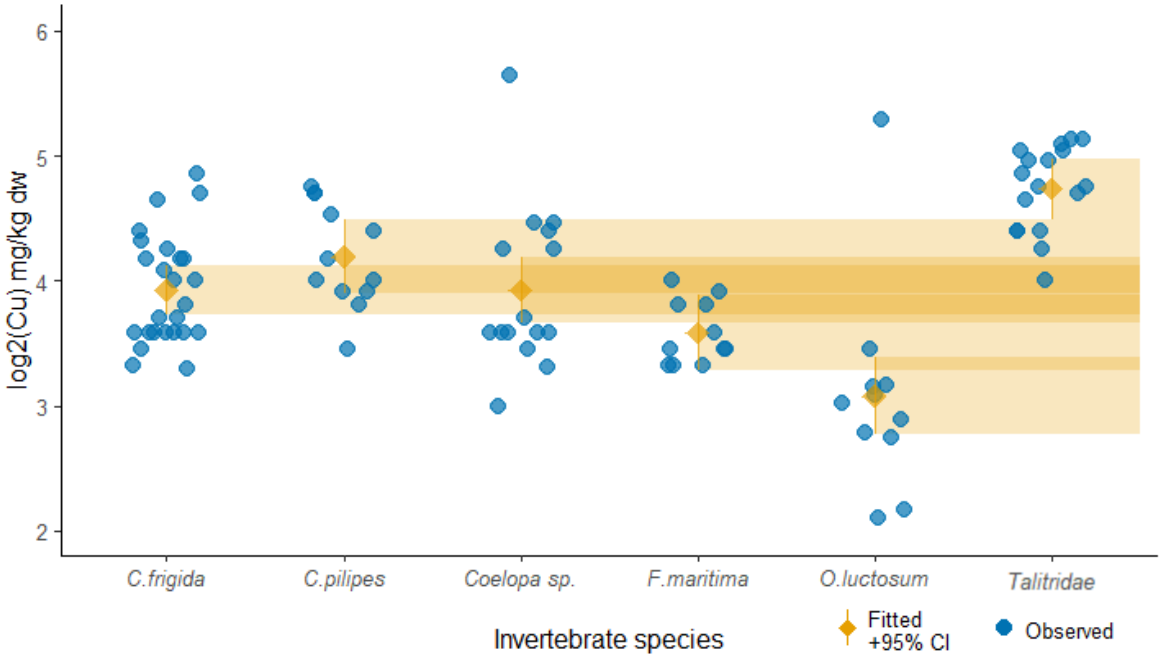


Figure 59. The estimated mean and 95%CI copper content of each species. Estimates modelled from observed data (blue circles). The 95%CIs have been projected right to allow easy pairwise comparison. A log_2 transformation was applied to the response variable Cu in order to normalise its distribution.

Table 17. P-values of each pairwise comparison of species' estimated copper content, visualised in Figure 59.

	Talitridae	*O.luctosum*	*F.maritima*	*Coelopa sp.*	*C.pilipes*
O.luctosum	<0.001				
F.maritima	<0.001	0.191			
Coelopa sp.	<0.001	<0.001	0.560		
C.pilipes	0.065	<0.001	0.059	0.744	
C.frigida	<0.001	<0.001	0.447	>0.999	0.667

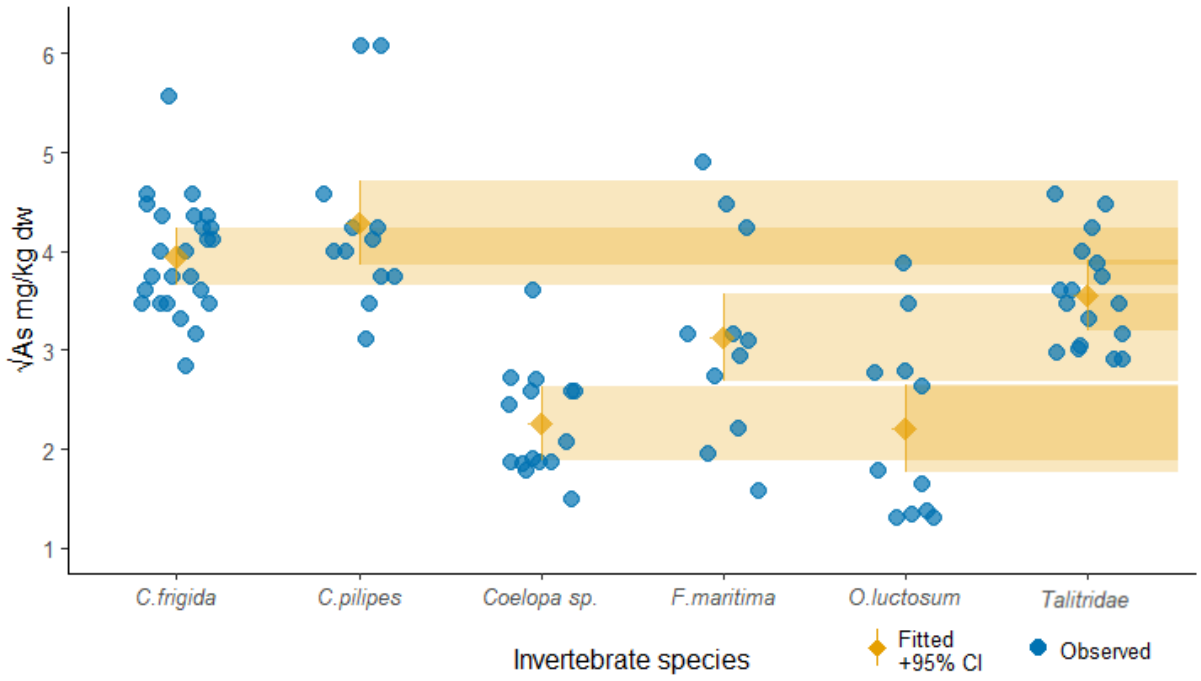


Figure 60. The estimated mean and 95%CI arsenic content of each species. Estimates modelled from observed data (blue circles). The 95%CIs have been projected right to allow easy pairwise comparison. A square root transformation was applied to the response variable As in order to normalise its distribution.

Table 18. P-values of each pairwise comparison of species' estimated arsenic content, visualised in Figure 60.

	Talitridae	*O.luctosum*	*F.maritima*	*Coelopa sp.*	*C.pilipes*
O.luctosum	<0.001				
F.maritima	0.686	0.046			
Coelopa sp.	<0.001	>0.999	0.043		
C.pilipes	0.096	<0.001	0.004	<0.001	
C.frigida	0.529	<0.001	0.034	<0.001	0.776

Species did not significantly affect the Pb concentrations, though substrate did. The effect of each substrate on Pb concentration is visualised in Figure 61 and the p-values of the associated pairwise comparisons between substrates are reported in Table 20. Assessing the species substrate interaction with *C.frigida*, *C.pilipes* and *F.maritima,* Fe (Figure 62), Cd (Figure 63) and Hg (Figure 64) had a significant interaction term.

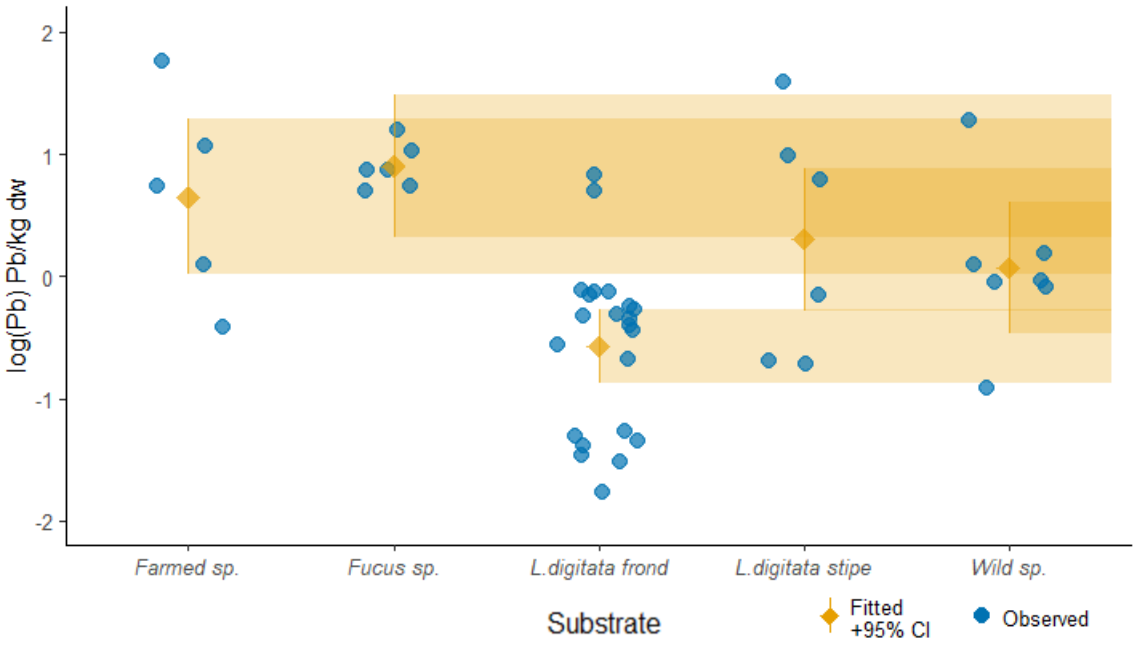


Figure 61. The estimated mean and 95%CI lead content of pooled larvae from C.frigida, C.pilipes and F.maritima reared on each substrate. Estimates modelled from observed data (blue circles). The 95%CIs have been projected right to allow easy pairwise comparison. A log transformation was applied to the response variable Pb in order to normalise its distribution. All the substrates comprised of farmed algae have been grouped in the substrate Farmed sp.

Table 19. P-values of pairwise comparisons of the lead content in larvae reared on each substrate, visualised in Figure 61.

	Wild sp.	***L.digitata stipe***	***L.digitata frond***	***Fucus sp.***
L.digitata stipe	0.975			
L.digitata frond	0.236	0.070		
Fucus sp.	0.228	0.585	<0.001	
Farmed sp.	0.626	0.925	0.009	0.975

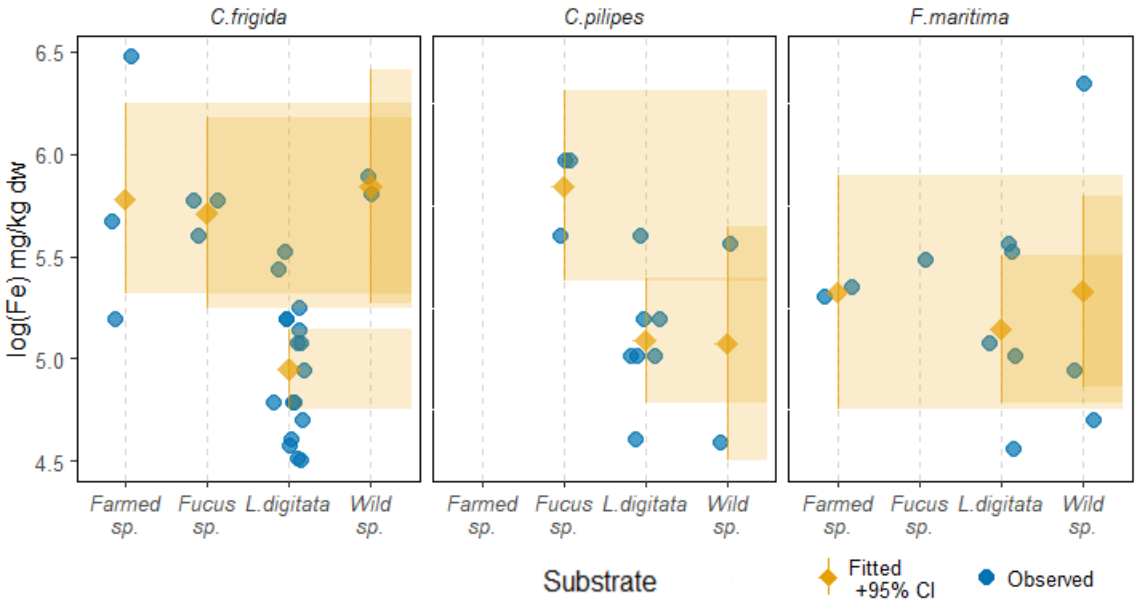


Figure 62. The estimated mean and 95%CI iron content of larvae reared on each substrate per species. Estimates modelled from observed data (blue circles). The 95%CIs have been projected right to allow easy pairwise comparison. A log transformation was applied to the response variable Fe in order to normalise its distribution. All the substrates comprised of farmed algae have been grouped in the substrate Farmed sp and the frond and stipe of L.digitata have also been grouped into species as no difference between them was modelled.

Table 20. P-values of pairwise comparisons of the iron content in larvae of reared on each substrate per species, visualised in Figure 62.

C.frigida	*Wild sp.*	*L.digitata*	*Fucus sp.*
L.digitata	0.023		
Fucus sp.	0.984	0.020	
Farmed sp.	0.998	0.010	0.997
C.pilipes			
L.digitata	>0.999		
Fucus sp.	0.165	0.044	
F.maritima			
L.digitata	0.920		
Farmed sp.	>0.999	0.950	

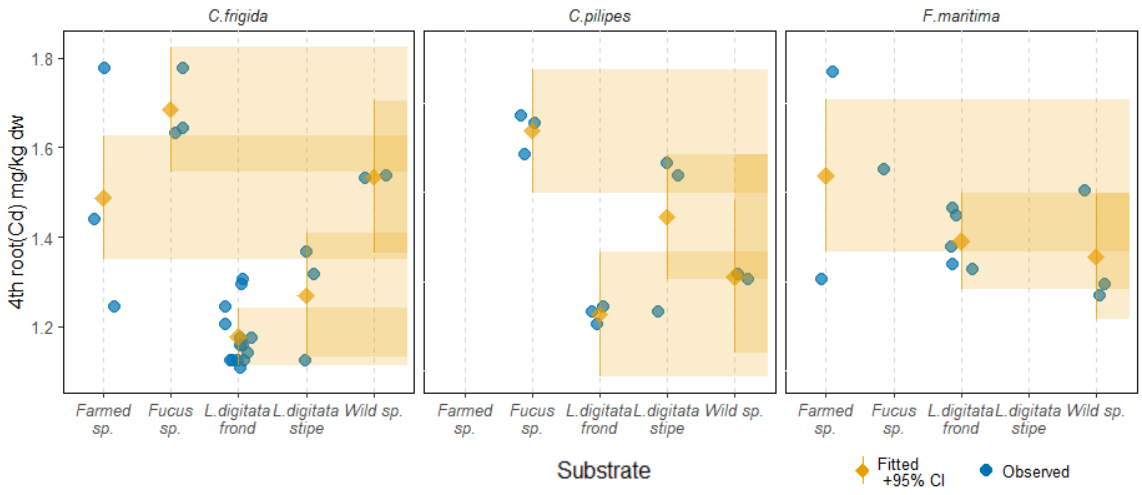


Figure 63. The estimated mean and 95%CI cadmium content of larvae reared on each substrate per species. Estimates modelled from observed data (blue circles). The 95%CIs have been projected right to allow easy pairwise comparison. A 4th root transformation was applied to the response variable Cd in order to normalise its distribution. All the substrates comprised of farmed algae have been grouped in the substrate Farmed sp.

Table 21. P-values of pairwise comparisons of the cadmium content in larvae reared on each substrate per species, visualised in Figure 63.

C.frigida	*Wild sp.*	*L.digitata stipe*	*L.digitata frond*	*Fucus sp.*
L.digitata stipe	0.124			
L.digitata frond	0.003	0.724		
Fucus sp.	0.639	0.001	<0.001	
Farmed sp.	0.992	0.183	0.002	0.269

C.pilipes	*Wild sp.*	*L.digitata stipe*	*L.digitata frond*
L.digitata stipe	0.725		
L.digitata frond	0.935	0.182	
Fucus sp.	0.036	0.294	0.001

F.maritima	*Wild sp.*	*L.digitata frond*
L.digitata frond	0.994	
Farmed sp.	0.457	0.583

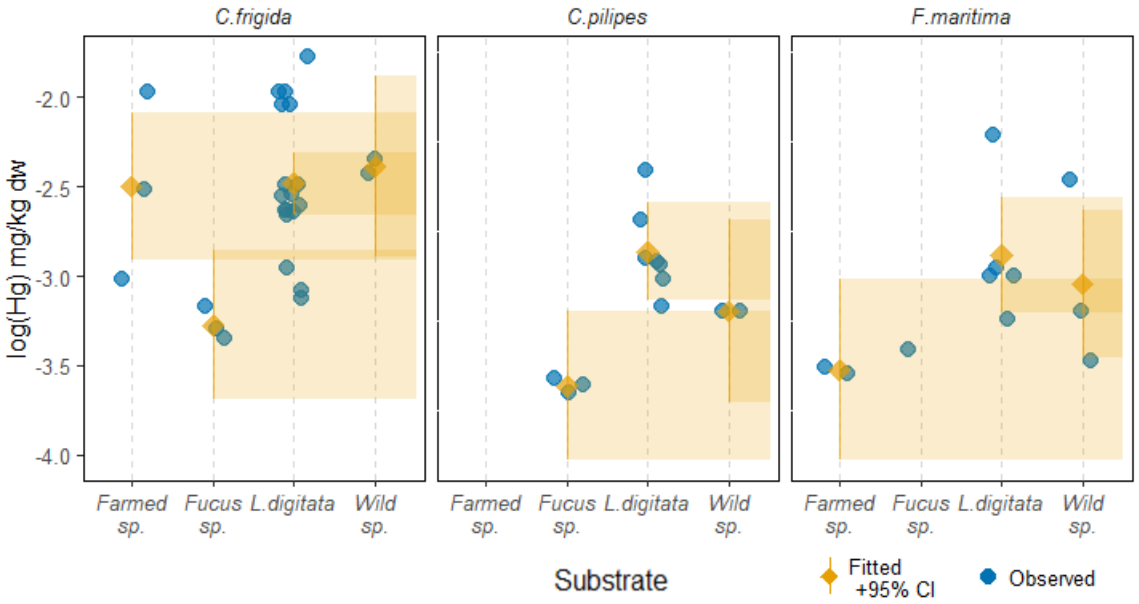


Figure 64. The estimated mean and 95%CI mercury content of larvae reared on each substrate per species. Estimates modelled from observed data (blue circles). The 95%CIs have been projected right to allow easy pairwise comparison. A log transformation was applied to the response variable Hg in order to normalise its distribution. All the substrates comprised of farmed algae have been grouped in the substrate Farmed sp and the frond and stipe of L.digitata have also been grouped back into species as no difference between them was modelled.

Table 22. P-values of pairwise comparisons of the mercury content in larvae reared on each substrate per species, visualised in Figure 64.

C.frigida	*Wild sp.*	*L.digitata*	*Fucus sp.*
L.digitata	0.984		
Fucus sp.	0.045	0.005	
Farmed sp.	0.986	>0.999	0.051
C.pilipes			
L.digitata	0.651		
Fucus sp.	0.573	0.020	
F.maritima			
L.digitata	0.920		
Farmed sp.	0.459	0.150	

Treatment

Lastly, in the larvae of *C.frigida* reared on *L.digitata* frond, following an independent samples t-test using the Welch's t-test method, the processing treatment was estimated to have a significant effect on the concentrations of manganese (Mn), phosphorus (P), magnesium (Mg) and Hg (Figure 65). As well as Fe, Pb and Cu (Figure 66). These effects and associated p-values are presented in their respective figures.

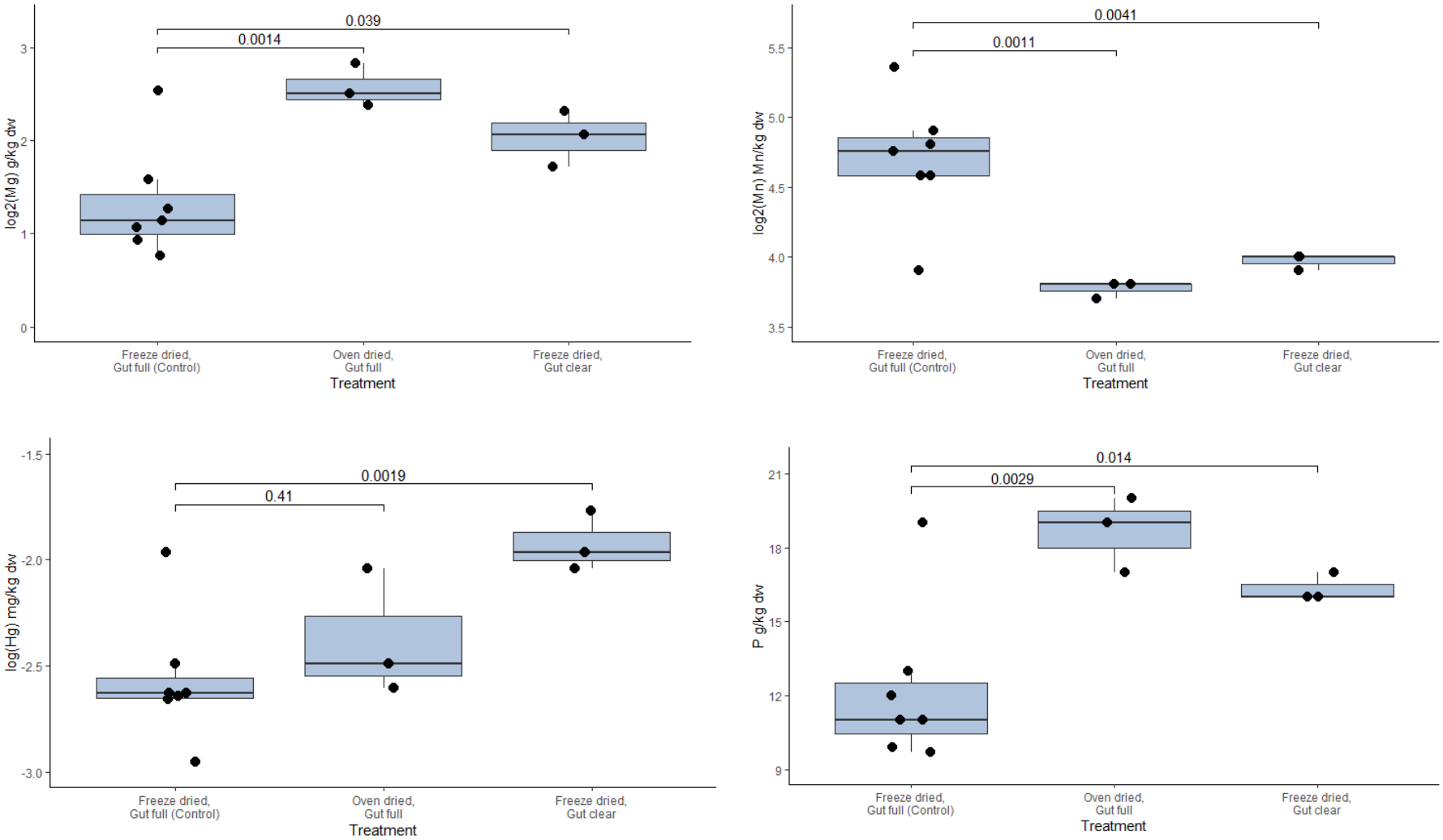


Figure 65. The observed differences in the concentration of Mg, Mn, Hg and P in the larvae of C.frigida reared on L.digitata frond per processing treatment. Each treatment was compared to those freeze dried with their guts full in an independent t-test (Welch's method). The corresponding p-values are presented on the tipped bars joining the control and treatment. Note some response variables have been transformed in order to normalise them.

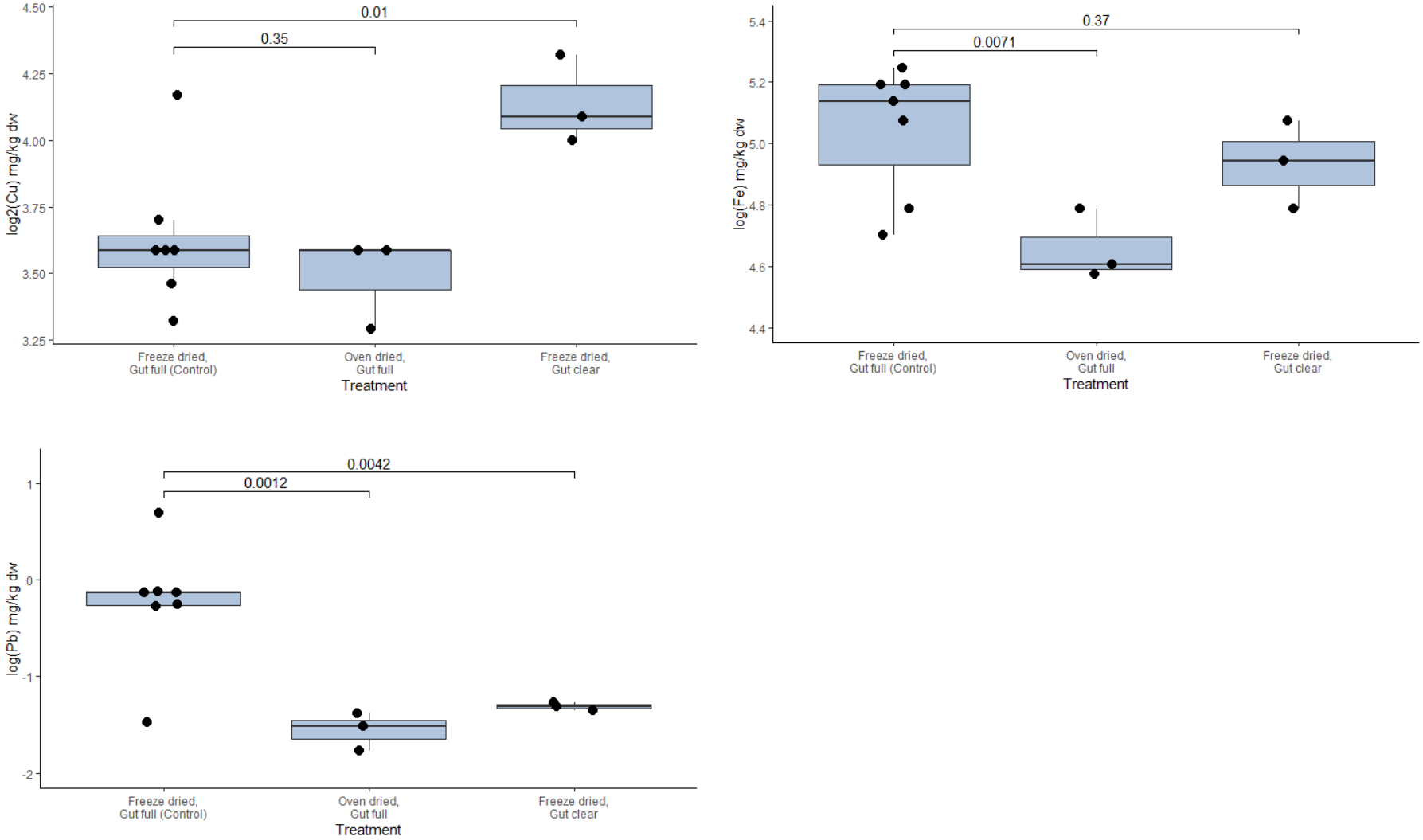


Figure 66. The observed differences in the concentration of Cu, Fe, Pb and As in the larvae of C.frigida reared on L.digitata frond per processing treatment. Each treatment was compared to those freeze dried with their guts full in an independent t-test (Welch's method). The corresponding p-values are presented on the tipped bars joining the control and treatment. Note some response variables have been transformed in order to normalise them.

Table 23. The mean observed concentration of each mineral in each invertebrate species (+/- 1 SD where n>1).

Species	Ca g/kg	Na g/kg	K g/kg	Mg g/kg	P g/kg	V mg/kg	Cr mg/kg	Mn mg/kg	Fe mg/kg	Co mg/kg
C.frigida	30.16 ± 7.7	9.94 ± 1.03	16.68 ± 1.65	3.92 ± 1.91	15.14 ± 3.49	0.50 ± 0.49	2.43 ± 2.31	61.64 ± 70.68	209.12 ± 124	0.46 ± 0.7
C.pilipes	49.58 ± 13.96	9.97 ± 1.83	15.33 ± 2.35	3.09 ± 0.54	13.86 ± 1.93	0.58 ± 0.35	3.55 ± 2.77	94.42 ± 79.18	215.67 ± 100.62	0.54 ± 0.55
Coelopa sp.	26.07 ± 12.54	9.40 ± 2.43	11.81 ± 2.07	2.75 ± 0.63	11.87 ± 1.06	0.60 ± 0.27	0.48 ± 0.34	27.07 ± 14.14	181.33 ± 52.76	0.18 ± 0.23
F.maritima	11.80 ± 7.23	5.17 ± 1.53	10.69 ± 3.6	2.18 ± 1.3	9.05 ± 3.27	0.53 ± 0.57	1.56 ± 2.26	62.18 ± 54.28	216.82 ± 129.77	1.03 ± 1.49
O.luctosum	116.73 ± 34.17	5.86 ± 2.82	8.73 ± 2.99	1.48 ± 0.64	7.72 ± 2.72	0.67 ± 0.6	0.45 ± 0.33	139.45 ± 62.6	176.18 ± 108.07	0.80 ± 0.64
Talitridae	63.12 ± 6.38	12.62 ± 3.12	6.42 ± 1.56	2.83 ± 0.39	12.71 ± 1.72	1.14 ± 0.54	0.83 ± 0.54	56.88 ± 49.34	315.88 ± 169.45	2.28 ± 0.99
Gammerus sp.	59.00 (n=1)	9.40 (n=1)	7.30 (n=1)	2.10 (n=1)	12.00 (n=1)	0.38 (n=1)	0.23 (n=1)	25.00 (n=1)	100.00 (n=1)	1.00 (n=1)
L.oceanica	72.00 (n=1)	29.00 (n=1)	8.70 (n=1)	4.90 (n=1)	6.90 (n=1)	3.20 (n=1)	2.20 (n=1)	93.00 (n=1)	820.00 (n=1)	0.87 (n=1)
Nematode sp.	2.20 (n=1)	11.00 (n=1)	4.00 (n=1)	1.20 (n=1)	7.90 (n=1)	0.17 (n=1)	0.37 (n=1)	13.00 (n=1)	120.00 (n=1)	0.71 (n=1)
Daphnia	44.33 (n=1)	5.00 (n=1)	6.60 (n=1)	4.13 (n=1)	8.37 (n=1)	14.67 (n=1)	11.67 (n=1)	183.33 (n=1)	5666.67 (n=1)	2.37 (n=1)

	Ni mg/kg	Cu mg/kg	Zn mg/kg	As mg/kg	Se mg/kg	Mo mg/kg	Ag mg/kg	Cd mg/kg	Hg mg/kg	Pb mg/kg
C.frigida	0.82 ± 0.64	15.91 ± 5.19	91.68 ± 33.56	15.88 ± 4.77	0.18 ± 0.07	0.48 ± 0.14	1.03 ± 0.551	3.50 ± 2.6	0.084 ± 0.037	1.45 ± 1.47
C.pilipes	1.13 ± 0.88	19.00 ± 5.41	104.00 ± 43.71	19.14 ± 8.85	0.15 ± 0.03	0.52 ± 0.12	1.088 ± 0.903	4.23 ± 2.22	0.048 ± 0.018	1.48 ± 0.99
Coelopa sp.	0.49 ± 0.22	16.99 ± 10.25	73.53 ± 24.24	5.40 ± 2.74	0.22 ± 0.04	0.58 ± 0.08	0.977 ± 0.451	1.61 ± 0.62	0.041 ± 0.011	1.93 ± 3.13
F.maritima	0.92 ± 0.48	12.18 ± 2.18	133.18 ± 39.13	10.81 ± 6.94	0.21 ± 0.09	0.4 ± 0.12	0.913 ± 0.429	4.35 ± 2.08	0.05 ± 0.026	2.30 ± 4.3
O.luctosum	0.83 ± 0.46	10.39 ± 9.69	72.27 ± 27.55	5.67 ± 4.61	0.16 ± 0.08	0.28 ± 0.08	0.468 ± 0.171	0.93 ± 0.54	0.026 ± 0.015	1.30 ± 1.09
Talitridae	1.33 ± 0.29	27.29 ± 6.03	127.65 ± 10.33	12.88 ± 4	0.34 ± 0.07	0.26 ± 0.03	0.421 ± 0.172	0.80 ± 0.16	0.032 ± 0.007	0.41 ± 0.17
Gammerus sp.	0.62 (n=1)	44.00 (n=1)	140.00 (n=1)	11.00 (n=1)	0.34 (n=1)	0.43 (n=1)	0.68 (n=1)	0.32 (n=1)	0.038 (n=1)	0.23 (n=1)
L.oceanica	2.7 (n=1)	55.00 (n=1)	71.00 (n=1)	19.00 (n=1)	1.40 (n=1)	<0.5 (n=1)	1.5 (n=1)	0.64 (n=1)	0.11 (n=1)	2.10 (n=1)
Nematode sp.	<0.3 (n=1)	7.40 (n=1)	220.00 (n=1)	24.00 (n=1)	0.15 (n=1)	0.23 (n=1)	0.62 (n=1)	1.60 (n=1)	0.016 (n=1)	0.31 (n=1)
Daphnia	5.6 (n=1)	7.57 (n=1)	51.00 (n=1)	5.67 (n=1)	2.23 (n=1)	<1 (n=1)	<0.1 (n=1)	0.09 (n=1)	<0.05 (n=1)	4.80 (n=1)

Table 24. The observed concentration of each mineral in each substrate type (n=1).

	g/kg					mg/kg													
Species	**Ca**	**Na**	**K**	**Mg**	**P**	**V**	**Cr**	**Mn**	**Fe**	**Co**	**Ni**	**Cu**	**Zn**	**As**	**Se**	**Mo**	**Ag**	**Cd**	**Hg**	**Pb**
A.esculenta	34.0	13.5	28	7.6	2.7	0.52	2.8	7.6	180	0.060	0.52	3.0	49	33	0.15	0.15	0.074	1.20	0.015	0.40
Fucus sp.	28.5	17.0	17	7.2	1.1	2.20	4.9	160.0	630	1.300	3.3	2.8	27	22	0.14	0.28	0.078	0.53	0.019	1.10
L.digitata farmed	32.0	14.0	29	7.3	1.9	0.66	1.4	5.5	110	0.170	0.52	3.6	53	48	0.062	0.14	0.280	0.26	0.018	0.19
L.digitata frond	25.0	20.0	26	6.6	3.1	2.70	13.0	26.0	800	0.600	1.8	4.4	34	61	0.23	1	0.240	0.34	0.063	0.88
L.digitata stipe	27.0	25.0	96	5.6	2.2	0.99	6.1	33.0	220	0.360	<3	2.9	24	63	<0.1	<1	0.280	0.39	<0.05	0.46
S.latissima	28.0	13.0	36	6.0	2.9	2.00	1.8	7.3	130	0.083	0.38	2.1	42	50	0.15	0.24	0.013	0.53	0.045	0.50
Wild sp.	24.0	31.0	30	6.9	2.4	4.40	11.0	130.0	800	1.200	3.8	8.5	36	40	0.25	0.86	0.190	0.55	0.032	1.40
Wrack.frass	31.0	24.0	20	6.9	2.7	11.00	37.0	150.0	3600	1.900	7	31.0	60	31	0.65	1.2	0.250	0.39	0.038	5.00

Amino acid composition

The composition (mg/g dw) of essential (Figure 67) and non-essential (Figure 68) amino acids in each of the substrate types were observed to differ with *A.esculenta* generally having the highest content of all amino acids followed by *L.digitata* frond then *S.latissima.* However, these values were obtained from one sample only, so the likelihood of the observations being true cannot be estimated. The data are visualised in bar charts to facilitate pairwise comparison.

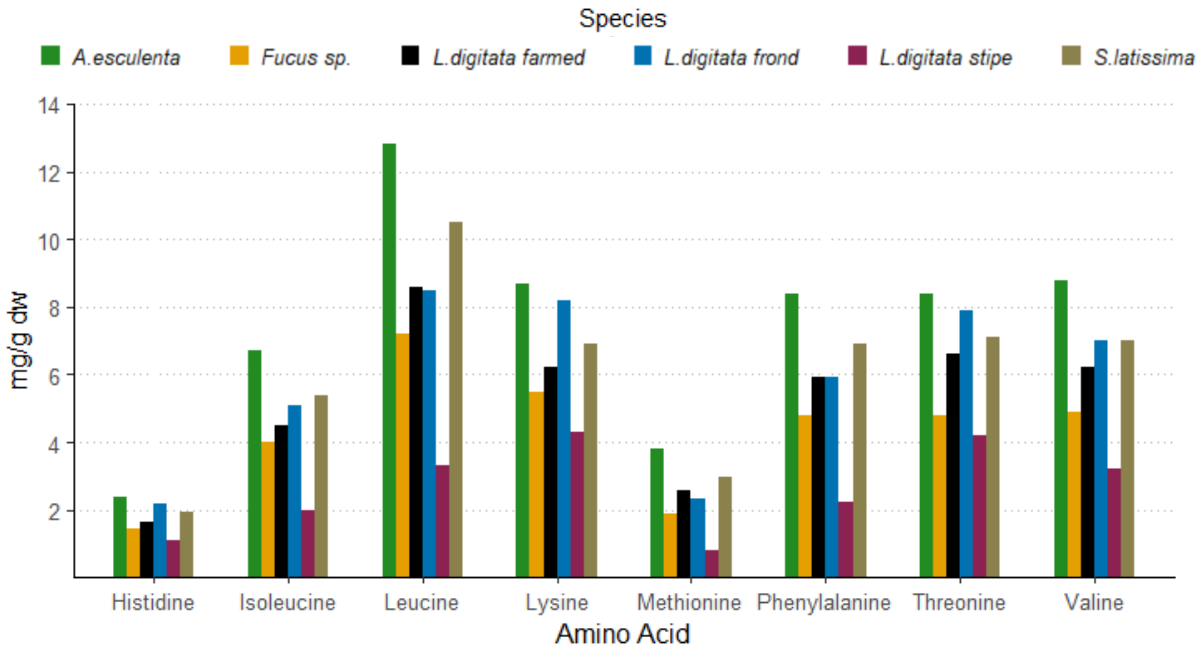


Figure 67. The observed content of essential amino acids in each of the substrates (n=1) in mg/kg dry weight.

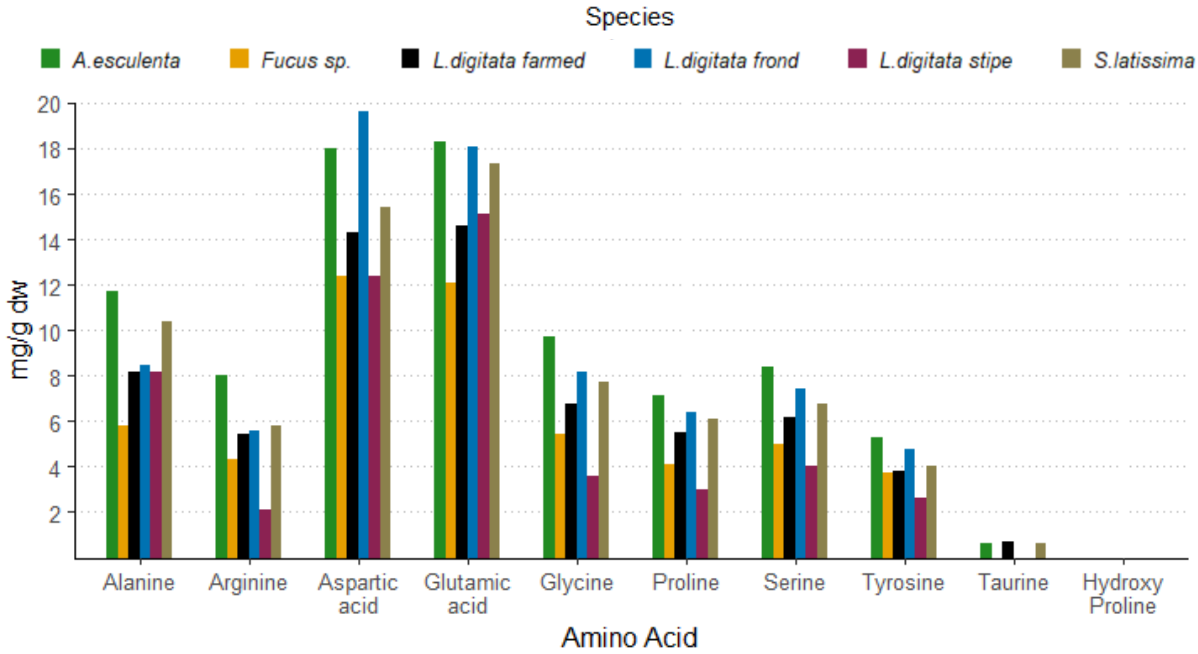


Figure 68. The observed content of non- essential amino acids in each of the substrates (n=1) in mg/kg dry weight.

The composition (mg/g dw) of essential (Figure 69) and non-essential (Figure 70) amino acids in each of the Diptera larvae were observed to only differ slightly between species. *C.frigida*, *C.pilipes* and *F.maritima* had similar levels of each amino acid while that of *O.luctuosum* was lower in all except taurine. Species was estimated to account for a significant amount of the variation in each essential amino acid but not in all non-essential. P-value tables of each pairwise comparison between species (including Talitridae) for essential and non-essential amino acids are presented in Table 26 and 27 respectively. The observed essential (Figure 71) and non-essential (Figure 72) amino acid content of Talitridae and the n=1 comparison samples are also presented.

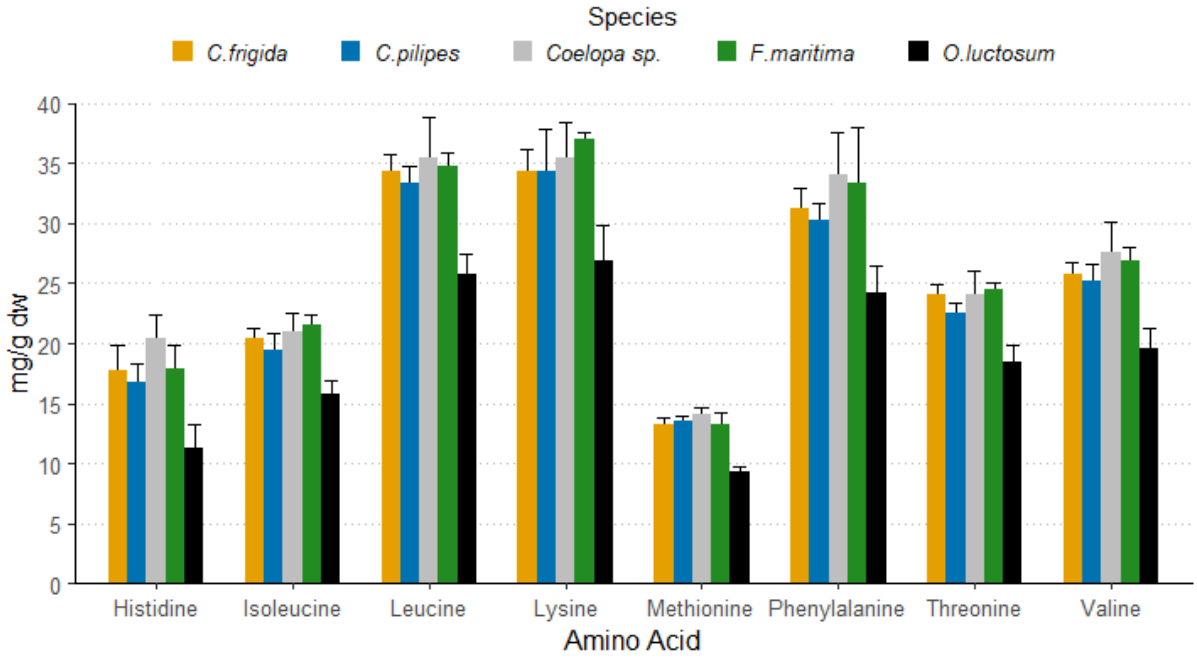


Figure 69. The observed content of essential amino acids in the larvae of each Diptera species in mg/kg dry weight. Error bars represent 1 SD.

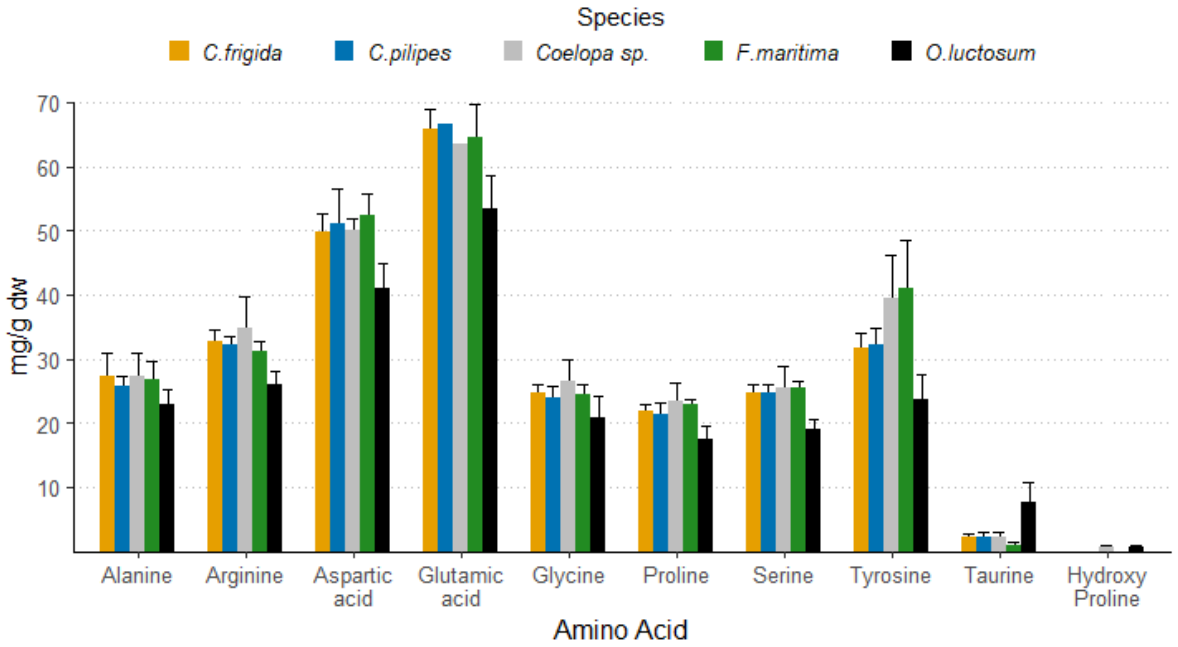


Figure 70. The observed content of non- essential amino acids in the larvae of each Diptera species in mg/kg dry weight. Error bars represent 1 SD.

Table 25. P-values quantifying the probability of observed differences in essential amino acid content of each species (n>1) occurring if no difference truly existed. Values in green denote those below the significance level of 0.05.

Pairwise Contrast	Histidine	Isoleucine	Leucine	Lysine	Methionine	Phenylalanine	Threonine	Valine
C.frigida* - *C.pilipes	0.979	0.999	>0.999	>0.999	>0.999	0.986	0.946	>0.999
C.frigida* - *Coelopa sp.	0.488	0.990	0.842	0.842	0.988	0.379	>0.999	0.678
C.frigida* - *F.maritima	>0.999	0.892	0.985	0.136	>0.999	0.524	>0.999	0.885
C.frigida* - *O.luctosum	<0.001	0.014	<0.001	<0.001	0.032	<0.001	<0.001	<0.001
***C.frigida* - Talitridae**	<0.001	<0.001	<0.001	<0.001	0.004	<0.001	<0.001	<0.001
C.pilipes* - *Coelopa sp.	0.224	0.944	0.767	0.980	>0.999	0.186	0.936	0.678
C.pilipes* - *F.maritima	0.976	0.776	0.950	0.488	>0.999	0.269	0.812	0.869
C.pilipes* - *O.luctosum	0.005	0.151	<0.001	<0.001	0.061	0.002	0.079	0.004
***C.pilipes* - Talitridae**	<0.001	0.006	<0.001	<0.001	0.009	<0.001	0.002	<0.001
Coelopa sp.* - *F.maritima	0.442	>0.999	0.991	0.885	0.986	0.998	>0.999	0.996
Coelopa sp.* - *O.luctosum	<0.001	0.003	<0.001	<0.001	0.008	<0.001	<0.001	<0.001
***Coelopa sp.* - Talitridae**	<0.001	<0.001	<0.001	<0.001	0.001	<0.001	<0.001	<0.001
F.maritima* - *O.luctosum	<0.001	<0.001	<0.001	<0.001	0.021	<0.001	<0.001	<0.001
***F.maritima* - Talitridae**	<0.001	<0.001	<0.001	<0.001	0.003	<0.001	<0.001	<0.001
***O.luctosum* - Talitridae**	0.026	0.546	0.744	<0.001	0.839	<0.001	0.524	0.439

Table 26. P-values quantifying the probability of observed differences in non-essential amino acid content of each species (n>1) occurring if no difference truly existed. Values in green denote those below the significance level of 0.05.

Pairwise Contrast	Alanine	Arginine	Aspartic	Glutamic	Glycine	Proline	Serine	Taurine	H.Proline
C.frigida* - *C.pilipes	>0.999	>0.999	0.974	0.989	>0.999	>0.999	>0.999	>0.999	>0.999
C.frigida* - *Coelopa sp.	0.976	0.940	>0.999	0.970	0.975	0.982	0.999	>0.999	>0.999
C.frigida* - *F.maritima	0.983	0.972	0.545	>0.999	>0.999	0.997	>0.999	0.973	>0.999
C.frigida* - *O.luctosum	0.706	0.008	<0.001	<0.001	0.346	0.258	0.043	>0.999	>0.999
***C.frigida* - Talitridae**	0.400	0.018	<0.001	<0.001	>0.999	0.922	0.048	0.679	>0.999
C.pilipes* - *Coelopa sp.	0.992	0.925	0.999	0.796	0.930	0.966	0.999	>0.999	>0.999
C.pilipes* - *F.maritima	0.996	0.997	0.986	0.934	>0.999	0.990	>0.999	0.958	>0.999
C.pilipes* - *O.luctosum	0.779	0.063	<0.001	<0.001	0.733	0.565	0.142	0.998	>0.999
***C.pilipes* - Talitridae**	0.483	0.073	<0.001	<0.001	>0.999	0.981	0.116	0.857	>0.999
Coelopa sp.* - *F.maritima	>0.999	0.545	0.838	0.997	0.949	>0.999	>0.999	0.938	>0.999
Coelopa sp.* - *O.luctosum	0.281	<0.001	<0.001	<0.001	0.089	0.068	0.023	0.997	>0.999
***Coelopa sp.* - Talitridae**	0.135	0.002	<0.001	<0.001	0.978	0.626	0.026	0.820	>0.999
F.maritima* - *O.luctosum	0.230	0.056	<0.001	<0.001	0.358	0.067	0.011	0.995	>0.999
***F.maritima* - Talitridae**	0.112	0.084	<0.001	<0.001	>0.999	0.711	0.017	0.251	>0.999
***O.luctosum* - Talitridae**	0.976	>0.999	0.015	0.996	0.575	0.960	0.997	0.498	>0.999

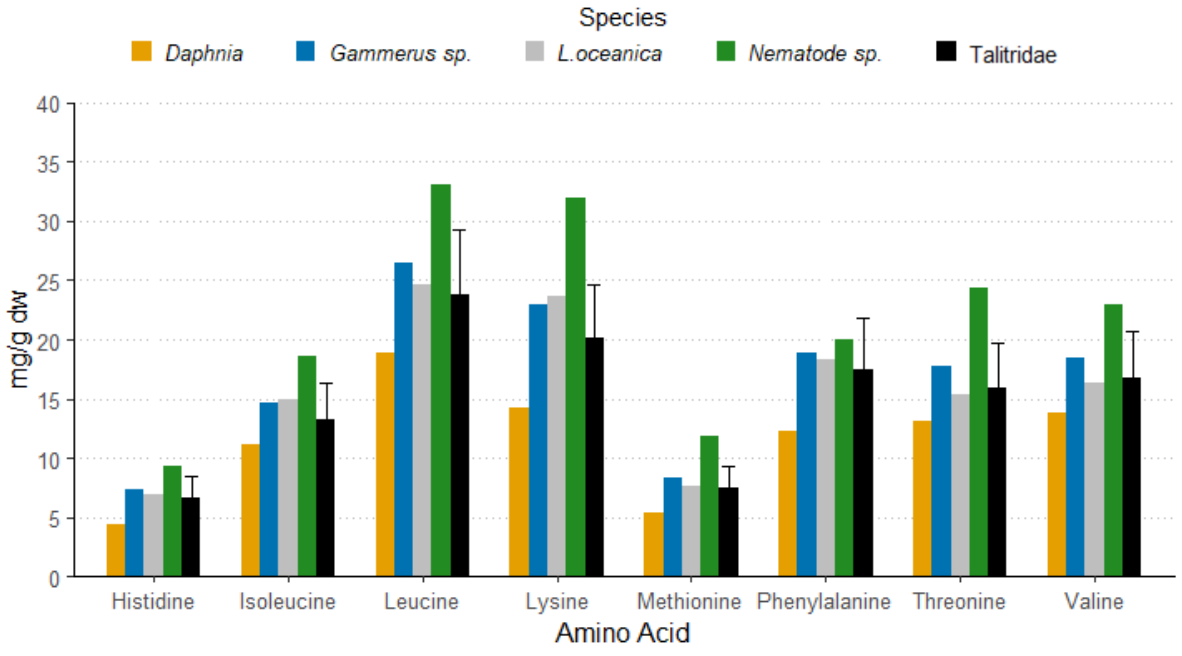


Figure 71. The observed content of essential amino acids in T.saltator and the four (n=1) reference species in mg/kg dry weight. Error bars represent 1 SD.

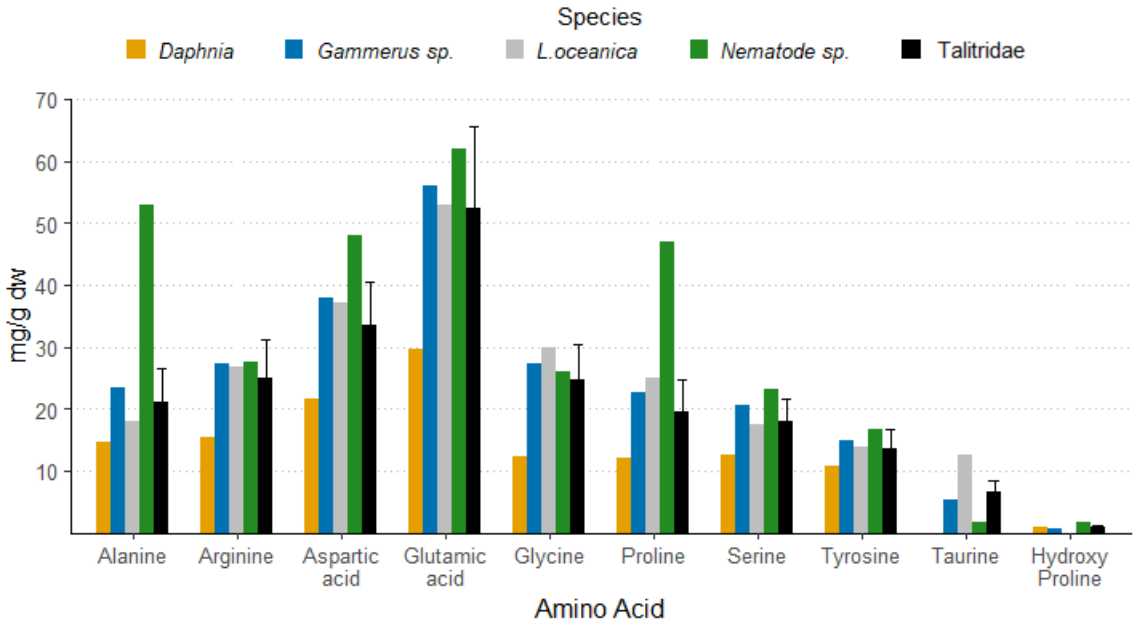


Figure 72. The observed content of non- essential amino acids in T.saltator and the four (n=1) reference species in mg/kg dry weight. Error bars represent 1 SD.

A significant amount of the variation in the content of amino acids in the larvae of *C.frigida*, *C.pilipes* and *F.maritima* was not explained by the substrate larvae were reared on. Similarly, the processing treatment of *C.frigida* larvae also had no significant effect. The observed mean concentration (+/- 1SD) of each amino acid in all species and substrate samples are available in Appendix 5.2.

Fatty Acid composition

A linear mixed-effects model was fitted to the data to estimate the effect of species, substrate, and their interaction on the content of each fatty acid. However, no effect of substrate was found (χ^2=2.64, df=6, p=0.852), so this was simplified to only the effect of species on FA. The model included a random intercept for each individual sample. The overall model was statistically significant (χ^2= 953.42, df = 136, $p < 0.001$). The main effect of species was significant (χ^2= 26.12, df = 4, $p < 0.001$), suggesting that different species had significantly different levels of fatty acid content. A lower AIC (Akaike Information Criterion) and BIC (Bayesian Information Criterion) value was obtained in the model with the random effect vs without. This evidences the necessity of the random effect to account for variability in the data not explained by the fixed effect(s). The random effect (sample ID) had an estimated variance of 1.534 and SD of 1.239 with a residual variance of 8.402 and SD of 2.899. These variance components suggest that the model is a good fit for the data.

The results of the model suggest that species was a useful predictor of fatty acid content. However, these results are specific to the data and conditions used in this study.

The observed mean concentration (+/- 1SD where appropriate) of each fatty acid in all species and substrate samples are available in Appendix 5.2. Here, the PUFA content in substrate types and fatty acids in species are compared before taking a closer look at any significant effects on the marine origin PUFAs specifically.

The composition (mg/g dw) of fatty acids in each of the substrate types was observed to differ with *Fucus* sp generally having the highest content. However, these values were obtained from one sample only, so the likelihood of the observations being true cannot be estimated. The observed content of PUFAs above the limit of detection (>LOQ) for each substrate are displayed in Figure 73.

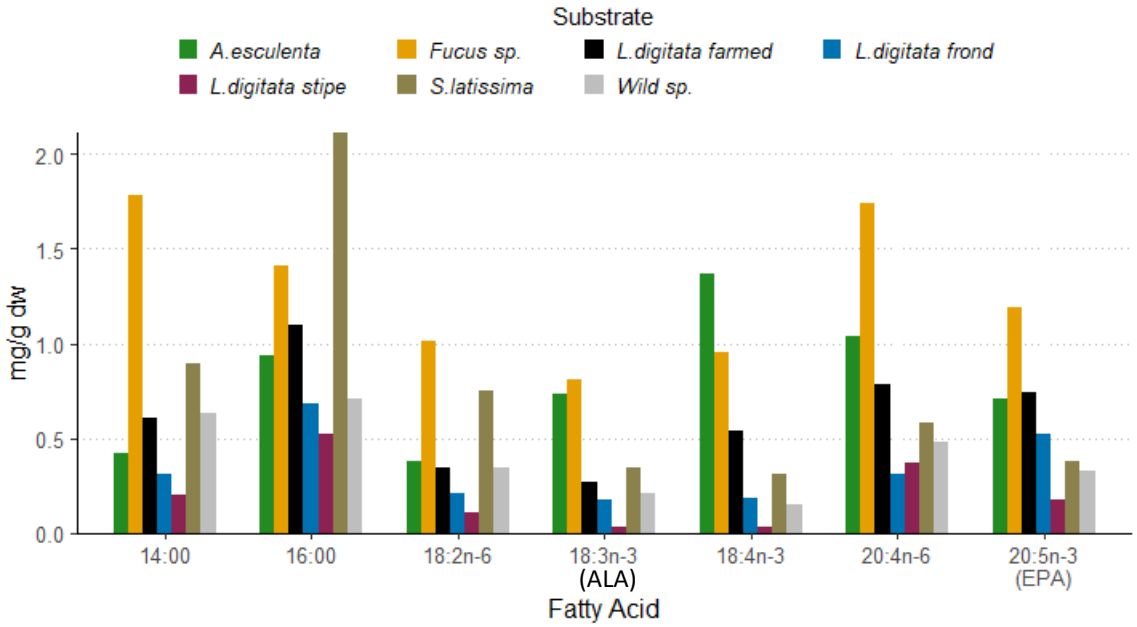


Figure 73. Observed content of poly-unsaturated fatty acids above the limit of detection in each substrate type.

There was a significant interaction between species and fatty acid type ($\chi^2 = 407.8634$, df = 35, $p < 0.001$), indicating that species influenced the composition of each fatty acid. The observed content of PUFAs >LOQ in each Diptera species are presented in Figure 74, and that of Talitridae and the comparison (n=1) samples in Figure 75, followed by a table of p-values of pairwise comparisons of all fatty acids for each species (including Talitridae) (Table 28). As can be seen by the relatively few significant p-values, while the model was significant, effect sizes were slight.

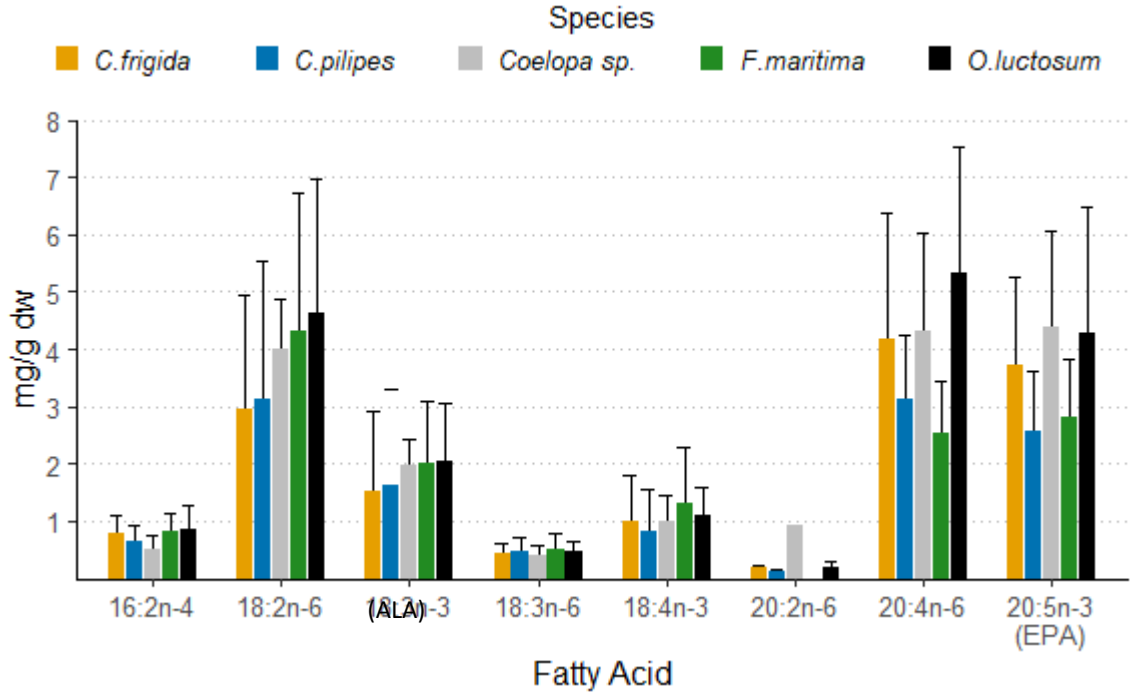


Figure 74. Observed content of poly-unsaturated fatty acids above the limit of detection in each Diptera species.

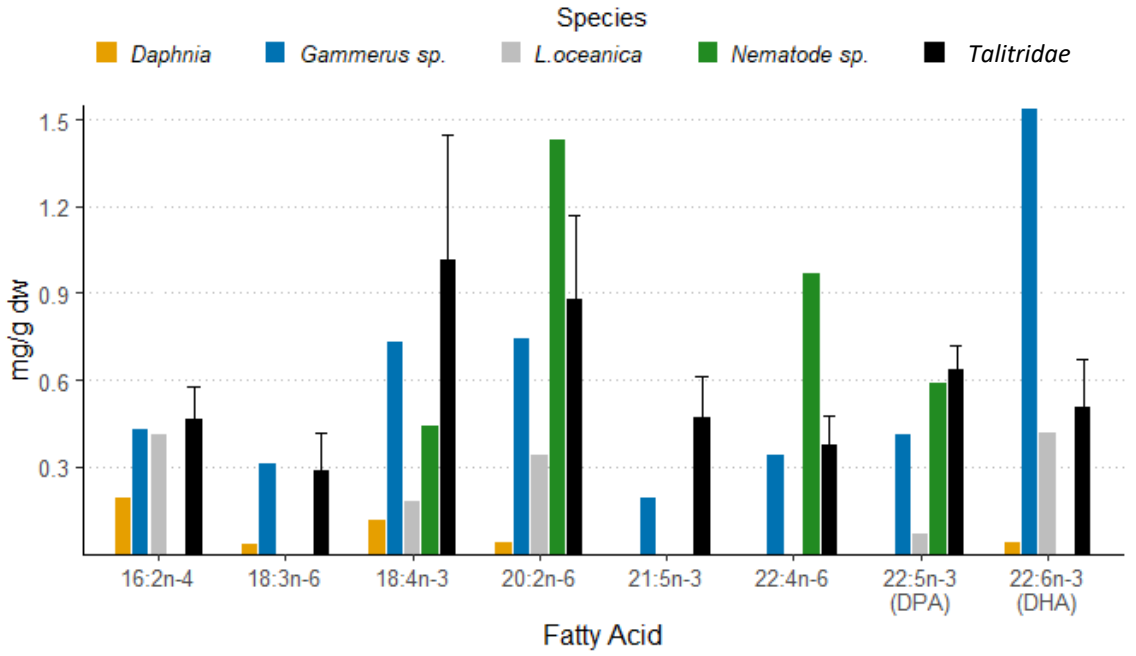


Figure 75. Observed content of poly-unsaturated fatty acids above the limit of detection in each T.saltator and the comparison (n=1) samples.

Table 27. P-values from each pairwise comparison of the fatty acid content between each invertebrate species. Values in green denote those below the significance level of 0.05. Part 1 of 2.

	Pairwise Contrast						
Fatty Acid	***C.frigida* - *C.pilipes***	***C.frigida* - *Coelopa sp.***	***C.frigida* - *F.maritima***	***C.frigida* - *O.luctosum***	***C.frigida* - Talitridae**	***C.pilipes* - *Coelopa sp.***	***C.pilipes* - *F.maritima***
12:00	>0.999	>0.999	>0.999	>0.999	<0.001	>0.999	>0.999
14:00	0.966	>0.999	0.996	0.865	<0.001	0.949	0.874
14:1n-9	>0.999	>0.999	>0.999	>0.999	0.922	0.997	>0.999
15:00	>0.999	>0.999	>0.999	0.999	>0.999	>0.999	>0.999
16:00	0.115	0.978	<0.001	0.928	0.186	0.042	<0.001
16:1n-7	<0.001	0.233	<0.001	0.033	<0.001	<0.001	<0.001
16:1n-9	>0.999	>0.999	0.571	0.989	0.909	>0.999	0.688
16:2n-4	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999
17:00	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999
18:00	>0.999	0.997	0.993	>0.999	0.995	0.998	0.995
18:1n-7	0.742	0.995	0.978	0.998	0.898	0.535	0.995
18:1n-9	0.541	0.006	<0.001	0.010	<0.001	<0.001	<0.001
18:2n-6	>0.999	0.985	0.968	0.929	0.414	0.997	0.991
18:3n-3	>0.999	>0.999	>0.999	>0.999	0.996	>0.999	>0.999
18:3n-6	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999
18:4n-3	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999
20:4n-6	0.988	>0.999	0.928	0.986	0.003	0.988	>0.999
20:5n-3[1]	0.982	0.998	0.995	>0.999	0.995	0.920	>0.999

[1] EPA

Table 28. P-values from each pairwise comparison of the fatty acid content between each invertebrate species. Values in green denote those below the significance level of 0.05. Part 2 of 2.

	Pairwise Contrast							
Fatty Acid	*C.pilipes* - *O.luctosum*	*C.pilipes* - Talitridae	*Coelopa sp.* - *F.maritima*	Coelopa sp. - O.luctosum	Coelopa sp. - Talitridae	F.maritima - O.luctosum	F.maritima - Talitridae	O.luctosum - Talitridae
12:00	>0.999	<0.001	>0.999	>0.999	<0.001	>0.999	<0.001	<0.001
14:00	0.553	<0.001	>0.999	0.950	<0.001	0.995	<0.001	0.003
14:1n-9	0.998	0.869	0.999	>0.999	0.983	>0.999	0.908	0.989
15:00	0.999	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999	0.996
16:00	0.790	<0.001	<0.001	0.672	0.737	<0.001	<0.001	0.059
16:1n-7	0.063	<0.001	0.020	<0.001	<0.001	<0.001	<0.001	<0.001
16:1n-9	0.992	0.963	0.720	0.997	0.915	0.956	0.178	0.716
16:2n-4	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999
17:00	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999
18:00	>0.999	0.997	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999
18:1n-7	0.973	0.999	0.880	0.958	0.709	>0.999	>0.999	0.998
18:1n-9	<0.001	<0.001	0.681	>0.999	<0.001	0.828	<0.001	<0.001
18:2n-6	0.976	0.684	>0.999	>0.999	0.900	>0.999	0.971	0.989
18:3n-3	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999
18:3n-6	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999
18:4n-3	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999
20:4n-6	0.878	0.003	0.935	0.995	0.015	0.739	0.001	0.147
20:5n-3[1]	0.956	0.882	0.959	>0.999	>0.999	0.979	0.935	>0.999

[1] EPA

Regarding the marine origin PUFAs, only EPA was consistently >LOQ in all species and the estimated mean EPA (+95%CI) of each species is visualised in Figure 76 with the p-values from associated pairwise comparisons presented in Table 29, three of which were significant. As can be seen in Figure 76, the effect sizes of species on EPA were marginal. While a species substrate interaction was estimated to have no significant effect on fatty acid concentrations overall, a pairwise comparison of each species' estimated EPA on each substrate found significant differences between *C.frigida* larvae. These are visualised in Figure 77 and the associated p-values presented in Table 29. Figure 78 visualises the estimated EPA values of the species from the 'experiment data' along with the observed values from the 'comparison data' (n=1) and the six substrate types.

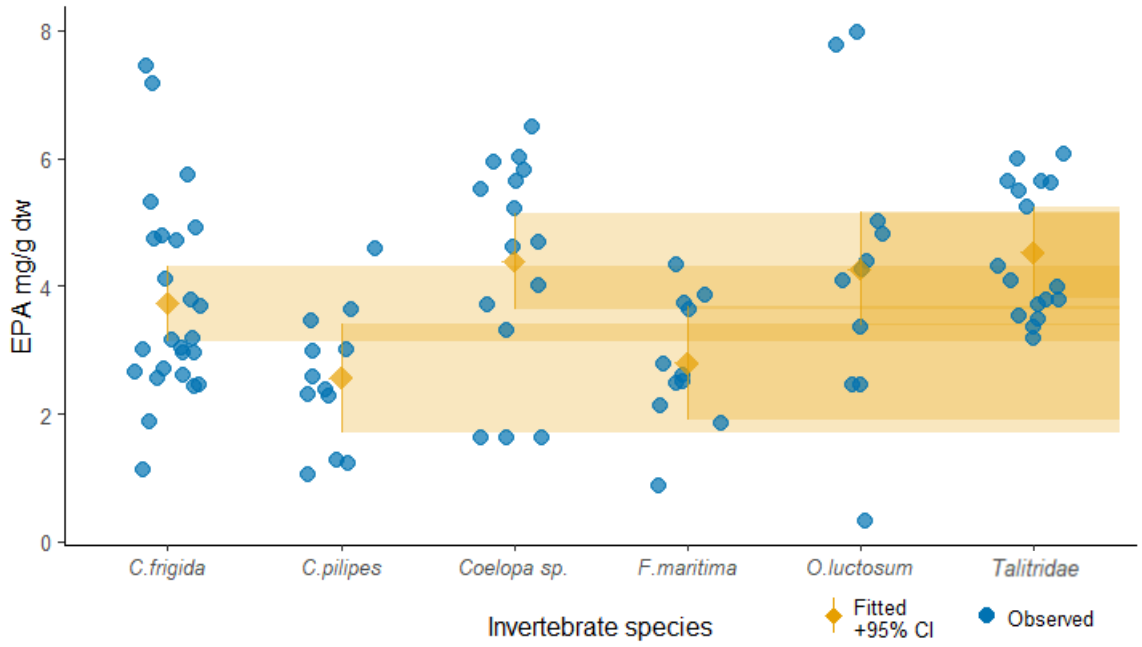


Figure 76. The estimated mean + 95%CI EPA content of each species. Estimates modelled from observed data (blue circles). The 95%CIs have been projected right to allow easy pairwise comparison.

Table 28. P-values from each pairwise comparison of EPA content between species, visualised in figure 75.

	Talitridae	*O.luctosum*	*F.maritima*	*Coelopa sp.*	*C.pilipes*
O.luctosum	0.998				
F.maritima	0.039	0.199			
Coelopa sp.	>0.999	>0.999	0.087		
C.pilipes	0.009	0.077	0.999	0.025	
C.frigida	0.523	0.913	0.522	0.746	0.239

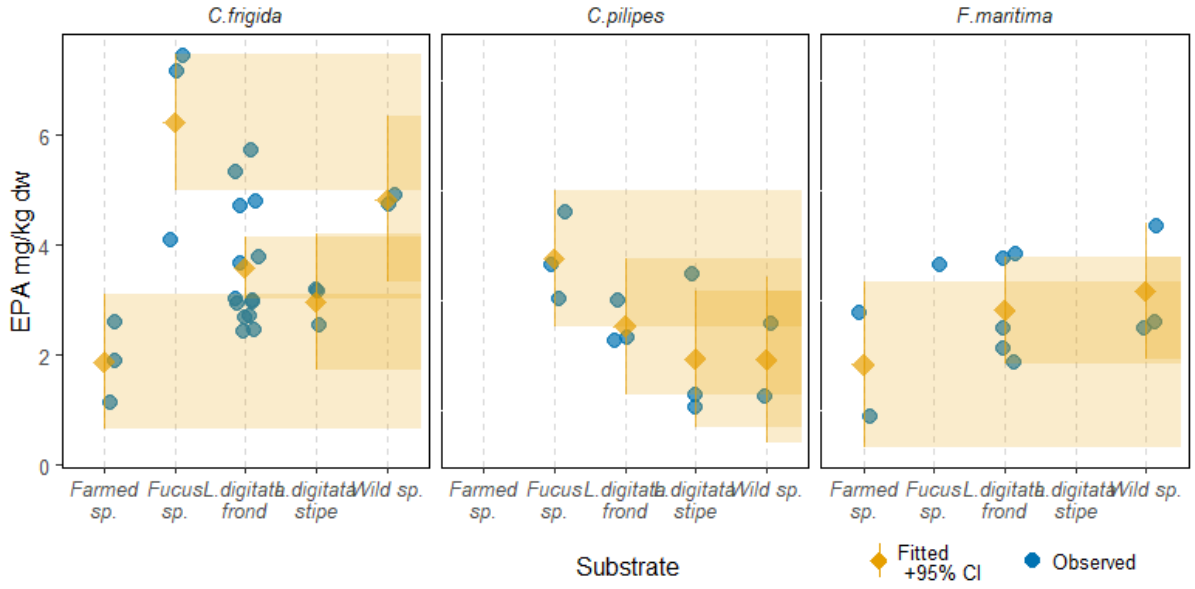


Figure 77. The estimated mean + 95%CI EPA content of larvae reared on each substrate per species. species. Estimates modelled from observed data (blue circles). The 95%CIs have been projected right to allow easy pairwise comparison. All the substrates comprised of farmed algae have been grouped in the substrate Farmed sp.

Table 29. The p-values of each pairwose comparison of the EPA content of C.frigida *larvae reared on each substarte.*

C.frigida	*Wild sp.*	*L.digitata stipe*	*L.digitata frond*	*Fucus sp.*
L.digitata stipe	0.316			
L.digitata frond	0.531	0.883		
Fucus sp.	0.595	0.005	0.003	
Farmed sp.	0.032	0.717	0.104	<0.001

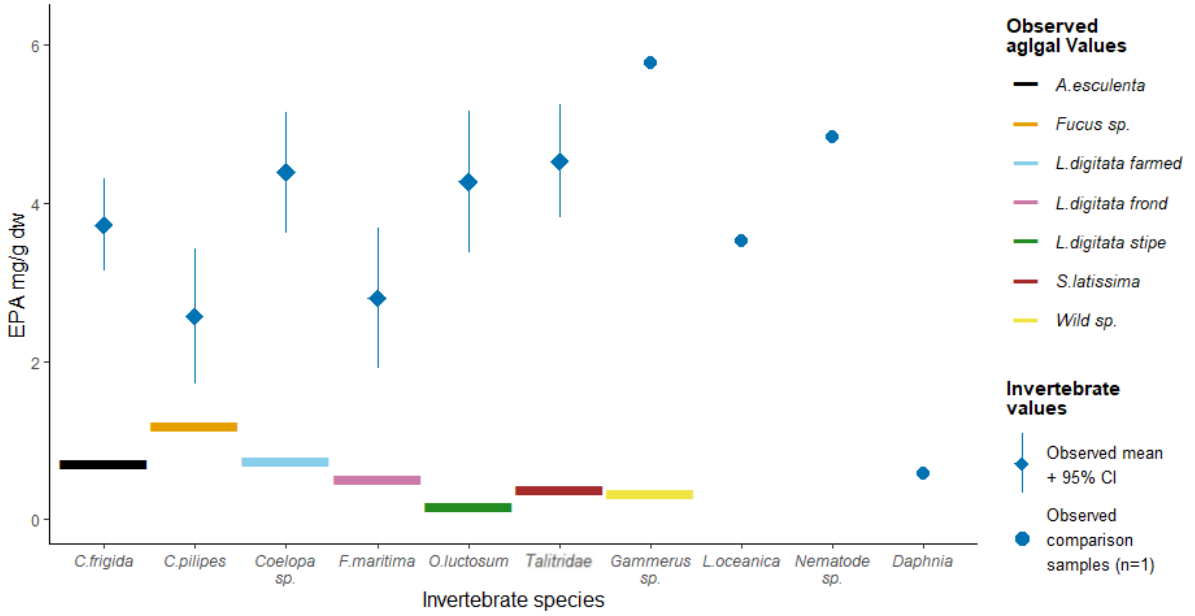


Figure 78. The EPA content in all 10 analysed invertebrate species as well as each of the substrates used.

The marine origin PUFAs: DPA and DHA were only present in wild harvested samples. Of the wild *Coelopa* samples (n=15) only one sample contained levels of DPA and DHA >LOQ. This was also the case for *O.luctuosum*. Conversely, Talitridae samples consistently had quantities >LOQ. The comparison samples of *Gammerus* sp and *L.oceanica* both had quantities >LOQ. The nematode sample had only DPA and the *Daphnia* sample only DHA >LOQ. The observed values are visualised in Figure 79.

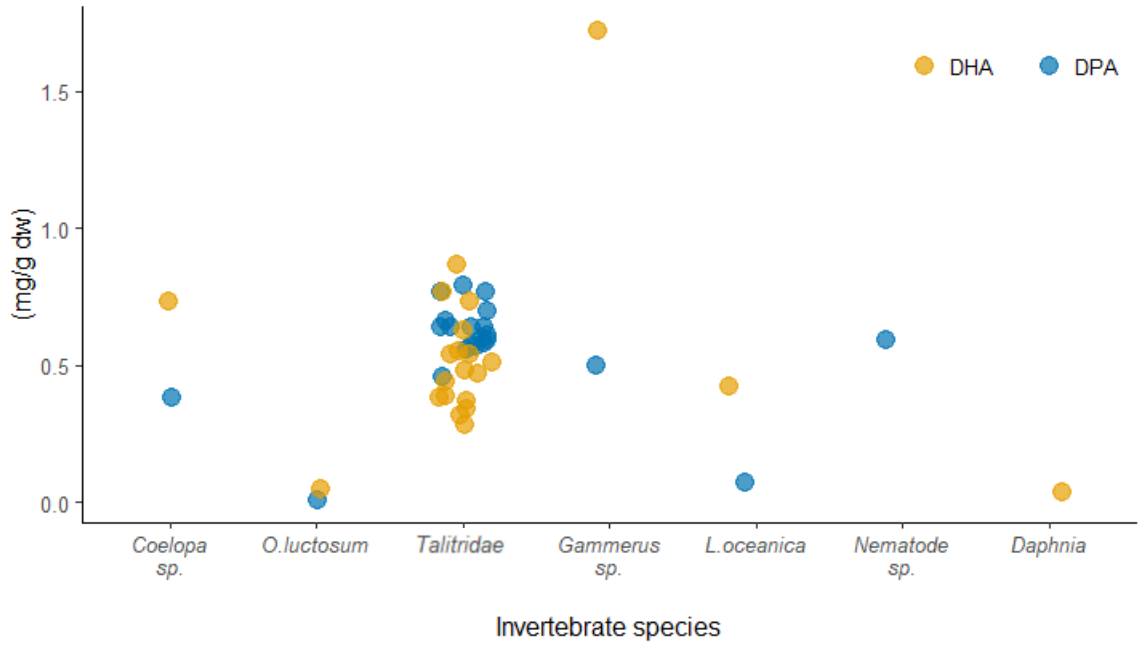


Figure 79. All observed values of DHA and DPA in each invertebrate sample.

Lastly, it was estimated that the 2nd generation of *C.frigida* larvae, reared on *L.digitata* frond, conditioned by a 1st generation, had a significantly higher EPA content than that of the 1st generation. This significance was found using a paired t-test visualised in Figure 80. This same significant effect was found in four other fatty acids. The t-values and p-values from a paired t-test of each fatty acid between 1st and 2nd generation larvae are presented in Table 30 (Left). The concentrations of each fatty acid in the frond of *L.digitata* pre and post rearing one generation of *C.frigida* larvae are presented in Table 30 (Right). In four out of the five fatty acids of which an increase was modelled, the content of said fatty acid decreased in the substrate.

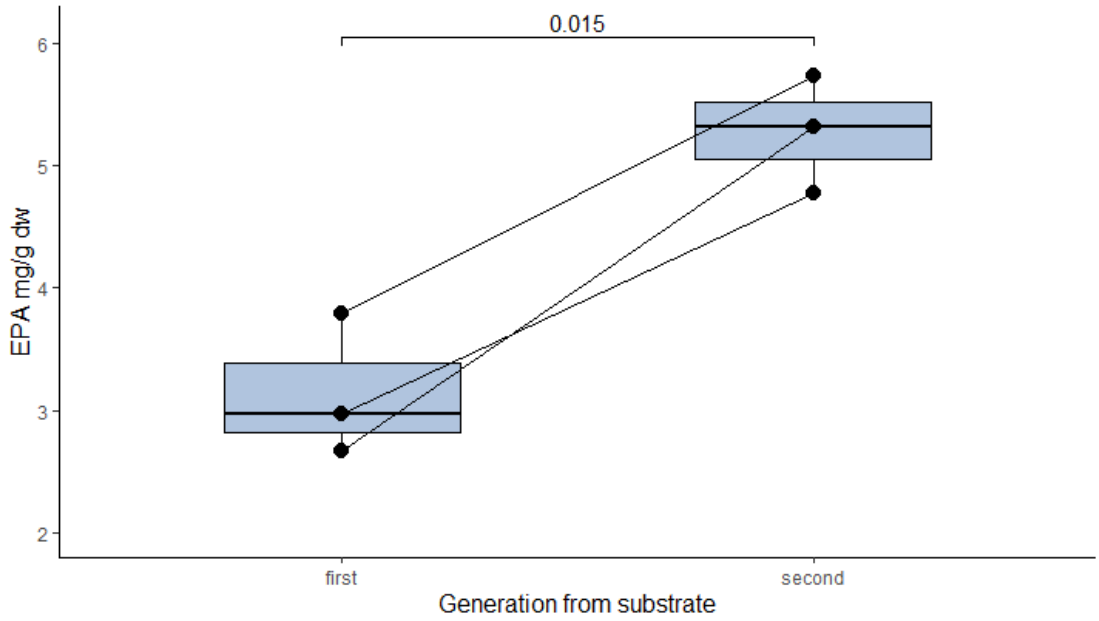


Figure 80. The observed differences in the concentration of EPA in the larvae of C.frigida reared on L.digitata frond per generation on substrate. Samples from the 1st and 2nd generation of three replicate cultures were compared using a paired t-test, the p-value of which is reported in the figure.

Table 30. Left. Results of paired t-tests comparing the fatty acid content of the 1st generation of C.frigida larvae to that of the 2nd generation reared afterwards in the same substrate. Direction of effect is therefore from 1st generation to 2nd generation. Right.Observed differences in the substrate L.digitata frond pre and post rearing one generation of C.frigida larvae.

Fatty Acid	t-value	p-value
12:00	0.47	0.659
14:00	-2	0.092
14:1n-9	-1.24	0.171
15:00	-3.46	0.037
16:00	0.82	0.750
16:1n-7	0.98	0.784
16:1n-9	0.11	0.539
16:2n-4	-2.56	0.062
17:00	-2.3	0.074
18:00	1.23	0.828
18:1n-11	-0.67	0.287
18:1n-7	-1.83	0.104
18:1n-9	1.45	0.858
18:2n-6	-2	0.092
18:3n-3	-3.26	0.041
18:3n-6	-0.77	0.261
18:4n-3	-5.17	0.018
20:1n-9	-1.99	0.092
20:4n-6	-6.7	0.011
20:5n-3 (EPA)	-17.94	0.002

	***L.digitata* frond**		
Fatty Acid	**pre rearing**	**post rearing**	**Change**
14:00	0.31	0.54	74%
15:00	0.01	0.06	533%
16:00	0.68	1.48	118%
16:1n-7	0.27	1.91	606%
18:00	0.05	0.15	207%
18:1n-7	0.03	0.52	1,633%
18:1n-9	0.73	1.15	58%
18:2n-6	0.21	0.23	11%
18:3n-3	0.17	0.15	−14%
18:3n-6	0.02	0.02	0%
18:4n-3	0.18	0.13	−30%
20:1n-9	0.01	0.03	167%
20:2n-6	0.01	0.02	100%
20:3n-6	0.01	0.02	67%
20:4n-3	0.02	0.02	−17%
20:4n-6	0.31	0.26	−15%
20:5n-3	0.52	0.44	−16%
22:5n-3 (EPA)	0.03	0.03	−11%

Finally, a summary table (Table 31) of the amino acid and fatty acid contents in each invertebrate species is provided.

Table 31. Summary of the observed amino acid and fatty acid content of each invertebrate species. Values are given as g/100g dry weight +/- 1SD where n>1. True protein represents the sum of dried amino acids.

	C.frigida	*C.pilipes*	*Coelopa sp.*	*F.maritima*	*O.luctosum*	Talitridae	*Gam*[a]	*L.oc*[b]	*Nem*[c]	*Daph*[c]
True Protein	47.8 +/- 1.2	47.6 +/- 1.9	50.5 +/- 2.9	49.8 +/- 1.2	37.7 +/- 2.2	33.5 +/- 6.5	37.1	36.2	47.9	22.3
Total FA[1]	10.53 +/- 2.44	8.42 +/- 3.16	12.34 +/- 5.51	14.38 +/- 2.93	11.61 +/- 4.1	15.65 +/- 5.58	20.3	3.79	7.3	2.18
Total SFA	2.97 +/- 0.73	2.41 +/- 0.98	3.25 +/- 1.41	4.68 +/- 1.13	3.15 +/- 1.12	5.41 +/- 2.2	5.75	0.98	2.5	0.98
Total MUFA	5.94 +/- 2.01	4.76 +/- 2.17	7.43 +/- 3.83	8.27 +/- 1.74	6.56 +/- 2.32	7.54 +/- 2.71	10.84	1.48	3.1	0.91
Total PUFA	1.61 +/- 0.83	1.24 +/- 0.68	1.66 +/- 0.47	1.42 +/- 0.54	1.89 +/- 0.8	2.69 +/- 0.89	3.7	1.32	1.7	0.28
Total n-3	0.66 +/- 0.42	0.5 +/- 0.33	0.75 +/- 0.25	0.61 +/- 0.27	0.75 +/- 0.34	0.92 +/- 0.27	1.28	0.64	0.78	0.18
Total n-6	0.89 +/- 0.44	0.68 +/- 0.36	0.89 +/- 0.28	0.74 +/- 0.31	1.07 +/- 0.47	1.75 +/- 0.61	2.33	0.64	0.89	0.07
Total un-id[2]	1.31 +/- 0.26	1.04 +/- 0.5	1.32 +/- 0.56	1.54 +/- 0.57	1.99 +/- 0.84	0.4 +/- 0.18	1.06	0.23	3.75	0.43

[a] *Gammerus sp.*
[b] *L.oceanica*
[c] *Nematode sp.*
[d] *Daphnia sp.*
[1] Total identified fatty acids
[2] Total un-identified fatty acids
All values in g/100g

5.4 Discussion

The results on the EPA and DHA contents support the statement that the EPA content does differ between species and within species between substrates. The size of these effects suggest EPA content is more influenced by substrate than species. Only Talitridae samples had more than one observation of DHA >LOQ, thus it seems the DHA content does differ between species. Any affects of substrate on DHA could not be assessed.

Across all the study species, of which n>1, mean estimated EPA content lay between 2-5mg/g dw, only 3 differences across all pairwise comparisons were significant, and the size of these effects were slight. Additionally, one of these significant differences was between *C.pilipes* and *Coelopa* sp the latter of which was comprised of wild harvested *C.frigida* and/or *C.pilipes.* Given no difference was estimated between laboratory reared *C.frigida* and *C.pilipes,* none should have been present between *C.pilipes* and *Coelopa* sp holding everything else constant. Naturally, many variables differed between reared and harvested samples; therefore, these data suggest that specific estimated difference is a result of environmental differences. A significant effect of the substrate-species interaction on the EPA content was found in *C.frigida* with *Fucus* sp rearing larvae with the highest estimated mean just above 6mg/g(dw) and Farmed sp the lowest just below 2mg/g(dw). *C.pilipes* were also highest in EPA when reared on *Fucus* sp though this difference was not

significant. Similarly, *F.maritima* reared on *Fucus* sp had the highest mean EPA, though this was from only one sample so could represent an outlier. These findings align with the observed EPA content in the substrates with *Fucus* sp containing the highest EPA content (Figure 78). While the significance of this could not be estimated (n=1), it is in line with the findings of Biancarosa *et al* (2017 and 2018). However, the significance of an interaction between species and substrate is in contrast to the findings of Biancarosa *et al* (2017) who found no significant difference between the EPA content of *C.frigida* or *C.pilipes* when reared on *L.digitata* or *F.serratus*. It seems probable that this is a result of them comparing percentage of total fatty acids rather than the absolute amount (mg/g).

The reporting of fatty acid results is often done in % of total fatty acids (% TFA) and can be misleading and deceptive as it does not provide the reader with the full picture of the data being presented. Reporting actual amounts, on the other hand, provides a clear and accurate picture of the data being presented. It allows the reader to see the real change that has occurred and make informed decisions based on that information. Thus, here it is argued fatty acid results should always be presented as actual amounts or at least both amount and %. Furthermore, assessing the significance of differences in percentages is far less useful than that of actual amounts. For example, finding a significant increase in the EPA% TFA between species A and species B suggests species B contains more EPA than species A, but the data would not necessarily show this.

Lastly, all laboratory reared samples had a DHA or DPA content <LOQ. Of the Diptera species, no wild harvested *F,maritima* samples contained DHA or DPA >LOQ and only one sample each from *Coelopa* sp (wild *C.frigida* and/or *C.pilipes*) and *O.luctuosum* did. This would suggest that on the occasion these two samples were collected, the feeding media of the larvae had been enriched with DHA and DPA from allochthonous material other than wrack deposited such as crab carcasses, which were often present in wrack deposits. This further suggests the fatty acid content of larvae is somewhat dependent on the substrate they are reared on. Talitridae samples consistently had DHA and DPA contents >LOQ and of the comparison samples both DHA and DPA were quantified in *Gammerus* sp and *L.oceanica* while only DPA and DHA were >LOQ in Nematode sp and *Daphnia* respectively. All DHA and DPA values ranged between LOQ and 1mg/g apart from the DHA in *Gammerus* sp, which was 1.79mg/g. These findings are in line with those of Ladhes *et al* (2010).

The true protein content of both *Coelopa* species and *F.maritima* is approximately 50% (Table 31). This is a considerable protein content and is comparable to that of BSFL IM and FM. Thus these Diptera larvae represent a potentially viable alternative protein source. This potential is furthered by the quality of this protein as they are not deficient in any of the essential amino acids (Figure 69)

including lysine and methionine, which Belghit *et al* (2019) highlight as the main limiting amino acids in aquafeeds.

Interest in the use of insects as food and feed has been rising in recent years. Figure 81 shows the number of times the term 'insects as food' was searched each month worldwide since January 2008, using Google search engine (Google 2023). This shows a clear trend of steadily increasing interest. It is important that as anything novel grows, regulation and safety standards grow with it. The recent growth of open access artificial intelligence is a prime example of this. As is the use of novel food and feed ingredients.

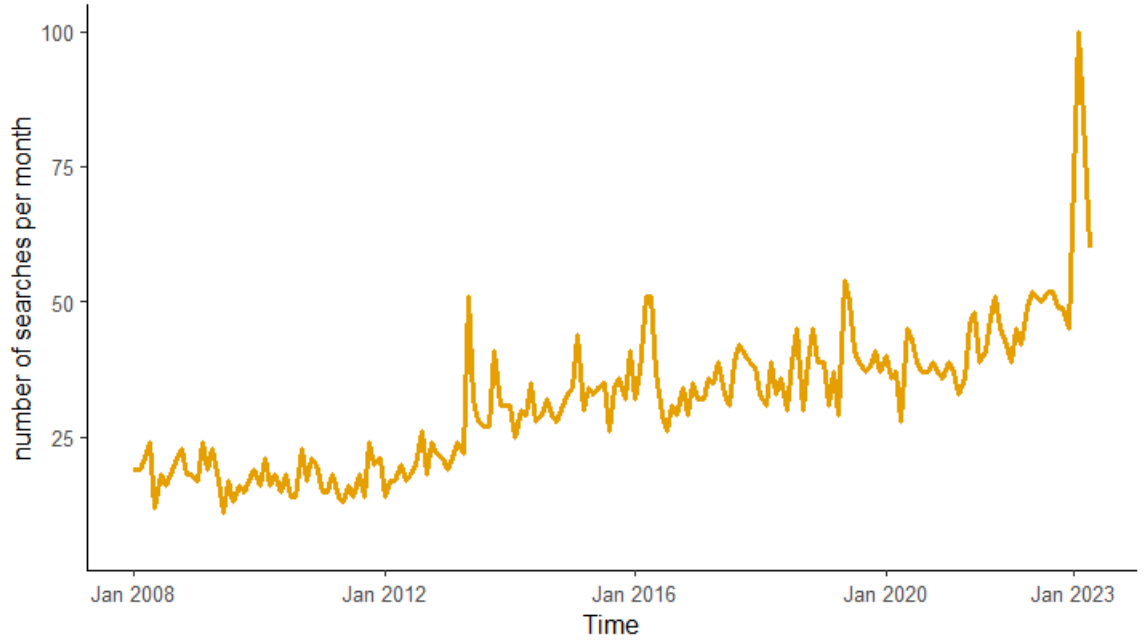


Figure 81. The number of times the term 'insects as food' was searched each month worldwide since January 2008 using Google search engine. Data from (Google 2023).

As such, any investigation into the potential of a novel food/feed ingredient must also assess its concentration of undesirable substances. To facilitate this, the European Commission (EC) set the maximum levels for a range of undesirable substances in animal feeds in Europe (EC 2019). Among these are the heavy metals As, Cd, Pb and Hg which the EC (2019) deem safe in animal feed ingredients up to the levels of 2mg/kg, 2mg/kg, 10mg/kg and 0.1mg/kg respectively. The mean values of these heavy metals for each invertebrate and substrate analysed are presented in Table 32.

Table 32. The mean observed value of heavy metal concentration in each invertebrate species (Left) and each substrate (right). All values presented in mg/kg and presented with +/- 1SD where applicable.

Species	As	Cd	Hg	Pb
C.frigida	15.88 ± 4.77	3.50 ± 2.6	0.084 ± 0.037	1.45 ± 1.47
C.pilipes	19.14 ± 8.85	4.23 ± 2.22	0.048 ± 0.018	1.48 ± 0.99
Coelopa sp.	5.40 ± 2.74	1.61 ± 0.62	0.041 ± 0.011	1.93 ± 3.13
F.maritima	10.81 ± 6.94	4.35 ± 2.08	0.05 ± 0.026	2.30 ± 4.3
O.luctosum	5.67 ± 4.61	0.93 ± 0.54	0.026 ± 0.015	1.30 ± 1.09
Talitridae	12.88 ± 4	0.80 ± 0.16	0.032 ± 0.007	0.41 ± 0.17
Gammerus sp.	11.00 (n=1)	0.32 (n=1)	0.038 (n=1)	0.23 (n=1)
L.oceanica	19.00 (n=1)	0.64 (n=1)	0.11 (n=1)	2.10 (n=1)
Nematode sp.	24.00 (n=1)	1.60 (n=1)	0.016 (n=1)	0.31 (n=1)
Daphnia	5.67 (n=1)	0.09 (n=1)	<0.05 (n=1)	4.80 (n=1)

Substrate	As	Cd	Hg	Pb
A.esculenta	33	1.20	0.015	0.40
Fucus sp.	22	0.53	0.019	1.10
L.digitata farmed	48	0.26	0.018	0.19
L.digitata frond	61	0.34	0.063	0.88
L.digitata stipe	63	0.39	<0.05	0.46
S.latissima	50	0.53	0.045	0.50

The Pb and Hg concentrations in all substrates and invertebrates are below safe limits apart from the Hg concentration of 0.11mg.kg in *L.oceanica*. Cd is also below the recommended level in all substrates and most invertebrates apart from *C.frigida*, *C.pilipes* and *F.maritima*. These latter three are considerably above the safe level of 2mg/kg with mean values of 3.5mg/kg, 4.23mg/kg and 4.35mg/kg respectively. However, these three also have the highest variance with all their SDs above 2mg/kg. The As concentrations appear to present a severe hurdle to wrack invertebrates use in animal feed as all are well above the safe limits with a range of 5.67-24.0mg/kg. This finding is mirrored by Biancarosa *et al* (2017) who also report high As and Cd levels in *C.frigida* and *C.pilipes* and highlight their concern over this. In the data presented here, the As values observed in the invertebrates pale in comparison to that of the substrates which, in some instances, exceed the safe As levels by more than 10x that of the invertebrates ranging from 22-63mg/kg. This brings into question the safety of all seaweed derived feedstuffs, not just wrack invertebrates, and these excessive As levels were observed in both farmed and wild harvested seaweed. These values are only obtained from one sample per substrate but are supported by other studies such as: Biancarosa *et al* (2017) who also report *L.digitata* and *F.serratus* levels considerably higher than 2mg/kg and by Biancarosa *et al* (2018) who evidence the significantly higher As content in brown algae species than red or green.

The high levels of As in the substrates in both lab reared and wild harvested larvae suggest invertebrates accumulate these heavy metals through the substrate they are reared on. Thus, management of the substrate may provide an approach to reduce the As levels in wrack invertebrates. This is also suggested by Biancarosa *et al* (2017) who found species substrate

interaction had a significant (p>0.01 < 0.05) effect on As concentration and this finding was also observed here (F(4, 37) = 2.66, p = 0.047). However, pairwise comparisons within this interaction show only one significantly (p=0.001) different As concentration between substrates within species, that of *F.maritima* larvae having a much lower concentration when wild harvested than reared on *L.digitata*. These pairwise comparisons are visualised in Figure 82. Furthermore, these high As levels could also be processed out of the feedstuff prior to incorporation into the animal feed through extraction of only the lipid content, for example.

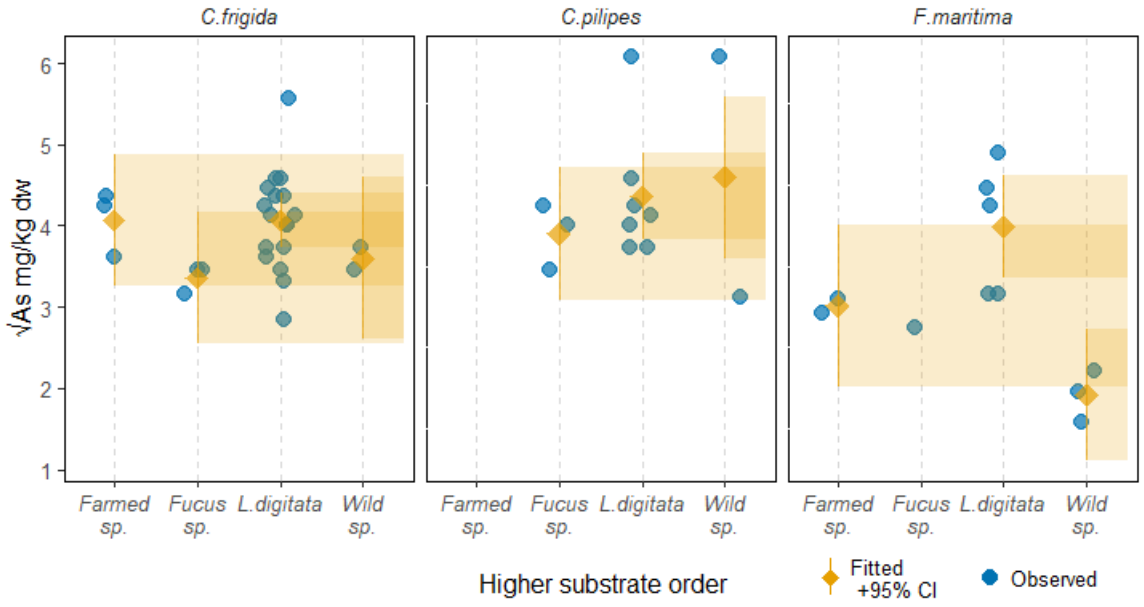


Figure 82. The estimated mean (+95%CI) arsenic concentration in the larvae of each species depending on the substrate on which they were reared. Cis have been projected right for easy pairwise comparison. A square root transformation was applied to the response variable (As) in order to normalise its distribution. In F.maritima the substrate 'Wild sp' refers to wild harvested samples. Where as in C.frigida and C.pilipes it refers to samples reared in the laboratory on wild harvested wrack substrate.

This observation of a much reduced As concentration in wild harvested samples compared to their lab reared counterparts is also evidenced in Figure 60, which shows the pairwise comparison of As concentration per species. In this, the significantly different (p<0.001) As concentration between *Coelopa* sp and both *C.frigida* and *C.pilipes* is evident. The *Coelopa* sp represent wild harvested *C.frigida* and/or *C.pilipes* as this was the lowest taxonomic level the larvae could be identified to. There are many different variables between lab reared and wild harvested samples, such as temperature, moisture and interspecific competition. However, as substrate has been shown to significantly effect As concentration, it is probable at least some of this difference between wild and lab reared comes from differences in substrate. Furthermore, the magnitude of this difference with a mean As of 5.4mg/kg in the wild *Coelopa* sp and a mean of 17.51mg/kg across both lab reared

Coelopa sp (Table 32), coupled with the observed content in substrate types being an order of magnitude higher than that of wild *Coelopa* sp could even suggest they were not feeding exclusively on seaweed substrates like those which were lab reared.

Overall, these findings support the statement that the accumulation of contaminants is significantly different among the study species and within species between substrates. The biggest differences within species appear to be between wild and laboratory reared samples suggesting a variable contained within this difference could be manipulated to reduce the unsafe observed As and Cd levels to those which are safe.

Similarly, the results presented above show that the method of treatment was estimated to significantly affect the contents of certain minerals (Figure 65, Figure 66) including that of lead and mercury. Supporting the statement that the method of larval processing does affect their contaminant composition. However no significant effect on the EPA or DHA content was found. The increased mercury content in larvae which had an empty gut compared to those with a loaded gut suggest larvae bioaccumulate mercury. Thus, monitoring the contaminant levels in any substrate used on an industrial scale would be necessary. The use of a substrate with a high mercury content could make wrack invertebrates an unsuitable omega-3 PUFA alternative.

The final question assessed by this study: "Is the EPA,DHA and/or contaminant composition of a 2nd generation of larvae different from that of the 1st reared on the same substrate?" found no significant effect of generation on the composition of any mineral and the estimated effect across all fatty acids was also insignificant ($F(20, 82) = 1.50$, $p = 0.104$). Although, five significant fatty acid specific effects were observed on 15:00, 18:3n-3 (ALA), 18:4n-3, 20:4n-6 and 20:5n-3 (EPA), and in all cases a significantly higher content was observed in the 2nd generation (Table 30). Surprisingly, this was in contrast to the observed difference in the content of the same fatty acids in the substrate pre and post rearing. Of those five FAs in which a significant effect was observed , in all except 15:00 (all the PUFAs), the content in the substrate decreased after rearing a generation (Table 30). These findings have three significant implications.

Firstly, the statistically non-significant effect of generation suggests there is no nutritional penalty to larvae reared on a substrate which has already reared one generation of larvae at ~optimum density. Above the optimum density, the relationship between conspecific *C.frigida* larvae appears to turn competitive with survival decreasing with increasing density (Leggett 1993, Chapter 3). The ability of a second generation to be reared on a substrate that has already reared one generation at optimum density with no adverse nutritional effects reported and no observed reduction in survival (visual

observation only) implies one of two things: Either the nature of this competition is not for the food resource or, larvae do not feed on the substrate directly, rather the microorganisms breaking it down. In the case of the latter, once the first generation are no longer grazing on the microorganisms, their populations would increase and become capable of supporting a second generation of larvae as they did the first. The question of what *C.frigida* larvae eat has been unanswered since work on the species began, though the consensus in the literature is that they feed on the microorganisms rather than the seaweed directly (Backlund 1945, Edward *et al* 2007, Orr 2013). The most convincing evidence for this comes from Edward *et al* (2008) whose stable isotope analysis suggest there is a trophic level between the *Coelopa* sp and seaweed substrate they are present in. However, these findings are based on isotopic analysis of the adult flies, not the larvae, and the stable isotope ratios of $\delta^{13}C$ and $\delta^{15}N$ are known to change between the larval, pupal and adult stages in other Diptera species (Doi *et al* 2007, Zehner and Gómez-Durán 2013). This is not accounted for by Edward *et al* (2008) leaving the question of larval food source unanswered. The finding of this study regarding the larval generations may add more evidence to the consensus that the larvae consume microorganisms which decompose the detached seaweed.

Secondly, this observed increase in some of the PUFAs in the 2nd generation of larvae despite the overall content in the substrate appearing to decrease may also have significant implications on the nature of conspecific interactions in *C.frigida* larvae. These results suggest the first generation modified the substrate in a way which increased the bioavailability of the PUFAs for the second generation. This multi generation facilitation makes sense as an evolutionary trait and would be naturally selected for as previous generations would improve the success of future generations. Though, the nature of this possible facilitation depends on what the larvae eat. Assuming it is the microorganisms that decompose the seaweed, the most obvious mechanism would be through the first generation increasing the surface area and aeration of the substrate through their mechanical action, promoting increased bacterial growth. However, it does seem unusual that this facilitation would only affect certain PUFAs as opposed to the broader larval nutrition. This highlights a potential limitation of this study briefly discussed in Chapter 3. If a multi-generational facilitative interaction which improves nutritional composition does exist, the results of this study may underestimate the potential of wrack flies as a PUFA alternative because each culture was setup as if it were the first colonisation of a fresh wrack deposit. However, these results suggest subsequent generations would have more potential as a PUFA alternative.

The third potentially significant implication of this result is the increased potential it gives *C.frigida* larvae as an alternative PUFA source. Firstly, the increased EPA value makes the larvae more likely to

be able to compete with the nutritional provision of fish oil. But secondly, it increases the sustainability of their use as a PUFA alternative as multiple generations could be reared from the same substrate, reducing the amount of waste created and increasing the quantity of feed generated from a given amount of substrate.

This effect of generation was only assessed in *C.frigida* reared on *L.digitata* frond and was done so with only three paired samples (n=6). The effect size (Cohen's d) was calculated to be δ=1.69 based on the standard deviation of differences within pairs (SD=1.26). The power achieved was 0.75, indicating that there was a 75% chance of detecting a true difference between the paired samples if it existed. Although, as each sample was comprised of three biological replicates the statistical power is technically higher than this. That being said, these results should still be replicated in a larger study to increase their validity and ensure they can be generalised to the wider population. Additionally, it would also be of great interest to see if the same results are obtained in the other wrack fly species. Here it is hypothesised that the same effect of generation would be observed in *C.pilipes* and possibly *O.luctuosum* but not the other wrack fly species. This theory is based on the physical adaptations and local distribution of each species previously discussed and proposes the adaptations which Egglishaw (1958) suggest make *Coelopa* sp and *O.luctuosum* suited to the consumption of wet wrack have actually been selected for the consumption of microorganisms. Thus explaining their occurrence in large wet banks, which have such high microbial activity they can maintain astounding temperatures independent of their environment (Leggett 1993). Conversely, the other species, with sharper mouth hooks, are adapted to consume the seaweed directly, and their distribution supports this as they are found in the smaller drier deposits in which microbial activity is much reduced.

Overall, this study suggests the concentrations of arsenic and cadmium in wrack invertebrates could represent a significant limiting factor in their use as an alternative PUFA source. Although management of the substrate and/or refining of the product may provide ways in which to improve this. Furthermore, the absence of DHA and, to a lesser extent DPA, in the lab reared Diptera samples limits their potential as a complete fish oil alternative. However, this is not true of the Crustacean species (Talitridae, *Gammerus* sp and *L.oceanica*), which have fatty acid contents capable of replacing fish oil. Comparisons of the overall potential of these wrack invertebrates, including PUFA composition, to fish oil are discussed in Chapter 7.

Chapter 6

PROTEOMIC SPECTRAL MATCHING AND PROTEIN QUANTIFICATION AS A TOOL FOR THE AUTHENTICATION OF NOVEL FOOD AND FEED.

6.1 Introduction

Concentration of undesirable substances, such as arsenic and cadmium, are one of many aspects that must be considered in assessing the safety of food and feed. A prime example of this is the spread of bovine spongiform encephalopathy (BSE), commonly called mad cow disease, in the United Kingdom during the 1980s and the agricultural and public health crisis which ensued. The spread of BSE is believed to have occurred as a result of recycling processed waste parts of cattle, or other bovines, into cattle feed (Smith and Bradley 2003). This highlights the need for regulation and legislation of food and feed. Such regulation is also critical in ensuring consumers can make informed decisions on their diets from trustworthy information (REGULATION (EC) No 1169/2011). Failure to provide this trustworthy information can lead to tangible negative economic effects such as those resulting from the 2013 'horse meat scandal' (Stanciu *et al* 2013). Despite this, occurrences of food fraud have increased in recent years (Bouzembrak *et al* 2018, Khaksar *et al* 2015, Moyer *et al* 2017). Though, none of these studies seem to account for an increase in detection capacity which seems likely. Nonetheless, it is vital that methods of food and feed analysis are accurate and reliable to ensure regulation is followed and can be enforced. Therefore, for novel ingredients, such as those derived from insects, to represent a viable food or feed alternative, methods must be developed to facilitate regulatory identification and quantification of the species present in the final product (Belghit *et al* 2019).

As evidenced in Chapter 5, wrack fly larvae possess a well-balanced amino-acid profile and true protein content of approximately 50%. Thus, while their main interest is as an alternative PUFA source, they also show potential as an alternative protein source to replace fishmeal and plant-based protein currently used in feed. Within the EU, protein derived from insects, insect meal (IM), falls under the same regulation as that of processed animal protein (PAP). As of January 2023, when *Alphitobius diaperionus* (lesser mealworm) was added to the list (REGULATION (EC) No 58/2023), four species of insect were approved on EU markets for direct human consumption and eight for use in the feed of fish, poultry and swine (REGULATION (EC) 2470/2017). To ensure the safety of insect-based PAP, it must meet the same regulatory demands as all other sources of PAP. However,

methods used to authenticate more traditional sources of PAP may not be effective in doing so for novel insect-based PAP. To ensure the use of PAP complies with regulations, the European Union Reference Laboratory for Animal Protein (RLAP) produce standard operating procedures for regulatory laboratories. For example, they have validated a light microscopy method to detect the presence of PAP in feed, which should contain none, and a polymerase chain reaction (PCR) method for the detection of ruminant DNA in feed that does contain PAP (Rasinger *et al* 2016). While light microscopy and PCR methods can be used to detect insects on a species level (Marein *et al* 2018, Ottoboni *et al* 2017), the growing number of insects permitted to be used as PAP coupled with the relative lack of molecular reference information on insect species makes this difficult (Belghit *et al* 2019). In 2018, the European Food Safety Authority identified HPLC-MS based proteomic analysis as a complementary tool for the regulation of PAP (European Food Safety Authority 2018). Some food and feed safety laboratories have developed methods using HPLC-MS to detect and quantify species present in PAP (Lecrenier *et al* 2016, Steinhilber *et al* 2018). These are targeted approaches, which rely on the detection of a protein with a documented amino acid sequence that is known to be present in the proteome of the target species. Thus, similar to the drawbacks of PCR, the targeted nature and limited database knowledge acts as a barrier to their use in the regulation of insect PAP (Belghit *et al* 2019, Varunjikar *et al* 2022a).

It is well known that DNA, except in the case of identical siblings or clones, is unique to an individual and contains the instructions needed for the development, survival and reproduction of an organism. This information is stored in genes, which are the most basic unit of inheritance; thus traits are passed on in the form of genes. In a basic sense, genes contain the code for the production of a protein, which performs a specific function(s) in the organism. These functions are required for the structure, function, and regulation of almost everything in an organism. Thus, the difference in appearance and function between organisms is a result of the particular genes the organism possesses and is expressed through the protein these genes code for. As a result, the combination of proteins present in an individual, known as the proteome, is equally, if not more unique than their genes and can be used for identification (Orr 2005, Stearns and Hoekstra 2000).

Proteins are the primary functional molecules in living organisms, and their amino acid sequences are directly determined by the genetic code. As organisms evolve, changes in their genetic code result in changes to this sequence and thus the structure and function of the protein, which can have a wide range of effects on the function and fitness of an organism. Where such changes improve the fitness of an organism, it will be able to survive and/or reproduce more effectively. Therefore, as stressors and environmental conditions change and species evolve, their proteome changes. The

rate at which this occurs reflects the evolutionary pressures that organisms experience, and the physiological role of the protein. Individuals with mutations that reduce fitness make them less able to reproduce, thus harmful mutations are often lost from the gene pool. Conversely, mutations, and the proteins they create, which increase an organism's fitness, are conserved in the gene pool (Lipman *et al* 2002).

Generally, conserved proteins remain unchanged as they define a competitive advantage in the species. For example, the use of O_2 in respiration facilitated the diversification of life. As O_2 respiration evolved, a mutation in the genes which coded for haemoglobin resulted in its function changing from one which detoxified O_2, into one which could transport it around larger organisms (Decker and Van Holde 2010). The essential nature of this bodily function (O_2 transport) means any mutation which alters it is likely to reduce fitness and thus not be inherited. As such, the protein haemoglobin has been conserved across a wide range of species, including mammals, birds, reptiles and fish. Therefore, the presence of conserved proteins can indicate shared ancestry and can be used to infer evolutionary relationships between organisms and to trace their evolutionary history. When two or more species have a conserved protein, it suggests that the protein was present in the common ancestor of those species and has been retained over evolutionary time. The degree of conservation can also provide information about the timing of divergence between different lineages. For example, if two species share a highly conserved protein, it suggests that they diverged relatively recently in evolutionary time. Each amino acid has a different mass-to-charge ratio (m/z) which can be quantified using mass spectrometry (MS). As each protein has a different number and abundance of amino acids and corresponding peptides, this can be used to identify proteins that are present. Thus, through the nature of protein conservation specie(s) present in a sample can be identified based on observed proteins. However, this can only be done with the aid of a database to which results can be matched. Such matching occurs via the comparison of observed spectra to database spectra and thus is known as spectral library matching (SLM). This technique has been suggested as a reliable method for identifying and differentiating between species (Belghit *et al* 2019, Rasinger *et al* 2016, Varunjikar *et al* 2022a).

The comparison of spectra produced from proteome-wide tandem MS to determine the presence of species-specific protein markers was used by Rasinger *et al* (2016) to designate ruminant PAP used in aquafeeds into the species from which they originated. The use of SLM to detect food and feed that does not conform to regulations has been described by several studies such as that by Belghit *et al* (2021), who demonstrate the accurate use of SLM in detecting the presence of sanctioned bovine PAP in the larvae of black soldier flies reared on contaminated substrate. Additionally, Nessen *et al*

(2016) demonstrate the use of SLM to identify closely related fish species in fresh, processed and even battered and deep-fried samples. Belghit *et al* (2019) used the same method as Rasinger *et al* (2016) to successfully differentiate insect meals into their respective taxonomic families and identified future areas of research as creating open access insect spectral library databases and developing highly sensitive tandem MS SLM assays. Building on the promise these studies evidence, and future research identified, Varunjikar *et al* (2022a) describe an optimised analytical flow (AF) high resolution (HR) HPLC-MS (AF-HPLC HR-MS) method for the authentication of insect species using SLM. Their results found that "AF-HPLC HR-MS provides data of sufficient quality to perform non-targeted species identifications of insects intended for use in food and feed." (Varunjikar *et al* 2022a). Therefore, in the absence of sufficient genomic information from which to identify fraudulent food and feed contents using established methods, according to the literature, SLM following untargeted proteomics using AF-HPLC HR-MS represents an effective tool to supplement existing methods in the detection, quantification and differentiation of insects in food and feed.

Previous studies evidence the capacity of SLM to differentiate taxonomically distant insect species to the family level (Belghit *et al* 2019) and closely related fish species to the species level (Nessen *et al* 2016, Varunjikar *et al* 2022b). The objective of this study, in collaboration with Varunjikar *et al* (2022a), was to assess the capability of SLM to differentiate between samples of closely related insect species. Specifically, between the wrack flies *C.frigida*, *C.pilipes*, *F.maritima* and *O.luctuosum*.

6.2 Methods

Larval samples were obtained following the same methods described in Chapter 5, and a total of fourteen samples were analysed. *C.frigida* (n=2), *C.pilipes* (n=2), *Coelopa* sp (n=2), *F.maritima* (n=4) and *O.luctuosum* (n=4). Of both the *F.maritima* and *O.luctuosum* samples, two were wild and two lab reared. The genus *Coelopa* represent the lowest taxonomic level wild harvested *C.frigida* and *C.pilipes* can be identified to in the larval stage, thus the *Coelopa* sp samples are wild harvested and the *C.frigida* and *C.pilipes* lab reared.

The following method was developed by Varunjikar *et al* (2022a) and is described in full there. Freeze dried samples were homogenised in test tubes over ice using lysis buffer and a pestle to break open the cells releasing the proteins. Keeping the mixture on ice helps to prevent protein degradation and maintain the integrity of the sample. To this, 1 M Dithiothreitol (DTT) was added to denature the proteins making them easier to extract. This created a final concentration of 0.1M. Samples were then centrifuged at 15'000 g for 10 minutes to separate the soluble protein from the insoluble material. The supernatant was collected and the protein in the sample further denatured

by heating it at 95°C for 5 minutes. The sample was again centrifuged before the supernatant collected and stored at -20°C. Following the Pierce 660 assay (Rasinger *et al* 2016), Bovine Serum Albumin was used as the standard curve from which the protein content in the extracted samples could be quantified.

These protein extracts were then broken down into peptides and individual amino acids (digested) with a filter-aided sample preparation (FASP) technique described by Belghit *et al* (2019). 150mg extracted protein was diluted with 200µL 8M urea solution to denature the protein and break down any protein-protein interactions. The solution was then centrifuged in an ultrafiltration spin column before being alkylated with 50 mM of iodoacetamide for 20 minutes to prevent the reformation of disulfide bonds during digestion and subsequent analysis. The mixture was washed with 200 µL of 8 M urea solution and 100 µL of 50 mM ammonium bicarbonate solution to remove any remaining impurities and to adjust the pH for optimal trypsin digestion. Proteins were then digested by adding trypsin at an enzyme to protein ratio of 1:50. The digested peptides were separated from any remaining impurities in a centrifuge and purified using a C18 spin column. The digested samples were eluted then evaporated into peptide pellets. These were dissolved in a solution of acetonitrile/formic acid/water (2/0.1/97.9, v/v/v) and stored at -20°C prior to analysis.

The AF-HPLC HR-MS workflow developed by Varunjikar *et al* (2022a) was used to analyse the amino acid and peptide contents of the fourteen wrack fly samples. The 200 resulting spectra with the greatest intensity were then converted into MGF files using msConvert and directly compared using the newly updated CompareMS2 (Marissen *et al* 2022). From these comparisons, distance matrices and phylogenetic trees were created. As the spectra were only compared to each other, this technique of species differentiation is database independent. Following this, as described by Belghit *et al* (2021), SpectraST (version 5.0) was used to create spectral libraries for each of the four insect species.

In addition to the database independent technique, a database dependent comparison was also made. When working with species whose proteome has not been modelled, it is a general practice to match spectra against the proteome of a modelled organism, preferably one closely related. The MS spectra collected from these samples were matched against the proteome of *Drosophila melanogaster* (Fruit fly), which were the closest taxonomically modelled species. Spectra from each sample were compared to the model species using the Trans-Proteomics Pipeline, which is a suite of tools used for analysing proteomics data in R via the database search engine: Comet as well as PeptideProphet and ProteinProphet (Deutsch *et al* 2015). From this, any peptides and corresponding proteins present in each sample that are also present in the modelled proteome of *Drosophila*

melanogaster were identified. Lastly, identified proteins were then compared between samples in Omics Explorer (v3.6) using two-way ANOVA, principal component analyses and hierarchical cluster analysis. Significantly (0.05) differentially expressed proteins were then used to cluster samples by perceived species.

HeLa cells are a type of human cervical cancer cell that was first isolated in 1951 from a woman named Henrietta Lacks (Gey 1952). HeLa cells can divide indefinitely and are easily cultured in laboratories, something no other human cell can currently claim. Owing to these properties, HeLa cells have become invaluable as a standard reference material enabling comparable studies between laboratories. They also facilitate the development of more robust and reproducible assays, as well as the validation and standardisation of new methods and techniques. As such, HeLa cell digest was used to optimize the HPLC and MS parameters by Varunjikar *et al* (2022a).

6.3 Results

From the database independent direct spectral comparison between samples using CompareMS2, samples were grouped according to the phylogenetic tree presented in Figure 83. The obtained mass spectra were only partly successful in arranging the samples into their respective species. Of the four species, *O.luctuosum* samples were most effectively clustered with three out of the four samples grouping together. Slightly less effectively grouped were three out of the four *F.maritima* samples, which also formed a cluster, but this also contained a *C.pilipes* sample. The laboratory reared and wild harvested *Coelopa* samples did not cluster close to each other.

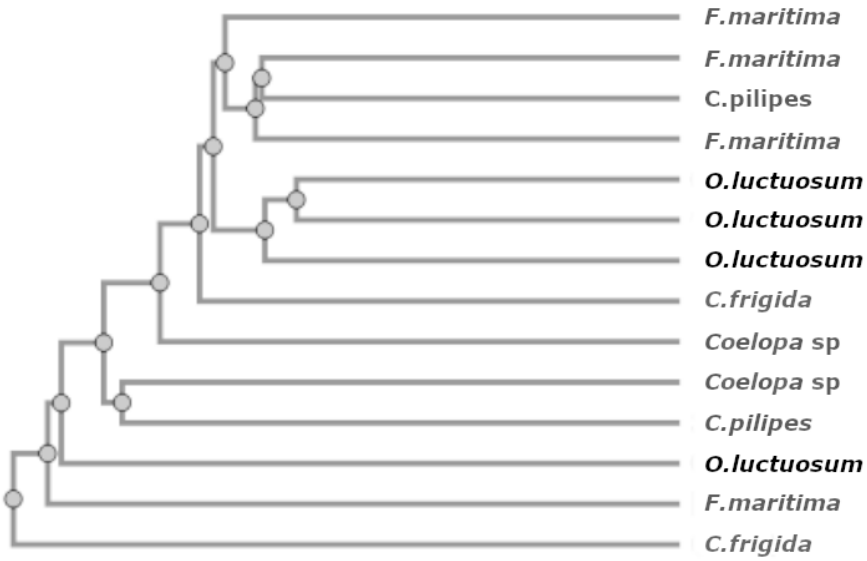


Figure 83. Phylogenetic grouping of insect samples based on direct comparison of mass spectra obtained by tandem mass spectrometry and compared using CompareMS2.

Using the database dependent workflow and comparing each sample's spectra to that of the modelled *Drosophila melanogaster* proteome, an average of 3057 proteins were identified per sample (Table 33). Of these, 38 were found to be significantly differentially expressed and were used to generate the principal component analysis (PCA) in Figure 84 and the heatmap in Figure 85.

Table 33. The total numbers of spectra, spectral matches and identified peptides and proteins for each sample. Plus the percentage of matched spectra from total observed. tSpectra - total spectra; PSM– protein spectra matches; Peptides – number of identified peptides; Proteins – number of identified proteins; % id – percentage of number of identified spectra divided by total number of spectra.

Sample species	**tSpectra**	**PSM**	**Peptides**	**Proteins**	**% id**
C.frigida	6478	6145	6076	4602	94.9
C.frigida	2774	2639	2620	2259	95.1
C.pilipes	2887	2704	2688	2376	93.7
C.pilipes	5406	5114	5073	4032	94.6
Coelopa sp	5000	4733	4688	3806	94.7
Coelopa sp	3851	3654	3623	3040	94.9
F.maritima	2843	2679	2664	2341	94.2
F.maritima	3165	2984	2962	2595	94.3
F.maritima	3668	3442	3416	2911	93.8
F.maritima	4479	4298	4287	3697	96.0
O.luctuosum	3221	2998	2972	2556	93.1
O.luctuosum	2796	2629	2268	2268	94.0
O.luctuosum	3045	2871	2846	2464	94.3
O.luctuosum	5142	4829	4777	3855	93.9

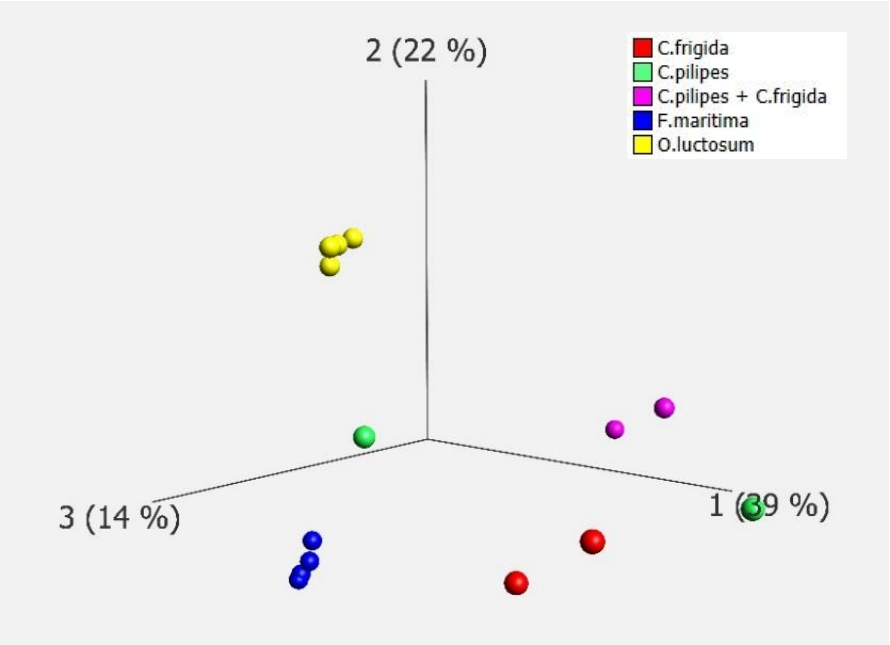


Figure 84. A principal component analysis grouping the samples by their significantly differentially expressed proteins.

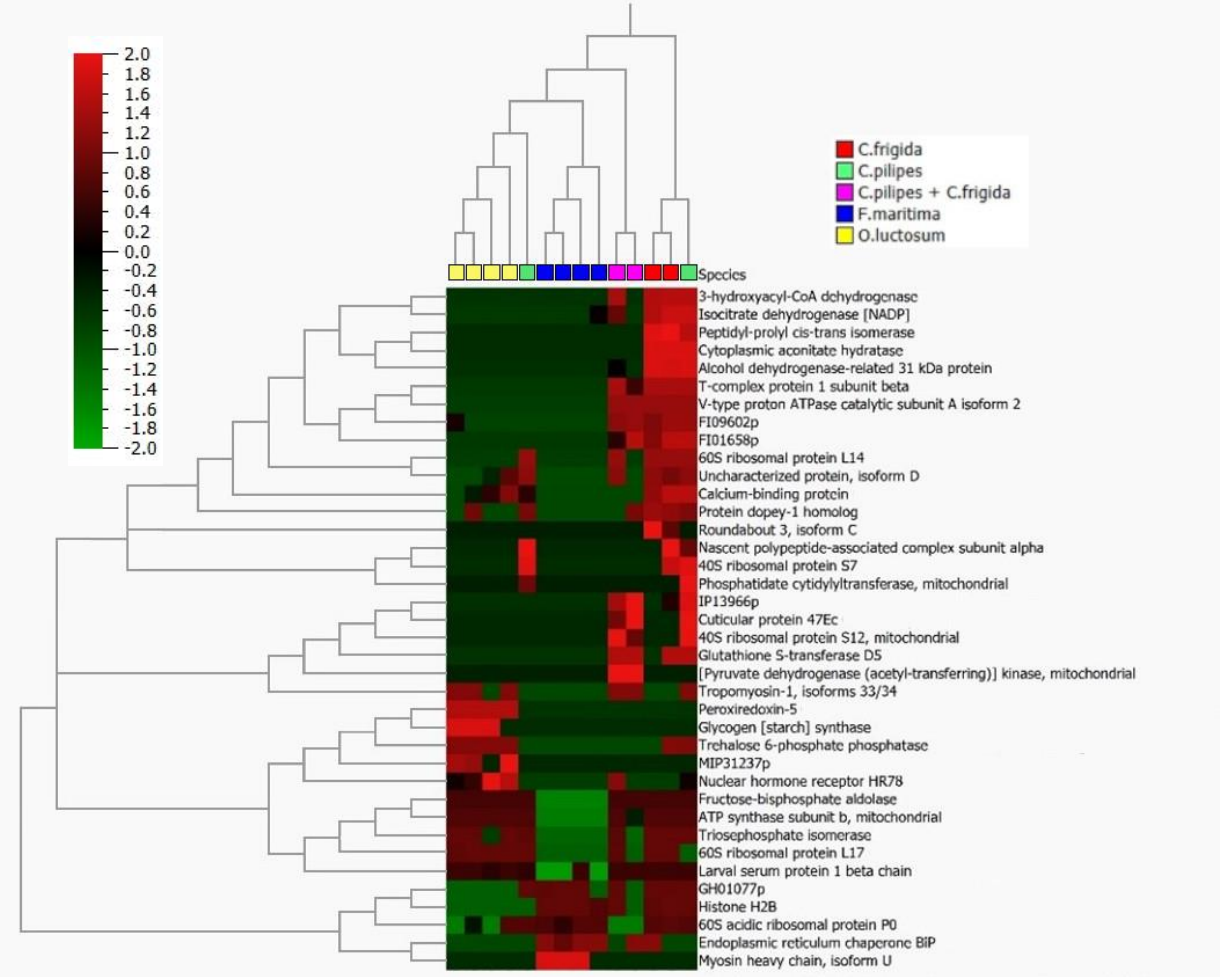


Figure 85. A semi-quantitative heatmap of the 38 significantly differentially expressed proteins across each of the fourteen samples. The samples (top) are dendrographically clustered based on their similarity and the proteins (sides) based on their evolutionary distances.

The results of the database dependent comparison of samples visualised in the PCA (Figure 84) and heatmap (Figure 85) were largely successful in grouping the samples into their correct species. In the PCA, all *O.luctuosum* and *F.maritima* samples were closely clustered and were done so away from all other species. The *C.frigida* and *Coelopa* sp samples were also clustered, though not as tightly, and the *C.pilipes* samples were widely dispersed. The same data are displayed in Figure 85, thus the grouping of samples is much the same.

6.4 Discussion

The increasing number of food and feed scandals violate consumer rights, are illegal in their breaching of legislation (Moyer *et al* 2017) and have negative economic impacts (Stanciu *et al* 2013). The AF-HPLC HR-MS database dependent and independent workflows developed by Varunjikar *et al* (2022a) have been suggested as an additional tool in the authentication of food and feed, particularly of those which are novel and have a limited reference database for other methods to

work with. Other studies demonstrate this technique's efficacy in differentiating between taxonomically distant insect species (Belghit *et al* 2019) and closely related fish species (Nessen *et al* 2016, Varunjikar *et al* 2022b). This study, to the best of current knowledge, is the first assessing the ability of this method to differentiate between closely related insect species. The current taxonomy of the four species of wrack fly used in this study are presented in Figure 86. This represents the desired grouping of the samples if the methods were wholly successful.

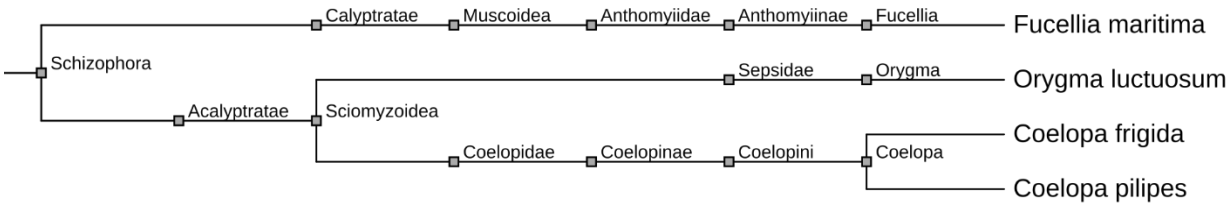


Figure 86. A phylogenetic tree of the four wrack fly species used in this study representing their taxonomy. Figure created using PhyloT (Letunic 2015).

The results of the database independent direct spectral comparison approach suggest this method may not currently be suitable for the differentiation of closely related insect species present in food or feed. The more closely related species are, the more genes and thus proteins will be shared. Naturally, this will produce similar mass spectra. The results in Figure 83 show this method was only partially successful in the taxonomically accurate grouping of samples. This suggests that obtaining higher resolution results would be required to differentiate between these similar spectra. These could be obtained through a more complete protein extraction and digestion and/or higher resolution MS. It is worth noting that in this study, the top 200 spectra of each sample were compared, where in studies using the same methods the top 500 were used (Belghit *et al* 2019, Varunjikar *et al* 2022a). This increased number of spectra could also provide the resolution needed to accurately group the samples by species. A greater number of spectra to compare would increase the likelihood of observing differential proteins between species.

Conversely, the database dependent workflow where the spectra of each sample were compared to that of the mapped proteome of *D.melanogaster* appears more effective in differentiating between closely related insect species. The results presented in Figure 84 and 85 show this technique tightly clustered two of the four species (*O.luctuosum* and *F.maritima*). However, these two species are not as closely related as the *Coelopa* sp with *O.luctuosum* coming from the *Sepsidae* (Dung fly) family and *F.maritima* the *Anthomyiidae* (Root maggot fly) making them easier to differentiate. The samples from the two *Coelopa* sp are closely related in sharing a genus making them harder to differentiate. Yet, this technique is still relatively successful in grouping these samples according to

their taxonomy based on the dendrographic tree presented in Figure 85. Both *C.frigida* samples and one of the *C.pilipes* samples were grouped two internal nodes away from each other. However, the other *C.pilipes* sample is relatively far from these with their closest connection being four internal nodes away. Similarly, the wild harvested *Coelopa* sp samples which represent *C.frigida* and/or *C.pilipes* are grouped next to *C.frigida,* although their closest connection is three internal nodes away. Based on the taxonomy, the *Coelopa* sp samples would be expected to be no more than two internal nodes from any of the *C.frigida* or *C.pilipes* samples. Nonetheless, these results suggest this method is mostly successful in differentiating between closely related insect species.

However, the inclusion of the *Coelopa* sp samples highlight a major limitation of this AF-HPLC HR-MS based approach in the authentication of novel food and feed. As discussed, studies such as those by Belghit *et al* (2019), Rasinger *et al* (2016) and Varunjikar *et al* (2022a) note the limited capability of more commonly used methods for the authentication of feedstuffs owing to their targeted approach and lack of reference database material. In turn, they suggest MS spectral comparison-based methods could be a supplementary technique to be used by regulatory laboratories to address this limitation. However, in the same paper as suggesting this, Belghit *et al* (2019) highlight the next research steps in the development of this technique as creating open access insect spectral library databases. This suggests this technique is also lacking reference database material. The need for this is evidenced by the *Coelopa* sp samples as this technique cannot quantify the contents of this mixed species sample without a complete modelled proteome of both species.

Like most animal feeds do, these *Coelopa* sp samples contained the protein from multiple species. Even if this technique where wholly successful in differentiating the species present in a feedstuff, without prior knowledge of what those species are, that actual species the differentiated proteins originate from is unknown. Given the ultimate goal in authenticating food and feed is determining if the species present are authorised or not, this creates something of a juxtaposition. So, while this technique may be able to evidence the presence of say three different insect species in an aquafeed, what those species are, and consequently if they are authorised or not, would be unknown.

The actual identification of species is possible with these methods but requires the presence of well-annotated reference proteomes of many insect species. This could be achieved by identifying species-specific proteins markers, the presence of which would be diagnostic of a species. However, to know if a protein is diagnostic of a species, one would have to be certain it is not present in any other species. In theory, this would require the mapping of every insect species' proteome, or at least of those which could conceivably be used fraudulently in food/feed. Identifying such species-specific proteins is known as mining and simply involves comparing the proteins present in all

recorded proteomes looking for those unique to a species. Varunjikar *et al* (2022a) mine for potential species-specific markers between the five insect species analysed in their study. From this, they suggest the larval cuticle protein A2B could be specific to the yellow mealworm (*T.malitor*) and the mitochondrial protein cytochrome c oxidase could be diagnostic of the house cricket (*A.domesticus*). However, the absence of these proteins in the four other insect species sampled does not make them likely to be species specific. Furthermore, if these are species specific and that is unknown, which reference proteome were they identified from?

If the efficacy of this technique to quantify the contents of food and feed containing insects is dependent on the building of a spectral library database, it is not much more of an effective tool than the commonly used methods such as PCR based techniques. As discussed, these are limited by a lack of genetic reference information to which insect samples can be matched to. However, the word 'genome' was coined by Hans Winkler in 1920 (Winkler 1920). Conversely, the first proteomics studies took place in 1975 (O'Farrel 1975, Klose 1975) and it wasn't until 1997 the term 'proteome' first appeared (Wilkins *et al* 1997) and did so in Functional Genomics. Therefore, as proteomics is a younger science, it seems more likely that the genetic insect database is in fact less sparse than that of the proteomic. In which case, the biggest advantage this proteomic based method has is its untargeted approach. Yet, similarly, without a thorough reference database, this method cannot identify species present, only differentiate between proteins from authorised species and not from authorised species. Even this cannot be done until the proteome of each authorised species is mapped, as to identify proteins not from authorised species all the proteins from authorised species must be known. If an unauthorised protein was then discovered, unless it happened to be a protein that was known to be species specific, its origin is entirely unknown. It could well be from the authorised substrate present in the larval gut. Thus, the proteome of every authorised substrate would also need to be mapped.

As such, while the spectral matching techniques described here are at least partially useful in quantifying the difference in protein composition between samples containing one known species, in the absence of a complete database for every authorised species and possibly substrate, this approach is unable to quantify the number or type of species present in a sample of mixed unknown species. Therefore, it can be argued that these techniques are just as unequipped as the commonly used methods to differentiate between authorised and unauthorised species present in a food or feed sample in the absence of a thorough reference database.

Perhaps, the use of stable carbon isotope patterns of amino acids known as $\delta^{13}C_{AA}$ fingerprinting, as suggested by Belghit *et al* (2021) and Wang *et al* (2018, 2019) could be a more useful supplementary

tool. $\delta^{13}C_{AA}$ fingerprinting is evidenced as being capable of differentiating between wild caught, organic or farmed salmon as well as those fed novel diets comprised of insects or algae and thus shows some promise (Wang *et al* 2018, 2019).

Lastly, as highlighted by Varunjikar *et al* (2022a), this quantification of the protein present in novel feed and especially food ingredients is useful in the identification of known allergenic proteins. As emphasized in the literature (EFSA 2021, Ribeiro *et al* 2021) extensive allergenic risk assessment is required before insects can be safely introduced to the food market. Those with a known allergy to proteins present in crustaceans (commonly called a shellfish allergy) have been identified as a group at particular risk owing to the cross reactivity and potentially shared proteins between many insects and crustaceans (Ribeiro *et al* 2021). While the detection of allergenic proteins requires a separate database comparison, the results presented here on the four species of wrack fly show the presence of a heavy chain myosin protein (Figure 85). This same type of protein has been shown to be involved in anaphylactic response to shrimp (Saenz de San Pedro *et al* 2023), highlighting the importance of thorough allergen detection in novel food and feed.

Overall, the potential use of wrack invertebrates as an alternative ω-3 PUFA source is somewhat dependent on the development of robust analytical techniques to be deployed by regulatory laboratories for the fast and effective authentication of their use in food or feed. Furthermore, prior to the incorporation of wrack invertebrates into the diets of people, any allergenic risk they could pose must be extensively researched.

Chapter 7

THE POTENTIAL OF WRACK MACRO-INVERTEBRATES AS AN ALTERNATIVE SOURCE OF THE MARINE ORIGIN OMEGA-3 FATTY ACIDS: EPA AND DHA

In the context of this research, the potential of wrack macro-invertebrates as a more sustainable source of the essential ω-3 PUFAs than fish oil can now be better assessed. As a result of globally increasing population and affluence, agricultural production is predicted to be significantly less than demand (FAO 2014, UN Department of Economic and Social affairs 2021, World Food Programme 2022). Increasing population not only increases demand for food directly, but also does so through competition for space. By requiring increased infrastructure, increasing population reduces the amount of land available for the production of food whilst also increasing the demand for food. As such, increasing the capability and sustainability of food production is a global priority (Tomberlin *et al* 2015). Space represents a major limiting factor to the increased output of conventional agriculture. Although, much more demand could be met from terrestrial agriculture by reducing food vs feed competition and consuming more food from the first trophic level.

Nonetheless, the demand for space, among other factors, results in many expecting a significant proportion of the increased demand for food to be produced from the ocean in the 'blue revolution' (Costello *et al* 2020,FAO 2020a, Free *et al* 2022, Little *et al* 2016). Furthermore, the absence of terrestrial sources of the essential PUFAs EPA and DHA creates a necessity to include aquatic life in the human diet/food chain as a source of these vital dietary constituents. As a result, the FAO (2020a) predict farmed fish production to increase significantly over coming decades and state *" the future sustainability of the fed aquaculture sector nevertheless remains intimately dependent on the sourcing of new and nutritionally balanced feed components"* (FAO 2020a, pg. 121). Historically reliant on fishmeal and fish oil produced from wild caught fish, aquafeed has contributed significantly to the overfishing of much of the world's ocean (Larsen and Rooney 2013). Initially replaced by vegetable-based protein, fishmeal demand has been steadily decreasing (Lock *et al* 2015). However, the food vs feed competition of vegetable-based protein is now seeing that replaced by insect meal in both terrestrial and aquatic animal feeds (WWF-UK 2021).

The potential benefits of insect-based livestock feeds are vast, and their use has less negative effects on the environment, economy and society thus increasing our sustainability. A recent report from WWF-UK (2021) titled; "The future of feed: a WWF roadmap to accelerating insect protein in UK

foods" makes this abundantly clear. This publication evidences how insect-based feeds, particularly from BSFL (black soldier fly larvae), improve all three aspects of sustainability. Briefly, where other feed production requires the input of valuable resources, IM (insect meal) is mostly created from organic side streams and resources considered 'waste'. Historically, the removal and processing of such 'waste' represented an economic cost. However, insects such as BSFL now turn this into a high value resource (IM). As highlighted by the WWF-UK (2021), it does so with economic efficiency as the cost of IM production is comparable to that of soymeal at an estimated £490/tonne and £380–£430/tonne respectively. Yet, the amino acid profile of IM makes it a superior final product than soymeal and nutritionally comparable to fishmeal which costs an estimated £1,000–£1,300/tonne. Furthermore, as this industry develops, the cost of production is expected to decrease making it even more cost-effective. This improved nutritional profile, when compared to the currently used soymeal, results in a better final product of the livestock being reared. Lastly, as discussed in Chapter 1, the environmental cost of IM production is orders of magnitude lower than that of soy or fish meal. It has lower land and water requirements, greenhouse gas emissions, feed in vs feed out ratios and land use competition (Giovanni 2015, van Huis 2020, WWF-UK 2021). Thus, this increase in environmental sustainability also comes with economic and social benefits.

However, of the numerous studies and trials on the use of insects as a more sustainable protein source in aquafeed, few acknowledge or discuss the need to also replace the essential ω-3 PUFAs sourced from fish oil (Liland *et al* 2021, Lock *et al* 2016, St-Hilaire *et al* 2007). Consequently, the nutritional value of farmed fish, in terms of the EPA and DHA content, has reduced over recent years, requiring double portion sizes, as compared to 2006, in order to satisfy recommended EPA and DHA intake levels endorsed by health advisory organisations (Sprague *et al* 2016). The aim of this research was to preliminarily assess the potential of eight species of wrack macro-invertebrates as one such replacement.

7.1 Feasibility of larvae as a feed ingredient

Above all else, any fish oil replacement must provide sufficient EPA and DHA to meet the dietary needs of whatever it is being fed to, be that livestock or humans directly. Of all farmed fish, Atlantic Salmon (*Salmo salar*) require the highest levels of EPA and DHA in their diets (Tocher 2010) and are the most (volume) farmed species in Europe (FAO 2020a). Thus, meeting the EPA and DHA requirements of Atlantic Salmon (herein referred to as Salmon) would serve as a proxy for all other species.

Obtaining absolute values of the dietary requirements of EPA and DHA in Salmon proves surprisingly difficult, though they can be estimated as below. Depending on their life cycle stage, Salmon require a daily feed intake of 1%-2.5% of their body weight (FAO 2020a) giving a median value of 1.75%. In 2020, the average weight of harvested farmed Salmon was 4.4 kg in Scotland (ISFA 2020). As can be seen in Figure 87, the weight of Salmon in the adult phase begins just below 1kg. Therefore, the estimated median feed requirement of adults (1.75% of body weight) will range from 17.5g to 77g as they grow. Figure 87 comes from a white paper recently produced by VERAMARIS® (2021) on "improving aquaculture productivity by meeting dietary omega-3 and omega-6 requirements of Atlantic Salmon". Their research finds, under commercial farm conditions, a minimum of 10% of the total fatty acids (TFA) should be comprised of EPA and DHA combined (VERAMARIS® 2021). Lastly, the total fat content in so called grower feeds, designed for the adult stage, is an average of ~ 25% in those sold by BioMar (BioMar 2023). From this, we can roughly estimate the absolute EPA and DHA requirements of adult Salmon to the point of harvest (1kg-4.4kg) as 0.44g – 1.92g per Salmon, per day, depending on weight.

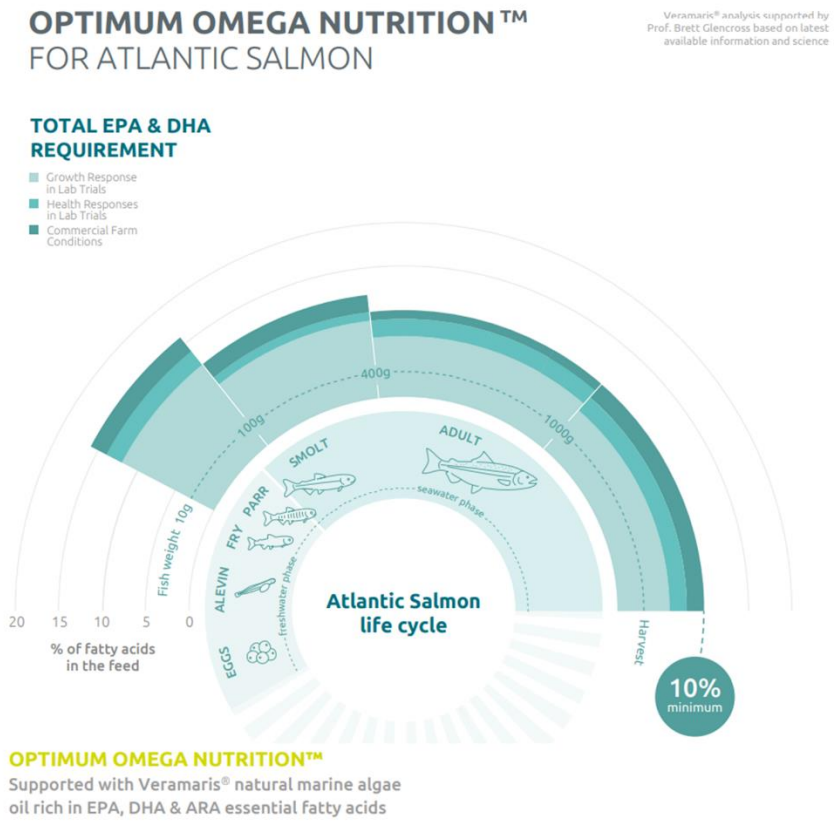


Figure 87. EPA and DHA requirements of Atlantic Salmon throughout their production cycle. Figure modified from (VERAMARIS® 2021).

Comparing these values to those obtained for wrack invertebrates (Figure 88) their potential as an alternative ω-3 source can be assessed. As suggested by Figure 88, and shown in Chapter 5, little difference was observed in the EPA content of any of the wrack species sampled with an approximate mean value of 4mg/g dw. While substrate was found to significantly affect the EPA content with *C.frigida* reared on *Fucus* sp having an estimated mean EPA content just above 6mg/g dw (Chapter 5), this was based on only three samples and needs further research to be confirmed. Regarding DHA, only those from the subphylum Crustacea consistently had a content above the limit of detection and ranged from 0.5-2mg/g dw.

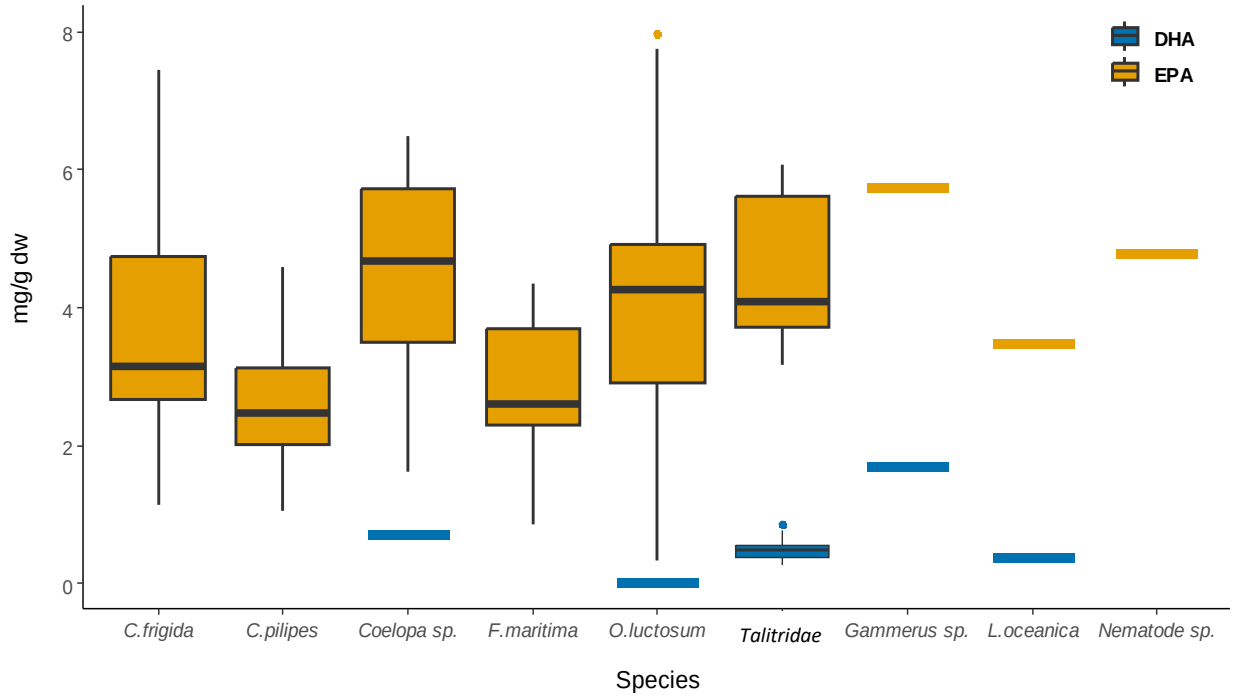


Figure 88. The observed EPA and DHA content of each studied wrack macro-invertebrate.

research so this cannot be quantitively assessed. Their development time is known to be measured in weeks or months (Sun and Patel 2019) rather than days as in the wrack fly larvae studied here. This increased development time likely represents an increased substrate requirement, however a complete study on their culturing is required to confirm this.

C.frigida have been shown to be the simplest and fastest to rear during this research, and higher densities could be more easily achieved. As such, they represent the most potential as an alternative feed ingredient and will be used in this analysis. *C.frigida* larvae have an optimum density of around 1 larva per gram of substrate (Leggett 1993, Chapter 3), and, when reared on *Laminaria* or *Fucus* sp, had an average wet weight of ~0.069g during this research. Thus, to obtain the 1.64kg of larvae needed to provide sufficient EPA for one adult Salmon for one day would require 23,780 individuals,

and at one larva per gram, this would require 23.78kg of seaweed. However, as evidenced in Chapter 5, at least one more generation of larvae can be reared on that substrate, after the first, without reducing the EPA content. In fact, it was estimated to increase significantly in the larvae of the second generation. Thus, the seaweed requirement can be halved to 11.89kg. The European Commission (2019) estimate the cost of farming brown macroalgae species at around £5/kg. This is supported in this research as all farmed seaweed was bought at £4-6/kg. Therefore, to provide the daily EPA requirement for a 2.7kg adult farmed Salmon in *C.frigida* larvae would cost £60 in substrate. However, the value of these larvae goes beyond their ω-3 provision.

As previously discussed, IM represents a viable source of protein for the use in animal feed, and the WWF-UK (2021) expect demand for IM from the UK's pig, poultry and salmon sectors to reach around 540,000 tonnes a year by 2050. As evidence in Chapter 5 and summarised in Table 31 (repeated below), *Coelopa,* larvae have an approximate true protein content of 50% dw and contain a balanced profile of all essential amino acids (Chapter 5). Thus, the quantity and quality of protein in the *Coelopa* larvae is comparable to that of BSFL and FM (WWF-UK). Therefore, as well as providing 1.18g of EPA, 295g (dw) of *Coelopa* larvae would also yield 147.5g protein. The FAO(2020a) state the protein requirement of adult Salmon as at least 30-40% of the diet. Applying the median value of 35% to the current example places the protein requirement at 16.5g per day. Thus, from the £60 substrate cost, the daily EPA requirement of one Salmon as well as the daily protein requirement of 8.9 Salmon could be met. The substrate cost of this protein production works out at £407/kg protein.

Table 31. Summary of the observed amino acid and fatty acid content of each invertebrate species. Values are given as g/100g dry weight +/- 1SD where n>1. True protein represents the sum of dried amino acids.

	C.frigida	*C.pilipes*	*Coelopa sp.*	*F.maritima*	*O.luctosum*	Talitridae	*Gam*[a]	*L.oc*[b]	*Nem*[c]	*Daph*[c]
True Protein	47.8 +/- 1.2	47.6 +/- 1.9	50.5 +/- 2.9	49.8 +/- 1.2	37.7 +/- 2.2	33.5 +/- 6.5	37.1	36.2	47.9	22.3
Total FA[1]	10.53 +/- 2.44	8.42 +/- 3.16	12.34 +/- 5.51	14.38 +/- 2.93	11.61 +/- 4.1	15.65 +/- 5.58	20.3	3.79	7.3	2.18
Total SFA	2.97 +/- 0.73	2.41 +/- 0.98	3.25 +/- 1.41	4.68 +/- 1.13	3.15 +/- 1.12	5.41 +/- 2.2	5.75	0.98	2.5	0.98
Total MUFA	5.94 +/- 2.01	4.76 +/- 2.17	7.43 +/- 3.83	8.27 +/- 1.74	6.56 +/- 2.32	7.54 +/- 2.71	10.84	1.48	3.1	0.91
Total PUFA	1.61 +/- 0.83	1.24 +/- 0.68	1.66 +/- 0.47	1.42 +/- 0.54	1.89 +/- 0.8	2.69 +/- 0.89	3.7	1.32	1.7	0.28
Total n-3	0.66 +/- 0.42	0.5 +/- 0.33	0.75 +/- 0.25	0.61 +/- 0.27	0.75 +/- 0.34	0.92 +/- 0.27	1.28	0.64	0.78	0.18
Total n-6	0.89 +/- 0.44	0.68 +/- 0.36	0.89 +/- 0.28	0.74 +/- 0.31	1.07 +/- 0.47	1.75 +/- 0.61	2.33	0.64	0.89	0.07
Total un-id[2]	1.31 +/- 0.26	1.04 +/- 0.5	1.32 +/- 0.56	1.54 +/- 0.57	1.99 +/- 0.84	0.4 +/- 0.18	1.06	0.23	3.75	0.43

[a] *Gammerus sp.*
[b] *L.oceanica*
[c] *Nematode sp.*
[d] *Daphnia sp.*
[1] Total identified fatty acids
[2] Total un-identified fatty acids
All values in g/100g

In 2020, Scottish Salmon producers received an average price of £4.58 per kilogram of farmed Salmon (SSPO 2021). Applying this to the median adult Salmon weight of 2.7kg gives a value of £12.36 per Salmon not including the weight lost during processing (removal of offal etc..). Comparing this to the estimated cost of substrate in *C.frigida* larvae at £60 for the daily EPA requirement of one Salmon and protein of 8.9, suggests they could never represent an economically viable alternative. However, this mismatch appears to come from the lack of transparency and open access information regarding the nutritional composition of aquafeeds and the nutritional requirements of farmed Salmon. While this information may represent 'trade secrets', the feasibility of innovative aquafeeds cannot be assessed without them. Thus, a more useful assessment of the potential of wrack macro-invertebrates use as a feedstuff may come from their comparison to the BSFL production model provided by WWF-UK (2021).

As previously discussed, this report by WWF-UK (2021) estimates the production cost of BSFL to be £490/tonne and the cost of substrate (bakery by-products and spent brewers grain) in this model was £35/tonne (WWF-UK 2021). In contrast, the current value of farmed seaweed is around £5000/tonne. Thus, the substrate cost alone represents a significant barrier to the use of *C.frigida* as an alternative feed ingredient. As the farmed seaweed industry develops, as it has done in ASIA, the cost of its produce will reduce. The FAO (2018) report costs of farmed seaweed in ASIA as ~50p/kg,

yet even this equates to £500/tonne. *Coelopa* larvae contain significant quantities of EPA which would make a more valuable final product compared to that of BSFL allowing a higher substrate cost. However, according to FAO (2022) the fish species *Peruvian anchoveta,* from the anchovy family, accounts for the biggest proportion of fishmeal and oil. Table 34, from Sørensen *et al* (2011), provides the fatty acid composition of some of the most frequently used fish in the production of FM and fish oil. Surprisingly, what these values are measured in is not reported by Sørensen *et al* (2011) and while they note the source of the values as De Silva *et al* (2011), this publication is not open access. Regardless, even taking the lowest EPA value of 7.6 in Anchovy and measuring this in mg/g or the less useful %TFA comes out higher than that of *C.frigida* larvae. As shown in Figure 88 their EPA ranges from 1.14-7.4mg/g and this represents a range of 1.4-7.7% of total fatty acids, Table 31 give these percentages context. Thus, whilst *C.frigida* larvae have a maximum EPA content comparable to the low end of that found in Anchovy oil, at the production cost of fish oil at £4 per kg (Norwegian Seafood Council 2021), this still would not make their use as a feed ingredient economically competitive.

Table 34. Fatty acid composition of fish oils frequently used as a feed ingredient for farmed salmon production. Table from (Sørensen et al 2011) values assumed to be given as % of total fatty acids.

Fatty acids	Anchovy	Herring	Capelin	Menhaden
14:0	6.5-9.0	4.6-8.4	6.2-7.0	7.2-12.1
16.0	17.0-19.4	10.1-18.6	10.0	15.3-25.6
18:0	4.2	1.4	1.2	4.2
16:1	9.0- 3.0	6.2-12.0	10.0-14.3	9.3-15.8
18:1 n-9	10.0-22.0	6.2-12.0	14.0-15-0	8.3-13.8
20:1	0.9-1.0	7.3-25.2	17.0	n.d.-1.0
22:1 n-11	1.0-2.1	6.9-30.6	15.4	n.d.-1.4
18.2 n-6	2.8	0.1-0.6	0.7	0.7-2.8
18.3 n-3	1.8	n.d.-2.0	0.2	0.8-2.3
20:5 n-3	7.6-22.0	3.9-15.2	6.1-8.0	11.1-16.3
22:5 n-3	1.6-2.0	0.8	0.6	2.0
22:6 n-3	9.0-12.7	2.0-7.8	3.7-6.0	4.6-13.8

As such, it can be concluded that the current inherent value of farmed seaweed acts as the most significant inhibitor to the potential of wrack macro-invertebrates as an alternative feed ingredient. However, if wild seaweed, which ordinarily creates a social and economic cost, such as the vast *Sargassum* deposits discussed in Chapter 2, were used as a substrate an economically superior feed ingredient could be produced.

7.2 Wrack macro-invertebrates as a reactive tool

News outlets are currently full of stories on the negative impacts of the *Sargassum* deposits occurring in Florida and the Caribbean (BBC 2023, CBS News 2023, CNN 2023). The BBC (2023) report that "Mexico has spent approximately $17 million (£14 million) on removing seaweed from its Caribbean beaches." Although they do not report the time scale over which this has been spent. Regardless, it evidences the economic and social cost seaweed deposits can have. Chapter 3 demonstrates the negative effects of removing wrack, and this is broadly applicable to all wrack deposits. However, when anthropogenic activity causes excessive deposition it has negative public health, environmental health and economic (tourism) effects such is the case with the Great Atlantic Sargassum Belt (GASB) (Wang *et al* 2019). Considering their negative impacts, these deposits could potentially be harvested for free or at the labour cost of doing so, thus overcoming the barrier of substrate price.

Between 2014 and 2018 the amount of *Sargassum* in this belt grew from 2.5 mega(million)-tonnes (Mt) to 20Mt, this continuous growth was unprecedented as prior to 2011 this belt had not been present all year round (Wang *et al* 2019). As a crude assessment, removing 17.5Mt of this would leave the initial amount of 2.5Mt present providing the ecosystem services of 'natural' floating *Sargassum*. From the prior calculations, using this 17.5Mt of *Sargassum* would yield 1736.75t and 0.22Mt of EPA and protein respectively from *C.frigida* larvae. To give this perspective, in 2018, 1.23Mt and 4.4Mt of fish oil and FM were produced globally (FAO 2020a). Assuming 10% of this fish oil is EPA, based on Table 34 (if it is in %), this equates to 123,000t EPA. This crude analysis suggests the use of economically, environmentally and socially costly macro algae blooms could feasibly replace a significant proportion of global FM and fish oil. If in 2018, 87.5% of the GASB had been harvested and used for this purpose, it could have been turned into 1736.75t and 0.22Mt of *C.frigida* larvae EPA and protein respectively. Applying a cost of fish oil at £4 per kg (Norwegian Seafood Council 2021) and of fishmeal at £1200/t (FAO 2020) to these amounts results in a combined cost of £267,460,036.

Using the WWF-UK model of BSFL production (which accounts for capital expenditure, operational costs, processing, the value of the waste as fertiliser etc…) as a crude estimate of the same for *C.frigida* larvae at £530 per ton of whole larvae. Producing this same amount of EPA and protein from the 17.5Mt of *Sargassum* would cost £110,284,750, saving £157,175,286. This is a very crude assessment but nonetheless evidences the potential *C.frigida* larvae have to turn seaweed into a FM and fish oil alternative while providing an environmental, economic and social benefit when the seaweed used as substrate represents a hazard and thus is free. However, while 'nuisance' wrack

deposits such as the *Sargassum* deposits can end up in landfill, the collected seaweed is also used in anaerobic digestion to create commercially valuable synthesis gas among other uses (Milledge and Harvey 2016). Thus, economic competition likely makes a wrack deposit with environmental approval for removal rarely/never free.

Furthermore, these wrack macro-invertebrates do not synthesise the ω-3 PUFAs so can never increase the amount that was initially present in the substrate. The undesirable substances present in the larvae, discussed in Chapter 5, such as arsenic would need to be processed out before they could be used as a feedstuff and this would likely be done through oil and protein extraction. If this processing is going to occur anyway, depending on the cost, it may well make more sense to simply extract the oil and protein from the seaweed directly. For example, *C.frigida* larvae reared on *Fucus* sp had an estimated mean EPA content of ~6mg/g (dw). The *Fucus* sp substrate had 1.19mg/g (dw). The optimum larval density is around 1 larvae per gram substrate, but the findings of Chapter 5 show at least two generations can be reared on each substrate thus the number of larvae reared per gram can be increased to two. Using the same values from the previous estimation to obtain 1g dw of *C.frigida* larvae (80.5 larvae ww) would require 40.25g of substrate. In the case of *Fucus* sp, this would contain 47.9mg EPA. Thus, applying the processing methods to the *Fucus* directly would result in a greater return of EPA, though at a larger amount and cost of material processed.

The same is true of the true protein content which at around 50% in *Coelopa* larvae would yield 500mg from 1g larvae compared to the 3.22g from the 40.25g *Fucus* sp substrate at a true protein content of 8%. While the amino acid profiles are different between the larvae and the substrate, none of the amino acids had an absolute value in 1g of larvae, which was greater than in 40.25g *Fucus* sp. Thus, assuming it is cost effective to process the protein and oil from the seaweed directly, a higher yield could be obtained in doing so. However, given the inherent value of seaweed, this does not represent an economically competitive alternative to FM and fish oil. Studies such as that by Wan *et al* (2019) or Gosch *et al* (2012), conclude that macroalgae/seaweed is a suitable or even superior aquafeed ingredient. Whilst this may be true in a nutritional sense, such studies lack a real-world application of their research and, as the inverse of many economic sectors' view on sustainability, fail to acknowledge that sustainability is holistic and a potential solution/innovation must be environmentally, economically and socially viable rather than just one of these.

Here it is argued that the inherent value of macroalgae in the production of many products such as plastics, chemicals, lubricants, biofuels, food additives and cosmetics (Ahmad *et al* 2022, Chapman 2013, Goswami *et al* 2015, Kandale *et al* 2011, Pooja 2014, Wang *et al* 2015) makes their use as an aquafeed ingredient economically unfeasible and thus unlikely to represent an alternative feed

ingredient. Building on this, the use of by-products from these industries as a feed ingredient or substrate could, however, represent an economically viable solution and thus is highlighted as an area of future research.

7.3 Trophic efficiency

Extracting the desired substances from the seaweed directly results in a higher yield because it represents a lower trophic level. As discussed in Chapter 1, the lower the trophic level the more efficient a feedstuff is. As a result, obtaining ω-3 PUFAs from the algae which produce it would be the most effective. The high market value of farmed seaweed prevents this from being economically viable in macroalgae, but the farming of microalgae could represent a holistically sustainable ω-3 PUFA replacement. Both autotrophic (primary producing) and heterotrophic (consuming) microalgae synthesise EPA and DHA de novo and the production of both for various applications have been studied (Cao *et al* 2012, Mendes *et al* 2009, Oliver *et al* 2020, Togarcheti *et al* 2017, Ward and Singh 2005).

Heterotrophic microalgae production is often considered to be more efficient than autotrophic production as it offers several advantages, including higher growth rates, higher biomass yields and greater consistency (Oliver *et al* 2020, Togarcheti *et al* 2017). However, similar to macroalgae, the main challenge in the use of heterotrophic microalgae as a source of omega-3 PUFAs is associated with the high cost of the carbon sources used as the food for these heterotrophic microorganisms. Generally, glucose is used as a carbon source, and can represent up to 80% of total cost of cultivation (Oliver *et al* 2020). Carbon sources from renewable side streams such as food waste hydrolysates, lignocellulosic biomass and sugarcane waste could provide a more economically viable alternative as discussed by Oliver *et al* (2020).

The use of heterotrophic microalgae would correspond to the 2nd trophic level which, as discussed, can inhibit efficiency. However, as these microalgae synthesize EPA and DHA from inputs which contain little to none, in the context of ω-3 PUFAs they can be thought of as primary producing heterotrophs. The true potential of microalgae production as a fish oil alternative needs to be assessed from a holistically sustainable context that accounts for environmental, economic and social outputs and then considers if these make the technology likely to be comprehensively adopted. Therefore, this is also highlighted as an important topic of future research. In reviewing alternative EPA and DHA sources, Tocher *et al* (2019) show that algal biomasses are currently used and formulated for farmed fish feed and human consumption on a commercial scale. This would

suggest doing so is economically viable, though the economic aspect of EPA and DHA alternatives is not discussed.

Lastly, the use of gene technology may also provide a more sustainable ω-3 PUFA rich alternative feed ingredient to fish oil. Amiri-Jami *et al* (2014) found introducing the gene cluster responsible for EPA and DHA synthesis to food grade micro-organisms, like lactic acid bacteria, allowed them to synthesise the PUFAs EPA and DPA. Similarly, Ruiz-Lopez *et* al (2012) produced transgenic oilseed crops, which were successfully engineered to accumulate ω-3 PUFAs at levels approaching those found in native marine organisms. Therefore, genetically modified strains of currently mass-produced species, are believed to be a reliable and economically attractive source for large-scale production of the ω-3 PUFAs (Cao *et al* 2012). Although, Ruiz-Lopez *et* al (2012) note that while the introduction of EPA and DHA biosynthetic pathways into oilseed crops was successfully demonstrated, reaching economically viable levels of EPA and DHA could prove challenging. Furthermore, the use of genetically engineered crop plants would exacerbate food vs feed competition for agricultural resources, arable land and potable water questioning its environmental and social sustainability. This could make genetically modified crop plants an unsuitable fish oil replacement, but again this requires future research to assess its holistic sustainability.

7.4 Increased seaweed habitat

These alternative PUFA sources could be struggling to economically compete with fish oil production as it is an established and developed industry. As novel methods become more refined, they may represent a more viable alternative. Or, perhaps instead, the effectiveness of harvesting these natural PUFA sources, is a result of the fundamentals of natural selection which, through trial and error, constantly work towards maximum efficiency. It could, therefore, be the case that the most realistically sustainable alternative involves the management of ecosystems through ecological modelling, mitigation and sustainable harvesting from the lowest, and thus most efficient, trophic levels. The importance and significance of algae in both the marine and global ecosystem has been evidenced during this research and the list of benefits it provides is long. Quantitative measures of this importance have only recently been deployed such as that by Eger *et al* (2023) who estimated the global value of the ecosystem services: fishery production, nutrient cycling and carbon removal, provided by the six seaweed genera which make up the majority of kelp forests. They conclude their economic worth to be a value between $465 and $562 billion/year worldwide (Eger et al 2023). This is the in situ value of these kelp forests, not their harvested value.

As such, it is suggested here that the most interesting topic of future research identified by this study is the potential for macroalgae farming to greatly increase the ecosystem services natural populations of macroalgae already provide. Given the importance of algae in the marine food web, the management of these seaweed farms as an ecosystem providing services akin to kelp forests could drastically improve the available habitat and thus productivity of many marine food chains. This increase in productivity would facilitate the growth of natural fish stocks and, combined with truly sustainable and well-regulated harvesting, may turn out to be the most holistically sustainable way to provide the dietary EPA and DHA, which are essential to the health and development of humans. The sustainability of this could be further increased by either or preferably both of: A - creating and EPA and DHA rich oil from species further down the food chain such as krill, which also shows promise as a more sustainable alternative to fish oil (Meyer *et al* 2020). B – encouraging the comprehensive adoption and normalisation of people consuming these EPA and DHA rich oils directly from the natural producers, rather than via the trophic link of farmed fish. Thus, future research which models the ecological effects of anthropogenically created seaweed habitats and/or seaweed farms, which are cyclically harvested, on natural fish population levels and estimates the carbon storage potential of this seaweed is highly recommended.

7.5 Conclusion

This research has evidenced the importance of the ecosystem services wrack provides: the invertebrates which thrive in wrack act as a valuable, EPA and protein rich, food source for the fry of several commercially valuable fish species and for many bird species, particularly those which are migratory. In breaking down the wrack, these invertebrates also facilitate primary and secondary production in the nearshore environment and the development of sand dunes and coastal succession providing natural sea defence among other ecosystem services. Some of these invertebrate species were found to be highly culturable giving them potential to be mass produced as a fish oil alternative. This potential is further evidenced in their nutritional composition containing significant quantities of EPA and a balanced and abundant amino acid profile. However, the presence of undesirable substances such as arsenic and cadmium act as a barrier to their use as an alternative feed source. Although, these undesirable substances were a result of their abundance in the seaweed used as substrate. Thus, all seaweed food and feed ingredients must have an effective monitoring scheme in place to ensure their safety as a feedstuff. Nevertheless, by far the most significant obstacle to the use of macro-invertebrates as a more holistically sustainable EPA and DHA rich fish oil alternative is the lack of economic sustainability deriving from the inherent value of the seaweed-based substrate. Insects as food and feed is a very promising industry which seems set to

significantly reduce the environmental impact of our food production systems (WWF-UK 2021). However, this is due to their holistic sustainability which also offers economic incentive by turning costly waste streams into valuable products facilitating their comprehensive adoption. Thus, unless similar side streams from seaweed processing industries are cheaply available, wrack macro-invertebrates are unlikely to be part of this future. The culturing of microalgae or of genetically engineered organisms which already have the infrastructure and knowledge to be mass produced are the most evidenced potential alternatives to fish oil. However, an assessment of their holistic sustainability is required. Lastly, this study identifies ecological modelling of the effects of anthropogenic seaweed habitats and/or seaweed farms as a potential solution to the unsustainable use of fish oil. Such habitats could mean there are, once again, plenty more fish in the sea.

References

Abbott, D.W., Aasen, I.M., Beauchemin, K.A., Grondahl, F., Gruninger, R., Hayes, M., Huws, S., Kenny, D.A., Krizsan, S.J., Kirwan, S.F. and Lind, V. (2020) Seaweed and seaweed bioactives for mitigation of enteric methane: Challenges and opportunities. *Animals*, 10 (12), p.2432.

Abdolshahi, A., Majd, M. H., Rad, J. S., Taheri, M., Shabani, A., & Teixeira da Silva, J. A. (2015) Choice of solvent extraction technique affects fatty acid composition of pistachio (*Pistacia vera* L.) oil. *Journal of Food Science and Technology*, 52(4), 2422-2427.

Abedi, E. and Sahari, M.A. (2014) Long-chain polyunsaturated fatty acid sources and evaluation of their nutritional and functional properties. *Food science & nutrition*, 2(5), pp.443-463.

Ackland, M., Bentley, H. and Brighton, P. (2017) The British Anthomyiidae. *Dipterist Forum.*

Ackman, R.G. (1980) Fish lipids. In: Connell, J.J. (ed.) Advances in fish science and technology. Papers presented at the Jubilee Conference of the Torry Research Station, Aberdeen, Scotland, July 23-27, 1979. *Fishing News Books*, Farnham, UK, pp. 87-103.

Adin, R. and Riera, P. (2003) Preferential food source utilization among stranded macroalgae by Talitrus saltator (Amphipod, Talitridae): a stable isotopes study in the northern coast of Brittany (France) Estuarine, *Coastal and Shelf Science*, 56, 91-98.

Afonso, C., Matos, J., Guarda, I., Gomes-Bispo, A., Gomes, R., Cardoso, C., Gueifão, S., Delgado, I., Coelho, I., Castanheira, I. and Bandarra, N.M. (2021) Bioactive and nutritional potential of Alaria esculenta and Saccharina latissima. *Journal of Applied Phycology*, 33, pp.501-513.

Ahmad, A., Banat, F., Alsafar, H. and Hasan, S.W. (2022) Algae biotechnology for industrial wastewater treatment, bioenergy production, and high-value bioproducts. *Science of The Total Environment*, 806, p.150585.

Alder, J., Campbell, B., Karpouzi, V., Kaschner, K. and Pauly, D. (2008) Forage fish: from ecosystems to markets. *Annual Review of Environment and Resources*, 33, pp. 153-166.

Allesina, S. and Levine, J.M., 2011. A competitive network theory of species diversity. *Proceedings of the National Academy of Sciences*, *108*(14), pp.5638-5642.

Amiri-Jami, M., LaPointe, G. and Griffiths, M.W. (2014) Engineering of EPA/DHA omega-3 fatty acid production by Lactococcus lactis subsp. cremoris MG1363. *Applied microbiology and biotechnology*, 98, pp.3071-3080.

Backlund, H.O. (1945) Wrack fauna of Sweden and Finland: ecology and chorology. *Entomologiska sällskapet i Lund.*

Baeza-Rojano, E., Calero-Cano, S., Hachero-Cruzado, I. and Guerra-García, J.M. (2013) A preliminary study of the Caprella scaura amphipod culture for potential use in aquaculture. *Journal of sea research*, 83, pp.146-151.

Ball, S. (2015) Introduction to the families of British Diptera. Part 2: Key to Families. *Dipterist Forum*.

Barabás, G., D'Andrea, R. and Stump, S.M., 2018. Chesson's coexistence theory. *Ecological monographs*, *88*(3), pp.277-303.

Baring, R.J., Fairweather, P.G. and Lester, R.E. (2014) Storm versus calm: Variation in fauna associated with drifting macrophytes in sandy beach surf zones. *Journal of Experimental Marine Biology and Ecology*, 461, pp.397-406.

Baweja, P., Kumar, S., Sahoo, D. and Levine, I. (2016) Biology of seaweeds. *In Seaweed in health and disease prevention* (pp. 41-106). Academic Press.

BBC (2023). "Seaweed invasion hits beaches." Available at: https://www.bbc.co.uk/newsround/64894471

Beal, C.M., Archibald, I., Huntley, M.E., Greene, C.H. and Johnson, Z.I. (2018) Integrating algae with bioenergy carbon capture and storage (ABECCS) increases sustainability. *Earth's Future*, 6(3), pp.524-542.

Behmer, S.T. and Nes, W.D. (2003) Insect sterol nutrition and physiology: a global overview. *Adv. Insect Physiol*, 31(1).

Beiras, R. (2018) Marine pollution: sources, fate and effects of pollutants in coastal ecosystems. Chapter 16 - Biological Tools for Monitoring: *Biomarkers and Bioassays*. Pages 265-291, Elsevier.

Belghit, I., Lock, E.J., Fumière, O., Lecrenier, M.C., Renard, P., Dieu, M., Berntssen, M.H., Palmblad, M. and Rasinger, J.D. (2019) Species-specific discrimination of insect meals for aquafeeds by direct comparison of tandem mass spectra. *Animals*, 9(5), p.222.

Belghit, I., Rasinger, J.D., Heesch, S., Biancarosa, I., Liland, N., Torstensen, B., Waagbø, R., Lock, E.J. and Bruckner, C.G., (2017) In-depth metabolic profiling of marine macroalgae confirms

strong biochemical differences between brown, red and green algae. *Algal research*, 26, pp.240-249.

Benjamini, Y. and Hochberg, Y. (1995) Controlling the false discovery rate: a practical and powerful approach to multiple testing. Journal of the Royal statistical society: *series B Methodological*, 57(1), pp.289-300.

Berdan, E.L., Mérot, C., Pavia, H., Johannesson, K., Wellenreuther, M. and Butlin, R.K., 2021. A large chromosomal inversion shapes gene expression in seaweed flies (Coelopa frigida). Evolution Letters, 5(6), pp.607-624.

Bernaerts, T.M., Gheysen, L., Foubert, I., Hendrickx, M.E. and Van Loey, A.M. (2019) The potential of microalgae and their biopolymers as structuring ingredients in food: A review. *Biotechnology Advances*, 37(8), p.107419.

Biancarosa, I., Belghit, I., Bruckner, C.G., Liland, N.S., Waagbø, R., Amlund, H., Heesch, S. and Lock, E.J. (2017a) 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), pp.2035-2042.

Biancarosa, I., Espe, M., Bruckner, C.G., Heesch, S., Liland, N., Waagbø, R., Torstensen, B. 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, pp.1001-1009.

Biancarosa, I., Liland, N.S., Day, N., Belghit, I., Amlund, H., Lock, E.J. and Gilburn, A.S. (2018) The chemical composition of two seaweed flies (Coelopa frigida and Coelopa pilipes) reared in the laboratory. *Journal of Insects as Food and Feed*, 4(2), pp.135-142.

Biancarosa, I., Liland, N.S., Day, N., Belghit, I., Amlund, H., Lock, E.J. and Gilburn, A.S. (2018) The chemical composition of two seaweed flies (Coelopa frigida and Coelopa pilipes) reared in the laboratory. *Journal of Insects as Food and Feed*, 4(2), pp.135-142.

BioMar. (2023) Available from https://www.biomar.com/en/denmark/product-and-species/salmon/growers/

Birch, E.E. (2010) The diamond study: a double-masked, randomized controlled clinical trial of the maturation of infant visual acuity as a function of the dietary level of docosahexaenoic acid. *Am. J. Clin, Nutr.*, 91, pp.848-859.

Blyth, J.E. and Gilburn, A.S. (2011) The function of female behaviours adopted during premating struggles in the seaweed fly, Coelopa frigida. *Animal Behaviour*, 81 (1), pp. 77-82.

Bolam, S.G., Fernandes, T.F., Read, P. and Raffaelli, D. (2000) Effects of macroalgal mats on intertidal sandflats: an experimental study. *Journal of Experimental Marine Biology and Ecology*, 249(1), pp.123-137.

Bondari, K. and Sheppard, D. (1987) Soldier fly, Hermetia illucens L., larvae as feed for channel catfish, Ictalurus punctatus (Rafinesque), and blue tilapia, Oreochromis aureus (Steindachner). *Aquaculture and Fisheries Management*, 18 (3), pp. 209-220.

Bouzembrak, Y., Steen, B., Neslo, R., Linge, J., Mojtahed, V. and Marvin, H.J.P. (2018) Development of food fraud media monitoring system based on text mining. *Food Control*, 93, pp.283-296.

Broadhurst, C.L., Wang, Y., Crawford, M.A., Cunnane, S.C., Parkington, J.E. and Schmidt, W.F. (2002) Brain-specific lipids from marine, lacustrine, or terrestrial food resources: potential impact on early African Homo sapiens. Comparative Biochemistry and Physiology Part B: *Biochemistry and Molecular Biology*, 131(4), pp.653-673.

Brown, a. C. & mclachlan, A. (1990) Ecology of sandy shores, Amsterdam, Elsevier Science Publishers.

Brown, A.C., and A. McLachlan. (2002) Sandy shore ecosystems and the threats facing them: some predictions for the year 2025. *Environmental Conservation*, 29: 62–77

Brusca, R.C., Coelho, V.R. and Taiti, S. (2007) Isopoda. *The Light and Smith Manual: Intertidal Invertebrates from Central California to Oregon* pp. 503-542

Bukkens, S.G. (1997) The nutritional value of edible insects. *Ecology of Food and Nutrition*, 36 (2-4), pp. 287-319.

Bunker, F., Brodie, J.A., Maggs, C.A. and Bunker, A.R., 2017. Seaweeds of Britain and Ireland (Vol. 9). Princeton University Press.

Burckle, L.H., 1998. Marine diatoms. *In Introduction to marine micropaleontology* (pp. 245-266). Elsevier Science BV.

Butler, J.N., Morris, B.F., Cadwallader, J. and Stoner, A.W., 1983. Studies of Sargassum and the Sargassum community (No. 589.304524 S7).

Butlin 1983 The maintenance of an inversion polymorphism in *Coelopa frigida.* unpublished PhD Thesis, University of Nottingham

Butlin, R.K., Collins, P.M. and Day, T.H. (1984) The effect of larval density on an inversion polymorphism in the seaweed fly *Coelopa frigida*. *Heredity*, 52(3), pp.415-423.

Calder, P.C. (2021) Health benefits of omega-3 fatty acids. *In Omega-3 Delivery Systems* (pp. 25-53). Academic Press.

Campbell, A.C. (1980) The Larousse Guide to the Seashore and Shallow Seas of Britain and Europe. Olympic Marketing Corporation.

Candela, C.G., López, L.B. and Kohen, V.L. (2011) Importance of a balanced omega 6/omega 3 ratio for the maintenance of health. Nutritional recommendations. *Nutricion hospitalaria*, 26(2), pp.323-329.

Cao, Y. and Cao, Y. (2012) Biotechnological production of eicosapentaenoic acid: from a metabolic engineering point of view. *Process Biochemistry*, 47(9), pp.1320-1326.

Cassidy, E.S., West, P.C., Gerber, J.S. and Foley, J.A. (2013) Redefining agricultural yields: from tonnes to people nourished per hectare. *Environmental Research Letters*, 8(3), p.034015.

Castro, L.F.C., Monroig, O., Leaver, M.J., Wilson, J., Cunha, I. and Tocher, D.R. (2012) Functional desaturase Fads1 (Δ5) and Fads2 (Δ6) orthologues evolved before the origin of jawed vertebrates. *PloS one*, 7(2), p.e31950.

CBS NEWS (2023). "Florida beaches overwhelmed by seaweed." Available at: https://www.cbsnews.com/news/florida-beaches-seaweed-sargassum-clumps-giant-blob/

Chakraborty, C., Sharma, A.R., Bhattacharya, M. and Lee, S.S. (2021) From COVID-19 to cancer mRNA vaccines: moving from bench to clinic in the vaccine landscape. *Frontiers in Immunology*, 12, p.2648.

Chan, J.C.C., Cheung, P.C.K. and Ang, P.O. (1997) Comparative studies on the effect of three drying methods on the nutritional composition of seaweed Sargassum hemiphyllum (turn.) C. Ag. *Journal of agricultural and food chemistry*, 45(8), pp.3056-3059.

Chandler, P. (1998) Checklists of insects of the British Isles (New Series). Part 1: Diptera. Royal Entomological Society. *Handbooks for the Identification of British Insects*, 12, pp.1-234.

Chapman, A.D. (2009) Numbers of living species in Australia and the world. Australian Biological Resources Study Canberra, Australia: Australian Biodiversity Information Services. Available

at: https://www.environment.gov.au/system/files/pages/2ee3f4a1-f130-465b-9c7a-79373680a067/files/nlsaw-2nd-complete.pdf [Accessed 14 March 2017].

Chapman, R.L. (2013) Algae: the world's most important "plants"—an introduction. *Mitigation and Adaptation Strategies for Global Change*, 18, pp.5-12.

Charlton, A.J., Dickinson, M., Wakefield, M.E., Fitches, E., Kenis, M., Han, R., Zhu, F., Kone, N., Grant, M., Devic, E. and Bruggeman, G. (2015) Exploring the chemical safety of fly larvae as a source of protein for animal feed. *Journal of Insects as Food and Feed*, 1(1), pp.7-16.

Charniaux-Cotton, H., 1957. Croissance, régénération et déterminisme endocrinien des caractères sexuels d'Orchestia gammarella Pallas (Crustacé Amphipode). Ann. Sci. Nat. Zool. Biol. Anim., 19, pp.411-559.

Charron, A.J. and Quatrano, R.S., 2009. Between a rock and a dry place: the water-stressed moss. Molecular Plant, 2(3), pp.478-486.

Chase, J.M., 2003. Community assembly: when should history matter?. *Oecologia*, *136*, pp.489-498.

Chesson, P., 1990. MacArthur's consumer-resource model. *Theoretical Population Biology*, *37*(1), pp.26-38.

Chesson, P., 1994. Multispecies competition in variable environments. *Theoretical population biology*, *45*(3), pp.227-276.

Chesson, P., 2000a. General theory of competitive coexistence in spatially-varying environments. *Theoretical population biology*, *58*(3), pp.211-237.

Chesson, P., 2000b. Mechanisms of maintenance of species diversity. *Annual review of Ecology and Systematics*, *31*(1), pp.343-366.

Chesson, P., 2003. Quantifying and testing coexistence mechanisms arising from recruitment fluctuations. *Theoretical population biology*, *64*(3), pp.345-357.

Chesson, P., 2011. Ecological niches and diversity maintenance. *Research in biodiversity: models and applications. InTech, Rijeka, Croatia*, pp.43-60.

Chesson, P., 2013. Species competition and predation. In *Ecological systems: Selected entries from the encyclopaedia of sustainability science and technology* (pp. 223-256). Springer New York.

Chesson, P., 2018. Updates on mechanisms of maintenance of species diversity. *Journal of ecology*, *106*(5), pp.1773-1794.

Chesson, P. and Huntly, N., 1997. The roles of harsh and fluctuating conditions in the dynamics of ecological communities. *The American Naturalist*, *150*(5), pp.519-553.

Chesson, P. and Kuang, J.J., 2008. The interaction between predation and competition. *Nature*, *456*(7219), pp.235-238.

Chesson, P.L. and Warner, R.R., 1981. Environmental variability promotes coexistence in lottery competitive systems. *The American Naturalist*, *117*(6), pp.923-943.

CNN, 2023. https://edition.cnn.com/2023/05/01/us/sargassum-seaweed-key-west-florida-tourism-scn-trnd/index.html

Cole, M.B., Augustin, M.A., Robertson, M.J. and Manners, J.M. (2018) The science of food security. *Science of Food*, 2(1), pp.1-8.

Coletta, J.M., Bell, S.J. and Roman, A.S. (2010) Omega-3 fatty acids and pregnancy. *Reviews in obstetrics and gynecology*, 3(4), p.163.

Colombini, I., Chelazzi, L., Gibson, R.N. and Atkinson, R.J.A. (2003) Influence of marine allochthonous input on sandy beach communities. *Oceanography and Marine Biology: An Annual Review*, 41, pp.115-159.

Costello, C., Cao, L., Gelcich, S., Cisneros-Mata, M.Á., Free, C.M., Froehlich, H.E., Golden, C.D., Ishimura, G., Maier, J., Macadam-Somer, I. and Mangin, T. (2020) The future of food from the sea. *Nature*, 588(7836), pp.95-100.

Crafford, J.E. and Scholtz, C.H. (1987) Phenology of stranded kelp degradation by the kelp fly Paractora dreuxi mirabilis (Helcomyzidae) at Marion Island. *Polar Biology*, 7(5), pp.289-294.

Crawford, M.A., Bloom, M., Broadhurst, C.L., Schmidt, W.F., Cunnane, S.C., Galli, C., Gehbremeskel, K., Linseisen, F., Lloyd-Smith, J. and Parkington, J. (1999) Evidence for the unique function of docosahexaenoic acid during the evolution of the modern hominid brain. *Lipids*, 34, pp.S39-S47.

Crawley, M. J. (2007) The R Book, Chichester, John Wiley & Sons.

Crawley, K.R., Hyndes, G.A., Vanderklift, M.A., Revill, A.T. and Nichols, P.D. (2009) Allochthonous brown algae are the primary food source for consumers in a temperate, coastal environment. *Marine Ecology Progress Series*, 376, pp.33-44.

Cury, P., Bakun, A., Crawford, R.J., Jarre, A., Quinones, R.A., Shannon, L.J. and Verheye, H.M. (2000) Small pelagics in upwelling systems: patterns of interaction and structural changes in "wasp-waist" ecosystems. *ICES Journal of Marine Science*, 57(3), pp.603-618.

Damude, H.G. and Kinney, A.J. (2007) Engineering oilseed plants for a sustainable, land-based source of long chain polyunsaturated fatty acids. *Lipids*, 42(3), pp.179-185.

Darwin, C. (1859) On the origin of species by means of natural selection, or the preservation of favoured races in the struggle for life. London: John Murray.

De Guevara, O.L., Padilla, P., García, L., Pino, J.M. and Ramos-Elorduy, J. (1995) Amino acid determination in some edible Mexican insects. *Amino Acids*, 9(2), pp.161-173.

Decker, H. and Van Holde, K.E. (2010) Oxygen and the Evolution of Life. *Springer Science & Business Media*.

Denlinger, D.L. (2009) Encyclopedia of insects. Chapter 72 - *Diapause*. Academic press, pp. 267-271.

Dept. of Agriculture, food, & forestry (2019) Available at: https://files.givewell.org/files/labs/animal-product-replacements/fs-cowweight.pdf

Deutsch, E.W., Mendoza, L., Shteynberg, D., Slagel, J., Sun, Z. and Moritz, R.L. (2015) Trans-Proteomic Pipeline, a standardized data processing pipeline for large-scale reproducible proteomics informatics. *PROTEOMICS–Clinical Applications*, 9(7-8), pp.745-754.

Dhillon, M.K., Hasan, F., Tanwar, A.K., Jaba, J., Singh, N. and Sharma, H.C. (2020) Genetic regulation of diapause and associated traits in Chilo partellus (Swinhoe). *Scientific Reports*, 10(1), pp.1-11.

Dierschke, V. (1998) High profit at high risk for juvenile Dunlins Calidris alpina stopping over at Helgoland(German bight). *Ardea*, 86(1), pp.59-69.

Dobson, T. (1976) Seaweed flies (Diptera: Coelopidae, etc.). *Marine insects*, p.447.

Dodds, W.K. (2002) Freshwater ecology: concepts and environmental applications. *Elsevier*.

Doi, H., Kikuchi, E., Takagi, S. and Shikano, S. (2007) Changes in carbon and nitrogen stable isotopes of chironomid larvae during growth, starvation and metamorphosis. Rapid Communications in Mass Spectrometry: *An International Journal Devoted to the Rapid Dissemination of Up-to-the-Minute Research in Mass Spectrometry*, 21(6), pp.997-1002.

dos Santos Aguilar, J.G. (2021) An overview of lipids from insects. *Biocatalysis and Agricultural Biotechnology*, 33, p.101967.

Doughman, S.D., Krupanidhi, S. and Sanjeevi, C.B. (2007) Omega-3 fatty acids for nutrition and medicine: considering microalgae oil as a vegetarian source of EPA and DHA. *Current diabetes reviews*, 3(3), pp.198-203.

Douglas Bates, Martin Maechler, Ben Bolker, Steve Walker (2015). Fitting Linear Mixed-Effects Models Using lme4. *Journal of Statistical Software*, 67(1), 1-48.

Drouin, G., Rioux, V. and Legrand, P. (2019) The n-3 docosapentaenoic acid (DPA): A new player in the n-3 long chain polyunsaturated fatty acid family. *Biochimie*, 159, pp.36-48.

Duffy, J.E. and Hay, M.E. (1990) Seaweed adaptations to herbivory.

Dugan, J.E., Hubbard, D.M., Page, H.M. and Schimel, J.P. (2011) Marine macrophyte wrack inputs and dissolved nutrients in beach sands. *Estuaries and Coasts*, 34(4), pp.839-850.

Dugan, J.E.,D.M.Hubbard,M.D.McCrary,and M.O. Pierson. (2003) The response of macrofauna communities and shorebirds to macrophyte wrack subsidies on exposed sandy beaches of southern California. *Estuarine, Coastal and Shelf Science*, 58: 25–40

Dunn, D.W., Sumner, J.P. and Goulson, D. (2005) The benefits of multiple mating to female seaweed flies, Coelopa frigida (Diptera: Coelpidae). *Behavioral Ecology and Sociobiology*, 58, pp.128-135.

Eaton, S.B. and Konner, M. (1985) Paleolithic nutrition: a consideration of its nature and current implications. *New England Journal of Medicine*, 312(5), pp.283-289.

Edward, D.A. and Gilburn, A.S. (2013) Male-specific genotype by environment interactions influence viability selection acting on a sexually selected inversion system in the seaweed fly, Coelopa frigida. *Evolution*, 67(1), pp.295-302.

Edward, D.A., Newton, J. and Gilburn, A.S. (2008) Investigating dietary preferences in two competing dipterans, Coelopa frigida and Coelopa pilipes, using stable isotope ratios of carbon and nitrogen. *Entomologia Experimentalis Et Applicata*, 127 (3), pp. 169-175.

EFSA Panel on Nutrition, Novel Foods and Food Allergens (NDA), Turck, D., Bohn, T., Castenmiller, J., De Henauw, S., Hirsch-Ernst, K.I., Maciuk, A., Mangelsdorf, I., McArdle, H.J., Naska, A. and Pelaez, C. (2021) Safety of frozen and dried formulations from whole house crickets (Acheta

domesticus) as a Novel food pursuant to Regulation (EU) 2015/2283. *EFSA Journal*, 19(8), p.e06779.

Eger, A.M., Marzinelli, E.M., Beas-Luna, R., Blain, C.O., Blamey, L.K., Byrnes, J.E., Carnell, P.E., Choi, C.G., Hessing-Lewis, M., Kim, K.Y. and Kumagai, N.H. (2023) The value of ecosystem services in global marine kelp forests. *Nature Communications*, 14(1), p.1894.

Egglishaw, H. J. 1958. The fauna of wrack-beds. PhD, University of Durham

Eibl, R., Meier, P., Stutz, I., Schildberger, D., Hühn, T. and Eibl, D. (2018) Plant cell culture technology in the cosmetics and food industries: current state and future trends. *Applied microbiology and biotechnology*, 102, pp.8661-8675.

EMBER (2022) Available at: https://ember-climate.org/app/uploads/2022/10/E3G-EMBER-Briefing-More-renewables-less-inflation.pdf

European Commission (2019) Sustainable Seaweed Farming in Europe: State of the Art, Innovative Practices and Future Perspectives. Brussels: European Commission. Available at: https://ec.europa.eu/research/participants/data/ref/h2020/other/publications/sea-farming-innovative-practices_en.pdf [Accessed 3 May 2023].

European Food Safety Authority, Aguilera, J., Aguilera-Gomez, M., Barrucci, F., Cocconcelli, P.S., Davies, H., Denslow, N., Lou Dorne, J., Grohmann, L., Herman, L. and Hogstrand, C. (2018) EFSA Scientific Colloquium 24–'omics in risk assessment: state of the art and next steps. *EFSA Supporting Publications*, 15(11), p.1512E.

Evershed, R.P., Davey Smith, G., Roffet-Salque, M., Timpson, A., Diekmann, Y., Lyon, M.S., Cramp, L.J., Casanova, E., Smyth, J., Whelton, H.L. and Dunne, J. (2022) Dairying, diseases and the evolution of lactase persistence in Europe. *Nature*, 608(7922), pp.336-345.

Field, C.B., Behrenfeld, M.J., Randerson, J.T. and Falkowski, P. (1998) Primary production of the biosphere: integrating terrestrial and oceanic components. *Science*, 281(5374), pp.237-240.

Filbee-Dexter, K. and Scheibling, R.E. (2012). Hurricane-mediated defoliation of kelp beds and pulsed delivery of kelp detritus to offshore sedimentary habitats. *Marine Ecology Progress Series*, 455, pp.51-64.

Filbee-Dexter, K. and Scheibling, R.E. (2014) Sea urchin barrens as alternative stable states of collapsed kelp ecosystems. *Marine ecology progress series*, 495, pp.1-25.

Food and Agricultural Organization (FAO). (2012) Balanced feeding for improving livestock productivity – increase in milk production and nutrient use efficiency and decrease in methane emission. *FAO Animal production and Health*, paper No. 173, Rome, Italy.

Food and Agriculture Organization of the United Nations (FAO). (2014) The State of World Fisheries and Aquaculture 2014. Rome. Available at: https://www.fao.org/3/i3720e/i3720e.pdf

Food and Agriculture Organization of the United Nations (FAO). (2020a) The State of World Fisheries and Aquaculture 2020. Sustainability in action. Rome. Available at: https://doi.org/10.4060/ca9229en

Food and Agriculture Organization of the United Nations (FAO). (2020b) Available at: https://www.fao.org/sustainability/news/detail/en/c/1274219/#:~:text=Global%20trends,of%20the%20global%20land%20surface.

Food Security Information Network. (2022) Global Report on Food Crises. Available at https://www.fsinplatform.org/

Ford, L., Stratakos, A.C., Theodoridou, K., Dick, J.T., Sheldrake, G.N., Linton, M., Corcionivoschi, N. and Walsh, P.J. (2020) Polyphenols from brown seaweeds as a potential antimicrobial agent in animal feeds. *Acs Omega*, 5(16), pp.9093-9103.

Free, C.M., Cabral, R.B., Froehlich, H.E., Battista, W., Ojea, E., O'Reilly, E., Palardy, J.E., García Molinos, J., Siegel, K.J., Arnason, R. and Juinio-Meñez, M.A. (2022) Expanding ocean food production under climate change. *Nature*, 605(7910), pp.490-496.

Friedl, T. (1997) The evolution of the green algae (pp. 87-101). Springer Vienna.

Froehlich, H.E., Afflerbach, J.C., Frazier, M. and Halpern, B.S. (2019) Blue growth potential to mitigate climate change through seaweed offsetting. *Current Biology*, 29(18), pp.3087-3093.

Fuller, R.A., Bearhop, S., Metcalfe, N.B. and Piersma, T. (2013) The effect of group size on vigilance in R uddy T urnstones A renaria interpres varies with foraging habitat. *Ibis*, 155(2), pp.246-257.

García, J.L., De Vicente, M. and Galán, B. (2017) Microalgae, old sustainable food and fashion nutraceuticals. *Microbial biotechnology*, 10(5), pp.1017-1024.

Garlock, T., Asche, F., Anderson, J., Bjørndal, T., Kumar, G., Lorenzen, K., Ropicki, A., Smith, M.D. and Tveterås, R. (2020) A global blue revolution: Aquaculture growth across regions, species, and countries. *Reviews in Fisheries Science & Aquaculture*, 28(1), pp.107-116.

Gasco, L., Belforti, M., Rotolo, L., Lussiana, C., Parisi, G., Terova, G., Roncarati, A. and Gai, F. (2014a) Mealworm (Tenebrio molitor) as a potential ingredient in practical diets for rainbow trout (Oncorhynchus mykiss). *Insects to Feed the World*. the Netherlands.14-17 May.

Gasco, L., Gai, F., Piccolo, G., Rotolo, L., Lussiana, C., Molla, P. and Chatzifotis, S. (2014b) Substitution of fish meal by Tenebrio molitor meal in the diet of Dicentrarchus labrax juveniles. *Insects to Feed the World*. the Netherlands.14-17 May.

Gerbault, P., Liebert, A., Itan, Y., Powell, A., Currat, M., Burger, J., Swallow, D.M. and Thomas, M.G. (2011) Evolution of lactase persistence: an example of human niche construction. Philosophical Transactions of the Royal Society B: *Biological Sciences*, 366(1566), pp.863-877.

Gerbens-Leenes, P.W., Nonhebel, S. and Krol, M.S. (2010) Food consumption patterns and economic growth. Increasing affluence and the use of natural resources. *Appetite*, 55(3), pp.597-608

Gey, G. (1952) Tissue culture studies of the proliferative capacity of cervical carcinoma and normal epithelium. *Cancer research*, 12, pp.264-265.

Gilburn, A.S. (2012) Mechanical grooming and beach award status are associated with low strandline biodiversity in Scotland. Estuarine, *Coastal and Shelf Science*, 107, pp.81-88.

Gilburn, A.S., Stewart, K.M. and Edward, D.A. (2009) Sex-biased phoretic mite load on two seaweed flies: Coelopa frigida and Coelopa pilipes. *Environmental entomology,* 38(6), pp.1608-1612.

Giovanni, S. (2015) Entomophagy and Italian consumers: An exploratory analysis. *Progress in Nutrition*, 17, no. 4: 311-316.

Gladyshev, M.I., Arts, M.T. and Sushchik, N.I. (2009) Preliminary estimates of the export of omega-3 highly unsaturated fatty acids (EPA+ DHA) from aquatic to terrestrial ecosystems. *Lipids in aquatic ecosystems*, pp.179-210.

Gladyshev, M.I., Sushchik, N.N., Anishchenko, O.V., Makhutova, O.N., Kolmakov, V.I., Kalachova, G.S., Kolmakova, A.A. and Dubovskaya, O.P. (2011) Efficiency of transfer of essential polyunsaturated fatty acids versus organic carbon from producers to consumers in a eutrophic reservoir. *Oecologia*, 165, pp.521-531.

Gladyshev, M.I., Anishchenko, O.V., Sushchnik, N.N., Kalacheva, G.S., Gribovskaya, I.V. and Ageev, A.V. (2012) Influence of anthropogenic pollution on content of essential polyunsaturated

fatty acids in links of food chain of river ecosystem. *Contemporary Problems of Ecology*, 5, pp.376-385.

Gladyshev, M.I., Sushchik, N.N. and Makhutova, O.N. (2013) Production of EPA and DHA in aquatic ecosystems and their transfer to the land. *Prostaglandins & other lipid mediators*, 107, pp.117-126.

Glazier, D.S. (2014) Amphipoda. Reference Module in Earth Systems and Environmental Sciences

Godfray, H.C.J., Beddington, J.R., Crute, I.R., Haddad, L., Lawrence, D., Muir, J.F., Pretty, J., Robinson, S., Thomas, S.M. and Toulmin, C. (2010) Food security: the challenge of feeding 9 billion people. *Science*, 327(5967), pp.812-818.

Google. (2023) available at: https://trends.google.com/trends/explore?date=all&q=%2Fg%2F11f3r48ldy&hl=en

Gosch, B.J., Magnusson, M., Paul, N.A. and De Nys, R. (2012) Total lipid and fatty acid composition of seaweeds for the selection of species for oil-based biofuel and bioproducts. *Bioenergy*, 4(6), pp.919-930.

Goswami, G., Bang, V. and Agarwal, S. (2015) Diverse applications of algae. *Int. J. Adv. Res. Sci. Eng,* 4(1), pp.1102-1109.

Graham LE, Wilcox LW (2000) Algae. Prentice Hall, New Jersey

Griffiths, C.L., and J.M.E. Stenton-Dozey. (1983) The fauna associated with kelp stranded on sandy beach. In Sandy beaches as ecosystems, ed. A. McLachlan and T. Erasmus, 557–568. The Netherlands: The Hague: Dr. W. Junk Publishers.

Griffiths, C.L., Stenton-Dozey, J.M.E. and Koop, K. (1983) Kelp wrack and the flow of energy through a sandy beach ecosystem. In Sandy beaches as ecosystems (pp. 547-556). Springer, Dordrecht.

Guschina, I.A. and Harwood, J.L. (2009) Algal lipids and effect of the environment on their biochemistry. *Lipids in aquatic ecosystems*, pp.1-24.

Haasova, S., Czellar, S., Rahmani, L. and Morgan, N. (2020) Connectedness with nature and individual responses to a pandemic: an exploratory study. *Frontiers in psychology*, 11, p.2215.

Hartmann, C. and Siegrist, M. (2017) Consumer perception and behaviour regarding sustainable protein consumption: A systematic review. *Trends in Food Science & Technology*, 61, pp.11-25.

Helland, I.B., Smith, L., Saarem, K., Saugstad, O.D. and Drevon, C.A. (2003) Maternal supplementation with very-long-chain n-3 fatty acids during pregnancy and lactation augments children's IQ at 4 years of age. *Pediatrics*, 111(1), pp.e39-e44.

Heymans, J.J., Coll, M., Libralato, S. and Christensen, V. (2012) Ecopath theory, modeling, and application to coastal ecosystems.

HilleRisLambers, J., Adler, P.B., Harpole, W.S., Levine, J.M. and Mayfield, M.M., 2012. Rethinking community assembly through the lens of coexistence theory. *Annual review of ecology, evolution, and systematics*, *43*, pp.227-248.

Hodge, S. & Arthur, W. (1997) Asymmetric interaction between species of seaweed fly. *Journal of Animal Ecology* 66, 743–754.

Hurd, C.L., Harrison, P.J., Bischof, K. and Lobban, C.S. (2014) Seaweed ecology and physiology. Cambridge University Press.

Innes, J.K., Calder, P.C. (2018) Omega-6 fatty acids and inflammation. *prostaglandin, leukotriene & essential fatty acids,* 132, pp.41-48

Itan, Y., Jones, B.L., Ingram, C.J., Swallow, D.M. and Thomas, M.G. (2010) A worldwide correlation of lactase persistence phenotype and genotypes. *BMC evolutionary biology*, 10(1), pp.1-11.

Jamieson, A.J., Lacey, N.C., Lörz, A.N., Rowden, A.A. and Piertney, S.B. (2013) The supergiant amphipod Alicella gigantea (Crustacea: Alicellidae) from hadal depths in the Kermadec Trench, SW Pacific Ocean. *Deep Sea Research Part II: Topical Studies in Oceanography*, 92, pp.107-113.

Jargin, S.V. (2022) The Conflict around Ukraine: Social and Environmental Aspects. *J Environ Stud*, 8(1), p.5.

Jeavons, J.C. (2001) Biointensive sustainable mini-farming: II. Perspective, principles, techniques and history. *Journal of Sustainable Agriculture*, 19(2), pp.65-76.

Jones, V.A. and Dolan, L. (2012) The evolution of root hairs and rhizoids. *Annals of botany*, 110(2), pp.205-212.

Judge, M.P., Harel, O. and Lammi-Keefe, C.J. (2007) Maternal consumption of a docosahexaenoic acid–containing functional food during pregnancy: benefit for infant performance on problem-solving but not on recognition memory tasks at age 9 mo. *The American journal of clinical nutrition,* 85(6), pp.1572-1577.

Julshamn, K., Dahl, L. and Eckhoff, K. (2001) Determination of iodine in seafood by inductively coupled plasma/mass spectrometry. *Journal of AOAC International*, 84(6), pp.1976-1983.

Kain, J.M. (1979) A review of the genus Laminaria. *Oceanographic*. Mar. Biol. 17, 101e161.

Kandale, A., Meena, A.K., Rao, M.M., Panda, P., Mangal, A.K., Reddy, G. and Babu, R. (2011) Marine algae: an introduction, food value and medicinal uses. *Journal of Pharmacy Research*, 4(1), pp.219-221.

Kaplan, E.L. and Meier, P. (1958) Nonparametric estimation from incomplete observations. *Journal of the American statistical association*, 53(282), pp.457-481.

Kaur, N., Chugh, V. and Gupta, A.K. (2014) Essential fatty acids as functional components of foods-a review. *Journal of food science and technology*, 51, pp.2289-2303.

Khaksar, R., Carlson, T., Schaffner, D.W., Ghorashi, M., Best, D., Jandhyala, S., Traverso, J. and Amini, S. (2015) Unmasking seafood mislabeling in US markets: DNA barcoding as a unique technology for food authentication and quality control. *Food Control*, 56, pp.71-76.

Kirkman, H. and Kendrick, G.A. (1997) Ecological significance and commercial harvesting of drifting and beach-cast macro-algae and seagrasses in Australia: a review. *Journal of Applied Phycology*, 9(4), pp.311-326.

Kirkpatrick, M. (2010) How and why chromosome inversions evolve. *PLoS biology*, 8(9), p.e1000501.

Klose, J. (1975) Protein mapping by combined isoelectric focusing and electrophoresis of mouse tissues: a novel approach to testing for induced point mutations in mammals. *Humangenetik*, 26, pp.231-243.

Koch, M., Bruckmoser, J., Scholl, J., Hauf, W., Rieger, B. and Forchhammer, K. (2020) Maximizing PHB content in Synechocystis sp. PCC 6803: a new metabolic engineering strategy based on the regulator PirC. *Microbial cell factories*, 19, pp.1-12.

Kolanowski, W., Stolyhwo, A. and Grabowski, M. (2007) Fatty acid composition of selected fresh water gammarids (Amphipoda, Crustacea): a potentially innovative source of omega-3 LC PUFA. *Journal of the American Oil Chemists' Society*, 84, pp.827-833.

Kolding, J., Bundy, A., van Zwieten, P. A. M. and Plank, M. J. (2016). Fisheries, the inverted food pyramid. *ICES Journal of Marine Science*, 73: 1697–1713.

Konner, M. and Eaton, S.B. (2010) Paleolithic nutrition: twenty-five years later. *Nutrition in Clinical Practice*, 25(6), pp.594-602.

Kotnala, G., Mandal, T.K., Sharma, S.K. and Kotnala, R.K. (2020) Emergence of blue sky over Delhi due to coronavirus disease (COVID-19) lockdown implications. *Aerosol Science and Engineering*, 4(3), pp.228-238.

Kris-Etherton PM, Harris WS, Appel LJ. (2002) Fish consumption, fish oil, omega-3 fatty acids, and cardiovascular disease. *Circulation*, 106, pp.2747–57.

Kroeckel, S., Harjes, A., Roth, I., Katz, H., Wuertz, S., Susenbeth, A. and Schulz, C. (2012) When a turbot catches a fly: Evaluation of a pre-pupae meal of the Black Soldier Fly (Hermetia illucens) as fish meal substitute—Growth performance and chitin degradation in juvenile turbot (Psetta maxima). *Aquaculture*,364, pp. 345-352.

Krumhansl, K.A. and Scheibling, R.E. (2011) Detrital production in Nova Scotian kelp beds: patterns and processes. *Marine Ecology Progress Series*, 421, pp.67-82.

Krumhansl, K.A. and Scheibling, R.E. (2012) Production and fate of kelp detritus. *Marine Ecology Progress Series*, 467, pp.281-302.

Kuczynska, P., Jemiola-Rzeminska, M. and Strzalka, K. (2015) Photosynthetic pigments in diatoms. *Marine drugs*, 13(9), pp.5847-5881.

Kump, L.R. (2008) The rise of atmospheric oxygen. *Nature*, 451(7176), pp.277-278.

Kvale, E.P. (2006) The origin of neap–spring tidal cycles. *Marine geology*, 235(1-4), pp.5-18.

Ladak, R.J., He, A.J., Huang, Y.H. and Ding, Y. (2022) The Current Landscape of mRNA Vaccines Against Viruses and Cancer–A Mini Review. *Frontiers in Immunology*, 13.

Lahdes, E.O., Farkas, T. and Lehtonen, K.K. (2010) Phospholipid characteristics and neutral lipid fatty acid composition related to temperature and nutritional conditions in ecologically important

amphipod species from the northern Baltic Sea. *Journal of Experimental Marine Biology and Ecology*, 390(2), pp.125-133.

Lane, K.E., Li, W., Smith, C. and Derbyshire, E. (2014) The bioavailability of an omega-3-rich algal oil is improved by nanoemulsion technology using yogurt as a food vehicle. *International Journal of Food Science & Technology*, 49(5), pp.1264-1271.

Larsen, J. and Roney, J.M. (2013) Farmed fish production overtakes beef. Washington, DC: Earth Policy Institute.

Lauritzen L, Hansen HS, Jorgensen MH, Michaelsen KF. (2001) The essentiality of long chain n-3 fatty acids in relation to development and function of the brain and retina. *Prog Lipid Res*v40:1–94.

Leblond, J.D., Evans, T.J. and Chapman, P.J. (2003) The biochemistry of dinoflagellate lipids, with particular reference to the fatty acid and sterol composition of a Karenia brevis bloom. *Phycologia*, 42(4), pp.324-331.

Lecrenier, M.C., Marbaix, H., Dieu, M., Veys, P., Saegerman, C., Raes, M. and Baeten, V. (2016) Identification of specific bovine blood biomarkers with a non-targeted approach using HPLC ESI tandem mass spectrometry. *Food Chemistry*, 213, pp.417-424.

Lecrenier, M.C., Planque, M., Dieu, M., Veys, P., Saegerman, C., Gillard, N. and Baeten, V. (2018) A mass spectrometry method for sensitive, specific and simultaneous detection of bovine blood meal, blood products and milk products in compound feed. *Food chemistry*, 245, pp.981-988.

Leggett, M. (1993) Competitive interactions between seaweed flies unpublished PhD Thesis University of Nottingham

Leliaert, F., Smith, D.R., Moreau, H., Herron, M.D., Verbruggen, H., Delwiche, C.F. and De Clerck, O. (2012) Phylogeny and molecular evolution of the green algae. *Critical reviews in plant sciences,* 31(1), pp.1-46.

Lenth R. (2023) _emmeans: Estimated Marginal Means, aka Least-Squares Means_. R package version 1.8.5, <https://CRAN.R-project.org/package=emmeans>.

Leonardi, M., Gerbault, P., Thomas, M.G. and Burger, J. (2012) The evolution of lactase persistence in Europe. A synthesis of archaeological and genetic evidence. *International Dairy Journal*, 22(2), pp.88-97.

Letunic, I. (2015) phyloT : Phylogenetic Tree Generator. [online] Phylot.biobyte.de. Available at: http://phylot.biobyte.de

Levine, J.M., Bascompte, J., Adler, P.B. and Allesina, S., 2017. Beyond pairwise mechanisms of species coexistence in complex communities. *Nature*, *546*(7656), pp.56-64.

Liland, N.S., Araujo, P., Xu, X.X., Lock, E.J., Radhakrishnan, G., Prabhu, A.J.P. and Belghit, I. (2021) A meta-analysis on the nutritional value of insects in aquafeeds. *Journal of Insects as Food and Feed*, 7(5), pp.743-759.

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), p.e0183188.

Lincoln, R.J. (1979) British marine amphipoda: Gammaridea (Vol. 818). British Museum (Natural History).

Lipman, D.J., Souvorov, A., Koonin, E.V., Panchenko, A.R. and Tatusova, T.A. (2002) The relationship of protein conservation and sequence length. *BMC evolutionary biology*, 2, pp.1-10.

Little, D. C., Edwards, P., & Demaine, H. (2016) Aquaculture and fisheries in the Blue Revolution: Challenges for a sustainable future. *Reviews in Aquaculture*, 8(3), 209-213.

Lock, E., Arsiwalla, T. and Waagbø, R. (2015) Insect larvae meal as an alternative source of nutrients in the diet of Atlantic salmon (Salmo salar) postsmolt. *Aquaculture Nutrition*, 22 (6), pp. 1202-1213.

Lock, E., Arsiwalla, T. and Waagbø, R. (2014) Insect meal: A promising source of nutrients in the diet of Atlantic salmon (Salmo salar). *Abstract Book Conference Insects to Feed the World*, p. 74.

Lock, E.R., Arsiwalla, T. and Waagbø, R. (2016) Insect larvae meal as an alternative source of nutrients in the diet of A tlantic salmon (S almo salar) postsmolt. *Aquaculture Nutrition*, 22(6), pp.1202-1213.

MacArtain, P., Gill, C.I., Brooks, M., Campbell, R. and Rowland, I.R. (2007) Nutritional value of edible seaweeds. *Nutrition reviews*, 65(12), pp.535-543.

MacMillan, M.R., and P.A. Quijón. (2012) Wrack patches and their influ- ence on upper-shore macrofaunal abundance in an Atlantic Canada sandy beach system. *Journal of Sea Research* 72: 28–37.

MacNeil, C., & Dick, J. T. (2013) The diet of the freshwater amphipod Echinogammarus stammeri (Karaman) in a stream of the Scottish Highlands. *Crustaceana*, 86(7-8), 851-867.

Mahata, S., Bhuiyan, A., Zaher, M., Hossain, M. and Hasan, M. (1994) Evaluation of silkworm pupae as dietary protein source for Thai sharpunti Puntius gonionotus. *Journal of Aquaculture in the Tropics*,9, pp. 77-85.

Makkar, H.P., Tran, G., Heuzé, V. and Ankers, P. (2014) State-of-the-art on use of insects as animal feed. *Animal Feed Science and Technology*, 197, pp.1-33.

Malm, T., Råberg, S., Fell, S. and Carlsson, P. (2004) Effects of beach cast cleaning on beach quality, microbial food web, and littoral macrofaunal biodiversity. *Estuarine, Coastal and Shelf Science,* 60(2), pp.339-347.

Manceron, S., Ben Ari, T. and Dumas, P. (2014) Feeding proteins to livestock: Global land use and food vs. feed competition.

Marien, A., Debode, F., Aerts, C., Ancion, C., Francis, F. and Berben, G. (2018) Detection of Hermetia illucens by real-time PCR. *Journal of Insects as Food and Feed*, 4(2), pp.115-122.

Marissen, R., Varunjikar, M. S., Laros, J. F., Rasinger, J. D., Neely, B. A., & Palmblad, M. (2022) compareMS2 2.0: An Improved Software for Comparing Tandem Mass Spectrometry Datasets. *Journal of Proteome Research*.

Mathis, W. N. and Mcalpine, D. K. (2011) A Catalog and Conspectus on the Family Coelopidae (Diptera: Schizophora). Available at: https://repository.si.edu/bitstream/handle/10088/18924/ent_MYIA12_Coelopidae.pdf (Accessed: 30 November 2018).

Mcalpine, D.K. (1991) Review of the Australian kelp flies Diptera: Coelopidae. *Systematic Entomology*, 16(1), pp.29-84.

McAlpine, J.F., Peterson, B.V., Shewell, G.E., Teskey, H.J., Vockeroth, J.R. and Wood, D.M. (1981)*nManual of Nearctic Diptera*. Volume 1 (No. 27).

McDougall, C.W., Brown, C., Thomson, C., Hanley, N., Tully, M.A., Quilliam, R.S., Bartie, P.J., Gibson, L. and Oliver, D.M. (2021) From one pandemic to another: emerging lessons from COVID-19 for tackling physical inactivity in cities. *Cities & Health*, 5(sup1), pp.S181-S184.

McNamara, R.K. and Carlson, S.E. (2006) Role of omega-3 fatty acids in brain development and function: potential implications for the pathogenesis and prevention of psychopathology. *Prostaglandins, Leukotrienes and Essential Fatty Acids*, *75*(4-5), pp.329-349.

Meader, S.J. and Gilburn, A.S. (2008) Asymmetrical costs of sexual conflict in the seaweed fly, Coelopa frigida. *Ecological Entomology*, 33(3), pp.380-384.

Mendes, A., Reis, A., Vasconcelos, R., Guerra, P. and Lopes da Silva, T. (2009) Crypthecodinium cohnii with emphasis on DHA production: a review. *Journal of applied phycology*, 21, pp.199-214.

Mereschkowsky, C. (1905) Uber natur und ursprung der chromatophoren im pflanzenreiche. *Biologisches Centralblatt*, 25, pp.293-604.

Merino, G., Barange, M., Mullon, C. and Rodwell, L. (2010) Impacts of global environmental change and aquaculture expansion on marine ecosystems. *Global Environmental Change*, 20(4), pp.586-596.

Mérot, C., Berdan, E.L., Babin, C., Normandeau, E., Wellenreuther, M., & Bernatchez, L. (2018). Intercontinental karyotype–environment parallelism supports a role for a chromosomal inversion in local adaptation in a seaweed fly. *Proceedings of the Royal Society B*, 285(1881), p.20180519.

Metcalfe, N.B., & Furness, R.W. (1984). Changing priorities: the effect of pre-migratory fattening on the trade-off between foraging and vigilance. *Behavioral Ecology and Sociobiology*, 15(3), pp.203-206.

Meyer, B., Atkinson, A., Bernard, K.S., Brierley, A.S., Driscoll, R., Hill, S.L., Marschoff, E., Maschette, D., Perry, F.A., Reiss, C.S., & Rombolá, E. (2020). Successful ecosystem-based management of Antarctic krill should address uncertainties in krill recruitment, behaviour and ecological adaptation. *Communications Earth & Environment*, 1(1), p.28.

Meyer, B.J., Mann, N.J., Lewis, J.L., Milligan, G.C., Sinclair, A.J., & Howe, P.R. (2003). Dietary intakes and food sources of omega-6 and omega-3 polyunsaturated fatty acids. *Lipids*, 38(4), pp.391-398.

Milledge, J.J., & Harvey, P.J. (2016). Ensilage and anaerobic digestion of Sargassum muticum. *Journal of applied phycology*, 28, pp.3021-3030.

Mittelbach, G.G. and Schemske, D.W., 2015. Ecological and evolutionary perspectives on community assembly. *Trends in ecology & evolution*, *30*(5), pp.241-247.

Moore, P.G., & Francis, C.H. (1985). Some observations on food and feeding of the supralittoral beach-hopper Orchestia gammarellus (Pallas)(Crustacea: Amphipoda). *Ophelia*, 24(3), pp.183-197.

Moroney, J.V., Bartlett, S.G., & Samuelsson, G. (2001). Carbonic anhydrases in plants and algae. *Plant, Cell & Environment*, 24(2), pp.141-153.

Morritt, D., & Stevenson, T.D. (1993). Factors influencing breeding initiation in the beachflea Orchestia gammarellus (Pallas)(Crustacea: Amphipoda). *Journal of Experimental Marine Biology and Ecology*, 165(2), pp.191-208.

Moyer, D.C., DeVries, J.W., & Spink, J. (2017). The economics of a food fraud incident–Case studies and examples including Melamine in Wheat Gluten. *Food Control*, 71, pp.358-364.

Mungkung, R., Aubin, J., Prihadi, T.H., Slembrouck, J., van der Werf, H.M., & Legendre, M. (2013). Life cycle assessment for environmentally sustainable aquaculture management: a case study of combined aquaculture systems for carp and tilapia. Journal of Cleaner Production, 57, pp.249-256.Nagy, K. and Tiuca, I.D., 2017. Importance of fatty acids in physiopathology of human body. *Fatty acids*. IntechOpen.

Nandeesha, M., Srikanth, G., Keshavanath, P., Varghese, T., Basavaraja, N. and Das, S. (1990) Effects of non-defatted silkworm-pupae in diets on the growth of common carp, Cyprinus carpio. *Biological Wastes*, 33 (1), pp. 17-23.

Nessen, M.A., van der Zwaan, D.J., Grevers, S., Dalebout, H., Staats, M., Kok, E., & Palmblad, M. (2016). Authentication of closely related fish and derived fish products using tandem mass spectrometry and spectral library matching. *Journal of agricultural and food chemistry*, 64(18), pp.3669-3677.

Ng, W., Liew, F., Ang, L., & Wong, K. (2001). Potential of mealworm (Tenebrio molitor) as an alternative protein source in practical diets for African catfish, Clarias gariepinus. *Aquaculture Research*, 32(s1), pp. 273-280.

NHS (2022). What should my daily intake of calories be? Available at: https://www.nhs.uk/common-health-questions/food-and-diet/what-should-my-daily-intake-of-calories-

be/#:~:text=Generally%2C%20the%20recommended%20daily%20calorie,women%20and%202%2C500%20for%20men. Accessed 27/11/2022.

Nijdam, D., Rood, T., & Westhoek, H. (2012). The price of protein: Review of land use and carbon footprints from life cycle assessments of animal food products and their substitutes. *Food policy*, 37(6), pp.760-770.

Norderhaug, K.M., & Christie, H.C. (2013). Lack of sea urchin settlement may explain kelp forest recovery in overgrazed areas in Norway. *Marine Ecology Progress Series*, 488, pp.119-132.

Norwegian Seafood Council (2021). Marine Fatty Acids: Omega-3 Oils and Fish Oil. Tromsø: Norwegian Seafood Council. Available at: https://en.seafood.no/globalassets/seafood/en/marine-fatty-acids/marine-fatty-acids-report-2021.pdf

Nova, P., Martins, A.P., Teixeira, C., Abreu, H., Silva, J.G., Silva, A.M., Freitas, A.C., & Gomes, A.M. (2020). Foods with microalgae and seaweeds fostering consumers' health: A review of scientific and market innovations. *Journal of Applied Phycology*, 32, pp.1789-1802.

O'Farrell, P.H. (1975). High-resolution two-dimensional electrophoresis of proteins. *Journal of biological chemistry*, 250(10), pp.4007-4021.

Office for National Statistics, 2021. How has lockdown changed our relationship with nature? Available at: https://www.ons.gov.uk/economy/environmentalaccounts/articles/howhaslockdownchangedourrelationshipwithnature/2021-04-26#:~:text=Along%20with%20the%20rise%20in,to%2046%25%20in%20July%202020.

Ojha, S., Bekhit, A.E.D., Grune, T., & Schlüter, O.K. (2021). Bioavailability of nutrients from edible insects*. Current Opinion in Food Science*, 41, pp.240-248.

Olabarria, C., Lastra, M., & Garrido, J. (2007). Succession of macrofauna on macroalgal wrack of an exposed sandy beach: effects of patch size and site*. Marine Environmental Research*, 63(1), pp.19-40.

Oliver, L., Dietrich, T., Marañón, I., Villarán, M.C., & Barrio, R.J. (2020). Producing omega-3 polyunsaturated fatty acids: A review of sustainable sources and future trends for the EPA and DHA market. *Resources*, 9(12), p.148.

Oonincx, D.G. and De Boer, I.J. (2012) Environmental impact of the production of mealworms as a protein source for humans–a life cycle assessment. *PloS One,* 7(12), e51145.

Oonincx, D.G., Van Itterbeeck, J., Heetkamp, M.J., Van Den Brand, H., Van Loon, J.J. and Van Huis, A. (2010) An exploration on greenhouse gas and ammonia production by insect species suitable for animal or human consumption. *PloS One*, 5(12), e14445.

Oppliger, L.V., Von Dassow, P., Bouchemousse, S., Robuchon, M., Valero, M., Correa, J.A., Mauger, S. and Destombe, C. (2014) Alteration of sexual reproduction and genetic diversity in the kelp species Laminaria digitata at the southern limit of its range. *PLoS One*, 9(7), e102518.

Orr, H.A. (2005) The genetic theory of adaptation: a brief history. *Nature Reviews Genetics*, 6(2), pp.119-127.

Orr, K.K. (2013) 'Predicting the ecosystem effects of harvesting beach-cast kelp for biofuel' A thesis presented for the degree of Doctor of Philosophy at the University of Aberdeen Declaration.

Orr, K.K., Wilding, T.A., Horstmeyer, L., Weigl, S. and Heymans, J.J. (2014) Detached macroalgae: its importance to inshore sandy beach fauna. *Estuarine, Coastal and Shelf Science*, 150, pp.125-135.

Ortuño, J., Ball, E., Linton, M., Richmond, A., Corcionivoschi, N. and Theodoridou, K. (2021) Effect of soyameal replacement by Black Soldier Fly (Hermetia illucens) meal in broiler diets on productive performance. *British Poultry Abstracts*, 17(1), pp.10-11.

Ottoboni, M., Tretola, M., Cheli, F., Marchis, D., Veys, P., Baeten, V. and Pinotti, L. (2017) Light microscopy with differential staining techniques for the characterisation and discrimination of insects versus marine arthropods processed animal proteins. *Food Additives & Contaminants: Part A*, 34(8), pp.1377-1383.

Öz, M., Ucak, İ. and Nayik, G.A. (2022) PUFA and MUFA. *Nutraceuticals and Health Care* (pp. 199-215). Academic Press.

Palmblad, M. and Deelder, A.M. (2012) Molecular phylogenetics by direct comparison of tandem mass spectra. *Rapid Communications in Mass Spectrometry*, 26(7), pp.728-732.

Pandey, K., de Bruijn, J., de Moel, H., & Aerts, J. (2023). Simulating the effects of sea level rise and soil salinization on migration and adaptation in coastal Mozambique (No. EGU23-409). Copernicus Meetings.

Patras, D., Moraru, C. V., & Socaciu, C. (2019). Bioactive ingredients from microalgae: food and feed applications. *BUASVMCN-FST*, 76, 1-9.

Paul, N. A., de Nys, R., & Steinberg, P. D. (2006). Chemical defence against bacteria in the red alga Asparagopsis armata: linking structure with function. *Marine Ecology Progress Series*, 306, 87-101.

Payne, C. L., Dobermann, D., Forkes, A., House, J., Josephs, J., McBride, A., Müller, A., Quilliam, R. S., & Soares, S. (2016). Insects as food and feed: European perspectives on recent research and future priorities. *Journal of insects as Food and Feed*, 2(4), 269-276.

Pernet, F., Tremblay, R., Demers, E., & Roussy, M. (2003). Variation of lipid class and fatty acid composition of Chaetoceros muelleri and Isochrysis sp. grown in a semicontinuous system. *Aquaculture*, 221(1-4), 393-406.

Persson, L. E. (1999). Growth and reproduction in two brackish water populations of Orchestia gammarus (Amphipoda: Talitridae) in the Baltic Sea. *Journal of Crustacean Biology*, 19, 53-59.

Peters, C. J., Picardy, J., Darrouzet-Nardi, A. F., Wilkins, J. L., Griffin, T. S., & Fick, G. W. (2016). Carrying capacity of US agricultural land: Ten diet scenarios. *Elementa: Science of the Anthropocene*, 4.

Phillips, D. S., & Arthur, W. (1994). Observations on the distribution of seaweed fly larvae and other invertebrates within a wrack-bed. *The Entomologist*, 113(2), 154-163.

Piccolo, G., Marono, S., Gasco, L., Iannaccone, F., Bovera, F., & Nizza, A. (2014). Use of Tenebrio molitor larvae meal in diets for gilthead sea bream Sparus aurata juveniles. *Insects to Feed the World*, the Netherlands, 14-17.

Plourde, M., & Cunnane, S. C. (2007). Extremely limited synthesis of long chain polyunsaturates in adults: implications for their dietary essentiality and use as supplements. *Applied physiology, nutrition, and metabolism*, 32(4), 619-634.

Pooja, S. (2014). Algae used as medicine and food-a short review. *Journal of Pharmaceutical Sciences and Research*, 6(1), 33.

Public Health England. (2021) “Seven in 10 adults are motivated to get healthier in 2021 due to COVID-19”. Available at: https://www.gov.uk/government/news/seven-in-10-adults-are-motivated-to-get-healthier-in-2021-due-to-covid-19

Purvis, B., Mao, Y., & Robinson, D. (2019). Three pillars of sustainability: in search of conceptual origins. *Sustainability Science*, 14(3), 681-695.

R Core Team. (2022). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Rahman, M., Zaher, M., Mazid, M., Haque, M., & Mahata, S. (1996). Replacement of costly fish meal by silkworm pupae in the diet of mirror carp (Cyprinus carpio L.). *Pakistan Journal of Scientific and Industrial Research*, 39(1-4), 64-67.

Ramos-Elorduy, J., Moreno, J. M. P., Prado, E. E., Perez, M. A., Otero, J. L., & De Guevara, O. L. (1997). Nutritional value of edible insects from the state of Oaxaca, Mexico. *Journal of Food Composition and Analysis,* 10(2), 142-157.

Rasinger, J. D., Marbaix, H., Dieu, M., Fumière, O., Mauro, S., Palmblad, M., Raes, M., & Berntssen, M. H. G. (2016). Species and tissue-specific differentiation of processed animal proteins in aquafeeds using proteomics tools. *Journal of Proteomics*, 147, 125-131.

Ray, D. K., Mueller, N. D., West, P. C., & Foley, J. A. (2013). Yield trends are insufficient to double global crop production by 2050. *PLoS One*, 8(6), e66428.

Refstie, S., Storebakken, T., & Roem, A. J. (1998). Feed consumption and conversion in Atlantic salmon (Salmo salar) fed diets with fish meal, extracted soybean meal or soybean meal with a reduced content of oligosaccharides, trypsin inhibitors, lectins, and soya antigens. *Aquaculture*, 162(3), 301-312.

REGULATION (EC) No 1069/2009 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 21 October 2009 laying down health rules as regards animal by-products and derived products not intended for human consumption and repealing Regulation (EC) No 1774/2002 (Animal by-products Regulation). Retrieved from https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2009:300:0001:0033:en:PDF

REGULATION (EC) No 1169/2011 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 25 October 2011 on the provision of food information to consumers, amending Regulations (EC) No 1924/2006 and (EC) No 1925/2006 of the European Parliament and of the Council, and repealing Commission Directive 87/250/EEC, Council Directive 90/496/EEC, Commission Directive 1999/10/EC, Directive 2000/13/EC of the European Parliament and of the Council, Commission Directives 2002/67/EC and 2008/5/EC and Commission Regulation (EC) No

608/2004. Retrieved from https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2011:304:0018:0063:en:PDF

Regulation (EC) No 2470/2017 of 20 December 2017 establishing the Union list of novel foods in accordance with Regulation (EU) 2015/2283 of the European Parliament and of the Council on novel foods. Available at http://data.europa.eu/eli/reg_impl/2017/2470/oj

Regulation (EC) No 58/2023 of 5 January 2023 authorising the placing on the market of the frozen, paste, dried and powder forms of Alphitobius diaperinus larvae (lesser mealworm) as a novel food and amending Implementing Regulation (EU) 2017/2470. Available at http://data.europa.eu/eli/reg_impl/2023/58/oj

Reka, A., Anovski, T., Bogoevski, S., Pavlovski, B., & Boškovski, B. (2014). Physical-chemical and mineralogical-petrographic examinations of diatomite from deposit near the village of Rožden, Republic of Macedonia. *Geologica Macedonica*, 28(2), 121-126.

Remize, M., Brunel, Y., Silva, J. L., Berthon, J. Y., & Filaire, E. (2021). Microalgae n-3 PUFAs production and use in food and feed industries. *Marine Drugs*, 19(2), 113.

Ribeiro, J. C., Sousa-Pinto, B., Fonseca, J., Fonseca, S. C., & Cunha, L. M. (2021). Edible insects and food safety: allergy. *Journal of Insects as Food and Feed*, 7(5), 833-847.

Rillig, M. C. (2004). Arbuscular mycorrhizae and terrestrial ecosystem processes. *Ecology Letters*, 7(8), 740-754.

Rizal, A. R. A., & Nordin, S. M. (2022). Getting ahead of the pandemic curve: A systematic review of critical determining factors for innovation adoption in ensuring food security. *Frontier in Nutrition*, 9.

Robert, S. S. (2006). Production of eicosapentaenoic and docosahexaenoic acid-containing oils in transgenic land plants for human and aquaculture nutrition. *Marine Biotechnology*, 8(2), 103-109.

Rockwood, L. L. (2015). Introduction to population ecology. John Wiley & Sons.

Roque, B. M., Venegas, M., Kinley, R. D., de Nys, R., Duarte, T. L., Yang, X., & Kebreab, E. (2021). Red seaweed (Asparagopsis taxiformis) supplementation reduces enteric methane by over 80 percent in beef steers. *PLoS One*, 16(3), e0247820.

Rothäusler, E., Gutow, L., et al. (2012). Floating seaweeds and their communities. *Seaweed Biology* (pp. 359, 380). Springer.

Ruiz-Delgado, M. C., Reyes-Martínez, M. J., Sánchez-Moyano, J. E., López-Pérez, J., & García-García, F. J. (2015). Distribution patterns of supralittoral arthropods: wrack deposits as a source of food and refuge on exposed sandy beaches (SW Spain). *Hydrobiologia*, 742, 205-219.

Ruiz-Delgado, M.C., Vieira, J.V., Reyes-Martínez, M.J., Borzone, C.A., Sánchez-Moyano, J.E. and García-García, F.J., 2016. Wrack removal as short-term disturbance for Talitrus saltator density in the supratidal zone of sandy beaches: an experimental approach. Estuaries and Coasts, 39(4), pp.1113-1121.

Ruiz-López, N., Sayanova, O., Napier, J.A. and Haslam, R.P. (2012) Metabolic engineering of the omega-3 long chain polyunsaturated fatty acid biosynthetic pathway into transgenic plants. *Journal of experimental botany*, 63(7), pp.2397-2410.

Rumpold, B.A. and Schlüter, O.K. (2013) Potential and challenges of insects as an innovative source for food and feed production. *Innovative Food Science & Emerging Technologies*, 17, pp.1-11.

Rustan, A.C. and Drevon, C.A. (2001) Fatty acids: structures and properties. e LS.

Ruxton, C.H.S., Reed, S.C., Simpson, M.J.A. and Millington, K.J. (2004) The health benefits of omega-3 polyunsaturated fatty acids: a review of the evidence. *Journal of Human Nutrition and Dietetics*, 17(5), pp.449-459.

Saenz de San Pedro, B., López Guerrero, A., Navarrete Del Pino, M. A., Alcántara Villar, M., Álvarez, F., López-Matas, M. A., & Carnés, J. (2023). Myosin Heavy Chain: An Allergen Involved in Anaphylaxis to Shrimp Head. *Journal of investigational allergology & clinical immunology*, 33(1), 66–68.

Saito, L., Johnson, B.M., Bartholow, J. and Hanna, R.B. (2001) Assessing ecosystem effects of reservoir operations using food web–energy transfer and water quality models. *Ecosystems*, 4(2), pp.105-125.

Salque, M., Bogucki, P.I., Pyzel, J., Sobkowiak-Tabaka, I., Grygiel, R., Szmyt, M. and Evershed, R.P. (2013) Earliest evidence for cheese making in the sixth millennium BC in northern Europe. *Nature*, 493(7433), pp.522-525.

Sampedro, L., Jeannotte, R. and Whalen, J.K. (2006) Trophic transfer of fatty acids from gut microbiota to the earthworm Lumbricus terrestris L. *Soil Biology and Biochemistry*, 38(8), pp.2188-2198.

Sánchez-Muros, M., Barroso, F.G. and Manzano-Agugliaro, F. (2014) Insect meal as renewable source of food for animal feeding: a review. *Journal of Cleaner Production*,65, pp. 16-27.

Sandison, F., Yeluripati, J. and Stewart, D. (2022) Does green vertical farming offer a sustainable alternative to conventional methods of production?: A case study from Scotland. *Food and Energy Security*, p.e438.

Sargent, J., Bell, G., McEvoy, L., Tocher, D. and Estevez, A. (1999) Recent developments in the essential fatty acid nutrition of fish. *Aquaculture*, 177(1-4), pp.191-199.

Sayanova, O.V. and Napier, J.A. (2004) Eicosapentaenoic acid: biosynthetic routes and the potential for synthesis in transgenic plants. *Phytochemistry*, 65(2), pp.147-158.

Scapini, F., L. Chelazzi, I. Colombini, and M. Fallaci. (1992) Surface activity, zonation and migrations of Talitrus saltator on a Mediterranean beach. *Marine Biology* 112: 573–581.

Schabel, H. G. (2010). Forest insects as food: A global review. Forest insects as food: Humans Bite Back. *Food & Agriculture Org*. pp. 37-64.

Scheiner, S.M. and Gurevitch, J. eds. (2001) Design and analysis of ecological experiments. Oxford University Press.

Scherbov, S., Lutz, W. and Sanderson, W.C. (2011) The uncertain timing of reaching 8 billion, peak world population, and other demographic milestones. *Population and Development Review*, 37(3), pp.571-578.

Schiel, D.R. and Foster, M.S. (2015) The biology and ecology of giant kelp forests. Univ of California Press.

Scottish Salmon Producers Organization. (2021). SSPO Fish Farm Production Survey 2020. Available at: https://scottishsalmon.co.uk/reports/salmon-farming-in-scotland/2020-production-survey/

Sealey, W.M., Gaylord, T.G., Barrows, F.T., Tomberlin, J.K., McGuire, M.A., Ross, C. and St-Hilaire, S. (2011) Sensory analysis of rainbow trout, Oncorhynchus mykiss, fed enriched black soldier fly prepupae, Hermetia illucens. *Journal of the World Aquaculture Society*, 42 (1), pp. 34-45.

Sheath, R.G. and Vis, M.L. (2015) Red algae. *Freshwater Algae of North America* (pp. 237-264). Academic Press.

Shetty, S.S., Kumari, N.S. and Varadarajan, R. (2023) The Ratio of Omega-6/Omega-3 Fatty Acid: Implications and Application as a Marker to Diabetes. *Biomarkers in Diabetes*, p.449.

Shipley, J.R., Twining, C.W., Mathieu-Resuge, M., Parmar, T.P., Kainz, M., Martin-Creuzburg, D., Weber, C., Winkler, D.W., Graham, C.H. and Matthews, B. (2022) Climate change shifts the timing of nutritional flux from aquatic insects. *Current biology*, 32(6), pp.1342-1349.

Shorrocks, B., Atkinson, W. and Charlesworth, P. (1979) Competition on a divided and ephemeral resource. *The Journal of Animal Ecology*, pp.899-908.

Shyama, S. and Keshavanath, P. (1993) Growth response of Tor khudree to silkworm pupa incorporated diets. *Colloques De l'INRA* (France).

Simopoulos, A.P. (1999a) Essential fatty acids in health and chronic disease. *The American journal of clinical nutrition*, 70(3), pp.560s-569s.

Simopoulos, A.P. (1999b) Evolutionary aspects of omega-3 fatty acids in the food supply. *Prostaglandins, Leukotrienes and essential fatty acids*, 60(5-6), pp.421-429.

Simopoulos, A. P. (2002) The importance of the ratio of omega-6/omega-3 essential fatty acids. *Biomedicine & pharmacotherapy* 56(8), pp.365-379.

Siva, N., 2022. COP27: a “collective failure”. *The Lancet*, *400*(10366), p.1835.

Slansky Jr, F. (1993) Nutritional ecology: the fundamental quest for nutrients. *Caterpillars: ecological and evolutionary constraints on foraging*, pp.29-91.

Smith, B.D. and Foreman, R.E. (1984) An assessment of seaweed decomposition within a southern Strait of Georgia seaweed community. *Marine Biology*, 84(2), pp.197-205.

Smith, P.G. and Bradley, R. (2003) Bovine spongiform encephalopathy (BSE) and its epidemiology. *British medical bulletin*, 66(1), pp.185-198.

Sørensen, M., Berge, G.M., Thomassen, M.S., Ruyter, B., Hatlen, B., Ytrestøyl, T., Aas, T.S. and Åsgård, T.E. (2011) Today’s and tomorrow's feed ingredients in Norwegian aquaculture. Nofima rapportserie.

Spaak, J.W. and Schreiber, S.J., 2023. Building modern coexistence theory from the ground up: the role of community assembly. *bioRxiv*, pp.2023-01.

Spiegel, M., Noordam, M.Y. and Van der Fels-Klerx, H.J. (2013) Safety of novel protein sources (insects, microalgae, seaweed, duckweed, and rapeseed) and legislative aspects for their application in food and feed production. *Comprehensive reviews in food science and food safety*, 12(6), pp.662-678.

Sprague, M., Dick, J.R. and Tocher, D.R. (2016) Impact of sustainable feeds on omega-3 long-chain fatty acid levels in farmed Atlantic salmon, 2006–2015. *Scientific reports*, 6(1), pp.1-9.

Spranghers, T., Ottoboni, M., Klootwijk, C., Ovyn, A., Deboosere, S., De Meulenaer, B., Michiels, J., Eeckhout, M., De Clercq, P. and De Smet, S. (2017) Nutritional composition of black soldier fly (Hermetia illucens) prepupae reared on different organic waste substrates. *Journal of the Science of Food and Agriculture,* 97(8), pp.2594-2600.

Stanciu, S., Stanciuc, N., Dumitrascu, L., Ion, R. and Nistor, C. (2013) The effects of horse meat scandal on Romanian meat market. *SEA-Practical Application of Science*, 1(1), pp.174-181.

Stark, A.H., Crawford, M.A. and Reifen, R. (2008) Update on alpha-linolenic acid. *Nutrition reviews,* 66(6), pp.326-332.

Stearns, S.C. and Hoekstra, R.F. (2000) Evolution, an introduction. Oxford University Press.

Steinhilber, A.E., Schmidt, F.F., Naboulsi, W., Planatscher, H., Niedzwiecka, A., Zagon, J., Braeuning, A., Lampen, A., Joos, T.O. and Poetz, O. (2018) Mass spectrometry-based immunoassay for the quantification of banned ruminant processed animal proteins in vegetal feeds. *Analytical chemistry*, 90(6), pp.4135-4143.

Steneck, R.S., Graham, M.H., Bourque, B.J., Corbett, D., Erlandson, J.M., Estes, J.A. and Tegner, M.J., (2002) Kelp forest ecosystems: biodiversity, stability, resilience and future. *Environmental conservation*, 29(4), pp.436-459.

Stenton-Dozey, J. and Griffiths, C.L. (1980) Growth, consumption and respiration by larvae of the kelp-fly Fucellia capensis (Diptera: Anthomyiidae). *African Zoology*, 15(4), pp.280-283.

Stephensen, K. (1925) Crustacea Malacostraca. VI.(Amphipoda II). *Danish Ingolf-Expedition*, 3(9), pp.98-180.

Stévant, P., Marfaing, H., Rustad, T., Sandbakken, I., Fleurence, J. and Chapman, A. (2017) Nutritional value of the kelps Alaria esculenta and Saccharina latissima and effects of short-term storage on biomass quality. *Journal of applied phycology*, 29, pp.2417-2426.

St-Hilaire, S., Sheppard, C., Tomberlin, J.K., Irving, S., Newton, L., McGuire, M.A., Mosley, E.E., Hardy, R.W. and Sealey, W. (2007) Fly prepupae as a feedstuff for rainbow trout, Oncorhynchus mykiss. *Journal of the World Aquaculture Society*, 38(1), pp.59-67.

Stoll-Kleemann, S. and Schmidt, U.J. (2017) Reducing meat consumption in developed and transition countries to counter climate change and biodiversity loss: a review of influence factors. *Regional Environmental Change*, 17(5), pp.1261-1277.

Sturaro, N., Caut, S., Gobert, S., Bouquegneau, J.M. and Lepoint, G. (2010) Trophic diversity of idoteids (Crustacea, Isopoda) inhabiting the Posidonia oceanica litter. *Marine biology*, 157(2), pp.237-247.

Subasinghe, R., Soto, D. and Jia, J. (2009) Global aquaculture and its role in sustainable development. *Reviews in Aquaculture*, 1(1), pp.2-9.

Summers, R.W., Smith, S., Nicoll, M. and Atkinson, N.K. (1990) Tidal and sexual differences in the diet of Purple Sandpipers Calidris maritima in Scotland. *Bird Study*, 37(3), pp.187-194.

Sun, D.A. and Patel, N.H. (2019) The amphipod crustacean Parhyale hawaiensis: An emerging comparative model of arthropod development, evolution, and regeneration. *Wiley Interdisciplinary Reviews: Developmental Biology*, 8(5), p.e355.

Tacon, A.G. and Metian, M. (2008) Global overview on the use of fish meal and fish oil in industrially compounded aquafeeds: Trends and future prospects. *Aquaculture*, 285(1-4), pp.146-158.

Taipale, S., Peltomaa, E. and Salmi, P. (2020) Variation in ω-3 and ω-6 polyunsaturated fatty acids produced by different phytoplankton taxa at early and late growth phase. *Biomolecules*, 10(4), p.559.

Tanaka, T., Ikita, K., Ashida, T., Motoyama, Y., Yamaguchi, Y. and Satouchi, K. (1996) Effects of growth temperature on the fatty acid composition of the free-living nematodeCaenorhabditis elegans. *Lipids*, 31(11), pp.1173-1178.

Tarr, J.G., Griffiths, C.L. and Bally, R. (1985) The ecology of three sandy beaches on the Skeleton Coast of South West Africa. *Madoqua*, 14(3), pp.295-304.

Terry M. Therneau & Patricia M. Grambsch (2000). Modeling Survival Data: Extending the Cox Model. Springer, New York. ISBN 0-387-98784-3.

Tilman, D., Balzer, C., Hill, J. and Befort, B.L. (2011) Global food demand and the sustainable intensification of agriculture. *Proceedings of the National Academy of Sciences*, 108(50), pp.20260-20264.

Tocher, D. R. (2010). Dietary requirements and metabolism of omega-3 fatty acids in fish. *Aquaculture Nutrition*, 16(2), pp.75-88.

Tocher, D.R., Betancor, M.B., Sprague, M., Olsen, R.E. and Napier, J.A. (2019) Omega-3 long-chain polyunsaturated fatty acids, EPA and DHA: bridging the gap between supply and demand. *Nutrients*, 11(1), p.89.

Togarcheti, S.C., kumar Mediboyina, M., Chauhan, V.S., Mukherji, S., Ravi, S. and Mudliar, S.N. (2017) Life cycle assessment of microalgae based biodiesel production to evaluate the impact of biomass productivity and energy source. *Resources, Conservation and Recycling*, 122, pp.286-294.

Tomberlin, J.K., Van Huis, A., Benbow, M.E., Jordan, H., Astuti, D.A., Azzollini, D., Banks, I., Bava, V., Borgemeister, C., Cammack, J.A. and Chapkin, R.S. (2015) Protecting the environment through insect farming as a means to produce protein for use as livestock, poultry, and aquaculture feed. *Journal of Insects as Food and Feed*, 1(4), pp.307-309.

Twining, C.W., Brenna, J.T., Lawrence, P., Shipley, J.R., Tollefson, T.N. and Winkler, D.W. (2016) Omega-3 long-chain polyunsaturated fatty acids support aerial insectivore performance more than food quantity. *Proceedings of the National Academy of Sciences*, 113(39), pp.10920-10925.

Twining, C.W., Shipley, J.R. and Winkler, D.W. (2018) Aquatic insects rich in omega-3 fatty acids drive breeding success in a widespread bird. *Ecology Letters,* 21(12), pp.1812-1820.

Ugolini, A., Perra, G., Focardi, S., Somigli, S., Martellini, T. and Cincinelli, A. (2012) Sandhopper Talitrus saltator (Montagu) as a bioindicator of contamination by polycyclic aromatic hydrocarbons. *Bulletin of environmental contamination and toxicology*, 89, pp.1272-1276.

Umen, J.G., 2020. Volvox and volvocine green algae. *EvoDevo*, 11(1), p.13.

UN. (2022). Global Compact for Safe, Orderly and Regular Migration. Available at: https://news.un.org/pages/wp-content/uploads/2022/08/GCRG_3rd-Brief_Aug3_2022_FINAL.pdf

UN Department of Economic and Social Affairs. Population Division (2014). World Urbanization Prospects: The 2014 Revision. Available at: https://www.un.org/development/desa/dpad/wp-content/uploads/sites/45/publication/PB_140.pdf

United Nations Framework Convention on Climate Change (UNFCCC), 2022. Available at https://unfccc.int/process-and-meetings/conferences/sharm-el-sheikh-climate-change-conference-november-2022/five-key-takeaways-from-cop27

United States Geological Survey (USGS). (2019). Water Science School: Earth's Surface. Available at:. https://www.usgs.gov/special-topic/water-science-school/science/earths-surface-water?qt-science_center_objects=0#qt-science_center_objects

USDA. (2011). Beef & Veal Nutrition Facts. Available at: https://www.fsis.usda.gov/sites/default/files/import/Beef_Veal_Nutrition_Facts.pdf

Uttaro, A.D. (2006) Biosynthesis of polyunsaturated fatty acids in lower eukaryotes. *IUBMB life*, 58(10), pp.563-571.

Van der Spiegel, M., Noordam, M. and Fels-Klerx, H. (2013) Safety of novel protein sources (insects, microalgae, seaweed, duckweed, and rapeseed) and legislative aspects for their application in food and feed production. *Comprehensive Reviews in Food Science and Food Safety*, 12 (6), pp. 662-678.

Van Huis, A. (2017) Edible Insects and Research Needs. *Journal of Insects as Food and Feed,* 3 (1), pp. 3-5.

Van Huis, A., 2020. Insects as food and feed, a new emerging agricultural sector: a review. *Journal of Insects as Food and Feed,* 6(1), pp.27-44.

Van Huis, A., Dicke, M. and Van Loon, J. (2015) Insects to feed the world. *Journal of Insects as Food and Feed,* 1 (1), pp. 3-5.

Van Huis, A., Van Itterbeeck, J., Klunder, H., Mertens, E., Halloran, A., Muir, G. and Vantomme, P. (2013) Edible insects: future prospects for food and feed security (No. 171). Food and Agriculture Organization of the United Nations.

van Scheltinga, C.T., Wilbers, G.J., Islam, F., Debrot, D., Verburg, C., Barrantes, M.N., Reinhard, S. and Veldhuizen, A. (2023) Food systems in the Bangladesh Delta: Overview of food systems in

Bangladesh with a focus on the coastal south west. Wageningen Environmental Research Report 3233- 57

Varunjikar, M.S., Belghit, I., Gjerde, J., Palmblad, M., Oveland, E. and Rasinger, J.D. (2022a) Shotgun proteomics approaches for authentication, biological analyses, and allergen detection in feed and food-grade insect species. *Food Control*, 137, p.108888.

Varunjikar, M.S., Moreno-Ibarguen, C., Andrade-Martinez, J.S., Tung, H.S., Belghit, I., Palmblad, M., Olsvik, P.A., Reyes, A., Rasinger, J.D. and Lie, K.K. (2022b) Comparing novel shotgun DNA sequencing and state-of-the-art proteomics approaches for authentication of fish species in mixed samples. *Food Control*, 131, p.108417.

Venables, W. N. & Ripley, B. D. (2002) Modern Applied Statistics with S. Fourth Edition. *Springer*, New York. ISBN 0-387-95457-0

Veramaris. (2021) Optimum omega nutrition for sustainable aquaculture [White paper]. Available at: https://veramaris.com/optimum-omega-nutrition-marine-fish.html

Walker, F.T. and Richardson, W.D. (1955) An ecological investigation of Laminaria cloustoni Edm.(L. hyperborea Fosl.) around Scotland. *The Journal of Ecology*, pp.26-38.

Walker, F.T. (1954) Distribution of Laminariaceae around Scotland. *Nature*, 173(4408), p.766.

Wall, R., Ross, R. P., Fitzgerald, G. F., & Stanton, C. (2010). Fatty acids from fish: the anti-inflammatory potential of long-chain omega-3 fatty acids. *Nutrition reviews*, 68(5), pp.280-289.

Wan, A.H., Davies, S.J., Soler-Vila, A., Fitzgerald, R. and Johnson, M.P. (2019) Macroalgae as a sustainable aquafeed ingredient. *Reviews in Aquaculture*, 11(3), pp.458-492.

Wang, H.M.D., Chen, C.C., Huynh, P. and Chang, J.S. (2015) Exploring the potential of using algae in cosmetics. *Bioresource technology*, 184, pp.355-362.

Wang, M., Hu, C., Barnes, B.B., Mitchum, G., Lapointe, B. and Montoya, J.P. (2019) The great Atlantic sargassum belt. *Science*, 365(6448), pp.83-87.

Wang, Y.V., Wan, A.H., Krogdahl, Å., Johnson, M. and Larsen, T. (2019) 13C values of glycolytic amino acids as indicators of carbohydrate utilization in carnivorous fish. *PeerJ*, 7, p.e7701.

Wang, Y.V., Wan, A.H., Lock, E.J., Andersen, N., Winter-Schuh, C. and Larsen, T. (2018) Know your fish: A novel compound-specific isotope approach for tracing wild and farmed salmon. *Food Chemistry,* 256, pp.380-389.

Ward, O.P. and Singh, A. (2005) Omega-3/6 fatty acids: alternative sources of production. *Process biochemistry*, 40(12), pp.3627-3652.

Webster, C.D., Yancey, D.H. and Tidwell, J.H. (1992) Effect of partially or totally replacing fish meal with soybean meal on growth of blue catfish (Ictalurus furcatus). *Aquaculture*, 103 (2), pp. 141-152.

Wehr, J.D. (2015) Brown algae. *Freshwater Algae of North America* (pp. 851-871). Academic Press.

Weiser, M.J., Butt, C.M. and Mohajeri, M.H. (2016) Docosahexaenoic acid and cognition throughout the lifespan. *Nutrients*, 8(2), p.99.

Wiencke, C. and Bischof, K. (2012) Seaweed biology. *Ecological studies*, 219.

Wilding, T.A. (2006) The benthic impacts of the Loch Linnhe artificial reef. *Marine Biodiversity* (pp. 345-353). Springer, Dordrecht.

Wilkins, M. R., Williams, K. L., Appel, R. D. and Hochstrasser, D. F. (Eds.), *Proteome Research: New Frontiers in Functional Genomics*, Springer Berlin, Heidelberg, pp.13-33.

Williams, J. A. (1985) The role of photoperiod in the initiation of breeding and brood development in the amphipod Talitrus saltator Montagu. *Journal of Experimental Marine Biology and Ecology* 86, pp.59-72.

Wilson, K., & Lee, K. P. (2000). Nutritional requirements of the medfly, Ceratitis capitata, and the use of chemically-defined diets for nutritional research and quality control. *Entomologia experimentalis et applicata*, 96(3), 303-316.

Winder, M. and Sommer, U. (2012) Phytoplankton response to a changing climate. Hydrobiologia, 698, pp.5-16.

Winkler, H. (1920) Verbreitung und ursache der parthenogenesis im pflanzen und tierreiche. G. Fischer.

Womeni, H.M., Linder, M., Tiencheu, B., Mbiapo, F.T., Villeneuve, P., Fanni, J. and Parmentier, M. (2009) Oils of insects and larvae consumed in Africa: potential sources of polyunsaturated fatty acids. Oléagineux, Corps gras, *Lipides*, 16(4-5-6), pp.230-235:

Wood, B. (1996) Human evolution. *Bioessays*, 18(12), pp.945-954.

World Food Programme. (2022) Global operational response plan, Update No. 5. Available at https://www.wfp.org/publications/wfp-global-operational-response-plan-update-5-june-2022.

World Health Organization. (2017) Preventing noncommunicable diseases (NCDs) by reducing environmental risk factors (No. WHO/FWC/EPE/17.01). World Health Organization.

WWF-UK. (2021) "The future of feed: a WWF roadmap to accelerating insect protein in UK foods." Available at https://www.wwf.org.uk/sites/default/files/2021-06/The_future_of_feed_July_2021.pdf

Ye, N., Zhang, X., Miao, M., Fan, X., Zheng, Y., Xu, D., Wang, J., Zhou, L., Wang, D., Gao, Y. and Wang, Y. (2015) Saccharina genomes provide novel insight into kelp biology. *Nature communications*, 6(1), p.6986.

Yen, A.L. (2015) Insects as food and feed in the Asia Pacific region: current perspectives and future directions. *Journal of Insects as Food and Feed*, 1(1), pp.33-55.

Yi, C., Qiuju, H., Lin, W. and Kuang, R. (2010) The utilization of insect-resources in Chinese rural area. *Journal of Agricultural Science*, 2 (3), pp. 146.

Yi N (2020). NBZIMM: Negative Binomial and Zero-Inflated Mixed Models_. R package version 1.0.

Zehner, R., & Gómez-Durán, A. (2013). Stable isotopes in forensic entomology: applications and limitations. *Invertebrate Survival Journal*, 10(1), 73-82.

Zhang, J., Zheng, L., Jin, P., Zhang, D. and Yu, Z. (2014) Fishmeal substituted by production of chicken manure conversion with microorganisms and black soldier fly. I*nsects to Feed the World*. the Netherlands. 14-17 May.

Zuk, M., 2013. *Paleofantasy: What evolution really tells us about sex, diet, and how we live*. WW Norton & Company.

Appendices

Appendix 3

Appendix 3.1. Model summary for model visualised in Figure 38

```
Call:
glm(formula = Successful2 ~ Sub, family = "binomial", data = df %>%
    filter(Species == "C.frigida"))

Deviance Residuals:
    Min       1Q   Median       3Q      Max
-2.1301  -0.9196   0.4673   0.9246   1.4823

Coefficients:
                     Estimate Std. Error z value Pr(>|z|)
(Intercept)           -0.5108     0.3651  -1.399   0.1618
SubFucus sp.           0.7185     0.5223   1.376   0.1689
SubL.digitata frond    2.6703     0.5650   4.726 2.29e-06 ***
SubL.digitata stipe    1.1394     0.5701   1.999   0.0456 *
SubL.digitata (farmed) -0.1310     0.5348  -0.245   0.8064
SubS.latissima         -0.1823     0.5323  -0.343   0.7320
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

(Dispersion parameter for binomial family taken to be 1)

    Null deviance: 274.45  on 200  degrees of freedom
Residual deviance: 226.09  on 195  degrees of freedom
AIC: 238.09
```

Appendix 3.2 model summary for model visualised in Figure 41

```
Call:
glm(formula = Successful2 ~ Sub, family = "binomial", data = df %>%
    filter(Species == "C.pilipes"))

Deviance Residuals:
    Min       1Q   Median       3Q      Max
-2.1981  -0.4265   0.2161   0.4325   2.4478

Coefficients:
                     Estimate Std. Error z value Pr(>|z|)
(Intercept)           -2.3514     0.7400  -3.177 0.001486 **
SubFucus sp.           2.8034     0.8152   3.439 0.000584 ***
SubL.digitata frond    1.9837     0.8577   2.313 0.020736 *
SubL.digitata stipe    4.6738     0.8759   5.336 9.51e-08 ***
SubL.digitata (farmed) -15.2147  1097.2473  -0.014 0.988937
SubS.latissima         -0.5931     1.2650  -0.469 0.639197
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

(Dispersion parameter for binomial family taken to be 1)

    Null deviance: 235.67  on 169  degrees of freedom
Residual deviance: 133.11  on 164  degrees of freedom
AIC: 145.11
```

Appendix 3.3. Model summary for model visualised in Figure 44 and 45

```
Call:
glm(formula = Successful2 ~ Sub + Cage, family = "binomial",
    data = df %>% filter(Species == "F.maritima"))

Deviance Residuals:
    Min       1Q   Median       3Q      Max
-2.4655  -0.9925  -0.3282   0.8582   2.0044

Coefficients:
                     Estimate Std. Error z value Pr(>|z|)
(Intercept)           -0.2208     0.5180  -0.426  0.66991
SubFucus sp.           0.2565     0.6885   0.373  0.70948
SubL.digitata frond    2.1813     1.1444   1.906  0.05665 .
SubL.digitata stipe   -2.6737     0.8774  -3.047  0.00231 **
SubL.digitata (farmed) -0.7827     0.6336  -1.235  0.21675
SubS.latissima        -1.2610     0.7150  -1.764  0.07782 .
CageO                  1.0299     0.4703   2.190  0.02854 *
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

(Dispersion parameter for binomial family taken to be 1)

    Null deviance: 153.44  on 110  degrees of freedom
Residual deviance: 116.71  on 104  degrees of freedom
AIC: 130.71
```

Appendix 3.4. Model summary for model visualised in Figure 46.

```
Call:
lm(formula = Survival ~ Density + Density.squ + Density.cub,
    data = frigida2)

Residuals:
    Min      1Q  Median      3Q     Max
-56.781 -19.512   4.387  15.357  58.708

Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept)   40.879      4.664   8.765 1.15e-13 ***
Density      103.608     25.570   4.052 0.000108 ***
Density.squ  -88.096     25.434  -3.464 0.000821 ***
Density.cub   18.343      6.630   2.767 0.006886 **
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 26.51 on 89 degrees of freedom
Multiple R-squared:  0.1907,    Adjusted R-squared:  0.1635
F-statistic: 6.992 on 3 and 89 DF,  p-value: 0.0002815
```

Appendix 3.5. Polynomial order selection.

```
> anova(poly1)
Analysis of Variance Table

Response: Survival
          Df Sum Sq Mean Sq F value Pr(>F)
Density    1     41   40.82  0.0481 0.8269
Residuals 91  77231  848.69
> anova(poly2)
Analysis of Variance Table

Response: Survival
            Df Sum Sq Mean Sq F value    Pr(>F)
Density      1     41    40.8  0.0541 0.8165991
Density.squ  1   9319  9319.4 12.3506 0.0006922 ***
Residuals   90  67911   754.6
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
> anova(poly3)
Analysis of Variance Table

Response: Survival
            Df Sum Sq Mean Sq F value    Pr(>F)
Density      1     41    40.8  0.0581 0.8100728
Density.squ  1   9319  9319.4 13.2639 0.0004541 ***
Density.cub  1   5378  5378.3  7.6547 0.0068863 **
Residuals   89  62533   702.6
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
> anova(poly4)
Analysis of Variance Table

Response: Survival
             Df Sum Sq Mean Sq F value    Pr(>F)
Density       1     41    40.8  0.0576 0.8109314
Density.squ   1   9319  9319.4 13.1427 0.0004829 ***
Density.cub   1   5378  5378.3  7.5848 0.0071510 **
Density.quar  1    133   132.8  0.1873 0.6662194
Residuals    88  62400   709.1
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

Appendix 4

Appendix 4.1. Model summary for model visualised in Figure 48.

```
> summary(frig.mod <- glmm.zinb(fixed = eggs ~ sub + day, random = ~ 1 | cage, data = frig2))
Computational iterations: 11
Computational time: 0.003 minutes
Linear mixed-effects model fit by maximum likelihood
  Data: frig2
  AIC BIC logLik
   NA  NA     NA

Random effects:
 Formula: ~1 | cage
        (Intercept)  Residual
StdDev:   0.3404975 0.7700856

Variance function:
 Structure: fixed weights
 Formula: ~invwt
Fixed effects:  eggs ~ sub + day
                       Value Std.Error DF   t-value p-value
(Intercept)          4.055689 0.6708695 73  6.045421  0.0000
subFucus sp.        -2.085131 0.2936669 73 -7.100326  0.0000
subL.digitata frond -0.646672 0.2326464 73 -2.779633  0.0069
subL.digitata stipe -1.266866 0.2674086 73 -4.737566  0.0000
subS.latissima       0.362402 0.2444097 73  1.482762  0.1424
day4                -0.386046 0.6498607 73 -0.594044  0.5543
day5                 0.775976 0.6367179 73  1.218712  0.2269
day6                 0.415997 0.6455260 73  0.644431  0.5213
day7                 0.321707 0.6694771 73  0.480535  0.6323
day8                 0.370260 0.6693887 73  0.553131  0.5819
day9                -0.180457 0.6726835 73 -0.268264  0.7893
day10                0.725829 0.6715647 73  1.080803  0.2833
day11               -0.641397 0.7040807 73 -0.910971  0.3653
day12               -0.406648 0.8266624 73 -0.491915  0.6243
day13               -1.733110 0.7322490 73 -2.366831  0.0206
 Correlation:
                    (Intr) sbFsp. sbL.df sbL.ds sbS.lt day4   day5   day6   day7   day8   day9
subFucus sp.        -0.153
subL.digitata frond -0.305  0.418
subL.digitata stipe -0.165  0.381  0.444
subS.latissima      -0.164  0.410  0.495  0.435
day4                -0.854  0.038  0.135  0.020 -0.008
day5                -0.861  0.011  0.136 -0.012 -0.006  0.873
day6                -0.871  0.054  0.136  0.063  0.008  0.868  0.877
day7                -0.866 -0.022  0.128 -0.005 -0.019  0.864  0.871  0.880
day8                -0.867 -0.022  0.128 -0.005 -0.019  0.864  0.872  0.880  0.904
day9                -0.862 -0.019  0.129 -0.004  0.003  0.858  0.866  0.873  0.896  0.896
day10               -0.784  0.049  0.130 -0.018 -0.004  0.797  0.815  0.798  0.774  0.774  0.769
day11               -0.730 -0.053  0.068 -0.005 -0.063  0.754  0.771  0.757  0.737  0.737  0.731
day12               -0.635 -0.011  0.102 -0.005 -0.113  0.657  0.670  0.657  0.640  0.640  0.633
day13               -0.723  0.043  0.127  0.039  0.061  0.725  0.741  0.729  0.704  0.705  0.701
                    day10  day11  day12

subFucus sp.
subL.digitata frond
subL.digitata stipe
subS.latissima
day4
day5
day6
day7
day8
day9
day10
day11               0.728
day12               0.635  0.607
day13               0.702  0.662  0.570

Standardized within-Group Residuals:
       Min         Q1        Med         Q3        Max
-1.5453341 -0.6598687 -0.3732312  0.2505720  3.0105020

Number of Observations: 90
Number of Groups: 3
```

Appendix 4.2. Model summary for model visualised in Figure 50.

```
> summary(mod <- glmm.zinb(fixed = eggs ~ sub + day, random = ~ 1 | cage, data = fucellia2))
Computational iterations: 19
Computational time: 0.009 minutes
Linear mixed-effects model fit by maximum likelihood
  Data: fucellia2
  AIC BIC logLik
   NA  NA     NA

Random effects:
 Formula: ~1 | cage
         (Intercept)  Residual
StdDev: 2.377474e-05 0.6066524

Variance function:
 Structure: fixed weights
 Formula: ~invwt
Fixed effects:  eggs ~ sub + day
                          Value Std.Error DF   t-value p-value
(Intercept)           2.0722315 0.5408846 62  3.831190  0.0003
subFucus sp.         -1.4551425 0.3429279 62 -4.243289  0.0001
subL.digitata frond -0.7839626 0.3337235 62 -2.349138  0.0220
subL.digitata stipe -1.0936436 0.3133645 62 -3.490004  0.0009
subS.latissima        0.5974183 0.3043919 62  1.962662  0.0542
day7                  2.0063623 0.6071669 62  3.304466  0.0016
day8                  1.5659935 0.6032125 62  2.596089  0.0118
day9                  2.0087109 0.5833443 62  3.443440  0.0010
day10                 1.3830958 0.5802689 62  2.383543  0.0202
day11                 1.3020133 0.6125390 62  2.125600  0.0375
day12                 0.8615750 0.6978902 62  1.234542  0.2217
 Correlation:
                     (Intr) sbFsp. sbL.df sbL.ds sbS.lt day7   day8   day9   day10  day11
subFucus sp.         -0.136
subL.digitata frond -0.127  0.353
subL.digitata stipe -0.130  0.368  0.369
subS.latissima       -0.110  0.403  0.416  0.414
day7                 -0.850 -0.071 -0.016 -0.072 -0.095
day8                 -0.855 -0.020 -0.081 -0.075 -0.099  0.783
day9                 -0.874 -0.077 -0.161 -0.083 -0.145  0.812  0.826
day10                -0.878 -0.110 -0.088 -0.114 -0.171  0.824  0.828  0.865
day11                -0.830 -0.105 -0.115 -0.108 -0.136  0.777  0.784  0.821  0.826
day12                -0.743 -0.006 -0.006 -0.108 -0.023  0.676  0.680  0.700  0.709  0.671

Standardized Within-Group Residuals:
       Min         Q1        Med         Q3        Max
-1.5462022 -0.5877935 -0.3984684 -0.2763484  3.6860145

Number of Observations: 75
Number of Groups: 3
```

Appendix 4.3. Model summary for the survival time analysis on C.frigida offspring success.

```
Call: survfit(formula = Surv(day - 6, eclosed) ~ sub, data = frig.df,
    conf.type = "log-log")

                sub=A.esculenta
     time n.risk n.event survival std.err lower 95% CI upper 95% CI

                sub=Fucus sp.
 time n.risk n.event survival std.err lower 95% CI upper 95% CI
    6    150      22    0.853  0.0289        0.786        0.901
    7    128      33    0.633  0.0393        0.551        0.705
    8     95      22    0.487  0.0408        0.405        0.564
    9     73       2    0.473  0.0408        0.392        0.551

                sub=L.digitata frond
 time n.risk n.event survival std.err lower 95% CI upper 95% CI
    1    150       3    0.980  0.0114        0.939        0.994
    2    147      11    0.907  0.0238        0.847        0.944
    3    136      72    0.427  0.0404        0.347        0.504
    4     64      47    0.113  0.0259        0.069        0.170

                sub=L.digitata stipe
 time n.risk n.event survival std.err lower 95% CI upper 95% CI
    4    150       7    0.953  0.0172        0.905        0.977
    5    143      26    0.780  0.0338        0.705        0.838
    6    117      28    0.593  0.0401        0.510        0.667
    7     89      12    0.513  0.0408        0.431        0.590

                sub=S.latissima
     time n.risk n.event survival std.err lower 95% CI upper 95% CI
```

Appendix 4.4. Model summary for the survival time analysis on C.pilipes offspring success.

```
Call: survfit(formula = Surv(day - 11, eclosed) ~ sub, data = pilipes.df,
    conf.type = "log-log")

                sub=A.esculenta
     time n.risk n.event survival std.err lower 95% CI upper 95% CI

                sub=Fucus sp.
 time n.risk n.event survival std.err lower 95% CI upper 95% CI
    4    150       1    0.993 0.00664        0.954        0.999
    5    149      27    0.813 0.03181        0.741        0.867
    6    122      56    0.440 0.04053        0.360        0.517
    7     66       7    0.393 0.03989        0.315        0.470

                sub=L.digitata frond
 time n.risk n.event survival std.err lower 95% CI upper 95% CI
    2    150       5    0.967  0.0147        0.922        0.986
    3    145      46    0.660  0.0387        0.578        0.730
    4     99      34    0.433  0.0405        0.353        0.511

                sub=L.digitata stipe
 time n.risk n.event survival std.err lower 95% CI upper 95% CI
    1    150      36    0.760  0.0349        0.683        0.821
    2    114      49    0.433  0.0405        0.353        0.511
    3     65      36    0.193  0.0322        0.135        0.260

                sub=S.latissima
     time n.risk n.event survival std.err lower 95% CI upper 95% CI
```

Appendix 4.5. Model summary for the survival time analysis on F.maritima offspring success.

```
all: survfit(formula = Surv(day - 15, eclosed) ~ sub, data = fucellia.df,
   conf.type = "log-log")

               sub=A.esculenta
time n.risk n.event survival std.err lower 95% CI upper 95% CI
   8    150       4    0.973  0.0132        0.931        0.990
   9    146      57    0.593  0.0401        0.510        0.667
  10     89      36    0.353  0.0390        0.278        0.430
  11     53      13    0.267  0.0361        0.199        0.339

               sub=Fucus sp.
time n.risk n.event survival std.err lower 95% CI upper 95% CI
   7    150       2    0.987 0.00937        0.948        0.997
   8    148       7    0.940 0.01939        0.888        0.968

               sub=L.digitata frond
time n.risk n.event survival std.err lower 95% CI upper 95% CI
   6    150      26    0.827  0.0309        0.756        0.879
   7    124      32    0.613  0.0398        0.530        0.686
   8     92      40    0.347  0.0389        0.272        0.423
   9     52       6    0.307  0.0376        0.235        0.381

               sub=L.digitata stipe
time n.risk n.event survival std.err lower 95% CI upper 95% CI
   1    150      13    0.913  0.0230        0.855        0.949
   2    137      11    0.840  0.0299        0.771        0.890
   3    126       7    0.793  0.0331        0.719        0.850
   4    119       6    0.753  0.0352        0.676        0.815
   5     89      15    0.626  0.0418        0.539        0.702

               sub=S.latissima
time n.risk n.event survival std.err lower 95% CI upper 95% CI
   9    150       1    0.993 0.00664        0.954        0.999
  10    149       2    0.980 0.01143        0.939        0.994
  11    147       9    0.920 0.02215        0.863        0.954
  12    138       2    0.907 0.02375        0.847        0.944
  13     48       7    0.774 0.05045        0.656        0.856
```

Appendix 5

Appendix 5.1

Calcium (Ca)

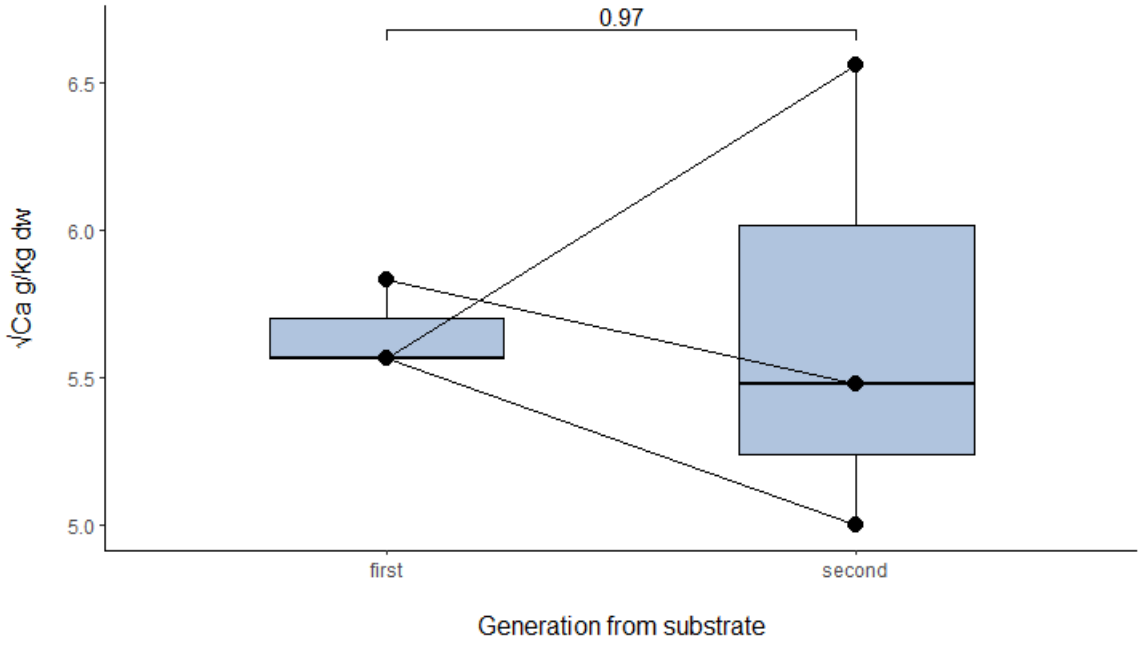


Figure 89. Paired t-test comparing composition of first and second generation of larvae reared on the same substrate.

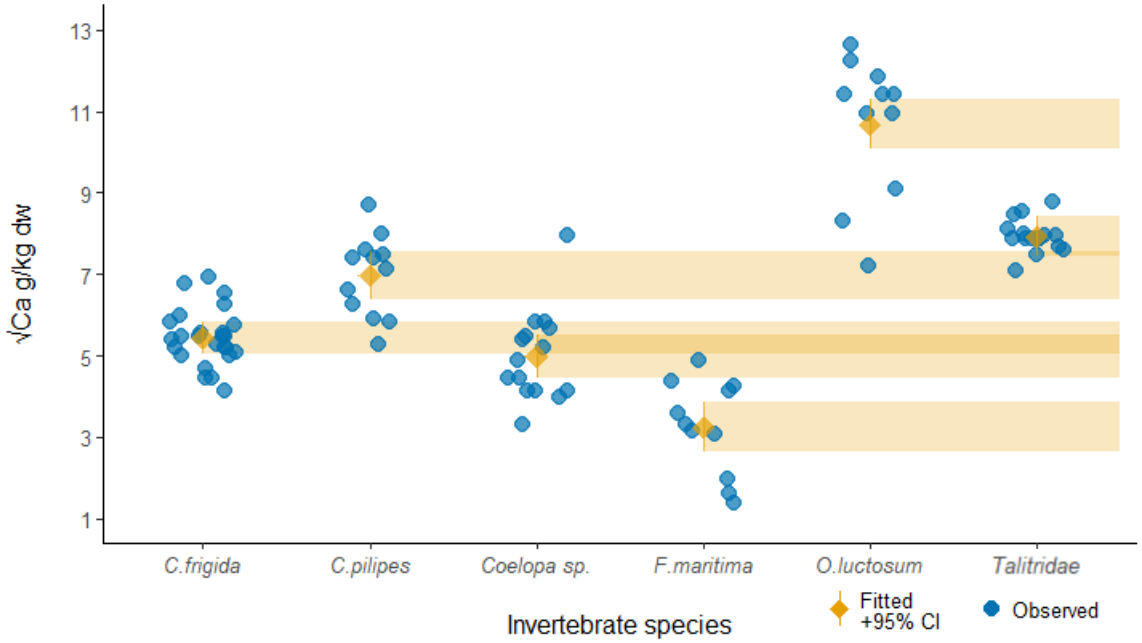


Figure 90. The estimated mean and 95%CI content of each species. 95%CIs have been projected right to allow easy pairwise comparison.

Table 35. P-values of each pairwise comparison visualised in Figure 90.

	Talitridae	*O.luctosum*	*F.maritima*	*Coelopa sp.*	*C.pilipes*
O.luctosum	<0.001				
F.maritima	<0.001	<0.001			
Coelopa sp.	<0.001	<0.001	<0.001		
C.pilipes	0.126	<0.001	<0.001	<0.001	
C.frigida	<0.001	<0.001	<0.001	0.725	<0.001

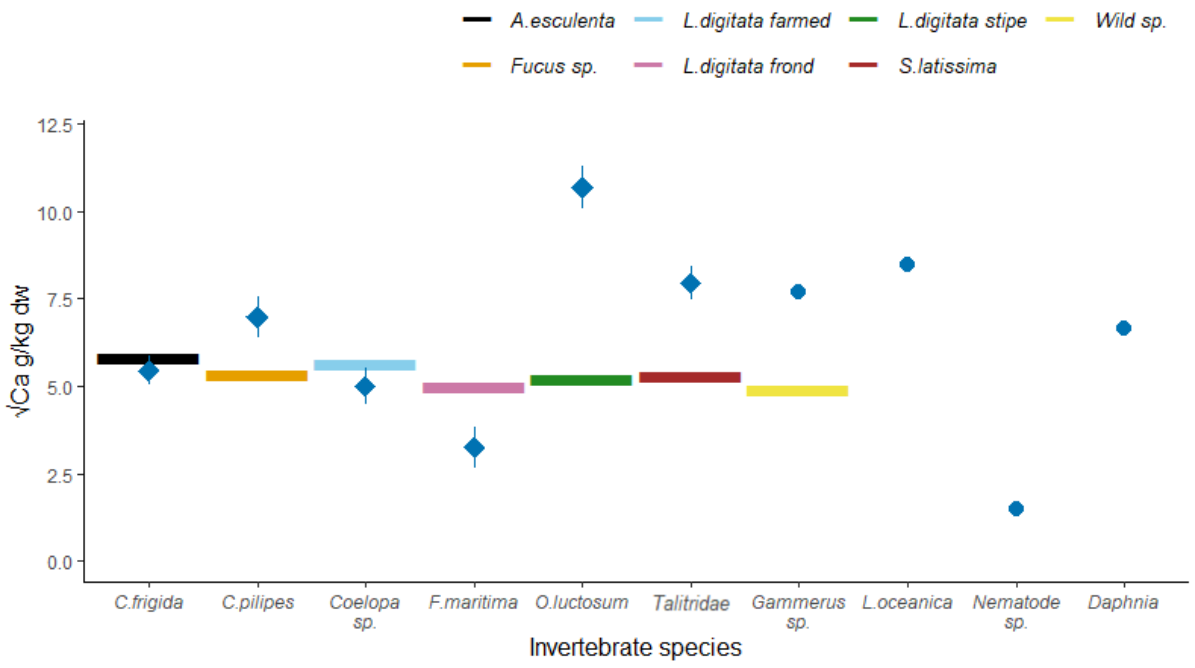


Figure 91. The content in all 10 analysed invertebrate species as well as each of the substrates used.

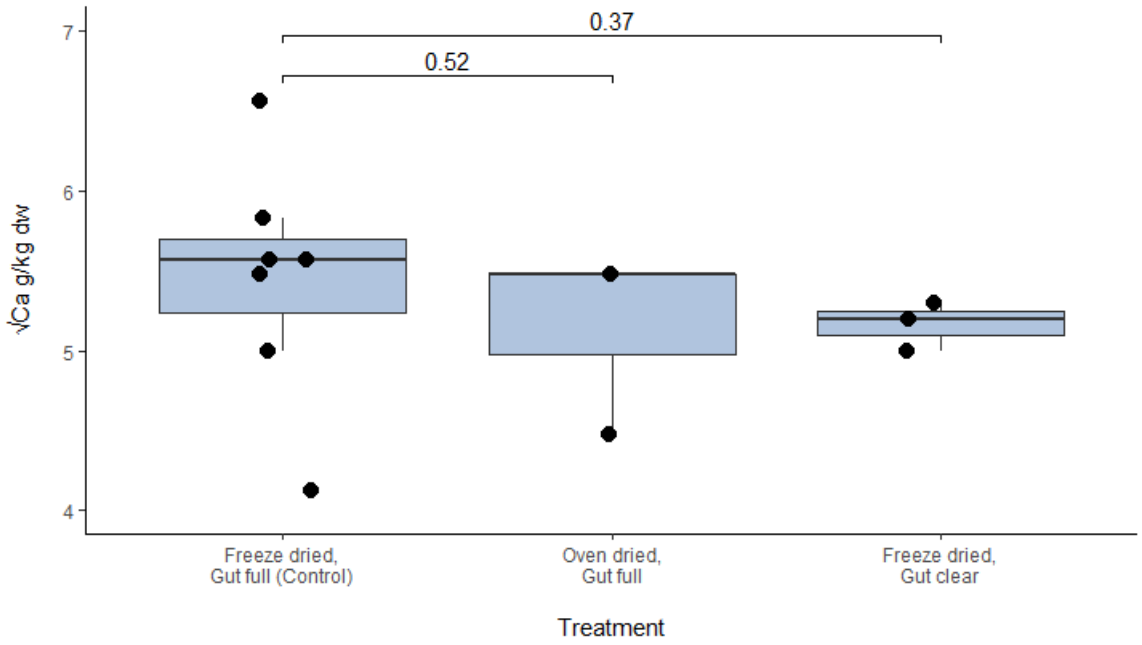


Figure 92. Welch's t-test comparing composition of larvae between processing methods.

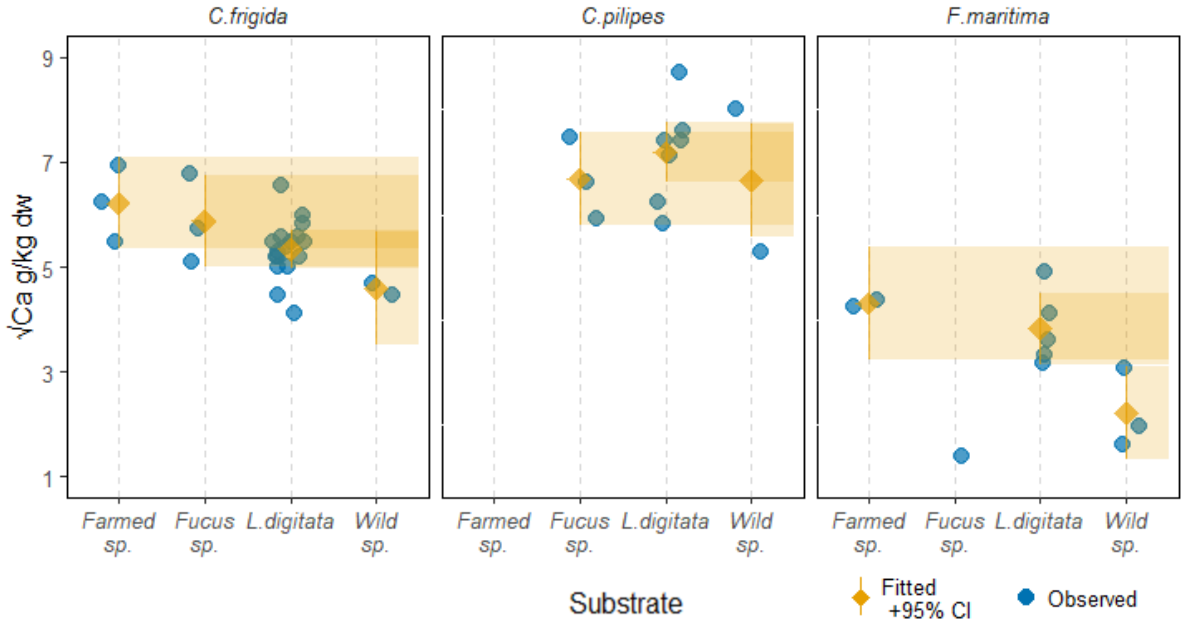


Figure 93. The estimated mean and 95%CI content of larvae reared on each substrate per species.

Table 36. P-values of each pairwise comparison visualised in Figure 93.

C.frigida	*Wild sp.*	*L.digitata*	*Fucus sp.*
L.digitata	0.533		
Fucus sp.	0.249	0.670	
Farmed sp.	0.096	0.261	0.944
C.pilipes	*Wild sp.*	*L.digitata*	*Fucus sp.*
L.digitata	0.795		
Fucus sp.	>0.999	0.747	
F.maritima	*Wild sp.*	*L.digitata*	*Fucus sp.*
L.digitata	0.028		
Farmed sp.	0.021	0.870	

Sodium (Na)

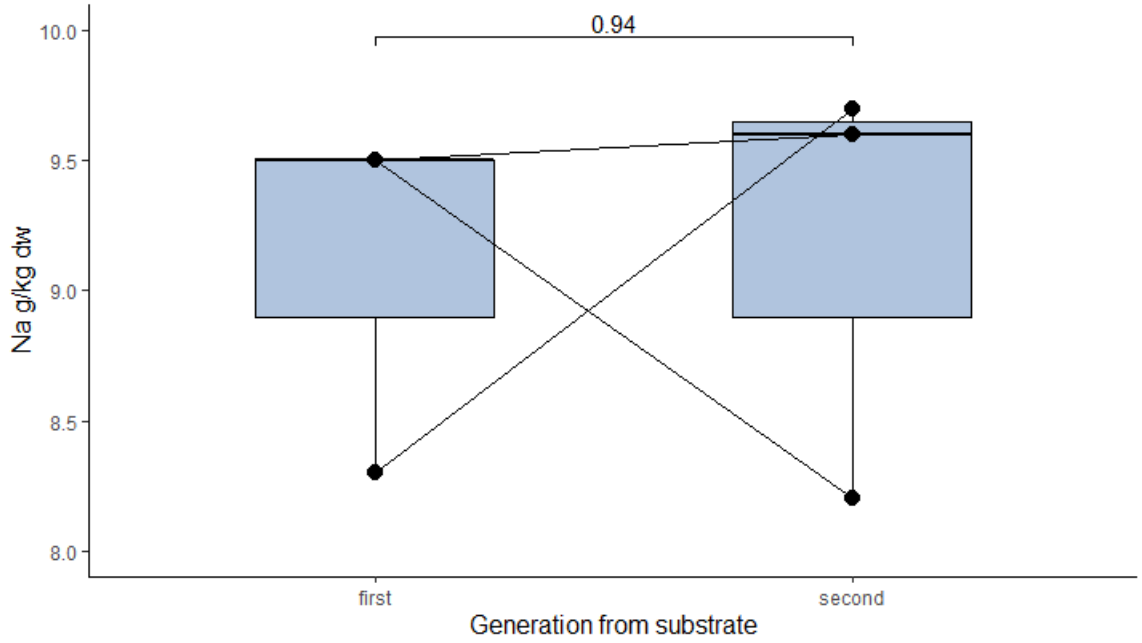


Figure 94. Paired t-test comparing composition of first and second generation of larvae reared on the same substrate.

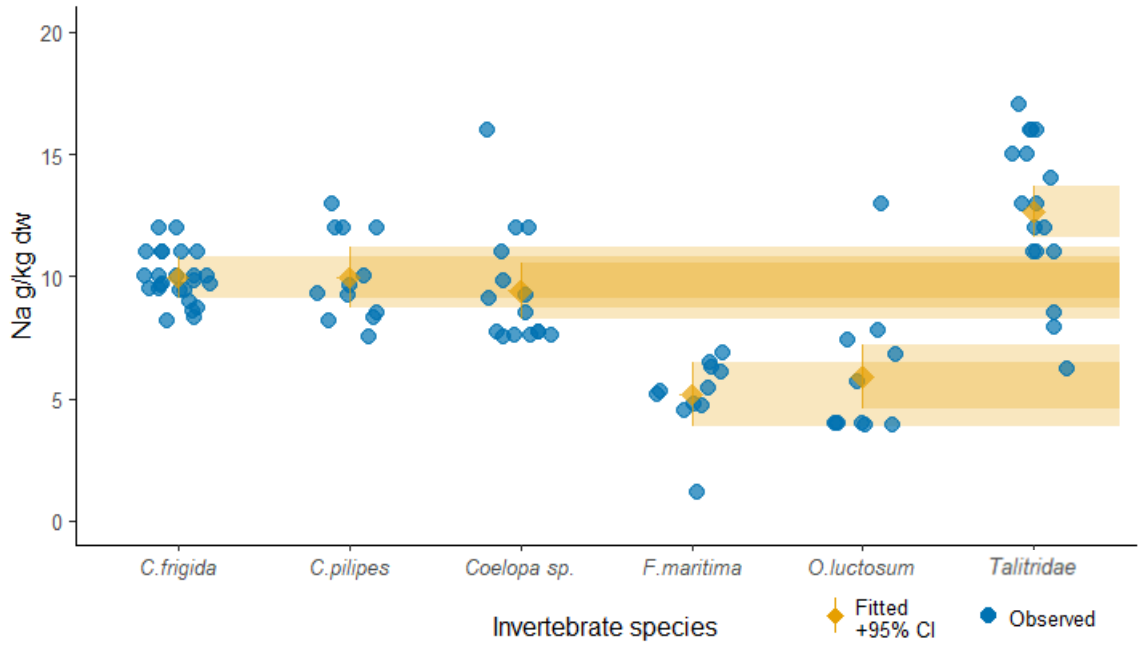


Figure 95. The estimated mean and 95%CI content of each species. 95%CIs have been projected right to allow easy pairwise comparison.

Table 37. P-values of each pairwise comparison visualised in Figure 95.

	Talitridae	*O.luctosum*	*F.maritima*	*Coelopa sp.*	*C.pilipes*
O.luctosum	<0.001				
F.maritima	<0.001	0.976			
Coelopa sp.	<0.001	0.001	<0.001		
C.pilipes	0.021	<0.001	<0.001	0.985	
C.frigida	0.002	<0.001	<0.001	0.974	>0.999

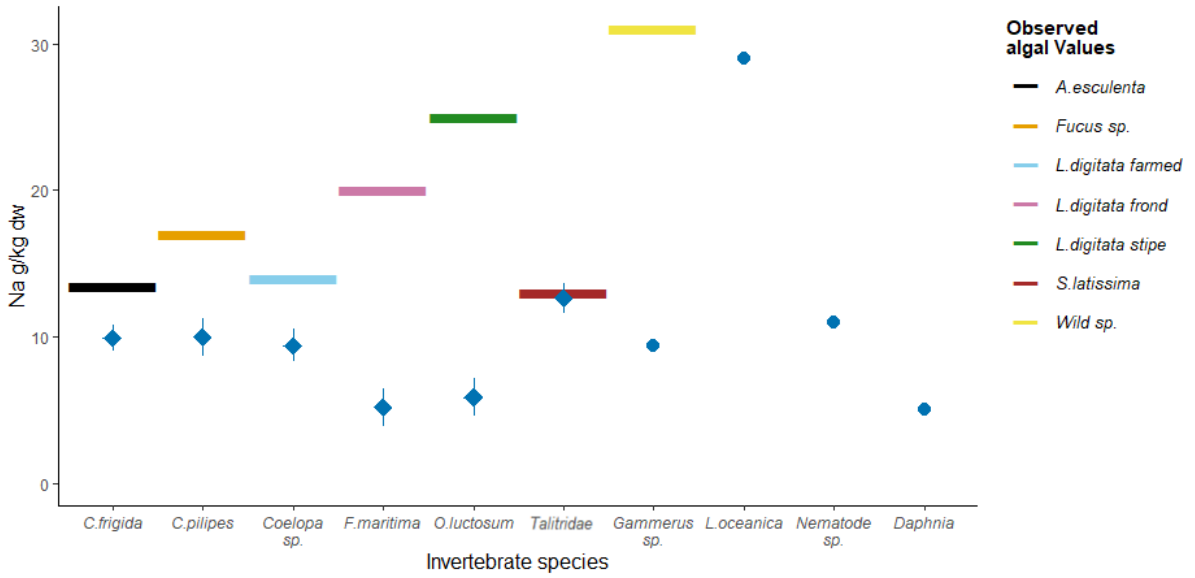


Figure 96. The content in all 10 analysed invertebrate species as well as each of the substrates used.

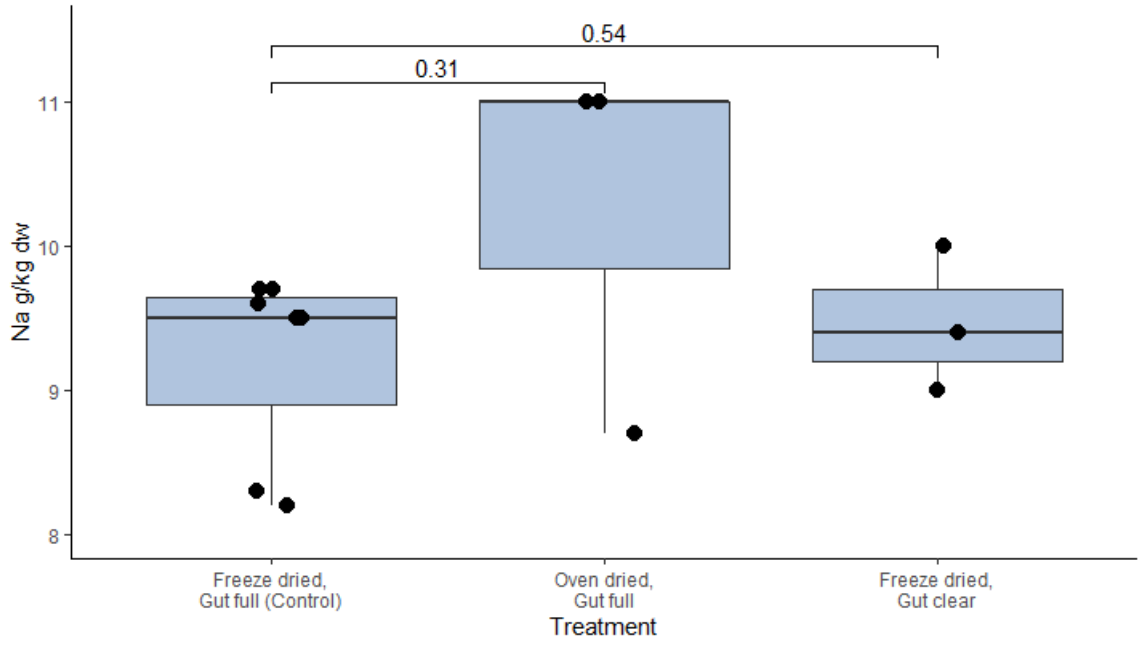


Figure 97. Welch's t-test comparing composition of larvae between processing methods.

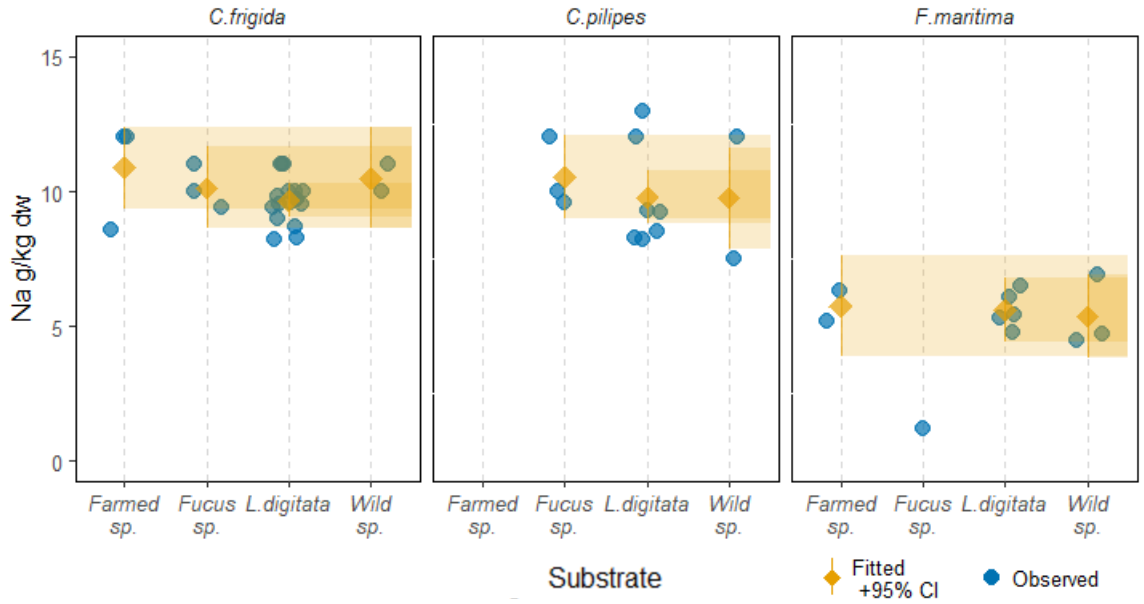


Figure 98. The estimated mean and 95%CI content of larvae reared on each substrate per species.

Table 38. P-values of each pairwise comparison visualised in Figure 98.

C.frigida	*Wild sp.*	*L.digitata*	*Fucus sp.*
L.digitata	0.828		
Fucus sp.	0.990	0.941	
Farmed sp.	0.990	0.464	0.899
C.pilipes	*Wild sp.*	*L.digitata*	*Fucus sp.*
L.digitata	>0.999		
Fucus sp.	0.911	0.838	
F.maritima	*Wild sp.*	*L.digitata*	*Fucus sp.*
L.digitata	0.993		
Farmed sp.	0.988	>0.999	

Potassium (K)

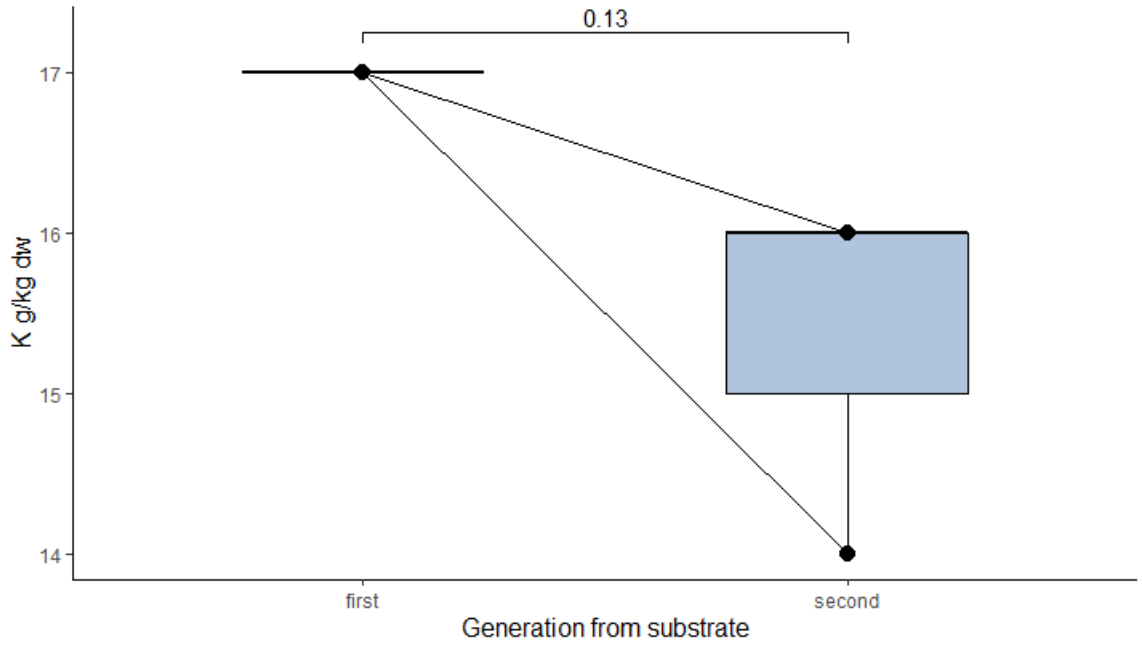


Figure 99. Paired t-test comparing composition of first and second generation of larvae reared on the same substrate.

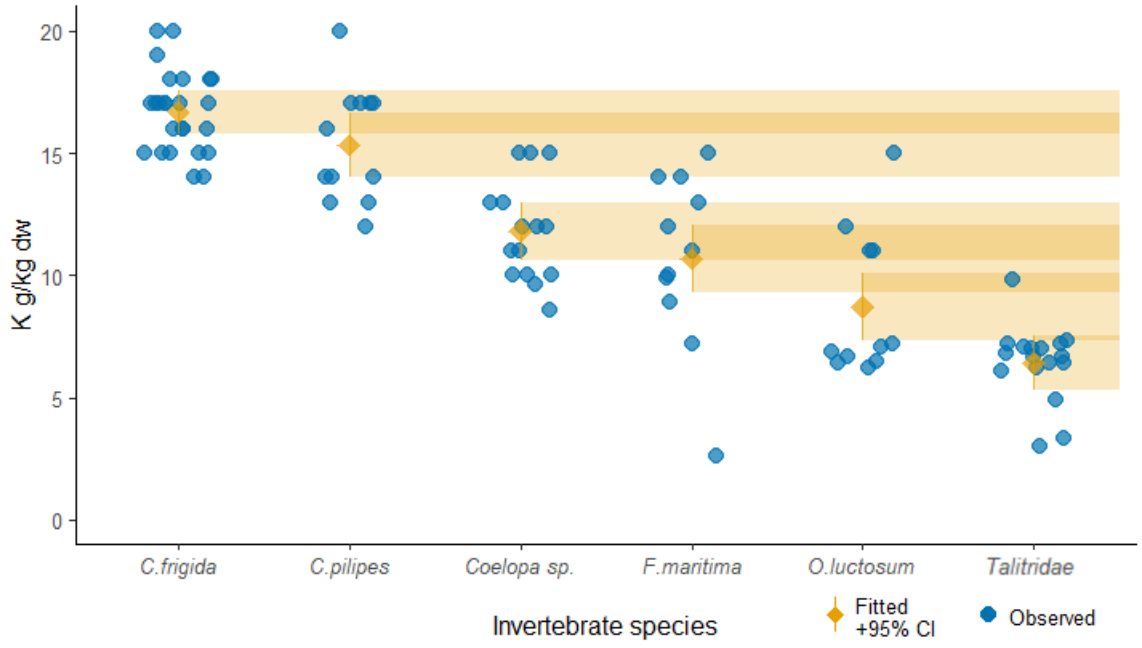


Figure 100. The estimated mean and 95%CI content of each species. 95%CIs have been projected right to allow easy pairwise comparison.

Table 39. P-values of each pairwise comparison visualised in Figure 100.

	Talitridae	*O.luctosum*	*F.maritima*	*Coelopa sp.*	*C.pilipes*
O.luctosum	0.105				
F.maritima	<0.001	0.342			
Coelopa sp.	<0.001	0.013	0.817		
C.pilipes	<0.001	<0.001	<0.001	0.002	
C.frigida	<0.001	<0.001	<0.001	<0.001	0.550

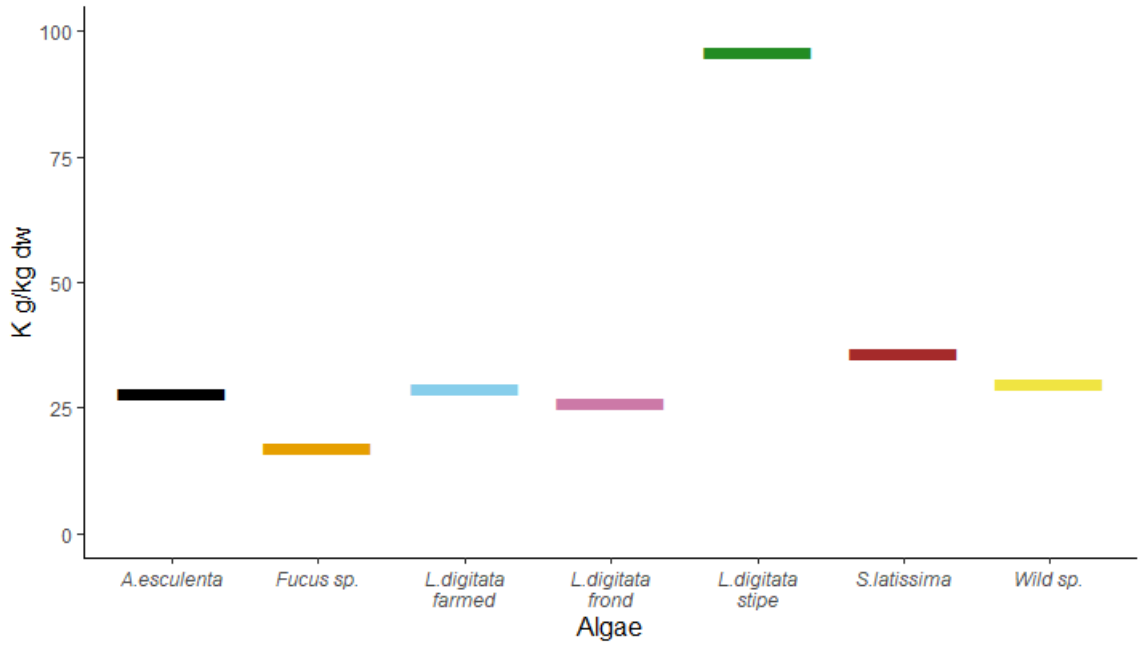


Figure 101. The content in each of the substrates used.

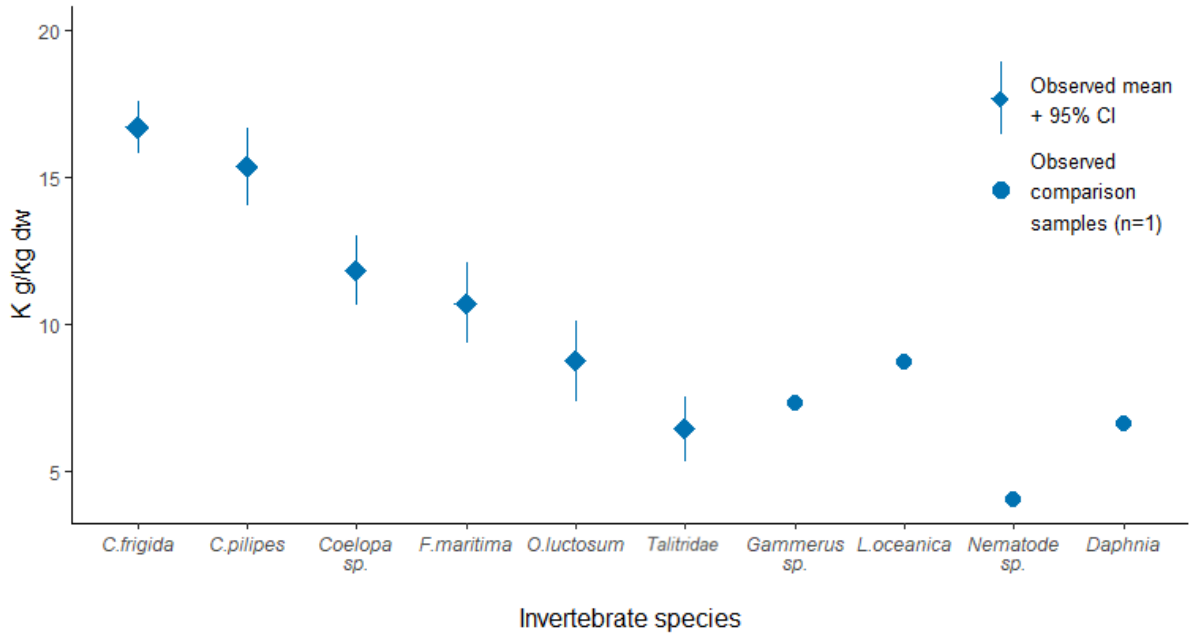


Figure 102. The content in all 10 analysed invertebrate species.

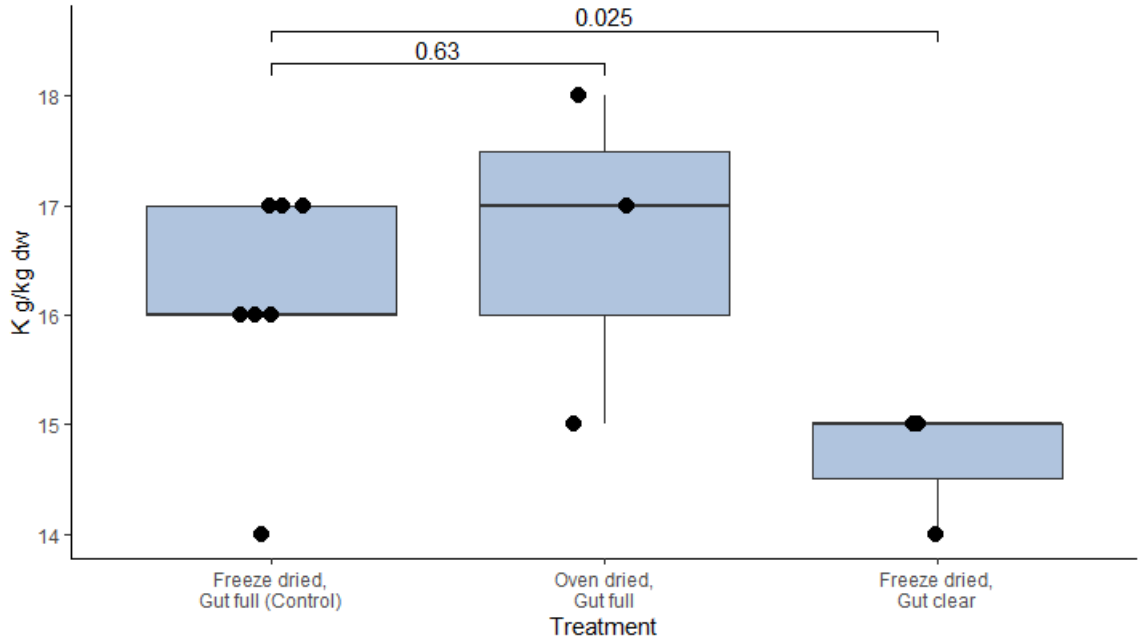


Figure 103. Welch's t-test comparing composition of larvae between processing methods.

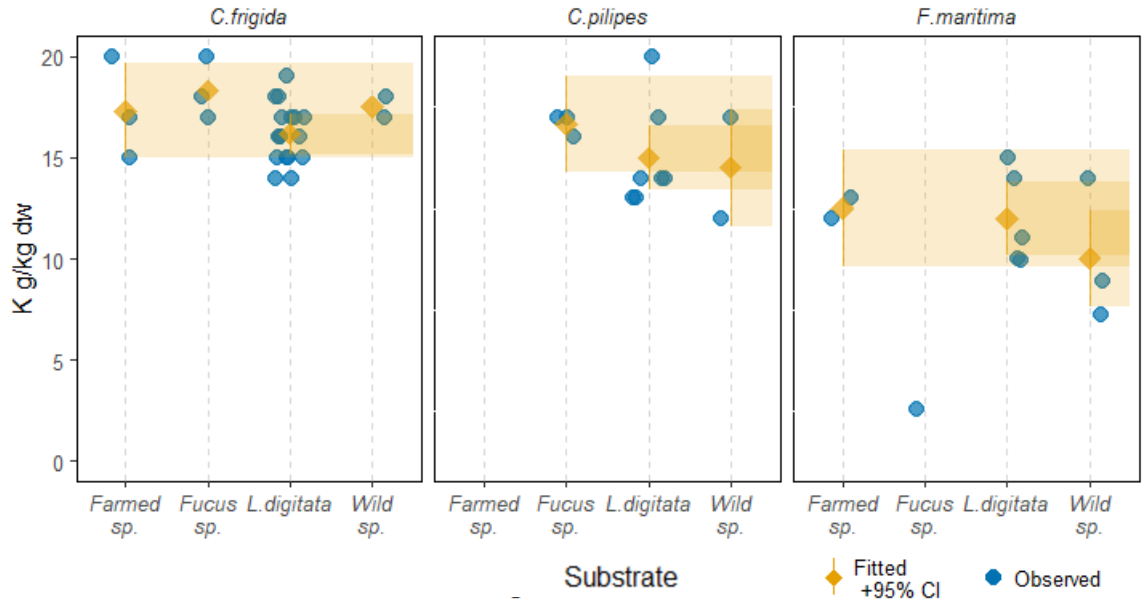


Figure 104. The estimated mean and 95%CI content of larvae reared on each substrate per species.

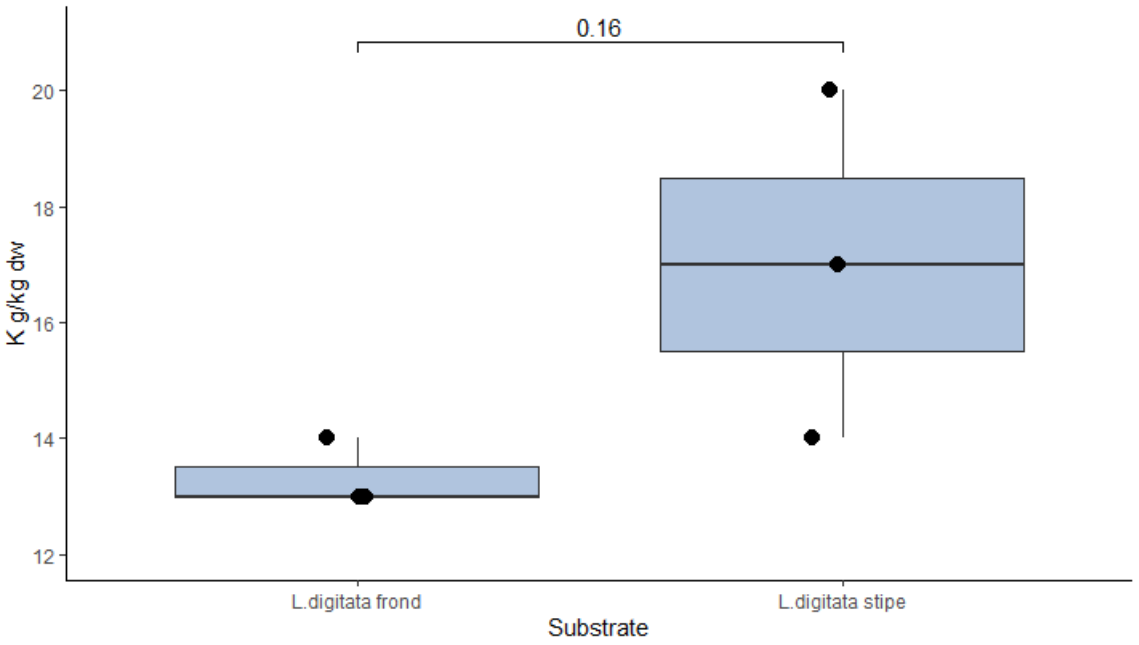


Figure 105. Paired t-test comparing composition of first and second generation of larvae reared on the same substrate.

Magnesium (mg)

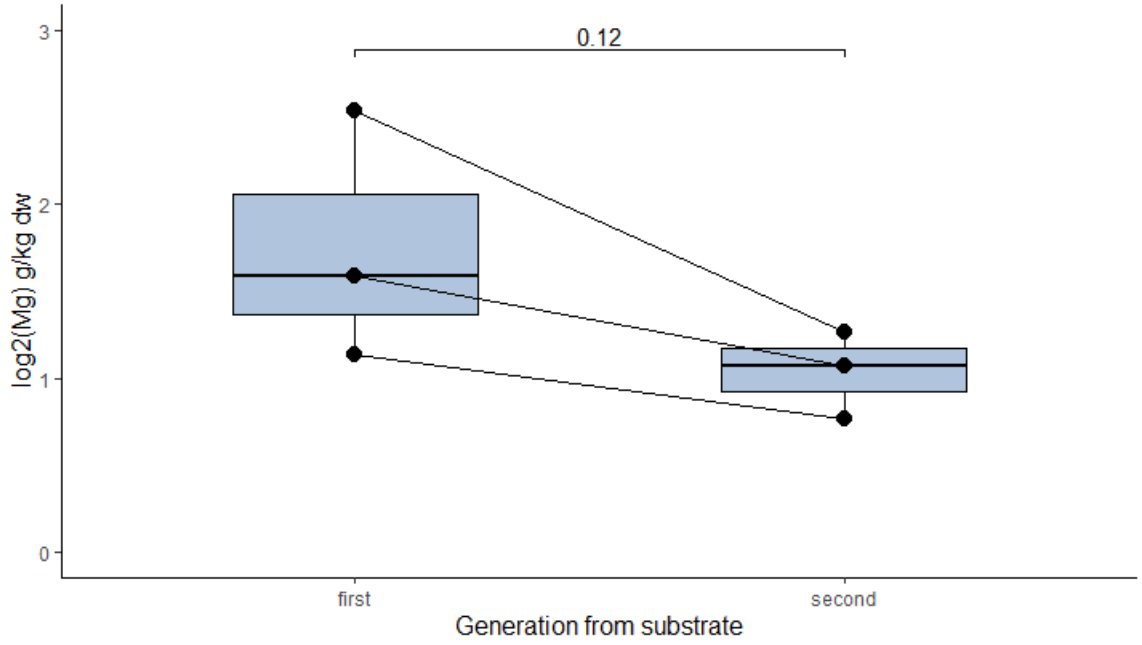


Figure 106. Paired t-test comparing composition of first and second generation of larvae reared on the same substrate.

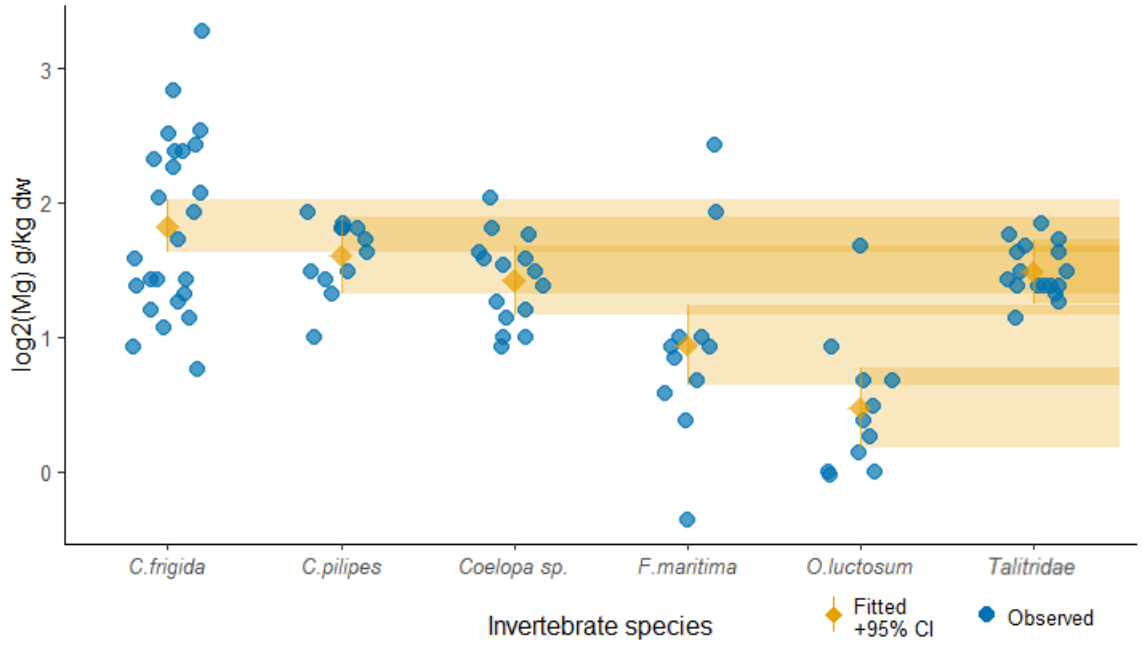


Figure 107. The estimated mean and 95%CI content of each species. 95%CIs have been projected right to allow easy pairwise comparison.

Table 40. P-values of each pairwise comparison visualised in Figure 107.

	Talitridae	*O.luctosum*	*F.maritima*	*Coelopa sp.*	*C.pilipes*
O.luctosum	<0.001				
F.maritima	0.062	0.251			
Coelopa sp.	>0.999	<0.001	0.157		
C.pilipes	0.989	<0.001	0.023	0.932	
C.frigida	0.274	<0.001	<0.001	0.146	0.812

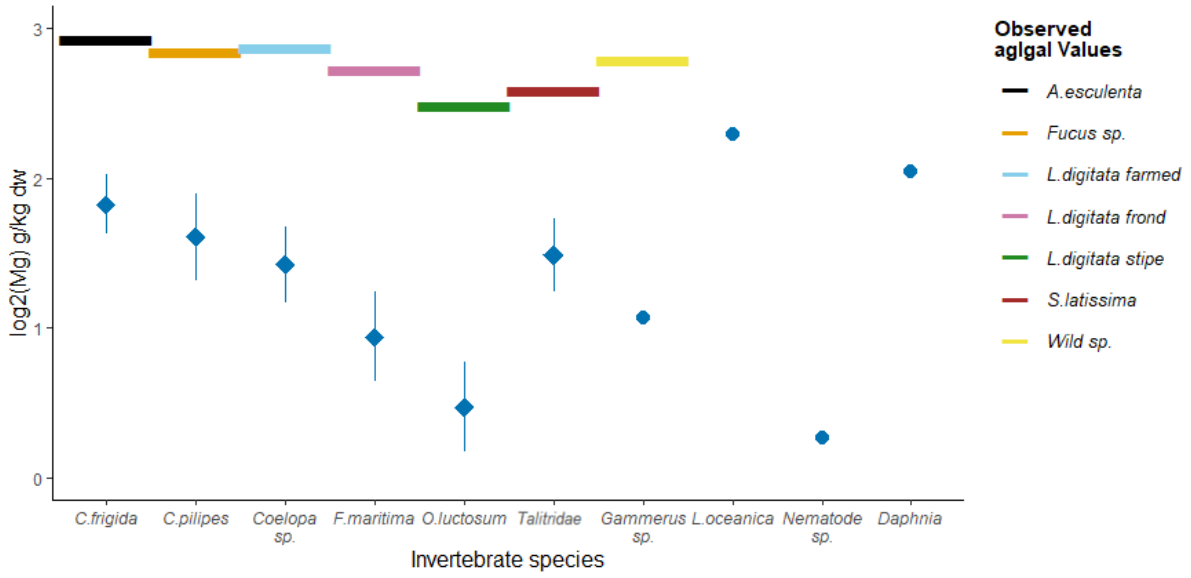


Figure 108. The content in all 10 analysed invertebrate species as well as each of the substrates used.

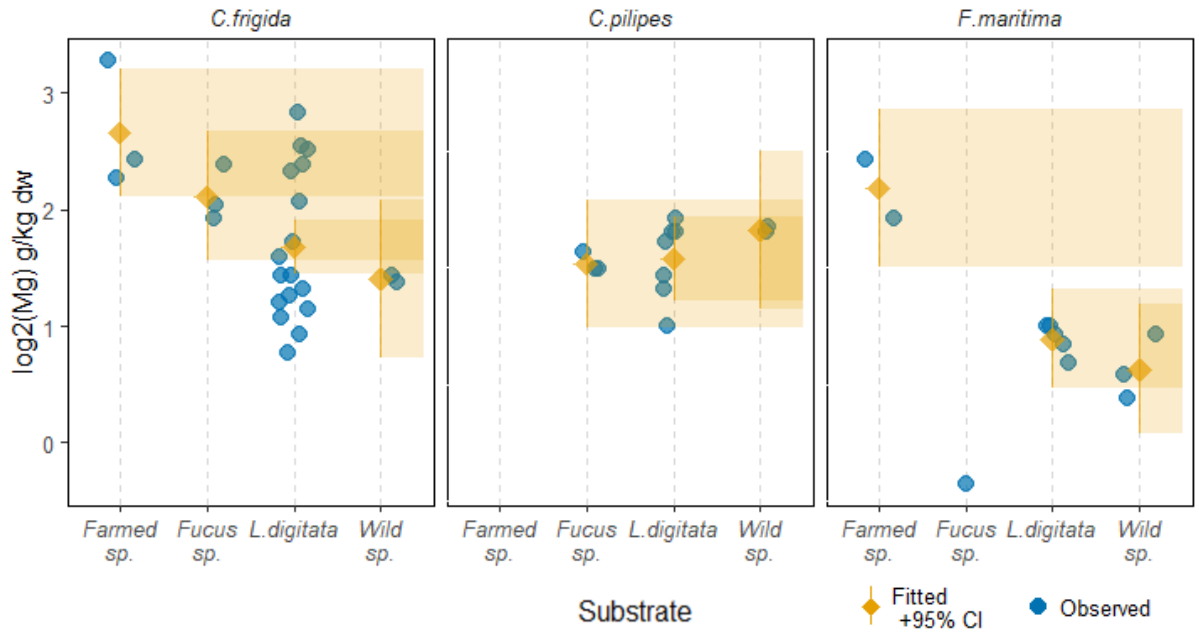


Figure 109. The estimated mean and 95%CI content of larvae reared on each substrate per species.

Table 41. P-values of each pairwise comparison visualised in Figure 109.

C.frigida	*Wild sp.*	*L.digitata*	*Fucus sp.*
L.digitata	0.869		
Fucus sp.	0.370	0.462	
Farmed sp.	0.030	0.011	0.501
C.pilipes	*Wild sp.*	*L.digitata*	*Fucus sp.*
L.digitata	0.908		
Fucus sp.	0.904	>0.999	
F.maritima	*Wild sp.*	*L.digitata*	*Fucus sp.*
L.digitata	0.874		
Farmed sp.	0.005	0.012	

Phosphorous (P)

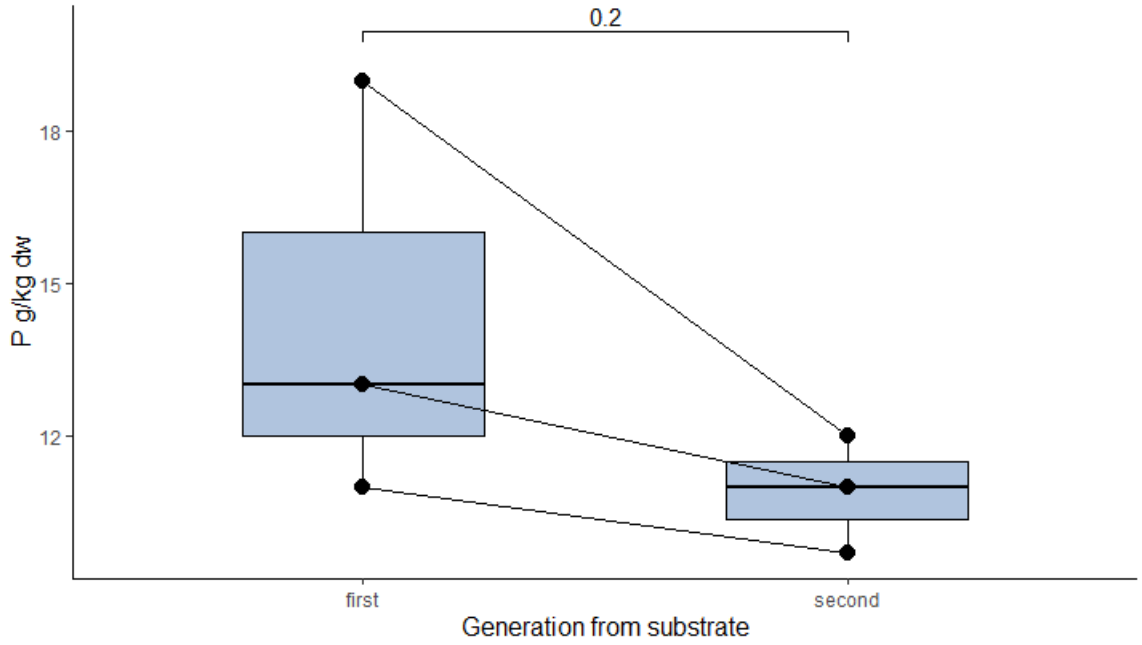


Figure 110. Paired t-test comparing composition of first and second generation of larvae reared on the same substrate

.

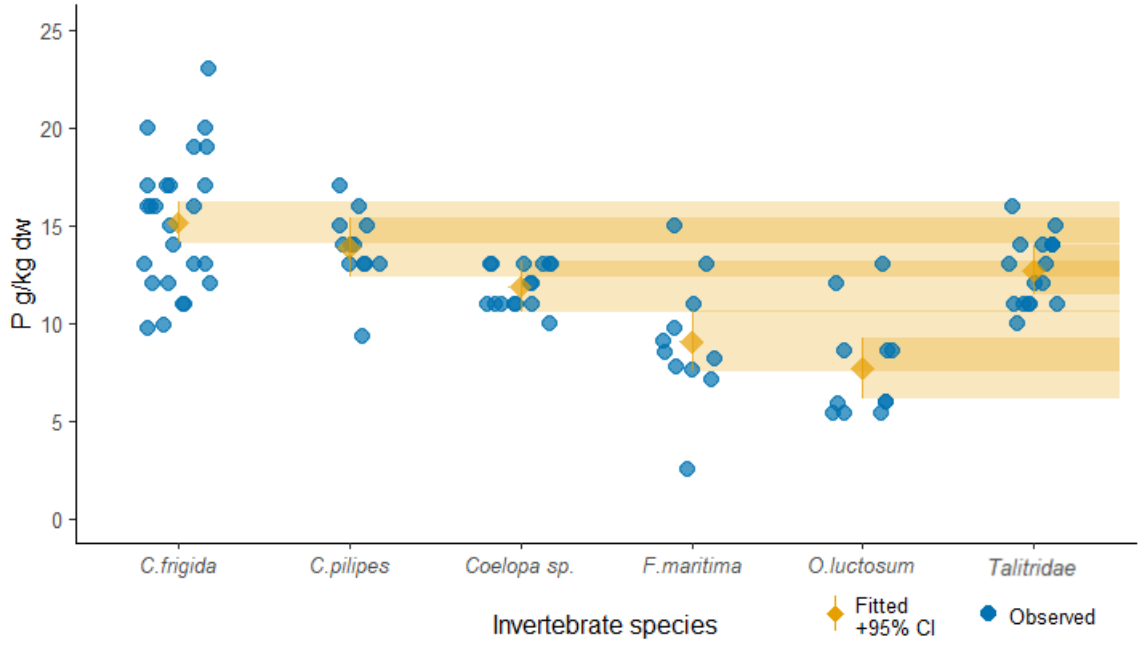


Figure 111. The estimated mean and 95%CI content of each species. 95%CIs have been projected right to allow easy pairwise comparison.

Table 42. P-values of each pairwise comparison visualised in Figure 111.

	Talitridae	*O.luctosum*	*F.maritima*	*Coelopa sp.*	*C.pilipes*
O.luctosum	<0.001				
F.maritima	0.006	0.838			
Coelopa sp.	0.943	0.002	0.080		
C.pilipes	0.848	<0.001	<0.001	0.365	
C.frigida	0.042	<0.001	<0.001	0.003	0.723

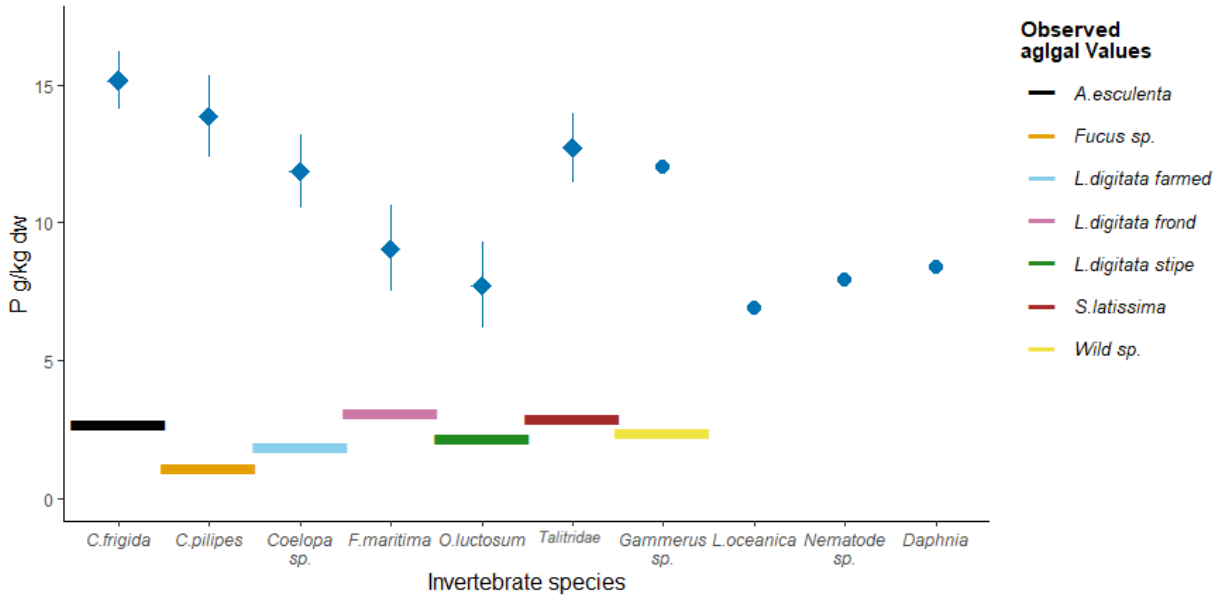


Figure 112. The content in all 10 analysed invertebrate species as well as each of the substrates used.

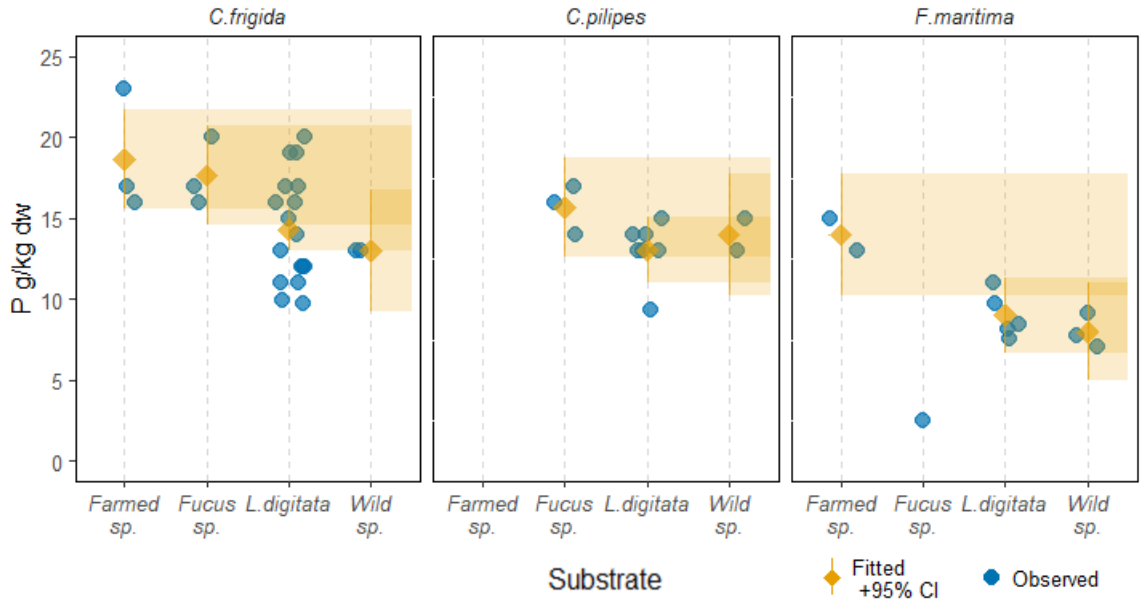


Figure 113. The estimated mean and 95%CI content of larvae reared on each substrate per species.

Chromium (Cr)

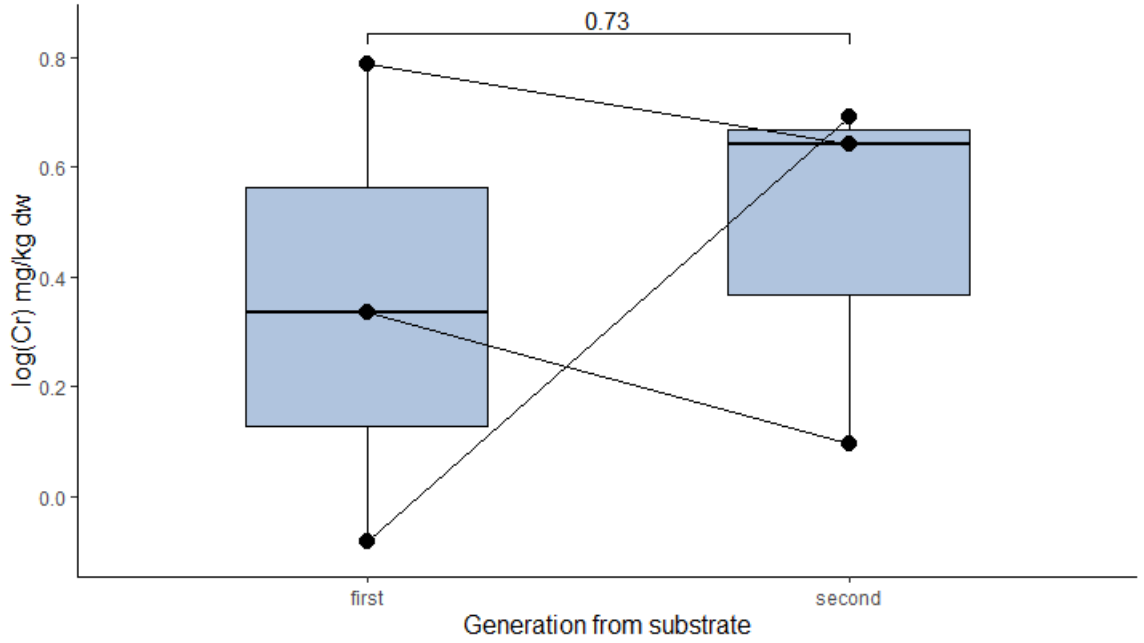


Figure 114. Paired t-test comparing composition of first and second generation of larvae reared on the same substrate.

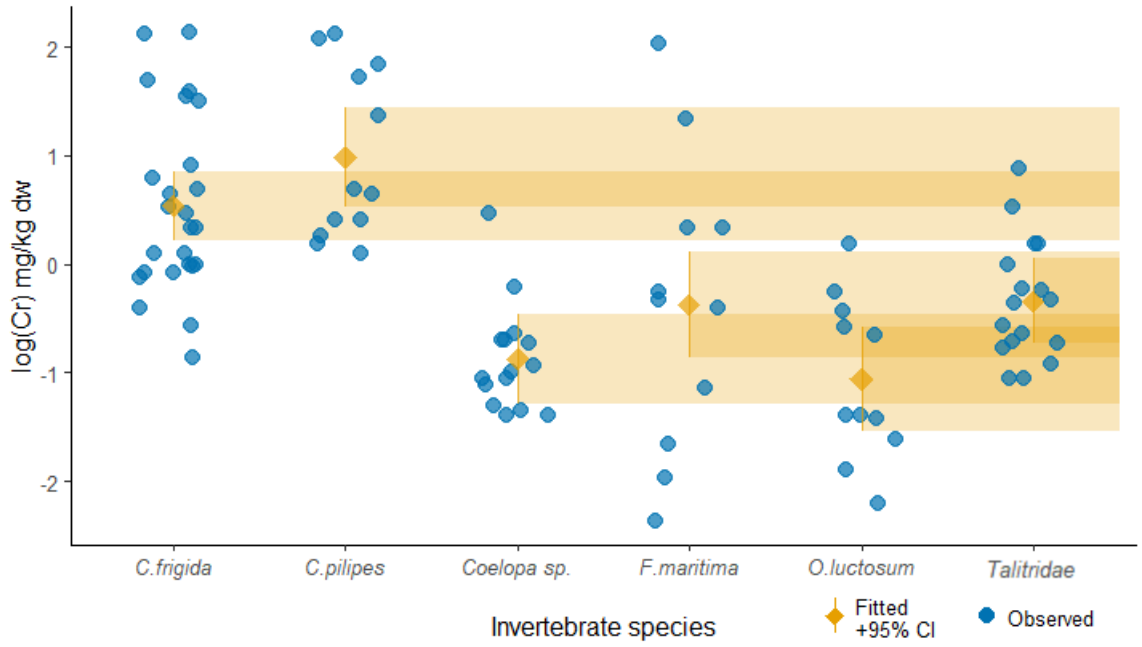


Figure 115. The estimated mean and 95%CI content of each species. 95%CIs have been projected right to allow easy pairwise comparison.

Table 43. P-values of each pairwise comparison visualised in Figure 115.

	Talitridae	*O.luctosum*	*F.maritima*	*Coelopa sp.*	*C.pilipes*
O.luctosum	0.207				
F.maritima	>0.999	0.351			
Coelopa sp.	0.440	0.992	0.626		
C.pilipes	<0.001	<0.001	0.002	<0.001	
C.frigida	0.011	<0.001	0.031	<0.001	0.611

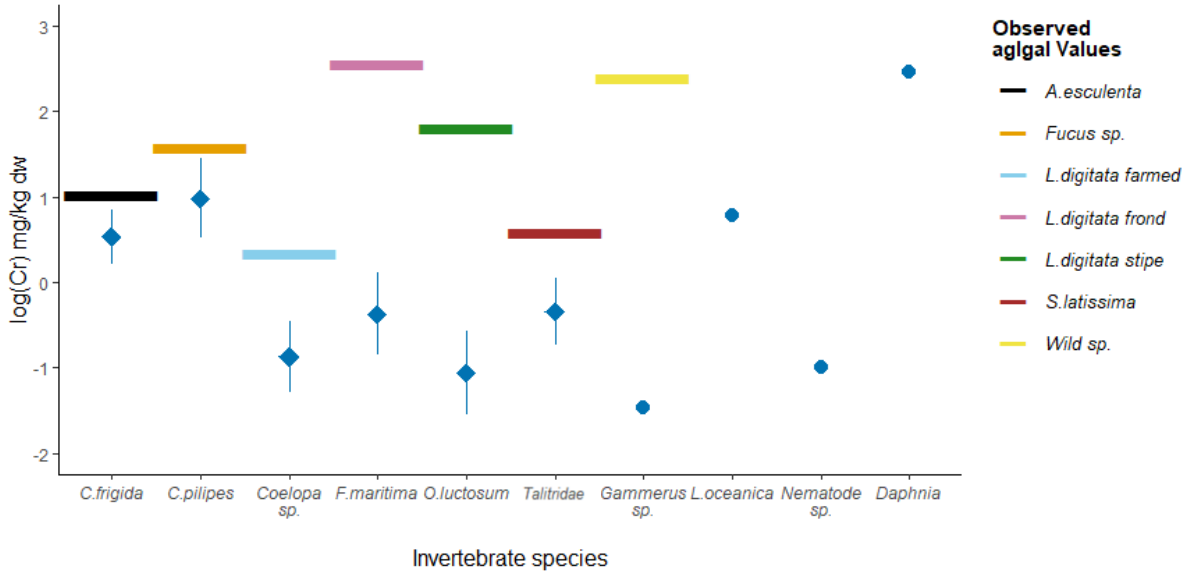


Figure 116. The content in all 10 analysed invertebrate species as well as each of the substrates used.

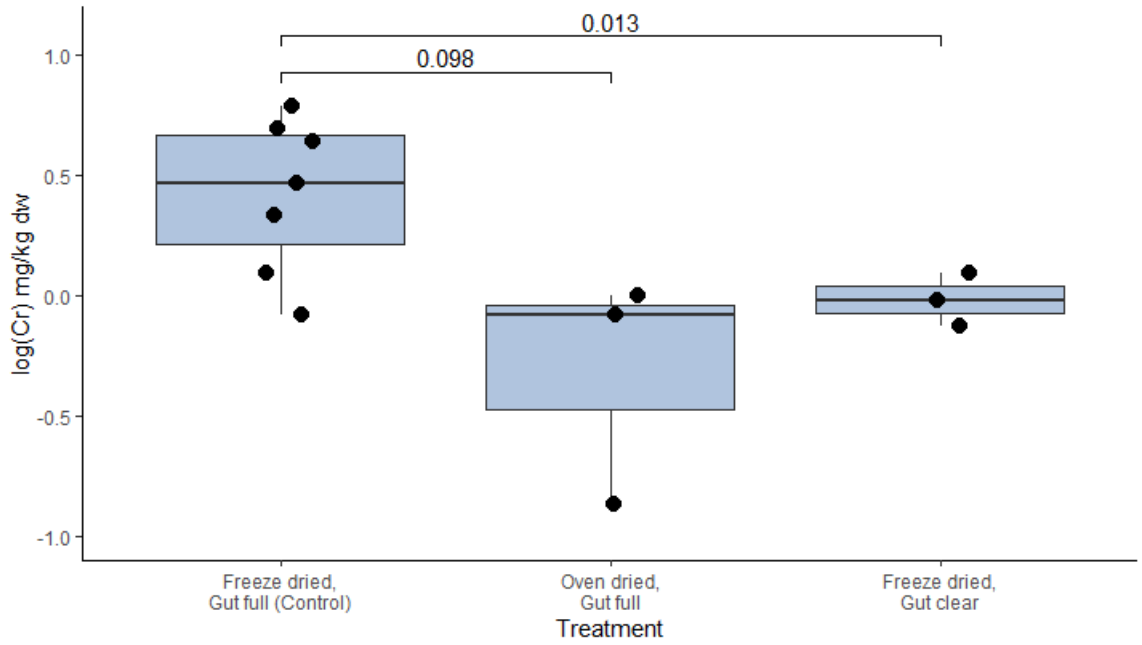


Figure 117. Welch's t-test comparing composition of larvae between processing methods.

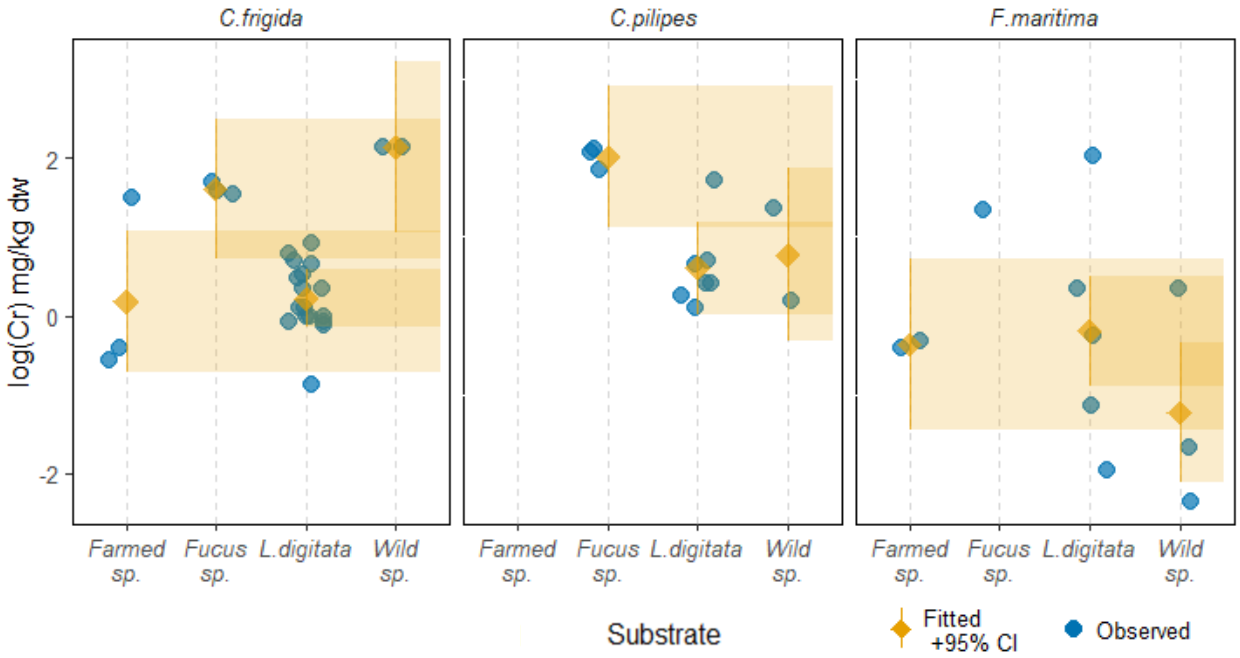


Figure 118. The estimated mean and 95%CI content of larvae reared on each substrate per species.

Table 44. P-values of each pairwise comparison visualised in Figure 118.

C.frigida	*Wild sp.*	*L.digitata*	*Fucus sp.*
L.digitata	0.009		
Fucus sp.	0.872	0.029	
Farmed sp.	0.037	>0.999	0.116

C.pilipes	*Wild sp.*	*L.digitata*	*Fucus sp.*
L.digitata	0.993		
Fucus sp.	0.295	0.050	

F.maritima	*Wild sp.*	*L.digitata*	*Fucus sp.*
L.digitata	0.263		
Farmed sp.	0.601	0.994	

Manganese (Mn)

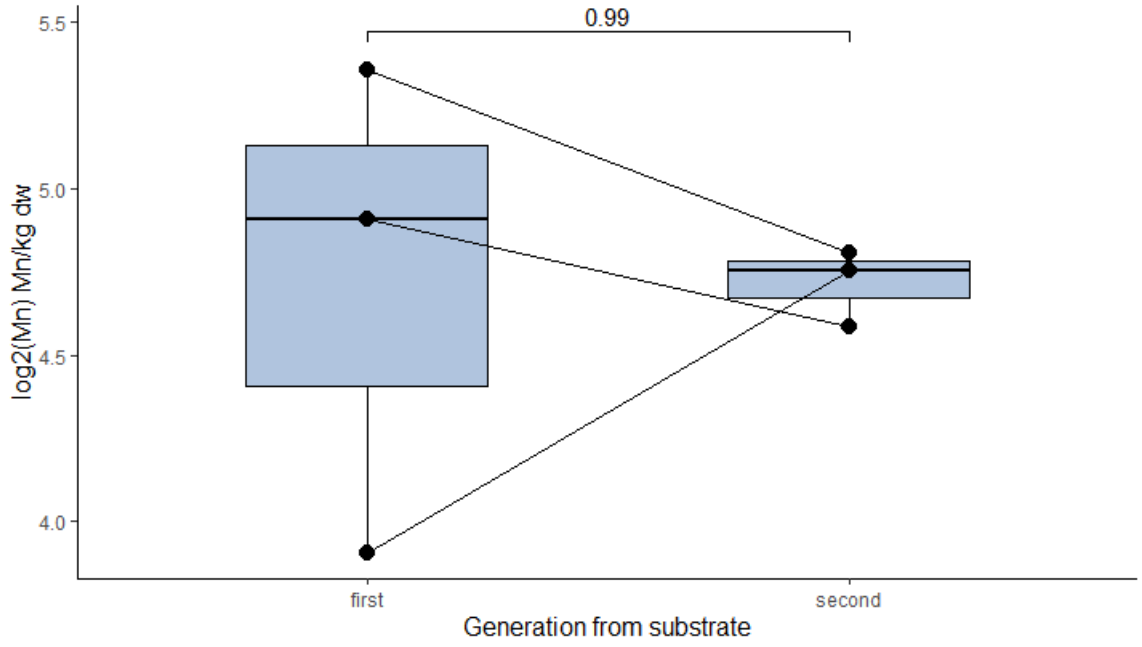


Figure 119. Paired t-test comparing composition of first and second generation of larvae reared on the same substrate.

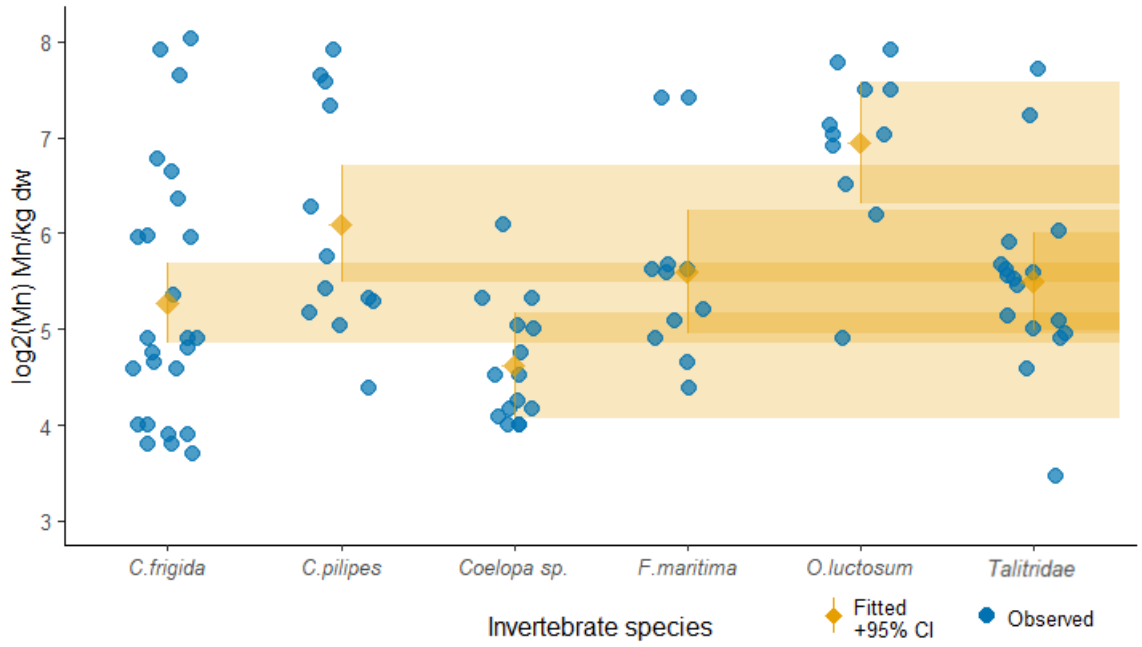


Figure 120. The estimated mean and 95%CI content of each species. 95%CIs have been projected right to allow easy pairwise comparison.

Table 45. P-values of each pairwise comparison visualised in Figure 120.

	Talitridae	*O.luctosum*	*F.maritima*	*Coelopa sp.*	*C.pilipes*
O.luctosum	0.009				
F.maritima	>0.999	0.043			
Coelopa sp.	0.193	<0.001	0.198		
C.pilipes	0.671	0.400	0.872	0.007	
C.frigida	0.985	<0.001	0.960	0.415	0.251

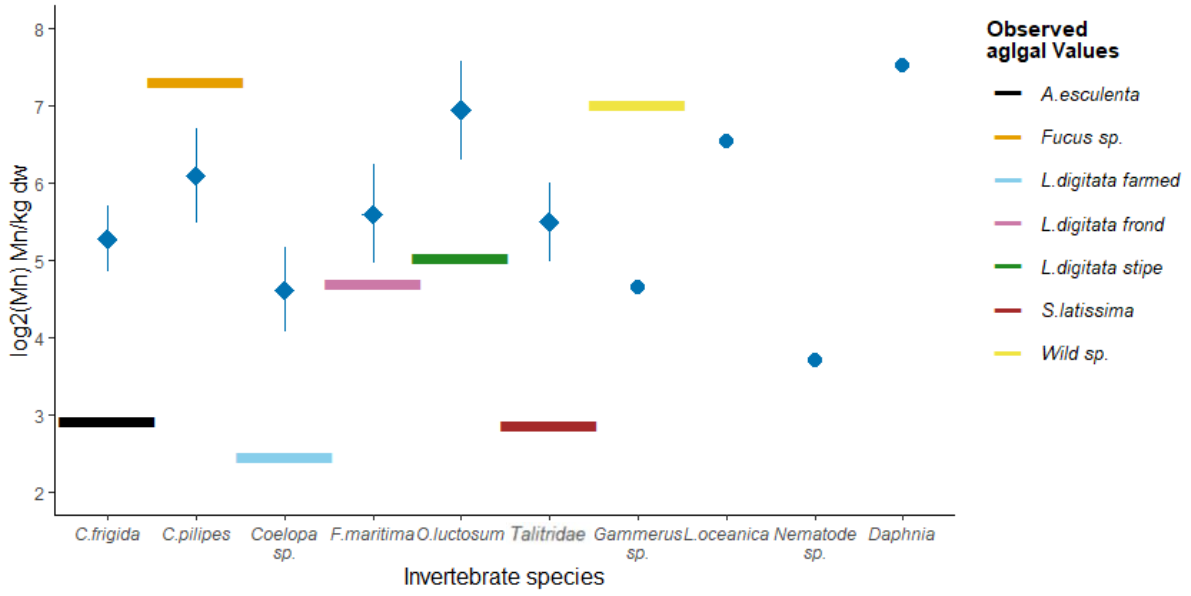


Figure 121. The content in all 10 analysed invertebrate species as well as each of the substrates used.

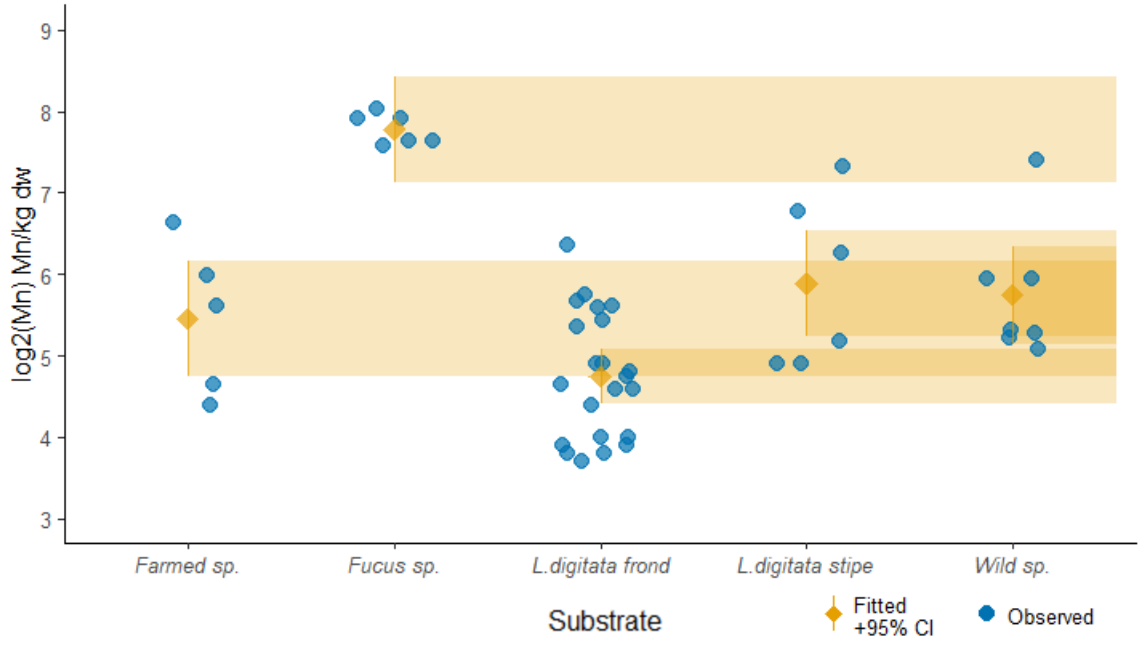


Figure 122. The estimated mean and 95%CI content of each substrate. 95%CIs have been projected right to allow easy pairwise comparison.

Table 46. P-values of each pairwise comparison visualised in Figure 122.

	Wild sp.	***L.digitata stipe***	***L.digitata frond***	***Fucus sp.***
L.digitata stipe	0.997			
L.digitata frond	0.043	0.024		
Fucus sp.	<0.001	0.001	<0.001	
Farmed sp.	0.969	0.888	0.384	<0.001

Iron (Fe)

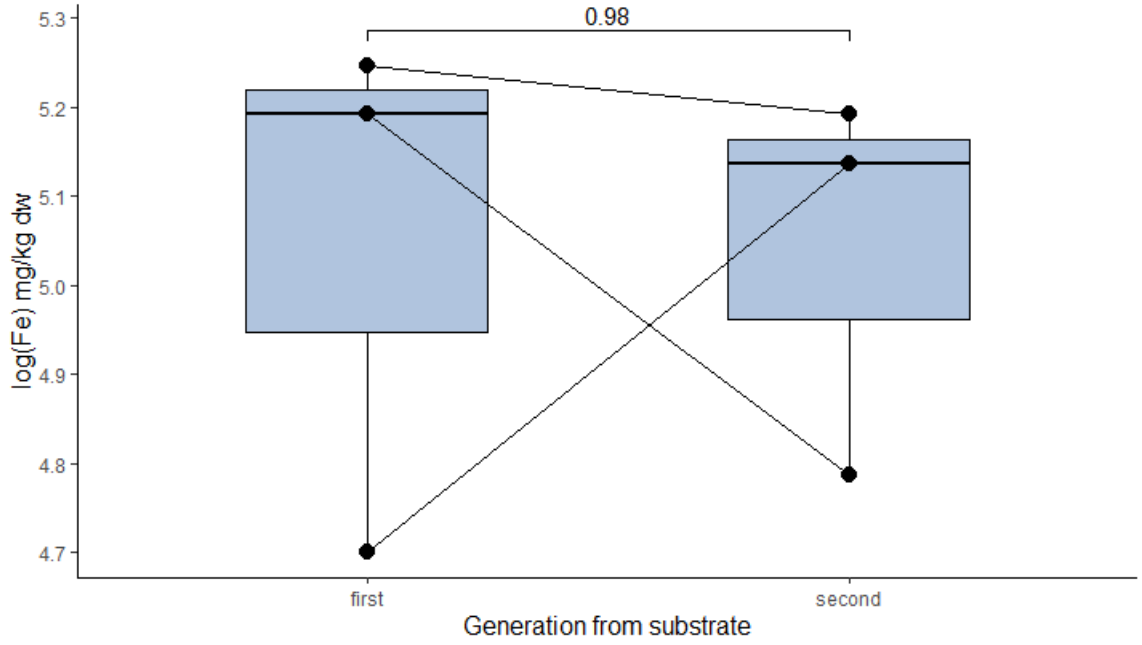


Figure 123. Paired t-test comparing composition of first and second generation of larvae reared on the same substrate.

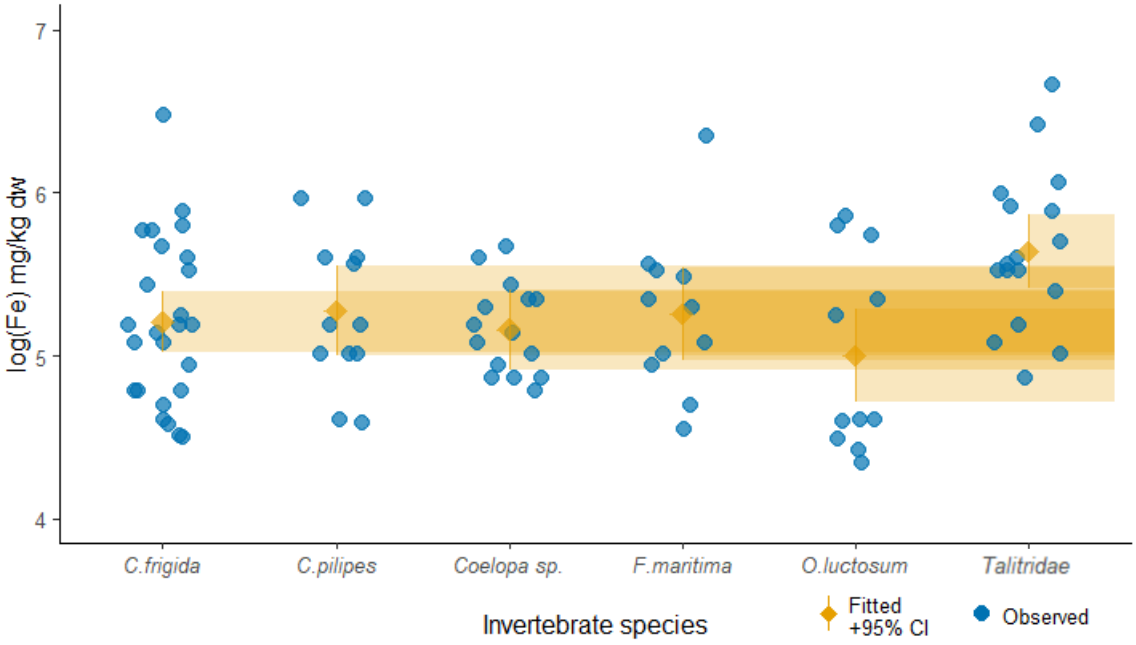


Figure 124. The estimated mean and 95%CI content of each species. 95%CIs have been projected right to allow easy pairwise comparison.

Table 47. P-values of each pairwise comparison visualised in Figure 124.

	Talitridae	*O.luctosum*	*F.maritima*	*Coelopa sp.*	*C.pilipes*
O.luctosum	0.011				
F.maritima	0.312	0.813			
Coelopa sp.	0.064	0.960	0.996		
C.pilipes	0.334	0.752	>0.999	0.990	
C.frigida	0.056	0.842	>0.999	>0.999	0.999

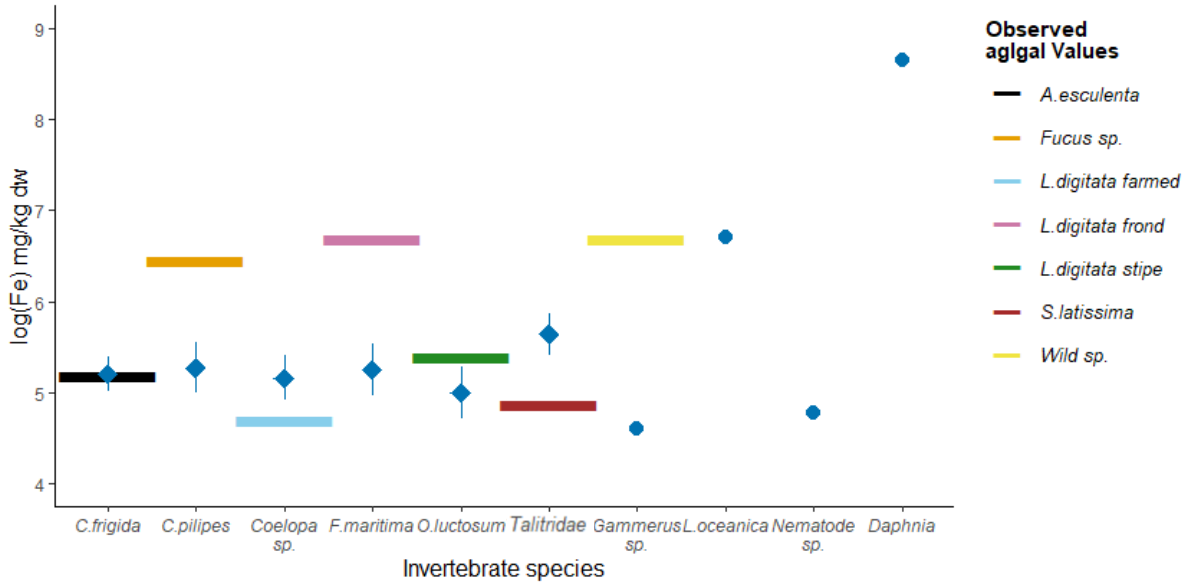


Figure 125. The content in all 10 analysed invertebrate species as well as each of the substrates used.

Copper (Cu)

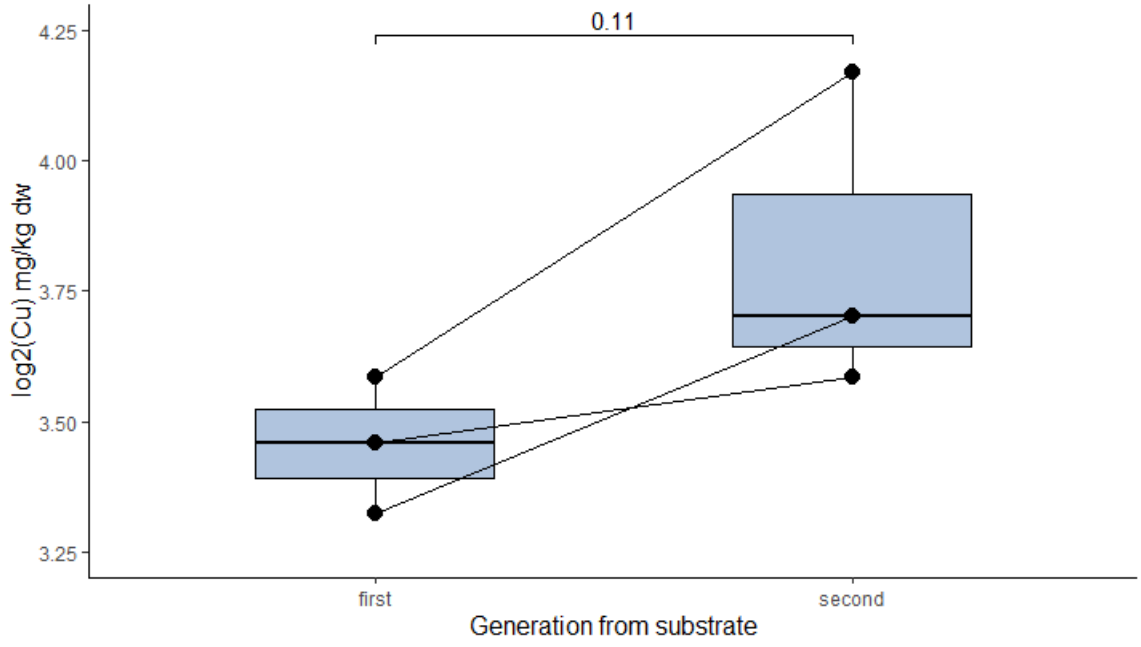


Figure 126. Paired t-test comparing composition of first and second generation of larvae reared on the same substrate.

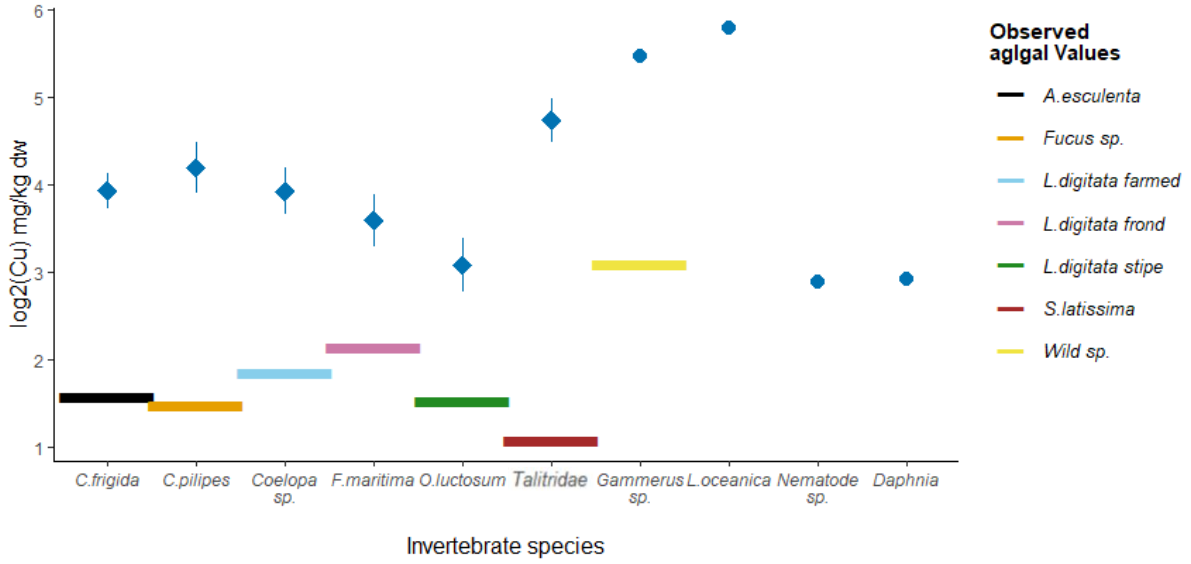


Figure 127. The content in all 10 analysed invertebrate species as well as each of the substrates used.

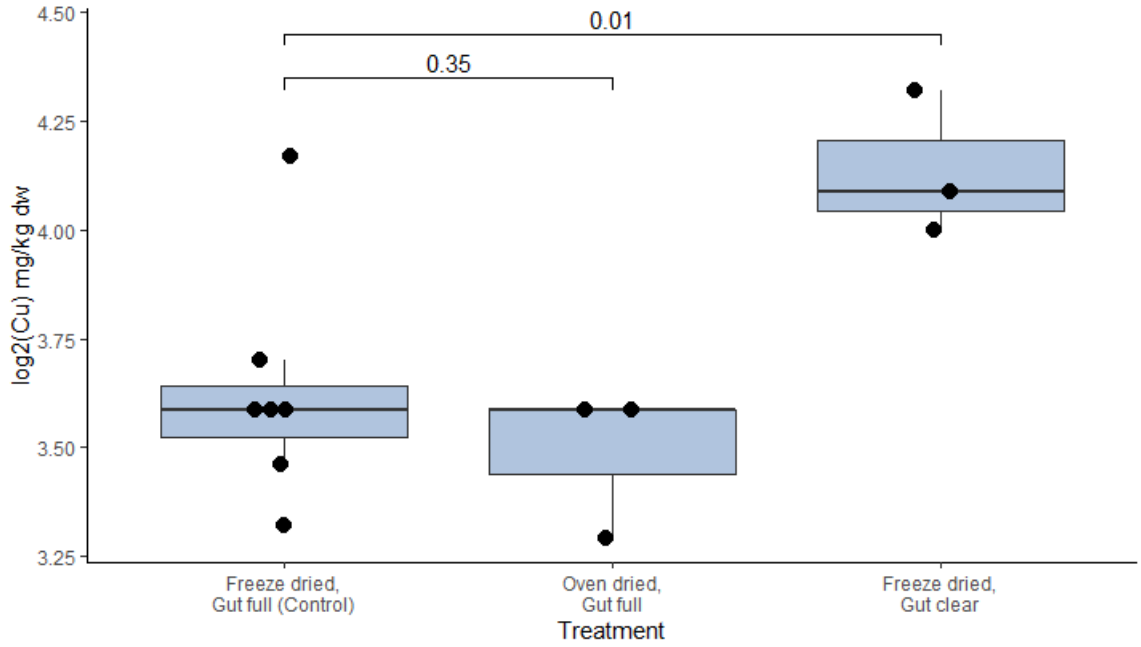


Figure 128. Welch's t-test comparing composition of larvae between processing methods.

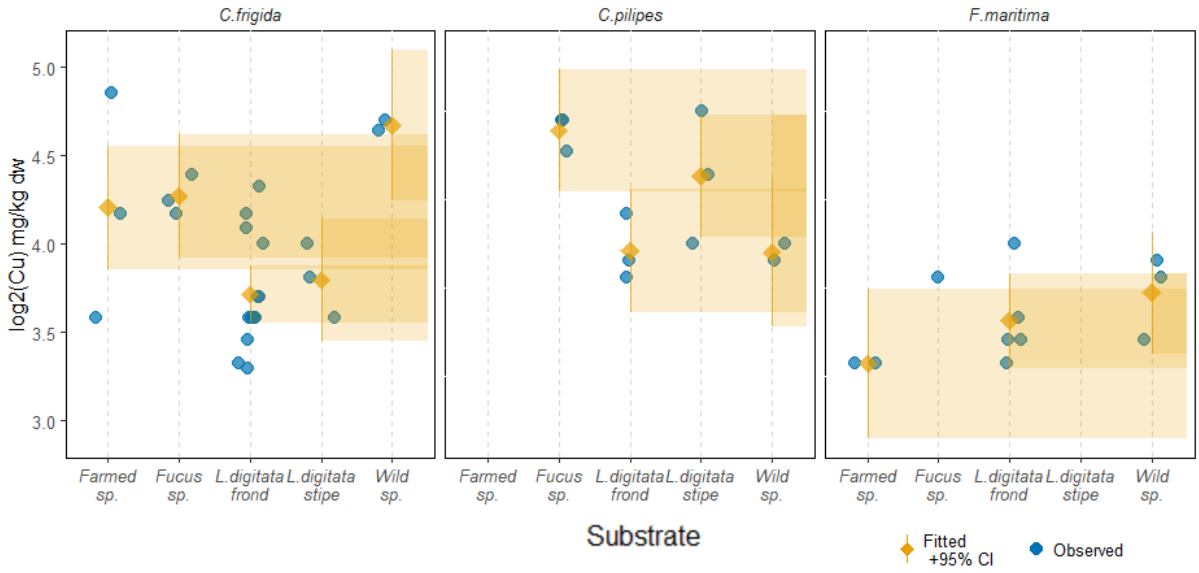


Figure 129. The estimated mean and 95%CI content of larvae reared on each substrate per species.

Table 48. P-values of each pairwise comparison visualised in Figure 129.

C.frigida	*Wild sp.*	*L.digitata*	*Fucus sp.*
L.digitata	0.002		
Fucus sp.	0.510	0.044	
Farmed sp.	0.378	0.092	0.994

C.pilipes	*Wild sp.*	*L.digitata*	*Fucus sp.*
L.digitata	0.967		
Fucus sp.	0.097	0.058	

F.maritima	*Wild sp.*	*L.digitata*	*Fucus sp.*
L.digitata	0.900		
Farmed sp.	0.509	0.794	

Zinc (Zn)

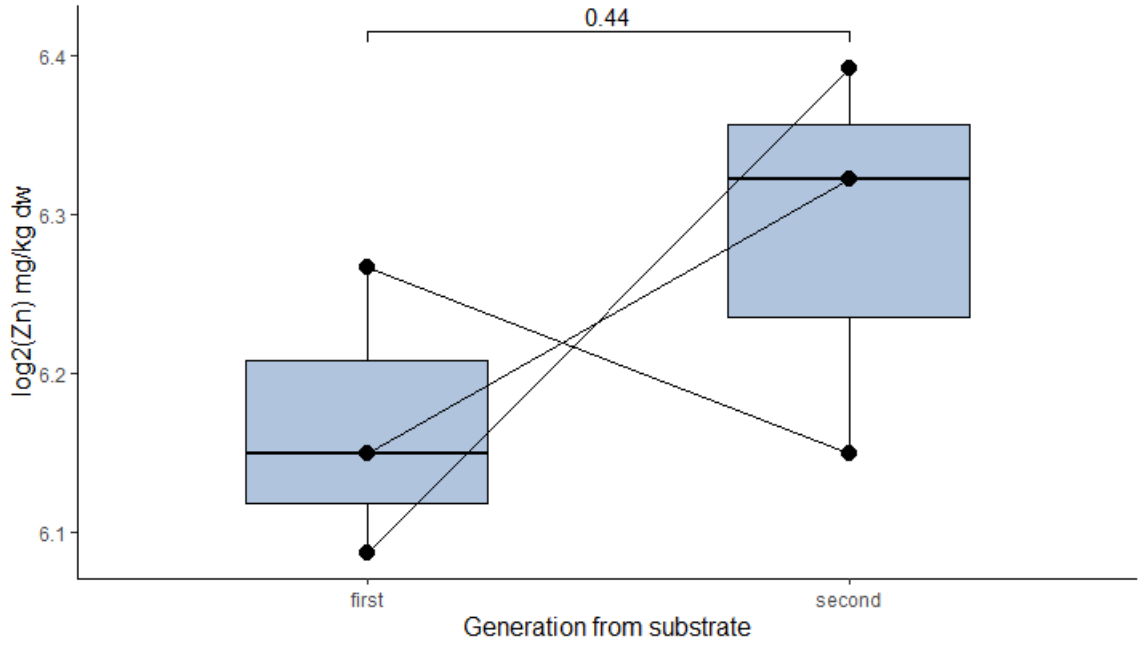


Figure 130. Paired t-test comparing composition of first and second generation of larvae reared on the same substrate.

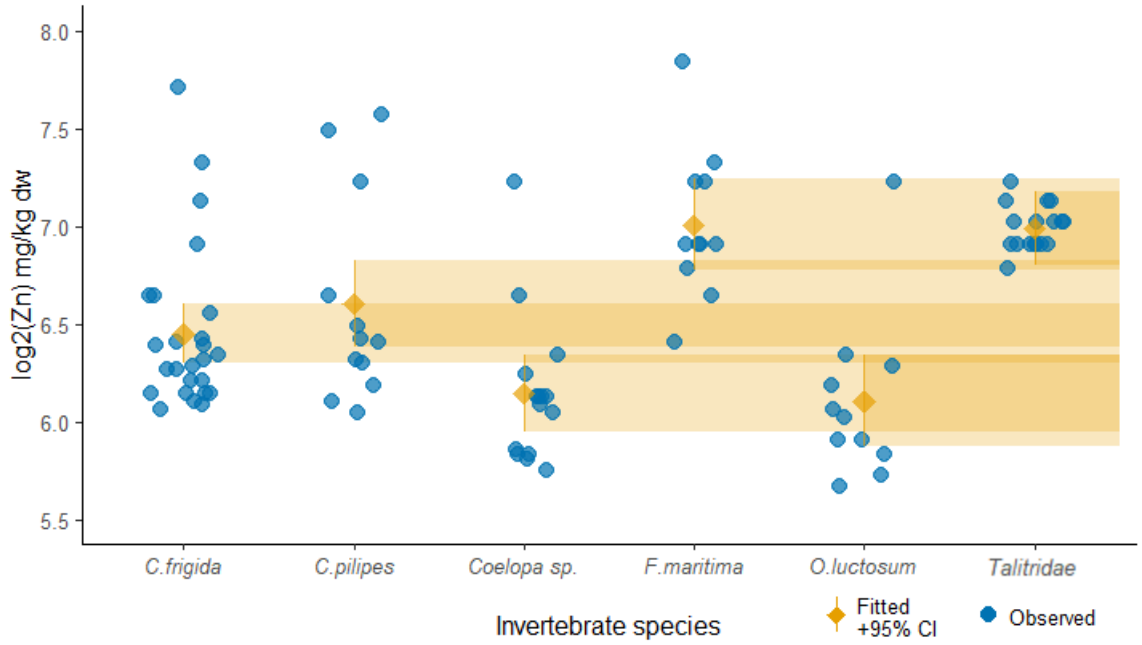


Figure 131. The estimated mean and 95%CI content of each species. 95%CIs have been projected right to allow easy pairwise comparison.

Table 49. P-values of each pairwise comparison visualised in Figure 131.

	Talitridae	*O.luctosum*	*F.maritima*	*Coelopa sp.*	*C.pilipes*
O.luctosum	<0.001				
F.maritima	>0.999	<0.001			
Coelopa sp.	<0.001	>0.999	<0.001		
C.pilipes	0.094	0.034	0.134	0.036	
C.frigida	<0.001	0.149	0.002	0.164	0.877

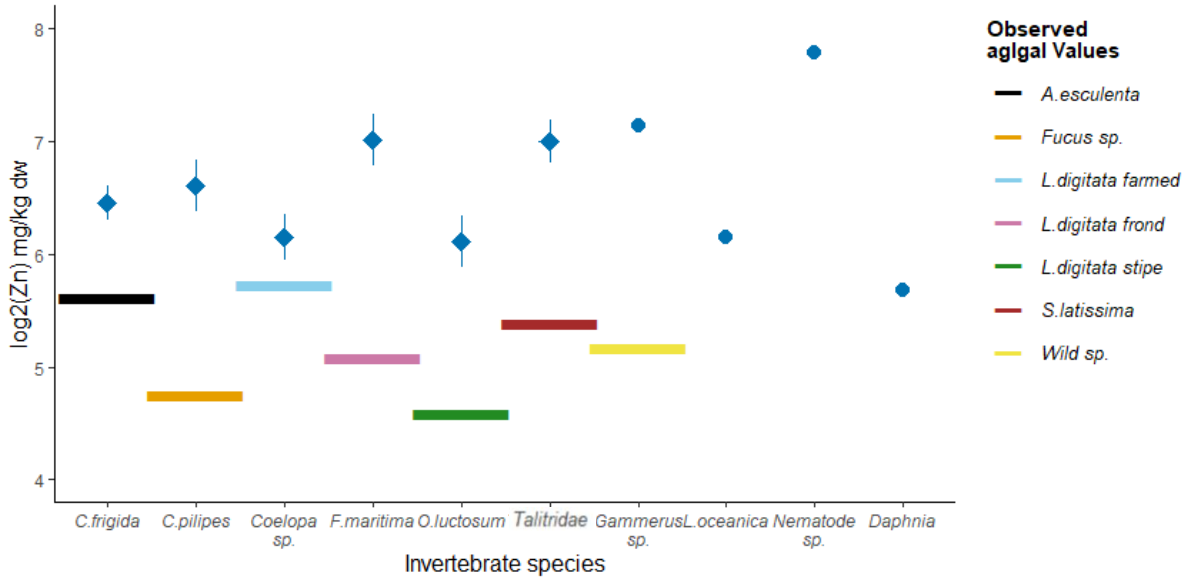


Figure 132. The content in all 10 analysed invertebrate species as well as each of the substrates used.

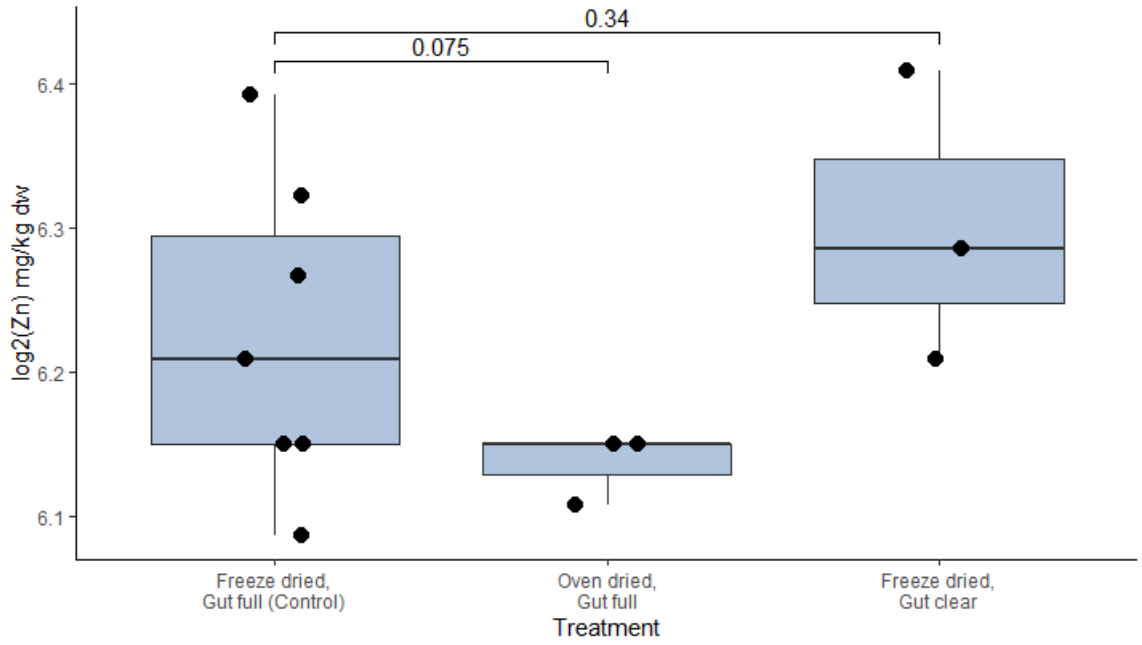


Figure 133. Welch's t-test comparing composition of larvae between processing methods.

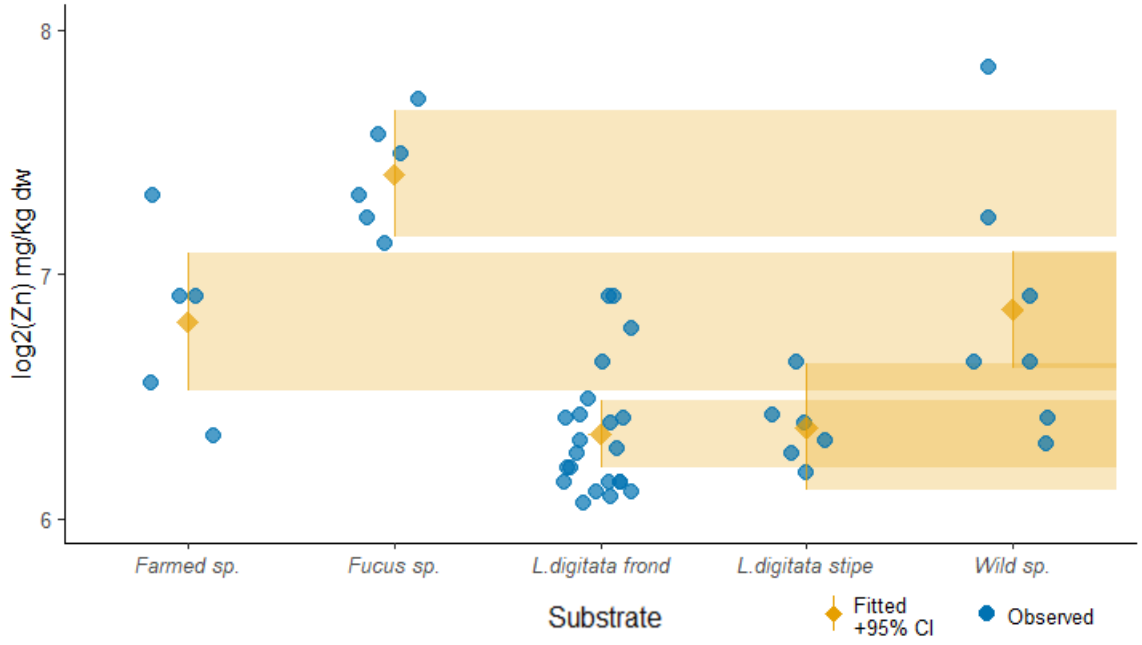


Figure 134. The estimated mean and 95%CI content of each substrate. 95%CIs have been projected right to allow easy pairwise comparison.

Table 50. P-values of each pairwise comparison visualised in Figure 134.

	Wild sp.	***L.digitata stipe***	***L.digitata frond***	***Fucus sp.***
L.digitata stipe	0.064			
L.digitata frond	0.005	>0.999		
Fucus sp.	0.023	<0.001	<0.001	
Farmed sp.	0.999	0.175	0.040	0.023

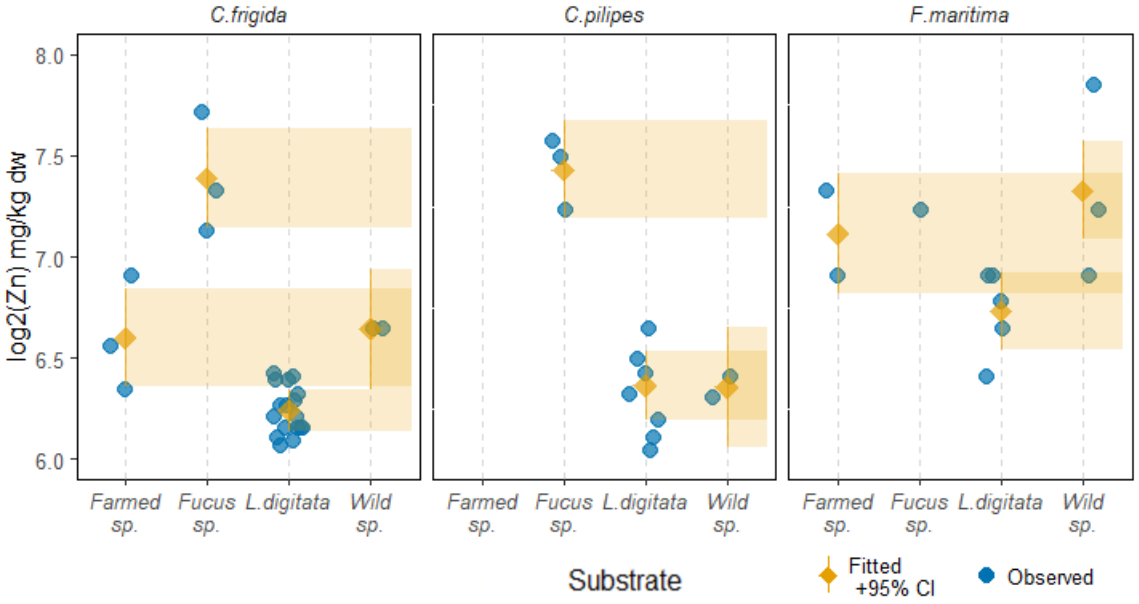


Figure 135. The estimated mean and 95%CI content of larvae reared on each substrate per species.

Table 51. P-values of each pairwise comparison visualised in Figure 135.

C.frigida	*Wild sp.*	*L.digitata*	*Fucus sp.*
L.digitata	0.059		
Fucus sp.	0.002	<0.001	
Farmed sp.	0.996	0.040	<0.001

C.pilipes	*Wild sp.*	*L.digitata*	*Fucus sp.*
L.digitata	>0.999		
Fucus sp.	<0.001	<0.001	

F.maritima	*Wild sp.*	*L.digitata*	*Fucus sp.*
L.digitata	0.002		
Farmed sp.	0.678	0.138	

Arsenic (As)

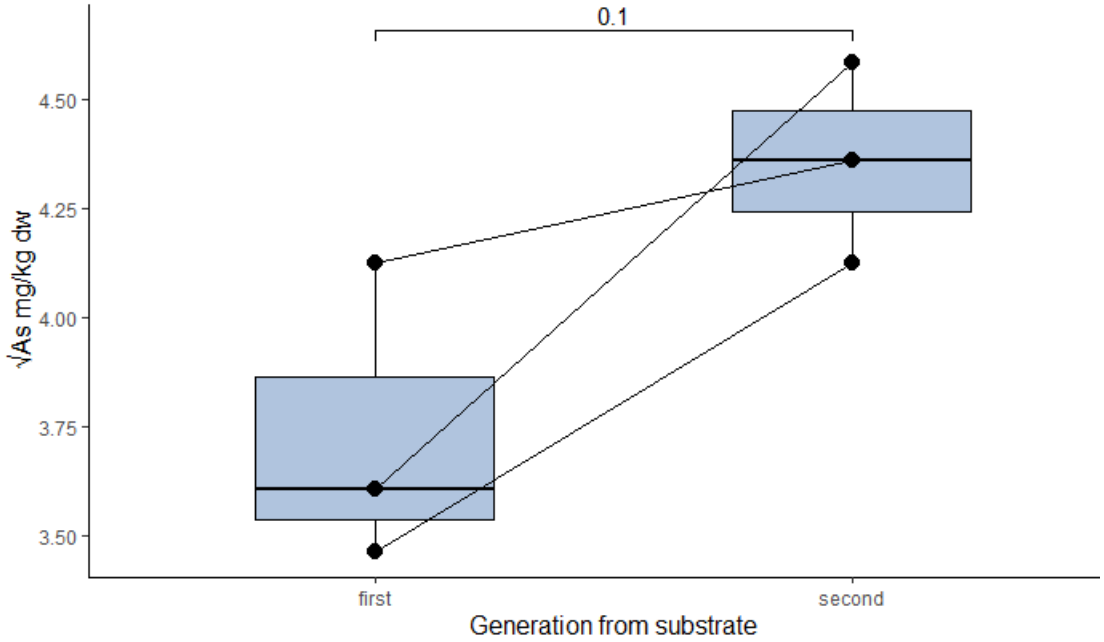


Figure 136. Paired t-test comparing composition of first and second generation of larvae reared on the same substrate.

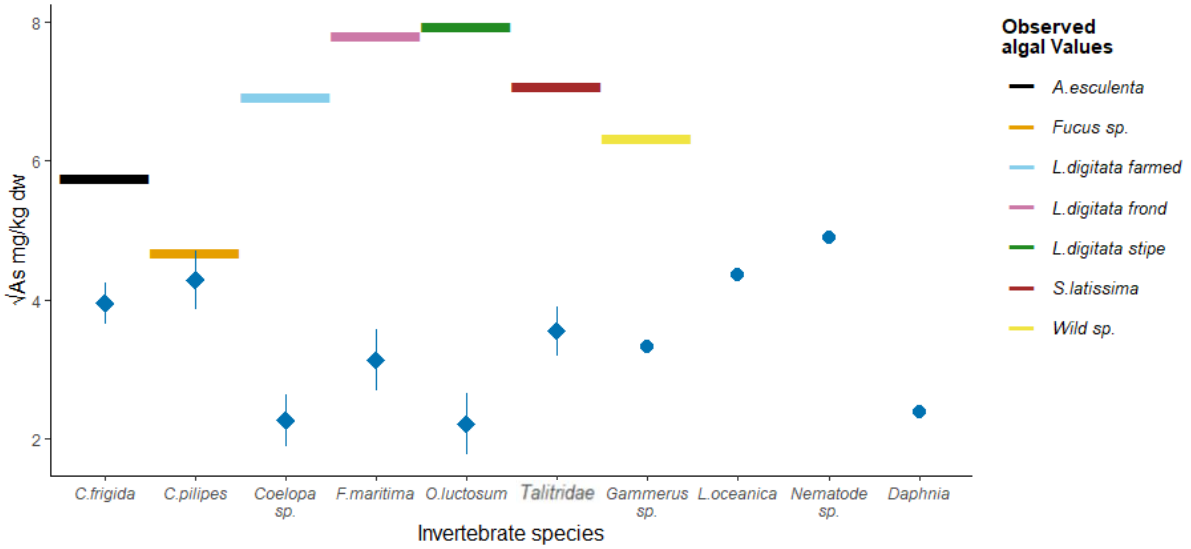


Figure 137. The content in all 10 analysed invertebrate species as well as each of the substrates used.

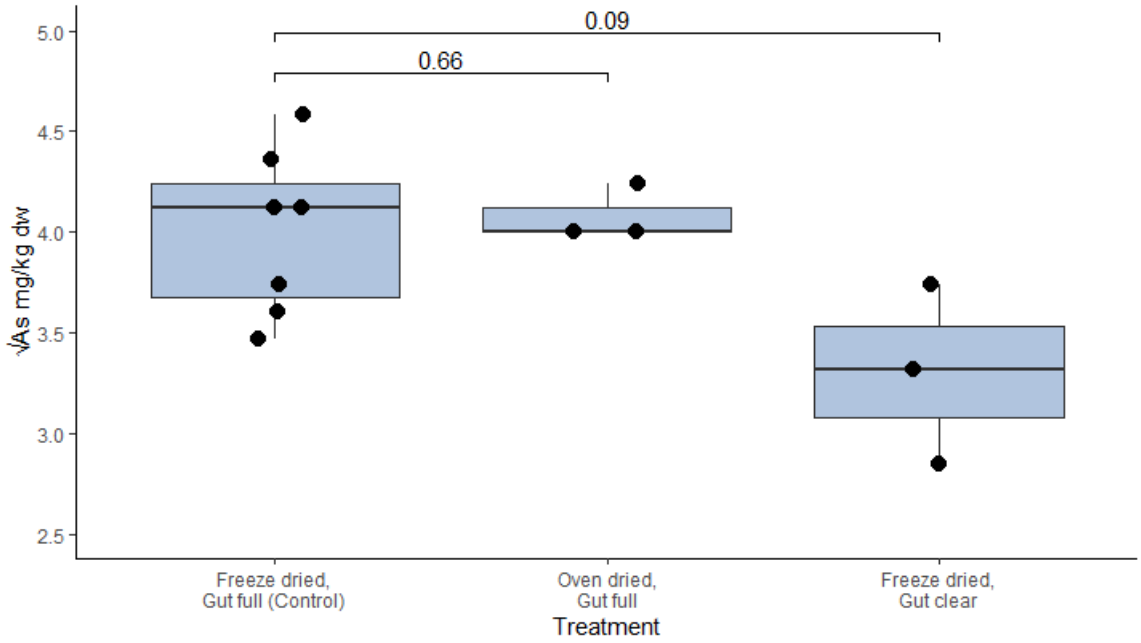


Figure 138. Welch's t-test comparing composition of larvae between processing methods.

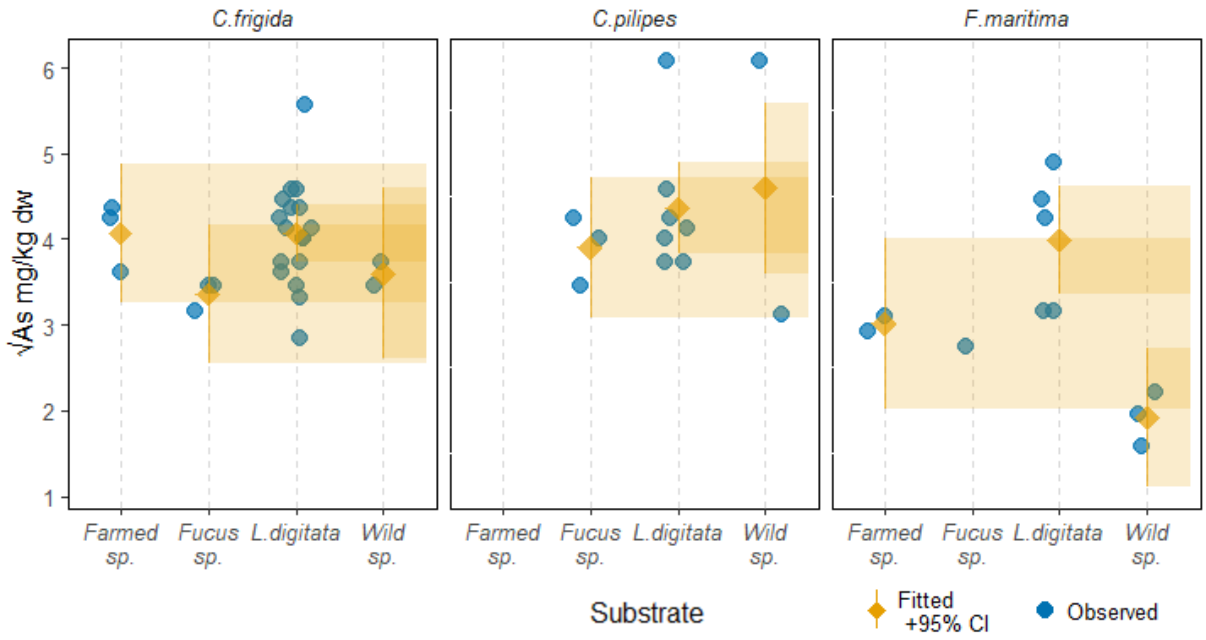


Figure 139. The estimated mean and 95%CI content of larvae reared on each substrate per species.

Table 52. P-values of each pairwise comparison visualised in Figure 139.

C.frigida	*Wild sp.*	*L.digitata*	*Fucus sp.*
L.digitata	0.809		
Fucus sp.	0.981	0.382	
Farmed sp.	0.882	>0.999	0.603
C.pilipes	*Wild sp.*	*L.digitata*	*Fucus sp.*
L.digitata	0.973		
Fucus sp.	0.693	0.776	
F.maritima	*Wild sp.*	*L.digitata*	*Fucus sp.*
L.digitata	0.001		
Farmed sp.	0.320	0.352	

Cadmium(Cd)

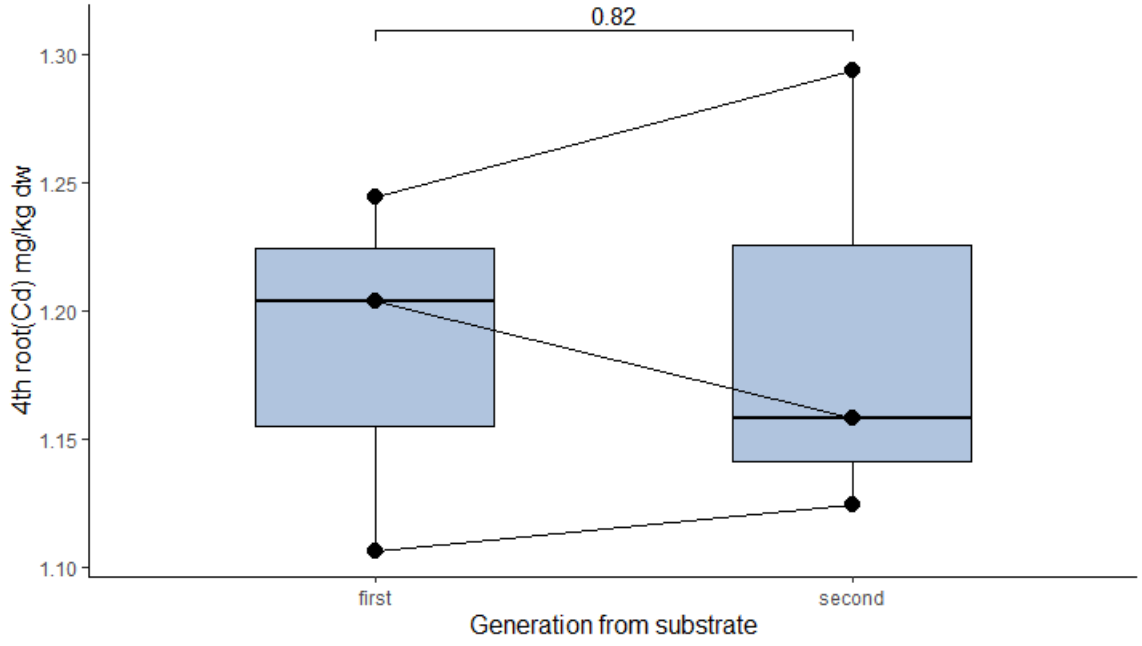


Figure 140. Paired t-test comparing composition of first and second generation of larvae reared on the same substrate.

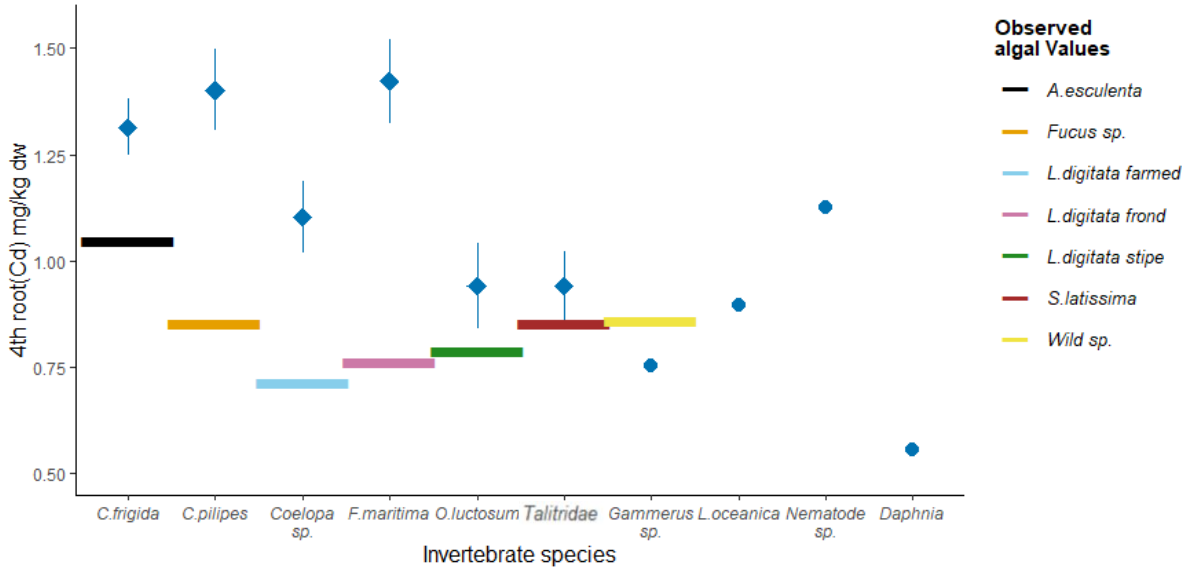


Figure 141. The content in all 10 analysed invertebrate species as well as each of the substrates used.

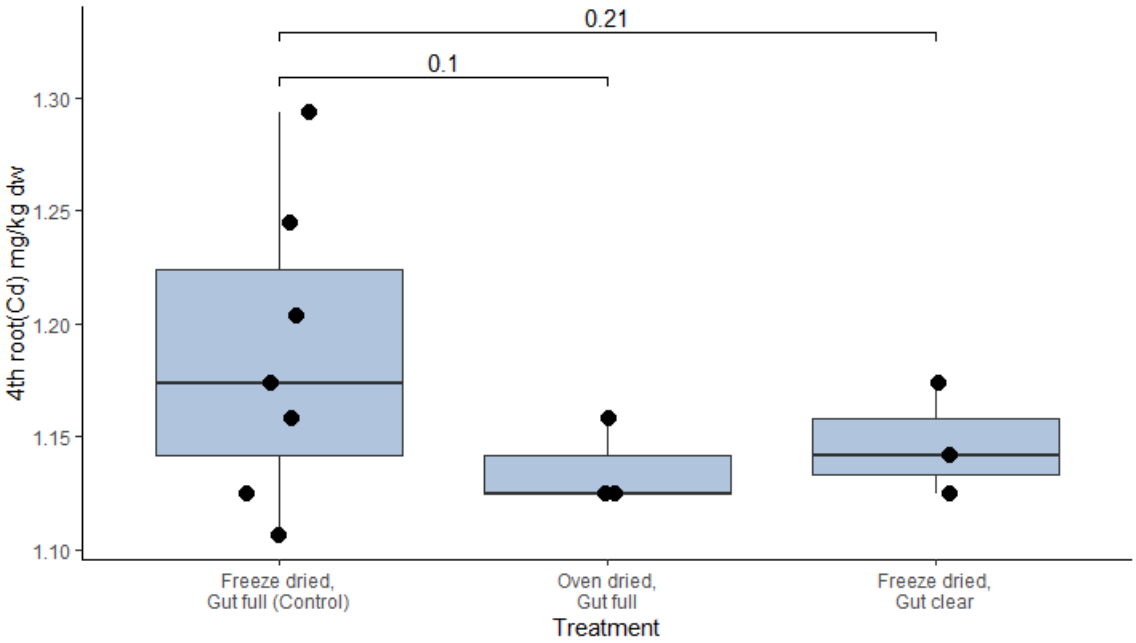


Figure 142. Welch's t-test comparing composition of larvae between processing methods.

Mercury (Hg)

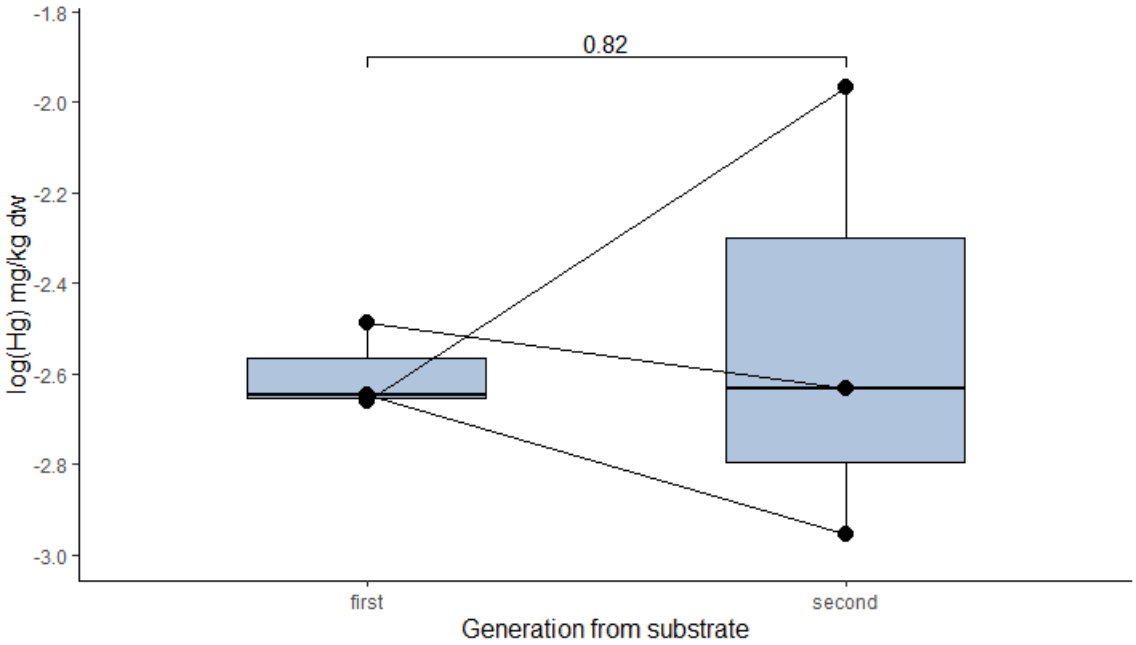


Figure 143. Paired t-test comparing composition of first and second generation of larvae reared on the same substrate.

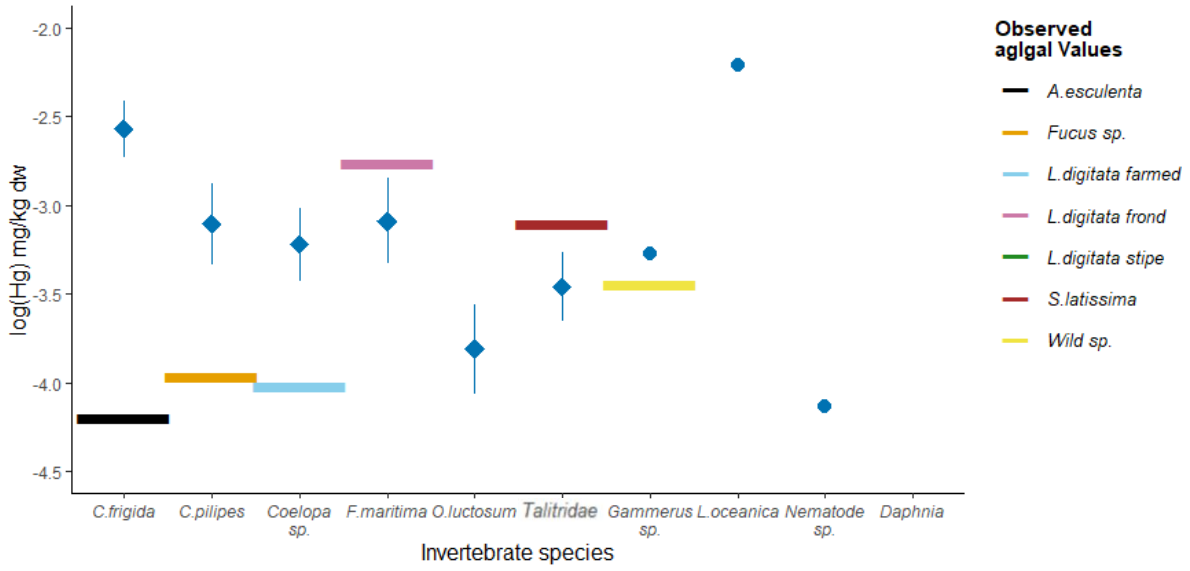


Figure 144. The content in all 10 analysed invertebrate species as well as each of the substrates used.

Lead (Pb)

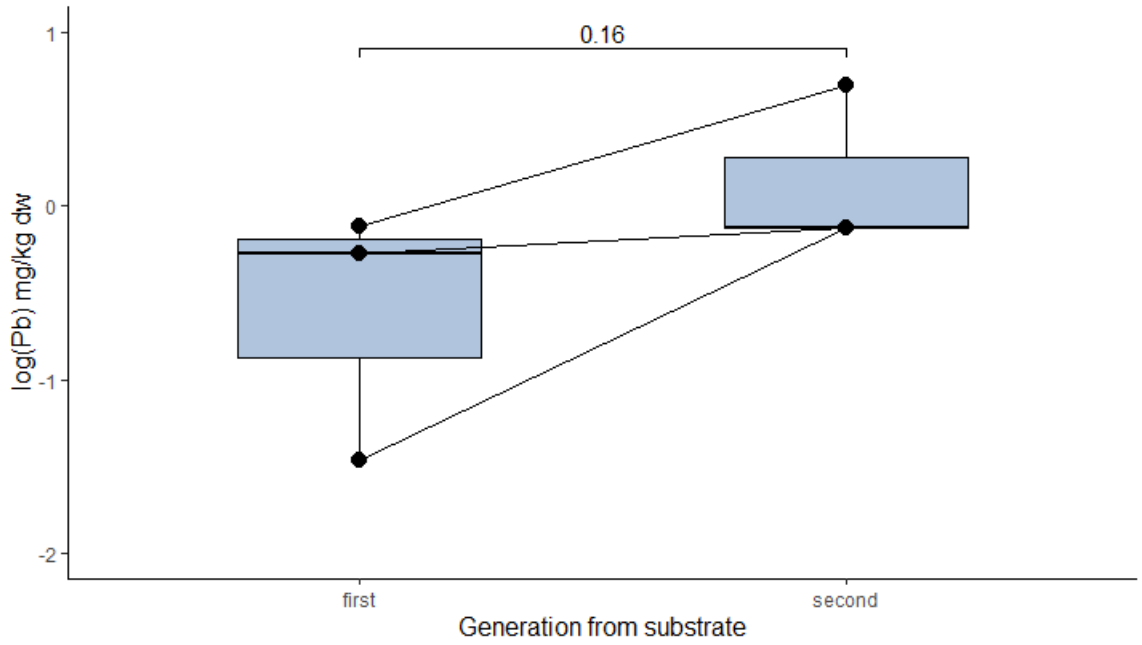


Figure 145. Paired t-test comparing composition of first and second generation of larvae reared on the same substrate.

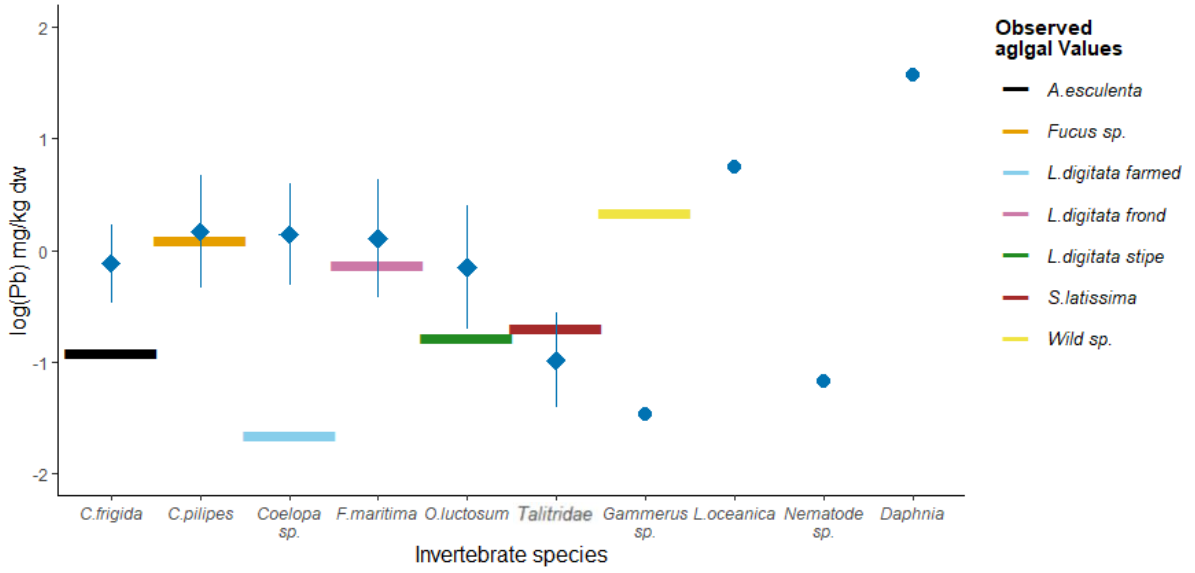


Figure 146. The content in all 10 analysed invertebrate species as well as each of the substrates used.

Appendix 5.2

Appendix 5.2.1. Mean amino acid content of each sample type.

INVERT SPECIES	ALANINE	PROLINE	LYSINE	TYROSINE	METHIONINE	VALINE	ISOLEUCINE	LEUCINE	PHENYLALANINE
C.FRIGIDA	25.45 ± 0.36	22.17 ± 1.25	33.5 ± 0.57	32.5 ± 2.38	13.32 ± 0.61	25.6 ± 1.02	20.25 ± 0.75	34 ± 1.41	31.67 ± 1.81
C.PILIPES	25.76 ± 1.53	21.43 ± 1.7	34.33 ± 3.51	32.33 ± 2.51	13.53 ± 0.49	25.16 ± 1.41	19.5 ± 1.4	33.33 ± 1.52	30.2 ± 1.38
COELOPA SP.	27.2 ± 3.74	23.42 ± 2.94	35.5 ± 2.88	39.5 ± 6.55	14.12 ± 0.62	27.57 ± 2.54	20.97 ± 1.49	35.5 ± 3.31	34 ± 3.55
F.MARITIMA	26.93 ± 2.62	22.83 ± 0.96	37 ± 0.63	41 ± 7.53	13.21 ± 1.09	26.86 ± 1.05	21.48 ± 0.9	34.66 ± 1.21	33.43 ± 4.58
O.LUCTOSUM	22.76 ± 2.49	17.65 ± 2.01	26.85 ± 2.85	23.6 ± 3.98	9.3 ± 0.46	19.55 ± 1.71	15.83 ± 1.11	25.83 ± 1.66	24.25 ± 2.27
T.ORCHESTIA	21.06 ± 5.34	19.56 ± 5.1	20.1 ± 4.46	13.5 ± 3.27	7.5 ± 1.75	16.76 ± 3.9	13.3 ± 3.08	23.76 ± 5.48	17.5 ± 4.3
GAMMERUS SP.[1]	23.4	22.6	22.9	15	8.3	18.5	14.7	26.4	18.9
L.OCEANICA[1]	18	24.9	23.7	13.8	7.7	16.4	14.9	24.6	18.3
NEMATODE SP.[1]	53	47	32	16.8	11.9	23	18.6	33	20
DAPHNIA[1]	14.5	12.1	14.3	10.7	5.4	13.8	11.1	18.9	12.3
ALGAL SPECIES[1]									
A.ESCULENTA	11.7	7.1	8.7	5.3	3.8	8.8	6.7	12.8	8.4
FUCUS SP.	5.8	4.1	5.5	3.7	1.91	4.9	4	7.2	4.8
L.DIGITATA FARMED	8.2	5.5	6.2	3.8	2.59	6.2	4.5	8.6	5.9
L.DIGITATA STIPE	8.2	3	4.3	2.6	0.82	3.2	2.01	3.3	2.25
S.LATISSIMA	10.4	6.1	6.9	4	2.99	7	5.4	10.5	6.9

INVERT SPECIES	H.PROLINE	HISTIDINE	TAURINE	SERINE	ARGININE	GLYCINE	ASPARTIC	GLUTAMIC	THREONINE
C.FRIGIDA	0 ± 0	18.27 ± 2.85	1.82 ± 0.77	24.87 ± 1.76	32.92 ± 2.29	25 ± 1.61	48.5 ± 1.73	64.5 ± 3.69	24.05 ± 1.04
C.PILIPES	0 ± 0	16.8 ± 1.45	2.24 ± 0.84	24.66 ± 1.3	32.33 ± 1.15	24 ± 1.75	51 ± 5.56	66.66 ± 3.78	22.6 ± 0.78
COELOPA SP.	0.17 ± 0.34	20.45 ± 1.99	2.25 ± 0.77	25.62 ± 3.22	34.75 ± 4.78	26.37 ± 3.45	50 ± 2	63.5 ± 6.65	24.12 ± 1.99
F.MARITIMA	0 ± 0	17.91 ± 1.89	0.3 ± 0.48	25.36 ± 1.24	31.23 ± 1.49	24.51 ± 1.57	52.5 ± 3.33	64.5 ± 5.31	24.48 ± 0.62
O.LUCTOSUM	0.11 ± 0.28	11.35 ± 1.85	1.28 ± 3.14	19.03 ± 1.46	25.93 ± 2.11	20.81 ± 3.53	41 ± 4	53.5 ± 4.96	18.51 ± 1.44
T.ORCHESTIA	0.25 ± 0.43	6.66 ± 1.78	5.3 ± 1.8	17.93 ± 3.72	25.1 ± 6.11	24.56 ± 5.69	33.46 ± 7	52.33 ± 13.27	15.93 ± 3.76
GAMMERUS SP.[1]	0.6	7.4	5.3	20.7	27.4	27.4	38	56	17.7
L.OCEANICA[1]	0	7	12.5	17.5	26.9	30	37	53	15.4
NEMATODE SP.[1]	1.8	9.3	1.69	23.1	27.6	25.9	48	62	24.3
DAPHNIA[1]	0.81	4.4	0	12.6	15.4	12.3	21.5	29.7	13.1
ALGAL SPECIES[1]									
A.ESCULENTA	0	2.37	0.61	8.4	8	9.7	18	18.3	8.4
FUCUS SP.	0	1.45	0	5	4.3	5.4	12.4	12.1	4.8
L.DIGITATA FARMED	0	1.66	0.7	6.2	5.4	6.8	14.3	14.6	6.6
L.DIGITATA STIPE	0	1.1	0	4	2.08	3.6	12.4	15.1	4.2
S.LATISSIMA	0	1.96	0.61	6.8	5.8	7.7	15.4	17.3	7.1

[1]N=1

Appendix 5.2.2. Mean fatty acid content of each sample type. Table extends over four pages

Species	10:00	12:00	14:00	14:1N-9	15:00	16:00	16:1N-7	16:1N-9	16:2-4	16:3N-3
C.frigida	<0.01	0.55 ± 0.24	6.05 ± 1.83	1.02 ± 0.57	0.82 ± 0.21	20.37 ± 4.99	35.49 ± 12.09	1.81 ± 0.49	<0.01	<0.01
C.pilipes	<0.01	0.22 ± 0.14	4.71 ± 2.06	0.47 ± 0.31	0.67 ± 0.28	16.17 ± 6.89	24.89 ± 12.95	1.74 ± 0.73	<0.01	<0.01
Coelopa sp.	<0.01	0.37 ± 0.17	6.34 ± 2.35	1.33 ± 0.92	0.95 ± 0.25	21.51 ± 10.16	38.91 ± 25.74	1.97 ± 0.58	<0.01	<0.01
F.maritima	<0.01	0.42 ± 0.12	6.91 ± 1.54	0.6 ± 0.26	0.98 ± 0.36	35.09 ± 10.25	44.84 ± 11.15	4.62 ± 2.52	<0.01	<0.01
O.luctosum	<0.01	0.3 ± 0.11	8 ± 3.35	1.34 ± 0.78	1.47 ± 0.63	18.72 ± 6.8	30.36 ± 10.85	2.89 ± 1.17	<0.01	<0.01
T.orchestia	<0.01	11.15 ± 5.22	14.86 ± 7.25	2.48 ± 1.55	0.53 ± 0.24	23.83 ± 8.57	12.71 ± 6.79	<0.01	<0.01	<0.01
Gammerus sp.[1]	<0.01	0.52	5.94	<0.01	0.61	15.25	10.19	1.03	0.43	0.25
L.oceanica[1]	<0.01	0.08	0.97	0.13	0.31	7.01	3.66	0.24	0.41	0.12
Nematode sp.[1]	<0.01	2.39	12.81	1.49	0.58	5.94	9.65	2.43	<0.01	0.2
Daphnia[1]	0.03	0.1	1.86	0.06	0.53	5.9	3.67	0.87	0.19	0.13
	16:4n-3	**17:00**	**18:00**	**18:1n-11**	**18:1n-7**	**18:1n-9**	**18:2n-6**	**18:3n-3**	**18:3n-6**	**18:4n-3**
C.frigida	<0.01	0.38 ± 0.09	2.05 ± 0.86	<0.01	5.53 ± 1.78	19.93 ± 5.54	2.96 ± 1.99	1.51 ± 1.43	0.45 ± 0.14	1.01 ± 0.8
C.pilipes	<0.01	0.32 ± 0.15	1.99 ± 1.11	<0.01	3.25 ± 1.7	17.13 ± 6.76	3.14 ± 2.41	1.62 ± 1.7	0.48 ± 0.25	0.82 ± 0.72
Coelopa sp.	<0.01	0.46 ± 0.16	2.77 ± 1.43	<0.01	6.35 ± 3.04	25.4 ± 9.6	4 ± 0.87	1.99 ± 0.43	0.42 ± 0.15	1.01 ± 0.43
F.maritima	<0.01	0.38 ± 0.16	3.03 ± 0.9	<0.01	4.28 ± 2.31	28.17 ± 7.49	4.33 ± 2.41	2 ± 1.11	0.52 ± 0.26	1.32 ± 0.96
O.luctosum	<0.01	0.53 ± 0.24	2.35 ± 0.95	<0.01	4.76 ± 1.71	25.71 ± 9.73	4.62 ± 2.36	2.05 ± 0.99	0.46 ± 0.19	1.12 ± 0.49
T.orchestia	<0.01	0.59 ± 0.31	2.82 ± 0.86	<0.01	3.96 ± 1.54	52.25 ± 17.36	5.76 ± 2.49	2.25 ± 1.04	<0.01	1.01 ± 0.43
Gammerus sp.[1]	<0.01	1.13	1.89	<0.01	4.3	21.73	5.6	3.25	0.31	0.73
L.oceanica[1]	<0.01	0.16	1.29	<0.01	3.15	7.54	3.06	1.99	<0.01	0.18
Nematode sp.[1]	0.12	0.95	2.34	0.26	11.08	2.4	1.73	1.17	<0.01	0.44
Daphnia[1]	<0.01	0.43	0.97	<0.01	1.33	3.2	0.56	1	<0.01	0.12

[1]n=1

Species	20:00	20:1N-11	20:1N-7	20:1N-9	20:2N-6	20:3N-6	20:3N-9	20:4N-3	20:4N-6	20:5N-3
C.frigida	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	4.19 ± 2.2	3.72 ± 1.55
C.pilipes	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	3.13 ± 1.12	2.57 ± 1.06
Coelopa sp.	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	4.31 ± 1.73	4.39 ± 1.69
F.maritima	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	2.53 ± 0.9	2.8 ± 1.02
O.luctosum	0.2 ± 0.08	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	5.32 ± 2.22	4.27 ± 2.23
T.orchestia	0.33 ± 0.13	<0.01	0.74 ± 0.27	2.92 ± 0.61	0.88 ± 0.29	0.47 ± 0.2	<0.01	0.23 ± 0.07	9.77 ± 2.96	4.53 ± 1.04
Gammerus sp.[1]	<0.01	<0.01	0.45	1.05	0.74 ± 0.6	0.22	<0.01	0.22	4.47	4.98
L.oceanica[1]	<0.01	<0.01	<0.01	0.15	0.34	0.09	<0.01	0.1	2.91	3.52
Nematode sp.[1]	<0.01	2.17	0.13	1.05	1.43	0.4	0.18	0.48	4.46	4.83
Daphnia[1]	<0.01	<0.01	<0.01	<0.01	0.04	<0.01	<0.01	<0.01	0.14	0.6
	21:5n-3	**22:00**	**22:1n-11**	**22:1n-9**	**22:4n-6**	**22:5n-3**	**22:5n-6**	**22:6n-3**	**24:1n-9**	**24:6n-3**
C.frigida	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
C.pilipes	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Coelopa sp.	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
F.maritima	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
O.luctosum	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
T.orchestia	<0.01	<0.01	<0.01	<0.01	0.37 ± 0.1	0.63 ± 0.09	<0.01	0.51 ± 0.17	<0.01	<0.01
Gammerus sp.[1]	<0.01	<0.01	<0.01	<0.01	<0.01	0.41	<0.01	1.54	<0.01	<0.01
L.oceanica[1]	<0.01	<0.01	<0.01	<0.01	<0.01	0.07	<0.01	0.42	<0.01	<0.01
Nematode sp.[1]	<0.01	<0.01	0.38	<0.01	0.97	0.59	<0.01	<0.01	<0.01	<0.01
Daphnia[1]	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

[1]N=1

SPECIES	10:00	12:00	14:00	15:00	16:00	17:00	18:00
A.ESCULENTA	<0.01	<0.01	0.42	0.02	0.94	0.03	0.06
FUCUS SP.	<0.01	<0.01	1.78	0.04	1.41	0.05	0.05
L.DIGITATA FARMED	<0.01	0.01	0.61	0.02	1.1	0.02	0.03
L.DIGITATA FROND	<0.01	<0.01	0.31	0.01	0.68	<0.01	0.05
L.DIGITATA STIPE	<0.01	<0.01	0.2	<0.01	0.52	<0.01	0.05
S.LATISSIMA	<0.01	<0.01	0.89	0.04	2.11	0.02	0.21
WILD SP.	<0.01	0.02	0.63	0.02	0.71	<0.01	0.05
WRACK.FRASS	<0.01	6.94	11.26	0.59	16.1	0.71	2
	16:1n-9	**16:1n-7**	**18:1n-11**	**18:1n-9**	**18:1n-7**	**20:1n-11**	**20:1n-9**
A.ESCULENTA	0.04	0.13	<0.01	0.79	0.03	<0.01	<0.01
FUCUS SP.	<0.01	0.19	<0.01	3.54	0.06	0.06	<0.01
L.DIGITATA FARMED	0.04	0.33	<0.01	1.01	0.03	<0.01	<0.01
L.DIGITATA FROND	<0.01	0.27	<0.01	0.73	0.03	<0.01	0.01
L.DIGITATA STIPE	<0.01	0.15	<0.01	0.55	0.02	<0.01	<0.01
S.LATISSIMA	<0.01	0.36	<0.01	2.68	0.06	<0.01	<0.01
WILD SP.	<0.01	0.23	<0.01	1.52	0.06	0.02	0.02
WRACK.FRASS	0.2	5.82	<0.01	41.49	1.95	0.16	1.76

SPECIES	24:1N-9	16:2N-4	16:3N-3	16:4N-3	18:2N-6	18:3N-6	18:3N-3
A.ESCULENTA	<0.01	0.02	<0.01	<0.01	0.38	0.07	0.73
FUCUS SP.	<0.01	0.02	<0.01	0.02	1.01	0.08	0.81
L.DIGITATA FARMED	<0.01	0.01	<0.01	<0.01	0.34	0.06	0.27
L.DIGITATA FROND	<0.01	<0.01	<0.01	<0.01	0.21	0.02	0.17
L.DIGITATA STIPE	<0.01	<0.01	<0.01	<0.01	0.11	<0.01	0.03
S.LATISSIMA	<0.01	0.02	<0.01	<0.01	0.75	0.11	0.34
WILD SP.	<0.01	<0.01	<0.01	<0.01	0.34	0.02	0.21
WRACK.FRASS	<0.01	<0.01	<0.01	<0.01	7.05	0.31	2.92
	20:3n-6	20:4n-6	20:4n-3	20:5n-3	22:4n-6	21:5n-3	22:5n-6
A.ESCULENTA	0.04	1.04	0.03	0.71	<0.01	<0.01	<0.01
FUCUS SP.	0.1	1.74	0.06	1.19	0.17	<0.01	<0.01
L.DIGITATA FARMED	0.02	0.78	0.03	0.74	<0.01	<0.01	<0.01
L.DIGITATA FROND	0.01	0.31	0.02	0.52	<0.01	<0.01	<0.01
L.DIGITATA STIPE	0.01	0.37	<0.01	0.17	<0.01	<0.01	<0.01
S.LATISSIMA	0.05	0.58	0.03	0.38	<0.01	<0.01	<0.01
WILD SP.	0.03	0.48	0.02	0.33	<0.01	0.07	<0.01
WRACK.FRASS	0.56	11.47	0.23	4.75	0.36	<0.01	<0.01